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# MICROBIOLOGIA BALKANICA 2011

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8<sup>th</sup> CONGRESS OF SERBIAN MICROBIOLOGISTS

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## Microbial consortia diversity in biodegradation of petroleum pollutants during bioremediation

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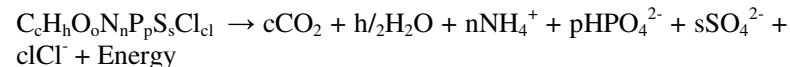
### Introduction

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; Gavrilesco, 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:



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<sub>10</sub>-C<sub>40</sub>), isoprenoids and among them the predominant C<sub>19</sub>, pristane (2,6,10,14-tetramethylpentadecane) and C<sub>20</sub>, 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.

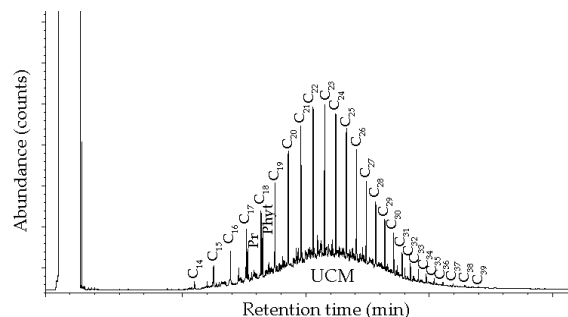


Figure 1. Gas chromatogram of TPH of the soil recently polluted with diesel oil (Pr: Pristane; Phyt: Phytane; UCM: Unresolved Complex Mixture).

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; Jovančičević 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.

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# 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. Vrvic**  
**Hemijski fakultet**  
**i**  
**Dr Vladimir P. Beškoski**  
**Centar za hemiju IHTM**

Beograd, 18. jul 2011.

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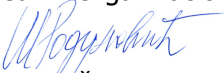
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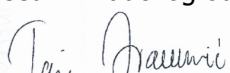
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