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# Microbiological Oxidation of High Viscosity Bitumen in Soil

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## Abstract

This paper presents the results of an investigation of microbiological oxidation in the model soil system of high viscosity bitumen from the Bayan-Erkhet deposit (Mongolia) with a high content of heteroelements. It is shown that bitumen, being a mixture of high molecular weight components, has no inhibitory effect on the indigenous soil microflora. Its active growth in the presence of oil products starts without adaptation and lasts for a good part of experiment resulting in 15–30 fold excess of microorganisms over its reference number. The enzymatic activity of the contaminated soil increases by a factor of 1.5–2.0, which indicates an assimilation of various hydrocarbon compounds. The weight analysis revealed that the biodegradation of oil products after 180 days of the experiment was 50% of the initial contamination at initial waste oil concentration 50 g/kg (5%). The analysis by IR spectroscopy revealed an accumulation of oxygen-containing compounds which are intermediate products of bio-oxidation of bitumen components. The method of chromatography-mass spectrometry (GC-MS) revealed the ability of aboriginal soil microflora to mineralize virtually all hydrocarbons contained in the bitumen under study. Their biodegradation ranges from 18 to 97%. It was shown by the GC-MS method that high-molecular heteroatomic components of bitumen (resins and asphaltenes) also undergo a microbial degradation, since their molecular structure changed after the destruction. Thus, the number of structural units in a hypothetical molecule and that of heteroatoms increased due to the high content of oxygen-containing structures. In addition, the ratio of hydrocarbons (oils), resins, and asphaltenes contained in the sample is also changed.

## 1. Introduction

The urgency of the problems is associated with a pollution of environment by various technogenic oil products (OP) [1]. Technogenic streams of oil hydrocarbons are formed in the course of production, transportation and processing of hydrocarbon raw materials. They play an essential role in anthropogenic changes of the natural cycle of matter [2]. The soil is the most environmentally sensitive part of ecosystem because of its vast adsorption surface. It is able to accumulate large amounts of contaminants, which results in changes in its physical-chemical properties, and as a consequence, loss of agricultural value [2]. The presence of oil and oil products in the soil represents a significant environmental risk, since they disturb biogeocoenose to the extent that the living soil organisms may die in the case of high concentrations of hydrocarbons [3].

The negative impact of liquid OP is due in large part to the violation of water-air regime of the soil. Transformation of OP may be nominally split into the abiotic phase (from fresh to mature pollution) and biotic degradation on the earth's surface and in the soils [4]. Microorganisms play the key role in decomposition of organic pollutants entering the environment [5]. Natural hydrocarbon-oxidyzing biocenoses have global distribution in soils and natural waters of all types in different edaphic-climatic zones [6]. However, the intensity of hydrocarbon biodegradation process depends upon the composition and concentration of OP, temperature, duration of the solar period, other limiting factors, and features of biocenosis [7].

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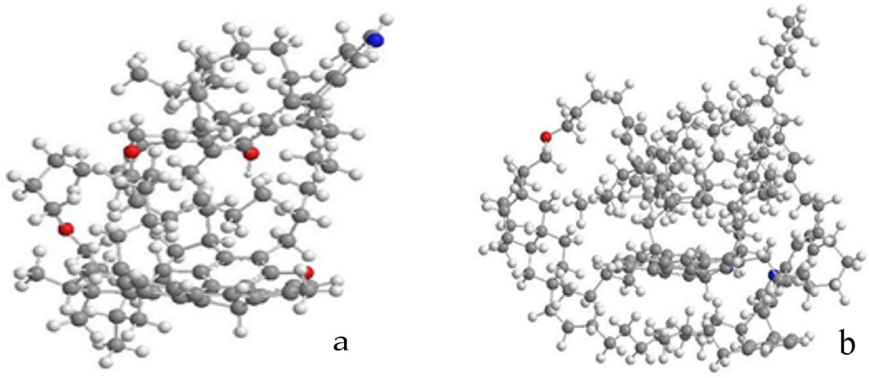


Fig. 1. Average hypothetical structures of molecules of resins (a) and asphaltenes (b).

Both free and stationary bound forms of OP release volatile fractions into the atmosphere and the soluble compounds – into the water. This process is not completely terminated over the course of time, since the microbial processes of transformation of hydrocarbons result in the formation of volatile and water-soluble products of their metabolism.

In recent years, a clear tendency towards reducing of light oil resources has emerged in the world [3]. The proportion of heavy high-viscosity oils and bitumens increases steadily in total amount of extracted and processed hydrocarbons. World resources of high-viscosity oils exceed significantly those of the light and low-viscosity oils. In the estimation of specialists, they are not less than one trillion tons. High-viscosity oils are characterized by a high content of polycycloaromatic hydrocarbons, hetero-organic compounds, and resin-asphaltene substances. High-viscosity bitumen is an oil product representing a viscous black liquid composed of naphthenic, aromatic, paraffinic hydrocarbons, and high molecular heteroatomic compounds (asphaltenes and resins) (Fig. 1) [8].

It is believed that the resin-asphaltene substances are not readily available to microorganisms, the process of their metabolism is slow and lasts sometimes for decades. When the seeping of oil product the resinous-asphaltene components are mainly absorbed in the upper humus horizon, sometimes firmly cementing it, which reduces the soil pore space. Resinous-asphaltene substances are hydrophobic, they envelope the plant roots blocking the water income, which results in plant drying. However, due to complicated biocenosis system (primarily to the content of hydrolyzing and oxidizing enzymes including polyphenol oxidases and peroxidases), the soils themselves are able to utilize a part of oil components by biochemical degradation and oxidative destruction to the point of conversion of certain part of them into organic matters consumed by plants [9–13].

The purpose of this experiment was to study the soil's ability to heal itself under laboratory conditions in case of its pollution with high viscosity bitumen from the Bayan-Erkhet deposit (Mongolia).

## 2. Experimental

The high viscosity bitumen from the Bayan-Erkhet deposit was taken as the object of the study. A brief description of the bitumen under study is presented in Table 1.

The experiment was carried out on artificially contaminated soil material. To prepare the model soil systems the universal 'Garant' soil produced by the production cooperative Temp-2 (Tomsk region, Russia) was used. The highly viscous bitumen in amounts of 50 g/kg was added to the weighted soil and thoroughly mixed. The soil contaminated with bitumen was placed into glass desiccators (soil layer thickness was 5.12 cm) and incubated at constant temperature +22 °C and humidity 25%. The soil was periodically loosened in order to improve the air regime and distribute the pollutants uniformly.

The design of experiment was as follows in Fig. 2.

Sequential composite samples from the experiment 1 were removed in order to determine the number of microorganisms and enzyme activity of soil. A number of heterotrophic bacteria was counted after the inoculation on meat-peptone agar (MPA). The catalase activity was determined by the gasometric method, while dehydrogenase, polyphenoloxidase, and peroxidase activities of the soil were determined by photocolorimetric method [14, 15]. The experiment was carried out during 180 days. The measurements were repeated three times.

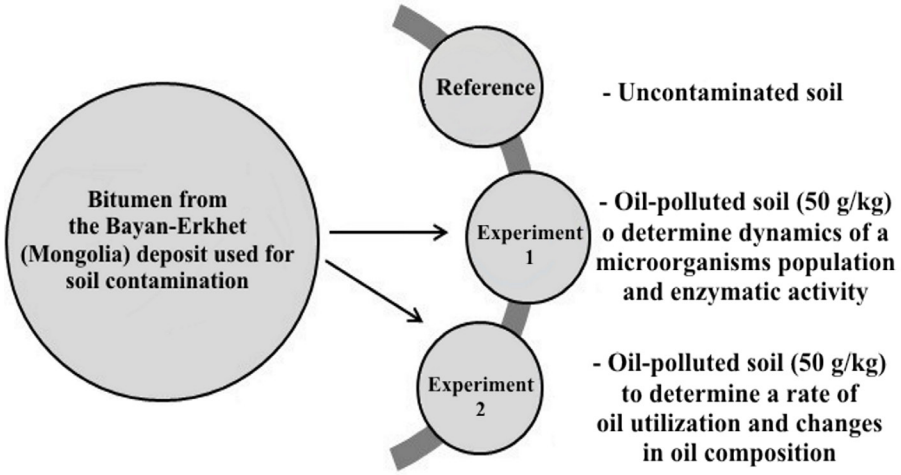


Fig. 2. Design of experiment in the degradation of high viscosity bitumen in the soil.

**Table 1**
Characteristics of bitumen from the Bayan-Erkhet deposit

| Characteristics | Value |
|---|---|
| H/C ratio | 1.82 |
| Average molecular mass, a.m.u. | 578 |
| Material composition, % wt. | |
| oils | 45.2 |
| resins | 52.5 |
| asphaltenes | 2.3 |
| Ultimate composition, % wt. | |
| carbon | 85.26 |
| hydrogen | 13.13 |
| sulfur | 0.45 |
| nitrogen | 0.98 |
| oxygen | 0.18 |

To evaluate the effectiveness of bioremediation, the residual content of oil products in the soil was determined by gravimetric method. The oil from contaminated soil of experiment 2 was extracted with chloroform in a Soxhlet apparatus. After removal of the chloroform using a rotary evaporator, the extracted oil product (bitumen) was weighed [16]. Changes in the structural composition of bitumen were determined by infrared spectroscopy using a NIKOLET 5700 Fourier spectrometer (FT-IR), Thermo Electron. A component composition of organic compounds was investigated by chromatography-mass spectrometry using a Thermo Scientific DFS magnetic GC-spectrometer (Germany). Physical and chemical characteristics of the initial bitumen were determined in the accredited laboratory of hydrocarbons and high-molecular oil compounds of the Institute of Petroleum Chemistry SB RAS (Accreditation certificate № ROSS RU.0001.510476).

The content of resins and asphaltenes in the initial and biodegraded bitumen samples was analyzed by standard methods [17]. Asphaltenes were isolated by diluting the sample with *n*-hexane in a volume ratio of 1:40. After sample diluting the resulting solution was kept for 24 h in the dark, and then the precipitate was filtered off. The resulting filter cake was placed in a paper cartridge and washed in a Soxhlet apparatus with *n*-hexane from oils and resins (maltenes), remained asphaltenes were then eluted with chloroform from the paper cartridge, the solvent was distilled off, and asphaltenes were dried to constant weight. After washing of precipitated asphaltenes and distilling off the solvent resulting maltene solutions were combined and applied to a bed of activated silica gel in a ratio of solution to the sorbent 1:15. The resulting mixture of silica gel with the adsorbed material was charged to a Soxhlet apparatus. The extracted oils were successively washed out with *n*-hexane, while resins with a mixture of ethanol and benzene (1:1) at boiling temperatures of these solvents. After removal of solvents the content of oils and resins in a sample was determined.

The structural-group analysis (SGA) of resins, asphaltenes, and oils extracted from the initial and biodegraded bitumens was performed by the method developed at the IPC SB RAS. This method is based on the use of combined results of determination of elemental composition, average molecular weights, and PMR spectroscopy data [17, 18].

Analysis of carbon, hydrogen, nitrogen, and oxygen was performed using an elemental Vario EL Cube analyzer (Germany). The amount of sulfur

was determined by its burning with subsequent absorption of the formed sulfur dioxides with sodium carbonate solution and titration with hydrochloric acid [8]. The molecular weights of substances were measured by method of cryoscopy in naphthalene with electrothermal measurement of the temperature depression using a "Cryon" device developed at IPC SB RAS. The error in determination of the molecular mass is not higher than 2% for substances with a molecular mass of 500–1000 a.m.u. and not higher than 5% for compounds having a molecular mass 1000–1500 amu at a concentration of substances under study not more than 0.5% wt. This method is sufficiently precise and accessible. Other methods (viscometry, osmometry, ultracentrifugation, method of molecular film, ebullioscopy, light scattering etc.) yield over-estimate values of molecular masses due to occurring processes of resin and asphaltene association.

Proton magnetic resonance spectra were registered using an AVANCE-AV-300 NMR Fourier spectrometer at concentration of the substances under study 1 wt.% using deuterochloroform as solvent and hexamethyldisiloxane as internal standard. The data of structural-group analysis (SGA) were represented by the same symbols of the structural parameters, which were used in previous studies [17, 18]. Namely, $C_a$, $C_n$, $C_\alpha$, $C_p$, $C_\gamma$ are the numbers of carbon atoms in the aromatic, naphthenic, and paraffinic structures of molecules, α-positions to heterofunctions and aromatic rings, and in terminal methyl groups ($C_\gamma$) not bounded with the latter, respectively; $f_a$, $f_n$ and $f_n$ are the fractions of carbon atoms in the corresponding structural fragments; $R_t$ is the total number of rings, $R_a$ and $R_n$ are the numbers of aromatic and naphthenic cycles per mean molecule; ma is the average number of structural units per molecule.

Average parameters of structural units are marked with accent asterisks, where C* is the total number of carbon atoms C in a structural unit and the other symbols are similar to these above mentioned ($C_a$*, $C_n$*, $C_p$*, $C_\alpha$*, $C_\gamma$*, $R_t$*, $K_a$*, and $K_n$*).

## 3. Results and discussion

The activity of microbiological processes and a combination of abiotic factors (light, humidity, temperature, mechanical and mineral soil compositions, etc.) largely determine the characteristics of chemical and biological transformations of oil hydrocarbons [1].

Figure 3 shows the dynamics of the population of heterotrophic bacteria in uncontaminated and contaminated soil. Changes in the number of heterotrophic microorganisms in the control group are represented by sinusoidal curve with a periodicity of 15–25 days. The number of heterotrophic bacteria at the points of maximum does not exceed 7–10 × $10^6$ CFU/g of soil and at the points of minimum – 3–4 × $10^6$ CFU/g (Fig. 3, curve 1).

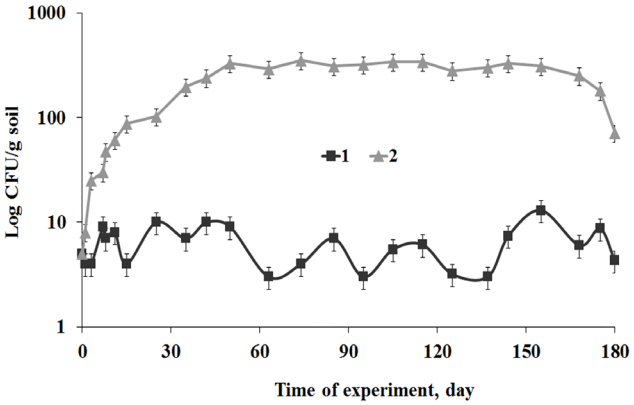


Fig. 3 Dynamics of the population of heterotrophic microorganisms in the reference soil (1) and in the soil contaminated with high viscosity bitumen (2).

In this experiment the dynamics of microflora population was represented by S-shaped curve without any adaptation period. The exponential growth of microorganisms lasted up to 50 days and then the population levelled off and lasted until 165$^{th}$ day. On the 70$^{th}$ day of cultivation, the number of microorganisms reached its maximum of 350 × $10^6$ CFU/g of soil. Although the population of microorganisms increased compared to that of reference, the biodiversity of microflora in the contaminated soil decreased. Hence, *Arthrobacter*, *Pseudomonas*, *Bacillus*, *Rhodococcus*, *Flavobacterium*, and *Micrococcus* species predominated.

It is known that the mineralization of oil hydrocarbons in the soil occurs with the participation of various enzymes. The enzymatic activity of soils is due not only to the quantitative content of microorganisms but also their diversity and physiological activity, thus the quantitative changes in the microbial cenosis of contaminated soil do not fully reflect the changes in its activity [2]. The most important and abundant enzymes of soil microorganisms are dehydrogenase, catalase, polyphenol oxidase, and peroxidase.

It is evident that an increase in activity for all enzymes under study begins right from the first day of the experiment. Figure 4a shows the dynamics of the formation of oxygen, which reflects the activity of catalase during an observation period.

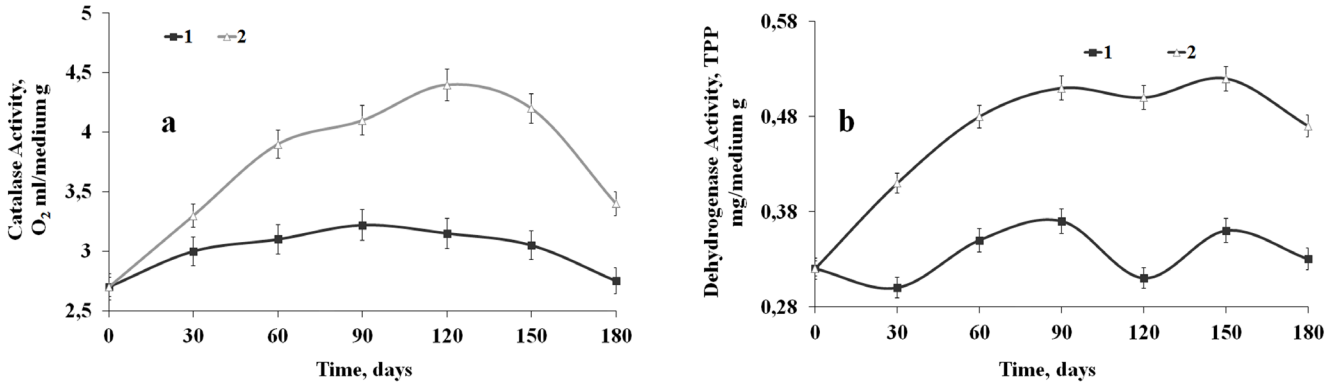


Fig. 4. Changes in catalase (a) and dehydrogenase (b) activities in uncontaminated (1) and contaminated soil (2).

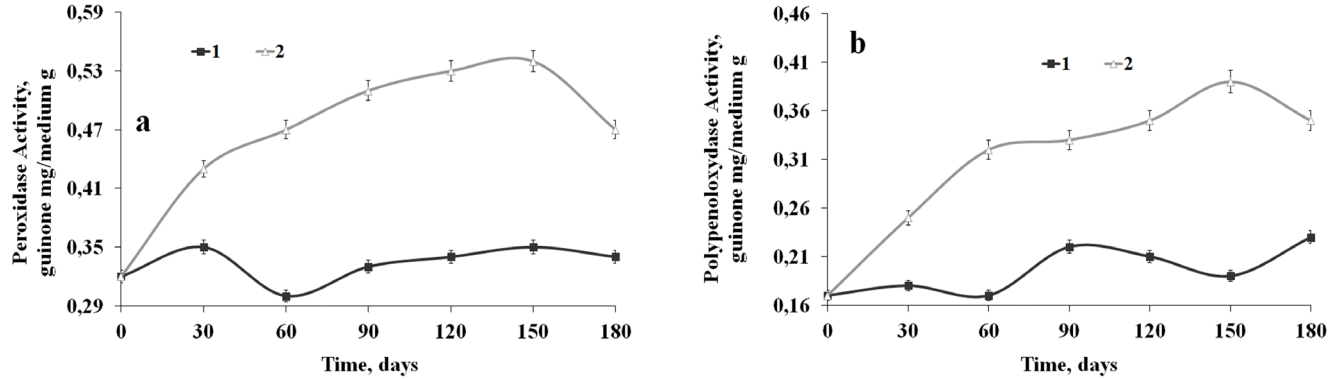


Fig. 5. Changes in peroxidase (a) and polyphenoloxydase activities (b) in uncontaminated (1) and contaminated soil (2).

Throughout the whole experiment its activity was still higher compared to that for the reference soil.

The maximum $O_2$ concentration reached 4.5 ml/g in contaminated soil, while in the reference sample (uncontaminated soil) the oxygen concentration was not higher than 3.1 ml/g throughout the whole experiment (Fig. 4a). Figure 4b shows the dynamics of the formation of triphenyl formazan (TPF) representing the activity of soil dehydrogenases. The activity of this enzyme increased during 150 days of the experiment, which indirectly indicates the biodegradation of *n*-alkanes and aliphatic chains in complex molecules. The amount of resulting TPF in the soil with bitumen increased to 0.53 mg/g, while in the reference version it was not higher than 0.37 mg/g (Fig. 4b). This indicates an increase in oxygenase activity of indigenous soil microflora and intensity of biodegradation of individual components contained in high viscosity bitumen.

In studies of biodegradation a considerable attention is paid to the investigation of soil phenol oxidases (peroxidases and polyphenol oxidases), which play an important role in the processes of humification and have a protective effect on the soil, decomposing various xenobiotics [2]. They are also involved in multi-stage processes of degradation and synthesis of organic aromatic compounds.

It is shown that the activity of these enzymes was growing to the end of experiment. This is indicative of the oxidation of polyaromatic hydrocarbons and structural framework of high-molecular compounds constructed by aromatic structures (Fig. 5).

The concentration of quinone exhibiting polyphenol and peroxidase activity increased in the soil with sludge at the end of the experiment to 0.54 mg/g and 0.4 mg/g, respectively (Fig. 5a, b). In the reference soil the activity of these enzymes was not higher than 0.34 mg/g and 0.22 mg/g, respectively. The results exceeded the control data by 1.6 to1.8 times.

The gravimetric analysis showed that the utilization of high viscosity bitumen in the model soil system over a period of 180 days was 25 g/kg, i.e. degradation was about 50%. With the degradation of OP, the products of incomplete oxidation of certain hydrocarbons are accumulated in soil. The products of partial oxidation have surface active properties which, in turn, promote the emulsionizing of hydrocarbons to droplets, increasing

their interfacial contact with the microbial cells and the degree of wetting of cell surface by hydrocarbons, which facilitates their diffusion across the cell membrane. The IR spectra of residual hydrocarbons extracted from the soil show additional absorption bands (a.b.) in regions of 3338, 1705, 1170, and 1032 $cm^{-1}$. The appearance of these absorption bands in these regions indicates the presence of various oxygen-containing compounds, which are intermediate products of hydrocarbon oxidation. This is consistent with classical pattern of HB oxidation involving the stages of formation of alcohols, ethers, ketones, saturated and aromatic carboxylic acids and further to $CO_2$ and $H_2O$.

The GC-MS analysis revealed the presence of a wide range of hydrocarbon compounds in the composition of initial and biodegraded bitumen (Table 2). With degradation of the bitumen under study the number of compounds of all groups decreases. The content of saturated hydrocarbons in a sample of biodegraded bitumen decreased by 63% against the content in sample of initial bitumen. Only solid high molecular paraffins remained unoxidized.

Steranes are representatives of tetracyclic saturated HCs, which are highly resistant to biooxidation, thus they are commonly used as geochemical biomarkers. However, as is shown in this paper, these compounds underwent rather intensive oxidation under aerobic conditions, hence their total destruction was 43%.

Aromatic hydrocarbons are more resistant to microbial oxidation than paraffins and naphthenes, but degradation of unalkylated fluoranthene and phenanthrene was 97%, while that of unalkylated pyrene 91%, respectively. The degree of degradation of naphthalenes was found to be 31%, while the degradation of their methyl- and dimethyl-substituted homologues was found to be 44–63%, respectively. Probably, the biodegradation of polar naphthalenes occurs much readily due to the oxidation at the point of side chain attachment. Biodegradation of alkyl phenanthrenes was 43–50%, while mono-aromatic compounds (*n*-alkyl benzenes) were utilized by 69%. The methyl-substituted fluoranthenes and pyrenes were biodegraded to a lesser extent – 18%. Total degradation of aromatic compounds was 62%.

Thus, not only the most accessible for the microbiological oxidation *n*-alkanes, but cyclanes and aromatic hydrocarbons were degraded with the microorganisms in the result of biochemical oxidation of bitumen (Table 2). This is probably due to the high initial diversity of soil microorganisms and multicomponent composition of substrate − hydrocarbons of different structure and soil humic substances were present in contamination.

Figure 6 shows the mass-fragmentograms of distribution of saturated, cyclic, mono- and biaromatic HC. In the initial sample of bitumen *n*-alkanes were identified in the homologous series from $C_{12}$ to $C_{36}$, while in a biodegraded sample – from $C_{13}$ to $C_{28}$ (m/z 71) with a significant decrease in the content of high molecular homologues. Homologous series of cyclohexanes (m/z 83) was reduced from $C_{13}$–$C_{30}$ in the initial sample to $C_{14}$–$C_{22}$ in that biodegraded.

Homologous series of *n*-alkyl benzenes (m/z 91) was $C_{12}$–$C_{33}$ in the initial sample and $C_{13}$–$C_{22}$ in that biodegraded (Fig. 6). Among naphthalenes, trimethyl-substituted homologues were the most degraded (m/z 170).

**Table 2**
GC-MS analysis of the initial and oxidized bitumen from the Bayan-Erkhet deposit

| Hydrocarbons | Initial sample, mcg/g | Oxidized sample, mcg/g | Biodegradation, % |
|---|---|---|---|
| Saturated hydrocarbons | | | |
| Alkanes | 2370.4 | 1001.6 | 58 |
| Cyclohexanes | 683.3 | 162.3 | 76 |
| Sesquiterpenes | 340.9 | 85.4 | 75 |
| Secohopanes | 254.9 | 104.7 | 59 |
| Total | 3649.5 | 1354.0 | 63 |
| Naphtenoarenes | | | |
| Monoaromatic sterenes | 135.2 | 76.1 | 44 |
| Triaromatic sterenes | 21.4 | 12.4 | 42 |
| Total | 156.6 | 88.5 | 43 |
| Aromatic hydrocarbons | | | |
| *n*-Alkylbenzenes | 16.4 | 5.1 | 69 |
| Naphtalenes | 1.2 | 0.8 | 31 |
| Methylnaphtalenes | 27.0 | 10.0 | 63 |
| Dimethylnaphtalenes | 72.5 | 40.3 | 44 |
| Trimethylnaphtalenes | 112.5 | 23.2 | 79 |
| Phenantrene | 52.9 | 1.7 | 97 |
| Methyl-phenantrenes | 19.3 | 11.1 | 43 |
| Dimethyl-phenantrenes | 41.5 | 20.6 | 50 |
| Fluoranthene | 20.9 | 0.5 | 97 |
| Pyrene | 12.0 | 1.1 | 91 |
| Methyl-fluoranthenes and pyrenes | 4.1 | 3.4 | 18 |
| Total | 360.9 | 223.1 | 62 |

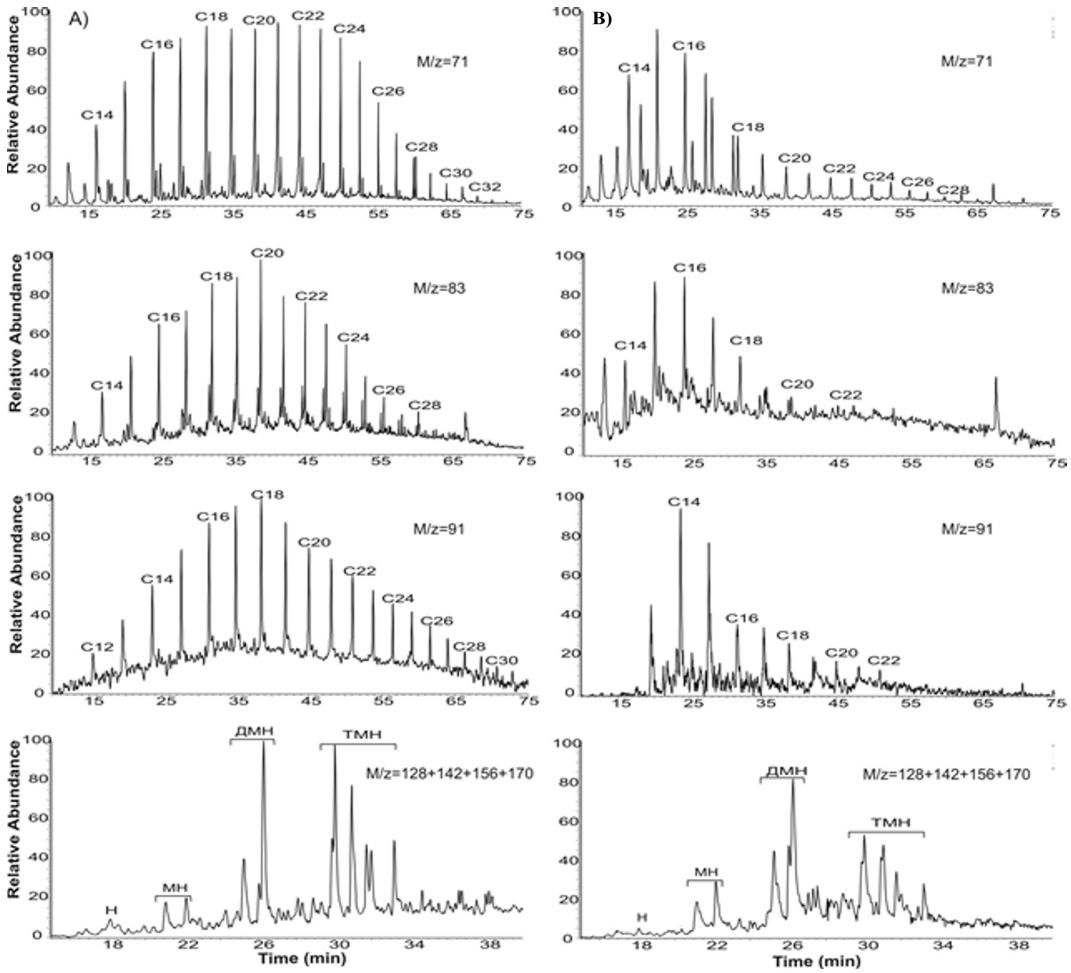


Fig. 6. Mass-fragmentograms of distribution of *n*-alkanes (m/z = 71), cyclohexanes (m/z = 83), *n*-alkyl benzenes (m/z = 91), naphthalene (m/z = 128) and its methyl-, dimethyl and trimethyl-substituted homologues (m/z = 142, 156, and 170) in the initial oil sample (A) and in the biodegraded sample (B).

Detailed analysis of biodegradation of high molecular heteroorganic compounds (HMHOC) showed that microbial oxidation results in the profound changes in the structural-group characteristics of resins and asphaltenes. The material composition of bitumen significantly changed in the course of its biodegradation. Structural-group characteristics of resins, asphaltenes of initial bitumen, and products of its biodegradation are presented in Table 3.

The total content of resins in the bitumen is reduced 3.10 times during biodegradation, while that of asphaltenes increases by factor of 2.96. Since a division into the resins and asphaltenes is conditional, i.e. it is determined by solubility of components in solvents, the increase in the asphaltene fraction can be explained by inclusion into their composition of more polar components, which are formed during biodegradation of resins and oils.

The relative fraction of resins in the initial bitumen decreased from 52.5 to 42.4 % wt in degradation products, while its total content in the soil decreased in the course of biodegradation from 26.25 to 8.48 g. Resins of initial bitumen had an average molecular weight of 580 a.m.u. and the average number of structural units per molecule $m_a = 1.02$. The percentage of atoms in aromatic structures was $f_a = 22.58\%$, while H/C ratio was 1.58. The mean molecule of resins in initial bitumen contained 42 carbon atoms, 9 of which belonged to aromatic ($C_a$) and 33 to saturated ($C_s$) and aliphatic ($C_p$) fragments.

The basis of structural units of resin molecules represented by two aromatic cycles ($R_a^* = 2.08$) includes two or three naphthenic cycles ($R_n^* = 2.91$) and alkyl substituents with the total number of carbon atoms in the chain up to 19–20 ($C_p^* = 19.56$). About 5 aliphatic carbon atoms in the structural unit ($R_p^* = 19.56$) are methyl substituents at the naphthene rings and terminal groups of aliphatic chains ($C_\gamma^* = 4.72$), which means that structural units of the mean molecule contain a number of low-branched alkyl substituents having a chain length from $C_3$ to $C_{14}$.

**Table 3**
Average structural parameters of molecules of resins and asphalthenes isolated from the initial and degraded bitumen

| Parameters | Resins | | Asphaltenes | |
|---|---|---|---|---|
| | initial | biodegraded | initial | biodegraded |
| Content, % wt | | | | |
| | 52.5 | 42.4 | 2.3 | 13.6 |
| Average molecular weight, a.m.u. | | | | |
| | 580 | 880 | 2130 | 2350 |
| Number of atoms in a mean molecule | | | | |
| C | 41.73 | 58.90 | 152.62 | 161.58 |
| H | 65.88 | 92.10 | 223.57 | 209.59 |
| N | 0.46 | 0.78 | 2.80 | 2.94 |
| S | 0.08 | 0.07 | 0.49 | 0.43 |
| O | 0.31 | 4.19 | 1.26 | 9.06 |
| H/C | 1.58 | 1.56 | 1.46 | 1.30 |
| Rings | | | | |
| $R_t$ | 5.09 | 6.17 | 21.58 | 29.64 |
| $R_a$ | 2.12 | 3.22 | 10.92 | 13.35 |
| $R_n$ | 2.97 | 2.95 | 10.66 | 16.30 |
| Aromaticity factor | | | | |
| $f_a$ | 22.58 | 22.04 | 26.94 | 32.01 |
| Parameters of average structural units | | | | |
| $R_t$* | 4.99 | 3.97 | 10.88 | 7.75 |
| $K_a$* | 2.08 | 2.07 | 5.50 | 3.49 |
| $K_n$* | 2.91 | 1.90 | 5.38 | 4.26 |
| C* | 40.89 | 37.90 | 76.96 | 42.27 |
| $C_p$* | 19.56 | 21.70 | 33.31 | 10.81 |
| $C_\alpha$* | 3.98 | 4.18 | 8.91 | 5.12 |
| $C_\gamma$* | 4.72 | 3.60 | 5.98 | 2.52 |

The average molecular weight of the resins increased 1.52-fold from 580 to 880 a.m.u., while the average number of carbon atoms in a molecule increased 1.41 times from 41.73 to 58.92. The percentage of atoms in aromatic structures ($f_a$) slightly decreased from 22.58% in initial resins to 22.04%. The H/C ratio changed from 1.58 in initial resins to 1.56 in resins after biodegradation. The average number of structural units in molecules of resins ($m_a$) increased from 1.02 to 1.55, while their average size decreased. The total number of rings per unit ($R_t$*) reduced from 4.99 to 3.97 mainly due to saturated cycles ($R_s$*), whose number decreased from 2.91 to 1.90. The number of aliphatic carbon atoms per unit ($C_p$*), on the contrary, increased from 19.56 to 21.70, while the number of methyl substituents at the naphthenic cycles and terminal groups of aliphatic chains decreased ($C_\gamma$*) from 4.72 in initial resins to 3.60 in resins after biodegradation. In addition, the number of sulfur atoms in initial mean molecules of resins decreased from 0.08 to 0.07, while the number of oxygen atoms increased 5.13 times from 0.31 to 4.19 atoms of oxygen per mean molecule.

The molecular weight of asphaltenes contained in the initial bitumen was 2.350 a.m.u., i.e. 4 times higher than that of resins. The mean molecule of asphaltenes from initial bitumen contained 153 carbon atoms and the average number of structural units per molecule was $m_a = 1.98$. The percentage of atoms in aromatic structures of asphaltene molecules was not substantially different from that of resin molecules ($f_a$ = 26.94% for asphaltenes) and H/C ratio was 1.46. The basis of the structural unit of asphaltene molecules was a naphthenic/aromatic core formed by five naphthenic ($R_s = 5.38$) and five aromatic rings ($R_a = 5.50$). The unit includes low-branched ($C_\gamma$* = 5.98) aliphatic structures ($C_P$* = 33.31).

Asphaltenes have undergone the most notable changes in the course of biodegradation, their relative fraction increased by 5.9 times from 2.3 (in the initial bitumen) to 13.6 wt.% (in biodegraded products), while their total content in the soil increased from 1.15 g to 3.40 g. Hovewer, the average molecular weight of asphaltenes was insignificantly changed from 2130 to 2350 a.m.u., while the average number of carbon atoms in the molecule changed from 152.62 to 161.58. The percentage of atoms in aromatic structures ($f_a$) increased from 26.94% (in the molecules of initial asphaltenes) to 32.01%. H/C ratio appreciably changed from 1.46 (in the initial asphaltenes) to 1.30 (after biodegradation). The number of oxygen atoms in a mean molecule increased from 1.26 to 9.06, while the proportion of other heteroatoms changed insignificantly. The average number of structural units ($m_a$) increased from 1.98 to 3.82, while the size of their cores decreased. The total number of rings per unit decreased ($R_o$*) from 10.88 (in the initial mean molecules of asphaltenes) to 7.75, that of saturated cycles ($R_s$*) from 5.38 to 4.26, and that of aromatic rings ($R_a$*) from 5.50 to 3.49. The number of aliphatic carbon atoms per unit ($C_p$*) decreased 3 times from 33.31 to 10.81.

Thus, the proportion of resins decreased in the course of degradation, while that of asphaltenes increased which is due to a more active degradation of oil and changes in the proportions of compo-

nents. In general, the process of biodegradation has resulted in an increased number of structural units ($m_a$) in a mean molecule of resins and asphaltenes (certainly, oxidized oil components were included into their composition). It was observed that the number of oxygen atoms in the mean molecules of resins and asphaltenes noteworthy increased, while the proportion of other heteroatoms changed insignificantly.

Results of structural-group analysis of high molecular heteroatomic components of bitumen are indicative of profound changes in the structure of these molecules in the course of degradation of the indigenous soil microflora.

In general, the degree of OP transformation and the rate of soil self-purification are not constant values, which depend on the composition and concentration of OP as well as on the properties of the soils themselves, the structure of landscape, climate conditions, and species composition of vegetation and soil microorganisms.

## 4. Conclusions

The data obtained suggest an adaptation of indigenous soil microflora to the relatively low concentration of an oil product under study. The number of microorganisms in the contaminated soil has increased by 1–1.5 order of magnitude, while the activity of investigated enzymes – by 1.5 to 2 times. As a result of intensive microbiological transformation of high-viscosity bitumen in the soil, after 180 days its mass loss was 50% of the initial pollution, all HC being oxidized by 58.6–100%.

Bio-oxidation of bitumen was accompanied by changes in its group and individual hydrocarbon compositions. Hence, the content of high-boiling fractions has been increased due to the residual accumulation of resins and asphaltenes. New oxygen-containing compounds of various structures have been also formed.

Asphaltenes and resins have also undergone qualitative and quantitative changes during biodegradation of bitumen. The total content of resins in the bitumen has been decreased 3.10 times during biodegradation (the total content in the soil has been decreased from 26.25 g to 8.48 g), while that of asphaltenes has been increased 2.96 times from 1.15 g to 3.40 g. Since the separation into resins and asphaltenes is conventional, i.e. it is determined by solubility of components in a solvent, an increase in the fraction of asphaltenes may be explained by inclusion into their composition of polar components, which are formed during biodegradation of resins and oils. These data have been confirmed by SGA, which has revealed an increase in the number of oxygen atoms in the mean molecules of resins and asphaltenes by 7–13 times. The number of structural units in molecules has been increased 1.5–1.9 times, while the number of carbon atoms in the structural units has been decreased. A decrease in paraffinic structures ($C_p$*) which were the most biodegradable is characteristic for asphaltene molecules. Their proportion has been decreased from 33.31 to 10.81 atoms per average structural unit. The resin molecules are characterized by pronounced changes in the relative proportion of atoms in naphthenic structures and a decrease in the number of cycles $R_s$* from 2.91 to 1.90.

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