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Original scientific paper

Investigation of the bioremediation potential of aerobic zymogenous microorganisms in soil for crude oil biodegradation

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Abstract: The bioremediation potential of the aerobic zymogenous microorganisms in soil (Danube alluvium, Pančevo, Serbia) for crude oil biodegradation was investigated. A mixture of paraffinic types of oils was used as the substrate. The laboratory experiment of the simulated oil biodegradation lasted 15, 30, 45, 60 and 75 days. In parallel, an experiment with a control sample was conducted. Extracts were isolated from the samples with chloroform in a separation funnel. From these extracts, the hydrocarbons were isolated by column chromatography and analyzed by gas chromatography–mass spectrometry (GC–MS). *n*-Alkanes, isoprenoids, phenanthrene and its derivatives with one and two methyl groups were quantitatively analyzed. The ability and efficiency of zymogenous microorganisms in soil for crude oil bioremediation was assessed by comparison between the composition of samples which were exposed to the microorganisms and the control sample. The investigated microorganisms showed the highest bioremediation potential in the biodegradation of *n*-alkanes and isoprenoids. A considerably high bioremediation potential was confirmed in the biodegradation of phenanthrene and methyl phenanthrenes. Low bioremediation potential of these microorganisms was proven in the case of polycyclic alkanes of the sterane and triterpane types and dimethyl phenanthrenes.

Keywords: bioremediation; soil zymogenous microorganisms; crude oil; hydrocarbons.

INTRODUCTION

Bioremediation is a process in which naturally occurring microorganisms, through their normal life functions, degrade or detoxify substances hazardous to

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human health and/or the environment.[1] As a natural cleaning process, bioremediation has been proven to be efficient in the removal of chlorinated solvents,[2] polyaromatic hydrocarbons,[3] pesticides,[4] and even some heavy metals.[5]

Bioremediation of the environment polluted by crude oil relies on the fact that indigenous microbial population can biodegrade most of the hydrocarbons present in oils, mineralizing them into carbon dioxide and water.[6] Compared to physico-chemical methods, bioremediation offers an effective technology for the treatment of oil pollution because the majority of molecules in the crude oil and refined products are biodegradable and oil-degrading microorganisms are ubiquitous.[7] However, some compounds present in oils can not be biodegraded, or the process is incomplete,[8] which restricts the usage of the microbial degradation as a bioremediation technique in oil pollution clean-up.

The aim of the research described in this paper was to investigate the bioremediation potential in crude oil biodegradation of zymogenous microorganisms which were isolated from soil in the vicinity of the wastewater canal (Oil Refinery Pančevo, Serbia). The water in the canal originates mainly from industrial effluents from the oil refinery and a nitrogen plant and is significantly polluted with petroleum hydrocarbons.[9]

The possibility of using zymogenous microorganisms from the wastewater canal for bioremediation purposes has already been investigated in detail.[10,11] Based on an experiment of simulated biodegradation under laboratory conditions, it was proven that the dominant microorganisms from the surface water of the wastewater canal have degradable effects on petroleum hydrocarbons.[10] However, the biodegradation was restrained to the *n*-alkanes and isoprenoids, while polycyclic alkanes of sterane and triterpane type remained unchanged both in their abundance and distribution. The investigations performed under the laboratory conditions confirmed certain bioremediation potential of zymogenous microorganisms isolated from the waste water canal sludge for the degradation of oil.[11] Nevertheless, these microbial cultures showed lower potential for oil degradation than those isolated from the waters of the canal.

The present research represents a continuation of our investigations on the bioremediation potential of zymogenous microorganisms from the area of the wastewater canal. Considering the fact that some components of the crude oil can be sorbed on soil particles and remain in the environment for a long time,[12] potentially influencing the quality of ground- and surface waters, these results are expected to help in the prediction of possibilities of bioremediation as a natural attenuation process of petroleum pollutants over the whole wastewater canal area.

EXPERIMENTAL

In this study, a series of laboratory experiments was conducted aimed at evaluating the bioremediation potential of zymogenous microorganisms in soil for crude oil biodegradation. The soil samples were taken in the vicinity of the waste water channel polluted primarily by

oil pollutants, located in the south industrial zone of the city of Pančevo (Danube alluvium, Serbia), where the oil refinery is situated. This area was described in detail by Kaisarevic *et al*., 2009.[13] Considering the fact that the efficiency of bioremediation depends not only on the characteristics of the microbial community present, but also on the crude oil composition and the environmental conditions,[7] all experiments were conducted under conditions similar to those existing in the area from which the soil samples were taken.

In the consortium of zymogenous microorganisms isolated from the soil samples the most numerous were bacteria from the genus *Bacillus* and actynomycetes, and species from the fungal genus *Penicillium.*

In order to isolate these organisms under the aerobic conditions, 1 g of soil was first seeded in 100 cm^3 sterile mineral medium, at 25 °C. The medium was prepared by dissolving 1.5 g NH_4Cl; 0.55 g KH_2PO_4; 0.25 g Na_2HPO_4; 0.25 g $MgSO_4$ $7H_2O$ and 5 cm^3 of a TSS (Trace Salts Solution) mixture in 1 dm^3 of distilled water. The TSS mixture was composed of: 500 mg dm^{-3} $Na_2EDTA{\cdot}2H_2O$; 200 mg dm^{-3} $FeSO_4$ $7H_2O$; 30 mg dm^{-3} H_3BO_3; 20 mg dm^{-3} $CoCl_2$ $6H_2O$; 10 mg dm^{-3} $ZnSO_4$ $7H_2O$; 3 mg dm^{-3} $MnSO_4$ H_2O; 3 mg dm^{-3} Na_2MoO_4 $2H_2O$; 2 mg dm^{-3} $NiSO_4$ $7H_2O$; 1 mg dm^{-3} $CuCl_2$ $2H_2O$ and 1 cm^3 NaOH, c = 10 mol dm^{-3}.[14]

Aimed at assessing the biodegradation effect of the microorganisms on crude oil, the solution of the mineral medium with microorganisms was added with the crude oil in quantity of *ca*. 100 μL in 100 cm^3, to achieve a concentration similar to that existing at the location of the wastewater canal.[10] For the present study, a mixture of paraffinic type of oils was used, carefully selected to be very similar to the oil pollutant previously identified in the wastewater canal area. A control sample included the sterile mineral medium without microorganisms and added mixture of oils.

The experiments of the simulated biodegradation were stopped after 15, 30, 45, 60, and 75 days by sterilization at 120 °C for 25 min, while the control experiment was stopped after 75 days. Organic substance from in total 6 samples was extracted with chloroform (HPLC, J.T., USA) by shaking in a separation funnel. From these extracts, the hydrocarbons (saturated and aromatic) were isolated by column chromatography: 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 followed by dichloromethane. A detailed description of the analytical procedure was discussed in previous papers.[15,16]

Hydrocarbons were analyzed by the gas chromatography–mass spectrometry (GC–MS) techniques. An Agilent 7890N gas chromatograph fitted with a HP5-MS capillary column (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 analyzes 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 the following ion chromatograms: *m*/*z* 57 (*n*-alkanes and isoprenoids), 217 (steranes), 191 (triterpanes), 178 (phenanthrene), 192 (methyl-phenanthrenes) and 206 (dimethyl-phenanthrenes). The most relevant peaks were identified according to organic geochemical literature data (*e.g*., Peters *et al*.[8]), 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). *n*-Alkanes and the isoprenoids pristane and phytane were quantified against squalane (supplied by Supelco) as an internal standard. Phenanthrene, methyl-phenanthrenes and dime-

thyl-phenanthrenes were quantified against phenanthrene-*d*10 (supplied by Supelco) as an external standard.

RESULTS AND DISCUSSION

The bioremediation potential of zymogenous microorganisms isolated from soil was investigated under controlled laboratory conditions using a mixture of paraffinic types of oils as a substrate. The ability and efficiency of these microorganisms in crude oil bioremediation was assessed by comparison between the composition of samples which were exposed to the microorganisms and a control sample which was prepared and treated in the same way, but containing no microorganisms. This study was focused on the transformations in the contents and distributions of the target compounds in the fraction of hydrocarbons.

The total ion current (TIC) chromatogram of the hydrocarbon fraction from the control experiment and TIC chromatograms of the hydrocarbon fractions of the samples after 15 and 75 days of the biodegradation experiment are shown in Fig. 1 (TIC chromatograms of the hydrocarbon fractions isolated from the extracts of the control sample and from the samples during the biodegradation ex-

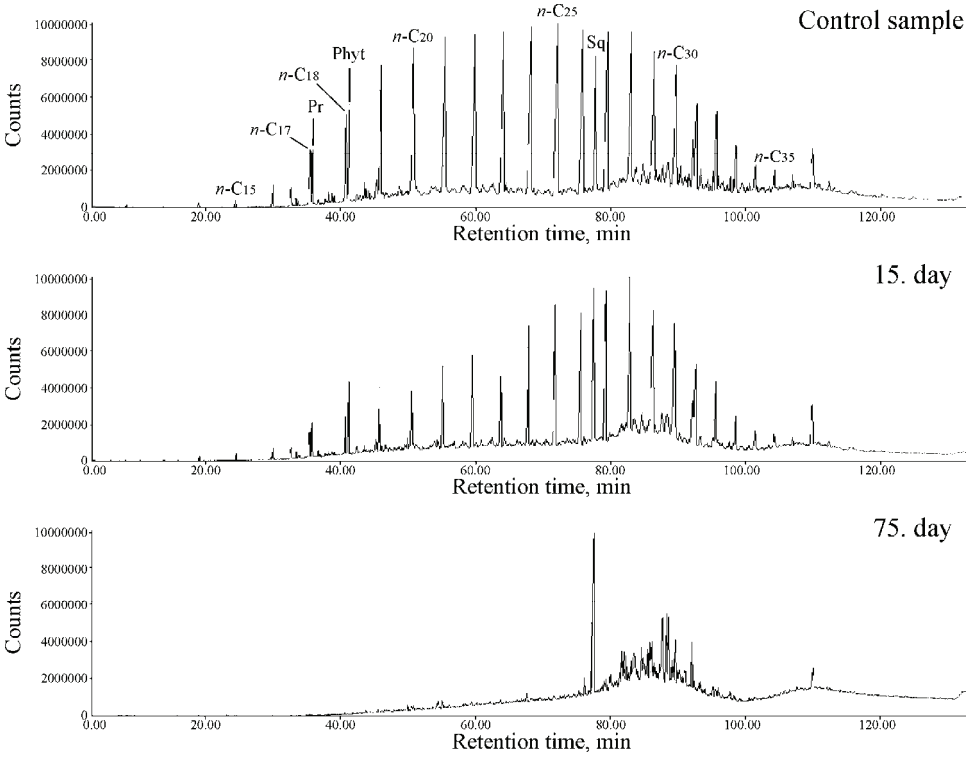

Fig. 1. TIC Chromatograms of the hydrocarbon fractions isolated from the extracts of the control sample and from the samples during the biodegradation experiment after 15 and 75 days (Pr = pristane; Phyt = phytane; Sq = squalane).

periment after 15, 30, 45, 60 and 75 days are shown in the Fig. 1-S in the Supplementary material). The dominant compounds in the hydrocarbon fraction of the control sample were *n*-alkanes and the isoprenoids pristane and phytane. These preliminary analyzes based on the TIC chromatograms showed a gradual decrease in the amount of *n*-alkanes and isoprenoids during 45 days. After 60 days of the experiment, *n*-alkanes and isoprenoids could not be observed in the TIC chromatograms indicating their possible complete degradation. At the end of the experiment, the TIC chromatograms were dominated by sterane biomarkers as the most abundant compounds in the fraction of hydrocarbons (Fig. 1).

n-Alkanes and isoprenoids

Detailed qualitative (Figs. 2 and 2-S in Supplementary material) and quantitative analyses (Fig. 3) of the *n*-alkanes and isoprenoids in the investigated samples enabled the calculation of the most characteristic biodegradation parameters (Table I) and provided a better insight into the dynamics of the biodegradation process.

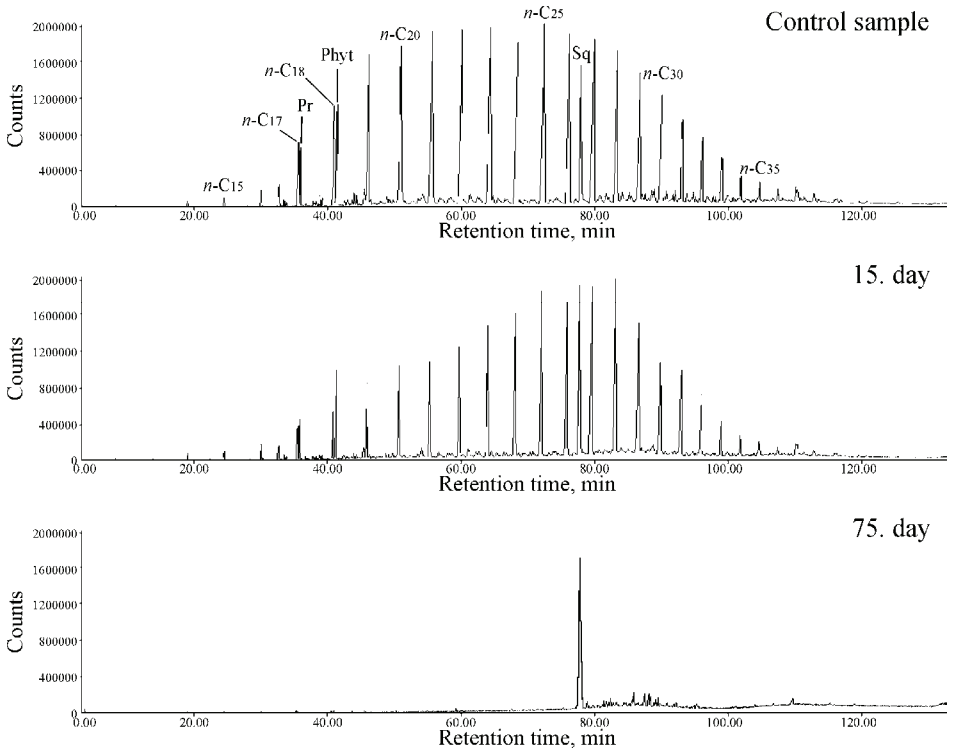

Fig. 2. GC–MS ion fragmentograms of the *n*-alkanes and isoprenoids (*m*/*z* 57) in the hydrocarbon fractions isolated from the extracts of the control sample and from the samples during biodegradation experiment after 15 and 75 days. (Pr = pristane; Phyt = phytane; Sq = squalane).

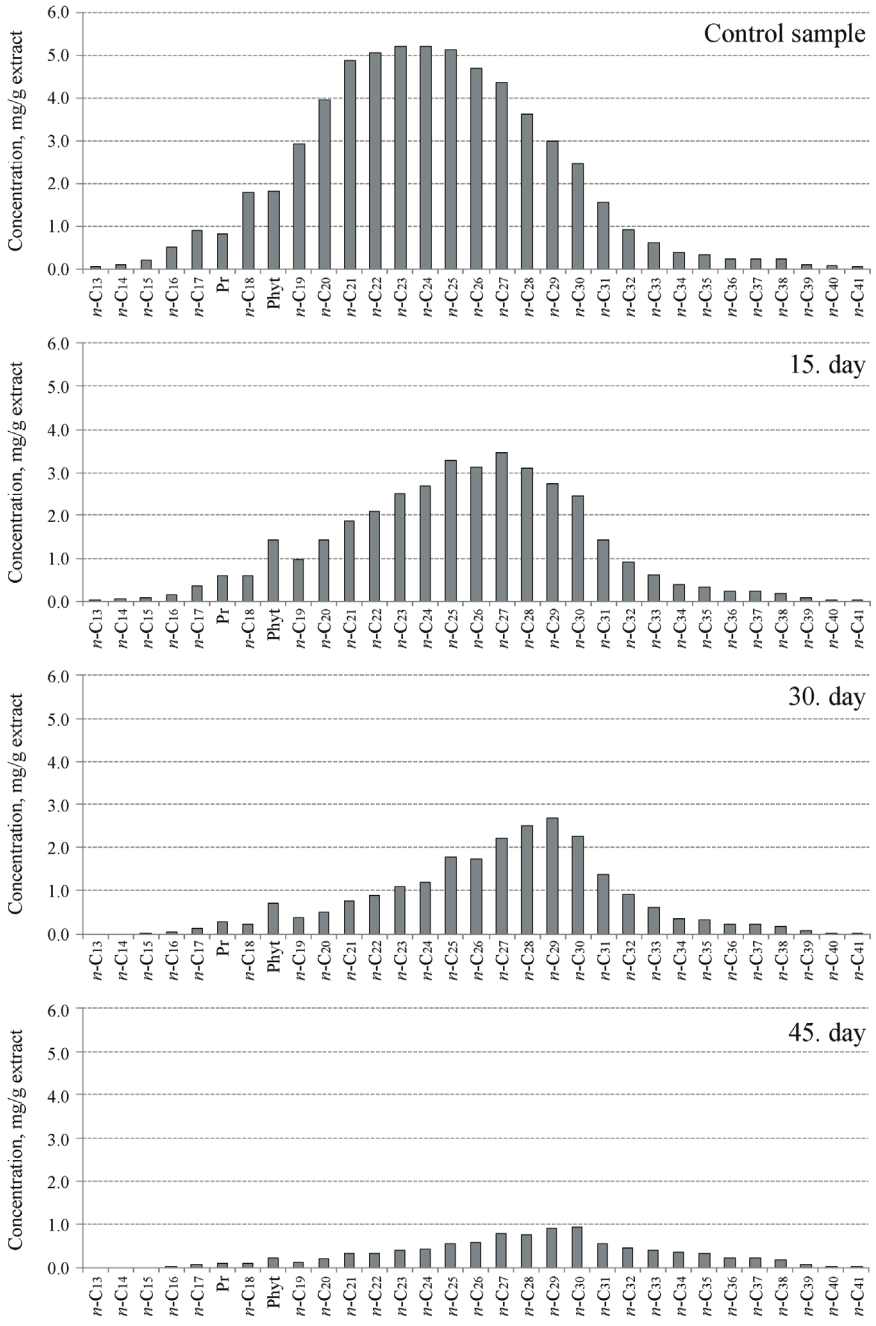

Fig. 3. Quantitative analysis of the *n*-alkanes and the isoprenoids pristane (Pr) and phytane (Phyt) in the hydrocarbon fractions isolated from the extracts of the control sample and from the samples during the biodegradation experiment after 15, 30 and 45 days.

TABLE I. Biodegradation parameters calculated from the quantitative analysis of *n*-alkanes and isoprenoids pristane and phytane in the control sample and in the samples during the biodegradation experiment (Pr – pristane; Phyt – phytane)

Duration of experiment, days	*n*-Alkanes range	Pr/*n*-C_{17}	Phyt/*n*-C_{18}	Concentration of *n*-alkanes mg per g of the extract	Loss of *n*-alkanes % of the control sample
Control sample	C_{13}–C_{41}	0.90	1.02	61.41	–
15	C_{13}–C_{41}	1.62	2.43	37.50	38.94
30	C_{13}–C_{41}	2.15	3.17	23.81	61.23
45	C_{16}–C_{41}	1.36	2.37	9.76	84.11
60	C_{26}–C_{41}	ND[a]	ND[a]	1.00	> 98.37
75	ND[b]	ND[a]	ND[a]	ND[b]	100

[a]Parameter was not calculated due to the degradation of the *n*-alkanes and isoprenoids; [b]parameter was not calculated due to the degradation of the *n*-alkanes

The ion chromatogram of the *n*-alkanes and isoprenoids in the hydrocarbon fraction of the control sample is characterized by the presence of *n*-alkanes in the C_{13}–C_{41} range with the uniform distribution of the even and odd homologues (Figs. 2 and 2-S in Supplementary material). The alkanes *n*-C_{17} and *n*-C_{18} and the closely eluting isoprenoids pristane and phytane are present in similar concentrations, as indicated by the values of the corresponding parameters Pr/*n*-C_{17} = = 0.90 and Phyt/*n*-C_{18} = 1.02 (Fig. 3; Table I).

Comparing the ion chromatogram of the alkanes in the hydrocarbon fraction of the sample after 15 days of the experiment with the control sample (Fig. 2), it can be easily concluded that *n*-alkanes lower than C_{30} were the most affected by the microorganisms. This observation was additionally confirmed by the quantitative analysis of these compounds (Fig. 3). The analysis of isoprenoids pristane and phytane indicated some decrease in their concentrations (Fig. 3). However, higher values of Pr/*n*-C_{17} and Phyt/*n*-C_{18} ratios (1.62 and 2.43 respectively; Table I) comparing to the values of these parameters in the control sample, confirmed a lower susceptibility of isoprenoids compared to *n*-alkanes during the first 15 days of the biodegradation process. According to these results, it can be concluded that during the first 15 days of the biodegradation processes by the microorganisms used in this experiment, a loss of 38.94 % of the *n*-alkanes occurred (Table I). Additionally, it can be concluded that this loss was mainly due to a decrease in the concentrations of *n*-alkanes in the C_{13}–C_{30} range (Figs. 2, 2-S in Supplementary material, and 3).

After 30 days of the experiment, a gradual decrease in the concentration of *n*-alkanes was observed (Fig. 3; Table I). The biodegradation was extended to the homologues up to C_{32}, resulting in loss of 61.23 % of the *n*-alkanes compared to the control sample (Fig. 3; Table I). A further increase in the Pr/*n*-C_{17} and Phyt/ /*n*-C_{18} ratios (2.15 and 3.17, respectively, Table I) compared to the sample after 15 days of the experiment, indicated that the isoprenoids were biodegraded at a

lower rate than the *n*-alkanes even if more than 60 % of the *n*-alkanes had been removed.

During the next 15 days, a steady biodegradation of *n*-alkanes continued. As a result, after 45 days of the experiment, *n*-alkanes lower than C_{16} had been completely removed. The concentration of the remaining *n*-alkanes (comprising homologues in the C_{16}–C_{41} range) was 84.11 % lower than in the control sample (Fig. 3; Table I). The relative decrease of the isoprenoid/*n*-alkane ratios (Pr/*n*-C_{17} = 1.36 and Phyt/*n*-C_{18} = 2.37, Table I) compared to the previous sample (Pr/*n*-C_{17} = 2.15 and Phyt/*n*-C_{18} = 3.17, Table I) indicates that during this phase of the experiment, a significant biodegradation of the isoprenoids occurred. Accordingly, it can be concluded that under the conditions used in this experiment, significant biodegradation of isoprenoids occurred after more than 80 % of the *n*-alkanes had been removed.

After 60 days of the experiment, an almost complete degradation of the *n*-alkanes and isoprenoids was observed (Fig. 3). Although *n*-alkanes in the C_{26}–C_{41} range were identified (Fig. 3), most of them could not be quantified. Their total concentration was lower than 98.37 % of the amount of *n*-alkanes in the control sample (Table I).

Finally, by the end of the experiment, after 75 days of exposure to the zymogenous microorganisms isolated from soil, the *n*-alkanes and isoprenoids were completely degraded.

Steranes and triterpanes

Polycyclic alkanes of the sterane and terpane types in crude oils in the subsurface are considered to be decomposed by microbial degradation after the degradation of acyclic hydrocarbons.[17] However, it was shown that crude oil biodegradation under aerobic conditions may follow completely different sequences than oil in reservoir rocks.[18] Additionally, an unusual biodegradation sequence of biomarker compounds was reported as a characteristic of biodegradation process during the bioremediation of refinery wastes.[19] Consequently, the hydrocarbon fractions of all samples exposed to microorganisms during the present experiment, as well as the corresponding control sample, were analyzed in detail for polycyclic alkanes of the sterane and terpane types. The corresponding ion chromatograms are shown in Figs. 4 and 3-S in Supplementary material.

In the control sample, biolipid and more stable geolipid isomers of the steranes and triterpanes types were identified with a distribution typical for crude oils (Figs. 4 and 3-S in Supplementary material). A detailed comparison of the abundances and distributions of these biomarkers in the control sample and in all samples during the biodegradation experiment showed no differences. Hence, it can be concluded that sterane and terpane biomarkers were not affected by biodegradation, and that the biodegradation of saturated hydrocarbons, under the

employed conditions, was restricted to the acyclic aliphatic compounds (*n*-alkanes and isoprenoids).

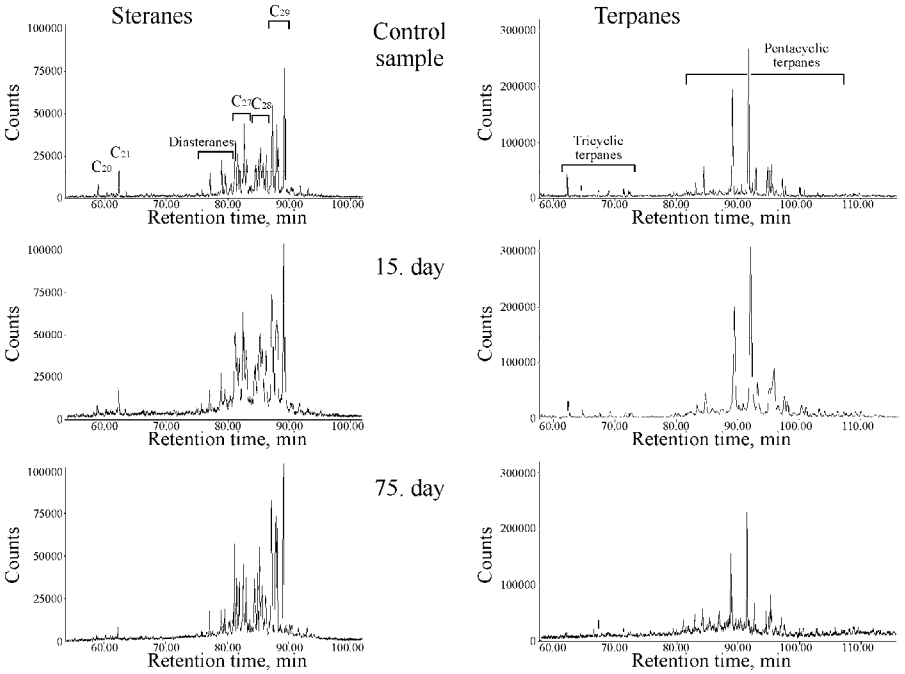

Fig. 4. GC–MS ion fragmentograms of steranes (*m*/*z* 217) and terpanes (*m*/*z* 191) in the hydrocarbon fractions isolated from the extracts of the control sample and from the samples during the biodegradation experiment after 15 and 75 days.

Aromatic hydrocarbons

Although the biodegradation of crude oil is often explained as a quasi-stepwise process in which various components are removed in a well-recognized sequence, it is well known that several compound classes are actually destroyed simultaneously but at different rates, reflecting differences in the rate of their catabolism under varying conditions.[8] Numerous studies showed that aromatic compounds can not only be degraded prior or concomitantly with sterane and terpane biomarkers in reservoir oils,[20] but also in the environmental conditions during oil spills.[21] Accordingly, aromatic compounds were carefully analyzed in all extracts during the present study.

Among aromatic hydrocarbons, the only compounds which could be identified and quantified in all samples investigated were phenanthrene and its derivatives with one and two methyl groups. The results of qualitative and quantitative analyses of these compounds are shown in Figs. 5, 4-S in Supplementary mate-

rial, and 6, respectively. Concentration changes of phenanthrene, methyl phenanthrenes and dimethyl phenanthrenes during the experiment are given in Table II.

The qualitative analyses of phenanthrene and its derivatives with one and two methyl groups showed that the hydrocarbon fraction of the control sample was characterized by the presence of these compounds in the distribution characteristic of mature crude oil (Figs. 5 and 4-S in Supplementary material).[8]

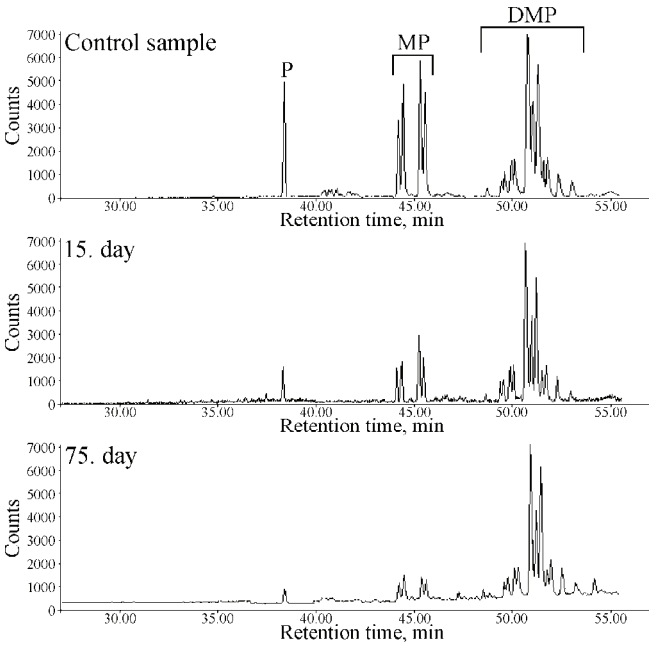

Fig. 5. Reconstructed GC–MS ion chromatograms of phenanthrene (P; *m*/*z* 178), methylphenanthrenes (MP; *m*/*z* 192) and dimethylphe-nanthrenes (DMP; *m*/*z* 206) in the hydrocarbon fractions isolated from the extracts of the control sample and from the samples during the biodegradation experiment after 15 and 75 days.

During the biodegradation experiment, the most distinct change in concentrations of these compounds was observed in the case of phenanthrene (Fig. 6; Table II). After only 15 days of the experiment, 61.90 % of phenanthrene had been degraded (Table II). However, during the rest of the experiment, the decrease in the concentration of phenanthrene was much less prominent. Compared to the control sample, after 30 days its loss was 75.13 %, and after 45 days it was 81.62 % (Table II). By the end of the experiment, 82.94 % of phenanthrene was degraded, leaving a residual concentration of 33.35 μg g^{-1} in the extract (Table II).

Methyl phenanthrenes underwent a significant decrease in the concentration during this experiment, but not as prominent as that of phenanthrene. After 15 days of the experiment, 48.49 % of methyl phenanthrenes had been degraded (Table II). During the rest of the experiment, a gradual loss of methyl phenanthrenes was observed (Fig. 6; Table II). Finally, at the end of the experiment, after 75 days of exposure to the zymogenous microorganisms isolated from soil, 74.74 % of the methyl phenanthrenes were degraded, leaving the residual con-

centration of 186.32 μg g^{-1} extract (Table II). Accordingly, it can be concluded that under the conditions used in this experiment, the zymogenous microorganisms exerted a considerably high bioremediation potential in the biodegradation of phenanthrene and methyl phenanthrenes. However, it must be emphasized that at the end of the experiment, the biodegradation of these compounds was not complete.

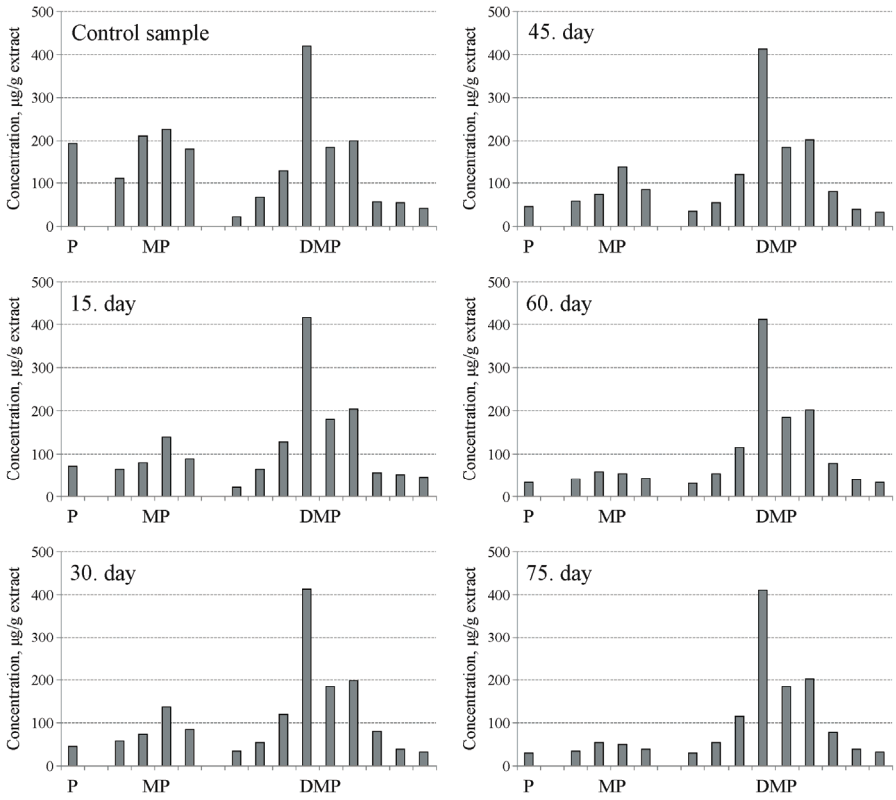

Fig. 6. Quantitative analysis of phenanthrene (P), methylphenanthrenes (MP) and dimethylphenanthrenes (DMP) in the hydrocarbon fractions isolated from the extracts of the control sample and from the samples during the biodegradation experiment after 15, 30, 45, 60 and 75 days.

During this biodegradation experiment no significant alteration in the concentration of dimethyl phenanthrenes was observed (Fig. 6). After 75 days, the concentration of these compounds was reduced by only 2.34 % (Table II), leaving them in almost unchanged amount compared to the control sample. Hence, it can be concluded that the zymogenous microorganisms isolated from soil have a low bioremediation potential for the degradation of dimethyl phenanthrenes under the conditions employed in this study.

TABLE II. Concentration changes of phenanthrene, methyl phenanthrenes and dimethyl phenanthrenes in the control sample and in the samples during the biodegradation experiment (P – phenanthrene; MP – methylphenanthrenes; DMP – dimethylphenanthrenes)

Duration of experiment	Concentration, μg per g of the extract			Loss, % of the control sample		
days	P	MP	DMP	P	MP	DMP
Control sample	195.50	737.67	1194.80	–	–	–
15	74.49	380.00	1193.68	61.90	48.49	0.09
30	48.62	363.82	1173.97	75.13	50.68	1.74
45	35.93	281.70	1170.22	81.62	61.81	2.06
60	34.08	204.39	1167.35	82.57	72.29	2.30
75	33.35	186.32	1166.82	82.94	74.74	2.34

CONCLUSIONS

In this study, the experiments were conducted with the aim of establishing the bioremediation potential of zymogenous microorganisms for crude oil biodegradation. The microorganisms were isolated from soil samples taken from within the oil refinery Pančevo (Danube alluvium, Serbia).

The investigated soil zymogenous microorganisms showed the highest bioremediation potential in biodegradation of *n*-alkanes and isoprenoids. During this experiment, a gradual decrease in the concentration of *n*-alkanes was observed. Significant biodegradation of isoprenoids occurred after more than 80 % of the *n*-alkanes had been removed. By the end of the experiment, after 75 days of exposure to the zymogenous microorganisms isolated from soil, the *n*-alkanes and isoprenoids had been completely degraded.

In the class of aromatic hydrocarbons, it was confirmed that the zymogenous microorganisms isolated from soil had a considerably high bioremediation potential in the biodegradation of phenanthrene and methyl phenanthrenes. However, the biodegradation of these compounds, under conditions used in this study, was not complete.

The low bioremediation potential of these microorganisms was proven in the case of polycyclic alkanes of the sterane and triterpane type and dimethyl phenanthrenes. After 75 days of the experiment, no significant alteration in the concentration of these compounds was observed.

The zymogenous microorganisms isolated from the soil in the vicinity of the wastewater canal showed somewhat a higher bioremediation potential for crude oil biodegradation than the microorganisms isolated from the water and from the sludge of this canal.[10,11] However, it must be stated that the biodegradation of crude oil by the soil aerobic zymogenous microorganisms at this location under natural conditions would be limited to the *n*-alkanes and isoprenoids. On the other hand, biodegradation of more complex polycyclic saturated and aromatic compounds, under the same conditions, would, most probably, not go to completion.

SUPPLEMENTARY MATERIAL

Complete data during the biodegradation experiments (Figs. 1-S–4-S) are available electronically at http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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И З В О Д

ИСПИТИВАЊЕ БИОРЕМЕДИЈАЦИОНОГ ПОТЕНЦИЈАЛА АЕРОБНИХ ЗИМОГЕНИХ МИКРООРГАНИЗАМА ИЗ ЗЕМЉИШТА У БИОДЕГРАДАЦИЈИ СИРОВЕ НАФТЕ

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Испитиван је биоремедијациони потенцијал аеробних зимогених микроорганизама из земљишта у биодеградацији сирове нафте (алувијална раван реке Дунав, Панчево). Смеша сирових нафти парафинског типа коришћена је као супстрат. Лабораторијски експеримент симулиране биодеградације трајао је 15, 30, 45, 60 и 75 дана. Паралелно је рађен и експеримент са контролним узорком. Екстракти су изоловани из узорака хлороформом у левку за одвајање. Из ових екстраката, угљоводоници су изоловани хроматографијом на колони и анализирани гаснохроматографски–масеноспектрометријски (GC–MS). *n*-Алкани, изопреноиди, фенантрен и његови деривати са једном и две метил групе квантитативно су анализирани. Способност и ефикасност зимогених микроорганизама из земљишта у биодеградацији сирове нафте процењена је поређењем састава узорака који су били изложени микроорганизмима и контролног узорка. Испитивани микроорганизми показали су највиши биоремедијациони потенцијал у биодеградацији *n*-алкана и изопреноида. Висок биодеградациони потенцијал уочен је при биодеградацији фенантрена и метилфенантрена. Низак биоремедијациони потенцијал ових микроорганизма доказан је у случају полицикличних алкана типа стерана и терпана, као и диметилфенантрена.

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