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## Biodestructive Processes in Oil-Contaminated Clay Soil

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### Abstract

The destruction of organic matter in oil-contaminated clay soil by native microflora was studied under laboratory conditions. Peat and the solution of a composition containing a surfactant and a nitrogenous substrate, as well as their combination with phytoremediation, were used as stimulating additives. After biodegradation, the residual oil was extracted and examined by means of IR spectroscopy, gas-liquid chromatography (GLC) and chromatography-mass spectrometry. According to the data of IR spectroscopy, peat and a solution of the composition promote the utilisation of paraffins, which is accompanied by an increase in the relative content of aromatic and carbonyl-containing structures. The isoprenoid coefficient  $K_i$ , which reflects the degree of hydrocarbon biodegradation, was calculated from GLC spectra as the ratio of *iso*- and *n*-alkanes. It increases with the introduction of stimulating substrates and soil loosening. The maximal biodegradation (biodestruction) coefficient is observed for the sample of oil-contaminated clay soil containing the surfactant composition, which contributes to oil emulsification and accelerates biodegradation. Acyclic (*n*- and isoalkanes), alicyclic (cyclohexanes, drimanes, cheilanthanes, regular and rearranged steranes and hopanes), naphthenoaromatic (mono- and triaromatic steranes), and aromatic (mono-, bi-, tri-, tetra- and pentacyclic) hydrocarbons were detected in the soil organic compound (SOC) by means of chromatography-mass spectrometry. As a result of biodestruction stimulated by the introduction of peat and the nutrient substrate, the concentration of hydrocarbons decreased significantly: *n*-alkanes – by 74–82 %, aromatic compounds – by 97–99 %, cyclohexanes – by 78–92 %, oil steranes and hopanes – by 88–97 %. The introduction of the composition containing a nitrogenous substrate and a surfactant into the soil leads to a significant decrease in the content of  $C_{12}$ – $C_{34}$  alkanes to the level of background soil. The introduction of peat additive veils (shades) the results of alkane oxidation due to their presence in peat itself. The use of stimulating substrates leads to a decrease in the content of aromatic hydrocarbons during biodegradation: monocyclic by 82–89 %, bicyclic – by 55–81 %, tricyclic – by 74–89 %, tetracyclic – by 54–77 %.

**Keywords:** oil-contaminated clay soil, microflora, hydrocarbons, biodegradation, stimulating substrates

### INTRODUCTION

Intense management of the natural resources of the West Siberian region under the conditions of the low stability of geosystems to various kinds of technogenic actions predetermines the existence of an entire complex of ecological problems. One of these problems is the contamination of clay soils. Clay soils are characterized by increased density, low oxygen concentration and insignificant bioproductive potential, which inhibits the vitality of microflora, an essential component of

the soil formation process [1]. Investigation of microbiological destruction processes is urgent for oil mining regions in West Siberia with widespread poorly drained clay soils with increased density and low fertility. The most widespread hydrocarbon-oxidizing microorganisms of polluted soils include the representatives of *Bacillus*, *Pseudomonas*, *Arthrobacter*, *Nocardia*, *Rhodococcus* genera and some others [2, 3].

The goal of the present work is investigation of biodestructive changes in the composition of hydrocarbons in oil-contaminated clay soil under

the action of substrates stimulating the activity of aboriginal microflora.

## EXPERIMENTAL

The objects of the physicochemical and microbiological investigation were soil samples collected in 2018, two years after the accident at the pipeline in the Tomsk Region, which resulted in the emergence of oil on the surface. The samples were taken in the centre of the oil spillage area. The background soil sample (BS) was taken at a distance of 100 m from the boundary of the spillage area. After transportation to the laboratory, the sample of initial oil-polluted clay soil (OCS<sub>i</sub>) was stored in a freezer.

The number of soil microflora organisms was determined using the classical method by inoculation on the meat-peptonic agar medium (MPA). Modelling of oil biodestruction in soil was carried out under laboratory conditions at room temperature for 22 days. Microflora isolated from OCS<sub>i</sub> and propagated on MPA in Petri dishes was used for biodestruction. To decrease soil density and to increase the enzymatic activity of aboriginal microflora, valley peat in the concentration of 10 % of soil mass and a 2.5 % solution of the composition containing surfactants and nitrous substrate (calculated for the mass of air-dry soil) were introduced into contaminated soil. Nitrous components of the composition served as an additional source of nutrition and energy for soil microflora. The experiment was carried out in several versions: the reference sample without additives (OCS<sub>r</sub>), with the addition of the composition (OCS<sub>i</sub> + C), peat (OCS<sub>i</sub> + P), composition and peat (OCS<sub>i</sub> + C + P), composition and peat in combination with phytoremediation (OCS<sub>i</sub> + C + P + Ph). Phytoremediation included seeding perennial grass. The samples under investigation were wetted and thoroughly mixed every 24 days to conserve water balance, optimal biodestruction and availability of oxygen for biological oxidation.

The concentration of initial pollution in sampled soil and after biodestruction for 3 weeks was determined by means of gravimetry. Oil extraction was carried out in Soxhlet's apparatus with a 7 % methanol solution in chloroform as an extracting agent. The extracts were dried, evaporated in a rotary evaporator and weighted after the constant mass was achieved. Thus

obtained oil extracts were analyzed by means of IR spectroscopy using a Nicolet 5700 Fourier transform IR spectrometer with the Raman unit (Thermo Electron, USA). The optical density of the functional groups of oil was used to calculate spectral coefficients [4]:  $C_{ar} = D_{1600}/D_{1465}$  is the aromaticity coefficient characterizing the relation between aromatic and paraffin structures;  $C_b = D_{1380}/D_{1465}$  is branching coefficient depicting the relative content of CH<sub>3</sub> groups;  $C_{ox} = D_{1730}/D_{1465}$  is oxidation coefficient characterizing the relative content of C=O groups of carboxylic acids, esters, ketones, aldehydes.

Hydrocarbons (HC) were isolated from extracts by means of liquid adsorption chromatography with a glass column filled with aluminium oxide of the IV degree of activity according to the Brockmann activity scale. The eluent was *n*-hexane. The composition of *n*- and isoalkanes was determined with a Khromos GKh-1000 gas-liquid chromatograph (Russia).

The entire set of saturated and aromatic HC was analyzed with the help of a quadrupole gas chromatograph-mass spectrometer Trace-DSQ (Thermo Scientific, Germany) in the full scan mode within mass range 50–550 Da. Chromatographic separation of the components was performed with a quartz capillary column of Agilent company, with the inner diameter of 0.25 mm, 30 m long, with DB-5MS 0.25 mm thick as the immobile phase; helium was used as a carrier gas. The samples under investigation were heated stepwise in helium flow according to temperature programme: at the first step with the isotherm at  $T = 80$  °C (2 min), then with the rate of 3 °C/min to 300 °C followed by exposure for 25 min at this temperature. The characteristics of the mass spectrometer were: ionization by electron impact; the energy of ionizing electrons: 70 eV; the temperature of ionization chamber and the interface: 270 °C.

The data of gas chromatography-mass spectrometry were obtained and processed using Xcalibur software. Identification of the peaks of components was based on mass spectral data including comparison with the mass spectral database of the National Institute of Standards NIST-05, as well as retention times of the components. Calculation of component content was carried out relying on the areas of the corresponding peaks in chromatograms using an internal standard (deuteroacenaphthene C<sub>12</sub>D<sub>10</sub>).

## RESULTS AND DISCUSSION

Initial oil content in the samples of polluted soil was 3 g/kg, oil content in BS did not exceed 0.2 g/kg, oil content in soil subjected to different versions of bioremediation was 0.6–1.0 g/kg.

The soil biocenosis was represented by *Bacillus*, *Pseudomonas*, *Rhodococcus*, *Arthrobacter* genera, by yeast and fungal cultures. The first several days were characterized by the low number of microorganisms in the soil microflora (Fig. 1), which is evidence of adaptation to the new conditions. Some microorganisms died, those surviving were mainly microorganisms of hydrocarbon-oxidizing group; they used oil HC as a source of nutrition and energy. The number of microflora was distributed non-uniformly, with more active accumulation of biomass at the 11th–22nd days of the experiment. The initial number of microflora in  $OCS_i$  did not exceed 1 thousand colony-forming units (CFU) in 1 g of soil. The number of microflora in  $OCS_k$  increased to 56 thousand CFU during biodestruction, and in the experiments involving the application of composition and with the joint application of the the composition and peat – to 170 thousand and 220 thousand CFU/g, respectively. The maximal number of aboriginal microflora in the experiment with the application of the stimulating solution, peat and phytoremediation was 453 thousand CFU/g of soil.

The IR spectra of soil extracts were used to calculate the coefficients of aromaticity ( $C_{ar}$ ), branching ( $C_b$ ) and oxidation degree ( $C_{ox}$ ), which

characterize the enzymatic activity of microflora in different versions of biodestruction (Table 1).

The background soil is characterized by the maximal values of  $C_{ar}$ ,  $C_b$ ,  $C_{ox}$  coefficients. Soil pollution with oil causes a decrease of these coefficients to minimal values, which is connected with the appearance of absorption bands related to methyl ( $1380\text{ cm}^{-1}$ ) and methylene ( $1465\text{ cm}^{-1}$ ) groups of oil HC in the IR spectra. The addition of peat and the solution of composition causes an increase in the number and activity of soil microflora, especially in the processes of paraffin utilization, and is accompanied by an increase in spectral coefficients. With phytoremediation in combination with the addition of peat and composition, spectral coefficients including  $C_{ox}$  increased to the maximal extent.

The addition of peat to heavy clay soil promotes its loosening and a decrease in density. Organic compounds that are present in soil may serve as an additional nutrition substrate for soil biocenosis [5–7]. Peat, which is composed of digested and pressed residues of moss, is used for radical and rapid improvement of soil properties. Its advantage is ecological safety and the absence of any aggressive features.

The results of GLC were used to calculate the isoprenoid coefficient:  $K_i = (Pr + Ph)/(C_{17} + C_{18})$ . It determines the activity of alkane biodestruction [8] (Table 2). This coefficient is the ratio of the sum of isoprenoid HC pristane (Pr) and phytane (Ph) to the sum of *n*-heptadecane ( $C_{17}$ ) and *n*-octadecane ( $C_{18}$ ). The larger is  $K_i$ , the more profound are biodestructive changes in the com-

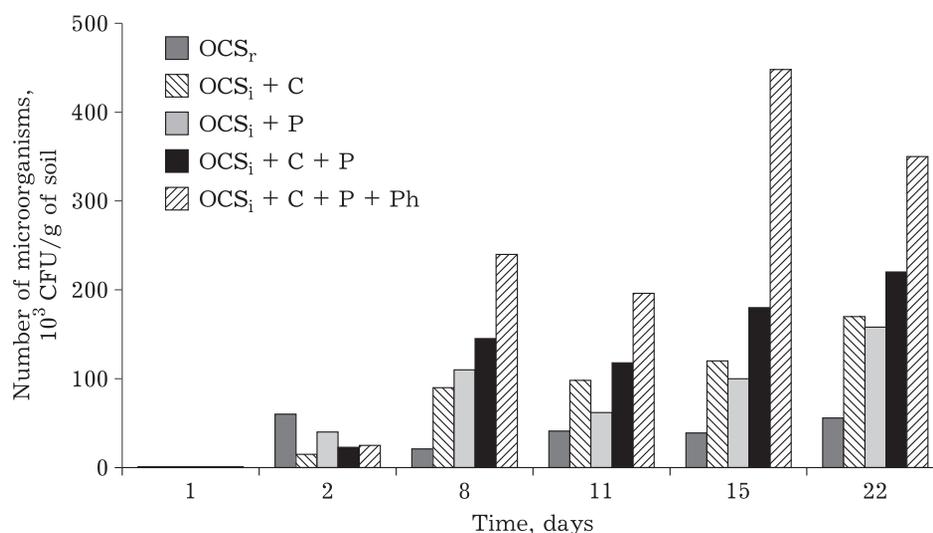


Fig. 1. Dynamics of the number of microorganisms during oil destruction.

TABLE 1

Spectral coefficients as indicators of the oxidative activity of soil microorganisms

Spectral coefficient	Soil sample						
	BS	OCS <sub>i</sub>	OCS <sub>r</sub>	OCS <sub>i</sub> + C	OCS <sub>i</sub> + P	OCS <sub>i</sub> + C + P	OCS <sub>i</sub> + C + P + Ph
C <sub>ar</sub>	0.30	0.11	0.12	0.16	0.15	0.14	0.19
C <sub>b</sub>	0.81	0.53	0.58	0.65	0.60	0.58	0.66
C <sub>ox</sub>	1.70	0.34	0.41	0.72	0.88	0.69	0.89

Note. C<sub>ar</sub> = D<sub>1600</sub>/D<sub>1465</sub>; C<sub>b</sub> = D<sub>1380</sub>/D<sub>1465</sub>; C<sub>ox</sub> = D<sub>1730</sub>/D<sub>1465</sub>, where D<sub>1600</sub> is optical density at 1600 cm<sup>-1</sup> characterizing the content of aromatic structures; D<sub>1465</sub> is optical density at 1465 cm<sup>-1</sup> characterizing the content of methylene groups; D<sub>1380</sub> is optical density at 1380 cm<sup>-1</sup> characterizing the content of methyl groups; D<sub>1730</sub> is optical density at 1730 cm<sup>-1</sup> characterizing the content of carbonyl groups.

position of saturated HC. The value of Pr/Ph parameter changes insignificantly during biodestruction (see Table 2), which is in agreement with the data reported in [9] concerning the conservation of this facial-genetic relation and allows bring the residual oil present in soil into correlation with possible pollution sources.

Oil-polluted soil is subjected to physicochemical and microbiological action. The destruction of separate oil components proceeds non-uniformly. The most active destruction is that of *n*-alkanes. Their carbon atoms are connected with each other through single bonds and do not require substantial energy consumption for enzymatic microbiological oxidation [10]. After the introduction of stimulating substrates and loosening, the activity of soil biocenosis increases. This is evidenced by an increase in K<sub>i</sub>. The maximal biodegradation coefficient is observed for the OCS<sub>i</sub> + C sample, in which the composition was added as the substrate stimulating the oxidation activity of soil microflora. Surfactants as a component of the solution of the composition promote oil emulsification, which accelerates biodestruction. The efficiency of biodestruction with the use of phytoremediation is lower, which is connected with the insufficient development of the rhizosphere – the root system of plants and soil around the roots. The rhizosphere is characterized by the high biological activity of microorganisms stimulated by

nutritional secretions of the root system [11]. A longer vegetation period (30–60 days, depending on soil type) is necessary in the clay soil for the development of the rhizosphere.

The composition of the organic matter (OM) was analyzed by means of gas chromatography-mass spectrometry. The list of identified compounds includes acyclic (*n*- and isoalkanes), alicyclic (cyclohexanes, drimanes, cheilanthanes, regular and rearranged steranes and hopanes), naphthoaromatic (mono- and triaromatic steranes) and aromatic (mono-, bi-, tri-, tetra- and pentacyclic) HC (Fig. 2).

The samples subjected to biodegradation exhibited a sharp decrease in the content of all HC classes in comparison with OCS<sub>i</sub>. This is true also for the reference soil sample in which the substrates were not added but the destruction was 34 %. The content of *n*-alkanes decreased by 33 %, aromatic compounds by 72 %, cyclohexanes by 44 %, oil steranes by 25 %.

Bioremediation in the reference experiment was promoted by regular loosening and wetting of soil, as well as the access of sunlight, which created favourable conditions for the vitality of soil microflora. Mixing may have increased the adsorption of microorganisms on the particles of clay soil, which results in the enhancement of their enzymatic activity.

TABLE 2

Geochemical parameters of *n*-alkanes in soils

Destruction coefficient	Soil sample						
	BS	OCS <sub>i</sub>	OCS <sub>r</sub>	OCS <sub>i</sub> + C	OCS <sub>i</sub> + P	OCS <sub>i</sub> + C + P	OCS <sub>i</sub> + C + P + Ph
K <sub>i</sub>	0.68	1.32	1.61	2.33	1.94	2.18	1.89
Pr/Ph	0.73	0.97	0.93	0.78	0.78	0.81	0.86

Note. K<sub>i</sub> is isoprenoid coefficient; Pr/Ph is pristine to phytane ratio.

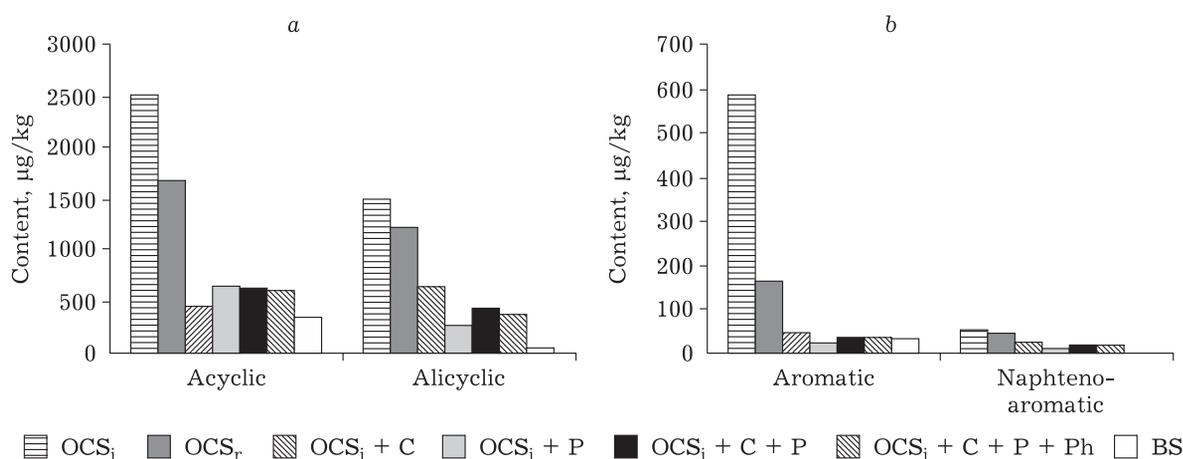


Fig. 2. Content of acyclic, alicyclic (a) and aromatic, naphthenoaromatic (b) hydrocarbons in the studied soil samples.

Oil biodestruction in the studied samples with the introduction of stimulating substrates was more intense than in the reference version by 62–69 %. The concentrations of all classes of compounds decreased substantially: *n*-alkanes by 61–73 %, aromatic compounds by 72–86 %, cyclohexanes by 61–86 %, oil steranes and hopanes by 42–76 %.

The features of the molecular mass distribution (MMD) of *n*-alkanes in soil extracts allow us to state that biodegradation proceeded intensely (Fig. 3), the content of individual *n*-alkanes C<sub>12</sub>–C<sub>24</sub> decreased sharply. The minimal content of alkanes with the maximum of distribution falling on odd homologues C<sub>25</sub>–C<sub>31</sub> was detected in the OM of the BS sample. In the OCS<sub>i</sub> sample, the content of even and odd homologues is the same, and the maximum falls on C<sub>15</sub>–C<sub>19</sub> alkanes. A similar (identical) distribution of alkanes is observed in the OCS<sub>r</sub> sample but their concentration decreases by a factor of 1.5 in comparison with the initial one. The application of stimulating substrates causes an increase in the activity of microorganisms, and the MMD of alkanes changes. Their concentration decreases by a factor of 2.6–3.7 in comparison with the reference version. The use of peat as a sorbent and stimulator of soil biocenosis causes a decrease in the content of C<sub>12</sub>–C<sub>22</sub> alkanes and an increase in the fraction of natural high-molecular alkanes C<sub>23</sub>–C<sub>33</sub>, which are present in peat and BS. The introduction of the composition into soil causes an increase in the oxidative activity of soil microflora, which leads to a substantial decrease in the content of the entire set of alkanes C<sub>12</sub>–C<sub>34</sub> to a level close to BS (see Fig. 3).

The processes involved in biodestruction of aromatic compounds were studied in a number of works [12–18]. The ability of mycobacteria to oxidize polycycloaromatic hydrocarbons (PAH) was demonstrated. The list of PAH includes phenanthrene, pyrene, fluoranthene, benz[a]pyrene. As a result of bioremediation of polluted water with the *Bacillus amyloliquefaciens* strain, polynuclear aromatic components were destroyed completely after incubation for 30 h. It was demonstrated that aromatic HC are more stable to microbiological oxidation than alkanes and naphthenes. The major factor of PAH degradation in the environment, especially in water and in air, is photolysis initiated by the ultraviolet radiation. Monocyclic aromatic compounds are the first to be oxidized, and alkylated homologues exhibit more rapid destruction than non-substituted ones because of the presence of side alkyl chains in the former compounds [19]. The oxidizing activity of microorganisms decreases with an increase in the number of condensed rings. With the use of a consortium of microorganisms, the degree of biodestruction of organic pollutants is higher by 5–10 % than in the case of their separate introduction.

The list of aromatic compounds detected in initial oil-polluted soil includes mono-, bi-, tri-, tetra- and pentacyclic HC (Table 3), which form a sequence according to their concentrations: tri- > bi- > mono- > tetra- > pentacyclic. The concentration of monocyclic *n*-alkylbenzenes is lower than the concentration of methylalkylbenzenes. Bi- and tricyclic HC form the following sequence according to their concentrations: non-substituted structure < methyl- < dimethyl- < trimethyl- < tetramethyl-homologues. The compounds de-

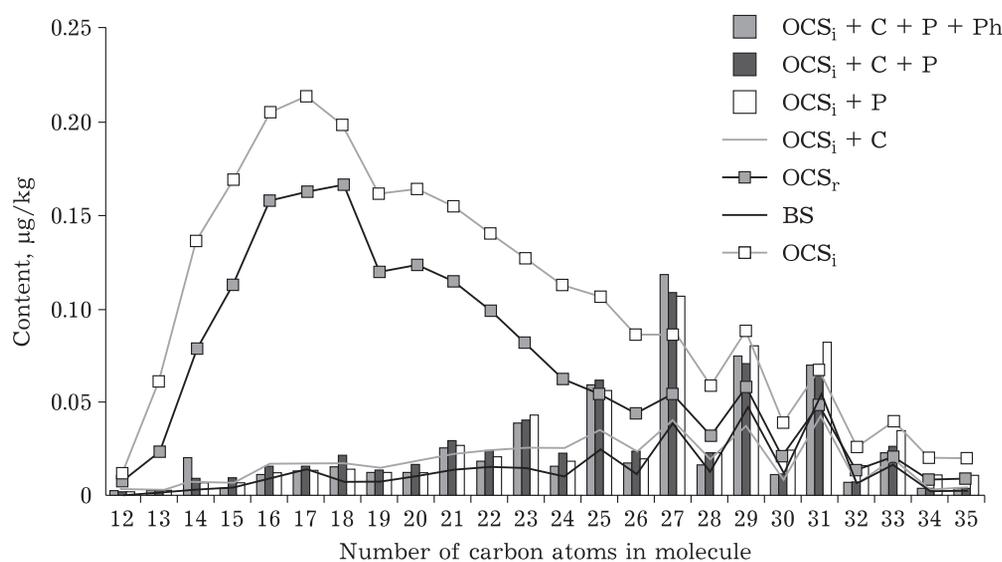


Fig. 3. Distribution of  $n$ -alkanes in the studied soil samples.

tected among tetracyclic HC are pyrene, fluoranthene and their methyl-substituted homologues, as well as benz[a]anthracene and its methyl- and dimethyl-derivatives. Among pentacyclic HC, benzfluoranthenes and benzpyrene were detected.

In the background soil, the content of aromatic HC is almost 20 times lower than in initial oil-polluted soil, but all groups of aromatic HC were also detected in the former (see Table 3), which is characteristic of the OM of soil subjected to technogenic pollution.

As a result of the experiment without the application of additional substrates, the content of  $n$ -alkyl- and methylalkylbenzenes decreased by 42 and 36 %, respectively, in comparison with the  $OCS_i$  sample. The concentration of unsubstituted naphthalene did not change, and the concentrations of its methyl-substituted homologues decreased by 35 %. The content of di-, tri- and tetramethyl-derivatives of naphthalene decreased by 79, 94 and 92 %, respectively, which is most probably connected with their oxidation due to side chains [19]. As a result, unsubstituted naphthalene appears, and is it likely to be summed up with partially degraded naphthalene which was present initially in the starting sample. Phenanthrenes are oxidized in a similar manner. Phenanthrene concentration in the reference soil decreased by 34 % in comparison with initial soil, while methyl-, dimethyl- and trimethyl-substituted homologues exhibited a decrease by 90, 81 and 61 %, respectively. The concentrations of pyrene and fluoranthene in the reference sample

decreased by 59 and 80 %, respectively, in comparison with the initial soil sample, the content of the methyl derivatives of these compounds decreased by 72 %. Biodestruction of benz[a]anthracene in the  $OCS_r$  sample was 12 % with respect to  $OCS_i$ , biodestruction of its methyl- and dimethyl-derivatives was 48–49 %, benzfluoranthenes and benzpyrene – 58 % (see Table 3).

It is known that the oxidation of pyrene using *Rhodococcus erythropolis* strain at 15 °C was 89 and 92 % after 28 and 48 days, respectively [20]. Active decomposer microorganisms of the *Pseudomonas* genus introduced into model soil systems provide complete utilization of naphthalene and phenanthrene in the concentrations of 2.5 and 1 mg/g of grey wood soil within 3–4 days, respectively [21, 22]. It was demonstrated that after 30 days of oil incubation in contact with aboriginal microflora, the naphthalene content in oil decreased by 82 % as average, phenanthrene content decreased by 57 %, fluoranthene and pyrene by 64 %, dibenzanthracene and chrysene by 45 % [23]. It was accepted previously that PAH compounds like indenopyrene and benz[k]fluoranthene are oxidized only by fungal cultures, but the data on their bacterial degradation under the action of microbial associations were presented in [24].

As a result of the application of stimulating substrates, biodestruction processes were more intense. In comparison with the reference sample, the concentration of  $n$ -alkylbenzenes in aromatic HC decreased by 87–91 %, methylalkylbenzenes by 79–88 %. Bicyclic HC were oxidized by 55–

TABLE 3

Effect of stimulating substrates and phytoremediation on the degree of biodestruction of aromatic, naphthenoaromatic and alicyclic compounds

Compounds	Content, µg/g of soil						
	Soil sample						
	OCS <sub>i</sub>	OCS <sub>r</sub>	OCS <sub>i</sub> + C	OCS <sub>i</sub> + P	OCS <sub>i</sub> + C + P	OCS <sub>i</sub> + C + P + Ph	BS
<b>Aromatic hydrocarbons</b>							
Monocyclic							
<i>n</i> -Alkylbenzenes	19.0	11.0	1.1	1.0	1.4	1.2	0.8
Methylalkylbenzenes	36.6	23.3	4.4	2.7	4.8	2.7	0.7
Bicyclic							
Naphthalene	0.4	0.4	0.2	0.03	0.1	0.2	0.1
Methylnaphthalenes	2.0	1.3	0.9	0.4	0.7	1.2	0.5
Dimethylnaphthalenes	11.6	2.4	1.1	0.4	0.8	1.2	1.6
Trimethylnaphthalenes	34.2	1.9	0.7	0.5	0.5	0.8	2.3
Tetramethylnaphthalenes	94.4	7.2	1.9	1.2	2.2	2.5	0.7
Tricyclic							
Phenanthrene	3.5	2.3	2.1	1.2	1.7	2.5	2.4
Methylphenanthrenes	42.6	4.4	3.3	0.8	1.2	2.3	2.7
Dimethylphenanthrenes	145.4	27.6	7.6	1.1	2.5	2.9	2.3
Trimethylphenanthrenes	148.6	57.5	11.0	7.3	7.5	8.0	1.1
Tetracyclic							
Pyrene	5.8	2.4	0.8	0.7	1.1	1.1	2.7
Fluoranthene	4.0	0.8	0.4	0.5	0.6	0.5	3.5
Methylfluoranthenes, methylpyrenes	13.4	3.7	1.4	1.4	2.0	1.8	7.4
Benz[a]anthracene	3.4	3.0	1.6	0.6	1.6	1.0	0.4
Methylbenz[a]anthracenes	11.2	5.8	2.2	1.0	2.3	2.2	0.4
Dimethylbenz[a]anthracenes	14.3	7.3	3.4	1.2	3.0	3.2	0.2
Pentacyclic							
Benzfluoranthenes	2.9	0.9	0.6	0.6	0.2	0.2	2.3
Benzpyrene	1.5	0.8	1.1	0.4	0.3	0.3	0.6
<b>Naphthenoaromatic hydrocarbons</b>							
MAS	13.8	13.1	7.4	3.1	5.0	4.7	0
TAS	37.2	31.7	16.6	7.2	12.3	12.1	0
<b>Alicyclic hydrocarbons</b>							
Cyclohexanes	210.5	118.0	46.2	15.8	27.6	21.0	8.3
Drimanes	342.7	138.7	28.8	16.9	25.2	15.7	0
Cheilanthanes	43.7	27.8	19.2	8.2	17.7	15.4	1.5
Regular steranes	195.1	152.4	95.9	46.2	61.7	54.9	4.8
Diasteranes	263.5	190.6	93.0	35.7	58.7	56.4	5.8
Hopanes	477.0	595.5	368.5	141.5	240.3	209.4	14.8

Note. MAS means monoaromatic steranes, TAS means triaromatic steranes.

81 %, and the maximal destruction of non-substituted naphthalene and its methyl-, di-, tri- and tetramethylsubstituted homologues was detected in the experiment with peat. The oxidation of tricyclic HC was 74–89 %; the destruction of phenanthrene and its homologues was most successful with the use of valley peat. Pyrene, fluoranthene

and their methyl-substituted homologues were utilized by 42–60 %, benz[a]anthracenes by 54–82 %. Benzfluoranthenes and benzpyrenes were detected only in trace amounts after the addition of stimulating substrates.

The minimal degree of destruction of naphthenoaromatic HC was detected with the compo-

sition, while the maximal one was observed in the case when peat was added to oil-polluted soil. Medium results were obtained for peat combination with the composition and additional introduction of plants into the substrate stimulating the development of microflora. The degree of elimination of saturated bicyclic HC (drimanes) is only weakly dependent on experimental conditions and is equal to 79–89 %. Biodegradation of saturated tri- (cheilanthanes), tetra- (steranes) and penta- (hopanes) cyclic HC is stimulated to a higher extent (71–81 %) by the introduction of peat into the soil. The addition of the composition and plants to peat causes a decrease in the extent of destruction to 36–70 %. With the use of the composition, the degree of destruction of alicyclic tri-, tetra- and pentacyclic HC decreases to 31–51 % and reaches 51 % only in the case of diasteranes.

## CONCLUSION

It is demonstrated that the introduction of nutrition substrates and peat into low-fertile oil-polluted clay soil stimulates the number and oxidative activity of soil biocenosis. Investigation of the dynamics of the content of oil hydrocarbons in soil revealed a low degree of biodestruction (0–8 %) during the first 3–10 days of cultivation, which is explained by the short period of remediation, small number and weak enzymatic activity of microorganisms in clay soil. After cultivation for 22 days using stimulating additives and phytoremediation, oil biodestruction reached 67–80 %, while in the reference version it was 33 %.

Destructive changes affect all groups of oil hydrocarbons; the maximal biodestruction was detected for mono-, bi-, and tricyclic aromatic hydrocarbons. A decrease in the content of  $C_{12}$ – $C_{34}$  *n*-alkanes to the level characteristic of background soil is observed after the introduction of the composition consisting of nitrogenous substrate and surfactants. The addition of peat to clay soil, causing its loosening and a decrease in density, promotes the destruction of mainly large polycyclic molecules by the microbes. The application of phytoremediation leads to succession changes in the soil biocenosis due to the root secretions from plants, which is promising in the case of the combined use of nutrition substrates for the development of biotechnology for recultivation of oil-polluted soils. These results demonstrate the possibility to use additives that promote the destruction of separate oil components, for

the optimization of bioremediation of soils polluted with oil and petroleum products with different chemical composition.

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## REFERENCES

- 1 Moskovchenko D. V., Ecogeochemistry of Oil and Gas Producing Regions of West Siberia [in Russian], Novosibirsk: Geo, 2013. 259 p.
- 2 Kulicheva N. N., Lysak L. V., Kozhevnikov P. A., Zvyagintsev D. G., Bacterial complexes in the soil, fallen leaves, and phylloplane of an urban ecosystem, *Microbiology (Mikrobiologiya)*, 1996, Vol. 65, No. 3, P. 366–370.
- 3 Milekhina E. I., Borzenkov I. A., Zvyagintseva I. S., Kostrikin N. A., Belyaev S. S., Characterization of a hydrocarbon-oxidizing *Rhodococcus erythropolis* strain isolated from an oil field, *Microbiology (Mikrobiologiya)*, 1998, Vol. 67, No. 3, P. 271–274.
- 4 Kalugina N. P., Glebovskaya E. A., Babaev F. R., Infrared Spectroscopy of Oil and Condensates [in Russian], Novosibirsk: Nauka, 1990, 271 p.
- 5 Svarovskaya L. I., Serebrennikova O. V., Duchko M. A., Strel'nikova E. B., Russkikh I. V., Changes in the composition of the bituminous components of valley peat under stimulated microbial action, *Solid Fuel Chemistry*, 2017, Vol. 51, No. 2, P. 67–77.
- 6 Serebrennikova O. V., Duchko M. A., Koronatova N. G., Strel'nikova E. B., Concentrations and composition of sphagnum peat lipids depending on temperature in the climatic zones of Western Siberia, *Solid Fuel Chemistry*, 2018, Vol. 52, No. 1, P. 36–43.
- 7 Serebrennikova O. V., Strel'nikova E. B., Russkikh I. V., Duchko M. A., Composition of the lipids of the sphagnum and cotton grass peats in the forest steppe and southern and middle taigas of West Siberia, *Solid Fuel Chemistry*, 2017, Vol. 51, No. 4, P. 195–204.
- 8 Goncharov I. V., Geochemistry of Oil in West Siberia [in Russian], Moscow: Nedra, 1987. 179 p.
- 9 Kashirtsev V. A., Indicators of the Settings for the Formation of Hydrocarbon Deposits [in Russian], Moscow: Nauka, 1988. 94 p.
- 10 Oborin A. A., Khmurchik V. T., Ilarionov S. A., Makarova M. Yu., Nazarov A. V., Oil-Polluted Biocenoses [in Russian], Perm: UrB RAS; Perm State Univ.; Perm State Techn. Univ., 2008. 511 p.
- 11 Emnova E. E., Merenyuk G. V., Slanina V. A., Tataru Yu. M., Rusnak T. N., Species composition of saprophytic fluorescent pseudomonadas in the rhizoplane of different species of agricultural plants [in Russian], *Mikrobiologiya*, 1995, Vol. 64, No. 6, P. 820–826.
- 12 Ivanova I. A., Ibragimov R. K., Ibragimova D. A., Petrov S. M., A review of microbiological means against the deposition of high-molecular components of oil [in Russian], *Vestn. Tekhn. Un-ta*, 2015, Vol. 18, No. 20, P. 137–143.
- 13 Kim S., Kweon O., Jones R. C., Freeman J. P., Edmondson R. D., Cerniglia C. E., Complete and integrated pyrene degradation pathway in *Mycobacterium vanbaalenii* PYR-1 based on systems biology, *Journal of Bacteriology*, 2007, Vol. 189, No. 2, P. 464–472.
- 14 Cheung P. Y., Kinkle B. K., *Mycobacterium* diversity and pyrene mineralization in petroleum-contaminated soils,

- Appl. Environ. Microbiol.*, 2001, Vol. 67, P. 2222–2229.
- 15 El Naggar A. Y., Kamel M. M., Aladly A. A., Ismail N. A., Bioremediation of paraffinic and polynuclear aromatic hydrocarbons using laser irradiated *Bacillus amyloliquefaciens*, *Journal of American Science*, 2010, Vol. 6, No. 10, P. 661–670.
  - 16 Kireeva N. A., Vodopyanov V. V., Miftakhova A. M., Biological Activity of Oil-Polluted Soils [in Russian], Ufa: Gilem, 2001. 377 p.
  - 17 Pikovkiy Yu. I. Natural and Technogenic Hydrocarbon Fluxes in the Environment [in Russian], Moscow: MGU, 1993. 208 p.
  - 18 Fedorova Yu. A., Akhmetova G. I., Korzhova L. F., Yagarova G. G., Studies of biodestruction of organic pollutants of aromatic series [in Russian], *Vestn. Tekhn. Un-ta*, 2017, Vol. 20, No. 15, P. 143–146.
  - 19 Rozanova E. P., Kuznetsov S. I., Microflora of Oil Deposits [in Russian], Moscow: Nauka, 1974. 197 p.
  - 20 Margesin R., Moertelmaier C., Mair J., Low-temperature biodegradation of petroleum hydrocarbons (n-alkanes, phenol, anthracene, pyrene) by four actinobacterial strains, *Int. Biodeter. Biodegr.*, 2013, Vol. 84, P. 185–191.
  - 21 Puntus I. F., Filonov A. E., Kosheleva I. A., Gayazov R. R., Karpov A. V., Boronin A. M., Isolation and characterization of microorganisms capable of degrading polycyclic aromatic hydrocarbons, *Microbiology (Mikrobiologiya)*, 1997, Vol. 66, No. 2, P. 222–225.
  - 22 Filonov A. E., Puntus I. F., Karpov A. V., Gaiazov R. R., Kosheleva I. A., Boronin A. M., Growth and survival of *Pseudomonas putida* strains degrading naphthalene in soil model systems with different moisture levels, *Process Biochemistry*, 1999, Vol. 34, P. 303–308.
  - 23 Altunina L. K., Svarovskaya L. I., Microbiological method of profound purification of oil slime polluted with viscous oil of the Tsagan-Els deposit (Mongolia) [in Russian], *Neft. Gas. Novatsii*, 2016, No. 6, P. 50–54.
  - 24 Ankudinova A. V., Malakhova D. V., Garabadzhiu A. V., Gorina A. S., Yankevich M. I., Investigation of PAH-destroying microorganisms isolated from soils under technogenous affection [in Russian], *Estestv. i Tekhn. Nauki*, 2008, No. 3, P. 114–119.