Supported by

Faculty of Biology
University of Belgrade

Serbian Society
for Microbiology

Serbian Society for
Medical Microbiology

MICROBIOLOGIA BALKANICA 2011

7th BALKAN CONGRESS OF MICROBIOLOGY

8th CONGRESS OF SERBIAN MICROBIOLOGISTS

PROCEEDINGS

October 25-29, 2011

Continental Hotel, Belgrade, SERBIA

CIP – Katalogizacija u publikaciji
Narodna biblioteka Srbije, Beograd

Udruženje mikrobiologa Srbije, Beograd.
Knjiga radova (Elektronski izvor) – Proceedings **/** MICROBIOLOGIA BALKANICA 2011 - 7th BALKAN CONGRESS OF MICROBIOLOGY & 8th CONGRESS OF SERBIAN MICROBIOLOGISTS, 25-29. oktobar 2011; (organizator) Udruženje mikrobiologa Srbije, Udruženje medicinskih mikrobiolga Srbije; (urednici: Dragojlo Obradović, Lazar Ranin, Špiro Radulović) – Beograd
1 elektronski optički disk (CD-ROM); 12cm

Sistemski zahtevi: Nisu navedeni. Nasl. sa naslovnog ekrana. –
Radovi na engleskom jeziku.-
Tekst latinica. –
Tiraž – 600.
Abstracts. – Registar

ISBN 978-86-914897-0-01
Udruženje mikrobiologa Srbije, Beograd.

KNJIGA RADOVA / PROCEEDINGS
MICROBIOLOGIA BALKANICA 2011 - 7th BALKAN CONGRESS OF MICROBIOLOGY & 8th CONGRESS OF SERBIAN MICROBIOLOGISTS, 25-29. oktobar 2011

Izdaje / Published by:
Udruženje mikrobiologa Srbije
Nemanjina 6, 11 080 Beograd, Srbija, tel/fax: 011 2199 711, dobradovic@agrifaculty.bg.ac.rs

Za izdavača / For Publisher:
Dragojlo Obradović, predsednik Udruženja

Urednici/Editors:
Dragojlo Obradović
Lazar Ranin
Špiro Radulović

ISBN 978-86-914897-0-01

Kompjuterska obrada teksta / Computer Layout:
Jelena Zovko Belić

Tiraž / Circulation
600 primeraka / 600 copy

Umnožavanje / Copying
Megaphone d.o.o., Vladimira Rolovića 105, Beograd, Srbija

Committees

Organizing Committee

President: Prof. dr Spiro Radulovic
Vice president: Prof. dr Dragojlo Obradovic
General Secretary: Prof. dr Lazar Ranin

Members:
Prof. dr Branislava Kocic
Prof. dr Branislava Savic
Prof. dr Ruzica Asanin
Prof. dr Veljko Mirovic
Prof. dr Vaso Taleski
Prof. dr Djordje Fira
Prof. dr Slavisa Stankovic

Scientific Committee

President: Prof. dr Tanja Jovanovic

Members:
Prof. dr Ruzica Asanin
Prof. dr Dejan Baskic
Prof. dr Sava Buncic
Prof. dr Angel Galabov
Prof. dr Zeynep Gulay
Dr Dobrila Jakic Dimic
Prof. dr Dragutin Djukic
Prof. dr Mirjana Jarak
Prof. dr Vera Katic
Prof. dr Jelena Knezevic Vukcevic
Prof. dr Dejan Krnjajic
Prof. dr Marija Kulauzov
Prof. dr Zoran Kulisic
Prof. dr Nada Kuljić Kapulica
Prof. dr Dusan Lalosevic
Prof. dr Nenad Milic
Prof. dr Gordana Mijovic
Prof. dr Sanja Mitrovic
Dr Jasmina Nedeljkovic
Prof. dr Viktor Nedovic
Prof. dr Marian Negut
Prof. dr Miomir Niksic
Prof. dr Aleksa Obradovic
Prof. dr Anna Papa
Prof. dr Milena Petrovska
Prof. dr Olga Petrović
Dr Milanko Sekler
Prof. dr Marija Skrinjar
Prof. dr Vaso Taleski
Prof. dr Ljubisa Topisirovic
Dr Branka Vasiljevic
Dr Branka Vidic
Prof. dr Jelena Vukojevic
Prof. dr Miroslav Vrvić
Dr Milan Kojić
Prof. dr Siniša Markov
Prof. dr Vera Raičević

Board of the Balkan Society for Microbiology

President:
Prof. dr Milena Petrovska (Skopje)
President – elect:
Prof. dr Spiro Radulovic (Belgrade)

Members:

Bulgaria:
Prof. dr Angel GALABOV (Sofia)
Prof. dr Todor KANTARDJIEV (Sofia)
Prof. dr Hristo NAJDENSKI (Sofia)

Greece:
Prof. dr Athanassios TSAKRIS (Athens)
Prof. dr Anna PAPA KONIDARI (Thessaloniki)

Macedonia:
Prof. dr Dzoko KUNGULOVSKI (Skopje)
Prof. dr Nikola PANOVSKI (Skopje)

Montenegro:
Dr Jelena ZINDOVIC (Podgorica)
Dr Zoran VRATNICA (Podgorica)
Doc. dr Vineta VUKSANOVIC (Podgorica)

Romania:
Prof. dr Gabriel IONESCU (Bucharest)
Prof. dr Marian NEGUT (Bucharest)
Prof. dr Alexandru RAFILA (Bucharest)

Serbia:
Prof. dr Branislava KOCIC (Nis)
Prof. dr Lazar RANIN (Belgrade)

Turkey:
Prof. dr Nezahat GURLER (Istanbul)
Prof. dr Zeynep GULAY (Istanbul)

Plenary speakers

Milton da Costa (Portugal)
Otto Haller (Germany)
Mirsada Hukic (BIH)
Gunnar Kahlmeter (Germany)
Anna Papa – Konidari (Greece)
Elisabeth Nagy (Hungary)
Bernhard Schink (Germany)
Vittorio Venturi (Italy)

Microbial consortia diversity in biodegradation of petroleum pollutants during bioremediation

Vladimir P. Beškoski[a], Miroslav M. Vrvić[a,b]

[a]Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Serbia

Introduction

Petroleum and its derivatives, are very widely used in all domains of work and everyday life. In spite of improvements in technology and equipment used by the petroleum industry, oil and oil derivatives represent a significant source of environmental contamination.

Both soil and water become contaminated by oil and oil derivatives due to accidental spills in their exploitation, transportation, processing, storing and utilization. In 2010, 3.91 billion tons of crude oil was produced (BP Statistical Review, 2011) and estimations are that annually 0.1% of produced petroleum is released into the environment (Ward et al., 2003) as a result of anthropogenic activities.

Bioremediation is a modern method in which the natural ability of microorganisms is employed for the reduction of the concentration and/or toxicity of various chemical substances (Singh & Ward, 2004). It has been proven to be efficient in the removal of crude oil and oil derivatives (Ollivier & Magot, 2005), chlorinated solvents (Bamforth & Singleton, 2005; Gavrilescu, 2005), and even some heavy metals (Seidel, 2004).

Bioremediation involves the use of microorganisms to reduce the complexity of organic molecules (biotransformation), or degradation to complete mineralization of organic substances (biodegradation) as shown in the general equation:

$$C_cH_hO_oN_nP_pS_sCl_{cl} \rightarrow cCO_2 + h/_2H_2O + nNH_4^+ + pHPO_4^{2-} + sSO_4^{2-} + clCl^- + \text{Energy}$$

A consortium (mixed culture) of microorganisms can conduct these complex processes, while at the same time, being more resistant, on average, to changes in the ecosystem than just a single microbial species (Brenner et al., 2008).

Aim

The aim of this study was to reveal the microbial consortia diversity and to present the use of various techniques and data to interpret their biochemical activities in biodegradation of different petroleum pollutants during bioremediation on industrial level.

Material and methods

Composition and activities of microbial consortia were monitored during *ex situ* biopile treatment of polluted soil. To increase the rate of biodegradation biostimulation, bioaugmentation and re-inoculation followed by intensive mixing/aeration were applied. In addition to classical cultivation techniques microorganisms were characterized by molecular methods and fatty acid methyl ester (FAME) analysis for identification. Intensity of biodegradation was determined by respirometry, enzyme activities and degradation studies with selected aliphatic and aromatic compounds. Particular attention has been focused on surfactant producing microorganisms.

Results

A gas chromatogram of the Total Petroleum Hydrocarbons (TPH) extract of soil polluted with diesel oil is presented in Figure 1. TPH chromatogram exhibit peaks of *n*-alkanes (C_{10}-C_{40}), isoprenoids and among them the predominant C_{19}, pristane (2,6,10,14-tetramethylpentadecane) and C_{20}, phytane (2,6,10,14-tetramethylhexadecane) and a hump of Unresolved Complex Mixture (UCM). In our study *n*-alkane fraction was dominant and most susceptible to bioremediation.

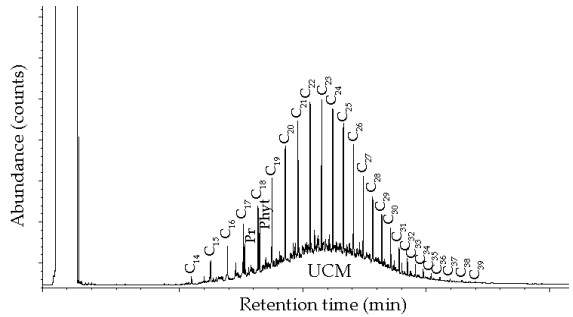

Figure 1. Gas chromatogram of TPH of the soil recently polluted with diesel oil (Pr: Pristane; Phyt: Phytane; UCM: Unresolved Complex Mixture).

During bioremediation of hydrocarbon contaminated soil a stable microbial community had been formed after initial fluctuations, and the microorganisms which decompose hydrocarbons were the dominant microbial population at the end of the *ex situ* bioremediation process, with a share of more than 80% (range 10^7 colony forming units g^{-1}). FAME analysis provided information leading to identification and quantification of viable bacterial biomass. Among isolated strains the most abundant genera were *Pseudomonas, Achromobacter, Sphingomonas, Acinetobacter*, *Bacillus*, *Staphylococcus*, *Micrococcus*, *Mycobacterium*, *Rhodococcus, Penicillium*, *Aspergillus* and *Rhodotorula*. Enzyme activity and respiratory tests were in accordance with biodegradation intensity and the metabolically active microbial community played the key role in the hydrocarbon biodegradation.

Biosurfactant producing strains were isolated and rhamnolipids produced by *Pseudomonas* strains were characterized.

Although the biodegradation of crude oil is often explained as a quasi-stepwise process in which various components are removed in a well-recognized sequence, in this study, all the components of the hydrocarbon mixture were consumed by microorganisms and several compound classes are actually degraded simultaneously but at different biodegradation rates. Average rate of decrease during biodegradation of heavy residual fuel oil-mazut were 23.7 for the aliphatic, 5.7 for the aromatic, and 3.3 mg/kg/day for nitrogen-sulphur-oxygen (NSO) and the asphaltene fraction. This reflects differences in the rate of catabolism of the components of the hydrocarbon group composition under varying conditions (Beškoski et al., 2010, Beškoski et al., 2011; Jovančićević et al., 2008).

Conclusion

Petroleum and petroleum products consist of thousand compounds and therefore the biodegradation of such a complex mixtures requires the participation of multiple cultures. Some defined bacterial species are able to degrade, to a limited extent, all hydrocarbons present in heavy fuel oil or oil sludge. Some of the polluting components may be dissolved only by the joint metabolic activity of multiple genera of microorganisms. An advantage to the use of mixed cultures is a broader degradation capacity, synergic effect and co-metabolism. A consortium of microorganisms can conduct processes of degradation, while at the same time, being more resistant, on average, to changes in the ecosystem than just a single microbial species.

Keywords: Microbial consortia, hydrocarbon-degraders, bioremediation.

Acknowledgments

This work was supported by the Ministry of Education and Science of the Republic of Serbia under Grant No. III 43004.

References

Bamforth, S. M. & Singleton, I. (2005). Review Bioremediation of polycyclic aromatic hydrocarbons: current knowledge and future directions. *Journal of Chemical Technology & Biotechnology* Vol.80, No.7, pp. 723–736

Beškoski, V.P.; Gojgić-Cvijović, G.; Milić, J.; Ilić, M.; Miletić, S.; Šolević, T. & Vrvić, M.M. (2011). *Ex situ* bioremediation of a soil contaminated by mazut (heavy residual fuel oil) – A field experiment, *Chemosphere*, Vol.83, No.1, pp. 34-40

Beškoski, V.P.; Takić, M.; Milić, J.; Ilić, M.; Gojgić-Cvijović, G.; Jovančićević B. & Vrvić, M.M. (2010). Change of isoprenoids, steranes and terpanes during *ex situ* bioremediation of mazut on industrial level, *Journal of the Serbian Chemical Society*, Vol.75, No.11, pp. 1605–1616

BP Statistical Review of World Energy, June 2011 In: *bp.com/statisticalreview*, 08.09.2011, Available from: http://www.bp.com/assets/bp_internet/globalbp/globalbp_uk_english/ reports_and_publications/statistical_energy_review_2011/ STAGING/ local_assets/pdf/statistical_review_of_world_energy_ full_report_2011.pdf

Brenner K.; You L. & Arnold F.H. (2008). Engineering microbial consortia: a new frontier in synthetic biology, *Trends in Biotechnology*, Vol.26, No.9, pp. 483–489

Gavrilescu, M. (2005). Fate of pesticides in the environment and its bioremediation. *Engineering in Life Sciences* Vol.5, No.6, (December 2005), pp. 497-526

Jovančićević, B.; Antić, M.; Pavlović, I.; Vrvić, M.; Beškoski, V.; Kronimus, A. & Schwarzbauer, J. (2008b). Transformation of petroleum saturated hydrocarbons during soil bioremediation experiments. *Water Air & Soil Pollution.* Vol.190, No.1-4, (December 2007), pp. 299–307

Ollivier B. & Magot M. (2005). *Petroleum Microbiology*, ASM Press, Washington

Seidel, H.; Löser, C.; Zehnsdorf, A.; Hoffmann, P. & Schmerold, R. (2004). Bioremediation process for sediments contaminated by heavy metals: Feasibility study on a pilot scale. *Environmental Science & Technology*, Vol.38, No.5, pp.1582–1588

Singh, A. & Ward, O.P. (2004). *Biodegradation and Bioremediation*. Springer-Verlag, Berlin

Ward, O.; Singh, A. & Van Hamme, J. (2003). Accelerated biodegradation of petroleum hydrocarbon waste. *Journal of Industrial Microbiology & Biotechnology*, Vol.30, No.5, (April 2003), pp. 260-270

MICROBIOLOGIA BALKANICA 2011
7th BALKAN CONGRESS OF MICROBIOLOGY

8th CONGRESS OF SERBIAN MICROBIOLOGISTS

Serbian Society for Medical Microbiology
Serbian Society for Microbiology

October 25-29, 2011
Continental Hotel Belgrade, Serbia

Prof. dr Miroslav M. Vrvić
Hemijski fakultet
i
Dr Vladimir P. Beškoski
Centar za hemiju IHTM

Beograd, 18. jul 2011.

Poštovane kolege,

Čast nam je da vas u ime Naučnog i Organizacionog odbora pozovemo da iz oblasti *primenjene mikrobiologije* održite uvodno predavanje na naučnom skupu medjunarodnog značaja:

"MICROBIOLOGIA BALKANICA 2011-7th BALKAN CONGRESS OF MICROBIOLOGY"

Na skupu će osim kolega iz Srbije, učestvovati i stručnjaci iz regiona, nekih evropskih zemalja i iz drugih krajeva sveta. Zvaničan jezik skupa je engleski.

Skup će se u organizaciji Udruženja medicinskih mikrobiologa Srbije i Udruženja mikrobiologa Srbije pod pokroviteljstvom Ministarstva zdravlja, Ministarstva prosvete i nauke i uz podršku Evropskog saveza mikrobioloških društava – FEMS, održati u Beogradu od **25. do 29. oktobra 2011. godine.**

Molimo vas da predavanje u formi rada obima ne manje od 3 strane i ne preko 5 strana, na engleskom sa izvodom na srpskom jeziku, prema priloženom uputstvu, pošaljete u elektronskom obliku na e-mail: office@aria.co.rs najkasnije do **09. septembra 2011 godine**.

Radovi će biti publikovani u CD-ROM Proceedings-u, koji će biti registrovan kod Narodne biblioteke Srbije i nadležnog Matičnog naučnog odbora Ministarstva prosvete i nauke.

Unapred zahvaljujemo i pozdravljamo vas.

Predsednik Organizacionog odbora
Prof. dr Špiro Radulović

Predsednik Naučnog odbora
Prof. dr Tanja Jovanović