https://doi.org/ 10.18321/ectj1034

Oxidation of a Mixture of Polyaromatic Hydrocarbons by a Mixed Culture of Hydrocarbon-Oxidizing Microorganisms

D.A. Filatov[1], M.A. Kopytov[1], V.S. Ovsyannikova[1]*, E.A. Elchaninova[1,2]

[1]Institute of Petroleum Chemistry SB RAS, 4, Academichesky Ave., Tomsk, Russia
[2]National Research Tomsk Polytechnic University, 30, Lenin Ave., Tomsk, Russia

Article info

Received:
19 July 2020

Received in revised form:
17 September 2020

Accepted:
25 November 2020

Keywords:
Polyaromatic hydrocarbons;
Biochemical oxidation;
Hydrocarbon-oxidizing microorganisms.

Abstract

The possibility of biochemical oxidation of polyaromatic hydrocarbon mixtures (PAHs) by the mixed culture of hydrocarbon-oxidizing microorganisms (HOM) in a liquid medium and soil was investigated. The mixed HOM culture was represented by *Pseudomonas stutzeri*, *Pseudomonas putida*, *Bacillus cereus*, and *Arthrobacter globiformis* genera. It was shown that during HOM cultivation of the microorganisms under study in the liquid medium their number increases from $0.25 \cdot 10^4$ to $11 \cdot 10^8$ CFU/ml, which is accompanied by an increase in their oxygenase activity. All PAHs identified were subjected to oxidation from 11.3 to 100%. The results of experiments on biodegradation of PAHs under natural conditions have shown that for 60 days the total utilization of oil products in soils was on the average 65% of the initial contamination. This suggests the prospects for the use of the mixed HOM culture under study for effective biodegradation of PAHs polluting soil and waste waters.

1. Introduction

Polyaromatic hydrocarbons (PAHs) are high on the list of ecotoxic substances by the harm caused to the environment [1]. They are extremely diverse, and their toxicity, carcinogenicity, and mutagenicity are well known [2–4]. Also, they are lipophilic, poorly soluble in water and volatile [5]. Many of these compounds belong to the superecotoxicants of the 1st hazard class (dimethylbenz(a)anthracene, benzo(a)pyrene, etc.). Human activities, including fuel combustion, burning of various waste and organic materials, and oil spills are the main sources of ingress of PAHs into the environment. The disposal of PAHs mainly consists of their burial in specialized landfills. While under natural conditions, polyaromatic compounds could be degraded by indigenous microbial communities or by photodegradation [6], their complete degradation takes years.

Nowadays, there is a wealth of information on the mechanism of biodegradation of aromatic hydrocarbons of different structures and on the stages, intermediates, and enzymes involved [7]. It is well known that bacteria of the *Pseudomonas*, *Rhodococcus*, and *Arthrobacter* genera and lignin mineralizing fungi such as *Trichoderma lignorum*, *Phanerochaete chrysosporium*, *Coriolus versicolor*, and *Chaetomium globosum* are active PAH degraders. It is also known that the degradation of PAHs is generally possible under cooxidation with an organic substrate that is most accessible to microbial oxidation. However, there is evidence that bacteria in pure cultures and mixed cultures are capable of oxidizing individual PAHs [8, 9].

Work is now underway toward the use of microbial strains that could decompose the ecotoxicants in treatment facilities, but the methods of biodegradation of toxic substances directly in the natural biocenoses (bioremediation) and industrial technologies for decontamination of natural landscapes from anthropogenic pollution by PAHs have been insufficiently developed [10, 11].

Most of the works on the degradation of PAHs deal with the investigation of oxidation of an individual substance or a mixture of PAHs containing not more than 5 components [12].

*Corresponding author. E-mail: varja@ipc.tsc.ru

Under real conditions, however, PAHs-ecotoxicants are more numerous. The foregoing proves that both the fundamental and applied investigations in this field are urgent and promising.

This work aims to investigate the oxidation of a multi-component mixture of PAHs by a mixed culture of hydrocarbon-oxidizing microorganisms (HOMs) a liquid mineral medium and in soil under natural conditions.

2. Experimental

The objects of the study were oil products, isolated from oil-contaminated soil in an oil field of Timan-Pechora oil and gas province (Russia). The concentration of oil products averaged 50 g/kg of soil. The bitumen was extracted from the contaminated soil via hot extraction with a chloroform/methanol mixture (93:7) and then the extractant was removed using a vacuum rotary evaporator. The total aromatics content amounted to 69.8, while that of resins and asphaltenes – 21.2 and 9%, respectively. To isolate the PAH fraction, the resins and asphaltenes were removed from the sample by filtration through a column containing a very active aluminum oxide (IV grade). The aluminum oxide was eluted with hexane.

We investigated the possibility of bio-oxidation of PAHs by a mixed HOM culture represented by *Pseudomonas stutzeri*, *Pseudomonas putida*, *Bacillus cereus*, and *Arthrobacter globiformis* species, previously isolated from oil polluted soils.

Laboratory experiments on bio-oxidation of PAHs were carried out in batch fermentation in a liquid Raymond's mineral medium in 300 ml glass flasks. The concentration of added PAHs (hexane fraction) was 0.5% (1 g per 200 ml of the medium). The HOM mixed culture was prepared by co-cultivation. The inoculum was introduced into the culture medium in a volume of 1 ml, while the content of the mixed HOM culture was $0.5 \cdot 10^6$ CFU/ml.

A 1 ml composition containing 16% of ammonium nitrate (NH_4NO_3), 32% of urea and 1.5 and 0.5% wt of surfactants – Neonol and Volgonat, respectively, was added to the culture medium as an additional mineral nutrient source. The flasks with the medium, PAHs, and HOM were incubated for 10 days at 20 °C under constant aeration and stirring in a magnetic stirrer with a rotation speed of 800 revolutions per minute. Use was made of three replicate flasks, two of them were test flasks containing the medium, PAHs and microorganisms and one reference flask containing the medium and PAHs only. The experiment was repeated 5 times.

Field experiments were carried out in the same oil field of the Timan-Pechora oil and gas province. The territory polluted with oil and oil products was divided into 10 plots, 5 reference plots and 5 experimental plots, each being 1 m^2 in area. Their total area was $10 \cdot 10$ m^2. We introduced 1 liter of the mixed culture at a concentration of $15–20 \cdot 10^7$ CFU/ml per 1 m^2 by sprinkling it over the experimental territory. Then 0.7 l of a 5% solution of the mixed culture per 1 m^2 was introduced into the soil. The reference plots were left without any treatment. To determine the number of microorganisms and enzyme activity, one sample was collected from each soil plot. The soil sample was composed of 10 random samples taken from different sites located in a checkerboard pattern. The experiment was carried out in summer for 60 days.

The number of colony-forming units were determined by the serial dilution followed by plating onto Petri dishes with an agar-based nutrient medium and counting of the resulting colonies.

The catalase activity was determined by the gasometric method from the rate of H_2O_2 decomposition. A soil sample (1 g) is introduced into a 100 ml flask and 0.5 g of $CaCO_3$ is added. Then a small glass with 5 ml of a 3% hydrogen peroxide solution is placed on the flask bottom. A sample of soil is moistened with 4 ml of distilled water. The flask is tightly closed with a stopper with a tube connected to a water-filled burette through a T-piece with a stopcock. The water level in the burette is balanced with a rubber bulb and then the stopcock is closed to make the device airtight. The time of the experiment beginning is noted by a stopwatch, the glass with hydrogen peroxide is overturned and the contents of the flask are shaken for 1 min. The evolved oxygen displaces water from the burette, the volume of which is marked. It was expressed in ml of O_2 released per 1 ml of medium and per 1 g of soil per 1 min.

To determine the dehydrogenase activity, use was made of colorless tetrazolium salts (2, 3, 5 triphenyltetrazolium chloride – TTC) as hydrogen acceptors, which are reduced to form red formazan compounds (triphenylformazan – TPF). A soil sample (1 g) is placed in a test tube to mix with 10 mg of $CaCO_3$, 1 ml of 0.1 M glucose solution and 1 ml of 1% TTC solution. The test tube is placed in an anaerostat to incubate under a vacuum at 30 °C for 24 h. The formed TPF is extracted with ethanol (25 ml) and filtered in the dark. The absorbance of the colored solution is determined at 540 nm and then the amount of formazan in milligrams is

calculated using the standard curve. The dehydrogenase activity was expressed in mg of TPF to 1 ml of medium per 1 g of soil per day.

The method of determining the polyphenoloxidase and peroxidase activities of soil is based on measuring the rate of hydroquinone oxidation by atmospheric oxygen from the color intensity of the resulting quinone. A soil sample (1 g) introduced in a 50 ml conical flask. To initiate the reaction to polyphenol oxidase 1 ml of phosphate buffer and 10 ml of 1% hydroquinone solution are added. To initiate the reaction to peroxidase 1 ml of phosphate buffer, 10 ml of 1% hydroquinone solution, and 1 ml of 0.5% H_2O_2 solution are added. The flasks are shaken and thermostated at 30 °C for 35 min. Then 10 ml of ethyl alcohol are added to the contents of flasks, stirred and filtered through a dense filter. The filtrate is photocolored at 430 nm. The polyphenoloxidase and peroxidase activities were expressed in mg of quinone per 1 ml of medium per 1 g of soil per 30 min [13, 14].

The residual contents of PAHs in the culture medium and soil were determined gravimetrically after their extraction with chloroform and removal of the solvent. The composition of individual PAHs in the initial fraction and that subjected to biodegradation were analyzed by a GC-MS analysis using a Thermo Scientific DFS magnetic GC-spectrometer (Germany).

The chromatograph was equipped with a Thermo Scientific quartz capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). The stationary phase was TR-5MS, and the carrier gas was helium. The GC oven temperature program was 80 °C (initial hold time 2 min), then 4 °C/min to 300 °C (initial hold time 30 min). The MS analysis was performed via electron impact ionization with the energy of ionizing electrons 70 eV. The temperature in the ionization chamber and that of the interface was 270 °C.

The chromatograms for organic compounds were obtained in two ways: from areas of the chromatographic peaks corresponding to the total ion current (TIC) and from the areas recorded for the main ion mass (m/z) in a SIM (Single Ion Monitoring) acquisition mode. The error in determining the peak area on this equipment is 3.2%.

The individual PAHs were identified using a computer search from among the literature data in the library of the National Institute of Standards NIST-05 and by the structure reconstruction according to the character of ionic fragmentation under electron impact. The content of a component was determined by the area of the corresponding peaks on the chromatograms using an internal standard – $C_{12}D_{10}$ deyteroacenaphthene.

The data in tables and graphs are presented as arithmetic means.

3. Results and discussion

Under the laboratory conditions the hydrocarbon-oxidizing microorganisms in a liquid mineral medium with PAHs as the sole carbon source began to actively grow on the first day of the experiment without any adaptation period. On the 4–5th day of cultivation, the number of microorganisms reached its maximum of $10–11 \cdot 10^8$ CFU/ml, i.e. their number increased 5 times from the initial value of $0.25 \cdot 10^4$ CFU/ml (Fig. 1). This indicates that HOBs under study immediately began to oxidize PAHs in the mixture. A certain role in the intensification of PAH bio-oxidation belongs to surfactants composing a stimulating nutrient supplement. The surfactants promote emulsification of the PAH mixture, increasing the reaction surface area of the substrate (PAHs) and the catalyst (HOM).

Microbiological oxidation of hydrocarbons is a series of enzymatic redox reactions. Some of them are carried out by oxidoreductases [15]. For example, catalase works to speed up the decomposition of peroxides with a release of highly active oxygen, which is subsequently used by microorganisms. Dehydrogenases are mainly responsible for the oxidation of normal alkanes and aliphatic chains in complex molecules. Phenoloxidases (polyphenol oxidases and peroxidases) play an important role in the decomposition of aromatic compounds [16, 17].

Changes in the activity of the above-mentioned enzymes during biodegradation of a PAH mixture are given in Fig. 2.

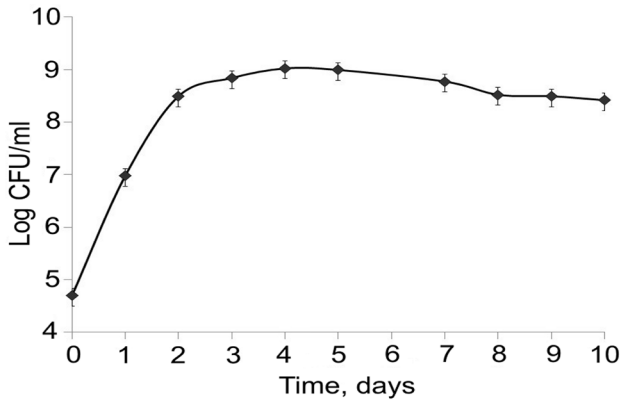

Fig. 1. Population dynamics for the mixed HOM culture during biodegradation of the PAH mixture in a liquid medium.

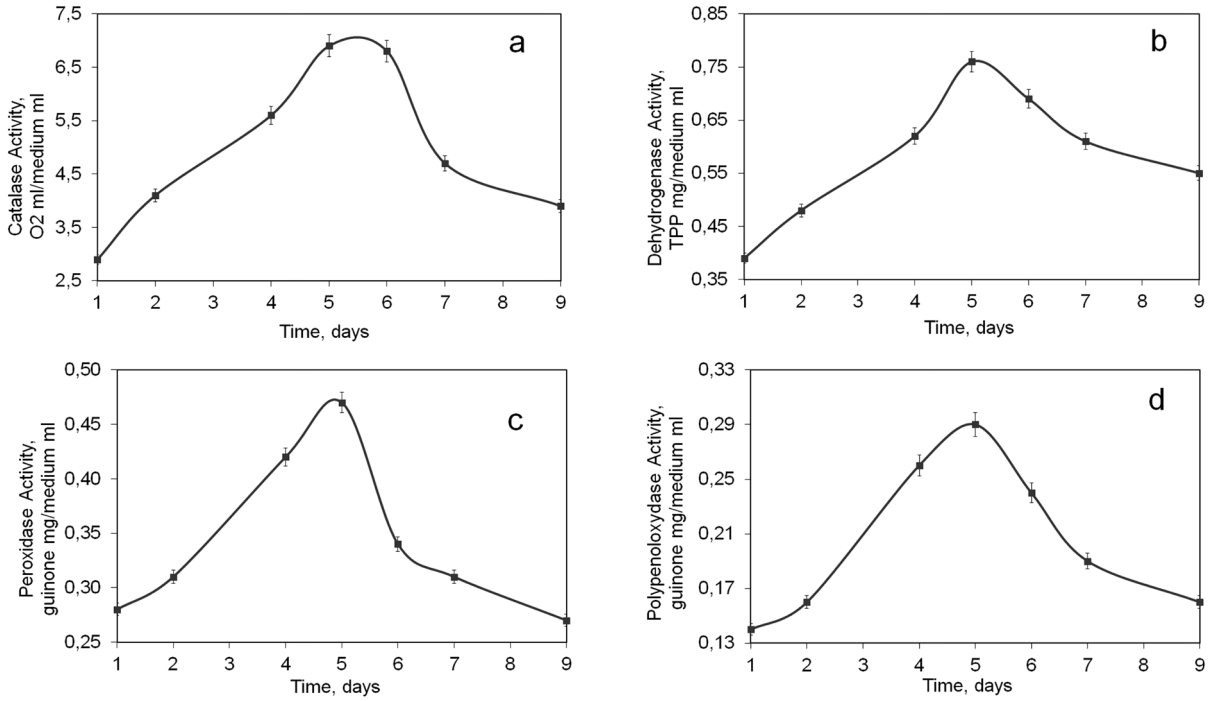

Fig. 2. Activity of catalase (a), dehydrogenase (b), peroxidase (c) and polyphenoloxydase (d) during biodegradation of PAHs by a mixed HOM culture in a liquid medium.

An increase in the activity of all enzymes under study begins immediately after the onset of metabolic activity, peaking on the 5th day of the experiment, and decreasing on the 6–9th day. The enzyme activity changed under the variations in the population dynamics of microorganisms. This decrease in the HOM activity in time might have resulted from the limit of mineral nutrition and accumulation of metabolites in the culture medium, which can decrease the size of the bacterial population.

GC-MS analysis revealed the presence of bi-, tri-, tetra-, penta- and hexacyclic aromatic hydrocarbons in the composition of the PAH mixture before and after biodegradation (Table 1). Heteroatomic compounds (dibenzthiophenes and benznaphtothiophenes were also identified, while no monoaromatic hydrocarbons were found. Polyarenes (phenanthrenes, naphthalenes, pyrenes and fluoranthenes) were found to prevail among arenes of the initial sample. The content of pentaarenes (benzopyrenes and perylene) was also high.

The rate of oxidation of organic substances is determined not only by their structure but also by the behaviour of the processes. An analysis of the experimental data on the PAH degradation in a liquid medium revealed that PAHs with different structures are degraded by HOM at different rates

Previously, it was shown that degradation of PAHs starts with hydroxylation of a single aromatic ring, but might involve more [18]. Therefore, the availability of PAHs for biooxidation would decrease as the number of benzene rings increases. For this laboratory experiment, the degradation of naphthalene was 100%, while that of compounds with higher molecular weight (phenanthrene, anthracene, fluoranthene, pyrene, and chrysene) was as low as 15–34% (Table 1). On the other hand, the degradation of substances with an even more complex structure (perylene, indenopyrene, and dibenzochrysene) ranged from 62 to 85%, which is inconsistent with the previously established principles/mechanisms. Thus, simultaneous oxidization of more than one ring is possible in PAH molecules with three or more rings.

Biodegradation of alkyl aromatics occurs more readily than that of unalkylated since the oxidation occurs mainly on the point of attachment of the side chain, and the primary oxidation is complicated due to the absence of open methyl groups [19]. For this experiment, the oxidation of some unalkylated arenes (fluorene, pyrene, and chrysene) was found to be 15–19%, while their methyl-substituted homologues were oxidized 23 to 57% (Table 1).

However, the degradation of unalkylated biphenyl and perylene was 65.9 and 80.3% respectively, while the degradation of their alkyl-substituted homologues was found to be only 11.3–12.2% and 47.6–49.4%, respectively. This indicates that the bioconversion of PAHs in the culture medium was nonuniform.

Table 1
Composition of aromatics in the initial PAHs fraction and in the fractions subjected to biodegradation with the HOM under study under the natural and laboratory conditions

Hydrocarbons	PAHs initial, mcg/g	Laboratory conditions		PAHs initial, mcg/g	Natural conditions	
		PAHs bio, mcg/g	Degradation, %		PAHs bio, mcg/g	Degradation, %
Biarenes						
Naphtalene	342.31	0.00	100.00	147.68	0.00	100.00
Methylnaphtalenes	717.35	54.93	92.34	496.23	5.21	98.95
Dimethylnaphtalenes	1208.72	459.00	62.02	934.81	1.86	99.80
Trimethylnaphtalenes	1073.93	492.11	54.17	875.32	1.66	99.81
Tetramethylnaphtalenes	214.45	92.84	56.70	203.25	0.99	99.51
Dibenzothiophene	267.96	212.28	20.77	186.49	22.65	87.85
Methyldibenzothiophenes	440.53	340.21	22.77	501.63	53.92	89.25
Dimethyldibenzothiophenes	356.67	181.87	49.00	297.46	25.10	91.56
Biphenyl	285.73	97.34	65.93	311.82	25.07	91.96
Methylbiphenyls	613.10	538.20	12.21	568.73	32.58	94.27
Dimethylbiphenyls	826.62	732.69	11.36	695.34	6.11	99.12
Fluorene	470.81	381.13	19.04	364.12	49.81	86.32
Methylfluorenes	864.53	663.00	23.31	901.23	58.21	93.54
Dimethylfluorenes	955.19	596.68	37.53	932.58	20.14	97.84
Triarenes						
Phenantrene	567.19	409.87	27.73	481.21	20.74	95.69
Anthracene	156.96	103.54	34.03	174.87	22.91	86.90
Methyl-phenantrenes and anthracenes	1691.66	1545.46	8.64	1875.96	240.12	87.20
Dimethyl-phenantrenes and anthracenes	2940.77	2319.49	21.12	2598.36	88.86	96.58
Terphenyls	72.54	34.33	52.67	87.36	8.62	90.13
Tetraarenes						
Fluoranthene	483.41	387.59	19.82	541.35	56.13	89.63
Pyrene	439.64	366.47	16.64	409.71	56.08	86.31
Methylfluoranthenes and pyrenes	1830.36	1400.69	23.47	1723.91	48.09	97.21
Dimethylfluoranthenes and pyrenes	1924.53	937.32	51.29	1451.36	7.11	99.51
Benzanthracene	235.07	149.79	36.27	296.57	30.42	89.74
Chrysene	276.26	232.17	15.95	261.71	36.69	85.98
Methylbenzanthracenes and chrysenes	825.19	348.01	57.82	934.27	36.24	96.12
Dimethylbenzanthracenes and chrysenes	315.50	138.93	55.96	297.31	12.28	95.87
Benzonaphtothyophenes	310.08	225.97	27.12	431.21	55.06	87.23
Pentaarenes						
Benz(e)acenaphtrilene	262.42	134.01	48.93	314.57	22.83	92.74
Benz(b)fluoranthene	83.24	48.79	41.38	101.44	6.57	93.52
Benz(k) fluoranthene	77.57	46.44	40.13	62.53	5.01	91.98
Benz(a)pyrene	199.83	89.64	55.14	253.11	6.17	97.56
Benz(e)pyrene	162.07	68.33	57.84	133.29	1.72	98.71
Perylene	14.48	2.85	80.31	20.46	0.25	98.76
Methylpentaarenes	940.85	476.09	49.40	783.24	26.94	96.56
Dimethylpentaarenes	467.38	244.56	47.67	561.29	13.81	97.54
Hexaarenes						
Dibenzochrysene	143.94	54.72	61.98	151.36	15.52	89.74
Benzoperylene	148.63	62.08	58.23	126.47	15.50	87.34
Indenopyrene	21.23	3.18	85.02	30.29	0.31	98.96

It was also noted that no penta- and hexaaromatic components were accumulated in the course of degradation of a PAH mixture by the mixed HOM culture in a liquid medium, hence no products of partial degradation were condensed during the bio-oxidation. The biodegradation of PAHs might follow different paths and depend on various factors both biotic (the composition of association of microorganisms, intermediates of oxidation, etc.) and abiotic (the content of oxygen and macroelements, temperature, etc.).

Thus, in a liquid medium, the HOMs under study to a greater or lesser extent destroyed all the components of the PAH mixture. The total biodegradation of bi-, tri-, tetra-, penta- and hexaarenes averaged 44.7, 28.8, 33.8, 52.6, and 68.4%, respectively (Table 1).

The degradation of a PAH mixture in the tested flask (medium and PAH, no microorganisms) was observed to be 1–2% maximum, i.e. within the error or device sensitivity. It is likely, that bioconversion of PAHs in a liquid medium is scarcely affected by abiotic factors (light, humidity, temperature, evaporation, etc.). Due to the fact that the mixed HOM culture demonstrated good results for the biodegradation of PAHs in a liquid medium, we carried out the experiments to utilize PAHs under natural conditions using the HOMs under study.

The initial number of heterotrophic microorganisms in the polluted soil was $1–3 \cdot 10^4$ CFU per 1 g of soil. This content of indigenous microorganisms was not enough for the biodegradation of the residual oil. After the introduction of bacterial inoculum and supplementary mineral nutrition, the number of HOMs started to grow already on the first day of the experiment and increased by 4–5 orders of magnitude. On the 35th day of the experiment, the number of microorganisms reached its maximum of $8–9 \cdot 10^8$ CFU per 1 g of soil (Fig. 3).

Their high number persisted to 50 days of the experiment. A reduction in the number of HOMs is likely due to the disposal of the majority of PAHs. The maximum number of microorganisms in the reference plots was $6–7 \cdot 10^4$ CFU per 1 g of soil. This suggests that the biodegradation was near zero in the reference plots of soil.

The activity of all investigated enzymes increased during the experiment, it was 5–6 times higher compared with that for reference plots, which is indicative of the intense transformation of hydrocarbons in the tested plots of soil (Table 2). In the reference plots, the enzyme activity was almost unchanged.

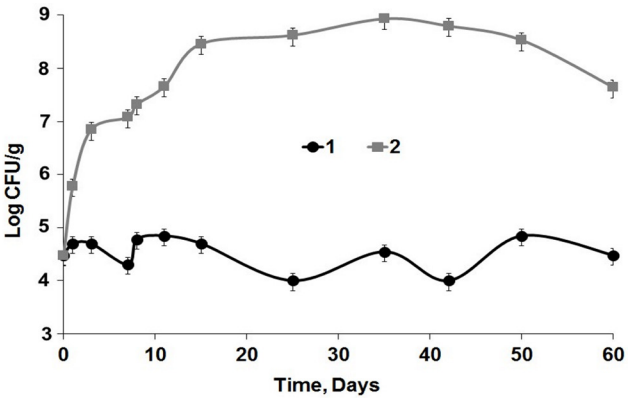

Fig. 3 Population dynamics for the mixed HOM culture during biodegradation of PAH mixture under natural conditions (1 – reference plot, 2 – tested plot).

The results demonstrated that the total degradation of oil products in the soil for 60 days averaged 32.5 g/kg, which was 65% of the initial contamination. Probably, the residual oil products in their chemical composition are similar to the bitumen of mother rock – resins and asphaltenes, which are high-molecular non-hydrocarbon components. These compounds are the most difficult to oxidize.

In the reference plots, the percentage of oil pollution remained the same and the composition of aromatic compounds was also unchanged.

The GC-MS data proved that the biodegradation of the PAHs under natural conditions using HOMs was much more intensive. The utilization of bi-, tri-, tetra-, penta-, and hexaarenes and their methyl substituted homologues averaged 94.98, 91.3, 91.95, 95.92, and 92.01%, respectively (Table 1). The data obtained are the results of a combination of several factors. These factors include a high concentration of active HOM and the introduction of nutrients and surfactants contained in the composition used.

Under the laboratory conditions, biodegradation occurred in a closed model system of a limited number of enzymes, specific to the microorganisms of the mixed culture in question, and a substrate of hydrocarbons of exclusively polyaromatic structure. Therefore, at a certain stage, the activity of microflora was suspended by the products of metabolism. The degree of degradation of separate hydrocarbons was on average 40–50%.

Under the field conditions, biodegradation occurred in an open system, where the number of enzymes was larger due to the soil indigenous microflora. An additional source of carbon and energy for the microflora was the soil organic matter and hydrocarbons of a different structure contained in the oil product. The products of metabolism were

Table 2
Enzymatic activity of soil in reference and tested plots

Soil	Enzymes			
	Catalase, ml O_2/g	Dehydrogenase, mg/g	Peroxidase, mg/g	Polyphenoloxydase, mg/g
Untreated	1.40±0.25	0.15±0.02	0.22±0.03	0.11±0.02
		10 days		
Reference	1.20±0.15	0.14±0.02	0.28±0.02	0.12±0.02
Tested	8.40±0.20	0.82±0.03	0.65±0.03	0.44±0.02
		30 days		
Reference	1.75±0.25	0.17±0.02	0.22±0.02	0.13±0.03
Tested	9.00±0.30	1.01±0.04	1.09±0.04	0.62±0.02
		50 days		
Reference	1.30±0.10	0.16±0.02	0.24±0.03	0.13±0.02
Tested	5.30±0.20	0.68±0.02	0.64±0.02	0.54±0.01

removed from the system by abiogenous factors or were incorporated into the food chain of indigenous microflora. The degree of degradation of separate PAHs varied from 85 to 100%.

4. Conclusion

In general, the investigated mixed HOM culture was capable of degrading PAHs of different structures and molecular weights and can thus be used for the treatment of PAH polluted areas. The results of field experiments demonstrate that it is very promising to use the HOM under study in combination with the compositions used for effective biodegradation of PAH pollutant waste water and soil.

References

[1]. J. Karl C. Nieman, Ronald C. Sims, Joan E. McLean, Judith L. Sims, Darwin L. Sorensen, *Chemosphere* 44 (2001) 1265–1271. DOI: 10.1016/S0045-6535(00)00304-0

[2]. G.J. Verrhiest, B. Clément, B. Volat, B. Montuelle, Y. Perrodin, *Chemosphere* 46 (2002) 187–196. DOI: 10.1016/S0045-6535(01)00064-9

[3]. S.K. Samanta, O.V. Singh, R.K. Jain, *Trends Biotechnol.* 20 (2002) 243–248. DOI: 10.1016/S0167-7799(02)01943-1

[4]. X.L. Zhang, S. Tao, W.X. Lio, Y. Yang, Q. Zuo, S.Z. Lio, *Environ. Sci. Technol.* 39 (2005) 9109–9114. DOI: 10.1021/es0513741

[5]. H. Habe, T. Omori, *Biosci., Biotech., Bioch*. 67 (2003) 225–243. DOI: 10.1271/bbb.67.225

[6]. K.T. Semple, B.J. Reid, T.R. Fermor, *Environ. Pollut.* 112 (2001) 269–283. DOI: 10.1016/S0269-7491(00)00099-3

[7]. N.G. Rojas-Avelizapa, E. Cervantes-Gonzalez, R. Cruz-Camarillo, L.I. Rojas-Avelizapa, *Bull. Environ. Contam. Toxicol.* 69 (2002) 835–842. DOI: 10.1007/s00128-002-0135-1

[8]. D.H. Pieper, M. Seeger, *J. Mol. Microbiol. Biotechnol.* 15 (2008) 121–138. DOI: 10.1159/000121325

[9]. R.A. Kanaly, S. Harayama, *J. Bacteriol.* 182 (2000) 2059–2067. DOI: 10.1128/JB.182.8.2059-2067.2000

[10]. I. Alkorta, C. Garbisu, *Bioresource Technol.* 79 (2001) 273–276. DOI: 10.1016/S0960-8524(01)00016-5

[11]. H.H. Richnow, E. Annweilr, M. Koning, J-C. Luth, R. Stegmann, C. Garms, W. Francke, W. Michaelis, *Environ. Pollut.* 108 (2000) 91–101. DOI: 10.1016/S0269-7491(99)00205-5

[12]. L. Launen, L. Pinto, C. Wiebe, M. Moore, *Can. J. Microbiol*. 41 (1995) 477–488. DOI: 10.1139/m95-064

[13]. L.I. Inisheva, C.H. Ivleva, T.A. Scherbakova. Handbook for anzymatic activity determination of peat soils and peats. Tomsk: Tomsk State University, 2003 (in Russian).

[14]. F.Kh. Khaziev Enzymatic activity of Soils. M.: Science. Nauka, 1976, p.180 (in Russian).

[15]. J. Jakoncic, Y. Jouanneau, C. Meyer, V. Stoyanov, *Biochem. Bioph. Res. Co* 352 (2007) 861–866. DOI: 10.1016/j.bbrc.2006.11.117

[16]. K. Shindo, Y. Shindo, T. Hasegawa, A. Osawa, O. Kagami, K. Furukawa, N. Misawa (2007) *Appl. Microbiol. Biot.* 75 (2007) 1063–1069. DOI: 10.1007/s00253-007-0928-6

[17]. B. Cao, K. Nagarajan, K-C. Loh, *Appl. Microbiol. Biot.* 85 (2009) 207–228. DOI: 10.1007/s00253-009-2192-4

[18]. D.A. Filatov, E.V. Gulaya, L.I. Svarovskaya, L.K. Altunina, *Petrol. Chem.* 53 (2013) 59–64. DOI: 10.1134/S0965544113010040

[19]. H. Huang, B.F.J. Bowler, T.B.P. Oldenburg, S.R. Larter, *Org. Geochem*. 35 (2004) 1619–1634. DOI: 10.1016/j.orggeochem.2004.05.009