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Degradability of *n*-alkanes during *ex situ* stimulated bioremediation of soil contaminated by heavy residual fuel oil (mazut)

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In this paper, the *ex situ* stimulated bioremediation of soil contaminated with heavy residual fuel oil (mazut) was conducted during the period of 6 months. The fate of *n*-alkanes in the pollutant was monitored.

According to our previous research [1] it was concluded that during natural biodegradation of oil pollutant, in the conditions of the reduced availability of certain class of compounds (first of all caused by their low amount), microorganisms opt for those which are more accessible, i.e. those which are found in the substrate in higher amount, even if these compounds are less biodegradable.

In the present research we investigated if in the conditions of stimulated bioremediation (with addition of biomass, nutrient substances and biosurfactant) the biodegradation of the compounds which are found in low amount can be stimulated as well.

The soil polluted with heavy residual fuel oil (mazut) was mechanically mixed with softwood sawdust and crude river sand to form a homogenized biopile. Reinoculation was performed periodically with biomasses of microbial consortia isolated from the mazut-contaminated soil. Biostimulation was conducted with addition of nutritional elements (N, P and K). Aeration was improved by systematic mixing. Biosurfactants were used as surface active agents to solubilize mazut.

During the interval of six months the samples were taken five times. Organic substance from in total 5 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 and analyzed by the gas chromatography–mass spectrometry (GC–MS) techniques. In the aromatic fraction phenanthrene, methyl-phenanthrenes, dimethyl-phenanthrenes and trimethyl-phenanthrenes were analyzed in detail. The saturated hydrocarbon mixture was separated into *n*-alkane and branched and cyclic alkane fractions by urea adduction. The *n*-alkanes in urea adducts were analyzed by gas chromatography (GC).

In the initial sample the fraction of total saturated hydrocarbons was characterized by a broad and prominent "hump" of an unresolved complex mixture (UCM), typical of oils altered by biodegradation. *n*-Alkanes were present in a very low abundance and their identification was possible only after concentration by urea adduction technique. According to these results the investigated oil pollutant was classified to be at the boundary between the third and the fourth biodegradation level.

During the experiment, a specific biodegradation pattern of methyl- phenanthrene homologues was observed (the most pronounced in comparison with the trimethyl-phenanthrenes and the least in comparison with the methyl-phenanthrenes). It was concluded that this process of applied bioremediation resulted in the increase in the availability of phenanthrene and its methyl derivatives to microorganisms and in that way increased the degradability of homologues with higher level of alkylation [2].

However, in the fractions of saturated hydrocarbons investigated in the present research, the removal of the remaining *n*-alkanes was not observed. The abundance of *n*-alkanes remained at the initial low level, even at end of the experiment, after six months of the intensive stimulated bioremediation.

According to these results it can be concluded that even in conditions of intensive stimulated bioremediation, biodegradation of individual components of oil pollutants will no proceed (not even in the case of *n*-alkanes which are the most biodegradable hydrocarbons in oils) unless they are present in some minimum "threshold" amount.

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