Treatmeanntation of methyl – phenanthrene by bioremediation of soil polluted by fuel oil

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Abstract

In this work, the ex situ natural bioremediation of soil polluted by heavy fuel oil from an oil refinery, was conducted on period about of six months. Phenanthrene and methyl-phenanthrenes are most Aromatic pollutants originating specially from fuel oil. Phenanthrene is usually degraded faster than methyl-phenanthrenes under different environmental conditions. Here, we noted a preferential and accelerated biodegradation of methyl –phenanthrenes versus phenanthrenes in soil contaminated by fuel oil. The polluted soil is mixed microbial consortia isolated from crude oil contaminated soil and treated by bio-surfactants and nutritive substances for biostimulation. In the present investigation, the ex situ natural bioremediation of soil for a steady increase in the relative abundances of phenanthrene compared to methyl phenanthrene was observed by gas chromatography – mass spectrometry the increase was the highest for trimethyl phenanthrenes.

Key wards: Gargaresh Formation, Calcarenite sediments.

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Introduction:

Bioremediation is a modern method in which the natural biodegradation 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 very efficient in the removal of crude oil and some oil derivatives (Ollivier, Magot, 2005), especially when biostimulation and bio-augmentation are applied (Beškoski et al., 2011).

The aim of this study was to investigate and define the influence of biostimulation factors on microbiological degradation of main components in petroleum-type pollutants. Oil is a very complex mixture of hydrocarbons, nitrogen, sulfur and oxygen compounds (NSO). More studies are required for each class of compounds to define the type of microorganisms and optimal conditions for microbial degradation (Fritshe and Hofrichter, 2008 and Van Hamme et al., 2003). The knowledge about the rate of microbial degradation of individual organic compounds in oil is mostly based on the organic geochemical research. Oils are classified into 9 groups according to their degree of biodegradation 3. Completed the classification of oils according to the biodegradation level, comparing the degradability of a larger number of organic compound classes (Head et al., 2003). First of all, they included mono- and triaromatic steroids as well as phenanthrene with its methyl isomers. Recently, more detailed research has been conducted on phenanthrene isomers. It was shown that of 29 types of bacteria in the soil, 11types use methyl phenanthrene (Lamberts et al. 2008). According to these authors. In this study, the changes in the distribution of phenanthrene and its methyl isomers (mono, di, and tri) within bioremediation of soil pollutants with heavy residual were investigated. The end of biodegradation experiment of soil that was treated with biomass (re inoculation) and nutrients (bio stimulation) were compared with the results of bioremediation of soil that was not subjected to these processes of stimulation.

Investigated soil

As already stated, a soil polluted with heavy fuel oil was considered the most appropriate medium for all investigation defined in the aim of this study. Accordingly, the soil from surroundings of an energy power

plant in was chosen as the most suitable for these criteria. Due to a break-down of the energy power plant facilities, this soil had been polluted with mazut and sediment from a mazut reservoir for a year. Because of that, we considered that this soil might contain high concentration of oil pollutant as well as a bioremediation potential high enough to satisfy the goals set by the aim of this research.

Environmental conditions during the experiment

The average daily temperature during the six months experiment was 7.6 ± 6.3 °C

(In the range from -2.3 to 23.5 °C). Although the experiment was conducted in autumn and winter, due to the intensive microbiological activity, the temperature of the soil was stable, above 25 °C. After each treatment the biopiles were covered with polyethylene foil to prevent reduction of the temperature and direct influence of precipitations and low temperatures (weather conditions) on the bioremediation substrate.

Experimental

During the period from August 2013 to February 2014, the ex situ natural biodegradation (unstimulated bio-remediation, without addition of biomass, nutrient substances and bio-surfactant) of soil pollutant with residual fuel oil was started. The crude oil-contaminated soil was excavated from an oil refinery which, due to a break-down, had been polluted with heavy fuel oil and sediment from a heavy oil reservoir for a year. The mazut polluted soil (approximately 150 t; 210 m3) was uniformly distributed over 300 m3 of not rinsed sand. 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 source as well. To ensure homogeneity, the components were mixed three times with a frontend, and finally, raked using a tractor fitted with a harrow. The entire homogenized material (volume of approx. 600 m3) defined as a substrate for bioremediation ,was then formed into a biopile shape with dimensions of 75 x 20 x 0.4 m (length, width, height), with bulldozers. Immediately after mixing, approximately 10 m3 of the biopile mixture was set aside on waterproof asphalt surface to be used as a substrate for monitoring natural biodegradation. The experiment was conducted in autumn and winter with average daily temperatures ranging from 25 °C to -10 °C. However, the biomass of microbial consortia, isolated from crude oil contaminated soil (re-inoculation) and nutritive substances (bio stimulation), was

applied on the biopile. Analytical Profile Index (API-Biomerieux) tests were used for identification of microorganisms conducted with isolated cultures of microorganisms identified: Pseudomonas aeruginosa, Rhodococcus sp., Pseudomonas sp., Pseudomonas fluorescens Achr-omobacter denitrificans, Stenotro-phomonas maltophilia and Aeromonas hydrophila. Biomass and nutritive substances were sprayed over the biopile using an agriculture sprayer fitted to a tractor with a trailer unit. biomass concentration was 1 .44 x 107 cells/mL in order to achieve an optimal ratio of C/N/P/K (approx., 100;10;0;1) was achieved by spraying a solution of dissolved ammonium nitrate(N), diammonium hydrogen phosphate(P and) and potassium chloride (K) with agricultural spraying. Aeration and mixing were performed each two weeks with powerful construction machinery. Biomass and nutritive substances were added one a month by turning a mixing the biopile.

Control pile

At the beginning of the study, immediately after mixing, but before the addition of sawdust, biomass, nutrient substances and biosurfactant, approximately 10 m3 of the biopile mixture was set aside on the same waterproof asphalt surface, to be used as a control pile. The complete analytical procedure that was applied to the samples was also applied to the control samples during an independent parallel non-biostimulated bio-degradation experiment (Novaković et al., 2012; Ramadan et al., 2012).

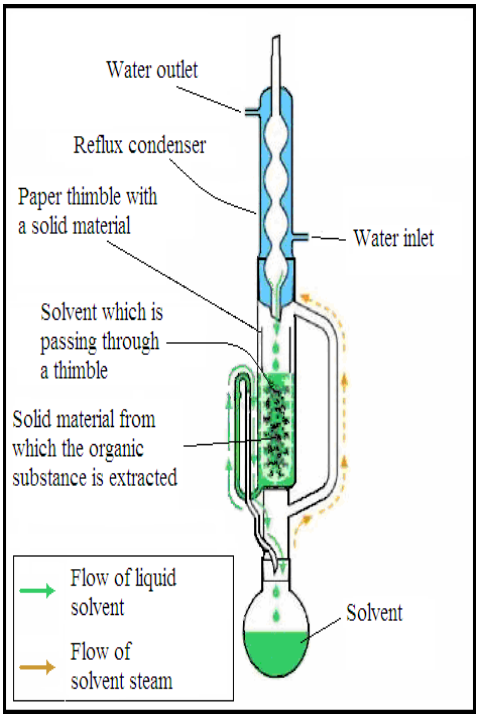
Sampling

About the six-month time interval the samples were taken five times (07/08/2013 – M1; 06/9/2013 – M2; 09/10/2013 – M3, 12/12/2013 – M4 and 18/2/2014 – M5). Samples taken from soil that were treated with sawdust, biomass, nutrient, and bio-surfactants were marked M1-M5. Control test samples, taken at the same time, were marked M1K-M5K.

Isolation of extracts

Organic substance from in total 10 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 chromate-graphy: the extracts were saponified with a 5 % solution of KOH in methanol, and neutralized (after standing overnight) with 10 % hydrochloric acid. The products were dissolved in a mixture of dichloromethane (containing 1 %

methanol) and hexane (1:40), and separated by column chromatography on alumina and silica gel. The hydrocarbon fractions were eluted with hexane (saturated hydrocarbons) followed by dichloromethane (aromatic hydrocarbons).



Picture 1. Soxhlet apparatus

Detailed analyses of the target compounds were conducted in the single ion monitoring mode (SIM), comprising the following ion chromatograms: 178 (phenanthrene), 192 (methyl-phenanthrenes), 206 (dimethyl-phenanthrenes) and 220 (trimethyl - phenanthrenes).Peaks of the phenanthre, methyl-phenanthrenes, and dimethyl – phenanthrenes were identified according to organic geochemical literature data (e.g., Peter et al. 2005),or based on the total mass specter, using mass spectra databases (NIST/EPA/NIH mass spectral LibraryNIST2000,Wiley/NBS

registry of mass spectral data, 7th electronic versions).

Picture 2. Hydrocarbons were analysed by GC–MS techniques. An Agilent 7890N gas chromatograph fitted with a HP5-MS capillary column (30 x 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.

Results and discussion

Mass fragmentograms of phenanthrene, methyl-, dimethyl- and trimethyl-phenanthrenes obtained by GC–MS analysis of aromatic fractions isolated from extracts of samples M1 – M5 are shown in Figure 1. These samples were subjected to re-inoculation and biostimulation with the addition of sawdust and biosurfactant. Fragmento-grams of control tests (samples M1K-M5K, without the addition of sawdust, biomass, nutrient substances and biosurfactant) are shown in (fig. 2.) In our work the changes in the distribution of phenanthrene and its methyl isomers (mono-, di- and tri-) were investigated. These aromatic hydrocarbons are not ate the so high level of toxicity (Simmon et al. 1999). However, they are in most of the oils, and therefore in most of the oil pollutants, dominant aromatic hydrocarbons. Moreover, in general, as well as polycyclic aromatic hydrocarbons, they represent a significant pollutant of all segments of the environment, including soils (Lichtfouse et al 2005; Bryselbout et al. 2000; Henner et al. 1997). Relative concentrations and values of methyl and trimethyl isomers were calculated (Novaković et al., 2012; Ramadan et al., 2012). it can easily be observed that during the process of biodegradation of soil pollutant by fuel oil (mazut), there was a uniform increase in the relative abundance of phenanthrene compared to (mono-, di- and tri-)methyl, this increase was pronounced in the case of trimethyl-phenanthrene. Based on these results can be drawn a general conclusion that the bioremediation process

under condition described results in increase in the concentration of phenanthrene but also its lower methyl homologue compared to the higher homologues. From a total of nine kinds of microorganisms identified in the zymogen consortium, six of them belong to the group of efficient petroleum hydrocarbons degraders. These are p. aeruginosa. Rhodococccus sp. Pseudomona sp., P. Rescans, P. luteola and A. denitrificans, S. maltophilia, and A. hydrophila (Bosser and Bartha 1984, Singh and ward 2004).

. This result could indicate that the interaction of phenanthrene isomers with bacterial cells is increase if phenanthrene contains a larger number of methyl-substituents. Monitoring change in the distribution of phenanthrene and its methyl isomers (mono-, di- and tri-) in five samples belonging to the control trials (samplesM1k-M5k), actually gives an estimate of the transformations that occur during the natural microbial bioremediation of oil contaminated. Contrary to the degradation process (samples M1-M5), sawdust, biomass, nutrient substances, and bio-surfactant were not added to the control samples.

Conclusion

1. The biodegradation trend among phenanthrene and its methyl-, dimethyl- and trimethyl-homologues observed in this research is opposite to the typical biodegradation trend of phenanthrene and its methyl isomers during the natural “unstimulated” biodegradation.
2. An increase in the availability of phenanthrene and its methyl derivatives to microorganisms can increase degradability of methyl-phenanthrenes compared to phenanthrene. In this study, an increased availability of phenanthrene and its methyl derivatives to microorganisms was accomplished by re-inoculation, bio-stimulation, as well as by the addition of sawdust and bio-surfactants.

Additionally, the level of degradability in these conditions depends on the number of methyl groups, that is, on the level of alkylation.

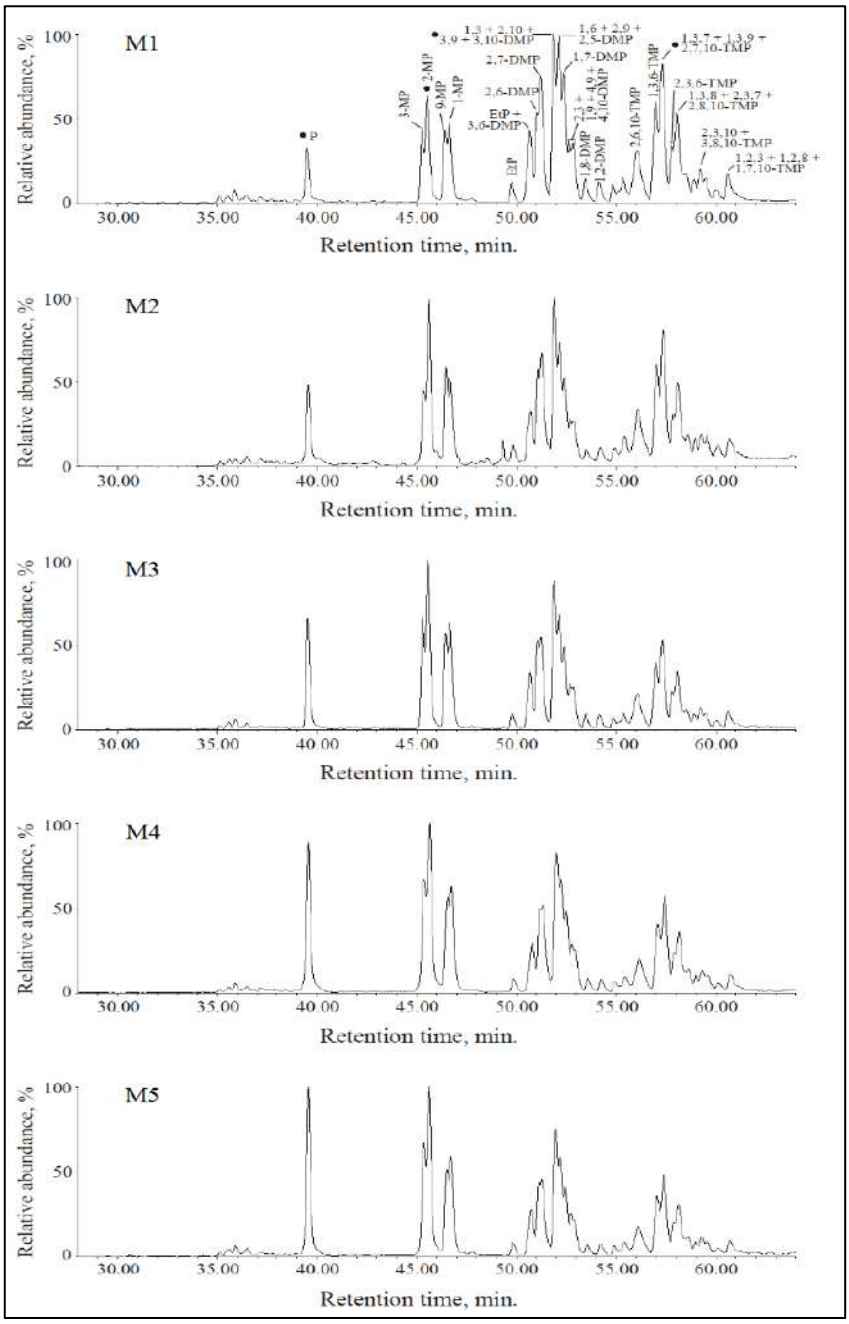


Figure 1. Mass fragmentograms of phenanthrene (m/z 178) methyl-phenanthrenes (m/z 192) dimethyl-phenanthrenes (m/z 206) and trimethyl-phenanthrenes (m/z 220), soil samples M1 – M5. Taken from soil that were treated. Not the uniform decrease in the relative abundance of methyl isomers compared to phenanthrene. This trend is opposite to the typical biodegradation sequence phenanthrene and its methyl isomers.

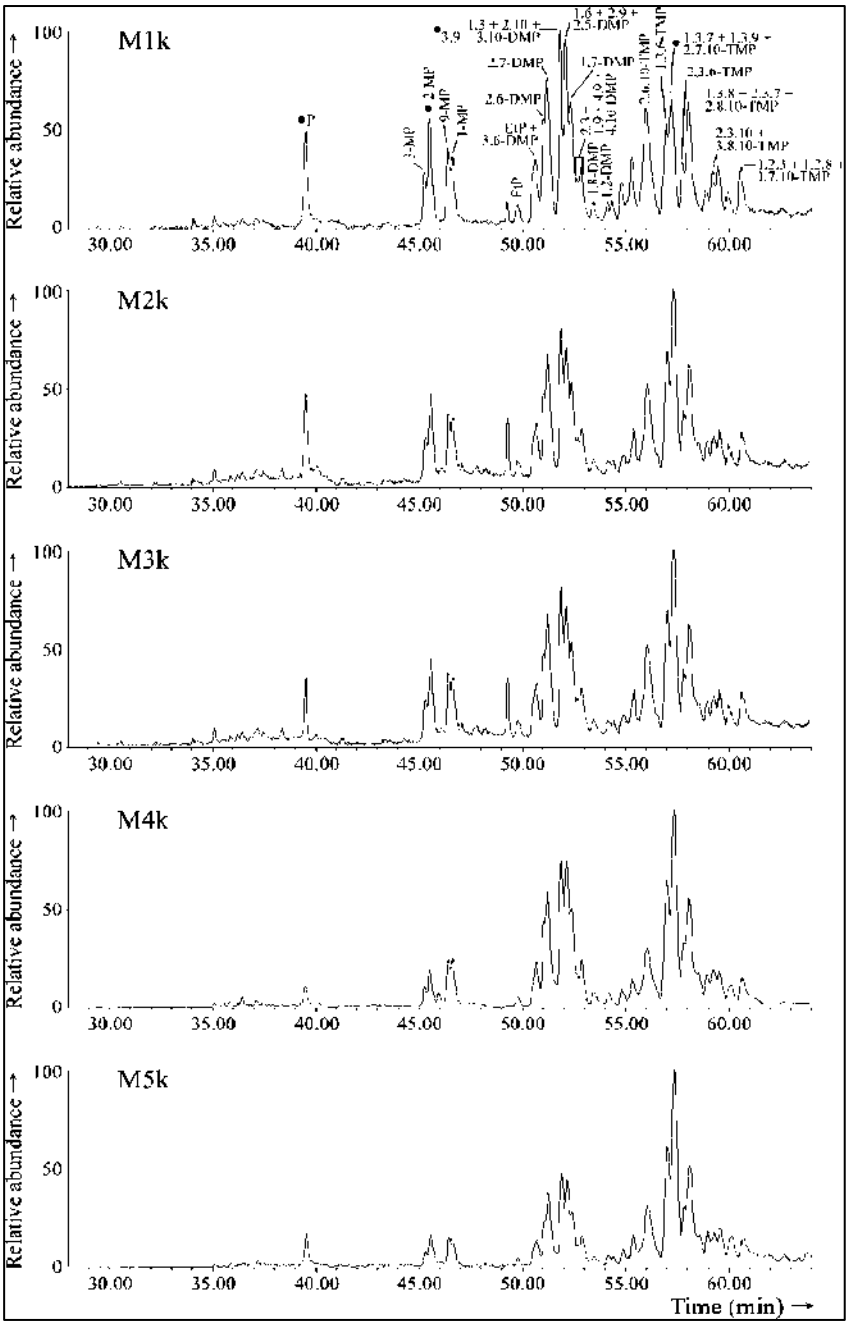


Figure 2. Mass fragmentograms of phenanthrene (m/z 178) methyl-phenanthrenes (m/z 192) dimethyl-phenanthrenes (m/z 206) and trimethyl-phenanthrenes (m/z 220), soil samples M1k – M5k. Taken from soil that were not treated. Note the uniform decrease in the relative abundance of phenanthrene compared to its methyl isomers.

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