September 2016 Vol.15 No. 9 *ISSN 1582 - 9596*

Environmental Engineering and Management Journal

An International Journal

Founding Editor: Matei Macoveanu

Editor-in-Chief: Maria Gavrilescu

Guest Editors:
Fabio Fava
Grazia Totaro
Maria Gavrilescu

Material & Energy Recovery and Sustainable Development
ECOMONDO 2015

“Gheorghe Asachi” Technical University of Iasi

Environmental Engineering and Management Journal

Environmental Engineering and Management Journal encourages initiatives and actions concerning the improvement of education, research, marketing and management, in order to achieve sustainable development. This journal brings valuable opportunities for those offering products, technologies, services, educational programs or other related activities, creating thus a closer relation with the request of the market in the fields of environmental engineering, management and education.

This journal address researchers, designers, academic staff, specialists with responsibilities in the field of environmental protection and management from government organizations (central and local administrations, environmental protection agencies) or from the private or public companies. Also, graduates of specialization courses or of the Environmental Engineering and Management profile, as well as other specialists may find in this journal a direct linkage between the offer and request of the market concerned with the protection of the environment and the administration of natural resources in the national and international context.

The journal was conceived as a means to develop scientific and technical relationships between people who offer and request solutions for environmental protection and conservation of natural resources, creating thus the premises to enhance the transfer of technology and know-how, the confirmation and implementation of ecological products and services.

Taking into consideration these aspects, we gladly welcome any persons or companies which correspond to the above-mentioned purposes and objectives to use our journal to identify potential collaborators.

For subscriptions and orders please contact us at:
OAIMDD (*Academic Organization for Environmental Engineering and Sustainable Development*),
73 Mangeron Blvd., PO 10, Box 2111, 700050 Iasi, Romania, CIF: 10150285

Phone/Fax: 0040-232-271759
E-mail: bmrobu.at.gmail.com, mgav_eemj.at.yahoo.com

In LEI:
ALPHA BANK ROMANIA (ABR)
SWIFT Code: BUCUROBU
IBAN: RO87BUCU1032235333663RON
Beneficiary: OAIMDD-EEMJ
Adress: PO 10, Box 2111, 700050 Iasi, Romania

In EURO:
ALPHA BANK ROMANIA (ABR)
SWIFT Code: BUCUROBU
IBAN: RO79BUCU1031215940036EUR
Beneficiary: OAIMDD-EEMJ
Adress: PO 10, Box 2111, 700050 Iasi, Romania

Department of Environmental Engineering and Management and
EcoZone Publishing House - O.A.I.M.D.D.
73 Prof.Dr.docent Dimitrie Mangeron Street, 700050-Iasi, Romania
Phone/Fax: 0040-232-271759

Environmental Engineering and Management Journal

EDITORIAL BOARD

Founding Editor:

Matei Macoveanu, *Gheorghe Asachi* Technical University of Iasi, Romania

Editor-in-Chief:

Maria Gavrilescu, *Gheorghe Asachi* Technical University of Iasi, Romania

Editorial Assistants:

Raluca-Maria Hlihor, *Gheorghe Asachi* Technical University of Iasi, Romania
Laura Bulgariu, *Gheorghe Asachi* Technical University of Iasi, Romania

SCIENTIFIC ADVISORY BOARD

Maria Madalena dos Santos Alves
University of Minho, Braga
Portugal

Abdeltif Amrane
University of Rennes, ENSCR
France

Ecaterina Andronescu
University *Polytechnica* of Bucharest
Romania

Robert Armon
Technion-Israel Institute of Technology, Haifa
Israel

Adisa Azapagic
The University of Manchester
United Kingdom

Hamidi Abdul Aziz
Universiti Sains Malaysia, Penang
Malaysia

Pranas Baltrenas
Vilnius *Gediminas* Technical University
Lithuania

Hans Bressers
University of Twente, Enschede
The Netherlands

Han Brezet
Delft University of Technology
The Netherlands

Dan Cascaval
Gheorghe Asachi Technical University of Iasi
Romania

Aleg Cherp
Central European University, Budapest
Hungary

Yusuf Chisti
Massey University, Palmerston North
New Zealand

Philippe Corvini
University of Applied Sciences Northwestern Switzerland,
Muttenz, Switzerland

Igor Cretescu
Gheorghe Asachi Technical University of Iasi
Romania

Silvia Curteanu
Gheorghe Asachi Technical University of Iasi
Romania

Andrew J. Daugulis
Queen's University Kingston
Canada

Valeriu David
Gheorghe Asachi Technical University of Iasi
Romania

Katerina Demnerova
University of Prague
Czech Republic

Gheorghe Duca
State University of Moldavia, Kishinew
Republic of Moldavia

Emil Dumitriu
Gheorghe Asachi Technical University of Iasi
Romania

Jurek Duszczyk
Delft University of Technology
The Netherlands

Anca Duta Capra
Transilvania University of Brasov
Romania

Fabio Fava
Alma Mater Studiorum University of Bologna
Italy

Eugenio Campos Ferreira
University of Minho, Braga,
Portugal

Cristian Fosalau
Gheorghe Asachi Technical University of Iasi
Romania

Anton Friedl
Vienna University of Technology
Austria

Anne Giroir Fendler
University *Claude Bernard* Lyon 1
France

Ion Giurma
Gheorghe Asachi Technical University of Iasi
Romania

Yuh-Shan Ho
Peking University
People's Republic of China

Arjen Y. Hoekstra
University of Twente, Enschede
The Netherlands

Nicolae Hurduc
Gheorghe Asachi Technical University of Iasi
Romania

Ralf Isenmann
Munich University of Applied Sciences
Germany

Marcel Istrate
Gheorghe Asachi Technical University of Iasi
Romania

Ravi Jain
University of Pacific, Baun Hall Stockton
United States of America

Michael Søgaard Jørgensen
Aalborg University
Denmark

Nicolas Kalogerakis
Technical University of Crete, Chania
Greece

Gheorghe Lazaroiu
University *Polytechnica* of Bucharest
Romania

Thomas Lindhqvist
International Institute for Industrial Environmental
Economics, Lund University, Sweden

Andreas Paul Loibner
University of Natural Resources and Life Sciences,
Vienna, Austria

Tudor Lupascu
Academy of Sciences, Institute of Chemistry, Kishinev,
Republic of Moldavia

Antonio Marzocchella
University of Naples *Federico II*,
Naples, Italy

José Mondéjar Jiménez
University Castilla-La Mancha, Cuenca
Spain

Shin' ichi Nakatsuji
University of Hyogo
Japan

Valentin Nedeff
Vasile Alecsandri University of Bacau
Romania

Alexandru Ozunu
Babes-Bolyai University of Cluj-Napoca
Romania

Yannis A. Phillis
Technical University of Crete, Chania
Greece

Marcel Ionel Popa
Gheorghe Asachi Technical University of Iasi
Romania

Marcel Popa
Gheorghe Asachi Technical University of Iasi
Romania

Valentin I. Popa
Gheorghe Asachi Technical University of Iasi
Romania

Tudor Prisecaru
University *Polytechnica* of Bucharest
Romania

Gabriel-Lucian Radu
Polytechnica University of Bucharest
Romania

Ákos Rédey
Pannon University, Veszprém
Hungary

Joop Schoonman
Delft University of Technology
The Netherlands

Dan Scutaru
Gheorghe Asachi Technical University of Iasi
Romania

Bogdan C. Simionescu
Gheorghe Asachi Technical University of Iasi
Romania

Florian Statescu
Gheorghe Asachi Technical University of Iasi
Romania

Carmen Teodosiu
Gheorghe Asachi Technical University of Iasi
Romania

Saulius Vasarevicius
Vilnius Gediminas Technical University
Lithuania

Angheluta Vadineanu
The University of Bucharest
Romania

Colin Webb
The University of Manchester
United Kingdom

Peter Wilderer
Technical University Munich
Germany

Petra Winzer
Bergische University Wuppertal
Germany

Environmental Engineering and Management Journal

Environmental Engineering and Management Journal is included and indexed in

CABI
Chemical Abstracts Service/SciFinder (ACS) (since 2002)
EBSCO Database (since 2002)
EVISA
ICAAP (International Consortium for Advancement of Academic Publications)
Index Copernicus Journal Master List (**ICV/2012=16.20**)
Journal Citation Reports® 2015 Edition (**IF=1.065**), (*Environmental Sciences*, Ranked **159 out of 221**; 5-Year Impact Factor: 0.806
Article Influence® Score: 0.069)
MedSci
ProQuest (since 2002)
The National University Research Council (RO)
Science Citation Index Expanded™ (Thomson ISI)
SJR (SCImago Journal&Country Rank) (*Environmental Sciences*, **SJR index/2014 = 0.311**; **SNIP index/2014 = 0.578;**
IPP index/2014=0.709; Ranked **653 out of 1174**, H=20)
SCOPUS (since 2008)
Thomson ISI Master Journal List
Web of Science® (Thomson ISI) (**H=22**)

Home page: http://omicron.ch.tuiasi.ro/EEMJ/
Full text: *http://www.ecozone.ro*

Environmental Engineering and Management Journal is edited by
***Gheorghe Asachi* Technical University of Iasi and *EcoZone* Publishing House of the *Academic Organization for Environmental Engineering and Sustainable Development* (O.A.I.M.D.D.)**

Editorial and Production Office:
Department of Environmental Engineering and Management - Faculty of Chemical Engineering and Environmental Protection and EcoZone-O.A.I.M.D.D., 73 *Prof.Dr.docent Dimitrie Mangeron* Street, 700050 Iasi, Romania
Phone: +40-232-278680, ext. 2259, 2137
Fax: +40-232-271759
e-mail: eemjournal.at.yahoo.com, eem_journal.at.yahoo.com, eemjeditor.at.yahoo.com, eemj_editor.at.yahoo.com, eemjournal.at.gmail.com, eemj.editor.at.gmail.com, eemj.office.at.gmail.com

Published 12 issues per year, **under the aegis of the** ***"Gheorghe Asachi" Technical University of Iasi*, Romania**
by *EcoZone* Publishing House of the *Academic Organization for Environmental Engineering and Sustainable Development* (O.A.I.M.D.D.), **http://www.ecozone.ro**

Annual subscription rate 2015 (12 issues)

Print:		**Electronic:**
EURO	500 per volume	400 per volume
EURO	50 per issue	40 per issue

Order directly to the Editorial Office
73 *Prof.Dr.docent Dimitrie Mangeron* Street, 700050 Iasi, Romania
Phone/Fax: Fax: +40-232-271759
e-mail: bmrobu.at.gmail.com, eemj.office.at.gmail.com

Electronic, full text:
Order or purchase on-line at: **www.ecozone.ro**

Bank account (EURO):
ALPHA BANK ROMANIA (ABR)
SWIFT Code: BUCUROBU,
IBAN: RO79BUCU1031215940036EUR
Beneficiary: OAIMDD-EEMJ, Adress: PO 10, Box 2111, 700050 Iasi, Romania

Bank account (LEI):
ALPHA BANK ROMANIA (ABR), IBAN: RO87BUCU1032235333663RON,
Beneficiary: OAIMDD-EEMJ

Environmental Engineering and Management Journal
September 2016, Vol.15, No. 9, 1905-2134
http://omicron.ch.tuiasi.ro/EEMJ/

"Gheorghe Asachi" Technical University of Iasi, Romania

CONTENTS

ICEEM09 – First Call

Editorial

Special issue papers

Current papers

ICEEM 09

Circular Economy and Environmental Sustainability

6 - 9 September 2017, Bologna, Italy

Location

The ICEEM 09 Conference will be hosted by the Alma Mater Studiorum Università di Bologna, School of Engineering and Architecture, Italy between 6 and 9 of September 2017

Abstract Submission

The **extended abstract submission** should be done **no later than 20 January 2017,** using the template available on www.iceem.ro. After the review process, authors will be notified for participation and the accepted abstracts (max 2 pages) will be published electronically in a Book of Abstracts (available in the Conference folder).

The 2nd Conference Call and decisions on **abstract acceptance will be available after 10 April 2017.**

Participant Registration and Fee Payment - starting from 15 May 2017

Selected papers by the International Scientific Committee will be published in special issues of the peer-reviewed international journals:

- New Biotechnology (ISI ranked, Elsevier) ;
- Process Safety and Environmental Policy (ISI ranked, Elsevier);
- Sustainable Production and Consumption (ISI, Elsevier);
- Environmental Engineering and Management Journal (ISI ranked, TUIASI).

ICEEM09 Conference language is English.

Fees

The fees for the ICEEM09 Conference will include the conference materials (Program, Abstracts book), coffee breaks, lunches and conference dinner.

- Full fee: **400 EUR until July 15 2017**
- Full fee: **450 EUR after July 16 2017**

ICEEM09 registration and fee payment details will be available on www.iceem.ro

Contacts

For details please visit the conference site: www.iceem.ro, or contact the Conference Organizing Committee: **conference@iceem.ro**

Gheorghe Asachi Technical University of Iasi
Faculty of Chemical Engineering and Environmental Protection
Department of Environmental Engineering and Management

Alma Mater Studiorum Università di Bologna
DICAM - Department of Civil, Chemical, Environmental and Materials Engineering

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA
DIPARTIMENTO DI INGEGNERIA CIVILE, CHIMICA, AMBIENTALE E DEI MATERIALI

European Federation of Biotechnology
Environmental Biotechnology Section

EUROPEAN FEDERATION OF
BIOTECHNOLOGY

envbiotech

9th INTERNATIONAL CONFERENCE ON ENVIRONMENTAL ENGINEERING AND MANAGEMENT

Circular Economy and Environmental Sustainability

1st Call for Papers

ICEEM / 09

6 - 9 September 2017, Bologna, Italy

www.iceem.ro

Partners:

- InterMEDIU - Information, Consultancy and DL Department, TUIASI, Romania
- Academic Organization for Environmental Engineering and Sustainable Development (OAIMDD), Iaşi, Romania

Foreword

The **International Conference on Environmental Engineering and Management (ICEEM)** is an important dissemination platform that strives at each edition to bring together specialists to discuss novel research and to develop inter- and multidisciplinary project ideas in the wide field of environmental sustainability.

The ninth edition of ICEEM will be held at the *Alma Mater Studiorum* Università di Bologna, Italy. ICEEM09 focuses on the themes of Circular Economy and Environmental Sustainability, the new European paradigms for economic development . Thus, ICEEM09 strongly encourages researchers, members of the academia, industry and society representatives to contribute with presentations that focus on innovation, multidisciplinarity and cross-sectorial approaches related to environmental problems and sustainability.

ICEEM09 fosters high quality research dissemination and thus participation is based on a peer-review abstract selection. Selected contributions by the International Scientific Committee will be published as full papers in Special Issues of 4 peer-reviewed ISI ranked international journals.

We hope that the plenary sessions, oral and poster presentations, workshops and side-events of ICEEM 09 will enhance multidisciplinarity, international cooperation, knowledge transfer and effective communication of scientists, engineers and managers.

Conference presidents

Prof.dr.,dr.h.c. Carmen Teodosiu
"Gheorghe Asachi" Technical University of Iasi

Prof.dr.,dr.h.c. Fabio Fava
Alma Mater Studiorum Università di Bologna

Prof.dr.,dr.h.c. Philippe Corvini
FHNW & European Federation of Biotechnology

Themes, Topics & Events

I. Environmental biotechnology in the circular economy
- Marine biotechnologies
- CO2 bio-fixation
- Biomineralization for remediation and valorisation
- Microbiome in the critical zone
- Bioelectrochemical systems

II. Water and wastewater cycles: technologies, recycling and valorisation
- Water and wastewater treatment
- Nutrient recovery
- Water and sludge reuse applications

III. Sustainable waste management and exploitation for chemicals, materials and energy recovery
- Waste treatment and valorisation
- Site bioremediation and rehabilitation
- End-of-waste alternatives for new by-products and fuels (biowaste, bio-refineries)

IV. Sustainability assessments and eco-innovation
- Life cycle assessments
- Footprint indicators
- Environmental and human health-related impact and risk evaluations
- Eco-design, eco-innovation and eco-entrepreneurship

V. Novel materials for environmental and energy applications
- Solar energy materials
- Materials, eco-processes and eco-products

VI. Monitoring and modelling of environmental pollution
- Process modelling, optimization and simulation;
- Investigation and prognosis tools for environmental issues;
- Pollution monitoring and control

VII. Events
- Key note lectures & Plenary Sessions
- Seminars & Project Meetings
- Thematic Workshops for mentoring young scientists,
- Posters Sessions & Exhibitions
- Social Events

International Scientific Committee

Prof.dr., dr.h.c. Maria Madalena dos Santos Alves
University of Minho, Portugal
Prof.dr. Spiros N. Agathos
University of Louvain, Belgium
Prof.dr, dr.h.c. Adisa Azapagic
The University of Manchester, UK
Dr. Federico Aulenta
Consiglio Nazionale delle Ricerche, Italy
Dr. Catia Bastioli
Novamont SpA, Novara, Italy
Prof. Dr. Alberto Bertucco
University of Padova, Italy
Prof.dr. Hans Bressers
University of Twente, The Netherlands
Prof.dr. Han Brezet
Delft University of Technology, The Netherlands
Prof. Dr. Alberto Brucato
University of Palermo, Italy
Prof.dr. Dan Cascaval
"Gheorghe Asachi"Technical University of Iasi, Romania
Prof.dr. Francesc Castells Piqué
University of Rovira i Virgili Tarragona, Spain
Prof. dr., dr.h.c. Yusuf Chisti
Massey University, New Zealand
Prof.dr. Cristina Costache
Politehnica University, Bucharest, Romania
Prof.dr. Igor Cretescu
"Gheorghe Asachi" Technical University of Iasi, Romania
Prof.dr. Daniele Daffonchio
King Abdullah University of Science and Technology, Kingdom of Saudi Arabia
Prof.dr. Ludo Diels
University of Antwerp, Belgium
Prof.dr. Francesco Fatone
Universita Degli Studi di Verona, Verona, Italy
Prof.dr. Silvia Fiore
Politecnico di Torino, Italy
Prof.dr.,dr.h.c. Anton Friedl
Vienna University of Technology, Austria
Prof.dr. Maria Gavrilescu
"Gheorghe Asachi" Technical University of Iasi, Romania
Prof.dr. Joan Garcia
Universitat Politecnica de Catalunya, Spain
Prof.dr. Almudena Hospido
University of Santiago de Compostela, Spain
Prof.dr.Nicolae Hurduc
"Gheorghe Asachi"Technical University of Iasi, Romania
Prof.dr. Ovidiu Gabriel Iancu
Al.I.Cuza University Iasi, Romania
Prof.dr., dr.h.c. Michael Jørgensen
Aalborg University, Denmark
Prof.dr. Nikolas Kalogerakis
Technical University of Crete, Greece
Prof.dr. Emmanuel G. Koukios
National Technical University of Athens, Greece
Prof.dr. Gabriel Lazar
Vasile Alecsandri University of Bacau, Romania
Dr. Markus Lenz
University of Applied Sciences and Arts Northwestern Switzerland, Switzerland
Prof.dr. Florica Manea
Politehnica University of Timisoara, Romania
Prof.dr. Mauro Majone
Universita degli Studi di Roma La Sapienza, Italy
Prof.dr. Antonio Marzocchella
University Federico II, Naples,Italy
Prof.dr. Rainer Meckenstock
Universität Duisburg-Essen, Germany
Prof.dr. Korneliusz Miksch
Silesian University of Technology, Poland
Prof.dr. Mircea Nicoara
Al.I.Cuza University, Iasi, Romania
Prof.dr. Alexandru Ozunu
Babeş Bolyai University, Cluj Napoca, Romania
Prof.dr., dr.h.c. Ákos Rédey
Pannonia University, Veszprem, Hungary
Prof.dr. Maria Reis
FCT, Universidade NOVA de Lisboa,Portugal
Prof.dr. Sebastien Royer
Universite de Lille, France
Prof.dr. Guido Saracco
Centre for Sustainable Futures CSF@PoliTo, Italy
Prof.dr., dr.h.c. Wilhelm Schabel
Karlsruhe Institute of Technology,Germany
Acad.prof.dr. Bogdan Simionescu
"Gheorghe Asachi" Technical University of Iasi, Romanian Academy, Romania
Prof.dr. Fabio Trincardi
ISMAR-CNR Headquarters, Italy
Dr. Magdalena Urbaniak
European Regional Centre for Ecohydrology, Polish Academy of Sciences, University of Lodz
Prof.dr., dr.h.c. Angheluta Vadineanu
University of Bucharest, Romania
Prof.dr. Ion Visa
Transilvania University of Brasov,Romania
Prof.dr. Wei Zhang
Flinders University, Australia

Local Scientific & Organising committees

Prof.Dr. Alessandra Bonoli
Prof.Dr. Alberto Montanari
Prof.Dr. Ezio Mesini
Prof.Dr. Alessandro Paglianti
Prof.Dr. Vittorio Di Federico
Assoc. Prof.Dr. Lorenzo Bertin
Assoc. Prof.Dr. Maria Bignozzi
Assoc. Prof.Dr. Alessandro Tugnoli
Dr. Dario Frascari
Dr. Giulio Zanaroli
Dr. Cristiana Boi
Dr. Noura Raddadi
Dr. Andrea Negroni
Assoc.Prof.Dr. Brindusa Sluser
Dr. George Barjoveanu
Dr. Adela Buburuzan
Dr. Daniela Gavrilescu
Dr. Daniela Fighir
Dr. Corina Musteret
Dr. Petru Apopei
Dr. Camelia Smaranda
Dr. Gabriela Soreanu
Dr. Raluca Hlihor
Dr. Isabela Simion
Drd. Irina Morosanu
Drd. Diana Comanita

Environmental Engineering and Management Journal
September 2016, Vol.15, No. 9, 1905-1906
http://omicron.ch.tuiasi.ro/EEMJ/

“Gheorghe Asachi” Technical University of Iasi, Romania

EDITORIAL

Material & Energy Recovery and Sustainable Development ECOMONDO 2015

19th International Trade Fair of Material & Energy Recovery and Sustainable Development

The contributions in the form of Full papers collected in this Special Issue of *Environmental Engineering and Management Journal* were selected from lectures and posters of the scientific and technical conferences hosted by *Ecomondo 2015* held in Rimini, Italy, 3–6 November, 2015 (http://en.ecomondo.com).

Ecomondo is the most recognized platform for the Mediterranean basin, in terms of materials exploitation and recycling for the large industry of the future, which will be part of *Green and Circular Economy*, ensuring a weighted and rewarding balance between sales dimension and technical-scientific dimension, with extensive room dedicated to research and innovation.

As with the previous editions, the aim of Ecomondo 2015 was to explore recent industrial advances and opportunities in waste production reduction, waste collection, fractionation, recycling, exploitation; biomass and biowaste exploitation via integrated biorefinery scheme, bioplastics, biobased products and biomethane; green and sustainable chemistry; water resources monitoring and protection; wastewater treatment and valorization with nutrients recovery and marine resources protection; sustainable remediation of contaminated sites and marine ecosystems; indoor and outdoor air monitoring and clean up; the state of the art and perspectives of the environmental footprint and the Industrial Symbiosis.

A special emphasis was given to European Eco- Innovation in the broad field of green and circular economy. Some of the initiatives were dedicated to the promotion of industrial symbiosis and networking i.e. the creation of partnerships between industries working in the complementary sectors or different countries and to the presentation of the most prominent international and EU RTD and industrial projects.

The areas of particular interest addressed within *Ecomondo 2015* are:

- waste characterization, management, recycling and exploitation (*Waste)*
- monitoring, management and treatment/ exploitation of industrial and civil wastewaters *(Blue Gold)*
- the sustainable and biomass-based chemical industry, with a special focus on the Join Technology Initiative Bio Based Industry (JTI BBI) and biomethane production (*Biobased Industry*)
- monitoring and treatment of air pollution (*Air*)
- the environmental footprint (*Eco-Innovation*)

Ecomondo 2015 hosted over than 100 conferences, more than 500 oral communications and almost 90 papers. This special issue provides some of the key information presented and discussed in the frame of some of such conferences. Over 103,000 delegates from 40 different nations were participating in the event.

We believe that this collection of papers will be useful to people who could not able to participate directly. It is primarily towards those individuals that it is directed, but it also aspires to provide permanent records in process of turning environmental problems and challenges into new opportunities for a green economy with a bright future.

Guest editors:
Prof. Fabio Fava
Alma Mater Studiorum - Università di Bologna
(University of Bologna), Bologna, Italy
Dr. Grazia Totaro
Alma Mater Studiorum - Università di Bologna
(University of Bologna), Bologna, Italy
Prof. Maria Gavrilescu
„Gheorghe Asachi” Technical University of Iasi, Romania

Fabio Fava, born in 1963, is Full Professor of “Industrial & Environmental Biotechnology” at the School of Engineering of University of Bologna since 2005.

He coordinated the FP7 projects (collaborative projects) NAMASTE (on the integrated exploitation of citrus and cereal processing byproducts with the production of food ingredients and new food products) and BIOCLEAN (aiming at the development of biotechnological processes and strategies for the bioremediation and the tailored depolymerization of major oil-deriving plastics). He also coordinates/coordinated the Unit of the University of Bologna participating/participated in the FP7 projects (collaborative projects) ECOBIOCAP and ROUTES (on the production of microbial polymers from different organic waste and food processing effluents), MINOTAURUS and WATER4CROPS (on the intensified bioremediation of contaminated waste- and groundwater and the integrated valorization and decontamination of wastewater coming from the food processing industry and biorefinery), and ULIXES and KILL SPILL (on the development of strategies for intensifying the in situ bioremediation of marine sediments contaminated by (chlorinated)hydrocarbons and the isolation and industrial exploitation of microbes from such contaminated matrices). He also participated in the FP7 BIORICE addressed to produce added value bioactive ingredients (semi-purified digestates and small molecular weight peptides) starting from protein by-products contained in the processing water of the rice starch production stream. He is vice-chairman of the “Environmental Biotechnology” section of the European Federation of Biotechnology (EFB). He is the Representative of the Italian Ministry of Education, University and Research in the "Working Party on Biotechnology, Nanotechnology and Converging Technologies" at OECD (Organisation for Economic Co-operation and Development), Paris, in the "European strategy for the Adriatic and Ionian Region" (EUSAIR) and in the "Western Mediterranean Initiative" (WEST MED). Further, he joined the "High Level Group on Key Enabling Technologies" and he is member of the "Expert Group on biobased products", both at the DG-GROW of European Commission (Bussels). Further, he is member of the Scientific Committee of the Joint Programming Initiative on Agriculture, Food Security and Climate Change (FACCE-JPI). Finally, he is the Italian Representative at the European Commission (Brussels) in the Horizon2020 Programme Committee of Societal Challenge 2: European Bioeconomy Challenges: Food Security, Sustainable Agriculture and Forestry, Marine, Maritime and inland water research" (DG RTD) and in the BLUEMED initiative (Chair of the Strategic Board) (DG RTD and DG MARE), and in the "State Representative Group" (as vice Chair) of the Public Private Partnership "Biobased Industry" (BBI JU) (Brussels).

Grazia Totaro, born in 1976, has a degree in Chemistry (University of Ferrara), a Master’s Degree in Science, Technology & Management with a specialization in Environmental Chemistry (University of Ferrara) and a PhD in Materials Engineering, about modification, characterization and applications of technopolymers (University of Bologna).

She worked at the R&D Centre of Basell Polyolefins in Ferrara for 2 years in the frame of a project addressed to the development of a novel methodology for qualitative and quantitative analysis of additives in polymers. She also worked at ARPA, Regional Agency for Environment in Ferrara, division Water Analysis. Then she started working at the school of Engineering of the University of Bologna for a Ph.D. in Materials Engineering (2007-2010). After that she had a scholarship "Spinner 2013" in cooperation with Reagens spa (San Giorgio di Piano) on novel PVC nanocomposites. Now she is post doc fellow at the same school on new polymer-based nanocomposites from renewable sources and inorganic fillers. She also worked at the laboratoire de Chimie et Biochimie Pharmacologique et Toxicologique (Université Réné Descartes) in Paris in 2001 and was visiting professor at the Ecole Nationale Superieure de Chimie (Université Blaise Pascal, Clermont Ferrand, FR) in 2012 and 2015.

Dr. Totaro has about 20 scientific papers and several participations at conferences and scientific schools. She collaborates on Ecomondo from 2013.

Maria Gavrilescu (born in 1956) is professor at the Department of Environmental Engineering and Management–Faculty of Chemical Engineering and Environmental Protection of the „*Gheorghe Asachi*” Technical University of Iasi, Romania, where she teaches courses in Chemical and Biological Process Engineering, Process Synthesis and Analysis, Sustainable Industrial Production, Environmental Risk Assessment and Management. She supervises doctoral programs in the field of Environmental Engineering.

Prof. Gavrilescu has authored more than 350 publications as books and articles in the fields of chemical and environmental engineering, pharmaceutical and environmental biotechnology, pollution prevention and cleaner production, risk assessment and management. Prof. Gavrilescu has also managed or participated in 18 national and 7 international research projects and 25 research projects with industrial companies. The scientific visibility is illustrated by over 2000 citations (WOS h-index 24, Scopus h-index 24).

The professional memberships includes the followings: 2012-Founder of the *Romanian Society of Environmental Science and Engineering*; Member of: the Editorial Advisory Board of six international journals; Romanian Society of Chemistry; European Federation of Biotechnology-*member of Experts Board* of the Environmental Biotechnology Section.

Prof. Gavrilescu is the Editor-in-Chief of the *Environmental Engineering and Management Journal* edited by the *Gheorghe Asachi* Technical University of Iasi, Romania; Editor-in-Chief of *Procedia-Environmental Science, Engineering and Management,* edited by the *National Society of Environmental Science and Engineering,* Romania; Series Editor of *Annals of the Academy of Romanian Scientists* – Series of *Chemistry Sciences*.

Environmental Engineering and Management Journal

September 2016, Vol.15, No. 9, 1907-1914
http://omicron.ch.tuiasi.ro/EEMJ/

"Gheorghe Asachi" Technical University of Iasi, Romania

VALORIZATION OF TYRES WASTE PYROLYSIS RESIDUE IN LIGHTWEIGHT MATERIALS

Fernanda Andreola*, Luisa Barbieri, Isabella Lancellotti

Department of Engineering "Enzo Ferrari", University of Modena and Reggio Emilia, via P. Vivarelli, 10, 41125 - Modena, Italy

Abstract

The aim of this study is to investigate an innovative way to utilize the char obtained from tyres pyrolysis, in order to realize light-weight materials for building. Pyrolysis residue, mixed with three types of waste glass, has been used as raw material to obtain lightweight materials, exploiting its capability to behave as foaming agent. The results demonstrate that the pyrolysis char is a good foaming agent for the mixtures 50/50 with glassy sand and packaging glass waste, thermal treated at 900°C for 45 minutes with degassing isotherm (500°C, 60 min). At these conditions, the samples obtained showed thermal behavior intermediate between an insulating commercially brick and a commercial refractory such as apparent density values lower than 1 g/cm^3, compared to traditional ceramic material where density values are higher than 2 g/cm^3. It can therefore be concluded that the materials produced have suitable properties for use in buildings as thermal and acoustic insulating.

Key words: char, lightweight materials, pyrolysis, tyre waste

Received: January, 2016; Revised final: June, 2016; Accepted: July, 2016

1. Introduction

The world-wide annual production of tyres is currently about 1.5 billion units which corresponds to an estimated 17 million tonnes of used tyres each year (ETRA, 2013; Sienkiewicz, 2012). These volumes are increasing due to both the number of vehicles produced and to the increased traffic in the developing countries as China. The management of waste tyres in the European Union has been regulated under the End of Life Vehicle Directive (EC Directive, 2000), which stipulates the separate collection of tyres from vehicle dismantlers and encourages their recycling. In addition, the EU Waste Landfill Directive has banned the disposal on landfill of used tyres (Sienkiewicz, 2012).

In each year in the EU Member States and Norway over 300 million of tyres (ETRA, 2013) are permanently removed from off-road vehicles and trucks and defined as waste. In some regions, End of Life Tyres (ELT) coming from agricultural, mining vehicles and airplanes are not included in the waste count. In other regions they are managed for recycling. Currently, recycling, recovery, reuse and retreading contribute to 96% of the total amount of used tyres recovered and only the 4% of ELT collected are dumped in landfills or have unknown recovery routes.

The recovery of tyre waste is divided into two different ways: material recovery and energy recovery. Today in Europe the recovery of materials is higher than the energy recovery in line with the European directive. In Italy it is still focused mainly on energy recovery, which currently accounts to about 59%. The recycling of the materials, which can be recovered from ELT, can be made from entire or from granulates tyres which is most utilized. Different techniques are available in order to crush the tyres to a suitable particle size: Mechanical Crushing, Cryogenic Crushing and Electro-thermal process (Reschner, 2015).

The best technological applications for

---

* Author to whom all correspondence should be addressed: e-mail: andreola.fernanda@unimore.it; Phone: +39- 059-2056237; Fax: +39- 059-2056243

material recovery are connected to road constructions, railroads and underground (asphalts, anti-vibrating materials utilization); building sector (acoustic isolation); street and pedestrian paving; sport sector (soccer field and athletic lanes) and fashion manufacturing (bags, belts, shoes soil etc.) (Bonoli et al., 2012; Moo-Young et al., 2003; Szodrai and Lakatos, 2014). Regarding energy recovery, the main applications are co-combustion in cement kilns (92%), incineration, co-combustion with coal (thermic central), co-combustion in paper manufacturing etc. (Miglore et al., 2015; Rada et al., 2012; http://www.ecopneus.it).

There is great interest in alternative treatment processes to convert scraps tyres in valuable products and energy source. Gasification and pyrolysis are emerging technologies. Gasification is a thermo chemical pathways used to convert carbonaceous feedstock into syngas (CO and H_2) in deprived oxygen environment. The process can accommodate conventional feedstock such as coal as well as discarded waste including plastics, rubber, and even municipal solid wastes (MSW) due to the high reactor temperature of 1000 to 1600°C.

Pyrolysis of tyres consists of a thermal decomposition of the organic components in an inert atmosphere, obtained by means of indirect heating, which occurs at about 400-600°C. The products obtained are a solid, containing carbon and ash (char), a liquid (oil) and a gas (syngas). The percentage of the three fractions depends on temperature, pressure, residence time in the reactor, condensing temperature and the type of plant (Williams, 2013). The gases produced in the process can be used to provide the heating energy for continuing the process.

There is no possibility to detect any heavy metals from the pyrolysis products since absent in-the fed material. Furthermore, the characteristic absence of oxygen in the process inhibits the formation of dioxins, carbon monoxide and carbon dioxide thus eliminating the problems of incineration and allow to reduce pollutants (Benelli et al., 2013).

For this study a char, produced by a pilot plant in Emilia Romagna (Italy), was used. The technology, that provided this material, is very innovative because it uses whole tires in the pyrolysis reactor, avoid the preventive shredding of material and reduce the financial and energy investments (Giorgini et al., 2013, 2015). Currently, the solid char material, obtained from the pyrolysis process, has high carbon content and it is usually used as substitute of carbon or undergone to a gasification process as combustible gas.

Foam glass is generally obtained by the action of a gas-generating agent (foaming agent), which is ground together with the starting waste glass to a finely divided powder. The mixture of glass powder, foaming agent, and occasionally other mineral agents is then heat treated at a suitable temperature (in a range around the glass softening point which depend on the glass typology) to promote viscous flow sintering and the thermal decomposition of the foaming agent which causes the expansion of the structure. The properties of finished foamed glass products depend strongly on the type and quantity of the added foaming agents, on the initial size of the glass particles and on the heat treatments (Fernandes et al., 2013). In the past, glass foam was manufactured from virgin glass with corresponding raw materials, glass making plant and energy costs. Nowadays, the growing availability of waste glass and a better knowledge of process parameters permit to obtain cheaper lightweight materials (Bayer and Koese, 1979; Scheffler and Colombo, 2006).

The aim of this study is to investigate a new way to use the char obtained from tyres pyrolysis, and three types of post consumers' glasses as raw materials to obtain recycled foams glass.

The selected waste glasses are: post-consumer silicate-sodium-calcium glass from end of life fluorescent lamp glass (EOL_FLG), packaging waste glass (PG), produced from the waste glass discarded by glass recyclers after primary treatment, and commercial glassy sand (GS) derived from secondary treatment plant of packaging glass.

The route here proposed to obtain lightweight materials involves the addition of pyrolysis char residue, which acts as foaming agent, due to oxidation (combustion) process of organic carbon during the heat treatment. The release of gas (CO_2) inside the softened glass leads to the foam formation.

2. Experimental

2.1. Materials used

During the testing phase the following materials were used:

- end of life fluorescent lamp glass (EOL_FLG): non hazardous residue (EWC: 19 12 05);
- commercial glassy sand (GS): End of Waste derived from secondary treatment plant for packaging glass after claiming;
- glass waste coming from packaging glass primary treatment (PG): non hazardous residue (EWC: 19 12 05);
- tyre pyrolysis residue (char): residue derived from a pilot plant of *Curti Costruzioni Meccaniche S.P.A*, *Divisione Energia* (Faenza and Castel Bolognese, Italy) able to process whole tyres (up to 35 kg). The reaction chamber is initially flushed with nitrogen in order to remove air. The operative conditions are:
  - from -15 to +20 mbar, pressure in the chamber;
  - 8°C/min, heating rate;
  - 150 min, residence time at the maximum temperature
  - maximum temperature ranging from 400 to 600°C (Georgini et al., 2015).
- polyvinyl alcohol (Poliviol G 26/140) solution 10% v/v.

2.2. Raw materials characterization

Chemical analysis of the waste glasses was conducted by X-ray fluorescence spectrometer (PW1414, Philips, Eindhoven, The Netherlands). A thermal study of the waste glasses used and their mixes with char pyrolysis was performed by heating optical microscopy (HSM Misura® 3.32, Expert System). The chemical char characterization was performed by Elemental Analyzer Thermo EA 1110, CHNS Carlo Erba, (data reported in dry and ash free basis) and by Scanning Electron Microscopy (ESEM, QUANTA 200 FEI) coupled with an energy dispersion spectroscopy equipment (X-EDS Oxford INCA-350). Mineralogy was determined by powder diffractometer (PW3710, Philips) with Ni-filtered CuKα radiation, 5^o–70^o 2θ range. The crystalline phases identification was made for comparison with data on the JCPDS files and supported by PANANALITICAL High Score Plus software. Thermal properties of the char was performed by thermal analysis (TG/DTA, Netzch DSC 409) with a heating rate of 20°C/min up to 1300°C in air. Particle size distribution was measured by Laser diffraction particle size analyzer (Mastersizer 2000, Malvern).

2.3. Sample preparation

The samples preparation followed the same procedure for all mixes and consists in milling, sieving, mixing, pressing, drying and thermal treatment.

2.3.1. Milling and sieving

Milling was carried out in order to reduce the particle size of the base materials and to achieve greater homogeneity. This treatment was performed on glass waste coming from packaging glass treatment and for end of life fluorescent lamps glass. Glassy sand was supplied as granules and it was only sieved. The glasses were ground in a ball mill jar for 40 min. After milling the glasses and the glassy sand were sieved in order to obtain a material with a particle size <125 μm. Char residue was ground for 3h in a PET container with alumina grinding media using a powder/balls ratio of 4/1. It was not necessary to sieve the char powder, because by using this kind of procedure the particle size distribution of the powder (determined by laser particle size analyzer) was comparable with the three sieved glasses.

2.3.2. Mixing, pressing and drying

The samples were obtained by mixing different glass powders with pyrolysis residue, humidified by 5 wt% of water. The cylindrical samples (D: 40 mm) were obtained by uniaxial pressing. The first samples, containing 10% of pyrolysis char, were pressed at 40 MPa. The other all compositions were pressed at 30 MPa because the lower pressure resulted more suitable for obtaining porous materials. The samples obtained were dried in oven at 110°C for 24h in order to remove the humidity. Table 1 summarizes the different compositions prepared. After characterization, only for the best compositions, corresponding to 2c and 3b, rectangular samples (100x50mm^2) were realized, in which the water was replaced by a binder (10% PVA solution) to favor their handling.

Table 1. Different compositions prepared for each type of glass

MIX EOL_FLG (1)	*PYROLYSIS CHAR (%)*	*GLASS (%)*
1.a	10	90
1.b	20	80
1.c	50	50
MIX PG (2)	*PYROLYSIS CHAR (%)*	*GLASS (%)*
2.a	10	90
2.b	20	80
2.c	50	50
MIX GS (3)	*PYROLYSIS CHAR (%)*	*GLASS (%)*
3.a	20	80
3.b	50	50

2.3.3. Thermal treatments

For the thermal treatments, an electric furnace (Lenton AWF13/12 Tmax: 1300°C, UK) has been used with a heating rate of 5°C/min until 500°C, and 10°C/min up to maximum (foaming) temperature. In order to optimize the samples, different temperatures and times have been tested (700-1000°C; 30, 45, 60 min). According to literature, the slowly heating was used in order to maintain a uniform temperature distribution within the poorly conductive sample materials (Bayer and Koese, 1979).

Different types of firing cycles were tested:

- thermal treatment with insertion of the samples at the foaming temperature (treatment in a hot furnace) with soaking time;
- thermal treatment with insertion of the samples at room temperature (treatment in a cold furnace) and heating up to foaming temperature with soaking time;
- thermal treatment with insertion of the samples in a cold furnace, step for degassing at 500°C for 60 min and heating up to foaming temperature with soaking time.

For all treatments the cooling step was inside furnace in order to avoid formation of cracks.

2.4. Fired samples characterization

The samples after firing were characterized performing an analysis of the apparent density (Envelope Density Analyzer Geo Pyc 1360, Micromeritics) and of the absolute density (gas (He) pycnometer AccyPy1330, Micromeritic), in order to calculate the porosity values (*P%*) of each sample (Eq. 1).

$$P\% = \frac{Dabs - Dap}{Dabs} * 100 \qquad (1)$$

For the best foam materials obtained, a test of water absorption (*WA%*), measured according to EN ISO 10545-3, and a thermal conductivity test, by comparison to other commercial products more or less insulating, were carried out. These tests were conducted using rectangular samples prepared ad hoc.

This last test was performed by subjecting the samples at 500°C (by constant heating with a furnace) in a face and measuring the behavior of the contact temperature in the other face using a thermocouple for flat surfaces. The values were recorded every 30 seconds up to 10 minutes, every minute up to 20 minutes, every 5 minutes up to 40 minutes and finally 10 minutes for a total duration of one hour.

For all the samples realized, a microstructural analysis using a stereo microscope and Scanning Electron Microscopy was carried out (ESEM, QUANTA 200 FEI) coupled with an energy dispersion spectroscopy equipment (X-EDS Oxford INCA-350).

3. Results and discussion

3.1. Characterization of raw materials

A chemical analysis (XRF) was carried out for the three glasses used and an elementary and EDS analysis were performed for the pyrolysis residue in order to evaluate the composition. The chemical composition for the glasses used is reported in Table 2.

All the three glasses are silicatic with high amounts of alkaline (Na_2O+K_2O), and alkaline earth oxides. The CaO amounts are different in each typology. The high percentage of calcium oxide (together with the presence of magnesium oxide) can favor the devitrification process of parent glass during the heat treatments.

From the elementary analysis resulted that the char is composed by carbon (87%), sulphur (2.90%), nitrogen (0.37%) and hydrogen (0.74%) while the EDS analysis confirms the presence of sulphur (S) and highlights zinc (Zn), copper (Cu) and iron (Fe) elements contained in the tyres blends. The data reported in Table 3 are the mean values of four sites analyzed.

Supplementary TG/DTA results evidenced an exothermic peak near 450°C associated to carbon oxidation in air and a weight loss of 12% up to 600°C indicates the not complete combustion of carbon. The weight loss is higher by increasing temperature (25% at 1000°C), which confirms the presence of a residual carbonaceous component equal to 35% after reaching the maximum temperature of analysis (1300°C). The second possible phenomenon is the pyrite oxidation in air to form hematite (Fe_2O_3) in agreement with Jorgensen and Moyle study (1982). These tests allowed identifying the foaming range in order to tailor the thermal treatments.

The mineralogical analysis carried out on the pyrolysis char as received, showed the presence of both an amorphous phase, due to the high concentration of amorphous carbon, highlighted by a broad band between 20° and 40° 2θ, and the crystalline phases as iron (pyrite) and zinc (wurtzite) sulfides and traces of calcium and potassium silicon aluminates. The predictable increase of calcium and potassium silicon aluminates concentrations in the material, calcined at 600°C and 1000°C, corresponding to the reduction of the amorphous carbon phase, contribute to the formation of a ceramic skeleton into the material. The particle size analysis on the pyrolysis char, after grinding, confirmed the good conditions performed to obtain a fine powder with a particle size below 125 μm. The characteristic diameters were D_{10} = 0.26μm; D_{50} = 16μm and D_{90} = 60 μm.

Table 2. Chemical analysis by XRF (wt% oxide) of the used glasses

Oxide (wt%)	***GS***	***PG***	***EOL_FLG***
SiO_2	71.30	66.80	67.20
Al_2O_3	2.00	2.25	2.37
Na_2O	12.68	11.22	12.30
K_2O	0.92	0.36	1.53
CaO	10.00	15.51	6.57
MgO	2.23	2.03	2.56
BaO	-	0.04	2.51
SrO	-	<0.02	0.13
PbO	0.04	0.04	3.12
Fe_2O_3	0.37	0.75	0.10
Sb_2O_3	-		1.36
ZrO_2	-	<0.02	539 ppm
SO_3	-		525 ppm
TiO_2	-	0.06	646 ppm
P_2O_5	-	-	225 ppm
Cl (element)	-	-	237 ppm
Y_2O_3	-	-	356 ppm
B_2O_3	-	0.07	-
L.O.I	-	-	-
TOTAL	99.54	99.17	99.75

Table 3. Chemical analysis by EDS (wt% oxide) of the char pyrolysis residue

Element	*wt%*
C	80.6
O	6.20
Si	0.15
Al	<0.10
Na	0.63
K	0.10
Ca	0.10
S	3.31
Fe	1.70
Cu	2.13
Zn	5.25

A thermal study was performed by heating optical microscopy in order to determine the characteristic temperatures (sintering, half sphere, sphere, softening, melting) of the waste glasses used and their mixes with maximum amount of char pyrolysis (50wt%).

From the data reported in Table 4 it is possible to observe that EOL_FLG shows the lower softening and melting temperatures with respect to the other glasses. It is probably due to the presence of high amount of alkali and alkali-earth oxides (~16%) and presence of PbO (~3%) into the composition. The other two glasses have comparable melting temperatures. The glass can be transformed in foam material by addition of compounds which generate gaseous decomposition at temperatures near or above the softening point where the viscosity is enough low to permits the expansion but not the collapse of the structure. So for this kind of glass, in agreement with other studies (Bayer and Koese, 1979), the best conditions for foaming will be higher than 850°C. Besides, the mixes with EOL-LFG and GS show sintering and softening temperatures higher than their respective parent glass. It is possible to hypothesise that in the mix compositions the alkaline oxide amount decreases due to the presence of char and this influences the shrinkage beginning (sintering temperature).

3.2. Characterization of mixes

Preliminary tests (not here reported) using the compositions disclosed in Table 1 highlighted that increasing the amount of tyre pyrolysis char the density values decreased, confirming its role as pores forming. Samples prepared by high pressure (40 MPa) showed a remarkable change of shape and dimensions and black core after firing. For this reason the successive samples were shaped by pressing at 30 MPa. No deformation was observed on samples pressed at 30 MPa, also in samples containing 50 wt% of char. After these tests the successive samples were prepared with 50wt% of pyrolysis char at 30 MPa.

In Fig. 1 the apparent density values for mixes containing 50/50 waste glass and pyrolysis residue after different type of thermal treatments (cold, hot (FC) and cold with degassing step (DEG)) are plotted. The mixes with EOL_FLG showed lower values of density with respect to the other compositions. On the other hand it was observed that the samples containing EOL_FLG treated both into a cold furnace (Fig. 2a) or into a hot furnace contained black core.

This phenomenon probably derives from the low softening temperature of the mix which makes difficult to complete the degassing of the organic carbon with the consequence of the presence of unreacted char inside the material (Andreola et al., 2014). So the use of this type of glass does not allow the scale-up to industrial production and was discarded. An example of the mix of glassy sand fired in hot furnace, where the defect is possible to observe, is shown in Fig. 2b. In general with all kind of glasses, the density measured for samples treated in hot furnace, resulted lower than those treated with cold furnace, but the fast treatment provokes an elevate percentage of un-combusted carbon material not suitable for industrial use.

According to the previously reported results, the best operative conditions for the others two glasses (PG and GS) were thermal treatment into a cold furnace (T_{max} = 900°C, 45 minutes) with degassing isotherm at 500°C for 60 min and char content of 50 wt%. Higher temperatures (950-1000°C) caused a decrease on material porosity due to the collapse of pores.

For these compositions a further characterization was completed. Different parameters were measured, water absorption (WA%), absolute and apparent density and calculated total porosity (Eq. 1), as shown-in Table 5. It is possible to observe from the data reported that the total porosity values are higher for packaging glass waste (PG) (sample 2c). The water absorption (WA%), related to the open porosity, is higher for sample containing glassy sand (GS) (sample 3b).

Table 4. Characteristic temperatures obtained by heating microscope for the waste glasses and the mixes (50/50)

Characteristic temperature T (°C)	*EOL_FLG glass*	*GS glass*	*PG glass*	*Mix EOL-_FLG1.c*	*Mix GS 3.b*	*Mix PG 2.c*
Sintering	682	730	715	754	753	693
Softening	826	837	845	901	948	1008
Sphera	902	947	976	924	1020	1020
Semi-sphera	980	1059	1069	972	1052	1038
Melting	1054	1124	1130	996	1079	1054

This behavior permits to hypothesize the presence of larger amount of close porosity into the sample 2c, very important characteristic for insulating materials (Andreola et al., 2014). The difference in closed porosity was confirmed by SEM images which shows for sample containing PG more uniformly distributed porosity (Fig. 3a) while for GS sample few large pores can be seen (Fig. 3b)

Regarding the thermal insulation, both samples analyzed showed a thermal behavior intermediate between a commercial insulating brick and a commercial refractory (Fig. 4).

The features of the products used are also reported in Table 5. Additionally, both samples show a heat transmission response significantly lower than a porcelain stoneware tile, typical sintered ceramic material, due to their porous nature (Effting et al., 2007).

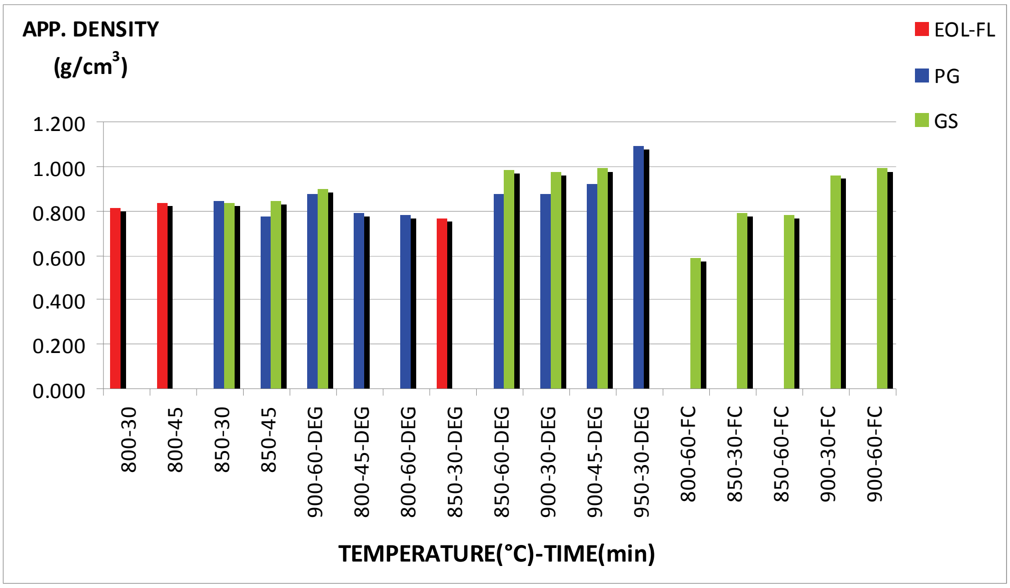


Fig. 1. Apparent density (g/cm^3), as a function of the different thermal treatments of mixes containing 50/50 waste glass and pyrolysis residue. DEG; degassing step; FC: hot furnace

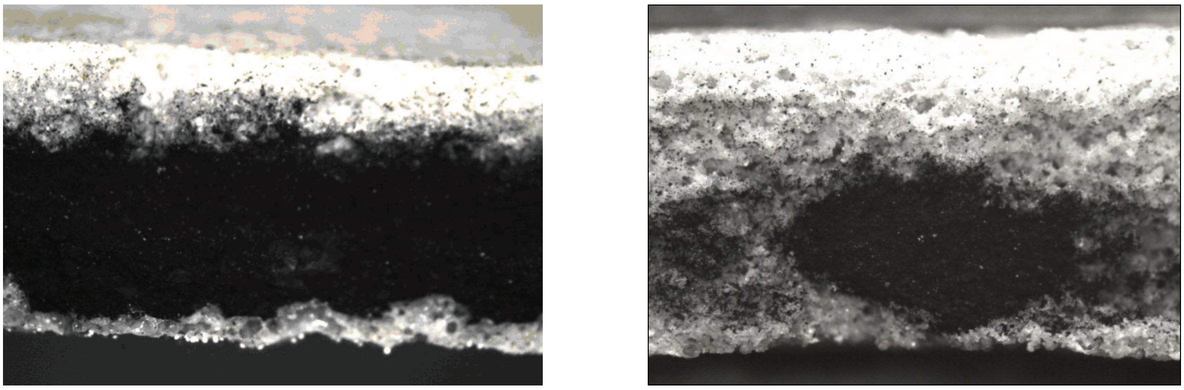
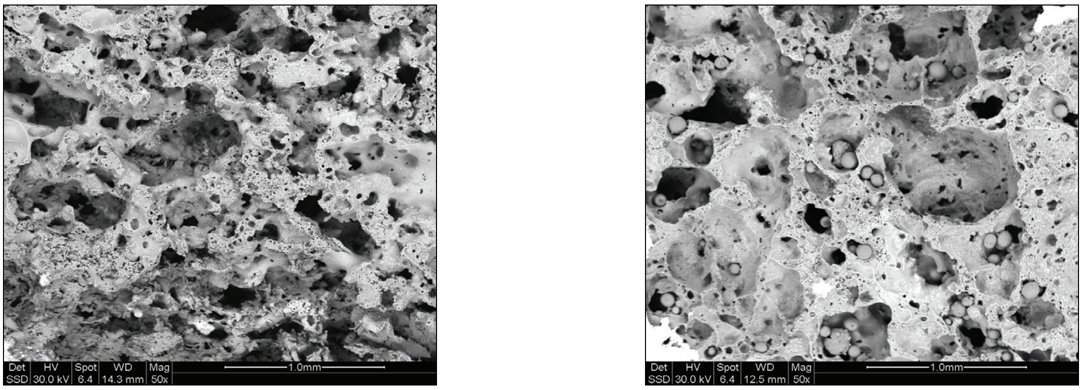

(a) **(b)**

Fig. 2. Stereo micrograph (10X): (a) mix 50/50 EOL_FLG fired in cold furnace at 850°C, 30 min; (b) mix 50/50 GS fired in hot furnace at 850°C, 30 min

Table 5. Physical properties for the best samples compared to commercial materials

Samples/comm. products	*Absolute density (g/cm^3)*	*Apparent density (g/cm^3)*	*Porosity (%)*	*WA (%)*
Mix PG	2.614	0.921	64.79	47.47
Mix GS	2.460	0.993	59.63	53.02
Insulating brick	2.411	0.623	74.18	n.d
Refractory	3.372	1.258	62.89	n.d
Porcelain Stoneware	2.410	2.210	8.298	n.d



(a) (b)

Fig. 3. SEM micrographs (50X) of fracture samples fired in cold furnace at 900°C, 45 min: (a) mix 50/50 PG; (b) mix 50/50 GS

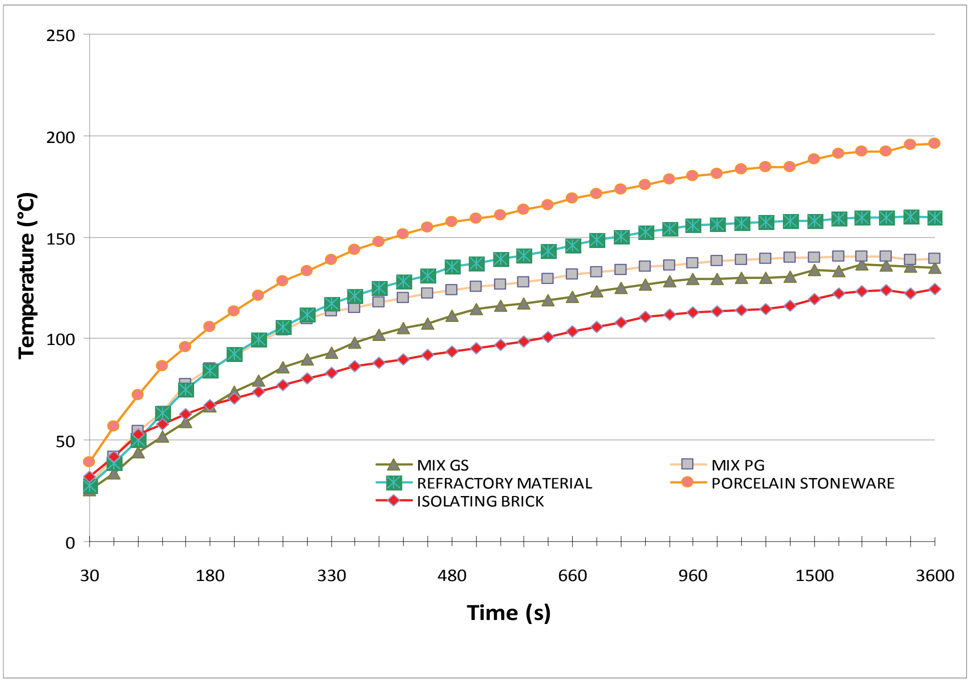


Fig. 4. Heat transmission behaviour of the samples containing packaging glass (■) and glassy sand (▲) compared to commercial materials: porcelain stoneware tile (●), refractory (■), isolating brick(♦)

4. Conclusions

From the studies carried out it is possible to argue that the pyrolysis char could be valorized as foaming agent for the obtainment of lightweight materials, when mixed with glassy sand and packaging glass waste. The samples obtained show apparent density values lower than 1 g/cm^3, while a traditional ceramic material has density values higher than 2 g/cm^3. Their thermal behavior, compared to commercial products, is between an isolating brick and a refractory material.

It can therefore preliminary concluded that the materials produced have suitable properties for use in buildings to contribute to thermal and acoustic insulating.

The advantages of these innovative materials are mainly:

- raw materials savings: samples are produced totally from waste materials, some of which are not currently reused, but are landfilled;
- energy saving: samples were obtained by thermal treatments at temperatures lower (900°C) than those used for the obtainment of commercial ceramic materials (1100-1250°C). Energy saving is also guaranteed by the fact that the pyrolysis residue derives from a plant, that does not require a process of tyres shredding, thus avoiding a high energy consumption.

References

Andreola F., Barbieri L., Lancellotti I., Tagliavini G., (2014), *Light-Weight Materials to Valorize Waste Tyres Pyrolysis Residue*, Proceedings SUM 2014, Second Symposium on Urban Mining; 19-21 May, Bergamo, Italy, vol. 154, 1-6.

Bayer G., Koese S., (1979), Reaction of foaming additives with waste glass powders in the preparation of lightweight materials, *Rivista Stazione Sperimentale del Vetro*, **5**, 310-320.

Benelli T., Giorgini L., Leonardi C., Bortolani G., Cavazzoni M., Tosi C., (2013), *Recovery of Materials and Energy from Pyrolysis of End-Life Tyres*, Proc. of European Polymer Congress (EPF), Editrice Pisana Publishing House, 5-14.

Bonoli A., Tondelli S., Bergonzoni M., (2012), *Technological Innovations in End of Life Tyres Recovery and Recycling*, In: *Urban Mining: A Global Cycle Approach to Resource Recovery from Solid Waste*, Cossu R., Salieri V., Bisinella V. (Eds.), Padue, Italy, CISA Publisher, 187-195.

EC Directive, (2000), Directive 2000/53/EC of the European Parliament and of the Council of 18 September 2000 (subsequently “ELV Directive”), *Official Journal of the European Communities*, L269, 21.10.2000, Brusselses.

Effting C., Güths S., Alarcon O.E., (2007), Evaluation of the thermal comfort of ceramic floor tiles, *Materials Research*, **10**, 301-306.

ETRA, (2013), Introduction of tyres recycling: Twenty years of tyre recycling in UE, European Tyre Recycling Association, Brussels, Belgium, On line at: http://www.etra-eu.org/joomla/libraries/articles/ETRA%20Introduction001.pdf.

Fernandes H.R., Andreola F., Barbieri L., Lancellotti I., Pascual M.J., Ferreira J.M., (2013), The use of egg shells to produce Cathode Ray Tube (CRT) glass foams, *Ceramics International*, **39**, 9071-9078.

Giorgini L., Leonardi C., Zattini G., Bortolani G., Tosi C., Cavazzoni M., (2013), *Pyrolysis of Waste Tires in a Novel not Dangerous Pilot Plant*, In: *The Chemistry in a Sustainable Society*, Proc. XIV National Congress of Environmental Chemistry and Cultural Heritage, Italian Chemical Society, Rimini, 127-128.

Giorgini L., Benelli T., Leonardi C., Mazzocchetti L., Zattini G., Bortolani G., Tosi C., Cavazzoni M., Montanari I., Tosi C., (2015), Efficient recovery of non-shredded tires via pyrolysis in an innovative pilot plant, *Environmental Engineering and Management Journal*, **14**, 1611-1622.

Jorgensen F.R.A., Moyle F.J., (1982), Phases formed during the thermal analysis of pyrite in air, *Journal of Thermal Analysis*, **25**, 473-485.

Migliore M., Campioli A., Lavagna M., Oberti I., Paganin G., Talamo C., (2015), intersectorial reuse of waste and scraps for the production of building products: strategies and valorization of waste, *Environmental Engineering and Management Journal*, **14**, 1675-1681.

Moo-Young H., Sellasie K., Zeroka D., Sabnis G., (2003), Physical and chemical properties of recycled tyres shreds for use in construction, *Journal of Environmental Engineering*, **129**, 921-929.

Rada E.C., Dal Maschio R., Ischia M., Ragazzi M., (2012), *Energy Valorization of Tyres Waste: A Multi-Step Approach*, In: *Urban Mining: A Global Cycle Approach to Resource Recovery from Solid Waste*, Cossu R., Salieri V., Bisinella V. (Eds.), CISA Publisher, Padue, Italy, 209-216.

Reschner K., (2015), Scrap Tire Recycling. A Summary of Prevalent Disposal and Recycling Methods, On line at: http://www.entire-engineering.de/Scrap_Tire_Recycling.pdf.

Scheffler M., Colombo P., (2006), *Cellular Ceramics: Structure, Manufacturing, Properties and Applications*, Chapter 2.7 Glass foams, John Wiley & Sons.

Sienkiewicz M., Kucinska-Lipka J., Janik H., Balas A., (2012), Progress in used tyres management in the European Union: A review , *Waste Management* , **32**, 1742-1751.

Szodrai F., Lakatos L., (2014), Measurements of the thermal conductivities of some commonly used insulating materials after wetting, *Environmental Engineering and Management Journal*, **13**, 2881-2886.

Williams P.T., (2013), Pyrolysis of waste tyres: A review, *Waste Management*, **33**, 1714-1728.

http://www.ecopneus.it

Environmental Engineering and Management Journal

September 2016, Vol.15, No. 9, 1915-1922
http://omicron.ch.tuiasi.ro/EEMJ/

"Gheorghe Asachi" Technical University of Iasi, Romania

ENVIRONMENTAL IMPACT ASSESSMENT OF ELECTRICITY GENERATION FROM BIOGAS IN PALESTINE

Jacopo Bacenetti[1*], Samer H. Baboun[2], Falah Demery[3], Iyad Aburdeineh[4], Marco Fiala[1]

[1]Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy, Università degli Studi di Milano,
[2]John Paul II Foundation - Palestine Branch
[3]Sustainable Energy and Climate Change, Palestinian Energy Authority, Palestine
[4]Iyad Aburdeineh, Joint Service Council, Bethlehem Governatorate, Palestine

Abstract

In Palestine, the energy situation is unique compared to other Middle Eastern Countries, as there are technical and political challenges for transporting, storing, importing and exporting energy. Actually, energy import from Israel meets the major share of Palestinian energy needs. Palestinian reliance on Israel concerning its energy supply is an issue due to the political situation. Furthermore, from an environmental point of view, Israel predominantly relies on fossil fuels for its own electricity production; therefore, renewable energy production in Palestine can represent a solution to environmental concerns. With regard to electricity (EE) generation, Anaerobic Digestion (AD) of biomass and/or Organic Fraction of Municipal Solid Waste (OFMSW) can be a particularly suitable solution when realized in small and local plants. In this study, the environmental impact of electricity generation from two Anaerobic Digestion (AD) plants in Palestine was evaluated, using the Life Cycle Assessment methodology. In both of them, the produced biogas is used to feed a CHP engine and the generated electricity is put into the grid. The first pilot plant, located in Dura (Governatorate of Hebron), is fed with animal manure and other urban waste; the second is in Bethlehem and mainly fed with OFMSW. For both AD plants, the results show that: 1) the produced EE has a lower environmental impact respect to that imported from Israel, 2) the main environmental hotspots are: digestate emissions, electricity and diesel consumption and emissions from biogas combustion.

Key words: anaerobic digestion, climate change, greenhouse gases, Life Cycle Assessment, renewable energy

Received: January, 2016; Revised final: April, 2016; Accepted: May, 2016

1. Introduction

In Palestine, the energy situation is somewhat unique compared to other Middle Eastern Countries, as there are technical and political challenges for transporting, storing, importing and exporting energy. Nowadays, energy import from Israel meets the major share of Palestinian energy needs. Palestinian reliance on Israel concerning its energy supply is an issue due to the political situation (Ismail et al., 2013). According to the Palestinian Energy Authority (2013), the consumption of electricity in Palestina in 2013 was 5.137 GWh, 87% of which imported Israel. The 9.7% of electricity is generated (Gaza Power Plant, 140 MW) in the Gaza Strip by the "Palestine Electric Company", 2.6% is imported from Jordan (to power Jericho area) and from Egypt (to power Rafah in the Gaza Strip). Among the Renewable Energy Sources, biomass represents an important source due to the local developing of the agricultural sector (Abualkhair, 2007). Several type of residues coming from agriculture (and agro-food industries as well) could be used for energy conversion; currently, renewable energy constitutes approximately 22% of Palestinian energy supply, mainly used for heating purposes. Among these wood (43%) and solar energy (56%) are predominant (Hamed et al., 2012).

According to Hamed et al. (2012), in Palestine, manure of cattle, goat and sheep from is

---

[*] Author to whom all correspondence should be addressed: e-mail: jacopo.bacenetti@unimi.it; Phone: +39 02 503 16869; Fax +39 02 503 16 845

one of the most promising feedstock for energy conversion by Anaerobic Digestion (AD); daily, about 13,000 m^3N of biogas could be produced from this biomass. In addition, an important volume of organic fraction of municipal solid waste (OFMSW) can be considered for biogas production.

Furthermore, from an environmental point of view, Israel predominantly relies on fossil fuels for its own electricity production; therefore, renewable energy production in Palestine can represent a solution to environmental concerns. With regard to electricity (EE) generation, Anaerobic Digestion (AD) of biomass and/or Organic Fraction of Municipal Solid Waste (OFMSW) can be a suitable solution, in particular when realized in small and local plants (Szabó et al., 2014; Zagorskis et al., 2012).

Renewable energy generation must deal two main issues: technical and economical feasibility and environmental sustainability. If the economic aspects are highly dependent on the public subsidies provided for the generation of electricity from renewable sources the environmental sustainability is related to technical and logistic aspects and can be previously estimated by applying appropriate methods of analysis (Tashima et al., 2014).

Nowadays, there are not AD plants in Palestine; nevertheless, the interest for this renewable energy source is growing in particular for biogas plants fed with residual biomass or waste. In this context, in 2013, the no-profit organization John Paul II Foundation – Dialogue, Cooperation and Development founded a research project entitled "*Renewable Energy Resources. Realization of pilot plants in Palestinian Territory*" aimed to the identification of pilot plants in order to generate energy (electrical, thermal), by means of renewable energy sources available in Palestinian Territory". Among the pilot plants, AD plants were identified.

In the last decade, the Life Cycle Assessment (LCA) method has become more and more employed for the environmental assessment of agricultural systems. LCA is a methodology that aims to analyze products, processes, or services from an environmental perspective, providing a useful and valuable tool for agricultural system evaluation (Bacenetti et al., 2013b). Over the years, several studies aiming to evaluate the environmental burdens of electricity from biogas were carried out (Bacenetti and Fiala, 2014, 2015; Bacenetti et al., 2015, Dressler et al., 2012; Meyer-Aurich et al., 2012; Lijo et al., 2014a, 2014b).

The goal of this study is to assess the environmental impact of electricity from biogas in Palestine in the two AD plants identified. In more details, the environmental performance of EE co-generated in CHP (Combine Heat and Power) systems fueled with biogas produced in two AD plants fed with different agricultural feedstock and OFMSW were assessed using the LCA methodology. Although the biogas produced by anaerobic digestion can be used in natural gas fueled cars, in this study, the attention was paid on electricity generation because: (i) this is by far the most widespread utilization pathway for the biogas produced by AD plants (Bacenetti et al., 2013a) and (ii) compared to the upgrading to biomethane, the biogas utilization in CHP engines is a solution technically mature requiring simpler devices and lower investment cost (Bacenetti et al., 2013b; Dressler et al., 2012).

2. Material and methods

Life Cycle Assessment (LCA) is a holistic method to assess the environmental impacts and resources used throughout the life of a product (process or activity) from raw material acquisition, production and use, to waste disposal (ISO 14040, 2006). This methodology has been widely considered to determine the environmental profile of numerous renewable energy systems (Dressler et al., 2012; Fusi et al., 2016; Lijò et al., 2014a; Meyer-Aurich et al., 2012).

2.1. Goal and scope definition

The primary goal of this LCA study is to assess the environmental impact of electricity produced by the Dura's and Bethlehem's AD pilot plants; secondary aims of the study are to identify the environmental hotspot of the process and to compare the environmental impact of electricity from biogas with the one of the Israel electricity mix.

In more details, the research questions can be summarized as follows:

1) What is the environmental impact of the EE produced in the two pilot AD plants?
2) What are the main hotspots associated with the process?

The outcomes of such an analysis can be helpful for technicians and local politicians involved in the biogas-bioenergy process.

2.2. Functional unit

The functional unit is an important step of any life cycle assessment since it provides the reference to which all other data in the assessment are normalized. For the renewable energy processes the most suitable functional unit is the produced energy. Therefore, in this study, 1 kWh electricity produced by the CHP engine and fed into the grid has been considered as FU.

2.3. System boundary and process description

The first plant is located in Dura (Governorate of Hebron), has an electrical power of 137 kW and is fed with animal manure. The second is located in Bethlehem, has an electrical power of 60 kW and is mainly fed with OFMSW. Table 1 reports the main characteristics of the CHP system in the two AD plants. Both the AD plants have been designed to operate in thermophilic conditions (55°C), with a total solid content ranging from 10 to 11% and are characterized by CSTR (Continuously Stirred Tank Reactor) digesters.

The analysis considers a gate-to-gate system boundary; therefore, all the processes (and the related inputs) that take place at the biogas plant has been taken into account. In more details, the biogas-to-electricity process has been divided into 6 sections (Fig. 1):

S1: feedstock loading into the digesters, this section involves the consumption of diesel fuel for feedstock transport from storage point to digesters as well as the electricity consumption for biomass loading by means of pumps and conveyor belts;

S2: anaerobic digestion, during this section electricity is consumed for mixers and pumps and biogas losses (0.5% of the produced volume) take places;

S3: digestate treatment (S3A) and storage (S3B). Only in the Dura's plant, the raw digestate is separated using a screw separator into a solid fraction (SF, characterized by 20.5% total solid, which represents about 20% of the raw digestate mass) and liquid fraction (LF, with 5.4% of total solid). In both the plants, the digestate is stored for 120 days in open tanks before to be used as organic fertilizer;

S4: biogas treatment, the raw biogas is filtered in a sandy filter, is dehumidified by means of an electric chiller and is desulphurized by a scrubber using NaOH;

S5: energy conversion, in this section the biogas feeds the CHP engine, and the produced EE is fed into the electrical grid, while the thermal energy is partially used to heat the digester and partially wasted.

Feedstock production has been excluded from the boundary because all the matrices are waste belonging to other production processes (Fusi et al., 2016; Lansche and Müller, 2012). Due to the high data variability, also feedstock transport (from collection point to the AD plant) has been excluded. Infrastructures such as digesters and CHP engines have been excluded due to their long life span (8-10 years for CHP and 15-20 years for digesters). The emissions from the digestate application on field are similar to the ones from manure application; therefore, this process has been excluded as well. The surplus heat is wasted.

In the Alternative Scenario (AS), the environmental benefits arising from the valorization of surplus heat has been assessed using the "credit approach". In this AS the surplus heat is fully valorized and substitute heat produced by coal combustion (Ecoinvent, 2013).

Credits, equal to environmental load of heat production from fossil fuel, were attributed to the AD process.

Table 1. Main characteristics of the two CHP systems

Biogas plant	*Electrical power*	*Efficiency*		*EE produced*	*TE produced*
		Electric	*Thermal*		
	kW	*%*	*%*	*MWh/year*	*MWh/year*
Dura	140	36.5	45.0	1150.5	1418.4
Bethlehem	60	35.0	45.0	469.4	603.6

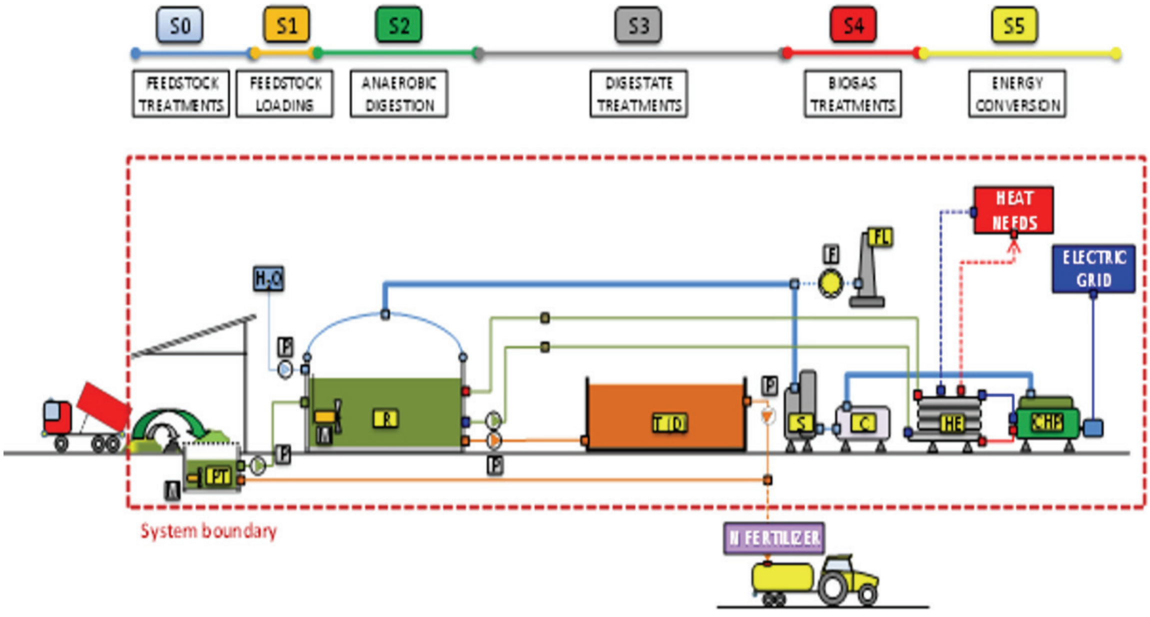


Fig. 1. System boundary and AD plant layout in Dura (top) and Bethlehem (bottom): R = reactor; T (D) - digestate tank; SP - separator (LF, SF); T (LF) - Liquid fraction tank; S - scrubber; C - chiller; FL - biogas flare; HE - heat exchanger; CHP - engine-generator; P- pump; M - mixer; F - fan

2.4. Inventory analysis

Primary data concerning the feedstock were obtained through surveys and questionnaires to local politicians and technicians, gathering the following information: (i) type of feedstock available for AD; (ii) feedstock availability (t/year); (iii) recoverable share for the different feedstock.

Based on feedstock availability (Table 2), the two AD plants size has been calculated considering: for the CHP, the characteristics reported in Table 1 and an annual running time of 8000 h and, for the feedstock, the characteristics reported in Table 3. Biogas losses, both from digesters (0.5%) and from the CHP (0.5%), were considered (Dressler et al., 2012) while losses during biogas treatment were considered negligible. Emissions data from biogas combustion in the CHP engines were accounted according to NERI (2010). The thermal energy consumption for digester heating have been calculated considering: (i) the difference between the temperature inside the digester (55°C) and the air temperature outside the digester, (ii) the amount of feedstock used to feed the digesters, (iii) the digester insulation.

Digestate emission of CH_4, NH_3 and N_2O during digestate storage in open tank were assessed in according to Edelmann et al. (2001) and Whiting and Azapagic (2014). Table 4 summarizes the main LCI data for the two biogas plants.

Background data for the production of diesel fuel, lubricating oil and chemicals were obtained from the Ecoinvent Database (Ecoinvent, 2013; Frischknecht et al., 2007; Nemecek and Käggi, 2007; Spielmann et al., 2007). The environmental impact of Israel electricity mix was assessed taking into account the Israel electric mix: 53% from hard coal, 42% from natural gas, 3.6% from crude oil and petroleum and 1.4% from renewable energy sources (IEA, 2015).

Table 2. Feeding of the two AD plants

Biogas plant	*OFMSW*	*Vegetal waste*	*Cattle manure*	*Sheep manure*	*Poultry manure*
	t/day	*t/day*	*t/day*	*t/day*	*t/day*
Dura	14.00	4.40	27.50	0.80	0.19
Bethlehem	5.56	0.29	0	0	0

Table 3. Feedstock characteristics

Feedstock	*Total solid*	*Volatil solid*	*Biogas production*	*Methane content*
	% of FM	*% of TS*	*m³/t of VS*	*% of biogas*
OFMSW	26%	96%	500	55%
VEGETAL VASTE	20%	85%	500	55%
CATTLE MANURE	17%	75%	360	55%
SHEEP MANURE	30%	75%	350	55%
POULTRY MANURE	40%	75%	400	55%

FM = fresh matter; TS = total solid; VS = volatile solid

Table 4. Main inventory data

Parameter		*Dura*	*Bethlehem*
FEEDING	COW MANURE SHEEP MANURE POULTRY MANURE VEG. RESIDUES OFMSW	27.5 t/day 0.8 t/day 0.2 t/day 4.4 t/day - -	- - - - 0.30 t/day 5.7 t/day
WATER		2372.5 t/year	1095 t/year
DIESEL CONSUMPTION		3832.5 kg/year	766.5 kg/year
LUBRICANT		29.2 kg/year	5.5 kg/year
NaOH CONSUMPTION		182.5 kg/year	180 kg/year
CH_4 PROD.		928 m^3_N/day	395 m^3_N/day
CH_4 LOSSES		9.28 m^3_N/day	3.95 m^3_N/day
DIGESTATE PROD.		61.4 t/day; TS = 8.6%	19.4 t/day; TS = 8.0%
PRODUCT OF DIGEST. SEP.		SF: 12.9 t/day LF: 48.5 t/day	No separation is carried out
ENERGY PRODUCTION	EE TE	1150.5 MWh/year 1418.4 MWh/year	469 MWh/year 603 MWh/year
ENERGY CONSUMPTION	EE[1] TE[2]	89.7 MWh/year 1010.6 MWh/year	42.21 MWh/year 325.6 MWh/year

[1]taken from the electric grid, [2]self-consumption: taken from CHP cooling jackets and exhaust gases.

2.5. LCIA

Nine impact categories were evaluated according the indication of ILCD handbook (Wolf et al., 2012): climate change (CC), ozone depletion (OD), particulate matter (PM), photochemical ozone formation (POF), terrestrial acidification (TA), terrestrial eutrophication (TE), freshwater eutrophication (FE), marine eutrophication (ME), and mineral and fossil resource depletion (MFRD).

2.6. Sensitivity analysis

Feedstock transport has been highlighted as an important variable in LCA studies focused on biogas production from waste (Berglund and Börjesson, 2006; Patterson et al., 2011). Based upon this consideration, a sensitivity analysis concerning the feedstock transport distance was performed. In more details, in the environmental assessment, transport distances equal to 2.5 – 5.0 km and 10-20 km were considered for the AD plant of Dura and Bethlehem, respectively. These transport distances have been assumed considering that both the plants are located in the middle of the area from which the feedstock arises. In particular, for the plant of Bethlehem, the distance takes into account that the OFMSW is produced in the towns involved in the Municipality of Bethlehem: Beit Lehem, Beit Sahour and Beit Jala. For the OFMSW a transport with truck has been considered while for manure and vegetables residues tractors and trailers have been taken into account.

3. Results and discussion

Table 5 reports the environmental impact for 1 kWh of produced electricity in the two AD plants compared with the environmental impact of the Israel electric mix. Fig. 2 shows the environmental hotspots for Dura's and Bethlehem's Plant.

Respect to the Israel electric mix, the electricity produced by the two AD plants shows a considerably lower environmental impact. The reduction ranges from -50% for TE up to - 89% for OD and FE.

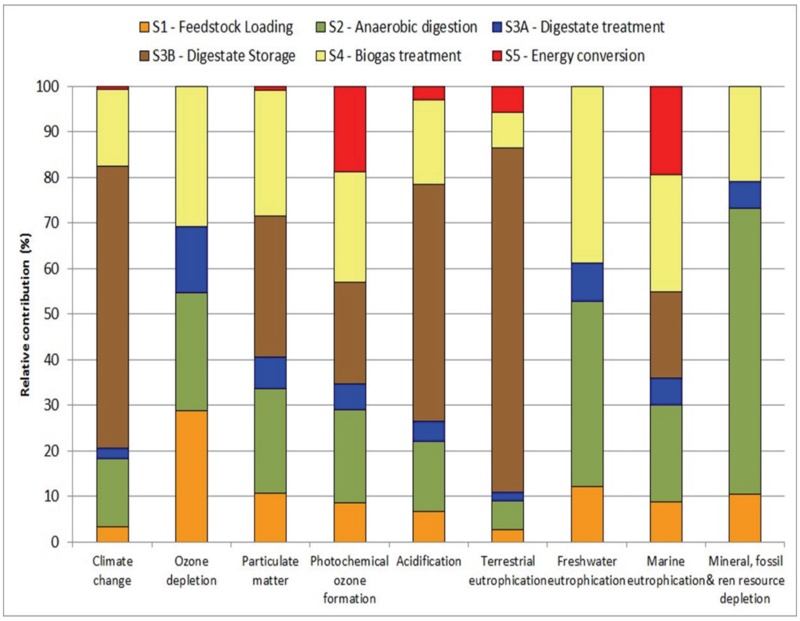
The feedstock loading (S1) does not represent an environmental hotspot. Its contribution to the environmental impact of the FU, mainly related to the consumption of diesel fuel, is lower than 12% for all the impact categories except for OD (28% in Dura's plant and 18% in Bethlehem's plant). The AD (S2), mainly due to high electricity and diesel fuel consumption, represents a hotspot for MFRD (63% in Dura, 77% in Bethlehem) and FE (41%). The share of CC related to this section is mainly due to fugitive methane emissions. In Dura's plant, the digestate separation (S3A) is responsible for about 15% of OD; for the other impact categories, the share is smaller. The GHG emissions that take place during digestate storage (S3B) are the main responsible for CC (about 62% in Dura and 59% in Bethlehem), TE (about 75% in both AD plants) and TA (31% in Dura and 49% in Bethlehem).

S4 is responsible for a remarkable impact for OD (about 31% in Dura and 50% in Bethlehem), PM (28% in Dura and 38% in Bethlehem), POF (25% in Dura and 33% in Bethlehem), FE (38% in Dura and 46% in Bethlehem) and ME (25% in Dura and 36% in Bethlehem). This impact, mainly related to the electricity and chemicals use during dehumidification and desulphurization, is proportionally higher for the Bethlehem's plant, where desulphurization requires a higher specific NaOH consumption. S5 does not represent an environmental hotspot, except for POF (about 17-18% in both the AD plants) and ME (18-19% in both the AD plants).

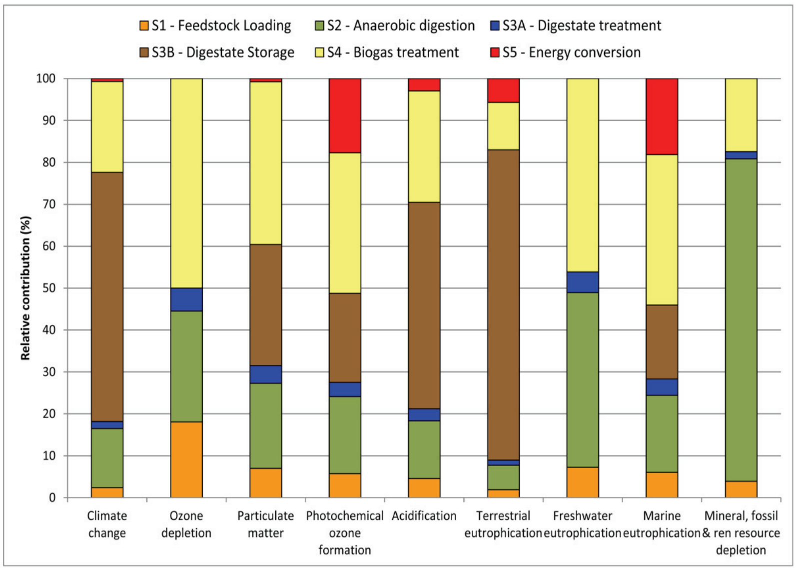
Table 6 reports the results of the sensitivity analysis carried out concerning the different feedstock transport distances. For the Dura's plant, even though shorter transport distances are considered, the transport with tractors and farm trailers involves a greater increase of the environmental impact respect to Bethlehem's plants where trucks are taken into account. Among the different evaluated impact categories, CC, PM, TA and TE are only slightly affected by transport distance while, for the other environmental effects, the impact increase is remarkable. In particular, for MFRD, the impact of transport is noticeable and, for the long distances, is capable to completely offsets the environmental benefits. When the surplus heat (TE produced – TE consumed to heat the digesters) is valorized to supply other thermal needing (e.g. greenhouse, absorption chiller, drying, etc.), the environmental performances of EE from AD plants improve, because additional environmental benefits are granted from heat production by no-fossil sources.

Table 5. Environmental impact for the FU

Impact category	*Unit*	*Dura*	*Bethlehem*	*Israel electricity mix*
CC	kg CO_2 eq/kWh	0.380	0.395	1.091
OD	kg CFC-11 eq/kWh	$7.43\cdot10^{-9}$	$6.91\cdot10^{-9}$	$6.14\cdot10^{-8}$
PM	kg PM2.5 eq/kWh	$4.95\cdot10^{-5}$	$5.31\cdot10^{-5}$	$3.66\cdot10^{-4}$
POF	kg NMVOC eq/kWh	0.000423	0.000447	0.002471
TA	molc H+ eq/kWh	0.00134	0.00141	0.00668
TE	molc N eq/kWh	0.004099	0.004193	0.008408
FE	kg P eq/kWh	$5.22\cdot10^{-7}$	$6.56\cdot10^{-7}$	$5.29\cdot10^{-6}$
ME	kg N eq/kWh	0.000112	0.00012	0.000767
MFRD	kg Sb eq/kWh	$9.63\cdot10^{-8}$	$1.65\cdot10^{-7}$	$4.55\cdot10^{-7}$



(a)



(b)

Fig. 2. Hotspots identification in Dura (a) and Bethlehem (b)

Table 6. Sensitivity analysis results: impact increase considering different feedstock transport distances

Impact category	***Dura***		***Bethlehem***	
	2.5 km	***5 km***	***10 km***	***20 km***
CC	2.2%	4.4%	1.4%	2.8%
OD	12.7%	25.5%	12.9%	25.8%
PM	9.6%	19.2%	3.8%	7.5%
POF	16.0%	32.0%	10.5%	20.9%
TA	4.4%	8.7%	2.6%	5.2%
TE	5.7%	11.4%	4.0%	7.9%
FE	41.1%	82.2%	5.8%	11.5%
ME	18.9%	37.8%	12.6%	25.2%
MFRD	423.3%	846.5%	111.2%	222.4%

Fig. 3 shows the results of the AS. The comparison is made among the environmental performances of Israel electricity mix, EE from Dura's and Bethlehem's AD plants with the surplus heat either wasted or recovered and used.

The surplus heat valorization involves a considerable impact reduction for all the evaluated impact categories. In particular, for the electricity of Dura's plant, in the case of some impact categories (OD, PM, POF, TA, ME and particularly FE), the use of the surplus heat allows to offset the environmental impact producing environmental benefits for the electricity produced at the AD plant.

The surplus heat valorization involves a considerable impact reduction for all the evaluated impact categories. In particular, for the electricity of Dura's plant, in the case of some impact categories (OD, PM, POF, TA, ME and particularly FE), the use of the surplus heat allows to offset the environmental impact producing environmental benefits for the electricity produced at the AD plant.

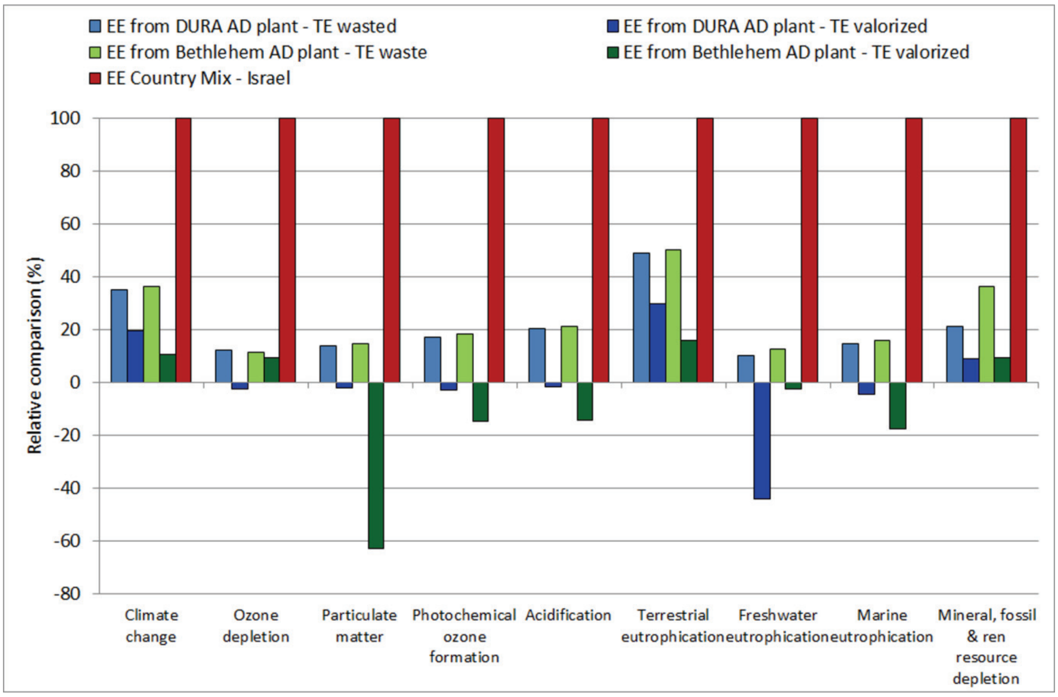


Fig. 3. Impact reduction from surplus heat valorization

A direct comparison with the previously carried out LCA studies focused on electricity generation from AD plants cannot be draft due to different LCIA methods, different system boundary, selected functional units and assumptions. Nevertheless, for what concern contribution analysis and, in particular, the identification of digestate emissions, methane losses and electricity consumption as the main environmental hotspots, the outcomes of this study are in agreement with Bacenetti et al. (2013b), Bacenetti and Fiala (2015), Fusi et al. (2016), Dressler et al. (2012) and Lijò et al. (2014a, 2014b, 2015). For what concern the impact for climate change impact category, the results for the two AD plants evaluated in this study (0.380 kg CO_2eq/kWh in Dura and 0.395 kg CO_2eq/kWh in Bethlehem), are slightly higher than the ones achieved by Bacenetti and Fiala (2014) for a AD plant fed only with cattle manure, Pucker et al. (2013) and Fusi et al. (2016) for AD plants fed with pig and cattle slurries. This higher impact is mainly related to the higher impact of consumed electricity. However, the electricity generated at the AD plants of Dura and Bethlehem shows a lower GHG emission if compared to one produced in biogas plants fed with energy crops and, in particular, with maize silage (Dressler et al., 2012; Ingrao et al., 2015; Lansche and Müller, 2012; Lijò et al., 2014a).

4. Conclusions

Palestine relies mostly on Israel for its fossil fuel and electricity imports; this involves high environmental, economic and political costs; nevertheless, it has the potential to reduce this reliance by producing its own energy from renewable sources.

The achieved results highlight the environmental sustainability of AD based on agricultural manure, vegetable waste and OFMSW as a bearable solution to produce electricity from renewable energy source in Palestine. Respect to the electricity generation from fossil fuels, benefits for the environment are achieved for all the evaluated impact categories. The electricity production via AD carried out at the Dura's AD plant allows producing EE with a lower environmental impact respect to the one produced in Israel. Although the two pilot plants are designed without considering the valorization of surplus heat, a remarkable improvement of the environmental performances can be achieved by means of its use. Furthermore, the storage of digestate in covered tanks could reduce methane emission and, consequently, improve the environmental performances, in particular for climate change. Based on the achieved outcomes, Palestine Authorities should enhance their efforts for the realization of the two AD plants. By their realization, the Palestinian Territories will create the opportunities for the development of technical trainings, for sharing scientific knowledge and latest technologies and will achieve also positive repercussions for other productive sectors.

References

Abualkhair A., (2007), Electricity sector in the Palestinian territories: Which priorities for development and peace?, *Energy Policy*, **35**, 2209-2230.

Bacenetti J., Fiala M., (2014), Life cycle assessment of electricity production from an aerobic digestion of animal slurry in a farm scale plant, *Procedia – Environmental Science, Engineering and Management*, **1**, 121-125.

Bacenetti J., Fiala M., (2015), Carbon footprint of electricity generation from anaerobic digestion plants in Italy, *Environmental Engineering and Management Journal*, **14**, 1495-1502.

Bacenetti J., Negri M., Cantore A., Cantarella P., Fiala M., (2013a), A detailed monitoring of an anaerobic digestion plant in Northern Italy, *Environmental Engineering and Management Journal*, **12**, 109-113.

Bacenetti J., Negri M., Fiala M., Gonzalez-Garcia S., (2013b), Anaerobic digestion of different feedstock: impact on energetic and environmental balances of biogas process, *Science of the Total Environment*, **463-464**, 541-551.

Bacenetti J., Duca D., Fusi A., Negri M., Fiala M., (2015), Mitigation strategies in the agro-food sector: the anaerobic digestion of tomato puree by-products. An Italian case study, *Science of the Total Environment*, **526**, 88-97.

Berglund M., Börjesson P., (2006), Assessment of energy performance in the life-cycle of biogas production, *Biomass and Bioenergy*, **30**, 254-266.

Dressler D., Loewen A., Nelles M., (2012), Life cycle assessment of the supply and use of bioenergy: impact of regional factors on biogas production, *The International Journal of Life Cycle Assessment*, **17**, 1104-1115.

ECOINVENT, (2013), Ecoinvent Database, On line at: www.ecoinvent.org.

Edelmann W., Schleiss K., Engeli H., Baier U., (2001), Life cycle assessment of electricity from agricultural biogas (in Geman), Project 35408, Vertrag 75187, On behalf of the Federal Office for Energy, Schlussbericht, November 2001.

Frischknecht R., Jungbluth N., Althaus H.J., Doka G., Heck T., Hellweg S., Hischier R., Nemecek T., Rebitzer G., Spielmann M., Wernet G., (2007), Overview and Methodology, Ecoinvent Report No. 1, Swiss Centre for Life Cycle Inventories, Dübendorf.

Fusi A., Bacenetti J., Fiala M., Azapagic A., (2016), Life Cycle Environmental Impacts of electricity from biogas produced by anaerobic digestion, *Frontiers in Bioengineering and Biotechnology*, **4**, Doi: 10.3389/fbioe.2016.00026.

Hamed T.A., Flamm H., Azraq M., (2012), Renewable energy in the Palestinian Territories: Opportunities and challenges, *Renewable and Sustainable Energy Reviews*, **16**, 1082-1088.

IEA Energy Statistics, (2015), Electricity generation by fuel: Israel, On line at: http://www.iea.org/stats/WebGraphs/ISRAEL2.pdf.

Ismail M.S., Moghavvemi M., Mahlia T.M.I., (2013), Energy trends in Palestinian territories of West Bank and Gaza Strip: Possibilities for reducing the reliance on external energy sources, *Renewable and Sustainable Energy Reviews*, **28**, 117-129.

Lansche J., Müller J., (2012), Life cycle assessment of energy generation of biogas fed combined heat and power plants: Environmental impact of different agricultural substrates, *Engineering in Life Sciences*, **12**, 313-320.

Lijó L., González-García S., Bacenetti J., Fiala M., Feijoo G., Lema J.M., Moreira M.T., (2014a), Life Cycle Assessment of electricity production in Italy form anaerobic co-digestion of pig slurry and energy crops, *Renewable Energy*, **68**, 625-635.

Lijó L., González-García S., Bacenetti J., Fiala M., Feijoo G., Moreira M.T., (2014b), Assuring the sustainable production of biogas from anaerobic mono-digestion, *Journal of Cleaner Production*, **72**, 23-34.

Meyer-Aurich A., Schattauer A., Hellebrand H.J., Klauss H., Plöchl M., Berga W., (2012), Impact of uncertainties on greenhouse gas mitigation potential of biogas production from agricultural resources, *Renewable Energy*, **37**, 277-284.

Nemecek T., Käggi T., (2007), Life cycle inventories of agricultural production systems, Final report Ecoinvent v2.0 No. 15a, Agroscope FAL Reckenholz and FAT Taenikon, Swiss Centre for Life Cycle Inventories, Zurich and Dübendorf, Switzerland.

NERI, (2010), Emissions from decentralized CHP plants, National Environmental Research Institute, On line at: http://www2.dmu.dk/pub/FR786.pdf.

Palestinian Energy Authority, (2013), Unpublished data, Ramallah, Palestina.

Patterson T., Esteves S., Dinsdale R., Guwy A., (2011), Life cycle assessment of biogas infrastructure options on a regional scale, *Bioresource Technology*, **102**, 7313-7323.

Spielmann M., Bauer C., Dones R., Tuchschmid M., (2007), Transport services, Ecoinvent Report No. 14, Swiss Centre for Life Cycle Inventories, Dübendorf, Switzerland.

Szabó G.Y., Fazekas I., Szabó S., Szabó G., Buday T., Paládi M., Kisari K., Kerényi A., (2014), The carbon footprint of a biogas power plant, *Environmental Engineering and Management Journal*, **13**, 2867-2874.

Tashima D., Asano Y., Tomomatsu S., Sugimoto Y., (2014), Thermal efficiency of small scale biogas plant for bio-methane production, *Environmental Engineering and Management Journal*, **14**, 745-749.

Wolf M.A., Pant R., Chomkhamsri K., Sala S., Pennington D., (2012), International Reference Life Cycle Data System (ILCD), Handbook – Towards more sustainable production and consumption for a resource-efficient Europe, JRC Reference Report, EUR 24982 EN, European Commission – Joint Research Centre, Luxembourg, Publications Office of the European Union.

Zagorskis A., Baltrėnas P., Misevičius A., Baltrėnaitė E., (2012), Biogas production by anaerobic treatment of waste mixture consisting of cattle manure and vegetable remains, *Environmental Engineering and Management Journal*, **11**, 849-856.

Environmental Engineering and Management Journal

September 2016, Vol.15, No. 9, 1923-1931
http://omicron.ch.tuiasi.ro/EEMJ/

"Gheorghe Asachi" Technical University of Iasi, Romania

ENVIRONMENTAL FOOTPRINT IN THE PRODUCTION OF RECYCLED WOOL

Silvia Bamonti, Rosangela Spinelli, Alessandra Bonoli*

Department of Civil, Chemical, Environmental and Material Engineering, University of Bologna, Italy

Abstract

We computed the Product Environmental Footprint (PEF) to identify the environmental performance of recycled wool by means of data belonging to a real case study in Italy. First, we analyzed inventory data quality by computing the data quality rating (DQR) index, according to the criteria provided by the PEF protocol. Quality of specific and generic data (selected from the Ecoinvent database) was evaluated as excellent and good, respectively. We then quantified the impacts related to each relevant production process via Life Cycle Assessment (LCA). Specifically, we identified four main processes involved in the production of recycled wool for the selected case study: (i) clippings supply, (ii) clippings preparation, (iii) ragging, and (iv) dyeing. In the production of recycled wool, the rate of incoming materials subject to dyeing decreases to about 40%. Nevertheless, LCA revealed that this process is the most critical due to the high energy consumption and chemical substances employed. This result was confirmed by further analysis based on the Eco-indicator approach and the single point eco-indicator score. In particular, we found that environmental impacts associated with the production of recycled wool are mainly related to the following categories: (i) depletion of abiotic resources, (ii) inorganic particles released into the air, (iii) climate change, and (iv) human toxicity. We quantified that the contribution of the dyeing process to the relevant impact categories is higher than 75%. These results revealed how the use of recycled materials strongly increases the environmental performance associated with wool production, reducing the impact of the most environmental demanding processes.

Key words: life cycle assessment, material recovery, product environmental footprint, textile sector

Received: January, 2016; Revised final: May, 2016; Accepted: August, 2016

1. Introduction

The Product Environmental Footprint (PEF) represents a measure of natural resources exploitation and environmental impacts for which a given product is responsible within its life-cycle (Bacenetti and Fiala, 2015; Ewing et al., 2011; Kjaer et al., 2015). This distinguishes the PEF approach from traditional carbon and water footprint, focused specifically on greenhouse gas emissions and freshwater consumption (directly/indirectly related to the product life cycle), respectively (e.g. Hertwich and Peters, 2009; Hoekstra, 2003). Another metric, the ecological footprint, captures the impacts on the biosphere's regenerative capacity, quantifying environmental consequences of pressures on the biosphere and related ecosystems (Wackernagel et al., 1999). In general, all these metrics may be applied at different scales (e.g., products, cities, regions, nations). Though partial overlaps exist among these approaches, the carbon, water and ecological footprints provide, in general, complementary information that can be jointly analyzed to account for pressures induced by human activities on the atmosphere, hydrosphere and biosphere compartments (Galli et al., 2012; Farcas et al., 2015).

The PEF approach was introduced by the European Commission (EU, 2013a) in the context of the Communication "Building the Single Market for Green Products" (EC, 2013) with the aim of enhancing comparability between products in order

---

* Author to whom all correspondence should be addressed: e-mail: alessandra.bonoli@unibo.it; Phone: +39 0512090234; Fax: +39 0512090308

to provide basis for environmental products declaration and promote the use of the Life Cycle Assessment (LCA) approach amongst industries (for further discussion see e.g., Finkbeiner, 2014; Lehmann et al., 2016, and references therein). Specifically, the PEF is a multi-criteria method aimed at identifying the environmental performance of a good or service throughout its life cycle. All the relevant supply chain activities, from extraction of raw materials to final waste management, are considered in this framework (EC, 2012; EU, 2013b). The PEF method relies on well-known guides among with the European Standards provided by the International Organization for Standardization (specifically ISO 14040, 2006 and ISO 14044, 2006), the International Reference Life Cycle Data System Handbook (ILCD, 2011), and the Greenhouse Gas Protocol (WRI and WBCSD, 2011). Environmental impacts of material/energy flows as well as emissions and wastes are computed via LCA (Corabieru et al., 2014; Ghinea et al., 2014).

Application of the PEF approach provides: (i) information about the environmental performance of a product or service, (ii) a support for eco-design strategies and for the optimization of the processes included in the product life cycle, and (iii) the identification of significant environmental impacts useful to the application of eco-labels criteria. When appropriate, incentives based on the environmental performance of a given product may be assigned according to the results provided by the PEF approach. This is practically realized by means of a set of environmental indicators related to the main categories of environmental impact (greenhouse gas emissions, resource efficiency, water footprint, etc.); in addition, stakeholders may employ these indicators, once validated, for marketing communication. For these reasons, the PEF approach may be relevant for an increasing number of industries interested in developing strategies for the reduction of environmental impacts associated with products life cycles (Kjaer et al., 2015).

In order to increase the environmental performance of a product, it may be relevant to use recycled materials as promoted by European regulations (EC Directive, 2008) and reports (e.g. ISPRA, 2011). Here, we explore this opportunity in the context of the textile sector for which a critical issue consists in the reduction of the environmental impact associated with the phases of a textile product's life cycle. Increasing attention is paid to improve sustainability in materials and processes, in government regulations and in the development of environmentally-friendly technologies. Recycling of end-of-life textile products may also play an important role (e.g. Blackburn, 2009). Some studies have been conducted to demonstrate the convenience of textile recycling relying on, e.g., the computation of the carbon footprint (Muthu et al., 2012) or the energy footprint (Woolridge et al., 2006) to assess benefit of recycling operations.

Here, we present a case study related to the production of recycled wool and we apply the PEF approach in order to (i) identify the most significant impacts and environmental demanding processes of the product life cycle, (ii) demonstrate how the use of recycled fibers significantly improves the environmental performance of wool production, reducing the amount of material involved with the processes associated with the most relevant impacts. To do this, we employ data belonging to a textile industry in the district of Prato (Italy). Our analysis may also represent a benchmark in the adoption of the PEF approach to assess the environmental impacts of a textile product.

In the following sections, we briefly revise some of the key steps of methods we employ, such as the analysis of the inventory and the selection of the environmental footprint impact categories (Section 2); we then describe the case study (Section 3) and discuss results provided by the LCA (Section 4). A set of conclusions closes the paper.

2. Materials and methods

Important steps of the PEF methodology consist in the building of the inventory and in the contextual assessment of data quality. Data have to be collected for each process involved in the product life cycle as a basis for modeling the PEF. Hence, a proper evaluation of the impacts associated with the diverse categories defined in the PEF framework, relies on the completeness and accuracy of the inventory. In this section, we first revise the criteria useful to pursue inventory analysis and data quality assessment; as a second step, we select proper EF impact categories to investigate a case study dealing with recycled wool production.

2.1. Analysis of inventory and data quality assessment in the PEF approach

In the framework of the PEF methodology, based on LCA (ISO 14040), the following steps are relevant: (i) goal and scope definition for the study, (ii) inventory analysis referred to input/output data associated with each process included in the product life cycle, (iii) impact assessment by means of a set of metrics/indicators, (iv) interpretation of the results in accordance with the aims of the study, as defined at the beginning. In this subsection, we focus on the analysis of the inventory that is divided into several steps in turn, as depicted in Fig. 1.

Data referred to the processes involved in the product life cycle are typically distinguished into two categories on the basis of the collection strategy. The first category includes specific data, i.e. input/output data that are directly measured in each process. As an example, energy, water and materials may fall into the category of specific input data, while emission measurements typically represent specific output data.

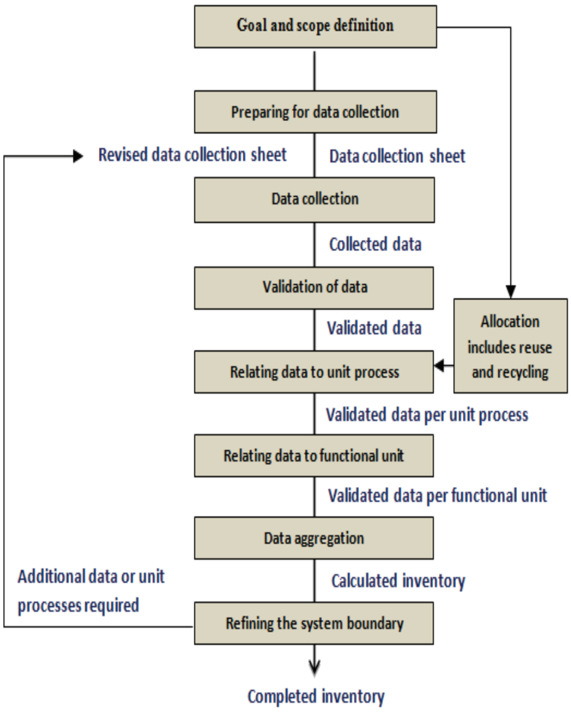


Fig. 1. Flow chart of the inventory analysis according to ISO 14041

The second category is that of generic data, resulting from literature, statistics, projects or databases and that should be properly employed to fill the gaps in the availability of specific data. Once collected relevant data, a set of metrics are used for data quality assessment. The PEF protocol requires a semi-quantitative assessment procedure based on expert judgment, according to a set of criteria listed in the following (EC, 2012; ISO 14044, 2006):

1. technological representativeness (TeR), representing the "*degree to which the dataset reflects the true population of interest regarding technology*";
2. geographical representativeness (G), representing the "*degree to which the dataset reflects the true population of interest regarding geography*";
3. time-related representativeness (Ti), representing the "*degree to which the dataset reflects the specific conditions of the system being considered regarding the time/age of the data*";
4. completeness (C), determined "*with respect to the coverage for each impact category and in comparison to a hypothetical ideal data quality*";
5. parameter uncertainty (P), determined for the resource use and emission data by means of the "*qualitative expert judgment or relative standard deviation as a % if a Monte Carlo simulation is used*";
6. methodological appropriateness and consistency (M), assessing if "*the applied Life Cycle Inventory methods and methodological choices are in line with the goal and scope of the dataset, especially its intended applications as support to decisions. The methods have also been consistently applied across all data*".

The first five criteria are directly referred to data, while the last one evaluates if the approach is PEF compliant. The value assigned to each of these parameters may range between 1 and 5, where 1 corresponds to a very good quality level, while 5 is associated to a very poor level of data quality according to given criteria (EC, 2012). By calculating the average of these values, it is possible to identify a unique parameter representing the overall data quality rating (*DQR*) (Eq. 1).

$$DQR = \frac{TeR + GR + TiR + C + P + M}{6} \tag{1}$$

According to the value assumed by the *DQR*, it is possible to classify data quality as reported in Table 1. A very good level of data quality is defined as the case in which the inventory "*meets the criterion to a very high degree, without need for improvements*" and this is typically achieved when the technological/geographical/time-related representativeness are evaluated as context-specific, a virtually negligible uncertainty is associated with parameters, a full compliance with the requirements of the PEF approach is observed and a very good completeness (>90%) is detected.

On the contrary, the overall data quality is very poor when completeness decreases below 50%, parameter uncertainty is high and none of the methods required by the PEF methodology (specifically: end of life modeling, system boundary following the approach from cradle to grave and multi-functionality according to ISO 14040/44) are employed (EC, 2012).

Table 1. Classification of the overall data quality based on the *DQR values*

Overall DQR	***Data quality level***
DQR ≤ 1.6	excellent quality
1.6 ≤ DQR ≤ 2.0	very good quality
2.0 ≤ DQR ≤ 3.0	good quality
3.0 ≤ DQR ≤ 4.0	fair quality
DQR ≥ 4.0	poor quality

The value of the *DQR* index is first computed for (i) each process involved in the life cycle analysis, and (ii) both specific and generic data. As a consequence, a total value is then calculated (for all the processes taken into account) as the average of all the ratings computed for specific and generic data. Completeness, related to the coverage of each EF impact category, respect to a hypothetical ideal data quality, is expressed by C as the share of elementary flows included in the inventory and is particularly important for the robustness of the method.

For PEF compliance, a "good quality" evaluation (*DQR* ≤ 3.0) should be associated at least to the 70% of the contributions to each EF impact category (EC, 2012).

2.2. Environmental Footprint impact categories

The PEF methodology defines specific impact categories to study the environmental footprint of a given product. Nevertheless, it is allowed to select additional impact categories related to environmental aspects of interest for the study. Table 2 collects the impact categories that we employed to analyze our case study. Note that, in the simulation code we used, SIMAPRO (Goedkoop et al., 2016), it was not implemented the method ILCD 2001; however, we derive useful information from other impact categories employing the same indicators. We also considered the impact category *Cumulative Energy Demand* (CED) developed by Frischknecht and Jungbluth (2007) and already included in the software.

Regarding to the other methods, we adopted the CML 2001 that proposes a set of impact categories and characterization methods for the impact assessment step. Specifically, the Eco-indicator 99 and the EPS method are selected as damage approaches; the CML 2001 impact assessment method implemented in Ecoinvent consists in the set of impact categories defined for the midpoint approach (Guinée et al., 2002, for more details). Based on the CML 2001 method we analyzed the following impact categories: (i) climate change, for which the characterization model is developed by the IPCC, related to emissions of greenhouse gases to air, and expressed as Global Warming Potential for time horizon 100 years (GWP100), (ii) stratospheric Ozone depletion, for which the characterization model is developed by the World Meteorological Organization (WMO), related to ozone depletion potential of different gasses, (iii) depletion of abiotic resources, related to extraction of minerals and fossil fuels due to inputs in the system, (iv) fresh-water aquatic eco-toxicity, related to the impact on fresh water ecosystems, as a result of emissions of toxic substances to air, water and soil and expressed as eco-toxicity potential (FAETP) based on USES-LCA, describing fate, exposure and effects of toxic substances.

We also employed the RECIPE method that implements both midpoint (problem oriented) and endpoint (damage oriented) impact categories. The midpoint characterization factors are multiplied by damage factors, to obtain the endpoint characterization values. This method is based on the integration of the problem-oriented approach of the CML method and the damage-oriented approach of Eco-indicator 99.

Based on the RECIPE method we analyzed the following impact categories: (i) natural land transformation, defined by the amount of natural land transformed and occupied for a certain time, (ii) ionising radiation, accounting for the level of exposure, (iii) photochemical oxidant formation, defined as the marginal change in the 24h-average European concentration of ozone due to a marginal change in emission of a given substance, (iv) freshwater eutrophication, accounting for the environmental persistence of the emission of P containing nutrients (v) marine eutrophication, accounting for the environmental persistence of the emission of N containing nutrients (vi) water depletion, define by the amount of fresh water consumption. These impact categories are characterized at midpoint level. The midpoint impact categories are aggregated into three endpoint categories, i.e. human health, ecosystems and resource surplus costs, that finally give rise to a single score (for more details refer to Goedkoop et al., 2009).

Among the other methods we employed, the USETOX model is used to account for human and eco-toxicological impacts and it is designed to describe the fate, exposure and effects of chemicals (Rosenbaum et al., 2011). TRACI is a tool for the reduction and assessment of chemical and other environmental impacts and consists in a stand-alone computer program developed by the U.S. Environmental Protection Agency.

Table 2. EF impact categories, methods and indicators employed to quantify the impacts

	Impact category	*Method*	*Indicator*
1	Climate change (GWP 100)	CML 2001	kg carbon dioxide/kg emission
2	Stratospheric Ozone depletion	CML 2001	kg CFC-11 equivalent/ kg emission
3	Depletion of abiotic resources	CML 2001	kg antimony equivalents/kg extraction
4	Natural land transformation	RECIPE	m^2
5	Human toxicity – cancer effects	USETOX	CTUh comparative toxic units
6	Human toxicity – non cancer effects	USETOX	CTUh comparative toxic units
7	Fresh-water aquatic eco-toxicity	CML 2001	1,4-dichlorobenzene equivalents/kg emission
8	Inorganic particles released into the air	IMPACT 2001	kg PM2.5 equivalent
9	Ionising radiation	RECIPE	kg (U^{235} to air)
10	Photochemical oxidant formation	RECIPE	kg (NMVOC to air)
11	Eutrophication	TRACI	kg N eq/kg substance
12	Freshwater eutrophication	RECIPE	kg (P to freshwater)
13	Marine eutrophication	RECIPE	kg (N to freshwater)
14	Water depletion	RECIPE	m^3 water eq
15	Acidification	TRACI 2	molc H+ eq
16	Cumulative Energy Demand	CED	MJ

It is a midpoint oriented life cycle impact assessment methodology that we adopted to account for eutrophication and acidification.

3. Case study

In this work, we analyze a case study referred to the production of recycled wool. Specifically, our aim is to assess the environmental performance of this product by employing the PEF methodology, with particular attention on the inventory analysis and selection of impact categories. Note that a comparison between the environmental performance of recycled and virgin wool goes beyond the aims of this work and it is not critical in the context of the PEF approach.

The system boundaries are defined by considering all the procurement activities associated with raw materials, resource consumption and emissions. The steps related to the product use and the end of life scenario are not analyzed in this study. This approach is not in contrast with what is reported in the reference guidelines, which require the system boundaries to include upstream activities (such as raw materials and energy production) and production activities (e.g. wool production).

Four main processes have been identified in the production of recycled wool for the selected case study. The first regards the supply of clippings, by means of materials coming from Italy and foreign countries (mainly Middle East countries). Environmental impacts associated with this process are mainly due to transport activities. As a second step, the incoming materials are selected and sorted by color and type before being sent to the subsequent process of ragging. The latter represents the third relevant process and the fibers, obtained at this stage, whose colors need to be modified, are finally sent to the dyeing process. The selection process is particularly relevant, reducing the material subject to dyeing. For this case study, it resulted that only the 42% of the incoming material is dyed. This contributes significantly to increase the environmental efficiency of the whole production of recycled wool. Fig. 2 depicts the workflow required to obtain recycled wool, based on the steps described above, together with the flows of energy and materials involved.

Data referred to the year 2014 are used to build the inventory for the application of the PEF methodology. The analysis of data quality, in line with the PEF Protocol, has led to the computation of the *DQR* index in (1) for both specific and generic data. Considering all the processes involved, the quality associated with the specific data is excellent (*DQR = 1.23*); note, that this evaluation is confirmed when analyzing the quality of specific data for each single process. Generic data are included in the inventory to integrate the information required by the analysis. Specifically, missed data are obtained resorting to the Ecoinvent database and their overall quality is evaluated as good (*DQR = 2.3*).

The functional unit chosen to evaluate the processes is 1 kg of recycled wool. The emissions considered as relevant are collected in Table 3; data are reported for each process.

Table 3. Emission inventory for the production of recycled wool for the selected case study

Emissions	***Unit***	***Total amount***	***Portion of each process (%)***			
			Clippings supply	***Clippings preparation***	***Ragging***	***Dyeing***
Sea transport	t km	1.54	100.0	0.0	0.0	0.0
Land transport	kg km	135.31	91.5	0.6	5.5	2.3
Electric energy	kWh	0.82	0.0	5.5	18.4	76.1
Methane	m^3	0.12	0.0	0.0	0.0	100.0
Water	kg	0.12	0.0	100.0	0.0	0.0
Water - well	m^3	0.002	0.0	0.0	0.0	100.0
Water treatment	m^3	2.14	0.0	0.0	0.0	100.0
Recycling	g	8.07	0.0	100.0	0.0	0.0
Iron	g	14.13	4.4	95.6	0.0	0.0
Polyethylene	g	9.00	6.8	93.2	0.0	0.0
Dye	g	16.80	0.0	0.0	0.0	100.0
Wetting agent	g	0.84	0.0	0.0	0.0	100.0
Acetic acet	g	2.10	0.0	0.0	0.0	100.0
Soda	g	2.78	0.0	0.0	0.0	100.0
Air Emissions	***Unit***	***Total amount***	***Clippings supply***	***Clippings preparation***	***Ragging***	***Dyeing***
Total Organic Carbon	mg	7.90	0.0	0.0	0.0	100.0
Dust	mg	159.92	0.0	0.0	0.0	100.0
Nitrogen oxides	mg	1061.82	0.0	0.0	0.0	100.0
Ammonia	mg	6.33	0.0	0.0	0.0	100.0
Sulfuric acid	mg	930.10	0.0	0.0	0.0	100.0
Formic acid	mg	744.09	0.0	0.0	0.0	100.0
Acetic acid	mg	10.98	0.0	0.0	0.0	100.0

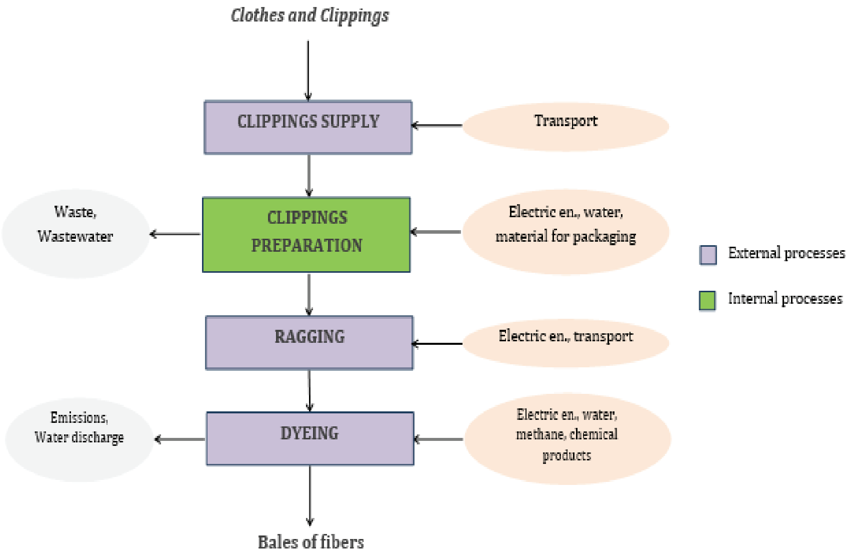


Fig. 2. Flow chart of recycled wool production

4. Results and discussion

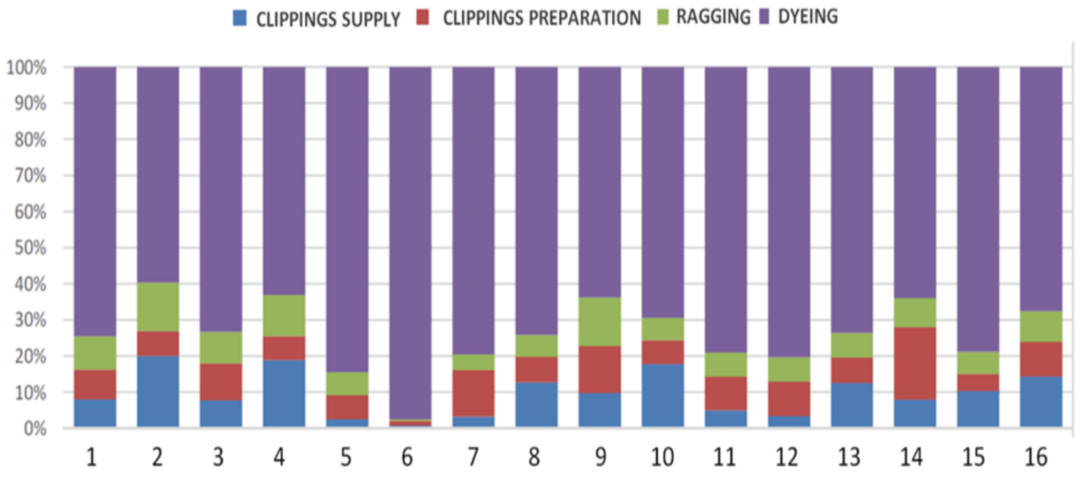
LCA has been realized by means of the code SIMAPRO (Goedkoop et al., 2016). The analysis reveals the contribution of the diverse processes to each impact category. Fig. 3 shows, for each selected impact category (see Table 2), the contribution (in percent) of the main processes involved in the production of recycled wool. The step that mainly produces significant impacts is dyeing, even if only the 42% of fibers is subject to this process. Indeed, dyeing operation is particularly onerous due to energy consumption and chemical substances employed in the process. Other high-impact processes are clippings supply, due to sea/land transport activities associated with not negligible emissions, and clippings preparation. On the contrary, the process of ragging produces the overall lower impact for the selected categories.

The Eco-indicator approach (Goedkoop and Spriensma, 2001) has been also employed in order to quantify the overall impact of the recycled wool production, exemplified by means of the selected case study. Fig. 4 shows analogue results obtained with this method and ordered for impact categories. It is possible to observe that previous analysis are confirmed by this approach; dyeing, in particular, is the process that primarily compromises the environmental performance of the entire production cycle.

Finally, the contribution (in percent) of each impact category to the *single point eco-indicator score* has been computed. The most relevant categories for this case study are: the depletion of abiotic resources (45.8%), the inorganic particles released into the air (30.8%), the climate change (18.5%), and the human toxicity – cancer effects (2.5%). This is due to the high electric/thermal energy consumption and to small quantities of carcinogens released mainly during dyeing. This leads to a value of the indicator equal to 0.127 with a maximum contribution of dyeing (77.3%); clippings preparation, clippings supply and ragging are responsible for the 13.3%, 5.6% and 3.8% respectively.

In the following, more details are provided for the most relevant impact categories identified by the previous analysis. Looking at the indicator accounting for climate changes, it is possible to observe that about the 75% of the impact is due to the process of dyeing; ragging is responsible for the 9%, while the processes of clippings supply and preparation are both responsible for the 8%. The high-impact of dyeing is mainly associated with the production/consumption of electric energy (42%) and the production/consumption of natural gas (27%). The indicator accounting for inorganic emissions into the atmosphere reveals that the impact of dyeing is about 74%, followed by clippings supply (13%), clippings preparation (7%) and ragging (6%). For this impact category, in the dyeing process, the consumption of electric energy and natural gas has the greatest influence together with the use of chemical substances. Similarly, the indicator accounting for the depletion of abiotic resources shows that the impact of dyeing is about the 73% mainly due to the energy production required by the process. Another important impact category is that related to human toxicity and, in particular, to carcinogens. The process of dyeing is responsible for the 84% of the total impact and also in this case, the consumption of electric energy (34.4%) and thermal energy (23.9%) play the main role. Nevertheless, the contribution of the use of chemical substances (4.2%) has to be taken into account for this impact category.

Our results confirm findings provided by other studies, showing the critical role played by the dyeing process (e.g. Kant, 2012; Parisi et al., 2015).



1	Climate change (GWP 100)	5	Human toxicity – cancer effects	9	Ionizing radiation	13	Marine eutrophication
2	Stratospheric Ozone depletion	6	Human toxicity – non cancer effects	10	Photochemical oxidant formation	14	Water depletion
3	Depletion of abiotic resources	7	Fresh-water aquatic eco-toxicity	11	Eutrophication	15	Acidification
4	Natural land transformation	8	Inorganic particles released into the air	12	Freshwater eutrophication	16	Cumulative Energy Demand

Fig. 3. Impacts of the main processes involved in the production of recycled wool for each impact category

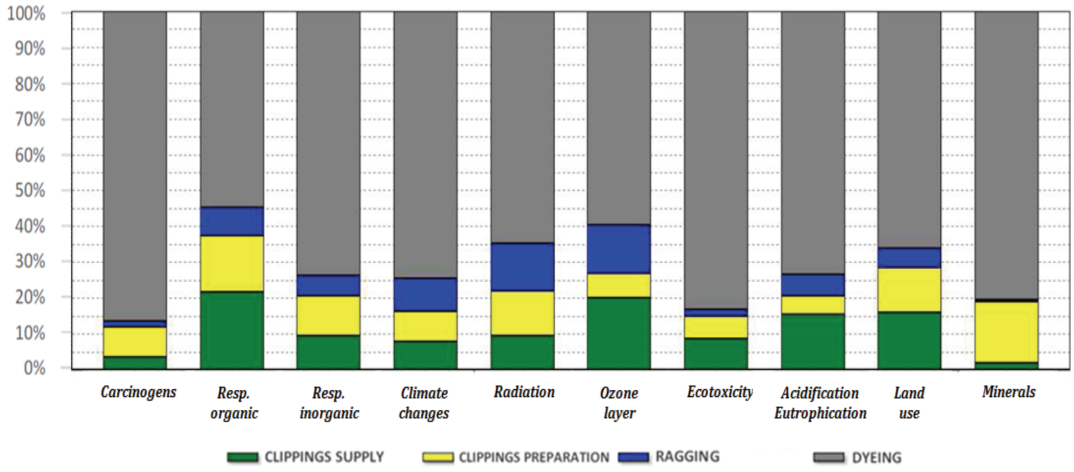


Fig. 4. Impacts of the main processes involved in the production of recycled wool by means of the Eco-indicator approach

Note that, while most of studies, regarding textile production, investigated the effectiveness of (i) alternative environmentally-friendly technologies or (ii) recycling of textile process waste, here we provide a quantitative analysis about the advantages associated with the recycling of end-of-life textile materials. In doing this, we obtained results consistent with other analysis (e.g. Blackburn, 2009; Muthu et al., 2012; Woolridge et al., 2006) based on other footprint approaches rather than the PEF.

5. Conclusions

In this study, we focused on recycling of end-of-life products in the textile sectors. Specifically, we analyzed a case study related to recycled wool production. Similar issues have been typically investigated by means of LCA in the context of traditional, e.g., carbon/water footprint. Here, we quantify the impacts associated with each relevant process in the life cycle of recycled wool relying on the PEF methodology, thus providing a benchmark in the use of this approach. Our results showed that dyeing is the most environmental demanding process inside the product life cycle, thus supporting previous analyses in literature. In addition, we highlighted how the use of recycled materials strongly increases the environmental performance of wool production, by reducing at the 42% the material involved in the dyeing process. This is relevant to support recycling as promoted by the EU directives and regulations on waste management. Our analysis also revealed that the principal impact categories (such as climate changes, inorganic emissions into the atmosphere, abiotic resources depletion and human toxicity) are mainly influenced by energy production and consumption. The use of chemical substances plays also a relevant role regarding to toxicity and soil/water pollution. Hence, strategies based on recycling of end-of-life textile materials may be properly combined with the use of environmentally-friendly technologies and with recycling of textile process waste, in order to further improve the environmental efficiency of production systems. In conclusion, the following actions are identified as relevant in order to increase the environmental performance of wool production (as exemplified by the selected case study): (i) improving the efficiency of the clippings supply process in order enhance the use of recycled materials, (ii) employing chemical substances with lower environmental impact, and (iii) reducing the energy demand associated with the product life cycle, introducing the use of renewable energy.

References

Bacenetti J., Fiala M., (2015), Carbon footprint of electricity from anaerobic digestion plants in Italy, *Environmental Engineering and Management Journal*, **14**, 1495-1502.

Blackburn R., (2009), *Sustainable Textiles: Life cycle and Environmental Impact*, Woodhead Publishing, Cambridge, UK.

Corabieru P., Corabieru A., Vasilescu D.D., (2014), New approaches in the design of plastic products for easy recycling, *Environmental Engineering and Management Journal*, **13**, 1997-2004.

EC Directive, (2008), Directive 2008/98/EC of the European Parliament and of the Council of 19 November 2008 on waste and repealing certain Directives on (Waste Framework Directive), *Official Journal of the European Communities*, L312/3, 22.11.2008, Brussels.

EC, (2012), European Commission - Joint Research Centre - Institute for Environment and Sustainability: Product Environmental Footprint (PEF) Guide, 17/07/2012, On line at: http://ec.europa.eu/environment/archives/eussd/pdf/footprint/PEF%20methodology%20final%20draft.pdf.

EC, (2013), Building the Single Market for Green Products, Facilitating Better Information on the Environmental Performance of Products and Organisations, Communication from the Commission to the European Parliament and the Council, COM(2013) 196 Final; European Commission: Brussels, Belgium; Luxembourg, Luxembourg.

EU, (2013a), Commission Recommendation of 9 April 2013 on the use of common methods to measure and communicate the life cycle environmental performance of products and organisations, On line at: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32013 H0179.

EU, (2013b), EU Environmental Footprint website, On line at: http://ec.europa.eu/environment/eussd/smgp/policy_footprint.htm.

Ewing A., Thabrew L., Perrone D., Abkowitz M., Hornberger G., (2011), Insights on the use of hybrid life cycle assessment for environmental footprinting – A case study of an Inland Marine Freight transportation company, *Journal of Industrial Ecology*, **15**, 937-950.

Farcas V., Ilies N., Cot R., (2015), CO_2 and energy footprint of different retaining walls solutions. Masonry retaining wall vs. cantilever retaining wall, *Environmental Engineering and Management Journal*, **14**, 1323-1326.

Finkbeiner M., (2014), Product environmental footprint-Breakthrough or breakdown for policy implementation of life cycle assessment?, *International Journal of Life Cycle Assessment*, **19**, 266-271.

Galli A., Wiedmann T., Ercin E., Knoblauch D., Ewing B., Giljum S., (2012), Integrating Ecological, Carbon and Water footprint into a "Footprint Family" of indicators: Definition and role in tracking human pressure on the planet, *Ecological Indicators*, **16**, 100-112.

Ghinea C., Petraru M., Simion I.M., Sobariu D., Bressers H.T.A., Gavrilescu M., (2014), Life cycle assessment of waste management and recycled paper systems, *Environmental Engineering and Management Journal*, **13**, 2073-2085.

Goedkoop M., Spriensma R., (2001), The Eco-indicator 99 A damage orientated method for Life Cycle Assessment, Methodology Report, Third edition, 22 June, PRé Consultants, Amersfoort, Netherlands, On line at: http://www.pre.nl/download/EI99_methodology_v3.pdf.

Goedkoop M., Heijungs R., Huijbregts M.A.J., De Schryver A., Struijs J., Van Zelm R., (2009), ReCiPe 2008 - A life cycle impact assessment method which comprises harmonised category indicators at the midpoint and the endpoint level, First edition, Report I: Characterisation, RIVM report.

Goedkoop M., Oele M., Leijting J., Ponsioen T., Meijer E., (2016), Introduction to LCA with SimaPro 8, Prè Consultants, On line at: https://www.pre-sustainability.com/download/SimaPro8IntroductionToLCA.pdf.

Guinée J.B., Gorrée M., Heijungs R., Huppes G., Kleijn R., de Koning A., van Oers L., Wegener Sleeswijk A., Suh S., Udo de Haes H.A., de Bruijn H., van Duin R., Huijbregts M.A.J., (2002), *Handbook on Life Cycle Assessment, Operational Guide to the ISO Standards, Part III: Scientific Background*, Kluwer Academic Publishers, Dordrecht.

Hertwich E.G., Peters G.P., (2009), Carbon footprint of nations: A global, trade-linked analysis, *Environmental Science and Technology*, **43**, 6414-6420.

Hoekstra A.Y., (2003), *Virtual Water Trade,* Proc. of the International Expert Meeting on Virtual Water Trade, Delft, The Netherlands, 12-13 December, Value of Water Research Report Series No. 12, UNESCO-IHE, Delft, The Netherlands, On line at: www.waterfootprint.org/Reports/Report12.pdf.

ILCD, (2011), European Commission-Joint Research Centre - Institute for Environment and Sustainability: International Reference Life Cycle Data System (ILCD) Handbook- Recommendations for Life Cycle Impact Assessment in the European context, First edition, EUR 24571 EN, Luxemburg, Publications Office of the European Union.

ISO 14040, (2006), Environmental management, Life cycle assessment - Principles and framework, *European Standard EN ISO 14040*, International Organization for Standardization, Geneva, Switzerland.

ISO 14044, (2006), Environmental management, Life cycle assessment - Requirements and guidelines, *European*

Standard EN ISO 14044, International Organization for Standardization, Geneva, Switzerland.

ISPRA, (2011), Waste Report, Edition 2011, Institute for the Protection and Environmental Research, On line at: http://www.isprambiente.gov.it/en/archive/news-and-other-events/ispra-news/year-2011/rapporto-rifiuti-2011

Kant R., (2012), Textile dyeing industry an environmental hazard, *Natural Science*, **4**, 22-26.

Kjaer L.L., Høst-Madsen N.K., Schmidt J.H., McAloone T.C., (2015), Application of environmental input-output analysis for corporate and product environmental footprints-learnings from three cases, *Sustainability*, **7**, 11438-11461.

Lehmann A., Bach V., Finkbeiner M., (2016*)*, EU Product environmental footprint-mid-term review of the pilot phase, *Sustainability*, **8**, 92, doi:10.3390/su8010092.

Muthu S.S., Li Y., Hu J.Y., Ze L., (2012), Carbon footprint reduction in the textile process chain: Recycling of textile materials, *Fibers and Polymers*, **13**, 1065-1070.

Parisi M.L., Fatarella E., Spinelli D., Pogni R., Basosi R., (2015), Environmental impact assessment of an eco-efficient production for coloured textiles, *Journal of Cleaner Production*, **108**, 514-524.

Rosenbaum R.K., Huijbregts M.A.J., Henderson A.D., Margni M., McKone T.E., van de Meent D., Hauschild M.Z., Shaked S., Li D.S., Gold L.S., Jolliet O., (2011), USEtox human exposure and toxicity factors for comparative assessment of toxic emissions in life cycle analysis: sensitivity to key chemical properties, *International Journal of Life Cycle Assessment*, **16**, 710-727.

Wackernagel M., Onisto L., Bello P., Linares A.C., Falfán L., García J.M., Suárez G.A.I., Suárez G.M.G., (1999), National natural capital accounting with the ecological footprint concept, *Ecological Economics*, **29**, 375-390.

Woolridge A.C., Ward G.D., Phillips P.S., Collins M., Gandy S., (2006), Life cycle assessment for reuse/recycling of donated waste textiles compared to use of virgin material: An UK energy saving perspective, *Resources Conservation and Recycling*, **46**, 94-103.

WRI, WBCSD, (2011), Greenhouse Gas Protocol Product Life Cycle Accounting and Reporting Standard, 2011, On line at: http://www.wri.org/publication/greenhouse-gas-protocol-product-life-cycle-accounting-and-reporting-standard.

1932

Environmental Engineering and Management Journal

September 2016, Vol.15, No. 9, 1933-1940
http://omicron.ch.tuiasi.ro/EEMJ/

"Gheorghe Asachi" Technical University of Iasi, Romania

VALORIZATION OF GLASS WASTES AS SUPPORT FOR LIPASE IMMOBILIZATION

Luisa Barbieri[1], Elena Bursi[1*], Maria Rita Cramarossa[2], Laura Ferroni[4], Luca Forti[2], Isabella Lancellotti[1], Chiara Ponzoni[1], Ivano Vassura[3,4]

[1]*Engineering Department "Enzo Ferrari", University of Modena and Reggio Emilia, Modena, Italy*
[2]*Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy*
[3]*Department of Industrial Chemistry "Toso Montanari", University of Bologna, Italy*
[4]*Interdepartmental Centre of Industrial Research CIRI Energy and Environment, University of Bologna, Italy*

Abstract

This work focuses on treatment of lead polluted waste glass from urban collection (Pb≈5% wt) for its valorization as support in the immobilization of lipase, because soda-lime glasses are used in enzymatic catalysis.
First of all, an extraction process of surface Pb was performed. The process is based on nitrilotriacetic acid (NTA) chelating agent. The operating conditions are: T=80°C, t=1h, pH 10, solid/liquid weight ratio=1/10, reagent concentration=0.1 M. Leaching tests at controlled pH performed on treated glass confirmed the typical inert nature of the glass and the effectiveness of the NTA treatment.
Afterwards lead polluted waste glasses, both untreated and treated with NTA, together with a commercial reference glass, were used as physical supports for the adsorption of *Rhizopus oryzae* lipase, in order to investigate their immobilization capability after specific washing/activation treatments to make functional their surface. The biocatalytic activity of the immobilized enzyme on the glasses was tested through the hydrolysis of *para*-nitrophenyl acetate to *para*-nitrophenol, quantifying the conversion percentage after 60 min of reaction by means of UV analyses at 410 nm.
From the first results, lipase immobilization on waste glasses appears to be encouraging. In particular, lipase immobilized on lead polluted glass from urban collection showed an activity comparable to that of reference glass.

Key words: chelating agents, enzymatic catalysis, leaching tests, lead extractive method, waste lead-glass

Received: January, 2016; Revised final: June, 2016; Accepted: June, 2016

1. Introduction

In 2014 approximately 2,298,484 tons of packaging glass have been put on the market in Italy and the 70.3% of them have been collected by separate collection. Around 1,615,000 tons of the packaging glass are recovered mainly (99%) in glassworks and 1% in alternative recovery (ceramic industry, building, others glass sectors) (Co.Re.Ve., 2015). The cycle of collection and recovery of this glass can be described in 5 phases:

- collection of glass packaging in the container for recycling,
- withdrawal and transfer of glass cullet to the treatment plant,
- particle size selection and subdivision of cullet glass,
- obtainment of scrap suitable for the glassworks (maximum 0.068% of impurities) (Co.Re.Ve., 2015),
- implementation of new packaging containing up to 90-95% of recycled glass.

Despite sophisticated equipment selection, total selection of "impure glass" is not guaranteed. The recycled glass portion unsuitable for glass smelting furnaces is made up of: a fine fraction (about 30%) with particle size less than 10 mm,

---

[*] Author to whom all correspondence should be addressed: e-mail: elena.bursi@unimore.it; Phone: +39 059 2056221

contaminated by ceramic granules and characterized by a high degree of organic pollutants; a coarser scrap, derived from the selection of automated optical readers (70%). An emerging problem related to this glass fraction is the presence of Pb coming from an incorrect separation of glass, which includes crystal glass and cathode ray tube glass (Taurino et al, 2015). In this paper the polluted glass fraction above reported is valorized as support for lipase immobilization after a pre-treatment for Pb extraction by means of chelating agents.

Lipases (triacylglycerol ester hydrolases, EC 3.1.1.3) are among the most popular enzymes in biocatalysis (Hasan et al., 2006; Reetz, 2002). In fact, owing to their usefulness in both hydrolytic and synthetic reactions, lipases show a broad variety of applications in fine chemistry, pharmaceutical industry, in the production of biodiesel and in the food industry (Aguieiras et al., 2015; Karimi M., 2016; Sharma et al., 2001).

The immobilized lipases is usually the preferred form in industrial process due to the better enzyme stability and activity, and the possibility of recovery and reuse of the biocatalyst at the end of the reaction (Mateo et al., 2007). One of the main lipases characteristic is their activation by hydrophobic interfaces, which could be mechanistically explained by the enzyme conformational change to an open, active structure (Brzozowski et al., 1991; Derewenda et al., 1992). For this reason numerous papers reported lipases immobilization by physical adsorption of the enzyme on a hydrophobic supports/carrier material (Bastida et al., 1998; Palomo et al., 2002; Pizarro et al., 2012).

One of the possible materials for lipase immobilization is glass (in fact soda-lime glasses are currently used in enzymatic catalysis) (Assis and Claro, 1999; Kim and Herr, 2013; Plant et al., 1991). Anyway, no studies concerning lipase immobilization on waste glasses are so far present in literature. As previously reported, a major issue concerning waste glasses is the presence of different kinds of pollutants, and in particular toxic metals, such as Pb, that could negatively affect the enzyme activity.

The aim of this study is therefore to investigate if the Pb contained in polluted waste glasses has an influence on lipase immobilization, and consequently on the enzyme catalytic activity. For this purpose, a comparison with a commercial soda-lime glass used for lipase immobilization has been carried out. The final aim is to valorize a worldwide produced waste, such as lead polluted glass, within the expansive and promising biotechnology field. To better understand the effect of Pb on biocatalysis efficiency, a chelating agent treatment based on NTA solution was besides performed on waste glass, in order to decrease the Pb content, particularly on the glass surface.

Few detailed studies have been made concerning the employment of chelating agents for lead extraction from waste glasses. Sasai et al. (2008) developed a mechano-chemical treatment based on Na_2EDTA, and recovered 99 wt% of Pb from waste funnel glass from cathode ray tubes (CRTs) after 20 hours of treatment at room temperature. Pant et al. (2014) combined chemical and biological leaching by using *Serratia plymuthica* and EDTA to extract several metals from waste CRTs.

In a previous study of screening of different chelating agents, it was assessed that NTA is an efficient chelating agent for the extraction of Pb from CRT funnel glass (PbO=12-25wt%). Furthermore, with respect to EDTA, NTA is more biodegradable (Barbieri et al., 2014). Performing NTA treatment in mild conditions (T=80°C, t=1h), Pb leachable from CRT glass in acidic medium decreased significantly. In particular, a 66-80% reduction was observed at pH 5. The main advantage of this treatment is that it can successfully extract Pb from the glass surface, meanwhile maintaining the glassy nature of the matrix (Bursi et al., 2015). Owing to this, in the present study the NTA treatment was performed on polluted waste glass from urban collection, which presents a lower Pb content than CRT glass (paragraph 2.1). Testing the treatment also on a glass with a low Pb content is a matter of concern, since it is well known that Pb concentration affects glass characteristics. In particular, until a concentration of about 20 wt%, Pb acts as network modifier, making glass more soluble, therefore a decrease of Pb should increase the resistance of glass (Schultz-Münzenberg et al., 1998).

2. Chelating agent glass treatment

2.1. Materials preparation

Lead contaminated packaging glass from urban collection was provided by a treatment plant in North of Italy. To get an index of the average content of lead present in this type of glass, a quartering of a representative sample was carried out, in order to obtain a final quantity of 100g. This was subsequently re-melted at 1450°C and quenched at room temperature to obtain a homogeneous sample, which was analyzed by means of EDS (microanalyzer Inca-350, Oxford Instruments) for semiquantitative lead analysis. The average content of lead resulted to be approximately 5% wt. The as-received glass was firstly dry-ball milled and sieved in order to separate 0.5-1 mm and 0.25-0.5 mm particle sizes. The chelating agent employed, 2,2',2''-Nitrilotriacetic acid (NTA) was used as sodium salt. NTA solution of 0.1 M was prepared keeping pH fixed around 10 with a buffer solution. NTA structure is reported in Fig. 1.

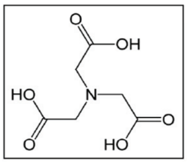


Fig. 1. NTA structure

2.2. Chelating agent treatment at T=80°C

For each of the two glass particle sizes (0.5-1 mm and 0.25-0.5 mm) a treatment with NTA chelating agent solution 0.1 M at pH 10 was performed. Conditions were chosen by a previous work, which demonstrated that alkaline environment favors the reaction (Barbieri et al., 2014). The glass characterized by a specific particle size was sealed in container with chelating agent solution, employing a solid/liquid weight ratio of 1/10. The system was heated at 80°C for 1 h. The treatment was carried out both in static and stirred conditions. The treated glass was separated from the chelating agent solution by settling and washed with distilled water in order to remove any chelating agent traces. The cleaned glass was collected and dried in oven at 110°C overnight.

2.3. Lead leaching tests

Leaching tests at controlled pH were performed to evaluate the effectiveness of the chelating agent treatments. The lead leachable was investigated at the natural pH of water/glass equilibrium (about 7) and at pH 5, since it was observed that this pH promotes the higher release considering different possible environmental scenarios (Bursi et al., 2015). Leaching tests were performed on both untreated and treated glass. The samples were sealed in suitable polypropylene vessels with a solid/liquid weight ratio of 1/10. The system was kept in stirring for 48 h at room-temperature. Set a pH value, this was kept constant (+/- 0.2) for the whole duration of the test. Control was achieved automatically by adding appropriate volumes of acid or base (HNO_3 0.1 M and NaOH 0.1 M) varying from 0.05 mL to over 1 mL. Lead concentration in eluates from leaching tests was determined by FA-AAS analysis (Perkin Elmer AAnalyst 400).

2.4. Chelating agent solution regeneration and lead recovery as lead sulphide

In a previous work, a treatment of regeneration of the spent NTA chelating agent solution was developed, allowing the recovery of the lead extracted from the glass as lead sulphide precipitate (Bursi et al., 2015). In the present study, in addition to the recovery of the metal as PbS, an evaluation of the chelating capability of the regenerated solution was further carried out.

Lead sulphide was precipitated at room temperature by adding to the stirred solution an appropriate quantitative of sodium sulphide (Na_2S), a highly water-soluble salt (470 g/L at 10°C), then it was separated from NTA solution by filtration. Ion-exchange reaction occurred instantly, because of PbS high insolubility ($K_{ps}=3.4*10^{-28}$). Considering that ideally each mole of NTA should have chelated a mole of Pb, and that to precipitate a mole of Pb a mole of Na_2S is needed (2 Na^+ replace Pb^{2+} in the coordination complex), the optimal molar ratio was found to be Na_2S/NTA=1.

The regenerated solution was then tested on 0.25-0.5 mm lead-polluted glass from urban collection, in the same treatment conditions reported in paragraph 2.2. Treated glass was then undergone to leaching tests at controlled pH (Paragraph 2.3).

3. Lipase immobilization on waste glasses

3.1. Materials preparation

Lead contaminated packaging glass from urban collection was ground and sieved to obtain two different particle sizes: a smaller one ranging from 200 to 300 µm and a bigger one ranging from 300 to 500 µm. To deeply understand the role of the compositions, the different surface morphologies and particle sizes of the supports, two Sigma Aldrich glass beads, widely used for enzymes immobilization, were also tested.

Controlled-pore glass beads (particle size 212-300 µm and particle size 425-600 µm) were purchased from Sigma Aldrich. Higly purified lipase from *Rhizopus oryzae* was purchased by Sigma Aldrich.

3.2. Glass treatments

In order to prepare the glass surfaces for the enzyme adsorption step all the glass supports were pre-treated following three different ways: Pb extraction, glass surface cleaning and silanization.

3.2.1. Pb extraction

In the Pb extraction (hereafter described as “Treatment N”), the glass support was undergone to the chelating agent treatment based on NTA solution previously described in paragraph 2.2. This treatment was performed only on lead contaminated glass from urban collection, since commercial soda-lime glass does not contain Pb.

3.2.2. Glass surface cleaning

This step consists of two parts, hereafter described as “Treatment A” and “Treatment B”.

- Treatment A: The glass support was washed with a CH_3COOH 5% v/v solution at T=80°C for 2h with solid/liquid ratio equal to 1/10 under stirring. Then, removed the solution by decantation, the glass was washed in doubly distilled water and ethanol and thereafter was dried in oven at T=110°C overnight.
- Treatment B: The glass, previously undergone to the Treatment A, was treated with a first washing with a solution of NH_4OH:H_2O_2:H_2O=1:1:5 at T=80°C for 5 min under stirring with solid/liquid ratio of 1/10. Then, removed the first solution by decantation, the glass was rinsed with doubly distilled water and then treated again with a second solution of HCl:H_2O_2:H_2O=1:1:5 at T=80°C for 5 min under stirring, with solid/liquid ratio of 1/10. After all the treatments the glass was washed in

doubly distilled water and ethanol, thereafter was dried in oven at T=110°C overnight.

3.2.3. Silanization

During this treatment (hereafter described as "Treatment C") the glass, previously undergone to the Treatment B, was rinsed twice in ethanol and three times in toluene. After that the support was immersed in 0.3% $Cl_2(CH_3)_2Si$ in toluene at T=room for 17 min under stirring with solid/liquid ratio equal to 1/10. After the treatment the solution was removed by decantation and the glass was washed three times with toluene and twice with ethanol and then dried in oven at T=110°C overnight. This treatment produced highly hydrophobic surfaces (Gunnlaugsdottir et al., 1998).

3.3. Lipase immobilization

The *Rhizopus oryzae* lipase was solubilized in a phosphate buffer solution 100 mM (pH=7) with a concentration of 2 mg/mL. The adsorption of crude lipase was carried out adding 5 mL of dissolved lipase to a specified amount of the glass support (160 mg), previously pre-treated. The adsorption was conducted for 22 h at T=35°C in an orbital shaker (330 rpm). The supernatant was removed then the lipase-adsorbed onto glass surface was washed twice with phosphate buffer solution and dried at 30°C. The immobilized lipase was stored at 4°C until use.

3.4. Quantification of the adsorbed lipase as *Protein Loading %*

The yield % of immobilized lipase was determined by measuring the activity of original lipase buffer solution (BS activity) and of the supernatant solution coming from the adsorption step (SS activity), according to Eq. (1). All data presented are the mean of three determinations.

[(BS activity - SS activity)/ (BS activity)]*100 (1)

3.5. Lipase activity in organic media

The activity of immobilized and/or suspended *Rhizopus oryzae* lipase was determined from the hydrolysis of *para*-nitrophenylacetate pNPP (Sigma, Germany) quantifying the amount of *para*-nitrophenol pNP formed by the absorption at 410nm (Pencreac'h and Baratti, 1996). In the reaction mixture, composed of 2 mL of n-hexane containing 10mM pNPP, 20 mg of biocatalyst (lipase immobilized on glass support) were added. The mixture was incubated at 30°C under reciprocal agitation 330 rpm. At fixed time intervals, usually every 5 min, agitation was stopped, the immobilized catalyst was allowed to settle for 30 sec, and 50 µl of the clear supernatant was withdrawn and mixed with 100 µl of NaOH 0.1 M and 2 mL of bidistilled water. The *para*-nitrophenol was extracted by the aqueous alkaline phase and it displayed a yellow color with an absorption at 410 nm.

3.5.1. Determination of hydrolytic activity of pNPP as Conversion %

The hydrolysis of pNPP by the lipase immobilized on glass supports was quantified measuring the absorbance at 410 nm as described previously. To quantify the pNP liberated in mmol a calibration curve was performed. Then the Conversion % was calculated based on the Eq. (2). All data presented are the mean of three determinations.

((initial pNPP conc. – conc. of pNPP not reacted) /initial pNPP conc.) *100 (2)

4. Results and discussion

4.1. Leaching tests at controlled pH

Leaching tests results are reported in Fig. 2. Lead leachable was investigated at the pH of natural equilibrium of water/glass system (about 7) and at pH 5. As expected, the release values were found to be very low both before and after the treatment, with a maximum of 0.23 mg/g for untreated glass with 0.5-1 mm particle size.

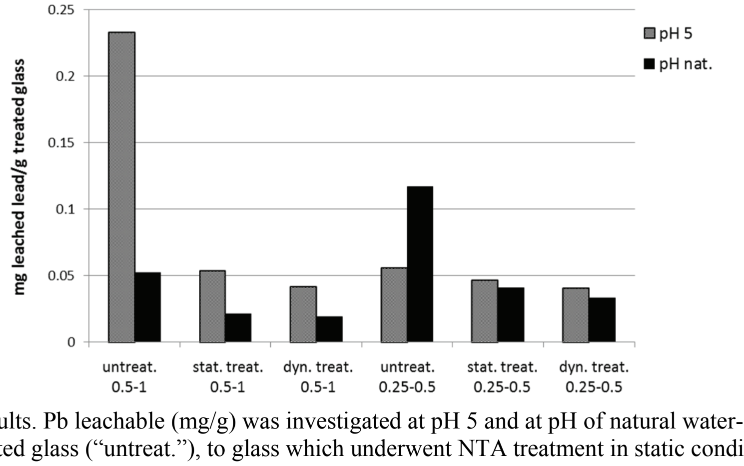


Fig. 2. Leaching tests results. Pb leachable (mg/g) was investigated at pH 5 and at pH of natural water-glass equilibrium. Data refer respectively to untreated glass ("untreat."), to glass which underwent NTA treatment in static condition ("stat. treat.") and to glass which underwent NTA treatment in stirred condition ("dyn. treat."). Both particle sizes (0.5-1 mm and 0.25-0.5 mm) were considered

This demonstrates the typical inert nature of the glass, considering also that the average lead content of this type of glass (Pb≈5% wt) is relatively low if compared for example to that of the funnel glass from cathode ray tube (PbO=12-25% wt).

Results show that in all the samples treated with NTA the release of lead is low as compared to untreated glass. This may indicate a lower lead availability on the glass surface. The samples treated under stirring in general presented lower values of release than those treated statically confirming a higher extraction. This is consistent with what was expected, since in the treatment under stirring the contact surface between glass and solution is continuously renewed. Anyway, it should be considered that these are centesimal differences (e.g. 0.041 mg/g released at natural pH by 0.25-0-5 mm particle size treated statically against 0.033 mg/g released in the same conditions after a treatment under stirring), as the starting release values are all extremely low.

The particle size seems to have not influenced significantly the effectiveness of the treatment and the leaching process, as the release values of 0.5-1 mm and 0.25-0.5 mm are comparable. It is noted that glass particle size is a significant factor affecting lead extraction, as the smaller is the particle size the forceful is the leaching. Anyway, leaching tests at controlled pH were performed after the NTA treatment at pH 10, which is likewise supposed to be forceful for the smallest particle size. Assuming therefore that the Pb extraction with NTA solution has been more effective for 0.25-0.5 mm particle size, some non-extracted Pb unavoidably remains into the glassy matrix, especially in the inner part of the grains, because of the superficial nature of the treatment. After NTA treatment, although 0.25-0.5 mm glass should have a minor quantitative of non-extracted Pb than 0.5-1 mm glass, it is reasonable to think that, owing to the smaller particle size, the non-extracted Pb can be more easily leached from the former during the subsequent leaching tests at controlled pH. Hence, the positive effect of the decrease in particle size may have been hidden by the double leaching. This behavior, previously observed also for CRT glass (Bursi et al., 2015), is currently object of further investigations.

Untreated glass with 0.25-0.5 mm particle size released more lead at natural pH than at pH 5. This result is in contrast with the behavior of glasses with a major lead content, such as CRT glass (Bursi et al., 2015), and may be related to the different characteristics of the two glasses, in particular to the more marked heterogeneity of the Pb polluted glass from urban collection.

4.2. Tests on regenerated chelating agent solution

Lead sulphide was precipitated by adding to the solution an appropriate quantitative of sodium sulphide (Na_2S), then it was separated from NTA solution by filtration. The reaction is reported in Eq. (3).

$$\text{NTA-Pb} + Na_2S \rightarrow Na_2NTA + PbS_{(s)} \qquad (3)$$

In order to test the effectiveness of the regeneration process, leaching tests at natural pH and at controlled pH 5 were carried out on 0.25-0.5 mm glass treated statically with the regenerated solution. Results are reported in Fig. 3, compared with the release values of untreated glass and of glass treated with the fresh solution. The glass treated with the regenerated solution released more lead than the glass treated with the fresh solution; anyway a decrease in lead leaching is still appreciable if compared to untreated glass. Therefore it may be concluded that the regeneration process only partially preserves the chelating agent solution effectiveness.

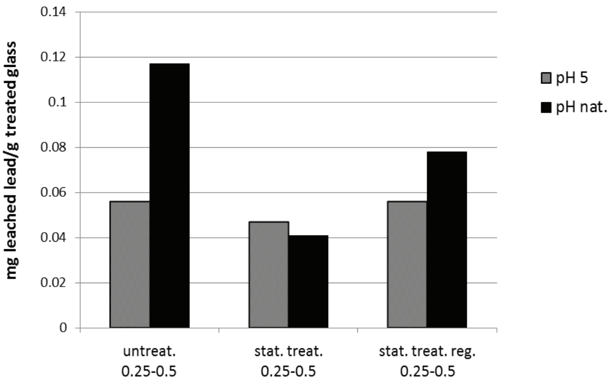


Fig. 3. Leaching tests results (glass treated with regenerated solution). Pb leachable (mg/g) was investigated at pH 5 and at pH of natural water-glass equilibrium. Data refer respectively to untreated glass ("untreat."), to glass which underwent NTA treatment in static condition ("stat. treat.") and to glass which underwent the treatment with the regenerated solution of NTA ("stat. treat. reg."). Only 0.25-0.5 mm particle size was considered

4.3. Catalytic activity of immobilized lipase

4.3.1. Time course of hydrolytic reaction

As shown in Fig. 4, Conversion (%) generally increases with time (min), both for reference glass and for waste glasses, i.e. the greater the contact time between enzyme and substrate and the greater the catalyzed product. In particular for the smaller particle sizes (212-300 μm and 200-300 μm), the reaction seems already quite fast at the beginning until reaching a maximum after 60 min, whereas for the coarser ones (425-600 μm and 300-500 μm) the reaction is slower in the first 30 min, then undergoes an increase of speed until reaching the maximum at 60 min. All data presented are the mean of three determinations. Since the error is very low as compared to the graph scale (it never exceeds the 2.5%), error bars are here omitted. Since all reactions showed a maximum Conversion % after 60 min, all the following results will refer to this time of reaction.

4.3.2. Grain sizes effect

The data about Protein loading (%) and Conversion (%) reported in Fig. 5 show a general trend: the smaller particle size of each type of supports allowed an increase of the enzyme immobilization. This is related to the major exposed surface.

The error bars reported in Fig. 5 represent the standard deviation of triplicated experiments.

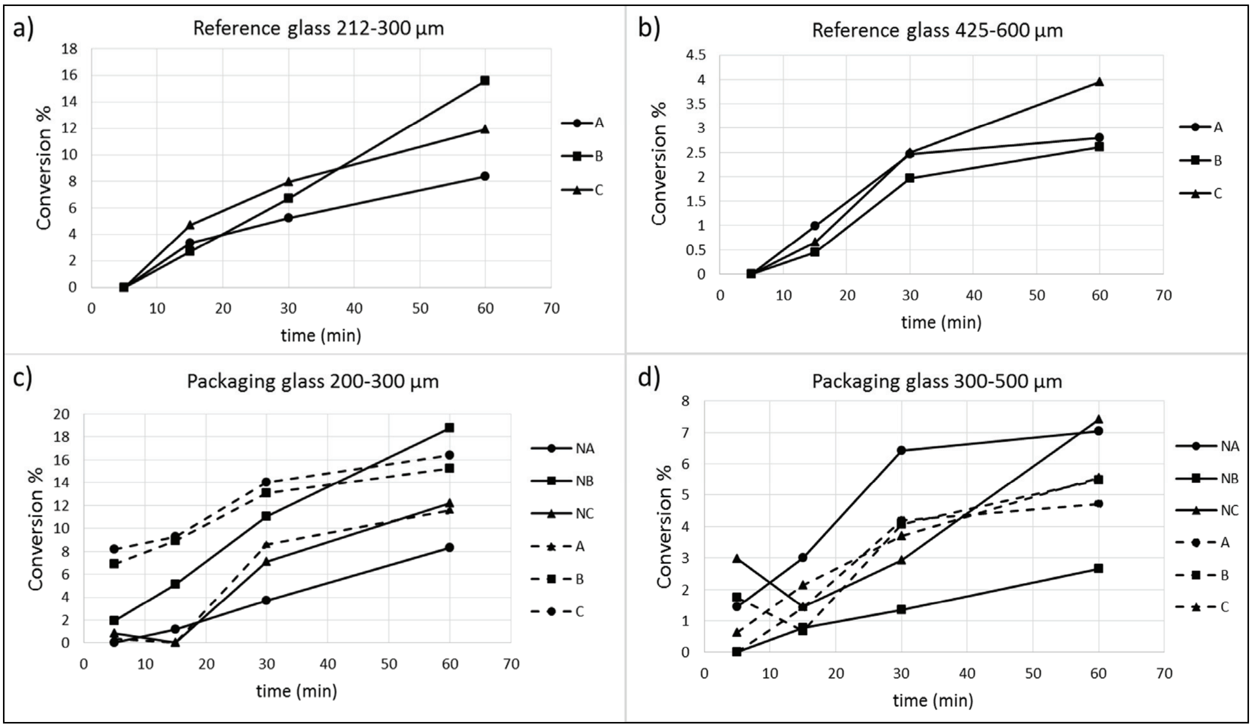


Fig. 4. Time course of hydrolytic reaction (Conversion %) for reference glass with 212-300 µm (a) and 425-600 µm (b) particle size, and for packaging glass with 200-300 µm (c) and 300-500 µm (d) particle size. Lines refer to the different treatments of the glass supports: Pb extraction (N), glass surface cleaning (A and B), and silanization (C)

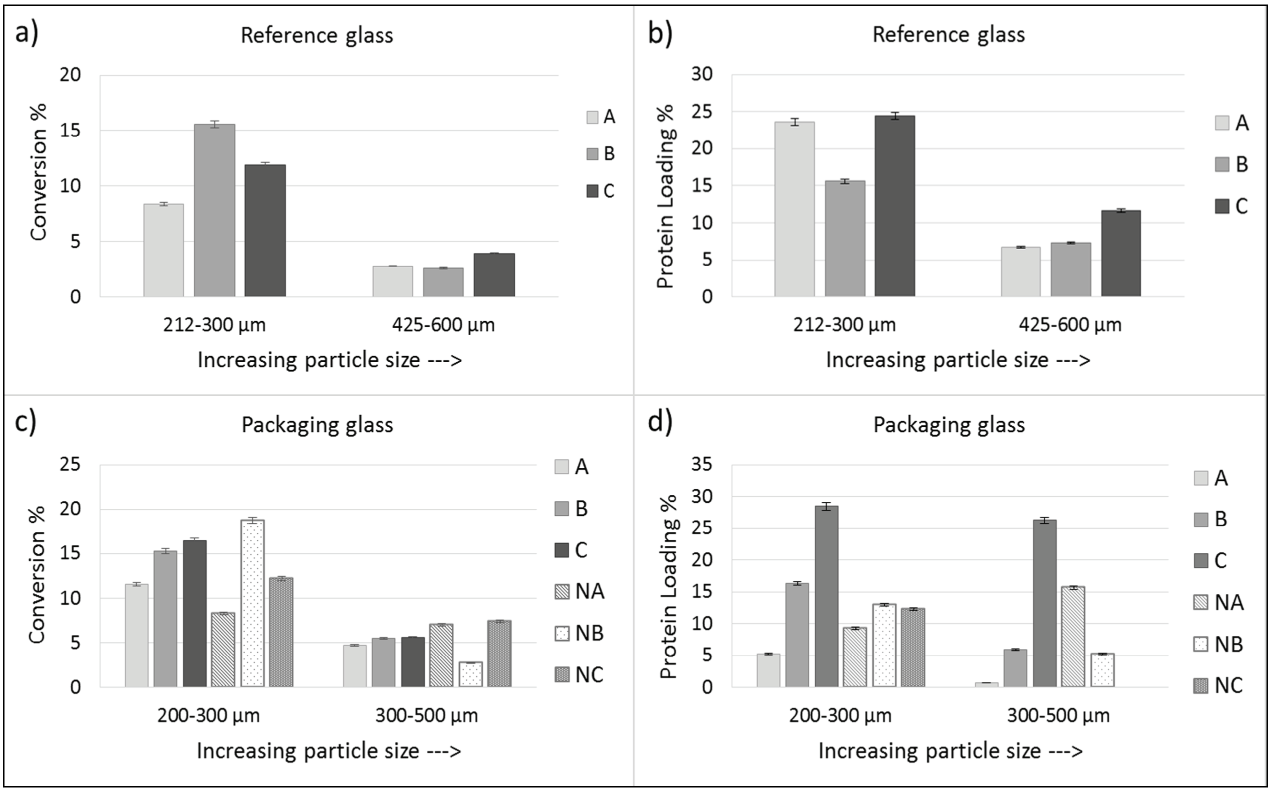


Fig. 5. Grain sizes effect: Conversion (%) and Protein loading (%) for reference glass (a, b) and packaging glass (c, d). Data refer to the different treatments of glass supports: Pb extraction (N), glass surface cleaning (A and B), and silanization (C)

4.3.3. Supports pre-treatment and lead effect

From results reported in Fig. 6, it is possible to observe that low concentrations of lead do not affect particularly catalysis. In fact lead contaminated packaging glass, both treated and untreated with NTA, showed activities similar to the reference glass. A particularly high conversion value is showed by glass treated with treatment B after NTA pre-treatment. It is noted that chemical treatments of the solid supports thoroughly affect the catalytic activity, and the obtained results seem rather in contrast with what was expected, namely that the glass treated with treatment C should ensure the best conversion, owing to the hydrophobic behavior of its surface. Anyway, the positive behavior of glass treated with treatment B is in accordance with what was reported by Reis et al. (2006). Indeed, they observed that the *Rhizomucor miehei* lipase favored the hydrolysis reaction when absorbed on a hydrophilic surface, and the condensation reaction when absorbed on a hydrophobic one. Since the reaction tested in the present study is actually a hydrolysis reaction, results are in agreement. They are, however, in contrast with the generally accepted view which considers hydrophobic surfaces as the most suitable for lipase adsorption, therefore further investigations are needed in this regard.

The error bars reported in the Fig. 6 represent the standard deviation of triplicated experiments.

4.3.4. Semiquantitative and microstructural analysis

Fig. 7 shows the SEM images of reference glass and packaging glass with 212-300 µm and 200-300 µm particle size, respectively, after lipase immobilization. Both glasses were undergone to treatment C. Morphologically, reference glass particles appear regular and rounded shaped, while packaging glass particles are more sharp and irregular. Well distributed dark spots are clearly visible on both glasses, and they were attributed to the immobilized enzyme.

This was confirmed by means of EDS analysis, that detected the presence of phosphorus, clear sign of the presence of the enzyme, that held back the phosphate buffer solution during immobilization.

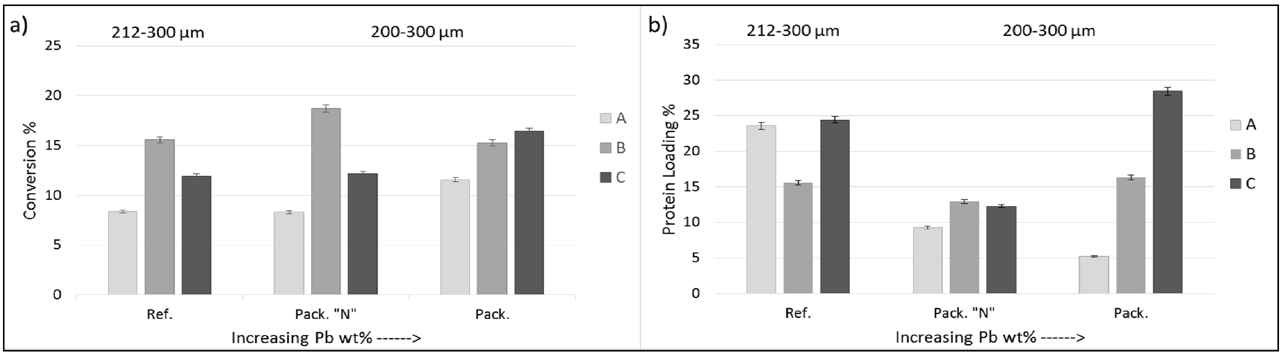


Fig. 6. Lead effect: Conversion (%) (a) and Protein loading (%) (b) for reference glass and packaging glass. Data refer to the different treatments of glass supports: Pb extraction (N), glass surface cleaning (A and B), and silanization (C)

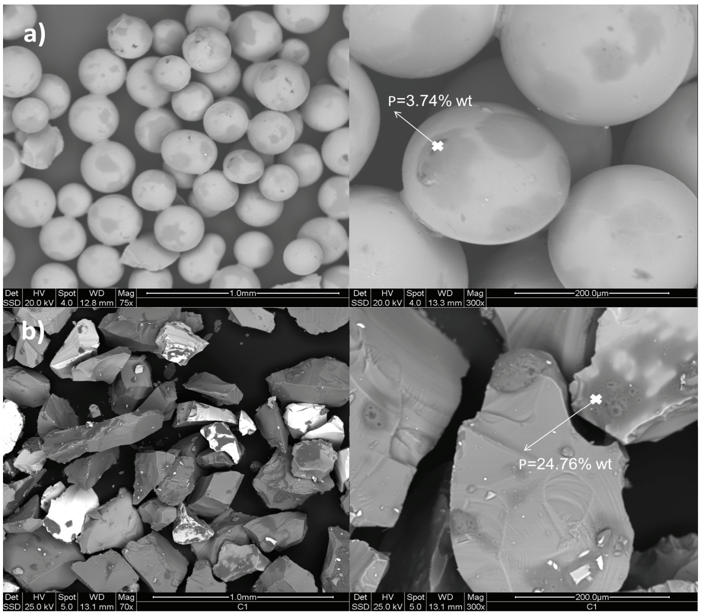


Fig. 7. SEM images of immobilized lipase on reference glass (a) and packaging glass (b).
Well distributed dark spots attributed to the immobilized enzyme are clearly visible on both glasses

5. Conclusions

In this paper lead contaminated packaging glass from urban collection was valorized as support for lipase immobilization after a pre-treatment for Pb extraction by means of NTA chelating agent solution.

Leaching tests were performed on both untreated and treated glass at pH 5 and at pH of natural equilibrium of water/glass system. Results confirmed the typical inert nature of the glass, since all the values of Pb release were relatively low. Moreover, the chelating agent treatment showed a positive effect, probably due to a decrease in the lead content on the glass surface.

Regarding lipase immobilization, notwithstanding the different micromorphology, lead contaminated packaging glass from urban collection, both treated and untreated with NTA, and the commercial soda-lime glass used as reference showed similar activities. Therefore it is reasonable to assume that supports with low lead content, such as lead contaminated glass from urban collection (PbO≈5% wt) do not affect particularly catalysis.

References

Aguieiras E.C.G., Cavalcanti-Oliveira E.D., Freire D.M.G., (2015), Current status and new developments of biodiesel production using fungal lipases, *Fuel*, **159**, 52-67.

Assis O.B.G., Claro L.C., (1999), Processing of soda–lime glass membranes by filler principle for the enzyme coupling applications, *Journal of Non-Crystalline Solids*, **247**, 237-240.

Barbieri L., Lancellotti I., Ponzoni C., (2014), Rapid screening of different chelating agents in the lead extraction from cathode ray tube (CRT) funnel glass, *Environmental Science and Pollution Research*, **21**, 13230-13236.

Bastida A., Sabuquillo P., Armisen P., Fernandez-Lafuente R., Huguet J., Guisan J.M., (1998), A single step purification, immobilization, and hyperactivation of lipases via interfacial adsorption on strongly hydrophobic supports, *Biotechnology and Bioengineering*, **58**, 487-493.

Bursi E., Ponzoni C., Lancellotti I., Vassura I., Ferroni L., Barbieri L., (2015), Cathode Ray Tube (CRT) lead glass: lead leaching study after a chelating agent treatment, *Environmental Engineering and Management Journal*, **14**, 1503-1509.

Brzozowski A.M., Derewenda U., Derewenda Z.S., Dodson G.G., Lawson D.M., Turkemburg J.P., Bjorkling F., Huge-Jensen B., Patkar S.A., Thim L., (1991), A model for interfacial activation in lipases from the structure of a fungal lipase-inhibitor complex, *Nature*, **351**, 491-494.

Co.Re.Ve., (2015), Specific Program of Prevention 2015 (Results of Collection and Recycling 2014).

Derewenda U., Byzozowski A.M., Lawson D.M., Derewenda Z.S., (1992), Catalysis at the interface: the anatomy of a conformational change in a triglyceride lipase, *Biochemistry*, **31**, 1532-1541.

Gunnlaugsdottir H., Wannerberger K., Sivik B., (1998), alcoholysis and glyceride synthesis with immobilized lipase on controlled-pore glass of varying hydrophobicity in supercritical carbon dioxide, *Enzyme and Microbial Technology*, **22**, 360-367.

Hasan F., Shah A.A., Hameed A., (2006), Industrial applications of microbial lipases, *Enzyme and Microbial Technology*, **39**, 235-251.

Karimi M., (2016), Immobilization of lipase onto mesoporous magnetic nanoparticles for enzymatic synthesis of biodiesel, *Biocatalysis and Agricultural Biotechnology*, **8**, 182-188.

Kim D., Herr A.E., (2013), Protein immobilization techniques for microfluidic assays, *Biomicrofluidics*, **7**, 41501, http://dx.doi.org/10.1063/1.4816934.

Mateo C., Palomo J.M., Fernandez-Lorente G., Guisan J.M., Fernandez-Lafuente R., (2007), Improvement of enzyme activity, stability and selectivity via immobilization techniques, *Enzyme Microbial Technology*, **40**, 1451-1463.

Palomo J.M., Muñoz G., Fernández-Lorente G., Mateo C., Fernández-Lafuente R., Guisán J.M., (2002), Interfacial adsorption of lipases on very hydrophobic support (octadecyl–Sepabeads): immobilization, hyperactivation and stabilization of the open form of lipases, *Journal of Molecular Catalysis B: Enzymatic*, **19-20**, 279-286.

Pant D., Singh P., Upreti M.K., (2014), Metal leaching from cathode ray tube waste using combination of Serratia plymuthica and EDTA, *Hydrometallurgy*, **146**, 89-95.

Pencreac'h G., Baratti J.C., (1996), Hydrolysis of p-nitrophenyl palmitate in n-heptane by the Pseudomonas cepacia lipase: a simple test for the determination of lipase activity in organic media, *Enzyme and Microbial Technology*, **18**, 417-422.

Pizarro C., Brañes M.C., Markovits A., Fernández-Lorente G., Guisán J.M., Chamy R., Wilson L., (2012), Influence of different immobilization techniques for *Candida cylindracea* lipase on its stability and fish oil hydrolysis, *Journal of Molecular Catalysis B: Enzymatic*, **78**, 111-118.

Plant A.L., Locascio-Brown L., Haller W., Durst R., (1991), Immobilization of binding proteins on nonporous supports, *Applied Biochemistry and Biotechnology*, **30**, 83-98.

Reetz M.T., (2002), Lipases as practical biocatalysts, *Current Opinion in Chemical Biology*, **6**, 145-150.

Reis P., Holmberg K., Debeche T., Folmer B., Fauconnot L., Watzke H., (2006), Lipase-Catalyzed Reactions at Different Surfaces, *Langmuir*, **22**, 8169-8177.

Sasai R., Kubo H., Kamiya M., Itoh H., (2008), Material recycling process for spent lead glass using a mechano-chemical process and Na2EDTA reagent, *Environmental Science & Technology*, **42**, 4159-4164.

Schultz-Münzenberg C., Meisel W., Gütlich P., (1998), Changes of lead silicate glasses induced by leaching, *Journal of Non-Crystalline Solids*, **238**, 83-90.

Sharma R., Chisti Y., Banerjee U.C., (2001), Production, purification, characterization, and applications of lipases, *Biotechnology Advances*, **19**, 627-662.

Taurino L., Andreola F., Leonelli C., Manfredini T., Gastaldi P., Barbieri L., (2015), Physical-mechanical properties of new green building materials based on glass waste, *Environmental Engineering and Management Journal*, **14**, 1735-1742.

Environmental Engineering and Management Journal
September 2016, Vol.15, No. 9, 1941-1948
http://omicron.ch.tuiasi.ro/EEMJ/

"Gheorghe Asachi" Technical University of Iasi, Romania

EVALUATION OF A PILOT-SCALE SEQUENCING BATCH BIOFILTER GRANULAR REACTORS (SBBGR) SYSTEM FOR MUNICIPAL WASTEWATER TREATMENT

Paolo Cirello*, **Giancarlo Cecchini**, **Biagio Eramo**

Acea Elabori SpA, Via Vitorchiano, 165 00189 Roma, Italy

Abstract

Sequencing Batch Biofilter Granular Reactors (SBBGRs) represent an innovative technology; coupling granular and attached biomass and operating in a batch mode. These systems allow good removal rates with low sludge production and space requirement. A particular application, consisting of an aeration unit and an upflow SBBGR in series, has been tested at pilot scale. The pilot system was installed at a conventional activated sludge plant and fed with average-strength urban wastewater after pretreatments. This works reports and discusses the experimental results, with reference to removal efficiencies and sludge production. Experimental results have shown a good flexibility of the system with respect to strong variations of influent loading, and adequate removal efficiencies for organic matter (as Chemical Oxygen Demand, COD), total suspended solids (TSS) and total nitrogen (TN): observed removal efficiencies were respectively 88%, 90% and 72% with volumetric loading rates ranging from 0.8 to 1.3 kg COD/m^3/d. Simultaneous nitrification/denitrification has been observed.

Key words: aerobic granular sludge, nitrogen removal, Sequencing Batch Biofilter Granular Reactor, sludge production, wastewater treatment

Received: January, 2016; Revised final: August, 2016; Accepted: September, 2016

1. Introduction

Aerobic granular sludge (AGS) systems are an innovative technology in biological wastewater treatment, as they allow, if compared with the conventional activated sludge process, to achieve higher biomass concentration and sludge retention time (SRT), and to handle greater organic loading rates with lower hydraulic retention time (HRT) (Checchini et al., 2015); due to these features, AGS processes are characterized by lower sludge production and space requirements (de Kreuk et al, 2006; Matran et al., 2015). Granular biomass is developed from bacteria found in sewage sludge, and selective environmental pressures are key elements in its formation (Barsan et al., 2014; Beun et al., 2002; Cecchini et al., 2014; McSwain et al., 2004). Granular aerobic biomass reactors are generally operated as a sequencing batch systems, thus allowing greater process flexibility.

Sequencing Batch Biofilter Granular Reactor (SBBGR), developed by the Water Research Institute of the Italian National Research Council (IRSA-CNR) is a more recent application combining granular and attached biomass, thus leading to a more efficient biomass retention; the reactor is filled by a support material and biomass mainly grows as high density granules (up to 40 kgVSS/m^3), entrapped inside the carrier (Di Iaconi et al., 2005, 2007, 2008 a, b). Three factors play a decisive role in SBBGR granular biomass generation: the trend of the hydrodynamic shear forces, the start-up operative conditions (i.e., the pattern of organic loading rate increase), and bed material features (Di Iaconi et al., 2005); the startup

---

* Author to whom all correspondence should be addressed: e-mail: paolo.cirello@aceaspa.it

period takes around three months, during which gradual shift from biofilm to granular biomass can be observed (De Sanctis et al., 2010).

The SBBGR technology is very promising both in terms of removal efficiencies and of low sludge production rates; the first pilot scale experiences are being carried out (Di Iaconi et al., 2008a, 2009, 2014), but are not very diffused yet.

The present paper reports and discusses pilot plant results of a particular SBBGR application; the tested technology is a Bioseq® system, (Bargna, 2009). The system consists of a SBBGR reactor coupled to an aeration tank, working in series. Aeration and biological reaction steps occur in two different compartments, aeration and backwashing can be operated in a more simple and efficient way.

2. Experimental

2.1. Pilot plant

The pilot plant (Fig. 1) is made up of two treatment units:

- aeration unit;
- SBBGR Biofilter.

Differently from other granular biomass sequential batch systems, aeration is carried out in a specific unit. The aeration unit consists of an open cylindrical steel tank (1.50 m diameter and 2.00 m high, with 3.50 m³ working volume); it is fed from the bottom and 4 discharge valves are located at different heights, thus allowing operational flexibility. Aeration is carried out by means of a blower connected with tube air diffusers.

The SBBGR reactor is also an open cylindrical steel tank (0.80 m diameter and 2.00 m high, with 1.00 m³ working volume); the reactor is partly filled with the biomass support material: wheel shaped plastic elements (10 mm diameter, with 700 m²/m³ specific surface and 1 g/mL specific mass). The porosity of the reactor, when filled with the carrier, is around 0.75 (supplier specifications). The reactor also is bottom fed and 5 valves are located at different heights (interdistance 0.4 m), in order to allow sampling and measures. An automation and control system makes it possible to perform continuous monitoring of operational parameters (flowrates, head losses etc.) and to handle cycles and phases management.

2.2. Treatment cycles

Each treatment cycle comprises the following phases:

- Static filling: influent wastewater is bottom fed to the aeration unit, to a pre-established level (half of the loading height), at which the air blower is started;
- Dynamic filling: the aeration unit continues to be fed to a second pre-established level (loading heights are shown in Table 2);
- Reaction: the recirculation pump is started; wastewater is bottom fed from the aeration unit to the biofilter, passes through the support material and is re-circulated back to the aeration unit from the top of the biofilter by gravity. In this phase wastewater is continuously circulated between the reactor and the aeration unit and biological reactions take place;
- Effluent discharge: air blower and recirculation pump are turned off, and the effluent is drawn by gravity from the aeration unit, using the appropriate discharge valve. In each treatment cycle, discharged volume coincides with loaded volume. The biofilter, totally filled with wastewater during the start-up of the system, is not withdrawn;

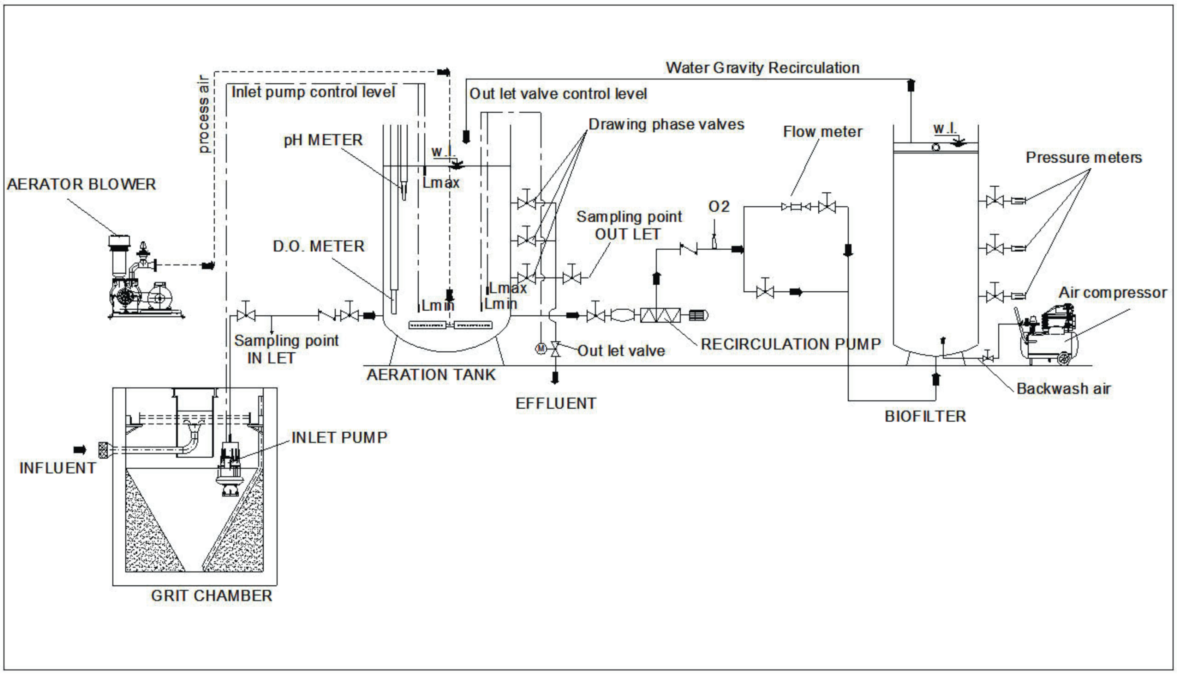


Fig. 1. Schematic diagram of the pilot plant

- Backwashing: operated with compressed air, when headloss exceeds a setpoint value.

Static/dynamic filling and effluent discharge overall take 15 to 30 minutes. The reaction phase duration can be varied in order to achieve the required organic loading. The system can be run in anaerobic/anoxic conditions by turning the air blower off.

2.3. Experimental conditions

Operating conditions are shortly summarized in Table 1. In the present work 3 daily cycles were carried out, each lasting 8 hrs. During the operation phase, as during startup, COD loading rate was progressively increased, as shown in Table 2, up to 1.3 kg COD/m^3/d. Recirculation rate was also progressively increased from 1.0 m^3/d to 1.6 m^3/d at the end of the experimentation. Organic loading rate applied to the reactor has been computed basing on the average influent COD concentration, about 400 mgO$_2$/L. During operation, organic loading rate was temporarily reduced (days 40-74) due to peak concentrations in the influent, which suggested to decrease loads, in order to avoid toxic shocks in the bioreactor. After this problem was overcome, organic loading rate could be increased again.

Aeration was run on a continuous base during the whole operation phase. Dissolved oxygen in the aeration unit was measured at the five discharge valves and in the effluent during sample collection; average concentrations of dissolved oxygen at the different heights from the bottom of the reactor are shown in Table 3. The aerated influent, fed from the bottom, shows rather high dissolved oxygen concentrations, gradually decreasing along the reactor height, due to consumption by the biomass.

2.4. Experimental tests

Experimental tests were aimed at assessing, at a pilot scale, the technical and operational performance of the system, and evaluating scale-up feasibility. The pilot plant has been installed at a small size activated sludge facility (9,000 P.E. capacity), treating urban wastewater. The pilot system was fed with wastewater after pretreatment (screening and degritting). The tested system was inoculated with mixed liquor from the aeration tank (Chiavola et al., 2010).

Table 1. Experimental condition during operation phase

Parameter	***UoM***	***Values***
Daily treated volume	m^3/d	1.1÷3.2
Hydraulic retention time (HRT)	h	7.5
Organic loading rate (U_{COD})	kgCOD/m^3/d	0.4÷1.3
Daily cycles during operation	-	3
Cycle duration	h	8
Biomass concentration*	gVSS/L	18÷24
Influent concentrations (average ± standard deviation)		
COD	mgO$_2$/L	412±265
TSS	mg/L	243±271
TN	mgN/L	54±40
NH$_4$-N	mgN/L	43±18
NO$_x$-N**	mgN/L	0.92±1.21

** determined at the end of the test; ** as sum of nitric and nitrous nitrogen (NO$_3$-N + NO$_2$-N)*

Table 2. Experimental condition during the operation phase

Period (days of operation)	***Phase***	***Daily cycles -***	***Volume fed m^3/d***	***U_{COD} kgCOD/m^3/d***	***Aerator Loading Heigth (m)***
1 ÷ 39	I	3	2.12	0.84	0.40
40 ÷ 53	II	3	1.05	0.42	0.20
54 ÷ 74	III	3	1.59	0.64	0.30
75 ÷ 95	IV	3	2.12	0.84	0.40
96 ÷ 140	V	3	2.66	1.06	0.50
141 ÷ 190	VI	3	3.18	1.27	0.60

Table 3. Dissolved Oxygen concentrations in the SBBGR biofilter (average ± standard deviation)

Valve	***Height from bottom (m)***	***DO (mgO$_2$/L)***
H1	0.0	7.5±1.3
H2	0.4	5.8±1.4
H3	0.8	2.5±1.7
H4	1.2	2.1±1.6
H5	1.6	1,9±1.4
EFFLUENT	2.0	1.3±1.5

Table 4. Influent and effluent concentrations and removal efficiencies

	COD mg/L O_2	***fCOD mg/L O_2***	***TSS mg/L***	***TN mg/L N***	***N-NH$_4$ mg/L N***	***N-NO$_x$ mg/L N***
Influent (avg ± st. dev.)	412.0±265.0	156.6±53.9	243.4±271.4	54.4±40.0	43.3±18.1	0.92±1.21
Effluent (avg ± st. dev.)	33.8±20.2	12.3±9.0	17.3±17.5	12.7±5.5	12.0±7.3	1.95±0.69
Removal efficiency (%) (range)	0÷98	66÷97	58÷99	37÷98	35÷100	-
Removal efficiency (%) (avg)	88	85	90	72	72	-

2.5. Analytical methods

Sampling of both influent and effluent were performed on a biweekly basis. The following parameters: COD, filtered COD (fCOD), Total Suspended Solids (TSS), Total Nitrogen (TN), NH_4-N, NO_3-N, NO_2-N were determined according to the Standard Methods (APHA, 1998). DO (Dissolved Oxygen), temperature and pH were measured online by means of standard probes.

3. Results and discussion

Results from the initial startup phase (120 days) and the first two months of the operation phase have been previously published (Chiavola et al., 2010). The present paper reports results of the whole operation phase (in total 190 days), started when the volumetric organic loading rate had reached values around 0.8 – 0.9 kg COD/m^3/d.

3.1. Removal efficiencies

In Table 4, influent and effluent average values and removal efficiencies observed during the whole operation phase are reported for COD, fCOD, TSS, TN, N-NH$_4$ and N-NO$_x$ (N-NO$_3$+N-NO$_2$). All the analyzed parameters presented high variability in the influent, and outlier values, higher than the average concentration found in urban wastewater, were observed. On those occasions, altered pH values (9÷10) and a high content of oil and grease (>10.0 mg/L) were found. The graphs below display the temporal trends of the analyzed parameters.

Chemical Oxygen Demand (COD) and filtered COD (fCOD)

COD and fCOD concentrations in influent and effluent and the respective removal efficiencies are shown in Figs. 2 and 3.

Influent COD concentration shows a high degree of variability, with several peaks exceeding 500 and in some cases even 1,000 mg O_2/L. These high loadings affected average removal rate and were probably due to factors such as anomalous discharges into the sewer system or first flush events; because of these anomalies, the organic load applied to the reactor was temporarily lowered.

Effluent COD concentrations were found in the range between the Detection Limit (10 mgO_2/L) and around 70 mgO_2/L, with an average concentration of about 30 mgO_2/L, more than compliant with the discharge limit of 125 mgO_2/L (EC, 1991). Removal efficiencies were found to fluctuate between 75 and 98% with average value 91% (computed excluding sampling days with influent COD>1,000 mgO_2/L, to be considered exceptional situations). The tested system has shown flexibility in working with a variable organic loading. Filtered COD (fCOD) is usually associated with the soluble component. Influent fCOD was found to be about 46% of COD, in a range of 30 to 350 mgO_2/L and with average concentration of 156 mgO_2/L. In the effluent fCOD represents about 80% of COD, ranging from 3 to 40 mgO_2/L, and 12 mgO_2/L is the average concentration. Average removal efficiency was 85%.

Effluent COD was substantially represented by the soluble fraction; in the removal of COD particulate fraction a role can be played by incorporation in the biofilm/granules (Adav et al., 2008).

Total Suspended Solids (TSS)

Figure 4 shows TSS concentrations and removal rates; influent concentration varied in the range 70 to 1,300 mg/L, with average value of about 250 mg/L. As for COD and associated with COD peaks, several very high values were found, exceeding 500 mg/L and sometimes even 1,000 mg/L. Effluent concentration were ranging from about 5 to 30 mg/L with average around 15; sporadically, slightly higher values were recorded (on one occasion, 90 mg/L). TSS therefore fully comply with the emission standards of (35 mg/L (EC, 1991). Removal efficiency was found in the 60÷99% range with average 90%.

In the case of TSS also, the tested experimental system has shown flexibility with respect to highly variable input concentration and to the gradual increase of organic load applied.

Total Nitrogen (TN)

TN concentrations and removal rates are represented in Fig. 5. TN influent concentrations were in the range 18÷100 mgN/L, with about 55 mgN/L as average, which is higher than typical values usually found in urban wastewater (Metcalf and Eddy, 2003). Effluent TN concentration during the operation period, with increasing organic loading, shows an average concentration around 13 mgN/L, compliant, except for some very sporadic cases, with the standards for 10,000÷100,000 p.e. capacity treatment plants discharging in sensitive areas (15 mgN/L), and slightly exceeding the limit of 10 mgN/L, for plants over 100,000 p.e. (EC, 1991).

As for removal efficiency, experimental data show values in the range 37÷98 %, with an average of about 72 %.

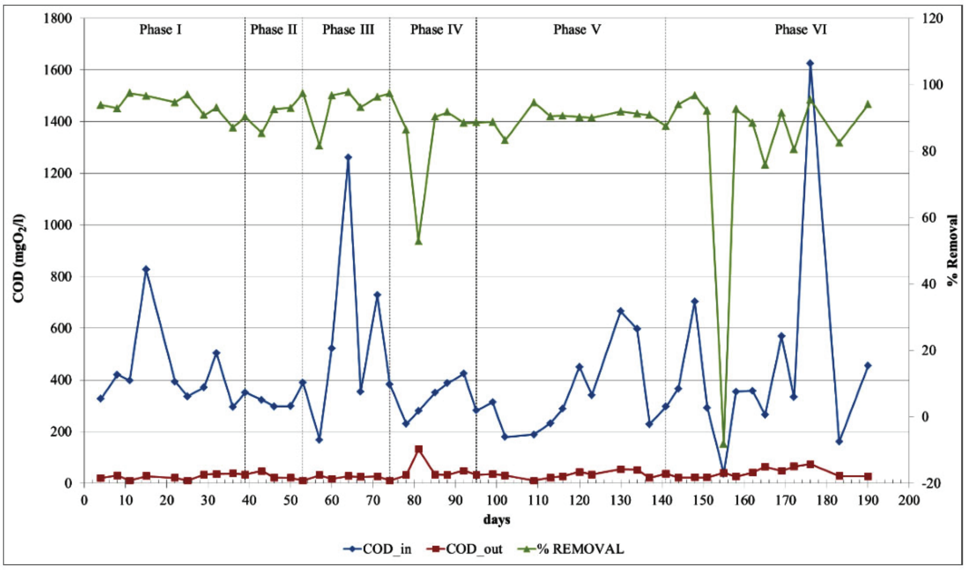


Fig. 2. COD concentrations and removal efficiency

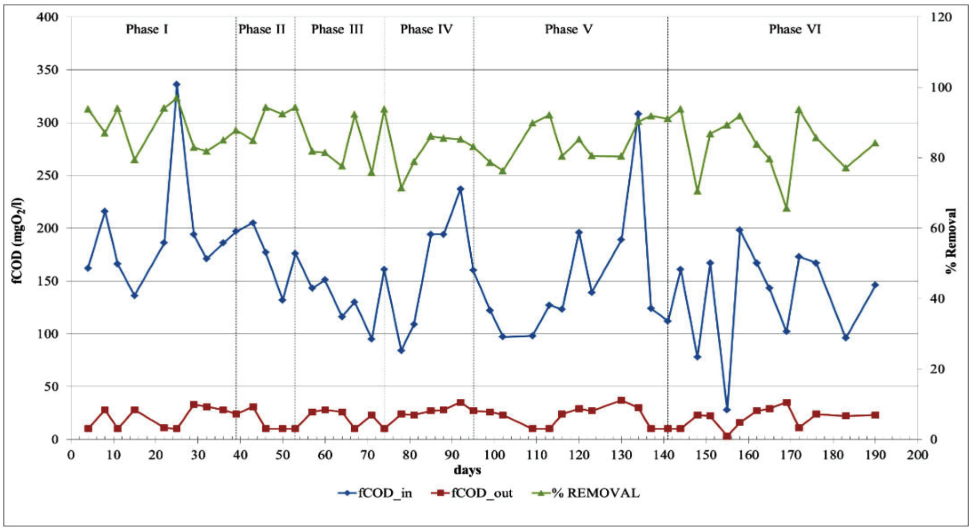


Fig. 3. fCOD concentrations and removal efficiency

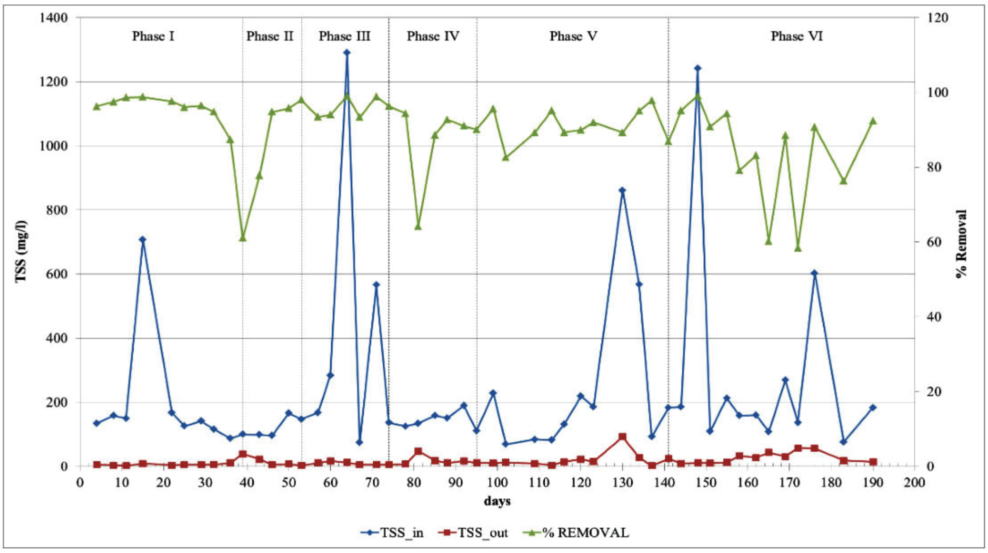


Fig. 4. TSS concentrations and removal efficiency

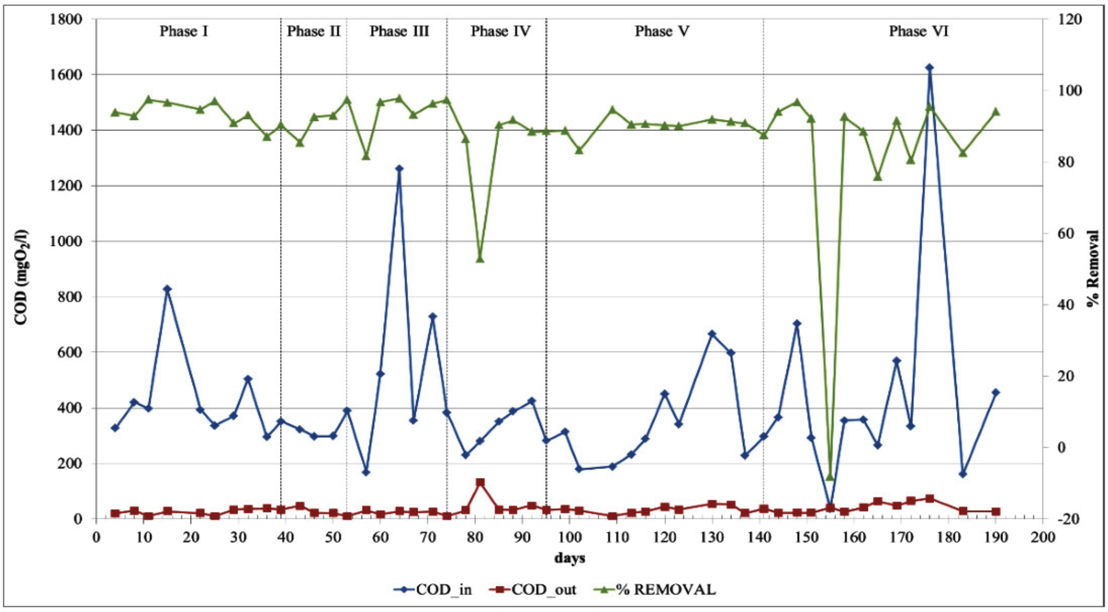


Fig. 5. TN concentrations and removal efficiency

3.2. Sludge production

Figure 6 displays sludge specific production rate over the course of the experiment; the average value observed was less than 0.2 kgTSS/KgCODremoved, and for most of the time around 0.1 kgTSS/kgCODremoved, very low when compared to typical values of 0.5÷0.6 reported for the conventional activated sludge processes (Metcalf and Eddy, 2003). This results are comparable and confirm other authors' observations (Di Iaconi et al., 2010).

At this purpose, it is important to remark that most recent sludge minimization technologies (such as Cannibal, physical, chemical or thermal treatments) do not seem able to reduce sludge production rates more than 30% (Roxburgh et al., 2006). In almost 200 days of plant operation, 41 backwashing procedures were carried out, that is, about one in four days, each time wasting 50÷200 l of sludge (100÷750 g TSS).

3.3. Headloss management and backwashing

Headloss was monitored by pressure sensors at the 5 valves located along the reactor height (H1, H2, H3, H4, H5); average values before and after backwashing are reported in Table 5. Backwashing, for few minutes with compressed air, was carried out when pressures measured at the H1 valve (bottom of the reactor) approached 1,000 mbar. During start-up, backwash had not been operated, and the gradual increase of pressures over time revealed that biomass growth was occurring during this period.

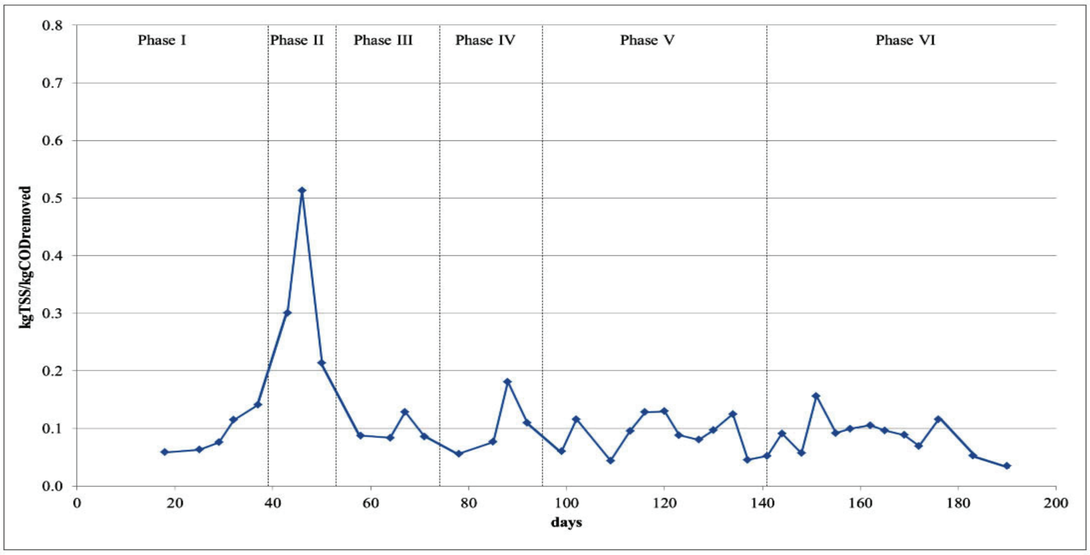


Fig. 6. Sludge specific production rate

Table 5. Average pressure in the SBBGR before and after backwashing

Valve	*H1*	*H2*	*H3*	*H4*	*H5*
Height from bottom (m)	0.0	0.4	0.8	1.2	1.6
Avg pressure, before backwash (mbar)	961±67	938±87	844±142	594±120	263±63
Avg pressure, after backwash (mbar)	738±125	703±125	579±135	348±101	143±47

4. Conclusions

This pilot scale experience confirms that the tested technology is adequate to obtaining good removal efficiencies with medium-high volumetric organic loadings; particularly, it has shown to be stable under variable loading rates, and nitrogen removal by simultaneous nitrification/denitrification has been observed. The system anyway needs a rather prolonged startup period, to allow granular biomass development.

A consistent advantage offered by this technology is the very low sludge production; indeed, SBBGR based technologies address a relevant issue of wastewater treatment, i.e. environmental, logistic and economic problems raised by sludge treatment and disposal. As pointed out above, the observed sludge specific production rate resulted much lower than those obtainable with the most recent sludge minimizing technologies. Other aspects to be mentioned are the simplicity and ease of management, being the facility totally automated, and the fact that odor emissions were found very low.

Experimental results obtained from this pilot study can represent the fundamentals for further developments, with the ultimate goal of transferring the technology to full scale application

References

Adav S.S., Lee D.J., Show K.J., Tay J.H. (2008), Aerobic granular sludge: Recent advances, *Biotechnology Advances*, **26**, 411-423.

APHA, (1998), Standard Methods for the Examination of Water and Wastewater, 20th edition, American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA.

Bargna R., (2009), Plant and process for the purification of wastewaters. European Patent, No. *EP2307323*.

Barsan N., Joita I., Stanila M., Radu C., Dascalu M., (2014), Modelling wastewater treatment process in a small plant using a sequencing batch reactor (SBR), *Environmental Engineering and Management Journal*, **13**, 1561-1566.

Beun J.J., van Loosdrecht M.C.M., Heijnen J.J., (2002), Aerobic granulation in a sequencing batch airlift reactor, *Water Research*, **36**, 702-712.

Cecchini G., Cirello P., Eramo B., (2014), Monitoring odour emissions from wastewater treatment plants by means of methodologies based on hydrogen sulfide distribution, *Procedia Environmental Science, Engineering and Management*, **1**, 83-86.

Cecchini G., Cirello P., Eramo B., (2015), Partitioning dynamics and fate of metals in an urban wastewater treatment plant, *Environmental Engineering and Management Journal*, **14**, 1511-1520.

Chiavola A., Sirini P., Cecili S., (2010), Technical and experimental evaluation of an innovative decentralized technology for the municipal wastewater treatment in the city of Rome, *Water Science & Technology*, **62**, 956-962.

de Kreuk M.K.,van LoosdrechtM.C.M., (2006), Formation of aerobic granules with domestic sewage, *Journal of Environmental Engineering*, **132**, 694-697.

de Kreuk M.K., Picioreanu C., Hosseini M., Xavier J.B., van Loosdrecht M.C.M., (2007a), Kinetic model of a granular sludge SBR: influences on nutrient removal, *Biotechnology and Bioengineering*, **97**, 801-815.

de Kreuk M.K., Kishida N., van Loosdrecht M.C.M, (2007b), Aerobic granular sludge-state of the art, *Water Science and Technology*, **55**, 75-81.

De Sanctis M., Di Iaconi C., Lopez A., Rossetti S., (2010), Granular biomass structure and population dynamics in Sequencing Batch Biofilter Granular Reactor (SBBGR), *Bioresource Technology*, **101**, 2152-2158.

Di Iaconi C., Ramadori R., Lopez A., Passino R., (2005), Hydraulic shear stress calculation in a Sequencing batch biofilm reactor with granular biomass, *Environmental Science and Technology*, **38**, 889-894.

Di Iaconi C., Ramadori R., Lopez A., Passino R., (2007), Aerobic granular sludge systems: the new generation of wastewater treatment technologies, *Industrial & Engineering Chemistry Research*, **46**, 6661-6665.

Di Iaconi C., De Sanctis M., Rossetti S., Ramadori R., (2008a), Technological transfer to demonstrative scale of sequencing batch biofilter granular reactor (SBBGR) technology for municipal and industrial wastewater treatment, *Water Science & Technology*, **58**, 367-372.

Di Iaconi C., Del Moro G., Lopez A., De Sanctis M., Ramadori R., (2008b), Municipal wastewater treatment by a periodic biofilter with granular biomass, *Water Science & Technology*, **58**, 2395-2401.

Di Iaconi C., Ramadori R., Lopez A., (2009), The effect of ozone on tannery wastewater biological treatment at demonstrative scale, *Bioresource Technology*, **100**, 6121-6124.

Di Iaconi C., De Sanctis M., Rossetti S., Ramadori R., (2010), SBBGR technology for minimising excess sludge production in biological processes, *Water Research*, **44**, 1825-1832.

Di Iaconi C., De Sanctis M., Lopez A., (2014), A single-stage biological process for municipal sewage treatment in tourist areas, *Journal of Environmental Management*, **144**, 34-41.

EC Directive, (1991), Directive 1991/271/EEC of the European Council of 21 May 1991, concerning urban waste water treatment, *Official Journal of the European Communities*, L 135/34, 30.05.1991, Brussels.

Matran R.-M., Blaga A.-C., Caşcaval D., Tucaliuc A., Galaction A.I., (2015), Comparative studies on kinetics of anaerobic and aerobic biodegradation of lipids from olive oil mill wastewaters with mixture of *Bacillus spp.* cells, *Environmental Engineering and Management Journal*, **14**, 575-579

McSwain B.S., Irvine R.L, Wilderer P.A., (2004), The influence of settling time on the formation of aerobic granules, *Water Science & Technology*, **50**, 195-202.

Metcalf I., Eddy C., (2003), *Wastewater Engineering: Treatment, Disposal, Reuse*, 4th Edition, Tchobanoglous G., Burton F.L., Stensel H.D. (Eds.), McGraw-Hill, New York.

Ni B.J., Yu H.Q., Sun Y.J., (2007), Modeling simultaneous autotrophic and heterotrophic growth in aerobic granules, *Water Research*, **42**, 1583-1594.

Roxburgh R., Sieger R., Johnson B., Rabinowitz B., Goodwin S., Crawford G., Daigger G., (2006), *Sludge Minimization Technologies-Doing More to Get Less*, Proc. 79th Water Environment Federation Technical Exposition and Conference, Dallas, Texas, WEFTEC 2006, Session 1÷10, 506-525.

Environmental Engineering and Management Journal

September 2016, Vol.15, No. 9, 1949-1961
http://omicron.ch.tuiasi.ro/EEMJ/

"Gheorghe Asachi" Technical University of Iasi, Romania

PROJECT *GREEN SYMBIOSIS* 2014 - II PHASE. RESULTS FROM AN INDUSTRIAL SYMBIOSIS PILOT PROJECT IN EMILIA ROMAGNA REGION (ITALY)

Laura Cutaia[1*], Claudia Scagliarino[2], Ugo Mencherini[3], Marco La Monica[4]

[1]ENEA, Centro Ricerche Casaccia - Via Anguillarese 301, Roma, Italy
[2]Free-lance environmental engineer – Via G. Molteni 141, Roma, Italy
[3]ASTER - Via Pietro Gobbetti 101, 40129, Bologna, Italy
[4]Tuscia University, - Via S.M. in Gradi 4, 01100, Viterbo, Italy

---

Abstract

The Project "Green - Industrial Symbiosis" (G-IS), in Emilia Romagna region (IT), took place in two phases: phase I, 05.2013 – 03.2014; phase II, 10. 2014 – 10.2015. During the first phase, it was completed the first part of the pilot project of industrial symbiosis (IS) in Emilia-Romagna, which involved 13 companies in the agro-industrial sector, 7 laboratories of the High Technology Network, with Unioncamere and Aster (promoters) and ENEA (technical and scientific coordinator). The first phase generated 90 potential synergies among the 10 companies that shared their input-output resources. During the second phase, promoted by ASTER and organized with the technical and scientific coordination of ENEA, some of the most interesting synergies of the first phase were selected, in order to go from the identification of potential synergies to its actual implementation. In particular, 3 pathways of industrial symbiosis were chosen, in which waste food industry outputs were destined to three different types of exploitation (production of biopolymers, nutraceuticals, energy recovery). The pathway that a resource must take to shift from being a company's output to another company's input, involves several steps that require compliance and verification of regulatory, technical, logistical and economic issues. All these factors have been examined and reported in 3 Operative Manuals for the companies involved, each one arranged for a different symbiosis' pathway. The manuals consist of two sections: an operative and a documental part (technical dossier). The operative part describes the transformation path of resources, with a layout in which each block and intermediate vector represent a passage of the resource (e.g. exit from the producing company, transport, valorisation). Under the layout, a synthesis table refers, for each stage, to the necessary requirements, reported in full in the technical dossier. Links on the synthesis table refer, for example, to laws or techniques that the specific flow, in each step, must comply with. These links also define, with a predefined color, if that aspect can be considered as an obstacle to the progress of the symbiosis.

Key words: circular economy, enhancement, restoration, symbiosis, synergy

Received: January, 2016; Revised final: August, 2016; Accepted: September, 2016

---

1. Introduction

Sustainability of production processes and the efficient and responsible use of resources are key issues, increasingly being seen as strategic for the economic development at European level: this supranational interest is stated within the "Europe 2020" strategy, for advancement of the economy of the European Union (EU Commission, 2010).

In particular, the transition towards a circular economy model is considered of fundamental importance for the achievement of a greater overall efficiency in the use of resources, increasing the competitiveness of European enterprises (EU

---

[*] Author to whom all correspondence should be addressed: e-mail: laura.cutaia@enea.it; Phone: +39 6 30483306

Commission, 2010). In the last few years, EU Commission presented several initiatives and documents regarding the transition towards this new paradigm: the "Resource Efficiency Flagship Initiative" (EU Commission, 2011a), the "Roadmap for a Resource Efficient Europe" (EU Commission, 2011b) and especially the new "Circular Economy Package" (EU Commission, 2015), consisting of an EU Action Plan for the Circular Economy that establishes a concrete and ambitious action, with measures covering the whole cycle: from production and consumption to waste management and markets for secondary raw materials.

Among the tools identified in the Action Plan as "key elements" for the transition, there is also Industrial Symbiosis (IS), a practice studied since almost twenty-five years and defined as "engaging traditionally separate industries in a collective approach to competitive advantage involving physical exchange of materials, energy, water, and by-products. The keys to IS are collaboration and synergistic possibilities offered by geographic proximity" (Chertow, 2000).

Also at Italian level, national and regional strategies encourage the application of IS: two particularly significant cases are those of Lazio (Regione Lazio, 2014) and Emilia-Romagna (Regione Emilia-Romagna, 2014) "S3-Smart Specialization Strategies". Emilia-Romagna region also included industrial symbiosis within the "Regional Waste Management Programme", as a tool able to reduce waste quantity and to increase regional sustainability, materials reuse and raw materials saving (Regione Emilia-Romagna, 2014).

In Emilia-Romagna an IS application was also developed: the "Green-Industrial Symbiosis" project (G-IS project, in short), promoted by Unioncamere Emilia-Romagna and ASTER, with the technical coordination of ENEA, aimed at the dissemination of an IS culture in the regional territory (Cutaia et al., 2014). It was the first application of IS in Emilia-Romagna and one of the firsts in Italy, with the involvement of 7 laboratories and 13 companies from the agro-industry sector. Since it was a pilot project, it was decided to focus it on the chain of reuse and enhancement of agro-industrial waste, with particular (but not exclusive) attention towards solutions aimed at the production of materials with high added value. At the end of the first phase, the project identified 8 main resource streams, 28 feasible destinations, and 90 potential synergies involving not only companies participating in the project, but also other companies in Emilia Romagna. The experience showed also that the industrial and research ecosystem are quite favorable and interested towards the application of IS, but there are some regulatory issues to overcome (Iacondini et al., 2015).

For this reason, the analysis of the most significant synergies, identifying economical, logistics, technical and legal conditions for the implementation of the results, was the object of the project's second phase described in this paper.

The objectives of this work are to describe the methodology and the results of the second phase of this IS pilot application, and to underline the significant efforts developed in Emilia-Romagna region to increase the application and the knowledge on IS and the involvement of stakeholders, in order to solve main obstacles and facilitate the application of successful processes in the industrial system.

2. Case studies

2.1. Foreword: project context

The project "Green - Industrial Symbiosis, was aimed at spreading the culture of Industrial Symbiosis in Emilia - Romagna, through the creation of integrated, sustainable and innovative management models for production areas, with focus on supply chains for the treatment and the utilization of biomass from agro-industrial waste.

In the first project phase (Cutaia et al., 2014), many companies of the area participated in the activities and, in addition, also the laboratories belonging to the High Technology Network of Emilia - Romagna were involved, with two objectives: to use know-how in the field of Industrial Symbiosis owned by research centers and encourage steps towards innovation (and subsequent collaborations) between companies and the research community.

The activity of the first part of the project, following the collection and analysis of data sent by companies and laboratories, allowed the identification of 90 possible pathways of symbiosis, of which a summary diagram is represented in Fig. 1.

The second phase of the project, named as "Symbiosis 2014" (but in the rest of the paper always reported as "Green - Industrial Symbiosis" project), continued more operationally the activities undertaken by the project "Green - Industrial Symbiosis", aiming at the actual realization of the Symbiosis pathways identified in the first phase, from potential to real synergies. Due to time and resources constraints, to achieve the objectives of the project it was necessary to choose only some of the symbiosis pathways identified. The activity was carried out through the following steps:

- Selection of a group of 3 "major" synergies based on criteria of numerical relevance based on criteria of maximization of resources, flows and companies involved, flows involved and participation of companies already present in the first project phase.
- Gathering of required information, through meetings with the participants of the second phase and bibliographic research.
- Preparation of an Operative Manual for each synergy, containing issues related to implementation of the symbiosis identified and possible technological solutions used in similar cases (taking also into account authorization and control of relevant government agencies, as well as characterization of the synergies by laboratories).

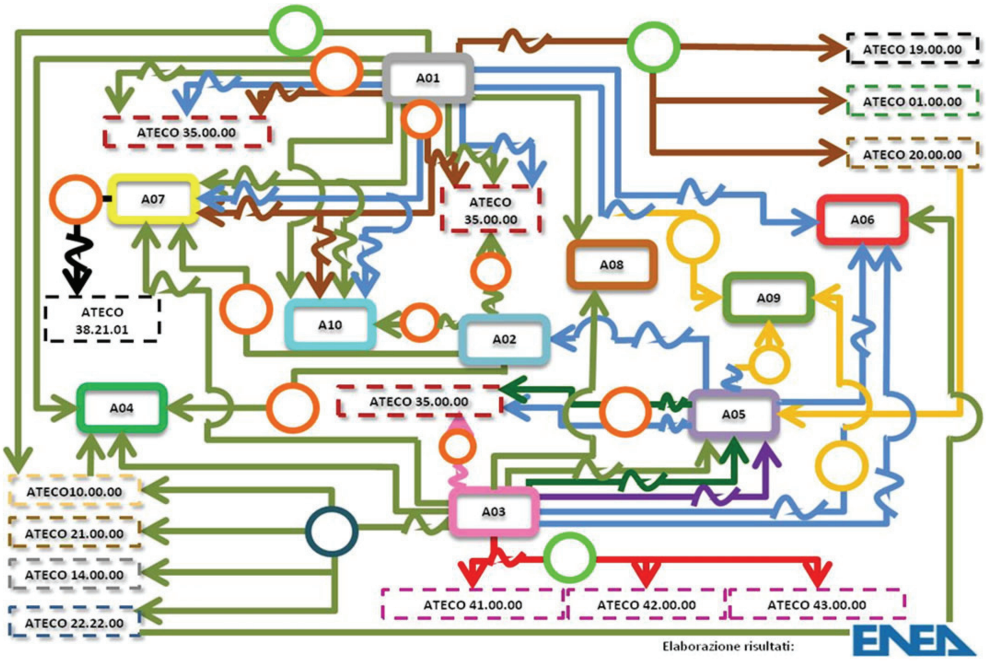


Fig. 1. Symbiosis pathways identified during the first phase of Project Green
A0X Participating companies, identified by codes for privacy reasons; ○ Laboratories proposing the symbiosis; ➔ Flows of identified resources: each colour identifies a different flow; ⌐ Productive sectors identified by the code ATECO (Italian correspondent of the NACE code) in which the resources can be used; ∿ Transformation required before reuse of a resource in another company

2.2. Selection of symbiosis pathways

The first step was the identification of the symbiosis pathways to be developed. The choice was based on the following considerations:

1. Production of nutraceuticals from agricultural and food waste: food scraps utilized are the skins and seeds of tomato. This synergy has been chosen for the significant impact it may have on the region. The Emilia - Romagna is among the top Italian region for tomato producers and companies processing tomatoes. For this reason it was decided to address the issue with one of the participating companies, in order to find viable solutions also for the other many companies in the industrial area. In Fig. 2 it is outlined the symbiosis pathway presented.
2. Energy production from agricultural and food waste: this solution was preferred to others especially for compliance with regulations, as in the same period in which the project was developed, Italy implemented the European Directive 2009/73/EC, which requires Member States to take measures to promote wider use of biogas and gas from biomass, the producers of which should get non-discriminatory access to the natural gas system. Developing this symbiosis, it was possible to analyze the new regulation and possible critical issues in its implementation. In Fig. 3 it is presented the diagram of this second symbiosis pathway.
3. Production of biopolymers from agricultural and food waste: this synergy was chosen because it involved the use of a technology not yet industrially developed. A present, in Italy the production of biopolymers from waste takes place only on a pilot scale, so it was considered important to conduct a focus on the subject, taking into account also the interest that is beginning to develop around the use of biopolymers in different industries. In Fig. 4 it has been schematically presented this last pathway of symbiosis.

2.3. Manuals

The three aforementioned pathways of symbiosis were analyzed and documentation, considerations and insights were collected and organized into 3 operative manuals. Objective of the manuals (and of the II part of the project G-SI) is to provide all relevant information to facilitate the implementation of symbiosis pathways identified during the two years of activity.

The "Operative Manual" is arranged in a first operating part (summary scheme) and a second documental part (technical dossier), to collect all the documentation necessary for helping to achieve the symbiosis pathway, trying to organize the material to be easily accessible and readable for non expert users.

The first part of the manual (summary scheme) consists of a Layout that summarizes the pathway of synergy identified and, in parallel, a Table describing the technical aspects of the pathway. The layout shows the pathway sequence for each phase of the synergy, from the generation of the output - waste - of a company until its use, with or without intermediate valorization process, by another receiving company. The Table shows, in correspondence with each block of the layout, specific aspects to consider for each stage of the pathway. In Fig. 5, an example of the layout can be seen with its corresponding table scheme reported.

Fig. 2. Symbiosis pathway I: production of nutraceuticals from agricultural and food waste

Fig. 3. Symbiosis pathway II: Energy production from agricultural and food waste

Fig. 4. Symbiosis pathway III: production of biopolymers from agricultural and food waste

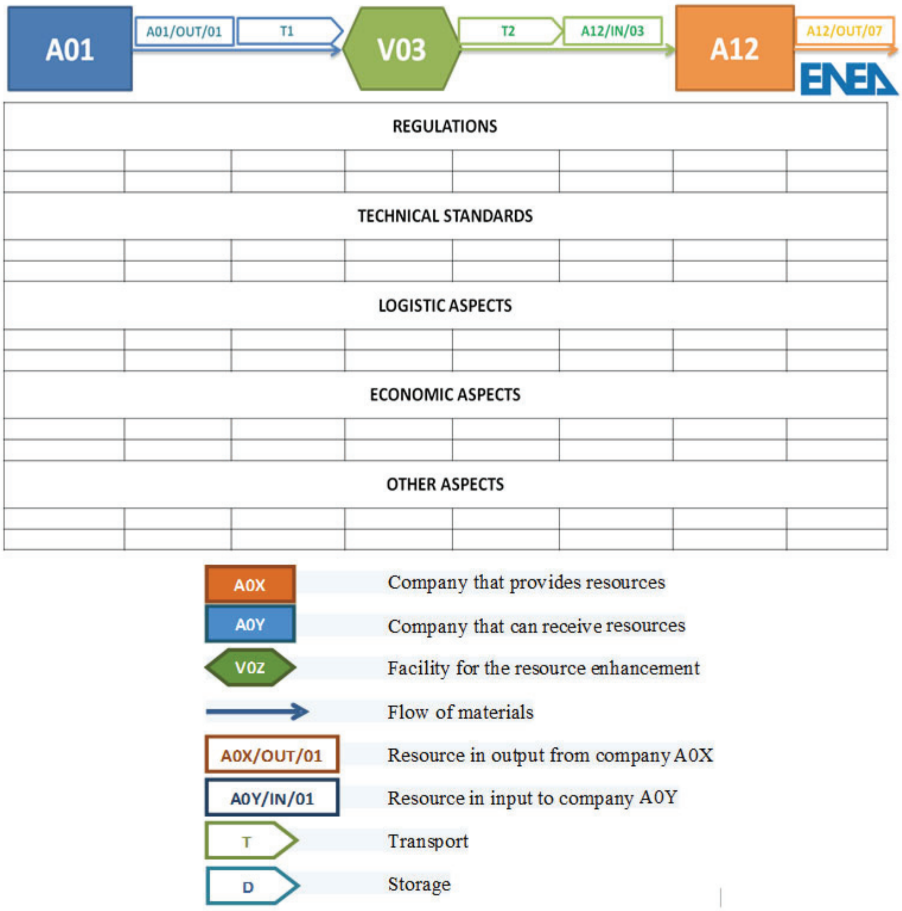


Fig. 5. Example of layout and summary Table included in the initial part of the Operative Manual

In detail, the layout is characterized by specific colors and shapes; the resources were codified for confidentiality reasons; arrows indicate the flow direction of resources.

The Table includes the basic elements of a synergy:

- Relevant norms and regulations;
- Technical Standards;
- Logistic aspects;
- Economic aspects;
- Other aspects.

"Other aspects" means specific situations that require detailed insights on a case by case basis.

These paragraphs were further divided to make the information more accessible and easy to understand. So, for example, norms and regulations paragraph is divided into: national, regional and local level. The Table was then filled with references to the documentation on the topic; each reference has a hyperlink to the document under consideration. Finally, the background of the cell presents different color based on the level of criticality of the topic: factors that may hinder the realization of synergy (red), that need to be further verified (yellow), that are compatible with the pathway (green), together with smart-tags (Fig. 6).

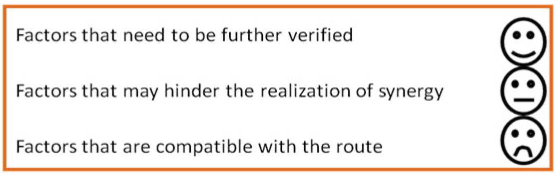


Fig. 6. Symbols used to identify the criticality within the Table in the Operative Manual

In the manual are also present cards describing the specific resources used in synergy, filled in by the companies, and a card filled in by the laboratory that summarizes the proposed valorisation process (or processes). This information was important to decide the type of synergy and figure out which type of valorisation intermediate process is necessary for the actual implementation of the synergy. The first part of the manual ends with a geo-referencing of involved companies that helps to assess the economic and regulatory feasibility of the proposed pathway: the geo-referencing was crucial in assessing both transportation costs for all resources involved in the synergy (economic feasibility) and compliance with local regulations of areas crossed by that pathway (regulatory feasibility). The technical dossier, which is the second part of the manual, is the collection of legislative, technical, scientific and other supplemental documents that are citied (and linked) in the summary table which is below the layout. Parts not strictly relevant to the subject were eliminated; a hyperlink is however present, for information completeness, to the complete documents.

The files can be used through the initial summary diagram, or read as a material in its own right. They were built with a logic that varies depending on the issues discussed but, in general, follow this pattern:

- Classification of the resource leaving the company providing the waste / resource;
- Resource Management: transportation, storage, collection etc.;
- Information about the valorization plant and process;
- Management of the resource by the receiving company;
- Output products;
- Economic considerations.

Each Manual can have all of these aspects or some of them, depending on the information collected and the steps required for the realization of synergy. The last part contains all sources used to prepare the technical dossier. Following, synthesis of the three manuals are presented.

2.3.1. Manual I: Production of nutraceuticals from agricultural and food waste

Wastes involved are skins and seeds of tomato. These types of waste, being classified as by-products, do not have problems of compliance with regulations. The laboratory "CIRI Agroalimentare" proposed to use these resources in a technological process of co-pressing of olives and tomato products that can transfer to the oily matrix lipophilic bioactive molecules, such as carotenoids by a mechanical-physical process. The optimized process will enhance the by-products of the tomato food industry enriching a product already known for its antioxidant content (olive oil) with bio-active compounds not naturally present (lycopene) by using only physical-mechanical processes without any solvents or chemicals. The procedure is simple and inexpensive and, once optimized the ratio olives/tomato by-product and identified the characteristics of the product and its conservation, will be applicable by oil mills already present on the national territory. Obtaining a new product made of olive oil and enriched in bioactive compounds of tomato could be very interesting not only for the food industry but also for dietary supplements companies. The option to use this product as a food requires complying with Italian and European legislation on food additives.

Issues identified for this synergy are of two types:

- Seasonal nature of the product;
- Preservation and storage.

In the first case the only viable solution is to program periods of production according to seasonal cycles of the resource. The second issue can be dealt with studies on storage periods and conditions to ensure that the product does not lose the required characteristics. For this concern, the time factor becomes important for the maintenance of the product. Logistically there are no major problems being the territory of Emilia - Romagna full of oil mills, thus allowing fast movements and ability to use more than one mill at a time.

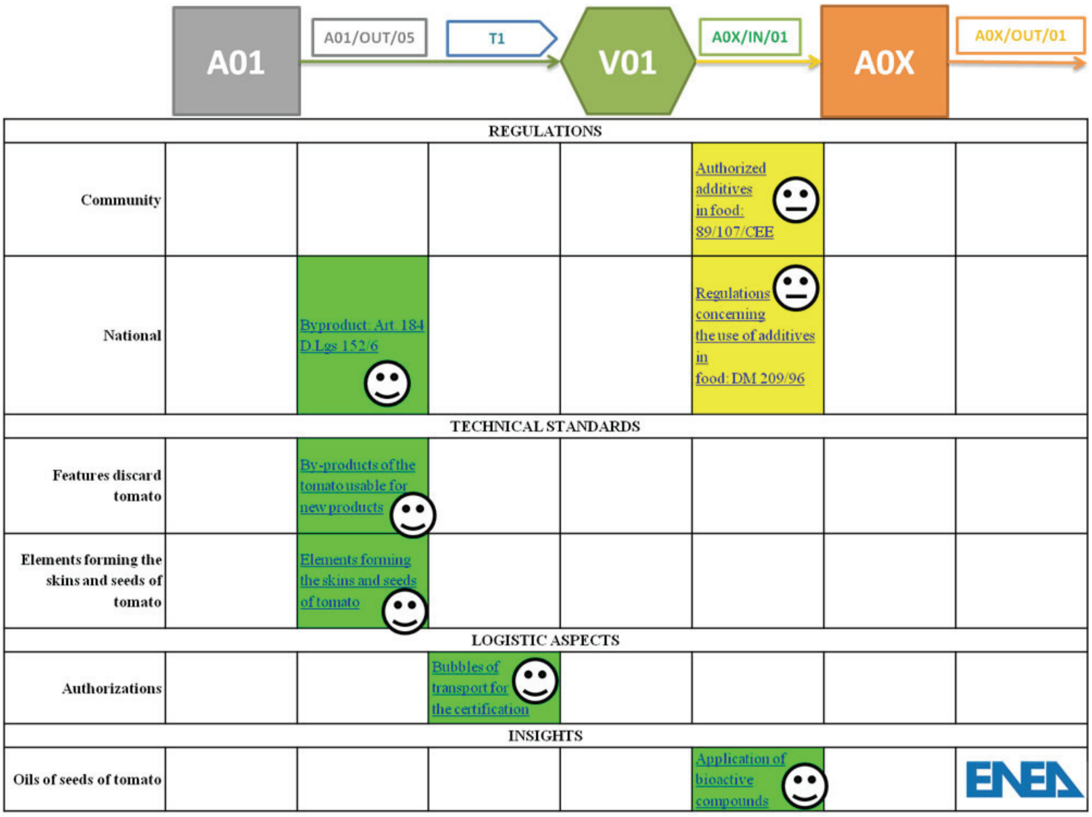
The summary Table with its related synergy layout is provided below in Fig. 7.

The parts in yellow refer to the need to obtain the necessary authorizations for the sale of a new food product. In fact, food in which the use of lycopene as a component added is approved are already allowed, therefore that after the approval process there are no identifiable reasons why the product should not be accepted.

2.3.2. Manual II: energy production from agricultural and food waste

Agricultural and food waste considered for this synergy are: grilled tomato, grilled pea, skins and seeds of tomato and grilled bean for a first company, middling and chopped of durum wheat for a second company. The receiving company deals with the transformation of fruit and vegetables, and has therefore already similar waste to handle, thus showing an interest for the building of a biogas plant for energy production. The ability to integrate its products with those of other nearby companies has led the business to participate in the project, in order to receive advice and information on new regulations and technological system, and arrange agreements with companies that produce food waste that do not reuse. This synergy has been proposed by the "LEAP laboratory" that evaluated the waste and the best type of enhancement for the production of energy. The enhancement process proposed is that of anaerobic digestion (AD). The laboratory, based on the data provided by the companies, has carried out a feasibility study for the application of the AD process the waste produced, then assessed separately all streams, in order to determine, through the matter and energy budget as well as by an economic cost evaluation, best feasible and more efficient solutions from both environmental and economic point of view. Research has thus analyzed the following steps:

- estimation of the quantity of waste and by-products in various stages of processing, approximating their availability and characterization throughout the year;
- for each waste flow, identification of the AD technology most suitable for the treatment;
- sizing of the anaerobic digestion process, with prediction of output (digestate and biogas);
- study regarding use of the digestate/soil conditioner downstream of the AD process/post-composting;
- study regarding the use of the biogas produced in AD;
- assessment of the environmental impact and sustainability of the supply chain of AD for a perspective of life cycle LCA (Life Cycle Assessment);
- preliminary assessment of economic feasibility.



REGULATIONS							
Community					Authorized additives in food: 89/107/CEE 😐		
National		Byproduct: Art. 184 D.Lgs 152/6 ☺			Regulations concerning the use of additives in food: DM 209/96 😐		
TECHNICAL STANDARDS							
Features discard tomato		By-products of the tomato usable for new products ☺					
Elements forming the skins and seeds of tomato		Elements forming the skins and seeds of tomato ☺					
LOGISTIC ASPECTS							
Authorizations			Bubbles of transport for the certification ☺				
INSIGHTS							
Oils of seeds of tomato					Application of bioactive compounds ☺		ENEA

Fig. 7. Layout and table of synergy for nutraceuticals production from agricultural and food waste

Finally, a conversion into biomethane was proposed, since it can be transported in gas pipeline and employed for all uses of natural gas, even as fuel for internal combustion engines, ensuring better economic yields, thanks also to state incentives. As initially mentioned, during project development, the Directive 2009/73/EC has been implemented in Italy, thus the biomethane can get direct access to the gas pipeline system, making development of this synergy very interesting economically.

Also for this synergy, a first criticality analyzed was the seasonality of the products, dealt with the use of animal manure and the programming of food waste during different periods of the year. The large quantities available have been an incentive for the realization of a plant but it has been necessary to take into consideration the variability of the quality of by-products (size, humidity, quantity of nitrogen etc.). To solve all the listed problems, the plant should encompass sections of receipt, storage and pre-treatment for different waste flows.

From the regulatory point of view, a research about national and regional regulations on construction and operation of a biogas plant was carried out. Finally, administrative procedures and technical regulations to comply with, in order to access government incentives for the construction of new biomethane plants, were reported. One of the issues that emerged from the study is the distance of one of the companies, more than 70 km away from the company that should receive its waste. This could be an actual limitation, not making cost-effective the symbiosis pathway proposed.

The two layouts with summary table (for both companies) are shown in Fig. 8. The aspects to which particular attention should be paid are the constraints on construction and management of the new plant that, once fulfilled, will pose no obstacle for the realization of synergy.

2.3.3. Manual III: production of biopolymers from agricultural and food waste

The resources considered for this synergy are wastes from agriculture, wood processing, packaging, rags and absorbent materials. The enhancement proposed, both by ENEA and the laboratories "Cipack" and "Siteia Parma", for these types of waste has been divided into two phases: an initial enhancement of the waste material should take place in a company dealing with thermoplastic compounds, then the enhanced material could be used in another company producing plastics, willing to convert some of its production in bioplastics. The two companies are already cooperating on other projects and in the past have developed new products testing innovative compounds together. Both companies are interested in trying to explore bioplastics market, supported by researchers who have developed, at least in pilot scale, the use of this type of waste for biopolymers production. The process to be used is currently being examined by experts of both the two companies and laboratory.

The major problem encountered in this synergy is on resources classification. At present, the materials described are classified by relevant norms as waste and landfilled.

The specific criteria needed for a waste to be classified as by-products are:

a. the substance or object shall be commonly used for specific purposes;

b. a market demand for such substance or object shall exists;

c. the substance or object fulfils specific technical requirements and meets existing legislation and standards applicable to products;

d. the use of the substance or object will not lead to overall effects adverse to environmental or human health.

A comparison with relevant public bodies (Environment Department of Emilia – Romagna region) was opened, to ask for a classification change of the resources, under article 184-ter of the fourth part of the Legislative Decree 152/2006 (Consolidated Environmental Law), which lists the features necessary for a waste to be considered as by-product.

Having considered the various aspects, it was concluded a change of classification for the concerned wastes could be requested. At present the request is still being considered by relevant authorities to allow the classification change; ENEA and ASTER are keeping open the dialogue for the resolution of this issue.

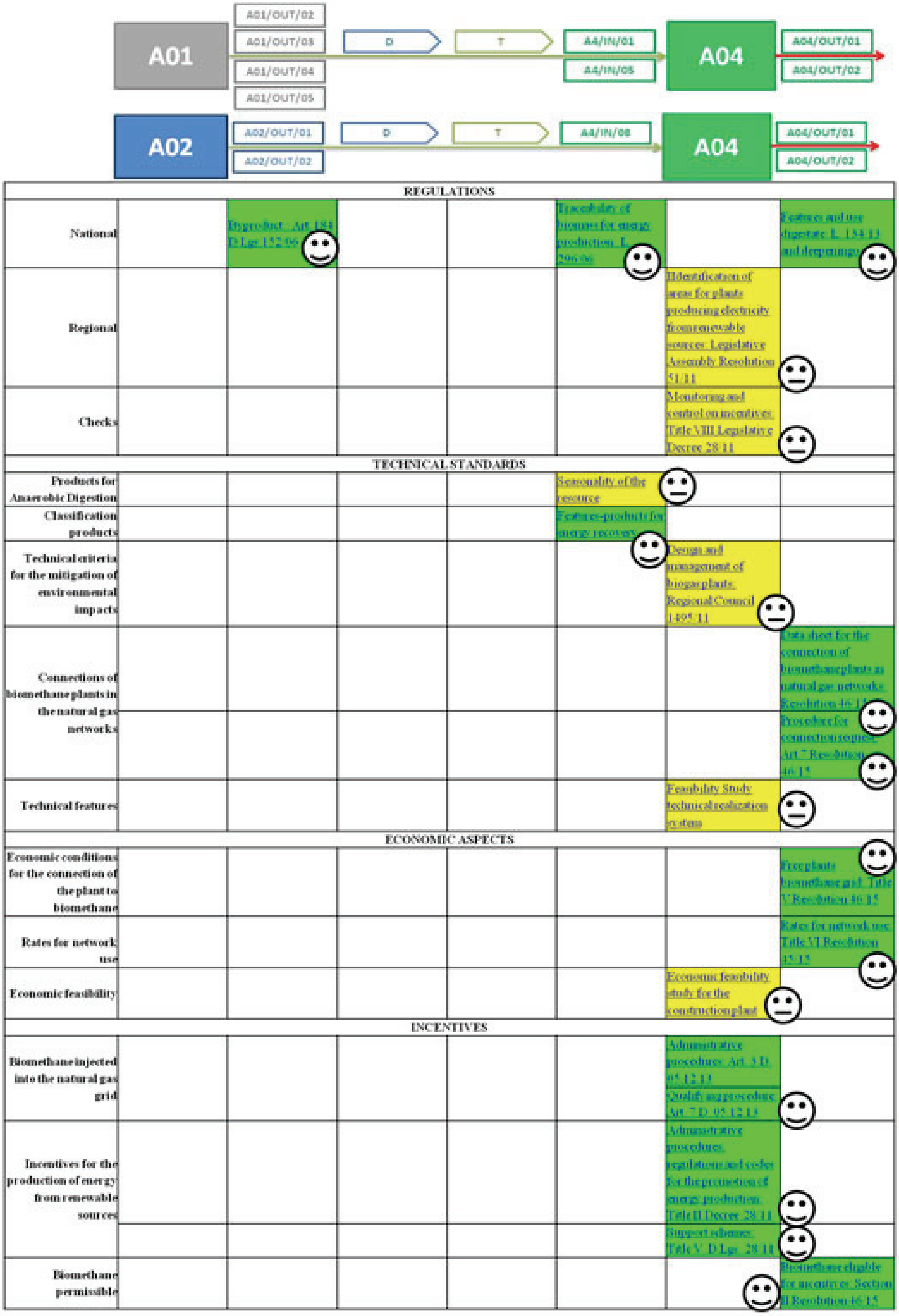
The layout and the summary table of the Manual are described in Fig. 9, pending aforementioned regulation change that will solve hindering aspects and unlock the synergy.

As the table shows, a very critical issue takes place from the classification of the stream as a "waste" instead of "byproduct"; in fact, for the Italian regulation, waste can be transported and treated only by authorized companies, limiting in this way the direct "industrial symbiosis" between waste producers and waste users.

3. Results and discussion

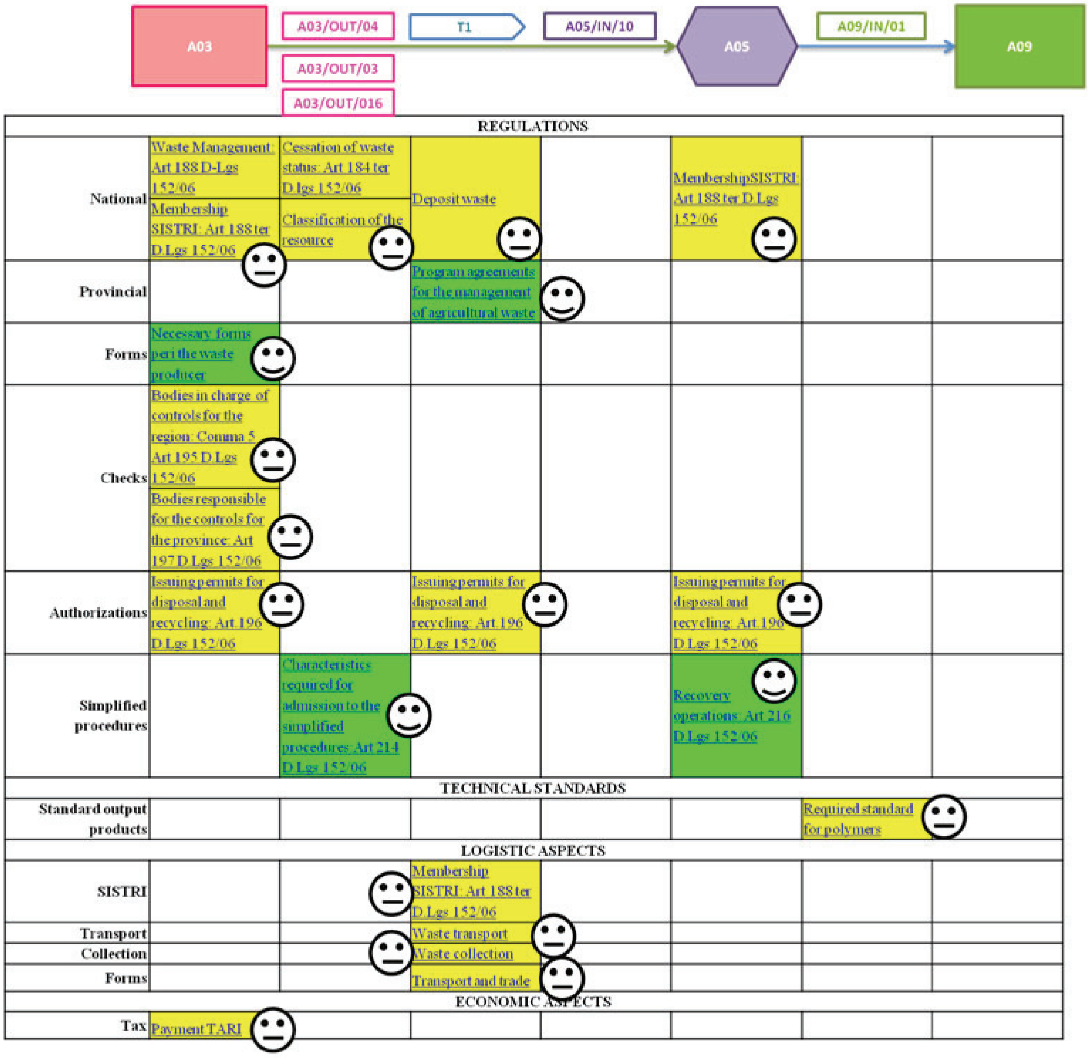
3.1. Strengths, weaknesses and potential improvements

The project began as a pilot project in Emilia - Romagna for testing and spreading the Industrial Symbiosis strategy within the region. In Italy in 2013, very few were aware of this tool for closing industrial cycles. ASTER, the promoter of the project in 2013, is a regional in-house company of the Chamber of Commerce (Unioncamere Emilia Romagna), working with local companies for technology transfer and innovation. ASTER main role, within the project, was networking, involving companies into the project, organizing meetings and workshops. At the beginning of the project ASTER asked ENEA (Environmental Technologies Technical Unit) to take part to the project in force of its experience on industrial symbiosis (Cutaia et al., 2015): ENEA took the role of scientific and technical coordinator of the project, setting the methodology, collecting and elaborating data, results and reports.



REGULATIONS							
National		Byproduct Art. 184 D.Lgs 152/06 ☺			Traceability of biomass for energy production L. 296/06 ☺		Features and use digestate L. 134/13 and dependings ☺
Regional						Identification of areas for plants producing electricity from renewable sources: Legislative Assembly Resolution 51/11 😐	
Checks						Monitoring and control on incentives: Title VIII Legislative Decree 28/11 😐	
TECHNICAL STANDARDS							
Products for Anaerobic Digestion					Seasonality of the resource 😐		
Classification products					Features-products for energy recovery ☺		
Technical criteria for the mitigation of environmental impacts						Design and management of biogas plants: Regional Council 1495/11 😐	
Connections of biomethane plants in the natural gas networks							Data sheet for the connection of biomethane plants in natural gas networks: Resolution 46/15 ☺
							Procedure for connection requests: Art 7 Resolution 46/15 ☺
Technical features						Feasibility Study technical realization system 😐	
ECONOMIC ASPECTS							
Economic conditions for the connection of the plant to biomethane							Free plants biomethane grid: Title V Resolution 46/15 ☺
Rates for network use							Rates for network use: Title VI Resolution 46/15 ☺
Economic feasibility						Economic feasibility study for the construction plant 😐	
INCENTIVES							
Biomethane injected into the natural gas grid						Administrative procedures: Art. 3 D. 05/12/13 Qualifying procedure: Art. 7 D. 05/12/13 ☺	
Incentives for the production of energy from renewable sources						Administrative procedures, regulations and codes for the promotion of energy production: Title II Decree 28/11 ☺	
						Support schemes: Title V D.Lgs. 28/11 ☺	
Biomethane permissible						☺	Biomethane eligible for incentives: Section II Resolution 46/15

Fig. 8. Layout and Table of the synergy for energy production from agricultural and food waste



REGULATIONS							
National	Waste Management: Art 188 D-Lgs 152/06 Membership SISTRI: Art 188 ter D.Lgs 152/06 😐	Cessation of waste status: Art 184 ter D.lgs 152/06 Classification of the resource 😐	Deposit waste 😐		MembershipSISTRI: Art 188 ter D.Lgs 152/06 😐		
Provincial			Program agreements for the management of agricultural waste 🙂				
Forms	Necessary forms peri the waste producer 🙂						
Checks	Bodies in charge of controls for the region: Comma 5 Art 195 D.Lgs 152/06 😐 Bodies responsible for the controls for the province: Art 197 D.Lgs 152/06 😐						
Authorizations	Issuing permits for disposal and recycling: Art 196 D.Lgs 152/06 😐		Issuing permits for disposal and recycling: Art 196 D.Lgs 152/06 😐		Issuing permits for disposal and recycling: Art 196 D.Lgs 152/06 😐		
Simplified procedures		Characteristics required for admission to the simplified procedures: Art 214 D.Lgs 152/06 🙂			Recovery operations: Art 216 D.Lgs 152/06 🙂		
TECHNICAL STANDARDS							
Standard output products						Required standard for polymers 😐	
LOGISTIC ASPECTS							
SISTRI		😐	Membership SISTRI: Art 188 ter D.Lgs 152/06				
Transport			Waste transport 😐				
Collection		😐	Waste collection				
Forms			Transport and trade 😐				
ECONOMIC ASPECTS							
Tax	Payment TARI 😐						

Fig. 9. Layout and table of synergy for production of biopolymers from agricultural and food waste

In addition, the Emilia-Romagna High Technology Network (HTN) was involved with the main goal of suggesting innovative reusing, recovery and recycling options for waste streams produced from involved companies. One of the strengths of the Project G-IS is having put together organizations with good knowledge of the area both from the industrial and the local government agencies point of view (ASTER), high expertise in research and international exposures (ENEA), together with a network of laboratories being very active in industrial research as well as in the study of regional issues (HTN).

The use of specific skills, cooperation and exchange of information has allowed a project started with limited funding and time to attract interest of companies, scientific research and industry sectors. This allowed the extension of the project in a second step, with the actual development of ideas only mentioned in the first part. The sharing of know-how of ENEA and HTN researchers allowed in some cases the development of new technologies for the exploitation of resources, in other cases, to find the most suitable and cost effective synergies, even though less technologically advanced.

Since the beginning of the project, a specific methodology was developed, which has showed its efficiency in both meetings with the participants and in information collection. In the meetings, steps necessary to achieve the proposed objectives and the tools to use were always explained. This allowed the continuous understanding by all participants of the requirements needed to achieve the objective. An ongoing dialogue with both laboratories and companies was kept constantly open, in order to dismiss doubts and get advice on how to optimize the process. The contacts were of various kinds: from meetings, to phone calls, to the intensive use of e-mails in order to minimize waiting times. Different data collection techniques were used: some formats, standards and tools already developed for a project by ENEA in Sicily have been reused (Eco-innovation Sicily, ENEA, 2011-2015) and adapted to the Emilia - Romagna context, while also other tools for situations that required specific material have been developed. The tools are designed to make the data more objective as possible and be able to collect the most important information in order to choose different synergies. The data collection forms, the arches <source, destination>, the synergies synthetic schemes and in general all the material produced during the project may be re-used and adapted in other programs of Industrial Symbiosis, citing ENEA.

An additional objective achieved by this project is to put together several companies in the area, various industrial sectors and local government authorities to share critical information. This allowed using the knowledge of both the companies and local authorities to find solutions, share ideas and develop projects where common objectives have been found. In addition to the synergies proposed by the project, cooperation between different companies was developed, that later allowed the launch of other projects, now completed.

The project begun in May of 2013 and ended in June 2015 suffered a time delay that created problems: changes in national laws, loss of interest of some individuals, development of new technologies. For the project G-IS, the inability to plan from the beginning time and resources to be used has certainly precluded the optimization of the different project steps, being in addition a pilot project among the first in Italy and thus has not able to benefit from the experience of previous cases studies. It has however created a new methodology, improvable indeed as hindered by inexperience, but which still had a good visibility in terms of publications and advertising.

During the project it became more evident a problem already known: the isolation between scientific research and industrial sectors. The Italian scientific research often suffers from little applicability of laboratory results in real industrial environment. Research is often brought to pilot scale and then abandoned for lack of funds, instead in this project the research results were proposed to business companies interested in developing new technologies. Two sectors that usually are not connected were linked, a small step forecasting possible future development.

3.2. Participation and interconnections created

In the last few months of the project we have submitted a questionnaire to the companies involved in order to assess: a) the main reasons impacting on the decision of a company to participate in industrial symbiosis pathways with other stakeholders; b) the main factors impeding the implementation of the industrial symbiosis; c) whether the methodology used in the project has fostered territorial cohesion. For achieving these objectives the questionnaire was divided into two parts.

First part: *"Industrial symbiosis between opportunities and obstacles"*. Through two matrix questions, the company delegates expressed their opinion on a set of items (respectively seventeen and sixteen) regarding to the objectives a) and b) using a rating scale from 1 (min) to 5 (max). In both cases, at the end of the two matrix questions the delegates could fill in few lines others important issues regarding the industrial symbiosis that were not present in the questionnaire.

Second part: *"Territorial cohesion"*. This part of the survey was based on concept of *territory* as "a system of economic and social relations, which make up the relational capital or the social capital of a certain geographical space" (Camagni, 2002) preferring a formal perspective of these relationships i.e. co-operation agreements among firms, among collective agents, among public institutions (Capello, 2007); and on concept of *cohesion* as density of relationships in a network (Salvini, 2005) focusing on the propensity to implement co-operation agreements among companies (Soda, 1998). On two lists of all companies participating in the project, the delegates had to mark, on the one hand, companies with which their company had already working relationships (production-subcontracting, services, marketing and export, logistics and transport, Research & Development) before the kickoff of the project; on the other hand, companies with which they had created new partnerships thanks to the project even beyond initiatives regarding to industrial symbiosis. We processed data through software UCINET 6 (Borgatti et al., 2002).

The questionnaire was submitted to the company delegates participating. Some delegates filled in the questionnaire during the conference *"Circular Economy: Experiences of Industrial Symbiosis in Emilia-Romagna"* which took place in Bologna, on the 5th of June 2015, as part of the fair *R2B - Research to Business*. The questionnaire with a short explaining text was emailed to other delegates which were not present at the conference. Data collection is over in September 2015. All delegates filled in and gave back their questionnaires.

3.2.1. Industrial symbiosis between opportunities and obstacles

According to the company delegates participating in the project G-IS the main reasons impacting on the decision of a company to participate in industrial symbiosis pathways with other stakeholders are in order of importance: 1) The opportunity to dispose of waste and by-products at a lower cost (4.08 points); 2) Higher revenues due to the opportunity of selling wastes and by-products (3.83 points); 3) The opportunity to buy at a lower cost scraps and by-products to be used in replacement of raw materials (3.58 points); 4) The creation of new partnerships and business networks (3.50 points).

On the contrary the main factors impeding the implementation of the Industrial Symbiosis are in order of importance: 1) The regulatory complexity and uncertainty (4.09 points); 2) The excessive bureaucracy (4.00 points); 3) The difficulty in finding other companies with which to realize the symbiosis (3.75 points); 4) The difficulty in estimating the costs and time of the investments and possible risks in planning and starting a partnership (3.50 points).

3.2.2. Territorial cohesion

Before the start of the project G-IS there were at least nine partnerships among the companies participated in the field of production (subcontracting), services, marketing and export, logistics and transport, R & D. Thanks to the project at least another six new relationships are created (See Fig. 10). Through a social network analysis an increase in the index of network cohesion from 0.1364 to 0.2273 (on a scale from 0 - no cohesion - to 1 - maximum cohesion) was measured. The project, therefore, has had a positive impact on strengthening the network of participating companies by 66% approximately. In particular LAB03 and A07 benefited most in participating in the project. If at the start of the project the two companies had only a relationship with the other participants, thanks to the project have created at least three more.

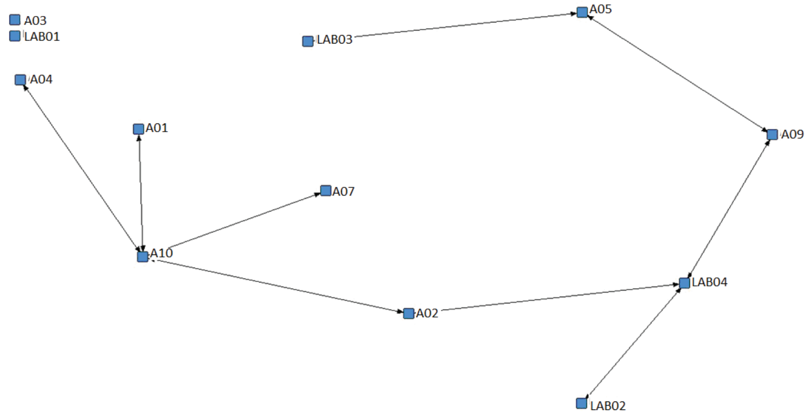
First of all it is important to note that the data collected through this survey are based on the companies having direct knowledge of many issues related to industrial symbiosis pathway thanks to the participation in the project G-IS. Analyzing the results we note that business opportunities push companies to implementation the industrial symbiosis as well as regulatory and information issues discourage them. On the basis of these results we emphasize that through the methodology used the project G-IS directly impacted a) on information obstacles reducing the difficulty in finding other companies with which to realize the industrial symbiosis; b) on the creation of new partnerships and business networks fostering in general a strengthening of territorial cohesion in the particular geographic space where it was made.

3.3. Next steps

The package of circular economy measures promoted by the European Commission implies that projects like the one proposed here can have a large development in the future. Industrial Symbiosis uses the resources available in a more intelligent and sustainable way. Many natural resources are not infinite: we must find a way to use them, behavior that is sustainable from an environmental and economic point of view, and it is also in the economic interests of companies to make the best use of their resources.

The possibility of solving problems of an area and the opportunity to find solutions even through sharing materials, energy, expertise and services fits perfectly with the vision of Circular Economy proposed by EU.

10a) Inter-firm network before the project



10b) Inter-firm network after the project

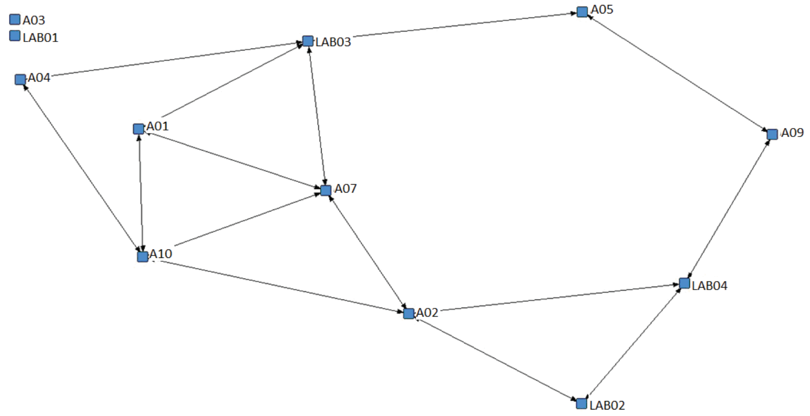


Fig. 10. Inter-firm network before (a) and after (b) the project G-IS

In a circular economy the value of products and materials is maintained as long as possible; waste and uses of resources are minimized and resources are maintained in the economy even when a product has reached the end of its life cycle, in order to reuse them several times and create additional value. This economical model can create jobs, promote innovations that can be a competitive advantage and a level of protection for people and the environment, while providing consumers with more sustainable and innovative products that can generate savings and improving life quality. In this context, Industrial Symbiosis promotion projects should find plenty of space and funding both at national and regional level.

The project G-IS has played the role of demonstrator of interest of companies in such an activity, as well as its technical feasibility. The strategy of industrial symbiosis is now addressed in the regional waste plan of Emilia Romagna Region and (RER, 2014a, b) in the regional regulation for Environmental equipped industrial areas (APEA) (Cavallo, 2013).

Now the project has ended. The authors hope that in this scenario of growing interest on industrial symbiosis new opportunities for the implementation of new projects on industrial symbiosis in Emilia Romagna will start, hopefully taking into account the experience already done and explained in this paper.

4. Conclusions

The work done within the G-IS project in Emilia Romagna shows a very good potential for the implementation of industrial symbiosis in such an area. Both industries as well as labs involved in the project did a really cooperative work, together with ASTER and ENEA, addressed at sharing resources and find valorization opportunities.

The approach used in the first part of the project was based on exchange of information on resources and on potential valorisation opportunities using specific data collection formats during several meetings and contacts between industries, labs and the working group During the first part of the project 90 potential synergies have been identified, starting from proposal made by companies directly during the meetings, from synergies proposed from labs (given shared resources) and from synergies proposed by the working group coordinated by ENEA.

In order to finalize potential synergies identified in the first phase of the project, potential synergies have been grouped and selected and for some of them an industrial symbiosis pattern has been traced and analytically described in operative manuals. This second part of the project had huge relations with local area conditions and specific applied regulations, as well as with local stakeholders and public bodies involved along industrial symbiosis patterns. The description of each part of industrial symbiosis pattern, from the production of the resource (waste or by-product) from one company till its utilization by one other company, has been “exploded” considering all possible aspects to be considered and respected by the “owner” or the “responsible” for the resource in each point of its pattern. The operative manuals, addressed at companies involved in the project and specifically in each industrial symbiosis pattern and at the moment fully available only for them, could be of general interest, for the replication of described synergies by other companies in the same area or even, could be used as a guide, for the implementation of such kind of synergies in other local conditions. Putting together and in a synthetic and systematic way all data, information and requirements needed for the actual implementation of a specific industrial symbiosis pattern has been considered really useful by companies involved in the synergies.

Moving from a pilot project, such as the Green Industrial Symbiosis project, towards a systematic, broad and continuous application of industrial symbiosis approach would require the availability of network connections and coordination, able to collect and valorize shared resources finding not only “one opportunity” but the “best opportunity” given the boundary conditions.

The EU Circular Economy package, published the 2nd of December 2015, could be a driver for implementing industrial symbiosis systematically as a strategy for saving resources.

References

Borgatti S.P., Everett M.G., Freeman L.C., (2002), UCINET 6 for Windows. Analytic Technologies, Harvard, On line at: https://sites.google.com/site/ucinetsoftware/home.

Camagni R., (2002), On the concept of territorial competitiveness: sound or misleading?, *Urban Studies*, **39**, 2395-2411.

Capello R., (2007), *Regional Economics*, Routledge, London.

Cavallo M., (2013), Industrial symbiosis and productive areas, *Environmental Engineering and Management Journal*, **12**, 265-268.

Chertow M.R., (2000), Industrial symbiosis: literature and taxonomy, *Annual Review of Energy and the Environmen*t, **25**, 313-337.

Cutaia L., Scagliarino C., Mencherini U., Iacondini A., (2014), Industrial symbiosis in Emilia-Romagna region: results from a first application in the agro-industry sector, *Procedia Environmental Science, Engineering and Management*, **2**, 11-36.

Cutaia L., Luciano A., Barberio G., Sbaffoni S., Mancuso E., Scagliarino C., La Monica M., (2015), The experience of the first industrial symbiosis platform in Italy, *Environmental Engineering and Management Journal*, **14**, 1521-1533.

Iacondini A., Mencherini U., Passarini F., Vassura I., Fanelli A., Cibotti P., (2015), Feasibility of industrial symbiosis in Italy as an opportunity for economic development: critical success factor analysis, impact and constrains of the specific Italian regulations, *Waste and Biomass Valorization Journal*, **6**, 865-874.

EU Commission, (2010), Europe 2020 Growth Strategy, EU Commission, On line at: http://ec.europa.eu/europe2020/index_en.htm.

EU Commission, (2011a), A resource-efficient Europe – Flagship initiative under the Europe 2020 Strategy, 26.7.2011, Brussels, On line at: http://ec.europa.eu/resource-efficient-europe/pdf/resource_efficient_europe_en.pdf.

EU Commission, (2011b), Roadmap to a Resource Efficient Europe, 20.9.2011, Brussels, On line at: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52011DC0571.

EU Commission, (2015), Closing the loop - An EU action plan for the Circular Economy, Brussels, On line at: http://ec.europa.eu/priorities/jobs-growth-investment/circular-economy/docs/communication-action-plan-for-circular-economy_en.pdf.

EC Directive, (2009), Directive 2009/73/EC of the European Parliament and of the Council of 13 July 2009 concerning common rules for the internal market in natural gas and repealing Directive 2003/55/EC, *Official Journal of the European Union*, L211, 94-136.

Regione Lazio, (2014), Smart specialization strategy (S3) Regione Lazio, On line at: http://lazioeuropa.it/files/140723/regione_lazio_smart_specialisation_strategy_luglio_2014.pdf.

RER, (2014a), Regional and Innovation Strategy for Smart Specialisation Regione Emilia-Romagna (RIS3ER), Regione Emilia-Romagna, On line at: http://www.regione.emilia-romagna.it/fesr/por2014-2020/documenti/documenti-approvati-ue/Allegato1ASmartSpecialisationStrategyEmiliaRomagna.pdf/at_download/file/Allegato%201A%20Smart%20Specialisation%20Strategy%20Emilia%20Romagna.pdf.

RER, (2014b), Regional Waste management Programme, Regione Emilia-Romagna, On line at: http://ambiente.regione.emilia-romagna.it/rifiuti/documenti/prgr-adottato/prgr-finale/at_download/file/PRGR%20Finale_alta%20risoluzione.pdf.

Salvini A., (2005), *Analysis of Social Networks: Resources and Mechanisms*, (in Italian), Ed PLUS, Pisa University Press, Pisa, Italy.

Soda G., (1998), *Networks between Companies: Models and Perspectives for a Theory of Coordination between Undertakings*, (in Italian), Carocci Editore, Rome, Italy.

1962

Environmental Engineering and Management Journal September 2016, Vol.15, No. 9, 1963-1969
http://omicron.ch.tuiasi.ro/EEMJ/

"Gheorghe Asachi" Technical University of Iasi, Romania

CHEMICAL CHARACTERIZATION OF ODOR ACTIVE VOLATILE ORGANIC COMPOUNDS EMITTED FROM PERFUMES BY GC/MS-O

Antonella Amenduni[1], Magda Brattoli[2], Gianluigi de Gennaro[1,2*], Federica Massari[1], Jolanda Palmisani[1], Maria Tutino[2]

[1]*Department of Biology, University of Bari, via Orabona 4, 70126 Bari, Italy*
[2]*ARPA, Agency for Environmental Protection of Apulia Region, Corso Trieste 27, 70126 Bari, Italy*

Abstract

The selection of proper compounds to include in perfumes' formulation is of particular interest for the perfume industry. Essential oils and perfumes are subject to quality control as well as to chemical characterization; therefore, GC/MS-O methodology can be considered a useful tool for research purposes and/or for improving industrial manufacturing processes. In this study, GC/MS-O methodology was applied to a commercial brand perfume and a natural-derived one, both characterized by a floral scent, with the main purpose of recognizing the odor active VOCs responsible of the characteristic and predominant notes. GC/MS-O analysis highlighted that sensory detection can be more efficient than the analytical one. Ocimene, α-Ionone and α-Isomethylionone were the most abundant odor compounds for the commercial-brand perfume, while β-Hydroxyethylbenzene was detected for the natural-derived one. Moreover, organic compounds of concern such as Toluene and Benzyl Alcohol were detected highlighting the need for quality control to reduce human risks for inhalation exposure and allergies.

Key words: human risk, odor compounds, perfume, sensitivity

Received: January, 2016; Revised final: September, 2016; Accepted: September, 2016

1. Introduction

In the recent years the scientific community has showed increasing interest in odor active Volatile Organic Compounds (VOCs). VOCs characterization, identification of sources and combination of chemical information with human-sensory perception were deeply investigated with the aim to increase knowledge in this research field and to develop direct applications in industry (Brattoli et al., 2013; de Gennaro et al., 2015). Some industrial applications focus on improving the quality of the formulation of scented products, such as perfumes, through the selection of natural or synthetic compounds (Andriani et al., 2013; Schilling et al., 2010). Others have the purpose to evaluate the impact of odorous compounds on Indoor Air Quality (IAQ) as well as the quality of human life and environment (Aatamila et al., 2011; Beghi et al., 2012; Li et al., 2013). Odor active VOCs may derive from industrial activities (landfills, wastewater treatment plants, refineries, tanneries) or may be emitted indoors from a variety of construction materials, furniture, consumer products as well as fragrance-containing products (Brattoli et al., 2014; Félix et al., 2013; González et al., 2013; Knudsen et al., 2007). Instrumental approaches based on Gas Chromatography coupled with Mass Spectrometry (GC/MS) have been widely developed in order to identify odorous compounds and to relate qualitative and quantitative information. The main limitation of these instrumental approaches is related to the complexity of the odor mixture and to the lack of information regarding human perception, so a correlation between identity and concentration of odorous compounds and the olfactory stimulus

---

[*] Author to whom all correspondence should be addressed: e-mail: gianluigi.degennaro@uniba.it; Phone: +39 0805443343

cannot be found (Brattoli et al., 2013). Moreover, many VOCs are often present at concentrations lower than the instrumental detection limit and lack of information about mechanisms of the human perception is still present, so a linear correlation between a quantified substance and an olfactory stimulus cannot be easily found (Di Francesco et al., 2001). Therefore, the implementation of sensory detection, performed by trained panelists, with GC/MS analysis allowed to overcome the limitations of the conventional analytical methodology, because the mammalian olfactory system is considered the most sensitive and inclusive odor detector (Delahunty et al., 2006).

A fragrance formula may be a complex mixture containing up to several hundred or more different ingredients. Perfumers work with several thousand natural or synthetically manufactured ingredients, mixed in order to create the fragrance composition with the overall desired smell (Curtis and Williams, 2001). Perfumes and essential oils are subject to quality control as well as chemical and odorous characterization in industry where the main goal is to make these products as pleasant as possible and to confer a characteristic odor. GC/MS-O is considered a reliable tool due to its ability to efficiently separate and characterize the principal compounds constituting the matrix of perfumes and essential oils such as Terpenes, Aldehydes and Alcohols (Costa et al., 2008; Curtis and Williams, 2001; Pybus and Sell, 2006). Despite the attention in this research field, the existing available information about the ingredients, the emissions and the allergenic potential of perfumes is not complete and further knowledge is needed.

The main aim of this study was the identification of the odor active VOCs responsible of the characteristic notes of two selected perfumes. The attention was also focused on the potential presence of VOCs of concern and EU-regulated fragrance substances, recognized as contact allergens. Information concerning their presence in cosmetic products should be provided to consumers (EC Directive, 2003). Measurements were carried out inside a test emission chamber. Air samples were collected on thermal desorption tubes and analyzed by means of GC/MS-O methodology to collect sensory data and to identify the released fragrance substances emitted by the two selected perfumes.

2. Materials and methods

2.1. Samples

The "commercial brand" perfume was purchased in a local perfumery. It is a female floral fragrance, made in France, with predominantly jasmine and musk notes (middle and base notes, respectively). This perfume was selected because is one of the most famous fragrances worldwide and used among women. The second perfume under investigation, a female and "natural-derived" fragrance made in Italy, is characterized by violet flowers scent. As declared by home producer, the ingredients used in the formulation are naturally derived by plants and flowers.

2.2. Emission testing and analysis

The characterization of odor active VOCs emitted from the selected perfumes required the use of a test emission chamber, a glass chemical reactor hermetically closed having a cylindrical shape characterized by the following dimensions: diameter =29 cm, height =61 cm and volume =0.05 m³ (Fig. 1). The chamber was supplied with ultrapure compressed air (VOCs-free air) and operated at controlled micro-environmental conditions (temperature=23±2°C, relative humidity=50 ± 5% and air exchange rate=0.05h^{-1}) (ISO 16000-9, 2006). These parameters simulate typical average indoor air conditions. Before the test, the chamber assemblies were cleaned with a specific detergent and rinsed with distilled water. In order to verify VOCs levels in the chamber, background samples were taken sampling the chamber air onto cartridges filled with a suitable adsorbent material. Thermal desorption and GC/MS analysis of the samples revealed that VOCs of interest were not present in the chamber.

Fig. 1. Test emission chamber

The cartridges consisted of a cylindrical stainless steel net (100 mesh) with an external diameter of 4.8 mm, containing 350 mg Carbograph™ 4 (35-50 mesh). Afterwards, a rectangular piece of laboratory filter paper was introduced inside the chamber and two sprays of the perfume were applied onto the surface. The perfume bottle, for each experiment, was weighed immediately before and after the application in order to calculate the mass of the product applied on the paper's surface. Immediately after the application, the lid was closed and the test started (t=0). VOCs emitted from the perfume inside the chamber were carried away by the air flow, mixed in the chamber and extracted via the outlet duct. Immediately after the start of the test (t=0), chamber air was sampled onto the cartridges in order to detect individuals VOC and by means of a photo-ionization detector to define the Total VOCs (TVOC) concentration-time profile (PhoCheck® Tiger, Ion Science Ltd, UK).

In accordance with the instructions provided by the manufacturer, the cartridges were conditioned and analyzed to verify the blank levels before the sampling. Conditioning procedure was carried out at 310°C for 20 minutes by means of a thermal desorber (Markes International Ltd, Unity 2™). Afterwards, GC/MS analysis of the cartridges allowed to verify that concentrations of the detected VOCs were lower than the detection limits.

For the chamber test, the sampling flow rate for the flow-controlled pump (Pocket Pump) was set at 50 ml/min and the final air volume collected was 5 liters. The aforementioned sampling conditions were selected according to the suggestions listed in the International Standard (ISO 16000-6, 2011). In order to avoid that the breakthrough could negatively affect the sampling procedure, two cartridges were put in sequence by means of a collecting tube. Although traces of the more volatile compounds were also detected on the back-up cartridges, no further information on the qualitative composition of the perfumes was obtained by the GC/MS analysis of the back-up cartridges. The analyses were carried out using a thermal desorber (Markes International Ltd, Unity 2™) connected to a gas chromatograph (GC Agilent 7890) equipped with an olfactometric port (ODP 3 Gerstel) and connected to a mass spectrometer (MS Agilent 5975). The thermal desorption consisted of a two-stage mechanism. VOCs were desorbed from the Carbograph adsorbent cartridge at 300°C and refocused onto a cold trap at -10°C. Primary desorption split was set and the inlet split ratio was equal to 3.2:1. Then the cold trap was flash heated to 300°C and the vapors were transferred via the heated transfer line (200°C) to the GC column and to the olfactometric port. The analytical column was HP5-MS (30mx250μmx0.25μm) and the flow of the carrier gas (Helium) was controlled by constant pressure and was equal to 1.7 ml min^{-1}. The GC oven was temperature programmed from 37°C to 100°C at 3.5°C min^{-1}, and from 100°C to 250°C at 15°C min^{-1}. The MS transfer line was 230°C and the MS was operated in electron impact (EI) ionization mode (70eV) with a mass range 20-250 m/z. Acquisition was carried out in SCAN mode. After the GC separation the column flow was splitted with the ratio 1:1, one part was led to the MS system and the other one to ODP. The effluent from the capillary column reached the olfactometric port through an uncoated transfer line (deactivated silica capillaries) and was sniffed by the assessor in a PTFE conical port, fitted to the shape of a nose. The transfer line was heated to prevent the condensation of compounds on the walls of the capillary. Auxiliary gas (make-up gas) was added to the GC effluent to prevent the drying of the assessors' nose mucous membranes, which could cause discomfort especially in longer analyses. Two trained panelists were asked to indicate when odor was perceived, pressing an electric push-button connected to the ODP, and to indicate with clear words a qualitative description of the odor. GC/MS-O analysis were carried out three times for each investigated perfume. The panelists were selected according to a standardized procedure used for the panel selection in Dynamic Olfactometry, the official methodology for odor emissions assessment standardized by a European technical law (CEN, 2003). The standardized procedure provides for individuals with average olfactory sensitivity that constitute a representative sample of the human population. The screening was performed evaluating the response to the most used reference gas, n-butanol. Only assessors who respected predetermined repeatability and accuracy criteria were selected as panelists. To perform GC/MS-O analysis, the panelists respect a specific code of conduct, including not smoking and eating/drinking strongly flavored foods for 1 h prior to GC/MS-O analysis and not wearing aftershave, perfume or deodorants the day of assessment (Delahunty et al., 2006; Etiévant et al., 1999). Moreover, since the sniff time could affect the human performance, a time of 25 – 30 min should be considered as the maximum sniff duration. The identification of odor active VOCs was performed by comparing the mass spectra of the unknown compounds with those listed in the NIST library (Agilent Technologies, coincidence of the spectra > 95%). Then, compounds were confirmed analyzing standard solutions for each detected compound.

3. Results and discussion

3.1. "Commercial-brand" floral perfume

Experimental data allowed a complete qualitative screening of VOCs useful to make a comparison between the investigated perfumes. The overlapping of GC/MS chromatogram (TIC) with aromagram (violet) for the "commercial brand" perfume is reported in Fig. 2. Aromagrams were registered by two assessors using the human trained nose as a detector. Aroma events resulting from separated compounds eluting from the gas chromatography column were characterized for perceived aroma intensity and odor duration. The intensity scale ranged from 0 (no odor perceived) to 4 (strong odor) while the duration is expressed in minutes. The assessors also provided a qualitative description of the perceived odor of each compound using suitable descriptors. Detected VOCs, the time when the assessor perceived the odorous stimulus (peak start expressed in minutes), odor description and intensity are listed in the Table 1.

VOCs detected at ODP port and identified by GC/MS are the following ones: Isoamylacetate, β-Pinene, β-Myrcene, 4-Methylanisole, α-Phellandrene, Methyl Benzoate, β-Hydroxyethylbenzene, Alloocimene, α-Ionone and α-Isomethylionone. Among them, Alloocimene, α-Ionone and α-Isomethylionone were identified as odor active

VOCs and perceived as the main characteristic notes for the "commercial brand" perfume. Alloocimene is a monoterpene and one of the isomeric forms of the Ocimenes. They are often found naturally as a mixture of the various forms, within a variety of plants and fruits. The mixture as well as the individual isomeric forms are characterized by a very pleasant floral smell and may be contained in the formulation of perfumes and essential oils. α-Ionone and α-Isomethylionone are widely used in perfumery, both associated to a strong floral violet-like smell (Anzaldi et al., 2000). The use of α-Isomethylionone in the fragrances' formulation is preferred to the use of the β-isomeric form, considered to be a strong sensitizer (Belsito et al., 2007; Lapczynski et al., 2007). The overlapping of the chromatogram with the aromagram (Fig. 2) shows that many odorous stimulus perceived by the assessors at the ODP (listed in Table 1 as "unidentified") were not identified as no chromatographic peaks were simultaneously visualized during the analysis.

In all the observed cases, the unsuccessful identification of odor active compounds is not related to a possible delay occurring from the elution of the compounds from the chromatographic column to the sensory detection by panelists (d'Acampora Zellner et al., 2008). More likely, the explanation is related to the weak retaining property of the stationary phase of the used chromatographic column for certain chemical compounds. Moreover, in certain cases, sensory detection is more efficient than the analytical one as odor active VOCs can be detected by human nose at concentrations lower than the detection limit (LOD) of the analytical technique.

Table 1. GC/MS-O analysis report for the "commercial brand" perfume. Peak start (min), odor intensity, odor description and identified compounds

Peak start (min)	*Intensity*	*Odor description*	*Identified compound*
05.52	2 (weak odor)	Minty	UNIDENTIFIED
08.42	2 (weak odor)	Minty	UNIDENTIFIED
10.65	3 (clear odor)	Fruity	Isoamylacetate
14.69	2 (weak odor)	Grass/Herbaceous	β-Pinene
15.24	3 (clear odor)	Grass/Herbaceous	β-Myrcene
15.90	3 (clear odor)	Fruity	α-Phellandrene
16.54	2 (weak odor)	Fruity	4-Methylanisole
19.22	3 (clear odor)	Acrid smell	UNIDENTIFIED
19.40	3 (clear odor)	Sweet	UNIDENTIFIED
19.90	2 (weak odor)	Sweet/Fruity	Methyl Benzoate
20.06	2 (weak odor)	Floral	β-Hydroxyethylbenzene
20.46	2 (weak odor)	Characteristic note of the perfume (Floral)	Alloocimene
21.04	2 (weak odor)	Floral (chrysanthemum)	UNIDENTIFIED
22.86	2 (weak odor)	Oriental incense	UNIDENTIFIED
23.03	2 (weak odor)	Burnt	UNIDENTIFIED
23.26	2 (weak odor)	Burnt	UNIDENTIFIED
26.10	2 (weak odor)	Characteristic note of the perfume (Floral)	α -Ionone
27.26	2 (weak odor)	Characteristic note of the perfume (Floral)	α-Isomethylionone

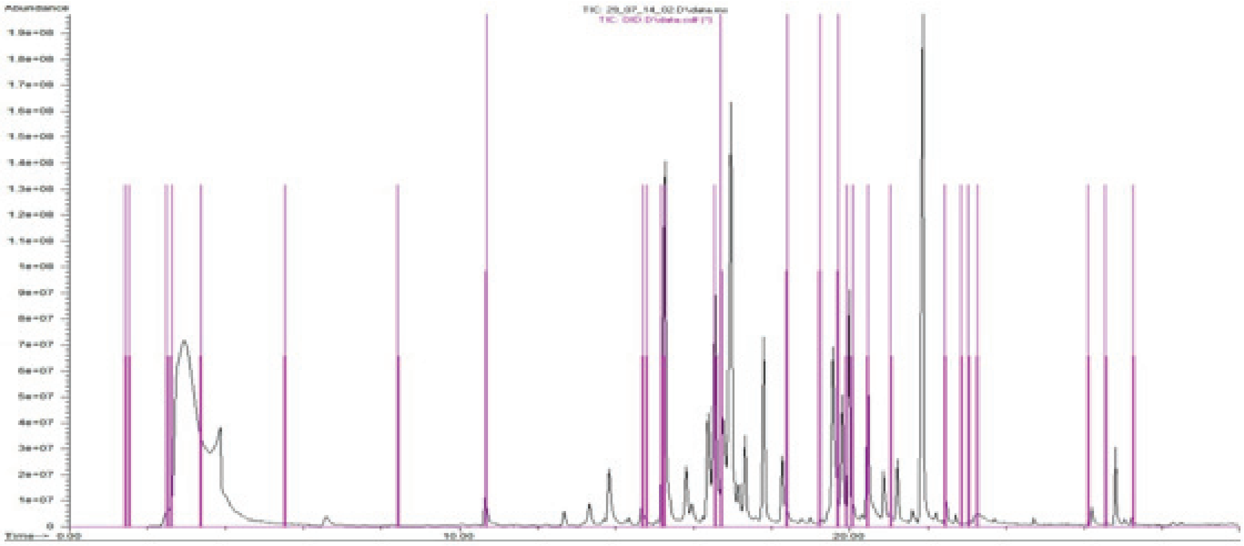


Fig. 2. The aromagram overlapped on GC/MS chromatogram (TIC) for the "commercial brand" perfume

Integration of the qualitative analysis data and sensory response also showed that many odorous stimuli panelists perceived corresponded to chromatographic peaks not sufficiently intense to allow accurate chromatographic identification or remained unrecognized due to the overlapping of co-eluting compounds resulting in a confidence rating of mass spectra comparison lower than the reference value (95%).

GC/MS analysis allowed identifying other VOCs emitted by the perfume but not generating odorous stimulus at ODP. A comprehensive qualitative screening is reported in Table 2 where identified odor active VOCs generating an olfactory stimulus or not are listed with the associated retention time.

Table 2. GC/MS analysis report for the "commercial brand" perfume. Retention times (min) and identified VOCs

Retention time (min)	*Identified VOC*
6.57	Toluene
10.09	m/p-Xylene
10.65	Isoamylacetate
12.67	α-Pinene
13.32	Camphene
13.82	Benzaldehyde
14.69	β-Pinene
15.26	β-Myrcene
15.80	α-Phellandrene
16.36	α-Terpinene
16.54	4-Methylanisole
16.95	d-Limonene
17.15	Benzyl Alcohol
17.79	α/β-Ocimene
18.26	γ-Terpinene
19.79	Methyl Benzoate
20.46	β-Hydroxyethylbenzene
21.12	Alloocimene
22.46	Terpineol
22.70	Dodecanal
26.19	α-Ionone
27.26	α-Isomethylionone

Among the identified compounds, there are Terpenes (α-Pinene, Camphene, d-Limonene, α-Phellandrene) and Aromatic Compounds of concern for inhalation exposure such as Toluene, m/p-Xylenes and Benzyl Alcohol. According to the quality standard's requirements of Code of Practice and Standards of the International Fragrance Association (IFRA), Toluene is classified as "prohibited" and should not be used as fragrance ingredient for any kind of application (IFRA, 2015). Benzyl Alcohol, associated to the jasmine note of the perfume (middle note) belongs to the list of fragrance chemicals, which according to the existing knowledge, are well-recognised contact allergens (Opinion on fragrance allergens in cosmetic products, Scientific Committee on Consumer Safety, 2011). European Scientific Committee on Consumer Safety (SCCS, 2011) recently highlighted the relevance of the presence of Benzyl Alcohol in perfumes and scented consumer goods.

3.2. "Natural-derived" perfume with violet fragrance

The second perfume under investigation was "natural-derived" characterized by a violet flowers fragrance. The overlapping of GC/MS chromatogram (TIC) with aromagram (violet) for the "natural-derived" perfume is reported in Fig. 3.

Odor active VOCs generating an odorous stimulus and identified via GC/MS were: Isoamylacetate, Benzaldehyde, β-Myrcene, Phenylacetaldehyde, β-Hydroxyethylbenzene and Dodecanal. GC/MS-O analysis allowed recognizing the β-Hydroxyethylbenzene, perceived by the assessors with an intensity level equal to 2, as the odor active compound responsible of the overall flavor of violets reported on the product's label. Some Terpenes, identified via GC/MS, were found in both the perfumes: Camphene, β-Pinene, β-Myrcene. Moreover, Ionone and α-Isomethylionone were detected while the presence of Eucalyptol and p-Cimenene was detected only in the "natural-derived" perfume (Table 3).

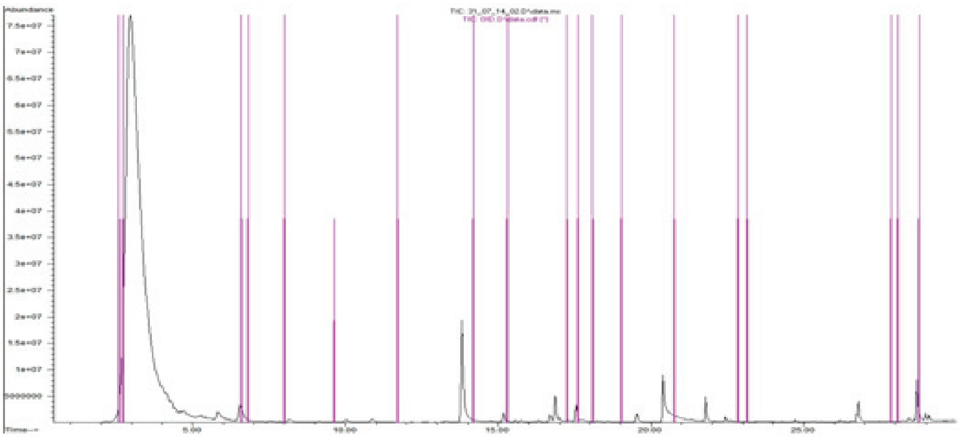


Fig. 3. The aromagram overlapped on GC/MS chromatogram (TIC) for the "natural-derived" perfume

Table 3. GC/MS-O analysis report for the "natural-derived" perfume. Peak start (min), odor intensity, odor description and identified compounds

Peak start (min)	*Intensity*	*Odor description*	*Identified compound*
06.81	2 (weak odor)	Solvent	Toluene
08.01	1 (just perceptable odor)	Caramel-like	UNIDENTIFIED
10.65	2 (weak odor)	Fruity	Isoamylacetate
13.81	2 (weak odor)	Sweet	Benzaldehyde
15.18	2 (weak odor)	Herbaceous/Floral	β-Myrcene
17.24	2 (weak odor)	Sweet	UNIDENTIFIED
17.55	2 (weak odor)	Floral	Phenylacetaldehyde
18.08	2 (weak odor)	Sweet	UNIDENTIFIED
19.03	2 (weak odor)	Sweet	UNIDENTIFIED
20.75	3 (clear odor)	Characteristic note of the fragrance (violet)	β-Hydroxyethylbenzene
22.85	2 (weak odor)	Floral	Dodecanal
23.15	2 (weak odor)	Sweet	UNIDENTIFIED
27.86	2 (weak odor)	Acrid	UNIDENTIFIED

Aromatic compounds of concern were also identified such as Toluene, m/p-Xylene and Styrene: the first two in common with the "commercial brand" floral perfume (Table 4).

Table 4. GC/MS analysis report for the "natural-derived" perfume. Retention times (min) and identified VOCs

Retention time (min)	*Identified VOC*
6.57	Toluene
10.02	m/p-Xylene
10.60	Isoamylacetate
10.87	Styrene
13.30	Camphene
14.19	Benzaldehyde
14.69	β-Pinene
15.76	β-Myrcene
17.01	Eucalyptol
17.61	Phenylacetaldehyde
18.59	Acetophenone
19.54	p-Cimenene
20.40	β-Hydroxyethylbenzene
22.36	Dodecanal
26.19	α-Ionone
26.79	α-Isomethylionone

4. Conclusions

The characterization of odor active VOCs emitted by a commercial brand and a natural-derived perfume was performed inside a test emission chamber. The odor evaluation and the simultaneous identification via GC/MS-O allowed to recognize the odor active VOCs responsible of characteristic notes of the overall fragrance: Ocimene, α-Ionone and α-Isomethylionone for the "commercial-brand" perfume and β-Hydroxyethylbenzene for the "natural-derived" one. GC/MS-O analysis also highlighted that, in certain cases, sensory detection can be more efficient than the analytical one. The investigation allowed the detection of the presence of organic compounds of concern such as Toluene and Benzyl Alcohol, highlighting the need for quality control in order to reduce human risks for inhalation exposure and allergies.

References

Aatamila M., Verkasalo P.K., Korhonen M.J., Suominen A.L., Hirvonen M.-R., Viluksela M.K., Nevalainen A., (2011), Odour annoyance and physical symptoms among residents living near waste treatment centres, *Environmental Research*, **111**, 164-170.

Andriani E., Brattoli M., Buono P., de Gennaro G., de Gennaro L., (2013), Development of a tool for industrial atmospheric emission management in the Apulia region, *Environmental Engineering and Management Journal,* **12**, 429-434.

Anzaldi M., Sottofattori E., Dusatti F., Ferro M., Pani M., Balbi A., (2000), Synthesis of ionones and carvone analogues: olfactory properties and preliminary toxicity assays, *European Journal of Medicinal Chemistry*, **35**, 797-803.

Beghi S.P., Santos J.M., Reis N.C., de Sá L.M., Goulart E.V., de Abreu Costa E., (2012), Impact assessment of odours emitted by a wastewater treatment plant, *Water Science and Technology*, **66**, 2223-2228.

Belsito D., Bickers D., Bruze M., Calow P., Greim H., Hanifin J.H., Rogers A.E., Saurat J.H., Sipes I.G., Tagami H., (2007), A toxicologic and dermatologic assessment of ionones when used as fragrance ingredients, *Food and Chemical Toxicology*, **45**, S130-S167.

Brattoli M., Cisternino E., Dambruoso P.R., de Gennaro G., Giungato P., Mazzone A., Palmisani J., Tutino M., (2013), Gas chromatography analysis with olfactometric detection (GC-O) as a useful methodology for chemical characterization of odorous compounds (review), *Sensors,* **13**, 16759-16800.

Brattoli M., Cisternino E., de Gennaro G. Giungato P., Mazzone A., Palmisani J., Tutino M., (2014), Gas Chromatography analysis with olfactometric detection (GC-O): An innovative approach for chemical characterization of odor active Volatile Organic Compounds (VOCs) emitted from a consumer product, *Chemical Engineering Transactions*, **40**, 121-126.

CEN, (2003), EN13725: 2003: Air Quality Determination of Odour Concentration by Dynamic Olfactometry, Committee for European Normalization, Brussels, Belgium.

Costa R., d'Acampora Zellner B., Crupi M.L., de Fina M.R., Valentino M.R., Dugo P., Dugo G., Mondello L., (2008), GC-MS, GC-O and enantio-GC investigation of the essential oil of *Tarchonanthus camphoratus* L., *Flavour and Fragrance Journal*, **23**, 40-48.

Curtis T., Williams D.G., (2001), *Introduction to Perfumery*, Micelle Press, New York USA.

d'Acampora Zellner B., Bicchi C., Dugo P., Rubiolo P., Dugo G., Mondello L., (2008), Linear retention indices in gas chromatographic analysis: A review, *Flavour and Fragrance Journal*, **23**, 297-314.

de Gennaro G., Dambruoso P.R., Di Gilio A., Marzocca A., Tutino M., (2015), Indoor and outdoor volatile organic compounds monitoring in a multi-storey car park, *Environmental Engineering and Management Journal,* **14**, 1563-1570.

Delahunty C.M., Eyres G., Dufour J.P., (2006), Gas chromatography-olfactometry, *Journal of Separation Science*, **29**, 2107-2125.

Di Francesco F., Lazzerini B., Marcelloni F., Pioggia G., (2001), An electronic nose for odor annoyance assessment, *Atmospheric Environment*, **35**, 1225-1234.

EC Directive, (2003), Directive 2003/15/EC of the European Parliament and of the Council of 27 February 2003 amending Council Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products, *Official Journal of the European Union*, L 66, 26-35.

Etiévant P.X., Callement G., Langlois D., Issanchou S., Coquibus J.N., (1999), Odor intensity evaluation in gas chromatography-olfactometry by finger span method, *Journal of Agricultural and Food Chemistry*, **47**, 1673-1680.

Félix J.S., Domeño C., Nerín C., (2013), Characterization of wood plastic composites made from landfill-derived plastic and sawdust: Volatile compounds and olfactometric analysis, *Waste Management*, **33**, 645-655.

González C.R.N., Björklund E., Forteza R., Cerdà V., (2013), Volatile organic compounds in landfill odorant emissions on the island of Mallorca, *International Journal of Environmental Analytical Chemistry*, **93**, 434-449.

Knudsen H.N., Clausen P.A., Wilkins C.K., Wolkoff P., (2007), Sensory and chemical evaluation of odorous emissions from building products with and without linseed oil, *Building and Environment*, **42**, 4059-4067.

IFRA, (2015), Code of Practice with Annexes (Final Version), (2015), International Fragrance association (IFRA), On line at: http://www.ifraorg.org/en-us/ingredients#.VoFaR_nNyM9.

ISO 16000-6, (2011), Indoor air - Part 6: Determination of volatile organic compounds in indoor and test chamber air by active sampling on Tenax TA sorbent, thermal desorption and gas chromatography using MS/FID, ISO 16000-6:2011, International Standard Organization, On line at: http://www.iso.org/iso/catalogue_detail.htm?csnumber=52213.

ISO 16000-9, (2006), Indoor air - Part 9: Determination of the emission of Volatile Organic Compounds from building products and furnishing-emission test chamber method, ISO 16000-9:2006, International Standard Organization, On line at: http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=38203.

Lapczynski A., Lalko J., McGinty D., Bhatia S., Letizia C.S., Api A.M., (2007), Fragrance material review on methyl-α-ionone, *Food and Chemical Toxicology*, **45**, S276-S279.

Li L., Han C., Guo B., Zhou Y, (2013), Comprehensive monitoring and assessment of odour emission sources from refinery I. monitoring of odorous sources, *Petroleum Processing and Petrochemicals*, **44**, 95-101.

Pybus D.H., Sell C.S., (2006), *Perfumery Materials of Natural Origin*, In*: The Chemistry of Fragrances: from Perfumer to Consumer*, 2nd Edition, RSC Publishing, Royal Society of Chemistry, Cambridge UK, 24-50.

Schilling B., Kaiser R., Natsch A., Gautschi M., (2010), Investigation of odors in the fragrance industry, *Chemoecology*, **20**, 135-147.

SCCS, (2011), Opinion on Fragrance allergens in cosmetic products, Scientific Committee on Consumer Safety (SCCS), Directorate-General for Health & Consumers, European Commission (SCCS/1459/11), On line at: http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm.

1970

Environmental Engineering and Management Journal

September 2016, Vol.15, No. 9, 1971-1980
http://omicron.ch.tuiasi.ro/EEMJ/

"Gheorghe Asachi" Technical University of Iasi, Romania

EXPERIMENTAL AND PREDICTED TOXICITY OF BINARY COMBINATIONS OF DICLOFENAC SODIUM, CARBAMAZEPINE AND CAFFEINE TO *Aliivibrio fischeri*

Valeria Di Nica*, Sara Villa, Antonio Finizio

Department of Earth and Environmental Sciences, University of Milano Bicocca, Piazza della Scienza 1, 20126 - Milano, Italy

Abstract

In this study, the toxic effects of binary mixtures of Pharmaceutical Active Compounds (PhACs) that are observed in the effluents from a wide range sewage treatment plants and surface water bodies were investigated using the bioluminescent bacterium *Aliivibrio fischeri* (Microtox® test). The selected chemicals were the nonsteroidal anti-inflammatory drug (NSAID) diclofenac sodium [DCF] and the anti-epileptic carbamazepine [CBZ]. In addition, caffeine [CFF], a psychoactive stimulant of the central nervous system, was also included in the study. Binary combinations were prepared at a predefined ratio that corresponded to the individual IC_{50} values of the investigated compounds (equitoxic ratio). The experimental results were compared with those obtained using the two most frequently used predictive models in aquatic toxicology: the Concentration Addition (CA) and Independent Action (IA) models. The results indicated that both models predict the observed mixture toxicity of the DCF-CBZ and DCF-CFF mixtures quite well. However, in the case of CFF-CBZ, both models slightly overestimated the experimental results, suggesting the presence of a potential antagonistic effect. The application of the Combination Index (CI) method, which allows us to identify and quantify the nature of the interactions between the chemicals present in a mixture (synergistic, additive or antagonistic effects), confirmed the additive behaviour of the DCF-CBZ and DCF-CFF combinations and the slightly antagonistic effect observed for the binary mixture of CFF-CBZ.

Key words: Aliivibrio fischeri, combination index, concentration addition, human pharmaceuticals, mixture toxicity

Received: January, 2016; Revised final: August, 2016; Accepted: August, 2016

1. Introduction

For both humans and for organisms living in the environment, chemical exposure rarely consists of single chemicals, but in many cases, it consists of mixtures of chemicals, often of fluctuating compositions and concentrations (van Gestel et al., 2011). There is accepted evidence demonstrating that mixture toxicities may be higher than the toxicity observed for their individual components (Cleuvers, 2003; Di Nica et al., 2016b; Gonzalez-Pleiter et al., 2013; Villa et al., 2012). Monitoring studies demonstrated the presence of residues of Pharmaceutical Active Compounds (PhACs) and their metabolites in water bodies in concentrations ranging from ng L^{-1} to μg L^{-1} (Daughton and Ternes, 1999; Halling-Sørensen et al., 1998; Heberer, 2002; Hilton and Thomas 2003; Kümmerer, 2001; López-Serna et al., 2010; Monteiro and Boxall, 2010). According to Voulvoulis et al. (2016), approximately one hundred pharmaceuticals from many classes of drugs and some of their metabolites were identified in treated sewage, rivers and creeks, seawater, groundwater and drinking water all around the world. Hence, in the last few years, the evaluation of the effects of mixtures of PhACs has become an emergent topic in ecotoxicological studies (Backhaus, 2014). Due to their large consumption,

---

* Author to whom all correspondence should be addressed: e-mail: v.dinica@campus.unimib.it

carbamazepine (CBZ), diclofenac sodium (DCF) and caffeine (CFF) are among the most commonly observed drug residues in aquatic systems. In fact, Zhang et al. (2008) estimated that the global consumption volumes of CBZ and DCF were 1014 tons and 940 tons per year, respectively. For CFF, Gokulakrishnan et al. (2005) estimated an average global consumption of 80-400 mg per person per day.

CBZ is typically used for the treatment of epilepsy and neuropathic pain. Together with its degradation products, 10,11-dihydrocarbamazepine and 10,11-dihydroxy-10,11-dihydrocarbamazepine (Hummel et al. 2006), this compound is regularly detected in sewage treatment plant (STP) effluents, freshwater (rivers and lakes) and even in seawater (Buser et al., 1998; Weigel et al., 2004). For this reason, CBZ has been proposed as an anthropogenic marker in water bodies (Clara et al., 2004). Thaker (2005) reported the presence of carbamazepine residues in forty-four rivers across the USA, with an average level of 60 ng L^{-1} in water and 4.2 ng mg^{-1} in sediments. In the Elbe River and its tributaries, Wiegel et al. (2004) found levels of CBZ up to 1.2 µg L^{-1}, whereas Monteiro and Boxall (2010) reviewed the presence of this compound and observed concentrations of up to 7.1 µg L^{-1} in Germany. In Italy, Zuccato et al. (2005) reported median concentrations of 291 ng L^{-1} and 175 ng L^{-1} in STPs and the Lambro River, respectively. Further evidence of the presence of CBZ in STP effluents, surface waters, drinking waters and groundwater in Europe, the United States and Canada are shown in the reports of Benotti and Brownawell (2007), Focazio et al. (2008), Hao et al. (2006), Loos et al. (2008) and Zhang et al. (2008).

DCF, a nonsteroidal anti-inflammatory drug (NSAID), is perhaps the most widely used analgesic (Cleuvers et al., 2004). In a long-term monitoring investigation of sewage and surface water samples, DCF was identified as one of the most relevant PhACs residues (Heberer, 2002). In the UK, DCF has been found in STP effluents (median concentration of 424 ng L^{-1}) (Ashton et al., 2004). Furthermore, it has also been measured in groundwater (Heberer et al., 2001). More recently, Iglesias et al. (2014) reported a mean concentration of 13.6 ng·L^{-1} in surface waters collected from rural areas in Northwestern Spain. More information about the presence of DCF residues in water bodies can be found in a recent review of Cherik et al. (2015). CFF is popularly consumed as a stimulant of the central nervous system (Ferreira, 2005). The presence of caffeine residues in water bodies is largely attributed to discharges of domestic wastewater (Martín et al., 2012; Metcalfe et al., 2003; Seiler et al., 1999; Wu et al., 2010), particularly from the disposal of unconsumed coffee, tea or soft drinks down household drains. Due to its high solubility in water, low octanol-water partition coefficient and low volatility, CFF fits the characteristics for a good chemical marker of pollution that is directly related to anthropogenic influences, with no potential biogenic sources (Siegener and Chen, 2002). The amount of CFF residues in water bodies is highly variable. For instance, in surface waters around Madrid (Spain), concentration ranges varying from 675 to 13167 ng L^{-1} were measured (Valcárcel et al., 2011). Based on this evidence, it is not surprising that residues of these compounds are present contemporaneously in surface waters. For instance, Heberer and Feldmann (2005) calculated that in total, 2.0 kg of carbamazepine per week (105 kg per year) and 4.4 kg of diclofenac sodium per week (226 per year) were discharged into Berlin's surface water. In a recent study, Al-Qaima et al. (2014) demonstrated the presence of the three PhACs, CFF, CBZ and DCF, in the Langat and Muar Rivers (Malaysia) at concentrations of 410, 15 and 39 ng L^{-1}, respectively. Consequently, they can potentially exert a joint effect on water organisms.

Assessed as individual compounds, the selected PhACs exert a moderate acute toxicity for aquatic organisms, at levels well above those measured in the aquatic compartment ($L(E)C_{50}$ ranging from tens to hundreds mg L^{-1}). For instance, the reported toxicity to aquatic organisms of DCF ranges from 22 mg L^{-1} (*D. magna*) to 167 mg L^{-1} (fish) (Cleuvers, 2003, 2004; Ferrari et al., 2003; Praskova et al., 2011). The same levels of toxicity are reported for CBZ and CFF (Cleuvers, 2003; Jos et al., 2003; Pounds et al., 2008; Selderslaghs et al., 2012; Van den Brandhof and Montforts, 2010). Anyway, the study of the joint effects and the potential interactions (e.g. synergism) of chemicals in mixture is of more concern for scientists and regulators. Currently, according to the EU legislation for the environmental risk assessment (ERA) of the human medicine products, no specific mandate for the assessment of mixtures exists. However, in 2011, the SCHER (Scientific Committee on Health and Environmental Risks; opinion on Toxicity and Assessment of Chemical Mixtures, 2011) specifically indicated, the need to take into account the existing scientific information on potential effects of combination of chemicals (including pharmaceuticals) in the environment. In this context, the aim of this study is to contribute to the improvement of the current knowledge in the field of the joint effects of human pharmaceuticals. In fact, the study of binary mixtures can be a suitable screening for the individuation of combinations of particular concern (Backhaus, 2014; Deneer, 2000).

To the best of our knowledge, there are very few data available in literature on the ecotoxicological effects of the mixtures composed of a combination of these compounds (Nieto et al., 2013; Stancova et al., 2014). In recognition of the lack of information concerning the joint effects of these compounds, in this study, we investigated the toxicity of their binary mixtures to *A. fischeri*. The binary mixtures were prepared at an equitoxic ratio corresponding to the individual IC_{50} values obtained in a previous study (Di Nica et al., 2016a). The combined experimental results were compared with those obtained by the application of two predictive models commonly used in ecotoxicology: Concentration Addition (CA) and Independent Action (IA) (Bliss, 1939; Greco et al., 1992; Loewe and Muischnek, 1926). Finally, the nature of the interactions between the PhACs was also investigated by applying the Combination Index (CI) method (Chou, 2006) to verify the existence of possible synergistic or antagonistic effects. The CI method, commonly applied in pharmacology, has been recently applied in ecotoxicological studies (Boltes et al., 2012; Di Nica et al., 2016b; Rodea-Palomares et al., 2010; Rosal et al., 2010).

2. Materials and methods

2.1. Chemicals

Diclofenac sodium [DCF] (CAS 15-307-79-6), carbamazepine [CBZ] (CAS 298-46-4), and caffeine [CFF] (CAS 58-08-2) were purchased at the highest available purity from Sigma-Aldrich (Milan, Italy). Information about their main physical-chemical properties are reported in Table 1. The stock solutions were prepared according to the method reported in Vighi et al. (2009).

2.2. Tested mixtures

Three binary mixtures (DCF-CFF, DCF-CBZ, and CFF-CBZ) were prepared by mixing the individual chemicals at equitoxic concentrations. The chemicals were mixed in a ratio corresponding to their individual IC_{50} values. The stock solutions of DCF-CFF mixture were directly prepared in the same saline solution used for the toxicity test (2% NaCl). On the contrary, mixtures stock solutions containing CBZ were prepared using the saline solution (2% NaCl) plus DMSO (2% v/v). DMSO was used to increase the solubility in water of CBZ. In the final test samples, the concentration of DMSO solvent did not produce any effect on bacteria. Tests were performed just after preparation of fresh solutions.

A fixed ratio design was used to determine the mixture toxicities (Backhaus et al., 2000). According to this approach, the mixture of interest is analyzed at a constant concentration ratio, while the total concentration of the mixture is systematically varied.

2.3. Testing procedure

Toxicity tests were performed according to the test conditions and operating protocol of the Microtox-system operating manual (Azur Environmental, 1998) using the luminescent marine bacterium *A. fischeri*. The reagents (the freeze-dried bioluminescent bacterium *A. fischeri*) and the other required test solutions were purchased from Ecotox LDS S.r.l. (Milan, Italy). The test was based on the reduction in luminescence after a short-term exposure to the pharmaceuticals (15 minutes). The reduction in luminescence reflected the acute toxic effect of the mixture (ICx) and was measured using a Microtox Model 500 analyzer. The described testing protocol was performed in duplicate using a control and nine different concentrations of the mixtures obtained by serial dilution from a stock solution (diluent = 2% NaCl solution at 20°C). The tested concentrations ranged from 0.39 mg L^{-1} to 100 mg L^{-1} for DCF-CBZ, from 1.42 mg L^{-1} to 726 mg L^{-1} for CFF-CBZ and from 0.035 mg L^{-1} to 2280 mg L^{-1} for DCF –CFF. Prior the tests no adjustment of pH was needed as the pH was in the range of 6–8 according to the EN ISO 11348–3:1999. The tests were repeated twice for DCF-CBZ and CFF-CBZ and three times for DCF-CFF.

2.4. Concentration-response curve fitting

The observed concentration-response data were fitted to a non-linear regression Weibull model (Eq. 1) to quantitatively describe the Concentration Response Curves (CRC) (R Core Team, 2015; drc package, Ritz and Streibig, 2005).

$$I = (c, \beta) = 1 - exp\{- exp\{ln(\beta_2(ln(c) - ln(\beta)))\}\} \qquad (1)$$

where: β_1 and β_2 were the parameters of the model, c was the concentration of the chemicals and I was the fractional response ($0 \leq E \leq 1$) in terms of the inhibition of the luminescence.

2.5. Statistical analysis

The analysis of the statistical parameters of the estimated regression coefficients was performed in order to check the goodness-of-fit of the selected model. The comparison of different fitting models gave negligible differences among models (application of the log likelihood functions and the Akaike Information Criterion). The high significance (*p-value*<0.001) of the statistic parameters ($\beta 1$ and $\beta 2$) clearly indicated the capability of the Weibull model to provide a very good estimation of bioluminescence inhibition (I). The null hypothesis of normality was not rejected (Kolmogorov-Smirnov test: *p-value* is >0.05). IC_x (IC_{50} and IC_{10}) values together with the corresponding confidence intervals (95%) were derived using the R software package" (R Core Team, 2015; drc package; Ritz and Streibig, 2005).

Table 1. Relevant physical chemical properties of the tested compounds

Pharmaceutical groups	***Compounds***	***CAS Nr.***	***Water Sol. (mg L^{-1})***	***pKa***
Anti-inflammatory	Diclofenac sodium [DCF]	15-307-79-6	2425 [a]	4.15 [b]
Anti-epileptic	Carbamazepine [CBZ]	298-46-4	112 [c]	13.9 [d]
SNC stimulant	Caffeine [CFF]	58-08-2	21600 [e]	14 [f]

[a] Ferrari et al., 2003; [b] Sangster, 1994; [c] Claessens et al., 2013; [d] Jones et al., 2002; [e] Yalkowsky and Dannenfelser, 1992; [f] Martin et al., 1969.

2.6. Theoretical calculation of the mixture response

The CA and IA models were applied to predict the effect of a mixture (ECx_{mix}). The first one is commonly used for chemicals with similar mode of action (Faust et al., 2003) (Eq. 2).

$$ECx_{mix} = \left(\sum_{i=1}^{n} \frac{p_i}{EC_{x_i}} \right)^{-1} \tag{2}$$

The mixture components are present in a fixed ratio; thus, it is possible to express the concentration of single chemical as a fraction of the total concentration (p_i). ECx_{mix} is the concentration of the mixture that causes *x%* of the effect, p_i is the fraction (C_i/C_{mix}; C_i = concentration of i^{th} component in the mixture and C_{mix} = total concentration of the mixture) of the i^{th} component in the mixture, ECx_i is the individual concentration of component *i* alone that provokes the same effect (*x%*) as the mixture.

The alternative IA model (Bliss, 1939; Faust et al., 2003) predicts the effect of concentration for those mixtures of chemicals showing different or dissimilar mechanism of action. According to this model, the mixture effect can be calculated using Eq. (3).

$$E(C_{mix}) = 1 - \prod_{i=1}^{n} (1 - E(C_i)) \tag{3}$$

in which $c_{mix}=\sum c_i$; $E(c_{mix})$ is the predicted joint concentration-response relationship provoked by the total concentration of the mixture, and $E(c_i)$ is the effect of the individual i^{th} component when applied individually at concentration c_i .

2.7. Application of Combination Index (CI) equations

The Combination index (CI) equations (Chou, 1976; 2006; Chou and Talalay, 1984) were used to investigate the nature of the possible interactions between chemicals that can lead to possible synergistic or antagonistic effects in *A. fischeri* in response to exposure to the binary mixtures. For *n* chemicals, these equations can be expressed as follows (Eq. 4).

$${}^{n}(CI)_x = \sum_{j=1}^{n} \frac{(D)_j}{(D_x)_j} = \sum_{j=1}^{n} \frac{(D_x)_{1-n}\left\{\dfrac{[D]_j}{\sum_{1}^{n}[D]}\right\}}{(D_m)_j\left\{\dfrac{(f_{a_x})_j}{[1-(f_{a_x})_j]}\right\}^{1/m_j}} \tag{4}$$

where: ${}^{n}(CI)_x$ is the combination index for *n* chemicals at *x%* inhibition, $(D_x)_{1-n}$ is the sum of the dose of *n* chemicals that exerts *x%* inhibition in combination, $\{([D]_j/\sum[D]\}$ is the proportionality of the dose of each of *n* drugs that exerts x% inhibition in combination, and $(D_m)_j \{(f_{ax})_j /[1 - (f_{ax})_j]\}^{1/mj}$ is the dose of each drug alone that exerts *x%* inhibition. D_m is the median-effect dose, f_{ax} is the fractional inhibition at *x%* inhibition, and *m* is the slope of the median-effect plot.

The synergistic, additive, and antagonistic effects are indicated by CI < 1, = 1, and > 1, respectively, obtained from Eq. (4). CI represents a special case of the CA model (Backhaus, 2014), where a deviation of CI values from 1 indicates a deviation from additive effects (CI is the ratio between the observed and predicted mixture effects using CA model). The computer program CompuSyn (Chou and Martin, 2005). (Compusyn Inc., USA) was used to calculate the CI values at different effect levels of the tested mixtures (from 0.05 to 0.95 of f_a). The F_a-CI plot (plot of CI values at different concentrations of mixtures versus the fraction affected, f_a) was also obtained.

3. Results and discussion

3.1. Toxicity of the tested mixture

Table 2 reports the individual effects of the concentrations (IC_{10} and IC_{50} values) of the investigated PhACs using the Microtox® test system (duration 15 min). In the study of Di Nica et al. (2016a), the authors indicated that these PhACs show a narcotic mechanism of action towards this organism.

Table 2. Toxicity data of chemicals tested with *A. fischeri* (mg L^{-1}; mean ± 95% confidence intervals in brackets) (Di Nica et al., 2016a)

Chemical	*IC_{10}*	*IC_{50}*
CBZ	4.2 (±1.3)	94.0 (±12)
CFF	24.5 (±6.6)	632.0 (±62.7)
DCF	5.9 (±0.8)	15.9 (±1.3)

The experimental bioluminescence response was inhibited by the binary mixtures of PhACs to *A. fischeri* (Weibull function) and is shown in Fig. 1, together with the curves predicted by the CA and IA models. The IC_{50} and IC_{10} (both experimental and predicted values) are reported in Table 3. All of the data were obtained by fitting the experimental results using the Weibull mathematical model, which gave us a good estimate of the effective concentrations obtained (*p-value*<0.05). For DCF-CBZ and CFF-CBZ combinations, the maximum percentage of inhibition was 65% and 57%, respectively. Nevertheless, the entire CRC was obtained with a good approximation with the regression model used here.

The obtained IC_{10} and IC_{50} values ranged between the highest and lowest toxicity of each mixture component (Tables 2 and 3). However, for the CFF-CBZ mixture, the toxicity at lower concentrations (e.g., IC_{10}) was lower than the values observed for the single components. Based on the experimental results, it seems that the tested combinations exert low toxicity towards *A. fischeri*, with DCF-CBZ > DCF-CFF > CFF-CBZ. These results are not surprising considering that both substances show a low level of toxicity to *A. fischeri* (Table 2). In addition, these substances act as narcotic-type compounds towards this organism (Di Nica et al., 2016a). It is well known that narcotics show an additive-type behaviour when are present in mixtures (Hermens, 1989).

For the DCF-CBZ and DCF-CFF combinations, the predictions of the CA and IA models are very close to the obtained experimental results. In addition, the prediction window (the ratio between IA/CA predictions) is <1, indicating that the two reference models predicted the toxicity of both mixtures equally well. In fact, the variation is very small (0.62 < IA/CA < 0.99) for all of the concentration-response relationships; this led to very small differences in the predictions of the IC_{10} and IC_{50} values between the two models (Table 3). As previously reported, Di Nica et al. (2016a) demonstrated that these compounds can be classified as exhibiting non-polar (CBZ) and polar (DCF) narcotic-type actions on *A. fischeri*. The narcotic-type behaviour of these chemicals could be equated to a similar mode of action towards *A. fischeri*. Könemann (1981) and Hermens et al. (1985) have shown that multicomponent mixtures of non-specifically acting organic substances (narcotics) induce combined effects in aquatic organisms and that the median effective concentrations of their combinations could be predicted fairly well using CA, even for multicomponent mixtures of chemicals. Consequently, this allows us to explain the ability of the CA model to predict the toxicity of binary combinations of DCF-CBZ and DCF-CFF.

On the other hand, in the literature, there are a number of studies reporting that the IA model can predict the toxicity of mixtures containing chemicals with the same or similar mechanisms of action (Backhaus et al., 2004; Syberg et al., 2008). In addition, it has been noted that the predictions of the CA and IA models will be more similar when there are fewer mixture constituents (Drescher and Boedeker, 1995; Villa et al., 2014).

Table 3. Experimental toxicity data (IC_{10} and IC_{50}; mg L^{-1}) for the binary mixtures, together with the 95% confidence intervals (in brackets) and predicted values from CA and IA models

Mixtures	*IC_{10} (Exp)*	*IC_{10} (CA)*	*IC_{10} (IA)*	*IC_{50} (Exp)*	*IC_{50} (CA)*	*IC_{50} (IA)*
DCF-CBZ	5.1 (±1.2)	4.0	4.0	59.2 (±5.4)	55.0	49.8
DCF-CFF	13.8 (±3.0)	14.3	13.3	275.5 (±20.7)	276.3	238.5
CFF-CBZ	47.0 (±10.2)	15.0	9.3	528.3 (±70.3)	362.9	224.2

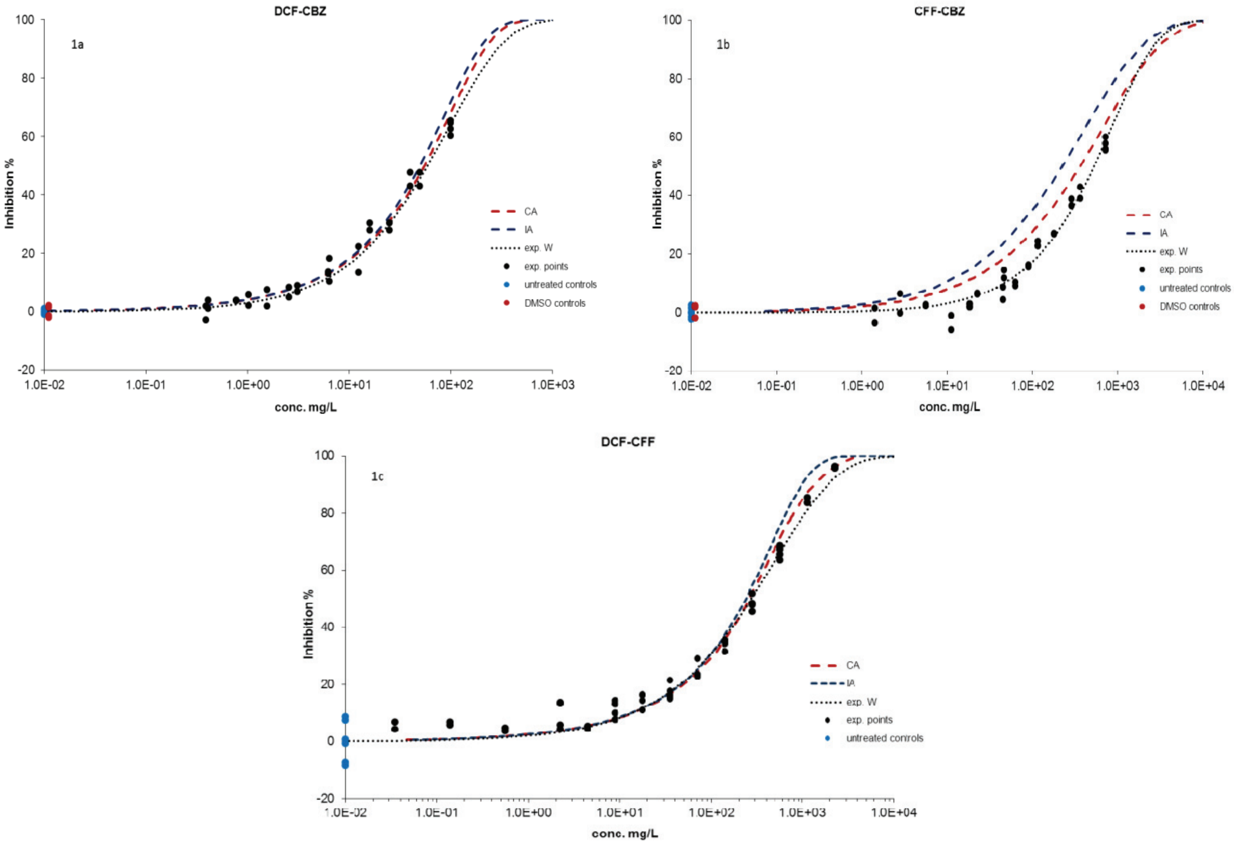


Fig. 1. Predicted values (CA in red lines and IA in blue lines) and observed toxicity values (black dashed line) of the tested binary mixtures of PhACs. (Each test was performed in duplicate and was repeated twice for DCF-CBZ (a) and CFF-CBZ (b) and three times for DCF-CFF (c))

Our results seem to be in contrast with those obtained in the study of Nieto et al. (2013). These authors tested a binary combination of CBZ and DCF on the freshwater shrimp *Atyaephyra desmarestii* and found that the experimental values did not fit with CA and IA models at lower concentrations. In particular, the measured effects were higher than the toxicity predicted by both models; on the contrary, at higher exposure concentrations, the tendency was better approximated by the IA model curve. The different results in the two studies suggest that mixtures can have different behaviours, depending on the tested organism. In some cases, they show dose-response variability: from synergy (at lower concentrations) to less than additive (at higher concentrations) in the case of *A. desmarestii*; in other cases, there is constancy in the dose-response relationship (additivity in the case of *A. fischeri*). For the CFF-CBZ combination, the IC_{10} and IC_{50} experimental values indicated a toxicity of the mixture that was lower than that predicted by both models, suggesting potential interactions between the two components that led to slight antagonistic effects. In the literature, there are a number of studies reporting observed mixture toxicities of PhCAs that significantly deviated from the conceptual expectation of the CA and/or IA models (synergism or antagonism) (Boltes et al., 2012; Brezovsěk et al., 2014; Cleuvers et al., 2003, 2004; Di Nica et al., 2016b; Gonzalez-Pleiter et al., 2013; Rodea-Palomares et al., 2010; Rosal et al., 2010; Shakya, 2011).

The effects resulting from interactions between chemicals (synergistic or antagonistic effects) seem to be independent of the mode of action of the chemicals. For instance, Cleuvers (2003) tested various PhACs that act as non-polar narcotic compounds on algae and *Daphnia*. The author found that the ibuprofen-diclofenac sodium combination produced an additive effect (well predicted by the CA model) on algae, while the effect of the same mixture on *Daphnia* was much higher than that predicted by the CA model (synergism). The same author (Cleuvers, 2004) obtained similar results with a combination of four anti-inflammatory compounds (all non-polar narcotic substances). In the study of Di Nica et al. (2016b), some synergistic and antagonistic effects on *A. fischeri* were observed using different binary combinations of polar and non-polar narcotic compounds (pharmaceuticals of veterinary use).

In an algal growth test using *Synechococcus*, Brezovsěk et al. (2014) observed antagonism with a binary combination of anti-neoplastic pharmaceuticals (5-fluoruracil and imatinib). In their study on the joint effects of fibrates towards *A. fischeri* and *Anabaena* CPB4337, Rodea-Palomares et al. (2010) highlighted a strong antagonistic effect of fenofibric acid and bezafibrate on *Anabaena*. The results obtained by Shakya (2011) indicated that the interaction between high concentrations of a binary mixture of metformin and metoprolol is antagonistic towards *Daphnia magna*. Di Nica et al. (2016b) in their studies on the bioluminescent bacteria *A. fischeri* observed a clearly antagonistic response with a binary combination of acetylsalicylic acid and chlortetracycline, and a dose-dependent antagonism behaviour in other cases, e.g. the binary combination of acetylsalicylic acid and sulfamethizole. For the last combination, the authors found an additive behaviour at lower doses, which became clearly antagonistic at the higher ones.

Based on this evidence, Backhaus (2014) concluded that the synergism and antagonism phenomena are rather specific for the tested mixture and bioassay; in addition, these phenomena are largely confined to mixtures of only a few compounds (two or three compounds). Backhaus argued that these phenomena might be explained by a presence of a sort of buffering effect in multicomponent mixtures, leading to a reduction of the impact of a few synergistic or antagonistic interactions.

The Combination Index (CI) method was applied to verify the nature of the interactions in the tested binary mixtures. Fig. 2 shows the so-called *f_a-CI plots* for the three tested mixtures, and the corresponding CI values at the main representative f_a levels (fractional inhibition with respect to the control) are reported in Table 4. The *f_a-CI plots* allow us to observe the trend of the nature of interactions present in the mixtures at any f_a levels (Chou, 2006).

The analysis of CI values and the *f_a-CI* plot allowed us to confirm the observations of the CA and IA models. In fact, the effects of the DCF-CBZ and DCF-CFF combinations were additive ($0.9<CI<1.10$: absence of interactions between the individual components). The slight synergism observed at low concentrations ($CI<1$) of DCF-CFF is likely due to the higher variability of the experimental data at lower concentrations, as fitted by the applied non-linear regression model. On the contrary, the application of the CI method to the CFF-CBZ combination confirmed the presence of interactions leading an antagonistic effect at low concentrations that becomes near additive behaviour at higher f_a levels. The CI graded symbols refer to the CI ranking that quantifies the magnitude of the synergistic or antagonistic effect, as refined by Chou (2006).

Table 4. Predicted data from the Concentration Addition (CA) and Independent Action (IA) models, as well as the Combination Index (CI) values and the corresponding graded symbols for 10% and the 50% inhibition concentration levels

Mixtures	***ICx***	***CA***	***IA***	***CI***	***CI graded symbols***
DCF-CBZ	IC10	4.03	3.98	0.97	±
	IC50	55.01	49.81	1.10	±
DCF-CFF	IC10	14.25	13.32	0.76	+ +
	IC50	276.28	238.50	1.09	±
CFF-CBZ	IC10	14.97	9.29	2.46	- - -
	IC50	362.94	224.92	1.89	- - -

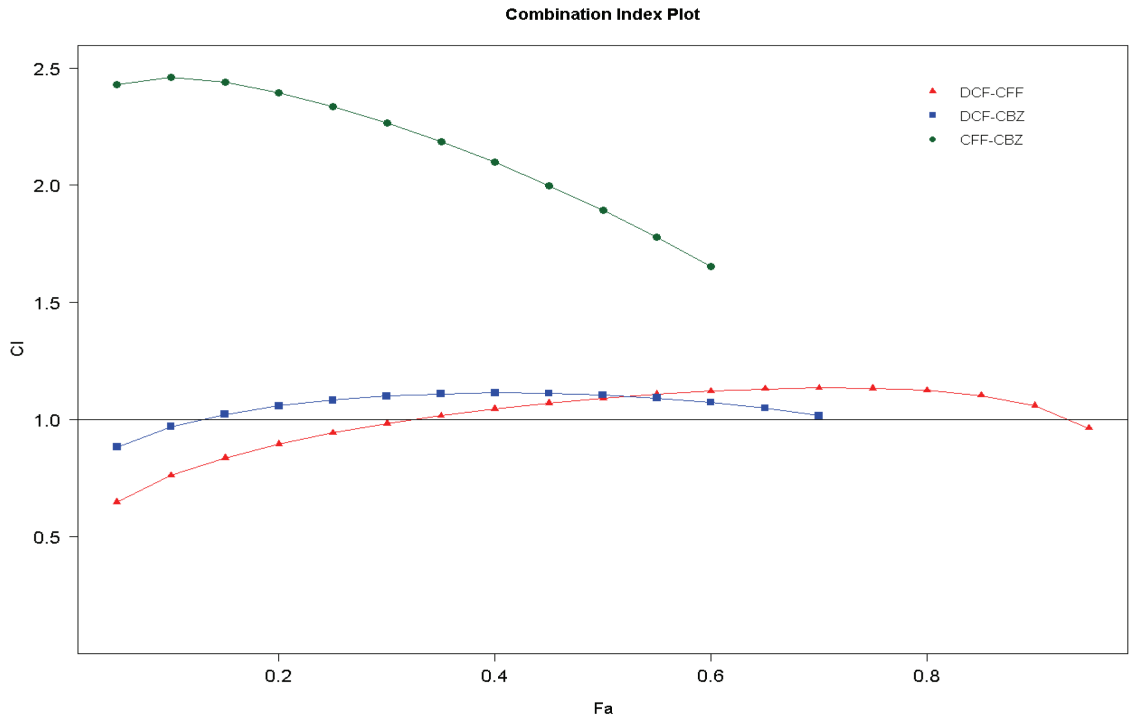


Fig. 2. CI plot (*f_a-CI plot*) for the effect of the binary mixtures of the tested PhACs on *A. fischeri*. The CI values are plotted as a function of the fractional inhibition of bioluminescence (*fa*) using a computer simulation (CompuSyn). CI < 1, = 1 and > 1 indicate the synergistic, additive and antagonistic effects, respectively (Chou and Martin, 2005)

4. Conclusions

In this study, binary combinations of DCF, CBZ and CFF (equitoxic ratio) were tested on the bioluminescent bacterium *Aliivibrio fischeri*. The resulting experimental values (IC_{50} and IC_{10}) indicated a low toxicity of the three tested mixtures (DCF-CBZ > DCF-CFF > CFF-CBZ).

Both the CA and IA models predicted the joint toxicities of DCF-CBZ and DCF-CFF equally well. These results are in accord with the narcotic behaviour of these compounds, which can be assimilated to exert a similar mechanism of action towards *A. fischeri*.

On the other hand, the CA and IA models overestimated the joint toxicity of CFF-CBZ, leading to the hypothesis of a potential interaction between the two components. The use of the Combination Index (CI) method allowed us to confirm both the additive behaviour (no interactions) for the DCF-CFF and DCF-CBZ mixtures (CI values near 1) and the antagonistic behaviour (stronger at low concentrations) for the CFF-CBZ combination.

However, from the obtained results, it can be concluded that there is no particular risk towards this organism with regard to the presence of these substances, at least in the form of their binary mixtures and when present at equitoxic concentrations. In fact, the concentrations of the individual compounds that produce the EC_{50} of the mixtures are at least 4 orders of magnitude higher than the median measured environmental concentrations in European surface waters.

References

Al-Qaima F.F., Abdullaha M.P., Othmana M.R., Latipa J., Zakariad Z., (2014), Multi-residue analytical methodology-based liquid chromatography-time-of-flight-mass spectrometry for the analysis of pharmaceutical residues in surface water and effluents from sewage treatment plants and hospitals, *Journal of Chromatography A*, **1345**, 139-153.

Ashton D., Hilton M., Thomas K.V., (2004), Investigating the environmental transport of human pharmaceuticals to streams in the United Kingdom, *Science of the Total Environment*, **333**, 167-184.

Azur Environmental, (1998), Microtox® acute toxicity basic test procedures, Carlsbad, CA, USA.

Backhaus T., Altenburger R., Boedeker W., Faust M., Scholze M., Grimme L.H., (2000), Predictability of the toxicity of a multiple mixture of dissimilarly acting chemicals to *Vibrio fischeri*. *Environmental Toxicology and Chemistry*, **19**, 2348-2356.

Backhaus T., Faust M., Scholze M., Gramatica P., Vighi M., Grimme L.H., (2004), Joint algal toxicity of phenylurea herbicides is equally predictable by concentration addition and independent action, *Environmental Toxicology and Chemistry*, **23**, 258-264.

Backhaus T., (2014), Medicines, shaken and stirred: a critical review on the ecotoxicology of pharmaceutical mixtures, *Philosophical Transactions of the Royal Society B*, **369**, 1-11.

Benotti M., Brownawell B., (2007), Distributions of pharmaceuticals in an urban estuary during both dry- and wet-weather conditions, *Environmental Science & Technology*, **41**, 5795-5802.

Bliss C.I., (1939), The toxicity of poisons applied jointly, *Annals of Applied Biology*, **26**, 585-615.

Boltes K., Rosal R., García-Calvo E., (2012), Toxicity of mixtures of perfluorooctane sulphonic acid with chlorinated chemicals and lipid regulators, *Chemosphere*, **86**, 24-29.

Brezovsěk P., Eleršek T., Filipič M., (2014), Toxicities of four anti-neoplastic drugs and their binary mixtures tested on the green alga *Pseudokirchneriella subcapitata* and the cyanobacterium *Synechococcus leopoliensis*, *Water Research*, **52**, 168-177.

Buser H.R., Poiger T., Müller M.D., (1998), Occurrence and fate of the pharmaceutical drug diclofenac in surface waters: Rapid photodegradation in a lake, *Environmental Science and Technology*, **32**, 3449-3456.

Cherik D., Benali M., Louhab K., (2015), Occurrence, ecotoxicology, removal of diclofenac by adsorption on activated carbon and biodegradation and its effect on bacterial community: A review, *World Scientific News*, **10**, 116-144.

Chou T.C., (1976), Derivation and properties of Michaelis-Menten type and Hill type equations for reference ligands, *Journal of Theoretical Biology*, **59**, 253-254.

Chou T.C., Talalay P., (1984), Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors, *Advances in Enzyme Regulation*, **22**, 27-55.

Chou T.C., Martin N., (2005), CompuSyn for drug combinations: PC software and user's guide: A computer program for quantification of synergism and antagonism in drug combinations and the determination of IC50 and ED50 and LD50 values, ComboSyn Inc., Paramus, NJ.

Chou T.C., (2006), Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies, *Pharmacological Review*, **58**, 621-681.

Clara M., Strenn B., Kreuzinger N., (2004), Carbamazepine as a possible anthropogenic marker in the aquatic environment: investigations on the behaviour of carbamazepine in wastewater treatment and during groundwater infiltration, *Water Research*, **38**, 947-954.

Claessens M., Vanhaecke L., Wille K., Janssen C.R., (2013), Emerging contaminants in Belgian marine waters: Single toxicant and mixture risks of pharmaceuticals, *Marine Pollution Bulletin*, **71**, 41-50.

Cleuvers M., (2003), Aquatic ecotoxicity of pharmaceuticals including the assessment of combination effects, *Toxicology Letters*, **142**, 185-194.

Cleuvers M., (2004), Mixture toxicity of the anti-inflammatory drugs diclofenac, ibuprofen, naproxen, and acetylsalicylic acid, *Ecotoxicology and Environmental Safety*, **59**, 309-315.

Daughton C., Ternes T., (1999), Pharmaceuticals and personal care products in the environment: agents of subtle change?, *Environmental Health Perspectives*, **107**, 907-938.

Deneer J.W., (2000), Toxicity of mixtures of pesticides in aquatic systems, *Pest Management Science*, **56**, 516-520.

Di Nica V., Villa S., Finizio A., (2016a), Toxicity of individual pharmaceuticals and their mixtures to *Aliivibrio fischeri*: Experimental results for single compounds, considerations of their mechanisms of action and potential harmful effects on aquatic organisms, *Environmental Toxicology and Chemistry*, Doi: 10.1002/etc.3568.

Di Nica V., Villa S., Finizio A., (2016b), Toxicity of individual pharmaceuticals and their mixtures to *Aliivibrio fischeri*. Part II: Evidences of toxicological interactions in binary combinations of veterinary pharmaceutical mixtures, *Environmental Toxicology and Chemistry*, Doi: 10.1002/etc.3568.

Drescher K., Boedeker W., (1995), Concepts for the assessment of combined effects of substances: the relationship between concentration addition and independent action, *Biometrics*, **51**, 716-730.

Jos A., Repetto G., Rios J.C., Hazen M.J., Molero M.L., Del Peso A., Salguero M., Fernández-Freire P., Pérez-Martín J.M., Cameán A., (2003), Ecotoxicological evaluation of carbamazepine using six different model systems with eighteen endpoints, *Toxicology in Vitro*, **17**, 525-532.

Faust M., Altenburger R., Backhaus T., Blanck H., Boedeker W., Gramatica P., (2003), Joint algal toxicity of 16 dissimilarly acting chemicals is predictable by the concept of independent action, *Aquatic Toxicology*, **63**, 43-63.

Ferrari B., Paxeus N., Lo Giudice R., Pollio A., Garric J., (2003), Ecotoxicological impact of pharmaceuticals found in treated wastewaters: study of carbamazepine, clofibric acid, and diclofenac, *Ecotoxicology and Environmental Safety*, **55**, 359-370.

Ferreira A.P., (2005), Caffeine as an environmental indicator for assessing urban aquatic ecosystem, *Cad Saude Publica*, **21**, 1884-1892.

Focazio M.J., Kolpin D.W., Barnes K.K., Furlong E.T., Meyer M.T., Zaugg S.D., Barbere L.B., Thurmand M.E., (2008), A national reconnaissance for pharmaceuticals and other organic wastewater contaminants in the United States – II. Untreated drinking water sources, S*cience of the Total Environment*, **402**, 201-216.

Gonzalez-Pleiter M., Gonzalo S., Rodea-Palomares I., Leganes F., Roberto Rosal R., Karina Boltes K., Marco E., Fernandez-Pinas F., (2013), Toxicity of five antibiotics and their mixtures towards photosynthetic aquatic organisms: Implications for environmental risk assessment, *Water Research*, **47**, 2050-2064.

Gokulakrishnan S., Chandraraj K., Gummadi S.N., (2005), Microbial and enzymatic methods for the removal of caffeine, *Enzyme and Microbial Technology*, **37**, 225-232.

Greco W., Unkelbach H.D., Pöch G., Suhnel J., Kundi M., Bödeker W., (1992), Consensus on concepts and terminology for combined-action assessment: the Saariselkä agreement, *Archives of Complex Environmental Studies*, **4**, 65-72.

Halling-Sørensen B., Nors Nielsen S., Lanzky P.F., Ingerslev F., Holten Lützhøft H.C., Jørgensen S.E., (1998), Occurrence, fate and effects of pharmaceutical substances in the environment-A review, *Chemosphere*, **36**, 357-393.

Hao C., Lissemore L., Nguyen B., Kleywegt S., Yang P., Solomon K.R., (2006), Determination of pharmaceuticals in environmental waters by liquid chromatography/electrospray ionization/tandem mass spectrometry, *Analytical and Bioanalytical Chemistry*, **384**, 505-513.

Heberer T., Fuhrmann B., Schmidt-Baumler K., Tsipi D., Koutsouba V., Hiskia A., (2001), *Occurrence of Pharmaceutical Residues in Sewage, River, Ground, and Drinking Water in Greece and Berlin (Germany)*, In: *Pharmaceuticals and Personal Care Products in the Environment*, Daughton C.G., Jones-Lepp T.L. (Eds.), ACS Symposium Series 791, American Chemical Society, Washington D.C., 70-83.

Heberer T., (2002), Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: A review of recent research data, *Toxicology Letters*, **131**, 5-17.

Heberer T., Feldmann D., (2005), Contribution of effluents from hospitals and private households to the total loads of diclofenac and carbamazepine in municipal sewage effluents-modeling versus measurements, *Journal of Hazardous Materials*, **122**, 211-218.

Hermens J., Busser F., Leeuwangh P., Musch A., (1985), Quantitative structure-activity relationships and mixture toxicity of organic chemicals in *Photobacterium phosphoreum*: the Microtox test, *Ecotoxicology and Environmental Safety*, **9**, 17-25.

Hermens J., (1989), *Quantitative Structure–Activity Relationships of Environmental Pollutants*, In: *Handbook of Environmental Chemistry*, Hutzinger O., (Ed.), Chemistry, Vol. 2E, Reactions and Processes, Springer-Verlag, Berlin, Germany, 111-162.

Hilton M.J., Thomas K.V., (2003), Determination of selected human pharmaceutical compounds in effluent and surface water samples by high-performance liquid chromatography-electrospray tandem mass spectrometry, *Journal of Chromatography A*, **1015**, 129-141.

Hummel D., Löffler D., Fink G., Ternes T.A., (2006), Simultaneous determination of psychoactive drugs and their metabolites in aqueous matrices by liquid chromatography mass spectrometry, *Environmental Science and Technology*, **40**, 7321-7328.

Iglesias A., Nebot C., Vázquez B.I., Coronel-Olivares C., Franco Abuín C.M., Cepeda A., (2014), Monitoring the presence of 13 active compounds in surface water collected from rural areas in Northwestern Spain, *International Journal of Environmental Research and Public Health*, **11**, 5251-5272.

Jones O.A.H., Voulvoulis N., Lester J.N., (2002), Aquatic environmental assessment of the top 25 English prescription pharmaceuticals, *Water Research*, **36**, 5013-5022.

Loewe S., Muischnek H., (1926) Effect of combinations: mathematical basis of problem (in German), *Naunyn-Schmiedebergs Archiv fur Experimentelle Pathologie und Pharmakologi*, **114**, 313-326.

Loos R., Locoro G., Huber T., Wollgast J., Christoph E.H., de Jager A., Gawlik B.M., Hanke G., Umlauf G., Zaldívar J.M., (2008), Analysis of perfluorooctanoate (PFOA) and other perfluorinated compounds (PFCs) in the River Po watershed in N Italy, *Chemosphere*, **71**, 306-313.

López-Serna R., Péreza S., Ginebreda A., Mira Petrovic M., Barceló D., (2010), Fully automated determination of 74 pharmaceuticals in environmental and waste waters by online solid phase extraction–liquid chromatography-electrospray–tandem mass spectrometry, *Talanta*, **83**, 410-424.

Könemann H., (1981), Fish toxicity tests with mixtures of more than two chemicals: a proposal for a quantitative approach and experimental results, *Toxicology*, **19**, 229-238.

Kümmerer K., (2001), Drugs in the environment: emission of drugs, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources - A review, *Chemosphere*, **45**, 957-969.

Martin A.N., Swarbrick J., Cammarata A., (1969), *Physical Pharmacy*, 2nd Edition, Lea & Febiger, Philadelphia, 194.

Martín J., Camacho-Muñoz D., Santos J.L., Aparicio I., Alonso E., (2012), Occurrence of pharmaceutical compounds in wastewater and sludge from wastewater treatment plants: Removal and ecotoxicological impact of wastewater discharges and sludge disposal, *Journal of Hazardous Materials*, **239-240**, 40-47.

Metcalfe C.D., Miao X.S., Koenig B.G., Struger J., (2003), Distribution of acidic and neutral drugs in surface waters near sewage treatment plants in the lower Great Lake, Canada, *Environmental Toxicology and Chemistry*, **22**, 2881-2889.

Monteiro S.C., Boxall A.B.A., (2010), Occurrence and fate of human pharmaceuticals in the environment, *Reviews of Environmental Contamination and Toxicology*, **202**, 53-154.

Nieto E., Blasco J., González-Ortegón E., Drake P., Hampel M., (2013), Is *Atyaephyra desmarestii* a useful candidate for lethal and sub-lethal toxicity tests on pharmaceutical compounds?, *Journal of Hazardous Materials*, **263**, 256-265.

Pounds N., Maclean S., Webley M., Pacoe D., Hutchinson T., (2008), Acute and chronic effects of ibuprofen in the mollusc *Planorbis carinatus* (Gastropoda: *Planorbidae*), *Ecotoxicology and Environmental Safety*, **70**, 47-52.

Praskova E., Voslarova E., Siroka Z., Plhalova L., Macova S., Marsalek P., Pistekova V., Svobodova Z., (2011), Assessment of diclofenac LC50 reference values in juvenile and embryonic stages of the zebrafish (*Danio rerio*), *Polish Journal of Veterinary Sciences*, **14**, 545-549.

R Core Team, (2015), R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, On line at: http://www.R-project.org/.

Ritz C., Streibig J.C., (2005), Bioassay analysis using R, *Journal of Statistical Software*, **12**, 1-22.

Rodea-Palomares I., Petre A.L., Boltes K., Leganés F., Perdigón-Melón J.A., Rosal R., Fernández-Piñas F., (2010), Application of the combination index (CI)-isobologram equation to study the toxicological interactions of lipid regulators in two aquatic bioluminescent organisms, *Water Research*, **44**, 427-438.

Rosal R., Rodea-Palomares I., Boltes K., Fernández-Piñas F., Leganés F., Petre A., (2010), Ecotoxicological assessment of surfactants in the aquatic environment: combined toxicity of docusate sodium with chlorinated pollutants, *Chemosphere*, **81**, 288-293.

Sangster J., (1994), Log KOW Databank, Montreal, Quebec, Canada, Sangster Research Laboratories.

SCHER, (2011), Opinion on toxicity and assessment of chemical mixtures, On line at: http://ec.europa.eu/health/scientific_committees/environmental_risks/docs/scher_o_155.pdf.

Seiler R.L., Steven Z.D., Thomas J.M., Howcroft D.L. (1999), Caffeine and pharmaceuticals as indicators of waste water contamination in wells, *Ground Water*, **37**, 405-410.

Selderslaghs I.W.T., Blust R., Witters H.E., (2012), Feasibility study of the zebrafish assay as an alternative method to screen for developmental toxicity and embryotoxicity using a training set of 27 compounds, *Reproductive Toxicology*, **33**, 142-154.

Shakya P., (2011), *Synergism and antagonism in toxicity of mixtures of pharmaceuticals to Daphnia magna*, Senior Theses, Trinity College, Hartford.

Siegener R., Chen R.F., (2002), Caffeine in Boston Harbor seawater, *Marine Pollution Bulletin*, **44**, 383-387.

Syberg K., Elleby A., Pedersen H., Cedergreen N., Forbes V.E., (2008), Mixture toxicity of three toxicants with similar and dissimilar modes of action to *Daphnia magna*, *Ecotoxicology and Environmental Safety*, **69**, 428-436.

Stancova V., Plhalova L., Bartoskova M., Zivna D., Prokes M., Marsalek P., Blahova J., Skoric M., Svobodova Z., (2014), Effects of mixture of pharmaceuticals on early life stages of tench (*Tinca tinca*), *BioMed Research International*, **14**, 1-10.

Thaker P.D., (2005), Pharmaceutical data elude researchers, *Environmental Science & Technology*, **139**, 193A-194A.

Valcárcel Y., González Alonso S., Rodrìguez-Gil J.L., Gil A., Catalá M., (2011), Detection of pharmaceutically active compounds in the rivers and tap water of the Madrid Region (Spain) and potential ecotoxicological risk, *Chemosphere*, **84**, 1336-1348.

Van den Brandhof E-J., Montforts M., (2010), Fish embryo toxicity of carbamazepine, diclofenac and metoprolol, *Ecotoxicology and Environmental Safety*, **73**, 1862-1866.

Vighi M., Migliorati S., Monti G.S., (2009), Toxicity on the luminescent bacterium *Vibrio fischeri* (Beijerinck). I: QSAR equation for narcotics and polar narcotics, *Ecotoxicology and Environmental Safety*, **72**, 154-161.

Wu J., Yue J., Hu R., Yang Z., Zhang L., (2010), Use of caffeine and human pharmaceutical compounds to identify sewage contamination, *International Journal of Civil Engineering and Environmental*, **2**, 98-102.

Van Gestel C.A.M., Jonker M.J., Kammenga J.E., Laskowski R., Svendsen C., (2011), *Mixture Toxicity Linking Approaches from Ecological and Human Toxicology*, CRC, Boca Raton.

Villa S., Migliorati S., Monti G.S., Vighi M., (2012), Toxicity on the luminescent bacterium *Vibrio fischeri* (Beijerinck). II: Response to complex mixtures of heterogeneous chemicals at low levels of individual components, *Ecotoxicology and Environmental Safety*, **86**, 93-100.

Villa S., Vighi M., Finizio A., (2014), Experimental and predicted acute toxicity of antibacterial compounds and their mixtures using the luminescent bacterium *Vibrio fischeri*, *Chemosphere*, **108**, 239-244.

Voulvoulis N., Barceló D., Verlicchi P., (2016), Pharmaceutical residues in sewage treatment works and their fate in the receiving environment (review), *Environmental Science and Technology*, **41**, 120-179.

Weigel S., Berger U., Jensen E., Kallenborn R., Thoresen H., Hühnerfuss H., (2004), Determination of selected pharmaceuticals and caffeine in sewage and seawater from Tromsø/Norway with emphasis on ibuprofen and its metabolites, *Chemosphere*, **56**, 583-592.

Wiegel S., Aulinger A., Brockmeyer R., Harms H., Loffler J., Reincke H., Schmidt R., Stachel B., Von Tumpling W., Wanke A., (2004), Pharmaceuticals in the river Elbe and its tributaries, *Chemosphere*, **57**, 107-126.

Yalkowsky S., Dannenfelser R., (1992), *Aquasol Database of Aqueous Solubility*, Version 5.

Zhang Y., Geißen S.U., Gal C., (2008), Carbamazepine and diclofenac: Removal in wastewater treatment plants and occurrence in water bodies, *Chemosphere*, **73**, 1151-1161.

Zhang Z., Hibberd A., Zhou J.L., (2008), Analysis of emerging contaminants in sewage effluent and river water: comparison between spot and passive sampling, *Analytica Chimica Acta*, **608**, 37-44.

Zuccato E., Castiglioni S., Fanelli R., (2005), Identification of the pharmaceuticals for human use contaminating the Italian aquatic environment, *Journal of Hazardous Materials*, **122**, 205-209.

Environmental Engineering and Management Journal September 2016, Vol.15, No. 9, 1981-1988
http://omicron.ch.tuiasi.ro/EEMJ/

"Gheorghe Asachi" Technical University of Iasi, Romania

OPTIMIZATION OF WASTEWATER TREATMENT PLANTS MONITORING IN FLOW VARIATION CONDITIONS DUE TO RAIN EVENTS

Carmine Fiorentino[1*], Maurizio Luca Mancini[1], Luca Luccarini[2]

[1]Department of Civil, Chemical, Environmental and Materials Engineering – DICAM, University of Bologna,
Viale Risorgimento, 2 - 40136, Bologna Italy
[2]ENEA - DTE-SEN-CROSS via Martiri di Monte Sole, 4 – 40129, Bologna, Italy

Abstract

The direct measurement of the nutrients largely contributes to understand how the quality of the information, coming from the instruments, may improve the performances of the plants. The advanced knowledge acquired on the meaning of the signals gives new possibilities to control the biological processes and estimate the operational state of the plant, improving the quality of the effluent and keeping the operational costs as low as possible. Such information, furthermore, can be used to understand what sensors to install and in what position of the plant.

Starting from an accurate literature update, this paper aims to propose some parameters, control sections and analytical methods indispensable to monitoring and automatically control full-scale plants, with urban sewage coming from combined sewer systems.

In particular, a measurement campaign has been carried out in the WWTP of Bologna (Italy), a traditional continuous flow Activated Sludge treatment without denitrification, during one year. Considering the influent in dry and rain conditions, the behaviour of the plant in different sections has been studied and characterized. Comparing the acquired data appears important to monitor the BOD, COD and TSS concentrations at the primary sedimentation outlet, the input and the output sections of the plant, as well as the TSS concentration in the aeration tank, measured using continuous optical methods. Finally, the study shows the need for a monitoring system related with the sewage system dimensions and how Hydraulic Retention Time plays a key role to identify and manage the inlet variations.

Key words: dilution, mixed drainage system, monitoring, nitrification, weak sewage

Received: January, 2016; Revised final: September, 2016; Accepted: September, 2016

1. Introduction

Nowadays, an efficient management of Wastewater Treatment Plants (WWTPs) of medium to large dimensions, is even more based on the use of innovative instrumentation, such as on-line in-situ sensors, and the adoption of control logics and policies by means of automatic control systems. Wastewater Treatment Plants (WWTPs) are complex systems and their management requests a multi-disciplinary approach, involving a wide variety of pollutants, biological process, management policies and control logics. In order to implement an automatic control system of a WWTP, the quantitative and qualitative knowledge of the most important variables and parameters is necessary, included the input and output of the plant. A new approach to this matter started in 1973 in London through the first ICA (Instrumentation Control and Automation) conference, under the sponsorship of IAWPR (International Association on Water Pollution Research). Later, new national and international regulatory, technological innovation and the need to save energy in the plants encouraged the

---

[*] Author to whom all correspondence should be addressed: e-mail: carmine.fiorentino2@unibo.it; Phone: +39 051 2093751

adoption of ICA tools. Today, a number of sensors are released by specialized producer making possible to deal with thousand of signals for a single WWTP (Olsson et al., 2014). Furthermore, the availability of this large amount pushed the scientific research to deeply study signal analysis to model, among the other things, fault detection techniques (Olsson et al., 2005), software sensors (Luccarini et al., 2002) and various control algorithms. An important emerging problem is the lack of connection between plant designing and plant management. Indeed, most often WWTPs are designed to work in static and fixed conditions, while is well known that the working conditions of the plants are variable. Based on those requirements, this paper focuses the use of the parameters for the control and the management of WWTPs, considering their relationship with the biological processes. The aim is to support the choice of the appropriate measurement instruments, identifying a correct position on the plant where install them. The case study concerns the WWTP of Bologna in Italy, with biological secondary treatment composed by an oxidation tank (Bragadin and Mancini, 2008), fed on sewage from urban combined sewer systems and whose general treatment scheme (Fig. 1) implies the usual subdivision in primary, secondary and tertiary treatments. In particular, the signals acquired by several sensors installed on WWTP, have been examined. The final destination of the treated effluent of the plant is a river, so the legal thresholds in the Italian code for the Environment (Legislative Decree No 152/2006) must be reached.

1.1. Parameters monitoring in WWTPs

Chemical analysis instruments, commonly used to better manage WWTPs are separated in five areas: off-line, at-line, on-line, in-line and non-invasive. In particular, the evolution of in-line analysis, involving four major types of in-line sensors, such as biosensors, optical sensors, sensors arrays and virtual sensors, is increasing steadily (Bonastre et al., 2005). Nowadays, large plants are regularly equipped with various sophisticated instruments and automatic control systems, whereas in the smaller ones the lack of technology is evident and generalized. This is due to a high cost/benefit ratio, being all the sensors expensive and need of much maintenance.

An economical alternative avoiding such constraints has arisen recently with the use of software sensors. Soft sensors are a valuable tool in many different industrial fields of application, including urban pollution and wastewater treatment plants monitoring. They are used to solve a number of different problems, such as real-time prediction for plant control, sensor validation and fault diagnosis strategies (Fortuna et al., 2007). A soft-sensor is conventionally described as an inpute-output process model. The model inputs consist of easy-to-measure secondary variables in the form of plant’s signals and measurements and, sometimes, numerically encoded expert knowledge. The model outputs consist of information associated with the hard-to-measure primary variables. In the soft-sensor, the input and output process information is modelled empirically and the internal model is used to return the outputs when only the inputs are available. The range of tasks that can be fulfilled by soft sensors is broad and mainly dictated by the nature of the available input information, by the information that we are interested to output and the typology of the input-output model. (Haimi et al., 2013). The soft sensors may supply information about the process otherwise directly measurable only with expansive hardware sensors. For this reason, they are assuming even more importance in WWTPs management, substituting in some cases the traditional hardware sensors. In the last years, dedicated sub-systems for the performances evaluation of soft sensors, to overcome their measurement accuracy weakness, have been proposed (Luccarini et al., 2010, 2012).

The search for information about the wastewater characteristics has to start from the sewer system. In particular, is important to know the typology of sewer system (mixed or separate), what measurements are executed along the system and their availability. Commonly, the mixed sewer system is used, since it is difficult to have an actual separate flow, even in separate systems. As regards the data availability, it is generally very poor or non-existent, and restricted to flow rate data. The desirable development of real-time management systems could increase the quality and the quantity of data, creating a greater inter-connection between the sewage system management, the WWTP and the receiving water body, leading to a reduction in management costs (Rathnayake, 2014; Schütze et al., 2004).

Flow rate variations in a WWTP influence the wastewater treatment processes, then its real-time monitoring is important to take decisions about the process management. These flow rate variations, that means also pollutants variations, depend on the type of the city and obviously on the habits of its citizen. It is therefore possible to have daily, weekly and seasonally variations (Bragadin and Mancini, 2007). The flowmeter instrument is commonly positioned at the beginning of the plant, where there is usually the inlet basin from the sewer system. In order to choose the appropriate flowmeter instrument it is important to know the arrival level of the sewer system and, in particular, if pumping the sewage is necessary (Bragadin and Mancini, 2007). The liquid flowmeters are commonly based on the change in water level due to an obstacle in the water flow path (Venturi Principle) (Vanrolleghem and Lee, 2003). Different ways can be chosen to measure it. For example some instruments are based on the Faraday induction law, other on the Von Karman theory, and Ultrasound instruments are based on the time delay of the ultrasound through the flow.

The pH measurement influences chemical and biological reactions. Its measurement and control in different sections of a WWTP could be very useful for its simplicity and cheapness of the measurement. As the pH measurement is normally carried on installing immersion probes with electrodes in sewage, the cleaning strategy is very important to get a measurement as real as possible. Nowadays, self-diagnosis systems are integrated in pH measurement systems. Sometimes the pH measurement fails or cannot give the right information as a consequence of a high buffering capacity of the sewage.

Urban sewage is made of a mixture of organic and mineral pollutants with a great size distribution, thus the measurement of the Total Suspended Solids (TSS) concentration is very useful in WWTPs management. Three types of measurement techniques are usually implemented: optical measurements, ultrasound measurements and dielectric spectrometry (Vanrolleghem and Lee, 2003). The first one is the most common method, based on the measure of the optic effects (absorption, transmission, scattering) in an illuminated sample. The size and quantity of solids are correlated to the absorption measurement and the scattering and scattering angle of the incident light on the sewage. Different sources of light emission are known: in lower visible, infrared range or laser (Azema et al., 2002). Interferences connected with air bubbles and fouling of probe tips are typical problems related to this method (Vanrolleghem and Lee, 2003). The simplicity and rapidity of the three common methods enable to measure the TSS parameter in different parts of the WWTP such as the input section, after the primary sedimentation, and after the secondary sedimentation.

In Activated Sludge processes the energetic cost of aeration is up to 40% the total costs of the plant (Marco et al., 2010; Olsson, 2015). Thus, the measurement of the dissolved oxygen (DO) and the control of the air compressor, play a key role (Luccarini et al., 2015; McCarty et al., 2011; Vanrolleghem and Lee, 2003). In particular, a PI (proportional – integral) controller to maintain DO concentration to a fixed set-point and an inverter to regulate the air flow insufflated by the compressor in the oxidation tank, may be used. Only for this simple control technique, between 30 and 50 per cent of the energy consumed could be saved (Luccarini et al., 2015). Since 1970's a huge amount of efforts has been directed towards improving DO concentration, driven by the desire to reduce the costs induced by this "energivorous process" (Olsson et al., 2014). In particular, worldwide use of DO control systems by the end of 1970's represents the beginning of process control in WWTPs. DO measurement is based on the electrochemical reaction of oxygen diffusing from the liquid through a permeable gas membrane in an amperometric or polarographic measurement cell (Vanrolleghem and Lee, 2003). Cleaning of the probes, consumption of the electrodes, long time of polarization (in case of polarized systems), as well as calibration, are common problems emerging from the measurement of this parameter. The proper location of the dissolved oxygen probes has to prevent fouling problems.

Despite the Biochemical Oxygen Demand (BOD) measurement is the most adopted parameter for water quality assessment (Bourgeois et al., 2001), the laboratory standard method has two main drawbacks: time-consuming and an uncertainty of 15-20% on the results. The time-consuming, in particular, prevents the use of this method for real-time control of WWTPs. Since 1977 a wide interest has grown in other innovative methods to assess the BOD and scientific articles have been published in this issue (Jouanneau et al., 2014). On the basis of those publications, Jouanneau et al. (2014) classify the assessment methods into six technological categories: 1) Modified standard methods, 2) Biosensors with redox-mediator, 3) Biosensors based on bioluminescent bacteria, 4) Biosensors with immobilized bacteria, 5) Microbial fuel cells, and 6) Bioreactors. Other methods for BOD determination developed in the last years are based on optical fiber biosensors (Bonastre et al., 2005). The parameter used for on-line estimation of BOD is "the short term BOD" (BODst), different from the BOD for the fewer time required for its analysis response. Finally, BOD measurement methods are even more technologically reliable with response time below the five days characteristic of standard method (only 70 s for the fastest system) but they are not adequate for automated monitoring yet.

The main problem to automate the Chemical Oxygen Demand (COD) analysis, for on-line and, as more as possible, real-time use, is to shorten the time of digestion in dichromate solution that is about two hours in laboratory methods. Different approaches have been proposed to oxidize the organic compound quickly using chemical compounds different from dichromate. Recently, the use of Ozone, characterized by a high oxidizing power, instead of dichromate or permanganate has also been investigated (Pisutpaisal and Sirisukpoca, 2014). Furthermore, methods based on the measurement of UV – VIS absorption are also developed and applied (Bourgeois et al., 2001). Further studies are based on thermal Biosensors (Yao et al., 2014). Nowadays, there are still a lot of drawbacks connected with an on-line use of COD measurement methods: time-consuming, use of toxic chemicals with production of hazardous liquid waste (e.g. Chromium(Cr) and Mercury(Hg)) or expensive chemical (e.g. Silver Sulfate(Ag_2SO_4)), clogging problems, or incomplete oxidation of the pollutants.

Activated sludge process is designed to achieve the biological nutrient removal, so nitrogen forms analysis and control is very important. For example the ammonium ($N\text{-}NH_4^+$) measurements are becoming important to calculate the variable DO set-point in the cascade Proportional – Integral controller (Olsson et al., 2014). There are three major types of on-line ammonium analyzer: colorimetric, ion-selective electrodes and spectrophotometers. The main problems of these analysis stay in the need to use chemical reagents, their calibration time and cleaning.

2. Materials and methods

The present study is based on data collected during the last annual measurement campaign in the WWTP of Bologna in Italy, started in January until December 2005. The plant is located in the outskirt of the city. The influent is raw sewage from a combined sewer system serving about 500,000 PE (population equivalent) from the city of Bologna and hinterland. The process is the traditional Activated Sludge treatment without denitrification (Fig. 1).

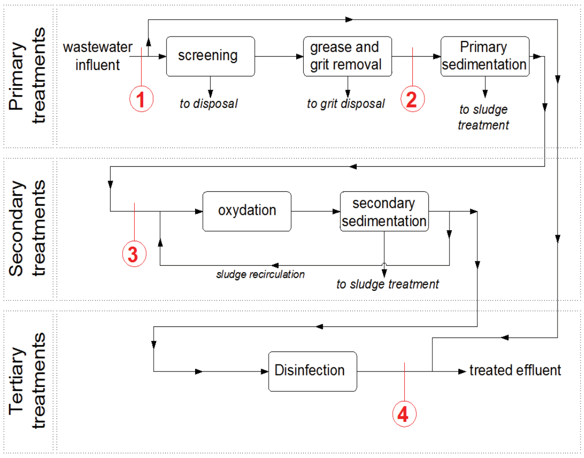


Fig. 1. Bologna WWTP scheme with the numbers related to the measurement sections listed in Table 1

The measurement campaign had the specific aim of understanding the plant behaviour under different input conditions along the plant sections due to differently diluted inlet. Further data analysis permits to verify the feasibility of continuous measurement in the plant, focusing on what are the necessary parameter measurements, the instruments availability and the feasibility of given measurements according to the wastewater characteristics in the plant sections, i.e. high solids concentration that could give measurement errors. The data come from four sections of the plant (see Fig. 1), identified with numbers, in which the control parameters (Table 1) were measured in accordance with APAT-IRSA methods (APAT IRSA CNR, 2003).

The COD analysis were carried out every day while the BOD only two or three times a week, as usual. In addition to those parameters, the solids concentration (Volatile Suspended Solids, VSS) has been measured every day in the sludge recirculation and in the biological tank.

The weather conditions have been noted every day during the campaign, in particular, temperature and rainfall measurement has been carried out through a monitoring central control unit.

Based on the data collected, four different cases have been analyzed and discussed in the following section 3. In particular, one month flow rate data have been discussed in connection with the most important parameters for the WWTPs management in case 1. In order to understand the behaviour of the plant in rain conditions, six days data have been studied in case 2. Finally the relation of the ammonium nitrogen in different sections and a comparison between the flow rate and the solids concentration in the biological tank and in the sludge recirculation have been studied in the cases three and four respectively.

Table 1. List of the control parameters measured in each section of the Bologna WWTP

Plant section	*Section number*	*Control parameters measured*
Input	1	BOD, COD, pH, Settleable Solids (SS), TSS, N-NH_4^+, Total Kjeldahl Nitrogen (TKN), Nitrate (N-NO_3^-), Nitrite (N-NO_2^-), Total Phosphorus (TP), Surfactants
Output grid removal	2	BOD, COD, pH, SS, TSS, N-NH_4^+, TKN, TP, Surfactants
Input biological treatment	3	BOD, COD, pH, SS, TSS, N-NH_4^+, TKN, N-NO_3^-, N-NO_2^-, TP, Surfactants
Output	4	BOD, COD, pH, SS, TSS, N-NH_4^+, TKN, N-NO_3^-, N-NO_2^-, TP, Surfactants

3. Results and discussion

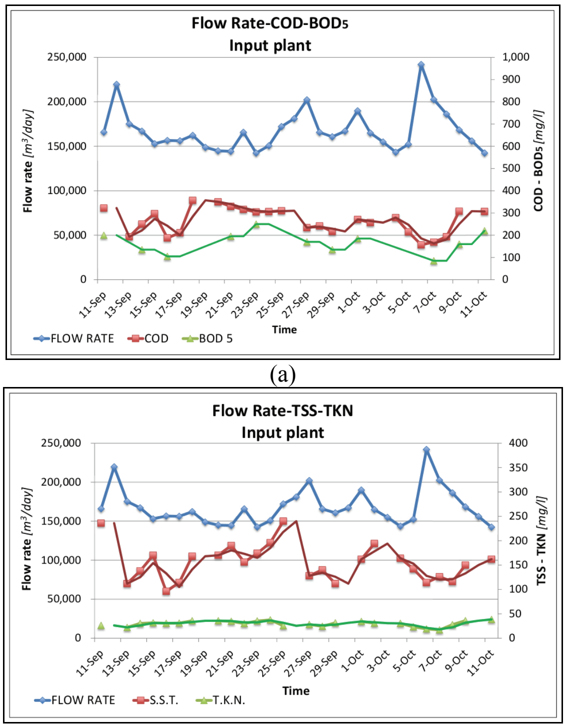
3.1. Case 1: flow rate variations in input

In this case 1, the relationship between the flow rate variations due to rain influence and the most relevant parameters in the input section of the plant has been studied. The considered parameters, measured in 31 consecutive days are: BOD, COD, TKN, TSS. This time has been chosen considering the rain events, in order to have as more variation of flow rate as possible. Fig. 2a and Fig 2b show the daily flow rate influent in the plant (blue line) related with BOD, COD, TSS and TKN measured in the same section 1. It has been observed that the flow rate variation due to rain events influences the concentration of all the studied parameters.

An increase of the flow rate corresponds to a decrease of the pollutants concentration, diluting the wastewater that enters in the plant. It means that a real time measurement of the influent sewer flow rate is essential for the WWTP management as it influences the pollutants concentrations and consequently the following processes.

3.2. Case 2: Behaviour during a rain event

In order to observe the behaviour of the plant during a single rain event, a comparison between flow rate in input and pollutants concentration in the sections 1, 3 and 4 has been studied. For this purpose, a representative time interval of six days without any rainfalls except on the third day with a single rain event that influences significantly the input flow rate to the plant has been chosen.



(a)

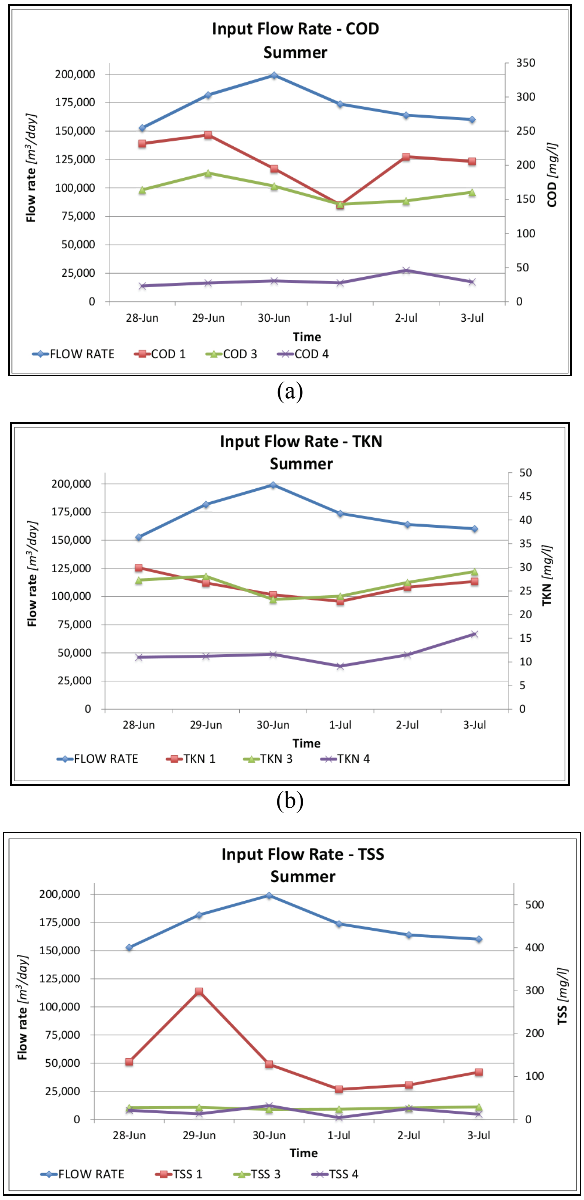
(b)

Fig. 2. Comparison between flow rate variations with BOD and COD (2a) and with TSS and TKN (2b)

In particular, two representative periods have been observed, in order to show scenarios both for summer (Fig. 3) and winter (Fig. 4) season, with the rain event on the third day. In the first case the plant's input is a typical "weak wastewater" exacerbated by the dilution effect of the rain events while in winter the influent wastewater could be considered "medium wastewater" (see blue and red lines in Fig. 3 and Fig. 4). The difference is due to the characteristics of the Bologna sewage system (mixed) that flow to the WWTP and the population behaviour.

Indeed, during summer the water use of the population is higher than in winter while the industrial discharge in winter is higher than in summer. Comparing the COD and TKN data, in summer and winter, the input flow variations influence the COD concentration and the TKN both in input (section 1) and output (section 4) still remaining under the legal thresholds.

Besides, the effect of a more dilute influent is observable from the day of the rainfall event until the further three days. In dilution conditions is also very important the monitoring of pH because the increase of pH could mean a decrease of the nitrification capacity. In effect, the process of oxidation of ammonia to nitrites, in a first step, and nitrates, subsequently, produces H^+ ions, causing a decrease of the pH value. In this case study, the increasing pH in the outlet section, corresponded to a reduction of the nitrification capacity due to a dilution in input, due to a reduction of the substrate.



(a)

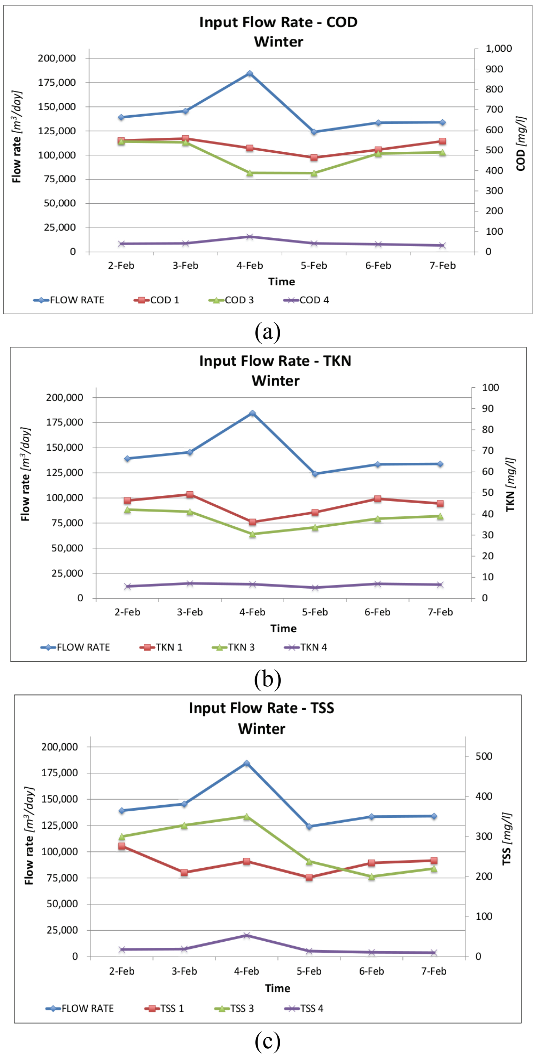
(b)

(c)

Fig. 3. Comparison between Input flow rate and COD (3a), TKN (3b), TSS (3c) in the sections 1,3,4 – Summer

3.3. Case 3: Ammonium in sections 1 and 3

As third case study, the relation between the ammonium nitrogen (NH_4^+) in sections 1 and 3, analyzing daily data during different seasons and input flow rate conditions, has been studied. As shown in Fig. 5, there is a good data correlation between ammonium concentration in the influent and ammonium concentration inlet to the biological sector. For this reason, the ammonium measurement in the section 3 will give the same results as in section 1. Nevertheless, ammonium measurement is recommended in section 3 rather than in section 1 as the wastewater solids content in this section is lower, allowing for a more accurate measurement.



(a)

(b)

(c)

Fig. 4. Comparison between Input flow rate and COD (4a), TKN (4b), TSS (4c) in the sections 1,3,4 - Winter

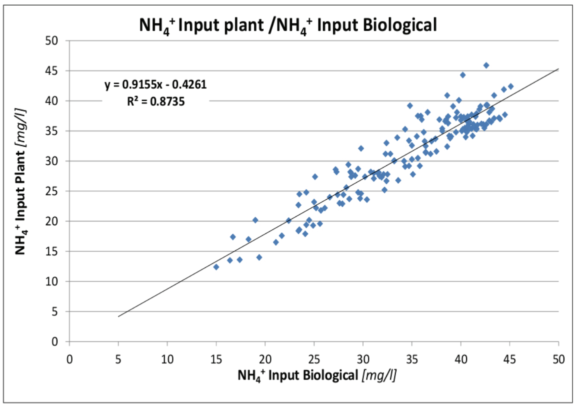
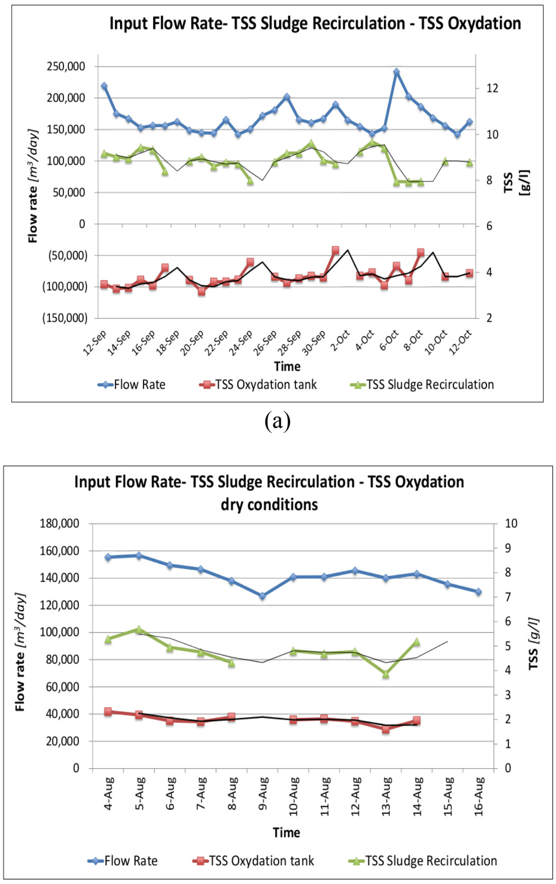


Fig. 5. Ammonium in Input plant (section 1) and Input Biological (section 3)

3.4. Case 4: Solids concentrations in biological tank and sludge recirculation

In the last case, the trends of three different signals have been compared: 1) the flow rate variations at the input section (blue line), 2) the solids concentration of the sludge recirculation (green line), and 3) the solids concentration in the biological tank (red line). The comparison has been investigated in two different situations: 31 days with some different rain events (Fig. 6a) and 13 days without any rain events (Fig. 6b). As in the previous cases, the flow rate increase in the input section leads to a decrease of the pollutants concentrations. The sludge recirculation concentration variations (green line) indicate the working state of the Secondary Sedimentation. Thus, the wastewater dilution in input influences Sedimentation, inducing a non-optimal sludge thickening and finally leading to a sludge recirculation concentration reduction. The variation of biomass concentration (red line) is due to two factors: the decrease of the Hydraulic Retention Time (HRT) and the variation of sludge recirculation concentration.

Furthermore, in dry conditions (Fig. 6b) the TSS concentration trend, both in oxydation tank and in sludge recirculation, is more stable than in the first situation (rainy conditions) with a clear effect of the more dilute inflow.



(a)

(b)

Fig. 6. Comparison between flow rate variations with TSS for rainy conditions (6a) and dry conditions (6b)

3.5. Discussion

Comparing the results of the monitoring campaign carried out on Bologna WWTP, it is possible to point out a way to change the biodegradation efficiency in dilution conditions. In similar WWTPs, in dry conditions the most important management policies are related to the insufflation of the oxygen in the aerobic tank and the sludge recirculation flow rate. This is always true as long as the input flow rate is three time the medium flow rate in dry conditions. When happen rain events, instead, and the input flow rate is four or five times the medium flow rate in dry conditions, due to, instead, as showed with the presented data, these policies are not sufficient to optimize the efficiency of the plant. In such conditions the most feasible interventions are dependent by the duration of the rain event. A part of the load in excess could be stored in an appropriate buffer, for example, waiting for to supply the plant in dry conditions.

Such management policies could be more effective if supported by an efficient monitoring system able to observe as soon as possible the input flow variations and then to estimate the operational state of the process. In particular, the monitoring campaign showed the importance of an accurate monitoring of the oxidation tank with reliable methods. As minimum requirement, pH, REDOX and DO real time probes are necessary in order to estimate the nitrification efficiency (Luccarini et al., 2015).

4. Conclusions

This study, starting from the state of the art of the existing instruments, suggests which parameters are important to adequately monitor a full scale WWTP, receiving combined sewage-rain urban drainage system.

By studying the data collected during a monitoring campaign in the Bologna real-scale plant and comparing them in dry and rain weather conditions, arise the need to monitor the BOD, COD and TSS parameters at the primary sedimentation outlet, as well as in the input and in the output section of the plant. In particular, the low values of TSS in the primary sedimentation outlet sections, allow for a reliable monitoring of nitrogen in the input sewage using Ion Selective Electrode (ISE) analysis. This continuous signal represents a crucial information to define and quantify the management operations.

Furthermore, analyzing the pollutant concentration variation during a single rain event (case 2), a variation of the measured parameters in all sections is noted and the effects of the dilution are visible for two or three days after the flow variation. Consequently, for an accurate evaluation of these variations a faster response of the instruments would be required.

It has been verified that an increase of the flow rate in input influences both TSS concentration in aeration tank and sludge thickening at secondary sedimentation. Thus, monitoring TSS concentration in aeration tank using continuous optical methods, in addition to the classic pH/ORP/OD probes, may result important.

Finally, the sewage dilution is a sudden and fast event, comparing with the usual monitoring response of a WWTP monitoring system, dependent on the sewage system dimension. In the case study the sewage system is large and its Hydraulic Retention Time (approximately two days) could permit to identify and manage the inlet dilution during rain events with an appropriate monitoring system.

References

APAT IRSA CNR, (2003), Analytical methods of water, APAT 29/2003, Manuals and guidelines (in Italian), Rome, Italy.

Azema N., Pouet M.F., Berho C., Thomas O., (2002), Wastewater suspended solids study by optical methods, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **204**, 131-140.

Bonastre A., Ors R., Capella J.V., Fabra M.J., Peris M., (2005), In-line chemical analysis of wastewater: present and future trends, *TrAC Trends in Analytical Chemistry*, **24**, 128-137.

Bourgeois W., Burgess J.E., Stuetz R.M., (2001), On-line monitoring of wastewater quality: A review, *Journal of Chemical Technology and Biotechnology*, **76**, 337-348.

Bragadin G.L., Mancini M.L., (2007), *Treatment and Disposal of Urban Waste* (in Italian), Pitagora Editrice Bologna, Italy.

Bragadin G.L., Mancini M.L., (2008), *Combined Sewage-Rain Urban Drainage System and Active Sludge WWTP Design*, Int. Symp. on Sanitary and Environmental Engineering-SIDISA 08-Proc. ANDIS-DICEA-IWA, Florence, Italy, vol. 1, 94/1-94/8.

Fortuna L., Graziani S., Rizzo A., Xibilia M.G., (2007*)*, *Soft Sensors for Monitoring and Control of Industrial Processes*, Springer Science & Business Media.

Haimi H., Mulas M., Corona F., Vahala R., (2013), Data-derived soft-sensors for biological wastewater treatment plants: An overview, *Environmental Modelling & Software*, **47**, 88-107.

Jouanneau S., Recoules L., Durand M.J., Boukabache A., Picot V., Primault Y., Lakel A., Sengelin M., Barillon B., Thouand G., (2014), Methods for assessing biochemical oxygen demand (BOD): A review, *Water Research*, **49**, 62-82.

Luccarini L., Porra` E., Spagni A., Ratini P., Grilli S., Longhi S., Bortone G., (2002), Soft sensors for control of nitrogen and phosphorus removal from wastewaters by neural networks, *Water Science and Technology*, **45**, 101-107.

Luccarini L., Bragadin G.L., Colombini G., Mancini M.L., Mello P., Montali M., Sottara D., (2010), Formal verification of wastewater treatment processes using events detected from continuous signals by means of artificial neural networks. Case study: SBR plant, *Environmental Modelling & Software*, **25**, 648-660.

Luccarini L., Pulcini D., Sottara D., Bragaglia S., Mello P., (2012), *Monitoring the performance of Soft Sensors Used in WWTPs by Means of Formal Verification*, In: *International Environmental Modelling and Software Society (iEMSs) 2012 International Congress on Environmental Modelling and Software Managing Resources of a Limited Planet: Pathways and Visions under Uncertainty*, Seppelt R., Voinov A.A., Lange S., Bankamp D. (Eds.), Sixth Biennial Meeting, Leipzig, Germany.

Luccarini L., Pulcini D., Sottara D., Spagni A., (2015), *Investigation of Energy Consumption and Costs Savings for A Pilot Scale SBR*, In: *Sewage Treatment Plants: Economic Evaluation of Innovative Technologies for Energy Efficiency*, Tsagarakis K.P., Stamatelatou K. (Eds), IWA Publishing, London, 311-326.

McCarty P.L., Bae J., Kim J., (2011), Domestic wastewater treatment as a net energy producer: Can this be achieved?, *Environmental Science and Technology*, **45**, 7100-7106.

Marco R., Menendez P.E., Black, Veatch, (2010), *How we Use Energy at Wastewater Plants and How we Can Use Less*, In: *Annual Conference Technical Paper*, NC AWWA-WEA (North Carolina American Water Works Association and the North Carolina Water Environment Association).

Olsson G., Nielsen M.K., Yuan Z., Lynggaard-Jensen A., Steyer J.P., (2005), *Instrumentation, Control and Automation in Wastewater Treatment Systems*, Vol. 4, Scientific and Technical Report Series No. 15, IWA Publishing, London, UK.

Olsson G., Carlsson B., Comas J., Copp J., Gernaey K.V., Ingildsen P., Jeppsson U., Kim C., Rieger L., Rodríguez-Roda I., Steyer J.-P., Takács I., Vanrolleghem P.A., Vargas A., Yuan Z., Åmand L., (2014), Instrumentation, control and automation in wastewater-from London 1973 to Narbonne 2013, *Water Science & Technology*, **69**, 1373-1385.

Olsson G., (2015), *Water and Energy. Threats and Opportunities-Second Edition*, IWA Publishing, London.

Pisutpaisal N., Sirisukpoca U., (2014), Development of rapid chemical oxygen demand analysis using ozone as oxidizing agent, *Energy Procedia*, **50**, 711-718.

Rathnayake U.S., (2014), *Optimal Control of Urban Sewer Systems-Where Do we Stand Today?*, Proc. SAITM Research Symp. on Engineering Advancements SAITM-RSEA 2014, 26th April, 2014, Malabe 10115, Sri Lanka.

Schütze M., Campisano A., Colas H., Schilling W., Vanrolleghem P., (2004), Real time control of urban wastewater systems-where do we stand today?, *Journal of Hydrology*, **299**, 335-348.

Vanrolleghem P.A., Lee D.S., (2003), On-line monitoring equipment for wastewater treatment processes: state of the art, *Water Science & Technology*, **47**, 1-34.

Yao N., Wang J., Zhou Y., (2014), Rapid determination of the chemical oxygen demand of water using a thermal biosensor, *Sensors*, **14**, 9949-9960.

Environmental Engineering and Management Journal *September 2016, Vol.15, No. 9, 1989-1995*
http://omicron.ch.tuiasi.ro/EEMJ/

"Gheorghe Asachi" Technical University of Iasi, Romania

SOLUBILITY AND DURABILITY OF CARDANOL DERIVED PLASTICIZERS FOR SOFT PVC

Antonio Greco[1*], Francesca Ferrari[1], Alfonso Maffezzoli[1], Pietro Delogu[2], Rosario Velardi[2], Alessia Timo[3], Antonella Tarzia[3], Alessandro Marseglia[3], Massimiliano Calò[4]

[1]Department of Engineering for Innovation - University of Salento, Via per Monteroni, Lecce, Italy
[2]Serichim, Udine, Italy
[3]Centro di Progettazione, Design & Tecnologie dei Materiali, Brindisi, Italy
[4]Kòmmi s.a.s., Lequile, Lecce, Italy

Abstract

This work was aimed to test the suitability of an epoxidized cardanol derived plasticizer for the production of soft PVC characterized by low environmental and toxicological impact soft PVC. Nowadays, the use of natural derived plasticizer in soft PVC industry is emerging as valid alternative towards conventional phthalate plasticizers, in order to reduce the environmental and toxicological impact of soft PVC. In facts, cardanol is a natural and renewable resource, characterized by a wide worldwide availability. In addition, being derived from cashew nut shell liquid, which is a by-product of cashew nut shell industry, it does not contribute to the subtraction of resources from the food chain, in contrast, for example, to epoxidized soybean oil.

To this purpose, soft PVC samples were produced in an industrial plant, using both cardanol derived and phthalate plasticizer. Thermal and mechanical characterization showed that the properties of PVC plasticized by cardanol derivative are comparable to those of soft PVC obtained by phthalate, which is a clear indication of the good plasticizing effectiveness of cardanol derivative, and highlights its potential for the production of soft PVC characterized by reduced environmental and toxicological impact.

Key words: cardanol, plasticizer, PVC

Received: January, 2016; Revised final: August, 2016; Accepted: August, 2016

1. Introduction

In recent years, due to their toxicity (Hileman, 2005; Wilkinson and Lamb, 1999), phthalate plasticizers, used in soft PVC industry, have been banned in some applications, as evidenced by the EC Directive (1999), and by other authors (Kampouris, 1975; Nass and Heiberger, 1986) of potential danger to human health. On the other hand, growing environmental awareness has motivated researchers from industry and academia to substitute oil-derived polymers and additives with products from bio-based materials (Bitinis et al., 2011; Cruz-Estrada et al., 2010). In a report prepared by the Institute of Environmental Sciences of the Leiden University in the framework of the "RiskCycle" project, founded by EU in the 7th Framework Program, different indicators (eco toxicity, global warming) show that phthalate plasticizers production accounts for about 25-30% of the total life cycle impact of soft PVC (Patel and Xanthos, 1995, 2001; Van Oers and Van der Voet, 2012).

The use of natural derived plasticizer in soft PVC industry represents a valid alternative towards the use of phthalate plasticizers, aimed to reduce both the environmental and toxicological impact of soft PVC. In facts, natural derived plasticizers, also referred to as bio-plasticizers, are nowadays commonly used in soft PVC industry. Most of these products are natural oil derived, properly modified to improve the compatibility with soft PVC (Bocque et al., 2016). The main drawback associated to the use

---

[*] Author to whom all correspondence should be addressed: e-mail: antonio.greco@unisalento.it; Phone: +39832297233; Fax: +39832297240

of bio-plasticizers is their high cost, which often is not compatible with the market price of soft PVC and its phthalate plasticizers (Bocque et al., 2016). Further, bio-plasticizes, making use of natural oil, contribute to the subtraction of resources to food chain. This is the case, for example, of epoxidated soybean oil, which is a widely used plasticizer and/or stabilizer for PVC in food contact applications (Bueno-Ferrer et al., 2010).

On the other hand, cardanol is an industrial grade oil obtained by vacuum distillation of "cashew nut shell liquid" (CNSL), which represents nearly 25% of the total nut weight, and its worldwide availability is estimated to be about 300,000 tons per year. Therefore, cardanol is a natural resource characterized by low cost and low toxicological impact.

Cardanol finds use in resins, coating and polymer industries (Voirin et al., 2014). It is also used in the chemical industry in oil and alcohol soluble resins, laminating resins, rubber compounding and coatings, serving as an excellent raw material for the preparation of high grade insulating varnishes, paints, enamels (Sathiyalekshmi and Gopalakrishnan, 2004). Cardanol can be employed in friction materials, and surfactants, as pigment dispersants for water-based inks and to make phenalkamines, which are used as curing agents for the durable epoxy coatings used on concrete floors. Cardanol can substitute phenol in up to 30% in phenolic resins used as plywood bonding resin and for the lamination industries (Yuliana et al., 2014).

The potential of cardanol derivatives as PVC plasticizers was demonstrated since the '70 (Neuse and Van Schalkwyk, 1977). In particular, it was shown that acetylation of the cardanol hydroxyl group improves the compatibility with PVC (Greco et al., 2010). Therefore, cardanol acetate (CA) can be used as secondary plasticizer, in addition to conventional plasticizers, for the production of soft PVC. Recently, it has been shown that also epoxidated cardanol glycidyl ether can be used as a secondary plasticizer, or at least as a primary plasticizer at very low contents (below 25 phr), for the production of soft PVC (Chen et al., 2015a; 2015b). Nevertheless, the migration of CA is very high, compared to the migration of phthalate plasticizers (Calò et al., 2011). Other approaches used to reduce the migration of plasticizer from soft PVC include the use of cardanol derivatives covalently bonded to PVC; nevertheless, in this case, the plasticizing effectiveness of the plasticizer is significantly reduced, and addition of 160 0 phr of plasticizer lead to a reduction of the glass transition comparable to that obtained by addition of 10 phr of DEHP (Yang at al., 2015).

Further epoxidation of cardanol acetate leads to the production of epoxidated cardanol acetate (ECA), a plasticizer characterized by a very good compatibility with PVC (Greco et al., 2010). The main limitation in the production of ECA is the chemical modification of cardanol. In the most conventional reaction routes, esterification requires the use of acetic anhydride as reagent and pyridine as solvent, whereas epoxidation requires m-chloroperbenzoic acid as reagent and di-chloromethane as solvent (Greco et al., 2010). Such conditions are not suitable for the production of a green product. On the other hand, in a recent work (Calò et al., 2011) it has been shown that esterification can be performed by the use of acetic anhydride, in the presence of hexahydrate zinc perchlorate, without the use of solvents. In contrast, complete epoxidation of cardanol side chain double bonds is very difficult to achieve in quantitative yields, avoiding the use of toxic solvents. Only recently, partial epoxidation of cardanol acetate to epoxidated cardanol acetate has been obtained by the use of enzymatic catalysts and hydrogen peroxide (Kim et al., 2007). However, the compatibility of the developed plasticizer with PVC is still quite low, due to the low yield of epoxidation attained by enzymatic catalyst. Other epoxidated cardanol esters have been shown their potential as plasticizers for PVC. It was shown that a high yield of epoxidation promotes the formation of a soft PVC characterized by thermal properties comparable to those of soft PVC plasticized by DEHP, and e better plasticizer retention (Yang et al., 2016).

Therefore, this work is aimed to study the suitability of epoxidated cardanol derived plasticizer as plasticizers for the production of soft PVC. The plasticizer was obtained by the use of peroxy-acetic acid in a solvent free reaction performed in a medium scale reactor, which allowed the production of batches about 20 kg. The plasticizer was tested in an industrial dry mixing/extrusion equipment. The thermal and mechanical properties of the soft PVC obtained by the use of epoxidated cardanol derived plasticizer were measured and compared with those of the soft PVC obtained with conventional phthalate plasticizer under the same processing conditions.

2. Materials and methods

PVC used is a commercial SOLVIN 271 (Solvay) in powder form, with a K-value 71. Cardanol derivative plasticizer (ECA) was provided by Serichim (Torviscosa, Udine, Italy). The ECA was obtained by a double reaction: acetylation was performed by acetic anhydride (molar ratio 1.2:1), at 110°C for 10 h, using Amberlyst 15, 3% weight as catalyst. Epoxidation was performed with the aim of obtaining epoxidated cardanol acetate (ECA). Epoxidation is actually composed of two different steps: in the first one, hydrogen peroxide is used in order to obtain a peroxyacetic acid by a proper catalyst system. Such reaction is an equilibrium reaction. The peroxyacetic acid is the oxidizing agent, which is used in an uncatalysed reaction to obtain conversion of double bonds to epoxies. The second reaction is a quantitative reaction. The peroxy-acetic acid is obtained by mixing acetic acid and hydrogen peroxide (molar ratio 1:2) with

Amberlist 15 resin catalyst (2.5 % weight of the total batch weight); after 1 h at 30°C the mixture is typically composed of 19% peroxy-acetic acid, 25% hydrogen peroxide, 15% acetic acid, 41% water. In the second step of reaction, cardanol acetate, peroxy-acetic acid (molar ratio to double bonds 1.4:1) are charged in the reactor (mechanical stirring at 480 rpm); reaction is run at 25°C for 7 h.

A scheme for the functionalization of cardanol is reported in Fig. 1.

The NMR spectrum of the developed ECA plasticizer is reported in Fig. 2. As a consequence of the reaction with the peroxy-acetic acid, the peaks for the CH=CH protons, at δ of 5.8, 5.4 and 5.0, decrease in intensity, whereas new peaks at δ of 3.1, 2.9 and 2.8, ascribed to the methine protons of the oxirane ring, are observed (Kim et al., 2007). NMR spectra were used to estimate a yield of epoxidation (ratio between the oxirane rings and the initial double bonds), equal to 81%, which is a values quite close to that found by other authors (Yang et al., 2016).

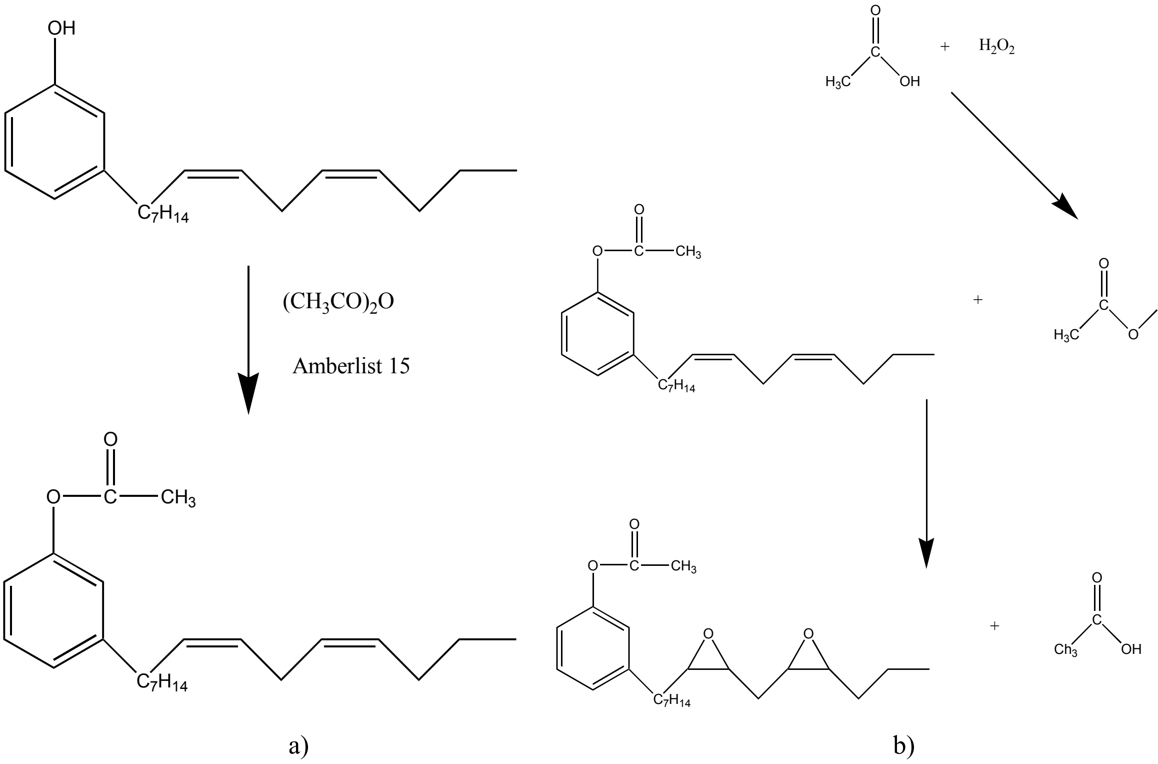


Fig. 1. Schemes for the reactions of a) acetylation, and b) epoxidation

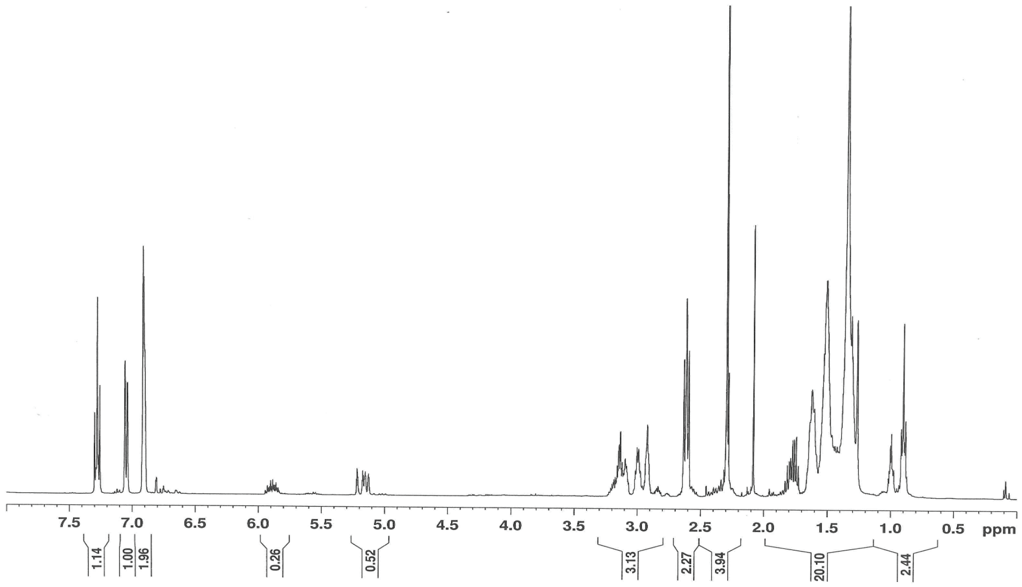


Fig. 2. NMR spectrum of ECA

55 phr PVC dry blends were obtained by mixing PVC and plasticizers according to the following procedure:

- the PVC was added inside the mixer CACCIA CP/300 (capacity of 300 L) combined with a cooler with a capacity of 1000 L at ambient temperature and mixed for 25 seconds at 1480 rpm;
- the speed of rotations was increased up to 2900 rpm;
- when the mixture inside the mixer reached 52°C, the plasticizer was added;
- when the blend reached the temperature of 95°C, the speed of rotation was decreased (1480 rpm);
- the mixer is turned off for 20 or 60 seconds, and wait for the cooling of the dry blend;
- at the end the material was discharged.

Therefore, two different dry blends were obtained, characterized by different times of mixing (20 and 60 second) at about 95°C. For comparison purposes, a PVC dry blend with di-(2-ethylhexyl)phthalate (DEHP) was also produced with the same composition and in the same processing conditions (20 seconds dry blending).

Afterwards, soft PVC was obtained by extrusion in a twin screw extruder Bausano MD72-24 (diameter: 24 mm; l/d: 24) Bausano extruder, using the following temperature profile from the feeding to the die: 135-140-145-150-110-110-100-100-100°C and a speed of rotation of 60 rpm. The processing conditions are the same used in the conventional processing of DEHP plasticized PVC (Kim et al., 2003).

Soft PVC plates about 8 mm thick were obtained by pre-heating the material in a forced convection oven up to 180°C, and then compression molding in a Campana hot press with a pressure of 20 tons, using cold plates.

Differential scanning calorimetry (DSC) was performed on PVC, ECA plasticizer, dry blends and soft PVC using a DSC Mettler, heating the material from -40 to 200°C at a heating rate of 10°C/min under an air flux. The existence of a single glass transition was considered indicative of the formation of a single phase between PVC and plasticizer, and therefore of an adequate compatibility between the materials. Thermogravimetric analysis (TGA) was performed on 20 mg samples (corresponding to about 1mm thickness) by a Mettler Toledo - TGA/SDTA 851 heating the materials from 50°C to 450°C at a heating rate of 10°C/min in air atmosphere.

The hardness of soft PVC was measured according to Shore A procedure on a Gibitre Instruments Digital Manual Hardness Check.

3. Results and discussion

DSC analysis on neat PVC, reported in Fig. 3, shows a typical glass transition signal (T_g) at about 80°C (T_g, midpoint = 87°C). At higher temperatures, about 250°C (T_{onset} = 260°C), thermal degradation of PVC starts. DSC analysis of PVC plasticized by ECA, also reported in Fig. 3, shows the presence of broad glass transition signal, ranging between -45°C and 50°C, with an inflection point at -15°C. DSC analysis on ECA plasticizer, reported in Fig. 4, shows a broad exothermic peak between 110 and 220°C, which disappears on a second heating scan: this indicates that the peak is due to some chemical reaction (irreversible process), which can occur in ECA. Rheological analysis was therefore performed on ECA before and after heating at 220°C (where the peak of Fig. 4 closes), which evidenced irrelevant changes of the viscosity of ECA, indicating that the peak cannot be attributed to a significant increase of the molecular weight of ECA. Also, FTIR analysis revealed that none of the characteristic peaks of ECA was modified after heating at 220°C. The irrelevant changes of the chemical and physical structure of ECA are confirmed by the very low values of the enthalpy associated to the peak, of about 22 J/g, correpsponding, for a molecular weight of 360 g/mole, to 7 KJ/mole, which is a negligible value compared to the theoretical enthalpy of reaction of epoxies, about 100 KJ/mole (Swier and Van Mele, 2003).

The glass transition temperature of the ECA plasticizer is expected to be about -75°C, as reported in a previous work (Greco et al., 2010).

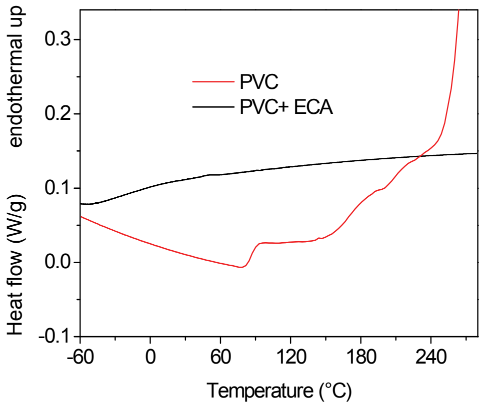


Fig. 3. DSC analysis on PVC

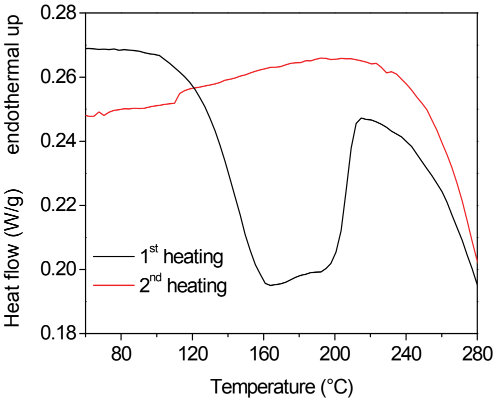


Fig. 4. DSC analysis on ECA plasticizer

The results from DSC characterization on PVC dry blends and extruded PVC are reported in Table 1. The value of the glass transition T_g after dry blending measured for PVC with ECA is much lower compared to that of PVC with DEHP This is to be attributed to the much faster absorption of ECA plasticizer compared to DEHP. The faster adsorption causes a reduction of the glass transition of PVC even after mixing at relatively low temperatures (95°C). In contrast, DEHP is not fully absorbed at such low temperatures, which causes a relatively high glass transition. On the other hand, there is no appreciable difference between the glass transition measured for the two samples blended at different times (20 or 60 s). As it can be observed in Table 1, after extrusion, the glass transition of PVC plasticized by ECA decreases to -32°C. This temperature is the same of that measured for PVC plasticized by DEHP. In facts, in the case of DEHP, extrusion at high temperatures causes a significant decrease of the glass transition measured for the dry blend.

TGA analysis results, reported in Fig. 5 for extruded materials, clearly show the thermo-oxidative degradation of PVC and plasticized PVC. Compared to neat PVC, addition of DEHP involves a significant decrease of the thermal stability, as evidenced in Table 2 by the decrease of the temperatures at 5% and 10% weight loss, and an increase of the weight loss measured at the end of the first degradation step, between 370 and 410°C. The decreased thermal stability of DEHP plasticized PVC compared to rigid PVC is attributed to the simultaneous decomposition of plasticizer and dehydrochlorination of PVC (Jimenez et al., 1999). In particular, the PVC significantly affects the starting decomposition temperature of the DEHP plasticizer, lowering it by around 100°C, from 250°C to 150°C (Saido et al., 2003).

In contrast, addition of ECA plasticizer involves a significant improvement of the thermal stability of soft PVC, as evidenced by the increase of the temperatures at 5% and 10% weight loss, and a reduction of the weight loss measured at the end of the first degradation step. Improvement of the thermal stability of soft PVC by epoxidated compounds has been shown to depend on the reaction between epoxy groups with HCl, reducing the polyene production rate (Benamiba and Massardier-Nargeotte, 2010). The thermal stability of PVC plasticized by ECA is better than that of PVC plasticized by DEHP, and also better than that of neat PVC.

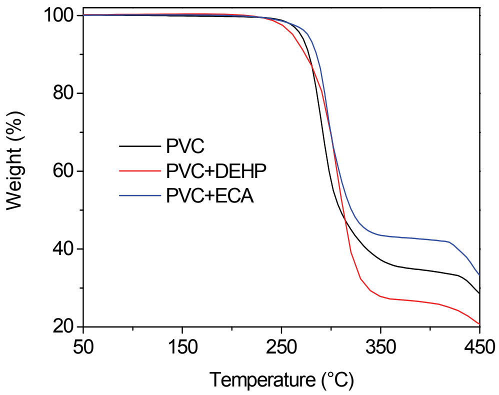


Fig. 5. Thermogravimetric analysis on PVC and soft PVC samples

Hardness test results, reported in Fig. 6, show that the PVC plasticized by ECA has substantially the same hardness of PVC plasticized by DEHP, in accordance with the equivalent value of the glass transition.

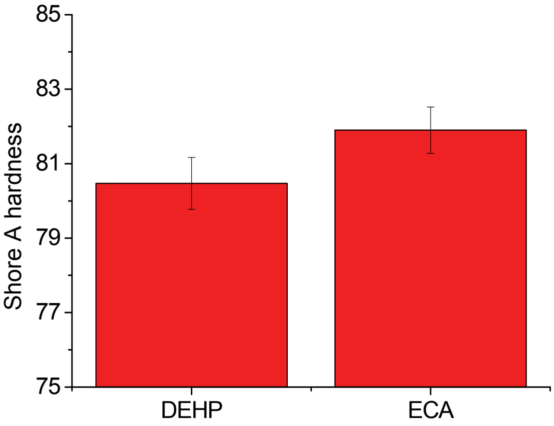


Fig. 6. Hardness tests results

Table 1. Glass transition temperatures of soft PVC and dry blend

Sample	*Time of dry blending (s)*	*T_g after dry blending (°C)*	*T_g after extrusion (°C)*
PVC + ECA	20	-15	-32
PVC + ECA	60	-10	-28
PVC + DEHP	20	33	-33

Table 2. Characteristic of thermogravimetric analysis

Sample	*T_5 (°C)*	*T_{10} (°C)*	*Weight loss at maximum degradation rate (%)*	*Weight loss at first step (%)*
PVC	268	276	26	66
PVC + DEHP	261	271	47	74
PVC + ECA	276	285	20	58

4. Conclusions

This work was aimed to test the suitability of a cardanol derived plasticizer for the production of soft PVC, in an industrial plant. The commercial plasticizer used for comparison was a phthalate, DEHP. Differential scanning calorimetry analysis showed that the developed cardanol derived plasticizer is able to significantly reduce the glass transition of PVC even after the dry blending stage, performed at relatively low temperatures. This indicates a faster absorption of plasticizer even at low temperatures, compared to DEHP. In addition, after extrusion at higher temperatures, the glass transition of soft PVC plasticized by ECA is comparable to that of soft PVC plasticized by DEHP, which indicates that the two plasticizers have the same plasticizing effectiveness.

Thermogravimetric analysis indicated the better thermal stability of soft PVC plasticized by ECA, which is a considerable advantage from the processing point of view, and could lead to a reduction of the amount of thermal stabilizer to be added to soft PVC. Finally, hardness tests showed similar values for the soft PVC obtained by ECA and DEHP, which is a further indication of the good plasticizing effectiveness of ECA, and highlights the potential of ECA plasticizer for the production of soft PVC characterized by reduced environmental and toxicological impact.

Acknowledgements

This study was conducted with funding support of the European Commission under the CIP Eco-Innovation -Pilot and Market Replication Projects, Call 2012 – Project PLACARD, Agreement Number – ECO/12/332833.
This publication has been produced with the assistance of the European Union. The contents of this publication are the sole responsibility of University of Salento, Kommi sas, Serichim srl and can in no way be taken to reflect the views of the European Union.

References

Benamiba M.T., Massardier-Nargeotte V., (2010), Evaluation effects of biobased plasticizer on the thermal, mechanical, dynamical mechanical properties, and permanence of plasticized PVC, *Journal of Applied Polymer Science*, **118**, 3499-3508.

Bitinis N., Verdejo R., Cassagnau P., Lopez-Manchado M.A., (2011), Structure and properties of polylactide/natural rubber blends, *Materials Chemistry and Physics*, **129**, 823-831.

Bocque M., Voirin C., Lapinte V., Caillol S., Robin J.J., (2016), Petro-based and bio-based plasticizers: Chemical structures to plasticizing properties, *Journal of Polymer Science Part A: Polymer Chemistry*, **54**, 11-33.

Bueno-Ferrer C., Garrigos M.C., Jimenez A., (2010), Characterization and thermal stability of poly(vinyl chloride) plasticized with epoxidized soybean oil for food packaging, *Polymer Degradation and Stability*, **95**, 2207-2212.

Calò E., Greco A., Maffezzoli A., (2011), Effects of diffusion of a naturally-derived plasticizer from soft PVC, *Polymer Degradation and Stability*, **96**, 784-789.

Chen J., Liu Z., Jiang J., Nie X., Zhou Y., Murray R.E., (2015a), A novel biobased plasticizer of epoxidized cardanol glycidyl ether: synthesis and application in soft poly(vinyl chloride) films, *RSC Advances*, **5**, 56171-56180.

Chen J., Liu Z., Li K., Huang J., Nie X., Zhou Y., (2015b), Synthesis and application of a natural plasticizer based on cardanol for poly(vinyl chloride), *Journal of Applied Polymer Science*, **132**, DOI: 10.1002/APP.42465.

Cruz-Estrada R.H., Martínez-Tapia E., Canché-Escamilla G., González-Chí P.I., Martín-Barrera C., Duarte-Aranda S., Guillén-Mallette J., Cupul-Manzano C.V., Martínez Dominguez O., García-Gómez C., (2010), A preliminary study on the preparation of wood-plastic composites from urban wastes generated in Merida, Mexico with potential applications as building materials, *Waste Management & Research*, **28**, 838-847.

EC Directive, (1999), Directive 1999/815/EC of the Comission Decision of 7 december 1999, *Official Journal of the European Communities*, L315/46, 9.12.1999, Brussels.

Greco A., Brunetti D., Renna G., Mele G., Maffezzoli A., (2010), Plasticizer for poly(vinyl chloride) from cardanol as a renewable resource material, *Polymer Degradation and Stability*, **95**, 2169-2174.

Hileman B., (2005), Panel ranks risks of common phthalate, *Chemical and Engineering News*, **83**, 32-36.

Jimenez A., Lopez J., Torre L., Kenny J.M., (1999), Kinetic analysis of the thermal degradation of PVC plastisols, *Journal of Applied Polyemr Science*, **73**, 1069-1079.

Kampouris E.M., (1975), The migration of plasticizers into petroleum oils, *European Polymer Journal*, **11**, 795-710.

Kim J.H., Kim S.H., Lee C.H., Nah J.W., Hahn A., (2003), DEHP migration behavior from excessively plasticized PVC sheets, *Bulletin of the Korean Chemical Society*, **24**, 345-349.

Kim Y.H., An E.S., Park S.Y., Song B.K., (2007), Enzymatic epoxidation and polymerization of cardanol obtained from a renewable resource and curing of epoxide-containing polycardanol, *Journal of Molecular Catalysis B: Enzymatic*, **45**, 39-44.

Nass L.I., Heiberger C.A., (1986), *Encyclopedia of PVC: Resin Manufacture and Properties*, Vol. 1, 2nd Edition, Marcel Dekker Inc., New York.

Neuse E.W., Van Schalkwyk J.D., (1977), Cardanol derivatives as PVC plasticizers. II. Plasticizer evaluation, *Journal of Applied Polymer Science*, **21**, 3023-3033.

Patel H., Xanthos M., (1995), Volatile emissions during thermoplastics processing - A review, *Advances in Polymer Technology*, **14**, 67-77.

Patel H., Xanthos M., (2001), Environmental issues in polymer processing: A review on volatile emissions and material/energy recovery options, *Advances in Polymer Technology*, **20**, 22-41.

Saido K., Taguchi H., Yada S., Ishihara Y., Kuroki T., Ryu I.J., Chung S.Y., (2003) Thermal decomposition products of phthalates with poly(vinyl chloride) and their mutagenicity, *Macromolecular Research*, **11**, 178-182.

Sathiyalekshmi K., Gopalakrishnan S., (2004), Evaluation of performance of polyurethanes based on hydroxy alkylated cardanol formaldehyde resins under aging conditions, *Advances in Polymer Technology*, **23**, 91-102.

Swier S., Van Mele B., (2003), Reaction thermodynamics of amine-cured epoxy systems: Validation of the enthalpy and heat capacity of reaction as determined by modulated temperature differential scanning calorimetry, *Journal of Polymer Science: Part B: Polymer Physics*, **41**, 594-608.

Van Oers L., Van der Voet E., (2012), Assessing plastics additives in Life Cycle Assessment, On line at: http://faa-tagungen-dresden.de/wp-content/uploads/V-Oers_RISKCYCLE_Presentation_ Dresden2012_CMLfinal.pdf.

Voirin C., Caillol S., Sadavarte N.V., Tawade B.V., Boutevin B., Wadgaonkar P.P., (2014), Functionalization of cardanol: towards biobased polymers and additives, *Polymer Chemistry*, **5**, 3142-3162.

Wilkinson C.F., Lamb J.C., (1999), The potential health effects of phthalate esters in children's toys: a review and risk assessment, *Regulatory Toxicology and Pharmacology*, **30**, 140-155.

Yang P., Sun H., Fan H., Shi B., (2016), Novel environmentally sustainable cardanol-based plasticizers: synthesis and properties, *Polymer International*, **65**, 464-472.

Yang P., Yan J., Sun H., Fan H., Chen Y., Wanga F., Shia B., (2015), Novel environmentally sustainable cardanol-based plasticizer covalently bound to PVC via click chemistry: synthesis and properties, *RSC Advances*, **5**, 16980-16985.

Yuliana M., Nguyen-Thi B.T., Faika S., Huynh L.H., Soetaredjo F.E., Ju Y.H., (2014), Separation and purification of cardol, cardanol and anacardic acid from cashew (*Anacardium occidentale* L.) nut-shell liquid using a simple two-step column chromatography, *Journal of the Taiwan Institute of Chemical Engineers*, **45**, 2187-2193.

1996

Environmental Engineering and Management Journal September 2016, Vol.15, No. 9, 1997-2002
http://omicron.ch.tuiasi.ro/EEMJ/

"Gheorghe Asachi" Technical University of Iasi, Romania

RAPESEED MEAL: AN AGRO-WASTE FOR CHEAP/LOW COST LACCASE PRODUCTION

Lucia Guarino[1]*, Giovanni Sannia[1,2], Vincenzo Lettera[1,2]

[1]*Department of Chemical Sciences University of Naples FEDERICO II Via Cintia, 4-80126 Napoli, Italy*
[2]*Biopox srl via Salita Arenella, 9 Napoli, Italy*

Abstract

Rapeseed meal (RM) is an abundant and cheap by-product of oil extraction process. RM is a rich source of nitrogen, carbon, and minerals. Thanks to these features, RM has a striking potential for eco-friendly production of high value products in biotechnological processes.

In this study, the possibility of using a low-cost medium based on the water soluble fraction of RM for laccase production by the fungus *Pleurotus ostreatus* has been evaluated and compared with the use of synthetic complex media. RM-based medium (RMM) led to extracellular laccase production comparable to that obtained in the presence of synthetic media. Moreover, the use of a raw material highly reduced the production costs. To further reduce cost and improve activity production the effect of the addition of nitrogen source, pure aromatic compounds and lignosulphonate, a low cost by-product of wood based industries, was evaluated. The optimized medium led to a laccase production level of more than 100,000 U/L at a cost of about $0.19*10^{-3}$ cent€/U. The obtained results demonstrate the effective recycle of a waste to produce an industrially relevant enzyme.

Key words: fermentation, laccase production, rapeseed meal

Received: January, 2016; Revised final: September, 2016; Accepted: September, 2016

1. Introduction

Rapeseed Meal (RM) is a by-product of oil extraction process from seeds. It is mainly composed of proteins, lignocellulose fibers, minerals and also oil residues, phenols and aromatic compounds (Lomascolo et al., 2012). In view of its large harvesting over the world, with a main production in EU of 14.4 millions of ton in 2014 (http://www.indexmundi.com/agriculture/), recovery, effective utilization and valorization of this residual meal is becoming a crucial issue in bio-economy.

RM is traditionally used as organic fertilizer or animal feed. However, the utilization of RM in feed industries is limited by some anti-nutritional or toxic constituents (Koutinas et al., 2007; Vig and Walia, 2001) and by a protein content not easily digestible (Kiran et al., 2012).

Recently, due to its high protein and lignocellulosic residues content, RM has been tested in biotechnological processes as an inexpensive, eco-friendly fermentative nutrient for the production of high-added value product such as enzymes (Żuchowski et al., 2013), antibiotics, antioxidants, vitamins, biogas and bio-oil (Lomascolo et al., 2012). Due to the presence of several nutrients that could not be directly assimilated by the majority of industrial bacteria and yeasts, at present, the utilization of RM as a renewable resource has been mainly used in solid state fermentation of filamentous fungi. In fact, agro-residues can represent an appropriate source of nutrient for white-rot basidiomycetes (Xin and Geng, 2011),

---

* Author to whom all correspondence should be addressed: E-mail: lucia.guarino@unina.it; Phone: +39081674475; Fax: +39081674313

stimulating production of different economically valuable hydrolytic and oxidative enzymes (Aro et al., 2005; D'Annibale et al., 2014; Eriksson et al., 1990) involved in lignocellulose component degradation. In this frame the white rot basidiomycete *Pleurotus ostreatus* (Jacq. Fr.) Kumm. Dikarya, Basidiomycota, Agaricomycotina, Agaricales) has been largely studied for its high potential to grow on agro-wastes and to produce high amount of laccases (benzenediol: oxygen oxidoreductase; EC 1.10.3.2), an industrially useful oxidoreductase having broad substrate specificity (Giardina et al., 2010). A lot of papers and patent show the importance of laccase in several industrial applications (Pezzella et al., 2015; Spina et al., 2015). For this reason it is essential to reduce laccase production costs. Optimization of fungal fermentation conditions by using cheap growth substrates, such as RM, represents one of the main and most viable approaches for laccase production improvement.

However, compared to solid state fermentation, submerged fermentation has been more widely applied in industrial processes because it has the advantages of high homogeneity of heat and mass transfer, easy control of oxygen and temperature, and more convenient handling (Chen et al., 2011; Mizumoto et al., 2007). Until now, only few study reports the utilization of RM as microbial growth media in submerged fermentation (Kiran et al., 2012).

The utilization of an agro-waste based culture medium can contribute to the low cost production of industrially valuable products. Various studies have been performed for laccase production by diverse white rot fungi using different lignocellulosic substrates as carbon sources (Bakkiyaraj et al., 2013; Birhanlı and Yeşilada, 2013; Lorenzo et al., 2002).

Furthermore, the overexpression of laccases can be induced by the presence of a wide variety of substances, mainly aromatic or phenolic compounds structurally related to lignin or lignin derivates. Thus, these chemicals are routinely added to fungal cultures to increase laccase production (Shraddha et al., 2011; Viswanath et al., 2014).

The objective of this study was the formulation of a cheap RM based media for the production of laccase from *P. ostreatus*. The final cost of the laccase produced in RMM added with lignosulphonate is lower than that obtained by fermentation in the presence of the most extensively used synthetic media based on potato dextrose/yeast extract.

2. Materials and methods

2.1. Organism

The fungus *Pleurotus ostreatus* strain 5ax3d, was obtained as described by Del Vecchio et al. (2012).

2.2. Growth conditions

P. ostreatus was maintained through periodic serial transfers on PDY agar plates (24 g/L potato dextrose (BD Difco^{TM}) and 5 g/L yeast extract (BD Difco^{TM})), incubated at 28°C for 5 days and kept at 4°C. Six 11-mm disks of active mycelium, excised from agar plates, were transferred to 250 mL Erlenmeyer flasks containing 75 mL PDY media. The cultures were maintained in continuous agitation at 125 rpm and 28°C in complete darkness. After five growth days, the entire culture was homogenized by Waring Blender 7011HS® (3 flash of 15 secondst maximum speed) and a portion of 15 mL was inoculated into 500 mL flasks containing 135 mL of different growth media: *i)* PDY, *ii)* Rapeseed Meal Medium(RMM) prepared mixing 200 g of RM powder in 1.2 L of distilled water, separating the liquid portion through centrifugation at 3220 g at room temperature for 15 minutes and sterilized at 120°C for 20 minutes, *iii)* RMM added with different concentration of yeast extract (5, 15 and 30 g/L), *iv)* RMM added with ferulic acid (1 and 2 mM) *v)* RMM added with gallic acid (1 and 2 mM) *vi)* RMM added with 2,5 xylidine (1 and 2 mM), *vii)* RM added with sodium lignosulphonate (15 and 30 g/L) (Borregaard AS-Sarpsborg, Norway). All media were supplemented with 150 μM $CuSO_4$. Cultures were incubated at 28°C under continuous agitation (125 rpm). Yeast extract and sodium lignosulphonate were added to RMM before heat sterilization. 250 mM ferulic acid and 2,5 xylidine were dissolved in 95% ethanol, 150 mM $CuSO_4$ was dissolved in distilled water. These inductors were sterilized by filtration and added to RMM after its heat sterilization.

2.3. Laccase assay

Laccase activity was determined spectrophotometrically measuring the oxidation of 2,2-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) at 420 nm ($e = 3.6 * 10^4$ cm^{-1} M^{-1}). The assays mixture contained 2mM ABTS and 0.1 M sodium citrate buffer, pH 3.0. The activities were expressed in U/L. When required, the sample was diluted in 50 mM sodium phosphate buffer (pH 6.0). One activity unit is defined as the amount of enzyme that oxidized 1 μmoL ABTS per min. All the values are the means of triplicate experiments.

2.4. Cost evaluation

Production costs were evaluated as the ratio between the cost of the culture medium and the amount of produced laccase activity. For the cost of culture media we used, the market prices of:

- Potato Dextrose and Yeast Extract were obtained by BD Difco^{TM} (NJ, USA);
- Rapeseed cake was obtained from UFOP Market Information Oilseeds and Biofuels

(http://www.ufop.de/files/9014/4949/9305/2015_12_E.pdf);

- Sodium lignosulphonate was obtained by Borregaard AS (Sarpsborg, Norway);
- All other used compounds were obtained by SIGMA–Aldrich® (St. Louis, USA).

3. Results and discussion

3.1. Growth media formulation based on RM

To investigate the potential application of low-cost RM on laccase production, the soluble fraction of RM has been used as nutrient source for *P. ostreatus* cultured under submerged fermentation, and the extracellular laccase production profile achieved was compared with that obtained in PDY broth, a synthetic rich medium already used for laccase production in *P. ostreatus* 5Ax3D (Del Vecchio et al., 2012). Since laccase is a copper-containing protein, $CuSO_4$, was added to both media, to allow the laccase correct folding (Baldrian and Gabriel, 2002; Birhanlı and Yeşilada, 2006; Piscitelli et al., 2011). The time-course analysis of extracellular laccase production was carried out during the fungal fermentation (Fig. 1). The fungus started to produce laccase on the third day (600 U/L) in both growth conditions and then laccase activities increase, peaking on the 7th day (42,000 U/L) and on the 9th day (47,000 U/L) in RMM and PDY medium, respectively. It is remarkable that although laccase production levels were comparable, the highest enzyme activity was reached in less time (thus increasing the productivity) for RMM, making the soluble fraction of RM potentially exploitable for the formulation of a low cost media for laccase production.

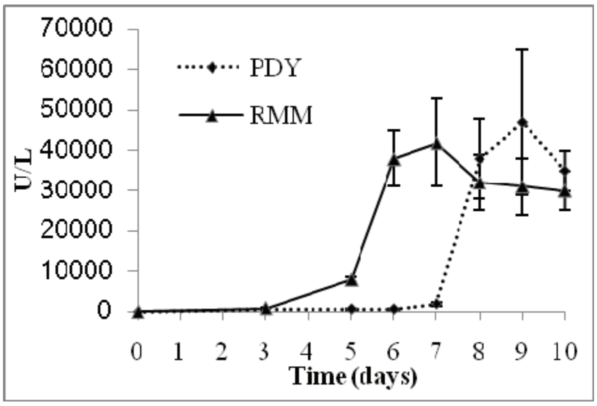


Fig. 1. Time course of extracellular laccase produced by *P.ostreatus* in PDY and RMM. Both media were supplemented with $CuSO_4$ 150 µM. Each experiment was performed in triplicate

It is extensively reported that the carbon/nitrogen ratio plays a key role in enzyme production with effects depending on its nature and concentration in the culture media (Piscitelli et al., 2011). Houet al. (2004) demonstrated that yeast extract increases laccase activity 1.40, 1.79, and 1.32 fold, in comparison with urea, ammonium sulfate, and ammonium tartrate respectively. Mishra and Kumar (2007) also showed that, yeast extract is about 2 times better than inorganic nitrogen source (ammonium sulfate and urea), in increasing laccase production by *P. ostretus* MTCC1804. Karp et al. (2015), reported that the use of the organic nitrogen source yeast extract provided an increase of 5.7-fold in laccase production from *P. ostreatus* (Pl 22 Em), in comparison with the use of inorganic ammonium sulfate. A positive effect on laccase production of the yeast extract addition has been achieved in other fungal strain such as *Coriolus versicolor* MTCC 138 (Revankar and Lele, 2006). In the present work yeast extract was added to RM-based medium at three different concentrations (5, 15 and 30 g/L). A remarkable enhancement of laccase activity was only observed at the lowest concentration of yeast extract, when compared to the RMM. In this condition the maximum laccase activities resulted to be 68,000 U/L at the 6th day, with a 1.6 fold increase in production. A higher concentration of yeast extract (15 g/L) did not further elicit laccase expression, whereas a concentration of 30 g/L leads to a reduction in extracellular enzyme level (Fig. 2). A positive effect of 5 g/L yeast extract addition on laccase production in another *P. ostreatus* strain has been also reported by Karp et al. (2012), whereas the negative effect linked to the highest concentration of yeast extract could be related to a catabolite repression, as reported by Kachlishvili et al. (2005) and by D'Agostini et al. (2011).

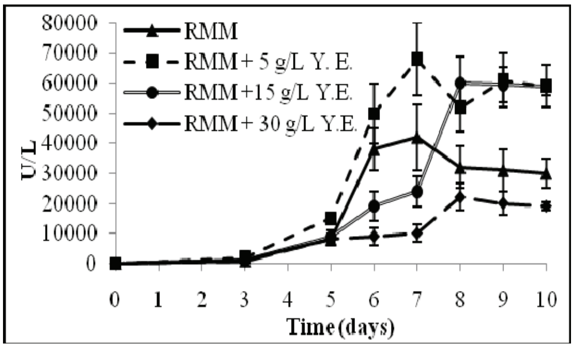


Fig. 2. Time course of extracellular laccase activity produced by *P. ostreatus* in RMM supplemented with different yeast extract (Y.E.) concentrations. All media were supplemented with $CuSO_4$ 150 µM. Each experiment was performed in triplicate

In this work, three aromatic compounds, already known as effective laccase inducers for *P. ostreatus*, gallic acid, 2,5-xylidine, and ferulic acid, and a low-cost lignin derivate, sodium lignosulphonate, were added to the RMM at two different concentrations and a time course analysis on laccase production in each growth medium was performed (Fig. 3).

Laccase levels in presence of 1 mM 2,5-xylidine resulted in 1.4 fold higher production than that obtained in the RM-basal medium. A positive effect of the addition of xylidine as inducer on laccase production has already been reported in other basidiomycetes strains such as *Trametes villosa, Trametes versicolor*, and *Pleurotus sajor-caju* (Viswanath et al., 2014) and in *Pycnoporus sanguineus* (Garcia et al., 2006; Ramirez-Cavazos et al., 2014). At higher concentration (2mM) 2,5-xylidine had a negative effect on laccase production maybe because of its toxicity at high concentration, as also reported by Lu et al. (1996).

Supplementation of gallic acid, both at 1 and 2 mM, did not affect the laccase production in *P. ostreatus* strain 5ax3d, in contrast with data reported by Patel et al. (2009) for a different *P. ostreatus* strain. This is not surprising due the variability of behaviours that different cultivars or subspecies can have in the same growth conditions (Piscitelli et al., 2013). However, it has already been reported by Ramírez-Cavazos et al. (2014) that laccase production is not affected by the presence of gallic acid in a native strain of *Pycnoporus sanguineus*.

Compared with non-induced cultures, ferulic acid supplementation at a 2 mM concentration enhanced laccase production (1.3-fold), with a maximum laccase activity of 58,000 U/L at the 7th growth day. This result is not unexpected being the ferulic acid eliciting effect extensively characterized in *P. ostreatus* and in other basidiomycetes such as *Pleurotus sajor-caju* (Zucca et al., 2011), *Ganoderma lucidum* (Kuhar and Papinutti, 2014) and *Pycnoporus cinnabarinus* (Meza et al., 2007). In fact, we have already reported a broad inductive effect on laccase activity in *P. ostreatus* submerged fermentation when ferulic acid was supplemented to the PDY medium (Del Vecchio et al., 2012). A positive effect of ferulic acid on laccase production has been also found in other *P. ostreatus* strains such as Pl 22 Em and IT 01 with a production of 89.18 U/g of dry substrate and 7,500 U/L that is 2 and 3-fold increased when compared to control, respectively (Karp et al., 2015; Vanhulle et al., 2007).

As far as sodium lignosulphonate presence in RMM is concerned, a few studies have been performed to assess the effect of lignosulfonates on laccase production in fungi. An increase in enzyme specific activity was observed when *Agaricus* strains are fermented in presence of lignosulfonate (Kaluskar et al., 1999). In this study a positive effect on laccase production in presence of of Ultrazine NA (lignosulphonate trade name) was observed. In particular a maximum laccase activity of 100,000 U/L was reached by adding 30 g/L of Ultrazine NA to RRM.

3.2. Cost analysis

Optimization of laccase production does not always result in a reduction of the final price of the marketable enzyme. Three main costs are generally taken into account at this scale: culture medium, equipment and operating costs. In all cases, the cost of the culture medium represents the highest contribution to the total costs (Osma et al., 2011). The cost of culture media and the cost of laccase produced in the different culture media are summarized in the Table 1. It is evident that the use of RMM instead of PDY reduces 20 times the cost of produced laccase even when the amount of produced enzyme is lower than that obtained in synthetic media. Although the addition of a supplementary nitrogen source or inducers increase the production of laccase, the laccase cost is reduced only when 1 mM 2.5 xylidine, or lignosulphonates (both 15 and 30 g/L) are added. The lowest price ($0.19*10^{-3}$ cent €/U) was obtained in the presence of 30 g/L lignosulphonates.

Although Del Vecchio et al. (2012) already optimized laccase production by *P. ostreatus* 5Ax3D obtaining similar levels of laccase production (110,000 U/L) in submerged fermentation using the synthetic medium PDY and ferulic acid, the final price ($4,06*10^{-3}$ cent€/L), is 22 times higher than that obtained in this study.

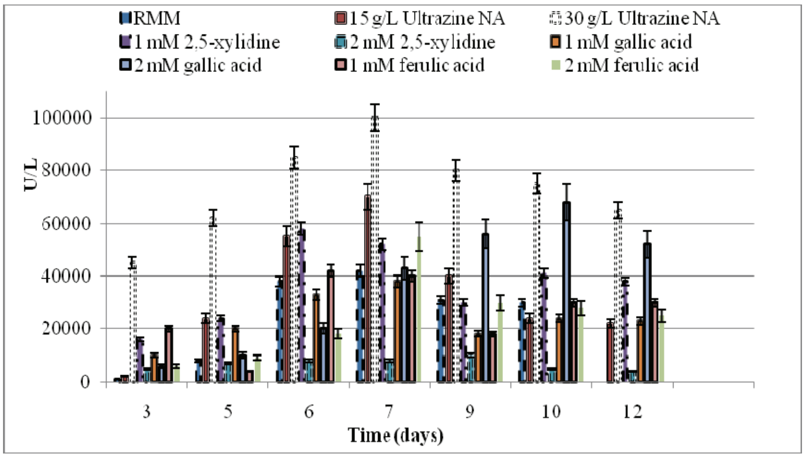


Fig. 3. Time course of extracellular laccase activity produced by *P. ostreatus* strain 5ax3d in RM supplemented with different inducers. Ultrazine NA is the lignosulphonate trade name. All media were supplemented with $CuSO_4$ 150 µM. Each experiment was performed in triplicate

Table 1. Cost analysis of laccase produced under different cultivation conditions

Culture medium		***Max activity (U/L)***	***Time (days)***	***Culture medium Cost (€/L)***	***Laccase Cost[$] (cent €/U)***
PDY	--	47000	9	4	$8.50*10^{-3}$
RMM	--	42000	7	0.1794	$0.42*10^{-3}$
RMM	5g/L Yeast extract	67,000	7	1.12	$1.67*10^{-3}$
RMM	1 mM ferulic acid	42,000	6	0.4177	$1.00*10^{-3}$
RMM	2 mM ferulic acid	55,000	7	0.6564	$1.19*10^{-3}$
RMM	1 mM gallic acid	38,000	6	0.2354	$0.62*10^{-3}$
RMM	2 mM gallic acid	43,000	7	0.2894	$0.67*10^{-3}$
RMM	1 mM 2,5 xylidine	58,000	6	0.2044	$0.35*10^{-3}$
RMM	2 mM 2,5 xylidine	10,000	9	0.2294	$2.29*10^{-3}$
RMM	15g/L lignosulphonate	70,000	7	0.1821	$0.26*10^{-3}$
RMM	30g/L lignosulphonate	100,000	7	0.1849	$0.19*10^{-3}$

[$] Laccase costs have been calculated considering only the costs of the culture media components and efficiency of production

Even though many researchers focused their attention on the optimization of laccase production, only few researchers are paying attention on the analysis of the production costs. Among these Osma et al. (2011) analyzed the cost of laccase produced by the white-rot fungus *Trametes pubescens* in more than 45 culture media. By comparing the best cost of laccase produced by *P. ostreatus* 5Ax3D ($0.19*10^{-3}$ cent €/U) and by *T. pubescens* (0.03 cent €/U), it is evident that the final cost of laccase produced by the fungus studied in this work in the formulated medium is the lowest reported up to date.

4. Conclusions

Laccases from white rot fungi can be applied in a great number of industrial applications, therefore, it is important to design and develop a robust and economic bio-based process for laccase production.

In the present work, formulation and optimization of production media, combined with the exploitation of a high laccase producer strain, has been a key factor to maximize the yield of laccase production and minimize production costs. Media were formulated testing an agro-industrial waste supplemented with lignosulphonates, a cheap by-product by paper industries.

The process carried out with the new medium resulted in allowing high levels of laccase production (100,000 U/L) at low costs of formulation ($0.19*10^{-3}$ cent €/U) respect to the semi-synthetic medium previously used for laccase production at laboratory scale.

References

Aro N., Pakula T., Penttilä M., (2005), Transcriptional regulation of plant cell wall degradation by filamentous fungi, *FEMS Microbiology Reviews*, **29**, 719-739.

Baldrian P., Gabriel J., (2002), Copper and cadmium increase laccase activity in *Pleurotus ostreatus*, *FEMS Microbiology Letters*, **206**, 69-74.

Bakkiyaraj S., Aravindan R., Arrivukkarasan S., Viruthagiri T., (2013), Lemon peelings: a potential substrate for laccase production by *Trametes versicolor* MTCC 138 in submerged fermentation, *International Journal of Current Chemical Sciences*, **2**, 17-24.

Birhanlı E., Yeşilada Ö., (2006), Increased production of laccase by pellets of Funalia trogii ATCC 200800 and *Trametes versicolor* ATCC 200801 in repeated-batch mode, *Enzyme and Microbial Technology*, **39**, 1286-1293.

Birhanlı E., Yeşilada Ö., (2013), The utilization of lignocellulosic wastes for laccase production under semisolid-state and submerged fermentation conditions, *Turkish Journal of Biology*, **37**, 450-456.

Chen K.Q., Zhang H., Miao Y.L., Wei P., Chen J.Y., (2011), Simultaneous saccharification and fermentation of acid-pretreated rapeseed meal for succinic acid production using *Actinobacillus succinogenes*, *Enzyme and Microbial Technology*, **48**, 339-344.

D'Agostini E.C., D'Agostini T.R., Silveira do Valle J., Paccola-Meirelles L.D., Colauto N.B., Linde G.A., (2011), Low carbon/nitrogen ratio increases laccase production from basidiomycetes in solid substrate cultivation, *Scientia Agricola*, **68**, 295-300.

D'Annibale A., Sampedro I., Federici F., Petruccioli M., (2014), Aqueous extract from dry olive mill residue as a possible basal medium for laccase production, *Environmental Engineering and Management Journal*, **13**, 3037-3044.

Del Vecchio C., Lettera V., Pezzella C., Piscitelli A., Leo G., Birolo L., Sannia G., (2012), Classical breeding in *Pleurotus ostreatus*: a natural approach for laccase production improvement, *Biocatalysis and Biotransformation*, **30**, 78-85.

Eriksson K.E.L., Blanchette R.A., Ander P., (1990), *Microbial and Enzymatic Degradation of Wood and Wood Components*, Springer-Verlag Berlin Heidelberg, New York, 89-180.

Garcia T.A., Santiago M.F., Ulhoa C.J., (2006), Properties of laccases produced by Pycnoporus sanguineus induced by 2,5-xylidine, *Biotechnology Letters*, **28**, 633-636.

Giardina P., Faraco V., Pezzella C., Piscitelli A., Vanhulle S., Sannia G., (2010), Laccases: a never-ending story, *Cellular and Molecular Life Sciences*, **67**, 369-385.

Hou H., Zhou J., Wang J., Du C., Yan B., (2004), Enhancement of laccase production by *Pleurotus ostreatus* and its use for the decolorization of anthraquinone dye, *Process Biochemistry*, **39**, 1415-1419.

Kachlishvili E., Penninckx M.J., Tsiklauri N., Elisashvili V., (2005), Effect of nitrogen source on lignocellulolytic enzyme production by white-rot basidiomycetes under solid-state cultivation, *World Journal of Microbiology and Biotechnology*, **22**, 391-397.

Kaluskar V.M., Kapadnis B.P., Jaspers C., Penninckx M.J., (1999), Production of laccase by immobilized cells of *Agaricus* sp.: induction effect of xylan and lignin derivatives, *Applied Biochemistry and Biotechnology*, **76**, 161-170.

Karp S.G., Faraco V., Amore A., Birolo L., Giangrande C., Soccol V.T., Pandey A., Soccol C.D., (2012), Characterization of laccase isoforms produced by *Pleurotus ostreatus* in solid state fermentation of sugarcane bagasse, *Bioresource Technology*, **114**, 735-739.

Karp S.G., Faraco V., Amore A., Letti L.A.J., Soccol V.T., Soccol1 C.D., (2015), Statistical optimization of laccase production and delignification of sugarcane bagasse by *Pleurotus ostreatus* in solid-state fermentation, *BioMed Research International*, ID 181204, http://dx.doi.org/10.1155/2015/181204.

Kiran E.U., Salakkam A., Trzcinski A.P., Bakir U., Webb C., (2012), Enhancing the value of nitrogen from rapeseed meal for microbial oil production, *Enzyme and Microbial Technology*, **50**, 337-342.

Koutinas A.A., Wang R.H., Webb C., (2007), The biochemurgist-bioconversion of agricultural raw materials for chemical production, *Biofuels Bioproducts & Biorefining*, **1**, 24-38.

Kuhar F., Papinutti L., (2014) Optimization of laccase production by two strains of *Ganoderma lucidum* using phenolic and metallic inducers, *Revista Argentina de Microbiologia* **46**, 144-149.

Lomascolo A., Uzan-Boukhris E., Sigoillot J.C., Fine F., (2012), Rapeseed and sunflower meal: A review on biotechnology status and challenges, *Applied Microbiology and Biotechnology*, **95**, 1105-1114.

Lorenzo M., Moldes D., Rodríguez Couto S., Sanromán A., (2002), Improving laccase production by employing different lignocellulosic wastes in submerged cultures of *Trametes versicolor*, *Bioresources Technology*, **82**, 109-113.

Lu S.X.F., Jones C.L., Lonergan G.T., (1996), *Correlation between Fungal Morphology and Laccase Expression under the Influence of Cellobiose Induction*, Proc. 10th Int. Biotechnology Symp. and the 9th Int. Symp. on Yeasts, Sydney, Australia.

Meza J.C., Auria R., Lomascolo A., Sigoillot J.-C., Casalot L., (2007), Role of ethanol on growth, laccase production and protease activity in *Pycnoporus cinnabarinus* ss3, *Enzyme and Microbial Technology*, **41**, 162-168.

Mishra A., Kumar S., (2007), Cyanobacterial biomass as N-supplement to agro-waste for hyper-production of laccase from *Pleurotus ostreatus* in solid state fermentation, *Process Biochemistry*, **42**, 681-685.

Osma J.F., Toca-Herrera J.L., Rodríguez-Couto S., (2011), Cost analysis in laccase production *Journal of Environmental Management*, **92**, 2907-2912.

Patel H., Gupte A., Gupte S., (2009), Effect of different culture conditions and inducers on production of laccase by a basidiomycete fungal isolate *Pleurotus ostreatus* HP-1 under solid state fermentation, *Bioresources*, **4**, 268-284.

Pezzella C., Guarino L., Piscitelli A., (2015), How to enjoy laccases, *Cellular and Molecular Life Sciences*, Doi: 10.1007/s00018-014-1823-9.

Piscitelli A., Giardina P., Lettera V., Pezzella C., Sannia G., Faraco V., (2011), Induction and transcriptional regulation of laccases in fungi, *Current Genomics*, **12**, 104-112.

Piscitelli A., Pezzella C., Lettera V., Giardina P., Faraco V., Sannia G., (2013,) *Fungal Laccases: Structure, Function and Application*, In: *Fungal Enzymes: Progress and Prospects*, de Lourdes M.T.M.P., Rai M. (Eds.), CRC Press, Boca Raton, 113-151.

Ramirez-Cavazos L.I., Junghanns C., Nair R., Cárdenas-Chávez D.L., Hernández-Luna C., Agathos S.N., Parra R., (2014), Enhanced production of thermostable laccases from a native strain of *Pycnoporus sanguineus* using central composite design, *Journal of Zhejiang University SCIENCE B Biomedicine & Biotechnology*, **15**, 343-352.

Revankar M.S., Lele S.S., (2006), Increased production of extracellular laccase by the white rot fungus *Coriolus versicolor* MTCC 138, *World Journal of Microbiology and Biotechnology*, **22**, 921-926.

Shraddha, Shekher R., Sehgal S., Kamthania M., Kumar A., (2011), Laccase: microbial sources, production, purification, and potential biotechnological applications, *Enzyme Research*, Article ID 217861, 1-11, Doi:10.4061/2011/217861.

Spina F., Fidaleo M., Nanni A., Romagnolo A., Varese G.C., (2015), Fungal laccases production using tomato-based medium: a factorial design approach, *Environmental Engineering and Management Journal*, **14**, 1743-1750.

Vanhulle S., Radman R., Parra R., Cui T., Bols C.-M., Tron T., Sannia G., Keshavarz T., (2007), Effect of mannan oligosaccharide elicitor and ferulic acid on enhancement of laccasesproduction in liquid cultures of basidiomycetes, *Enzyme and Microbial Technology*, **40**, 1712-1718.

Vig A.P., Walia A., (2001), Beneficial effects of *Rhizopus oligosporus* fermentation on reduction of glucosinolates, fibre and phytic acid in rapeseed (Brassica napus) meal, *Bioresource Technology*, **78**, 309-312.

Viswanath B., Rajesh B., Janardhan A., Kumar A.P., Narasimha G., (2014), Fungal laccases and their applications in bioremediation, *Enzyme Research,* Article ID 163242, 1-21, Doi: 10.1155/2014/163242.

Xin F., Geng A., (2011), Utilization of horticultural waste for laccase production by *Trametes versicolor* under solid-state fermentation, *Applied Biochemistry and Biotechnology*, **163**, 235-246.

Zucca P., Rescigno A., Olianas A., MacCioni S., Sollai F.A., Sanjust E., (2011), Induction, purification, and characterization of a laccase isozyme from *Pleurotus sajor-caju* and the potential in decolorization of textile dyes, *Journal of Molecular Catalysis B: Enzymatic*, **68**, 216-222.

Żuchowski J., Pecio Ł., Jaszek M., Stochmal A., (2013), Solid-state fermentation of rapeseed meal with the white-rot fungi *Trametes versicolor* and *Pleurotus ostreatus*, *Applied Biochemistry and Biotechnology*, **171**, 2075-2081.

Environmental Engineering and Management Journal

September 2016, Vol.15, No. 9, 2003-2010
http://omicron.ch.tuiasi.ro/EEMJ/

"Gheorghe Asachi" Technical University of Iasi, Romania

PRODUCTION OF BIOPLASTIC FROM WASTE OILS BY RECOMBINANT *Escherichia coli*: A PIT-STOP IN WASTE FRYING OIL TO BIO-DIESEL CONVERSION RACE

Cinzia Pezzella*, Marco Vastano, Angela Casillo, Maria Michela Corsaro, Giovanni Sannia

Dipartimento di Scienze Chimiche, Complesso Universitario Monte S. Angelo, via Cintia 4, 80126 Napoli

Abstract

Waste frying oil (WFO) is a complex and heterogeneous waste collected mainly from restaurants and, to a lesser extent, from food processors and households. In the last decades, interest for this waste has deeply grown and a strong competition in grabbing this raw material has begun. About 90% of WFOs collected in Europe is recycled for biodiesel production, being the high free fatty acids (FFAs) concentration in WFOs one of the main drawback limiting the yield of the conversion process. In this study we proposed a pit stop in the WFO to biodiesel conversion race by introducing an upstream microbial fermentation step aimed at reducing the FFAs content through its conversion into biopolymers, i.e. Polyhydroxyalkanoates (PHA). A properly engineered *Escherichia coli* strain, able to produce PHA exclusively from FFAs, but not secreting lipases, was tested in this process. Recombinant production of a near P(HHx) homopolymer was achieved in a process requiring an aqueous pretreatment step aimed to reduce the content of non-lipid carbon sources, which compete with FFAs for microbial growth impairing the PHA production. A WFO with a halved FFAs content was recovered after fermentation, thus rendering it more attractive for further conversion in biodiesel. Despite the quite low production yield achieved at this stage, the idea of no-competitiveness behind the process was verified, leaving space to further strain improvement strategies to boost PHA yield.

Key words: biodiesel, circular process, polyhydroxyalkanoates, FFAs reduction, WFO

Received: January, 2016; Revised final: September, 2016; Accepted: September, 2016

1. Introduction

1.1. Waste frying oils (WFO): current uses and supply chain

According to the UK Environment Agency (Environment Agency, 2009), Waste cooking (frying) oils (WCO-WFO) are purified fats of plant or animal origin, which are liquid at room temperature. Like all fats, WFOs are a water-insoluble mixture of triacylglycerol (TGA), diacylglycerol (DGA), monoacylglicerol (MGA) and free fatty acid (FFA). Moreover, WFO are typical characterized by the presence of small pollutants as small pieces of fried food and a higher content of FFAs compared to unused equivalent (Spöttle et al., 2013).

WFO is a complex and heterogeneous waste collected from diverse food preparations. WFOs have been traditionally recycled in several processes including: *i)* animal feeding; *ii)* oleochemical industry; and *iii)* energy production (incineration and biodiesel). However, following BSE scandals in the early 2000s and the ABPR 1069/2009 regulation amended in 2013, which authorizes the use of animal fats of all categories for oleochemical products, the share of WFOs allocated for biodiesel industry has increased noteworthy. As fact, 90% of WFO in the EU-27 is allocated for biodiesel vs 10% for oleochemical industry in 2013 (Spöttle et al., 2013).

---

* Author to whom all correspondence should be addressed: e-mail: cpezzella@unina.it; Phone: +39081674475; Fax: +39081674313

The use of WFOs for biodiesel represents a sustainable outlet for the disposal of this problematic waste, with the potential to offer significant Green House Gas (GHG) savings in accordance with the target imposed by the EU Renewable Energy Directive 2009/28/EC.

WFOs addressed to biodiesel production are usually collected from a large number of relatively small feedstock producers and often undergo several stages of basic filtering and pre-processing to remove impurities such as water and food residues. The quality of WFOs is determined by its cleanliness, the level of FFAs and water content which are dependent on the type of fried foods, the frequency of replacing the cooking oil with fresh one and the nature of the vegetable oil used for cooking. The major technical challenge for biodiesel production from WFOs is linked to FFAs concentration (Chai et al., 2014). It has been claimed that, when the FFAs level exceeds 5%, during the alkaline trans-esterification process, undesirable formation of soap becomes substantial (Banani et al., 2015). Soap formation results in yield loss and increased difficulty in biodiesel/glycerol separation (Kulkarni et al., 2006; Ma et al., 1998). A two-step conversion process has been generally applied to address this issue. In this process, an acid-catalyzed methanol demanding esterification precedes the traditional alkaline-catalyzed trans-esterification in order to lower the FFAs content (Agnew et al., 2009; Javidialesaadi and Raeissi, 2013).

Due to its interesting potential, WFO has acquired a real market value in the past few years. For restaurants or food processors WFO is primarily a waste that needs to be properly disposed. However, WFO is becoming an attractive raw material that some collectors are starting to pay for to receive from catering premises, even if prices paid for WFO are reported to vary widely, 380-910 €/ton in Europe and about 150 €/ton in the USA, depending on the oil quality (Smith et al., 2013). In addition, an increasing number of WFO thefts have been reported in the EU, and also in the USA (Spöttle et al., 2013).

The WFO supply chain has not been highly regulated. Restaurants are the major source of WFO followed by food processors and households. The main differences in composition between wastes from catering premises and households mainly consists in the complexity of the oil (one type *vs* a mixture of different oils respectively), and the FFAs content. As regard to this latter parameter, higher and more variable values ranging from < 1% to > 40% have been reported for food industry derived wastes in comparison with homemade ones (Banani et al., 2015; Chai et al., 2014; Corro et al., 2016; Javidialesaadi and Raeissi, 2013).

1.2. New routes for WFO valorisation: microbial fermentation to biopolymer production

Among the possible routes for WFOs exploitation, a new emerging area is represented by its utilization as raw material for microbial fermentation. Several studies have already proposed the microbial conversion of fatty acids rich media in renewable fuels and chemicals (Dellomonaco et al., 2010; Fernández et al., 2015; Jeon et al., 2012), suggesting that fatty acids could become a sustainable feedstock for industrial production (Doi et al., 2014; Sathesh and Lee, 2015).

In this frame, biopolymers are among the high-added value products obtainable through microbial conversion of waste oils. Biopolymers market, as fact, is forecasted to a Compound annual growth rate (CAGR) over than 10 % reaching a market size of 4.55 billion Euros by 2021. This strongly increasing trend is due to not only by the petroleum prices fluctuations but especially by the worldwide stringent environmental regulations, forcing the companies to search for new strategies to reduce the carbon content in their products. Although some example of innovative materials have already seduced the customers (i.e. Bio-PET), the demand for eco-friendly plastics made from bio-based polymers is growing at a high rate. This is due to products such as PLA, PHA, and starch-based polymers finding increased applications thanks to their biodegradability (Comaniță et al., 2015; Research and Market, 2016).

Polyhydroxyalkanoates (PHAs) are polyesters, synthesized and stored as intracellular granules by numerous prokaryotes, which have attracted industrial attention as environmentally friendly and biodegradable alternatives to petroleum-based plastics. Two main groups of polymers can be classified depending on the number of carbons in the monomer units: short-chain length PHAs (scl-PHAs) having from 3 to 5 carbon atoms and medium-chain length PHAs (mcl-PHAs) having from 6 to 14 carbon atoms. Differences in repeating unit composition influence the physical properties of PHAs (Doi, 1990). scl-PHAs show thermoplastic material properties similar to polypropylene, while mcl-PHAs possess elastic material properties similar to rubber (Liu et al., 2011; Muhr et al., 2013). Due to their high elasticity and low crystallinity, mcl-PHAs polymers have emerged as suitable materials for novel applications in cosmetics, paint formulations, other coatings, medical devices and tissue engineering (Park and Lee, 2004).

Monomer composition of PHAs depends on the nature of the supplied carbon source and on the metabolic pathway fuelling precursors for PHAs biosynthesis (Tan et al., 2014) (Fig. 1). In view of process sustainability, the use of waste materials as carbon sources represents a valuable route to reduce mcl-PHAs production costs. However, the heterogeneous and not-reproducible nature of these wastes, together with the complexity of the metabolic routes fuelling PHA precursors, make the achievement of a biopolymer with defined composition a challenging goal to be pursued. As a fact, despite the high production levels achieved, 20-40% of cell dry weight (CDW), only polymers with mixed and variable compositions (from C8 to C16) have been produced from different *Pseudomonas* species fed with WFO as related C-sources (Cruz et al., 2016; Fernandéz et al., 2005; Follonier et al., 2014; Haba et al., 2007; Song et al., 2008).

Examples of PHAs production from fats rich waste raw material have been also reported for wild type and mutant variants of *Ralstonia eutropha* (Cruz et al., 2016; Riedel et al., 2014). In this case, the high production yields achieved (up to 100g/L) are linked to the production of P(3HB) or copolymer with only small amount of mcl monomers. Metabolic engineering strategies have been also applied to *Pseudomonas* species (Chung et al., 2011; Wang et al., 2011) as well as to *Escherichia coli* recombinant strains (Davis et al., 2008; Tappel et al., 2012), to achieve the production of mcl-homopolymers and/or mixed copolymers. However, only the feeding with defined fatty acids has shown to allow constant polymer composition.

An example of recombinant system able to convert WFO from homemade preparation in mcl-PHA with constant composition has been previously reported by our research group (Vastano et al., 2015). In this *E. coli* recombinant strain (*sPha*), monomers for PHA synthesis are fuelled only through the activity of recombinant ketoacyl-CoA reductase (PhaB). The lack of any other enzymes involved in the PHA pathways described by Tan et al. (2014) (PhaA, PhaJ, PhaG, etc.) allows the biopolymer production only when free fatty acids are used as carbon source. In this strain, a strict control of mcl-PHA repeating unit composition has been achieved by the presence of biosynthetic enzymes endowed with peculiar selectivity towards polymer precursors. As a fact, a P(HHx) near homopolymer has been produced by the developed recombinant system fed with different fatty acids in pure free form and also in complex matrices as homemade WFO preparations.

In this work, we aim to study the exportability of the above mentioned recombinant system on WFO deriving from catering services, characterized by high FFAs content. Different strategies have been tested for WFO valorisation into a high-added value product (mcl-PHA), including strain improvement and waste pretreatment. The idea behind the work is that microbial fermentation of WFO by properly designed strain, able to produce PHA from FFAs, but not secreting lipases, may turn out in a twofold advantage: allowing biopolymer production, and generating an “exhausted waste oil” with reduced FFAs content, thus more sustainingly exploitable for conversion in biodiesel.

2. Material and methods

2.1. Bacterial strains and growth conditions

Bacillus cereus 6E/2 was provided by University of Naples, Italy, culture collection. *Escherichia coli* Top 10 (Life Technologies, Monza, Italy) was used as host for gene cloning. *Escherichia coli* BL21 (DE3) (Novagen, Germany) was chosen as host for recombinant protein production (Table 1).

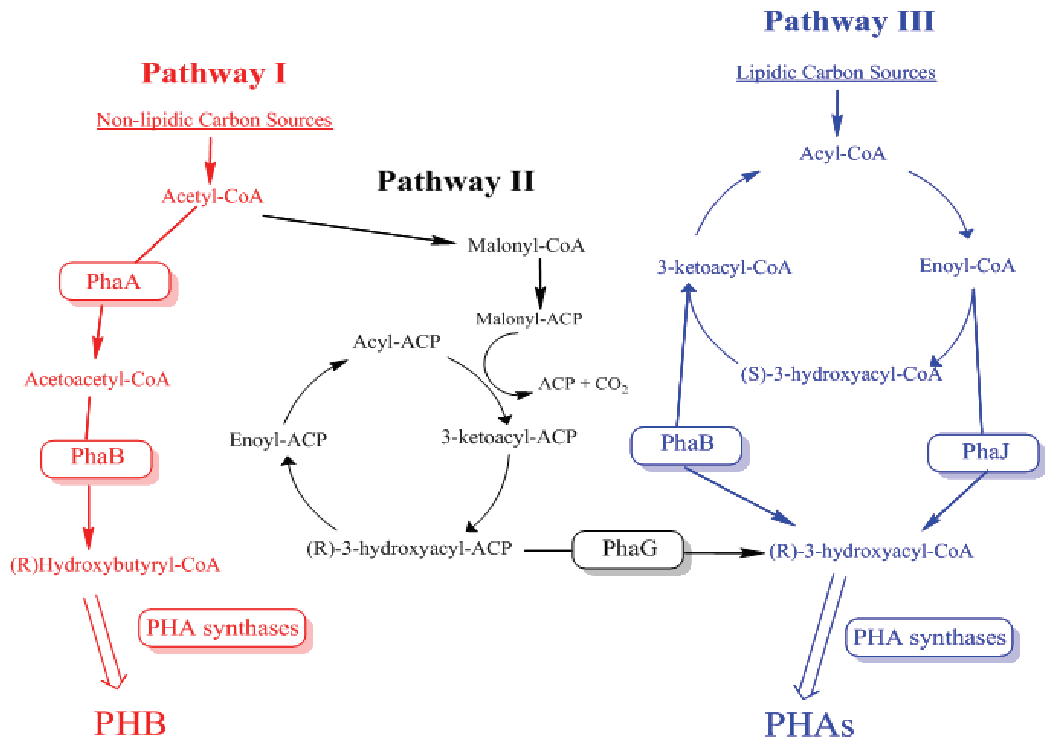


Fig. 1. The most representative metabolic pathways for PHA biosynthesis: Pathway I leads to the biosynthesis of PHB polymers by furnishing 3-hydroxybutyryl-CoA precursors from metabolism of non-lipidic carbon sources; Pathway II and III supply precursors for mcl-PHAs by channeling intermediates from fatty acid synthesis and degradation metabolisms respectively. The key enzymes responsible for diverting precursors towards polymer biosynthesis are highlighted. PhaA: β-ketothiolase; PhaB: ketoacyl-CoA reductase; PhaC: PHA synthases; PhaG: 3-hydroxyacyl ACP-CoA transacylase; PhaJ: enoyl-CoA hydratase

Table 1. Plasmids and bacterial strains

Plasmid or strain	***Relevant characteristics***	***Source or reference***
Plasmids		
pET40	kan^r, P_{T7lac}, pBR322 origin	Novagen
pET16SPHA	pET16b derivative, *B. cereus* 6E/2 *sphaRBC*$_{Bc}$	Vastano et al.(2015)
pET40A	pET40 derivative, *B. cereus* 6E/2 *phaA*$_{Bc}$	This study
DUETTOASPHA	Dual promoter vector pET16b-pET40 derivative, *B. cereus* 6E/2 *sphaRBC*$_{Bc}$ and *phaA*$_{Bc}$	This study
Strains		
B. cereus 6E/2	Wild-type	
Escherichia coli Top10	F^- *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara leu*) 7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G	Life technologies
E. coli BL21(DE3)	F^- *ompT hsdS*$_B$ (r_B^- m_B^-) *gal dcm* (DE3)	Novagen
sPha	*E. coli* BL21(DE3), pET16SPHA	Vastano et al.(2015)
A-sPha	*E. coli* BL21(DE3), DUETTOASPHA	This study

Table 2. Oligos sequences with highlighted restriction enzymes sites: *NdeI*, underlined; *KpnI*, double underlined; ***SphI***, bold

Oligonucleotide	***Sequence***
phaAfw	GCAATTACATATGAGAGAAGCTGTCATTGTTGCGGG
phaArev	CGTAAT**GCATGC**GGTACCTTATAGTAATTCAAACACTCCTGCTG
pET40-SphIfw	GGAATGGT**GCATGC**AAGGAGATGG

All bacterial strains were routinely grown in Luria-Bertani (LB) broth (Sambrook and Russell, 2011) at 37°C, supplemented with ampicillin (100 µg/mL) if transformed, unless otherwise specified. For PHA production, strains were grown in Minimal Medium (MM) broth boosted with 6 % v/v of WFOs (MM: 1g/L Bacto Tryptone, 1g/L Sodium Chloride, 0.5g/L Yeast Extract).

2.2. PHA production and analysis

For PHAs production, recombinant *E. coli* strains were cultured in 250 mL Erlemeyer flasks containing 50 mL sterile MM growth media. Flasks were incubated at 37°C on a rotary shaker at 250 rpm. 0.5 mM IPTG induction was performed at a defined time (5 h from the inoculum) due to the interference in optical density measurements caused by the turbidity of oil containing growth media. Effective induction of protein expression was verified by performing SDS-PAGE analyses according to standard methods (Ausubel et al., 1998).

For PHAs analysis, cells were harvested by centrifugation (11,120 rcf for 10 min), washed twice with hexane, and lyophilized. PHAs were converted to the corresponding monomer-esters by combining 1 mL of chloroform and 1 mL of 15% H_2SO_4 in methanol (v/v) with 2-5 mg of lyophilized cells in a 6 mL pyrex glass tube (Agnew et al., 2012). The mixture was heated at 100°C in a heat block for 3 h followed by neutralization with 2 mL of 100 mg/mL $NaHCO_3$ in water. The mixture was vortexed and centrifuged 797 rcf for 10min and the aqueous layer was removed by aspiration. The organic phase (1 µL) was analyzed using an Agilent Technologies 6850A gas chromatograph equipped with a mass spectrometer 5973N (Agilent Technologies Italia, Milan, Italy) and a Zebron ZB-5 Phenomenex, 30 x 0.25 mm i.d. column (He 1mL/min) (Phenomenex, Bologna, Italy). The injection was done in splitless mode with Tinjector at 230°C. The temperature programme used was as follows: 40°C → 240°C at 20°C /min, 240°C for 10 min. The MS was operated in scanning mode between 40 and 360 m/z. The identification of compounds was performed by comparison of mass spectra with standard ones. PHA contents (mg/L) were calculated by using calibration curves, which in turn were obtained by using either 3-hydroxybutyrate or 3-hydroxyhexanoate (Larodan Fine Chemicals, Sweden) supplemented with benzoic acid as internal standard. %PHAs was expressed as the ratio between produced PHAs (mg) and CDW (mg) of lyophilized cell material.

2.3. Construction of A-sPha strain

A PhaA coding gene (*phaA*$_{Bc}$) was amplified from *B. cereus* genomic DNA, extracted as previously described (Vastano et al., 2015). The oligonucleotide primers were designed on the basis of those reported by Davis and coauthors (Davis et al., 2008) for a *phaA* homologue (Table 2). Sequences for proper restriction sites were included in primer design for cloning in pET40 vector (Life Technologies, Monza, Italy).

The *promphaA*$_{Bc}$ sequence was then amplified from the cloning vector with the oligo pairs pET40-SphIfw/phaArev (Table 3), in order to add the T7 promoter sequence at the 5' extremity, and finally cloned in *SphI* digested pET16SPHA, obtaining DUETTOASPHA plasmid. Amplification was performed using High Fidelity Q5^R polymerase (New England Biolabs, Ipswich, Massachusetts, USA) according to manufacturer's instruction. All cloned PCR products were checked by sequencing (PRIMM Sequencing Service). Recombinant plasmids were transformed in *E. coli* strains using standard transformation methods (Sambrook and Russell, 2001).

2.4. WFOs suppliers

Yellow fluid (YF) WFO was collected by a local restaurant. Brown dense (BD) WFO has been supplied by FARGECO Srl, a regional service for waste oils collection from catering premises and food-factories.

2.5. Water extraction of WFOs

A defined amount of WFO was twice extracted with the same volume of room temperature water. The two phase solution was shacked until mixture results opaque. WFO-water dispersion was resolved by centrifugation (5,524 rcf for 10 min). Upper lipidic phase was collected, avoiding the white opalescence phase at interlayer, and a second step of extraction was undertaken. WFOs was autoclaved before use. Tap water, Demineralised water and Elix® water (Merck, s.p.a, Milan, Italy) have been tested in the extraction process.

2.6. FFAs calculation

Sample of 1mL of WFO was dissolved in 10mL of isopropanol with Phenolphthalein as pH indicator. %FFAs value was calculated according the Eq. (1) considering oleic acid as standard:

$$\%FFAs = (v - b) \times N / w \times 282 \times 100 \qquad (1)$$

where: *v* is the volume of titration solution; *b* is the volume of the titrant for isopropanol neutralization; N is the Normality of the titration solution; *w* is the weight of the oil sample; *282* is the molecular weight of oleic acid (g/mol). NaOH 0.025N solution was used as titration solution.

3. Results and discussion

3.1. PHA production from industrial WFOs

Two WFOs, furnished by two restaurants, were tested as raw materials for mcl-PHAs production. Although their characterization was out of the scope of this work, the two oil variants were labelled as YF (Yellow Fluid preparation; 4.2 % FFAs) and BD (Brown Dense preparation; 8.5 % FFAs) on the base of their different colour and density. The *E. coli sPha* recombinant system, already proved to be able to produce P(HHx) homopolymer from several household cooking oil preparations (up to 5% cell dry weight) (Vastano et al., 2015), was tested for polymer production in Minimum Medium boosted with 6% of both WFOs. However, no PHAs production was achieved in these conditions. Of course, differences between homemade and catering oil preparations can affect the polymer yield, as already observed among different domestic preparations (Vastano et al., 2015). This is probably due to several and uncontrolled parameters such as time and temperature of cooking, initial oil composition, compounds originated by cooked foodstuffs, etc.. In particular, "deep frying", the typical cooking technique for catering fries, might have generated a waste with peculiar composition.

In spite of the absence of PHAs production, microbial growth was not hampered by the presence of WFOs. This behaviour suggests that, in addition to FFAs, these WFOs may contain a higher amount of alternative C-sources (carbohydrates and/or amino acids) compared to WFOs from household preparations. These preferred nutrients may be utilized instead of FFAs, preventing their fuelling towards PHAs metabolic route.

In order to verify this hypothesis and promote biopolymer production, two alternative approaches have been carried out: *1)* genetic improvement of the producing strain, aimed at supporting PHA biosynthesis from non lipidic C-sources; *2)* implementation of the raw material, including a pre-treatment step to extract undesired carbon sources. These strategies are not exclusive alternatives allowing the production of different kind of biopolymers. As fact, according to the metabolic routes involved in the process (Fig. 1), the first approach may promote the conversion of WFOs into scl-mcl co-polymers (through Pathway 1 and 3), the second one, instead would produce a mcl-PHAs near-homopolymer (through Pathway 3).

3.2 Approach 1: engineering sPha strain for conversion of untreated WFOs

A new system allowing PHAs production from carbohydrate carbon sources, named *A-sPha,* was constructed. In addition to the *phaRBC* operon, this system expresses a β-ketothiolase coding gene from *B. cereus* (*phaA$_{B.c.}$*), whose product has been shown to be involved in the production of P(3HB) when expressed in *E. coli*. Considering the metabolic routes activated in the new engineered strain, a higher 3HB fraction is expected to be incorporated in the produced polymer with respect to *sPha* (Fig. 1).

The productive performances of *A-sPha* were tested in MM with each WFOs variant. However, as for *sPHA*, no PHA production was observed when the system was fed with YF or BD. The poor efficiency of PhaB$_{B.c.}$ in reducing acetoacetyl-CoA generated by PhaA$_{B.c}$ from pathway 1, may explain the absence of differences in *sPha* and *A-sPha* behaviour when fed with WFOs. As a fact, a high specificity towards the incorporation of 3-ketoacids with 6 carbon atoms into the growing polymer has been previously suggested for PhaB from *B.cereus* (PhaB$_{B.c.}$).

3.3. Approach 2: water extraction of the raw material

With the aim to remove the non-lipidic carbon sources from the waste, a pre-treatment step of WFOs consisting of an aqueous extraction, has been performed. Three kinds of water, with different pH and salts composition, were tested for the extraction step (Table 3) to assay the effects of these parameters in solubilizing non-lipidic compounds from WFOs.

Table 3. Properties of extraction waters

Water	*pH*	*Conductivity [µS/cm]*
Tap Water	8.3	696
Demineralised Water	7.1	28
Elix®Water	6.8	3

The pre-treated WFOs were added to MM medium and PHA production by *sPha* strain analyzed (Table 4).

Table 4. PHA production from *sPha* strain in the presence of WFO. ND, not detected; CDW, cell dry weight; PHA, polyhydroxyalkanoates; YF and BD, industrial waste frying oils preparations. The SD of each series of results was less than ±15%

WFOs	*Extraction Water*	*CDW [mg/L]*	*PHA amount [mg/L]*	*PHA [%]*
YF	Untreated	709.75	ND	ND
	Tap water	359.00	0.6	0.2
	Demineralized Water	377.63	0.6	0.2
	Elix®Water	362.75	1.4	0.4
BD	Untreated	466.25	ND	ND
	Tap water	409.87	3.5	0.8
	Demineralised Water	437.50	4.5	1.0
	Elix®Water	495.63	3.7	0.8

A reduction in microbial biomass was observed as a consequence of water extraction for YF WFO, independently of the water properties. This result is due to the removal of competitive nutrients. As expected, in this way the FFAs consumption was promoted, determining biopolymer production.

Comparing the biomass amount between the two untreated wastes, a lower CDW was obtained for BD WFO. The complexity and heterogeneity of these raw materials may explain this data. Moreover, in contrast with YF WFO, the water extraction treatment was effective in allowing PHA production but did not alter microbial growth.

No significant effect of the different extraction waters was observed in terms of CDW and PHA production. Up to 1% PHA production could be achieved after the pre-treatment using BD WFO. WFOs were easily recovered after microbial fermentation and characterized for the FFAs content. A FFAs reduction ranging of about 40 and 60% respectively for YF and BD WFOs has been verified.

Independently from WFOs and extracting water, a P(3HHx) near-homopolymer, with a quantity of 3-hydroxybutyrate not exceeding 1%, was produced. Despite the low level of accumulated biopolymer, this is the first reported example of an *ad hoc* designed recombinant strain able to produce an almost homopolymeric mcl-PHA from industrial WFOs. These results demonstrate the effectiveness of the pretreatment step in generating a raw material deprived of those competing C-sources (mainly carbohydrates and amino acids) that may have prevented the triggering of PHA synthesis in the untreated WFOs.

3.4. Design of a circular process for WFO valorization

The introduction of an upstream microbial fermentation step in the WFO to biodiesel conversion route was found to satisfy a two-fold purpose: converting the WFO into a high-added value mcl-PHA, and generating an "exhausted waste oil" more suitable for biodiesel production. The choice of a non-lipase secreting microorganism allowed to generate a waste with reduced FFAs without impairing tryacylglycerol content, with a potential advantage in terms of trans-esterification yield. Despite the lower PHA production level achieved in comparison with native biopolymer producers, such as *R. eutropha* or *Pseudomonas* species (Lee et al., 2012; Riedel et al., 2012; Riedel et al., 2014), the idea of no-competitiveness with respect to WFO conversion in biodiesel has been verified.

In addition, being potentially rich in soluble carbohydrates and proteins, the extraction water could be used as growing medium in alternative to MM medium tested in this study. Although only preliminary experiments have been carried out in this frame, extraction water did not hamper microbial growth and in some specific conditions, it allowed biopolymer production (data not shown). In Fig. 2 a possible circular process for WFOs valorisation is depicted. According to the idea of zero-waste process, a pyrolysis of residual biomass after biopolymer extraction has been proposed for production of bio-oil and bio-chair (Wei et al., 2015).

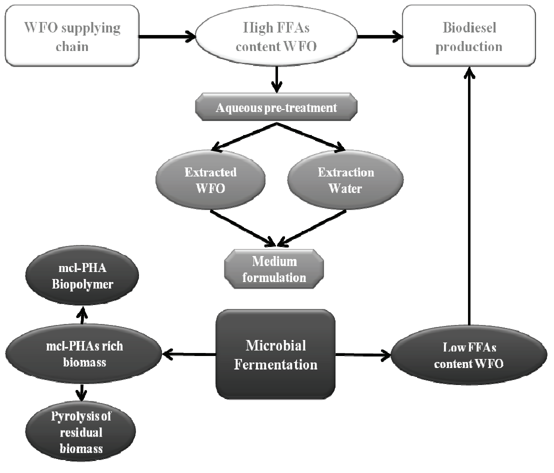


Fig. 2. Proposed circular process for WFOs valorization

4. Conclusions

In this study, a recombinant *E. coli* strain was tested for PHA production on two different catering oil preparations. Two different strategies were tested for WFO valorisation, including strain improvement and waste pretreatment. The two approaches would potentially result in the valorisation of WFOs into two kind of biopolymers differing in their monomeric composition.

Biopolymer production was achieved only after a WFO pretreatment step, which was helpful to remove most of the competing C-sources for microbial growth. Engineering the *E. coli* genetic makeup introducing a PhaA coding gene, did not turn out into polymer production and is a point that needs to be further investigated. Replacing the *B. cereus* - ketoreductase PhaB, endowed with a peculiar specificity towards C 6 monomeric precursors from fatty acid metabolism with a homologue able to act also on keto intermediate with 4 carbon atoms, could promote polymer production even in the presence of non lipidic carbon sources. The study also lays the bases for the development of a circular process allowing PHA production from WFO as well as a more advantageous reuse of the "exhausted waste oil" for biodiesel production. Optimization of polymer production yield can be achieved by further improvement of the recombinant *E. coli* genetic background, or in alternative by testing native producers after carefully knock-out of their lipase coding genes.

In conclusion, validation of the proposed process will require a strict setting up of all the process steps. Optimization of the water extraction protocol, the verification of the use of the extracted water as bacterial growth medium, as well as the effect on the transesterification process from the "exhausted waste oil" will be the key points to be further evaluated in terms of the overall economicity of the process.

Aknowledgments

This work was supported by grants from the Fondazione CARIPLO, Industrial Biotechnology Project BEETOUT - Sugar Beet biorefinery for the integrated production of biofuel and polyesters- FARGECO s.r.l.

References

Agnew D.E., Stevermer A.K., Youngquist J.T., Pfleger B.F., (2012), Engineering *Escherichia coli* for production of C12-C14 polyhydroxyalkanoate from glucose, *Metabolic Engineering*, **14**, 705-713.

Agnew R., Chai M., Lu M., Dendramis N., (2009), Making biodiesel from recycled cooking oil generated in campus dining facilities, *Sustainability*, **2**, 303-307.

Ausubel F.M., Brent R., Kingston R.E., Moore D.D., Seidman J.G., Smith J.A., Struhl K., (1998), *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc., NY.

Banani R., Youssef S., Bezzarga M., Abderrabba1 M., (2015), Waste frying oil with high levels of free fatty acids as one of the prominent sources of biodiesel production, *Journal of Materials and Environmental Science*, **4**, 1178-1185.

Chai M., Tu Q., Lu M., Yang Y.J., Chai M., Tu Q., Lu M., Yang Y.J., (2014), Esterification pretreatment of free fatty acid in biodiesel production, from laboratory to industry, *Fuel Processing Technology*, **125**, 106-113.

Chung A., Jin H., Huang L., Ye H.M., Chen J.C., Wu Q., Chen G.Q., (2011), Biosynthesis and characterization of poly(3-hydroxydodecanoate) by *β*-oxidation inhibited mutant of *Pseudomonas entomophila* L48, *Biomacromolecules*, **12**, 3559-3566.

Comaniţă E.D., Ghinea C., Hlihor R.M., Simion I.M., Smaranda L., Favier L., Roşca M., Gostin I., Gavrilescu M., (2015), Challenges and oportunities in green plastics: an assessment using the ELECTRE decision-aid method, *Environmental Engineering and Management Journal*, **14**, 689-702.

Corro G., Sanchez N., Pal U., Banuelos F., (2016), Biodiesel production from waste frying oil using waste animal bone and solar heat, *Waste Management*, **47**, 105-113.

Costa S., Lepine F., Milot S., Deziel E., Nitschke M., Contiero J., (2009), Cassava wastewater as a substrate for the simultaneous production of rhamno-lipids and polyhydroxyalkanoates by *Pseudomonas aeruginosa*, *Journal of Industrial Microbiology and Biotechnology*, **36**, 1063-1072.

Cruz M.V., Freitas F., Paiva A., Mano F., Dionisio M., Ramos A.M., Reis M.A.M., (2016), Valorization of fatty acids-containing wastes and byproducts into short- and medium - chain length PHAs, *New Biotechnology*, **33**, 206-215.

Davis R., Anilkumar P.K., Chandrashekar A., (2008), Biosynthesis of polyhydroxyalkanoates co-polymer in *E. coli* using genes from *Pseudomonas* and *Bacillus, Antonie Van Leeuwenhoek*, **94**, 207-216.

Dellomonaco C., Rivera C., Campbell P., Gonzalez R., (2010), Engineered respiro-fermentative metabolism for the production of biofuels and biochemicals from fatty acid-rich feedstocks, *Applied Environmental Microbiology*, **76**, 5067-5078.

Doi H., Hoshino Y., Nakase K., Usuda Y., (2014), Reduction of hydrogen peroxide stress derived from fatty acid beta-oxidation improves fatty acid utilization in *Escherichia coli*, *Applied Microbiology and Biotechnology*, **98**, 629-639.

Doi Y., (1990), *Microbial Polyesters*, VCH Publishers, New York.

Environment Agency, (2009), Quality Protocol – Biodiesel: End of waste criteria for the production and use of biodiesel derived from waste cooking oil and rendered animal fat, On line at: http://www.environment-agency.gov.uk/static/documents/Leisure/090612_Biodiesel_QP_V5_final.pdf

Fernandéz D., Rodriguez E., Bassas M., Vinas M., Solanas A.M., Llorens J., Marques A.M., Manresa A., (2005), Agro-industrial oily wastes as substrates for PHA production by the new strain *Pseudomonas aeruginosa* NCIB 40045: Effect of culture conditions, *Biochemical Engineering Journal*, **26**, 159-167.

Fernández C.M., Solana M., Fiori L., Rodríguez J.F., Jesús Ramos J.M., Pérez A., (2015), From seeds to biodiesel: extraction, esterification, transesterification and blending of *Jatropha curcas* oil, *Environmental Engineering and Management Journal*, **14**, 2855-2864.

Follonier S., Goyder M. S., Silvestri A., Crelier S., Kalman F., Riesen R., Zinn M., (2014), Fruit pomace and waste frying oil as sustainable resources for the bio-production of medium-chain-length polyhydroxyalkanoates, *International Journal of Biological Macromolecules*, **71**, 42-52.

Haba E., Vidal-Mas J., Bassas M., Espuny M.J., Llorens J., Manresa A., (2007), Poly 3-(hydroxyalkanoates) produced from oily substrates by *Pseudomonas aeruginosa* 47T2 (NCBIM 40044): Effect of nutrients and incubation temperature on polymer composition, *Biochemical Engineering Journal*, **35**, 99-106.

Hiroe A., Tsuge K., Nomura C.T., Itaya M., Tsuge T., (2012), Rearrangement of gene order in the *phaCAB* operon leads to effective production of ultra-high-molecular-weight poly[(R)-3-hydroxybutyrate] in genetically engineered *Escherichia coli*, *Applied and Environmental Microbiology*, **78**, 3177-3184.

Javidialessadi A., Raeissi S., (2013), Biodiesel production from high free fatty acid-content oils: experimental investigation of the pretreatment step, *APCBEE Procedia*, **5**, 474-478.

Jeon E.Y., Lee J.H., Yang K.M., Joo Y.C., Oh D.K., Park J.B., (2012), Bioprocess engineering to produce 10-hydroxystearic acid from oleic acid by recombinant *Escherichia coli* expressing the oleate-hydratase gene of *Stenotrophomonas maltophilia*, *Process Biochemistry*, **47**, 941-947.

Kulkarni M.G., Dalai A.K., (2006), Waste cooking oil an economical source for biodiesel: A review, *Industrial and Engineering Chemistry Research*, **45**, 2901-2913.

Lee J.H., Asbhy R.D., Needleman D.S., Lee K., Solaiman D.K.Y., (2012), Cloning, sequencing, and characterization of lipase genes from a polyhydroxyalkanoate (PHA)-synthesizing *Pseudomonas resinovoran, Applied Microbiology and Biotechnology*, **96**, 993-1005.

Leong Y.K., Show P.L., Ooi C.W., Ling T.C., Lan J.C., (2014), Current trends in polyhydroxyalkanoates (PHAs) biosynthesis: Insights from the recombinant *Escherichia coli*, *Journal of Biotechnology*, **180**, 52-65.

Liu Q., Luo G., Zhou X.R., Chen G.Q., (2011), Biosynthesis of poly(3-hydroxydecanoate) and 3-hydroxydodecanoate dominating poly-hydroxyalkanoates by *β*-oxidation pathway inhibited *Pseudomonas putida*, *Metabolic Engineering*, **13**, 11-17.

Ma F., Clements L.D., Hanna M.A., (1998), The effects of catalyst free fatty acids and water on transesterification of beef tallow, *Transactions of ASAE*, **41**, 1261-1264.

Muhr A., Rechberger E.M., Salerno A., Reiterer A., Schiller M., Kwiecien M., Adamus G., Kowalczuk M., Strohmeier K., Schober S., Mittelbach M., Koller M., (2013), Biodegradable latexes from animal-derived waste: Biosynthesis and characterization of mcl-PHA accumulated by *Ps. Citronellolis*, *Reactive and Functional Polymers*, **73**, 1391-1398.

Park S.J., Lee S.Y., (2004), New FadB homologous enzymes and their use in enhanced biosynthesis of medium-chain-length poly-hydroxyalkanoates in *fadB* mutant *Escherichia coli*, *Biotechnology and Bioengineering*, **86**, 681-686.

Research and Markets, (2016), Bioplastics & Biopolymers Market-Trends & Forecast to 2021, On line at: http://www.researchandmarkets.com/research/745tc2/bioplastics.

Riedel S.L., Bader J., Brigham C.J., Budde C.F., Yusof Z.A.M., Rha C., Sinkey A.J., (2012), Production of Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) by *Ralstonia eutropha* in high cell density palm oil fermentations, *Biotechnology and Bioengineering*, **109**, 74-83.

Riedel S.L., Lu J., Stahl U., (2014), Lipid and fatty acid metabolism in *Ralstonia eutropha*: relevance for the biotechnological production of value-added products, *Applied Microbiology and Biotechnology*, **98**, 1469-1483.

Rochester Institute of Technology, (2012), Biodiesel Workshop October 5, 2012 in Rochester, NY, How to Start Your Own Biodiesel Fuel Program, On line at: https://www.rit.edu/affiliate/nysp2i/sites/rit.edu.affiliate.nysp2i/files/biodiesel_workshop_presentation_2012-10-05.pdf.

Sambrook J., Russell D.W., (2001), *Molecular Cloning: A Laboratory Manual*, 3rd Edition, Cold Spring Harbor Laboratory Press, New York.

Sathesh-Prabu C., Lee S.K., (2015), Production of long-chain α,ω-dicarboxylic acids by engineered *Escherichia coli* from renewable fatty acids and plant oils, *Journal of Agricultural and Food Chemistry*, **63**, 8199-8208.

Smith H., Winfield J., Thompson L., (2013), The market for biodiesel production from used cooking oils and fats, oils and greases in London, Leading Resource Sustainability Consultancy, On line at: https://www.london.gov.uk/sites/default/files/the_market_for_biodiesel_production_from_ucos_and_fogs_in_london_-_september_2013.pdf.

Song J.H., Jeon C.O., Choi M.H., Yoon S.C., Park W., (2008), Poly-hydroxyalkanoate (PHA) production using waste vegetable oil by *Pseudomonas* sp. strain DR2, *Journal of Microbiology Biotechnology*, **18**, 1408-1415.

Spöttle M., Alberici S., Toop G., Peters D., Gamba L., Ping S., van Steen H., Bellefleur D., (2013), Low ILUC potential of wastes and residues for biofuels: straw, forestry residues, UCO, corn cobs, © Ecofys 2013 by order of: German Federal Ministry for the Environment, Nature Conservation and Nuclear Safety, German Federal Environment Agency, German Fachagentur Nachwachsende Rohstoffen, Dutch Ministry of Infrastructure and the Environment, Danish Energy Agency.

Tan G., Chen C., Li L., Ge L., Wang L., Razaad I.M. N., Li Y., Zhao L., Mo Y., Wang J., (2014), Start a research on biopolymer polyhydroxyalkanoate (PHA): A review, *Polymers*, **6**, 706-754.

Tappel R.C., Wang Q., Nomura C.T., (2012), Precise control of repeating unit composition in biodegradable poly(3-hydroxyalkanoate) polymers synthesized by *Escherichia coli*, *Journal of Bioscience and Bioengineering*, **113**, 480-486.

Toop G., Alberici S., Spoettle M., van Steen H., (2013), *Trends in the UCO market - Input to DRAFT PIR* –, © Ecofys 2013 by order of: Department for Transport of United Kingdom (DfT).

Vastano M., Casillo A., Corsaro M.M., Sannia G., Pezzella C., (2015), Production of medium-chain-length polyhydroxyalkanoates from waste oils by recombinant Escherichia coli, *Eng in Life Sci.*, **15**, 700-709.

Wang H., Zhou X., Liu Q., Chen G., (2011), Biosynthesis of polyhydroxyalkanoate homopolymers by *Pseudomonas putida*, *Applied Microbiology and Biotechnology*, **89**, 1497-1507.

Wei L., Liang S., Guho N.M., Hanson A.J., Smith M.W., Garcia-Perez M., McDonald A.G., (2015), Production and characterization of bio-oil and biochar from the pyrolysis of residual bacterial biomass from a polyhydroxyalkanoate production process, *Journal of Analytical and Applied Pyrolysis*, **115**, 268-278.

Environmental Engineering and Management Journal

September 2016, Vol.16, No. 9, 2011-2018
http://omicron.ch.tuiasi.ro/EEMJ/

"Gheorghe Asachi" Technical University of Iasi, Romania

EFFECT OF METHYLENE BLUE ON ELECTRON MEDIATED MICROBIAL FUEL CELL BY *Saccharomyces cerevisiae*

Ruggero Rossi*, Leonardo Setti

Department of Industrial Chemistry "Toso Montanari", University of Bologna, Bologna 40136, Italy

Abstract

Microbial fuel cells (MFCs) are bioelectrochemical reactors that directly convert the chemical energy stored in chemical bonds of organic substrates, to electrical energy. *Saccharomyces cerevisiae* was implemented as biocatalyst in the anaerobic anode compartment of an MFC using glucose as carbon source and methylene blue as electron mediator. Our results showed the effect of methylene blue on the microbial metabolism and the reversibility of the redox reaction of the shuttle mediator in the presence and the absence of a carbon source.

The implementation of this reaction in a yeast catalysed MFC suggested that the electron transfer from the cells to the anode was certainly limited by the mediator concentration in the solution.

However, a higher influence on the biopower generation of the MFC seemed to be linked to the yeast adsorption on the electrode surface than to the reduced methylene blue concentration in solution.

Key words: methylene blue, microbial fuel cell, *Saccharomyces cerevisiae*

Received: January, 2016; Revised final: July, 2016; Accepted: August, 2016

1. Introduction

Nowadays, one of the biggest technological challenges of our society is to obtain energy and chemical building blocks from renewable feedstock. Due to the discovery of the "electrogenicity" of some microbial communities (Potter, 1911) and of the capability of the latter to gain energy by transferring electrons from an electron donor, such as the organic matter, to an electron acceptor, such as oxygen (Logan et al., 2006), the international community was stimulated by the opportunity to produce sustainable energy from wastewater and renewable biomass (Duteanu et al., 2010). However, only a small fraction of the potential energy of wastewater can be exploited by the usual treatment (Mathuriya, 2014; McCarty et al., 2011).

A fascinating alternative to the traditional technologies is represented by the microbial electrochemical technologies (METs) in which microorganisms are used to convert the chemical energy stored in biodegradable materials directly to electric energy by specific microbial fuel cell (MFC) (Logan and Rabaey, 2012).

Literature reports MFC catalysed by eukaryotic microorganisms (Potter, 1911), even though the efficiency of direct electron transfer by the prokaryotic cells made the scientists concentrate their efforts on the characterization of the electron transfer mechanism only of bacteria (Cohen, 1931; Logan, 2009). Thus, two main mechanisms of electron transfer between cells and electrode have been identified: direct electron transfer (DET) and mediated electron transfer (MET). The first one is accomplished through the physical contact of the cellular membrane or organelle with the electrode while in the second one the electron transfer is carried out thanks to the presence of mediator molecules, which operate in extracellular conditions as well as in endocellular one (Schröder, 2007).

Has been demonstrated that prokaryotic bacteria like *Shewanella putrefaciens* (Baron et al.,

---

[*] Author to whom all correspondence should be addressed: e-mail: ruggero.rossi2@unibo.it; Phone: +0039 0512093672

2009), *Geobacter sulfurreducens* (Bond and Lovley, 2002) and *Rhodoferax ferrireducens* (Chaudhuri and Lovley, 2003) are able to transfer the electrons directly on the electrode through cytochromes into the outer cell membrane or microbial nanowires (Lovley, 2008).

Because of the higher physiological complexity, as well as of the absence of nanowires, eukaryotic cells need a mediator for the electrochemical connection of cells to the anode in MFC (Gunawardena et al., 2008; Villaseñor Camacho et al., 2014). However, the regular addition of exogenous mediators is a deeply criticized practice, mainly due to the poisoning of the medium (Schröder, 2007). Nevertheless, in recent years, the development of microbial fuel cells catalysed by eukaryotic microorganism, like yeasts, has attracted attention. Yeasts, in fact, could represent the perfect biocatalyst for MFC because of their wide substrate ranges, their robustness and their being widely used in industry (Mao and Verwoerd, 2013; Schaetzle et al., 2008). Nowadays the performances of the yeast fuel cells are doubtless not comparable with those of the prokaryotic based MFC (Hubenova and Mitov, 2015), even though a large improvement could be possible if the electron transfer mechanism between cells and electrode was better explained (Mao and Verwoerd, 2013). Many publications have dealt with a lot of different strains of eukaryotic cells in the presence or absence of mediators (Hubenova and Mitov, 2015).

Methylene blue has been commonly used as electron shuttle in yeast-based biofuel cell (Hubenova and Mitov, 2015; Rossi et al., 2015) since it is electrochemically active, not toxic to the microorganism and soluble and chemically stable in water (Babanova et al., 2011).

However, the effect of methylene blue on the microbial metabolism of the common baker's yeast, *Saccharomyces cerevisiae*, has not been previously reported. In this study we have evaluated the effects of the presence of methylene blue on the fermentation of the *S. cerevisiae* cells and the influence of this mediator in the anodic chamber of a lab-scale MFC. We have used two separate glass chambers and salt bridge to connect the half-cells of the MFC in order to better understand the electron transfer mechanism in the anode compartment, rather than on the power production of the cell.

2. Materials and methods

All chemicals and reagents used for the experiments were analytical grades and supplied by Sigma-Aldrich.

2.1. Chromatographic studies

Chromatographic studies were performed at the same operative conditions of the electrochemical cell. Crude samples of the fermentation broth were filtered using 0.20 μm Sartorius Stedim Minisart® syringe tip filter after different times of starvation. Filtered aliquots of 10 μL were injected in an HPLC operating at a flow rate of 0.6 $mLmin^{-1}$ and the HPLC column was heated to 60°C. All analyses were run on an Agilent 1260 Infinity Quaternary LC equipped with UV DAD (G4212B) and RID (G1362A) detector. Rezex ROA-Organic acid, dimensions: 300 x 7.8 mm (Phenomenex Inc., Torrance, California) was used as column.

2.2. Spectrophotometric studies

The cells (0.25 gL^{-1} dry weight) were harvested by centrifugation and washed twice with 0.1 M phosphate buffer pH 7.8 then methylene blue was added until got to 9 μM. The solution was then stored at 30°C with different glucose concentrations in sealed quartz cuvette (Hellma). After 19, 20, 43, 44, 67, 68, 91 h the absorbance was measured either at 750 nm and 664 nm. After 19, 43 and 67 h the solution was aerated.

2.3. Construction of the Fuel Cell and electrochemical measurements

The fuel cell presented two glass chambers (0.10 L) connected by a salt bridge, the working volume was 0.05 L (50% of total). The salt bridge was a solution of KCl 10 gL^{-1} in phosphate buffer 0.1M at pH 7.8, stirred and heated until it reached 85°C, then Agar was added until it got to 2.5 gL^{-1}. The solution was poured into a silicon tube 29 cm long, with an 8 cm internal diameter. The anodic chamber was purged with gaseous nitrogen (14 Lh^{-1}) during all the experiment. Anodic and cathodic solutions were stirred continuously at the same rotational speed for all the experiments and the temperature of each chamber was 27.5 ± 2.5°C. Sampling was avoided in the MFC in order to limit the volume alteration.

MFCs operated under different conditions in the cathode compartment using oxygen or hydrogen peroxide as electron acceptor. An aerated solution of HCl 0.2 M or hydrogen peroxide 4.41 M in phosphate buffer 25 mM pH 6.0 was used as cathodic solution. Saccharomyces cerevisiae cells (baker's yeast, CLECA S.p.a. Mantova Italy) were used as a biocatalyst. Anodic solution was 5 gL^{-1} sample of the dried yeast and glucose 27.8 mM in phosphate buffer 0.1 M pH 7.8 in presence of methylene blue in concentration from 0 to 5 mM.

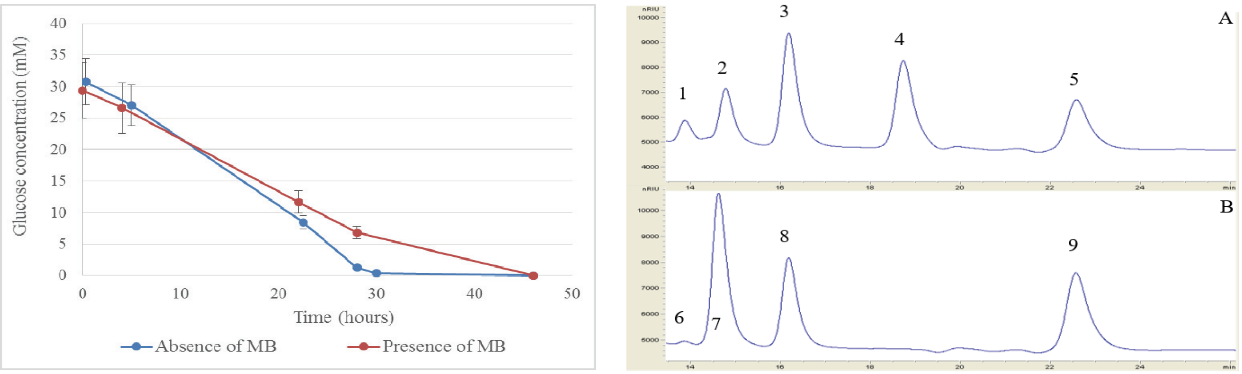
After different times of starvation the methylene blue was completely reduced and the anodic solution resulted completely discolored. The cell circuit was then closed and i-V curves were recorded. The electrode was inserted immediately after the inoculum or after the completely discoloration of the methylene blue solution. As far as the experiments carried out in the absence of methylene blue, the cell performances were recorded after having waited the same period of time. The electrodes in the two chambers were 7.8 cm^2 open area graphite rods connected with copper wire to the electrochemical system.The current-voltage, i-V, characteristics were measured from -0.1 V to round up OCV at the scan rate of 10 mVs^{-1} by an electrochemical measurement system (Keithley series 2400 Multimeter, Keithley Instrument Inc.).

3. Results and discussion

3.1. Effects of Methylene Blue on Saccharomyces cerevisiae metabolism

The effects of the mediator on the *Saccharomyces cerevisiae* metabolism were tested by HPLC analysis on fermentation reactors with yeast incubated both in the presence and in the absence of the methylene blue, using glucose (27.8 mM) as electron donor (Fig. 1). Chromatographic analysis at different times of incubation demonstrated that the glucose was completely depleted after 42 h both in the presence and absence of methylene blue (Fig. 1). Furthermore, preliminary chromatographic analysis of the end-products showed different products of the yeast metabolism in the presence or absence of methylene blue (Table 1). This result suggested that methylene blue could affect the yeast metabolism by activating new metabolic pathways to be deeply investigated in the next future.

In biochemistry, methylene blue is a well-known redox indicator; in fact, the solution of the dye results blue, with a maximum adsorption at 664 nm, in oxidizing conditions while it is colourless when it is exposed to reducing agents (Mowry and Ogren, 1999). The reduction of the methylene blue by yeast cells was followed by recording the decolourization of the blue solution in the absence of oxygen as shown in Fig. 2.



a) b)

Fig. 1. Glucose consumption (a) and end-products of fermentation (b) in the absence (A) and in the presence (B) of methylene blue (MB)

Table 1. HPLC analysis data of crude samples of fermentation broth in the presence or the absence of methylene blue after 42 hours of incubation

Peak number	***Presence/Absence of methylene blue***	***Compound***	***Retention time (min.)***	***Concentration (mM)***
1	Absence	Lactic acid	13.8	1.17 ± 0.03
2	Absence	Glycerol	14.7	2.0 ± 0.4
3	Absence	Acetic acid	16.1	11.40 ± 0.06
4	Absence	Unknown	18.7	/
5	Absence	Ethanol	22.5	1.00 ± 0.01
6	Presence	Lactic acid	13.8	0.14 ± 0.09
7	Presence	Formic acid	14.6	2.27 ± 0.03
8	Presence	Acetic acid	16.1	8.7 ± 0.2
9	Presence	Ethanol	22.5	1.54 ± 0.05

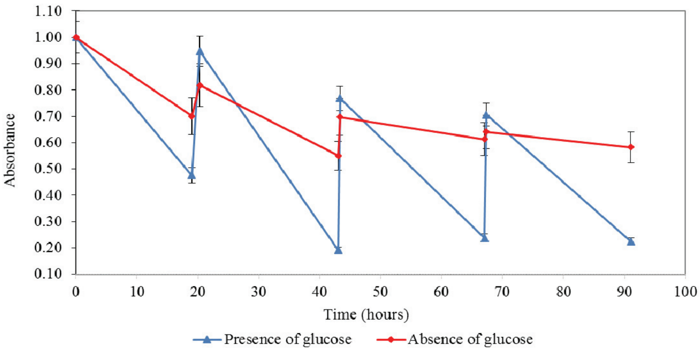


Fig. 2. Normalized values of absorbance at 664 nm carried out at different incubation times in the presence (blue line) or in the absence (red line) of glucose. After 19, 43 and 67 hours the solution was aerated

Suspension of yeast cells in phosphate buffer at pH 7.8 showed a fast reduction of methylene blue even in the absence of glucose as electron source. After 19h, the yeasts cell suspension was aerated causing the instantaneous oxidation of methylene blue. The suspension was then sealed again observing, consequently, a new reduction of methylene blue. This redox cycle was repeated almost every 20h and the results in terms of normalized absorbance values of the solution were reported in Fig. 2. Alternation of methylene blue reduction and oxidation reactions into the yeast cell suspension in the absence of glucose suggested the presence of an unknown reduced compound in the solution which could be probably attributed to an accumulated residual carbon source from the yeast production or the presence of glycogen inside the cells (François and Parrou, 2001). After four cycles the reduction step of methylene blue in the absence of glucose did not take place anymore as a consequence of the exhaustion of the accumulated carbon source into the yeast cells.

Furthermore, after each redox cycle a progressive decrease in the concentration of the oxidized form of the methylene blue was observed. This result was probably due to a biochemical transformation of the methylene blue during the redox cycles as well as to its accumulation into the cells as demonstrated in previous paper (Rossi et al., 2016).

This experiment shows that an electron acceptor like oxygen can immediately oxidize the reduced methylene blue in solution and it is possible to continuously repeat the redox cycle of methylene blue until there is enough carbon source in solution. This original redox cycle was used in all electrochemical experiments in order to prepare the yeast before the use in the MFC, thus avoiding the interference due to the accumulated carbon sources.

3.2. Microbial fuel cell using oxygen at the cathode

Yeast worked as biocatalyst in the MFC and used the glucose in the anode chamber as the only carbon source. The electrons derived from the oxidation of glucose were consumed in the cathode chamber reacting with oxygen.

Open Circuit Voltage (OCV) and power density (PD) resulted closed to zero in the presence of only glucose while, in the presence of only methylene blue, increasing values of the voltage and power were observed reaching 0.24 Volt and 10 μWcm^{-2} after 400 minutes, respectively (Fig. 3).

The contemporary presence of glucose and methylene blue led to a further increase of OCV and of the power density at 0.27 Volt and 15 μWcm^{-2}, respectively. These trends could be explained by a progressive accumulation of reduced methylene blue in solution due to both the reductive condition in the anode chamber and the presence of reducing sugars such as glucose.

In the presence of the yeast, the reduction of methylene blue increased largely as demonstrated by the complete discoloration of the solution in the anode chamber. OCV and power density reached the maximum values of 0.46 V and 45 μWcm^{-2}, respectively. The yeast had great potential in reducing methylene blue, showing an important role in enhancement of the bioelectricity generation of the MFC.

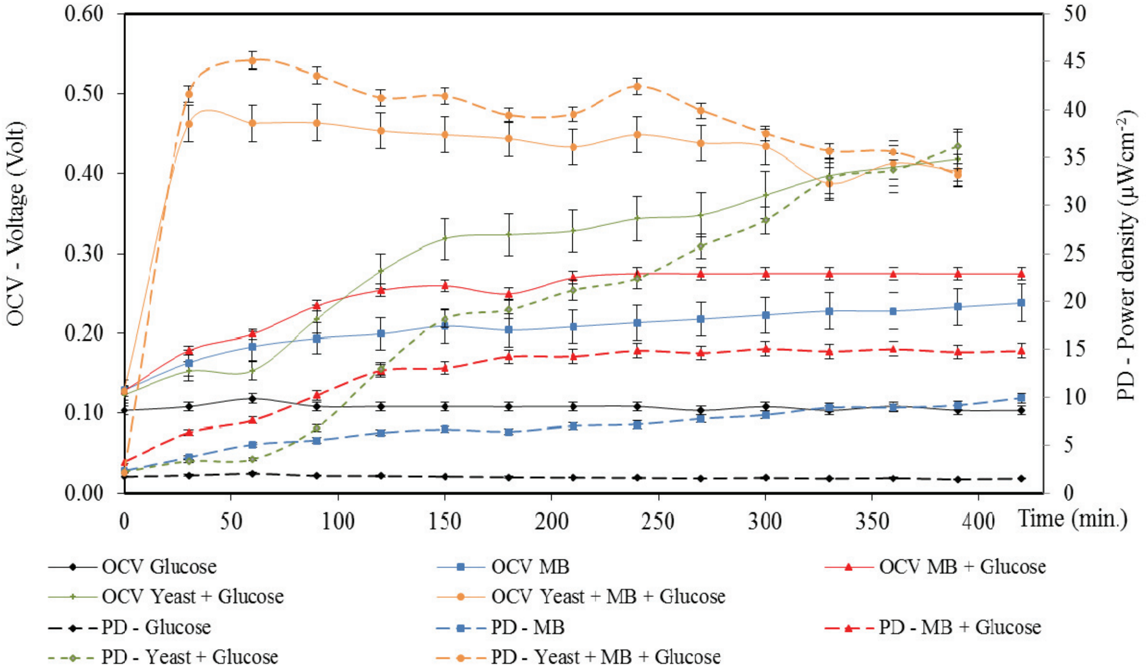


Fig. 3. Comparison of Open Circuit Voltage (OCV) and power density (PD) values carried out in the presence of each single component (glucose, methylene blue (MB) 5.00 mM) and yeast) or in different combinations

Electron mediators, such as methylene blue, were demonstrated necessary to improve the power output of the MFC (Najafpour et al., 2010), even if methylene blue showed a positive answer to the current generation also in the absence of the yeast.

However, the solution in the anode chamber did not turn blue when the circuit of the electrochemical cell was closed as shown in Fig. 2, suggesting that the slow step of the reaction was related to a limited diffusion of the reduced methylene blue to the electrode in the anode compartment and/or to a weak electrons transfer from the electrode to the oxygen in the cathode compartment. Furthermore, the negative slope of the power density of the MFC in the presence of yeast, methylene blue and glucose was related to the depletion of oxygen in the cathode chamber and the slow rate of oxygen diffusions in solution.

3.3. Microbial fuel cell using hydrogen peroxide at the cathode

In the previous experiment, the presence of oxygen in the cathode chamber showed a limiting factor in the control of the electron acceptor concentration. On this basis, oxygen was replaced by hydrogen peroxide in the cathode chamber and the open circuit voltage (OCV) and the power density (PD) under different operative conditions of the MFC were recorded. In order to evaluate the performances of the MFC with the anode to its full potential all the experiments were conducted starting with methylene blue completely reduced. The operative conditions of the microbial fuel cells were chosen in order to have a similar behaviour to that observed using oxygen as electron acceptor even if OCV and power density of methylene blue and glucose in the absence of yeast resulted higher in the presence of oxygen at the cathode (Fig. 4). The positive response under reducing conditions was then further confirmed using hydrogen peroxide as electron acceptor. The values of power density were double in the presence of methylene blue and yeasts in comparison with the values without the electron mediator. However, using hydrogen peroxide at the cathode, the performances of the MFC were more stable and higher OCV and power density values were reached at the end of the experiment in respect to oxygen as electron acceptor.

Our findings showed that the bioelectricity production of the MFC was dependent upon the methylene blue concentration even though the capability of the yeast to favour the electron transfer to the electrode was also observed in the absence of mediator (Figs. 5 and 6). The increase in the methylene blue concentration in the anode chamber increased the values of both OCV and PD.

When the electrode was submerged into the anode chamber after the complete reduction of methylene blue by yeasts (not incubated electrode), OCV and power density progressively increased until they reached the same values obtained while maintaining the electrode submerged into the solution during the reduction step (incubated electrode) (Figs. 5 and 6). These results suggested a diffusive regime depending on the migration of the yeast instead of the methylene blue onto the electrode surface.

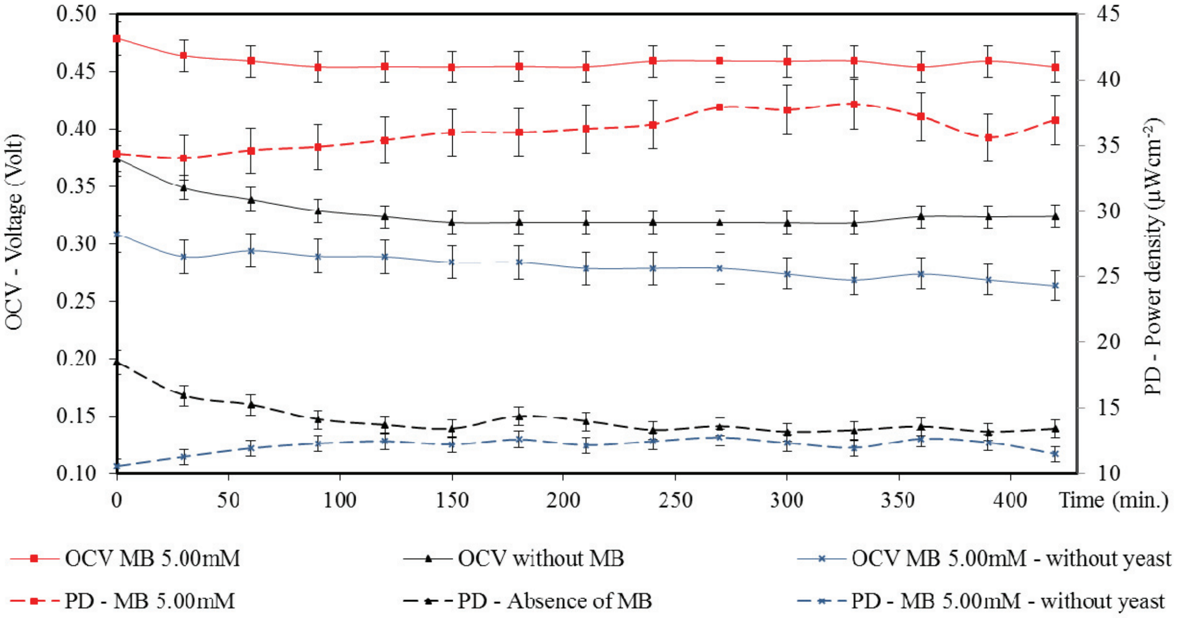


Fig. 4. Comparison of Open Circuit Voltage (OCV) and power density (PD) values carried out at different times of incubation with different concentration of methylene blue (MB) and yeast cells

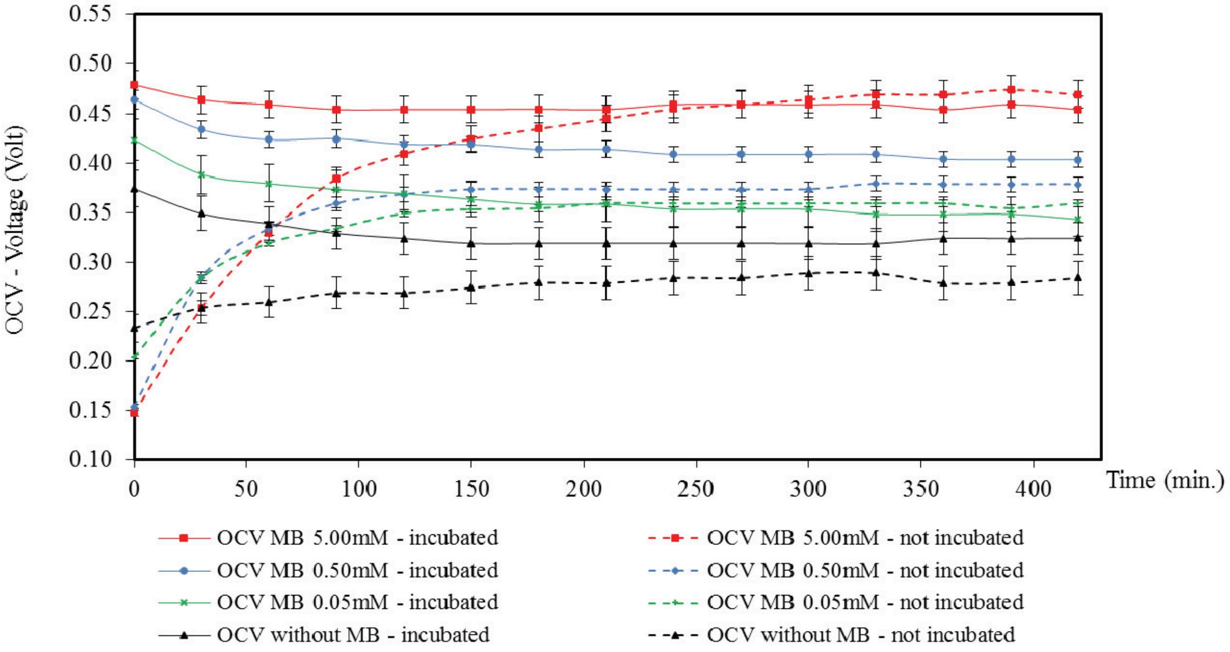


Fig. 5. Open Circuit Voltage (OCV) values carried out at different time of incubation with the electrodes incubated in solutions during the methylene blue (MB) reduction step (incubated) and electrodes inserted only 30 min. before the first measurement (not incubated)

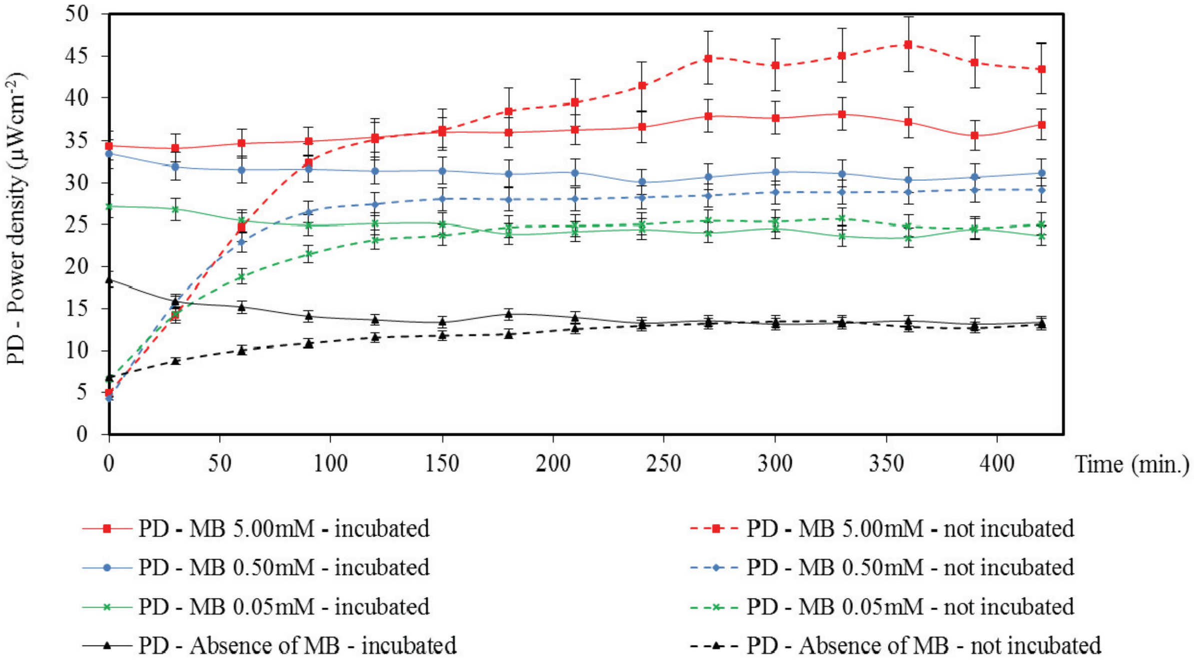


Fig. 6. Power density values carried out at different time of incubation with the electrodes incubated in solutions during the methylene blue (MB) reduction step (incubated) and electrodes inserted only 30 min. before the first measurement (not incubated)

The diffusive regime outlined that the electron transfer depended more on the adhesion of the cells on the surface of the electrode than by the diffusion of the reduced mediator on the electrode.The consistency of the OCV and PD values of the incubated and not incubated electrode after 420 min of operation was demonstrated in Fig. 7. Increasing the methylene blue concentration showed a non-linear trend in respect to the values of OCV and PD. This behavior could be explained by a stable micro-environment reached on the surface of the anode, probably due to the adsorption of cells.

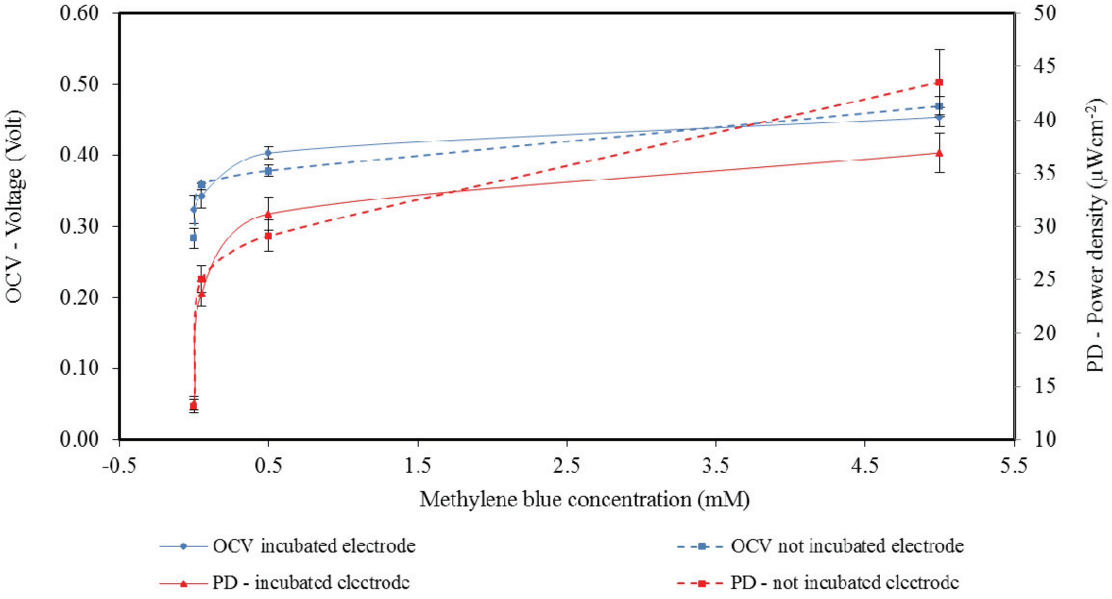


Fig. 7. Open Circuit Voltage (OCV) and power density values of incubated and not incubated electrode at different methylene blue concentrations after 420 min. of MFC reaction

4. Conclusions

Our results showed that the presence of methylene blue did not greatly affect the glucose consumption by the yeast cells such as *Saccharomyces cerevisiae*, but the presence of the mediator had a large impact on the final products of the fermentation.

Our findings demonstrated the reversibility of the redox reaction of methylene blue in the presence and in the absence of oxygen as electron acceptor. This effect was observed both in the presence and in the absence of glucose as energetic source. We have attributed this phenomena to an accumulated carbon source into the yeasts such as glycogen. After several redox cycles, no further reduction of the methylene blue was observed in the absence of glucose due to the exhaustion of the accumulated carbon source.

The application of the reaction between methylene blue and yeasts to the MFC in the presence of oxygen as electron acceptor showed a diffusion-limited behaviour both in the anode and in the cathode compartment.

The electron transfer mechanism depended on the capability of the yeasts to adhere on the electrode; in fact, when the electrode was not incubated in the anodic chamber during the methylene blue reduction, the current density reached its maximum after 100 min. with respect to immediate response obtained by the incubated electrode. Today, the activity of methylene blue is known to mediate the electron transfer between cells and electrode; however, our findings demonstrated that the limiting factor is probably due to the cells adhesion on the electrode surface.

Finally, using hydrogen peroxide as electron acceptor in the cathode compartment improved the stability of performances and the relation between the time of incubation and the power density of the MFC was showed.

Our findings could open new interesting perspectives on the effect of methylene blue in the yeast metabolic pathway to be investigated.

References

Babanova S., Hubenova Y., Mitov M., (2011), Influence of artificial mediators on yeast-based fuel cell performance, *Journal of Bioscience and Bioengineering*, **112**, 379-387.

Baron D., LaBelle E., Coursolle D., Gralnick J.A., Bond D.R., (2009), Electrochemical measurement of electron transfer kinetics by *Shewanella oneidensis* MR-1, *The Journal of Biological Chemistry*, **284**, 28865-28873.

Bond D.R., Lovley D.R., (2002), Electricity production by *Geobacter Sulfurreducens* attached to electrodes, *Science*, **295**, 483-485.

Chaudhuri S., Lovley D.R., (2003), Electricity oxidation by direct oxidation of glucose in mediatorless microbial fuel cell, *Nature Biotechnology*, **21**, 1229-1232.

Cohen B., (1931), The bacterial culture as an electrical half-cell, *Journal of Bacteriology*, **21**, 18-19.

Duteanu N.M., Ghangrekar M.M., Erable B., Scott K., (2010), Microbial fuel cells – an option for wastewater treatment, *Environmental Engineering and Management Journal*, **9**, 1069-1087.

François J., Parrou J.L., (2001), Reserve carbohydrates metabolism in the yeast *Saccharomyces cerevisiae*, *FEMS Microbiology Reviews*, **25**, 125-145.

Gunawardena A., Fernando S., To F., (2008), Performance of a yeast-mediated biological fuel cell, *International Journal of Molecular Science*, **9**, 1893-1907.

Mowry S., Ogren P.J., (1999), Kinetics of methylene blue reduction by ascorbic acid, *Journal of Chemical Education*, **76**, 970-973.

Hubenova Y., Mitov M., (2015), Extracellular electron transfer in yeast-based biofuel cells: A review, *Bioelectrochemistry*, **106**, 177-185.

Logan B.E., (2009), Exoelectrogenic bacteria that power microbial fuel cells, *Nature Reviews Microbiology*, **7**, 375-381.

Logan B.E., Hamelers B., Rozendal R., Schröder U., Keller J., Freguia S., Aelterman P., Verstraete W., Rabaey R., (2006), Microbial fuel cells: methodology and technology, *Environmental Science and Technology*, **40**, 5181-5192.

Logan B.E., Rabaey K., (2012), Conversion of wastes into bioelectricity and chemicals by using microbial electrochemical technologies, *Science*, **337**, 686-690.

Lovley D.R., (2008), The microbe electric: conversion of organic matter to electricity, *Current Opinion in Biotechnology*, **19**, 564-571.

Mathuriya A.S., (2014), Enhanced tannery wastewater treatment and electricity generation in microbial fuel cell by bacterial strains isolated from tannery waste, *Environmental Engineering and Management Journal*, **13**, 2945-2954.

Mao L., Verwoerd W.S., (2013), Selection of organisms for systems biology study of microbial electricity generation: a review, *International Journal of Energy and Environmental Engineering*, **4**, 1-18.

McCarty P.L., Bae J., Kim J., (2011), Domestic wastewater treatment as a net energy producer-can this be achieved?, *Environmental Science & Technology*, **45**, 7100-7106.

Najafpour G.D., Rahimnejad M., Mokhtarian N., Wan Ramli Wan Daud, Ghoreyshi A.A., (2010), Bioconversion of whey to electrical energy in a biofuel cell using *Saccharomyces cerevisiae*, *World Applied Sciences Journal*, **8**, 01-05.

Potter M.C., (1911), Electrical effects accompanying the decomposition of organic compounds, *Royal Society B*, **84**, 260-276.

Rossi R., Fedrigucci A., Setti L., (2015), Characterization of electron mediated microbial fuel cell by *Saccharomyces cerevisiae*, *Chemical Engineering Transactions*, **43**, 337-342.

Rossi R., Cavina M., Setti L., (2016), Characterization of electron transfer mechanism in mediated microbial fuel cell by entrapped electron mediator in *Saccharomyces cerevisiae*, *Chemical Engineering Transactions*, **49**, 559-564.

Schaetzle O., Barrière F., Baronian K., (2008), Bacteria and yeasts as catalysts in microbial fuel cells: electron transfer from micro-organisms to electrodes for green electricity, *Energy & Environmental Science*, **1**, 607-620.

Schröder U., (2007), Anodic electron transfer mechanism in microbial fuel cells and their energy efficiency, *Physical Chemistry Chemical Physics*, **9**, 2619-2629.

Villaseñor Camacho J., Montano Vico M.C., Andrés M., Rodrigo R., Fernández Morales F.J., Cañizares Cañizares P., (2014), Energy production from wastewater using horizontal and vertical subsurface flow constructed wetlands, *Environmental Engineering and Management Journal*, **13**, 2517-2523.

Environmental Engineering and Management Journal *September 2016, Vol.15, No. 9, 2019-2027*
http://omicron.ch.tuiasi.ro/EEMJ/

"Gheorghe Asachi" Technical University of Iasi, Romania

PRODUCT ENVIRONMENTAL FOOTPRINT IN THE OLIVE OIL SECTOR: STATE OF THE ART

Carlo Russo[1]*, Giulio Mario Cappelletti[1], Giuseppe Martino Nicoletti[1], George Michalopoulos[2], Claudio Pattara[3], Juan Antonio Polo Palomino[4], Hanna L. Tuomisto[5]

[1]Department of Economics, University of Foggia, Italy
[2]RodaxAgro Ltd, Kottou 48, Metamorphosis, Attikis, Athens, Greece
[3]Department of Economics, University "G. d'Annunzio", Pescara, Italy
[4]CO_2 Consulting, Córdoba, Spain
[5]European Commission, Joint Research Centre, Institute for Environmental and Sustainability

Abstract

As a part of the development of the Environmental Footprint (EF) guidelines, in June 2014 the European Commission started 11 pilot projects for the development of Product Environmental Footprint Category Rules (PEFCRs) for food, feed and beverage products. The PEFCRs are developed by technical secretariats involving various stakeholders from industries, academia, governments, trade unions and non-governmental organisations. This paper presents the state of the art of developing the PEFCR for the olive oil sector. The functional unit is defined as a litre of packed olive oil and the system boundaries cover the whole supply chain from cradle-to-grave. A screening study that estimated the EF of the average olive oil consumed in the European markets showed that olive production phase had the highest contribution to most environmental impact categories. The results of the screening study provide a benchmark that will be further adjusted after the current draft PEFCR will be tested in case studies for real products.

Key words: environmental impacts, life cycle assessment, olive oil, product environmental footprint

Received: January, 2016; Revised final: September, 2016; Accepted: September, 2016

1. Introduction

1.1. Environmental Footprint

In the context of the Communication "Building the Single Market for Green Products" (COM 196, 2013), the European Commission (EC) recommends a method to measure the environmental performance of products, named the Product Environmental Footprint (PEF) (EC, 2013 a, b). The PEF is a multi-criteria measure of the environmental performance of goods and services from a life cycle perspective. PEF studies are produced for the overarching purpose of seeking to reduce the environmental impacts associated with goods and services, taking into account supply chain activities (from extraction of raw materials, through production and use, to final waste management). As the "Guidelines for the implementation of the PEF (EC, 2016) are overall guidelines that have to be applicable to all products, PEFCRs aim to define specific aspects for calculating and reporting life cycle environmental impacts of products in a harmonised way. For these reasons, 25 PEF pilot projects started in 2014 by involving various stakeholders for the development of PEFCRs in a process that includes public consultations, reviews and approvals by the Environmental Footprint pilot Steering Committee that includes representatives from each pilot, European Union (EU) Member States and NGOs. In June 2014, 11 pilots for the sectors food, feed and beverage started (Benini et al.,

---

* Author to whom all correspondence should be addressed: e-mail: carlo.russo@unifg.it; Phone: +39 0881 781732

2014). They include beer, coffee, dairy, feed, pet food, seafood, meat, pasta, packed water, olive oil and wine.

1.2. EF Olive oil pilot project

The Technical Secretariat (TS) of the olive oil pilot, consisting or representatives from industry, academia, governments and NGOs, is responsible for the design and drafting of the PEFCR. The first task of the TS was to define the scope of the PEFCR and the representative product that describes the average product consumed in the European markets. A public consultation for the scope and representative product of olive oil was organised in October-November 2014, and the SC approved the documents in February 2015.

The second phase involved carrying out a screening study in order to identify the most contributing life cycle stages, processes, environmental impact categories and elementary flows of the representative product defined in the scope document. The screening report was submitted to the reviewers appointed by the EC, and after the revising phase, it was approved in November 2015.

The results of the full screening study were used as a basis for the draft PEFCR for olive oil, which went through a public stakeholder consultation in November-December 2015, and was approved by the EF SC in January 2016. Successively, the draft PEFCR are in testing in supporting studies, which are applying the PEFCR for real products. During the supporting studies, various ways of communicating the environmental footprint results to consumers and businesses will be tested, including a SME tool based on ICT (application on smartphone). The PEFCR will be revised based on the lessons learned from the supporting studies, after which the stakeholders have another opportunity to provide comments on the PEFCR. Before final approval of the PEFCR by the EF SC, the PEFCR will be reviewed by external reviewers. The final PEFCRs are scheduled to be released by beginning of 2017.

In this paper, we present an overview of the methods and results of the screening study that modeled the impact of an average olive oil consumed in the European markets. In particular, the paper aims to describe the methodological issues behind the definition of the life cycle assessment of a 'virtual olive oil' (representative product) defined as average of European data for each phase. More detailed description of the screening study is available in the technical report of Tuomisto et al. (2015) study.

2. Methods

2.1. Scope and representative product

The functional unit was set as a liter of packed olive oil used by consumers as salad dressing and for cooking. The system boundaries were defined from cradle to grave as shown in Fig. 1.

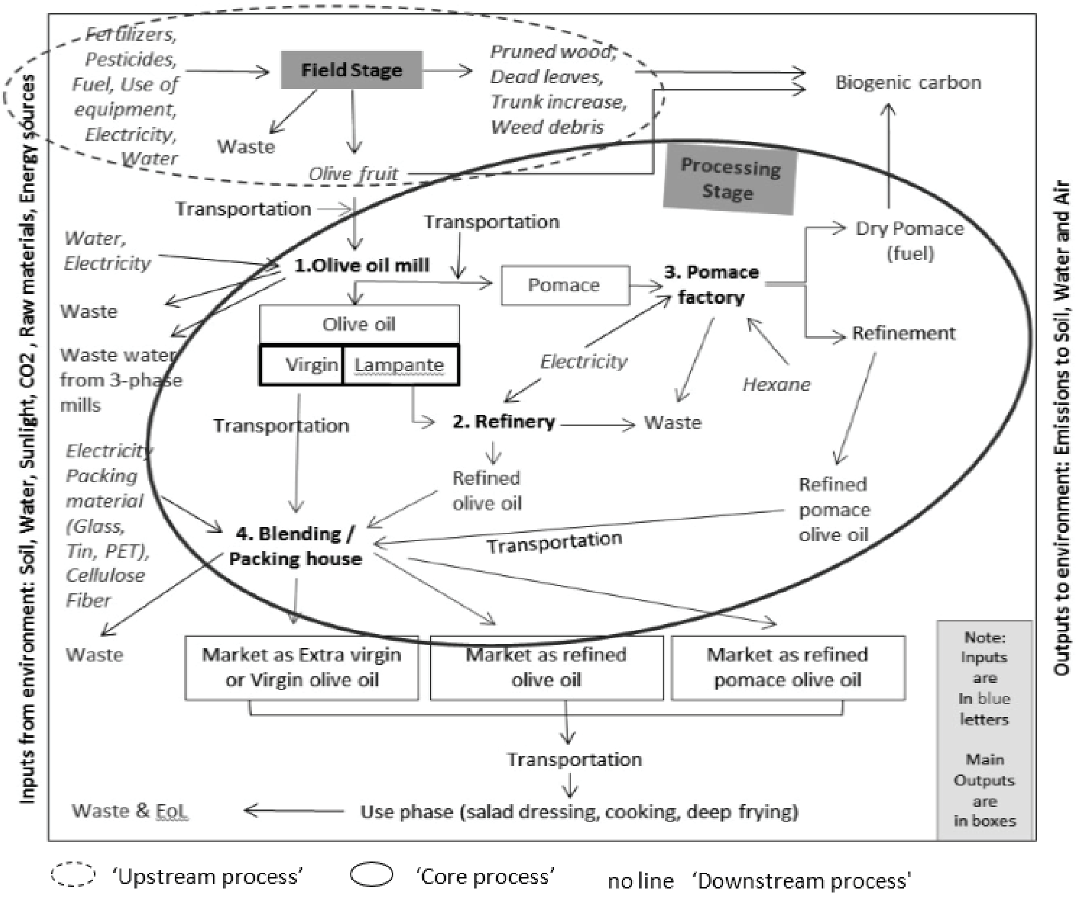


Fig. 1. System boundaries

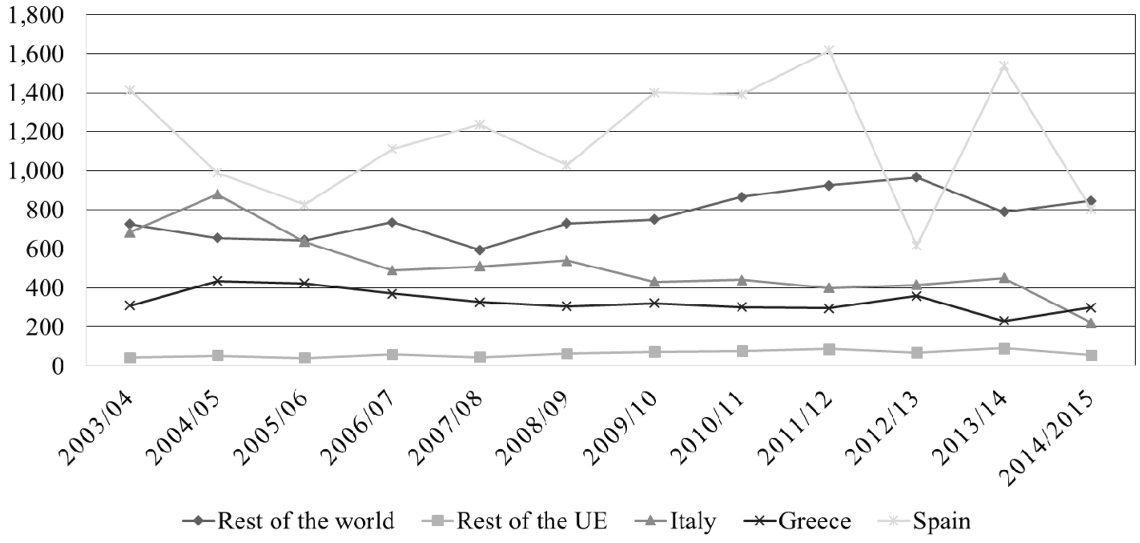


Fig. 2. Olive oil world production (1,000 tonnes). *Source:* International Olive Oil Council (IOOC)

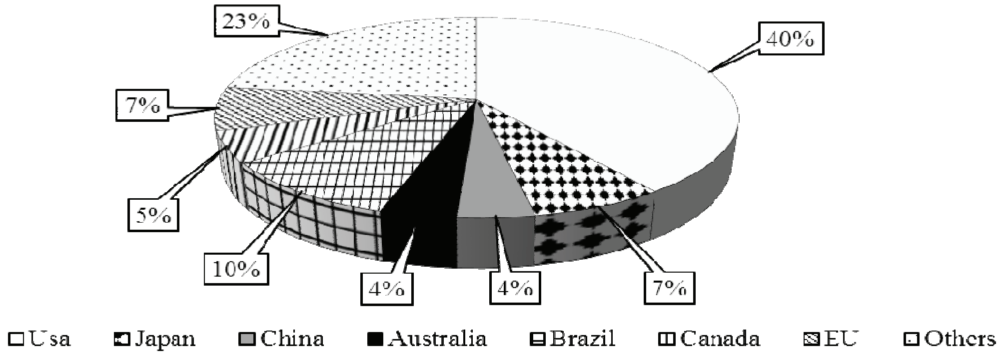


Fig. 3. Olive oil world imports. *Source:* IOOC (2012)

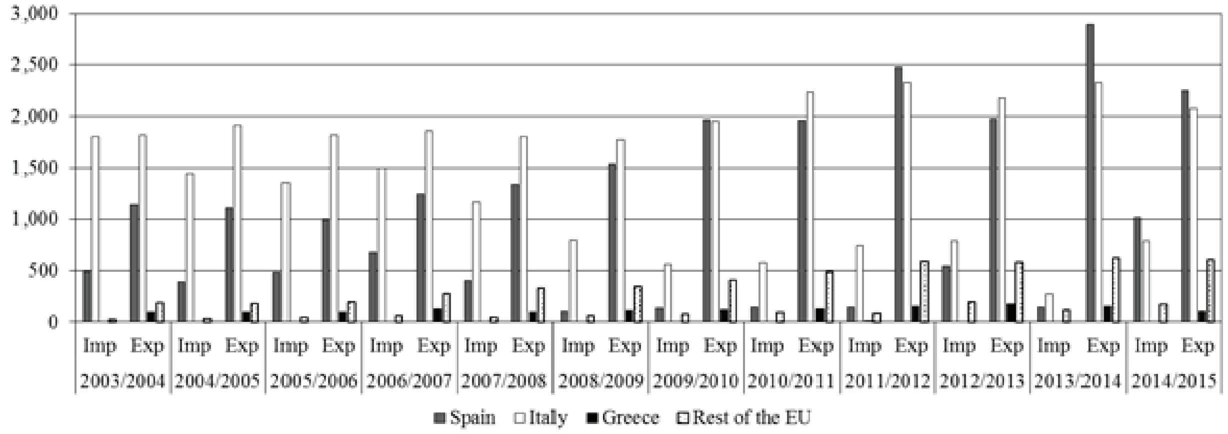


Fig. 4. Olive oil imports and exports in EU (1,000 tonnes). *Source:* IOOC (2016)

The representative product was defined as a virtual product describing the average olive oil in the European markets (IOOC, 2012). As approximately 75% of all olive oil is produced in Europe, and as Spain, Italy and Greece account for over 90% of the European production (Fig. 2), the representative product was modelled based on data from Spain, Italy and Greece. According to the aims of the PEF Pilot, export and import flows were excluded from the study. However, Fig. 3 shows that import flows towards the European countries are not so relevant, while, Fig. 4 shows that Italy and Spain are the main exporting countries (D'Annibale et al., 2014; IOOC, 2016).

As shown in the flow chart in Fig. 1, efforts were done to calculate average data for the European context by describing a system with a great degree of complexity and high spatial heterogeneity due to the contribution of many factors both exogenous and endogenous to LCA methodology.

The representative product (virtual product) was assumed to be consisting of the following olive oil types classified on the base of European legislation (EC, 2013a):

- virgin olive oil (including extra virgin and virgin olive oils);
- refined olive oil;
- refined pomace oil.

Fig. 5 shows the contribution of different olive oil types to the representative product calculated as a sum the contribution of the three main European producer countries: Spain, Italy and Greece.

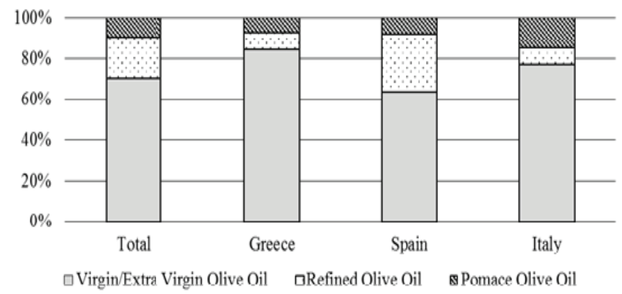


Fig. 5. Percentage of different olive oil type defined as representative product

As for the allocation procedure, it was based on economic allocation calculated by multiplying the mass of each fraction per its market value. Table 1 shows the three allocation factors referred to the production of extra virgin, virgin and lampante olive oil (first allocation), the production of pomace olive oil (second allocation) and the production of refined olive oil (third allocation).

2.2. Data sources and LCA modelling

GaBi software and Ecoinvent dataset (Frischknecht et al., 2007; IKP and PE, 2002; Weidema et al., 2013) were used for the life cycle assessment modelling. As for the geographical context, Italian data for the screening study was mainly taken from past olive oil LCA studies (Cappelletti et al., 2014; De Luca et al., 2015; Proietti et al., 2014; Rinaldi et al., 2014; Salomone et al., 2010; Salomone et al., 2015; Tsarouhas et al., 2015) and Environmental Product Declarations (Apolio, 2012; Assoproli, 2012; De Cecco, 2012; Monini, 2012a, 2012b, 2012c, 2012d), and some data were collected directly from the industry. As far as Greece, data from the LIFE+ project OLIVE CLIMA (LIFE project OLIVE CLIMA, 2013) and a LIFE Project SAGE10 (LIFE project SAGE10, 2011) were used.

Spanish system is based on the average data from representative Andalusian farms. This choice is due to the relevance that this region has on the Spanish olives production (EC, 2012).

The life cycle assessment (LCA) was carried out according to the ISO 14040 and 14044 standards (ISO 14040, 2006; ISO 14044, 2006), the guidance provided by JRC-IES (2010), the PEF guide (EC, 2013b) and specific guidance provided for the pilot projects (EC, 2016). The EF impact categories and assessment methods were those presented in the PEF guide (Table 2).

2.2.1. Field stage

According to the Eurostat data referred to the years 2010-2012 for Greece and Spain and to the years 2010-2011 for Italy, the olive production was modelled by considering the following average yield in kg per hectare: Spain 2,436; Italy 2,720; Greece 2,157 (Eurostat, 2014).

As concerning the biogenic carbon sequestrated by the olive fruits, an average value of 0.25 kg of carbon per kg of olives was assumed (Nardino et al., 2013), despite, the CO_2 sequestrated is released in the atmosphere in the other phases of the life cycle. The impacts related to land use change were not included in the base model, due to the fact that olive groves in Europe have been established more than 20 years ago.

Table 1. Allocation factors

		Mass	***Unit value***	***Allocation factor***
		%	***Euro/kg***	***%***
First allocation after pressing olive fruit	Extra virgin olive oil	9.14	1.88	46.14
	Virgin olive oil	6.5	1.73	30.34
	Lampante olive oil	4.67	1.61	20.26
	Olive pits	9.3	0.07	0.95
	Wet pomace (70% moisture)	70.4	0.01	2.32
	Total	**100**		**100**
Second allocation (content of wet pomace)	Crude pomace	91.6	0.79	100
	Dry pomace	8.4	0	0
	Total	**100**		**100**
Third allocation (crude pomace)	Refined pomace olive oil	2.8	1.11	86.92
	Dry pomace	26	0.01	9.47
	Olive pit	1.8	0.07	3.61
	Waste water	69.4	0	0
	Total	**100**		**100**

Table 2. Impact categories

Impact Category	***Unit***
Climate change midpoint, excl biogenic carbon (v1.06)	kg CO_2-eq
Climate change midpoint, incl biogenic carbon (v1.06)	kg CO_2-eq
PEF-IPCC global warming (biogenic)	kg CO_2-eq
Ozone depletion, WMO model, ReCiPe	kg CFC_{-11}-eq
Human toxicity cancer effects, USEtox (without long-term)	CTU_h
Human toxicity non-canc. effects, USEtox (without long-term)	CTU_h
Acidification, accumulated exceedance	Mole of H^+-eq
Particulate matter/Respiratory inorganics, RiskPoll	kg $PM_{2.5}$-eq
Ecotoxicity for aquatic fresh water, USEtox (without long-term)	CTUe
Ionising radiation, human health effect model, ReCiPe (corrected)	kg ^{235}U-eq
Photochemical ozone formation, LOTOS-EUROS model, ReCiPe	kg NMVOC
Terrestrial eutrophication, accumulated exceedance	Mole of N-eq
Freshwater eutrophication, EUTREND model, ReCiPe (without long-term)	kg P-eq
Marine eutrophication, EUTREND model, ReCiPe	kg N-eq
Land use, Soil Organic Matter (SOM, Ecoinvent & Hemeroby - EMS-19May2015)	kg C deficit-eq
Resource depletion water, midpoint, Swiss Ecoscarcity (v1.06 - EMS- 19May2015)	m^3-eq.
Resource depletion, mineral, fossils and renewables, midpoint (v1.06)	kg Sb-eq

Source: European Commission (2016)

As for the agricultural practices, it is worth nothing that the representative product referred to average data about farming systems adopted in the three main producer countries (Spain, Italy and Greece). Large differences can be observed in the olive orchard management for each country, due to many factors such as agricultural systems (traditional, intensive, super-intensive, organic, conventional), farming techniques (ratio of irrigated/rain-fed olive groves, soil cultivation, fertilisers and pesticides use) and genetic resources (cultivars). As above specified, efforts was simply describing a complex system with a great degree of variability.

The following agricultural practices were considered for olive production: harvesting, irrigation, use of plant protection products and herbicides, soil management, pruning and fertilising. The harvesting process was modeled by considering the principal techniques of harvesting adopted in the main producer countries. As for Italy, the Ecoinvent processes of using fertilising broadcaster was adopted in order to calculate the diesel consumption and the emissions of the use of air compressor activated by the tractor gimbal. For Spain and Greece, the two stroke engine vibrator was considered, so the emissions of the combustion of two stroke blend petrol were modeled by applying a correction factor to the emissions referred to a process of use of a car petrol (Aminu, 2006; EPA, 2009).

As for the irrigation process, mean electricity consumption was calculated per m^3 of water used for irrigation, and also the plastic material of drip irrigation system was investigated. The scenario includes an electric pump that withdraws ground water from a well to the irrigation system (localized drip-irrigation system that adopts drip-sprinklers of polyethylene). The ground water flows were regionalized according to the different countries. The pesticides dispersion in air, soil and water was calculated according to the Mackay's fugacity model (Mackay, 1991).

The practices of soil management were modelled according to the Ecoinvent dataset. The pruning operation were modeled considering the use of chainsaw, so the same assumption of the two-stroke engine emissions done in the harvesting phase were considered. In this case, the lube oil consumption was relatively high.

The fertilization phase was modeled by considering the N-P-K content required per hectare, so a generic N, P and K fertilizer was taken into account. The nitrogen losses were estimated by using the Bentrup's model (Brentrup et al., 2000). The NO_x and phosphorous emissions were calculated according to the Ecoinvent report no.15 on Agricultural production systems (Nemecek and Kägi, 2007).

2.2.2. Processing stage

As concerning the industrial phase of extra virgin, virgin and lampante olive oil production, the main technologies adopted in the three main producer countries Spain, Italy and Greece were considered (75% three phases and 25% two phase systems). According to the Reg. CEE 1513/2001 and International Olive Oil Council standard (IOOC/T.15/NC No 3), the process for obtaining theese three categories of olive oil is the same, and differences are only due to chemical-physical and organoleptics characteristics (e.g free acidity, expressed as oleic acid). Indeed, extra virgin and virgin olive oils are used for human consumption, while lampante is intended for further refining process or for technical use.

As concerning the the pomace olive oil (oil obtained from the residues of olive oil extraction composed of husk, pit fragments and pulp of the olives after olive oil extraction process), the extraction process was modeled by considering an average process of chemical extraction by using hexane as solvent. Exhausted pomace was assumed to be used as fuel to dry the virgin pomace before the pomace olive oil extraction and produce steam able to recover the hexane. The refining process was modeled by considering a physical refining.

2.2.3. Packaging

The packaging for the virtual olive oil (representative product) was constructed from the average European mix of three types of packaging: 60% glass, 20% polyethylene terephthalate (PET) and 20% metal cans (composed by aluminum, tin and steel).

2.2.4. Transportation

As concerning transports, average transportation distances were assumed according to data collected for each phase of the life cycle. Due to the fact that imports and exports flows were excluded, transports referred to olives and olive oil production phases. As for distribution of the packed product to the consumer, both sea and road transportation were assumed.

2.2.5. Use phase

Due to lack of official data, the assumptions of the use phase consider 55% salad dressing, 7% deep-frying and 38% cooking. As concerning the deep-frying, an average industrial process with the use of an electric fryer was modelled according to literature (Wu et al., 2010). The cooking phase was modelled by considering a natural gas powered stove, a temperature around 150°C and a time of cooking of 600 seconds. The emissions of natural gas burning were modelled according to the Ecoinvent dataset.

2.2.6.End of Life

The waste oil was assumed to be disposed of only in the case of deep-frying, as it was assumed that all olive oil used as salad dressing or for cooking is consumed by human. Due to the long self-life of olive oil, it was assumed that disposal of unused olive oil is negligible. Regarding the End of Life (EoL) of the packaging materials, the default formula provided in the PEF guide was used.

As for the EoL of the waste oil after deep frying the biogenic CO_2 emission was also taken into account in the process of energy recovery, while a biogenic methane emission was included if the waste oil was considered disposed in landfill. All information regarding background and activity data of the inventory considered in the study are available in the in the technical report by Tuomisto et al. (2015).

3. Results and discussion

3.1. Life Cycle Impact Assessment

The results of the LCA indicated in the screening report and referred to the virtual product show that the olive production has the highest contribution to most impact categories, except for human toxicity (cancer effects), which is dominated by packaging (Fig. 6).

The major impacts of the phase of olives cultivation is principally due to the production and use of fertilisers and plant protection products. Soil management, pruning and harvesting practices have a relative high contribution in Particulate Matter/Respiratory inorganics and photochemical ozone formation impact categories. Irrigation dominates the total freshwater consumption –impact category. The negative biogenic carbon removal during olive production stage includes the carbon that is removed by harvesting the olive fruit. This carbon is released back to the atmosphere during consumption of olive oil and end of life management.

However, according to the End of Life formula the biotic global warming potential is slightly positive due to biogenic methane emissions from the end of life stage.

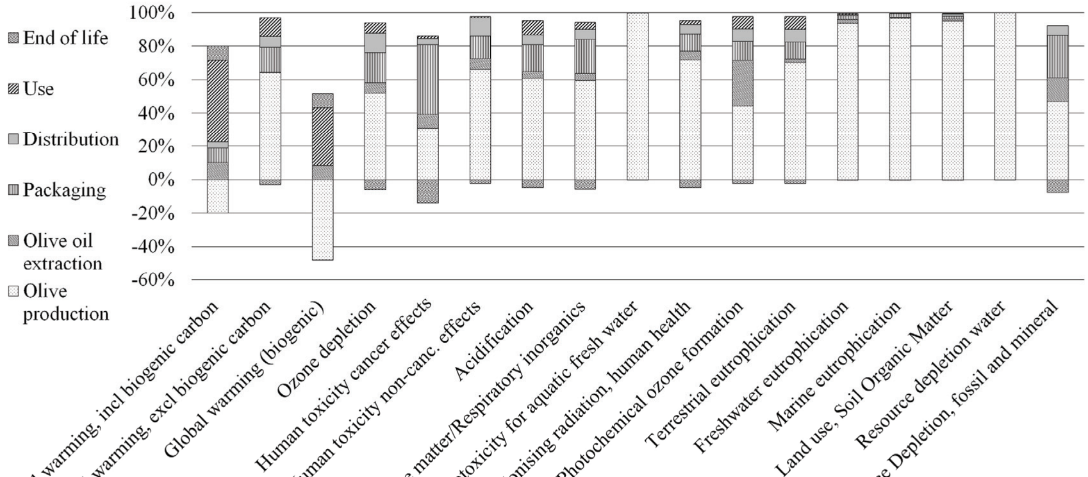


Fig. 6. Contribution of different life cycle stages on the total impact

As concerning the different olive oil extraction steps, pomace olive oil extraction has the highest contribution to most impact categories. Electricity use is the most contributing processes for most impact categories. At the same way, electricity production mainly affects the virgin olive oil extraction phase, despite some credits are derived from using disposed leaves as fuel for energy generation. Electricity use has also a high contribution to the impacts of the refining process. However, upstream impacts from chemical production (including phosphoric acid, sodium hydroxide and activated bentonite) and steam production have also relative high contribution.

In the packaging phase, glass bottle production is the most contributing process. The impact of distribution of the final product from the olive oil mill to the consumer is mainly dominated by lorry transportation. The end of life impacts is mainly negative due to the credit from energy generation from waste or recycling. The biogenic carbon emissions from waste olive oil management are mainly due to release of biogenic carbon that is absorbed from the atmosphere by the olive fruit.

3.2. Open issues and benchmark

A significant must be devoted to the methodological issues in order to standardize the datasets quality, the procedures and the calculations of the environmental impacts through LCA. PEFCR for the Olive Oil PEF Pilot are close to be finalized, with the contribution of major market players. It is also true that the future of PEF for olive oil sector will depend on the assurance that it will provide to all the stakeholders that any comparisons to be made will be unequivocally fair, equally for large players and for SMEs, large countries and less developed areas.

The difference between large companies and SMEs is the availability of resources for the development of their own PEF. Considering that data collection is the most costly phase of an LCA and that overcoming data confidentiality is a challenge for the transparency required for product comparisons, it is obvious that the trueness of the activity data is maybe the most sensitive issue for PEF. In other terms, PEF usefulness is proportional to the quality, completeness and credibility of the data it relies on. If this is achieved, PEF can become the vehicle for designing olive oil chain on a new basis, just because it is offered for comparisons on how it is performing, based on quantified information. Apart from price per product unit and income per year, it is the first time a quantity can be the base for evaluation of efficiency of the sector in economic and environmental terms, offering opportunities for side objectives that can be pursued by one or more of the partners.

For these reasons the definition of benchmark is a key issues of the pilot. Due to many uncertainties and data gaps of the virtual product identified in the screening report, the benchmark will be revised after supporting studies. In particular the environmental performances of the worst and best case will be defined and the environmental performance classes will be determined between this range. Three benchmarks will be created for the three olive oil categories marketed for human consumption (virgin olive oil, olive oil and pomace olive oil). Furthermore, the current draft PEFCR will be revised and default datasets will be provided by distinguising different types of olive grove management systems, industrial processes and type of packaging.

4. Conclusions

LCA and environmental performance of products enters a new daring era with PEF. Daring, just because the concept of product comparison and comparative assertions is introduced, creating a strong drive for producers of final products, expandable to intermediate ones, even on raw materials etc, as long as PEFCRs will be made available.

The screening study of the average olive oil consumed in the European markets provide an initial benchmark for the PEFCR development, despite many uncertainties and data gaps. PEFCR will be tested on real products in the supporting studies, in order to determine environmental performance classes. Furthermore, the current draft PEFCR will be revised and default datasets will be provided.

Despite the efforts of the members of the Technical Secretariat many issues remain opened and the future challenge must be addressed to reduce uncertainity and harmonize the data collection procedures for LCA, especially for the use of inputs. For these reasons the olive oil pilot’s TS is attempting to provide specific default datasets able to represent the huge variability of the systems that characterize the olive oil sector in Europe, especially for the complexity of the field stage (mainly due to the different agricultural systems, farming techniques, and genetic resources) and the accounting of the net carbon sequestration by soils of olive plantations.

Acknowledgements

The study was carried out according to the involvement of the authors in the Olive Oil PEF pilot. All information could be find at the following link:
https://webgate.ec.europa.eu/fpfis/wikis/display/EUENVFP/PEFCR+Pilot%3A+Olive+oil

References

Aminu J., (2006), *Pollution by 2-Stroke Engines*, The Nigerian Conference on Clean Air, Clean Fuels and Vehicles, 2-3 May, Abuja, Nigeria.

Apolio, (2012), Environmental Product Declaration (EPD) of the De-Pitted Extra Virgin Olive Oil (in Italian), On line at: http://www.environdec.com/en/Detail/epd021.

Assoproli, (2012), Environmental product declaration for oasis extra virgin olive oil packaged in glass bottles of 0.5 L, On line at: http://www.environdec.com/en/Detail/epd367.

Benini L., Mancini L., Sala S., Manfredi S., Schau E.M., Pant R., (2014), *Normalization method and data for Environmental Footprints*, Report EUR 26842 EN, European Commission Joint Research Centre, Ispra, Italy.

Brentrup F., Küsters J., Lammel J., Kuhlmann H., (2000), Methods to estimate on-field nitrogen emissions from crop production as an input to LCA studies in the agricultural sector, *International Journal of LCA*, **5**, 349-357.

Cappelletti G.M., Ioppolo G., Nicoletti G.M., Russo C., (2014), Energy requirement of extra virgin olive oil production, *Sustainability*, **6**, 4966-4974.

COM 196, (2013), Communication from the Commission to the European Parliament and the Council. Building the Single Market for Green Products. Facilitating Better Information on the Environmental Performance of Products and Organizations, European Commission, Brussels, 2013, On line at: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52013DC0196&from=EN.

D'Annibale A., Sampedro I., Federici F., Petruccioli M., (2014), aqueous extract from dry olive mill residue as a possible basal medium for laccase production, *Environmental Engineering and Management Journal*, **13,** 3037-3044.

De Cecco, (2012), Environmental Product Declaration (EPD) of the 'De Cecco Extra Virgin Olive Oil', On line at: http://gryphon.environdec.com/data/files/6/9226/epd410_Dececco_olive_oil.pdf.

De Luca A.I., Molari G., Seddaiu G., Toscano A., Bombino G., Ledda L., Milani M., Vittuari M., (2015), Multidisciplinary and innovative methodologies for sustainable management in agricultural systems, *Environmental Engineering and Management Journal*, **14,** 1571-1581.

EC, (2012), Economic analysis of the olive sector, European Commission, Directorate-General for Agriculture and Rural Development, On line at: http://ec.europa.eu/agriculture/olive-oil/economic-analysis_en.pdf.

EC, (2013a), Commission Implementing Regulation (EU) No. 1348/2013 of 16 December 2013 amending Regulation (EEC) No. 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis, *Official Journal of the European Union*, L338, 31-67.

EC, (2013b), Recommendations. Commission Recommendation of 9 April 2013 on the use of common methods to measure and communicate the life cycle environmental performance of products and organizations (Text with EEA relevance) (2013/179/EU), *Official Journal of the European Union*, L124, 1-210.

EC, (2016), Guidance for the implementation of the EU Product Environmental Footprint (PEF) during the Environmental Footprint (EF) Pilot Phase, Guidance for the implementation of the EU PEF during the EF pilot phase - Version 5.2 - February 2016, On line at: http://ec.europa.eu/environment/eussd/smgp/pdf/Guidance_products.pdf.

EPA, (2009), *Stationary Internal Combustion Sources*, In: *AP 42 Compilation of Air Pollutant Emission Factors*, Fifth Edition, Vol. I, Agency U.S.E.P., On line at: http://www.epa.gov/ttnchie1/ap42/ch03/.

Eurostat, (2014), *Agriculture, Forestry and Fishery Statistics - 2014 edition*, Forti R., Henrard M. (Eds.), Eurostat, Luxembourg: Publications Office of the European Union, Doi: 10.2785/59171, On line at: http://ec.europa.eu/eurostat/documents/3217494/6639628/KS-FK-14-001-EN-N.pdf/8d6e9dbe-de89-49f5-8182-f340a320c4bd.

Frischknecht R., Jungbluth N., Althaus H.-J., Doka G., Heck T., Hellweg S., Hischier R., Nemecek T., Rebitzer G., Spielmann M., Wernet G., (2007), Overview and methodology - Ecoinvent report No. 1, Swiss Centre for Life Cycle Inventories, Dübendorf, On line at: https://www.ecoinvent.org/files/200712_frischknecht_jungbluth_overview_methodology_ecoinvent2.pdf.

IKP and PE, (2002), GaBi 4 - Software-system and databases for life cycle engineering, Stuttgart, Echterdingen.

IOOC, (2012), International Olive Oil Council, Overview of the olive and olive oil sector itemised by country and based on the official replies to IOC questionnaires,, nternational Olive Oil Council, On line at: http://www.internationaloliveoil.org/estaticos/view/222-standards.

IOOC, (2016), International Olive Oil Council, Olive Oil Figures, On line at: http://www.internationaloliveoil.org/estaticos/view/131-world-olive-oil-figures.

ISO 14040, (2006), *Environmental Management - Life Cycle Assessment - Principles and Framework*, ISO 14040:2006, International Standard Organization, Geneva.

ISO 14044, (2006), *Environmental Management - Life Cycle Assessment - Requirements and Guidelines*, ISO 14044:2006, International Standard Organization, Geneva.

JRC-IES, (2010), *International Reference Life Cycle Data System (ILCD) Handbook - General Guide for Life Cycle Assessment - Detailed Guidance*, Publications Office of the European Union, Luxembourg, On line at: http://publications.jrc.ec.europa.eu/repository/bitstream/JRC48157/ilcd_handbook-general_guide_for_lca-detailed_guidance_12march2010_isbn_fin.pdf.

LIFE project OLIVE CLIMA, (2013), LIFE11 ENV/GR/942 – OLIVE CLIMA, Introduction of new OLIVE crop management practices focused on CLIMAte change mitigation and adaptation, On line at: http://ec.europa.eu/environment/life/project/Projects/index.cfm?fuseaction=search.dspPage&n_proj_id=4194&docType=pdf

LIFE project SAGE10, (2011), LIFE09/ENV/GR/000302 - SAGE10, Establishment of Impact Assessment Procedure as a tool for the sustainability of agroecosystem: the case of Mediterranean olives, On line at: http://ec.europa.eu/environment/life/project/Projects/index.cfm?fuseaction=search.dspPage&n_proj_id=3681&docType=pdf.

Mackay D., (1991), *Multimedia Environmental Models: The Fugacity Approach*, Lewis Publishers, Inc., Chelsea. MI.

Monini, (2012a), Environmental Product Declaration (EPD) of the Extra Virgin Olive Oil 'Classico' (in Italian), On line at: http://www.environdec.com/en/Detail/epd384.

Monini, (2012b), Environmental Product Declaration (EPD) of the Extra Virgin Olive Oil 'Delicato' (in Italian), On line at: http://www.environdec.com/en/Detail/?Epd=8857.

Monini, (2012c), Environmental Product Declaration (EPD) of the Extra Virgin Olive Oil 'Granfruttato' (in Italian), On line at: http://www.environdec.com/en/Detail/?Epd=8854.

Monini, (2012d), Environmental Product Declaration (EPD) of the Extra Virgin Olive Oil 'Poggiolo' (in Italian), On line at: http://www.environdec.com/en/Detail/?Epd=8856.

Nardino M., Pernice F., Rossi F., Georgiadis T., Facini O., Motisi A., Drago A., (2013), Annual and monthly carbon balance in an intensively managed mediterranean olive orchard, *Photosynthetica*, **51**, 63-74.

Nemecek T., Kägi T., (2007), Life Cycle Inventories of Agricultural Production Systems. Data v2.0, Ecoinvent report No. 15, ART, Zurich and Dubendorf, On line at: https://db.ecoinvent.org/reports/15_Agriculture.pdf.

Proietti S., Sdringola P., Desideri U., Zepparelli F., Brunori A., Ilarioni L., Nasini L., Regni L., Proietti P., (2014), Carbon footprint of an olive tree grove, *Applied Energy*, **127**, 115-124.

Rinaldi S., Barbanera M., Lascaro E., (2014), Assessment of carbon footprint and energy performance of the extra virgin olive oil chain in Umbria, Italy, *Science of the Total Environment*, **482-483**, 71-79.

Salomone R., Cappelletti G.M., Ioppolo G., Mistretta M., Nicoletti G.M., Notarnicola B., Olivieri G., Pattara C., Russo C., Scimia E., (2010), *Italian Experiences in Life Cycle Assessment of Olive Oil: A Survey and Critical Review*, Proc. 7th Int. Conf. on Life Cycle Assessment in the Agri-Food Sector, Bari, Italy, 265-270.

Salomone R., Cappelletti G.M., Malandrino O., Mistretta M., Neri E., Nicoletti G.M., Notarnicola B., Pattara C., Russo C., Saija G., (2015), *Life Cycle Assessment in the Olive Oil Sector*, In: *Life Cycle Assessment in the Agri-food Sector. Case Studies, Methodological Issues and Best Practices*, Notarnicola B., Salomone R., Petti L., Renzulli P.A., Roma R., Cerutti A.K. (Eds.), Springer, London, 57-122.

Tsarouhas P., Achillas C., Aidonis D., Folinas D., Maslis V., (2015), Life Cycle Assessment of olive oil production in Greece, *Journal of Cleaner Production*, **93**, 75-83.

Tuomisto H.L., Russo C., Michalopoulos G., Pattara C., Polo Palomino J.A., (2015), PEF screening report in the context of the EU Product Environmental Footprint Category Rules (PEFCR) Olive Oil Pilot, On line at: https://www.researchgate.net/publication/288826282_PEF_screening_report_in_the_context_of_the_EU_Product_Environmental_Footprint_Category_Rules_PEFCR_Olive_Oil_Pilot.

Weidema B.P., Bauer C., Hischier R., Mutel C., Nemecek T., Reinhard J., Wernet G., (2013), Overview and methodology: Data quality guideline for the ecoinvent database version 3, Swiss Centre for Life Cycle Inventories, Ecoinvent Report; No. 1, Vol. 3, On line at: https://www.ecoinvent.org/files/dataquality guideline_ecoinvent_3_20130506.pdf.

Wu H., Tassou S.A., Jouhara H., Karayiannis T.G., (2010), *Analysis of Energy Use in Crisp Frying Processes*, Proc. of SEEP2010 Conference, Bari, Italy, On line at: http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.426.9004&rep=rep1&type=pdf.

Environmental Engineering and Management Journal
September 2016, Vol.15, No. 9, 2029-2039
http://omicron.ch.tuiasi.ro/EEMJ/

"Gheorghe Asachi" Technical University of Iasi, Romania

SANITATION RESPONSE IN EMERGENCIES: LESSONS LEARNT FROM PRACTITIONERS IN POST-EARTHQUAKE HAITI

Daniela Giardina, Sabrina Sorlini*, Luca Rondi

CeTAmb LAB - Research Laboratory on Appropriate Technologies for Environmental Management in Resource-Limited Countries, Department of Civil Engineering, Architecture, Land, Environment and of Mathematics, University of Brescia, Via Branze 43, 25123 Brescia, Italy

Abstract

The January 2010 earthquake in Haiti caused catastrophic damage, and the displacement of about 1.5 million people in urban and peri-urban areas of Port-au-Prince. A web-based questionnaire was developed and sent to international organizations working in Haiti through the Water, Sanitation and Hygiene (WASH) Cluster in order to assess how humanitarian actors responded to the sanitation needs for Internally Displaced People (IDP). The findings showed different sanitation systems being implemented during humanitarian relief and the transition to the reconstruction/development phase. Humanitarian practitioners faced specific challenges to sanitation provision related to the urban type of the emergency response. However, experts' opinions highlighted innovative solutions that have not been implemented in the field, due mostly to land tenure issues, donors' restrictions and lack of political will. Practitioners provided some suggestions for more resistant sanitation technologies as well as some recommendations for sanitation provision in the early recovery phase.

Key words: disaster risk reduction, earthquake, emergency sanitation, Haiti

Received: January, 2016; Revised final: September, 2016; Accepted: September, 2016

1. Introduction

The transition between disaster response and recovery represents a critical stage in which strategic decisions need to be taken, in order to re-establish or up-grade the situation of low income and displaced communities (Wisner and Adams, 2002). In the immediate aftermath of a disastrous event, which often includes displacement, the access to safe and enough water, appropriate sanitation and hygiene is crucial in order to reduce the risk of water-borne diseases spreading, especially in crowed camps (Cronin et al., 2008; Harvey, 2007; Pollmann Gomez et al., 2014; Rondi et al., 2014; Sorlini et al., 2014; Toole and Waldman, 1997; Wisner and Adams, 2002).

Moreover, urban emergencies are on an increasing trend posing different challenges to the humanitarian community especially when they imply a significant number of people displaced (IFRC, 2010a; IASC, 2011).

On 12th of January 2010, the earthquake that hit Haiti, killing over 220,000 people, caused the displacement of about 1.5 million persons in Port-au-Prince metropolitan area which settled in over 1,342 internally displaced camps (IASC, 2010). The Government of Haiti lost buildings and civil servants and over 25 million USD funding were committed for the recovery and reconstruction (IASC, 2010).

Before the earthquake, Haiti did not have any form of sewerage or waste treatment system. Existing sanitation included uncontrolled simple pit latrines, often placed over or connected to storm-water drains. In the high income areas of the city the use of large volume septic tank facilities was common. According to the Joint Monitoring Programme only 19% of Haitians had access to improved sanitation (WHO/UNICEF, 2010), a figure that has even

---

* Author to whom all correspondence should be addressed: e-mail: sabrina.sorlini@unibs.it

decreased from the 26% of the 1990's (McLeod, 2009). Open defecation in Haiti was widespread and hygiene practices were randomly practiced (IFRC, 2010b).

The poor sanitation conditions of the country prior to the earthquake, coupled with the devastation and crowded camps in which people who lost their properties settled, posed health risks to communicable waterborne diseases, especially for the most vulnerable. An outbreak of cholera, though not directly related to the disaster, spread in late October 2010 killing 6,942 people until December 2011, the cases of cholera grew up to 515,699 (OCHA, 2011). To date cholera remains one of the major health threats in Haiti, due to the lack of access to WASH systems and with the vulnerability of the country to other natural disasters such as hurricanes and earthquakes that could cause further displacement and overcrowded unsanitary conditions.

The aim of this paper is to document the sanitation response, through measuring actions, views and opinions of experienced humanitarian workers involved in the delivery of water and sanitation during the humanitarian response to the 2010 earthquake in Haiti. Moreover, through the experts' responses, the survey aim is to highlight some of the challenges faced in providing appropriate sanitation solutions in urban areas, and withdraw some lessons learnt from the Haitian disaster response.

2. Materials and methods

2.1. Data collection

The research findings are built on a web-based survey that was developed in English and was available to be compiled online between November 15th and December 15th 2011. Literature review on available reports for sanitation planning and available options in post-disasters constituted the basis to develop a preliminary questionnaire (Harvey, 2007; SuSanA, 2008). A fieldwork conducted during October-December 2010 by the author allowed to better target the questions for the specific situation in Haiti, through observational surveys on Internally Displaced People (IDP) camps, and unstructured interviews with beneficiaries and water and sanitation professionals.

The questionnaire is structured in different sections: i) information about the respondents, ii) information about funding, project activities hardware and software (hygiene promotion), iii) sanitation planning criteria, iv) sanitation technology choice, v) constraints to sanitation provision (close and open questions), and vi) disaster risk reduction techniques applied. It was first trialled with few people, before sending it out to a wider audience. Key areas for sanitation sustainability were also assessed through different groups of questions. The sample was selected through purposive sampling in order to produce the most valuable data about the specific event of the sanitation provision in Haiti after the quake and to be informative towards the research purpose. In fact, an invitation email to undertake the survey was sent out to the 92 international organizations part of the Water Sanitation and Hygiene (WASH) Cluster set up in Haiti after the earthquake.

2.2. Data analysis

EpiInfoTM7- a free public health software developed by the Centre of Disease Control (CDC) - was used for quantitative data analysis, and Excel utilized for the presentation of the results. The replies to open questions related to personal experiences of the informants were analysed through applied basic qualitative analysis techniques, with the use of coding data according to specific categories. The responses, for both quantitative and qualitative analysis techniques, were evaluated with equal importance since the selection of the humanitarian respondents was done purposively targeting water and sanitation practitioners with varied experience working in Haiti, as such considered key informants.

3. Results and discussion

3.1. Information on respondents and project activities

All the 92 organizations part of the WASH cluster were contacted through emails and 30 unique respondents compiled the online questionnaire (33%); the gender breakdown included 19 male practitioners (63%) and 11 female practitioners (37%). The response rate is 33% and is in line with similar type of surveys. Not all respondents answered every question; thus, the data responsive to some questions are more robust than others.

Most of the respondents (26) were working with international Non-Governmental Organizations (86.67%), and 4 of them with UN organizations or other donors involved in water, sanitation and hygiene activities in Haiti. Most organizations represented by the survey (18) were involved in development projects in Haiti for over 5 years (60%), 2 organizations (7%) between 2 and 5 years, whilst one third, 10 organizations (35%) started working in Haiti after the disastrous event in 2010.

Out of the 30 respondents 12 (40%) had over 5 years' experience in working in emergencies, 7 (23%) between 3 and 5 years, and 11 (37%) between 1 and 3 years. 5 respondents reported they worked in Haiti less than one year; 22 (73%) reported they worked in the island longer than one year though less than 2 years, and only 3 reported they worked longer than 5 years. The title that best describe their work is project manager (13 respondents, 44%) or technical advisor (10 respondents, 33%). Almost all practitioners that replied to the survey carried out activities in the sectors of water, sanitation and hygiene; one reported to deal also with solid waste management, and another one to carry out solid waste recycling activities, whilst one reported to be actively involved in the coordination of interventions.

Respondents were asked through which donors they received funding for their water, sanitation and hygiene activities. 22 people (73%) responded from private donations and internal funding of their organizations; 15 (50%) indicated also Government funds and 14 (47%) from UN agencies. In Table 1, on 28 valid responses, it is reported the number of beneficiaries which varied according to the type and size of the organization and the entity of available funds.

Table 1. Number of beneficiaries reported

Beneficiaries	*Number of respondents*	*% of respondents*
< 10,000	5	18
10,000 - 49,999	6	21
50,000 - 99,999	5	18
100,000 - 199,999	6	21
> 200,000	6	21

International organizations carried out activities mainly directed to support the Internally Displaced People (IDP) in camps (22 respondents), 11 supporting schools, and 4 institutions. There were some respondents that, for the specific mission and vocation of the respective organizations, supported prisons and hospitals in the water and sanitation sector. Furthermore, 17 out of the 22 respondents, who indicated to support IDPs, also dedicated support to the not displaced local community.

The reference guidelines predominantly used by the respondents (16 respondents, 53%) was the Humanitarian Charter and Minimum Standards in Disaster Response, known as SPHERE (The Sphere Project, 2011), and documents that the WASH Cluster produced together with the Directorate of Potable Water and Sanitation (DINEPA - *Direction Nationale de l'Eau Potable et de l'Assainissement*), whilst the rest reported to have used internal guidelines from their or other organizations available for emergency response.

3.2. Sanitation planning criteria

To understand how humanitarian workers took decisions in the selection of a sanitation system, the respondents were asked to rank 10 criteria, on a scale from one to three (1 low importance, 2 medium importance, and 3 high importance). The criteria are listed, ranked by ordering the ones rated as most important:

1. Protection of water sources (90%)
2. Health protection (87%)
3. Cultural Attitudes (80%)
4. Technical feasibility (77%)
5. Institutional framework (67%)
6. Availability of spare parts and operators (57%)
7. Financial Resources (53%)
8. Quick to install (47%)
9. Resistant to further disasters (40%)
10. Cost-recovery mechanism (23%)

Some of the respondents mentioned other criteria they deemed to be useful in deciding which sanitation facilities to implement. One respondent noted the importance of considering the impact of latrines after the emergency phase. The absence of a clear monitoring system let organizations implement different types of sanitation facilities, with different approaches and methodologies and often without assessing the geographical location of the facilities.

In Fig. 1, the criteria ranked from more important to less important from the respondents' point of view are reported. Cost-recovery mechanism ranked as the least important factor for sanitation choices; respondents reported that availability of materials for spare parts and operators did not constitute a main concern for emergency response.

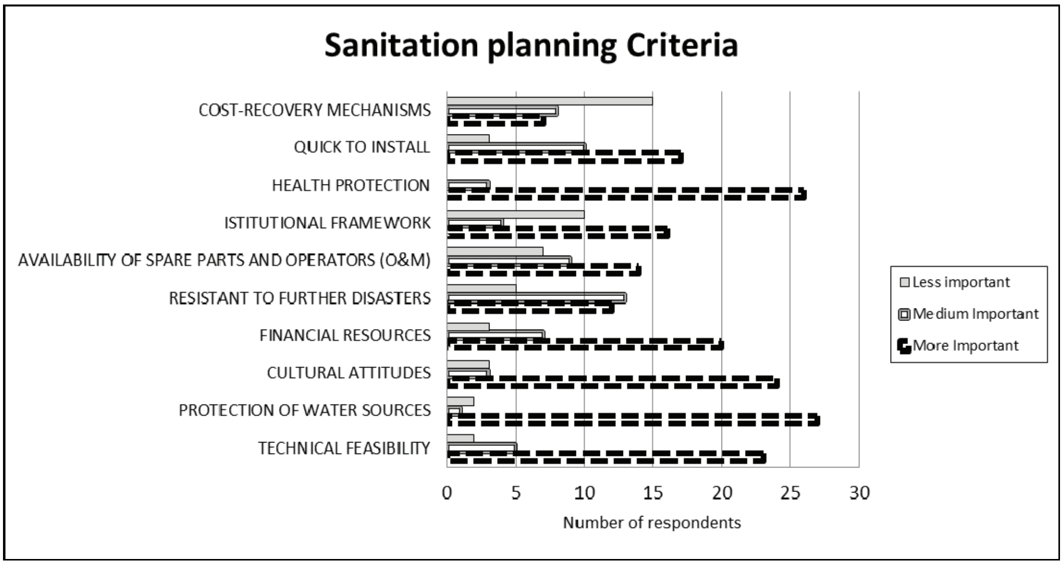


Fig. 1. Criteria for the selection of a sanitation technology

Even financial resources available for the sanitation projects were not deemed to be a criterion from which to select sanitation facilities. It was also highlighted that the availability or not of sludge disposal and/or treatment facilities was a criterion used to choose the type of sanitation system to implement. Another respondent indicated the importance of the participation component by the community from the sanitation planning to the implementation phase. Several respondents stressed the institutional component and the land tenure issues, collaboration and agreement with, and support and permissions by local and national Governments.

3.3. Sanitation technology choice

To find out which sanitation system was implemented during the different phases of emergency response, from the relief phase (0-6 months) until almost two years after the disastrous event, the respondents were asked to specify the most used technology by their respective organizations.

Herein after, we report some brief description of the main sanitation choices used/installed in the camps in Haiti after the earthquake:

- Packet latrines consist of single use disposable plastic bag in which the users defecate into. There are several types of bags that can be used for this purpose, from plastic to biodegradable bags and they can contain also enzymes in order to speed up the degradation process. One type that was employed in Haiti is the “pee-poo” bag, which consists of a double biodegradable bag system containing powdered urea. For its ease of use, it should be placed in a container of the right size. It can be directly used at household level, or in communal facility with a superstructure. After use, the bag is removed, tied and placed in a storage container for further transport and disposal (Patel et al., 2011).
- Portable toilets are single prefabricated plastic units incorporating a sit-down pan, lockable door and a sealed tank for excreta and urine. Some of them can contain chemicals to aid excreta digestion and reduce odours. The types used in Haiti were portaloos with no additional chemicals (Eyrard, 2011; Reed, 2010).
- Latrines with holding tanks made of polyethylene (PE) can be of different volumes (usually in Haiti of about 1,000 L), and are placed underneath the latrine superstructure. The superstructures consist mainly in a wooden frame with plastic or corrugated iron roofing.
- The emergency latrines are by far the most used in emergency, since the development of the plastic slab, which is the interface that protects humans from the contact with faeces. They are specifically designed for rapid deployment in emergencies (San Plat, Nga magic, etc.). Superstructures consist mainly of a wooden frame with plastic sheeting, and it does require digging of a simple pit.
- Raised latrines are used in areas where it is not allowed to dig for providing sanitation facilities, or where the geological conditions do not permit an easy digging (rocky soil) or even where the water table is high. They can be constructed with different type of materials, provided that they are resistant and watertight (Harvey et al., 2002).
- Compost toilets consist of pits where the human waste is treated by composting and dehydration to produce a usable product, which is a soil additive.
- Bio-digester is a dry type of sanitation interface with a treatment plant that stores excreta for about 30 days. When excreta are decomposed in anaerobic conditions, the treatment produces biogas that can be used as source of energy.
- EcoSan is a specially designed pan with two different holes to separate the urine from the faeces at source. No water is used to flush and ash, dry soil or sawdust are added to the faeces to absorb excess moisture.

As reported in Table 2, for the relief phase, 67% of the respondents replied to have built pit latrines, 60% emergency kit latrines with plastic slabs, and 47% Ventilated Improved Pits (VIP). For the recovery phase, the most used were pit latrines (73%), secondly VIP (60%), latrines with holding tanks (33%) and raised latrines in concrete or bricks.

Table 2. Sanitation technologies implemented by the respondents (respondents could indicate more than one type)

Element	*Sanitation facility*	*Relief phase 0-6 months, N=30 (%)*	*Recovery phase 6-12 months, N=30 (%)*	*Reconstruction phase after 1 year, N=30 (%)*
Excreta Containment	Portable toilets	12 (40)	5 (17)	3 (10)
	Emergency kit latrines	18 (60)	1 (3)	0
	Packet latrines	5 (17)	3 (10)	0
	Latrines with holding tanks	8 (27)	10 (33)	4 (13)
	Pit latrines	20 (67)	22 (73)	22 (73)
	Ventilated improved pits (VIP)	14 (47)	18 (60)	18 (60)
	Pour-flush latrines	4 (13)	4 (13)	4 (13)
	Raised latrines	7 (23)	10 (33)	10 (33)
Containment and primary treatment	Bio-digesters	1 (3)	2 (7)	5 (17)
	Compost latrines	2 (7)	4 (13)	5 (17)
	Septic tanks	3 (10)	7 (23)	11 (37)
	EcoSan	4 (13)	4 (13)	4 (13)

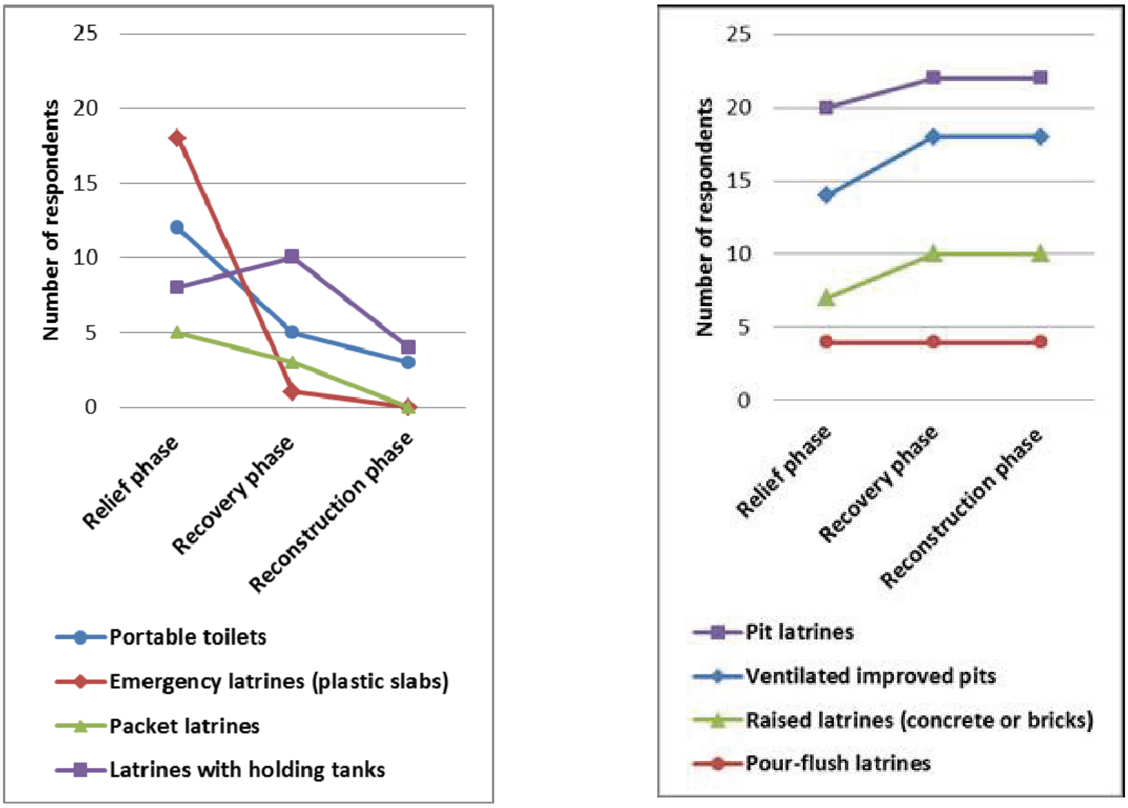


Fig. 2. Type of sanitation containment facility implemented by respondents

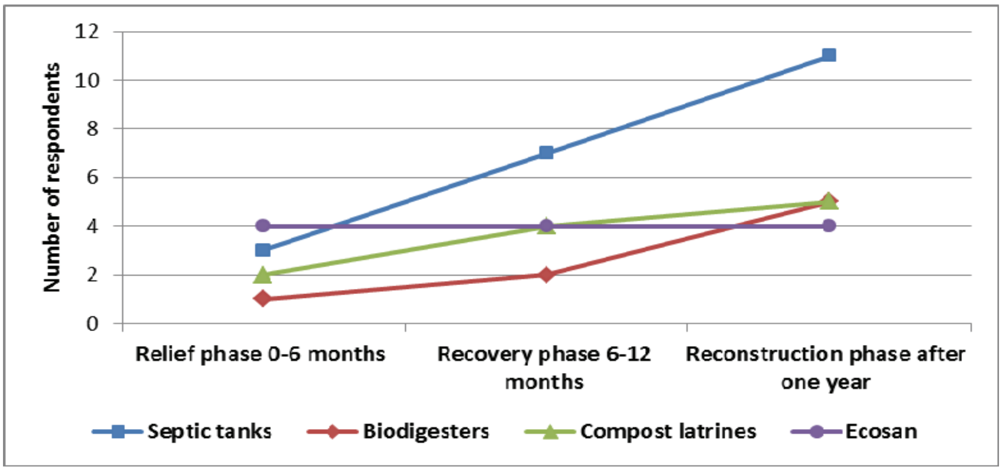


Fig. 3. Type of onsite sanitation treatment implemented by the respondents

For the reconstruction phase, 73% reported to have built pit latrines, 60% VIP, and 37% septic tanks. None indicated to have provided emergency latrines or packed latrines during the reconstruction phase. As reported in Table 2, most respondents relied on the well-known and widely implemented on-site conventional technology such as pit latrines and Ventilated Improved Pits (VIP) in all phases of the response, from emergency to reconstruction phase.

By comparing, the different technologies used and at what time-scale after the emergency, the analysis shows that there was a decrease on the use of packet latrines, porta-loos, emergency kit latrines and holding tanks, as the emergency was progressing to a more stable phase, which meant a shift from the more provisional sanitation facilities (Fig. 2) to more permanent ones. Amongst the onsite sanitation facilities that include a partial excreta treatment (reported in Fig. 3), septic tanks were reported as the most used, especially for respondents that implemented projects for schools and institutions.

In the northern region of Haiti, EcoSan systems were already implemented, through the work of SOIL, a US based NGO. When the earthquake struck, the organization installed this technology for some IDP campsites in Port-au-Prince. DINEPA built latrines connected to bio-digesters, in few campsites together with UNEP, IOM and other NGOs, but at pilot scale.

3.4. Opinions and view about the most appropriate sanitation technology

The respondents, when asked their opinions on which would be the most suitable options for IDP's camps and for re-settlement areas, showed a more innovative thinking in terms of technologies choice that was not put in practice when compared to the previous replies (Figs. 2 and 3). Fig. 4 shows how raised latrines, EcoSan, Bio-digesters and compost toilets were considered appropriate for re-settlement options, but in reality, they were not amongst the mostly chosen for the project implementation.

If we consider the whole sanitation chain, which includes defecation interface, collection of sludge and its final disposal, the sludge disposal was rarely taken into account. The respondents were asked to report how the emptying of the latrines was performed: the majority stated that it was done by *bayakous* (informal manual private workers who are paid for pits desludging in Haiti) (22 respondents); others reported that the emptying was done by vacuum tankers (14) or by trucks (13); only few reported not to have previewed sludge emptying.

The disposal was mainly done at the only official solid waste landfill site outside Port-au-Prince metropolitan area, called Truitier (20 respondents, 67%); whilst four reported to perform disposal in drainage/channels or rivers, 2 at the seaside, and 2 at specifically designed composting sites, installed after the quake. Four respondents replied that they were not aware about the location of the disposal sites.

The official landfill site did not have the appropriate treatment facilities for sludge disposal (two separate holes were dug for the sludge emptying after the quake), though it was the safest available site in proximity of the capital city Port-au-Prince. Not even with the cholera outbreak it was possible to agree on a legal site, where to build a sludge treatment facility. Eventually in May 2012, a wastewater/sludge system was built for the metropolitan area of Port-au-Prince at *Morne a Cabrit*, which even if it is a good improvement, has not the capacity to receive and treat all the wastewater/sludge produced in the metropolitan area.

3.5. Constraints to sanitation provisions

The survey data suggested that providing appropriate sanitation in Haiti was seen as a difficult task by most of the respondents, which highlighted, even in the open-ended questions, several challenges that they had to confront with, during the implementation of their projects.

During the field trips to Haiti, a number of important constraints for the delivery of emergency sanitation were identified. The respondents were asked to rate the degree of importance of 12 specific factors that inhibited certain aspects of sanitation provision in Internally Displaced Camps. The respondents rated the level of importance on a scale from five to one: most important (5 to 4), medium important (3), and low important (2 to 1). The most important factors perceived by the respondents that constrained the provision of sanitation facilities in IDP camps were the lack of hygiene awareness (70%), and the impediment of building permanent sanitation structures by landowners (70%). The factors that had less impact on the achievement of sanitation coverage were the accessibility and availability of materials.

The increase on tax importations was not strictly considered as a hindering factor (15 respondents), demonstrating the high availability of project funds during the emergency relief phase and the capacity for projects to absorb inflated market costs. Respondents to the questionnaire were also asked to explain responding to an open question, which had been, in their opinion, the main impediments in providing sanitation facilities during their project implementation.

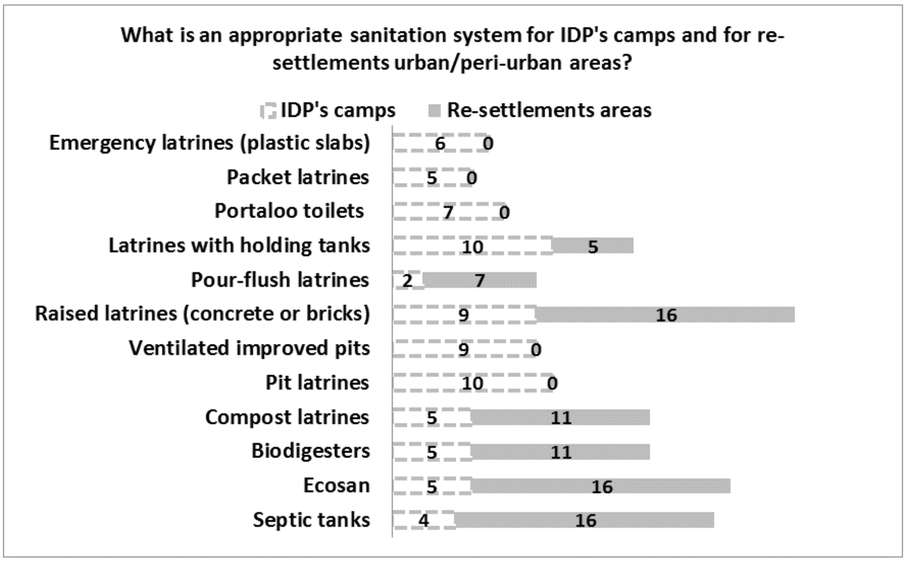


Fig. 4. Views on appropriate sanitation systems for IDP's camps and re-settlements areas

Table 3. Challenges and constraints to provide sanitation facilities in urban areas in Haiti

Criteria	*Main problems reported by the respondents*
Institutional / WASH Cluster	Landowners blocking infrastructure installations: technical choice limited to temporary latrines installation.
	Lack of governance from DINEPA and in general national and local authorities.
	Coordination challenges at the cluster level.
	Duplication of activities in areas with high densities of NGOs.
	Lack of long-term vision. Government often looked at the "immediate gain" rather than longer term solutions.
	Government, local authorities DINEPA timelines and restraints.
	Security issues.
Social and cultural	No clear communication to populations on government policy on camps.
	Camps committees and local population worked mainly with Cash for Work mechanism.
	Lack of awareness of cholera disease and hygienic issues.
Environmental and health	Implementation slowed down by several extreme events (hurricanes and heavy rains) causing flash floods.
	High groundwater table in many areas limited technology choice.
	Cholera outbreak.
Technical and operational	Not enough space to build latrine up to the standards required by SPHERE and National Authorities.
	Difficulties in procurement of some construction materials.
	Desludging machinery not always available.
	Hiring desludging machinery (operation) highly expensive.
	No safe disposal site available.
	Lack of interest to develop and scale-up innovative sanitation solutions.
Financial and economic	Donors' restraints on funding us in terms of time.
	Restriction in use of funding by donors.
	Heavy assets losses for local community.

The open questions collected replies from 15 respondents, who pointed out several issues they had to tackle. The responses were qualitatively analysed, and then categorized under the five sustainability criteria: Institutional/WASH Cluster, social and cultural, environmental and health, technical and operational, financial and economic, and reported in Table 3.

3.6. Vulnerable and gender needs

The experts were asked to report which actions they performed in order to facilitate access to sanitation services for elderly and disabled people. Out of 30 organizations, 25 respondents provided separate facilities for men and women, though other gender issues were not particularly taken into account, such as menstruation: distribution of sanitary pads and the equipment of latrines with appropriate washing points for women needs were performed by 11 organizations replying to the current survey. Latrines for disabled were not always provided: 33% noted that they did not adapt the latrines to give access to disabled people, and 17% declared to be not aware. On the other hand, respondents reported to have taken more in consideration children needs in terms of specific distribution of potties (63%). Sanitation facilities were adapted to elders or people injured or with reduced movements by 43% of respondents.

3.7. Hygiene promotion

According to the available funding, almost half the respondents (43%) reported that hygiene promotion activities (both soft and hard) were between 20% and 30% of the total available budgets for WASH activities. Out of all the 30 organisations, hygiene promotion was performed by 21 during all implementation phases (before, during and after); whilst the remaining organisations responded either before or during construction with no follow up. Out of 27 valid replies, 25 organizations provided hand-washing points, 23 provided showers, and five organizations laundry posts.

In emergency responses, the use of participatory approaches is not mainstreamed yet or is anyhow limited, especially considering that a participatory approach needs a process to build trusting relationship with the affected population. Moreover, people in camps might have come from different areas and did not always have a structured network amongst them. The Participatory Hygiene and Sanitation Transformation approach, PHAST approach (Simpson-Hebert et al., 2000), which focuses on behavioural changes through participatory approaches, was used by most of the respondents (73%), though one expert pointed out the lengthy process to develop appropriate tools (such as posters, cards, leaflets) and to organize tailored focus groups. PHAST approach was promoted by the WASH Cluster, and ad-hoc trainings were also organized for local staff. Moreover, PHAST approach was already used in Haiti, and some hygiene promoters had previous knowledge about it.

Organizations that targeted schools, hospitals or institutions (13 respondents, 43%) reported to have used Health Focus Groups and Community Health clubs. Community Health Clubs (CHC) proved to be successful in other emergencies, such as after the cholera outbreak in Zimbabwe (Whaley and Webster, 2011) especially when used for schools or rural communities, with the use of dedicated activities and public rewards for the active members promoting hygiene.

One respondent highlighted the use of the Community Led Total Sanitation (CLTS), for internally displaced and local community, which is a method developed in Bangladesh (Kar and Chambers, 2008) on moving people from Open Defecation to an Open Defecation Free (ODF) environment. However, this has proven to be successful more in rural areas than in urban and peri-urban contexts. Another respondent noted the singularity of addressing hygiene promotion in prisons and the use of a specific manual developed by its international agency.

In camp management, the cleaning and maintenance committees were instituted by 25 of the respondents, and of those, 17 reported to have established a cash for work mechanism through which the committees were paid for the latrines cleaning by the international organization. The usage of latrines was reported as positive by 23 respondents (77%), 3 reported that people moved out of the IDP camps, and 3 reported that they were abandoned after first period of usage.

In terms of health outcomes, the majority of respondents stated that they did not measure the reduction of diarrhoeal diseases related to the improvement in the camps of sanitation access. When asked if they found any reduction in the water-borne disease incidence of their beneficiaries, 10 respondents (33%) gave a positive answer, but only 4 could indicate a reduction of diseases between 40 and 65%. Ten declared they did not collect baseline data and eight (27%) reported not to be aware of any reduction, whilst two informants replied negatively to this question. The ones that measured health outcomes were the ones who stated to have the highest number of beneficiaries, over 200,000. Those organizations had more available funds for baseline assessments and data collection than smaller organization with limited programmatic capacity.

3.8. Cholera response activities

When the cholera outbreak started in late October 2010, all the respondents engaged in cholera response activities. Staff targeted trainings were carried out for health promoters and for community mobilizers of NGOs by the medical organizations present in Haiti (22 reported to have sent their staff). At first, the cholera response focused primarily on prevention measures through a disinfection campaign of water and sanitation facilities. Additionally, the distribution of chlorine in the form of Aquatabs (chlorine tablets) and local made products (*Dlo lavi*) for household water treatment was performed by 28 (93%) respondents. Distributions of soap for personal use and other cleaning/disinfecting products for latrines were carried out (22 respondents, 73%). The prevention actions included daily latrine disinfection (12 respondents, 40%), chlorination of reservoirs and water supply systems (13 respondents, 43%). Another activity that respondents carried out was the support to the WASH component of the new established Cholera Treatment Centres (CTC) and Cholera Treatment Units (CTU) in all the country, in order to receive patients and give the appropriate treatment (16 respondents, 53%). Additionally, the respondents participated in speeding up the construction of family latrines (16 respondents, 53%) along with cholera awareness campaigns (16 respondents, 53%) that were carried out using different means of communication.

Ministry of Public Health and Population (MSPP, *Ministère Santé Publique et Population*), National Directorate for Potable Water and Sanitation (DINEPA - *Direction Nationale de l'Eau et de l'Assainissement*) together with UNICEF/WHO and the WASH Cluster developed a set of 12 short key messages to prevent, recognize, and treat an ill person, to be disseminated by different media forms: radio, mobile phones, leaflets, etc. Haitians were not familiar with this infectious disease, thus they also feared its spreading. The disease spread quickly from the Artibonite region, killing many people in few hours. Population has a low perception to be at health risk by not performing hand-washing (Curtis et al., 2009), however fear could be a driver for behaviour change in the short-term and related to disease such as cholera; however long term monitoring is needed to prove sustainability after the epidemic is over. The communication campaigns made use of mobile phones: the 12 messages were sent by SMS and they were able to reach a higher number of people. It was perceived that short messages straight to the point were easily understood and remembered.

Out of the 30 respondents, 12 (40%) acknowledged that they coordinated with the MSPP in the dissemination of above-mentioned messages. All the respondents engaged in the cholera response carried out activities mostly related to information, education and communication (IEC):

- hygiene promotion with community groups (26 respondents, 87%);
- leaflets distribution (24 respondents, 80%);
- radio programmes (17 respondents, 57%);
- trucks (or car) with megaphones (16 respondents, 53%);
- mobile phones messages (6 respondents, 20%);
- door to door education in camps and neighbourhoods (1 respondent, 3%);
- theatre performances (1 respondent, 3%).

3.9. Coordination

During the height of the relief phase, the WASH cluster coordination amongst all the actors was not an easy task. In the survey, only 11 (37%) responded positively to the coordination provided by the WASH Cluster, 15 (50%) responded that it could have been better organized, whilst four (13%)

responded the WASH cluster was not of help during the response or did not participate to it. The guidance in terms of technical design was positively perceived only by nine respondents, who then felt there was a lack in phasing out mechanisms for sanitation provision. In general, there was a lack of knowledge on government strategic directions, and a critical perception that the standards for sanitation, decided by the DINEPA authorities, were unachievable in some areas (17 respondents, 57%).

One respondent pointed out that there was not a clear division of activities amongst the water and the health sectors. Moreover, coordination and information sharing, also with the shelter cluster, revealed to be a huge challenge.

3.10. Disaster Risk Reduction strategies in sanitation

In reconstructing sanitation systems after a disaster, the focus should be on building systems that can endure future hazards; the ability to maintain proper operation of sanitation systems in the aftermath of a disaster is fundamental for the protection and recovery of health of the affected population (PAHO, 2006). In terms of resistance to extreme weather events, in the recovery/reconstruction phase, 23 respondents (77%) reported to have built sanitation facilities that are more resistant to disasters, 4 did not know, and 3 did not put in place any measures. Several respondents specified what measures they implemented to strengthen sanitation facilities and reduce the risks of the effects, using some simple construction techniques that to some extent can resist to flooding and hurricanes. For example, one respondent pointed out the usefulness of having latrines with increased sludge storage capacity, to increase their filling up time, and reduce the desludging rate and maintenance in case of reduced access to service providers. Haiti had neither safety building code, nor earthquake proof construction code, so each of the measures here reported was adopted and chosen by the organizations that responded to this survey, not by the Government.

Respondents highlighted some measures that had put in place towards protection against disasters; these are listed in Table 4, according to the type of disaster addressed. After the Hyogo framework (UNISDR, 2005) that set out the International Strategy for Disaster Reduction, the Sendai agreement (UNISDR, 2015) is stressing specific actions for enhancing disaster preparedness for effective response.

The international community have to address not only practical measures to mitigate the impact of a disaster that it is not possible to predict (such as an earthquake), but also have to call upon working with local governments in the building of a culture of risk, in which the community is aware of what can happen and what to do in the first phases.

In this sense, the respondents stressed out the priority of:

- training of committees on risk prevention and mitigation;
- public awareness campaigns and alert campaigns (short messages on mobile phones, etc.);
- having a preparedness plan: stocks of materials such as plastic slabs, and other hygiene kits, alert communication plan with the local civil protection, arrangements with providers for latrine construction and maintenance (desludging).

Table 4. Sanitation protection measures per disaster type

Type of disaster	***Protection measures put in place***
Flooding	Site selection measures (avoid flood prone)
	Raised pit latrines (cement block work)
	Earth banks to divert flash flood water
Hurricane	Site selection measures (avoid flood prone, hilly tops)
	Securing roofs to walls with straps to avoid wind damage
	Strengthen structural design (block work)
Earthquake	Site selection measures (avoid steep slopes, ground condition)
	Strengthen structural designs, using reinforced concrete on the basis of seismic events

3.11. Recommendations

The respondents were also asked to provide, if any, some recommendations and lessons learnt from the Haitian case that could be useful to improve next urban disaster response. The responses to this open-ended question gave interesting inputs. The analysis of the responses was carried out dividing the contribution of the 15 respondents according to four out of five sustainability categories. The aim is not to provide a sustainability framework, but only to strengthen some important inputs influencing different aspects (from the social-cultural to the technical ones). Regarding the institutional aspect, the answers provided by the respondents were:

- Government and WASH Cluster should provide clearer guidelines (type of latrines, available designs etc.) for the WASH implementing actors;
- Coordination measures of the WASH Cluster should be enhanced and facilitated in order that all organization participate to it;
- The attention should not be focused on the number of latrines built, since this does not guarantee access to everyone, neither the actual use by people;
- The level of monitoring and accountability for NGOs should be increased. This process was already started with the establishment of specific partnerships and initiatives that focus on this, but it needs strengthening of the monitoring mechanism;
- Addressing land tenure issues. Typical to the Haitian urban displacement situation, the issue of land ownership became very stringent not only related to plan for resettlements and more permanent housing, but also in terms of sanitation services. During the earthquake the few cadastral documents

that were registered, were lost; as a consequence, people claimed ownership of land but it was difficult to ascertain their truthfulness. Improving the cadastral system and including regulations that in time of catastrophe would allow for allocation of displaced and for their sanitation systems as well;

- A hygiene and sanitation contingency and recovery plan that can be followed also in post-emergency settings should be developed at institutional level;
- Clearer roles of the different Institutions should be defined. Often sanitation responsibilities are divided amongst different ministries and government bodies, making confusion on who is responsible for what tasks: having clearer designed responsibilities will enable to plan for future sanitation facilities.

Two responses considered the environmental and health protection aspects:

- it is crucial to avoid the direct disposal of faecal sludge in rivers or drainage channels;
- proper solid waste management, especially in terms of household rubbish and emergency food rations packaging, has to be addressed.

The suggestions that can be classified as technical were:

- faecal sludge collection and treatment facilities have to be in place;
- the distribution of toilet paper or the water availability should be guaranteed, in order to avoid clogging of latrines for indiscriminate usage of other anal cleansing materials (e.g. plastic bags);
- a monitoring system on how the facilities are used and maintained should be provided;
- the promotion of the re-use of resources from composting, ecological sanitation or bio-digesters has to be strengthened.

Regarding the cultural and social issues, the respondents provided the following recommendations:

- communication with the displaced population has to be clear to improve accountability;
- available media (mobile phones, radio, TV) should be used to reach a wider audience;
- the community participation should be promoted from the planning to the implementation phase;
- the most vulnerable people have to be protected through safer access to sanitation facilities and better lighting.

5. Conclusions

The purpose of this article was to draw on a survey about sanitation technologies applied in Haiti for the IDP's camps and relocation sites, in different phases of the emergency. According to the results of this survey, during an emergency in an urban area, the sanitation solutions provided should primarily address the protection of water resources (90% of respondents), the health protection (87%) and the cultural attitudes (80%).

The most appropriate sanitation containment system for the IDP's camps, according to the respondents, varies depending on the specific phase of emergency: in the relief phase, the recommended technology is the emergency latrine (e.g. plastic slab); in the recovery and in the reconstruction phases, the most appropriate solution in this case is the holding tank latrine, considering the risks to floods in the areas

Regarding the onsite sanitation treatment, the use of septic tanks is the most recommended for the re-settlements areas together with Ecosan and raised latrines The economic aspect, which was not outlined as a constraint by the respondents, however needs to be considered since operational and maintenance costs of the sanitation facilities should be affordable in order to guarantee the sustainability of the action after the emergency phase.

Most of the respondents declared to have provided hygiene promotion activities during all the disaster phases, mainly using the PHAST approach (even because it was already applied in Haiti). This aspect is extremely important and often overlooked in order to support good WASH practices in the long term. Recommendations provided by the practitioners suggest interesting inputs such as including some technical protection measures for the reconstruction phase of the sanitation systems had been identified as critical in order to reduce the risk of collapse of the systems in future disasters.

One of the biggest challenges highlighted by respondents is unclear land tenure: addressing it is a delicate point, extremely dependent on the type of emergency and on the country/city where the emergency happens. Protection of the most vulnerable people (especially elders and disabled) should be carefully assessed and managed to minimize risks.

In this study, it was not taken into consideration the role of the private sector and in general markets for the recovery/recontruction phase, including different financing arrangements, which indeed could play a role in urban emergencies. The conclusions here presented refer to a single case study and sanitation requirements and systems should be tailored to the needs and situations.

However, the results of the survey conducted amongst the practitioners involved in the earthquake in Haiti provided some information and suggestions that could give some implementable indications for the WASH community in urban emergencies.

Acknowledgments

Authors wish to thank the Organizing Committee of the 19th International Trade Fair of Material & Energy Recovery and Sustainable Development, ECOMONDO 2015, for the opportunity given for presenting this research activity. Authors want even to thank the NGO CESVI (Bergamo, Italy) for the technical and economic support during the research activity. Daniela Giardina carried out field data collection and analysis and was responsible for the paper drafting; Sabrina Sorlini supervised the research activity and the paper drafting; Luca Rondi contributed to the paper drafting.

References

Cronin A.A., Shrestha D., Cornier N., Abdalla F., Ezard N., Aramburu C., (2008), A review of water and sanitation provision in refugee camps in association with selected health and nutrition indicators - the need for integrated service provision, *Journal of Water and Health*, **6**, 1-13.

Curtis V.A., Danquah L.O., AungerR.V, (2009), Planned, motivated and habitual hygiene behaviour: An eleven country review, *Health Education Research*, **24**, 655-673.

Eyrard J., (2011), *Portable Toilets in Emergencies: Lessons Learned from Port-au-Prince, Haiti*, Proc. 35th WEDC Int. Conference "The Future of Water, Sanitation and Hygiene: Innovation, Adaptation and Engagement in a Changing World", Loughborough University of Technology, UK, Briefing paper, **1261**, 1-4.

Harvey P.A., (2007), *Excreta Disposal in Emergencies: A Field Manual*, WEDC - Water, Engineering and Development Centre, Loughborough University of Technology, UK.

Harvey P.A., Baghri S., Reed R.A., (2002), *Emergency Sanitation: Assessment and Programme Design*, WEDC - Water, Engineering and Development Centre, Loughborough University of Technology, UK, ISBN 1-84380-005-5.

IASC, (2011), IASC Strategy, Meeting Humanitarian Challenges in Urban Areas, Inter-Agency Standing Committee, On line at: https://interagencystandingcommittee.org/meeting-humanitarian-challenges-urban-areas/documents-public/iasc-strategy-meeting-humanitarian

IASC, (2010), Response to the humanitarian crisis in Haiti - Following the 12 January 2010 Earthquake - Achievements, challenges and lessons to be learned, Inter-Agency Standing Committee, On line at: http://www.unicef.org/evaldatabase/files/IASC-Haiti_6Mos_Review_USA-2010-005-1.pdf.

IFRC, (2010a), World Disaster Report 2010: focus on urban risk, International Federation of Red Cross and Red Crescent Societies (IFRC), Geneva, Switzerland.

IFRC, (2010b), Haiti, from sustaining lives to sustainable solutions: the challenge of sanitation, International Federation of Red Cross and Red Crescent Societies (IFRC), Geneva, Switzerland, On line at: http://www.ifrc.org/Global/Publications/disasters/199600-haiti-sanitation-report-july-2010-EN.pdf

Kar K., Chambers R., (2008), *Handbook on Community-Led Total Sanitation*, Institute of Development Studies, Plan International, London, UK.

McLeod C., (2009), *Haiti: Exploring Water and Sanitation,* University of Pennsylvania, On line at: http://www.pgwi.org

OCHA, (2011), Humanitarian Bulletin (19 November-19 December 2011), United Nations Office for the Coordination of Humanitarian Affairs, Port-au-Prince, Haiti; Online at: http://reliefweb.int/sites/reliefweb.int/files/resources/Full_Report_3147.pdf

PAHO, (2006), The challenge in disaster reduction for the water and sanitation sector: improving quality of life by reducing vulnerabilities, Pan American Health Organization (PAHO/WHO), United Nations Children's Fund (UNICEF), International Strategy for Disaster Reduction (ISDR), International Federation of Red Cross and Red Crescent Societies (IFRC), Washington D.C., USA.

Patel D., Brooks N., Bastable A., (2011), Excreta disposal in emergencies. bag and peepoo trials with internally displaced people in Port-au-Prince, *Waterlines*, **30**, 61-77.

Pollmann Gomez A., Sorlini S., Collivignarelli C., (2014), Water safety of improved source: the case study of Vilanculos (Mozambique), *Procedia Environmental Science, Engineering and Management*, **1**, 47-51.

Reed B., (2010), *Emergency Excreta Disposal Standards and Options for Haiti*, DINEPA & Global WASH Cluster, WEDC - Water, Engineering and Development Centre, Loughborough University of Technology, UK.

Rondi L., Sorlini S., Collivignarelli M.C., (2014), Water safety plan: an approach to reduce drinking water contamination in a rural area of Senegal, *Procedia Environmental Science, Engineering and Management*, **1**, 47-51.

SuSanA, (2008), Towards more sustainable sanitation solutions - SuSanA Vision Document, Sustainable Sanitation Alliance (SuSanA), Germany.

The Sphere Project, (2011), The Sphere project: humanitarian charter and minimum standards in disaster response (Sphere handbook), Sphere Project, Geneva, Switzerland.

Toole M.J., Waldman R.J., (1997), The public health aspects of complex emergencies and refugee situations, *Annual Review of public Health*, **18**, 283-312.

UNISDR, (2005), Hyogo Framework for Action 2005-2015: Building the Resilience of Nations and Communities to Disasters, United Nations International Strategy for Disaster Reduction (Extract from the final report of the World Conference on Disaster Reduction - A/CONF.206/6).

UNISDR, (2015), Sendai Framework for Disaster Risk Reduction 2015-2030, United Nations International Strategy for Disaster Reduction, On line at: http://www.unisdr.org/we/inform/publications/43291

Simpson-Hebert M., Sawyer R., Clarke L., (2000), The PHAST Initiative. Participatory Hygiene and Sanitation Transformation - A New Approach to Working with Communities, World Health Organisation and the United Nations Development Program - World Bank Water and Sanitation Program.

Sorlini S., Rondi L., Pollmann Gomez A., Collivignarelli C., (2015), Appropriate technologies for drinking water treatment in Mediterranean countries, *Environmental Engineering and Management Journal*, **14**, 1721-1733.

Whaley L., Webster J., (2011), The effectiveness and sustainability of two demand driven sanitation and hygiene approaches in Zimbabwe, *Journal of Water, Sanitation and Hygiene*, **1**, 20-36.

WHO/UNICEF, (2010), Progress on sanitation and drinking water: 2010 update. Joint Monitoring Programme for Water Supply and Sanitation, World Health organization and United Nations Children's fund.

Wisner B., Adams J., (2002), *Environmental Health in Emergencies and Disasters: A Practical Guide*, World Health Organization, Geneva (Switzerland), ISBN 92-4-154541-0.

Environmental Engineering and Management Journal

September 2016, Vol.15, No. 9, 2041-2048
http://omicron.ch.tuiasi.ro/EEMJ/

"Gheorghe Asachi" Technical University of Iasi, Romania

BIOGAS PRODUCTION FROM BIODEGRADABLE BIOPLASTICS

Ciro Vasmara*, Rosa Marchetti

Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria - Unità di ricerca per la suinicoltura,
345 Via Beccastecca, 41018 San Cesario sul Panaro (MO), Italy

Abstract

In this study, the potential for biogas production from biodegradable bioplastics was evaluated. Mater-Bi® (a family of maize-starch based flexible films) and PLA (PolyLactic Acid; a rigid, polylactide-based, polymer) bioplastics were digested in laboratory batch reactors, alone or in co-digestion with pig slurry or scotta (partially deproteinized cheese whey), at 35°C or 55°C. Methane (CH_4) and hydrogen (H_2) production were monitored during the incubation period. Maximum CH_4 (Mmax) or H_2 (Hmax) production per reactor, potential CH_4 (BMP) or H_2 (BHP) production g^{-1} volatile solids (VS), and residual VS in the digestates were determined. Methane was produced when bioplastics were digested alone or with pig slurry, whereas H_2 was produced only in co-digestion with scotta. Mmax, BMP, Hmax and BHP were on average higher at 55°C than at 35°C (+69%, +158%, +51% and +45%, respectively). At 35°C, in monodigestion, small amounts of CH_4 (33 mL g^{-1} VS) were produced with Mater-Bi® only. At 55°C, the BMP for Mater-Bi® and for PLA were equal to 113 mL and 282 mL CH_4 g^{-1} VS, respectively. Monodigestion of Mater-Bi® and PLA at 55°C reduced the initial VS content by 51%. When PLA was in co-digestion with pig slurry, Mmax was 12% higher than the theoretical one, with a synergistic effect. In co-digestion with scotta, a nearly significant 12% increase in H_2 production was observed for Mater-Bi® incubated at 35°C. The exploitation of bioplastic waste in anaerobic digestion for biogas production, together with or in alternative to conventional composting, appears a promising possibility for a successful waste management.

Key words: co-digestion, dark fermentation, deproteinized cheese whey, hydrogen, methane, pig slurry

Received: January, 2016; Revised final: August, 2016; Accepted: August, 2016

1. Introduction

Bioplastics (or bio-based plastics) are plastics based on renewable resources (Comaniță et al., 2015; Pilla, 2011) and their use in substitution of fossil fuel-based plastics can reduce the depletion of fossil fuels, as well as the pollution from emissions generated by the conventional plastics production processes.

Bio-based plastics may be biodegradable or non-biodegradable. When they are biodegradable, their disposal is simpler and faster than that of conventional plastics. For this reason, the use of biodegradable bioplastics is widely spreading. The most widespread biodegradable bioplastics are: polylactide biopolymers (PLA; 12.2% global production), starch blends (10% global production), polyhydroxyalkanoates (PHAs; 2% global production), cellulose acetate (2% global production) (Aeschelmann et al., 2015).

PLA is a thermoplastic, high-strength polymer, mainly obtained from polymerization of lactic acid produced by bacterial fermentation of hexose sugars (Garlotta, 2002). The PLA bioplastics are used for rigid packaging (industrial packaging, food packaging, biocompatible/bioabsorbable medical devices). Mater-Bi® is a family of liquid impervious, thermoplastic, biodegradable films, which comprises a blend of an interpenetrated network of destructurized maize-derived starch with ethylene/acrylic acid copolymers or ethylene/vinyl alcohol copolymers, and an aliphatic polyester such as polycaprolactone (Toms and Wnuk, 1999). The Mater-Bi® plastics are used for flexible packaging (as shoppers, or backsheets in articles such diapers, sanitary napkins).

---

* Author to whom all correspondence should be addressed: e-mail: ciro.vasmara@crea.gov.it; Phone: +39 059926268; Fax: +39 059928371

In recent years the production of bioplastics has increased, especially as consequence of the EU pressure for reducing industrial and green-house gas emissions. In 2014 the global production capacities of bioplastics was of 1,697,000 tons and the forecasts indicate that in 2019 a production capacity of 7,848,000 tons will be reached, 1,287,000 of which will be biodegradable. As the use of bioplastics has dramatically increased, the amount of bioplastics requiring disposal has also increased. Biodegradable bioplastics are usually subject to separate collection within the organic fraction of urban waste (OFMSW), followed by composting. The final product from composting enters the fertilizer market (Centemero et al., 2012). A further increase in the value of bioplastics to be disposed of could result from their use as substrates in anaerobic digestion for the production of methane or in dark fermentation for the production of hydrogen. Garaffa (2014) studied the combination of anaerobic digestion and composting for biowaste treatment. This combination, beside the production of energy, permits a reduction in the solid residues amount, an improvement of compost quality and a reduction in odour emissions (Bacenetti and Fiala, 2015). However, pre-treatments are needed for the sorting-out of non-biodegradable biowaste components. Bioplastics degradability could facilitate the biowaste anaerobic digestion and allow a reduction of the pre-treatment costs.

The aim of this study was to evaluate the potential for CH_4 or H_2 production using bioplastics as carbon source. In particular, we evaluated at laboratory scale the rate and yield of H_2 or CH_4 production using two types of biodegradable bioplastics: Mater-Bi® and PLA, in mesophilic or thermophilic conditions, in monodigestion or in co-digestion with other organic waste. Co-digestion was considered as the more realistic hypothesis, since bioplastics are usually mixed with OFMSW. Mater-Bi® and PLA were chosen as widespread representatives of flexible and rigid packaging, respectively. Selected substrates for co-digestion were pig slurry and scotta. Pig slurry was chosen because animal effluents are the material most frequently exploited in anaerobic digestion of agricultural waste. Scotta is a partially deproteinized cheese whey coming from the production of ricotta, a typical Italian milk-derived product. Scotta, like cheese whey, contains high amounts of lactose. For this reason it has a high pollution potential, if not exploited in re-use systems. In this work scotta has been selected as representative of waste with highly fermentable carbohydrate content and therefore particularly suitable for hydrogen production (Kapdan and Kargy, 2006).

2. Material and methods

2.1. Bioplastics and co-digestion materials

In this study, two types of bioplastics products were used: shoppers and plastic cups. Shopper bags in Mater-Bi® were produced by Plastica Marconi Srl licenced by Novamont; the plastic cups in PLA Ingeo™ were produced by ILPA S.r.l. (Fig. 1). They were manually cutted with scissors in small pieces (<1 cm^2) before use in anaerobic digestion and for analyses.

Pig slurry was collected from the storage tank of our swine husbandry experimental farm, in S. Cesario sul Panaro (MO), after mechanical homogenisation. Scotta was obtained from a cheese-making factory located near our Research Unit. Both pig slurry and scotta were frozen immediately after collection, stored at -20°C and thawed just before use. Selected analytical characteristics of these materials are reported in Table 1.

Fig. 1. Bioplastic material used in the experiment

Table 1. Selected composition characteristics of the materials used in the experiment

Parameter	*Unit*	*Pig slurry*	*Scotta*	*Mater-Bi®*	*PLA*
Total solids (TS)	%	1.52	6.37	98.5	99.6
Volatile solids	% FW	1.19	5.74	94.5	99.5
pH		7.6	6.8	8.0	8.1
Total N	% FW	0.126	0.093	0.060	0.012
NH_4-N	% FW	0.070	0.008	ND	ND
Organic carbon	% TS	46.25	36.40	50.64	49.4
Lactose monohydrate	% FW	ND	5.8	ND	ND

ND= not determined; FW= fresh weight

2.2. AD inoculum preparation

Pig slurry withdrawn from the farm storage tank collecting the liquid fraction of pig manure after solid separation was digested, and the digestate was used as the inoculum source (Vasmara et al., 2015). The inoculum was prepared following a lab consolidated procedure (patent pending; Marchetti et al., 2015).

2.3. Analytical methods

Hydrogen and CH_4 concentrations in the biogas were determined by means of a MicroGC Agilent 3000 gaschromatograph (GC), equipped with 2 columns: Molsieve and Plot U; detector: TCD. Carrier gas: argon.

Total solids (TS), volatile solids (VS), total N and pH were determined on pig slurry, scotta and digestates according to APHA (1992). Total solids were determined gravimetrically by thermal treatment at 105°C at constant weight. Volatile solids (VS) were determined as the difference between TS and ashes. Ashes were determined by incineration in a muffle furnace at 550°C for 10 hrs. Total N was determined with the Kjeldahl apparatus after acid digestion with H_2SO_4 and copper as catalyzing agent. Ammonium N was determined on pig slurry samples and digestates by distillation. The pH was determined after suspension, 2-h stirring and sedimentation of 1 g fresh matter in 50 mL distilled water.

Volatile solids and residual VS in bioplastics after anaerobic digestion (AD) were determined as follows. Each digestate was sieved with the objective of separating the remaining plastic pieces greater than 2 mm. The recovered fragments were washed with distilled water, dried at 60°C and weighed for calculating the corresponding biodisintegration degree, according to Sarasa et al. (2009). Total solids were determined gravimetrically by thermal treatment at 105°C at constant weight, ashes were determined in a muffle furnace at 500°C for 4 h, according to Gómez and Michel (2013).

3. Experimental

3.1. Experimental design

Treatments applied were as follows: 2 bioplastic materials (Mater-Bi® and PLA) in monodigestion or in co-digestion with pig slurry (PS) or scotta (Sc) at two temperature levels: 35 and 55°C, in a completely randomized block design with 3 replications, for a total of 48 reactors. Reactors without energy source were included as controls. Digestions were carried out in batch conditions. Methane volume from plastics alone and plastics in co-digestion with pig slurry, or hydrogen volume from plastics or plastics in co-digestion with scotta were measured during the incubation period.

3.2. Digestion experiment

Anaerobic digestion was carried out in 100-mL reactors (118.5 mL effective volume) according to Owen et al. (1979). Besides the substrates (see below) the reaction mixture included 5 mL inoculum. The headspace of the reactors was gassed with 100% N_2 throughout the preparation steps before inoculation. Reactors were plugged with butyl rubber stoppers and aluminium seals and they were incubated at 35°C and 55°C for 98 days. During the incubation period they were randomly distributed on the incubator shelves. Biogas was collected by means of 100 mL glass syringes. The syringes were equipped with latex tubes (internal diameter, 3 mm) for connection to the CG inlet. The tubes were tightened with clamps, when not in use. A gas aliquot was let out before each injection, in order to purge the gas line. The incubation period was completed when there was no more biogas production in any of the reactors. No methane and hydrogen production was detected in the control reactors at 35°C and 55°C, where the inoculum had been suspended in hydration medium without energy source.

3.3. Monodigestion conditions

Anaerobic digestion was carried out using plastics as substrate (Mater-Bi® or PLA). The reaction mixture included 1 g (fresh weight) of plastics in 50 mL sterilized phosphate buffered basal medium (PBBM), without energy sources ("hydration medium"). The initial pH of the mixture was on average equal to 7.4.

3.4. Co-digestion conditions

Mater-Bi® and PLA were utilized in co-digestion with pig slurry (PS+), to check the methane production, or with scotta (Sc+) to check hydrogen production. In each PS+ reactor, 1 g of plastics was added to 50 mL non-sterilized pig slurry. In each Sc+ reactor, 1 g of plastics was added to 50 mL non-sterilized scotta. Pig slurry or scotta alone were inoculated as controls. The average pH value in presence of pig slurry was 7.5, whereas it was 6.9 in the presence of scotta.

3.5. Hydrogen production potential and biomethanation potential

Methane production was measured 2 days after the start of the incubation and then weekly for 3 months. The comparison of the cumulative CH_4 production curves was based on the parameters: maximum cumulative CH_4 production, Mmax (mL CH_4), expressed as the maximum amount of CH_4 cumulated over time that can be produced in the reactor, including the amounts of CH_4 released in the syringe at each measurement date as well as the CH_4

volume remaining within the reactor; daily rate of CH_4 accumulation in the linear phase of CH_4 accumulation, R (mL CH_4 d^{-1}); and lag time duration (λ, d) that is the time of microbial adaptation before the starting of CH_4 production. These parameters were estimated by fitting a modified Gompertz equation to measured data (Lay et al., 1997). Measurements from 3 replicates were merged for the parameter value estimation. Fitting was performed using the PROC NLIN of the SAS package (SAS Institute, 1987); the parameter values were estimated according to the Gauss-Newton method. Biomethanation potential (mL CH_4 g^{-1} VS) was expressed as the maximum amount of CH_4 cumulated over time (Mmax), that can be produced by a given substrate per g of volatile solids.

3.6. Hydrogen production potential

The amount of H_2 that can be released by dark fermentation depends on the concentration of the fermentable substrate (Mohanty and Das, 2012), lactose, in our case. However, in batch conditions and in the absence of buffers, dark fermentation of carbohydrates involves a drop of the pH values, which may inhibit microbial activities. Stuck fermentations may occur even when the substrate has been only partially utilized. Consequently, the amount of H_2 produced in these conditions is not the one potentially producible. However, fermentation can be restored by adjusting pH values to suitable values (Kim et al., 2011). In our batch conditions, hydrogen production potential (BHP) was calculated as the sum of the amounts of H_2 released 24 hours after the start of incubation, and at each occasion after pH adjustment at neutrality. The pH measurements were made within an anaerobic workstation (Whitley DG250 Workstation, Don Whitley Scientific) using a Crimson pH meter (Titromatic 1S). The pH adjustment was made with NaOH 32%, using a syringe equipped with a sterile filter (Sartorius Minisart RC15, pore size 0.2 μm). Measurements of H_2 release were stopped when no H_2 production occurred in spite of the pH adjustment. The maximum cumulated H_2 production per reactor (Hmax) was calculated as the sum of H_2 volumes measured after each pH correction, including the amounts of H_2 released in the syringe at each measurement date as well as the H_2 volume remaining within the reactor. Hydrogen production potential (mL H_2 g^{-1} VS) was expressed as the maximum cumulated amount of H_2 that can be produced by a given substrate per g of volatile solids.

3.7. Statistical analysis

Analysis of variance (ANOVA) was performed using the PROC MIXED procedure of the SAS statistical package (Littell et al., 1996). Multiple comparisons of the means were carried out using the SAS LSMEANS statement. Factors and factor interaction effects were considered significant at $P < 0.05$ The Tukey or Tukey-Kramer test ($P < 0.05$) was used to compare treatment mean values.

4. Results and discussion

In monodigestion, and in co-digestion with pig slurry, bioplastics produced almost exclusively methane, whereas only hydrogen was produced with bioplastics in co-digestion with scotta. The effect of the temperature was the most significant on biogas production. The presentation of the results will take into consideration these evidences.

4.1. Bioplastics and methane production: monodigestion

At 35°C, reactors with PLA did not produce gas, whereas those with Mater-Bi® produced only 33 mL of methane (Table 2). At 55°C, the Mmax value for Mater-Bi® was 108 mL. For the PLA treatment, the lag phase was longer than for Mater-Bi® but the final Mmax (285 mL) was nearly twice than that for Mater-Bi®.

At 55°C, physical changes occurred both in Mater-Bi® and in PLA during the incubation period: after a week, PLA became folded and opaque. Sixty days after the start of the incubation, PLA was completely dissolved whereas Mater-Bi® was nearly disintegrated into very small pieces (Fig. 2). No physical changes were observed at 35°C.

The temperature effect on the physical properties of the PLA cups was expected, because Kale et al. (2007) described the same physical changes for PLA bottles in composting conditions at 65°C. The fragmentation causes the breaking of the long polymer chains into shorter oligomers chains and monomers more easly digestible for microorganisms.

Yagi et al. (2009) compared the anaerobic degradation of PLA at 35°C and at 55°C and observed a 90% degradation in 60 d at 55°C, whereas at 35°C the PLA degradation started 55 d after the start of the incubation and was much more slower than that at 55°C. As far as Mater-Bi® is concerned, disintegration was observed only at 55°C, whereas no disintegration occurred at 35°C. On the one hand, Mater-Bi® fine shredding at 55°C (Fig. 2) could explain the higher methane yield observed at 55°C, that was nearly three times the yield at 35°C. On the other hand, the lack of physical degradation for PLA at 35°C can explain the lack of methane production, which was instead abundant at 55°C, where the PLA complete dissolution occurred.

4.2. Bioplastics and methane production: co-digestion with pig slurry

The use of pig slurry in co-digestion with plastics remarkably increased the rate and amount of biogas production, in comparison with the plastics in monodigestion. The methane production rate was higher at 35°C (Fig. 3a) than at 55°C (Fig. 3b) but the total amount of methane produced at 35°C was much lower than at 55°C (285 mL vs 483 mL, on average). At 55°C, PLA produced 68% more methane than Mater-Bi®.

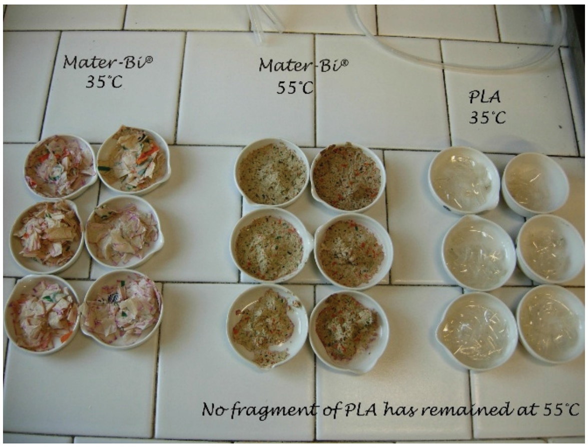


Fig. 2. Bioplastic residues in the digestates

When considering the theoretical maximum amount of methane production, that is the sum of Mmax for the individual ingredients in the recipe (pig slurry and bioplastics), the theoretical production was quite similar to the actual production, except for PLA (Table 2). In fact, at 55°C, PLA produced 12% more methane than expected. Therefore, a synergistic effect can be invoked for co-digestion. A synergistic effect of pig slurry in co-digestion with other substrates has been reported (Esposito et al., 2012). The reason may be in the capacity of pig slurry to improve substrate quality mainly by buffering and improvement of the equilibrium among nutrients.

4.3. Biomethanation potential

In the previous paragraphs, we referred the production of methane to the reaction volume (55 mL) of the reactors, that is, the Mmax value in Table 2 is the maximum amount of methane produced *per reactor*. On this basis, the amount of methane produced in the reactor with PLA in co-digestion with pig slurry at 55°C was more than twice that produced by pig slurry alone (Table 2), the substrate weight (50 g in monodigestion, 51 g in co-digestion) and volume being almost the same in the two treatments. In practical situations, we can obtain higher amounts of methane per reactor volume when adding bioplastics to pig slurry in AD.

The biomethanation potential of PLA in monodigestion reached 282 mL CH_4 (STP) g^{-1} VS. This amount can compete with that of other more conventional organic substrates.

Table 2. Parameter values of the Gompertz model for the methane accumulation curves. The theoretical value of Mmax is the sum of the Mmax of the individual recipe ingredients. All the models were significant for P<0.001

Temperature (°C)	*Material*	*λ (d)*	*R (mL d^{-1})*	*Mmax (mL)*	*Theoretical value of Mmax in co-digestion (mL)*
Monodigestion					
35	Mater-Bi®	1.26	1.3	33	
35	PLA	no methane production			
55	Mater-Bi®	0.24	2.1	108	
55	PLA	17.4	4.1	285	
35	Pig slurry	0.53	12.1	264	
55	Pig slurry	1.91	13.2	256	
Co-digestion					
35	Mater-Bi®	0.32	12.8	303	296
35	PLA	0.44	12	267	264
55	Mater-Bi®	2.88	11.6	362	365
55	PLA	-0.02	9.2	605	542

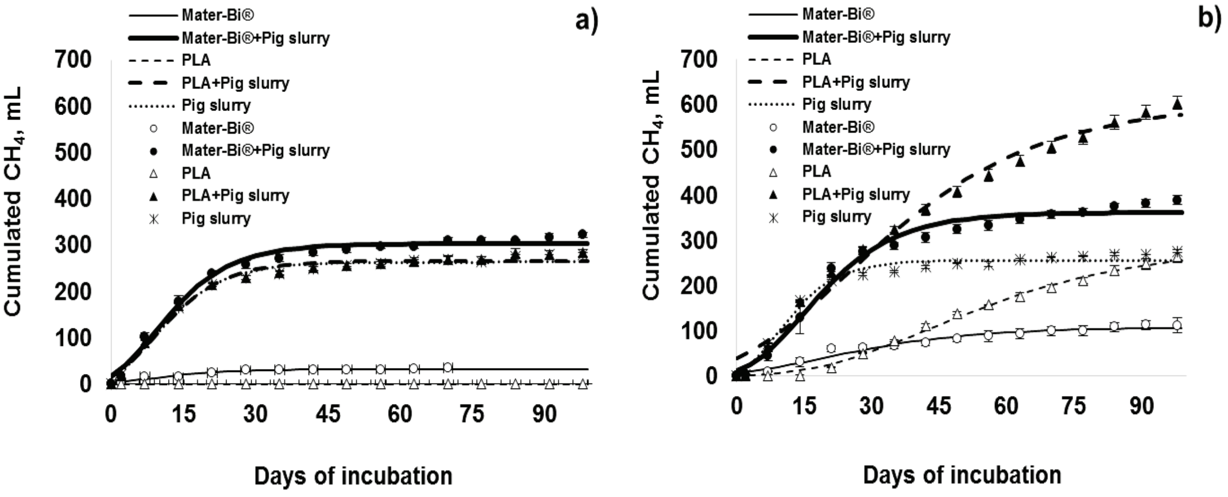


Fig. 3. Gompertz-estimated curves of methane accumulation from bioplastics in monodigestion or in co-digestion with pig slurry, with incubation: a) at 35°C; b) at 55°C. Lines: estimated values. Dots: measured values. Vertical bars are the standard deviations

In fact, reported BMP values for pig slurry and cattle slurry are, respectively, 321 and 247 mL CH_4 g^{-1} VS and, for grass silage, 320 mL CH_4 g^{-1} VS (Luna-delRisco et al., 2011); for wheat straw, 276 mL CH_4 g^{-1} VS (Bauer et al., 2010); for maize residues, 317 mL CH_4 g^{-1} VS, for barley straw, 229 mL CH_4 g^{-1} VS, and rice straw, 195 mL CH_4 g^{-1} VS (Dinuccio et al., 2010).

Substrate degradation occurred as consequence of biogas production (Fig. 4). At the end of the digestion process, at 55°C, the residual VS content in digestates was 44% of the initial, in co-digestion, and 63%, in monodigestion. The highest VS reduction (i.e., the lowest value in the residual VS percentage) was observed at 55°C for PLA in co-digestion with pig slurry (36% of the initial VS content). The VS reduction was much lower at 35°C (80% of the initial VS content, either in monodigestion or in co-digestion). This reduction in waste organic load fulfils the general need to pursue environmental quality preservation.

4.4. Bioplastics and hydrogen production: co-digestion with scotta

We already mentioned that negligible H_2 production in monodigestion was obtained at 55°C only, by using Mater-Bi® as substrate. We therefore will limit our comments to the results for bioplastics in co-digestion with scotta (Table 3). The incubation temperature showed the strongest effect on H_2 production. In fact, Hmax was on average 1.5 times higher at 55°C than at 35°C, with no significant differences between recipes. The BHP value was also significantly higher at 55°C (104 mL H_2 g^{-1} VS, on average) than at 35°C (70 mL H_2 g^{-1} VS). For BHP, however, the H_2 production with scotta alone was higher than for bioplastics in co-digestion with scotta. The main source of organic C in scotta, lactose, is definitely more easily digestible than complex polymers of amylose and amylopectine (Mater-Bi®), or of lactic acid (PLA). No significant differences were found among treatments in the residual VS content of the fermented broths.

Even though no significant differences could be detected among recipes, it should however be noticed that at 35°C Hmax with Mater-Bi® was higher than that of scotta alone, and closer to Hmax at 55°C, meaning a possible contribution of Mater-Bi® to H_2 production. Hydrogen production from starch-derived bioplastics has been reported by Russo et al. (2009), and can be explained by the suitability of starch degradation products (glucose) to dark fermentation.

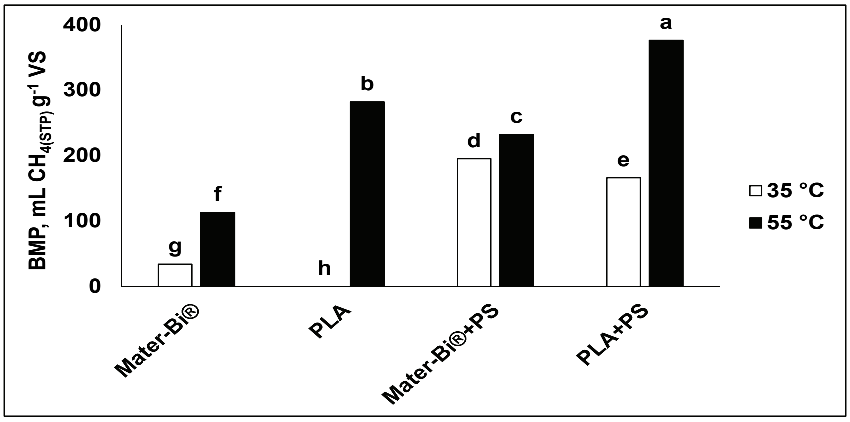


Fig. 4. Biomethanation potential (BMP) of Mater-Bi® and PLA in monodigestion and in co-digestion, at 35°C and at 55°C. Equal letters above histograms indicate the lack of significant differences among treatments

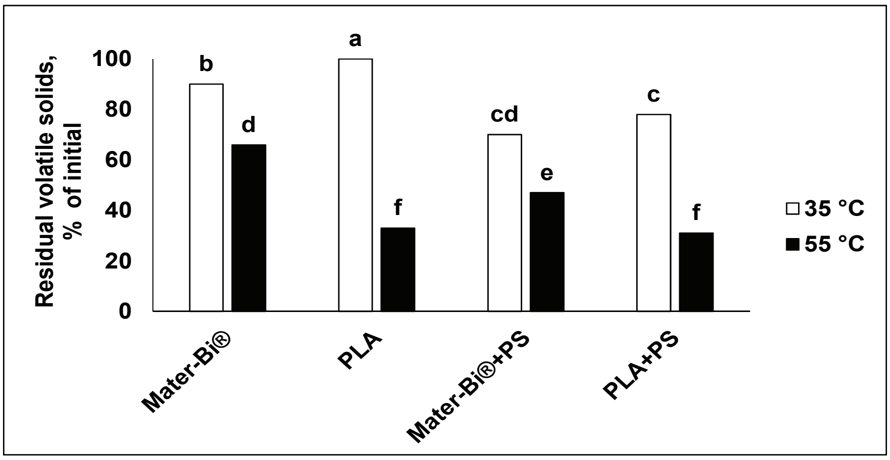


Fig. 5. Residual volatile solids as percentage of initial after anaerobic digestion of Mater-Bi® and PLA, in monodigestion and in co-digestion at 35°C and at 55°C. Equal letters above histograms indicate the lack of significant differences among treatments

Table 3. Mean values of maximum H_2 production (Hmax), biohydrogen production potential (BHP) and residual volatile solids (VS) in the fermented broths of bioplastics in co-digestion with scotta, compared with scotta alone

Temperature (°C)	***Recipe***	***Hmax (STP) cum, mL***		***BHP, mL H_2(STP) g^{-1} VS***		***Residual VS, % initial[1]***	
35	Mater-Bi®, co-digestion	267	abc	70	bc	49.2	a
35	PLA, co-digestion	224	c	58	c	56.2	a
35	Scotta, monodigestion	239	bc	83	bc	49.7	a
	Mean	243		70		51.7	
55	Mater-Bi®, co-digestion	369	a	96	ab	53.7	a
55	PLA, co-digestion	345	ab	89	bc	52.5	a
55	Scotta, monodigestion	364	a	126	a	50.3	a
	Mean	360		104		52.1	

[1] *Initial VS content in the reactors was: 2.89 g, for scotta; 3.83 g, for Mater-Bi® in co-digestion with scotta, and 3.88 g for PLA in co-digestion with scotta.*

The presence of lactose from scotta may have favoured the start of the Mater-Bi® decomposition. Conversely, PLA is derived from lactic acid: the possible release of this molecule does not imply any H_2 production by fermentation. The incubation at 55°C could have affected the inoculum performances for H_2 production. In fact, inocula used in our laboratory are suitable for mesophilic conditions, and no acclimatation was applied before use at 55°C.

5. Conclusions

The biomethanation potential of bioplastics can compete with that of other more conventional organic substrates, actually used in biogas plants, since with PLA in monodigestion it was possible to obtain up to 282 mL CH_4 (STP) g^{-1} VS.

Biogas production using Mater-Bi® or PLA as substrates benefits from thermophilic conditions. These conditions also favor the physical degradation of the bioplastics: anaerobic digestion at 55°C permitted the total degradation of PLA and a partial degradation of Mater-Bi®, with a substantial reduction of the size of the plastic fragments. Conversely, mesophilic conditions did not induce any physical change.

Co-digestion of Mater-Bi® or PLA with pig slurry was more productive than monodigestion (384 mL, on average, in co-digestion and 142 mL, on average, in monodigestion), also due to contribution of the pig slurry VS. In fact, methane yields per reactor in co-digestion at 55°C were on average more than twice (PLA, 605 mL) or 3 times (Mater-Bi®, 362 mL) those in monodigestion (285 mL and 108 mL, respectively). A synergistic effect of pig slurry with PLA was evidenced in thermophilic conditions.

Bioplastics in monodigestion did not produce any H_2. In co-digestion with scotta, a slight increase in H_2 production in comparison with scotta in monodigestion was observed for Mater-Bi® incubated at 35°C (267 mL vs 239 mL from scotta in monodigestion). Further research seems worthwhile in order to understand if the observed H_2 production increase in codigestion of Mater-Bi® with scotta, although not significant in the actual experimental conditions, may be proven significant in specific “ad hoc” experiments.

The exploitation of bioplastic waste in anaerobic digestion for biogas production, together with or in alternative to conventional composting, appears a promising possibility for a successful waste management.

Acknowledgements

We are grateful to Anna Orsi and Lidia Sghedoni for their contribution to the laboratory analyses.

References

Aeschelmann F., Carus M., Baltus W., Blum H., Busch R., Carrez D., Ißbrücker C., Käb H., Lange K.-B., Philp J., Ravenstijn J., von Pogrell H., (2015), *Bio-based Building Blocks and Polymers in the World*, 3rd Edition, Nova-Institut GmbH, Hürth, Germany.

APHA, (1992), Standard Methods for the Examination of Water and Wastewater, 18th Edition, American Public Health Association, Washington D.C.

Bacenetti J., Fiala M., (2015), Carbon footprint of electricity from anaerobic digestion plants in Italy, *Environmental Engineering and Management Journal*, **14**, 1495-1502.

Bauer A., Leonhartsberger C., Bösch P., Amon B., Friedl A., Amon T., (2010), Analysis of methane yields from energy crops and agricultural by-products and estimation of energy potential from sustainable crop rotation systems in EU-27, *Clean Technologies and Environmental Policy*, **12**, 153-161.

Centemero M., Zanardi W., Ricci M., Dell'Anna D., Bianconi L., Ghiringhelli G., Giavini M., (2012), *Consorzio Italiano Compostatori Technical Report 2012*, (In Italian), 4th Edition, Papiergraph, Roma.

Comaniţă E.-D., Ghinea C., Hlihor R.M., Simion I.M., Smaranda C., Favier L., Roşca M., Gostin I., Gavrilescu M., (2015), Challenges and oportunities in green plastics: an assessment using the ELECTRE decision-aid method, *Environmental Engineering and Management Journal*, **14**, 689-702.

Dinuccio E., Balsari P., Gioelli F., Menardo S., (2010), Evaluation of the biogas productivity potential of some Italian agro-industrial biomasses, *Bioresource Technology*, **101**, 3780-3783.

Esposito G., Frunzo L., Giordano A., Liotta F., Panico A., Pirozzi F., (2012), Anaerobic co-digestion of organic wastes, *Reviews in Environmental Science and Bio/Technology*, **11**, 325-341.

Garaffa C., (2014), Anaerobic digestion and bioplastics: a strong alliance for optimal food waste capture rates, higher biogas yields and quality compost production, AD Europe 2014 - 20-21 February, Dublin, Ireland.

Garlotta D., (2002), A literature review of Poly(Lactic Acid), *Journal of Polymers and the Environment*, **9**, 63-83.

Gómez E.F., Michel Jr.F.C., (2013), Biodegradability of conventional and bio-based plastics and natural fiber composites during composting, anaerobic digestion and long-term soil incubation, *Polymer Degradation and Stability*, **98**, 2583-2591.

Kale G., Auras R., Singh S.P., Narayan R., (2007), Biodegradability of polylactide bottles in real and simulated composting conditions, *Polymer Testing*, **26**, 1049-1061.

Kapdan I.K., Kargy F., (2006), Bio-hydrogen production from waste materials, *Enzyme and Microbial Technology*, **38**, 569-582.

Kim D.H., Kim S.H., Jung K.W., Kim M.S., Shin H.S., (2011), Effect of initial pH independent of operational pH on hydrogen fermentation of food waste, *Bioresource Technology*, **102**, 8646-8652.

Lay J.J., Li Y.Y., Noike T., (1997), Influences of pH and moisture content on the methane production in high solids sludge digestion, *Water Research*, **31**, 1518-1524.

Littell R.C., Milliken G.A., Stroup W.W., Wolfinger R.D., (1996), *SAS System for Mixed Models*, SAS Inst. Inc., Cary, NC.

Luna-delRisco M., Normak A., Orupõld K., (2011), Biochemical methane potential of different organic wastes and energy crops from Estonia, *Agronomy Research*, **9**, 331-342.

Marchetti R., Vasmara C., Aleandri R., (2015), Plant and method for the production of microorganisms for biogas, Patent, No. *UB2015A000235*.

Mohanty K., Das D., (2012) *Kinetics of Biohydrogen Production by Dark Fermentation Processes*, In: *State of the Art and Progress in Production of Biohydrogen*, Bentham Science Publishers, Sharjah, 127-136.

Owen W.F., Stuckey D.C., Healy Jr.J.B., Young L.Y., McCarty P.L., (1979), Bioassay for monitoring biochemical methane potential and anaerobic toxicity, *Water Research*, **13**, 485-492.

Pilla S., (2011) *Handbook of Bioplastics and Biocomposites Engineering Applications*, Wiley, Hoboken, New Jersey.

Russo M.A.L., O'Sullivan C., Rounsefell B., Halley P.J., Truss R., Clarke W.P., (2009), The anaerobic degradability of thermoplastic starch: Polyvinyl alcohol blends: Potential biodegradable food packaging materials, *Bioresource Technology*, **100**, 1705-1710.

Sarasa J., Gracia J.M., Javierre C., (2009), Study of the biodisintegration of a bioplastic material waste, *Bioresource Technology*, **100**, 3764-3768.

SAS Institute, (1987), *SAS/STAT™ Guide for Personal Computers*, Version 6, 2nd Edition, SAS Inst., Cary, NC.

Toms D., Wnuk A.J., (1999), Biodegradable, liquid impervious films, EU Patent, No. *EP0591409 B1*.

Yagi H., Ninomiya F., Funabashi M., Kunioka M., (2009), Anaerobic biodegradation tests of poly(lactic acid) under mesophilic and thermophilic conditions using a new evaluation system for methane fermentation in anaerobic sludge, *International Journal of Molecular Sciences*, **10**, 3824-3835.

Vasmara C., Cianchetta S., Marchetti R., Galletti S., (2015), Biogas production from wheat straw pre-treated with ligninolytic fungi and co-digestion with pig slurry, *Environmental Engineering and Management Journal*, **14**, 1751-1760.

Environmental Engineering and Management Journal September 2016, Vol.15, No. 9, 2049-2056
http://omicron.ch.tuiasi.ro/EEMJ/

"Gheorghe Asachi" Technical University of Iasi, Romania

EXTRACTION OF BIOCHEMICALS FROM THE WINE INDUSTRY BY-PRODUCTS AND THEIR VALORIZATION

Paola Zitella[1*], Barbara La Licata[1], Alexia Boulanger[1], Vincenzo Cuzzola[1], Simone Morra[2], Francesca Valetti[2], Gianfranco Gilardi[2]

[1]Bioenergy Lab, Environment Park, Via Livorno 60, 10144 Torino, Italy
[2]Department of Life Sciences and Systems Biology, University of Torino, Via Accademia Albertina 13, 10123 Torino, Italy

Abstract

The production process of wine and distilled generates several by-products (20-30% of total production). Currently, most of the solid by-products (pomace, stalks, lees and seeds) obtained as downstream of the vinery industry, are conferred to the distillery or, less frequently, used in agriculture and for energy production. As a consequence, much of the antioxidant compounds contained in the grape is unused in the products of processing and is lost. These substances (among which the most important are polyphenols, anthocyanins and resveratrol) are a heterogeneous group of compounds particularly known for their beneficial effects on human health. In this article we present the results arising from a pilot scale research devoted to the evaluation of the extraction of such important compounds from the by-products of four varieties of Italian grape varieties. The pomaces obtained after wine production were extracted by innovative technologies, such as steam explosion and enzymatic extraction, without the use of organic solvents. The results show that it is possible to recover relevant amounts of polyphenols (up to 1383±50 mg GAE/L), anthocyanins (up to 148±2 mg/L) and resveratrol (up to 0.064 mg/L) from such by-products. Moreover, the recovered biochemicals are functional and act as radical scavenger, suggesting possible future applications in the cosmetics industry. The novel approach proposed here supports the possible application of steam explosion as industrial techniques to recover valuable compounds from grape pomace in a sustainable perspective from the economic and environmental standpoint.

Key words: cosmetics, Italian vineyard, polyphenol, wine industry by-products

Received: January, 2016; Revised final: September, 2016; Accepted: September, 2016

1. Introduction

Agricultural by-products contain a variety of biologically active compounds that are usually discarded. Particularly, most plant derived materials are rich in antioxidant polyphenols that might be recovered and used as a source of natural added value compounds (Balasundram et al., 2006; Colibaba et al., 2015; Schieber et al., 2001).

Grape is the world's largest fruit crop with an annual production of more than 67 million tons, having a noticeable economic value. The production process of wine and distilled generates several by-products: pomace, grape stalks, lees, grape seeds, washing water and sludge, up to 20-30% of total production (Teixeira et al., 2014). The management of these residues represents a challenge from an economic, environmental and technological point of view; grape pomaces are actually managed as a crop fertilizer even though this use is limited by the inhibitory effects caused by the polyphenols content (Piotrowski et al., 2008; Wacker et al., 1990) and so they represent a waste to be managed with additional costs for industry in the most of cases (Devesa-Rey et al., 2011). From a different point of view, grape pomace represents a low cost source for the extraction of phytochemicals suitable for application in different sectors: pharmaceutical, cosmetic and food industries. One of the most important family of valuable compounds that can be found in grapes are a

---

[*] Author to whom all correspondence should be addressed: e-mail: paola.zitella@envipark.com; Phone: +39 011 2257255; Fax: +39 011 2257225

variety of polyphenols, including resveratrol (Frémont, 2000; Rivière et al., 2012) with high acknowledged health promoting properties thanks to their antioxidant characteristics as reducing agents that delay and inhibit lipids oxidation (Furlan et al., 2014). Polyphenols in grapes are largely concentrated in the seeds (60%) and in the skin (30%), and a lower amount in the pulp and stems (less than 10%) (Mendes et al., 2013). Only a part of the polyphenols are extracted from grape during wine production; they provide sensory qualities to wine (color, flavour, astringency). Quantity of polyphenols in wine basically changes with vinification process: the average total polyphenol content, measured by the Folin-Ciocalteau method and by similar spectrophotometric assays, is in the range 180-400 (averaging 250) mg GAE/100mL for red wine, approximately 30-40 mg/100mL for rosé and between 16 and 72 mg GAE/100mL for white wine (Colibaba et al., 2015; Dobrinas et al., 2015; Frankel et al., 1995; Sato et al., 1996; Teissedre and Landrault, 2000). Beside this, several studies have showed that grapes skin from winemaking of red wine contain several types of colorants in relevant concentration that make these wastes valuable as a raw material for organic dyes production (Castillo-Muñoz et al., 2009; He and Giusti 2010; Zhu et al., 2015). Moreover, it must be considered that around 38 and 52% of grapes are made of seeds, with high lipid fraction (grape seeds oil) and have also high phenol content till now only partially exploited (De Marchi et al., 2012; Ky et al., 2014; Thorngate and Singleton, 1994).

Grape seeds extracts showed also neuroprotective effects (Narita et al., 2014) and antibacterial effect against a widespread typology of bacteria (Furiga et al., 2014), justifying their integration in food systems to prevent food deterioration; in this sense these products are a promising source with low cost and high availability, for compounds useful to be added for food preservation, as safer food antioxidants with added beneficial functional properties.

Many pharmacological and therapeutic features of grape products such as antioxidant, anti-inflammatory and antimicrobial activities, as well as cardio-, hepato-, and neuroprotective properties have been primarily attributed to grape tannins content (Bastianetto et al., 2015; Bitterman and Chung, 2015; Ras et al., 2013). Moreover the antioxidant action of low molecular weight polyphenols has been recently questioned due to their low bioavailability (Manach et al., 2004). Therefore, even though most of the health benefits of wine have been ascribed to polyphenols, it's actually unknown how much of their original content in grapes remains in pomace after enological fermentation, but the estimation refers about 70% of the original content (Alonso et al., 2002; Kammerer et al., 2004). Hence, significant amounts of valuable bioactive phenolic compounds could be recovered and exploited by the development of a clean and effective separation process.

During the last years, following the increasing attention to the sustainability and environmental impact of agro-industrial processes, a strong effort has been applied in the exploitation of grapes transformation industry wastes and by-products (Barros et al., 2015; Teixeira et al., 2014). One of the most important key points that must be improved in order to foster the growth of this sector is the optimization of extraction techniques to achieve high production yield with low costs and environmental impact.

The most common technique reported for the recovery is solid/liquid extraction (Alonso et al., 1991; Amendola et al., 2010; Pinelo et al., 2005; Yilmaz and Toledo, 2006). Solvent type is one of the main factors affecting the efficiency of the process. Due to the polar nature of polyphenols, they are easily solubilized in polar protic media. When alcohols are used as solvents, a progressive release of polyphenols from grape pomace as a function of the extraction time is observed, whereas contact time is not so important when water is used (Rajha et al., 2014). The pH of extraction solution also affects the process yields, increasing by adding acidified solvent mixture at high ethanol percentage or in basic conditions in extracting media with low ethanol percentage (Librán et al., 2013). HCl 2M at 100°C has also been used as an efficient extraction method for anthocyanins (Popescu et al., 2011). On the other hand, the application of ultrasound technology seems to be effective as replacement to conventional stirring in solvent extraction increasing the efficiencies and leading to reduce the steps to a single stage. Other emerging techniques of extraction are the supercritical fluid extraction (Aliakbarian et al., 2012) the accelerated solvent extraction (Kurabachew et al., 2015), the enzymatic processes (Aliakbarian et al., 2012; Chamorro et al., 2012; Landbo and Meyer2004).

This work reports the results obtained by two innovative extraction techniques: steam explosion and enzymatic treatment. These have been tested to recover valuable biochemicals from different types of grape pomaces.

2. Materials and methods

2.1. Extraction

Trial tests were performed by using pilot scale reactors and laboratory scale conditions (both facilities of Environment Park SpA). The starting biomass was supplied by local producers and it is from four different Italian grape varieties: Barbera, Freisa, Grignolino and Aleatico.

The steam explosion (SE) reactor (Fig. 1) consists of two vessels: one for the pressurization of the biomass (R101, total volume 30L) and the other for the following expansion of the biomass at atmospheric pressure (V101).

Fig. 1. Steam explosion pilot plant

The steam explosion process consists of several steps (Fig. 2). After feeding the fresh biomass in the reactor, the vessel is heated by steam until the reaction conditions are reached and then kept constant for the reaction time. Subsequently, the pressure is rapidly released and the biomass is recovered. The pressure in the reactor is maintained by an automatic gauge that controls the steam flow generated in a high pressure boiler. The boiler generates steam at a pressure that is slightly higher than that set for the test in order to maintain the correct parameters.

In these experiments, the biomass was not treated with any organic solvent; the final extraction of biochemical was obtained simply by the water condensed from the steam used during the stem explosion treatment. The main parameter that influences the outcome of a steam explosion treatment is severity (*Ro*). Severity depends linearly on time and exponentially on the temperature inside the pressurization vessel as reported in the following equation (Overend and Chornet, 1987):

$$Ro = \int exp\left[\frac{Tr - Tb}{14.75}\right]dt \qquad (1)$$

where: *Ro* – severity; *Tr* - reaction temperature, °C; *Tb* - base temperature (100°C); *t* - retention time, minute.

On the basis of the biomass availability, every pomace sample was treated with a selection of different severity conditions. In most cases, the different conditions were obtained varying the reaction temperature, and a single test was performed varying the time of treatment.

After the steam explosion treatment, some of the samples having high polyphenol content were selected for an additional extraction assay by enzymatic digestion. The enzyme treatments were carried out in glass bottles of 500 mL, using 10 g of biomass and 42 g of 0.1M citrate buffer pH 5.5. The enzymes used are a mixture of non commercial cellulases and hemicelluloses obtained from Novozymes, Denmark. The bottles were maintained in a thermostat at the optimum temperature for enzyme activity (50°C) for 24h or 48h, as suggested by the manufacturer.

Before the subsequent analyses, all extracts were clarified by centrifugation at 16300 *g*, 4°C for 10 minutes.

2.2. Total polyphenols quantification

The total polyphenols were quantified via the Folin-Ciocalteau method using gallic acid as standard (Ho et al., 2010).

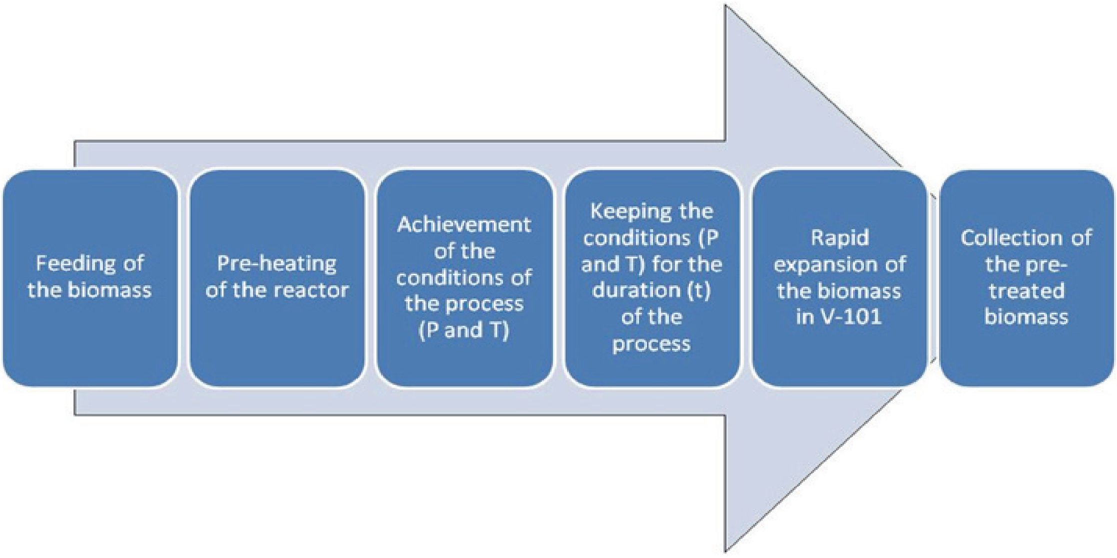


Fig. 2. Steam explosion cycle

Briefly, 250 μL of appropriately diluted sample or standard was mixed with 250 μL of 50% v/v Folin-Ciocalteau reagent; after 5 minutes at room temperature, 500 μL of 20% w/v sodium carbonate was added; after 10 minutes at room temperature the absorbance was read at 730 nm using an Ultrospec 2000 spectophotometer (Pharmacia). The results are expressed as gallic acid equivalent (GAE) milligrams per L of sample.

2.3. Anthocyanins quantification

The total anthocyanins were quantified by a spectrosphotometric method as previously described (Segade et al., 2011). Briefly, in a 96-well plate, 20 μL of sample were mixed with 280 μL of hydroalcoholic solution (70 volumes of ethanol, 30 volumes of deionised water, 1 volume of 37% HCl). Absorbance was read at 544 nm using a SPECTROstar Nano microplate reader (BMG Labtec). Results are expressed as malvidin-3-glucoside chloride milligrams per L of sample.

2.4. Resveratrol quantification

Resveratrol was quantified by HPLC, adapting previously described methods (Prajitna et al., 2007; Lamuela-Raventos et al., 1995). Briefly, an Agilent 1260 Infinity was equipped with MWD detector and a Zorbax Eclipse plus C18 column (4.6x100 mm 3.5 μm). The samples were acidified with 1% w/v acetic acid (final concentration). The column was equilibrated with 90% of 1% acetic acid + 10% acetonitrile at a flow rate of 0.5 mL/min. Separation was obtained by a linear gradient up to 100% acetonitrile in 90 minutes. Detection wavelength was at 280 nm; the quantification was obtained by correlating the samples to a standard curve, built with a commercial compound (Sigma) in the range 0-0.4 mg/L.

2.5. Radical scavenging activity determination

The radical scavenging activity was determined adapting previously described methods (Le et al., 2007). Briefly, the radical $ABTS^{\cdot+}$ was prepared by mixing 5 mL of 7 mM 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) with 88 μL potassium persulfate and incubating over night in the dark. The solution was diluted with ethanol until the absorbance at 734 nm was approximately 0.7. 297 μL of the radical solution were mixed with 3 μL of appropriately diluted sample in a 96-well plate and incubated for 20 minutes in the dark at room temperature.

The disappearance of the radical was recorder by measuring the absorbance ay 734 with a SPECTROstar Nano microplate reader (BMG Labtec) and correlating to a standard curve of vitamin C. The results are expressed as vitamin C equivalent antioxidant capacity (VCEAC) milligrams per L of sample.

3. Results and discussion

3.1. Extraction

In order to avoid the use of organic solvents and to provide an industrially scalable technology, the extraction was assayed by applying steam explosion to grape pomaces. Steam explosion is a process in which a biomass is treated with hot steam (120-240°C) under pressure followed by an explosive decompression that results in a de-structuration of fiber and consequent release of the biomass components (Chen and Liu, 2015; Huang et al., 2008). Various tests on a steam explosion pilot scale plant were performed in order to verify the real possibility of waste biomass fractionation for improved consequent polyphenols extraction.

The wine cultivar considered in this study were four Italian varieties: *Barbera, Freisa, Grignolino* and *Aleatico*. A variety of steam explosion parameters was tested, as summarized in Table 1. The parameters were selected keeping a low severity (*ie* the combination between pressure and treatment time), in order to minimize the heat treatment to the biomass and to avoid damage to the biochemicals.

On the basis of the results obtained by steam explosion alone, enzymatic extraction tests were assayed on selected samples (see below). These were performed with a mixture of enzymes (cellulases and hemicellulases) that can contribute to break the cell wall of the biomass. This assay was carried out to assess the potential further release of polyphenols, as expected from enhancements reported in literature (Aliakbarian et al., 2012; Chamorro et al., 2012; Landbo and Meyer, 2004). Likewise to what upper reported, the reduction of particle size has a positive effect on the recovery of polyphenols from the matrix, promoting an increase in surface contact between solid biomass and solvent (water), and so increasing the extraction efficiency.

3.2. Characterization of the recovered biochemicals

The extracts were analysed to determine the amount of some key compounds or class of compounds that are of interest for the cosmetics: polyphenols, anthocyanins and resveratrol. Moreover, the radical scavenging activity of the extracts was determined (Fig. 3).

From the general point of view, it is possible to observe that the extraction treatment was successful and allowed the recovery of several compounds of interest. The results show treatment-dependent features: the less intense extraction conditions (*ie* lower temperature for shorter time in steam explosion) are the best for most vineyards tested.

Table 1. Steam explosion conditions

		Sample weight (kg)	***Pressure (bar)***	***Temperature (°C)***	***Time to reach the treatment condition (min)***	***Time of treatment (min)***	***Severity***
Barbera Superiore pomace	Sample 1	2.2	5.03	150.31	1.77	5.13	156.7
	Sample 2	2.2	10.04	176.71	1.14	5.00	913.51
	Sample 3	2.2	10.12	177.03	0.53	10.24	1906.72
	Sample 4	2.2	15.15	195.61	0.60	5.30	3494.76
	Sample 5	2.2	20.05	210.19	0.80	5.03	8910.25
Freisa pomace	Sample 6	1.5	20.07	201.25	0.87	5.07	9011.70
	Sample 7	1.5	15.03	195.21	0.26	5.00	3203.37
	Sample 8	1.5	5.05	150.40	0.17	5.10	156.65
Grignolino pomace	Sample 9	2.7	5	150	0.87	5.07	148.3
	Sample 10	2.7	15	195.3	0.26	5.00	3198.5
	Sample 11	2.7	20	210.3	0.17	5.10	8843.1
Aleatico pomace	Sample 12	1.5	20	210.3	0.87	5.07	8843.1
	Sample 13	1.5	15	195.3	0.26	5.00	3198.5
	Sample 14	1.5	5	150	0.17	5.10	148.3

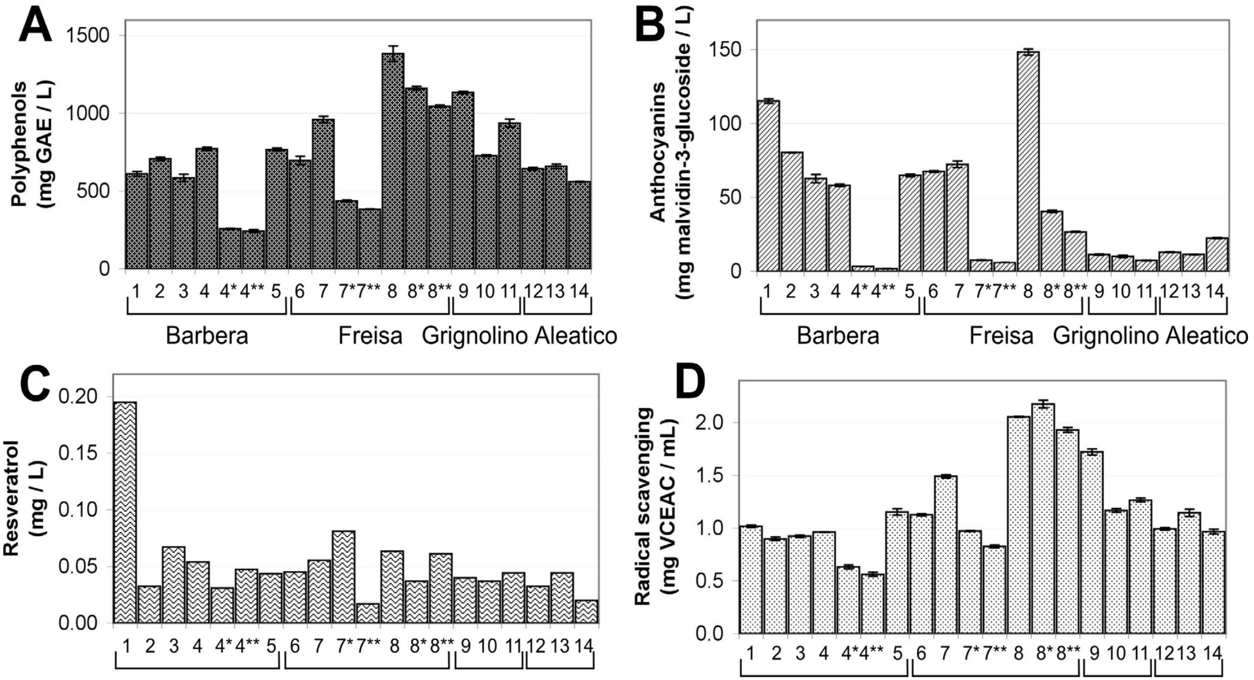


Fig. 3. Characterization of the extracts: A) total polyphenols content; B) anthocyanins content; C) resveratrol content; D) radical scavenging activity. Asterisks refer to a subsequent enzymatic treatment of 24h (*) or 48h (**)

This is particularly evident in the *Barbera* samples 1-2-4-5. This trend is reasonably a consequence of temperature-induced degradation of the compounds of interest. Nevertheless, it has to be highlighted that steam explosion, at the lowest severity conditions (5 bar, 150°C, 5 minutes), allows the recovery of certain amounts of polyphenols, anthocyanins and resveratrol that are functional, acting as radical scavenger into *in vitro* assays (Fig. 3). On the contrary, resveratrol recovery is not much influenced by the treatment conditions and the concentration is very low, with the only exception of the *Barbera* sample 1, probably because the high temperatures used in the other samples caused its degradation (Zupančič et al., 2015). Despite the highest resveratrol content, sample 1 does not display the highest radical scavenging rate, which is observed in sample 8, probably because other polyphenols are responsible for this functional activity.

After steam explosion, enzymatic extraction was tested on some of the samples (4, 7 and 8) having high polyphenols content, but it was unfruitful: the compound extracted by steam explosion were degraded during this treatment, probably because the process is longer and causes oxygen-driven or microbial-catalyzed degradation of these organic molecules.

More importantly, it is possible to observe similar trends between the various compounds; for example, as expected, polyphenols content correlates very precisely with the radical scavenging activity.

For most of the tested parameters, the *Freisa* sample 8 is the best, containing 1383±50 mg/L polyphenols, 148±2 mg/L anthocyanins, 0.064 mg/L resveratrol and having a radical scavenging capacity of 2.05±0.01 mg VCEAC/mL. In comparison to red wine, the amount of compounds recovered by our method is generally lower, but in the same order of magnitude. In the case of polyphenols, concentration in red wine can be as high as 1365-3326 mg GAE/mL (Simonetti et al., 1997), while anthocyanins range between 200-350 mg/L (Manach et al., 2004). Concerning resveratrol, the concentration in wine ranges between 0.1 and 15 mg/L (Frémont, 2000). This is particularly relevant because the extraction from pomaces allows the recovery of a relevant amount of functional antioxidants that can be valorized in various future applications, especially in cosmetics.

A cost analysis on the basis of the local cost of pomace for distilleries (www.regione.piemonte.it/agri/politiche_agricole/viticoltura/dwd/por140909.pdf) highlighted a maximum value of by-products of 7 €/100 kg, with a mean value to the winemakers of 2.5 €/100 kg. The cost of pomace waste disposal to the wine producers, for grape stalks-containing wastes, was up to 5€/100 kg.

Taking into account the cosmetics development costs (5 products were considered,: shower gel 200 mL and face cream with Freisa 50 mL, body cream 300mL, face serum 30 mL and hand soap 300 mL with Barbera) a sustainable investment can result in calculated break-even-point (Brealey et al., 2009) after the sale of only 5800 pieces (on the overall 5 products considered). In this respect the final cost of cosmetics was also taken into account. A plausible cost of pomace that the cosmetic producing company could sustain and pay to the winemaker in this scenario ranges from 15 to 20 €/100 kg, with a gain for the winemakers from 2 to 10 times compared to selling to distilleries.

The commercial implementation of phenolic compound from grape processing residues is a complex approach depending on several parameters that should be considered. Stating the actual progress and results in this research field, the next challenges for industrialization of this applications are the scaling up of the process without affecting the efficiency and the quality standards, the market developments of these organic bio-chemicals, the proof of physiological activities of these phenolics in the final products by *in vivo* tests.

4. Conclusions

The utilization of bio-wastes derived from wineries directly to biochemical extraction requires tailor-made pre-treatment processes in order to increase the mobilization of polyphenols in terms of chemical extraction amount. The sustainable utilization of grape pomace will be a valuable approach for wineries with the purpose of decreasing environmental impact and as an alternative to reduce the carbon footprint in the whole production chain. In this context, simplified processes (with few extraction and purification steps) will be the choice with the aim of both an easier scale-up as well as a cheaper production.

The entire process efficiency must be balanced against the impact on the cost of the downstream processing steps and the trade-off between operating costs, capital costs and biomass costs. The extraction based on the treatment with steam explosion has been proven effective in the extraction of polyphenols and anthocyanins in particular at low values of severity (5 bar 5 min.), especially with cultivars of Freisa, while resveratrol was extracted in greater quantity ever with treatments with low severity from cultivar of Barbera Superiore.

Preliminary tests of obtained extracts in cosmetic preparations were performed starting from samples 1 and 8 by a company involved in the study: Reynaldi SrL, Pianezza, Italy. The technical methods and results of the cosmetic preparations strategy are part of a proprietary process. The obtained cosmetic preparations have been found suitable for the required standards in view of market sale.

The study proved very promising for applicative outcomes, providing results for further development of research in the field of enhancement of pomace extraction of compounds with high added value without the use of chemical solvents.

Acknowledgements

This research was funded by the “Poliwine” project co-founded by Piedmont Region (E.U. Structural Funds N.1083/2006 F.E.S.R. 2007-2013) and was realized in cooperation with Metec Srl, as grape pomace provider and for cultivar information, and Reynaldi Srl for the cosmetic development.

References

Aliakbarian B., Fathi A., Perego P., Dehghani F., (2012), Extraction of antioxidants from winery wastes using subcritical water, *Journal of Supercritical Fluids*, **65**, 18-24.

Alonso Á.M., Guillén D.A., Barroso C.G., Puertas B., García A., (2002), Determination of antioxidant activity of wine byproducts and its correlation with polyphenolic content, *Journal of Agricultural and Food Chemistry*, **50**, 5832-5836.

Alonso E., Bourzeix M., Revilla E., (1991), Suitability of water/ethanol mixtures for the extraction of catechins and proanthocyanidins from Vitis vinifera seeds contained in a winery by-product, *Seed Science and Technology*, **19**, 542-552.

Amendola D., De Faveri D.M., Spigno G., (2010), Grape marc phenolics: Extraction kinetics, quality and stability of extracts, *Journal of Food Engineering*, **97**, 384-392.

Balasundram N., Sundram K., Samman S., (2006), Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses, *Food Chemistry*, **99**, 191-203.

Barros A., Gironés-Vilaplana A., Texeira A., Baenas N., Domínguez-Perles R., (2015), Grape stems as a source of bioactive compounds: application towards added-value commodities and significance for human health, *Phytochemistry Reviews*, **14**, 921-931.

Bastianetto S., Ménard C., Quirion R., (2015), Neuroprotective action of resveratrol, *Biochimica et Biophysica Acta*, **1852**, 1195-1201.

Bitterman J.L., Chung J.H., (2015), Metabolic effects of resveratrol: addressing the controversies, *Cellular and Molecular Life Sciences*, **72**, 1473-1488.

Brealey R., Myers S., Marcus A., Maynes E., Mitra D., (2009), *Fundamentals of Corporate Finance*, McGraw-Hill Ryerson. USA. 284.

Castillo-Muñoz N., Fernández-González M., Gómez-Alonso S., García-Romero E., Hermosín-Gutiérrez I., (2009), Red-color related phenolic composition of Garnacha Tintorera (Vitis vinifera L.) grapes and red wines, *Journal of Agricultural and Food Chemistry*, **57**, 7883-7891.

Chamorro S., Viveros A., Alvarez I., Vega E., Brenes A., (2012), Changes in polyphenol and polysaccharide content of grape seed extract and grape pomace after enzymatic treatment, *Food Chemistry*, **133**, 308-331.

Chen H.Z., Liu Z.H., (2015), Steam explosion and its combinatorial pretreatment refining technology of plant biomass to bio-based products, *Biotechnology Journal*, **10**, 866-885.

Colibaba L.C., Cotea V.V., Rotaru L., Nechita B., Niculaua M., Tudose-Sandu-Ville S., Luchian C., (2015), Volatiles in *Tămâioasă românească* via supercritical fluid extraction (SFE) analysis, *Environmental Engineering and Management Journal*, **14**, 297-302.

De Marchi F., Seraglia R., Molin L., Traldi P., De Rosso M., Panighel A., Dalla Vedova A., Gardiman M., Giust M., Flamini R., (2012), Seed oil triglyceride profiling of thirty-two hybrid grape varieties, *Journal of Mass Spectrometry*, **47**, 1113-1119.

Devesa-Rey R., Vecino X., Varela-Alende J.L., Barral M.T., Cruz J.M., Moldes A.B., (2011), Valorization of winery waste vs. the costs of not recycling, *Waste Management*, **31**, 2327-2335.

Dobrinas S., Soceanu A., Artem V.D., (2015), Determination of polyphenolic compounds in murfatlar wines by validated spectrophotometric method, *Environmental Engineering and Management Journal*, **14**, 863-870.

Frankel E.N., Waterhouse A.L., Teissedre P.L., (1995), Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins, *Journal of Agricultural and Food Chemistry*, **43**, 890-894.

Frémont L., (2000), Biological effects of resveratrol, *Life Sciences*, **66**, 663-673.

Furiga A., Roques C., Badet C., (2014), Preventive effects of an original combination of grape seed polyphenols with amine fluoride on dental biofilm formation and oxidative damage by oral bacteria, *Journal of Applied Microbiology*, **116**, 761-771.

Furlan A.L., Jobin M.L., Buchoux S., Grélard A., Dufourc E.J., Géan J., (2014), Membrane lipids protected from oxidation by red wine tannins: A proton NMR study, *Biochimie*, **107 Pt A**, 82-90.

Huang H.J., Ramaswamy S., Tschirner U.W., Ramarao B.V., (2008), A review of separation technologies in current and future biorefineries, *Separation and Purification Technology*, **62**, 1-21.

He J., Giusti M.M., (2010), Anthocyanins: natural colorants with health-promoting properties, *Annual Review of Food Science and Technology*, **1**, 163-187.

Ho S.-T., Tung Y.-T., Cheng K.-C., Wu J.-H., (2010), Screening, determination and quantification of major antioxidants from *Balanophora laxiflora* flowers, *Food Chemistry*, **122**, 584-588.

KammererD., Claus A., Carle R., Schieber A., (2004), Polyphenol screening of pomace from red and white grape varieties (*Vitis vinifera* L.) by HPLC-DAD-MS/MS, *Journal of Agricultural and Food Chemistry*, **52**, 4360-4367.

Kurabachew S.D., Casazza A.A., Mohamed H.B., Perego P., Fiori L., (2015), Extraction of polyphenols from grape skins anddefatted grape seeds using subcritical water: Experiments and modeling, *Food and Bioproducts Processing*, **94**, 29-38.

Ky I., Lorrain B., Kolbas N., Crozier A., Teissedre P.L., (2014), Wine by-products: phenolic characterization and antioxidant activity evaluation of grapes and grape Pomaces from six different french grape varieties, *Molecules*, **19**, 482-506.

Lamuela-Raventos R.M., Romero-Perez A.I., Waterhouse A.L., de la Torre-Boronat M.C., (1995), Direct HPLC analysis of *cis*- and *trans*- resveratrol and piceid isomers in Spanish red *Vitis vinifera* wines, *Journal of agricultural and food chemistry*, **43**, 281-283.

Landbo A.-K., Meyer A.S., (2004), Effects of different enzymatic maceration treatments on enhancement of anthocyanins and other phenolics in black currant juice, *Innovative Food Science and Emerging Technologies*, **5**, 503-513.

Le K., Chiu F., Ng K., (2007), Identification and quantification of antioxidants in *Fructus lycii*, *Food Chemistry*, **105**, 353-363.

Librán C.M, Mayor L., Garcia-Castello E.M., Vidal-Brotons D., (2013), Polyphenol extraction from grape wastes: Solvent and pH effect, *Agricultural Sciences*, **4**, 56-62.

Manach C., Scalbert A., Morand C., Rémésy C., Jiménez L., (2004), Polyphenols: food sources and bioavailability, *The American Journal of Clinical Nutrition*, **79**, 727-747.

Mendes J.A.S., Prozil S.O., Evtuguina D.V., Cruz Lopes L.P., (2013), Towards comprehensive utilization of winemaking residues: Characterization of grape skins from red grape pomaces of variety Touriga Nacional, *Industrial Crops and Products*, **43**, 25-32.

Narita K., Hisamoto M., Okuda T., Takeda S., (2011), Differential neuroprotective activity of two different grape seed extracts, *PLoS One*, **6**, e14575, Doi:10.1371/journal.pone.0014575.

Overend R.P., Chornet E., (1987), Fractionation of lignocellulosics by steam-aqueous pretreatments, *Philosophical Transactions of the Royal Society A*, **321**, 523-536.

Pinelo M., Del Fabbro P., Marzocco L., Nunez M.J., Vicoli M.C., (2005), Optimization of continuous phenol extraction from *Vitis vinifera* byproducts, *Food Chemistry*, **92**, 109-117.

Prajitna A., Dami I.E., Steiner T.E., Ferree D.C., Scheerens J.C., Schwartz S.J., (2007), Influence of cluster thinning on phenolic composition, resveratrol, and antioxidant capacity in Chambourcin wine, *American Journal of Enology and Viticulture*, **58**, 346-350.

Piotrowski J.S., Morford S.L., Rillig M.C., (2008), Inhibition of colonization by a native arbuscular mycorrhizal fungal community via *Populus trichocarpa* litter, litter extract, and soluble phenolic compounds, *Soil Biology and Biochemistry*, **40**, 709-717.

Popescu C., Fitigau F. , Segnean A.-E., Balcu, I., Martagiu R., Vaszilcsin C.G., (2011), Separation and characterization of anthocyanins by analytical and electrochemical methods, *Environmental Engineering and Management Journal*, **10**, 697-701.

Rajha H., Darra N., Hobaika Z., Boussetta N., Vorobiev E., Maroun R., Louka N., (2014), Extraction of total phenolic compounds, flavonoids, anthocyanins and tannins from grape byproducts by Response Surface Methodology. Influence of solid-liquid ratio, particle size, time, temperature and solvent mixtures on the optimization process, *Food and Nutrition Sciences*, **5**, 397-409.

Ras R.T., Zock P.L., Zebregs Y.E., Johnston N.R., Webb D.J., Draijer R., (2013), Effect of polyphenol-rich grape seed extract on ambulatory blood pressure in subjects with pre- and stage I hypertension, *British Journal of Nutrition*, **110**, 2234-2241.

Rivière C., Pawlus A.D., Mérillon J.M., (2012), Natural stilbenoids: distribution in the plant kingdom and chemotaxonomic interest in Vitaceae, *Natural Product Reports*, **29**, 1317-1333.

Sato M., Ramarathnam N., Suzuki Y., Ohkubo T., Takeuchi M., Ochi H., (1996), Varietal differences in the phenolic content and superoxide radical scavenging potential of wines from different sources, *Journal of Agricultural and Food Chemistry*, **44**, 37-41.

Schieber A., Stintzing F.C., Carle R., (2001), By-products of plant food processing as a source of functional compounds – recent developments, *Trends in Food Science & Technology*, **12**, 401-413.

Segade S.R., Giacosa S., Gerbi V., Rolle L., (2011), Berry skin thickness as main texture parameter to predict anthocyanin extractability in winegrapes, *LWT Food Science and Technology*, **44**, 392-398.

Simonetti P., Pietta P., Testolin G., (1997), Polyphenol content and total antioxidant potential of selected Italian wines, *Journal of Agricultural and Food Chemistry*, **45**, 1152-1155.

Teissedre P.L., Landrault N., (2000), Wine phenolics: contribution to dietary intake and bioavailability, *Food Research International*, **33**, 461-467.

Teixeira A., Baenas N., Dominguez-Perles R., Barros A., Rosa E., Moreno D.A., Garcia-Viguera C., (2014), Natural bioactive compounds from winery by-products as health promoters: A review, *International Journal of Molecular Sciences*, **15**, 15638-15678.

Thorngate J.H., Singleton V.L., (1994), Localization of procyanidins in grape seeds, *American Journal of Enology and Viticulture*, **45**, 259-262.

Wacker T.L., Safir G.R., Stephens C.T., (1990), Effects of ferulic acid on *Glomus fasciculatum* and associated effects on phosphorus uptake and growth of asparagus (*Asparagus officinalis* L.), *Journal of Chemical Ecology*, **16**, 901-909.

Yilmaz Y., Toledo R.T., (2006), Oxygen radical absorbance capacities of grape/wine industry byproducts and effect of solvent type on extraction of grape seed polyphenols, *Journal of Food Composition and Analysis*, **19**, 41-44.

Zhu Z., Wu N., Kuang M., Lamikanra O., Liu G., Li S., He J., (2015), Preparation and toxicological evaluation of methyl pyranoanthocyanin, *Food and Chemical Toxicology*, **83**, 125-132.

Zupančič Š., Lavrič Z., Kristl J., (2015), Stability and solubility of trans-resveratrol are strongly influenced by pH and temperature, *European Journal of Pharmaceutics and Biopharmaceutics*, **93**, 196-204.

Environmental Engineering and Management Journal *September 2016, Vol.15, No. 9, 2057-2062*
http://omicron.ch.tuiasi.ro/EEMJ/

"Gheorghe Asachi" Technical University of Iasi, Romania

PRODUCTION CAPACITY OF LEACHATE FROM BIHOR LANDFILL

Mihai-Cosmin Belciu[1], Emilian Florin Moşneguţu[1*], Valentin Nedeff[1], Silvia Fiore[2], Alexandra-Dana Chiţimuş[1], Narcis Bârsan[1]

[1] "Vasile Alecsandri" University of Bacau, Faculty of Engineering, Calea Marasesti, 157 Bacău – 600115, România
[2] Politecnico di Torino, Department of Environmental Engineering, Land and Infrastructure, corso Duca degli Abruzzi 24, Torino - 10129, Italy

Abstract

Landfilling is one of the most important issues that can be discussed nowadays related to waste management, mainly concerning the importance of what we have to do with waste quantities and how energy can be recovered. The paper proposes a three-dimensional mathematical model applied to calculate the production capacity of leachate from Bihor landfill, situated in Bihor County, Romania. The leachate production capacity is related to the total amount of waste disposed in landfill during active phase. The parameters taken into consideration in the research refer only to the municipal solid waste quantity disposed and the life of the landfill. Leachate production time was divided into two well-defined periods: active phase, in which the waste was stored in landfill and the post-closure phase – the period of time until the landfill is definitively closed (30 years). The mathematical model has been elaborated and tested using TableCurve 3D software based on data provided by SC EcoBihor SA and based on the assessment of leachate quantity that can be collected from the landfill. The mathematical model proposed offers a viable solution to determine the maximum leachate production capacity during municipal solid waste landfilling.

Key words: landfill, leachate, mathematical model, Municipal Solid Waste, time

Received: March, 2016; Revised final: September, 2016; Accepted: September, 2016

1. Introduction

A coherent policy on waste recycling and reuse has been developed in European Union Member States, in recent decades. This fact has already shown a 20% w/w average decrease of the total amount of municipal solid waste disposed in landfills between 2002-2012 in the EU-27. However, an amount of 32% w/w of waste was disposed in landfills in 2012 (Eurostat, 2015a, 2015b).

In the last years (2010 - 2014), a maximum quantity of 300 kg of waste generated/capita was registered in Romania (Eurostat, 2015c), while the amount of waste disposed in landfills represents 78% from the total amount of generated waste (Ionescu et al., 2015; Pop et al., 2015).

Bihor Landfill is located in Oradea town, Bihor County and is divided into two cells (Fig. 1). Cell 1 covers an area of 3.8 ha and it was designed for a total capacity of 756,436.8 tonnes of disposed waste (during August 2005 and May 2011) (Fig. 2). Cell 2 occupies a total area of 3.8 ha and it was designed for the same capacity for the period between June 2011 and December 2014 (Fig. 2) (Bihor, 2015).

During the active phase of landfilling, the following categories of waste were stored in the landfill (Bihor, 2015):

- non-hazardous industrial waste;
- non-hazardous waste from construction/ demolition - used for the coverage and also used to build and to stabilize the access road on the landfill;
- non-hazardous municipal solid waste;
- excavated stripped soil – used to build the slope.

---

[*] Author to whom all correspondence should be addressed: e-mail: emos@ub.ro; Phone: +40-234-542.411; Fax: +40-234-545.753

Fig. 1. Bihor Landfill site

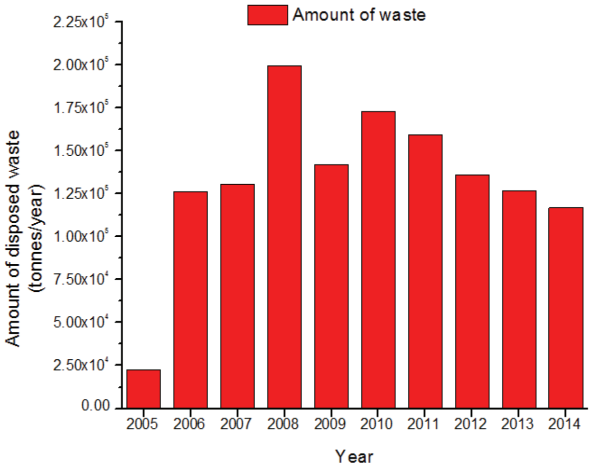


Fig. 2. Total amount of waste disposed in Bihor landfill

Concerning Bihor landfill, leachate is collected and stored in a tank used for leachate collection, until the treatment process. Leachate treatment takes place in the wastewater treatment plant which is located in the landfill area and is based on the reverse osmosis process. After treatment, permeate is transported in a temporarily storage pool. The permeate is used in technological purposes as treated water, without contaminants, while the concentrate is disposed in a tank, then is stabilized with fireplace ashes and applied over waste disposed layers (Bhattacharya et al., 2015; Bihor, 2015; Pohontu et al., 2010; Şchiopu and Ghinea, 2013).

Several types of waste are stored in Bihor landfill. For matemathical modeling purposes, only municipal solid waste was taken into consideration. Concerning the other types of waste stored in the landfill (industrial waste or demolition waste), these weren't taken into account, because the composition of each fraction from these types of waste was impossible to be identified. Moreover, the quantities of these fractions weren't monitored by the administrator of landfill, EcoBihor. In the specialized literature, in European landfills, only Municipal Solid Waste (MSW) was taken into consideration to identify the leachate production and also the biogas production (Chiţimuş et al., 2014).

The aim of this paper consists in identifying the leachate production capacity from Bihor landfill. This is an important activity, which can allow the waste managers and environmental authorities to prevent disasters, but also to monitor the production capacity of biogas from landfills.

2. Estimation of leachate production capacity

The elemental analysis for each fraction of disposed waste was performed in order to estimate the leachate production capacity of Bihor landfill. The moisture content and the density of each fraction of disposed waste in landfill and used in the calculations are provided in Table 1 and Fig. 3 (during the active phase).

Since Bihor landfill does not have any own weather station, data concerning weather-related parameters (temperature and precipitation) were collected from the Department of Climatology of Weather Romania. Also, based on literature we have calculated the average monthly variation of precipitation and temperature for the period 2005÷2013 (Weather, 2015).

In order to achieve the hydrological balance, it was calculated the average monthly variation of runoff and the average monthly variation of evapotranspiration. Surface runoff was calculated according to the amount of precipitation and considering an empirical coefficient, which was evaluated at maximum flow during heavy rainfall of short duration (Vasilica et al., 2015). This coefficient is influenced by the soil texture, the slope and the vegetation type. Evapotranspiration was calculated using Thornthwaite equation that takes into account the latitude and the monthly precipitation rates. Considering that during some months, the monthly average calculated temperatures are negative, it was not considered the values for evapotranspiration for those months (Chiţimuș et al., 2012; Cossu and Christensen, 1989; Cossu et al., 1989; Thornthwaite, 1948).

For each fraction of waste, it can be estimated the total amount of liquid which can be formed in the landfill. Fig. 4 presents the quantity of liquid which can be formed from moisture content of each fraction of waste disposed in Bihor landfill. It was impossible to make a comparison between the estimated quantity and the real quantity of collected leachate because the administrator of landfill doesn't monitor the leachate quantity collected. For Bihor landfill, the leachate is stored in a tank without knowing the quantity generated daily.

The estimation of liquid quantity that can be generated inside the landfill was performed considering the general formula of Hydrological Mass Balance (Cossu and Christensen, 1989; Cossu et al., 1989), which takes into consideration only the moisture content of each fraction from waste, the runoff, the evaporation and the evapotranspiration. We did not considered different chemical or biological parameters. The leachate production was related to the total amount of water from landfill considering a good impermeabilization of the landfill. Climatological parameters are one of the most important parameters that contributes to the leachate production from landfills for Municipal Solid Waste. The estimation of liquid volume that can be formed inside the Bihor landfill from climatological parameters values is 395.394 m^3/year and, reported to the whole period of its activity it results 3953.94 m^3. The total amount of leachate that can be formed inside the Bihor landfill during its active phase is 458485.6 m^3.

Table 1. Elemental analysis of each fraction of waste disposed in Bihor landfill (fraction of waste, moisture content and fraction density) (Bihor, 2015; Ionescu, 2012; Panepinto and Genon, 2012)

Waste fraction	***Type of waste percentage (%)***	***Moisture content (%)***	***Fraction density (kg/m^3)***
Paper and cardboard waste	8.2	8	750
Leather and textile waste	4.2	10	550
Wood waste	2.7	2.5	900
Glass and inert waste	2.2	3	750
Metal waste	0.6	3	2800
Plastic waste	6.9	3	50
Organic waste	33.2	60	800
Fine particles waste	42.1	2	700
Other types of waste	n.a.	2	700

**n.a. – does not exist.*

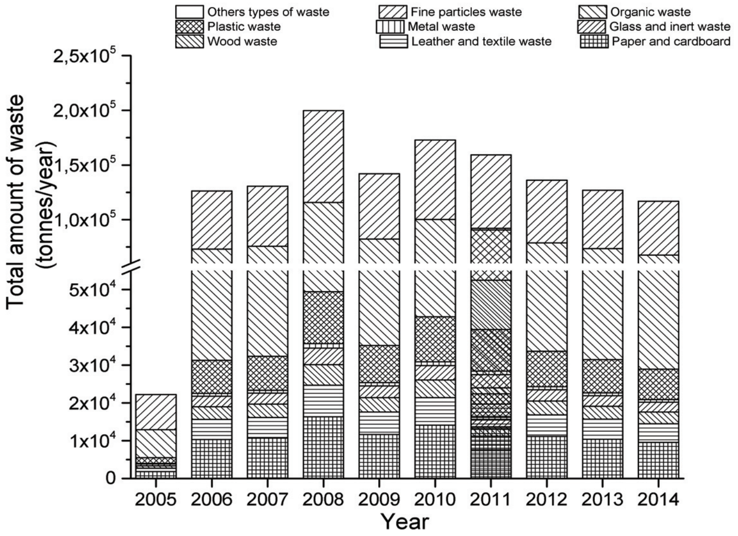


Fig. 3. Representation of quantitative composition for disposed waste in active phase of Bihor landfill

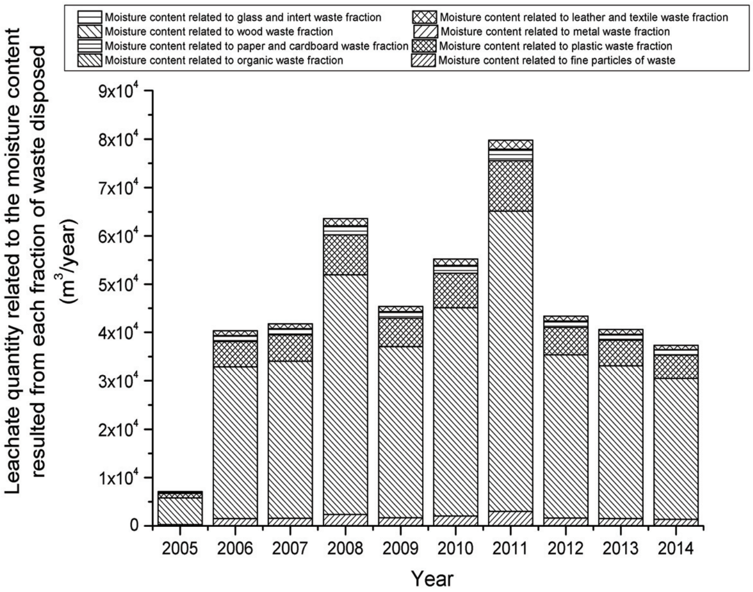


Fig. 4. Estimation of leachate quantity related to the moisture content resulted from each fraction of waste disposed in Bihor landfill

The total amount of leachate that can be formed in the body of the Bihor landfill, Romania is given by considering the following parameters:

- estimation of the moisture content resulted from summing of each fraction of waste disposed in active phase (Fig. 4);
- estimation of leachate quantity that can be formed from climatological phenomena.

It was considered that the post-closure phase had begun on 1st of January 2015, after ten years since the waste started to be disposed in landfill. In mathematical calculations there were considered the following assumptions:

- landfill opening – first year – year 2005;
- closure of landfill – year number ten –2014;
- first year of leachate collection (calculated and estimated) – the fourth year –2009;
- final closure of the landfill – after 41 years - 2045.

Fig. 5 presents a comparative analysis between the amount of leachate determined through calculation and the assessment of leachate quantity using a mathematical model for Bihor landfill. The assessment of leachate quantity using mathematical model had used a Gaussian equation as follow (Eq. 1):

$$y = y_0 + \left[\frac{A}{w \cdot \sqrt{\left(\frac{\pi}{2}\right)}} \cdot \exp\left(-2 \cdot \left(\frac{x - xc}{w} \right)^2 \right) \right] \text{ (m}^3\text{/year)} \quad (1)$$

where: y represents the leachate quantity, annually estimated; y_0, w, xc, x and A – equation parameters.

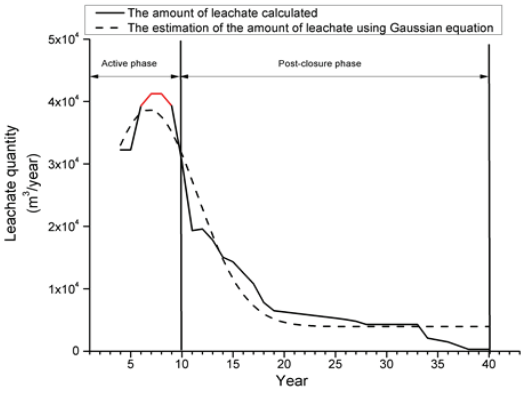


Fig. 5. Comparative analysis between the amount of leachate determined through calculation and assessment of leachate quantity using Gaussian equation landfill in Bihor landfill

The equation parameters developed to calculate the leachate quantity from Bihor landfill are shown in Table 2. For Gaussian equation, it was found the correlation coefficient of R^2=0.95516. The mathematical model with Gaussian equation was generated using Origin Pro 8.5 software.

3. Mathematical modeling of leachate production capacity from Bihor landfill

3.1. Basis of mathematical modeling development

The results obtained from the leachate production capacity calculation reflect the fact that the leachate quantity is directly influenced by the waste quantity disposed in landfill and by the life time within the landfill. Using TableCurve 3D software, linear and nonlinear equations were generated, which shows, through three-dimensional mathematical model the dependence of leachate production capacity in time (active and post-closure phases) and the disposed waste quantity.

Table 2. Gaussian equation parameters used for estimating the amount of leachate that can be collected from Bihor landfill

Parameter	*Value*
y_0	3953.91212
xc	6.81093
w	9.41047
A	409667.90769

Fig. 6 presents the variation of leachate production capacity for Bihor landfill for a period of 38 years. For this mathematical model was considered the first year of leachate production 2006, after one year of landfill activity. It was considered the first year of leachate collection as 2008 (the fourth year used in mathematical modeling) and the final closure of the landfill happened in 2045 (year number 41 used in mathematical modeling).

The surface generated by the applied mathematical model is characterized by Eq. (2):

$$z = a + b \cdot \ln x + c \cdot (\ln x)^2 + d \cdot (\ln x)^3 + e \cdot (\ln x)^4 + f \cdot y \quad \text{(m}^3\text{/year)} \quad (2)$$

where: z represents leachate volume which can be collected from landfill, annually, (m³/year); x – time, landfill activity year, (years); y – waste quantity, annually disposed in landfill, (tonnes/year); *a, b, c, d, e* and *f* – equation constants.

Table 3 indicates the values of the constants from Eq. (2) for the production of leachate calculated for Bihor landfill. The constant values were generated by the software Table Curve 3D, considering the best percentage for correlation coefficient (R^2). The correlation coefficient for Eq. (2) is R^2 = 0.93 (Table 3).

3.2. Validation of mathematical model

It was verified the mathematical model obtained using the TableCurve 3D program to generate linear and non-linear equations for production capacity of leachate which can be collected from Bihor landfill.

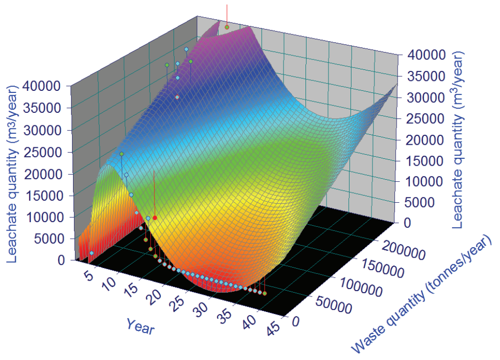


Fig. 6. Variation of leachate production capacity for the quantity of leachate that can be collected from Bihor landfill

Table 3. The values of the constants which describe the mathematical model used to calculate the leachate production from Bihor landfill

Equation constants	*Constant value*	*Correlation coefficient*
a	-2022.50542621047	0.93
b	-77821.010989026	
c	121442.679510803	
d	-50981.6085053701	
e	6494.18972518707	
f	0.081792082432437	

To check the model, it was validated the equation (3) for the variation of production capacity of leachate which can be collected from Bihor landfill.

$$P_{levigat} = -2022.50542621047 - 77821.010989026 \cdot \ln t_{Bi} + 121442.679510803 \cdot \left(\ln t_{Bi}\right)^2 - 50981.6085053701 \cdot \left(\ln t_{Bi}\right)^3 + 6494.18972518707 \cdot \left(\ln t_{Bi}\right)^4 + 0.081792082432437 \cdot m_{Bi} \quad (3)$$

Model validation showed that there are errors with different values, lower in the period which it wasn't collected leachate and in some parts of post-closure period (Figs. 7, 8). It is important to observe that higher discrepancies between values appear in the first years of active phase, which can be explained byy the fact that, in some landfills, leachate collection starts after few years of activity because an important water quantity accumulates in landfill after a period of time without leaching. Also, it can be observed an important variation between values during the post-closure phase. This fact can be explained through the fact that the evapotranspiration phenomena influence these parameters and also, after the final closure of the landfill the water content diminished in time.

The differences between the estimation and the mathematical model applied using Table Curve 3D Software can be observed in the graphical representation (Fig. 8). Considering that in the passive phase (after landfill closure) the waste quantity remains constant and also the climatological parameters are different than in the active phase, some differences can appear between the values.

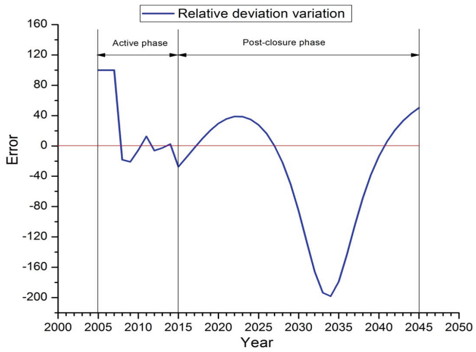


Fig. 7. Variation of relative deviation from mathematical model elaboration for leachate production capacity collected from Bihor landfill

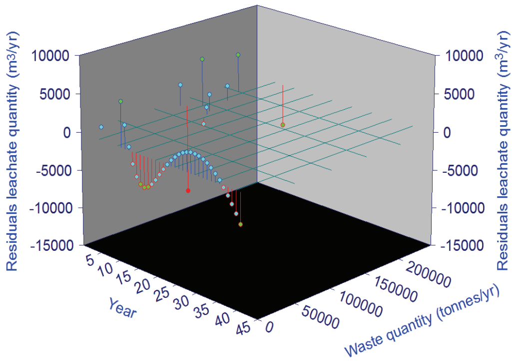


Fig. 8. Graphical representation of the differences between the leachate production obtained using the mathematical model and estimated leachate production

It can be supposed that in Romania landfilling can induce threats in the environment, because ecological landfills were constructed in the last 10-15 years according to European provision and using European funding. For this reason, not all parameters are monitored and also, there aren't so many studies for Romanian landfills. In specialized literature are some papers or works that are representative for waste management, which For example presents some mathematical models applied on municipal solid waste landfills (Ghinea et al., 2016, Olatunde, 2001; Schiopu and Ghinea, 2013).

4. Conclusions

In the last years, the waste disposal in landfills presented a paramount importance. This aspect should be treated complexly, first of all from a technologically point of view and also concerning economic and environmental issues.

The estimations of leachate quantities that can be collected from landfills are important to avoid the dangers that may arise and the fractures that can appear. Also is important to recirculate the leachate to increase the biogas production and to reduce the operating costs. Concerning Bihor landfills, is known the real leachate quantity only for year 2014. Using moisture content method to calculate leachate presents the fact that for year 2014, the leachate calculated has the value as in reality, namely 30315.6 m^3. The Gaussian equation used for estimation presents an overestimation of the real value, but keeping the variation curve given by calculated values. The difference between the values obtained by estimation and the values obtained with mathematical model generated with TableCurve 3D is insignificant for Bihor landfill during 2008-2030 and 2038-2045. The difference is significant for the period of time 2005-2007 (the leachate wasn't collected) and from 2031 to 2037.

It is important to identify the total quantity of leachate that can be formed in landfill because of the consequences of remaining of leachate in the landfill and also the contribution of leachate in biogas formation.

Using the mathematical model described above helps landfill administrators to solve some important aspects that are dangerous for population. Although, using mathematical models and estimative models for leachate contribute in identifying the environmental aspects that may have potential impact. Knowing leachate quantities may prevent a disaster and also, the total amount of the landfill product may be recirculated in landfill body to increase the biogas production capacity.

The administrators of Romanian landfills must be informed that the construction, the landfill impermeabilization, the leachate and biogas monitoring and the post-closure activity of the landfill are the most important aspects that influence the landfills and the communities that are near landfills.

Acknowledgments

The authors addressed thanks to Bihor County Council and to SC EcoBihor SA Oradea in providing data for Bihor landfill.

References

Bhattacharya P., Ghosh S., Majumdar S., Roy S.N., Somendra N., Bandyopadhyay S., (2015), Ceramic membrane based microfiltration for treatment of highly contaminated tannery wastewater, *Environmental Engineering and Management Journal*, **14**, 2139-2148.

Bihor C.C., (2015), Bihor County Council, Technical Department for Environment Compartment, On line at: http://www.cjbihor.ro/despre-noi/direc%C8%9Bia-general%C4%83-tehnic%C4%83/compartimentul-mediu.

Chițimuș A.D., Moşneguțu E.F., Nicolescu M., Turcu M., Belciu M.C., Ardeleanu G., (2014), Mathematical modelling of water migration time in soil, *Environmental Engineering and Management Journal*, **13**, 1581-1586.

Chițimuș A.D., Nedeff V., Moşneguțu E.F., Panainte M., (2012), In situ soil flushing-studies on remediation efficiency of polluted sandy soils with organic acids, *Environmental Engineering and Management Journal*, **11**, 2161-2166.

Cossu R., Christensen T., (1989), *Sanitary Landfilling: Process, Technology and Environmental Impact*, Ed. Academic Press.

Cossu R., Christensen T.H., Stegmann R., (1989), *Role of Landfilling in Solid Waste Management*, In: *Sanitary Landfilling: Process. Technology and Environmental Impact*, Harcourt Brace Jovamovich Publishers, Academic Press, Vol. 3.

Eurostat, (2015a), European Commission Statistics, Environmental Data Centre on Waste, Key waste streams, On line at: http://ec.europa.eu/eurostat/tgm/refreshTableAction.do?tab=table&plugin=1&pcode=tsdpc240&language=en

Eurostat, (2015b), European Commission Statistics Environmental Data on Municipal Waste management, On line at: http://ec.europa.eu/eurostat/web/waste/transboundary-waste-shipments/key-waste-streams/municipal-waste.

Eurostat, (2015c), European Commission Statistics Environmental Data centre on waste, Waste generation, On line at: http://ec.europa.eu/eurostat/web/waste/waste-generation-and-management.

Ghinea C., Petraru M., Simion I.M., Sobariu D., Bressers H.T.A., Gavrilescu M., (2014), Life cycle assessment of waste management and recycled paper systems, *Environmental Engineering and Management Journal,* **13**, 2073-2085.

Ionescu G., (2012), *Critical analysis of pyrolysis and gasification applied to waste fractions with growing energetic content*, PhD Thesis, Polytechnic University of Bucharest, Romania.

Ionescu G., Rada E.C., Cioca L.I., (2015), Municipal solid waste sorting and treatment schemes for the maximization of material and energy recovery in a latest EU member, *Environmental Engineering and Management Journal,* **14**, 2537-2544.

Olatunde O., (2001), *Modeling Leachate Production in Heterogeneous Municipal Solid Waste Landfills*, Department of Chemical and Petroleum Engineering, University of Calgary, Calgary, Alberta, Italy.

Panepinto D., Genon G., (2012), Carbon dioxide balance and cost analysis for different solid waste management scenarios, *Waste and Biomass Valorization*, **3**, 249-257.

Pohontu C., Cretescu I., Secula M.S., Paduraru C., Tofan L., Macoveanu M., (2010), Integrated treatment of leachate from municipal waste solid landfill, *Environmental Engineering and Management Journal*, **9**, 95-101.

Pop I.N., Baciu C., Bican-Brisan N., (2015), Survey on household waste composition generated in Cluj-Napoca, Romania during the summer season, *Environmental Engineering and Management Journal*, **14**, 2643-2651

Şchiopu A.M., Ghinea C., (2013), Municipal solid waste management and treatment of effluents resulting from their landfilling, *Environmental Engineering and Management Journal*, **12**, 1699-1719.

Thornthwaite C.W., (1948), An approach toward a rational classification of climate, *Geographical Review*, **38**, 55-94.

Vasilica M., Capsa D., Covaci I., Nedeff V., Barsan N., Panainte M., (2012), A case study on heavy rainfall in the Central-Southern Moldavia Region, Romania, *Environmental Engineering and Management Journal*, **11**, 2151-2158.

Weather D.C., (2015), Weather Romania - Climatological Department, On line at: http://www.meteoromania.ro/anm/?lang=ro_ro.

Environmental Engineering and Management Journal *September 2016, Vol.15, No. 9, 2063-2074*
http://omicron.ch.tuiasi.ro/EEMJ/

"Gheorghe Asachi" Technical University of Iasi, Romania

COMPARATIVE ANALYSIS OF WATER LEAK DETECTION SYSTEMS AND METHODOLOGIES

Pedro Rosas Quiterio*, Florencio Sánchez Silva, Ignacio Carvajal Mariscal, Jesús Alberto Meda Campaña

Instituto Politécnico Nacional, Escuela Superior de Ingeniería Mecánica y Eléctrica, Av. IPN, Edif. 5, Third Floor, SEPI ESIME Zac, Col. Lindavista, C.P. 07738, D.F., México

Abstract

In this paper the problem of water leakage is shown widely as well as the impact in Mexico resources, which is reflected in a huge economic and environmental impact. Also several leak detection systems and methodologies are presented in a comparative analysis that was carried out in order to provide the reader a clear idea of how the operation of the systems are affected by means of various factors which in turn leads the reader to know about the requirements of systems and methodologies and addresses quantitatively their advantages and disadvantages. Finally the comparative analysis, leads the authors to develop a leak detection system based on pressure gradient according to the analyzed features, capabilities and needs.

Key words: detection, leak, location, methodologies, systems

Received: August, 2013; Revised final: April, 2014; Accepted: April, 2014

1. Introduction

When fluids are transported by pipelines usually leakage problematic is presented into the domestic area as well as in the industry, which represent economic and environmental impact. The Comisión Nacional del Agua (CONAGUA) in 2011 reported that the main problem was the water leakage supplied to Valley of Mexico, which is around 34%. This problematic is reflected in 3 aspects: a big index of visible leaks, the uncertainty of not visible leaks in both locations and magnitude, and a lack of a water leak control program (CONAGUA, 2011). That is why it is important to count on with real time leak detectors, without the necessity of personal supervisors.

According to the report presented by the Instituto Mexicano de Tecnología del Agua (IMTA, 2010), leakage losses were 43.2% of the total supply, in the other hand the federal government estimates that physical loss is around of 85% and this is reflected in a monthly economic loss approximately of $436,000, and without mentioning the severe environmental impact. Therefore, the leak detection in a shorter time is essential, in order to decrease the economic and environmental impact. Thus, in the present study an analysis of several systems and methodologies for leak detection in function of their mean features.

The water shortage at the Valley of Mexico caused by leaks has as scoop, to know leak magnitudes in order to determine the best solution for each particular case. Based on this CONAGUA conducted an investigation in 2005, and the results shown that 35 m^3/s are supplied to Mexico City, which the 27% are lost by leaks (around 8 m^3/s), in many cases, these leaks can take up to 24h to be reported with a rate of 70 leaks per day. In turn, supply pipes have a diameter of 1m with leaks up to 0.05 m^3/s, while house pipes have a diameter of 0.02m (Martínez, 2005). Moreover, CONAGUA and the Comisión Nacional de Población (CONAPO)

---

[*] Author to whom all correspondence should be addressed: e-mail: riddle_ESIME@hotmail.com; Phone: +(52)55 53563354; Fax: +(52) 55 5729 6000 Ext: 54754

carried out a water availability projection from 2007 to 2030, in function of the population growth and water shortage rate in the country as shown Fig. 1 (CONAGUA, 2008).

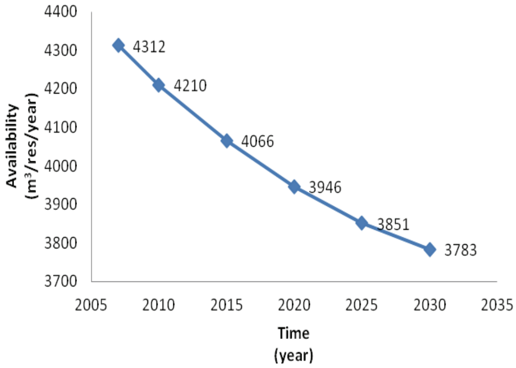


Fig. 1. Projection of natural water per capita availability in Mexico from 2007 to 2030

In 2009, the researcher Durán E.P. from the Escuela Superior de Ingeniería y Arquitectura (ESIA) from Instituto Politécnico Nacional determined that wasted water from Mexico City should be enough to supply water to an 8.4 million population about a country like Honduras with rate of 0.25 m^3/day. At the same time, it was reported that a resident of upper or middle class consumes 0.567-0.399 m^3/day and that a resident of lower class consumes 0.12 m^3/day, while the expected consumption should be 0.17 m^3/day. This research shows how the water is distributed among the population in the Valley of Mexico (Durán, 2009).

Afterwards Ph.D. Salazar A.E. of Mexican Academy of Science (AMC) carried out a research where he noticed that from 100% of water distribution costs only 80% are collected, and which in turn the physical efficiency is 59% (amount of water coming into the houses of the total amount produced), while in the first world it comes to be 95% (AMC, 2011).

In 2012 the CONAGUA announced that it will invest $37 million to supply water to the Valley of Mexico from other aquifers in order to stop extracting deep wells that generate sagging in Mexico City. Losses in Mexico City rise up 40% and losses in cities like Tijuana and Monterrey rise up 19% and 28% respectively, while cities like Tokyo presents leaks under 10% (Luege, 2012). Prospecting of investment for water supply project for Valley of Mexico is shown in Table 1.

For this reason it is a priority to attack the leakage problem with the most appropriate method for each particular case. Nowadays CONAGUA is employing water leak detection and locations systems like: acoustical correlation system (static system) or trained inspector equipped with megaphones, but these solutions have some disadvantages like: initial cost, in case of failure only manufactures can fixed it, the sensors robbery, dry channels are required for installation and in mobile system the inspector requires to be experimented to obtain correct results.

Leaks can be classified according to their exposition as visible or hidden. Visible leaks sprout to the surface and soak the ground and are identified and reported by an observer. Hidden leaks usually are filtered and their location is unknown, these kinds of leaks are small at the beginning and grow with the time. Leaks can occur in the pipe itself or in the accessories (Bucur and Rafa, 2014; CONAGUA, 2011).

O'Day (1982), carried out an investigation which classifies the leaks by their shape and cause. Leak classification by shape is: longitudinal, circumferential, combined fissures and holes. Leak classification by cause is: overload temperature and corrosion. As can occur when some of the following phenomena appear:

- ✓ High pressure fluid inside of the pipe.
- ✓ External corrosion in metallic pipes due to soil contact.
- ✓ Internal corrosion due to transported fluid.
- ✓ Transit of heavy vehicles on the ground shallow pipes.
- ✓ Poor quality of materials and accessories of pipes.
- ✓ Poor quality of workmanship that install or repair the pipes.
- ✓ Old pipes.
- ✓ Earthquakes.

To address the problematic of leakage, CONAGUA implemented an inspection program consisting of three parts as follows: in first place to obtain the current level of physical causes for leaks, in second place to evaluate the leak-control programs and finally generate tree-problems of leakage. Then the current level of leaks and its physical causes had been calculated by CONAGUA based on a water balance of the historical record and must be expressed in percentage of annual volume supply of potable water (CONAGUA, 2011).

Table 1. Investment for water supply Project for Valley of Mexico

System Name	*Valley of Mexico*	*Mezquital*	*Temazcaltepec*
Aquaduct length (km)	140	80	80
Treatment plants	1	1	-
Pumping plants	4	3	3
Wells	-	150	-
Volumetric flow (m^3/s)	9	6.5	3.5
Invest (mdp)	21000	9600	6700
Dams	-	1	-

Additionally to the results of the mentioned statistical study, this study provides the physical origin of occurrence as shown in Fig. 2, i.e. determining the leak rates based on:

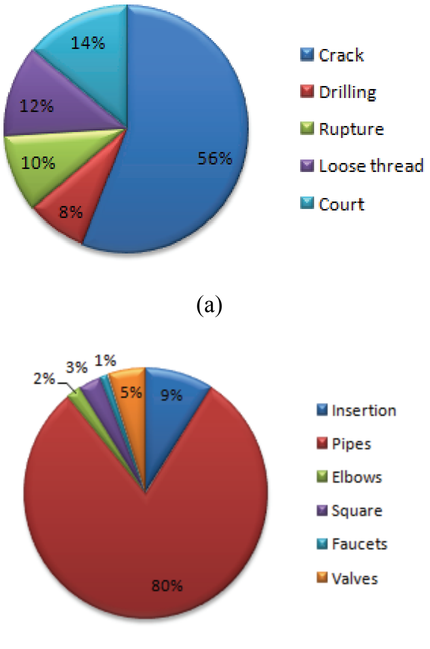
a) The origin of the leak (junctions, boxes, pipes, couplings).

b) Leak type (cutting, drilling, cracks, loose parts).

c) Pipe material (cooper, PVC, cement, cast iron).

d) Suddenly pressure gradient.

e) Change in pipe diameter and pipe networks.



(a)

(b)

Fig. 2. Leak analysis schemes presented for CONAGUA: (a) Leak percentage according to their types; (b) Leak percentage according to their location

2. Cases studied

2.1. Commercial systems for leak detection

Several systems for leak detection are available but such systems have many advantages and disadvantages over each other, so that, having a wide range of needs involved a wide range of systems. The most common water leak detection systems are described below:

2.1.1. Acoustical system

The acoustical system is one of the most frequently used due to its easy implementation in both domestic and industrial leaks. The system consists in an inspection made by a human operator that employs an equipment, which allows to hear the sound emitted by a leaking fluid (Hunaidi, 2000).

The equipment consists of a pair of hydrophones that initially capture the acoustical signal and transmits it to an amplification system (46dB), which finally transmits the acoustical signal it to the operator through the headphones. The amplification system has a volume control for protection of the operator, because he can watch the acoustical level into the screen, the variations of the acoustical level depend on the hydrophone position related to the leak, as it is shown in Fig. 3 (SEWERIN, 2012).

Detection time depends on the operators-experience and the pipe length inspection, also the leak location by means of this system becomes difficult if the pipe pressure is under 15psi and in plastic pipes frequency might be lower than 50Hz which is not audible for humans and the recommended measurement distance for this system is 1m (Hunaidi, 2000). This system has the advantage of requiring only one operator for the entire equipment, but the disadvantage is it requires that the operator has some experience operating it and lowers vibrational levels on surface where the leak noise is censed (Hunaidi, 2000). This system has the capability of detecting and locating more than one leak, due to the fact that it depends on the operator's rute, but if the new leak appears in a tested position of the operator's route, then it will not be able to be detectable nor locatable.

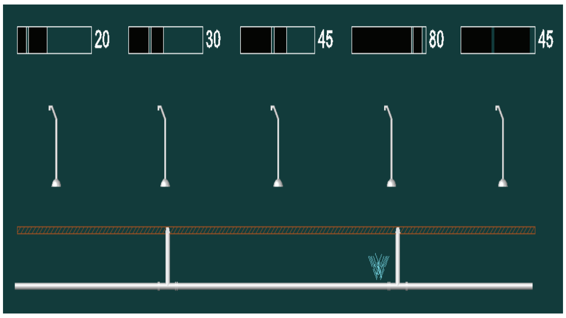


Fig. 3. Operation for leak detection employing the acoustical system

2.1.2. Acoustical correlation system

The acoustical leak detection system had been used around the world due to the possibility of recovering the initial cost in a short time. This system consists of a pair of hydrophones o accelerometers (depending of the accuracy needed) which are attached to two hydraulic pipe terminals between which a leak is expected and serve as preamplifiers. This equipment will carry out a correlation process based on distances and arrival times of the acoustical signal in pipe, this signal is received by the hydrophones (Fujitecom, 2010) (Fig. 4).

Fig. 4 shows that correlation signals are possible due to the difference "N" between the leak position and the distances from both terminals, generating a delay time "TD" in both signals. Knowing the sound speed propagation in the fluid inside the pipe and the delaying time, the leak position respect both distances can be determined. When the leak is located, the system displays the leak position regarding both terminals and the delaying time (Fujitecom, 2010).

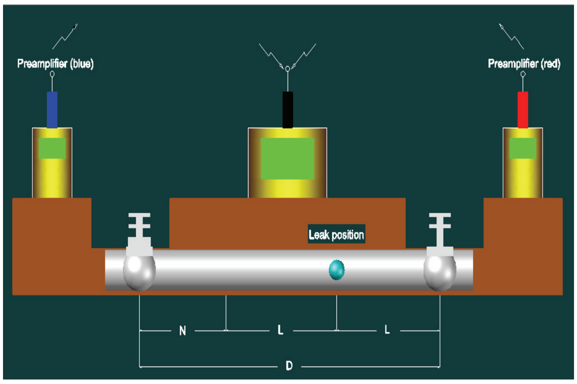


Fig. 4. Operation for leak detection employing the acoustical correlation system

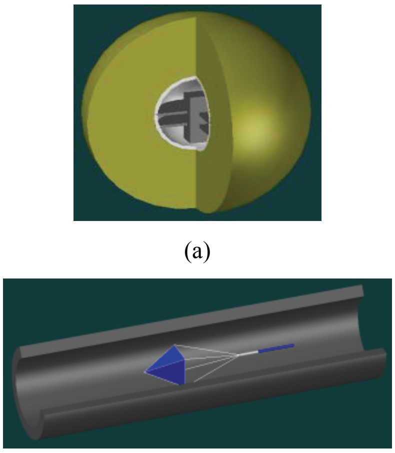
The system does not have the capability of detecting more than one leak, and it would not be able to detect the leak if its position is outside of the terminal boundaries. However, if the system is operating and there is not a leak between the terminals but while the test is running a leak appears, the system could be capable of detecting and locating it.

Morefield and Carlyle (2007) conducted an experiment that implements the acoustical correlation in pipes with leaks and implements a verification of the leak position with a direct inspection by means of acoustic hydrophones. Leaks are usually located to within 1% of the sensor spacing (HWM, 2013): in one of the cases, the sensors were placed to 193m and a leak of 12.61E-03 m^3/s was detected and for other case the sensors were placed to 15.2 m and a leak of 780E-06 m^3/s was detected. Finally, Morefield and Carlyle (2007), observed that leak detection time and leak location time in a 1000 m were 30s and 20mins respectively. In the other hand, this system was implemented on a PVC pipe with a diameter of 0.66 m with a distance between sensors of 137.16 m with a variation in leak position of 0.3 m, which represents an absolute error of 0.22%.

2.1.3. Tracer probe system

Tracer probe system had been widely used for industrial community due to the fact that it is applied on systems where long distance inspections are required. This system consists of a probe, which is inserted in a hydraulic intake and when the tracking is over the probe is extracted in an outtake by means of a web (Puretechltd, 2013a) (Figs. 5a-6a). The inserted probe could be wire or wireless (Puretechltd, 2013a). The probe record the acoustic signal while it is traveling along the pipe and when the track is over, the probe is extracted to analyze the recorded data. Inside of the pipe the probes displace by a parachute in wire probe case, in the other side the wireless probe travels freely (Figs. 5b-6b).

The equipment is located inside a wagon that provides supporting to the probe while it is tracking and reading the recorded data, the equipment also displays the leak position (Puretechltd, 2013b).

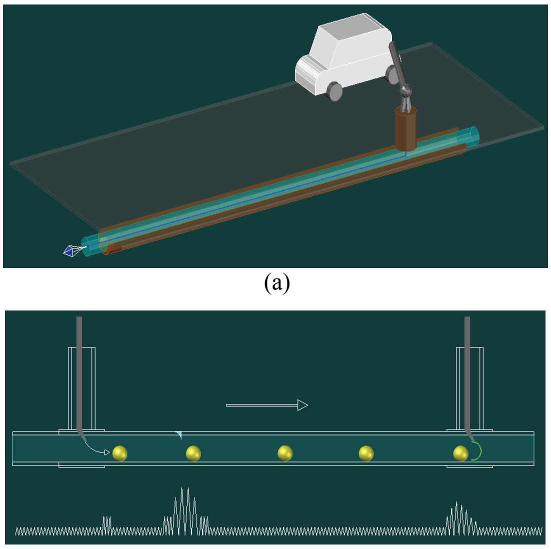


(a)

(b)

Fig. 5. Tracer Probes: (a) Wireless; (b) Wire

For wireless probe the maximum inspection distance is 20000 m, its battery has a duration of 12hrs and can operate under high pressures up to 3445600 Pa, an operation condition for this systems should have an intake and outtake which must have at least 0.15 m and the velocity flow must be at least 0.5 m/s. For wire probe the maximum inspection distance is 1600m and it can detect leaks up to 310E-09 m^3/s, an installation condition for this systems is that inlet and outlet must have at least 0.1m for wireless probe and 0.05 m for wire probe, and the velocity flow must be at least 0.5 m/s (Puretechltd, 2013c). These systems can detect and find as many leaks as there might be along the inspected pipe distance, and it could also detect a new leak that might suddenly appear forward the probe but if a leak is generated in an inspected position after the probe has passed over there, then the system could not detected nor located the leak.



(a)

(b)

Fig. 6. Operation for leak detection employing the tracer probe system: (a) Wire probe; (b) Wireless probe

Kurtz (2006) developed an investigation in field using wireless tracer probe, which tracked along 60640.3 m with a velocity of 0.47 m/s. The insertion and extraction of the probe took 1hr and 3hr respectively. Because of this investigation it was possible to detect a leak with a magnitude of 63.08E-06 m^3/s, generated by a valve of 0.0127m of diameter. Afterwards another case was studied where the system was capable of detect a leak with a magnitude of 6.3E-06 m^3/s (Kurtz, 2006).

2.1.4. Thermographic System

The thermographic system for leak detection is one of the most effective in terms of implementation speed and specially is applied in buildings for identify intramural water leaks as air leaks positioned in windows, roofs and other inaccessible places (FLUKE, 2013). This system consists on detecting the leak by means of thermographic picture. The radiation emitted by an object is detected and measured by an infrared radiometer, which has a transducer that becomes the emitted radiation in electrical signals which in turn are expressed in a scale of colors, showing the leak position (TT, 2010) (Fig. 7). This system can detect and locate as many leaks as they appear into the display, and it could also detect and locate an appearing leak in the right moment that the picture is taken.

The possibility of detecting more than one leak in less than 2s with a single picture is an advantage of the thermographic system and also does not require any experience to operate the camera, which can operate in a temperature range from -20°C to 600°C of the photographed object with an accuracy of 2%. The disadvantages of the system are that it cannot determine the leak magnitude and in case the operator has to inspect a pipe network, a lot of pictures will have to be taken (FLUKE, 2013).

Fig. 7. Operation for leak detection employing the thermographic system

Finally it is necessary to point out that leak detection by means of thermographic system (Fig. 8) is very versatile and it can be applied in the construction industry (leak detection in roofs, walls, windows, etc.), in the thermal industry (convection in boilers, leak detection in air conditioning and in capacitors, etc.), in the electrical industry (energy losses by malfunction in motors bearings and in ventilation systems, etc.) (TT, 2010). Fahmy and Moselhi (2009) who inspected underground pipes in Montreal with 1.5m to 2m of diameter and with lengths from 48m to 300m and a depth of 1.8m. The authors employed the thermographic system and a leak could have been detected and located by aerial shots. At the same time leak detection by means of the acoustic correlation system was carried out, which allows to compare both results, acoustic correlation and thermographic.

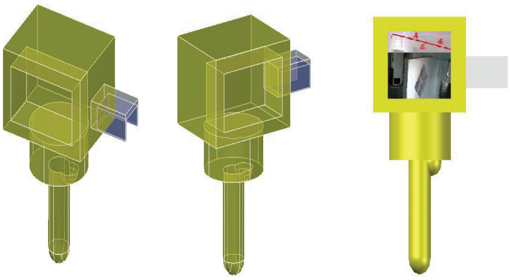


Fig. 8. Camera of the Termographic System

An specific case where the studied pipe had a length of 48.7m with a leak that was located to 16.8m from the end of the pipe and the leak located by acoustic correlation system and thermographic system were 15.41m and 14.9m, respectively from the end of the pipe, these results are interpreted as an absolute error of 2.85% and 3.9% respectively (Fahmy and Moselhi, 2009). This work also reveled that from 6 am to 8 am was the best time for take the aerial shots.

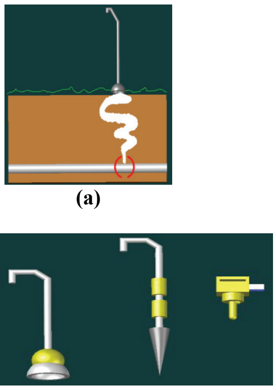
2.1.5. Tracer Gas System

Tracer gas system has been widely used by the industry due to its application in underground pipelines and in pipe networks. This system consists on a tracer gas which is injected into the pipeline, then the gas moves along the pipe with the fluid and it escape through the leak and then an operator employs a gas detector that indicate the leak position. When the pipelines are buried, the tracer gas is colored in order to show the leak position by a stain on the surface after 2hrs or less, depending of the depth (SEWERIN, 2013) (Fig. 9a).

Basically the tracer gas system has a tracer gas compound by hydrogen and helium (because they are not flammable, corrosive or toxic, also the system has several sampling probes types according to sampling needs (Fig. 9b), which record the sensed information into a portable processor that displays the leak magnitude in parts per million allowing the operator to know the leak position (SEWERIN, 2013). This system is able to detect and locate more than one leak since it depends on the operator's route, but if the new leak appears in a tested position of the operator's route, then it could not be detectable or locatable.

2.1.6. Comparative analysis between leak detection systems

The features analysis of leak detection systems before mentioned, was carried out in function of the following factors: installation and operation time, inspection length, sensibility and number of operators, and finally a recommendation of how to select a leak detection system based on the advantages and disadvantages. These factors are listed in order to specify their function and relevance in present study.


(a)

(b)

Fig 9. Tracer Gas System: (a) Tracer gas system operation; (b) Tracer gas equipment

Installation time

Installation time becomes relevant due to the necessity to stop the process in order to insert the sensors of leak detection inside of the pipe, therefore, it is essential to have the shortest installation time, because it directly affects on the cost of process. In agreement with the present investigation, the systems with the shortest installation or assembly time were: thermographic (180s), acoustical (300s) and tracer gas (1800s).

Operation time

Operation time becomes relevant due to leaking time has an impact in maintenance cost and fluid losses. Also operation time involves two different aspects: detection and location; because the first action is to detect if the leak really exist, then the next step is locate the leak position in order repair it.

According to the present investigation, the systems with the shortest time for leak detection were: thermographic system (2s), acoustical correlation (30s) and acoustical (180s). Notice that this time is related to the leak position, if the leak is not in the segment of the inspected pipe, the system will need to displace along the pipe, until the leak has been detected. According to the present investigation, the systems with the shortest time for leak location were: thermographic system (2s), acoustical correlation (20s). Notice that this time is function of the leak position, if the leak is not in the segment of the inspected pipe, the system needs to displace along the pipe, until the leak has been detected.

Inspection distance

The inspection distance becomes the most compensable factor because the greater inspection distance inspection take a shorter operation time and also it allows to reduce the installation time. In agreement with the present investigation, the systems with the longest inspection distance were: tracer wireless probe (20000m), tracer wire probe (1600m) and correlation acoustic (1200m).

Sensitivity

The sensitivity in the leak detection systems turns essential due to the fact that the higher sensitivity system allows detecting very small leaks, but also it can prevent great damages of the pipelines because it can detect small faults as leaks due to corrosion. According to the present investigation, the systems with the highest sensitivity were: tracer wire probe (310E-09 m^3/s), acoustical correlation (780E-09 m^3/s) and tracer wireless probe (6300E-09 m^3/s).

Number of operators

The number of operators working in a process increase the costs and the probability of making errors, therefore, while fewer personal has been required lower the losses will be. In agreement to the present investigation, the systems with the fewer operation number were: acoustical (1 operator), thermographic (1 operator) and tracer gas (2 operators).

Capability of detect more than one leak

If several small leaks appear into a pipe, the waste of fluid could be similar to a big leak. Therefore, the capability of detecting more than one leak may be an important factor to choose the appropriate system which might be suitable to detect and locate more than one leak. Among these systems we could find for instance: the acoustical, tracer probes, tracer gas and thermographic.

Capability of detecting a new leak while the test is already running

Some systems cannot detect a leak that it is originated while the test is already running in consequence locate it, therefore, even if the water leak detection system is working correctly, a leak could be ignored. Therefore, the capability of detecting a new leak that is generated while the system is being tested, depends on the new position of the leak. For example in the case of the acoustical and tracer gas systems, if the leak appears in a pipe previously inspected by the operator, then the leak becomes undetectable and it could happen the same with the tracer probes. In an acoustical correlation case, if the test was running without a leak, it would detect it and finally, in a termographical system the leak could be detected if it is big enough to be displayed in a termographic picture.

Recommendations for selection of the leak detection system

When a leak detection system needs to be selected, the first step is to identify the needs of the system which were listed before. From the above list, it is possible to summarize that the best system for leak detection is the one that fits to the environment and in second plane, must be in function of the cost generated by time, personal, transportation, etc. For example, if it is necessary to detect a leak in a long pipeline the best option must be the tracer probe, on other hand if the leak is placed inside of a house, the best option must be a thermograchic camera, but if the leak is inside a pipe network the tracer gas is recommended. It is important to highlight that these systems cannot be applied in a continuous monitoring in order to detect or locate leaks.

2.2. Leak detection methodologies

Several methodologies for leak detection in pipes have been developed and classified by means of flow properties due to the leak, in three groups:

- ✓ Stationary state methodologies.
- ✓ Transient state methodologies.
- ✓ Stationary-transient state methodologies.

2.2.1. Stationary state methodologies

The stationary state methodologies for leak detection, as its name mentions, are those in which the flow properties are unaffected respect the time. The most used methodology is based on Hydrostatic (Scheinfeld et al., 1986), it also can be applied in housing, tanks etc.

Hydrostatic

This methodology detects a leak in a pipe, which has a fluid in stationary state and it is frequently used for pipes verification before these have been in operation. Schwendeman carried out a leak detection in a pipe by means of pressure measurement for a period of time, in order to observe if it exists a decomposition when leak occurs (Schwendeman, 1987).

Nowadays in Mexico the hydrostatic methodology has been widely used to test pipe networks, applying the norm NOM-013-CNA-2000, which indicates that pipe must be pressured at 150% of the normal operational range of internal pressure and must stay that way for 5 days (NOM, 2000).

Vista Research (VLD, 2013) developed the HT-100 which allows to detect leaks in underground pipelines which transport fuel in long distances in airports, the company informed that the HT-100 system could detect leaks around 0.0021% of pipeline volume in 1hr (VistaResearch, 2001). So this methodology is a great tool, when the leak detection in stationary state is the target. Although this methodology has the advantage of detecting small leaks, it has the disadvantage of a big operation time and cannot be employed into transient state. This methodology cannot locate leaks, but it allows detecting a leak while a test is running, due to the fact that it operates by means of several sensors and a instantaneous pressure drop will be sensed even if it is small.

2.2.2. Transient state methodologies

Transient state methodologies for leak detection, are those in which the flow properties change respect the time, for example, the waterhammer is generated when a leak occurs in pipeline.

Damping transient analysis

This methodology is based on observing how the transient phenomena propagates along the pipe due to friction and other external factors as the leak case (Liou, 1998). Liou (1998) carried out an experiment monitoring the system in real time, and such experiment shows that the damping depends on the pipe friction and the leak magnitude.

Wang et al. (2002) developed an experiment at Adelaide University in an experimental facility which is composed by two tanks connected by a pipeline, with a valve on each one of the extremes. In the pipeline there is a discharge valve, which generates the perturbation and the leak. Tank 1 has 2m elevation related to tank 2. This facility has 5 pressure transducers connected to an acquisition and processing system, also the pressure in tanks are electronically controlled. For a graphical description please refer to Wang et al. (2002). From the experimentation it was determined that leak can be detected by examining the induced damping wave in pipe flow due to the leak and comparing it to the damping wave in the same pipe without the leak (for a graphical description please refer to Wang et al., 2002). In turn the experimentation showed that the damping depends of leak magnitude, also, the leak position was determined by means of different damping ratios of various Fourier components.

The experiment also reveled from the analytical solution of the damping wave induced by the friction is exponential and damping wave induced by the leak is approximately exponential. The damping rate of the induced leak not only depends of the leak magnitude and position, but also depends on the pressure in the pipe, the transient position measure point and the transient initial condition (transient shape) (Wang et al., 2002).

This methodology was successful in detecting, locating and quantifying a leak with 0.1% with respect to the transversal section in the pipe, based on the numerical and experimental tests (Wang et al., 2002). Nowadays this methodology is being improved in order to be applied into a pipe network. This methodology can detect and locate more than one leak and it can also detect and locate a new leak while a test is running.

Finally this methodology is a useful tool not only to detect leaks but also but to monitor the installation also, it is necessary to point out that if the system can detect small leaks in transient state is unable to detect leak in stationary state, also, the operation of this methodology cannot used to detect leaks in pipe networks due to accessories such as elbows, junctions, valves, etc.

Negative reflected waves

When a transient source generates a wave, this travels along the pipe and reaches a leak point, the wave is partially reflected and partially transmitted (Lee et al., 2007). If the wave reflected (usually the first one) can be identified from the transient measure, the leak location can be possible by means of the multiplication of half the time reflection for the propagation velocity of the wave inside the pipe (Lee et al., 2007).

This methodology consists basically on observing the wave speed, in order to determine the position of the leak within the pipeline from the arrival time of the leak reflected signals, under the condition that the starting time of the beginning of transient event must be shorter than the time between the reflection between the leak and measure point, in order to observe correctly the phenomena (Lee et al., 2007).

Lee et al. (2007) carried out an experiment in Adelaide University, which consists on two electronically regulated tanks connected by a pipe with inline ball valves, this installation allows the generation of sharp pulses. The flow travels from tank 1 to tank 2 (for a graphical description please refer to Lee et al., 2007), and the transient source was a solenoid valve which was exchanged along the pipe in order to generate 3 different configurations:

A) the leak is set between the source of transient and the pressure transducer.

B) the source of transient is set between the leak and pressure transducer.

C) the pressure transducer is set between the leak and the source of transient.

In stationary test a leak of 1.5mm of diameter was located and represents 0.465% of the pipe area (Lee et al., 2007). In addition to the three configurations before mentioned, Lee determined that when a transient is generated, the wave takes two possible paths: in the first one, the operation mechanism travels from the source of transient to the leak and in the other one the operation mechanism travels from the source of transient until the end of the pipe and it goes trough the pipe to the leak position, in both cases the phenomena is registered by means of pressure transducer, for a graphical description please refer to (Lee et al., 2007). This methodology based on waves propagation in pipes was usually discarded due to the absorption of the wave, but Silva et al. (1996) experimented in two PVC pipes with 433m and 124m respectively, showing that a leak with 5% of the total flow into the pipe could be located.

Brunone (1999) experimented in a polyethylene pipe with a diameter of 0.0938m, a length of 352m and a leak of 0.5% of the transversal section of the pipe which was detected. This methodology can detect and locate more than one leak and it can also detect and locate a new leak while a test is running.

2.2.3. Stationary-transient leak detection methodologies

The stationary-transient leak detection methodologies as their name mentions, are those which can operate when the flow properties are or not unaffected respect the time. For example, a big leak should be identified for the transient leak detection program, but if the leak generates a small disturbance that cannot be detected for a change on flow properties then it might be detected by a statistic method.

Mass balance methodology

This methodology consists of detecting the leak by means of a mass balance or a volume balance. Mears (1988) carried out an investigation where a leak of 1% and 4% were detectable for stationary and transient flow, respectively.

Newell (2006) carried out a research about the sampling frequency and the data asymmetry and obtained that both factors drastically affect the leak detection quality. Also, the author observed that scanning every 2 min may conform fine block valves, however detection works better with faster rates due to time reduction between the leak appearance and the leak alarm. Although at first sight it would seem to be a simple method, in application it tends to be more complex because there are several temperature variations in flow along the whole/entire pipe.

Emerson Process, EP (2008) corrected the temperature variation by means of implementing a system based on Coriolis effect (Fig. 10) and it was corrected by a real time correction of fluid properties (by implementing thermostats, flowmeters, etc) carrying out a mass or volume conversion, with an accuracy of 0.01% and density accuracy of 0.5 kg/m^3 (EP, 2008).

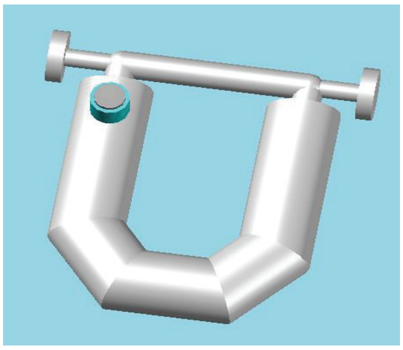


Fig. 10. Emerson Process Flowmeter

Then a deviation between the measured and calculated values is made in order of activate the alarm when the limits are exceeded (for a graphical description please refer to Silva et al., 2004). Leak quantification depends on the accuracy of properties sensors: flow, temperature, density; and also has to do with leak magnitude that in turn depends of the mathematical algorithm. Leak location and detection depends on leak magnitude and pipe physical condition (Newell, 2006).

The most advanced systems for leak detection generate programs that inherently have an analyzing algorithm capable of discarding false alarms. It is a priority that alarm trigger should not be based on efficiency process (because its change with the operation conditions), therefore the alarm trigger should be in function of the minimal liberation flow rate, which belongs to the inspected pipe. It is also important mentioning that the program generates a panoramic view of the monitoring pipe, in order to easily locate the leak position (Newell, 2006). This system allows to detect and quantify a leak in real time while a test is running, although this system cannot quantify multiple leaks because the system employs flowmeters that provide mass difference in a pipe section, therefore the leak value will be the total leak flow produced by the multiple leak flows.

Inverse transient analysis

This methodology consist on detecting the leak by means of an inverse analysis, because usually the system features are known (length, diameter, etc) and the fluid properties are unknown (pressure, flow velocity, etc), but in this methodology is reversed (Vítkovský et al., 2000; Vítkovský, 2001), due to the fact that operation conditions and fluid properties are known but the position and magnitude of the leak, and friction coefficient are unknown.

Pudar carried out an analysis for leak detection in stationary state by comparing the measured data against the calculated data for minimizing the sum of the squared differences of pressures, using the Levenberg-Marquardt method (Ligget and Chen, 1994). Pudar concluded that the error variations were fewer than the estimation error of friction coefficient of the pipe and he concluded that this kind of analysis could not be applied to pipe networks (Pudar and Ligget, 1992).

Afterwards, Ligget and Chen (1994) implemented a numerical verification of Pudar experimentation considering the same installation, but the problem was worked out by a numerical simulation, which involved a zero error, and he also came to the conclusion that the convergence between the measured and calculated data, should be faster if the friction coefficients of the pipe are correct.

Pudar and Ligget (1992) introduced the leak detection method of inverse transient analysis by means of two paths: Levenberg-Marquardt that gives direction to the Generic Algorithm Method. Kapelan et al. (2003a) observed that Levenberg-Marquardt tends to have faster results but also in the inflection point it was stacked, but on the other hand the Generic Algorithm Method tends to provide good results but in a long period of time because it was necessary to run the program a lot of times (Kapelan, et al., 2002, 2003b, 2004).

Therefore, Vítkovský et al. (2000, 2002) generated and hybrid algorithm called Systematic-Levenberg-Marquardt (SLM), this program places the leak position in all possible positions along the pipe, in order to obtain the leak position by means of a convergence between the simulated signal and the measure signal, as shows.

On the other hand Kapelan et al. (2002) led the development of an algorithm with the advantages of both algorithms: Levenberg-Marquardt and Generic Algorithm; calling it as Hybrid Algorithm (Kapelan et al., 2002; Vítkovský et al., 2002). This algorithm was split in two parts: the first part converged by iterating the measured signal and the simulated signals (a global and faster convergence is generated by means of Levenberg-Marquardt) and the second part search for the solution within the previously established limits (the stacking is avoided and good results are guaranteed by means of Generic Algorithm) (Kapelan et al., 2002; Vítkovský et al., 2002).

Covas et al. (2006) implemented an inverse transient analysis in Scotland into pipe network to detect a leak with a pipe length of 5900m and the detection and quantification of the leak could be done with an error of about 1% of the total pipe length. This experimentation was necessary to characterize pipe factors as: leaks, change of diameter, air bags, pipe networks, accessories, etc.

In 2007, Vítkovský et al. (2007) conducted an experimentation about a leak detection, quantification and location by means of inverse transient analysis. This experimentation was modeled and it obtained as a result the satisfactory detection, quantification and location of several leak size: 0.001m, 0.0015m, 0.002m; i.e. a perceptual relation leak diameter-pipe of 4.5%, 6.8% and 9% respectively. The experimentation and simulation was even applied into a 2 leaks in order to test multiple leaks of 1E-03m. A comparison between the experimental data and the inverse transient analysis solution was carried out by Vítkovský et al. (2003), which indicates that the pressure decaying is inverse to the time decaying. The perturbation was originated by a 1mm × 1mm leak and represented on adimensionalized pressure head (pressure head divided by the Joukowsky pressure head) versus valve closure time (relation pipe-length/wave-speed), in 3 cases: 2.5, 25 and 50 times the time decaying. For a graphical description please refer to Vítkovský et al. (2007). In order to understand in depth on this methodology the reference Colombo et al. (2009) has been recommended.

From the above, the conclusion is that the methodology inverse transient analysis for leak detection results a powerful tool, which could apply to pipe networks with a previously characterization, in order to observed the perturbation into the signal recorded by the sensors and in this way, to get exclude them by comparing the measure and simulated signals. This methodology can detect and locate more than one leak and it can also detect and locate a new leak while a test is running.

2.2.4. Comparative analysis between methodologies for leak detection

The feature analysis of leak detection methodologies before mentioned, was carried out in function of the following factors: time detection, inspection distance, monitoring and sensibility. Also a recommendation of what methodology should be employed has been made in function of their advantages and disadvantages. These factors are listed in order to specify their function and relevance in present study.

Location time

From the current investigation about leak detection methodologies it was observed that the shortest time for leak detection was the mass balance methodology, because it has a real time monitoring and also conducted by sampling every 2min, therefore, it allows to locate the leak in both stationary and transient state.

Distance of inspection

On this research about methodologies it was observed that the longest inspection distance for leak detection was obtained with the negative reflected waves with 1200 m, considering that can reach that distance with only 2 sensors. Also the inverse transient analysis and mass balance could detect and quantify the leak in larger distances, but they have the disadvantage of requiring more sensors although they can detect leaks even in pipe networks.

Monitoring

From the present investigation about methodologies it was observed that the best monitoring system for the leak detection time was the mass balance, due to its ability of continuously generate a data base, which in turn generates patron of: flows, pressures, etc. These patrons allow to detect, quantify and locate the leak when is slightly disturbed.

Sensitively

From the present investigation about methodologies it was observed that the system with the highest sensitively system for the leak detection time was mass balance, since it complements the flow-meters with pressure transducers, allowing the improvement of the sensitively, due to several factors such as: response time, readability and databases. Also the need of employ the same terminology and units in each methodology is indispensable.

Capability of detecting and locating more than one leak

When a leak appears into a pipe due to corrosion or a failure in different sections of a pipeline, it usually tends to cause multiple small leaks and the waste of fluid could be similar to a big leak. Therefore, the capability of detecting more than one leak may be an important factor to choose the appropriate methodologies which might be suitable to detect and locate more than one leak. According to the present study the methodologies with capability of detecting more than one leak are: damping transient analysis, negative reflected waves and inverse transient analysis.

The capability of detecting a new leak while the test is already running.

Some methodologies cannot detect a leak that is originated while the test is already running; therefore, even if the water leak detection methodology is working correctly, a leak could be ignored. So the capability of detecting a new leak that is generated while the system is being tested depends on the new position of the leak. For example in the case of the hydrostatic and mass balance, if the leak appears while the test is running, the leak becomes detectable but it could be not locatable. The methodologies with the capability of detecting a new leak while the test is already running according to the present study are: damping transient analysis, negative reflected waves and inverse transient analysis.

3. Discussion

Based on the aim of this paper, which is to investigate the advantages and disadvantages of the systems and methodologies for leak detection, in order to apply the best solution to the current problematic about water leak detection in Mexico, a system with the lowest costs, higher efficiency, low rate false alarm, real time monitoring capability and easier implementation, the authors make all the following recommendations:

The leak location systems tend to be more accurate when the sampling frequency increases, therefore the first challenge is to generate a system with lowest accuracy using a short sampling time. On other hand, the leak detection systems, tend to be more accurate when the leak already appears, avoiding losses due to time operation and human resources to repair the leak and indirectly improve the cost reduction.

The present study also observed that leak location systems differ in terms of congruence about inspection distance and magnitude of the leak, therefore the second challenge is to generate a system which has a detailed sensitively study, in order to predict the error of locating and quantifying the leak, in function of several factors as the distance between sensors, leak magnitude respect flow and transversal section of pipe, transient generation time, to mention some. In turn, it was observed a third challenge which is to generate a leak detection system with a low false alarms rate, avoiding using human resources for unnecessary personalized inspection.

It is necessary to take in consideration that systems of leak detection should quantify the leak in dimensionless numbers, in order to avoid the disagreements between authors and thus assess comparatively and more effectively defined which the correct system is depending on the needs. Normalization should be considered for quantifying the leak magnitude in areas ratio or flows ratio and should be solidly justified taking the operation pressure into account.

The systems need to have the capability of self adjustment after the transient phenomena appears, allowing that if a new event disturbed the system (i.e. if a succession of leak appears in the system) the system should set the limits over and over again, automatically without intervention of an operator. It is important to point out, that the capability of a system to detect and locate a multiple leak scenario is a must nowadays, due to a multiple leak phenomena which appears because of a waterhammer originated by a normally closed valve in case of an electrical failure.

The possibility of detecting a new leak while a test is running is an important quality for a leak detection methodology to avoid the need of to carry out a second test in order to verify that the pipeline is leak free. This becomes important due it represents a cost reduction but not only because the leaking time is reduced, but also the testing time and human resources are minimized.

Finally, the importance of an efficient leak detection, quantification and location system, lies not only in the economy for maintenance or for the fluid escape, but it is much more important to pay attention to the environmental impact, as the vital liquid waste (approached from the future availability) as the excessive humidity that would lead to generate a decomposition of an organic element and whether the contaminants came to a river, resulting in an evident environmental risk of epidemic or diseases, this pollution could reach a river and cause further damage.

4. Conclusions

A conclusion is that there is still a wide range of opportunities concerning research in the field of detection, quantification and location of leaks in pipes, for a diversity of fluids, operation conditions and external factors affecting the phenomena.

Main parameters to optimize were also determined: inspection distance, operation time and sensitivity. Factors like false rate alarm, implement cost and also the need of cutting off the process to inspect the pipeline are very important but they were underestimated by the original authors.

Finally, this study lead the authors to develop a water leak detection system based on pressure gradient, according to the observed parameters, capabilities and needs.

Acknowledgements

To the Consejo Nacional de Ciencia y Tecnología for the support of resources to conduct this research which allows to the Escuela Superior de Ingeniería Mecánica y Eléctrica del Instituto Politécnico Nacional, to develop a system for detecting, quantifying and locating leaks in real time. Therefore the aim of the proposed system will consider the advantages and disadvantages reviewed at this study, in order to develop a system which satisfies the current needs for leakage problematic of hydraulic supply for Mexico City and in this way to preserve human, material and economic resources, which indirectly have an impact on favorable environment for all of us.

References

AMC, (2011), Leaks, the main problem of water shortage in country (in Spanish), AMC/178/11, Mexican Academy of Sciences, Mexico.

Brunone B., (1999), Transient test-based technique for leak detection in outfall pipes, *Journal of Water Resources Planning and Management*, **125**, 302-306.

Bucur A., Rafa V., (2014), Detection of accidental leaks in natural gas main pipelines by fuzzy logic tools, *Environmental Engineering and Management Journal,* **13**, 1533-1536.

Colombo A.F., Lee P., Karney B.W., (2009), A selective literature review of transient-based leak detection methods, *Journal of Hydro-Environmental Research*, **2**, 212-227.

CONAGUA, (2008), Water Statistics in Mexico, National Water Comission & National Population Comission (Comisión Nacional del Agua y Comisión Nacional de Población), On line at: http://www.conagua.gob.mx/CONAGUA07/Publicaciones/Publicaciones/EAM_2008.pdf.

CONAGUA, (2011) Manual of Physical Efficiency Increase, Potable Water Systems of Hydraulic and Energetic, National Water Comission, On line at: http://www.conagua.gob.mx/conagua07/publicaciones/publicaciones/sgp-1-11-eam2011.pdf.

Covas D., Ramos H., Lopes N., Almeida A., (2006), *Water by Transient Pressure Signals*, Eighth Annual Water Distribution Systems Analysis Symposium, Cincinnati, Ohio, United States, 1-19.

Durán E.P., (2009), Leaks consume 30% of water in DF (in Spanish), Sistema de Aguas de la Ciudad de México, On line at: http://www.eluniversal.com.mx/notas/616762.html.

EP, (2008), Micro Motion. Coriolis Flow and Density Measurement in the Petroleum Refining Industry, Emerson Process Management Brochure, On line at: http://www.spartancontrols.com/~/media/resources/micro%20motion/brochure/289_micro-motion_brochure.pdf

Fahmy M., Moselhi O., (2009), *Detecting and Locating Leaks in Underground Water Mains Using Thermography*, 26th Int. Symp. of Automation and Robotics in Construction, Canada.

FLUKE, (2013), Industrial Thermographic Camera or Comercial FLUKE Ti27, FLUKE (in Spanish), On line at: http://www.fluke.com/Fluke/mxes/Termografía/Fluke-Ti27.htm?PID=72281.

Fujitecom, (2010), How to work Leak Noise Correlator LC-2500, On line at: http://www.fujitecom.com/howto/LC2500.html

Hunaidi O., (2000), Detecting leaks in water distribution pipes, *Journal of American Water Works Association*, **92**, 82-94.

HWM, (2013), Advanced electronic acoustic leak detection, Halma Water Management, On line at: http://www.watermarksolutions.ca/uploads/Xmic_CA_En.pdf.

IMTA, (2010), "43.2% of supplied water is lost in leaks: UN" (in Spanish), Mexican Institute of Water Technology, On line at: http://www.imta.mx/internacional/index.php/noticias-internacionales/noticias-internacionales-deagua/243-el-agua-se-pierde-432-en-fugas-onu.html.

Kapelan Z.S., Savic D.A., Walters G.A., (2002), *Hybrid GA for Calibration of Water Distribution Models*, Proc. 1st Annual Environmental & Water Resources

Systems Analysis (EWRSA) Symp. EWRI, Roanoke, Virginia, USA.

Kapelan Z.S., Savic D.A., Walters G.A., (2003a), Optimal sampling design for calibration of transient network models using multi-objective gas, *Journal of Hydraulic Engineering*, **131**, 190-200.

Kapelan Z.S., Savic D.A., Walters G.A., (2003b), A hybrid inverse transient model for leakage detection and roughness calibration in pipe networks, *Journal of Hydraulic Research*, **41**, 481-492.

Kapelan Z.S., Savic D.A., Walters G.A., (2004), Incorporation of prior information on parameters in inverse transient analysis for leak detection and roughness calibration, *Urban Water Journal*, 129-143.

Kurtz D.W., (2006), Developments in free swimming. Acoustic leak detection systems for water transmission pipelines, *American Society of Civil Engineers Journal*, **211**, 1-8.

Lee P.J., Vítkovský J.P., Lambert M.F., Ligget J.A., (2007), Leak location in pipelines using the impulse response function, *Journal of Hydraulic Engineering*, **45**, 643-652.

Ligget J.A., Chen L.C., (1994), Inverse transient analysis in pipe network, *Journal of Hydraulic Engineering*, **120**, 934-955.

Liou C.P., (1998), Pipeline leak detection by impulse response extraction, *Journal of Hydraulic Engineering*, **120**, 833-838.

Luege T.J.L., (2012), Investing more than 37mmdp in city water plans (in Spanish), Comisión Nacional del Agua, On line at: http://www.eluniversal.com.mx/ciudad/112887.html.

Martínez P.A., (2005), The hydraulic network suffers 70 leaks a day (in Spanish), Sistema de Aguas de la Ciudad de México, On line at: http://www.eluniversal.com.mx/ciudad/72107.html.

Mears M.N., (1988), Major pipeline installs system to pinpoint leak size, location, *Oil & Gas Journal*, 37-42.

Morefield S., Carlyle J., (2007), Acoustic leak survey of the underground potable water system at a CONUS army installation, *Advanced Materials Research*, **38**, 143-154.

Newell R.D., Greenwood B., (2006), Mass balance leak detect, can it work for you, Auspex, On line at: http://www.auspex-inc.com/images/Entelec%202006.pdf.

O'Day K., (1982), Organizing and analyzing leak and break data for making main replacement decisions, *Journal of American Water Works Association*, **74**, 588-594.

Pudar R.S., Ligget J.A., (1992), Leaks in pipe networks, *Journal of Hydraulic Engineering*, **118**, 1031-1046.

Puretechltd, (2013a), In-Line Detection Services for Large-Diameter Pipelines, Pure Technologies, On line at: http://www.puretechltd.com/solutions/leak_detection/index.shtml.

Puretechltd, (2013b), Sahara Leak & Gas Poket Detection, Pure Technologies, On line at: http://www.puretechltd.com/products/sahara/sahara_leak_gas_pocket.shtml.

Puretechltd, (2013c), Smartball for Water and Wastewater Water Mains, Pure Technologies, On line at: http://www.puretechltd.com/solutions/leak_detection/index.shtml

Scheinfeld R.A., Robert J.B., Schwendenman T.G., (1986), Underground storage tank monitoring observation well based systems, *Groundwater Monitoring and Remediation*, **6**, 49-56.

Schwendeman T., (1987), Detecting underground piping leaks, *Civil Engineering*, **57**, 56-58.

SEWERIN, (2012), Receptor AQUAPHON@ A 100, On line at: http://www.sewerin.com/cms/fileadmin/redakteure/Prospekte/pro_aquaphon_a100_es.pdf

SEWERIN, (2013), Variotec 460 Tracergas, SEWERIN, On line at: http://www.sewerin.co.uk/products/water-leak-location/variotec-460/.

Silva R.A., Buiatti C.M., Cruz S.L., Pereira J.A.F.R., (1996), *Pressure Wave Behavior and Leak Detection in Pipelines*, 6th European Symp. on Computer Aided Process Engineering, Computers & Chemical Engineering, vol. 20, 491-496, Rhodes, Greece.

Silva H.V., Moroka C.K., Guilherme I.R., Mendes J.R.P., Fonseca T.C., (2004*)*, *Leak Detection Using a Fuzzy System*, ABCM Symp. Series in Mechatronichs, vol. 1, 625-634.

SSLD, (2009), Leak noise correlator Model LC-2500, Sub Surface Leak Detection Inc., On line at: http://www.subsurfaceleak.com/PDFs/LC-2500_brchr.pdf.

TT, (2010), Top 6 places to look for energy losses in commercial buildings, Techni-Tool & FLUKE, USA, On line at: http://www.techni-tool.com/ARTICLES/Fluke-Top-6-places-to-look-for-energy-losses-in-commercial-buildings.

Vítkovský J.P., Simpson A.R., Lambert M.F., (2000), Leak detection and calibration using transients and genetic algorithms, *Journal of Water Resources Planning and Management*, **126**, 262-265.

Vítkovský J.P., (2001), *Inverse analysis and modelling of unsteady pipe flow: theory. application and experimental verification*, PhD Thesis, Adelaide University, Australia.

Vítkovský J.P., Simpson A.R., Lambert M.F., (2002), Minimization Algorithms and Experimental Inverse Transient Leak Detection, On line at: https://www.researchgate.net/profile/Angus_Simpson/publication/256117151_Minimization_Algorithms_and_Experimental_Inverse_Transient_Leak_Detection/links/00b49521d4fe4b58a0000000.pdf.

Vítkovský J.P., Lambert M.F., Simpson A.R., Ligget J.A., (2003), Optimal measurement site locations for inverse transient analysis in pipe networks, *Journal of Water Resources Planning and Management*, **E 129**, 480-491.

Vítkovský J.P., Lambert M.F., Simpson A.R., Ligget J.A., (2007), Experimental observation and analysis of inverse transients for pipeline leak detecition, *Journal of Water Resources Planning and Management*, **133**, 519-530.

VistaResearch, (2001), Leak detection and Location Equipment: HT-100 and SPAL system, Vista Research.

VLD, (2013), Leak Detection in Large Pipeline Systems & Airport Hydrant Fuel Systems HT-100, Vista Leak Detection, On line at: http://www.vistaleakdetection.com/solutions/2011-10-04-07-38-32/ht-100.

Wang X.J., Lambert M.F., Simpson A.R., Ligget J.A. Vítkovský J.P., (2002), Leak detection in pipeline systems using fluid transients, *Journal of Hydraulic Engineering*, **128**, 697-711.

Environmental Engineering and Management Journal September 2016, Vol.15, No. 9, 2075-2084
http://omicron.ch.tuiasi.ro/EEMJ/

"Gheorghe Asachi" Technical University of Iasi, Romania

SUSTAINABLE USE OF METALLIC MINERAL RESOURCES OF SERBIA FROM AN ENVIRONMENTAL PERSPECTIVE

Radule Tošović[1], Predrag Dašić[2]*, Ivica Ristović[3]

[1]*University of Belgrade, Faculty of Mining and Geology, Department of Economic Geology*
Djušina Street No. 7, 11000 Belgrade, Serbia
[2]*SaTCIP Publisher Ltd., Tržni Centar Pijaca Street No. 101, 36210 Vrnjačka Banja, Serbia*
[3]*University of Belgrade, Faculty of Mining and Geology, Mining Department, Djušina Street No. 7, 11000 Belgrade, Serbia*

Abstract

Sustainable use of metallic mineral resources of Serbia, being a part of natural resources, represents the basis for the planning, functioning, development and growth of numerous sectors of the economy as well as the basis for assessing the positions and perspectives for further socio-economic development of the country. Following a two-decade long period of socio-economic regression and economic and financial decline in Serbia, as well as problems in the functioning of institutions and strategic planning and development, the application of concepts for sustainable development is of great importance for the further development of Serbia's mineral sector. Defining the conditions and ways of using metallic mineral resources in the transitional conditions of economic growth and the European integration process represents a delicate geological, mining, technological and economic undertaking, among other things, important for the further functioning of the mineral sector and successful development of the country's mineral economics. Sustainable use of Serbia's metallic mineral resources should enable the implementation of a long-term mineral strategy and mineral policy of sustainable use of mineral resources and is focused on, among other things, increasing the efficiency of mineral resource use on one hand, while decreasing the negative impact of economic utilisation of mineral resources on the environment on the other. The geological and economic analysis in this paper is focused on observing the aspect of Serbia's sustainable economic growth, based on the sustainable use of metallic mineral resources while simultaneously mitigating the negative impact on the environment.

Key words: metallic mineral resources, mineral economics, Serbia, sustainable development

Received: November, 2015; Revised final: June, 2016; Accepted: July, 2016

1. Introduction

Assessing the sustainability of metallic mineral reserves and resources represents the partial achievemnt of Serbia's strategic orientation to implement the concept of sustainable growth. Satisfying the existing needs of numerous sectors of the economy, especially the manufacture of metal goods, followed by the construction, automotive and electric power industries and planned economic growth based on metallic mineral resources, requires an appropriate rate of consumption of various metals from existing reserves. Activities in mineral economics and the mineral sector of Serbia, in the aforementioned period which lasted two decades, were followed by a crisis in the functioning of the country's economy and economics.

The degree of economic development and activity of the country in this time period may best be observed through gross domestic product per capita. According to the Statistical Office of the Republic of Serbia, the value of this macroeconomic indicator, in the years 1995-1999 ranged from 2,012 to 3,342 USD, in the years 2000-2004 ranged from 1,648 to 3,336 USD, in the years 2005-2009 ranged from 3,520 to 6,697 USD and in the years 2010-2014 ranged from 5,400 to 6,351 USD. It is estimated that approximately 2% of Serbia's GDP (Pavlović, 2011)

---

* Author to whom all correspondence should be addressed: E-mail: dasicp58@gmail.com; Phone: +381 60 6926690; Fax: +381 37 692669

belongs to the mineral sector. Structure-wise, approximately 90% of the estimated 2% comprises energy resources including coal, oil and natural gas, followed by copper being a metallic mineral resource. The remaining 10% comprises the production of lead and zinc and non-metallic mineral resources. Instances of socio-economic regression, economic and financial decline as well as problems in the functioning of institutions and strategic planning and development all require the correction of their after-effects for future economic and developmental activity. Because of that, it is why the implementation of the concept for sustainable development in mineral economics and in the mineral sector of Serbia is especially important.

The question of sustainability of metallic mineral resources of Serbia is linked to multiple, partial subquestions (Pactwa et al., 2015; Pengda et al., 2008; Zhong et al., 2013), e.g.: (a) available metallic mineral reserves and resources; (b) the rate of their exploitation and the degree of their exhaustion; (c) discovering new reserves within the metallic mineral resource base; (d) the planning and realisation of new geological explorations; (e) the required investment resources for geological exploration, maintaining current mining facilities and the construction of new ones; (f) obtaining metals from secondary sources through the process of recycling; and (g) the costs of environmental protection and pollution prevention. A particular specificity of metallic mineral resources, which directly affects their sustainable use, is the fact that they are limited, non-renewable and exhaustible. The economic option of sustainability, which is directly constrained in Serbia, refers to the limited possibility of satisfying needs with imported metals according to available mineral types. Imports are conducted in conditions of a relatively high trade deficit, a chronic deficit of financial resources and the need to actuate domestic production, to employ the domestic workforce and to use domestic production resources (Tošović et al., 2009). The starting point for assessing the conditions and methods for sustainable use is the current state of metallic mineral resources management in Serbia.

During the last two decades, the scope and structure of the production of metallic mineral resources in Serbia changed depending on the economic, financial and political conditions in the country (Tošović et al., 2009). These were the conditions which also impacted the scope and structure of the imports and exports of metallic mineral resources. These highly significant indicators of sustainability are part of a larger set of 22 indicators which shall be defined and analysed in a forthcoming discussion on the sustainability of metallic mineral resources.

According to the current scope of exploration and level of knowledge, mineral resources in Serbia possess a different spatial, qualitative, quantitative and regional disposition, which is directly dependent upon the geological setting of the earth, belonging to certain metallogenic units and genetically suitable conditions for formation. However, the determined disposition also depends on the success, efficiency and effectiveness of geological exploration aimed at locating and defining the mineral resources of Serbia quantitatively, qualitatively, geologically and economically (Tošović et al., 2009).

The most significant metallic mineral resources of Serbia are located in the area of the Dinaric, Serbian-Macedonian and Carpatho-Balkan metallogenic province i.e. in their taxonomic subregions: ore zones, areas, districts and fields (Jelenković, 2014). They were created by complex processes of geological structuring and shaping of the terrain and are linked to endogenous processes, especially hydrothermal activity as well as the physical, mechanical and chemical weathering of primary deposits of ore-bearing rocks in exogenous conditions.

The greatest number of metallic mineral deposits within Serbia's territory were formed in the Caledonian, Hercynian and Alpine metallogenic epochs (Jelenković, 2014). Deposits and occurrences of Fe ores were formed during the Caledonian metallogenic epoch, deposits and occurrences of: Fe, Mn, W, Mo, As, Sb, Cu, Au, U ores etc., during the Hercynian epoch. Notable mineralisations formed in the Early Alpine metallogenic epoch are of: Fe, As, Cu, Al, Pb-Zn ores etc. During the Late Alpine metallogenic epoch deposits of: Cu, Pb-Zn, Al, Mo, W, Sb ores were also formed with occurrences and deposits of: Hg, Au, Fe, Mn ores and other metallic mineral resources (Jelenković, 2014).

Metallic mineral resources of Serbia belong to different genetic and morpho-structural types of mineralisations and are characterised by the presence of different mineral parageneses and element associations. Predominant among them, according to amount and economic significance, are hydrothermal deposits (Jelenković, 2014), including the following genetic subtypes: (1) bound to granitoid complexes (U, Pb, Zn); (2) bound to vulcanogenic sheeted dyke complexes (Mn, Cu, Sb, Au); (3) porphyry deposits (Cu, Mo); (4) hydrothermal subvolcanic, impregnation and stockwork-impregnation deposits (Fe, Cu, Pb-Zn, Sb, Au); (5) volcanogenic hydrothermal-sedimentary deposits (Fe, Mn, Cu, Pb-Zn, Au); (6) greisen deposits (Sn, W); (7) skarn/metasomatic deposits in carbonate rocks (Fe, Pb-Zn, Sb) etc. Apart from those, numerous underlying deposits of Cr and Fe ores, segregation deposits of Ni and Cu ores, volcanogenic hydrothermal-sedimentary deposits of Cu and Mn ores, laterite deposits of Ni, part Ni-Fe ores as well, whose genesis is related to ophiolite complexes.

Under current conditions, the solving of the question regarding the sustainability of metallic mineral resources is accomplished by means of a complex analysis of the developmental courses of the global economy (Marker, 2005), in the context of sustainable economy (Bermejo, 2014) and with a special focus on sustainable growth in mineral economics (Auty and Mikesell, 1999; Dubiński, 2013; Krzak, 2012; Szamalek, 2011). Current analyses of sustainable use of metallic mineral resources involve the issues of managing metallic mineral resources (Camus, 2002), the development of mining (Franks, 2015), followed by sustainable managing of mining activities (Botin, 2009; Giurco and Cooper, 2012), as well as future development based on metallic mineral resources (Rankin, 2011).

The main aim of this paper is a to develop a qualitative and quantitative analysis and to define Serbia's metallic mineral resources from the standpoint of a concept for sustainable development, i.e. the conditions and possibilities for their sustainable use, taking into consideration the essential impacts of the domains of the economy, social development and environmental protection. Based on an analytical assessment of the importance of metallic mineral resources for many industrial sectors in Serbia, concerning both economics and the economy, the aim was also to perform their economic classification. The basis for assessing sustainability lies in the fact that metallic mineral resources represent the initial material link in the chain of production. The production process connected to metallic mineral resources starts with geological exploration and exploitation, technical processing and metal production, followed by intermediate goods and, finally, consumer goods with mineral components. Hence, we took into consideration that the sustainability of metallic mineral resources is the basis of economic development and production planning regarding the functioning, development and growth of numerous sectors of the economy which depend upon them (Tošović et al., 2009). Indirectly, this sustainability, when appreciated as part of the pillar of development as one of the three pillars of sustainable growth, represents the basis for observing the perspectives for the further development of the country's society, economics, economy and social security.

The analysis of metallic mineral resources within the concept of sustainability in this paper is based on the aforementioned positive experiences abroad, therefore our elaboration originates from the relationship between the current state of metallic mineral reserves (Milovanović and Tošović, 2006; Ristović, 2008), the mineral economics of the country (Tošović, 2014), modern geo-economic evaluations (Milovanović and Tošović, 2007) which take into account the elements of sustainable development and indicators of sustainability within specific projects. The basic framework when conducting the analysis of the sustainability of metallic mineral resources is generally defined by the National Strategy for Sustainable Use of Natural Resources of Serbia (Tošović et al., 2009).

2. Case study support and methods

Determining the sustainability of Serbia's metallic mineral resources required prior collection of basic data, geological analysis and the defining of the current state of metallic mineral reserves and resources. That was the most extensive part of the work, requiring the collection of data from approximately 2,500 occurrences and deposits of metallic mineral resources discovered so far in Serbia (Jelenković, 2014). Next, the following were carried out, an analysis of a huge number of collected data sorted by type, upon which the method of synthesis for defining the relevant cross section of the state of mineral reserves and resources was subsequently applied. The economic analysis and evaluation were conducted in the part encompassing the assessment of the economic significance and evaluation of the subject reserves of metallic mineral resources. The economic analysis comprises a qualitative and quantitative analysis, a trend analysis and a static and dynamic economic assessment. The data obtained through the qualitative and quatitative economic analysis of the metallic mineral reserves served for the application of the classification method and the distinguishing of economic ore types of Serbia's metallic mineral resources. Based on an elaborate data analysis of the complex structure, concerning geological, mining, technological, economic, environmental and other aspects, a set of sustainable use indicators were defined for each chosen metallic mineral resource (Sadeghi et al., 2015).

In defining the set of indicators of sustainable use the following steps were implemented: (a) defining groups of indicators by defining indicator goals; (b) broadening and defining individual indicators and (c) assessing the state and determining the value of individual indicators. In accordance with the presented characteristics which apply to the defined indicators, the geological, mining, economic (production) and ecological aspects of the sustainable use of metallic mineral resources are described.

Given the relatively low level of detail of the official records and statistics regarding the metallic mineral sector of Serbia, 22 indicators have been defined and encompassed by the main framework with varying degrees of possible representation. In the domain of the classification of mineral resources and reserves (categories of reserves: A, B, C_1, C_2, D_1 i D_2), certain indicators are noteworthy because they are based on current legislation, regulations and terminology which have not been aligned with international practice in this area. The entire set encompasses the following indicators (Tošović et al., 2009): (1) Geological Reserves, (2)Balance Reserves (corresponding to: Economic mineral reserves or Proved + Probable reserves (UNFC-2009, PERC-2008)), (3) Off-balance Reserves (corresponding to: Potential economic reserves or Measured + Indicated resources), (4) Ore Quality, (5) Content of Metal/Components, (6) Reserves of Metal/Components, (7) Exploitation Reserves (Exploitable mineral reserves without loss of exploitation), (8) Industrial Reserves (Mineral reserves usable in industrial processing without loss of processing), (9) Excavated Reserves (Mineral reserves excavated from the beginning of exploitation), (10) Potential Ore Resources, (11) Potential Metal/Component Resources, (12) Current Needs of the Economy, (13) Expected Needs of the Economy, (14) Scope of Exploitation, (15) Scope of Import, (16) Scope of Export, (17) Ore Import/Export Ratio, (18) Preparation Scope, (19) Processing Scope, (20) Industrial Production in Serbia, (21) Scope of Reusability, and (22) Storage and Disposal. The data collection process was particularly problematic for certain indicators of certain metallic mineral resources due to the absence of data.

In the end, a comparative analysis was conducted between the existing metallic mineral reserves and resources, and the current and expected degree of metallic mineral resource use, eventually defining the possible degree of their sustainable use in certain time periods. The trend analysis method was applied to monitor the production, import and export of metallic mineral resources. A regression model for the import and export of metallic mineral resources in Serbia was also created. For the graphical representation of data and analysis polynomial regression models, standard statistical methods were used, shown in papers (Dašić, 2001, 2011, 2012), among which the regression analysis with a 2nd and 5th degree polynomial was particularly significant during analytical processing with the accompanying correlation analysis.

Certain objective obstacles were also present in the exploration and analytical work regarding the assessment of the sustainability of Serbia's metallic mineral reserves and resources, from which the following may be singled out (Tošović, 2014): (a) lack of an official mineral strategy and mineral policy; (b) lack of a complete economic evaluation of Serbia's mineral reserves and resources; (c) different temporal, geological and economic cross sections regarding mineral reserves and resources; (d) limited access and selective availability of data regarding certain categories of metallic mineral resources; and (e) unavailable official data regarding the consumption of certain metallic mineral resources. These limitations, present for certain metallic mineral resources, though being a hindering factor, did not directly impact the overall assessment of the conditions for sustainable metallic mineral resources in Serbia.

3. Results and discussion

3.1. State of production activation, potential and economic types

Based on the geological state analysis of metallic mineral resources and an economic analysis and assessment of economic significance of Serbia's metallic mineral resources, it may be ascertained that they can be divided into five groups according to production activation and potential (Jelenković, 2014) and according to analysis in the study performed by (Kharitonova et al., 2013): (a) deposits in operation with considerable reserves; (b) deposits out of exploitation with considerable reserves; (c) potential deposits with reserves which are not fully defined; (d) expected but undiscovered deposits; (e) exhausted or economically unprofitable deposits.

Metallic mineral resources in exploitation with considerable ore reserves primarily include deposits of Cu and Pb-Zn ores. They are characterised by a markedly low metal content, large reserves and considerable potentials. The most significant deposits from this group are deposits of Cu ores: Majdanpek, Bor, Veliki Krivelj etc.; and deposits of Pb-Zn ores: Stari Trg, Kiževak, Belo Brdo, Rudnik etc. (Jelenković, 2014).

Metallic mineral resources with identified reserves out of exploitation primarily include deposits of Sn, Mn, U, Mo, Ti and W ores. They are characterised by small reserves, markedly limited economic significance, with the possibility of the expansion of the reserves, especially of U ores. The most significant deposits from this group are deposits of Sn ores: Cer and Bukulja; deposits of Mn ores: Laznica, Novo Brdo and Drača; deposits of U ores: Cigankulja, Paun Stena, Mezdreja etc.; deposits of Mo ores: Mačkatica; deposits of W ores: Osanica, Blagojev Kamen and Tanda (Jelenković, 2014).

Potentially significant metallic mineral resources with reserves which have not been fully defined primarily include deposits of Ni, Co, Sb, Al and naturally alloyed Fe ores. The valorising of these mineral resources is conditioned by techno-economic parameters. The basic limiting factor is economic profitability, though there are natural prerequisites for reserve increase. The most significant deposits from this group are deposits of Ni and Co ores: Čikatovo, Glavica etc.; deposits of Sb ores: Zajača district, Bujanovac massif deposits etc.; deposits of Fe ores: Lipovac and Mokra Gora; deposits of Al ores: deposits in Western Serbia (Aluge etc.) (Jelenković, 2014).

Metallic mineral resources, whose deposits can be expected in Serbia, include deposits of Au, Ag, rare and scattered metals ores. Based on the conducted metallogenetic analyses of geological environments, it is realistic to expect new reserves, for which additional exploration is required. The most significant deposits from this group are deposits of Au: Blagojev Kamen, Železnik, Brodica, Majdanpek, Bor-Timok and Lece complex etc.; deposits of Ag within sulphide deposits of Pb-Zn and Cu; deposits of rare and scattered metals ores within deposits of Pb-Zn and Cu ores (Jelenković, 2014).

Mostly exhausted or economically unprofitable metallic mineral resources include deposits of Cr and Fe ores. The prospects for discovering new reserves are low, mainly in deeper levels of peridotite massifs with deposits which are already known. The most significant deposits from this group are deposits of Cr ores: peridotite complexes of Orahovac, Trnava, Lojan etc.; deposits of Fe ores: Duge Njive, Suvo Rudište, oolithic ores of Šumadija etc. (Jelenković, 2014).

Table 1. Partial depiction of sustainable use indicators of Serbia's metallic mineral resources

Ore/Metal Type	***Geological Reserves****	***Balance Reserves****	***Off balance Reserves****	***Prospective Reserves****	***Annual Production***
	Mineral resources**	***Proved + Probable reserves*****	***Measured + Indicated resources*****	***Inferred resources*****	
Cu Ore (Mt)	2,576	1,199	1,377	470	7.8
Pb and Zn Ore (Mt)	105.03	37.48	19.04	46.17	0.272
Fe Ore (Mt)	119.39	3.97	115.42	52.6	-
Cr Ore (Mt)	0.089	0.089	-	0.1	-
Ni and Co Ore (Mt)	38.65	19.92	18.73	8	-
Mo Ore (Mt)	1,115	1,09	25.16	1,645	-
W Ore (Mt)	0.330	-	0.330	0.420	-
Sb Ore (Mt)	4.198	0.978	3.22	3.1	-
Al Ore (Mt)	3.89	2.69	1.2	19.9	-
Hg Ore (Mt)	0.083	-	0.083	0.050	-
U Ore (Mt)	9.241	-	2.603	6.638	-

Note: State of mineral reserves and resources on 31.12.2009, based on the data of the National Strategy for Sustainable Use of Natural Goods and Resources (2012), Official Gazette of the Republic of Serbia, 33/2012

** terms according to: The Book of Regulations on Classification and Categorization of Reserves of Solid Mineral Raw Materials and Keeping a File on Them (issued in former SFR Yugoslavia, 1979)*

*** terms according to: UNFC (2009) - United Nations Framework Classification for Fossil Energy and Mineral Reserves and Resources (UNFC-2009) and PERC code (2008) - Pan European Code for Reporting of Exploration Results, Mineral Resources and Reserves ("The Perc Reporting Code")*

Metallic mineral resources of Serbia include more than 30 metallic mineral resources, 16 of which are economically especially significant and are included in this sustainability analysis in a notable way. Based on geo-economic characteristics, economic assessment and current economic significance, with the aim of providing clarity of the analytic depiction and the accompanying interpretations of sustainable use and sufficiency, metallic mineral resources have been divided into three groups according to economic significance: (a) primary; (b) secondary and (c) tertiary.

Metallic mineral resources of primary economic significance include: Cu, Pb, Zn, Au and Ag. Metallic mineral resources of secondary economic significance include: Fe, Cr, Ni, Co, Mo, W, Sn, Sb, bauxite, Hg, U and technogenic metallic mineral resources. These two groups were especially the subject of analysis taking into account the implementation of the concept of sustainability. Metallic mineral resources of tertiary economic significance include: Hg, As, PGEs, rare elements, rare earths and other metals which were not explored in more detail.

A thorough economic analysis followed by economic evaluation of metallic mineral resources enabled the assessment of the especially significant five primary metallic mineral resources (Cu, Pb, Zn, Au, Ag) from the economic point of view. They have been exploited and economically valorised for a long time, and, taking into account their reserves and mineral potential, they will realistically be strategically important in the following decades. Metallic mineral resources of Serbia of secondary importance possess economic potential i.e. partly defined economic mineral reserves, various qualitative, quantitative and other characteristics, and may potentially be exploited in varying scopes. They partly appear as basic economically profitable components of ores, and partly as accompanying components which are sometimes of secondary economic significance but are important for the overall economic effects of business and the complex utilisation of Serbia's available metallic mineral resources. Metallic mineral resources of Serbia of tertiary importance mainly encompass lower occurrences of mineral raw materials with undefined mineral reserves; therefore, from the standpoint of geology, economics and sustainable growth, they are currently not especially important. There are some however, such as PGE and REE, possessing a certain level of geological potential which needs to be proved via extensive and costly geological exploration. The final result should be properly defined mineral reserves which could, after economic assessment, condition the re-categorisation of these metals into the secondary or primary economic type.

3.2. Indicators of sustainable growth and sustainability/sufficiency of mineral reserves

The set of 22 indicators required for the strategic assessment of the current state, conditions prospects and methods of sustainable use of Serbia's metallic mineral resources has been defined (Tošović et al., 2009), and presented above. It has served as the basis for the assessment of sustainability/sufficiency and its monitoring through time. The developed sustainable growth indicators should secure a quality basis for decision-making at all levels of the country's mineral sector and contribute to the integrated environmental and developmental system's self-regulation, in accordance with Agenda 21 (Tošović and Milovanović, 2007). However, due to non-existent systematised and reliable data for certain metallic mineral resources according to all of the aforementioned indicators, especially as they are scattered throughout various individual sources, many of which are unofficial, interpretations regarding the sustainability of mineral resources are based on key geological indicators (from indicator 1 to indicator 6, partly up to indicator 14). This had a direct effect on scope completeness and the quantitative properties of current interpretations regarding sustainability, especially the lack of a precise numerical depiction of the group of conducted indicators, based on basic de facto unavailable indicators. One of the analytic variants was consideration of the indicator of the geo-economic evaluation as an indicator of sustainability, however, in this analytic phase, due to the difficult collection of a large amount of data and considerably larger analytic scope, it was not implemented. A depiction of the basic indicators deemed most important is given in Table 1.

3.3. Analysis of trends in metallic mineral ores production, imports and exports

The economic analysis of quantitative index data regarding the production of metallic mineral resources in the period 1990-2013, as a significant indicator of sustainability, showed overall stability and improvement of the production trend in the last decade of the included time period (Fig. 1). During the period of economic sanctions and wartime, economic problems reflected on the constrained functioning of the mineral sector and the mineral economy of Serbia, as evidenced by lower production of metallic mineral resources.

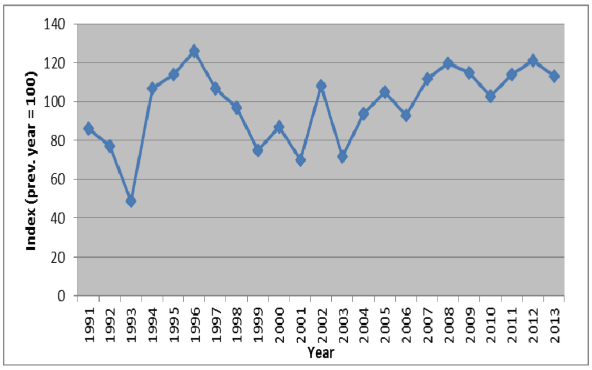


Fig. 1. Trend line of the production of metallic mineral ores in Serbia in the years 1990-2013 (according to the data of the Statistical Yearbook of Serbia 2002-2014)

The economic analysis of the effects of metallic mineral resource production regarding imports/exports illustrates a marked dependence on imports and a disproportion of imported as opposed to exported metallic mineral resources (Fig. 2). Imports are significantly overshadowed by exports in the analysed period including a pronounced growth of imports, especially in the years 2004-2008. That is followed by a period of sharp decline of imports due to economic and financial problems, after which exports increased, coinciding with a gradual increase in production. Metals produced in Serbia, Cu, Pb and Zn, dominate the export structure. Apart from the aforementioned, there are small-scale exports of Al, Fe and steel, all made from imported ores. The majority of metals required for the manufacture of various metal goods are imported and their sustainability has been analysed. Furthermore, the trade deficit is exceedingly high, becoming even worse during periods of crises, due to insufficient financial resources as well as the impeded functioning of consumer goods companies which imported certain metallic mineral resources. In general, the above indicates a mineral and raw material dependence of the Serbian economy on imported metallic mineral resources. This is also significant because of the additional negative impact on the country's trade balance and further economic development within the European integration process.

From the strategic aspect, the analytic conclusions in this paper should serve as the basis for the planning and implementation of short-term, medium-term (5-10 years) and long-term (15-25 years) strategic measures in the mineral sector of Serbia. These measures should not only secure the sustainable use of existing metallic mineral resources but also plan new geological exploration on prospective metallogenic (explored) areas, in order to discover new mineral reserves and halt, change or at least mitigate current trends.

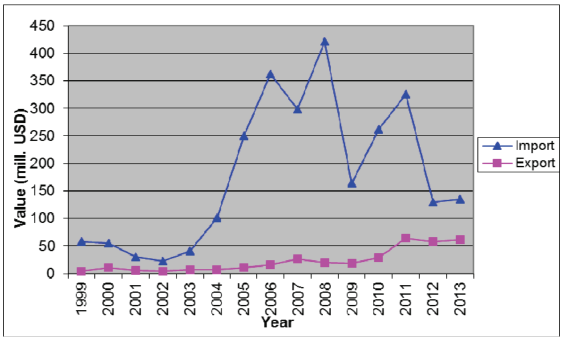


Fig. 2. Import/Export trends of metallic mineral resources in Serbia in the years 1999-2013 (according to the data of the Statistical Yearbook of Serbia 2002-2014)

An approximative export dependence value has been calculated based on the data regarding the export of metallic mineral resources monitored over a certain period of time. The export trends of Serbia's metallic mineral resources in the period 1999-2013 may be approximated using a 2nd degree polynomial (Eq. 1):

$$y = 1.90373 \cdot 10^6 - 1902.1713 \cdot x + 0.47515 \cdot x^2 \qquad (1)$$

with a coefficient of correlation R=0.9466, and a coefficient of determination R^2=0.8961 and an adjusted coefficient of determination AdjR^2=0.8788 (Fig. 3).

The correlation analysis shows that the value of the correlation coefficient is high, meaning that there is a pronounced positive dependence on the export of metallic mineral resources in the defined time period. The determination coefficient's high value indicates that the approximation curve corresponds to the data i.e. the variation in the value of exports is relatively small in relation to the approximation curve.

Despite economic, financial and production constraints on the economy, the calculated approximation indicates a trend of growth in the export of Serbia's metallic mineral resources. Given its positive economic and financial effect, this is a trend that should persist and be maintained in the upcoming period through adequate measures at the macroeconomic level.

An approximative import dependence value has been calculated based on data regarding the import of metallic mineral resources monitored over a certain period of time. The import trends of Serbia's metallic mineral resources in the period 1999-2013 may be approximated using a 5th degree polynomial (Eq. 2):

$$y = -5.0032 \cdot 10^{14} + 1.2482 \cdot 10^{12} \cdot x - 1.2456 \cdot 10^{9} \cdot x^{2} + \\ + 621514.1444 \cdot x^{3} - 155.0549 \cdot x^{4} + 0.01547 \cdot x^{5} \qquad (2)$$

with a coefficient of correlation R=0.8778, and a coefficient of determination R^2=0.7705 and an adjusted coefficient of determination AdjR^2=0.6430 (Fig. 4). The correlation analysis shows that the value of the correlation coefficient is high, meaning that there is a pronounced positive dependence on the import of metallic mineral resources in the defined time period. The determination coefficient's high value indicates that the approximation curve corresponds to the data i.e. the variation in the value of imports is relatively small in relation to the approximation curve.

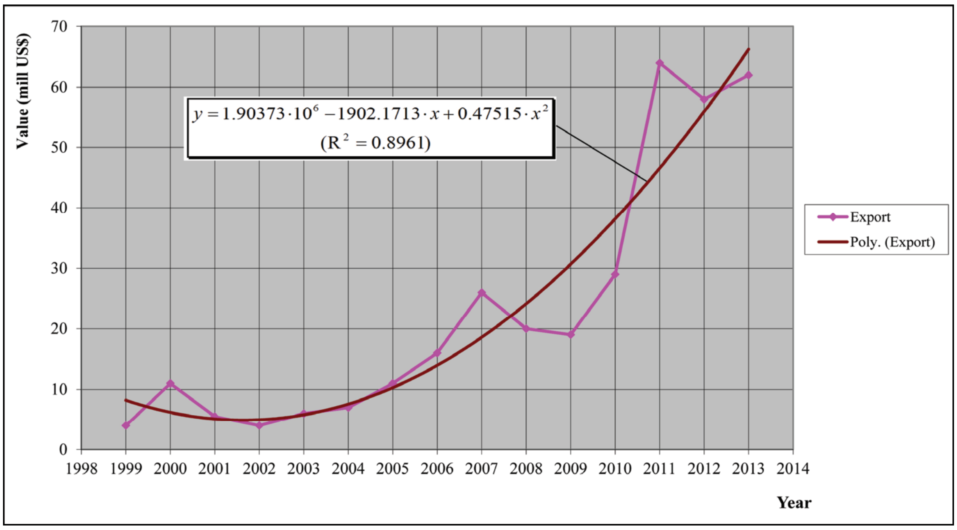


Fig. 3. Approximation of the export trend of Serbia's metallic mineral resources in the period 1999-2013 using a 2nd degree polynomial

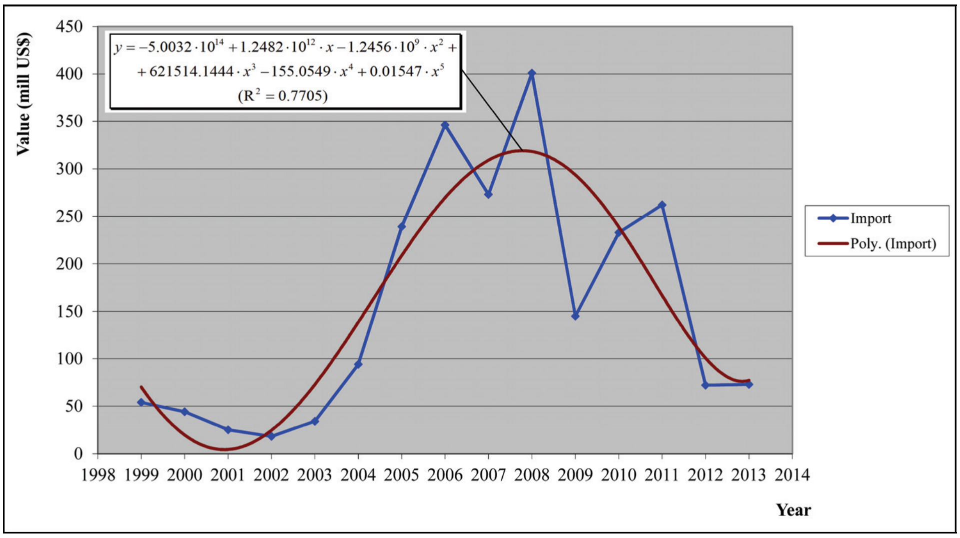


Fig. 4. Approximation of the import trend of Serbia's metallic mineral resources in the period 1999-2013 using a 5th degree polynomial

3.4. Analysis of sustainability of metallic mineral resources in Serbia

Due to a lack of data which are complete, precise and reliable for specific mineral resources regarding average annual consumption in Serbia, data regarding imports/exports, as well as projections of consumption according to economic and development plans, in this phase of the analytic work it was not possible to provide precise quantitative parameters of sustainability/sufficiency of metallic mineral resources for a certain number of years. Therefore, a solution was implemented by assessing, dividing and depicting three classes of sustainability of metallic mineral resources: (a) insufficient (IS); (b) partially sufficient (PS) and (c) sufficient (SF), which is depicted in the final analytic and synthetic expression according to specific metallic mineral resources in Table 2.

In the subject sustainability analysis two groups of metallic mineral resources are singled out, (a) the first group with active mines and existing production (Cu, Au, Pb-Zn) and (b) the second group with no current production (the remaining metals). The sustainability analysis for the first group is based on the proportion between existing mineral reserves and annual production. The sustainability analysis for the second group is based on expert evaluations founded in assessments of potential medium production capacity and existing mineral reserves. Mineral reserves are divided into small, medium and large reserves where the median quality of the mineral raw material has been taken into account. Small reserves of metallic mineral resources belong to the 'insufficient' class of sustainability, medium reserves to the 'partially sufficient' class and those with large reserves to the 'sufficient'. Mineral resources of analysed metallic mineral resources were also taken into account for long-term sustainability.

Certain metals e.g. Ni, Co, Sb, Al and U, of which there are medium reserves and when appreciated from the standpoint of medium-term sustainability, are classified as 'partially sufficient'. When appreciated in the long-term, the same metals, given the scarcity of reserves in the long run, are classified as 'insufficient'. In order to change the class of sustainability it is necessary to conduct new geological explorations and locate new mineral reserves. In the case where reserves are classified as medium both in the medium-term and the long-term e.g. Au, Pb, Zn and Mo, they are likewise classified as 'partially sufficient' in both instances. Cu is the only metal which is classified as 'sufficient' both in the medium-term and the long-term. The remaining metals, including Fe, Cr, W and Hg, are classified as 'insufficient' both in the medium-term and the long-term due to small reserves or low-quality reserves (e.g. Fe).

A case study of sustainability in the temporal dimension was considered for two characteristic periods: (a) 10 years, representing medium-term sustainability and (b) 25 years, representing long-term sustainability. The choice was made in accordance with the potential requirements for strategic planning and the realisation of geological explorations in case there is a lack in sufficient reserves of certain metallic mineral reserves and the fact that geological explorations, on average, last at least 5 years, and from the moment of discovery to the beginning of production, on average, another 5 to 10 years pass.

Mineral reserves of Cu ores are characterised by long-term sustainability and mineral reserves of Au, Pb-Zn and Mo ores by partial sustainability.

Table 2. Serbia's metallic mineral resource sufficiency in the period of 10 and 25 years

Metallic mineral resources	***10 years***			***25 years***		
	Insufficient	***Partially Sufficient***	***Sufficient***	***Insufficient***	***Partially Sufficient***	***Sufficient***
	IN	***PS***	***SF***	***IN***	***PS***	***SF***
Cu			●●●●●			●●●●●
Au		●●●			●●●	
Pb		●●●			●●●	
Zn		●●●			●●●	
Fe	○			○		
Cr	○			○		
Ni		●●●		○		
Co		●●●		○		
Mo		●●●			●●●	
W	○			○		
Sb		●●●		○		
Al		●●●		○		
Hg	○			○		
U		●●●		○		

Except for the aforementioned long-term sustainable metals, Ni, Co, Sb, Al and U are characterised by medium-term partial sustainability. For other metals, including Fe, Cr, W and Hg, medium-term sustainability is insufficient. The stated analytic conclusions regarding the sustainability of metallic mineral resources are significant both for the management of mineral resources, as well as the strategic and operative planning of geological explorations (Xu and Wei, 2004), and finally the defining of objects which shall be the subject of concessions and more significant attraction of foreign investment into the mineral sector and Serbia's mineral economics.

4. Conclusions

The activity of Serbia's metallic mineral sector is very significant both strategically and economically for current business activities of particular companies, activities within certain regions and for the further and all-encompassing development of the country's economy and economics. Serbia's planned economic recovery and growth require an adequate rate of consumption of different metals needed to satisfy the need of various sectors of the economy, among which the manufacture of metal goods is the most significant, followed by the construction, automotive and electric power industries and the production of various consumer goods. In conditions of a relatively high trade deficit, a chronic deficit of financial resources and the need to actuate domestic production, employ the domestic workforce and use domestic resources for production, it is necessary to use available metallic mineral resources. All assessments regarding their use must be carried out in accordance with the concept for sustainable use with the application of the necessary geological, mining, economic, environmental protection and other areas of expertise.

The economic analysis of sustainable use of primary and secondary mineral resources of Serbia encompassed a 10-year medium-term and a 25-year long-term period. It has shown the sustainability of the use of mineral resources of Cu and partial sustainability of the use of the mineral resources of Au, Pb-Zn and Mo, while the state of the rest of the metallic mineral resources are not characterised by sustainability. From the given property of sustainability there is a clear implication that the development of the mineral sector and the growth of mineral economics in the foreseeable future can be based on mineral reserves of Cu ores, and partially on mineral reserves of Au and Pb-Zn ores. Other metals ores require a strategic decision regarding the preparation of a plan for geologic explorations, discovery of adequate metallic mineral reserves and economic evaluation, so as to define the mineral resource base as the foundation for concessions and investments in mineral economics. In this way, the precondition for the successful management of mineral resources would be created, as well as the complete analysis of all three pillars of the sustainability of metallic mineral resources within the realisation of all future economic and development plans in Serbia.

References

Auty R.M., Mikesell R.F., (1999), *Sustainable Development in Mineral Economies*, 1st Edition, Clarendon Press, London.

Bermejo R., (2014), *Handbook for a Sustainable Economy*, Springer, Berlin-Heidelberg-New York.

Botin J.A., (2009), *Sustainable Management of Mining Operations*, Society for Mining, Metallurgy and Exploration, Colorado, USA..

Camus J.P., (2002), *Management of Mineral Resources: Creating Value in the Mining Business*, Society for Mining, Metallurgy and Exploration, Colorado, USA.

Dašić P., (2001), *The Analysis of Possibilities of the Approximation of Machining Process Characteristics by Power Polynomial*, Proc. 16th Int. Conf. on Production Research, vol. 5, Prague, 43.

Dašić P., (2011), Approximation of cutting tool wear function using polynomial regression equation, *Journal of Research and Development in Mechanical Industry*, **3**, 171-180.

Dašić P., (2012), Application of polynomial regression models for approximation of time series, *Journal of Economic and Management Based on New Technologies*, **1**, 81-160.

Dubiński J., (2013), Sustainable development of mining mineral resources, *Journal of Sustainable Mining*, **12**, 1-6.

Franks D., (2015), *Mountain Movers: Mining, Sustainability and the Agents of Change* (*Routledge Studies of the Extractive Industries and Sustainable Development*), Routledge-London-New York.

Giurco D., Cooper C., (2012), Mining and sustainability: Asking the right questions, *Minerals Engineering*, **29**, 3-12.

Jelenković R., (2014), *Exploration of Mineral Resources*, In: *Serbian Mining and Geology in the Second Half of the Twentieth Century* (in Serbian), Vujić S. (Eds.), Academy of Engineering Sciences of Serbia, Matica Srpska, Mining Institute, Belgrade, 145-212.

Krzak M., (2012), Dilemmas of mineral resources use in selected economic theories, *Mineral Resources Management*, **28**, 25-41.

Kharitonova M., Mikhailov A., Matsko N., (2013), Influence of the time factor on the availability of deposits of nonferrous metals, *Resources Policy*, **38**, 490-495.

Marker B.R., (2005), *Sustainable Minerals Operations in the Developing World*, In: *Geological Society of London, Special Publication*, Petterson M.G., McEvoy F., Stephenson M.H. (Eds.), London, 1-4.

Milovanović D., Tošović R., (2006), Metallic mineral resources in the world mineral economy (in Serbian), *Tehnika: Rudarstvo,Geologija i Metalurgija*, **1**, 1-11.

Milovanović D., Tošović R., (2007), Geological-economic aspects of sustainable development in the mineral-raw material complex, *Ecologica*, **14**, 19-26.

Pactwa K., Blachowski J., Górniak-Zimroz J., (2015), GIS as a support tool in regional management of rock mineral resources-Experiences from SW Poland, *Procedia Earth and Planetary Science*, **15**, 488-494.

Pengda Z., Qiuming C., Qinglin X., (2008), Quantitative prediction for deep mineral exploration, *Journal of China University of Geosciences*, **19**, 309-318.

Pavlović V., (2011), *Basis for Design of Management Strategy of Mineral Resources of the Republic of Serbia* (in Serbian), Faculty of Mining and Geology, Belgrade.

Rankin W.J., (2011), *Minerals, Metals and Sustainability: Meeting Future Material Needs*, CSIRO Publishing, Melbourne.

Ristović I., (2008), *The Current State and Development of the Energy Mining in Serbia*, Proc. 2th Int. Symp. of Energy Mining, Tara, vol. 1, 1-10.

Sadeghi B., Madani N., Carranza E.J.M., (2015), Combination of geostatistical simulation and fractal modeling for mineral resource classification, *Journal of Geochemical Exploration*, **149**, 59-73.

SORS, (2002-2014), Statistical Yearbook of Serbia, Years from 2002 to 2014, Statistical Office of the Republic of Serbia, Belgrade, On line at: http://webrzs.stat.gov.rs/WebSite/

Szamalek K., (2011), Rational mineral deposit management in the light of mineral resources theory, *Mineral Resources Management*, **27**, 5-15.

Tošović R., Milovanović D., (2007), Sustainable development indicators of non-metallic mineral complex in Serbia (in Serbian), *Tehnika: Rudarstvo, Geologija i Metalurgija*, **62**, 1-7.

Tošović R., Jelenković R., Seke L., (2009), National strategy for sustainable use of mineral resources as part of natural resources (in Serbian), *Nature Protection*, **60**, 489-499.

Tošović R., (2014), *General Review of Mineral Economy in Modern Conditions of Sustainable Development*, Proc. 15th Symp. on Serbian Geological Congress, Donji Milanovac, vol. 1, 756-761.

Xu W.-X., Wei Y.-M., (2004), Multiple objective-integrated methodology of global optimum decision-making on mineral resources exploitation, *Computers & Industrial Engineering*, **46**, 363-372.

Zhong M.-R., Chen J.-Y., Zhu X.-H., Huang J.-B., (2013), Strategic equilibrium price analysis and numerical simulation of preponderant high-tech metal mineral resources, *Transactions of Nonferrous Metals Society of China*, **23**, 3153-3160.

Environmental Engineering and Management Journal

September 2016, Vol.15, No. 9, 2085-2094
http://omicron.ch.tuiasi.ro/EEMJ/

"Gheorghe Asachi" Technical University of Iasi, Romania

ENVIRONMENTALLY FRIENDLY PHYTOSYNTHESIS OF SILVER-BASED MATERIALS USING *Cornus mas* L. FRUITS

Ioana-Raluca Suica-Bunghez[1,A], Marcela Elisabeta Barbinta-Patrascu[2,A]*, Ovidiu Dumitrescu[3]*, Camelia Ungureanu[3,A], Irina Fierascu[1], Stefan Marian Iordache[4], Rodica-Mariana Ion[1]

[1]National R&D Institute for Chemistry and Petrochemistry – ICECHIM, Bucharest, 060021, Romania
[2]University of Bucharest, Faculty of Physics, Department of Electricity, Solid state Physics and Biophysics, Bucharest-Magurele, 077125, Romania
[3]University Politehnica of Bucharest, Faculty of Applied Chemistry and Materials Science, Polizu Street No 1, Bucharest, 011061, Romania
[4]University of Bucharest, Faculty of Physics, 3Nano-SAE Research Centre, PO Box MG-38, Bucharest-Magurele, 077125, Romania

Abstract

The aim of the present study was to prepare environmentally friendly silver-based materials using *Cornus mas* L. fruits extract. For this purpose, silver nanoparticles (*Cornus mas*-AgNPs) were green synthesized using this plant extract. The bioreduction of silver ions was analyzed by UV–VIS and ATR-FTIR spectroscopic studies. Scanning Electron Microscopy (SEM) analysis revealed the formation of spherical phyto-nanosilver particles with size ranging between 12 and 40 nm. Different amounts of double walled carbon nanotubes (DWCNTs) were mixed with these herbal silver nanoparticles to obtain stable hybrids. The *Cornus mas*-AgNPs-(1 mg) DWCNTs hybrid presented the highest physical stability with zeta potential value of -31.1 mV. The DPPH radical-scavenging assay revealed high antioxidant activity (AA%) values (more than 93%) of all samples. The silver-based materials showed significant antibacterial activity, the most potent biocides being *Cornus*-AgNPs which provides inhibition zones of 23 mm, 20.3 mm and 15.3 mm against *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212) and *Escherichia coli* (ATCC 8738), respectively. The results obtained in this study open the perspective of applying these eco-friendly materials based on phytonanosilver, in the biomedical field (as drug delivery systems carrying bioactive cornelian principles) or in bionanotechnology (as antimicrobial and antioxidant coating).

Key words: antimicrobial activity, antioxidant properties, *Cornus mas* L., environmentally friendly, silver-based material phytosynthesis

Received: September, 2013; Revised final: October, 2014; Accepted: October, 2014

1. Introduction

Building materials using environmentally friendly methods gained a great interest in the last years. Development of green processes in nanotechnology offers the advantages of being rapid, cost-effective and benign to environment, using nontoxic materials (Barbinta-Patrascu et al., 2013, 2016; Kora and Arunachalam, 2012; Malarkodi et al., 2013; Otelea and Rascu, 2015; State et al., 2015; Virkutyte and Varma, 2013). In the last decade, a lot of progresses have been registered in drug delivery system development (Barbaresso et al., 2013; Lacatusu et al., 2012). A lot of research work was focused on nanoparticles as drug carriers. Thus, nanoparticles, the building blocks for interesting

---

[*] Authors to whom all correspondence should be addressed: e-mail: elipatras@gmail.com; Phone: +40730134895; e-mail: ovidiu.dumitrescu@upb.ro; Phone: +40.722.430.189 ; [A]All three authors contributed equally to this work.

nanomaterial construction, are used in many areas (Bao et al., 2013; Jahanshahi and Babaei, 2008), especially in diverse compositions in organic/inorganic materials very effective in drug releasing to target places, resulting in high therapeutic efficiency (Lim et al., 2013).

Many nanostructured hybrid materials based on carbon nanotubes (CNTs), noble metal nanoparticles (AuNPs, PtNPs, AgNPs) or liposomes have been suggested as effective drug delivery systems. Over the past decades, there has been an important interest in developing drug delivery systems using metal nanoparticles as carriers for different types of bio-active molecules (Bao et al., 2013; Jahanshahi and Babaei, 2008). Thus, the silver nanoparticles, AgNPs, have become one of the most important topics in the nanotechnological research area due to their applications in different fields (drug delivery, catalysis, optics, nanobiotechnology, medicine, materials science). The biocidal properties of silver, known from ancient times, were exploited to build materials for antimicrobial purposes (Malarkodi et al., 2013; Podila et al., 2012; Prabhu and Poulose, 2012). The successful production of silver nanoparticles is determined by the ability to synthesize noble metal particles with long stability, given their tendency to aggregate in aqueous media (Rifai et al., 2006). The stability is considered very important for biomedical applications (Korbekandi and Iravani, 2012).

The classical methods of silver nanoparticle synthesis involve many steps and use chemical reducing substances (such as hydrazine, sodium borohydride) and also capping agents (citrate, sodium dodecyl sulfate, and polyvinylpyrrolidone). Because the physical and chemical processes were costly and use energy, high pressure or heating steps, it was necessary to introduce ‘‘green’’ approaches (Dichiarante et al., 2010; Nasrollahzadeh et al., 2014). Marteel-Parrish and Abraham (2014) showed the economic benefits and the benign environmental impact of the green strategies, comparing the traditional chemistry methods with green chemistry approaches.

In the last years, a significant number of papers reported the green synthesis of metal nanoparticles using plant or fruit extracts (Awwad et al., 2013; Barbinta-Patrascu et al., 2013; Kamal et al., 2010; Kiruba et al., 2011; Sathyavathi et al., 2010). In contrast to classical methods, “green” synthesis procedures using plant extracts offers many advantages: low cost, rapidity, simplicity, eco-friendliness and biocompatibility (Baharara et al., 2014; Barbinta Patrascu et al., 2014a, 2014b, 2016; Makarov et al., 2014; Phanjom et al., 2012; State et al., 2015; Vanaja et al., 2013). In this respect, Makarov et al. (2014) emphasized the economical and the environmental advantages of “green” synthesis over traditional methods of metal nanoparticle production.

In this paper, a “green” approach to design silver-based materials using an aqueous *Cornus mas* L. fruit extract is presented. *Cornus mas* L. belonging to the *Cornaceae* family which is a small tree originated from Southern Europe and Southwest Asia, with red fruits used to produce different kinds of alcohol drinks, sweets, gels, jams, compotes. The cornelian cherry fruit extracts are widely used for cosmetic and dermatological purposes, replacing the synthetic astringent substances (Ercisli et al., 2008; Ersoy et al., 2011; West et al., 2012). The cornelian fruits present high antioxidant activity due to their high concentration in polyphenols, ascorbic acid, anthocyanin and carotenoid pigments (Ercisli, 2004; Gulcin et al., 2005; Hashempour et al., 2010; Pantelidis et al., 2007; Rosu et al., 2011; Tesevic et al., 2009; Tural and Koca, 2008). Therefore, these fruits have also antibacterial, antiallergenic, antimicrobial, antimalarial and antihistaminic properties (Ercisli et al., 2008; Pawlowska et al., 2010; Rop et al., 2010; West et al., 2012).

The first goal of our study was to phytosynthesize silver nanoparticles using *Cornus mas* L. fruits extract. This plant material was chosen due to the special properties of the cornelian cherries. The second goal of this work was to design stable cornelian silver nanoparticles/double walled carbon nanotubes hybrid materials. All samples were characterized by spectral (UV-VIS and ATR-FTIR spectroscopy) and microscopy (SEM) methods. The resulting silver-based materials exhibited good antioxidant and antimicrobial properties.

2. Experimental

2.1. Materials

Silver nitrate ($AgNO_3$, 99%) and sodium hydroxide (NaOH, 97%) were supplied by Merck (Germany). 2,2-Diphenyl-1-picryl-hydrazyl-hydrate stable free radical (DPPH) and double walled carbon nanotubes (DWCNTs) were purchased from Sigma-Aldrich. Methanol was procured from Scharlau. All solutions were prepared with bidistilled water in-house production (Double Distiller GFL 2102, Germany). Cornelian fruits were collected from a mountain area on Prahova Valley (Romania).

2.2. Preparation of Cornus mas extract

Cornelian (*Cornus mas* L.) fruits were washed in bidistilled water and dried at 50°C (Fig. 1). An amount of 50 g of these fruits were weighed and transferred into a 500 mL Erlenmeyer flask containing 250 mL of bidistilled water and then boiled for 5 minutes. The extract obtained from aqueous *Cornus* fruits was cooled and then passed through a filter paper in order to obtain a clear red extract.

2.3. Phytosynthesis of cornelian silver nanoparticles

The cornelian silver nanoparticles (*Cornus mas*-AgNPs) were obtained by adding 10 mL of 10^{-3} mol/L $AgNO_3$ aqueous solution over 10 mL of *Cornus mas* fruit extract and the resulting mixture was kept overnight at room temperature.

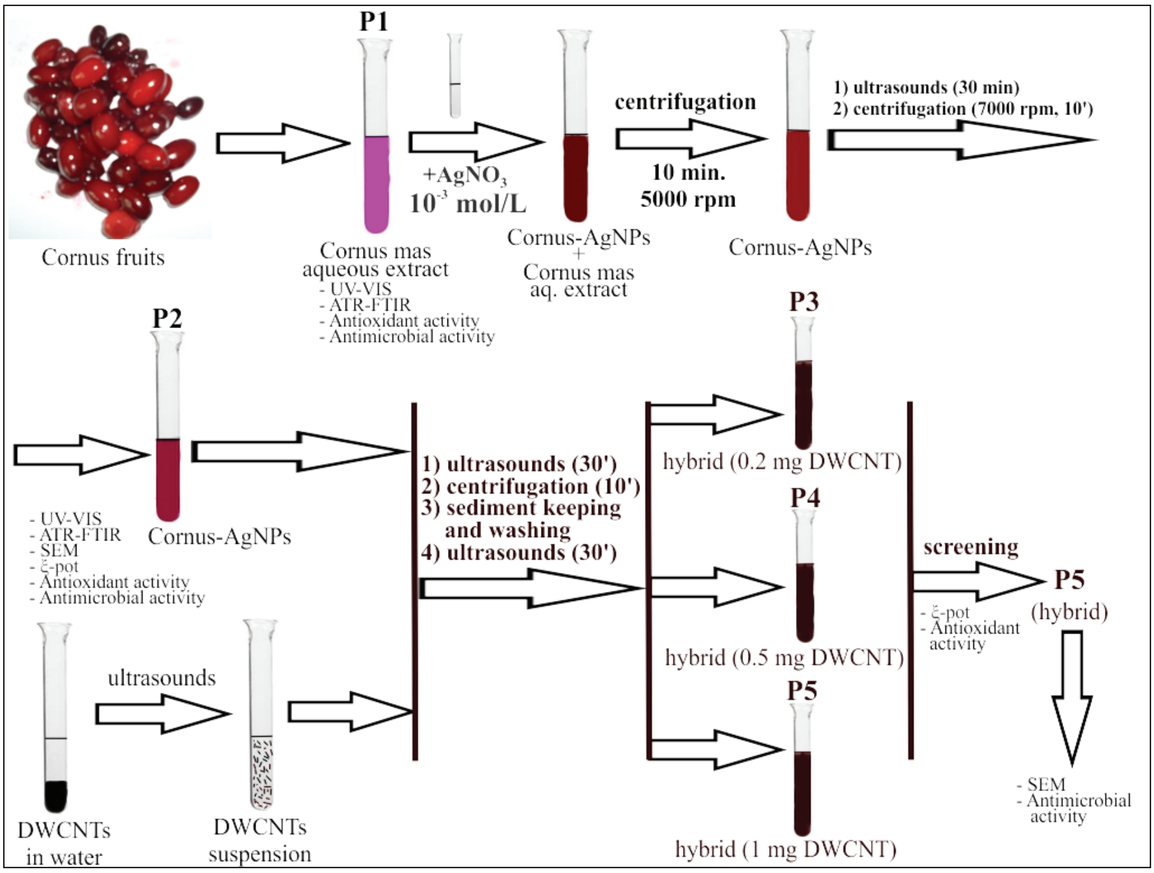


Fig. 1. Schematic representation of silver nanoparticles and hybrids preparation

The colloidal silver nanoparticles were separated from the biomass using centrifugation (10 min, 5000 rpm, SIGMA 2-16 K centrifuge). In order to obtain much smaller silver nanoparticles, the prepared *Cornus mas*-AgNPs were subjected to 30 min ultrasonic irradiation in an ultrasonic bath (Bioblock Scientific). After ultrasound treatment, the samples were centrifuged (10 min, 7000 rpm, SIGMA 2-16 K centrifuge) and the supernatants (which contain a population of AgNPs smaller in size than in sediment) were kept and used in further experiments.

2.4. Preparation of hybrids based on carbon nanotubes and Cornus mas-AgNPs

Different amounts (0.2, 0.5 and 1 mg) of DWCNTs were immersed each in 5 mL bidistilled water and sonicated for 30 minutes in an ultrasonic bath (Fig. 1). To each resulting suspension 10 mL *Cornus mas*-AgNPs and 10 mL NaOH 0.01 mol/L were added. This step was followed by an ultrasound treatment (30 minutes). The resulting suspensions were centrifuged for 10 minutes and each supernatant was discarded and the sediments washed. The centrifugation and washing steps were repeated. The final products were sonicated for 30 min and then dried at 50°C for 1h. The codes of the samples prepared are shown in the Table 1.

Table 1. Sample codes

Sample code	***Description***
P1	*Cornus mas* fruits aqueous extract
P2	*Cornus mas*-AgNPs
P3	*Cornus mas*-AgNPs-(0.2 mg) DWCNTs hybrid
P4	*Cornus mas*-AgNPs-(0.5 mg) DWCNTs hybrid
P5	*Cornus mas*-AgNPs-(1 mg) DWCNTs hybrid

2.5. Characterization methods

2.5.1. Antioxidant activity assay

The antioxidant activity of all the samples was evaluated spectrophotometrically using the DPPH method described in the scientific literature (Ersoy et al., 2011; Mosquera et al., 2009) with minor changes. Each sample was evaluated at 100 mg/L by mixing 0.5 mL of it with 1 mL of 0.02 mg/mL DPPH solution and kept in the dark for 30 minutes, at room temperature. Each mixture was then tested for the DPPH radical-scavenging assay by evaluating the absorbance at 517 nm on a Specord M 400 UV-VIS spectrophotometer. For each sample, the antioxidant activity percentage (*AA%)* was calculated using the expression (1) (Mosquera et al., 2009):

$$AA\% = [(A_{Control} - A_{Sample} / A_{Control}] \times 100 \qquad (1)$$

where: $A_{Control}$ is the absorbance of a DPPH solution without sample (a mixture from 0.5 mL of bidistilled water and 1 mL of 0.02 mg/mL DPPH solution), A_{Sample} is the absorbance of the sample mixed with 0.02 mg/mL DPPH solution. The measurements were performed in triplicate and the mean values of antioxidant activities are presented together with standard deviation (SD).

2.5.2. Zeta potential measurements

The zeta potential (ξ-potential) determinations were carried out on Zetasizer Nano ZS (Malvern Instruments Ltd., U.K.) by applying an electric field across the aqueous dispersions of the samples. These measurements were performed at 25°C temperature, in triplicate.

2.5.3. UV-VIS spectroscopy

A M400 Carl Zeiss Jena double beam UV-VIS spectrophotometer was used to record the absorption spectra of the samples, in the wavelength range of 200-800 nm, at 1 nm resolution and 0.3 nm/s scan rate.

2.5.4. ATR-FTIR spectroscopy

Fourier transform IR (FT-IR) spectra were collected using a Perkin Elmer Spectrum GX spectrometer with Attenuated Total Reflectance (ATR) diamond crystal. Scans in the range of 600–4000 cm^{-1} were acquired for each spectrum at a spectral resolution of 4 cm^{-1}.

2.5.5. Scanning Electron Microscopy (SEM) analysis

SEM analysis has been performed with a Quanta 200 Scanning Electron Microscope (SEM), which produces enlarged images of a variety of specimens, achieving magnifications of over 100000x providing high resolution imaging in a digital format. One drop of each sample was placed on an aluminum support and left to dry overnight. SEM images were recorded using a secondary electron detector.

2.5.6. X-Ray Fluorescence (XRF)

The X-ray fluorescence analysis was performed on a PW4025 – MiniPal – Panalytical type energy dispersive XRF Spectrometer with rhodium anode by using a 3.6 μm Mylar tissue.

2.5.7. Antibacterial assay

The antibacterial activity of the samples was evaluated using the *agar well* diffusion assay against three microbial strains such as *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 8738. The bacterial strains were grown in Luria Bertani Agar (LBA) plates at 37°C with the following medium composition: peptone, 10 g/L; yeast extract 5 g/L, NaCl 5 g/L and agar 20 g/L. The stock culture was maintained at 4°C.

Sterile LBA plates were prepared by pouring the sterilized media in sterile Petri dishes under aseptic conditions. 1 mL of each test organism was spread on agar plates.

For agar well diffusion method (Tyagi and Malik, 2011; Ungureanu and Ferdeş, 2012), a well was prepared in each plate using a cork-borer (6 mm). 50 μL of each sample were introduced into the well. The plates were incubated overnight at 37°C (Laboshake Gerhardt). Microbial growth was determined by size of inhibition zone (IZ, mm). For each bacterial strain, controls were maintained where pure solvents were used instead of samples. The results were obtained by inhibition zone diameter measurement (Fig. 7).

The experiment was performed in triplicate and the mean values are presented as mean ± standard deviation (SD). Standard deviation was calculated as the square root of variance using STDEV function in Office *Excel* 2010 (Microsoft).

3. Results and discussion

3.1. Evaluation of chemical and physical stability of samples. Screening of the most stable carbon-based hybrids

In order to check their antioxidant properties which are related to chemical stability, the samples (*Conus mas* L. fruits extract, *Cornus mas*-AgNPs and *Cornus mas*-AgNPs-DWCNTs hybrids) were subjected to the DPPH radical-scavenging assay. The experimental results revealed that all the samples exhibited antioxidant activity.

The aqueous *Conus mas* extract (sample P1) exhibited strong antioxidant properties (AA = 93.5%) due to the polyphenolic compounds and other bioactive ingredients present in *Cornus mas* L. fruits extract (Isaak et al., 2013; Vareed et al., 2006; Yilmaz et al., 2009). A slight increase in the antioxidant activity value (AA = 94%) was observed in the case of silver nanoparticles (sample P2) synthesized using cornelian cherry fruit extract.

The DPPH radical-scavenging activity was enhanced by increasing the amount of carbon nanotubes in the hybrids (Fig. 2). The antioxidant activity of *Cornus mas*-AgNPs-DWCNTs hybrid (sample P5) reached a value of 94.3%. In recent years, scientific studies revealed the antioxidant properties of carbon nanotubes (Galano, 2008, 2010; Lucente-Schultz et al., 2009). The free-radical scavenging action of CNTs could be explained by their high electron affinity (Fenoglio et al., 2006); the free radicals are trapped by CNTs surface via radical addition to the carbon framework. This explains why carbon nanotubes increased the antioxidant activity of the nano-scaled silver particles.

The physical stability of cornelian silver-based materials was evaluated in terms of ξ-potential. Zeta potential is a physical property essential for the characterization of nanosuspension stability. A minimum of ±30 mV zeta potential value is required for indication of stable suspension (Jacobs et al., 2002; Shameli et al., 2012).

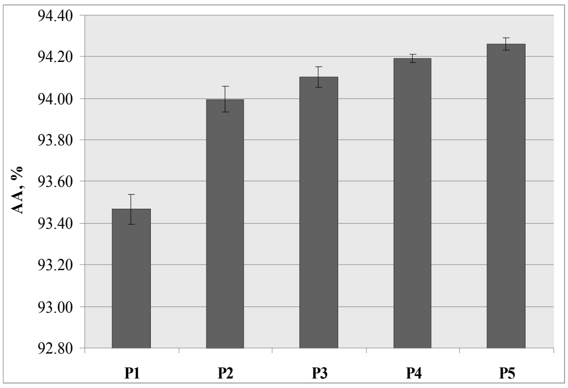


Fig. 2. Antioxidant activity of samples by using the DPPH radical-scavenging assay

High negative or positive zeta potential values indicate strong electrostatic repulsive forces between particles, thus preventing their aggregation (Wissing and Müller, 2002). The *Cornus mas*–AgNPs exhibited a moderate stability in time (ξ = –23.4 mV). The zeta potential value enhanced from –25.4 mV to –31.1 mV as the DWCNT amount increased in silver-based hybrids (Table 2).

Table 2. ξ potential values of the silver-based materials

Sample	***Zeta potential [mV] (mean ± SD)***
P2	–23.4 ± 0.2
P3	–25.3 ± 0.6
P4	–26.4 ± 0.4
P5	–31.1 ± 0.1

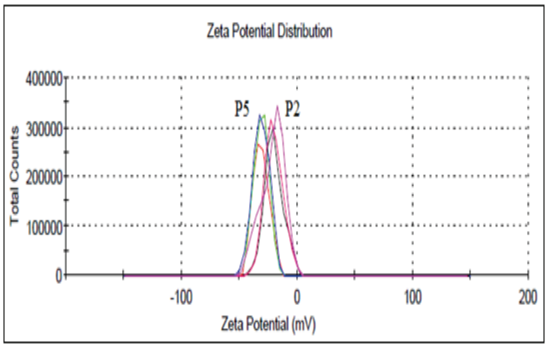


Fig. 3. Comparison of zeta potential distribution of cornelian silver nanoparticles (P2) and *Cornus mas*-AgNPs-(1 mg) DWCNTs hybrid (sample P5)

Fig. 3 represents a comparison of the zeta potential distribution for cornelian silver nanoparticles (sample P2) and *Cornus mas*-AgNPs-(1 mg) DWCNTs hybrid (sample P5). The P5 hybrid exhibited the highest physical stability in time (ξ = –31.1 mV) between all samples. This sample was used in further experiments.

3.2. Spectral characterization of Cornus-AgNPs cornelian AgNPs-(1 mg) DWCNT hybrids

The UV-VIS absorption spectrum of *Cornus mas* aqueous extract (Fig. 4) was compared to the absorption spectrum of *Cornus mas*-AgNPs, *Cornus mas*-AgNPs-(1 mg) DWCNTs hybrids and 10^{-3} mol/L silver nitrate solution.

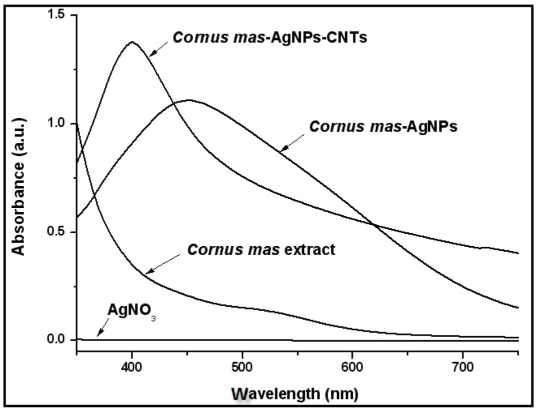


Fig. 4. The UV–VIS absorption spectra of the 1mM $AgNO_3$ solution, *Cornus mas* extract (sample P1), *Cornus mas*-AgNPs (sample P2) and of the *Cornus mas*-AgNPs-DWCNTs hybrid (sample P5)

The bioreduction of silver ions by cornelian extract was confirmed by the appearance of a peak at 450 nm in the spectrum of *Cornus mas*-AgNPs, characteristic for the silver nanoparticle formation (Ramteke et al., 2013; Šileikaitė et al., 2006). This band doesn't appear in the spectrum of the aqueous fruit extract, but in the case of *Cornus mas*-AgNPs-CNTs hybrids, the peak was blue-shifted with 50 nm indicating the interaction of silver nanoparticles with carbon nanotubes resulting in the hybrid material formation.

The presence of a single SPR band suggests that the Ag-NPs were spherical in shape (Vodnik et al., 2008), assumption further confirmed by the SEM results. Fourier transform infrared spectroscopy (FTIR) was used to study the phytosynthesis of cornelian nano-scaled silver particles and to investigate what biocompounds were responsible for the silver ions reduction. The formation of *Cornus mas*-AgNPs-DWCNTs hybrid was also studied.

The ATR-FTIR transmission spectra (Fig. 5) were recorded in the wavenumber region of 600-4000 cm^{-1} in order to obtain good signal/noise ratio. The peaks located at 1720, 1599 and 1237 cm^{-1} in the cornelian extract spectrum, can be assigned to amides, proteins and enzymes which seem to be responsible for the Ag^+ reduction (Podila et al., 2012).

The peak at 1075 cm^{-1} present in the ATR-FTIR spectrum of the cornelian aqueous extract can be assigned to C-O, C-N stretching vibrations of the aliphatic amines or alcohols/phenols (Kora and Arunachalam, 2012), indicating the presence of polyphenols in the *Cornus mas* extract.

The sharp peak at 1385 cm^{-1} (corresponding to asymmetric and symmetric stretching vibrations of the nitrate group) which is present in the spectrum of $AgNO_3$ was weakened after nano-scaled silver particle phytosynthesis.

ATR-FTIR results revealed that phytocompounds responsible for silver bioreduction could be the polyphenols and the proteins which are present in the cornelian aqueous extract; these bioactive compounds presumed to act as reducing and as capping agents for the AgNPs, preventing the agglomeration of the silver nanoparticles. The FTIR spectrum of *Cornus mas*-AgNPs-DWCNTs hybrid (Fig. 5) revealed strong interactions between cornelian silver nanoparticles and carbon nanotubes resulting in hybrid material formation. Thus, some IR bands in the *Cornus mas*-AgNPs spectrum centered at 3401 cm^{-1} (O–H stretching) and 2936 cm^{-1} (alkyls C–H stretching) were shifted to 3422 cm^{-1} and 2923 cm^{-1}, respectively. The peak at around 1630 cm^{-1} in the AgNPs spectrum, assigned to the amide I bonds of proteins (Shivshankar et al., 2003), was shifted to 1632 cm^{-1} with a high transmittance level.

Some ATR-FTIR bands (1727 cm^{-1}, 818 cm^{-1}) of *Cornus mas*-AgNPs FTIR spectrum disappeared, others bands (1076 cm^{-1}) were weakened after interaction with DWCNTs.

The carbonyl groups arising from phytocompounds present in the cornelian extract like: flavonoids, tannins (Hashempour et al., 2010; Pantelidis et al., 2007) were sonochemical reduced in the presence of sodium hydroxide. These phytochemicals could undergo an aldol condensation through a sonochemical route resulting in total or partial loss of carbonyl groups in the final reaction products, fact that explains the disappearance in composite of the bands at 818 cm^{-1} and 1727 cm^{-1} related to the stretching of –C=O (carbonyl) groups (Han et al., 2014; Moulisha et al., 2009). Ultrasound-assisted chemistry used in this study in order to obtain AgNPs/DWCNTs hybrids is a "green" method, associated with some key characteristics: safety, energy saving, waste prevention (Pazdera, 2012).

X-ray fluorescence analysis was performed in order to identify the silver existence in the DWCNTs/*Cornus*-AgNPs hybrid materials. Using this technique, the presence of silver in the samples P2 and P5 was observed (Fig. 6), emphasizing the formation of *Cornus mas*-AgNPs (sample P2) and of *Cornus mas*-AgNPs-DWCNTs hybrid materials based on fruit extracts, silver nanoparticles and DWCNTs. As expected, the control, cornelian aqueous extract used as a reference, doesn't contain silver.

3.3. Characterization of Cornus-AgNPs and Cornus-AgNPs-CNTs by SEM analysis

Scanning Electron Microscopy (SEM) was used to provide useful information about the morphology of the samples.

Thus, the SEM image shown in Fig. 7a displays high density of silver nanoparticles synthesized by an aqueous extract of cornelian fruits (*Cornus*-AgNPs) mainly spherical in shape ranging in size from 12 to 40 nm. The results are in close correlation with the absorption spectra that were predicted the form of AgNPs.

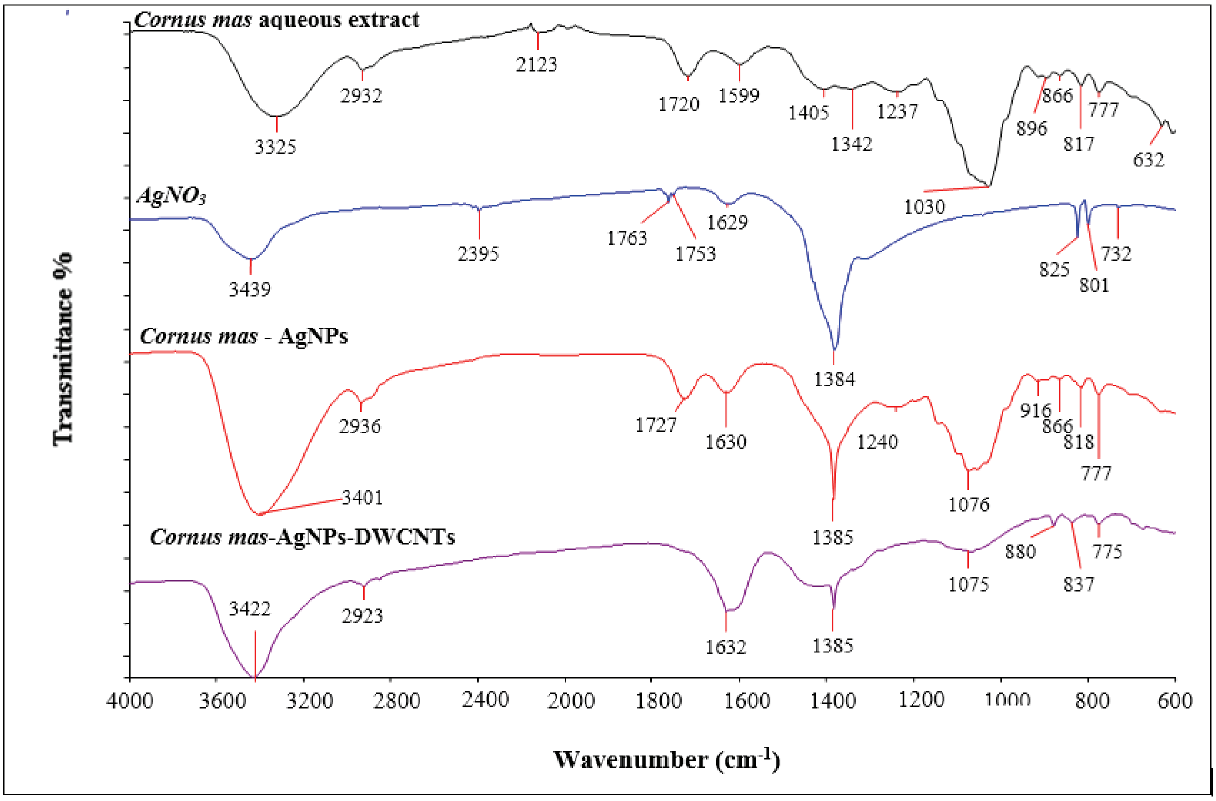


Fig. 5. Comparison of ATR-FTIR spectra of *Cornus mas* fruit aqueous extract, 10^{-3} mol/L AgNO$_3$ aqueous solution, *Cornus mas*-AgNPs and *Cornus mas*-AgNPs-(1 mg) DWCNTs hybrid

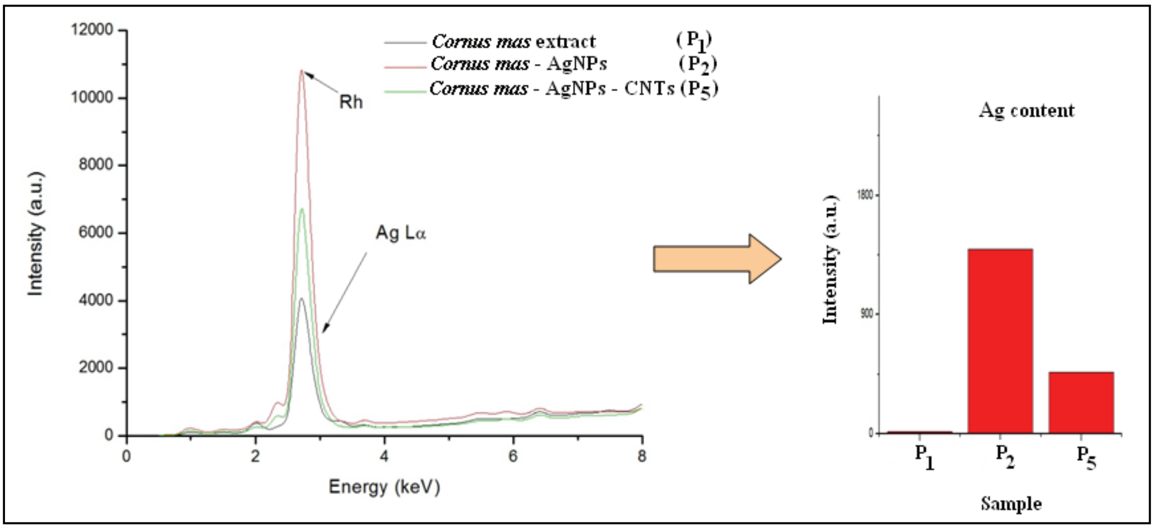
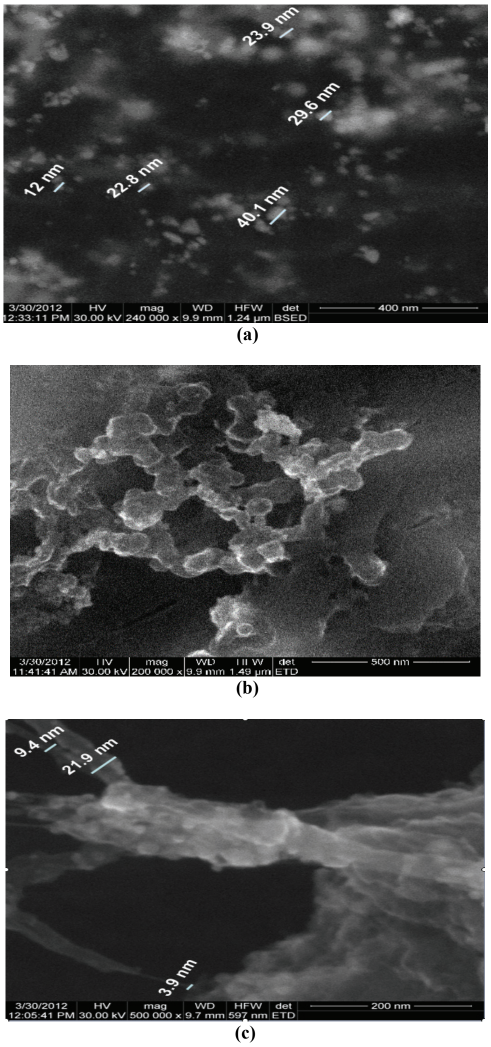


Fig. 6. XRF investigation of silver presence in *Cornus mas*-AgNPs (P2) and *Cornus mas*-AgNPs–DWCNTs hybrids (P5); the absence of silver in the sample P1 (*Cornus mas* fruit extract) used as a reference was observed

Cornus mas L. fruit extract proved to have strong capability as a reducing and capping agent for silver nanoparticle phytosynthesis.



(a)

(b)

(c)

Fig. 7. SEM micrographs of *Cornus*-AgNPs (a) and of *Cornus*-AgNPs-CNTs hybrids (b) and (c)

SEM images of *Cornus*-AgNPs-CNTs hybrids (Figs. 7b and c) revealed carbon nanotubes decorated with cornelian silver nanoparticles. The phytosynthesized nano-scaled silver particles were uniformly deposited on carbon nanotube surface. The successful formation of hybrids based on silver nanoparticles and carbon nanotubes was confirmed by these SEM images.

3.4. Evaluation of antibacterial properties of cornelian samples

The diameters of the growth inhibition zones for all strains studied, ranged between 8.2 to 23.0 mm (Fig. 8). Considering the results of the agar well diffusion method, all of the silver-based materials exhibited high values of antimicrobial activity.

Among treatments, maximum *in vitro* inhibition of tested microorganisms *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212) and *Escherichia coli* (ATCC 8738) was scored in *Cornus*-AgNPs which offered an inhibition zone of 23 mm, 20.3 mm and 15.3 mm respectively (Fig. 8).

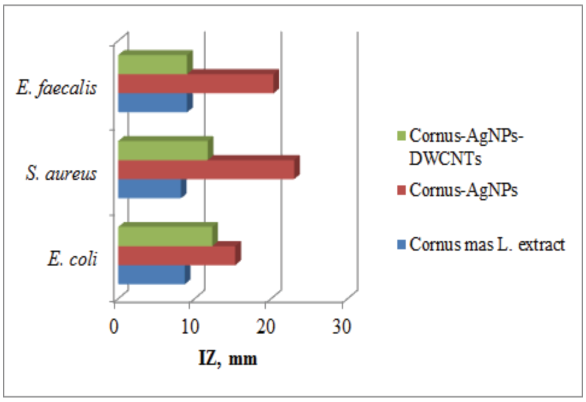


Fig. 8. Comparative inhibition zone diameters of all samples against different bacterial strains

The cornelian aqueous extract presented the same antimicrobial activity against all three tested bacterial strains offering diameters of inhibition zones ranging between 8.2 and 9 mm. Natural products are currently in great demand for research purposes due to the huge and extensive biological properties of medicinal and commercialization values. Hybrids based on carbon nanotubes decorated with small quantity of cornelian silver nanostructures exhibited good antimicrobial activity offering an inhibition zone of 12.3 mm, 11.7 mm and 9.0 mm against *E. coli*, *S. aureus* and *E. faecalis* respectively (Fig. 8).

The phytosynthesized silver nanoparticles using *Cornus mas* extract present many advantages over the AgNPs obtained through classical methods: less time-consuming, safety in biomedical applications, environmental-friendly, antioxidant properties. The "green" silver nanoparticles exhibit Chemical synthesis procedures use toxic chemicals which can be absorbed on the surface of AgNPs making them difficult to use in medical applications (Phanjom et al., 2012).

4. Conclusions

A simple, cost-effective and eco-friendly approach to build stable hybrid materials based on carbon nanotubes and cornelian silver nanoparticles was presented in this work.

Cornus mas L. fruit extract was used to phytosynthesize spherical silver nanoparticles with size ranging between 12 and 40 nm, possessing strong antioxidant (AA = 94%) and antibacterial properties offering inhibition zones of 23 mm, 20.3 mm and 15.3 mm against *Staphylococcus aureus*, *Enterococcus faecalis* and *Escherichia coli*, respectively. The method is simple, rapid, inexpensive and non-toxic.

Cornus mas-AgNPs-(1 mg) DWCNTs hybrid showed good physical stability in time (ξ = -31.1 mV), strong antioxidant properties (AA = 94.3%) and good antimicrobial activity, opening the perspective of their application in the biomedical or biotechnological field.

Acknowledgements

S.-M. Iordache was supported by the strategic grant POSDRU/159/1.5/S/133652, "Integrated system to improve the quality of doctoral and postdoctoral research in Romania and promotion of the role of science in society" cofinanced by the European Social Found within the Sectorial Operational Program Human Resources Development 2007 – 2013.

References

Awwad A.M., Salem N.M., Abdeen A.O., (2013), Green synthesis of silver nanoparticles using carob leaf extract and its antibacterial activity, *International Journal of Industrial Chemistry*, **4**, 1-6.

Baharara J., Namvar F., Ramezani T., Hosseini N., Mohamad R., (2014), Green synthesis of silver nanoparticles using *Achillea biebersteinii* flower extract and its anti-angiogenic properties, *Molecules*, **19**, 4624-4634.

Bao G., Mitragotri S., Tong S., (2013), Multifunctional nanoparticles for drug delivery and molecular imaging, *Annual Review of Biomedical Engineering*, **15**, 253-282.

Barbaresso R., Rau I., Zgarian R., Meghea A., Ghica M.V., (2013), Niflumic acid-collagen delivery systems used as anti-inflammatory and analgesic in dentistry, *Comptes Rendus - Chimie*, **17**, 12-17.

Barbinta-Patrascu M.E., Bunghez I.R., Iordache S.M., Badea N., Fierascu R.C., Ion R.M., (2013), Antioxidant properties of biohybrids based on liposomes and sage silver nanoparticles, *Journal of Nanoscience and Nanotechnology*, **13**, 2051-2060.

Barbinta Patrascu M.E., Ungureanu C., Iordache S.M., Iordache A.M., Bunghez I.R., Ghiurea M., Badea N., Fierascu R.C., Stamatin I., (2014a), Eco-designed biohybrids based on liposomes, mint–nanosilver and carbon nanotubes for antioxidant and antimicrobial coating, *Materials Science and Engineering C*, **39**, 177-185.

Barbinta Patrascu M.E., Ungureanu C., Iordache S.M., Bunghez I.R., Badea N., Rau I., (2014b), Green silver nanobioarchitectures with amplified antioxidant and antimicrobial properties, *Journal of Materials Chemistry B*, **2**, 3221-3231.

Barbinta-Patrascu M.E., Badea N., Ungureanu C., Constantin M., Pirvu C., Rau I., (2016), Silver-based biohybrids "green" synthesized from *Chelidonium majus* L., *Optical Materials*, **56**, 94-99.

Bunghez I.R., Barbinta Patrascu M.E., Badea N., Doncea S. M., Popescu A., Ion R.M., (2012), Antioxidant silver nanoparticles green synthesized using ornamental plants, *Journal of Optoelectronics and Advanced Materials*, **14**, 1016-1022.

Bunghez I.R., Fierascu R.C., Dumitriu I., Ghiurea M., Ion R.M., (2011), Biosynthesis of silver and gold nanoparticles via pigments extracted from *Spinacia oleracea*, *Environmental Engineering and Management Journal*, **10**, 1582-1596.

Dichiarante V., Ravelli D., Albini A., (2010), Green chemistry: state of the art through an analysis of the literature, *Green Chemistry Letters and Reviews*, **3**, 105-113.

Ercisli S., (2004): Cornelian cherry germplasm resources of Turkey, *Journal of Fruit and Ornamental Plant Research*, **12**, 87-92.

Ercisli S., Orhan E., Esitken A., Yildirim N., Agar G., (2008), Relationships among some cornelian cherry genotypes (*Cornus mas* L.) based on RAPD analysis, *Genetic Resources and Crop Evolution*, **55**, 613-618.

Ersoy N., Bagci Y., Gok V., (2011), Antioxidant properties of 12 cornelian cherry fruit types (*Cornus mas* L.) selected from Turkey, *Scientific Research and Essays*, **6**, 98-102.

Gulcin I., Beydemir S., Sat I.G., Küfrevioğlu O.I., (2005), Evaluation of antioxidant activity of cornelian cherry (*Cornus mas* L.), *Acta Alimentaria*, **34**, 193-202.

Han H., Pan D., Wu X., Zhang Q., Zhang H., (2014), Synthesis of graphene/methylene blue/gold nanoparticles composites based on simultaneous green reduction, in situ growth and self-catalysis, *Journal of Materials Science*, **49**, 4796-4806.

Hashempour A., Ghazvini R.F., Bakhshi D., Ghasemnezhad M., Sharafti M., Ahmadian H., (2010), Ascorbic acid, anthocyanins, and phenolics contents and antioxidant activity of Ber, Azarole, Raspberry, and Cornelian

Cherry fruit genotypes growing in Iran, *Horticulture, Environment and Biotechnology*, **51**, 83-88.

Isaak C.K., Petkau J.C., Karmin O., Ominski K., Rodriguez-Lecompte J.C., Siow Y.L., (2013), Seasonal variations in phenolic compounds and antioxidant capacity of *Cornus stolonifera* plant material: Applications in agriculture, *Canadian Journal of Plant Science*, **93**, 725-734.

Jacobs C., Müller R.H., (2002), Production and characterization of a budesonide nanosuspension for pulmonary administration, *Pharmaceutical Research*, **19**, 189-194.

Jahanshahi M., Babaei Z., (2008), Protein nanoparticle: A unique system as drug delivery vehicles, *African Journal of Biotechnology*, **7**, 4926-4934.

Kamal S.S.K., Sahoo P.K., Vimala J., Premkumar M., Ram S., Durai L., (2010), A novel green chemical route for synthesis of silver nanoparticles using *Camellia Sinensis*, *Acta Chimica. Slovenica*, **57**, 808-812.

Kiruba D.S.C.G., Kumar R., Sathish V., Sivakumar M., Sunitha S., Sironmani A.T., (2011), Green synthesis (*Ocimum tenuiflorum*) of silver nanoparticles and toxicity studies in Zebra Fish (*Danio rerio*) model, *International Journal of NanoScience and Nanotechnology*, **2**, 103-117.

Kora A.J., Arunachalam J., (2012), Green fabrication of silver nanoparticles by Gum Tragacanth (*Astragalus gummifer*): A dual functional reductant and stabilizer, *Journal of Nanomaterials*, **2012**, ID 869765, Doi:10.1155/2012/869765.

Korbekandi H., Iravani S., (2012), *Silver Nanoparticles*, In: *The Delivery of Nanoparticles*, Hashim A.A. (Ed.), InTech, Rijeka, Croatia, 3-36.

Lacatusu I., Badea N., Oprea O., Bojin D., Meghea A., (2012), Highly antioxidant carotene-lipid nanocarriers: synthesis and antibacterial activity, *Journal of Nanoparticle Research*, **14**, 902-918.

Lim E.K., Jang E., Lee K., Haam S., Huh Y.M., (2013), Delivery of cancer therapeutics using nanotechnology, *Pharmaceutics*, **5**, 294-317.

Makarov V.V., Love A.J., Sinitsyna O.V., Makarova S.S., Yaminsky I.V., Taliansky M.E., Kalinina N.O., (2014), "Green" nanotechnologies: synthesis of metal nanoparticles using plants, *Acta Naturae*, **6**, 35-44.

Malarkodi C., Rajeshkumar S., Vanaja M., Paulkumar K., Gnanajobitha G., Annadurai G., (2013), Eco-friendly synthesis and characterization of gold nanoparticles using *Klebsiella pneumoniae*, *Journal of Nanostructure in Chemistry*, **3**, 1-7.

Marteel-Parrish A.E., Abraham M.A., (2014), *Green Chemistry and Engineering, a Pathway to Sustainability*, John Wiley & Sons, Inc., Hoboken, New Jersey.

Mosquera O.M., Correra Y.M., Nino J., (2009), Antioxidant activity of plant extracts from Colombian flora, *Brazilian Journal of Pharmacognosy*, **19**, 382-387.

Moulisha B., Bikash M.N., Partha P., Kumar A.G., Sukdeb B., Kanti H.P., (2009), *In vitro* anti-leishmanial and anti-tumour activities of a pentacyclic triterpenoid compound isolated from the fruits of *Dregea volubilis* Benth *Asclepiadaceae*, *Tropical Journal of Pharmaceutical Research*, **8**, 127-131.

Nasrollahzadeh M., Babaei F., Sajadi S.M., Ehsani A., (2014), Green synthesis, optical properties and catalytic activity of silver nanoparticles in the synthesis of N-monosubstituted ureas in water, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **132**, 423-429.

Otelea M., Rascu A., (2015), Genomics and proteomics techniques in nanoparticles studies – new approach in environmental research, *Environmental Engineering and Management Journal*, **14**, 2283-2291.

Phanjom P., Sultana A., Sarma H., Ramchiary J., Goswami K., Baishya P., (2012), Plant-mediated synthesis of silver nanoparticles using *Elaeagnus latifolia* leaf extract, *Digest Journal of Nanomaterials and Biostructures*, **7**, 1117-1123.

Pantelidis G.E., Vasilakakis M., Manganaris G.A., Diamantidis G., (2007), Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and cornelian cherries, *Food Chemistry*, **102**, 777-783.

Pawlowska A.M., Camangi F., Braca A., (2010), Quali-quantitative analysis of flavonoids of *Cornus mas* L. (Cornaceae) fruits, *Food Chemistry*, **119**, 1257-1261.

Pazdera P., (2012), *Emerging Ubiquity of Green Chemistry in Engineering and Technology*, Chen D., Sharma S.K., Mudhoo A. (Eds.), In: *Handbook on Applications of Ultrasound: Sonochemistry for Sustainability*, CRC Press, Taylor & Francis Group, US, 1-22.

Podila R., Chen R., Ke P.C., Brown J.M., Rao A.M., (2012), Effects of surface functional groups on the formation of nanoparticle-protein corona, *Applied Physics Letters*, **101**, 1-26.

Prabhu S., Poulose E.K., (2012), Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications and toxicity effects, *International Nano Letters*, **2**, 1-10.

Ramteke C., Chakrabarti T., Sarangi B.K., Pandey R.A, (2013), Synthesis of silver nanoparticles from the aqueous extract of leaves of *Ocimum sanctum* for enhanced antibacterial activity, *Journal of Chemistry*, **2013**, ID 278925, Doi: 10.1155/2013/278925.

Rifai S., Breen C.A., Solis D.J., Swager T.M., (2006), Facile *in situ* silver nanoparticle formation in insulating porous polymer matrices, *Chemistry of Materials*, **18**, 21-25.

Rop O., Mlcek J., Kramarova D., Jurikova T., (2010), Selected cultivars of cornelian cherry (*Cornus mas* L.) as a new food source for human nutrition, *African Journal of Biotechnology*, **9**, 1205-1210.

Rosu C.M., Olteanu Z., Truta E., Ciornea E., Mânzu C., Zamfirache M.M., (2011), Nutritional value of *Rosa Spp*. L. and *Cornus mas* L. fruits, as affected by storage conditions, *Scientific Annals of „Alexandru Ioan Cuza" University*, **XII**, 147-155.

Sathyavathi R., Krishna B.M., Rao V.S., Saritha R., Rao N., (2010), Biosynthesis of silver nanoparticles using *Coriandrum Sativum* leaf extract and their application in nonlinear optics, *Advanced Science Letters*, **3**, 1-6.

Shameli K., Ahmad M.B., Jazayeri S.D., Shabanzadeh P., Jahangirian H., Mahdavi M., Abdullahi Y., (2012), Synthesis and characterization of polyethylene glycol mediated silver nanoparticles by the green method, *International Journal of Molecular Science*, **13**, 6639-6650.

Shivshankar S., Ahmad A., Sastry M., (2003), Geranium leaf assisted biosynthesis of silver nanoparticles, *Biotechnology Progress*, **19**, 1627-1631.

Šileikaitė A., Prosyčevas I., Puišo J., Juraitis A., Guobienė A., (2006), Analysis of silver nanoparticles produced by chemical reduction of silver salt solution, *Materials Science*, **12**, 287-291.

Soare L.C., Ferdes M., Stefanov S., Denkova Z., Nicolova R., Denev P., Ungureanu C., (2012), Antioxidant and antimicrobial properties of some plant extracts, *Journals of Chemistry*, **63**, 432-434.

State R., Papa F., Dobrescu G., Munteanu C., Atkinson I., Balint I., Volceanov A., (2015), Green synthesis and characterization of gold nanoparticles obtained by a direct reduction method and their fractal dimension, *Environmental Engineering and Management Journal*, **14**, 587-593.

Tesevic V., Nikicevic N., Milosavljevic S., Bajic D, Vajs V., Vuckovic I., Vujisic L., Dordevic I., Stankovic M., Velickovic M., (2009), Characterization of volatile compounds of "Drenja", an alcoholic beverage obtained from the fruits of cornelian cherry, *Journal of the Serbian Chemical Society*, **74**, 117-128.

Tural S., Koca I., (2008), Physico-chemical and antioxidant properties of cornelian cherry fruits (*Cornus mas* L.) grown in Turkey, *Scientia Horticulturae*, **116**, 362-366.

Tyagi A.K., Malik A., (2011), Antimicrobial potential and chemical composition of *Mentha piperita* oil in liquid and vapour phase against food spoiling microorganisms, *Food Control*, **22**, 1707-1714.

Vanaja M., Gnanajobitha G., Paulkumar K., Rajeshkumar S., Malarkodi C., Annadurai G., (2013), Phytosynthesis of silver nanoparticles by *Cissus quadrangularis*: influence of physicochemical factors, *Journal of Nanostructure in Chemistry*, **3**, 1-8.

Vareed S.K., Reddy M.K., Schutzki R.E., Nair M.G., (2006), Anthocyanins in *Cornus alternifolia*, *Cornus controversa*, *Cornus kousa* and *Cornus florida* fruits with health benefits, *Life Sciences*, **78**, 777-784.

Virkutyte J., Varma R.S., (2013), *Green Synthesis of Nanomaterials: Environmental Aspects*, In: *Sustainable Nanotechnology and the Environment: Advances and Achievements*, ACS Symposium Series, vol. 1124, Shamim N., Sharma V.K. (Eds.), American Chemical Society, USA, 11-39.

Vodnik V.V., Božanić D.K., Bibić N., Šaponjić Z.V., Nedeljković J.M., (2008), Optical properties of shaped silver nanoparticles, *Journal of Nanoscience and Nanotechnology*, **8**, 3511-3515.

West B.J., Deng S., Jensen C.J., Palu A.K., Berrio L.F., (2012), Antioxidant, toxicity, and iridoid tests of processed Cornelian cherry fruits, *International Journal of Food Science & Technology*, **47**, 1392-1397.

Wissing S.A., Müller R.H., (2002), Solid lipid nanoparticles as carrier for sunscreens: *in vitro* release and *in vivo* skin penetration, *Journal of Controlled Release*, **81**, 225-233.

Environmental Engineering and Management Journal
September 2016, Vol.15, No. 9, 2095-2107
http://omicron.ch.tuiasi.ro/EEMJ/

"Gheorghe Asachi" Technical University of Iasi, Romania

THE EFFECT OF Si CONTENT ON FERRIHYDRITE SORPTION CAPACITY FOR Pb(II), Cu(II), Cr(VI), AND P(V)

Gabriela Pieczara, Grzegorz Rzepa*

AGH University of Science and Technology, Faculty of Geology, Geophysics and Environmental Protection,
Department of Mineralogy, Petrography and Geochemistry, al. Mickiewicza 30, 30-059 Krakow, Poland

Abstract

In this study, the impact of silicate admixture in 2-line ferrihydrite on its sorption properties is presented. Synthetic ferrihydrites with broad Si content in the range of 0 to 1.5 Si/Fe molar ratios were prepared and characterized using X-ray diffractometry (XRD), Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM-EDS). With an increase in the Si/Fe ratio, a typical XRD peak at 2.5Å broadens and shifts toward 3 Å; the position of Si-O stretching band in FTIR spectrum shifts from 930 to 1003 cm^{-1}; the pH$_{pzc}$ value strongly decreases from 8.41 to 1.99. The surface area, equal to 280 m^2/g for pure ferrihydrite, increases to 332 m^2/g for the lowest Si/Fe molar ratio but decreases to 245 m^2/g for the highest Si/Fe molar ratio. Si-bearing ferrihydrites exhibit higher cation sorption capacities and lower anion sorption capacities compared to Si-free oxyhydroxide. Regarding sorption behavior, Si-ferrihydrites were divided into two groups: low-Si ferrihydrites (LSFs) with Si/Fe molar ratios up to 0.2 and high-Si ferrihydrites (HSFs) with Si/Fe molar ratios above 0.2. These two groups reveal completely different surface chemistry. Langmuir isotherms fit better to the experimental results of anion adsorption onto pure ferrihydrite and LSFs, while the anion adsorption on HSFs fits the Freundlich isotherm in a better manner. The opposite results from cation adsorption experiments: data fit the Freundlich isotherm better for LSFs but the Langmuir model is usually preferred for cation sorption on HSFs. Si-ferrihydrite appears to be an effective adsorbent, for instance in water treatment, thanks to the enhanced cation sorption efficiency and higher stability compared to those of pure ferrihydrite.

Key words: adsorption, iron oxyhydroxides, silicate, surface chemistry

Received: November, 2015; Revised final: May, 2016; Accepted: July, 2016

1. Introduction

Ferrihydrite is an extremely poorly-crystalline iron oxyhydroxide, ubiquitous in many near-surface environments, where it plays an important role in the biogeochemical cycling of various major and trace elements (Guo and Barnard, 2013; Hasselöv and von der Kammer, 2008; Hemmingsson and Chi Fru, 2015; Jambor and Dutrizac, 1998; Waychunas et al., 2005). Since its discovery in 1973, ferrihydrite has been extensively studied due to its environmental significance. These studies have focused on the properties which are important from the environmental point of view such as solubility, thermodynamic features, surface chemistry, adsorption properties and catalytic activity (Cornell and Schwertmann, 2003). In a majority of these studies, synthetic ferrihydrites of chemical composition close to ideal have been used (Antelo et al., 2010; Carbante et al., 2010; Manceau, 1995; Wang et al., 2013; Yu et al., 2002). However, natural ferrihydrite virtually always contains numerous impurities, including silicate, phosphate, arsenate, sulfate, organic matter, calcium, aluminum, manganese, and various trace elements (Carlson and Schwertmann, 1981; Cismasu et al., 2011, 2013; Filip et al., 2007; Senn et al., 2015; Wang X. et al., 2015; Zhu et al., 2014), which strongly affect the oxyhydroxide surface chemistry, reactivity, physical properties, transformation pathways etc. (Cornell and

---

[*] Author to whom all correspondence should be addressed: e-mail: gprzepa@cyf-kr.edu.pl; Phone: (4812) 617-21-48; Fax: (4812) 633-43-30

Schwertmann, 2003; Guo and Barnard, 2013; Paige et al., 1997; Pokrovski et al., 2003; Senn et al., 2015). Probably the most common admixture is silicate, which was shown to decrease ferrihydrite crystallinity, hamper its transformation to stable phases (i.e., goethite and/or hematite), and modify morphology, magnetic properties, and so on (Campbell et al., 2002; Cismasu et al., 2014; Dyer et al., 2010, 2012; Francisco et al., 2016; Glasauer et al., 2000; Kukkapudu et al., 2004; Parfitt et al., 1992; Rzepa et al., 2016; Vempati and Loeppert, 1989).

A large number of studies have attempted to characterize the sorption of various metals and oxyanions on ferrihydrite using a variety of methods (Antelo et al., 2015; Arai and Sparks, 2001; Brinza et al., 2008; Neupane et al., 2014; Raven et al., 1998; Rout et al., 2012; Tzou et al., 2003; Wang et al., 2013; Zhu et al., 2014). However, studies that take into account the influence of impurities on ferrihydrite sorption properties are still scarce (Adra et al., 2016; Anderson and Benjamin, 1985; Cismasu et al., 2013; Johnston and Chrysochoou, 2016; Swedlund and Webster, 1999). Therefore, the survey dealing with sorption properties of Si-ferrihydrites would have shed new light on this issue, and therefore on inorganic weathering processes and biochemical cycling of many major and trace elements. It would have also provided important insights into pollution transfer and transformations in near-surface environments. Environmental engineering implications might be important as well. Since Si-ferrihydrite is much more stable (Cornell and Schwertmann, 2003; Rzepa et al., 2016), it would not transform into goethite and/or hematite (on dewatering, aging, etc.) as easily as pure ferrihydrite. The transformation is undesirable because goethite and hematite reveal better crystallinity, hence lower surface area than ferrihydrite. A previous study (Zeng, 2003) showed that although granulated "Fe(III)-Si binary oxide" (i.e., Si-ferrihydrite) possessed reduced arsenic adsorption capacity, it was much more resistant to disintegration in water than Si-free oxyhydroxide. Therefore, as Si-ferrihydrite appears to be an interesting adsorbent, more data on its sorption properties are needed prior to potential application in, for example, water treatment.

The main objective of this study was to establish anion and cation sorption capacities of the Si-ferrihydrite series with increasing Si/Fe molar ratios from 0.00 to 1.50. For this purpose, batch sorption experiments on the uptake of lead(II), copper(II), phosphate(V), and chromate(VI) from aqueous solution were carried out. These ions are environmentally important and were previously found to have strong affinities to iron oxyhydroxides. Adsorption onto these minerals, especially ferrihydrite, is regarded as one of the most effective methods of immobilization of harmful pollutants. To assess the effect of Si content in ferrihydrite on the extent of the ions removal, sorption isotherms were generated. To characterize all the sorbents and to compare the differences in their features that may influence the sorption properties, comprehensive mineralogical analyses as well as specific surface area and zeta potential measurements were carried out.

2. Experimental

2.1. Materials and analytical methods

Ferrihydrite samples having different Si/Fe molar ratios: 0.00, 0.05, 0.10, 0.20, 0.50, 0.75, 1.00, and 1.50 (referred below as FHYD-XXX, where XXX denotes the Si/Fe ratio), were synthesized according to the method described in Vempati et al. (1990) and characterized using X-ray diffractometry (XRD), scanning electron microscopy (SEM-EDS), and diffuse reflectance Fourier-transform infrared spectroscopy (DRIFT). All the samples were obtained by simultaneous adding to polypropylene bottles containing 0.1 dm^3 of distilled water, 0.25 dm^3 0.35 M of $Fe_2(SO_4)_3$ and 0.15 dm^3 $Na_2SiO_3 \cdot 5H_2O$ with required concentration under constant stirring. The suspensions pH was adjusted to 8.2 by dropwise adding of 1 M NaOH. The suspensions were further stirred for 12 h with a magnetic stirrer. The precipitates were aged for four days at room temperature, dialyzed to remove an excess of salt until a conductivity of 2-5 μS/cm was achieved, and finally freeze dried. Freeze-drying was applied because dehydration of ferrihydrite during air-drying or oven-drying usually leads to the formation of aggregates which are hardly redispersable in water. In such preparation, a loose powder or soft aggregates are obtained. Therefore, prior to sorption experiments the samples were only gently ground in an agate mortar. All reagents and solutions were prepared and stored in polypropylene vessels to prevent any Si contamination.

Powder X-ray diffraction analysis was carried out using a Philips ADP PW 3020 X'Pert diffractometer equipped with Cu anode (35 kV and 30 mA generator settings) and graphite monochromator. The XRD patterns were recorded in the 2–73°2θ range with 0.05°2θ step and counting time of 1 second per step.

The micromorphology of the precipitates was characterized using a FEI Quanta 200 field emission scanning electron microscope (SEM) operated at 15 kV in low vacuum. For elemental analysis the energy dispersive X-ray spectroscopy (EDS) was applied. All synthetic samples were analyzed without coating.

The infrared spectra were collected using diffuse reflectance technique (DRIFT) on Thermo Scientific Nicolet 7600 spectrophotometer in the range 4000-400 cm^{-1} at a resolution of 4 cm^{-1}. The samples were mixed with KBr prior to analysis.

The specific surface area (SSA) was measured using the Brunauer-Emmet-Teller (BET) method. The N_2 adsorption/desorption experiments were performed with a Nova 1200e Quantachrome instrument. Prior to analysis, the samples were outgassed for 20 h at 363 K. These experimental conditions were used to avoid alteration of the samples (Weidler, 1997).

Zeta potentials were measured using a Zetasizer Nano ZS Malvern instrument at room temperature. Samples were dispersed in 0.1 M KNO_3 to maintain constant ionic strength. Solid-to-solution ratio was 2 g/dm^3. As titrants, 0.1 M and 0.01 M HNO_3 were used.

The initial and equilibrium concentrations of anions were determined using UV-Vis Hitachi U-1800 spectrophotometer. Due to the possible interference between silica and phosphate (Neal et al., 2002), the standard ammonium molybdate method (blue complex method, Lenoble et al., 2003) was used for low range of P(V) concentrations while the vanadomolybdophosphoric method was used at a higher concentration range (yellow complex method, APHA, 1997). We have tested both methods for the interference with silicate. The results indicate that the yellow complex method is less sensitive to Si interference than the blue complex method, particularly in the presence of high-Si ferrihydrite. Cr(VI) concentrations were analyzed by the diphenylcarbazide method (APHA, 1997). The concentrations of Pb(II) and Cu(II) were determined using the GBC SavantAA absorption atomic spectrometer. Calculated LOD values (mg/dm^3) were as follows: P(V) – 0.02, Cr(VI) – 0.006, Cu(II) – 0.09, and Pb(II) – 0.24. RSD values were < 2% and < 5% for UV-Vis and AAS measurements, respectively.

2.2. Adsorption studies

Batch sorption experiments were carried out in single sorbate systems at room temperature and suspension concentration of 20 g/dm^3, and these conditions were applied in many previous studies (e.g. Koteja and Matusik, 2015; Matusik, 2014; Matusik and Bajda, 2013; Rzepa et al., 2009). An aliquot of 100 ±1 mg of ferrihydrite was placed in a polypropylene centrifuge tube and 5 cm^3 of solution containing dissolved $Pb(NO_3)_2$, $Cu(NO_3)_2$, $K_2Cr_2O_7$, or K_2HPO_4 was added. Initial concentrations were as follows: 0.24–13.5 mM of Pb(II); 0.51–18.9 mM of Cu(II); 0.05–18.4 mM of Cr(VI); and 0.09–22.0 mM of P(V). In the instance of P(V) and Cr(VI), the results are expressed as elements, but as all the concentrations are molar, these are equal to phosphate(V) and chromate(VI), respectively. The initial pH was adjusted to 4.5 in Pb(II) and Cu(II) solutions, 5.0 in Cr(VI) and 6.5 in P(V) solution, using KOH and HNO_3. Phreeqc modeling revealed that in such pH regime, the dominant species were as follows: $PbNO_3^+$, $CuNO_3^+$, $HCrO_4^-$, and $H_2PO_4^-$/HPO_4^{2-}. The suspensions were shaken for 24 h and then centrifuged for 20 min at 14,000 rpm. The sorbate-sorbent reaction time of 24 hours was chosen because: (i) it has been often applied in adsorption studies on various materials (e.g. Bajda and Kłapyta, 2013; Matusik, 2013), (ii) the results of earlier works indicate that the time is sufficient to reach equilibrium in the case of iron (oxyhydr)oxide materials (e.g. Mohapatra et al., 2010; Rout et al., 2012; Rzepa et al., 2009), and (iii) the results of preliminary experiments revealed that the equilibrium was achieved after 24 hours.

The pH and equilibrium concentration of ions were determined in the supernatants. The adsorbed amounts of ions were estimated as a difference between the initial and the equilibrium concentrations. Sorption efficiency (S_e) and capacity (S) were calculated using the following equations (Eqs. 1-2):

$$S_e = \frac{C_0 - C_{eq}}{C_0} \cdot 100\% \qquad (1)$$

and

$$S = \frac{C_0 - C_{eq}}{m} \cdot V \qquad (2)$$

where C_0 and C_{eq} are initial and equilibrium concentrations (mM), respectively, m is the weight of the sorbent (kg), and V is the sample volume (dm^3). The experiments were carried out in duplicates and the mean values are reported below. RSD of the repeated analyses were < 5% in most cases.

2.3. Adsorption isotherm models

The adsorption data were fitted using Langmuir and Freundlich isotherm equations. The linear form of the Langmuir isotherm is given as (Eq. 3):

$$\frac{C_{eq}}{q} = \frac{1}{q_{\max} \cdot K_L} + \frac{C_{eq}}{q_{\max}} \qquad (3)$$

where C_{eq} is the concentration of an ion at equilibrium (mM), q is the amount of sorbate adsorbed per unit weight of adsorbent (mmol/kg), q_{max} (mmol/kg) is the maximum monolayer adsorption capacity, and K_L is the Langmuir constant related to the adsorption energy (dm^3/mmol). K_L enables the calculation of the dimensionless separation factor (Eq. 4) (Foo and Hameed, 2010):

$$R_L = \frac{1}{1 + K_L \cdot C_0} \qquad (4)$$

where C_0 (mM) is the highest initial ion concentration. It is a measure of adsorption favorability: the adsorption is favorable if $0 < R_L < 1$ and unfavorable if $R_L > 1$.

The linear form of the Freundlich isotherm is given as (Eq. 5):

$$\log q = \log K_F + \frac{1}{n} \log C_{eq} \qquad (5)$$

where K_F (dm^3/kg) and $1/n$ are Freundlich constants related to the sorption capacity and sorption affinity of the sorbent, respectively.

The *n* constant indicates the favorability of adsorption, and when its value exceeds unity, a favorable nature of adsorption is expected. The Langmuir isotherm describes homogeneous monolayer adsorption with all adsorption sites possessing the same affinity for the adsorbate (Kyzioł-Komosińska et al., 2014), while the Freundlich model shows adsorption-complexation reaction taking place during the adsorption process, which could be monolayer and the adsorbent surface may be heterogeneous in nature. The Freundlich isotherm does not reach a plateau as C_{eq} increases (Limousin et al., 2007).

3. Results and discussion

3.1. Characteristics of synthetic ferrihydrites

SEM observations of freshly synthesized ferrihydrite indicate that in all cases the synthesis results in homogeneous precipitate (Fig. 1). Si-free ferrihydrite forms fine spherical aggregates with very smooth surface devoid of any crystalline features. The aggregation increases with the Si/Fe molar ratio.

Elemental SEM/EDS analyses of the ferrihydrites indicate that the actual Si/Fe molar ratios are close to theoretical values. Additionally, small amounts of sulfur have been detected in pure ferrihydrite and low-Si samples (up to Si/Fe = 0.2), while the samples with Si/Fe ratios ≥ 0.5 exhibit sodium impurities. These impurities resulted from incomplete removal during dialysis and did not affect the results of the sorption experiments significantly.

The XRD patterns obtained from all ferrihydrite samples are presented in Fig. 2.

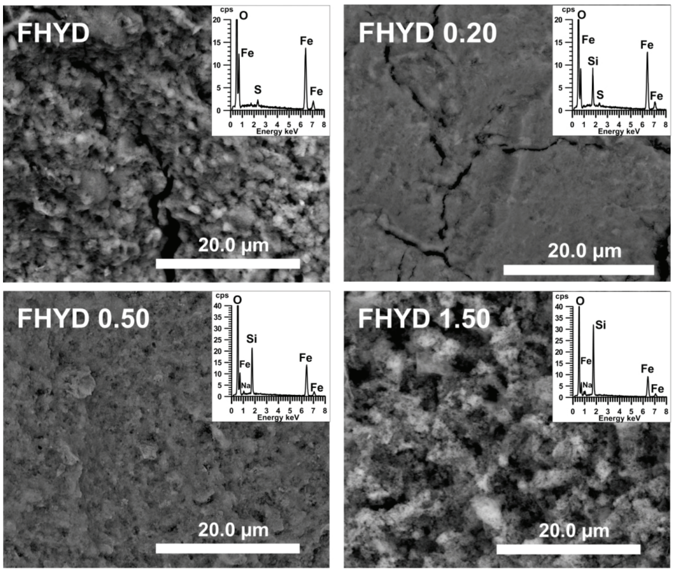


Fig. 1. Secondary electron images and EDS spectra of selected ferrihydrite samples

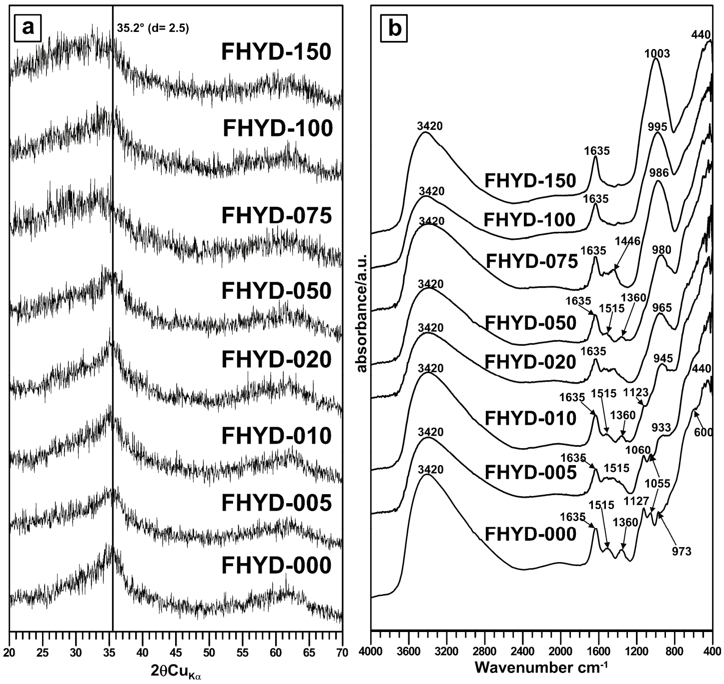


Fig. 2. X-ray diffraction patterns (a) and FTIR spectra (b) of the ferrihydrite sample

Two broad asymmetric peaks at 2.5 Å and 1.5 Å are apparent for FHYD-000, which is typical for 2-line ferrihydrite (Cornell and Schwertmann, 2003). With the increase in the Si/Fe molar ratio, the first peak gradually broadens, becomes less asymmetric, and shifts toward the lower two-theta angles (from 35 to 29° 2Θ, which corresponds to an increase in d spacing from 2.5 up to 3 Å). A similar shift resulting from Si presence has been reported previously for natural (Carlson and Schwertmann, 1981; Cismasu et al., 2011) and synthetic (Dyer et al., 2010; Kaegi et al., 2010; Seehra et al., 2004) Si-ferrihydrites.

In the FTIR spectrum of FHYD-000 sample (Fig. 2), typical ferrihydrite broad bands attributable to Fe-O stretching vibrations are visible at 440 cm^{-1} and 600 cm^{-1} (Russell and Fraser, 1994). The presence of adsorbed or lattice water is reflected by the bands at 1635 cm^{-1} and 3420 cm^{-1}. The bands at 1360 cm^{-1} and 1515 cm^{-1} were identified as a bending mode of Fe-OH near the surface of ferrihydrite nanoparticles (Seehra et al., 2004). The weak features at 973 cm^{-1}, 1060 cm^{-1}, and 1127 cm^{-1} are attributed to the binuclear-bridging surface complex between sulfate and ferrihydrite surface (Vempati et al., 1990). For the samples of Si/Fe ≥ 0.10, the ~600 cm^{-1} shoulder is hardly recognizable and sulfate peaks disappear. The intensity of the 930 cm^{-1} band (Si-O stretching) increases with an increase in the Si/Fe molar ratio. Simultaneously, the position of this band is shifted toward higher wavenumbers, reaching 1003 cm^{-1} for FHYD-150. Similar features were reported in previous studies (Vempati et al., 1990; Wang X. et al., 2015). The shift of the Si-O stretching band is associated with siloxane linkages between adjacent orthosilicate molecules adsorbed at the surface of ferrihydrite (Hansen et al., 1994).

The results of SSA measurements are consistent with those of previous studies (Cismasu et al., 2011; Dyer at al., 2010; Filip et al., 2007; Jambor and Dutrizac, 1998; Schwertmann at al., 2004). The surface area of FHYD-000 sample is lower (280 m^2/g) compared to low-Si ferrihydrites (Fig. 3, Table 1).

Table. 1. Specific surface areas and pH_{pzc} values of ferrihydrite series

Sample	***Surface area [m²/g]***	***pHpzc***
FHYD 0.00	280.29 ± 0.59	8.41 ± 0.13
FHYD 0.05	331.60 ± 1.14	n.d.
FHYD 0.10	323.51 ± 0.64	5.42 ± 0.21
FHYD 0.20	331.32 ± 0.61	3.15 ± 0.06
FHYD 0.50	309.73 ± 0.65	2.67 ± 0.06
FHYD 0.75	311.57 ± 0.66	2.62 ± 0.14
FHYD 1.00	245.24 ± 0.65	2.18 ± 0.09
FHYD 1.50	245.21 ± 1.40	1.99 ± 0.04

However, the SSA does not correlate linearly with an increase in the Si/Fe molar ratio. A sample having the lowest Si content (FHYD-005) reveals the highest SSA, while FHYD-150 with the highest Si concentration has the lowest SSA. The measured SSA is distinctly higher (323-332 m^2/g) for low-Si ferrihydrites (Si/Fe=0.05-0.2) and the lowest (245 m^2/g) for samples FHYD-100 and FHYD-150. BET measurements of the samples FHYD-050 and FHYD-075 revealed intermediate values (309 and 311 m^2/g, respectively). Therefore, small amounts of Si increase the SSA of ferrihydrite and this phenomenon can be explained by a decline in the oxyhydroxide crystallinity (Schwertmann et al., 2004). On the other hand, high contents of Si result in decrease of SSA (Fig. 3). This is related to the aggregation of ferrihydrite nanoparticles due to the grouping of adsorbed silicate species, formation of siloxane bridges, and further polymerization on the surface (Dyer al., 2012; Swedlund and Webster, 1999). According to Cismasu et al. (2014), at high Si contents, the ferrihydrite nanoparticles are possibly embedded in a Fe-bearing siliceous matrix. Hence, the precipitate aggregates are more compact, which impedes the penetration of nitrogen molecules during BET measurements. However, technical factors might play a role here-prior to SSA measurements, the samples are outgassed. Since it is possible that high-Si samples bind water more strongly, some of the water molecules might remain on the surface leading to partial inaccessibility of the surface to N_2 and therefore to the underestimation of the SSA (Wang et al., 2013).

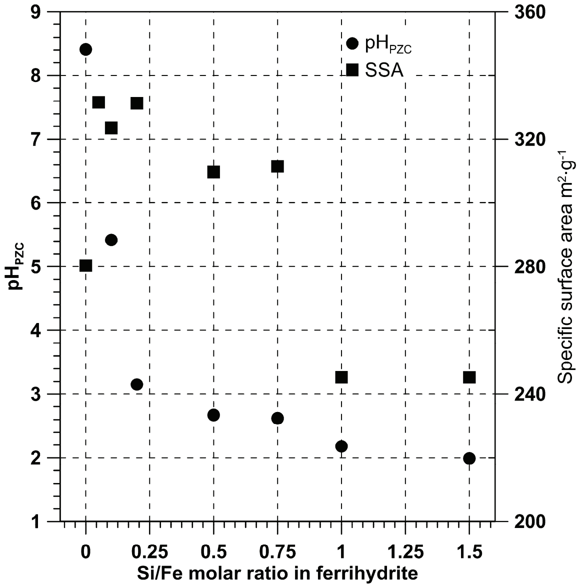


Fig. 3. Specific surface area and pH_{pzc} values of Si-ferrihydrites

FHYD-000 sample has pH_{pzc} at 8.41. This value is close to the one previously reported for synthetic ferrihydrite (Antelo et al., 2015; Brinza et al., 2008; Rout et al., 2012), but higher when compared to the value of natural ferrihydrites (Schwertmann and Fechter, 1982). This is because the natural oxyhydroxide always contains admixtures such as silica, phosphate, or organic matter, resulting in a drop in the pH_{pzc} value. It can be seen that the increasing Si/Fe molar ratio in ferrihydrite results in a significant lowering of the pH_{pzc} values (Fig. 3). Even in relatively low-Si material (FHYD-010) pH_{pzc} drops to 5.42. A similar trend was observed previously (Anderson and Benjamin, 1985; Schwertmann and Fechter, 1982). The differences in the pH_{pzc} can be explained by the partial displacement of Fe-OH groups on the ferrihydrite surface, which are proton acceptors, by silanol groups (Si-OH), which are proton donors (Schwertmann and Fechter, 1982). Si adsorbed onto ferrihydrite particles forms a negative surface complex and thus increases the negative surface charge. Therefore, a further increase in Si content causes further decrease in pH_{pzc}, down to 3.15 for FHYD-020 and to 2.67 for FHYD-050. The pH_{pzc} of the highest Si-ferrihydrite (FHYD-150) amounts to 1.99, a value typical for pure SiO_2 (Kosmulski, 2011). This probably reflects the final stage of the polymerization process of silica onto ferrihydrite surface, as proposed by Dyer et al. (2012).

3.2. Adsorption of cations

Because among the studied ferrihydrite series different samples revealed distinct differences in sorption properties, when discussing the results below, three groups of the samples are referred to. The groups are as follows: pure (i.e., Si-free) ferrihydrite (PF, sample FHYD-000), low-Si ferrihydrites (LSFs, samples FHYD-005, FHYD-010, and FHYD-020), and high-Si ferrihydrites (HSFs, samples FHYD-050, FHYD-075, FHYD-100, and FHYD-150). The results of sorption experiments are interpreted with respect to the Si content and final pH (Figs. 4-9). Both Pb and Cu adsorption appears to be favored by increasing the Si content in ferrihydrite (Figs. 4, 5).

The highest amount of Pb(II) was sorbed by HSFs (in the range of 282-616 mmol Pb/kg), lower amounts by LSFs (in the range of 166-199 mmol Pb/kg), and the lowest amount by PF (145 mmol Pb/kg). An almost linear relationship was observed between the Si admixture in ferrihydrite and its Pb sorption capacity (Fig. 8). The adsorption capacity of PF is comparable with that expressed in previous studies (e.g. Zhu et al., 2010). Relatively similar relations were found for Cu(II), with maximum uptake by HSFs (280-589 mmol Cu/kg), slightly lower by LSFs (139-189 mmol Cu/kg), and the lowest by PF (131 mmol Cu/kg). Cu is sorbed by PF in lower amounts than Pb, which is consistent with previous works (Zhu et al., 2010) despite higher than previously observed adsorption capacity. Again, Cu adsorption increases almost linearly with increasing Si/Fe ratio (Fig. 8). Therefore, the addition of Si systematically enhances ferrihydrite cation adsorption capacity.

The HSF samples remove almost 100% of aqueous Pb(II) at the initial concentrations below 6.9 mM Pb(II) (FHYD-050 and FHYD-075), below 11.6 mM Pb(II) (FHYD-100), and below 13.5 mM Pb(II) (FHYD-150). At the highest Pb(II) concentrations (13.5 mM), sorption efficiency of FHYD-050, FHYD-075, FHYD-100, and FHYD-150, reached 42%, 47%, 65%, and 91%, respectively (Fig. 4). Surprisingly, in low-concentrated solutions, all the HSFs exhibit a slight increase in Pb(II) removal efficiency with the increase in initial Pb(II) concentration.

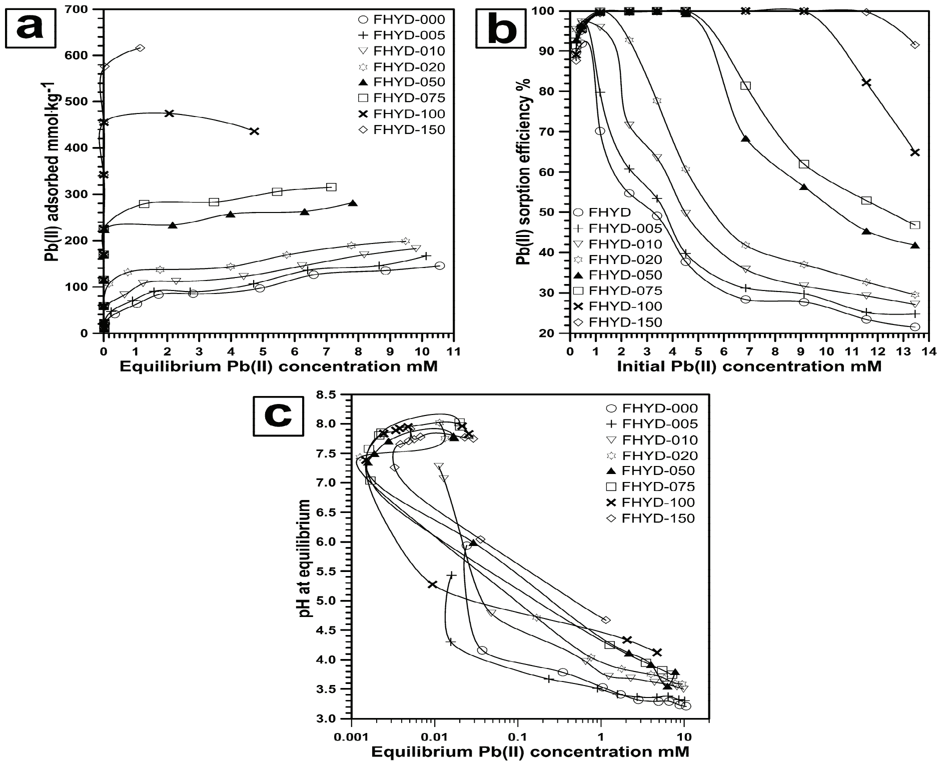


Fig. 4. Results of Pb(II) sorption experiments: sorption capacities (a), sorption efficiencies (b) and final pH values (c)

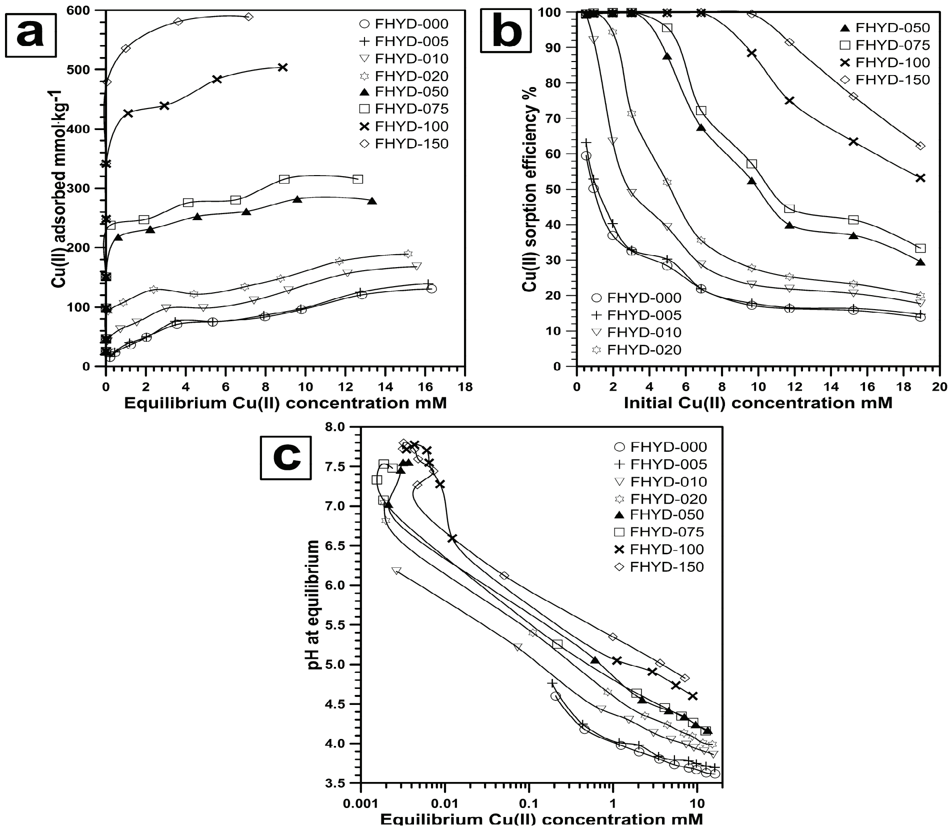


Fig. 5. Results of Cu(II) sorption experiments

In Cu(II) experiments, 95-100% removal of Cu was achieved for HSFs at the initial concentrations below 5.0 mM Cu(II) (FHYD-050 and FHYD-075), below 6.9 mM Cu(II) (FHYD-100), and below 11.7 mM Cu(II) (FHYD-150). At the highest Cu(II) concentration (19.0 mM), sorption effectiveness in the range of 30–62% was attained (Fig. 5).

LSF samples show lower cation sorption effectiveness than HSF samples (Figs. 4, 5). The removal of Pb(II) is distinctly more efficient (above 90%) at an initial concentration below 1.2 mM Pb (II) (FHYD-005) and below 2.3 mM Pb(II) (FHYD-010 and FHYD-020) than for the maximum metal concentration in solution. In this case, only approximately 25-30% of the initial Pb content is bound by the sorbent. The highest sorption effectiveness of Cu(II) (above 90%) was attained for Si-low samples at initial concentrations below 0.9 mM Cu(II) (FHYD-010) and below 2.0 mM Cu(II) (FHYD-020). At the maximum Cu concentration, sorption effectiveness did not exceed 20% (Fig. 5).

PF exhibits much lower affinity to cations than Si-ferrihydrites. At the lowest initial concentration (0.5 mM) of Pb(II) or Cu(II) the sorption effectiveness reaches 92% and 60%, respectively. At the highest metal concentrations, only 22% of initial Pb(II) and 14% of initial Cu(II) is immobilized (Figs. 4, 5).

Sorption of Pb(II) and Cu(II) is correlated also with the final pH of the solution (Figs. 4, 5, 9). The maximum amount (90-100%) of cations is sorbed at final pH in the range of 5.0-8.0. In more acidic solutions (final pH < 5), the amount of cations removed gradually decreases for all equilibrium concentrations of the adsorbate. High effectiveness of cation removal at high pH suggests that the adsorption may be accompanied by the precipitation of metal hydroxides (Matei et al., 2015). A coprecipitation of Pb and Cu with iron oxyhydroxide nanoparticles at pH above 6.5 and 5.5, respectively, was reported by Lu et al. (2011). A fine precipitate of Pb-containing particles was encountered in SEM images of selected HSFs after the experiments with maximum Pb(II) concentrations of 14.5 mM. This could also explain lower sorption efficiency of PF than those of Si-ferrihydrites: final pH in this case (Figs. 4, 5, 9) is in the range of 6.0-3.2 for Pb(II) and 4.6-3.6 for Cu(II). At this pH range, which is far below the point of zero charge (pH_{pzc}= 8.41), cation adsorption decreases with a decrease in pH due to strong competition with protons and to electrostatic repulsion between positively charged ferrihydrite surface and cationic species (Rout et al., 2012). In contrast, final pH in all the experiments with Si-ferrihydrites is above their points of zero charge: pH ranges from 4.5 for LSFs to 2.4 for HSFs (Figs. 4, 5, 9). Therefore, in such a pH regime, Si-ferrihydrites exhibit a strong affinity for cations. However, sorption efficiency of HSFs increases slightly with decreasing pH in lower concentrated Pb(II) solutions: this indicates that the mechanisms of Pb(II) removal are probably more complex.

An additional effect of pH can be related to the solubility of ferrihydrite. Increased solubility of PF by protonation-induced dissolution at pH below 4 may result in the release of Pb(II) and Cu(II) and iron to the solution (Schwertmann, 1991). On the other hand, pH above 7 may increase the possibility of decomposition of HSFs. The concentration of Fe was measured in selected supernatants resulting from cation adsorption experiments with PF and LSFs (which exhibit a relatively low final pH) as well as experiments with HSFs (relatively high final pH). Fe concentrations in PF solutions were generally low but increased slightly with the decrease in pH from 6.0 to 3.0, indicating the increase in solubility. A similar trend was observed for LSFs, although the concentrations of iron were lower. In contrast, in the case of HSFs, Fe levels decreased with the decrease in pH. These observations confirm that solubility of PF and LSFs increases in acidic solutions, while HSFs are more soluble in alkaline conditions. This might explain, at least partially, lower uptake of cations by PF, as well as complex behavior of HSFs-Pb(II) system at lower Pb concentrations.

Irrespective of the details of the process, the results presented above indicate that ferrihydrite containing even relatively small amounts of Si exhibits a distinctly higher cation sorption capacity than PF.

3.3. Adsorption of anions

Phosphate sorption correlates well with Si content in ferrihydrite (Figs. 6, 8). The highest amount of P(V), comparable to other studies (e.g. Liu et al., 2016; Wang H. et al., 2015), was sorbed by PF (875 mmol/kg), lower amounts by LSFs (in the range of 876-662 mmol/kg), and the lowest by HSFs (in the range of 468-178 mmol/kg). A similar relation has been found for Cr(VI): the maximum uptake was recorded for PF (655 mmol/kg), slightly lower for LSFs (in the range of 585-332 mmol/kg), and the lowest for HSFs (in the range of 154-36 mmol/kg). Therefore, the presence of Si impedes anion adsorption on ferrihydrite, which is consistent with the findings of previous works (Anderson and Benjamin, 1985; Zachara et al., 1987).

The uptake of P(V) and Cr(VI) from the solution by PF is higher than 93% for almost the entire range of anion concentrations (Figs. 6, 7). Sorption effectiveness decreases below 80% for initial concentrations of P(V) above 17.4 mM (Fig. 6) and below 71% for initial concentrations of Cr(VI) above 13.1 mM (Fig. 7).

All Si-ferrihydrites show lower affinity toward anions than PF. LSFs sorbed 90-100% of P(V) for the initial concentrations up to 5 mM, and in the case of FHYD-005 and FHYD-010 samples up to 8.7 mM (Fig. 6). Sorption effectiveness of HSFs is distinctly lower – 42-58% for FHYD-050, 22-39% for FHYD-075, 15-30% for FHYD-100, and only 2-19% for FHYD-150. Surprisingly, an expected reduction of sorption efficiency with increasing initial sorbate concentration was not observed for HSFs. Instead, the efficiency appears to increase slightly with increasing initial P(V) concentration from 0.09 to 0.9-1.8 mM and then it remains at roughly the same level for higher phosphate concentrations (Fig. 6). A possible explanation of this observation is the replacement of silicate by phosphate on the ferrihydrite surface (Vempati et al., 1990). Similar trends are observed in the case of Cr(VI) experiments. The adsorption efficiency of FHYD-005 and FHYD-010 is in the range of 95-100% for initial chromate concentrations up to 4.7 mM and 2.8 mM, respectively, and it decreases to 64% and 50% in the most concentrated solutions (18.4 mM).

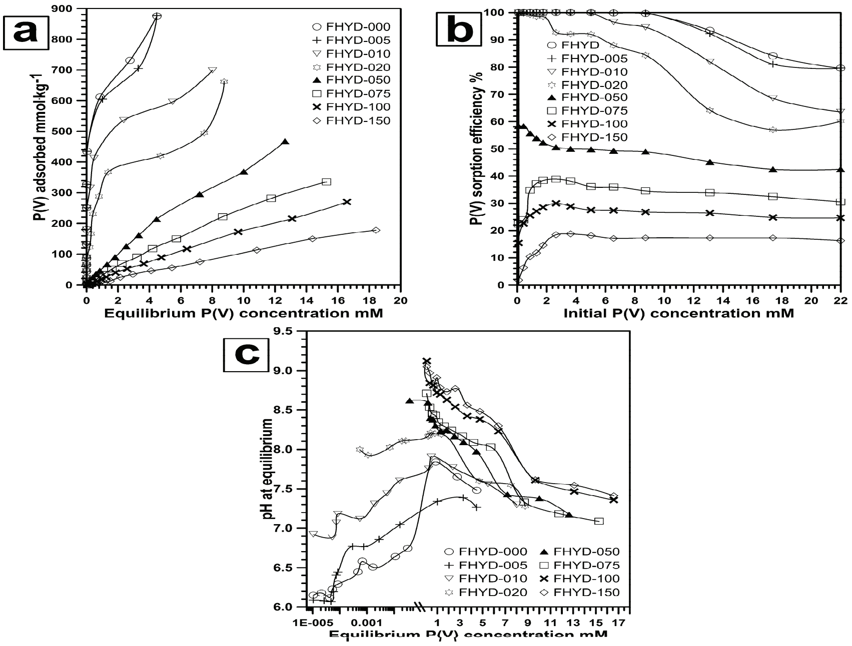


Fig. 6. Results of P(V) sorption experiments

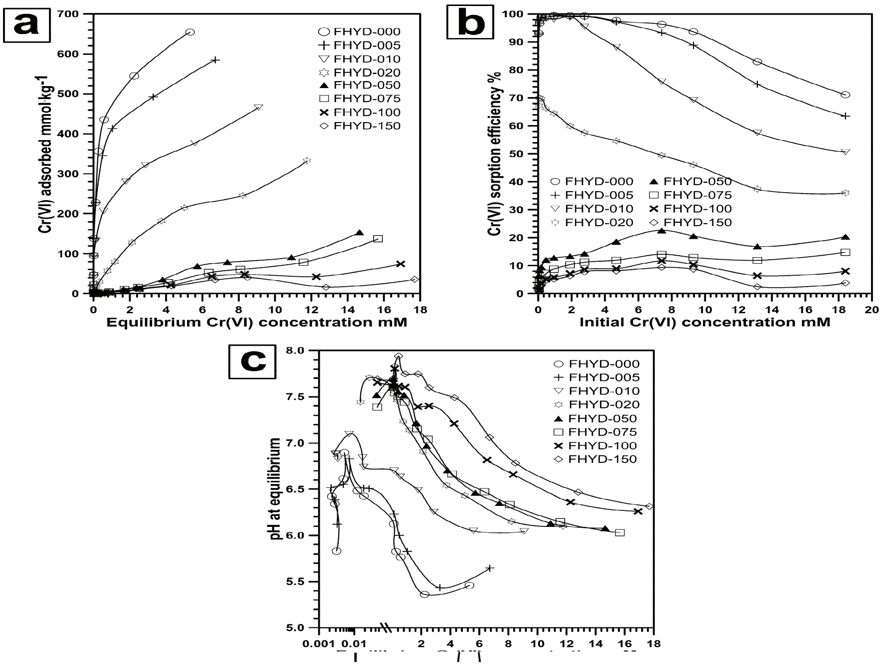


Fig. 7. Results of Cr(VI) sorption experiments

The FHYD-020 sample differs from other LSFs and removes only 60-70% at low Cr(VI)concentrations (up to 1.9 mM), while at the highest concentration the sorption efficiency is lower than 36%. The highest sorption effectiveness of HSFs is much lower, that is, from a few to about a dozen percent. As in the case of P(V), a slight increase in Cr(VI) uptake with increasing chromate concentration was noticed (Fig. 7).

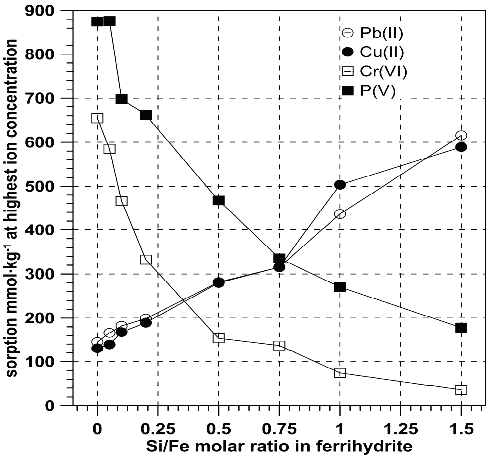


Fig. 8. The relation between sorption capacities and Si/Fe molar ratios in ferrihydrites

In the experiments with PF, final pH increased from ca. 6.2 to 7.5 with an increase in P(V) equilibrium concentration from ca. 0.0 to 4.5 mM (Figs. 6, 9). At the same time, phosphate sorption efficiency decreased from 100% to 80% (Fig. 6). Similar relations were found previously (Arai and Sparks, 2001; Warry and Kramer 1976). At pH below pH_{pzc}=8.41, the surface of ferrihydrite is positively charged and it easily adsorbs P(V) oxyanions. The decreasing trend of P(V) sorption with an increase in the final pH observed for PF may be caused by P(V) speciation, which strongly depends on the pH value. This is observed mostly at pH 7. Below this value, the $H_2PO_4^-$ form prevails, while in alkaline conditions, more negatively charged HPO_4^{-2} dominates. Thus, phosphate sorption onto PF decreases with decreasing $H_2PO_4^-$ species and simultaneously increasing HPO_4^{-2} species in an aqueous phase.

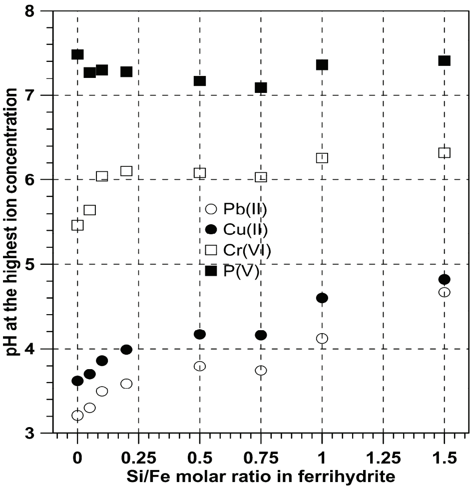


Fig. 9. The relation between final pH and Si/Fe molar ratios in ferrihydrites

The final pH of the samples FHYD-005 and FHYD-010 increased slightly as well from ca. 6.1 to ca. 7.3 in the former and from ca. 6.9 to ca. 7.5-7.9 in the latter (Figs. 6, 9). At the same time, the P(V) sorption efficiency was lowered to approximately 80% and 63%, respectively. However, in the case of higher-Si samples, a decrease in pH value with increasing phosphate concentrations was observed.

Table 2. Langmuir and Freundlich model coefficients for cation and anion sorption on Si-ferrihydrites.
S_{max} – maximum experimental sorption capacity

Sample	***S_{max}***	***Langmuir isotherm***				***Freundlich isotherm***		
		q_{max}	***K_L***	***R_L***	***R^2***	***n***	***K_F***	***R^2***
Pb(II)								
FHYD 0.00	145.00	149.03	0.88	0.078	0.9594	2.57	59.14	**0.9750**
FHYD 0.05	166.30	162.87	0.98	0.070	0.9483	2.84	69.60	**0.9578**
FHYD 0.10	182.20	176.68	1.51	0.047	**0.9641**	3.03	86.88	0.9085
FHYD 0.20	198.63	193.05	2.73	0.026	**0.9818**	4.03	110.36	0.6581
FHYD 0.50	281.61	276.24	6.58	0.011	**0.9955**	5.77	168.11	0.2828
FHYD 0.75	315.27	316.46	6.08	0.012	**0.9944**	6.46	178.65	0.2189
FHYD 1.00	436.23	462.96	5.54	0.013	**0.9499**	8.74	209.51	0.0574
FHYD 1.50	615.63	781.25	2.91	0.025	0.2125	12.50	198.43	0.0102
Cu(II)								
FHYD 0.00	130.55	145.56	0.28	0.159	0.9372	2.07	33.57	**0.9925**
FHYD 0.05	139.15	151.06	0.28	0.159	0.9194	2.13	36.07	**0.9893**
FHYD 0.10	167.98	166.67	0.62	0.079	0.9378	4.72	77.89	**0.9540**
FHYD 0.20	189.44	181.16	1.28	0.040	**0.9633**	5.71	107.99	0.9211
FHYD 0.50	279.81	275.48	5.26	0.010	**0.9919**	5.67	187.85	0.6584
FHYD 0.75	315.28	309.60	5.21	0.010	**0.9910**	5.50	212.81	0.6864
FHYD 1.00	503.66	497.51	11.82	0.004	**0.9980**	4.03	358.92	0.6042
FHYD 1.50	589.11	591.72	28.17	0.002	**0.9998**	3.73	486.41	0.5514
P(V)								
FHYD 0.00	875.21	833.33	24.00	0.002	**0.9886**	3.37	885.73	0.8688
FHYD 0.05	876.06	806.45	24.80	0.002	**0.9830**	3.56	843.53	0.7191
FHYD 0.10	698.54	662.25	9.44	0.005	**0.9892**	3.58	517.97	0.9170
FHYD 0.20	661.63	581.40	2.10	0.021	**0.9505**	2.34	280.54	0.9394
FHYD 0.50	467.60	1030.9	0.06	0.431	0.8812	1.14	53.36	**0.9983**
FHYD 0.75	335.39	-5555	-0.00	1.096	0.0532	0.93	23.82	**0.9828**
FHYD 1.00	270.39	-2326	-0.01	1.282	0.0195	0.93	16.14	**0.9891**
FHYD 1.50	178.26	-58.75	-0.06	3.125	0.1203	0.71	5.03	**0.9696**
Cr(VI)								
FHYD 0.00	654.60	675.7	3.44	0.016	**0.9721**	1.59	520.12	0.7754
FHYD 0.05	584.92	591.72	3.31	0.016	**0.9830**	1.68	394.00	0.7932
FHYD 0.10	466.02	452.49	2.15	0.025	**0.9740**	1.85	214.98	0.8352
FHYD 0.20	333.42	414.94	0.23	0.191	0.9622	1.26	59.99	**0.9896**
FHYD 0.50	153.65	-110.38	-0.05	12.50	0.4393	0.82	7.68	**0.9970**
FHYD 0.75	136.79	-44.09	-0.06	9.615	0.2579	0.77	4.28	**0.9945**
FHYD 1.00	74.30	-2.33	-0.63	0.094	0.1915	0.74	2.58	**0.9824**
FHYD 1.50	35.84	-1278.8	-0.00	1.019	0.0166	0.85	2.31	**0.9569**

This effect is particularly pronounced in HSFs, where final pH values decrease from ca. 8.6-9.0 to 7.1-7.4 and it might be another factor causing the above-mentioned increase in phosphate adsorption by HSFs in more concentrated P(V) solutions (Fig. 6) because anion adsorption on ferrihydrite at pH above pH_{pzc} is distinctly lowered (Brinza et al., 2008).

In Cr(VI) experiments, a slight increase in final pH values in the lowest concentrated solutions followed by a distinct decrease was observed for all of the samples (Fig. 7). However, the pH values themselves vary significantly among the samples, being lower for LSFs and higher for HSFs (Figs. 7, 9). Because it was shown previously (Ajouyed et al., 2010) that chromate adsorption on iron (oxyhydr)oxides is highest between pH 2 and 6 and falls off rapidly in more alkali conditions, the above-mentioned distinct final pH decrease in HSFs might cause somewhat higher adsorption capacities in more concentrated solutions.

3.4. Langmuir and Freundlich models

The adsorption data were fitted by linearized forms of Langmuir and Freundlich equations (Table 2). A comparison of the regression coefficients indicates that the adsorption data of Pb(II) and Cu(II) fit in a slightly better manner the Freundlich model for PF ($R^2 > 0.97$) and FHYD-005 ($R^2 > 0.95$) than the Langmuir model ($R^2 > 0.95$ and $R^2 > 0.94$, respectively). Although R^2 values of cations sorption on PF and LSFs are higher for the Freundlich model, the sorption capacity values obtained from Langmuir isotherms are close to the maximum experimental sorption capacity, suggesting that the monolayer chemisorption mechanism rules the interactions between cations and the ferrihydrite surface. The Langmuir model describes the adsorption behaviors of Pb(II) on all remaining Si-ferrihydrite samples much better than the Freundlich equation (R^2 = 095-0.99 vs 0.06-0.66), besides the FHYD-150 sample, for which Pb adsorption does not follow either the

Langmuir or the Freundlich model. Similarly, the Langmuir equation produced excellent fits ($R^2 > 0.99$) for Cu(II) adsorption on the HSFs, while Cu sorption on PF as well as FHYD-005 and FHYD-010 samples fits better to the Freundlich model. Langmuir constants (K_L) for sorption of both Pb and Cu appear to increase with increasing Si content in ferrihydrite. Since the constant is related to the adsorption energy, this trend suggests increasing bond strength. In the case of Cu sorption, the increase is observed in the whole Si/Fe range. For Pb sorption, it is observed only in LSFs, and then a distinct decrease in K_L values with increasing Si/Fe is apparent. At the same time, the HSFs sorption data for Cu fit better to the Langmuir isotherm than those for Pb. These observations are an effect of the above-mentioned increase in Pb removal efficiency by HSFs with the increase in initial metal concentration, which was found in relatively low-concentrated solutions. The basic assumptions of the Langmuir model are not met then.

The Langmuir isotherm matches the experiment data of P(V) adsorption on PF and LSFs ($R^2 > 0.97$) better than the Freundlich isotherm. However, in the case of HSFs, the Langmuir model does not work at all and results in negative values of sorption capacities and adsorption energies. Here, the Freundlich model gives much better fits. The Freundlich *n* constant values indicate the decrease in phosphate adsorption favorability with the increase in the Si/Fe molar ratio in ferrihydrites. Moreover, *n* is below 1 for LSFs, suggesting that chemisorption is a dominant sorption mechanism. This is not the case for HSFs.

A similar tendency is observed for the Cr(VI) sorption. Experimental data for PF and FHYD-005 and FHYD-010 fit to the Langmuir isotherm. On the other hand, chromate adsorption on FHYD-020 and HSFs follows the Freundlich isotherm ($R^2 > 0.95$). Again, a drop in *n* values suggests that chromate adsorption is not favorable onto HSFs. In the P(V) and Cr(VI) systems, which are better described by the Langmuir model (i.e., PF and LSFs), the K_L values systematically decrease with increasing Si/Fe ratios. Even though the Langmuir equation does not describe well all the experimental data, the separation factor R_L calculated from the isotherm confirms that the cation adsorption is more favorable, whereas the anion adsorption is much less favorable onto HSFs than onto LSFs (Table 2).

4. Conclusions

Our work shows that the presence of Si in ferrihydrite strongly affects the surface chemistry and sorption properties of the oxyhydroxide. Silicon, which is majorly adsorbed onto ferrihydrite in the form of silicate, distinctly lowers pH_{PZC}, thereby increasing the net negative charge of the surface. Specific surface area of ferrihydrite is also modified: low silicate loads appear to increase the SSA, but high Si contents reduce it. In comparison to pure ferrihydrite, those containing silicate exhibit distinctly higher affinity toward cations and distinctly lower affinity toward anions. This is an effect of the alteration of ferrihydrite surface chemistry by adsorbed silicate. It forms continuous and highly polymerized coating on HSFs. Therefore, in terms of the surface chemistry, LSFs and HSFs represent virtually different substances. The results showed that Langmuir isotherms fit better experimental data of anion adsorption onto PF and LSFs than the Freundlich equation. On the other hand, the Freundlich isotherm appeared to describe anion adsorption on HSFs in a better manner. The opposite tendency was noticed in the case of cation adsorption – the data fit the Freundlich isotherm in a slightly better way for LSFs but the Langmuir model is usually preferred for HSFs. Despite fairly good fits of the experimental data to the models, the actual sorption mechanisms are complicated by surface precipitation as well as by partial desorption of silicate and partial dissolution of ferrihydrite.

From the point of view of environmental engineering, Si-ferrihydrite appears to be a more convenient adsorbent than the pure ferrihydrite. Sorption properties can be controlled by an appropriate Si/Fe molar ratio. Also, enhanced stability of Si-ferrihydrite should facilitate the preparation of the adsorbent (e.g., granulation), its further maintenance, and disposal.

Acknowledgments

This work was supported by AGH-UST statutory grant no. 11.11.140.319. The authors are indebted to Prof. Maciej Manecki for his help during preparation of the manuscript and to Dr. Tomasz Bajda for fruitful discussion. Four anonymous reviewers are also thanked for constructive remarks which allowed improving the quality of the article.

References

Adra A., Morin G., Ona-Nguema G., Brest J., (2016), Arsenate and arsenite adsorption onto Al-containing ferrihydrites. Implications for arsenic immobilization after neutralization of acid mine drainage, *Applied Geochemistry*, **64**, 2-9.

Ajouyed O., Hurel C., Ammari M., Allal L.B., Marmier N., (2010), Sorption of Cr(VI) onto natural iron and aluminum (oxy)hydroxides: Effects of pH, ionic strength and initial concentration, *Journal of Hazardous Materials*, **174**, 616-622.

Anderson P.R., Benjamin M.M., (1985), Effect of silicon on the crystallization and adsorption properties of ferric oxides, *Environmental Science and Technology*, **19**, 1048-1053.

Antelo J., Fiol S., Pérez C., Marino S., Arce F., Gondar D., López R., (2010), Analysis of phosphate adsorption onto ferrihydrite using the CD-MUSIC model, *Journal of Colloid and Interface Science*, **147**, 112-119.

Antelo J., Arce F., Fiol S., (2015), Arsenate and phosphate adsorption on ferrihydrite nanoparticles. Synergetic interaction with calcium ions, *Chemical Geology*, **410**, 53-62.

APHA, (1997) *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, Washington DC.

Arai Y., Sparks D.L., (2001), ATR-FTIR spectroscopic investigation on phosphate adsorption mechanisms at the ferrihydrite-water interface, *Journal of Colloid and Interface Science*, **241**, 317-326.

Bajda T., Kłapyta Z., (2013), Adsorption of chromate from aqueous solutions by HDTMA-modified clinoptilolite, glauconite and montmorillonite, *Applied Clay Science*, **86**, 169-176.

Brinza L., Benning L.G., Statham P.J., (2008), Adsorption studies of Mo and V onto ferrihydrite, *Mineralogical Magazine*, **72**, 385-388.

Campbell A.S., Schwertmann U., Stanjek H., Friedl J., Kyek A., Campbell P.A., (2002), Si incorporation into hematite by heating Si-ferrihydrite, *Langmuir*, **18**, 7804-7809.

Carbante I., Grahn M., Holmgren A., Hedlund J., (2010), In situ ATR–FTIR studies on the competitive adsorption of arsenate and phosphate on ferrihydrite, *Journal of Colloid and Interface Science*, **351**, 523-531.

Carlson L., Schwertmann U., (1981), Natural ferrihydrites in surface deposits from Finland and their association with silica, *Geochimica et Cosmochimica Acta*, **45**, 421-429.

Cismasu A.C., Michel F.M., Tcaciuc A.P., Tyliszczak T., Brown Jr G.E., (2011), Composition and structural aspects of naturally occurring ferrihydrite, *Comptes Rendus Geoscience*, **343**, 210-218.

Cismasu A.C., Levard C., Michel F.M., Brown Jr G.E., (2013), Properties of impurity-bearing ferrihydrite II: Insights into the surface structure and composition of pure, Al- and Zn K-edge X-ray absorption spectroscopy, *Geochimica et Cosmochimica Acta*, **119**, 46-60.

Cismasu A.C., Michel F.M., Tcaciuc A.P., Brown Jr G.E., (2014), Properties of impurity-bearing ferrihydrite III. Effects of Si on the structure of 2-line ferrihydrite, *Geochimica et Cosmochimica Acta*, **133**, 168-185.

Cornell R.M., Schwertmann U., (2003), *The Iron Oxides: Structure, Properties, Reactions, Occurrences and Uses*, Wiley-VCH Verlag, Weinheim.

Dyer L.F, Fawell P.D., Newman O.M.G., Richmond W.R., (2010), Synthesis and characterization of ferrihydrite/silica co-precipitates, *Journal of Colloid and Interface Science*, **348**, 65-70.

Dyer L.G., Chapman K.W., English P., Saunders M., Richmond W.R., (2012), Insight into the crystal and aggregate structure of Fe^{+3} oxide/silica co-precipitates, *American Mineralogist*, **97**, 63-69.

Filip J., Zboril R., Schneeweiss O., Zeman J., Cernik M., Kvapil P., Otyepka M., (2007), Environmental applications of chemically pure natural ferrihydrite, *Environmental Science and Technology*, **41**, 4367-4374.

Foo K.Y., Hameed B.H., (2010), Insights into the modeling of adsorption isotherm systems, *Chemical Engineering Journal*, **156**, 2-10.

Francisco P.C.M., Sato T., Otake T., Kasama T., (2016), Kinetics of Fe^{3+} mineral crystallization from ferrihydrite in the presence of Si at alkaline conditions and implications for nuclear waste disposal, *American Mineralogist*, **101**, 2057-2069.

Glasauer S.M., Hug P., Weidler P.G., Gehring A.U., (2000), Inhibition of sintering by Si during the conversion of Si-rich ferrihydrite to hematite, *Clays and Clay Minerals*, **48**, 51-56.

Guo H., Barnard A.S., (2013), Naturally occurring iron oxide nanoparticles: morphology, surface chemistry and environmental stability, *Journal of Materials Chemistry A*, **1**, 27-42.

Hansen H.C.B., Wetche T.P., Raulund-Rasmussen K., Boggaard O.K., (1994), Stability constants for silicate adsorbed to ferrihydrite, *Clay Minerals*, **29**, 341-350.

Hasselöv M., Von Der Kammer F., (2008), Iron oxides as geochemical nanovectors for metal transport in soil-river systems, *Elements*, **4**, 401-406.

Hemmingsson C., Chi Fru E., (2015), *A Silica & Arsenic Controlled Early Proterozoic P Cycle*, In: *Goldschmidt Abstracts*, 25th Anniversary of Goldschmidt Conference, Prague 16-21 August, 1229.

Jambor J.L., Dutrizac J.E., (1998), Occurrence and constitution of natural and synthetic ferrihydrite, a widespread iron oxyhydroxide, *Chemical Reviews*, **98**, 2549-2585.

Johnston C.P., Chrysochoou M., (2016), Mechanisms of chromate, selenate, and sulfate adsorption on Al-substituted ferrihydrite: implications for ferrihydrite surface structure and reactivity, *Environmental Science and Technology*, **50**, 3589-3596.

Kaegi R., Voegelin A., Folini D., Hug S., (2010), Effect of phosphate, silicate, and Ca on the morphology, structure and elemental composition of Fe(III)-precipitates formed in aerated Fe(II) and As(III) containing water, *Geochimica et Cosmochimica Acta*, **74**, 5798-5816.

Kosmulski M., (2011), The pH-dependent surface charging and points of zero charge V. Update, *Journal of Colloid and Interface Science*, **353**, 1-15.

Koteja A., Matusik J., (2015), Di- and triethanolamine grafted kaolinites of different structural order as adsorbents of heavy metals, *Journal of Colloid and Interface Science*, **455**, 83-92.

Kukkapudu R.V., Zachara J.M., Fredrickson J.K., Kennedy D.W., (2004), Biotransformation of two-line silica-ferrihydrite by a dissimilatory Fe(III)-reducing bacterium: Formation of carbonate green-rust in the presence of phosphate, *Geochimica et Cosmochimica Acta*, **68**, 2799-2814.

Kyzioł-Komosińska J., Rosik-Dulewska C., Dzieniszewska A., Pająk M., Krzyżewska I., (2014), Removal of Cr(III) ions from water and wastewater by sorption onto peats and clays occurring in an overburden of lignite beds in central Poland, *Environment Protection Engineering*, **40**, 5-22.

Lenoble V., Deluchat V., Serpaud B., Bollinger J.-C., (2003), Arsenite oxidation and arsenate determination by the molybdene blue method, *Talanta*, **61**, 267-276.

Limousin G., Gaudet J.-P., Charlet L., Szenknect S., Barthes V., Krimissa M., (2007), Sorption isotherms: A review on physical bases, modeling and measurement, *Applied Geochemistry*, **22**, 249-275.

Liu J., Zhu R., Xu T., Xu Y., Ge F., Xi Y., Zhu J., He H., (2016), Co-adsorption of phosphate and zinc(II) on the surface of ferrihydrite, *Chemosphere*, **144**, 1148-1155.

Lu P., Nuhfer N.T., Kelly S., Li Q., Konishi H., Elswick E., Zhu C., (2011), Lead coprecipitation with iron oxyhydroxide nano-particles, *Geochimica et Cosmochimica Acta*, **75**, 4547-4561.

Manceau A., (1995), The mechanism of anion adsorption on iron oxides: evidence for the bonding of arsenate tetrahedral on free $Fe(O,OH)_6$ edges, *Geochimica et Cosmochimica Acta*, **59**, 3647-3653.

Matei E., Predescu C., Badanoui A., Predescu A., Ficai D., (2015), Application of magnetite nanoparticles as adsorbent for Cr, Cd, Ni and Cu from aqueous solutions, *Environmental Engineering and Management Journal*, **14**, 1001-1010.

Matusik J., Bajda T., (2013), Immobilization and reduction of hexavalent chromium in the interlayer space of positively charge kaolinites, *Journal of Colloid and Interface Science*, **398**, 74-81.

Matusik J., (2014), Arsenate, orthophosphate, sulfate, and nitrate sorption equilibria and kinetics for halloysite and kaolinites with an induced positive charge, *Chemical Engineering Journal*, **246**, 244-253.

Mohapatra M., Mohapatra L., Singh P., Anand S., Mishra B.K., (2010), A comparative study on Pb(II), Cd(II), Cu(II) and Co(II) adsorption from single and binary aqueous solutions on additive assisted nano-structured goethite, *International Journal of Engineering, Science and Technology*, **2**, 89-103.

Neal C., Neal M., Wickhman H., (2000), Phosphate measurement in natural waters: two examples of analytical problems associated with silica interference using phosphomolybdic acid methodologies, *Science of the Total Environment*, **251/252**, 511-522.

Neupane G., Donahoe R.J., Arai Y., (2014), Kinetics of competitive adsorption/desorption of arsenate and phosphate at the ferrihydrite-water interface, *Chemical Geology*, **368**, 31-38.

Paige C.R., Sondgrass W.J., Nicholson R.V., Scharer J.M., He Q.H., (1997), The effect of phosphate on the transformation of ferrihydrite into crystalline products in alkaline media, *Water, Air and Soil Pollution*, **97**, 397-412.

Parfitt R.L., Van der Gaast S.J., Childs C.W., (1992), A structural model for natural siliceous ferrihydrite, *Clays and Clay Minerals*, **40**, 675-681.

Pokrovski G.S., Schott J., Farges F., Hazemann J.-L., (2003), Iron (III)-silica interactions in aqueous solution: Insight from X-ray absorption fine structure spectroscopy, *Geochimica et Cosmochimica Acta*, **19**, 3559-3573.

Raven K.P., Jain A., Loeppert R.H., (1998), Arsenite and arsenate adsorption on ferrihydrite: kinetics, equilibrium, and adsorption envelopes, *Environmental Science and Technology,* **32**, 344-349.

Rout K., Mohapatra M., Anand S., (2012), 2-Line ferrihydrite: synthesis, characterization and its adsorption behavior for removal of Pb(II), Cd(II), Cu(II) and Zn(II) from aqueous solutions, *Dalton Transactions*, **41**, 3302-3312.

Russell J.D., Fraser A.R., (1994), *Infrared Methods*, In: *Clay Mineralogy: Spectroscopic and Chemical Determinative Methods*, Wilson M.J. (Ed.), Chapman & Hall, London, 11-67.

Rzepa G., Bajda T., Ratajczak T., (2009), Utilization of bog iron ores as sorbents of heavy metals, *Journal of Hazardous Materials*, **162**, 1007-1013.

Rzepa G., Pieczara G., Gaweł A., Tomczyk A., Zalecki R., (2016), The influence of silicate on transformation pathways of synthetic 2-line ferrihydrite, *Journal of Thermal Analysis and Calorimetry*, **125**, 407-421.

Schwertmann U., Fechter H., (1982), Point of zero charge of natural and synthetic ferrihydrites and its relation to adsorbed silicate, *Clay Minerals*, **17**, 471-476.

Schwertmann U., (1991), Solubility and dissolution of iron oxides, *Plant and Soil*, **130**, 1-25.

Schwertmann U., Friedl J., Kyek A., (2004), Formation and properties of continuous crystallinity series of synthetic ferrihydrites (2- to 6-line) and their relation to FeOOH forms, *Clays and Clay Minerals*, **52**, 221-226.

Seehra M.S., Roy P., Raman A., Manivannan A., (2004), Structural investigations of synthetic ferrihydrite nanoparticles doped with Si, *Solid State Communications*, **130**, 697-601.

Senn A.-C., Kaegi R., Hug S.J., Hering J.G., Mangold S., Voegelin A., (2015), Composition and structure of Fe(III)-precipitates formed by Fe(II) oxidation in water at near-neutral pH: independent effects of phosphate, silicate and Ca, *Geochimica et Cosmochimica Acta*, **162**, 220-246.

Swedlund P.J., Webster J.G., (1999), Adsorption and polymerisation of silicic acid on ferrihydrite, and its effect on arsenic adsorption, *Water Research*, **33**, 3413-3422.

Tzou Y.M., Wang M.K., Loeppert R.H., (2003), Sorption of phosphate and Cr(VI) by Fe(III) and Cr(III) hydroxides, *Archives of Environmental Contamination and Toxicology*, **44**, 445-453.

Vempati R.K., Loeppert R.H., (1989), Influence of structural and adsorbed Si on the transformation of synthetic ferrihydrite, *Clays and Clay Minerals*, **37**, 273-279.

Vempati R.K., Loeppert R.H., Cocke D.L., (1990), Mineralogy and reactivity of amorphous Si-ferrihydrites, *Solid State Ionics*, **38**, 53-61.

Wang X., Liu F., Tan W., Li W., Feng X., Sparks D.L., (2013), Characteristic of phosphate adsorption-desorption onto ferrihydrite: comparison with well-crystalline Fe (hydr)oxides, *Soil Science*, **178**, 1-11.

Wang X., Zhu M., Lan S., Ginder-Vogel M., Liu F., Feng X., (2015), Formation of secondary mineralization of ferrihydrite in the presence of silicate and Mn(II), *Chemical Geology*, **415**, 37-46.

Wang H., Zhu J., Fu Q.-L., Xiong J.-W., Hong C., Hu H.-Q., Violante A., (2015), Adsorption of phosphate onto ferrihydrite and ferrihydrite-humic acid complexes, *Pedosphere*, **25**, 405-414.

Warry N.D., Kramer J.K., (1976), Some factors affecting the synthesis of cryptocrystalline strengite from an amorphous phosphate complex, *The Canadian Mineralogist*, **14**, 40-46.

Waychunas G.A., Kim C.S., Bandfield J.F., (2005), Nanoparticulate iron oxides minerals in soils and sediments: unique properties and contaminant scavenging mechanism, *Journal of Nanoparticle Research*, **7**, 409-433.

Weidler P.G., (1997), BET sample pretreatment of synthetic ferrihydrite and its influence on the determination of surface area and porosity, *Journal of Porous Materials*, **4**, 165-169.

Yu J.Y., Park M., Kim J., (2002), Solubilities of synthetic schwertmannite and ferrihydrite, *Geochemical Journal*, **36**, 119-132.

Zachara J.M., Girvin D.C., Schmidt R.L., Resch C.T., (1987), Chromate adsorption on amorphous iron oxyhydroxide in the presence of major groundwater ions, *Environmental Science and Technology*, **21**, 589-594.

Zeng L., (2003), A method for preparing silica-containing iron(III) oxide adsorbent for arsenic removal, *Water Research*, **37**, 4351-4358.

Zhu J., Pigna M., Cozzolino V., Caporale A.G., Violante A., (2010), Competitive sorption of copper(II), chromium(III) and lead(II) on ferrihydrite and two organomineral complexes, *Geoderma*, **159**, 409-416.

Zhu M., Northrup P., Shi C., Billinge S.J.L., Sparks D.L., Waychunas G.A., (2014), Structure of sulfate adsorption complexes on ferrihydrite, *Environmental Science and Technology Letters*, **1**, 97-101.

Environmental Engineering and Management Journal

September 2016, Vol.15, No. 9, 2109-2117
http://omicron.ch.tuiasi.ro/EEMJ/

"Gheorghe Asachi" Technical University of Iasi, Romania

INNOVATIVE METHOD FOR THE EVALUATION OF PROFESSIONAL RISK DURING CONTROLLED DEMOLITION WITH EXPLOSIVES OF CIVIL USE

Gabriel Dragos Vasilescu[1], Attila Kovacs[1], Tiberiu Attila Csaszar[1], Constantin Baciu[2]*, Raluca Elena Baciu[3], Iuliana Silvia Georgescu[2]

[1]National Institute for Research and Development in Mine Safety and Protection to Explosion – INSEMEX Petroşani, 32-34 G-ral Vasile Milea St., 332047 Petroşani, Hunedoara, Romania
[2]"Gheorghe Asachi" Technical University of Iaşi, 67 D. Mangeron Bd., 700050 Iaşi, Romania
[3]"Gr.T. Popa" University of Medicine and Pharmacy from Iasi, 16 Universităţii St., 700115 Iaşi, Romania

---

Abstract

This paper presents an analytical approach to the evaluation concept of professional risk specific to activities of controlled demolition with civil use explosives of industrial/civil objectives, ensuring the appropriate level of safety to the effects generated by performing blasting to these types of constructions. The scientific research highlighted in this article was performed within the Project PN 7:45 1:28 in the frame of The National Programme for Research and Development CORE/2014. The main purpose of evaluating occupational risk is to prevent the likelihood of injuries and occupational diseases. When there is no any possibility to eliminate the risks, it is mandatory to reduce it up to the level of residual risks, which must be adequately controlled.

Key words: blasting operations, demolition activity, explosives for civil use, risk assessment, technical demolition documentation

Received: March, 2015; Revised final: September, 2015; Accepted: October, 2015

---

1. Introduction

The Legislation forces employers to take all measures in order to prevent workers exposure to multiple risks which could cause accidents or professional diseases. Thereby, methodological Regulations for applying Health and Safety Law no. 319/2006 regulates the mechanism that the employer must use to prevent an increase of these risks (Law 319, 2006).

Each industrial branch generates specific risks that must be considered both by the employer and workers. During demolition works performed by authorized companies using specific explosives, there are different types of risk, which can be found on construction sites, together with risks involved in working with explosive materials (Conte et al., 2011; Covello et al., 1993; Vasilescu et al., 2014).

In many situations, on the same site, at least two companies are involved in the demolition works, such as a general contractor, a sub-contractor who brings and uses the special equipment required on the site, and the authorized company hired for blasting works. According to the law, each company is required to organize its own prevention and protection activities. At the same time, all companies have to work together, collaborate and create an emergency plan in order to reveal and avoid the risks that could be generated by each company and create difficulties for the others.

Besides the risks for the employees working on the site, there must also be considered the risks that could affect other persons exposed, living in the vicinity of the demolition area. Inside a working

---

* Author to whom all correspondence should be addressed: e-mail: constantin_baciu@yahoo.com; Phone: +40729969619

system, the general obligation of an employer is to ensure workers health and safety. An evaluation process of professional risks must provide the possibility to establish proper measures of prevention, protection and insurance so as to avoid accidents and professional disease, but also to inform employees and implement an efficient system for professional safety management (Cruz, 2004; Platon and Hionis, 2014; Risk Management Guidelines, 2011; Smid, 2001).

The main reason for evaluating professional risks is to prevent the accidents and professional diseases; when the elimination of these risks is not possible, it is required to reduce their level to the value of residual risk, when this can be adequately controlled. During the evaluation process of professional risks and also along the implementation of multiple safety measures, a special attention must be directed to the possibility that the professional risk might not be moved from one area to another of the work system. The technical and organizational solutions adopted in order to decrease or eliminate these risks must not create additional situations.

From a structural point of view, the main stages of evaluating the professional risks are: identification of dangerous situations causing accidents and professional disease; identification of persons exposed to these dangerous situations; estimation and assessment of professional risks; studies for finding possibilities to eliminate professional risks; opportunity and necessity analysis for decreasing the need to adopt additional measures for eliminating the occupational hazards (CPCCDE 3016A, 2014; Papadopoulos et al., 2010; Pollution Prevention Guidelines, 2012; Programme – Based Engagement, 2009; Vasilescu, 2008 a, b: Vasilescu et al., 2008).

The assessment methodology for professional risks has two essential requests in this area (Moraru et al., 2002; Moraru et al., 2014; NUCLEU Project, 2014):

- the evaluation procedure must be able to analyze all dangerous situations, possible accidents and professional disease, whatever their manifestation (obvious or potential dangers);
- elimination, if possible, of all risk of injury and professional disease, identified during the evaluation process.

The structure of the assessment process is presented below:

- identification of all factors of accidents risk and professional disease from the analyzed work system;
- identification of all persons exposed to injury and professional disease;
- estimation of professional risks;
- establishment and adoption of decisions regarding the new applicable measures for elimination and reduction of professional risk;
- analysis of the prevention measures adopted to establish the order of their application;
- subsequently undertaken actions in the evaluation process.

Risk evaluation represents a permanent preoccupation for all the leading personnel of a company, from the beginning of a project, continuing with preparatory works, demolition works and monitoring their effects on human health and safety, the integrity of materials and goods and also effects of environment (Antonov et al., 2014; Kovacs et al., 2014; Law 319, 2006; MacDonald et al., 2000).

2. Materials and methods

The development of blasting works and demolition of civil and industrial constructions, for certain affected buildings, involves different operations which could generate health and safety risks for the employees working in the company providing the project; also these operations could affect the vicinity of the site and the environment (Nisipeanu et al., 2014; Law 319, 2006; Lupu et al., 2014; Vasilescu, 2008).

2.1. Graphical and analytical representation and assessment of professional risk on controlled demolition of industrial and civil objectives, using civil use explosives

The existence of risk in a work system appears due to the risk factors of injury and professional disease. Thereby, the elements which could properly characterize the risk are: (i) the probability of an accident influenced by a risk factor and (ii) the severity of risk action consequences on the victim. Using both scales of probability and consequences severity of risk factor actions, each factor of risk from a system can be associated with characteristic elements, for each element having a certain level of risk (Fig. 1).

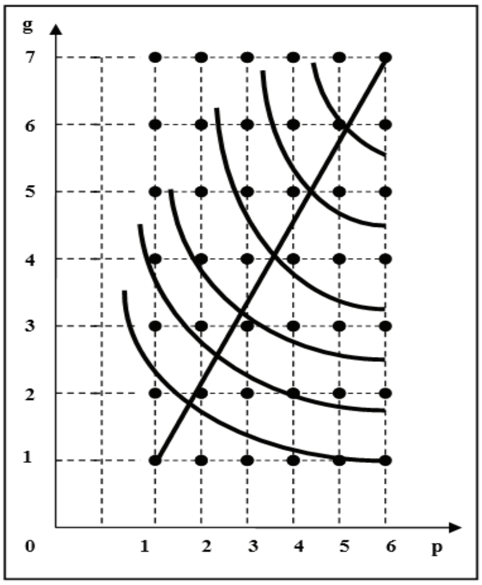


Fig. 1. Curves of professional risk level

From an analytical point of view, the system formed by six curves dividing the integral field of risk in seven distinct areas presented in Table 1 and represents the general solution of the differential equation (degree 2), with second differentiation order, and which variable is the gravity parameter g, (Eq. 1):

$$g'' + 0.28574\, g' + 0.020408\, g = 0 \qquad (1)$$

This equation can be solved based on the characteristic equation (Eq. 2):

$$r^2 + 0.2857\, r + 0.020408 = 0 \qquad (2)$$

which has a general solution (Eq. 3):

$$g_i = [c_{1i}(p+1) + c_{2i}]e^{-(p+1)r} \qquad (3)$$

g - variable of differential equation which defines the gravity parameter, (G);
p - variable of general solution g_i (where i=1,6 represents the index of risk curve i), which defines the probability parameter, (P);
r - variable of characteristic equation for solving the differential equation;
c_{1i}, c_{2i} - integration constant, determined from the initial conditions c_1 and c_2, corresponding to the curve of minimum risk.

Considering the values of both integration coefficients, the general solution of the differential equation is (Eq. 4):

$$g_i = \left[\begin{vmatrix} 1.000000000 \\ -0.024965858 \\ -0.013377423 \\ -0.010971558 \\ -0.019157306 \\ -0.004349399 \end{vmatrix}(p+1) + \begin{vmatrix} 1.000000000 \\ 0.716544186 \\ 0.537294492 \\ 0.395512740 \\ 0.262815264 \\ 0.128874756 \end{vmatrix}\right] e^{-(p+1)/7} \qquad (4)$$

Normative regulations from most countries do not allow employers the achievement of critical standard. Thereby, generally, for each risk factor there are established either maximum limits in the form of values, in cases when factors manifestation could be characterized through measurable elements, or interdictions (factors which cannot be measured).

The respective regulations correspond to a maximum level of risk, which is different from a country to another, depending on the economic and social conditions. According to the specialized literature, the acceptable level of risk for our country is about 3.5 (22÷29), which actually requires an attitude in relation to the potential risk , mainly characterized by measures to monitor and control dangerous situations manifestation of risk factors (Moraru et al., 2002; Pece, 1997). That means in the first place that the operating permit of the economic agents, from environmental point of view, should be issued only if the risk evaluation on workplaces confirms the acceptable level; this fact should be demonstrated even by an analyze and reduction of evaluated risk found in the unacceptable area; their normalization is developed by applying proper measures for prevention and protection.

2.2. Analysis and reduction of professional risk on controlled demolition of industrial/civil objectives, using civil use explosives

In cases of evaluated risks situated in unacceptable areas, it must be applied and analyze and reduction procedure, by applying a suitable program of technical and organizational measures, in order to prevent the causes of unexpected events production (working accidents and/ occupational disease, also the effects of demolition on the vicinity of sites) (Lee et al., 2012; Murè et al., 2006; Nor Rizman Bin Abas, 2010).

In this case, it is used "the professional risk analyzer" (presented in Fig. 2), which was built based on the provided grids with value classes corresponding to the following parameters: the probability of producing an unexpected event, P presented in Table 3 and the gravity of maximum consequences, G presented in Table 2. In the following, there are introduced the grids correspondent to the parameters of health and safety at work, respectively P, G and R/S and the scale of attitude towards the level of professional risk presented in Tables 4 and 5 (Waddell and Burton, 2001). If after assessing the health and safety risk in the field of controlled demolition of industrial/ civil buildings using civil use explosives, there is a hazard for which an unacceptable risk situation has been evaluated: (i) the risk will be highlighted in the index to risk records through the corresponding risk factor (Table 6); (ii) a form of analysis and reduction of professional risk will be completed; (iii) a diagram of risk reduction will be built (Table 7 and Fig. 2) (Construction Phase Plan - Demolition, 2011).

Table 1. Equations of risk curves

No. crt.	*Name of equation*	*Equation*
1.	Equation of minimum risk curve	$g_1 = [(p+1) + 1]e^{-(p+1)/7}$
2.	Equation of low risk curve	$g_2 = [-0.024965858\,(p+1) + 0.716544186]e^{-(p+1)/7}$
3.	Equation of medium risk curve	$g_3 = [-0.013377423\,(p+1) + 0.537294492]e^{-(p+1)/7}$
4.	Equation of high risk curve	$g_4 = [-0.010971558\,(p+1) + 0.395512740]e^{-(p+1)/7}$
5.	Equation of very high risk curve	$g_5 = [-0.019157306\,(p+1) + 0.262815264]e^{-(p+1)/7}$
6.	Equation of maximum risk curve	$g_6 = [0.004349399\,(p+1) + 0.128874756]e^{-(p+1)/7}$

Table 2. The grid of the gravity parameter of maximum consequences

Classes of severity	*Consequences*	*Severity of consequences G*
1	**Negligible**	Consequences minor unable to work up to 3 days (healing without treatment)
2	**Small**	Consequences minor unable to work up to 3 – 45 days, that need of medical treatment
3	**Averages**	Reversible consequences with a predictable work incapacity between 45 – 180 days, requiring medical treatment and hospitalization
4	**High**	Irreversible consequences, with a decrease of working capacity of 50%, the individual being able to provide a professional activity (disability grade III)
5	**Serious**	Consequences 100% irreversible, loss of labour capacity but with the possibility of self-service and spatial orientation (disability grade II)
6	**Severe**	Irreversible consequences with total loss of ability to work, the self-conduction or spatial orientation (disability grade I)
7	**Maximum**	Death

Table 3. The grid of the probability parameter of producing an unexpected event, P

Classes of probability	*Events*	*The likelihood of consequences P*
1	**Extremely rare**	**(extremely low), P > 10 years**
2	**Very rare**	**(very low), 5 years < P < 10 years**
3	**Rare**	**(low), 2 years < P < 5 years**
4	**Less frequent**	**(average), 1 year < P < 2 years**
5	**Frequently**	**(high), 1 month < P < 1 year**
6	**Very frequently**	**(very high), P < 1 month**

Table 4. The assessment grid of professional risk level

Levels of risk / safety	*Risk assessment values R*	*Appreciation level of professional risk*	*Appreciation level of occupational safety*
1 / 7	**1 ÷ 7**	**Minimum risk**	**Maximum security**
2 / 6	**8 ÷ 13**	**Very small risk**	**Very high security**
3 / 5	**14 ÷ 21**	**Low-risk**	**High security**
4 / 4	**22 ÷ 29**	**Medium risk**	**Medium security**
5 / 3	**30 ÷ 35**	**High-risk**	**Small security**
6 / 2	**36 ÷ 39**	**Very high risk**	**Very small security**
7 / 1	**40 ÷ 42**	**Maximum Risk**	**Minimum security**

Legend:

□ □ □	Represents the field of acceptable risk
▨ ▨ ▨ ■	Represents the field of unacceptable risk

Table 5. Scale of attitude towards professional risk

Levels of risk	*Appreciation level of professional risk*	*Attitude towards the professional risk*
1	**Minimum risk**	No special action is taken
2	**Very small risk**	
3	**Low-risk**	
4	**Medium risk**	Shall be made the monitoring of dangerous situations, event control of risk factors, additional corrective measures can be applied taking into account the cost-effectiveness
5	**High-risk**	Efforts will be made to reduce the level, but the costs of prevention should be carefully measured. Measures to reduce the risk level is implemented strictly determined period of time. Where is associated with greater risk of serious consequences, should be set exactly the probability of manifestation of risk factors responsible, and measures will be taken to mitigate them.
6	**Very high risk**	Activities can not continue until the risk is not reduced. Resources should mitigate the risk. At occurrence of other risks in the work, take immediate action.
7	**Maximum Risk**	Activities can not start until the risk is not reduced. If not immediately possible to decrease the level of risk, then work in this environment is PROHIBITED!

Table 6. Index of professional risk records

No. Doc	***Description of occupational risk factors identified the concrete form of manifestation***	***Workplace (subsystem)***
1.	Professional risk generated by the preparatory works.	″Coș de Fum - Zlatna″
2.	Professional risk generated by the execution of blasting works.	

3. Experimental

For applying the innovative methodological instrument on the demolition achieved by blasting combined with classical works of the objective ″Cos de Fum – Zlatna″, presented in Table 7, the following steps were taken (NUCLEU Project, 2014):

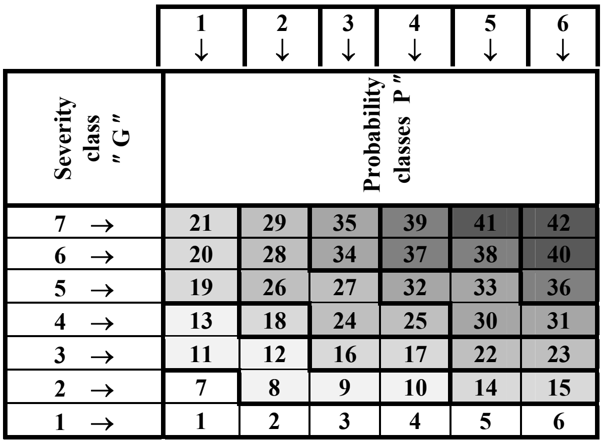
I. The general information on the objective subjected to demolition, from the documentation sent to INCD INSEMEX Petrosani for approval, which was developed by SC WEST OGS IMPEX SRL Timisoara, named Demolition achieved by blasting combined with classical works of the objective ″Cos de Fum – Zlatna″;

II. Identifying the factors of professional risk based on predetermined control lists;

III. Estimation and assessment of occupational risk to evaluate the level of risk in both cases;

IV. The analysis and reduction of professional risk that could be unacceptable;

V. The hierarchy of professional risk and the establishment of the appropriate measures for prevention and protection.



	1 ↓	**2 ↓**	**3 ↓**	**4 ↓**	**5 ↓**	**6 ↓**
Severity class ″G″	**Probability classes ″P″**					
7 →	**21**	**29**	**35**	**39**	**41**	**42**
6 →	**20**	**28**	**34**	**37**	**38**	**40**
5 →	**19**	**26**	**27**	**32**	**33**	**36**
4 →	**13**	**18**	**24**	**25**	**30**	**31**
3 →	**11**	**12**	**16**	**17**	**22**	**23**
2 →	**7**	**8**	**9**	**10**	**14**	**15**
1 →	**1**	**2**	**3**	**4**	**5**	**6**

Fig. 2. Matrix of professional risk assessment, built using numeric variables (matrix of professional risk analyzer)

Table 7. Form for analysis and risk reduction in professional controlled demolition of the industrial / civil with explosives for civil uses

<table>
<tr><td colspan="5">*ANALYSIS AND PROFESSIONAL RISK REDUCTION*</td></tr>
<tr><td colspan="5">**Economic agent:** SC WEST OGS IMPEX SRL
Headquarter: Timisoara/Romania
Workplace (subsystem): ″Cos de Fum – Zlatna″</td></tr>
<tr><td>**Document no. 1**</td><td colspan="2">**Risk: Medium**</td><td colspan="2">**Level: 4**</td></tr>
<tr><td colspan="5">1. Professional risk generated by the preparatory works.</td></tr>
<tr><td rowspan="2">**Hazard, determined:** Injury or disease of personnel during the preparatory works for controlled demolition of constructions using explosives.</td><td>**P**</td><td>**G**</td><td colspan="2">**Estimate / risk assessment R**</td></tr>
<tr><td>5</td><td>3</td><td>22</td><td>Medium</td></tr>
<tr><td colspan="5">**Identification of potential risk factor: $R_{ig}(p_i,g_i)=(\sum r_iR_i)/\sum r_i \rightarrow R_{ig}(5,3)=22$, i=1,10 (Medium risk)**
F1. Improper condition of access routes: $R_1(p_1,g_1) \rightarrow R_1(5,5)=33$
F2. Unprotected/deteriorated electrical plant: $R_2(p_2,g_2) \rightarrow R_2(2,7)=29$
F3. Obstacles present on the evacuation routes: $R_3(p_3,g_3) \rightarrow R_3(6,3)=23$
F4. Falls from height: $R_4(p_4,g_4) \rightarrow R_4(1,7)=21$
F5. Objects falling from height: $R_5(p_5,g_5) \rightarrow R_5(1,7)=21$
F6. Lack of specific elements PSI: $R_6(p_6,g_6) \rightarrow R_4(5,3)=22$
F7. Dark/narrow spaces: $R_7(p_7,g_7) \rightarrow R_5(5,3)=22$
F8. Harmful and toxic environment: $R_8(p_8,g_8) \rightarrow R_8(5,3)=22$
F9. Environment with extreme or variable temperatures: $R_9(p_9,g_9) \rightarrow R_9(4,2)=10$
F10. Improper lighting: $R_{10}(p_{10},g_{10}) \rightarrow R_{10}(4,2)=10$

Description of the risk factor identified concrete form of manifestation:
- The affecting of stability and solidity of access routes
- Direct touching of electrical plant
- Obstruction of evacuation routes for personnel
- Usage of improvised devices or inadequate scaffold
- Lack of insurance or supervision of objects left on different heights
- Removal of elements regarding FSC
- Inadequate ventilation
- Presence of gas, steam, dust and noise
- High temperatures during summer time and low during winter time
- Limited and insufficient natural lighting
Cause:
- Performance of preparatory works for a controlled demolition of industrial/ civil constructions subjected for decommissioning
Dysfunction:</td></tr>
</table>

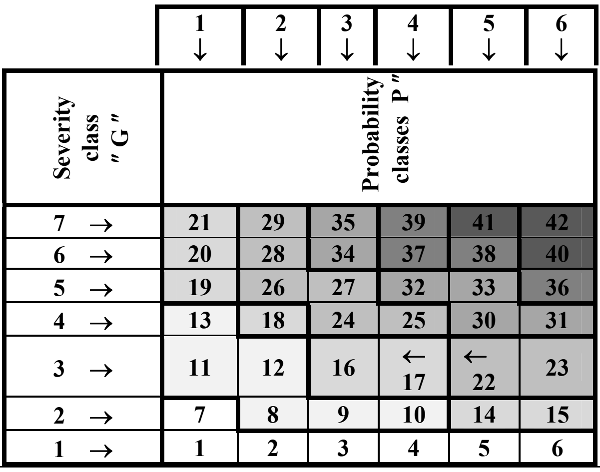
- Favoring the phenomenon of injury or professional disease as a result of exposure to the identified risks.

Technical and organizational measures possible:	References:
<u>Technical measures</u>: - Choosing safe walking routes - Verification and decoupling of electrical plant from the network - Verification of qualitative condition of air from the work environment - Suitable work and PPE equipment - Usage of proper equipment for measuring the quality of air from the work environment <u>Organisational measures</u>: - Proper training of personnel - Business bureaucracy - Proper coordination and allocation of personnel on jobs/ work posts	-Law 319/2006 for Safety and Health at work and the methodological implementing rules with further changes and additions -Law 126/1995 re-published and the technical implementing rules -Advised technical demolition documentation -OHS instructions -SR ISO 31000:2010 Risk management – Principles and guidelines

Residual hazard identification:	P	G	Estimate / risk assessment, R	
-Nature and configuration of constructions subjected to controlled demolition using explosives and the work environment related to it	4	3	17	Low

Residual risk:	Actions:
-The Performance of preparatory works for a controlled demolition of constructions using explosives, in terms of compliance of the organizational and technical measures specified above.	Reduction of risk from value 22 (medium risk) to value 17 (low risk), by applying organizational and technical measures.

Diagram of professional risk reduction



Severity class " G "	1 ↓	2 ↓	3 ↓	4 ↓	5 ↓	6 ↓
	Probability classes P"					
7 →	21	29	35	39	41	42
6 →	20	28	34	37	38	40
5 →	19	26	27	32	33	36
4 →	13	18	24	25	30	31
3 →	11	12	16	← 17	← 22	23
2 →	7	8	9	10	14	15
1 →	1	2	3	4	5	6

Document no. 2	Risk: High	Level: 5

2. Professional risk generated by the execution of blasting works.

Hazard, determined:	P	G	Estimate / risk assessment R	
Hazard, determined: Injury or disease of personnel during the blasting works for controlled demolition of constructions using explosives.	5	4	30	High

Identification of potential risk factor: $R_{ig}(p_i,g_i)=(\sum r_iR_i)/\sum r_i \rightarrow R_{ig}(5,4)=30,0$ (High risk)

F1. Improper use of explosives: $R_1(p_1,g_1)\rightarrow R_1(3,7)=35$

F2.Presence of energy sources, open fire, thunder, electromagnetic field: $R_2(p_2,g_2)\rightarrow R_2(3,7)=35$

F3. Lack of possibility to keep separate (in different storage spaces) the disruptive explosives and the initiation resources: $R_3(p_3,g_3)\rightarrow R_3(2,7)=29$

F4. Falls from height: $R_4(p_4,g_4)\rightarrow R_4(2,7)=29$

F5. Objects falling from height: $R_5(p_5,g_5)\rightarrow R_5(2,7)=29$

F6. Improper correlation or mismatch of work charge with qualified human potential: $R_6(p_6,g_6)\rightarrow R_6(3,6)=34$

F7. Incorrect execution of loading operation and achievement of the initiation circuit: $R_7(p_7,g_7)\rightarrow R_7(2,7)=29$

F8. Appearance of misfires and their elimination: $R_8(p_8,g_8)\rightarrow R_8(3,7)=35$

F9. Noise produced by performing blasting operations: $R_9(p_9,g_9)\rightarrow R_9(5,3)=22$

F10. Uncontrolled detonation (inside the initiation grids) on the use of detonated electric staple with low intensity, in the presence of foreign electric sources: $R_{10}(p_{10},g_{10})\rightarrow R_{10}(2,7)=29$

F11. Network interruption or the failure to initiate the charges from the holes, on the use of detonating fuse with reduced linear charge: $R_{11}(p_{11},g_{11})\rightarrow R_{11}(2,7)=29$

F12. Appearance of partial misfires as a result of delayed detection of fitting mistakes, in cases of using non-electric elements: $R_{12}(p_{12},g_{12})\rightarrow R_{12}(2,7)=29$

F13. Accidental detonation of explosive residues remained unexploded during the charge of rubbish (manually or mechanically): $R_{13}(p_{13},g_{13})\rightarrow R_{17}(2,7)=29$

F14. Illicit pernancy by the unauthorized personnel of the rest of unexploded material: $R_{14}(p_{14},g_{14})\rightarrow R_{14}(2,7)=29$

Description of the risk factor identified concrete form of manifestation:
- Lack of measures or ineffective measures for restricting the access to the blasting area
- Close distances towards the residential area
- Storage of explosives in improper conditions before the performance of blasting works
- Lack of lightning conductor and grounding ring for the explosive deposits
- Inadequate location of temporary storage deposits in the job site
- Usage of improvised devices or inadequate scaffold
- Lack of insurance or supervision of objects left on different heights
- Inadequate allocation of work tasks in relation to the training and potential of personnel
- Tiredness installed in different stages of the loading operation and performance of blasting network
- Performing additional operations required for clearing the misfire
- Inadequate control of work environment after blasting works
- Presence of gas, steam, dust and noise
- High temperatures during summer time and low during winter time

Cause:
- Performance of blasting works for a controlled demolition of civil/ industrial constructions subjected for decommissioning

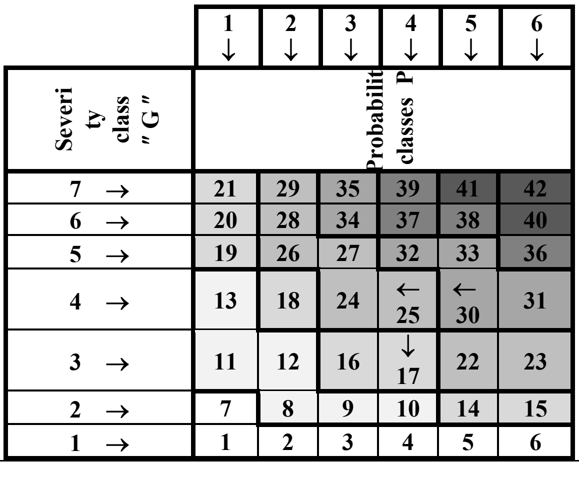
Dysfunction:
- Favoring the phenomenon of injury or professional disease as a result of exposure to the identified risks.

Technical and organizational measures possible:	**References:**
Technical measures: -The compliance of working technology found in the technical documentation for the advised demolition - Respecting OHS instructions Organizational measures: - Proper training of personnel - Business bureaucracy - Proper coordination and allocation of personnel on jobs/ work posts	- Law 319/2006 for Safety and Health at work and the methodological implementing rules with further changes and additions - Law 126/1995 re-published and the technical implementing rules - Advised technical demolition documentation - OHS Instructions - SR ISO 31000:2010 Risk management – Principles and guidelines

Residual hazard identification:	**P**	**G**	**Estimate / risk assessment, R**	
- Nature and configuration of all remains resulted from demolition constructions through blasting.	4	3	17	Low

Residual risk:	**Acțions:**
- Control of work environment after blasting works.	Reduction of risk from value 30 (high risk) to value 17 (low risk), by applying organizational and technical measures.

Diagram of professional risk reduction



	1 ↓	2 ↓	3 ↓	4 ↓	5 ↓	6 ↓
Severity class ″ G ″	**Probability classes P**					
7 →	21	29	35	39	41	42
6 →	20	28	34	37	38	40
5 →	19	26	27	32	33	36
4 →	13	18	24	← 25	← 30	31
3 →	11	12	16	↓ 17	22	23
2 →	7	8	9	10	14	15
1 →	1	2	3	4	5	6

From the occupational point of view, the main risks unique to the demolition activities of ″Cos de Fum - Zlatna″ using civil use explosives are the professional risk generated by the preparatory works and execution of blasting works.

4. Results and discussion

After the assessment of risk on the controlled demolition of ″Cos de Fum – Zlatna″ using explosives for civil uses were obtained the following results (Table 8).

5. Conclusions

The execution of blasting of civil and industrial constructions for buildings which must be dismantled, involves a series of operations which generate risks in health and safety domain (both for the employees of the economic operator performing the project, and also for humans living in the vicinity and the environment).

Table 8. Results of risk assessment

Appraisal and assessment of professional risk on preparatory works	
Improper condition of access routes	$R_1(p_1,g_1)\rightarrow R_1(5,5)=33$
Unprotected/ deteriorated electrical plant	$R_2(p_2,g_2)\rightarrow R_2(2,7)=29$
Obstacles present on the evacuation routes	$R_3(p_3,g_3)\rightarrow R_3(6,3)=23$
Falls from height	$R_4(p_4,g_4)\rightarrow R_4(1,7)=21$
Objects falling from height	$R_5(p_5,g_5)\rightarrow R_5(1,7)=21$
Lack of specific elements PSI	$R_6(p_6,g_6)\rightarrow R_4(5,3)=22$
Dark/ narrow spaces	$R_7(p_7,g_7)\rightarrow R_5(5,3)=22$
Harmful and toxic environment	$R_8(p_8,g_8)\rightarrow R_8(5,3)=22$
Environment with extreme or variable temperatures	$R_9(p_9,g_9)\rightarrow R_9(4,2)=10$
Improper lighting	$R_{10}(p_{10},g_{10})\rightarrow R_{10}(4,2)=10$
$R_{ig}(p_i,g_i)=(\sum r_iR_i)/\sum r_i\rightarrow R_{ig}(5,3)=22$ (Medium risk)	
Appraisal and assessment of professional risk on blasting works	
Improper use of explosives	$R_1(p_1,g_1)\rightarrow R_1(3,7)=35$
Presence of energy sources, open fire, thunder, electromagnetic field	$R_2(p_2,g_2)\rightarrow R_2(3,7)=35$
Lack of possibility to keep separate (in different storage spaces) the disruptive explosives and the initiation resources	$R_3(p_3,g_3)\rightarrow R_3(2,7)=29$
Falls from height	$R_4(p_4,g_4)\rightarrow R_4(2,7)=29$
Objects falling from height	$R_5(p_5,g_5)\rightarrow R_5(2,7)=29$
Improper correlation or mismatch of work charge with qualified human potential	$R_6(p_6,g_6)\rightarrow R_6(3,6)=34$
Incorrect execution of loading operation and achievement of the initiation circuit	$R_7(p_7,g_7)\rightarrow R_7(2,7)=29$
Appearance of misfires and their elimination	$R_8(p_8,g_8)\rightarrow R_8(3,7)=35$
Noise produced by performing blasting operations	$R_9(p_9,g_9)\rightarrow R_9(5,3)=22$
Uncontrolled detonation (inside the initiation grids) on the use of detonated electric staple with low intensity, in the presence of foreign electric sources	$R_{10}(p_{10},g_{10})\rightarrow R_{10}(2,7)=29$
Network interruption or the failure to initiate the charges from the holes, on the use of detonating fuse with reduced linear charge	$R_{11}(p_{11},g_{11})\rightarrow R_{11}(2,7)=29$
Appearance of partial misfires as a result of delayed detection of fitting mistakes, in cases of using non-electric elements	$R_{12}(p_{12},g_{12})\rightarrow R_{12}(2,7)=29$
Accidental detonation of explosive residues remained unexploded during the charge of rubbish (manually or mechanically)	$R_{13}(p_{13},g_{13})\rightarrow R_{17}(2,7)=29$
Illicit pernancy by the unauthorized personnel of the rest of unexploded material	$R_{14}(p_{14},g_{14})\rightarrow R_{14}(2,7)=29$
$R_{ig}(p_i,g_i)=(\sum r_iR_i)/\sum r_i\rightarrow R_{ig}(5,4)=30,0$ (High risk)	

From the occupational point of view, the main risks specific to the demolition of industrial/civil objectives using civil use explosives are the professional risk arising from the preparatory and blasting works. For the appraisal and assessment of risk on the demolition of industrial/civil objectives using civil use explosives, it has been designed an innovative methodological instrument for diagnosis and prognosis of professional risk specific to explosive activities.

This graphical analytical innovative instrument has as basis an analytical approach of the explicating concept of risk parameters, offering the possibility of listing the occupational risk (specific to demolition activities with explosives for civil use), both for evaluating levels, also in function by the position occupied within the same level.

From the research performed on controlled demolition of the objective ″Cos de Fum″, using civil use explosives, it can be appreciated that the obtained results clearly and objectively reflect the possibility to harm the workers engaged in hazardous activities and their health and safety.

References

Antonov A.E., Buica G., Beiu C., (2014), Management and control of occupational risk related to maintenance activities of work equipment in companies by using software tools, *Environmental Engineering and Management Journal*, **13**, 1361-1364.

Covello V.T., Merkhofer M.W., (1993), *Risk Assessment Methods. Approaches for Assessing Health and Environmental Risks*, Springer, Berlin-Heidelberg-New, Berlin-Heidelberg-New York.

Cruz M., (2004), *Operational Risk Modelling and Analysis. Theory and Practice*, Risk Book, Incisive Financial Publishing Ltd, London.

Conte J.C., Rubio E., Garcia A.I., Cano F., (2011) Occupational accidents model based on risk-injury affinity groups, *Safety Science*, **49**, 306-314.

Construction Phase Plan (Demolition), (2011), NSL/PF/018, Demolition Method Statement, Northem Safety Ltd, 109B, Allison Avenue, Teesside Industril Estate, Thornoby, Stockton on Tees.

CPCCDE 3016A, (2014), Identify hazards on demolition sites and apply risk management strategies, Construction & Property Services Industry Skills Council, Australian Government, January 2014.

Desroches A., (1995), *Concepts and Probabilistic Methods of Security Base* (in French), Lavoisier-TEC&DOC, Paris.

Kovacs M., Toth L., Gheţie G., Drăghici A., Vasiu T., Laurenţiu G., (2014), Best management practices applied to prevent and reduce concentrations of dust and gases released from power plants, *Environmental Engineering and Management Journal*, **13**, 1421-1426.

Lee H.S., Kim H., Park M., Ai Lin Teo E., Lee K.-P., (2012), Construction risk assessment using site influence factors, *Journal of Computing in Civil Engineering*, **26**, 319-330.

Law 319, (2006), Law No.319/2006 on occupational health and safety, *Romanian Official Monitor*, Part I, No. 646 from of 26th of July 2006.

Law 126, (1995), Law 126/1995 on the status of explosive materials, republished in 2014, *Romanian Official Monitor*, Part I, No. 177 from 12th of September, 2014.

Lupu L., Ghicioi E., Jurca A., Păun F., (2014), Ensuring security and environmental safety at blasting workplaces, *Environmental Engineering and Management Journal*, **13**, 1517-1522.

MacDonald E.B., Baranski B., Wilford J., (Eds.), (2000), *Occupational Medicine in Europe: Scope and Competencies*, WHO European Centre for Environment and Health, Bilthoven, On line at: http://www.who.int/occupational_health/regions/en/oeheuroccmedicine. pdf?ua=1.

Moraru R., Babut G., Matei I., (2002), *Professional Risk Assessment Guide*, Publisher FOCUS.

Moraru R.I., Băbuţ G.B., Cioca L.I., (2014), Rationale and criteria development for risk assessment tool selection in work environments, *Environmental Engineering and Management Journal*, **13**, 1371-1376.

Murè S., Demichela M., Piccinini N., (2006), Assessment of the risk of occupational accidents using a "fuzzy" approach, *Cognition, Technology and Work*, **8**, 103-112.

Nisipeanu S.E., Pece S., Ivan I.M., Chiurtu E.R., Haiducu M., Mănuc D., (2014), Application of specific models and software for identification, assessment and prevention of occupational risks in the romanian healthcare sector, *Environmental Engineering and Management Journal*, **13**, 1537-1541.

Nor Rizman Bin Abas, (2010), *Risk assessment for demolition works in Malaysia*, PhD Thesis, Faculty of Civil Engineering and Earth Resources, University Malaysia Pahang.

NUCLEU Project, (2014), Risk assessment of industrial demolition / civil with explosives for civil in order to prevent unwanted seismic effects induced, NUCLEU Research Program, Project PN 07 45 01 28, INSEMEX, 2014.

Papadopoulos G., Georgiadou P., Papazoglou C., Michaliou K., (2010), Occupational and public health and safety in a changing work environment: An integrated approach for risk assessment and prevention, *Safety Science*, **48**, 943-949.

Pece Şt., (1997), *Method for assessment of occupational safety in human-machine system*, PhD Thesis, University of Petrosani, Romania.

Platon S.N., Hionis C.A., (2014), Preventing risk of noise exposure in working environment using noise mapping, *Environmental Engineering and Management Journal*, **13**, 1349-1354.

Pollution Prevention Guidelines, (2012), *Working at Construction and Demolition Sites: PPG6, Pollution Prevention Guidelines 2012*, Environment Agency, Horizon House, 2nd edition.

Programme-Based Engagement, (2009), Technical Advisory for Demolition, WSH COUNCIL, Published by the Workplace Safety and Health Council and Ministry of Manpower.

Risk Management Guidelines, (2011), (RSA), RMG07-v05-02/11, Royal & Aliance Insurance plc.

Smid T., (2001), *Risk Perception and Risk Communication in Occupational Health*, Presentation at the occasion of the first EASOM Summer School, Dresden, On line at: http://www.easom.eu/index.php/past-summer-schools/13-summerschools/summerschools-detailed/18-summer-school-2001.

SR ISO 31000, (2010), Risk management - Principles and guidelines, On line at: https://www.theirm.org/media/886062/ISO3100_doc.pdf.

Vasilescu G.D., (2008a), *Unconventional Methods for Occupational Risk Analysis and Assessment* (in Romanian), INSEMEX Publishing House, Petroşani.

Vasilescu G.D., (2008b), *Probabilistic Calculation Methods Used for Industrial Risk Prognosis and Diagnosis* (in Romanian), INSEMEX Publishing House, Petroşani, Romania.

Vasilescu G.D., Pleşea V., Baciu C., (2008), Establishing the acceptable risk level in occupational accidents and diseases based on a formal analysis, *Environmental Engineering and Management Journal*, **7**, 785-789.

Vasilescu G.D., Ghicioi E., Drăghici A., Mija N., (2014), Risk assessment of whole-body vibrations generated by industrial activities with environmental impact, *Environmental Engineering and Management Journal*, **13**, 1453-1458.

Waddell G., Burton A.K., (2001), Occupational health guidelines for the management of low backpain at work: Evidence review, *Occupational Medicine*, **51**, 124-135.

Environmental Engineering and Management Journal

September 2016, Vol.15, No. 9, 2119-2126
http://omicron.ch.tuiasi.ro/EEMJ/

"Gheorghe Asachi" Technical University of Iasi, Romania

HETEROGENEOUS PHOTO-FENTON DEGRADATION OF QUINOLINE WITH A NOVEL INTERNAL CIRCULATING FLUIDIZED-BED REACTOR

Ran Zhang[1], Hong You[1*], Ting Liu[1], Donghai Wu[2], Weiming Wang[1]

[1]*State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, P.O. Box 2606, 202 Haihe Road, Harbin 150090, China*
[2]*Key Laboratory for Integrated Regulation and Resources Development on Shallow Lakes, China Ministry of Education, College of Environment, Hohai University, 1 Xikang Road, 210098 Nanjing, China*

Abstract

The removal of quinoline from synthetic wastewater by heterogeneous photo-Fenton in a novel internal circulating fluidized-bed reactor was investigated systematically. The flow field in the reactor was simulated by FLUENT software conjoined with tracer particles experiment, and the influence of reaction parameters such as initial quinoline concentration, catalysts dosage and H_2O_2 dosage were evaluated. Results indicated that the designed reactor was suitable for the heterogeneous photo-Fenton process, and the catalysts could be fully fluidized. Moreover, the quinoline degradation reactions were well described with pseudo first-order kinetics, the removal rate was negatively correlated with the initial concentration of quinoline, whereas properly increasing catalysts and H_2O_2 dosage showed a positive effect. The optimal dose of catalysts and H_2O_2 were 1.0 g/L and 14.70 mmol/L, respectively, and resulted in 94.2% removal efficiency after 120 min reaction. The results suggest that heterogeneous photo-Fenton in an internal circulating fluidized-bed reactor is potentially applicable for organic wastewater treatment.

Key words: FLUENT software, internal circulation fluidized-bed reactor, photo-Fenton, quinoline

Received: July, 2013; Revised final: July, 2013; Accepted: September, 2013

1. Introduction

Water pollution is a worldwide problem at present. Quinoline, a typical toxic and refractory nitrogen heterocyclic compound, is widely used as a raw material in different industries. Consequently, it has been frequently detected in the environment, which poses a threat to the ecosystems and organisms (Cermenati et al., 1997; Jing et al., 2012). The increase in stringent wastewater regulations and the increasingly water recycling demand efficient technologies for the quinoline degradation.

Photo-Fenton process has been recognized as one of the most efficient wastewater treatment technologies for high reaction yields with low treatment costs. Evidences have shown that the application of UV in combination with Fenton brings about enhanced formation of hydroxyl radicals (•OH), which are unselective and extremely reactive species (Modenes et al., 2012; Simion et al., 2015; Trovo et al., 2012b; Vilar et al., 2012; Zhong et al., 2012; Zhang et al., 2015). Moreover, compared with homogeneous photo-Fenton process the heterogeneous system has more advantages from the viewpoints of the reaction pH range and the removal of iron ions (Feng et al., 2006; Gonzalez-Olmos et al., 2012; Idel-aouad et al., 2011). However, efficient reactors for heterogeneous photo-Fenton and optimization of processing parameter are still two important barriers that impede its commercial.

In recent years, several types of reactors have been applied for photocatalytic treatment of

---

[*] Author to whom all correspondence should be addressed: e-mail: youhong@hit.edu.cn; Phone: +86 13304608828

wastewater (Chong et al., 2009; Chan et al., 2003; Favier et al., 2015; Ochuma et al., 2007; Tony et al., 2015). According to the catalysts state, these reactors can be classified into fixed-bed and slurry-type. The fixed-bed reactor is often associated with mass transfer limitation over the immobilised layer of photocatalysts. In contrast, the later type reactor usually performs a high total surface area of photocatalyst per unit volume (Pareek et al., 2008). Nevertheless, avoiding the separation of catalysts in effluent, and recirculation use of catalysts is critical for slurry reactors.

Compared to traditional slurry reactors, internal circulating fluidized-bed reactor prevents the use of a coagulation, flocculation or sedimentation unit to separate the catalyst particles from the treated water stream. Other benefits include further energy saving and size of process installation and site area required. Thus, internal circulating fluidized-bed reactor with direct light photon distribution may be a technically promising solution for solving the downstream separation and recycling use of photocatalyst particles.

In this paper, the effectiveness of heterogeneous photo-Fenton treatment for degradation of quinoline in a novel three-phase internal circulating fluidized-bed reactor was evaluated. The state of water, gas, and catalysts in the reactor were investigated systematically, and the effect of reaction parameters were examined and optimized.

2. Materials and methods

2.1. Materials

The commercial TiO_2/Al_2O_3 supporter was purchased from Yixing YiPu Catalyst Co., LTD. H_2O_2 (30%, w/w) were purchased from Tianjin Jizhun Co., LTD). Analytical grade quinoline was purchased from Tianjin Guangfu fine chemical industry research institute. The deionized water was utilized throughout the experiment. All other reagents used in the experiment are of analytical grade and used without further purification.

2.2. Catalysts preparation

Cu–Fe mixed oxides catalysts were prepared by the hydrothermal method, and the detailed process is described as follows. 20 g TiO_2/Al_2O_3 (40~60 mesh) carrier was first added into 500mL aqueous solution containing 0.1 mol/L $FeSO_4$ and 0.02 mol/L $CuSO_4$, and kept vigorous stirring for 2 h. Then 600mL of 0.2 mol/L NaOH aqueous solution was added dropwise. After been stoved for 40 h at 105°C in the drying oven, the solid products were separated and washed several times with deionized water until sulfate was washed out and dried at 80°C. The dried samples were calcined at 200°C for 5 h in a muffle furnace, and the bimetal oxidates catalysts were obtained.

2.3. Experimental setup

Experiments were conducted in a poly internal circulating fluidized-bed reactor (1070 mm height, 150 mm wide and 280-654 mm length) as shown in Fig. 1. Two vertical clapboards (150 mm wide, 587 mm height) divided the fluidized bed into four zones: up flow zone, sedimentation zone, down flow zone, and recirculation zone. To get the internal circulation and enhance the efficiency of solid - liquid separation, the sedimentation zone and recirculation zone were designed to be divergent. A total of 36 UV-C lamps (Philips, 11W) with an emission maximum at 254 nm were positioned horizontally and parallel to each other with an adjacent interval of 69 mm. Compressed air was sparged into reaction system by a micropore aeration titanium plate (aperture 10-100 μm) from the bottom of the reactor. Under the effect of air flotation and gravity, the catalysts and reaction liquid flow from the up flow zone to sedimentation zone firstly, and then reverse through the down flow zone and recirculation zone, creating an internal circulating flow within the reactor.

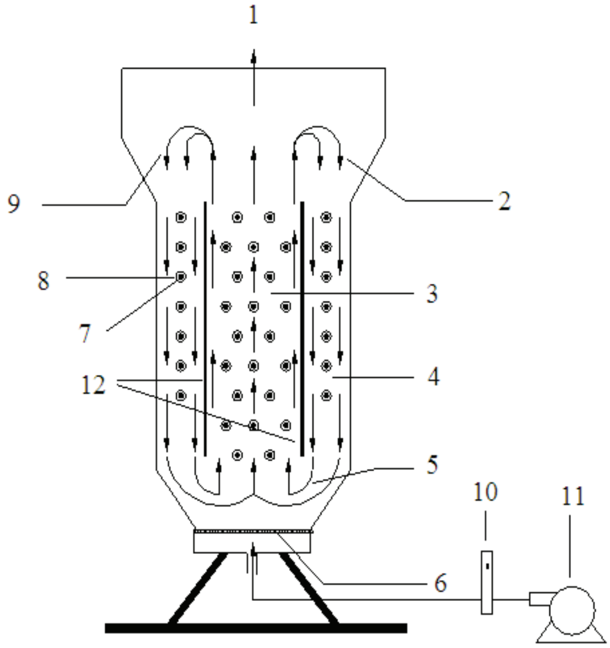


Fig. 1. Schematic diagram of internal circulation fluidized-bed reactor: (1) exhaust; (2) sedimentation zone; (3) up flow zone; (4) down flow zone; (5) recirculation zone; (6) aeration titanium plate; (7) UV lamp; (8) quartz tube; (9) recirculation water; (10) flow meter; (11) air compressor pump; (12) baffles

2.4. Heterogeneous photo-Fenton removal of quinoline

The heterogeneous photo-Fenton degradation of quinoline solutions was examined. Quinoline solution was filled in the reactor. At the beginning, Fe/Cu-TiO_2/Al_2O_3 catalysts and H_2O_2 (30%, w/w) were fed into the reactor, and the UV lamp power and compressed air supply were switched on simultaneously. This was considered as the initial time for reaction. Samples were withdrawn at time intervals for testing, and 0.1mol/L sodium sulfite was added as terminator. The reaction was carried out at room temperature (25±2°C) and natural pH. Batch experiments were performed to investigate the effects of initial quinoline concentration, catalysts dosage and H_2O_2 dosage.

The percentage of quinoline removal was calculated by following Eq. (1):

$$\text{Removal percentage} = (C_0 - C_t) / C_0 \qquad (1)$$

where, C_0 is the initial quinoline concentration, C_t is the quinoline concentration remaining at time t.

2.5. Analysis method

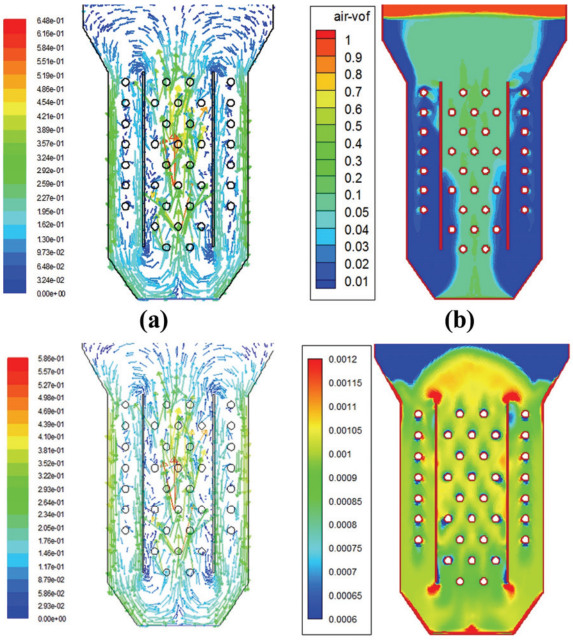
The flow field in the reactor was simulated by FLUENT software; The photos of tracer particles experiments were taken by Cannon EOS 30D camera with exposure time of 1/30 s; The oxidation state of Fe and Cu on the surface of catalysts was characterized by X-ray photo-electron spectroscopy (XPS) using a PHI 5700 spectrometer; The Brunauer–Emmett–Teller (BET) specific surface area was observed by adsorption of nitrogen at 77 K, by using automated volumetric adsorption instrument (model Quantachrome Autosorb-1); Quinoline concentrations were analyzed using a high performance liquid chromatography (HPLC) system (Bai et al., 2009); The total organic carbon reduction was measured with a TOC-VCPN analyzer (TOC-5000A, Shimadzu, Japan).

3. Results and discussion

3.1. Flow field in the reactor

The flow field in the internal circulating fluidized-bed reactor was stimulated by FLUENT software using Euler-Euler-Euler model, standard K - ε turbulence model, and Syamlal & O'Brien particle viscosity model (Ali et al., 2008; Ali and Pushpavanam, 2011; Kozic et al., 2011; Rahimzadeh et al., 2012; Zhao and Fernando, 2007). The solid phase was completely fluidized in the reactor, and its velocity distribution was similar to the liquid phase (Fig. 2). In the sedimentation zone, the solid holdup decreased with the increasing of height, and was much lower in sides than that in the middle, implying that the reactor was efficient for solid phase circulation.

Experiment using black tracer particles was also carried out to further reveal the flow field in the reactor, and results were consistent with the simulation (Fig. 3). The gas phase in the reactor was limited in the up flow zone for the compression by water from recirculation zone, and resulted in a good recirculation of liquid phase and solid phase (Fig. 3a and Fig. 3b). The simulated average solid velocity in the sedimentation zone was about 0.109 m/s (Fig. 3c), and the average tracer particles velocity in the experiment, which was calculated by camera exposure time and displacement of particles, was about 0.112 m/s (Fig. 3d).



(a) **(b)**

(c) **(d)**

Fig. 2. Three-phase simulation using FLUENT software (air flow = 1.62 m^3/h). (a) The velocity profile of liquid-phase; (b) The contour of air volume fraction; (c) The velocity profile of solid-phase; (d) The contour of solid volume fraction

Both the simulation and experiment results showed that solids near the reactor wall had faster settling rate than that nearby the baffle in the down flow zone, about 0.20 m/s and 0.15 m/s were detected, respectively (Fig. 3e and Fig. 3f). Compared to the down flow zone, solid velocities in the recirculation zone declined a little, about 0.07 to 0.15 m/s were obtained (Fig. 3g and Fig. 3h).

These observations suggest that the developed reactor was suitable for heterogeneous photo-Fenton process, and the reaction system could be fully fluidized. Compared with traditional cylindrical reactors, which hold UV lamps vertically in the center, scale-up of the reactor used in this work maybe more feasible as the reactor size would not be restricted by UV intensity and lamp length.

3.2. Degradation tests

The removal efficiency of quinoline by heterogeneous photo-Fenton process was compared to each individual unit process under identical conditions (Fig. 4). H_2O_2/catalyst and UV/catalyst tests just yielded 17% and 22% degradation at 120-min treatment, respectively. On the other hand, the removal percentages for 120-min UV/H_2O_2 or UV/H_2O_2/carrier tests were about 38%, implying that the carrier had no catalytic activity. In contrast, the presence of catalyst during UV/H_2O_2 treatment significantly enhanced the quinoline degradation, with 94% removal percentage after 120min.

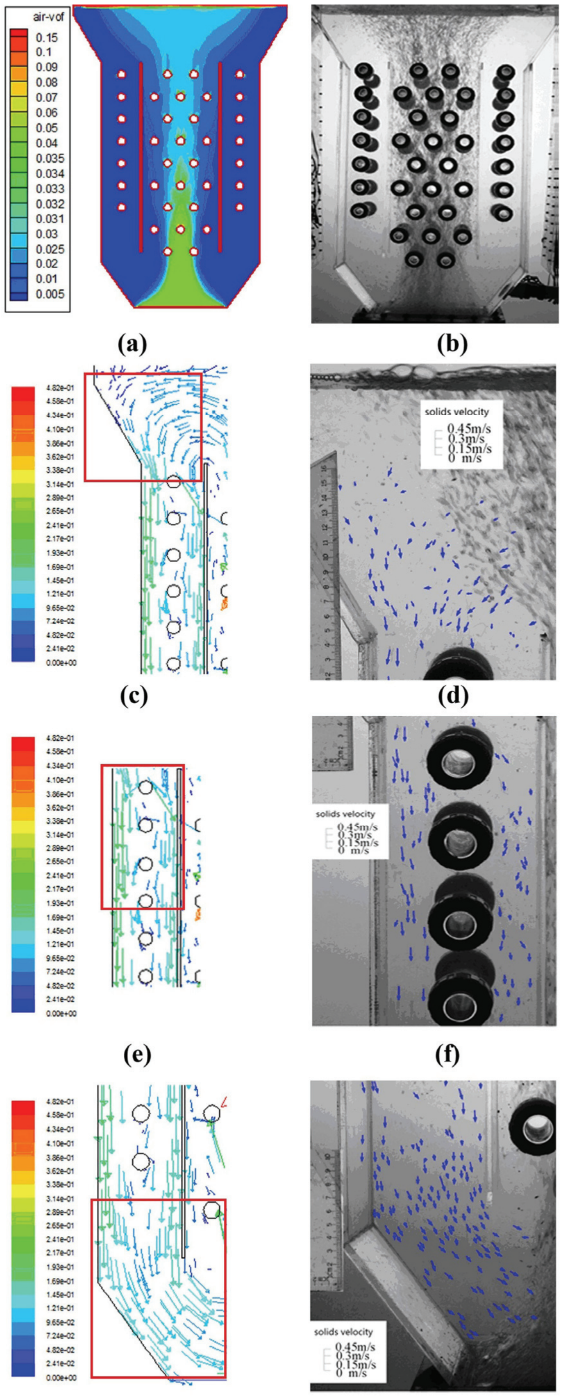


Fig. 3. The contrast between numerical simulation and experiment results (air flow = 0.75 m^3/h). Images of FLUENT simulation were labeled with a (integral), c (sedimentation zone), e (down flow zone), g (recirculation zone) and the corresponding tracer particles experiments photos were shown respectively in b, d, f, and h

The present experimental results showed that heterogeneous photo-Fenton process performed better than other processes assessed, and was efficient for quinoline degradation. The XPS and BET analysis showed that the prepared catalyst contains FeOOH, Fe_2O_3, CuO, and Cu_2O, with BET surface area of 144.2 m^2/g. As suggested earlier (Iara et al., 2008; Pham et al., 2013), in the photo-Fenton process iron oxides or copper oxides could serve as photocatalysts and H_2O_2 as oxidizing agent, thus producing more •OH in comparison with the UV/H_2O_2 or Fenton systems, which eventually leads to higher oxidation rates. In addition, it should be pointed out that air was employed in this work from the practical application point of view. Therefore, in the presence of UV light the oxygen in air might be transformed into reactive oxygen species, which participate efficiently in the degradation process with a different mechanism from •OH (Jing et al., 2012).

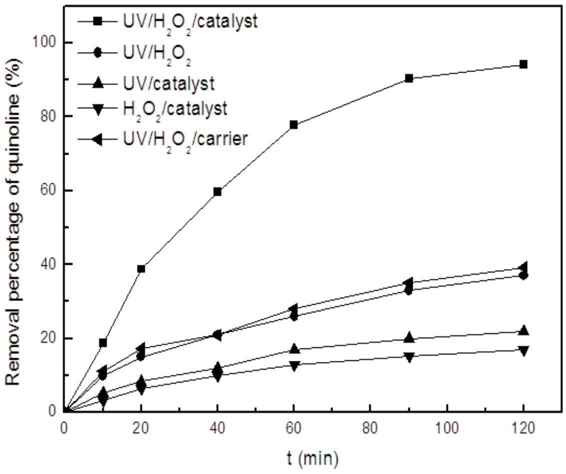


Fig. 4. Rremoval percentages of quinoline (catalysts dosage of 1.0 g/L; H_2O_2 of 14.70 mmol/L and initial quinoline concentration = 50 mg/L)

3.3. Effect of initial quinoline concentration

To evaluate the effect of initial quinoline concentration on the treatment efficiency of heterogeneous photo-Fenton process, a series of quinoline degradation experiments were carried out with catalysts dosage of 1.0 g/L and H_2O_2 of 14.70 mmol/L (Fig. 5).

It can be seen that the degradation rates fit a pseudo first-order model, and the observed rate constant k_{obs} is inversely proportional to the initial concentration of quinoline. The detected k_{obs} for initial concentration of 25 mg/L and 100 mg/L were 0.038 and 0.006 min^{-1}, respectively. The decrease of k_{obs} as increasing initial quinoline concentration indicates that there was a competition between the instantaneous intermediates and quinoline for the active sites on the catalysts.

3.4. Effect of H_2O_2 dosage

Select an optimal H_2O_2 dosage for the heterogeneous photo-Fenton reaction is important. The effect of H_2O_2 dosage on quinoline degradation was investigated by varying H_2O_2 dosage (7.35, 9.80, 12.25, 14.70, 19.60 mmol/L) (Fig. 6).

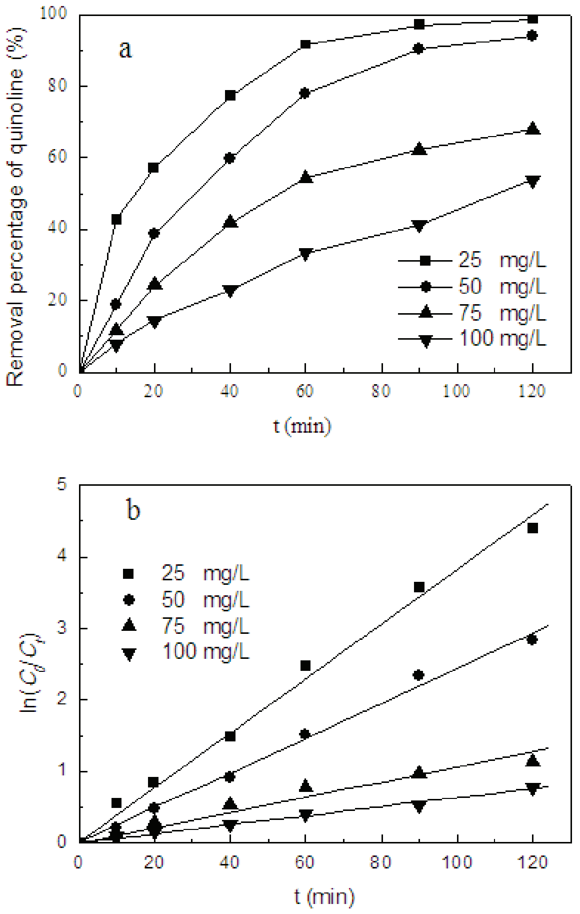


Fig. 5. Effect of initial quinoline concentration on quinoline degradation (catalysts dosage =1.0 g/L; H_2O_2 dosage =14.70 mmol/L)

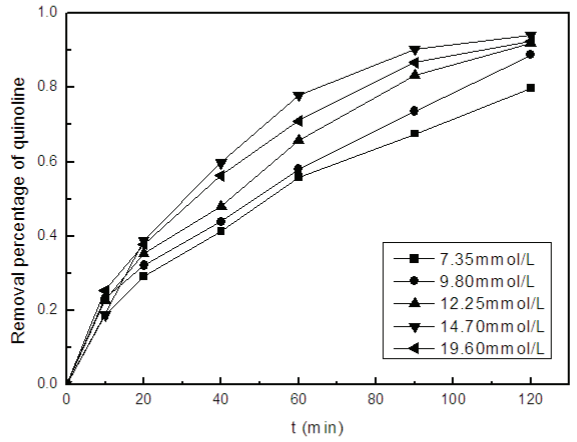


Fig. 6. Effect of H_2O_2 dosage on quinoline degradation (catalysts dosage =1.0 g/L; initial quinoline concentration = 50 mg/L)

The addition of H_2O_2 dosage improved treatment efficiency at first. When H_2O_2 dosage increased from 7.35 to 14.70 mmol/L, the quinoline removal percentages increased correspondingly from 79.7% to 94.2% after 120 min reaction. The increase in H_2O_2 concentration in the solution leads to an increase in the formation of •OH (Idel-aouad et al., 2011). However, when given more H_2O_2, the degradation rate was declined. This may be due to high concentration H_2O_2 acts as a scavenger of the •OH radicals, and would lead to a competition of •OH consumption between the substrate and H_2O_2 (Dutta et al., 2001).

3.5. Effect of catalysts dosage

To investigate the effect of catalysts dosage on the treatment efficiency of heterogeneous photo-Fenton process, a series of experiments were carried out by varying the catalysts dosage from 0 to 1.5 g/L with H_2O_2 of 14.70 mmol/L. It can be seen that the catalysts dosage had a significant influence on the degradation rate (Fig. 7). Similar to the effect of H_2O_2 dosage, the removal efficiency increased with the dosage of catalysts at first, then showed a plateau. After 120 min, only approximately 37% removal percentage was obtained without the presence of the catalyst, whereas about 80% removal was observed with catalysts dosage of 0.5 g/L. When the catalysts dosage was over 1.0 g/L, no significant difference was observed between various catalysts dosage reactions.

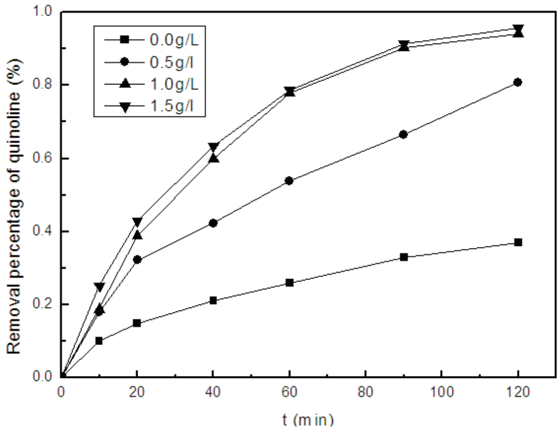


Fig. 7. Effect of catalysts dosage on quinoline degradation. (H_2O_2 dosage =14.70 mmol/L; quinoline concentration = 50 mg/L)

The influence of catalysts dosage on mineralization of quinoline was also investigated (Fig. 8). TOC removal percentage was positively correlated with catalysts dosage, which was consistent with degradation results. After 120 min, 26% and 61% removal were obtained for catalysts dosage of 0.5 and 1.5 g/L, respectively.

Heterogeneous photo-Fenton in the three-phase internal circulating fluidized-bed reactor avoided the outflow of catalyst, and realizes its recycle and reuse. Thus increased catalysts dosage improved the total surface area of catalysts, and provided more active sites, which could decompose H_2O_2 into high concentration of •OH radicals in solution. The enhanced formation of •OH radicals leads to higher oxidation rates. However, turbidity of suspension in the presence of excessive catalysts would decrease the penetration of UV-light, resulting in a loss of available radiation via light scattering (Iurascu et al., 2009). Consequently, the generation of effective photons was decreased and then the photo-catalytic activity would not be enhanced or even reduced.

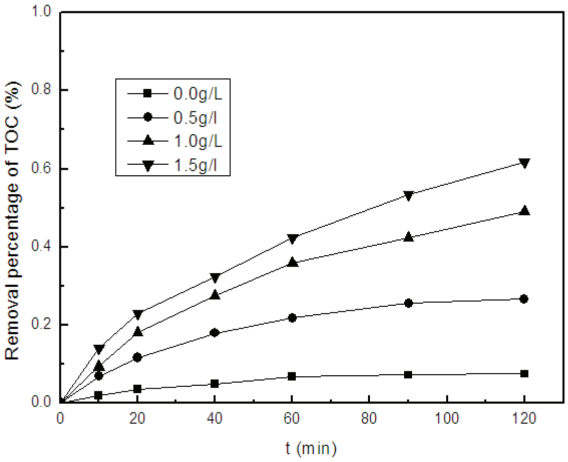


Fig. 8. Effect of catalysts dosage on quinoline mineralization (H_2O_2 dosage =14.70 mmol/L; quinoline concentration = 50 mg/L)

3.6. The stability of catalysts

The chemical stability is a very important feature for the catalyst when it is used in a practical industry application. The stability of the prepared catalysts was evaluated by the additional fresh quinoline solution with used catalyst from the previous run. In this procedure, the sample was reused over 10 times and its catalytic activities were evaluated by detecting the TOC remove of quinoline solution. As shown in Fig. 9, the catalyst can keep catalytic activities in the multiple runs and no noticeable loss of the activity was observed during the whole reaction process. It indicates that the activity of the prepared catalyst was stable.

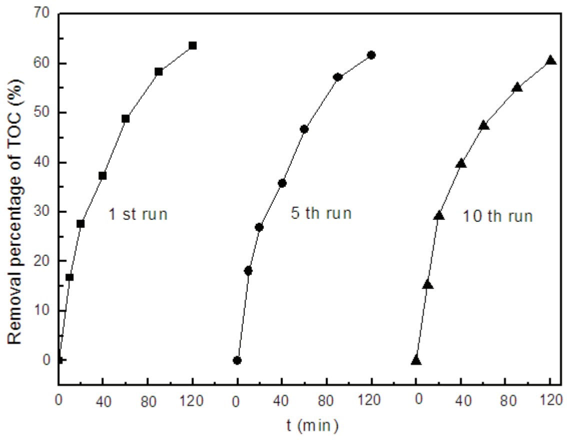


Fig. 9. Cyclic degradation of quinoline as a function of time (H_2O_2 dosage =14.70 mmol/L; quinoline concentration = 50 mg/L; catalysts dosage =1.0 g/L)

During the quinoline degradation reaction, iron and copper ions leached from the catalysts were also determined (Fig. 10). Results indicated that only very little iron and copper ions leached from the catalysts during the whole reaction. After 120 min, the detected concentrations of iron and copper ions were 0.089 and 0.019 mg/L, and the calculated leaching percentages were 0.030% and 0.016%, respectively. To further evaluate the influence of homogeneous photo-Fenton reaction owing to the iron and copper ions leaching from the catalyst, the simulated experiments were performed using iron and copper salts in similar concentration (0.1 mg/L Fe^{2+} and 0.02 mg/L Cu^{2+}) based on the maximal amount of metal leaching from catalysts.

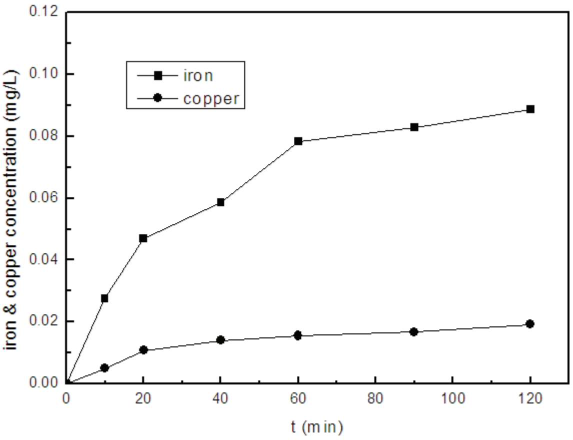


Fig. 10. Metal ion leaching concentration (catalysts dosage =1.0 g/L; H_2O_2 dosage =14.70 mmol/L, quinoline: concentration= 50 mg/L)

Only 38.0% degradation efficiency was obtained after 120min reaction, which is similar to the system without catalysts, suggesting that the homogeneous reaction could be ignored (Fig. 11). Therefore, in this study, the degradation of quinoline was mainly due to the heterogeneous photo-Fenton reaction.

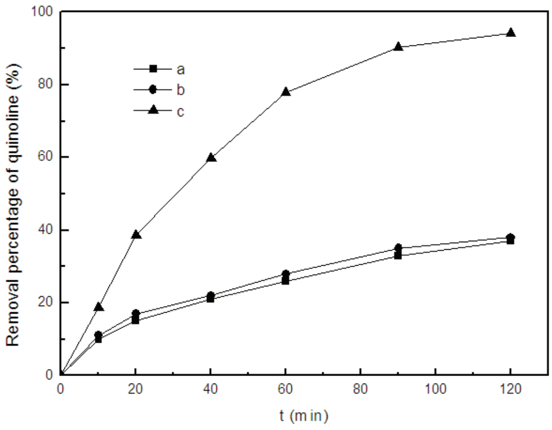


Fig. 11. The effect of homogeneous Fenton on quinoline degradation (a. without catalyst; H_2O_2 dosage =14.70 mmol/L; b. 0.1 mg/L Fe^{3+} and 0.02 mg/L Cu^{2+}, H_2O_2 dosage =14.70 mmol/L; c. catalysts dosage =1.0 g/L; H_2O_2 dosage =14.70 mmol/L; quinoline concentration = 50 mg/L)

4. Conclusions

This study established the heterogeneous photo-Fenton system with the internal circulating fluidized-bed reactor for quinoline degradation. Both the FLUENT simulation and tracer particles experimental results suggested that the designed reactor was suitable for the heterogeneous photo-Fenton reaction.

Compared to individual process, the combination of UV and homogeneous Fenton with Cu-Fe mixed oxides catalysts gives higher removal efficiency. The degradation rates fit a pseudo first-order model, and increased with decreasing initial quinoline concentration, whereas properly increasing catalysts and H_2O_2 dosage enhanced quinoline removal. The catalyst was stable and the effect of iron and copper ions leached from the catalysts could be ignored.

The heterogeneous photo-Fenton process using the internal circulating fluidized-bed reactor is efficient for organic wastewater treatment when considering cost/space constraints and the potential for catalysts recycle and reuse.

Acknowledgements

This work was financial support from the State Key S&T Special Projects (Grant No. 2012ZX07201003-01).

References

Ali B.A., Kumar C.S., Pushpavanam S., (2008), Analysis of liquid circulation in a rectangular tank with a gas source at a corner, *Chemical Engineering Journal*, **144**, 442-452.

Ali B.A., Pushpavanam S., (2011), Experimental and computational investigation of two phase gas-liquid flows: point source injection at the center, *Industrial & Engineering Chemistry Research*, **50**, 13220-13229.

Bai Y.H., Sun Q.H., Zhao C., Wen D.H., Tang X.Y., (2009), Simultaneous biodegradation of pyridine and quinoline by two mixed bacterial strains, *Applied Microbiology and Biotechnology*, **82**, 963-973.

Chan A.H.C., Chan C.K., Barford J.P., Porter J.F., (2003), Solar photocatalytic thin film cascade reactor for treatment of benzoic acid containing wastewater, *Water Research*, **37**, 1125-1135.

Chong M.N., Lei S.M., Jin B., Saint C., Chow C.W.K., (2009), Optimisation of an annular photoreactor process for degradation of Congo Red using a newly synthesized titania impregnated kaolinite nano-photocatalyst, *Separation and Purification Technology*, **67**, 355-363.

Cermenati L., Pichat P., Guillard C., Albini A., (1997), Probing the TiO_2 photocatalytic mechanisms in water purification by use of quinoline, photo-Fenton generated OH·radicals and superoxide dismutase, *The Journal of Physical Chemistry B*, **101**, 2650-2658.

Dutta K., Mukhopadhyay S., Bhattacharjee S., Chaudhuri B., (2001), Chemical oxidation of methylene blue using a Fenton-like reaction, *Journal of Hazardous Materials*, **84**, 57-71.

Favier L., Simion A.L., Rusu L., Pacala M.L., Grigoras C., Bouzaza A., (2015), Removal of an organic refractory compound by photocatalysis in batch reactor-kinetic studies, *Environmental Engineering and Management Journal*, **14**, 1327-1338.

Feng J.Y., Hu X.J., Yue P.L., (2006), Effect of initial solution pH on the degradation of Orange II using clay-based Fe nanocomposites as heterogeneous photo-Fenton catalyst, *Water Research*, **40**, 641-646.

Gonzalez-Olmos R., Martin M.J., Georgi A., Kopinke F.D., Oller I., Malato S., (2012), Fe-zeolites as heterogeneous catalysts in solar Fenton-like reactions at neutral pH, *Applied Catalysis B-Environmental*, **125**, 51-58.

Iara R.G., Luiz C.A.O., Paulo F.Q., Teodoric C.R., Marcio P., Jose D.F., Jose D.A., (2008), Modified goethites as catalyst for oxidation of quinoline: Evidence of heterogeneous Fenton process, *Applied Catalysis A*: General, **347**, 89-93.

Idel-aouad R., Valiente M., Yaacoubi A., Tanouti B., Lopez-Mesas M., (2011), Rapid decolourization and mineralization of the azo dye CI Acid Red 14 by heterogeneous Fenton reaction, *Journal of Hazardous Materials*, **186**, 745-750.

Iurascu B., Siminiceanu I., Vione D., Vicente M.A., Gil A., (2009), Phenol degradation in water through a heterogeneous photo-Fenton process catalyzed by Fe-treated laponite, *Water Research*, **43**, 1313-1322.

Jing J.Y., Li W.Y., Adina B., Zhang Y., Vicki L.C., William W.Y., (2012), Photocatalytic degradation of quinoline in aqueous TiO_2 suspension, *Journal of Hazardous Materials*, **237-238**, 247-255.

Kozic M.S., Ristic S.S., Puharic M.A., Katavic B.T., (2011), Numerical simulation of multiphase flow in ventilation mill and channel with louvers and centrifugal separator, *Thermal Science*, **15**, 677-689.

Modenes A.N., Espinoza-Quinones F.R., Manenti D.R., Borba F.H., Palacio S.M., Colombo A., (2012), Performance evaluation of a photo-Fenton process applied to pollutant removal from textile effluents in a batch system, *Journal of Environmental Management*, **104**, 1-8.

Ochuma I.J., Fishwick R.P., Wood J., Winterbottom J.M., (2007), Optimisation of degradation conditions of 1,8-diazabicyclo 5.4.0 undec-7-ene in water and reaction kinetics analysis using a cocurrent downflow contactor photocatalytic reactor, *Applied Catalysis B-Environmental*, **73**, 259-268.

Pareek V., Chong S.H., Tade M., Adesina A.A., (2008), Light intensity distribution in heterogenous photocatalytic reactors, *Asia-Pacific Journal of Chemical Engineering*, **3**, 171-201.

Pham A.N., Xing G.X., Christopher J.M., David W., (2013), Fenton-like copper redox chemistry revisited: Hydrogen peroxide and superoxide mediation of copper-catalyzed oxidant production, *Journal of Catalysis*, **301**, 54-64.

Rahimzadeh H., Maghsoodi R., Sarkardeh H., Tavakkol S., (2012), Simulating flow over circular spillways by using different turbulence model, *Engineering Applications of Computational Fluid Mechanics*, **6**, 100-109.

Simion V.A., Cretescu I., Lutic D., Luca C., Poulios I., (2015), Enhancing the fenton process by UV light applied in textile wastewater treatment, *Environmental Engineering and Management Journal*, **14**, 595-600.

Tony M.A., Purcell P.J., Zhao Y.Q., Tayeb A.M., El-Sherbiny M.F., (2015), Kinetic modeling of diesel oil wastewater degradation using photo-Fenton process, *Environmental Engineering and Management Journal*, **14**, 11-16.

Trovo A.G., Nogueira R.F.P., Aguera A., Fernandez-Alba A.R., Malato S., (2012), Paracetamol degradation intermediates and toxicity during photo-Fenton treatment using different iron species, *Water Research*, **46**, 5374-5380.

Vilar V.J.P., Moreira F.C., Ferreira A.C.C., Sousa M.A., Goncalves C., Alpendurada M.F., Boaventura R.A.R., (2012), Biodegradability enhancement of a pesticide-containing bio-treated wastewater using a solar photo-Fenton treatment step followed by a biological oxidation process, *Water Research*, **46**, 4599-4613.

Zhang H., Fu H., Wang Y., Chen L., (2015), Decolorization of orange ii by heterogeneous Fenton process using goethite as catalyst, *Environmental Engineering and Management Journal*, **14**, 737-744.

Zhao Z.H., Fernando H.J.S., (2007), Numerical simulation of scour around pipelines using an Euler-Euler coupled two-phase model, *Environmental Fluid Mechanics*, **7**, 121-142.

Zhong Y.H., Liang X.L., Zhong Y., Zhu J.X., Zhu S.Y., Yuan P., He H.P., Zhang J., (2012), Heterogeneous UV/Fenton degradation of TBBPA catalyzed by titanomagnetite: Catalyst characterization, performance and degradation products, *Water Research*, **46**, 4633-4644.

Environmental Engineering and Management Journal

September 2016, Vol.15, No. 9, 2127-2133
http://omicron.ch.tuiasi.ro/EEMJ/

"Gheorghe Asachi" Technical University of Iasi, Romania

REMOVAL OF PHTHALIC ACID DIESTERS IN THE MUNICIPAL SOLID WASTE INCINERATION PLANT LEACHATE TREATMENT PROCESS

Hongyuan Liu[1], Yixuan Chu[2], Chengran Fang[2]*

[1]College of Civil Engineering and Architecture, Zhejiang University of Technology, Hangzhou 310014, China
[2]Key Laboratory of Recycling and Eco-treatment of Waste Biomass of Zhejiang Province, Zhejiang University of Science and Technology, Hangzhou 310023, China

Abstract

The removal of phthalic acid diesters PAEs, specifically di-*n*-butyl phthalate (DBP) and di(2-ethylhexyl) phthalate (DEHP), from leachates of a municipal solid waste incineration plant treatment process was studied. The leachates originated from the adjusting unit, the biochemical unit, the ultrafiltration membrane unit, and the reverse osmosis membrane unit. The initial concentrations of DBP and DEHP were 250.5 and 332.3 μg/L, respectively, which were correspondingly reduced to 4.2 and 2.2 μg/L in the effluent, representing 98.3% removal of DBP and 99.3% removal of DEHP. DBP was removed stepwise during the process, mainly by degradation by microorganisms. Approximately 70.1% of the DEHP was removed by the membrane processes, owing to physical retention of the compound by the membranes. Large amounts of PAEs can accumulate over long periods of time in the concentrated leachate product, which may increase the difficulty of further leachate treatment and create greater environmental issues.

Key words: di-*n*-butyl phthalate, di(2-ethylhexyl) phthalate, leachate, removal efficiency

Received: January, 2016; Revised final: September, 2016; Accepted: September, 2016

1. Introduction

Phthalic acid diesters (PAEs) are a class of organic compounds, which are widely used in plastics, coatings, and cosmetics. Some of PAEs are suspected mutagens and carcinogens (Mo et al., 2008; Wu et al., 2011). The US Environmental Protection Agency and the China National Environmental Monitoring Center have classified PAEs as a top-priority environmental pollutant (Lu et al., 2009; Wu et al., 2011). Di-*n*-butyl phthalate (DBP) and di(2-ethylhexyl) phthalate (DEHP) are the most commonly PAEs. They are used mainly as coalescing aid in latex adhesive and plasticiare for plastics (Lu et al., 2012; Wang et al., 2012). Therefore, DBP and DEHP have been frequenly identified in various environmental samples including river water, groundwater, seawater, soil, sediments, and biota (Cai et al., 2007; Chang et al., 2007; Fang et al., 2015; Liang et al., 2008; Liu et al., 2010; Wang et al., 2008; Xu et al., 2008).

Because PAE-containing synthetic products (such as plastics, cosmetics, and paints) are disposed of in municipal solid waste (MSW) landfills, high concentrations of DBP and DEHP have been detected in leachates from MSW landfill sites (Asakura et al., 2004; Fang et al., 2009; Zheng et al., 2009). Studies focused on their removal from such leachates have included conventional biological treatments, advanced oxidation processes, newly developed processes, and some combined process (Boonnorat et al., 2014; Cui et al., 2012; He et al., 2009; Li et al., 2014; Lu et al., 2012; Özyaka et al., 2015; Zhang and Wang, 2009; Zheng et al., 2009). Removal efficiencies for DBP and DEHP differ significantly for the different leachate treatment processes. Zheng

---

* Author to whom all correspondence should be addressed: e-mail: fangchengr@163.com; Phone: +86-571-85070529; Fax: +86-571-85070529

et al. (2009) found that less than 32% of PAEs could be removed using a coagulation and flocculation process and their removal was related to the molecular weight of dissolved organic matter in the leachate. He et al. (2009) found that more than 40% of DBP and DEHP were removed from the leachate by the Fenton treatment. A combined landfill leachate biotreatment process was found to reduce the DBP and DEHP concentrations in leachate by up to 92.9% and 95.2%, respectively (Lu et al., 2012).

Existing studies have, however, mainly focused on leachates from landfill sites and few have been conducted on the leachate from MSW incineration plants. In addition, previous studies were often conducted by retrieving leachate from a landfill and treating it in a laboratory-scale process. The removal of DBP and DEHP from real leachates produced by MSW incineration plants has not been considered. Additionally, some leachate treatment processes that exhibit high efficiencies for DBP and DEHP removal at a laboratory scale may not be applicable to real sites. The transformation and removal of DBP and DEHP from real leachates is therefore worthy of study. The objectives of the present study were to investigate the removal of DBP and DEHP from MSW incineration plant leachates and to discuss their removal mechanisms and risks. Leachate was sampled from each treatment unit in a typical MSW incineration plant in China.

2. Materials and methods

2.1. Reagents

The dichloromethane and methanol used in this work were of high-performance liquid chromatography grade, while all other reagents were of analytical grade. Water was generated using a Milli-Q system. DBP and DEHP (both ≥ 99%) and all other reagents including dichloromethane and methanol were obtained from Tianjin Siyou Co. (Tianjin, China). The physical properties of DBP and DEHP are listed in Table 1.

2.2. Sample collection

The leachate samples were collected from a waste incineration plant in Shanghai that had been operating since July 2014. The sampling sites included the raw leachate in the adjusting pool (ADJ) (i.e., the feed to the leachate treatment process), and the effluents from the biochemical pool (BIO), the ultrafiltration membrane unit (UFM), and the reverse osmosis membrane unit (ROM). Leachate treatment capacity was 600 ton each day. The BIO system consisted of two reactors. The first anaerobic reactor was Up-flow Anaerobic Sludge Blanket (UASB) and had a total volume of 4200 m^3. The second aerobic reactor was Membrane Bioreactor (MBR) and had a total volume of 1800 m^3. The hydraulic retention time (HRT) in UASB and MBR were maintained at 7 day and 3 day respectively. The effluents from the BIO were further treated by UFM and ROM. The diagram of leachate treatment process is shown in Fig. 1. The leachate was collected using clean glass bottles and then promptly transferred to dark flasks. These samples were immediately sent to the laboratory for storage at 4°C. Chemical analyses were carried out in triplicate during the subsequent 2 days.

2.3. Sample extraction

ODS-C_{18} cartridges (500 mg × 3 mL, Supelco) were conditioned with 6 mL dichloromethane and 6 mL methanol, and then with 3 × 6 mL pure water. Leachate samples (50 mL) were then passed through the activated cartridge at a flow rate of 20 mL/min. The cartridges were dried by pumping air through them for 30 min. The extracted compounds were eluted with 2 × 3 mL of a 1:9 (v:v) mixture of dichloromethane:methanol, and the eluate was concentrated to 1.0 mL under a gentle nitrogen stream.

2.4. Analytical methods

The conventional leachate indices were determined using standard methods (EPA of China, 1989). The target phthalic acid esters were analyzed by high-performance liquid chromatography (Waters e2695). The extracted samples were injected onto an Eclipse XDB-C18 column (4.6 × 250 mm) using a methanol:water (90:10 v:v) mixture at a flow rate of 1.0 mL/min as the mobile phase and determined using an ultraviolet (UV) detector set to 242 nm.

Table 1. Physical properties of the two phthalate esters (Staples et al., 1997)

Compound	*Phthalate ester*	*Alkyl chain length*	*Log K_{ow}*	*Water solubility (mg/L)*
DBP	Di-*n*-butylphthalate	4	3.74-5.15(4.45)^a	1.5-13(11.2)
DEHP	Di(2-ethylhexyl)phthalate	8	4.20-8.39(7.50)	0.00024-0.09(0.0003)

^{a}( ) values of reference materials

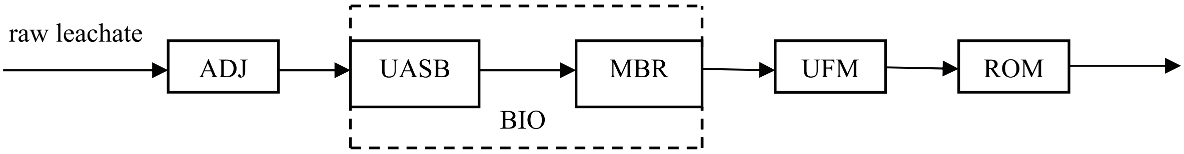


Fig. 1. Diagram of leachate treatment process

Table 2. Properties of raw leachate from the waste incineration plant
(n = 3; reported in mg/L except for pH and chromaticity)

Parameter	***Raw leachate***	***Concentrated leachate***^{a}	***Parameter***	***Raw leachate***	***Concentrated leachate***
pH	5.92	6.74	Cu	0.74	1.71
Chromaticity	800	1600	Zn	1.83	0.475
Volatile phenol	6.5	0.54	Ni	0.76	1.48
SS	2400	40	Cd	0.23	1.15
COD	120 224	2171	Pb	0.80	0.21
BOD_5	50 681	104	Fe	13.7	3.71
TN	226	73.3	Cr (VI)	0.10	0.02
NH_4^+-N	44.4	26.2	Cr (Total)	0.17	0.16
TP	25.4	6.94	Ca	25.5	368
CN^-	0.114	0.083	Mg	416	800
SO_4^{2-}	670	1391	DMP	ND	ND
As	0.012	26.7	DBP	0.25	0.08
Mn	2.46	10.07	DEHP	0.33	0.17

^{a}*Concentrated leachate was generated from the reverse osmosis membrane process (ROM); ND: not detected*

The recoveries of DBP and DEHP were 84.2 to 98.7% and 83.2 to 102.5%, respectively, while their detection limits were 0.1 and 0.2 μg/L, respectively.

3. Results and discussion

3.1. Characteristics of the raw leachate

The physicochemical characteristics of the raw leachate sample are presented in Table 2. These values fall in similar ranges to those of previous studies (Calli et al., 2006; Reinhart and Grosh, 1998). Chemical oxygen demand (COD) of the raw leachate was very high, with a value of 120 200 mg/L. The pH of the raw leachate was below neutral. The ratio of biological to chemical oxygen demand (BOD_5/COD) was around 0.4, which reveals its recalcitrant characteristics. Three types of PAEs-dimethyl phthalate (DMP), DBP, and DEHP were detected in this leachate, but DMP could not be quantified as it was below the detection limit of the analytical technique. The concentration of DEHP (332.3 μg/L) was a little higher than that of DBP (250.5 μg/L).

3.2. Removal of leachate contaminants during the steps of the leachate treatment process

A biological treatment is used to treat the leachate from the adjusting pool owing to its high values of COD and BOD_5. The effluent is then sequentially treated by UFM and ROM. As shown in Fig. 2, the removal efficiencies of COD, BOD_5, and SS from the leachate by the biological treatment were 94.7%, 95.6%, and 8.3%, respectively, but this process had no effect on chromaticity. The chromaticity removal efficiency achieved 50.0% after the UFM treatment.

Following the ROM treatment, removal efficiencies of approximately 99% from the leachate were achieved for COD, BOD_5, SS, and chromaticity, with respective values of 89, 26, and 15 mg/L, and 2, all of which meet the standard for pollution control for MSW incineration (China's Environmental Protection Department, 2014) (18).

From Fig. 3, it can been seen that the removal efficiencies of NH_4^+-N, total nitrogen (TN), total phosphorus (TP), and volatile phenol content of the leachate were 98.0%, 88.7%, 90.9%, and 99.4% respectively, with corresponding values of 0.9, 25.5, 2.3, and 0.04 mg/L after the leachate had been processed through all of the treatments. The values of all these parameters also meet the standards for pollution control for MSW incineration.

3.3. Removal of PAEs by leachate treatment process

The mean concentrations of DBP and DEHP in the leachate decreased from 250.6 to 4.2 μg/L and from 332.3 to 2.2 μg/L, respectively, after the leachate treatment, which included the ADJ, BIO, UFM, and ROM units (Fig. 4). This indicates that this treatment process has high simultaneous removal efficiencies for the two pollutants. DBP was degraded in a stepwise manner on passing through each unit, achieving 64.0% removal efficiency in the biological treatment unit, and up to 98.3% removal in the ROM effluent.

The removal efficiency of DEHP was not as high in the biological treatment process, achieving only 29.0%, but the overall removal improved to 83.7% after the UFM treatment unit, and eventually achieving 99.3% removal in the ROM effluent. Although there is no specification for PAE concentrations in the pollution control standard for MSW incineration, the DBP and DEHP concentrations in the final effluent were lower in this study than those of other studies (Boonnorat et al., 2014; Cui et al., 2012; Zhang and Wang, 2009). This confirms that DBP and DEHP present in the leachate are effectively removed in a leachate treatment process that includes the unit operations of ADJ, BIO, UFM, and ROM.

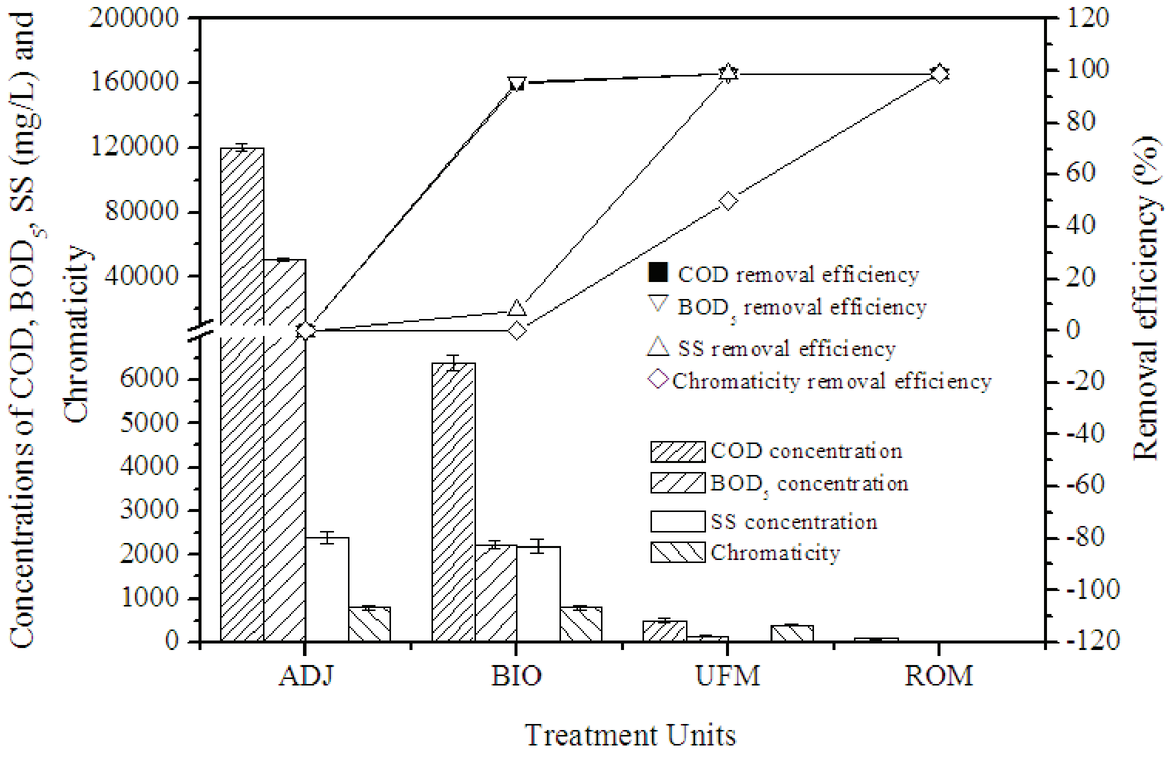


Fig. 2. Effects of leachate treatment process on COD, BOD$_5$, SS, and chromaticity of leachate

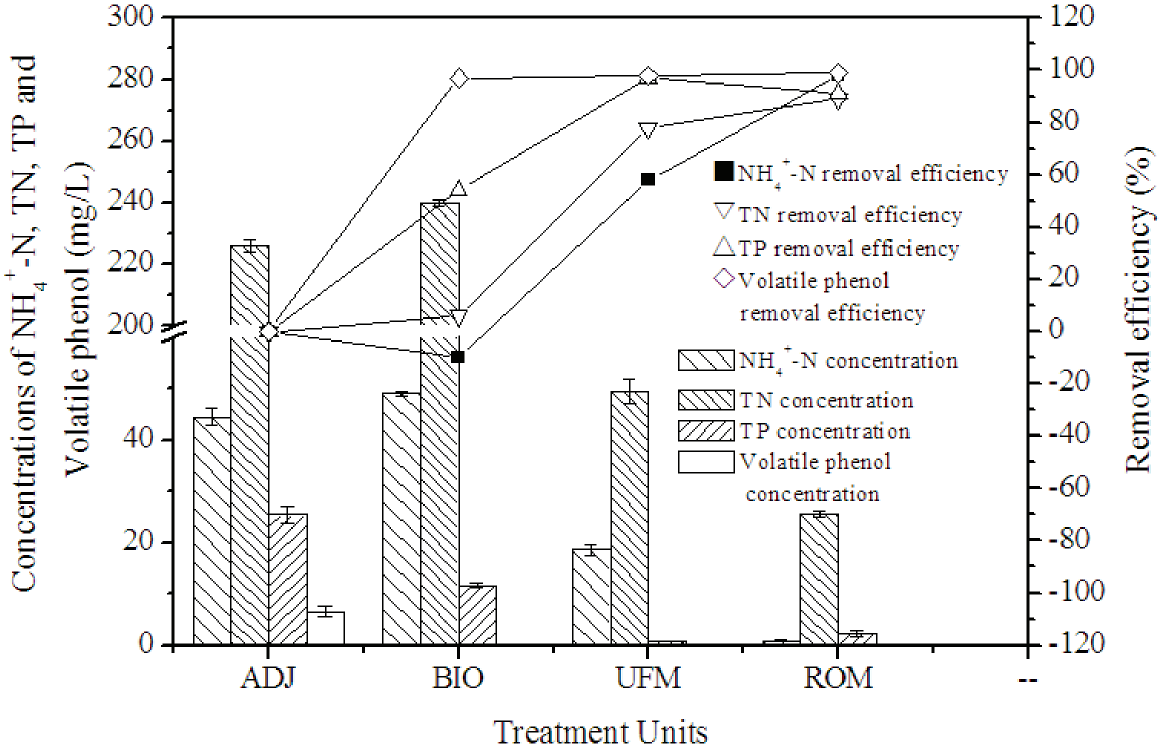


Fig. 3. Effects of leachate treatment process on NH$_4^+$-N, TN, TP, and volatile phenol content of leachate

3.4. Removal pathway, mechanism, and risk of phthalic acid esters

The percentage removal of DBP and DEHP in each of the leachate treatment units is shown in Fig. 5. The individual extents of DBP removal by the BIO, UFM, and ROM units were 64.2%, 7.0%, and 27.1%, respectively, leaving approximately 1.7% of the DBP in the ROM effluent. The extents of DEHP removal by the BIO, UFM, and ROM units were 29.1%, 54.5%, and 15.6%, respectively; 0.8% of the DEHP was not removed. It is evident that a certain amount of DBP was removed in each of the treatment units; however, the extent of removal by the biological treatment was much higher than that by the other two processes. UFM was least effective in DBP removal but resulted in much greater removal of DEHP, although the extent of removal of this compound was still lower than that achieved by the BIO unit.

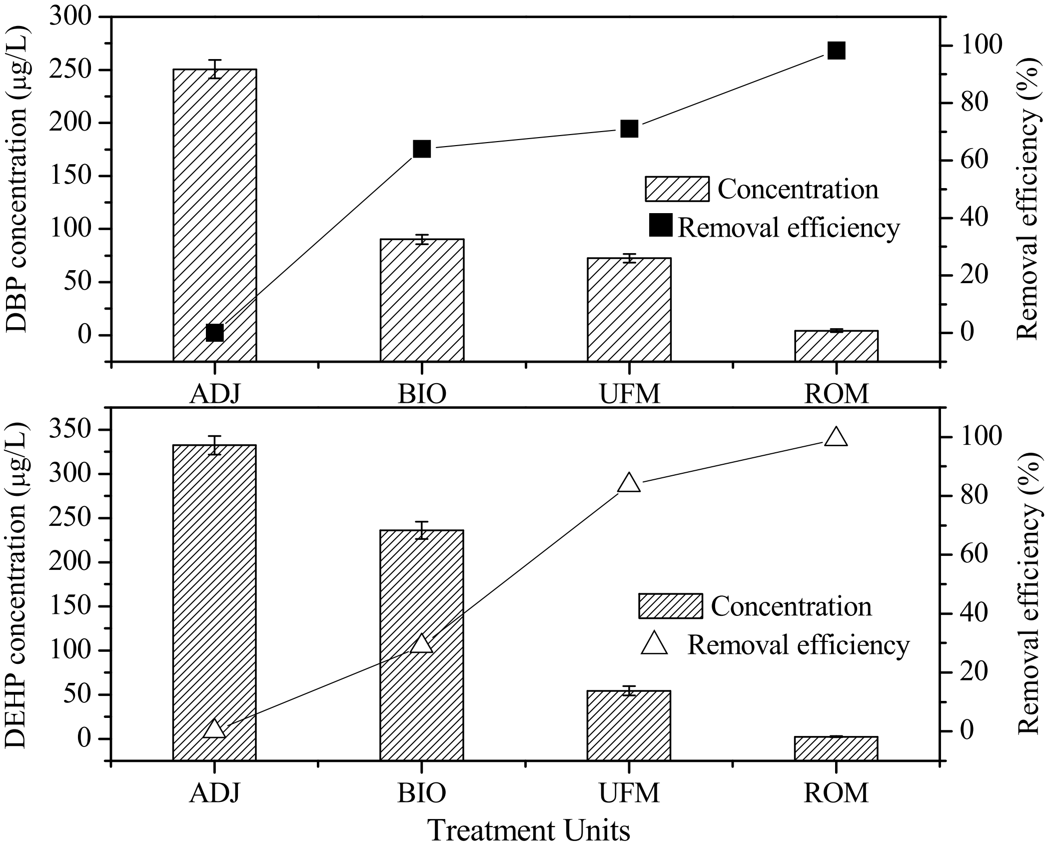


Fig. 4. Concentration and removal efficiency of DBP and DEHP in each leachate treatment unit

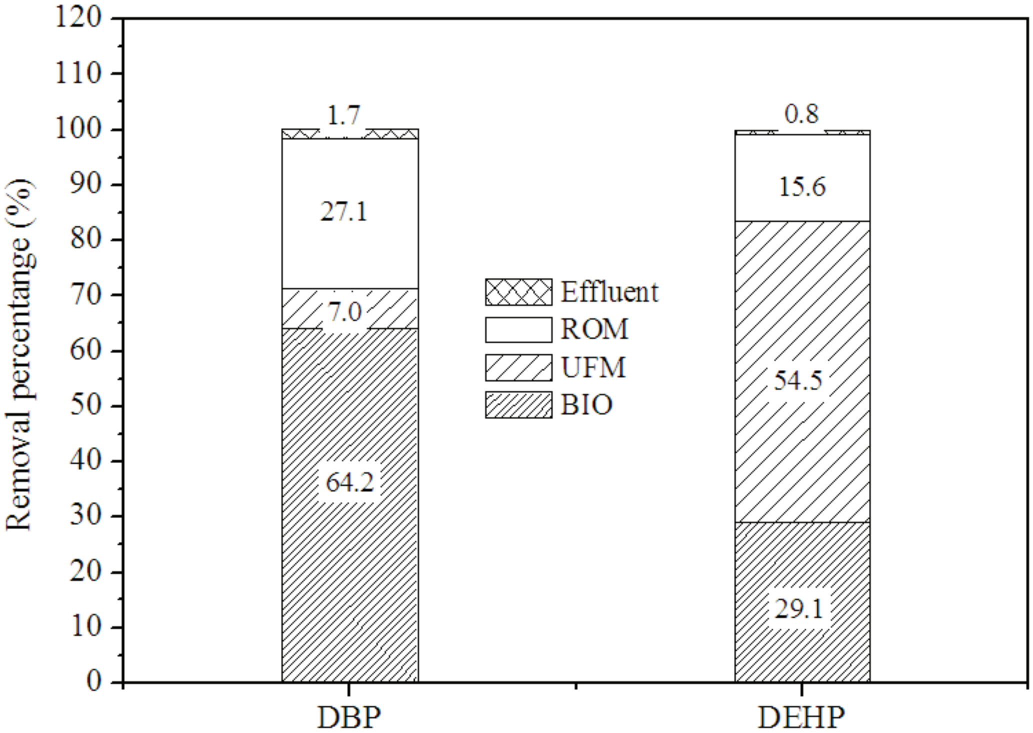


Fig. 5. Percentage removal of DBP and DEHP in each of the leachate treatment units

Table 3. Removal of PAEs during leachate treatment of previous studies and this experiment

Leachate characteristic	***Process***[a]	***Removal efficiency***		***Reference***
		DBP	***DEHP***	
Fresh	CF	29%	32%	Zheng et al., 2009
Mature	Fenton	50%	20%	He et al., 2009
Mature	UASB+BAF+ANO+MBR	92.9%	95.2%	Lu et al., 2012
Partially stabilized	Two-stage MBR	96.8%	95.7%	Boonnorat et al., 2014
Fresh	UASB+MBR+UFM+ROM	98.3%	99.3%	Present study

[a] *CF: Coagulation and Flocculation; UASB: Up-flow Anaerobic Sludge Blanket; BAF: Biological Aerated Filter; ANO: Anoxic; MBR: Membrane Bioreactor; UFM: Ultrafiltration Membrane; ROM: Reverse Osmosis Membrane*

Comparing the extents of removal of DBP and DEHP in the biological treatment process, it can be seen that DEHP is much more difficult to remove than DBP. This indicates that biodegradation of DEHP is limited and that the physical processes of UFM and ROM play key roles in DEHP removal. These results confirm that shorter carbon chain PAEs have higher biodegradability (Bauer et al., 1998; Ejlertsson et al., 1997). DBP has been shown to be more hydrophilic, more readily leached from waste into the aqueous phase, and more easily degraded under anaerobic conditions compared with more hydrophobic PAEs (Ejlertsson et al., 1997). The different removal mechanisms of the two PAEs observed in this work may therefore be related to their different molecular structures: DEHP is hydrophobic and is therefore not degraded as easily as hydrophilic diesters, such as DBP. The concentrations of both DBP and DEHP in the process effluent were both very much lower than in the original leachate, which indicates that the entire treatment process-comprising the unit operations of ADJ, BIO, UFM, and ROM-removes both DBP and DEHP with high efficiency although their removal pathways and mechanisms are different. DBP is removed mainly by biological treatment, while UFM and ROM removed 70.1% of the DEHP owing to the physical retention of this molecule by the membranes.

The overall removal efficiency of PAEs in this study was higher than that reported in most previous studies (Table 3). This difference may have occurred due to differences in the leachate treatment process and condition of the experimental operation in each study. Specially, ROM process has a much stronger ability to remove pollutants than other process. In addition, the condition of the laboratory-scale process and the real sites also differ greatly.

DBP and DEHP concentrations and other parameters measured in the concentrated leachate are given in Table 2. The DEHP concentration (173.5 μg/L) was much higher than that of DBP (76.8 μg/L) in this stream, confirming that DEHP is filtered by the membranes and accumulates in the concentrated leachate. Metals and SO_4^{2-} are difficult to remove during the leachate treatment process and also accumulate in concentrated leachate. The biodegradability of this concentrated leachate is low, indicated by a BOD_5/COD ratio of 0.048. This is likely owing to its high concentrations of refractory organic materials, including aromatic compounds, long-chain hydrocarbons, and halohydrocarbons, as well as toluene, ethylbenzene, and chlorobenzene (Zhang et al., 2013). Although the PAEs, and especially the long alkyl chain PAEs such as DEHP, were effectively removed from the aqueous phase by the filtration membranes and transferred to the concentrated leachate in the treatment process, large amounts of PAEs can accumulate over long periods of time.

These types of organics degrade with difficulty in this solution and will remain in the refuse and leachate. This not only enhances the difficulty of leachate treatment, but these pollutants are toxic to the microorganisms present in refuse. Degradation of refuse leachate can therefore be inhibited, which raises questions about possible environmental damage.

5. Conclusions

The removal of DBP and DEHP in the MSW incineration plant leachate treatment process was studied. About 98.3% of DBP and about 99.3% of DEHP were removed from the raw leachate. The principle mechanism of DBP removal is biodegradation, while membrane processes are the major removal steps for DEHP. PAEs, especially long alkyl chains such as DEHP, are transferred in this process to the concentrated leachate and may increase the environmental risk of this solution.

Acknowledgments

This work was funded by the National Natural Science Foundation of China (51678531), the Zhejiang Open Foundation of the Most Important Subjects of Zhejiang University of Technology (20130310), the Innovative Team Foundation of Zhejiang Province (2013TD12), the International Science and Technology Cooperation Program of China (2014DFE90040) and the Significant Science and Technology Project of Zhejiang Province (2015C33006).

References

Asakura H., Matsuto T., Tanaka N., (2004), Behavior of endocrine-disrupting chemicals in leachate from MSW landfill sites in Japan, *Waste Management*, **24**, 613-622.

Bauer M.J., Herrmann R., Martin A., (1998), Chemodynamics, transport behaviour and treatment of phthalic acid esters in municipal landfill leachate, *Water Science and Technology*, **38**, 185-192.

Boonnorat J., Chiemchaisri C., Chiemchaisri W., Yamamoto K., (2014), Removals of phenolic compounds and phthalic acid esters in landfill leachate by microbial sludge of two-stage membrane bioreactor, *Journal of Hazardous Materials*, **277**, 93-101.

Cai Q.Y., Mo C.H., Wu Q.T., Zeng Q.Y., Katsoyiannis A., (2007), Occurrence of organic contaminants in sewage sludges from eleven wastewater treatment plants, China, *Chemosphere*, **68**, 1751-1762.

Calli B., Mertoglu B., Roest K., Inanc B., (2006), Comparison of long term performances and final microbial compositions of anaerobic reactors treating landfill leachate, *Bioresource Technology*, **97**, 641-647.

Chang B.V., Wang T.H., Yuan S.Y., (2007), Biodegradation of four phthalate esters in sludge, *Chemosphere*, **69**, 1116-1123.

China's Environmental Protection Department, (2014), Standard for pollution control on the municipal solid waste incineration (GB 18485-2014), China Environmental Science Press, Beijing, China.

Cui F., Lu L., Liu W., Xu S., Xu N., (2012), Removal of DBP and DEHP with DOM in combined landfill leachate biotreatment process (in Chinese), *Acta Scientiarum Naturalium Universitatis Pekinensis*, **48**, 792-800.

Ejlertsson J., Alnervik M., Jonsson S., Svensson B.H., (1997), Influence of water solubility, side-chain degradability and side-chain structure on the degradation of phthalic acid esters under methanogenic conditions, *Environmental Science & Technology*, **3**, 2761-2764.

EPA of China, (1989), Standard for waste water analysis, China Environmental Science Press, Beijing, China.

Fang C.R., Long Y.Y., Shen D.S., (2009), Comparison on the removal of phthalic acid diesters in a bioreactor landfill and a conventional landfill, *Bioresource Technology*, **100**, 5664-5670.

Fang C., Long Y., Shen D., (2015), Degradation and adsorption behavior Of dibutyl phthalate in methanogenic phase refuse, *Environmental Engineering and Management Journal*, **14**, 709-717.

He P.J., Zheng Z., Zhang H., Shao L.M., Tang Q.Y., (2009), PAEs and BPA removal in landfill leachate with Fenton process and its relationship with leachate DOM composition, *Science of the Total Environment*, **407**, 4928-4933.

Liang D.W., Zhang T., Fang H.H., (2008), Phthalates biodegradation in the environment, *Applied Microbiology and Biotechnology*, **80**, 183-198.

Li P., Gao X., Wu J.H., Zhu N.W., Wu P.X., (2014), Characteristics of dissolved organic matters in waste incineration plant leachate treatment process (in Chinese), *China Environmental Science*, **34**, 2279-2284.

Liu H., Liang H.C., Liang Y., Zhang D., Wang C., Cai H.S., Shvartsev S.L., (2010), Distribution of phthalate esters in alluvial sediment: A case study at JiangHan Plain, Central China, *Chemosphere*, **78**, 382-388.

Lu L., Liu W., Cui F., Xu N., Xu S., Ni J.R., (2012), Removal of DBP and DEHP in combined landfill leachate treatment process (in Chinese), *Chinese Journal of Envionmental Engineering*, **6**, 2295-2302.

Lu Y., Tang F., Wang Y., Zhao J.H., Zeng X., Luo Q.F., Wang L., (2009), Biodegradation of dimethyl phthalate, diethyl phthalate and di-n-butyl phthalate by *Rhodococcus* sp. L4 isolated from activated sludge, *Journal of Hazardous Materials*, **168**, 938-943.

Mo C.H., Cai Q.Y., Li Y.H., Zeng Q.Y., (2008), Occurrence of priority organic pollutants in the fertilizers, China, *Journal of Hazardous Materials*, **152**, 1208-1213.

Özyaka V.S., Çakmakcı M., Yaman F.B., Özkaya B., Karadağ D., (2015), Treatment of compost leachate by membrane processes, *Environmental Engineering and Management Journal*, **14**, 2237-2241.

Reinhart D.R., Grosh C.J., (1998), Analysis of Florida MSW landfill leachate quality data, Report 97-3, Florida Center for Solid and Hazardous, On line at: http://www.bvsde.paho.org/bvsacd/cd48/analysis-florida.pdf.

Şchiopu A.M., Piuleac G.C., Cojocaru C., Apostol I., Mămăligă I., Gavrilescu M., (2013), Reducing environmental risk of landfills: leachate treatment by reverse osmosis, *Environmental Engineering and Management Journal*, **12**, 2319-2331.

Staples C.H., Peterson D.R., Parkerton T.F., Adams W.J., (1997), The environmental fate of phthalate esters: A literature review, *Chemosphere*, **35**, 667-749.

Wang F., Xia X.H., Sha Y.J., (2008), Distribution of phthalic acid esters in Wuhan section of the Yangtze River, China, *Journal of Hazardous Materials*, **154**, 317-324.

Wang Y.Y., Miao B., Hou D.M., Wu X.L., Peng B., (2012), Biodegradation of di-n-butyl phthalate and expression of the 3,4-phthalate dioxygenase gene in *Arthrobacter* sp. ZH2 strain, *Process Biochemistry*, **47**, 936-940.

Wu X.L., Wang Y.Y., Liang R.X., Dai Q.Y., Jin D.C., Chao W.L., (2011), Biodegradation of an endocrine-disrupting chemical di-n-butyl phthalate by newly isolated *Agrobacterium* sp. and the biochemical pathway, *Process Biochemistry*, **46**, 1090-1094.

Xu G., Li F.H., Wang Q.H., (2008), Occurrence and degradation characteristics of dibutyl phthalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP) in typical agricultural soils of China, *Science of Total Environment*, **393**, 333-340.

Zhang C.X., Wang Y.X., (2009), Removal of dissolved organic matter and phthalic acid esters from landfill leachate through a complexaton-flocculation process, *Waste Management*, **29**, 110-116.

Zhang Q.Q., Tian B.H., Zhang X., Ghulam A., Fang C.R., He Ruo., (2013), Investigation on characteristics of leachate and concentrated leachate in three landfill leachate treatment plants, *Waste Management*, **33**, 2277-2286.

Zheng Z., Zhang H., He P.J., Shao L.M., Chen Y., Pang L., (2009), Co-removal of phthalic acid esters with dissolved organic matter from landfill leachate by coagulation and flocculation process, *Chemosphere*, **75**, 180-186.

Environmental Engineering and Management Journal

"Gheorghe Asachi" Technical University of Iasi, Romania

73 Mangeron Blvd., 700050, Iasi, Romania
Tel./Fax. +40-232-271759, PO 10, BOX 2111,
URL: *http://omicron.ch.tuiasi.ro/EEMJ/*
e-mail: *eemjournal@yahoo.com*, *eemj.office@gmail.com*

INSTRUCTIONS FOR AUTHORS

1. Introduction

Environmental Engineering and Management Journal (EEMJ) is an international medium for publication of Original Papers, Reviews, Case Studies, Book Reviews on the fundamentals, applications in environmental engineering and technologies, applied environmental sciences, environmental health, management, sustainable development, education for sustainability. Advertising is also accepted with contractual payment forms.

Submission of a manuscript implies that the work described has not been published before (except in the form of an abstract or as part of a published lecture, or thesis); that it is not under consideration for publication elsewhere; that its publication has been approved by all coauthors, if any, as well.

Papers, books for review, offers to review, suggestions and commercials (advertising) should be submitted to the *Editor-in-Chief* All papers will be published in English. Non-English speaking authors should seek the advice of a professional language expert or an English speaker to help translate the paper.

2. Legal requirements

The author(s) guarantee(s) that the manuscript is/will not be published elsewhere in any language without the consent of the copyright holders, that the rights of third parties will not be violated, and that the publisher will not be held legally responsible should there be any claims for compensation.

Authors wishing to include figures or text passages that have already been published elsewhere are required to obtain permission from the copyright holder(s) and to include evidence that such permission has been granted when submitting their papers. Any material received without such evidence will be assumed to originate from the authors.

The author(s) are encouraged to transfer the copyright of the article to the publisher upon acceptance of an article by the journal, using the Authors' Warranty and Assignment of Copyright agreement. This transfer enables the Editor to protect the copyrighted material for the authors, but does not relinquish the author's proprietary rights. The publication of an article is conditioned by the signature of the author to whom correspondence should be addressed on this Authors' Warranty and Assignment of Copyright that is provided by the Editor.

Ethics in Publishing

For information on ethics in publishing for journal publication see http://omicron.ch.tuiasi.ro/EEMJ/plagiarism.htm

Conflict of interest

All authors are requested to disclose any actual or potential conflict of interest such as any financial, personal or other relationships with other people or organizations concerning the submitted work that could inappropriately influence, or be perceived to influence, their work.

3. Editorial procedure

For original papers, an upper limit of 7000 words is recommended (including Abstract, Keywords, References, Figures and Tables), processed with MS editing facilities.

For review papers (critical evaluation of existing data, defined topics or emerging fields of investigation, critical issues of public concern), an upper limit of 15000 words is recommended (including Abstract, Keywords, References, Figures and Tables).

Manuscripts should be written in English (American or British usage is accepted, but not a mixture of these) and submitted **electronically**, in .doc format (please do not use .docx) to the *Editor-in-Chief*, at one (and only one) of the following e-mail addresses: **eemjournal at yahoo dot com**; **eem_journal at yahoo dot com**; **eemjeditor at yahoo dot com**; **eemj_editor at yahoo dot com**; **eemjournal at gmail dot com**; **eemjeditor at gmail dot com; eemjoffice at gmail dot com.**

Please be sure to include your full affiliation and e-mail address (see Sample manuscript).

When submitting the manuscript, it is mandatory to include a cover letter to the editor. The cover letter must state:

- that all authors mutually agree that the manuscript can be submitted to EEMJ;
- that the manuscript contains the original work of the authors;

- the novelty in results/findings, or significance of results;
- that the manuscript has not already been published, or is not under consideration for publication elsewhere.

Manuscripts are evaluated first in the Editorial Office (as a preliminary condition for acceptance) in terms of meeting the requirements of the journal, including attempts of plagiarism. The authors are responsible for the accuracy of the whole paper and references. Authors will be notified about the registration of their contribution. Only those contributions, which conform to the following instructions, can be considered for the peer-review process. Otherwise, the manuscripts are returned to the authors, with observations, comments and annotations.

The peer review process is decisive for paper acceptance. It could be done in several stages, depending on the revision quality of the manuscript in accordance with the requirements of paper evaluators.

Please do not transmit electronic data or requirements to the publisher until your manuscript has been reviewed and accepted for publication. Please follow the instructions below.

A minimum of four suitable *potential reviewers* should be provided by the authors. Please provide their name, email addresses, and institutional affiliation. When compiling this list of potential reviewers please consider the following important criteria: they must be knowledgeable about the manuscript subject area of the manuscript; they must not be from the authors' own institution or country; they should not have recent (less than five years) joint publications with any of the authors. However, the final choice of reviewers is at the editors' discretion.

4. Manuscript preparation

General:

Authors must follow the Instructions for authors strictly, failing which the manuscripts would be rejected without review. Editors reserve the right to adjust the formatting style to conform to the standards of the journal.

Manuscripts should be concise, in 1.5 line spacing, and should have 2 cm all over margins. The font should be Times New Roman of size 12 points. The numbering of chapters should be in decimal form. Ensure that each new paragraph is clearly indicated, using TAB at 1.25 pts.

The text layout should be in single-column format. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the manuscript. However, do use bold face, italics, subscripts, superscripts etc. Add line numbering and page numbers. To avoid unnecessary errors it is strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Title page will contain:

- A concise and informative title (Times New Roman bold, all caps of size 14 points); the maximum length of the title should be maximum 100 letters and spaces; The full name(s) of the author(s) (first name, then last name, with Times New Roman bold 12 points) should be written below the title. The affiliation(s) and complete postal address(es) of the author(s) will be provided immediately after the full name of the authors and will be written with Times New Roman 12 points. When the paper has more than one author, their name will be followed by a mark (Arabic numeral) as superscript; for the corresponding author, an asterix will be added using *Word_Insert_Reference_Footnote_Symbol* sequence. Also, the full and e-mail addresses, telephone and fax numbers of the corresponding author will be provided in the footer of the first page, as: Author to whom all correspondence should be addressed: email….., Phone…….., Fax…………
- **Abstract:** each paper must be preceded by an abstract presenting the most important results and conclusions in no more than 250 words. Do not include citations in the Abstract.
- **Keywords:** three to five keywords should be supplied after the Abstract for indexing purposes, separated by comma, **ordered alphabetically,** using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, "and", "of"). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible.
- The text of the paper should be divided into **Introduction, Materials and methods (**or **Experimental), Results and discussion, Conclusions, References** (for papers dealing with environmental management, policy, education etc., the Experimental part can be replaced by case-studies presentation).

1. Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

2. Material and methods (or 2. Experimental)

Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

3. Results and discussion

Results should be clear and concise. Discussion should explore the significance of the results of the work, not repeat them. Avoid extensive citations and discussion of published literature.

4. Conclusions

The main conclusions drawn from results should be presented in a short Conclusions section. Do not include citations in this section.

Formulae, symbols and abbreviations: Formulae will be typeset in Italics (preferable with the Equation Editor of Microsoft Office 2003) and should be written or marked as such in the manuscript, unless they require a different styling. The formulae should be numbered on the right side, between brackets:

$$a^3 = 3M / 4N \quad (1)$$

Always refer in the text to Equations as (Eq. 1), Eqs. (1-4) etc.
The more complex Chemical Formulae should be presented as Figures.
Abbreviations should be defined when first mentioned in the abstract and again in the main body of the text and used consistently thereafter.

SI units must be used throughout.

Footnotes should be avoided.

References:
The list of References should only include works that are cited in the text and that have been published. References should be cited in the text in brackets ***(Harvard style)*** as in the following examples:

(Chisti, 1989), (Gavrilescu and Roman, 1996), (Moo-Young et al., 1999).
References should be alphabetically listed at the end of paper, with complete details, as follows:

Books: Names and initials of authors, year (between brackets), chapter title, title of the book (italic), editors, edition, volume number, publisher, place, page number:
Mauch K., Vaseghi S., Reuss M., (2000), *Quantitative Analysis of Metabolic and Signaling Pathways in Saccharomyces cerevisiae*, In: *Bioreaction Engineering*, Schügerl K., Bellgardt K.H. (Eds.), Springer, Berlin Heidelberg New York, 435-477.
Faber K., (2000), *Biotransformations in Organic Chemistry – A Textbook*, vol. VIII, 4th Edition, Springer, Berlin-Heidelberg-New York.
Handbook, (1951), *Handbook of Chemical Engineer*, vol. II, (in Romanian), Technical Press, Bucharest, Romania.

Symposia volumes: Names and initials of authors, year (between brackets), paper title, symposium name, volume number, place, date, page numbers:
Clark T.A., Steward D., (1991), *Wood and Environment*, Proc. 6th Int. Symp. on Wood and Pulping Chemistry, Melbourne, vol. 1, 493-498.

Journal papers: Names and initials of authors, year (between brackets), full title of the paper, full name of the journal (italic), volume number (bold), first and last page numbers:
Tanabe S., Iwata H., Tatsukawa R., (1994), Global contamination by persistent organochlorines and their ecotoxicologcial impact on marine mammals, *Science of the Total Environment*, **154**, 163-177.

Patents: Names and initials of authors, year (between brackets), patent title, country, patent number (italic):
Grant P., (1989), Device for Elementary Analyses. USA Patent, No. *123456*.

Dissertations: Names and initials of authors, year (between brackets), title, specification (PhD Thesis, MSc Thesis), institution, place:
Aelenei N., (1982), *Thermodynamic study of polymer solutions*, PhD Thesis, Institute of Macromolecular Chemistry Petru Poni, Iasi, Romania.
Star K., (2008), *Environmental risk assessment generated by natural hazards*, MSc Thesis, Institute of Hazard Research, Town, Country.

Legal regulations and laws, organizations: Abbreviated name, year (between brackets), full name of the referred text, document type, author, URL address:
ESC, (2007), Improving access to modern energy services for all fundamental challenge, Economic and Social Council, ENV/DEV/927, On line at: http://www.un.org/News/Press/docs/2007/envdev927.doc.htm.
EPA, (2007), Biomass Conversion: Emerging Technologies, Feedstocks, and Products, Sustainability Program, Office of Research and Development, EPA/600/R-07/144, U.S. Environmental Protection Agency, Washington, D.C., On line at: http://www.epa.gov/Sustainability/pdfs/Biomass%20Conversion.pdf.
EC Directive, (2000), Directive 2000/76/EC of the European Parliament and of the Council of 4 December 2000, on the incineration of waste, Annex V, *Official Journal of the European Communities*, L 332/91, 28.12.2000, Brussels.
GD, (2004), Governmental Decision No. 1076/2004 surnamed SEA Governmental Decision, regarding the procedure for strategic environmental impact assessment for plans or programs, *Romanian Official Monitor*, Part I, No. 707 from 5th of August, 2004.

Web references
The full URL should be given in text as a citation, if no other data are known. If the authors, year, title of the documents are known and the reference is taken from a website, the URL address has to be mentioned after these data:
Burja C., Burja V., (2008), Adapting the Romanian rural economy to the European agricultural policy from the perspective of sustainable development, MPRA, Munich Personal RePEc Archive, On line at: http://mpra.ub.uni-muenchen.de/7989/1/MPRA_paper_7989.pdf

Web references must not be listed separately, after the reference list.

All references must be provided in English with a specification of original language in round brackets.

Citation in text
Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Do not cite references in the abstract and conclusions.
Unpublished results, personal communications as well as URL addresses are not recommended in the references list..
Citation of a reference as "in press" implies that the item has been accepted for publication.

Papers which have been accepted for publication should be included in the list of references with the name of the journal and the specification "in press".

References style

Text: All citations in the text may be made directly (or parenthetically) and should refer to:

- *single author:* the author's name (without initials, unless there is ambiguity) and the year of publication: “as previously demonstrated (Smith, 2007)”; “as Smith (2007) demonstrated”
- *two authors:* both authors' names and the year of publication: (Arnold and Sebastian, 2008; Smith and Hansel, 2006; Stern and Lars, 2009)
- *three or more authors:* first author's name followed by "et al." and the year of publication: “As has recently been shown (Werner et al., 2005)…”, “Kramer et al. (2000) have recently shown ...."

Citations of groups of references should be listed first alphabetically, then chronologically.

Examples: "…..as demonstrated (Aden, 1996a, 1996b, 1999; Allan and Jones, 1995; Han et al., 2007; Weiss and Schmidt, 1988)”.

Abbreviations

Define all abbreviations at their first mention in the text. Ensure consistency of abbreviations throughout the article.

Acknowledgements

Include acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, funding supports etc.).

Footnotes

Footnotes must be avoided. Do not include footnotes in the Reference list.

Table footnotes

Indicate each footnote in a table with a superscript lowercase letter.

Tables

Draw the Tables in grid format using a basic, solid line style without shadows.

Ensure that the data presented in Tables do not duplicate results described in Figures or elsewhere in the paper.

Figures

Number Figures consecutively in accordance with their appearance in the text. All illustrations should be provided in camera-ready form, suitable for reproduction, which may include reduction without retouching. Photographs, charts and diagrams are all to be referred to as Figure(s) and should be numbered consecutively, in the order to which they are referred.

Figures may be inserted preferably as black line drawings. They should be pasted on, rather than taped, since the latter results in unclear edges upon reproduction.

Ensure that each illustration has a caption, placed below the Figure. Supply also captions separately, not attached to the figure. A maximum limit of 8 Figures are allowed per manuscript.

A caption should comprise a brief title (**not** on the Figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used. Multiple Figures can be expressed as one Figure (for e.g. 1a, 1b, 1c etc...), while retaining the maximum limit of 6.

ALL Figures must be submitted in either .jpg or .tiff format with a very good resolution (but do not submit graphics that are disproportionately large for the content).

Figures and Tables must be embedded in the text.

Proofs

Proofs will be sent to the corresponding author (by e-mail) and should be returned within 72 hours of receipt. Corrections should be restricted to typesetting errors; any other changes may be charged to the authors.

Paper in Electronic Format:

Authors are asked to submit their final and accepted manuscript as an attachment to one of the above-mentioned e-mail addresses. Use the **.doc** format (not .docx !).

5. Page charge

There is no charge per printed page for regular papers.

6. Reprints

The corresponding author will be provided with a .pdf file of the paper via e-mail, free of charge. A hard copy Of the issue containing the paper can be provided on request, for a fee. This request will be formulated when the final form of the manuscript (the electronic one) is provided.

7. Additional procedures for the editorial management of *Environmental Engineering and Management Journal*

Starting with volume 13/2014, the members of the Scientific Advisory Board and the Corresponding authors will receive each issue of the journal in electronic .pdf format. Printed copies can be delivered on request.

An author cannot appear on more than two papers per regular issue. An author cannot appear on more than three papers in a Guest Editor/ Conference issue.

The evaluation/peer-review process of manuscripts submitted for Guest Editor / Conference issues published according to journal *Editorial Procedure and Policy* will be handled and evaluated as with regular manuscripts, so that only consistent papers will be published. The manuscripts must be sent at least six months before the presumed data of publication. Those manuscripts which do not fulfill the journal requirements will be rejected. This is to discourage superficiality in the development of the manuscript, from formal and scientific points of view. No amount of money will be refunded following the rejection of manuscripts.

No more than 160 pages and no more than 20 papers are acceptable for regular issues. No more than 180 pages and no more than 25 papers are opportune for special issues. The number of pages is based on the published version of an article, not on the submitted version (for instance, when page breaks are changed and when images are enlarged for easier viewing).

An issue can include, as an exception, a number of 25-30 papers as a maximum. This situation could appear when the authors of an already accepted paper need and ask for early publication, from the position in the list of accepted papers. In this circumstance, a yearly submission to our journal (500 Euro) will be asked for each already accepted paper, for its publication in advance.

A newly-received manuscript can be evaluated by priority, when a deposit of 200 Euro shall be paid by authors in advance. If the manuscript is deemed unsuitable for publication, the authors will not be eligible for a refund.

Failure to pay mandatory charges may result in the paper being withheld from early publication.

In all cases, the corresponding author will sign an agreement with the *Editor-in-Chief*, and the *Director* of the *EcoZone* Publishing House according to journal *Editorial Procedure and Policy.*

Automated software is used for checking against plagiarism. In order to avoid false results, the generated reports are cautiously checked and confirmed by journal staff. We do not send plagiarism reports to authors. Any manuscripts which do not prove to be at least 90% original will be rejected. The rejection for plagiarism may arise at any phase of the editorial / review process, even if the manuscript was accepted for publication. The authors are solely responsible for the originality of their submission. Therefore, any manuscript should be carefully checked for such inconsistencies before submitting to EEMJ.

The authors are required to follow academic publishing rules, to adhere to the principles of scientific ethics and to sign the Authors' warranty and Copyright transfer.

Detailed information concerning these issues may be found of the EEMJ website, under *Editorial Procedure and Policy.*