REMOVAL OF DIBENZOTHIOPHENE AND ITS ALKYL HOMOLOGUES DURING *ex situ* STIMULATED BIOREMEDIATION OF CONTAMINATED SOIL

Tatjana Šolević Knudsen[1,*], Mila Ilić[1], Jelena Milić[1], Vladimir Beškoski[2], Gordana Gojgić-Cvijović[1], Branimir Jovančićević[2] and Miroslav M. Vrvić[2]

[1]Center of Chemistry, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Njegoševa 12, P.O. Box 473, 11001 Belgrade, Serbia
[2]Faculty of Chemistry, University of Belgrade, Studentski trg 12-16, P.O. Box 158, 11001 Belgrade, Serbia
(* corresponding author: tsolevic@chem.bg.ac.rs)

INTRODUCTION

Dibenzothiophene (DBT) and its alkylated homologues are common sulfur heterocyclic constituents of crude oils. In oil pollutant biodegradation studies they keep attracting great concern due to their environmental persistence but also due to their toxicity. Additionally, they seem to be more resistant to aerobic microbial degradation then polycyclic aromatic compounds (PAHs) of similar weight.

The aim of this research was to investigate the changes in the distribution of DBT and its C1 and C2 alkyl homologues during ex situ stimulated bioremediation of a soil contaminated with heavy residual fuel oil. The results of this experiment were compared with the results of natural biodegradation of the same soil that was not subjected to the processes of stimulation.

BIOREMEDIATION EXPERIMENT

During the period from September 2009 to March 2010, the *ex situ* stimulated bioremediation of soil contaminated with heavy residual fuel oil (mazut) was conducted. The crude oil–polluted soil was excavated contaminated soil from an energy power plant. Due to a break-down of the energy power plant, the soil had been polluted with heavy fuel oil and sediment from a heavy oil reservoir for a year. The crude oil–polluted soil (approximately 150 t; 210 m^3) was uniformly distributed over 300 m^3 of not rinsed sand from the Sava River (settlement Ostružnica, Serbia). The sawdust from poplar, beech, and oak (approx. 60 m^3) was added in order to increase the retention water capacity, but as alternative additional carbon (C) substrate as well. The entire material (volume of approx. 600 m^3), defined as a bioremediation substrate, was homogenized and then formed into a biopile shape with dimensions of 75 m × 20 m × 0.4 m (length, width, height), with bulldozers. After formation, the biopile was continuously sprayed with biomass, from the tank of 5 m^3. The biomass of microbial consortia, isolated from the crude oil–contaminated soil (re-inoculation) and nutritive substances (biostimulation), was applied on the biopile. Analytical profile index (API-Biomerieux) tests conducted with isolated cultures of microorganisms identified *Pseudomonas aeruginosa*, *Rhodococcus* sp., *Pseudomonas* sp., *Pseudomonas fluorescens*, *Sphingomonas paucimobilis*, *Pseudomonas luteola*, *Achromobacter denitrificans*, *Stenotrophomonas maltophilia* and *Aeromonas hydrophila*.

An optimal ratio of C/N/P/K (approx. 100:10:1:0.1) was achieved by spraying a solution of dissolved ammonium nitrate (N), diammonium hydrogen phosphate (P and N) and potassium chloride (K) with agricultural spraying. Aeration and mixing were performed each 2 weeks with powerful construction machinery. Biomass and nutritive substances were added once a month by turning and mixing the biopile. Biosurfactant of Biosolve type was applied on the biopile at a concentration of 70 mL of the original solution per cubic meter of soil. After preparation, the biopile was covered with plastic foil to prevent direct influence of precipitation and low temperatures on the bioremediation material.

The average daily temperature during the sixmonth experiment was 7.6 ± 6.3 °C (in the range from -2.3 to 23.5 °C). However, due to the intensive microbiological activity, the temperature of the soil was stable, above 25 °C.

A detailed description of this procedure was discussed in the previous paper (Beškoski et al. 2011). Simultaneously with the sampling from the biopile, at the beginning of the experiment, immediately after mixing, but before the addition of sawdust, biomass, nutrient substances, and biosurfactant, samples were taken from the control pile. The complete analytical procedure that was applied to the samples was also applied to the control samples. During the six-month time interval, samples were taken several times.

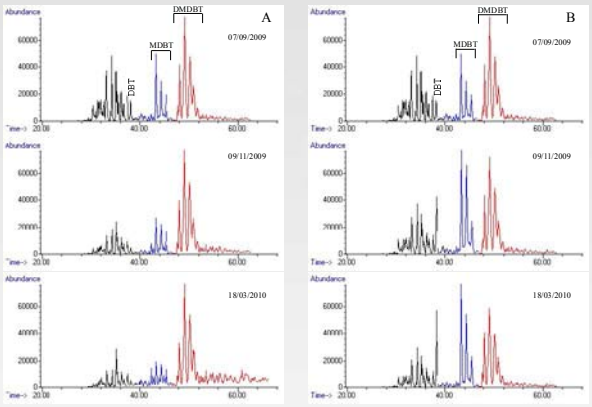

Figure 1. Reconstructed ion chromatograms of dibenzotiophene DBT, *m/z* = 184) + methyldibenzotiophenes (MDBT, *m/z* = 198) + dimethyldibenzotiophenes (DMDBT, *m/z* = 212).

A – natural biodegradation; B – stimulated bioremediation

EXPERIMENTAL

Organic substance from the soil samples was extracted with chloroform (HPLC, J.T., USA) using a Soxhlet apparatus. From these extracts, the hydrocarbons (saturated and aromatic) were isolated by column chromatography. A detailed description of the analytical procedure was discussed in previous papers (Jovančićević et al. 2003; 2005). Hydrocarbons were analyzed by the gas chromatography–mass spectrometry (GC–MS) techniques. An Agilent 7890N gas chromatograph fitted with a HP5-MS capillary column (30 × 0.25 mm, 0.25 μm film; temperature range: 80 °C for 0 min; then 2 °C min^{-1} to 300 °C and held for 20 min) with helium as the carrier gas (flow rate 1 cm^3 min^{-1}) was used. The GC was coupled to a Hewlett- Packard 5972 MSD operated at 70 eV in the 45–550 scan range. Preliminary analyses of the investigated samples were conducted in the full-scan mode. Detailed analyses of the target compounds were conducted in the single-ion monitoring mode (SIM), comprising following ions: 184 (dibenzotiophene), 198 (methyl-dibenzotiophenes) and 212 (dimethydibenzotiophenes).

The peaks were identified according to the literature data or based on the total mass spectra, using mass spectra databases (NIST/EPA/NIH mass spectral library NIST2000, Wiley/NBS registry of mass spectral data, 7th ed., electronic versions.

CONCLUSIONS

Based on these results, a general conclusion can be drawn that the conditions applied during this stimulated bioremediation process of contaminated soil result in more pronounced reduction in the concentrations of alkyl DBT homologues comparing to their parent molecules. Moreover, under the conditions applied, higher alkyl DBT homologues were more degradable than the lower ones. However, the extent and the applicability of this process, as well as the further fate of these persistent pollutants, still remain to be investigated.

REFERENCES:
V. Beškoski, G. Gojgić-Cvijović, J. Milić, M. Ilić, S. Miletić, T. Šolević, M. M. Vrvić, Chemosphere 83 (2011) 34
B. Jovančićević, P. Polić, M. M. Vrvić, G. Sheeder, M. Teschner, H. Wehner, Environ. Chem. Lett. 1 (2003) 73
B. Jovančićević, M. Antić, T. Šolević, M. Vrvić, A. Kronimus, J. Schwarzbauer, Environ. Sci. Pollut. Res. 12 (2005) 205

ACKNOWLEDGMENTS:
We thank the Ministry of Education, Science and Technological Development of the Republic of Serbia (Projects 176006 and III 43004) for supporting this research.