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Change of isoprenoids, steranes and terpanes during *ex situ* bioremediation of mazut on the industrial scale

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Abstract: This paper presents the results of an *ex situ* bioremediation of soil contaminated by mazut (heavy residual fuel oil) in the field scale (600 m^3). The treatment-bed (thickness 0.4 m) consisted of mechanically mixed mazut-contaminated soil, softwood sawdust as an additional carbon source and crude river sand, as a bulking and porosity increasing material. The inoculation/reinoculation was conducted periodically using a biomass of a consortium of zymogenous microorganisms isolated from a bioremediation substrate. The biostimulation was performed through addition of nutritious substances (N, P and K). The aeration was improved by systematic mixing of the bioremediation system. After 50 days, the number of hydrocarbon degraders had increased a 100 fold. Based on the changes in the group composition, the average biodegradation rate during bioremediation was 24 mg kg^{-1} day^{-1} for the aliphatic fraction, 6 mg kg^{-1} day^{-1} for the aromatic fraction and 3 mg kg^{-1} day^{-1} for the nitrogen–sulphur–oxygen compounds (NSO)–asphaltene fraction. In the saturated hydrocarbon fraction, gas chromatography–mass spectrometry (GC–MS) in the single ion-monitoring mode (SIM) was applied to analyse isoprenoids pristane and phytane and polycyclic molecules of sterane and triterpane type. Biodegradation occurred during the bioremediation process, as well as a reduction of the relative quantities of isoprenoids, steranes, tri- and tetracyclic terpanes and pentacyclic terpanes of the hopane type.

Keywords: mazut; bioremediation; field experiment; zymogenous microbial consortia; isoprenoids, steranes and terpanes.

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INTRODUCTION

Mazut is a low quality, heavy residual fuel oil (chain length 12–70 C atoms).[1,2] In the United States and western Europe, heavy fuel oil is blended or broken down with the end product being diesel. Mazut is widely used in eastern Europe as a heating agent. Its long-term storage and use leads to the formation of a reservoir of hydrocarbon sediment with a high content of various mechanical impurities and water, which may potentially be hazardous for the environment.

The accumulated sediment is periodically taken off by mechanical means. Common practice dictates that the removed sediment should be placed into metal barrels or discarded reservoirs. In the case of incidents and mazut spills onto concrete surfaces, it is habitual to add sand to prevent further spreading, which is then covered and discarded along with the removed contaminated soil on a watertight material. Improper disposal of such material leads to contamination of the environment, in particular contamination of soil, and represents a grave hazard for the subterraneous waters.

Among numerous technologies for the cleansing of contaminated sites, bioremediation by means of zymogenous microorganisms[3] is the most commonly used method.[4,5]

Oil-degrading mixed cultures can be constructed either by combining a number of strains with known complementary degradative capabilities (defined consortia) or by direct enrichment procedures (non-defined consortia).[6]

Enrichment procedures with selected oil products or with some specific components can provide non-defined, metabolically specialized microbial consortia. The result is a microbial population naturally selected by its metabolic cooperation in the degradation of each mixture, with potentially higher efficiency in degrading identified and non-identified components.[7]

Studies conducted to date of bioremediation of soil polluted by mazut, performed in laboratories on the level of model systems, have proven the presence of bioremediation potential for stimulated self-purification.[8]

Although the most suitable criteria for optimising the bioremediation process are known (careful control of temperature, aeration, particle size, moisture, macro and micronutrients in the mass to be composted, C/N ratio of the materials, *etc*.),[9] so that the microbial activity necessary for treating this organic matter can be encouraged, very few studies have attempted to treat mazut and heavy fuel oil on the industrial scale.[10–14]

The fraction of saturated hydrocarbons is dominant in most oils compared to aromatic hydrocarbons and nitrogen-sulphur-oxygen compounds (NSO).[15–17] The fraction shows the highest presence of *n*-alkanes and isoprenoid aliphatic alkanes. The *n*-alkanes in oils may be present in different ranges, most frequently *n*-C_{10}–C_{35}, while the most represented isoprenoids are C_{19}, pristane and C_{20}, phytane.

Polycyclic hydrocarbons of the sterane (C_{27}–C_{29}) and terpane type (tri-, tetra-, and pentacyclic; C_{19}–C_{35}) are most commonly found in much smaller quantities. Due to their great biomarker potential, these compounds are highly important in both organo-geochemical and bioremediation testing.

Previous field scale application showed that the oil pollutant mixture in the soil treated by *ex situ* bioremediation behaved in a complex way: different degradation rate and time evolution were observed for the different fractions of the hydrocarbon mixture, which are characterised by different molecular weight and structure.[18,19] It was also concluded that a stable microbial community had been formed after initial fluctuations and that the microorganisms which decompose hydrocarbons were dominant in the microbial population at the end of the bioremediation process, with a share of more than 80 % (range 10^7 CFU/g).[20]

This paper presents transformations of saturated hydrocarbons of petroleum type pollutants (isoprenoids and polycyclic alkanes of the sterane and terpane type) during *ex situ* bioremediation of soil contaminated by mazut of approximately 600 m^3. Dominant hydrocarbon utilizing strains from mazut-contaminated soil were selected and used for reinoculation as the active zymogenous microbial consortium in this study. Experiments of biostimulation, bioventilation and reinoculation were performed during five months.

EXPERIMENTAL

Preparation of the biomass of the zymogenous consortium

The consortium of microorganisms was obtained from a mazut-contaminated soil by the method of enrichment using a mineral medium (10 mass %),[21] in which the mazut was used as the sole source of energy and carbon at a concentration of 2000 ppm in one-litre Erlenmeyer flasks with 200 mL medium.

Suspensions of the microorganisms were concentrated and used as inoculum to seed four 5-litre Erlenmeyer flasks containing 2000 mL of a medium containing 23 g of nutritious bouillon, 100 mL of soil extract and 20 g of mazut.[22] A commercial non-toxic and readily biodegradable surfactant, Biosolve® Clear® supplied by the Westford Chemical Corporation, (Westford, MA, USA) was used as a surface active agent to solubilise the mazut. The original solution supplied by the manufacturer was used at a concentration 1 mL L^{-1}. The growth conditions were as follows: temperature, 28 °C; 120 rpm; pH 7.0 (adjusted with 1.0 M HCl or NaOH); duration of growth: 96 h.

The microbial populations from all four flasks were used to inoculate (approx. 1 % v/v) a self-designed and produced mobile bioreactor (total volume 1000 L) with a working volume of 800 L, to produce the microbial consortium. The medium was 12 g L^{-1} of meat peptone (Torlak, Belgrade, Serbia); 0.2 g L^{-1} $(NH_4)_2HPO_4$; 25 g L^{-1} of an in autoclave sterilized soil sample from an undisturbed deciduous woodland; and 10 g L^{-1} of mazut. The growth conditions: non-sterile, 25 °C, aeration and agitation 0.70 volume of air/volume of medium/min, pH 7.0 (adjusted with 10 M HCl or NaOH), duration 48 h and sunflower oil (1 mL L^{-1}) as an antifoaming agent.

Experimental treatment bed, design and treatment

The treatment bed for bioremediation was set up on a watertight asphalt base with an approximate area of 1500 m^2 and a 1 % slope. A quantity of 270 m^3 of contaminated soil was mixed with 300 m^3 of crude river sand and 60 m^3 of softwood sawdust as the additional carbon source and bulking component. To ensure homogenization, the components were mixed 3 times with a front-end loader and finally fitted with a harrow. The final dimensions of the treatment bed at the bioremediation site were about 75 m×20 m with a height of 0.4 m, which means that the bioremediation substrate had a volume of approximately 600 m^3. A perimeter drain enclosed the entire treatment area and directed all leachate and runoff to a joint vessel, from where they were pumped back to the treatment bed. Based on the analysis, the optimal ratio C:N:P:K (approx.100:10:1:0.1) was secured by spraying a solution of ammonium nitrate, diammonium phosphate and potassium chloride using a tractor-powered agricultural sprinkler. This also provided for the required level of moisture (40–60 % of the water holding capacity--WHC). Moisture and aeration during bioremediation were maintained by 15-day watering, tumbling and mixing of the treatment-bed. Reinoculation with the prepared microbial biomass was performed in 30-day intervals, in the same manner as the moistening substrate was applied.

The treatment bed was also amended with BioSolve® Clear®, applied at a volume of 70 mL of original solution per cubic meter.

After mixing, the heaps were covered with polyethylene foil to prevent the direct influence of the weather conditions on the bioremediation substrate.

The bioremediation experiment was conducted from March to August 2009 and in this period, the average temperature was 18.2 °C (min. 0.3, and max. 35.4 °C).

Chemical and microbiological indicators of the bioremediation process were monitored immediately after application of the microbial biomass (time zero, sample S-0) and every 50 days over a period of 150 days (designations S-50, S-100, S-150).

Determination of the number of microoorganisms

The number of microorganisms was determined by the method of a serial dilution on agar plates incubated at 28 °C. For the total microorganisms, a nutrient agar was used and for hydrocarbon degraders, a mineral based medium with 2000 ppm diesel fuel.[21,23]

Determination of hydrocarbon fractions and GC–MS analysis

The organic substance of the bioremediation substrate was extracted by the Soxhlet method and the components of the group composition were quantified after chromatographic separation on an adsorbent column. The NSO-asphaltene fraction was calculated arithmetically.[24]

Isoprenoid aliphatic alkanes, pristane and phytane, and polycyclic alkanes of sterane and triterpane types in saturated hydrocarbon fractions were analysed by gas chromatography––mass spectrometry (GC–MS). An Agilent GC System 6890N gas chromatograph with an Agilent 7673 Series injector and Series 5973 mass detector fitted with J & W Scientific DB--5ms-ITD column (30 m, 0.25 mm id, 0.25 μm film) was used. Helium was employed as the carrier gas (flow rate 1 mL min^{-1}). The temperature program of the column was 50 to 285 °C at 10 °C min^{-1}. Isoprenoids were identified from the *m/z* 183, steranes from *m/z* 217 and triterpanes from *m/z* 191 fragmentograms obtained from analysis in the single ion-monitoring mode (SIM). The most relevant peaks were identified based on organic geochemical literature data,[17] or based on total mass spectra, using mass spectra databases.[25]

All the results were calculated to dry substance.

RESULTS AND DISCUSSION

Basic microbiological indicators

The initial concentration of the zymogenous population of hydrocarbon degrading bacteria in the sample S-0 was approximately 10^4 CFU g^{-1} (Fig. 1). Previously conducted studies had determined that the bioremediation would not be realised to a significant extent if the population of the microorganisms capable of degrading the target contaminant was smaller than 10^5 CFU g^{-1} of soil.[4] The concentration of these microorganisms was increased through reinoculation of the zymogenous microbial biomass and multiplication in the bioreactor. Following biostimulation and reinoculation, the number of hydrocarbon degrading microorganisms after 50 days had increased by as much as 100 times (from 10^4 to 10^6). Midway through the process, the proportion of oil hydrocarbon degraders in the total number of microorganisms had increased to 75 %. This proportion amounted to only < 1 % at the beginning of the process. A decreased hydrocarbon concentration brought this proportion down to 25 %. The population of the bacterial consortium able to consume diesel oil as a sole source of carbon was stable even after 150 days following the first application of the microbial consortium, indicating the very high survivability of the introduced strains.

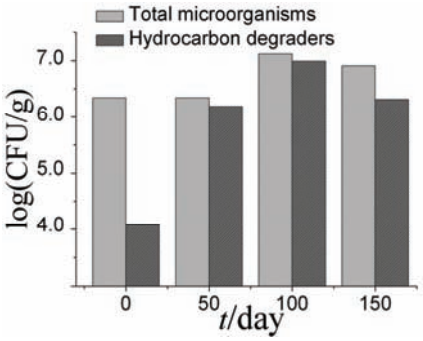

Fig. 1. Changes in the number of total microorganisms and hydrocarbon degraders during the bioremediation process.

Due to the toxic effect of the contaminant, the biodiversity of the contaminated soil decreased, whereas the concentration of the population of microorganisms degrading the contaminating substance rose.[26] Along with a decrease in the concentration of the contaminant towards the end of the remediation process, the share of hydrocarbon utilizing microorganisms in the total number decreased. Due to the reduced toxicity, the microbial population in the soil became more diverse.

Analysis of the hydrocarbon fractions

Biodegradation and the decreased concentration of the individual fractions within the hydrocarbons is shown in Fig. 2. The highest degree of degradation was noted in the most extensive aliphatic fraction. In sample S-0, the proportions

of aliphatic, aromatic and NSO-asphaltene fraction were 71.6, 17.0 and 11.4 %, respectively. Due to the intensive biodegradation processes, the share of individual fractions after 50 days compared to the initial concentration decreased to 27.0, 5.2 and 7.5 %. Since the *n*-alkane fraction was dominant and the most prone to bioremediation, the trend continued so that the percentages of the individual fractions amounted to 15.4, 3.3 and 6.4 after 100 days, *i.e.*, 3.1, 0.6 and 1.9 after 150 days.

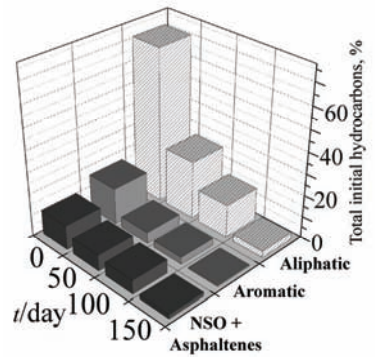

Fig. 2. Percent and decrease of the concentration of group composition components within the total initial hydrocarbons during *ex situ* bioremediation.

The average rate of decrease during the biodegradation of the heavy residual fuel oil was 23.7 mg kg^{-1} day^{-1} for the aliphatic fraction, 5.7 mg kg^{-1} day^{-1} for the aromatic fraction, and 3.3 mg^{-1} kg^{-1} day^{-1} for NSO and the asphaltene fraction. As expected, the asphaltene fraction was the most recalcitrant and the most persistent in the environment, which is the reason why it has the slowest rate of decomposition.[12] Considering the decomposition rate of the individual fractions, the relative ratios within the group composition change at the expense of decreased aliphatic and aromatic fractions and increased proportion of NSO and the asphaltene fraction. The increase in the proportion of NSO and the asphaltene fraction was followed by a decrease in the absolute value of all fractions in the group composition.

GC–MS Analysis of the aliphatic hydrocarbon fraction

Changes in the content of the alkane fraction were monitored by means of GC–MS analysis of samples S-0, S-50, S-100 and S-150. The total ion current (TIC) chromatograms of the alkane fraction of samples S-0 and S-150 are shown in Fig. 3. The SIM-chromatograms of isoprenoids (m/z = 183), terpanes (m/z = = 191) and steranes (m/z = 217) are presented in Figs. 4–6, respectively.

Based on the appearance of the TIC-chromatograms (for samples S-0 and S-150, Fig. 1), it may be concluded that alkane fractions of the tested samples are characterized by a high quantity of an unresolved complex mixture (UCM). This is an expected result, knowing that the soil tested in this study had been polluted

by mazut, a heavy oil fraction. The presence of peaks of individual *n*-alkanes is also visible, which is in accordance with the fact that the alkane fraction makes up over 75 % of the total hydrocarbons. As judged from the gas chromatograms, the hydrocarbons left in the soil had already been degraded to some extent during natural biodegradation processes since the abundances of C_{17} and C_{18} *n*-alkanes at time zero was somewhat smaller than the abundances of pristane (C_{19}) and phytane (C_{20}).

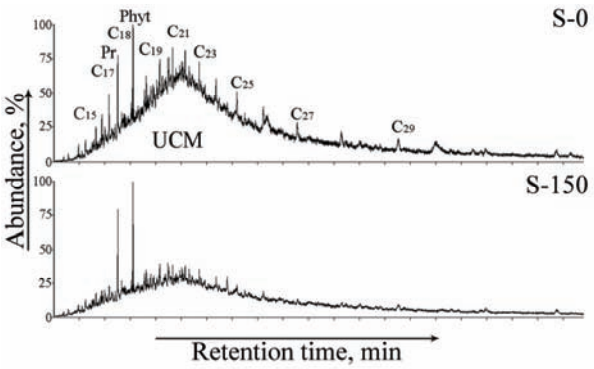

Fig. 3. TIC of the alkane fractions of extract samples S-0 and S-150; Pr: pristane; Phyt: phytane.

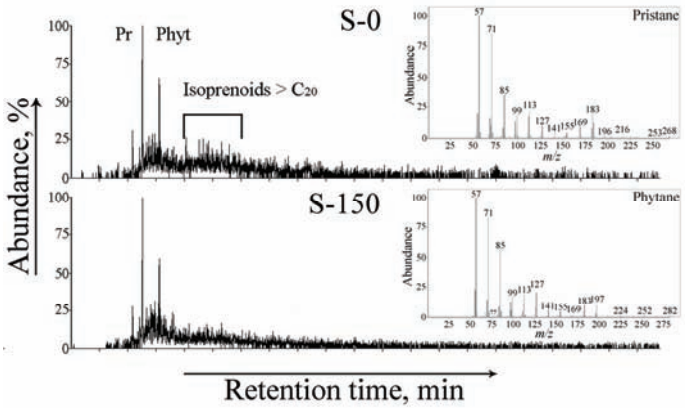

Fig. 4. Fragmentograms of the isoprenoids (SIM, *m/z* 183) of the alkane fractions in the samples S-0 and S-150 (full mass spectra corresponding to peaks of pristane C19 and phytane C20 for S-0 sample are also presented); Pr: pristane; Phyt: phytane.

The ratios Pr/*n*-C_{17} and Phyt/*n*-C_{18} may be used for differentiating between physical weathering and bioremediation.[27] Since the volatility of *n*-C_{17} and pristane are similar, as is the case with the volatility of *n*-C_{18} and phytane, a decrease in the concentrations of these substances over time should be attributed to weathering if their ratio remains the same. If with time, the ratios Pr/*n*-C_{17} and Phyt/

/*n*-C_{18} increase, the conclusion is that this occurs due to bioremediation, which is a consequence of the fact that bioremediation removes *n*-C_{17} and *n*-C_{18} at higher rates than pristane and phytane, respectively.[28]

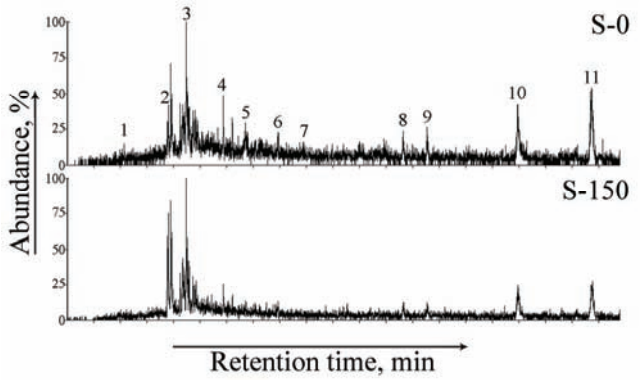

Fig. 5. Fragmentograms of the terpanes (SIM, *m/z* 191) of the alkane fractions in the samples S-0 and S-150. 1: C19 – tricyclic terpane; 2: C_{21} – tricyclic terpane; 3: C_{22} – tricyclic terpane; 4: C_{24} – tricyclic terpane; 5: C_{25} – tricyclic terpane; 6: C_{24} – tetracyclic terpane; 7: C_{28} – tricyclic terpane; 8: C_{27} – 18α(*H*)-22,29,30-trisnorhopane (Ts); 9: C_{27} – 17α(*H*),18α(*H*),21β(*H*)--25,28,30-trisnorhopane; 10: C_{29} – 17α(*H*),21β(*H*)-hopane; 11: C_{29} – 18α(*H*),21β(*H*)-30-norneohopane.

A decrease of the quantity of isoprenoid molecules over the course of the bioremediation process may also be noted based on the abundance of the peaks in chromatograms of the ion *m/z* 183, characteristic for these molecules (samples S-0 and S-150; SIM method; Fig. 4). In the chromatogram *m/z* 183 of all the samples, the pristane and phytane peaks are clearly differentiated, being the most intensive in the sample S-0. In this sample, individual peaks originating from the homologue strain > C_{20} isoprenoids are also observed, while in sample S-150 they are biodegraded.

The chromatogram of terpane in the initial sample S-0 (SIM, *m/z* 191, Fig. 5) is dominated by peaks originating from C_{19}–C_{28} tricyclic terpanes, C_{24} tetracyclic terpane and C_{27}–C_{29} pentacyclic terpanes, with a distribution not typical for raw heavy fuel oil (data not shown) rather for samples that had been exposed to weathering and biodegradation over extended periods of time.[17] This is in accordance with the ratio Pr/*n*-C_{17} and Phyt/*n*-C_{18} of the SIM isoprenoid fraction. Bearing in mind that terpanes are, generally speaking, more resistant to microbiological degradation compared to *n*-alkanes and isoprenoid aliphatic alkanes, their presence in the oil pollutant that had previously been exposed to natural biodegradation is not surprising. During the bioremediation process, the quantity of all tricyclic and tetracyclic terpanes decreased and in the sample S-150, the quantity of tricyclic (C_{19}, C_{24}, C_{25}, and C_{28}) and tetracyclic terpanes (C_{24}) is accord-

ing to their intensity virtually no different from the peaks overlapped by the background noise. In relation to them, the quantity of pentacyclic terpanes of the hopane type also decreased at an intensity that conforms to degradation of oil and oil derivatives under natural conditions.

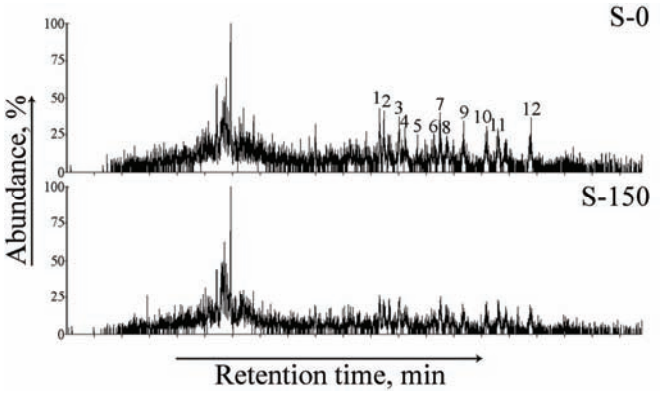

Fig. 6. Fragmentograms of the steranes (SIM, *m/z* 217) of the alkane fractions in the samples S-0 and S-150. 1: C_{27} – 13β(*H*),17α(*H*)-diasterane (20S); 2: C_{27} – 13β(*H*),17α(*H*)-diasterane (20R); 3: C_{27} – 13α(*H*),17β(*H*)-diasterane (20S); 4: C_{27} – 13α(*H*),17β(*H*) diasterane (20R); 5: C_{28} – 13β(*H*),17α(*H*)-diasterane (20R); 6: C_{28} – 13α(*H*),17β(*H*)-diasterane (20S) + C_{27} – – 14α(*H*),17α(*H*)-sterane (20S); 7: C_{29} – 13β(*H*),17α(*H*)-diasterane (20S) + C_{27} – – 14β(*H*),17β(*H*)-sterane (20R); 8: C_{27} – 14β(*H*),17β(*H*)-sterane (20S) + C_{28} – – 13α(*H*),17β(*H*)-diasterane (20R); 9: C_{29} – 13β(*H*),17α(*H*)-diasterane (20R); 10: C_{29} – 13α(*H*),17β(*H*)-diasterane (20R) + C_{28} –14β(*H*),17β(*H*)-sterane (20R); 11: C_{28} – 14α(*H*),17α(*H*)-sterane (20R); 12: C_{29} – 14α(*H*),17α(*H*)-sterane (20R).

Steranes and diasteranes are polycyclic alkanes that are degraded more quickly and easily than terpanes.[29] The decreased concentration due to intensive bioremediation processes makes their identification by the SIM method more difficult. Over the course of conducted bioremediation experiments, the fate of steranes and their more stable structural isomers, diasteranes, C_{27}–C_{29}, is similar to the fate of the terpanes (Fig. 6), and biodegradation, decrease and loss of resolution of the individual signals, was observed in the sample S-150.

CONCLUSIONS

This research monitored the effects of bioremediation of soil contaminated by mazut over a period of 150 days (March – July 2009). The bioremediation was stimulated through a two-week reinoculation with a zymogenous microbial consortium, along with mixing, watering and biostimulation. The analysis of change in the group composition evidenced that the average rate of decrease during the biodegradation of mazut was 23.7 mg kg^{-1} day^{-1} for the aliphatic fraction, 5.7 mg kg^{-1} day^{-1} for the aromatic fraction and 3.3 mg kg^{-1} day^{-1} for the NSO-asphaltene fraction. These findings indicate that the microorganisms

consumed all the components of the compounds of the hydrocarbon mixture, although at different biodegradation rates.

The participation of oil hydrocarbon degraders to the total number of microorganisms had increased to 75 % by the mid-cycle, compared to < 1 % during early bioremediation. The population of microorganisms capable of using diesel as their sole source of carbon remained stable even 150 days after the first reinoculation, which indicates that the natural ability of microorganisms to adapt to the environmental conditions significantly contributes to their extremely high survival rate.

The fate of the saturated hydrocarbons was monitored through changes in the composition of the alkane fraction. The abundance of *n*-C_{17}- and *n*-C_{18}-alkanes at time zero was somewhat smaller than the abundance of pristane (C_{19}) and phytane (C_{20}). This indicates that the process of hydrocarbon degradation had started before the design and application of the bioremediation procedure.

Polycyclic alkanes, steranes and diasteranes, as well as terpanes, were biodegraded in the sample S-150 and did not differ from peaks overlapped by the background noise. The zymogenous bacterial consortium used in this bioremediation study degraded isoprenoids with > C_{20}, and to a certain extent pristane and phytane as well, which are recalcitrant compounds resistant to biodegradation and hence commonly used as chemical markers against which the degree of biodegradation of the other compounds and oil maturation are measured.[27,30] Biodegradation of pristane and phytane means that these compounds are not suitable internal standards for monitoring bioremediation. Therefore, by comparing the content of other hydrocarbons relative to pristane and phytane, their degree of biodegradation may be underestimated, because branched pristane and phytane are also biodegradable, although in most cases with a lower biodegradation rate. However, the ratio Pr/*n*-C_{17} and Phyt/*n*-C_{18} may serve to differentiate between physical weathering and bioremediation.[27] The ratio Pr/*n*-C_{17} and Phyt/*n*-C_{18} increased over time of bioremediation because bioremediation removes *n*-C_{17} and *n*-C_{18} at a higher rate than pristane and phytane, respectively.

TIC confirmed a significant degradation of alkanes. This was followed by a decreased concentration of isoprenoids (> C_{20}), tricyclic (C_{19}–C_{26}) and tetracyclic terpanes (C_{24}), pentacyclic triterpanes (C_{27}–C_{30}) as well as by decreased sterane and diasterane contents (C_{27}–C_{29}).

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ИЗВОД

ПРОМЕНА ИЗОПРЕНОИДНЕ, СТЕРАНСКЕ И ТЕРПАНСКЕ ФРАКЦИЈЕ ТОКОМ *EX SITU* БИОРЕМЕДИЈАЦИЈЕ МАЗУТА НА ИНДУСТРИЈСКОМ НИВОУ

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Приказани су резултати *ex situ* биоремедијације земљишта контаминираног мазутом на индустријском нивоу (600 m^3). Биоремедијациони материјал (дебљине 0,4 m) се састојао од механички помешаних мазутом загађеног земљишта, чамове пиљевине као додатног извора угљеника и непречишћеног речног песка, додатог у циљу мешања и повећања порозности. Инокулација/реинокулација (биоаугментација) је периодично рађена са биомасом конзорцијума зимогених микроорганизама изолованих из супстрата за биоремедијацију. Биостимулација је реализована додатком хранљивих супстанци (N, P и K). Аерација је побољшавана систематским мешањем биоремедијационог система. Након 50 дана број микроорганизама који деградирају угљоводонике повећан је 100 пута. На основу промена у групном саставу просечна стопа биодеградације током биоремедијације је била за алифатичну фракцију 24 mg kg^{-1} по дану, за ароматичну 6 mg kg^{-1} по дану и 3 mg kg^{-1} по дану за HCO-асфалтенску фракцију. У засићеној угљоводоничној фракцији методом GC–MS (SIM метод) анализирани су изопреноиди пристан и фитан и полициклични молекули стеранског и тритерпанског типа. Током биоремедијационог процеса дошло је до биодеградације и смањења релативних количина изопреноида, стерана, три- и тетрацикличних терпана и пентацикличних терпана хопанског типа.

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REFERENCES

1. *ASTM D396-09a, Standard Specification for Fuel Oils*, 2009
2. *ISO 8217:2005, Petroleum products – Fuels (class F) – Specifications of marine fuels*, 2005
3. U. Langer, L. Böhme, F. Böhme, *J. Plant Nutr. Soil Sci.* **167** (2004) 267
4. J. V. Forsyth, Y. M. Tsao, R. D. Bleam, in *Bioaugmentation for Site Remediation*, R. E. Hinchee, J. Fredrickson, B. C. Alleman, Eds., Battelle Press, Columbus, OH, 1995, p. 1
5. S. J. MacNaughton, J. R. Stephen, A. D. Venosa, G. A. Davis, Y. J. Chang, D. C. White, *Appl. Environ. Microbiol*. **65** (1999) 3566
6. M. Viñas, M. Grifoll, J. Sabaté, A. M. Solanas, *J. Ind. Microbiol. Biot.* **28** (2002) 252
7. F. M. Ghazali, R. N. Z. A. Rahman, A. B. Salleh, M. Basri, *Int. Biodeter. Biodegr*. **54** (2004) 61
8. A. M. Boronin, V. G. Grishchenkov, A. V. Karpov, S. G. Seleznev, V. G. Tokarev, M. U. Arinbasarov, R. R. Gajazov, N. P. Kuzmin, *Process Biochem*. **32** (1997) 13
9. A. Singh, O. P. Ward, *Biodegradation and bioremediation*, Springer-Verlag, Berlin, 2004, p. 1
10. J. A. Marín, J. L. Moreno, T. Hernández, C. García, *Biodegradation* **17** (2006) 251
11. S. J. McMillen, A. G. Requejo, G. N. Young, P. S. Davis, P. N. Cook, J. M. Kerr, N. R. Gray, in *Microbial Process for Bioremediation*, R. R. Hinchee, F. J. Brokman, C. M. Vogel, Eds., Battelle Press, Columbus, OH, 1995, p. 91

12. M. Nocentini, D. Pinelli, F. Fava, *Chemosphere* **41** (2000) 1115
13. K. Sugiura, M. Ishihara, T. Shimauchi, S. Harayama, *Environ. Sci. Technol*. **31** (1997) 45
14. S. Mishra, J. Jyot, R. C. Kuhad, B. Lal, *Curr. Microbiol*. **43** (2001) 328
15. B. P. Tissot, D. H. Welte, *Petroleum formation and occurrence*, Springer, Berlin, 1984, p. 1
16. D. Waples, *Geochemistry in petroleum exploration*, International Human Resources Development Corporation, Boston, USA, 1985, p. 1
17. K. E. Peters, C. C. Walters, J. M. Moldowan, *The biomarker guide*, Cambridge University Press, Cambridge, 2005, p. 1
18. B. Jovančićević, M. Antić, I. Pavlović, M. Vrvić, V. Beškoski, A. Kronimus, J. Schwarzbauer, *Water Air Soil Poll*. **190** (2008) 299
19. B. Jovančićević, M. Antić, M. Vrvić, M. Ilić, M. Novaković, R. M. Saheed, J. Schwarzbauer, *J. Serb. Chem. Soc*. **73** (2008) 577
20. J. S. Milić, V. P. Beškoski, M. V. Ilić, S. A. M. Ali, G. Dj. Gojgić-Cvijović, M. M. Vrvić, *J. Serb. Chem. Soc*. **74** (2009) 455
21. C. Loser, H. Seidel, A. Zehnsdorf, U. Stottmeister, *Appl. Microbiol. Biotechnol*. **49** (1998) 631
22. http://www.ccap.ac.uk/media/recipes/SE.htm (01.05.2010)
23. I. D. Bossert, L. M. Shor, D. S. Kosson, in *Manual of Environmental Microbiology*, 2nd Ed., C. J. Hurst, R. L. Crawford G. R. Knudsen, M. J. McInerney, L. D. Stetzenbach, Eds., ASM Press, Washington, DC, 2006, p. 934
24. B. S. Jovančićević, *Practical Organic Geochemistry with Fuel Chemistry*, Faculty of Chemistry, Belgrade, 1999, p. 13 (in Serbian)
25. *NIST/EPA/NIH Mass spectral library NIST2000*, Wiley
26. E. Katsivela, E. R. B. Moore, D. Maroukli, C. Strömpl, D. Pieper, N. Kalogerakis, *Biodegradation* **16** (2005) 169
27. Z. Wang, M. Fingas, S. Blenkinsopp, G. Sergy, M. Landriault, L. Sigouin, J. Foght, K. Semple, D. W. S. Westlake, *J. Chromatogr. A* **809** (1998) 89
28. M. P. Antic, B. S. Jovancicevic, M. Ilic, M. M. Vrvic, J. Schwarzbauer, *Environ. Sci. Pollut. Res*. **13** (2006) 320
29. W. K. Seifert, J. M. Moldowan, G. J. Demaison, *Org. Geochem*. **6** (1984) 633
30. N. L. Olivera, M. G. Commendatore, A. C. MorWan, J. L. Esteves, *J. Ind. Microbiol. Biot*. **25** (2000) 70.