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Transformation of a petroleum pollutant during soil bioremediation experiments

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Abstract: The experiment of *ex situ* soil bioremediation was performed at the locality of the Oil Refinery in Pančevo (alluvial formation of the Danube River, Serbia) polluted with an oil type pollutant. The experiments of biostimulation, bioventilation and reinoculation of an autochthonous microbial consortium were performed during the six-month period (May–November 2006). The changes in the quantity and composition of the pollutant, or the bioremediation effect, were monitored by analysis of the samples of the polluted soil taken in time spans of two weeks. In this way, from the beginning until the end of the experiment, 12 samples were collected and marked as P_1–P_{12} (Pančevo 1–Pančevo 12). The results obtained showed that more significant changes in the composition of the oil pollutant occurred only during the last phases of the experiment (P_8–P_{12}). The activity of microorganisms was reflected in the increase of the quantity of polar oil fractions, mainly fatty acid fractions. In this way, the quantity of total eluate increased, and the quantity of the insoluble residue was reduced to a minimum, whereby the oil pollutant was transformed to a form that could be removed more efficiently and more completely from the soil, as a segment of the environment.

Keywords: bioremediation; oil type pollutant; soil; Pančevo Oil Refinery; alkanes; fatty acids.

INTRODUCTION

The fate of an oil type pollutant in the environment can be monitored most accurately by determining its quantity and studying its composition in polluted samples from the same or a neighbouring locality during different time periods. For example, in this way, oil microbiological transformations were monitored in

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samples of ground waters during a period of almost three years in the locality of the Oil Refinery in Pančevo (Serbia), which belongs to an alluvial formation of the Danube River.[1–4] The samples of ground waters were taken from the same piesometer. An identical method was applied for determining the quantity of hydrocarbons and the composition at the molecular level was defined using the instrumental techniques, gas chromatography (GC) and gas chromatography combined with mass spectrometry (GC–MS). However, these studies obviously lasted too long.

Simulation of natural conditions in the laboratory with the simultaneous intensification of only specified factors affecting the intensity and rate of transformation enabled time saving and relevant conclusions to be reached. Thus, the course of the biodegradation of an oil pollutant in a sample of surface water (alluvial formation of the Danube River, Serbia) was accelerated under the laboratory conditions during ninety days by exposing the pollutant to the influence of those microorganisms identified in the natural environment but at a much higher concentration.[5] There are also numerous other examples. Rosa and Trigüis[6] assessed the behaviour of oil pollutants during a laboratory investigation of bioremediation (using nutrients) in tropical coast sediments. Bioremediation experiments on oil polluted soils were also performed using white rot fungus, *Pleurotus tuberregium* (Fr.) Sing.[7] A similar method was used to monitor the fate of other non-oil organic pollutants, *e.g.*, polycyclic aromatic hydrocarbons (PAHs),[8,9] polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-*para*-dioxins (PCDD/Fs).[10,11]

The mentioned experiments of bioremediation offered the opportunity to not only gain fundamental knowledge of the fate of pollutants in the environment, but also to asses to what extent bioremediation as a technique may be applied to refine polluted water, soil or sediments.

In this paper, the results of a study of the decomposition of an oil-type pollutant during *ex situ* bioremediation of soil from the pilot heap (halde) of approximately 150 m^3 at the Petroleum Refinery Pančevo (Serbia) are presented. Experiments of biostimulation, bioventilation and re-inoculation of autochthonous microbial consortium were performed during a period of six months.

EXPERIMENTAL

The experiment of soil bioremediation was performed at the locality of the Oil Refinery in Pančevo (alluvial formation of the Danube River, Serbia) polluted with an oil type pollutant. It lasted for 6 months (May–November 2006). The changes in the quantity and composition of the pollutant, *i.e.*, the bioremediation effect, were monitored by analysis of the polluted soil samples taken in time spans of two weeks. In this way, from the beginning until the end of the experiment, 12 samples were collected and denoted as P_1–P_{12} (Pančevo 1–Pančevo 12).

Approximately, a quantity of 150 m^3 of polluted soil was collected from five locations from the refinery's property. The samples were mechanically homogenized. Poplar cutting was added to the soil (10 % of total volume) in order to increase its aeration. Esters of long

chain alcohols and fatty acids were added as biodegradable substrates active at the surface (0.20 kg m^{-3}), and manure from poultry farms as a source of nitrogen and phosphorus for biostimulation (approx. 5 %). Thereafter, the soil was formed into a halde shape and aeration pipes and a gutter system for collecting seepage water were installed. Manual stirring of the soil material every two weeks ensured homogenization and additional aeration of the halde content. The halde was protected from direct external influences by a green house.

The titre of microorganisms was determined by serial dilution on agar plates incubated at 26 °C. Nutrient agar was used for bacteria, Sabouraud glucose agar with chloramphenicol for yeasts and moulds and a mineral base medium with 2000 ppm diesel fuel was used for hydrocarbon-degrading microorganisms.

The population growth of the microorganisms and intensification of the biodegradation process in addition to biostimulation was achieved by re-inoculation of zymogenous microorganisms. Bacteria strains which are able to decompose hydrocarbons were spawned in the laboratory bioreactor. 10 dm^3 of biomass of high density (up to 10^9 CFU cm^{-3}) of activated microorganisms were deposited on the experimental halde once a week during the monitoring of the bioremediation.

The organic substance from samples P_1–P_{12} was Soxhlet extracted with chloroform for 36 h and quantified. The group composition in the extracts was determined and fractions of saturated hydrocarbons, aromatic hydrocarbons, alcohols and fatty acids were isolated by column chromatography. GC–MS was applied for analysis of some of the compounds in fractions. Fatty acids were detected as methyl esters formed during the elution step. Detailed descriptions of the analytical procedure and applied analytical techniques were discussed in previous papers.[3,12]

RESULTS AND DISCUSSION

The contents of the organic substance in the samples P_1–P_{12}, as well as the results obtained by chromatographic separation of the isolated extracts are given in Table I.

TABLE I. Extracted content of organic matter in samples P_1–P_{12} and the results obtained by column chromatography of the isolated extracts

Sample	Date of sampling	Organic substance from soil, %	Saturated HC in eluate, %	Aromatics in eluate, %	Alcohols in eluate, %	Fatty acids in eluate, %
P_1	10/05/2006	5.9	46.4	9.5	27.4	16.7
P_2	24/05/2006	4.8	53.3	13.0	26.0	7.8
P_3	12/06/2006	5.9	45.9	10.8	32.4	10.8
P_4	28/06/2006	5.7	54.4	14.4	21.1	10.0
P_5	12/07/2006	5.5	51.2	12.8	24.4	11.6
P_6	26/07/2006	4.6	45.5	17.0	26.1	11.4
P_7	14/08/2006	4.2	53.2	13.9	21.5	11.4
P_8	30/08/2006	6.6	58.1	6.5	25.8	9.7
P_9	13/09/2006	4.5	53.6	10.1	21.7	14.5
P_{10}	04/10/2006	6.1	49.4	19.5	23.4	7.8
P_{11}	19/10/2006	5.3	39.2	19.6	26.8	14.4
P_{12}	10/11/2006	4.1	33.3	17.1	32.4	17.1

The extractable contents of organic substance ranged from 4.1 to 6.6 %. However, although the last analyzed sample contained the least amount in the

extract, a regular decreasing trend in the total extract amount with the increasing time of bioremediation was not observed.

Changes in the contents of the four isolated fractions of P_1 to P_{12} also showed no significant regularity. Only for the content of saturated hydrocarbons was perhaps an evenly decreasing trend from 58.1 → 33.3 % for P_8 (sample taken on 30/08/2006) to P_{12} (10/11/2006) observed (Table I). Based on this observation, it may be concluded that the microbiological decomposition of the alkane fraction occurred in the second part of the bioremediation experiment. It is very well known that saturated hydrocarbons, compared to other fractions in oil, are the least resistant to biodegradation.[13] Therefore this reduction in the contents of the alkane fraction may be considered as an expected and logical result.

The results of the group composition of the extracts given in Table I represent the quantities of some fractions recalculated to the total eluate. However, a much more realistic picture on the changes in composition of the oil pollutant during the performed bioremediation experiment (especially for samples P_8 to P_{12}) can be obtained if the calculation includes also the residue remaining on the column during the chromatographic separation. Then the results of the contents of the four isolated fractions in the 12 examined samples become obviously different (Table II).

TABLE II. Results obtained by column chromatography of the isolated extracts (including the column residue)

Sample	Saturated HC in extract, %	Aromatics in extract, %	Alcohols in extract, %	Fatty acids in extract, %	Column residue, %
P_1	37.1	7.6	21.9	13.3	20.0
P_2	38.7	9.4	18.9	5.7	27.4
P_3	32.7	7.7	23.1	7.7	28.8
P_4	47.6	12.6	18.4	8.7	12.6
P_5	41.5	10.4	19.8	9.4	18.9
P_6	38.8	14.6	22.3	9.7	14.6
P_7	40.8	10.6	16.5	8.7	23.4
P_8	35.3	3.9	15.7	5.9	39.2
P_9	33.3	6.3	13.5	9.0	37.8
P_{10}	34.9	13.8	16.5	5.5	29.4
P_{11}	35.0	17.5	23.9	12.9	10.7
P_{12}	32.4	16.7	31.5	16.7	2.8

This calculation avoids the detected reduction of the alkane fraction from P_8 to P_{12}. In this phase of the bioremediation process, an obvious increase in the content of the fatty acid fraction (5.9 → 16.7 %, Table II) occurred. GC–MS analysis confirmed that these fractions were dominated by *n*-fatty acids (Fig. 1).

The quantities of the aromatic and alcohol fractions increased, with certain deviations, from sample P_8 to sample P_{12}. If, however, the values for these two fractions are summed, the increasing trend was even more obvious. The detected regularities are very clear if the values are presented as a histogram (Fig. 2).

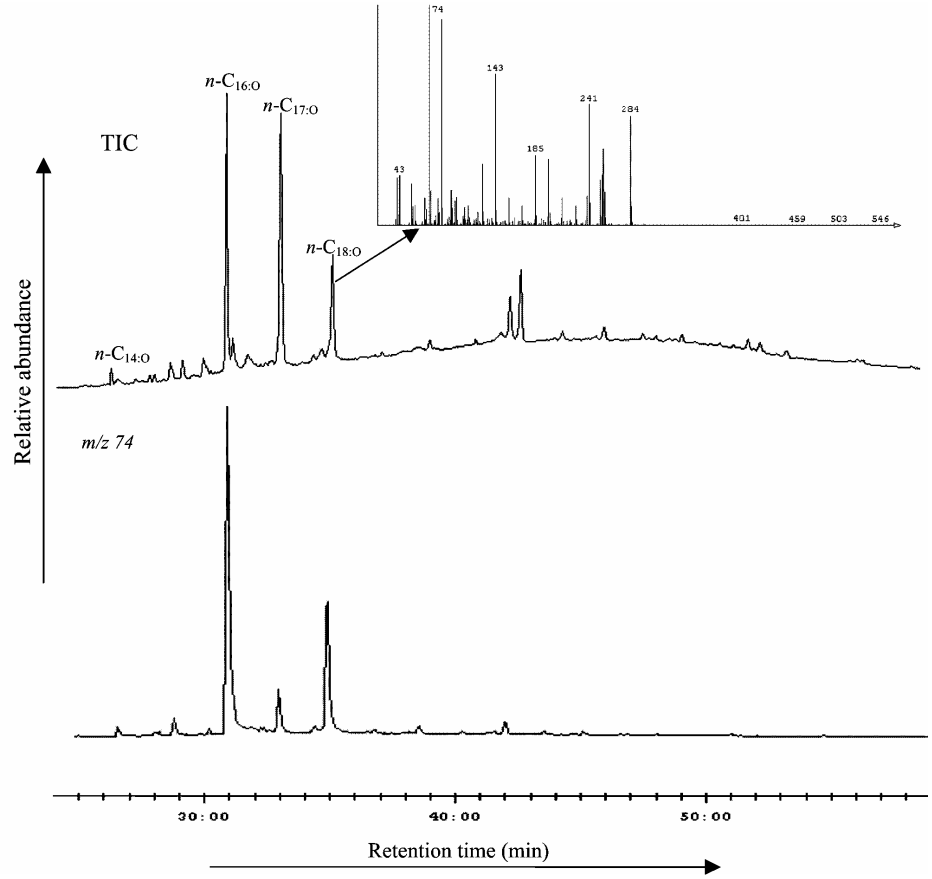

Fig. 1. Total ion chromatogram (TIC) of the fatty acid fraction and related chromatogram of mass *m*/*z* 74 (typical for fatty acid esters) of the P_{10} extract, characteristic for all the investigated samples. As additional proof of the presence of fatty acids (or their esters), the entire mass spectrum which matches the peak of *n*-C_{18} fatty acid esters is also given.

For the part of the bioremediation experiment covered by samples P_8 to P_{12}, the most obvious results are the reduction of the amount of residue remaining on the column (39.2 → 2.8 %) and the increase in the total amount of eluate (60.8 → → 97.2 %, Table II; Fig. 2). Based on this observation, it may be concluded that the activity of the microorganisms contributed to the minimization of the insoluble part in the oil pollutant. The results of the group composition of the extracts showed that this activity also resulted in an increase of all polar fractions (aromatics, alcohols and fatty acids) and that the quantity of the saturated hydrocarbon fraction remained approximately constant. The detected reduction in the quantity of this fraction for samples P_8–P_{12} based on the results presented in Table I (calculated only for the eluate mass) is only apparent and is the consequence of the increased quantities of the other fractions.

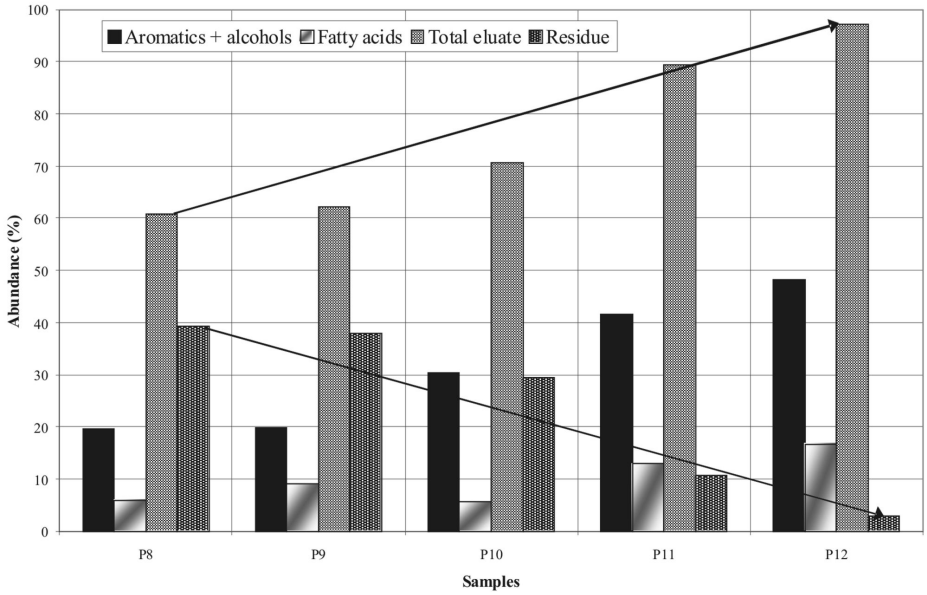

Fig. 2. Contents of total aromatics and alcohols, fatty acids, total eluate and the column residue for samples $P_8 - P_{12}$.

CONCLUSIONS

An *ex situ* experiment of the bioremediation of oil polluted soil from the locality of the Oil Refinery in Pančevo (alluvial formation of the Danube River, Serbia) was performed during a period of 6 months (May–November 2006). The effect of the bioremediation was monitored by analysis of samples of the polluted soil taken in time intervals of two weeks (12 samples in total; samples P_1–P_{12}). The obtained results showed that significant changes in the composition of the oil pollutant occurred only during the last phases of the experiment (samples P_8–P_{12}). The activity of the microorganisms was reflected in an increase in the quantity of the polar oil fractions, most obvious for the fatty acid fractions. In this way, the quantity of the total eluate increased (60.8 → 97.2 %) and the quantity of the insoluble residue diminished (39.2 → 2.8 %). Thus, the oil pollutant was transformed into a form which could be removed more efficiently and more completely from the soil, as a segment of the environment.

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ИЗВОД

ТРАНСФОРМАЦИЈЕ ЗАГАЂИВАЧА НАФТНОГ ТИПА ЗА ВРЕМЕ ЕКСПЕРИМЕНТА БИОРЕМЕДИЈАЦИЈЕ

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Извођени су експерименти *ex situ* биоремедијације земљишта на локалитету Рафинерије нафте Панчево (алувијална формација реке Дунав). Експерименти су извођени у току периода од шест месеци (мај–новембар 2006. године). Промене у количини и саставу нафтног загађивача (биоремедиациони ефекат), праћене су анализом узорака који су узимани у временским размацима од две недеље. На тај начин, у току шест месеци сакупљено је 12 узорака означених са P_1–P_{12} (Pančevo 1–Pančevo 12). Добијени резултати показали су значајне промене у саставу нафтног загађивача само у последњој фази експеримента (P_8–P_{12}). Активност микроорганизама огледала се у повећању количине поларних нафтних компонената, на првом месту фракције масних киселина. На тај начин, у овој фази експеримента, повећана је количина укупног елуата, а количина нерастворног остатка сведена је на минималну вредност, чиме је нафтни загађивач доведен у облик који се ефикасније може уклонити из земљишта, као сегмента животне средине.

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