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Analysis of Dibenzothiophene Biodegradation Products During ex situ Bioremediation of Soil Contaminated with Oil Pollutant

Tatjana Šolević Knudsen; Jelena Milic, Mila Ilić,; Srđan Miletić; Vladimir Beškoski, Gordana Gojgić-Cvijović, Miroslav M. Vrvić

The aim of this work was to investigate dibenzothiophene (DBT) biodegradation products formed during ex situ stimulated bioremediation of a soil contaminated with heavy residual fuel oil. A detailed description of the contaminated soil used, bioremediation experiment and microbial community employed is given in our previous paper (Beškoski et al., 2011). Presence of DBT and its methylated homologues in this soil has already been confirmed, as well as microbial activity in biodegradation of these compounds (Šolević Knudsen et al., 2015).

During six-month long ex situ bioremediation experiment, soil samples were taken five times in regular intervals. The soil samples were extracted in a Soxhlet apparatus with dichloromethane as a solvent. The extracts were cleaned up and fractionated using column chromatography. Target compounds were analysed by gas chromatography – mass spectrometry (GC-MS). The GC-MS analysis comprised numerous DBT oxygenated and hydroxylated derivatives, which are known as intermediary products of different DBT biodegradation pathways (Monot and Warzywoda, 2008). Of all DBT derivatives investigated in this research, 2-hydroxybiphenyl (HBP) was the only one identified in the soil samples analyzed. HBP was identified in the soil samples in the final phases of the experiment, when DBT was present in a low abundance, and later, when DBT was almost completely degraded. Detection of HBP, which is a product of, and marker for the 4S DBT biodegradation pathway indicates that corresponding metabolism might also be operational in the microbial community employed in this research. Additionally, presence of HBP in the soil samples when DBT was almost completely degraded indicates that this compound might be useful indicator of former DBT presence in soil even when DBT is completely degraded. Finally, these results indicate that biodesulfurization processes, which have already found application in removal of sulfur-containing compounds from crude oil and its derivatives, aiming at improving their quality, might also play a significant role in reduction of the environmental pollution from the fossil fuel contamination.

[1] Beškoski et al., (2011) Ex situ bioremediation of a soil contaminated by mazut (heavy residual fuel oil) – A field experiment, Chemosphere 83, 34–40; [2] Monot et al., (2008). Microbial desulfurization of petroleum products, In: Petroleum Microbiology: Concepts, Enviromental Implications, Industrial Applications, Vol. 2, Ed.: Vandecasteele J.P. (Paris: Editions Technip), 757–775; [3] Knudsen et al., (2015) Removal of dibenzothiophene and its alkyl homologues during ex situ stimulated bioremediation of contaminated soil. The 16th European Meeting on Environmental Chemistry – EMEC16, Torino, Italy, 30 November – 3 December 2015. Book of Abstracts 151.

Notes

ANALYSIS OF DIBENZOTHIOPHENE BIODEGRADATION PRODUCTS DURING *ex situ* BIOREMEDIATION OF SOIL CONTAMINATED WITH OIL POLLUTANT

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INTRODUCTION

The aim of this work was to investigate dibenzothiophene (DBT) biodegradation products formed during *ex situ* bioremediation of a soil contaminated with heavy residual fuel oil.

Presence of DBT and its methylated homologues in this soil has already been confirmed, as well as microbial activity in biodegradation of these compounds (Šolević Knudsen et al., 2015).

EXPERIMENTAL

During the period from September 2009 to March 2010, the ex situ bioremediation of soil contaminated with heavy residual fuel oil (mazut) was conducted. The crude oil–polluted soil (approximately 150 t; 210 m3) was excavated contaminated soil from an energy power plant, and 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 m3) 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.

Simultaneously with the sampling from 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 six-month long ex situ bioremediation experiment, soil samples were taken five times in regular intervals. The soil samples were extracted in a Soxhlet apparatus with dichloromethane as a solvent. The extracts were cleaned up and fractionated using column chromatography. Target compounds were analysed by gas chromatography – mass spectrometry (GC-MS). The GC-MS analysis comprised numerous DBT oxygenated and hydroxylated derivatives, which are known as intermediary products of different DBT biodegradation pathways (Monot and Warzywoda, 2008).

The compounds were identified by coinjection of authentic standards and matching of their spectra with those from the spectral library (NIST11).

RESULTS

Of all DBT derivatives investigated in this research, 2-hydroxybiphenyl (HBP) was the only one identified in the soil samples analyzed. This compound was identified only in the control pile. HBP was identified in the soil samples in the final phases of the experiment, when DBT was present in a low abundance, and later, when DBT was almost completely degraded (Figures 1 and 2).

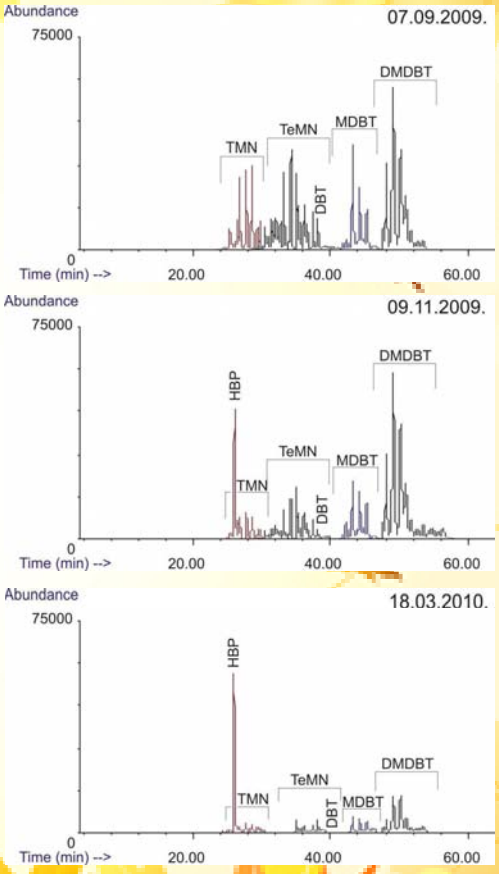

Figure 1. GC-MS chromatograms of ions *m/z* = 170 (2-hydroxybiphenyl – HBP and trimethyl-naphthalenes – TMN)), *m/z* = 184 (tetramethyl-naphthalenes – TeMN and dibenzotiophene – DBT), *m/z* = 198 (methyl-dibenzotiophenes – MDBT), and *m/z* = 212 (dimethyl-dibenzotiophenes – DMDBT) of the aromatic fractions isolated during the experiment.

CONCLUSIONS

Detection of HBP, which is a product of, and marker for the 4S DBT biodegradation pathway indicates that corresponding metabolism might also be operational in the microbial community employed in this research.

Additionally, presence of HBP in the soil samples when DBT was almost completely degraded indicates that this compound might be useful indicator of former DBT presence in soil even when DBT is completely degraded.

Finally, these results indicate that biodesulfurization processes, which have already found application in removal of sulfur-containing compounds from crude oil and its derivatives, aiming at improving their quality, might also play a significant role in reduction of the environmental pollution from the fossil fuel contamination.

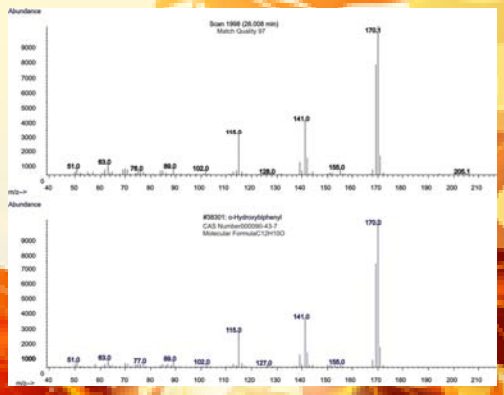

Figure 2. Mass spectrum of HBP identified in the samples (up) and the best matching spectrum from the NIST library (down) .

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Beškoski V., Gojgić-Cvijović G., Jelena 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 83, 34–40.

Monot F., Warzywoda M. (2008). Microbial desulfurization of petroleum products, In: Petroleum Microbiology: Concepts, Enviromental Implications, Industrial Applications, Vol. 2, Ed.: Vandecasteele J.P. (Paris: Editions Technip), 757–775.

Šolević Knudsen T., Ilić M., Milić J., Beškoski V., Gojgić-Cvijović G., Jovančićević B., Vrvić M.M. (2015) Removal of dibenzothiophene and its alkyl homologues during ex situ stimulated bioremediation of contaminated soil. The 16th European Meeting on Environmental Chemistry – EMEC16, Torino, Italy, 30 November – 3 December 2015. Book of Abstracts 151.

ISO/TR 11046:1994 International standard: soil quality - Determination of mineral oil content - Method by infrared spectrometry and gas chromatographic method.

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