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Study of Transformation of Tar and Its High Molecular Mass Components in Soil

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Abstract

The paper presents the results of biochemical oxidation of Novokuybyshevsk refinery tar with high contents of heteroelements in a model of the soil system. The microorganism population is restored to the initial level and then increases in 20–30 times on the 4–5th day after pollution, as demonstrated. Enzyme activity of polluted soil increases in 1.5–1.8 times, which attests to an increase in oxygenase activity of microorganisms and consequently assimilation of different hydrocarbon compounds. The formation and accumulation of incomplete HC oxidation products happen, as determined during biodegradation of tar components. The disposal of the studied oil product was 50 % of the initial pollution on the 180th day of the experiment Native soil microflora is able to mineralize almost all HC in the tar composition, as established by the GC/MS method. High molecular mass heteroatom tar components are also subjected to microbial degradation, as demonstrated. The structure of these species undergoes profound changes during degradation.

Keywords: oil pollution, biodegradation, native soil microflora, hydrocarbon-oxidizing microorganisms, enzyme activity, saturated and aromatic hydrocarbons, tar

INTRODUCTION

The relevance of the issue is related to environmental pollution with different species of oil products (OP) of technogenic origin [1]. Technogenic HC flows are generated during producing, transporting and processing of hydrocarbon (HC) raw materials. They play a significant part in anthropogenic changes in natural cycles of matter [2]. The presence of OP in soil is of environmental danger, as it is a cause of violations of biogeocenosis until the death of living organisms of soil at high HC concentrations [3].

Negative effects of liquid OP are increasingly associated with violation of the water-air regime of soil. Transformation of OP can be conditionally broken down into the abiotic phase (from fresh to mature pollution) and biotic degradation on the earth's surface and in soils [4]. The leading part in the degradation of organic pollutants entering into the environment belongs to microorganisms [5]. Hydrocarbon-oxidizing natural biocenoses are developed everywhere in all types of soils and natural water of different climatic zones [6]. However, the intensity of HC degradation process depends on temperature, summer period duration and other limiting factors, and also biocenosis peculiarities [7].

Both free and sedentary bound OP forms return volatile fractions into the atmosphere, and soluble compounds into the water. This process is not fully completed with time as microbiological transformation processes of HC

lead to the formation of volatile and water-soluble metabolism products.

Tars that are formed resulting from oil distillation up to fractions boiling out at 450 °C are some of such products. Naphthenic, aromatic, paraffinic HC, and besides, high-molecular heteroatom compounds (HMHC) enter into tar composition. Almost all metals present in oil are usually concentrated in tar. It is mainly used for the production of the road, roofing and construction bitumen, low ash coke, and lubricants. Tar under study has high contents of resin-asphaltene components (39.3 %) [8]. It is believed that resin-asphaltene substances are stable to oxidation with microorganisms; their metabolism process proceeds slowly, sometimes dozens of years. Resin-asphaltene components of oil products are mainly sorbed in the upper humus level during leakage from above, sometimes firmly cementing it, which decreases the first space of soils. Resin-asphaltene substances are hydrophobic; enveloping the roots of plants, they greatly worsen the admission of moisture thereto, with the result that plants may die. However, soils are able to dispose of a part of falling oil components *via* biochemical degradation and oxidative destruction of some of their part into organic matter assimilated by plants, owing to a complex biocenosis system (primarily, hydrolyzing and oxidative enzymes including polyphenol oxidases and peroxidases) [9, 10].

The work goal is the study of the ability of soil to self-regeneration during its pollution with oil processing product (tar) under laboratory conditions.

EXPERIMENTAL

Tar (the Novokuibyshevsk refinery JCS) was selected as a study object. The elemental composition of tars, mass %: C 82.28, H 10.73, S 3.95, N 0.19, O_2 1.14; H/C ratio is 1.56. Tar material composition, %: oils 60.7, resins 33.6, asphaltenes 5.7.

Soil pollution was carried out *in vitro*. To prepare model soil systems universal soil-ground Garant was used. Soil was weighed and tar was introduced therein in the amount of 50 g/kg, the mixture thoroughly mixed. Then polluted soil was placed into glass desiccators (soil layer thickness is 12.5 cm) and incubated at 22 °C. Moisture content was maintained ranging from 25 to 27 %. Spudding was carried out periodically during experiments. Experiment duration was 180 days. Selection of the above experimental parameters was driven by the previous experiments on the biodegradation of high viscosity oils and bitumens [11, 12].

Experimental scheme:

– Blank experiment: clean soil for comparative assessment of population dynamics of microorganisms and enzyme activity;

– Experiment 1: soil polluted with oil products to determine population dynamics of microorganisms and enzyme activity;

– Experiment 2: soil polluted with oil products to determine the degree of disposal and study a change in tar composition.

To determine the population of microorganisms and enzyme activity of soil during experiment 1 averaged samples from which heterotrophic microflora was allocated by sowing in multiple dilutions at meat infusion agar (MIA) were selected during the experiment. Calculations of colony-forming units (CFU) were carried out after thermostatting, and then recalculated per 1 g of dry soil [13]. Catalase activity was determined by the gasometrical method. Dehydrogenase, polyphenoloxidase, and peroxidase activity of soil was defined by photocolorimetric methods [14].

Tar degradation degree was determined by the gravimetric method according to the content of residual oil products in soil. For this purpose, oil from polluted soil (experiment 2) was extracted with chloroform in Soxhlet apparatus and then filtered off. The extracted oil product (tar) was freed from chloroform using a rotary evaporator and weighed [15].

Rheological measurements of samples of initial and biodegraded tar were carried out in dynamic mode (*i.e.* at oscillating shear stress) at plane/plane measuring geometry. The values of elastic modulus (G'), the phase shift angle (δ), and sample viscosity (η) were measured using HAAKE RheoStress rheometer.

A change in the structural composition of tar was determined by IR spectroscopic method using Thermo Electron NIKOLET 5700 (FT-IR) spectrometer (USA).

Componential analysis of organic compounds of initial and biodegraded tar was carried out

by GC/MS spectrometry using DFS Thermo Scientific magnetic spectrometer (Germany) with a quartz capillary chromatographic column, 30 m length, TR-5MS stationary phase.

The contents of resins and asphaltenes were determined by the standard technique [16]. Those isolated from initial and biodegraded tars were subjected to structural group analysis (SGA) by the technique developed at the Institute of Petroleum Chemistry (IPC SB RAS, Tomsk) and based on shared use of the results of elemental composition determination, average molecular masses and PMR-spectroscopy data [17, 18]. The contents of carbon, hydrogen, nitrogen, and oxygen were determined using Vario EL Cube elemental analyzer (Germany manufacturing). Sulfur content was determined by the double combustion method. Molecular masses of substances were measured by the cryoscopic method in naphthalene using KRION microcalorimeter developed at IPC SB RAS (Tomsk). Proton nuclear magnetic resonance spectra were removed using AVANCE-AV-300 NMR Fourier spectrometer in deuterochloroform as solvent and hexamethyldisiloxane as an internal standard at a 1 % concentration of substances under study.

To compare SGA data the same designations of structural parameters that were used in previous works [17, 18] were utilised, namely: C_a, C_n, C_p, C_α, C_γ are the number of carbon atoms in aromatic, naphthenic and paraffinic molecular structures in α-positions to heterofunctional groups and aromatic nuclei and not bound to the latter of terminal methyl groups (C_γ), respectively; f_a, f_n и f_p are the proportions of carbon atoms in appropriate structural fragments; K_t is a total number of rings, K_a and K_n are amounts of aromatic and naphthenic rings in average size species (similar average parameters of the structural units are labelled with superscript asterisks); m_a is the average number of structural units in species.

Measurement repeatability in experiments is triple. Figures and tables present data as arithmetic average values.

RESULTS AND DISCUSSION

The activity of microbiological processes and the entire set of abiotic factors (light, humidity, temperature, mechanical and mineral composition of soil *etc.*) in many respects determine the conditions under which the chemical and biological transformations of oil hydrocarbons (HC) proceed.

Figure 1 presents the population dynamics of heterotrophic bacteria in pure (monitoring) and oil-polluted soil (curve 2). There is a slight decrease in the microorganism population from $(5–5.5) \cdot 10^6$ CFU/g soil to $(2–2.5) \cdot 10^6$ CFU/g soil. Apparently, this is due to the destruction of unstable groups of microorganisms, which proceeds resulting from toxic action of some HC on soil microflora. The microorganism population increases to the initial level 4–5 days after pollution. Apparently, there is the rearrangement of soil microbiocenosis, at which hydrocarbon oxidizing microorganisms (HOM) able to destruct oil HC obtain an advantage. In a similar way, some microorganisms may acquire the ability to HC oxidation, for example, due to polymorphism or genetic material transfer (transduction, conjugation, *etc.*).

The maximum population of heterotrophic bacteria was reached 60 and 105 days of the experiment and amounted to $(120–130) \cdot 10^6$ CFU/g soil. Their high abundance persisted up to 150 days of the experiment. The maximum population of microorganisms in monitoring was much lower and did not exceed $(11–12) \cdot 10^6$ CFU/g soil (see Fig. 1, curve *1*).

Soil microflora biodiversity in polluted soil samples decreases during experiment and representatives of the following species prevail: *Arthrobacter*, *Pseudomonas*, *Bacillus*, *Rhodococcus*, *Flavobacterium*, and *Micrococcus*.

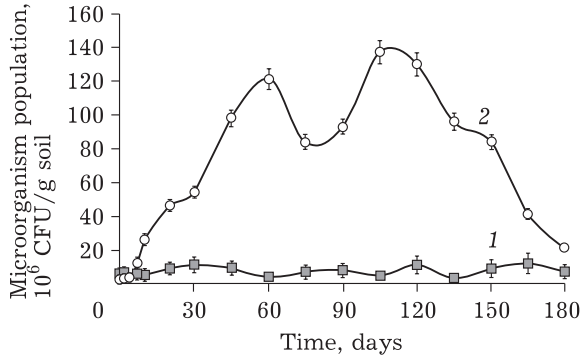

Fig. 1. Population dynamics of heterotrophic microorganisms in reference soils (1) and soils polluted with oil products (*2*).

Mineralization of oil HC in soil proceeds with participating of various ferments, as known. Soil enzyme activity is driven by not only different microorganism contents but also their diversity and physiological activity, therefore, quantitative changes proceeding in microbial cenosis of polluted soils do not reflect a change in their activity [2]. Dehydrogenase, catalase, oxidase, and peroxidase are the most important and widespread enzymes in soil microorganisms.

An increase in the activity of all enzymes under study proceeds during the first days of the experiment, as established. Figure 2, *a* presents oxygen formation dynamics that reflects catalase activity during observation. Its activity remains higher than that of reference soils during the entire experiment. The maximum concentration of O_2 reached 4.4 mL/g in polluted soil; oxygen concentration in the reference sample (pure soil) did not exceed 3.2 mL/g during the entire experiment (see Fig. 2, *a*). Figure 2, *b* presents the formation dynamics of triphenylformazon (TPF) reflecting the activity of soil dehydrogenases. This enzyme activity increases to 150 experimental days, which indirectly testifies biodegradation

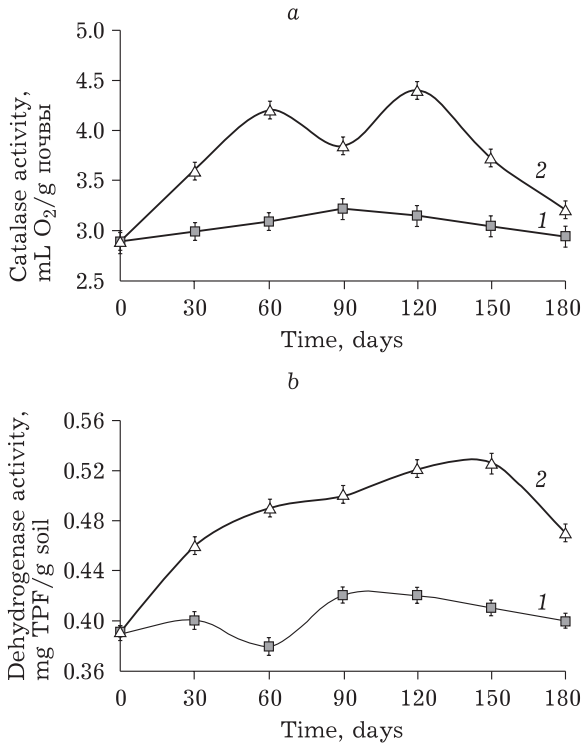

Fig. 2.Change of catalase (*a*) and dehydrogenase (*b*) activity in pure (*1*) and polluted (*2*) soils.

of *n*-alkanes and aliphatic chains in the composition of complex species. The amount of TPF in soil with tar increases to 0.52 mg/g, it does not exceed 0.42 mg/g in the reference option (see Fig. 2, *b*). This testifies an increase in the oxigenase activity of native soil microflora and the intensity of biodegradation processes of individual components entering tar composition.

Considerable attention in the exploration of biodegradation is paid to the study of resulting phenoloxidases (peroxidase and polyphenol oxidases). The latter play an important part in humification processes, have a protective effect on soil, decomposing various xenobiotics [2], participate in multistage processes of decomposition and synthesis of aromatic organic compounds.

The activity of these enzymes increases until the experiment end, as established. This may indicate oxidation of polyaromatic HC, and also the structural frame of high-molecular-mass compounds that compose aromatic structures. Quinone concentration that demonstrates the activity of peroxidase and polyphenol oxidase in soil with sludge increases at the end of experiment to 0.54 and 0.4 mg/g, respectively (Fig. 3). In the control soil, the activity of these enzymes does not exceed 0.34 and 0.22 mg/g, respectively. The findings exceed the control data in 1.6–1.8 times.

The gravimetric method has demonstrated that sludge disposal in a model of the soil system was 25 g/kg (50 %) for 180 days.

Incomplete oxidation products of some hydrocarbons are accumulated during degradation of oil products. The former have surfactant-active properties that, in turn, contribute to HC emulsification into small drops, which contributes to their adsorption by microbial cells, and also increases the degree of wetting of the cell surface by hydrocarbons and ensures HC diffusion *via* a cell membrane.

Additional absorption bands (AB) in the 3338, 1705, 1170, and 1032 cm^{-1} range (Fig. 4) appear in IR spectra of residual hydrocarbons extracted from soil. The appearance of AB in these ranges evidences to the presence of a large amount of various oxygen compounds that are metabolism intermediates. This is in agreement with the classic scheme of oxidation of hydrocarbons that are first oxidized to

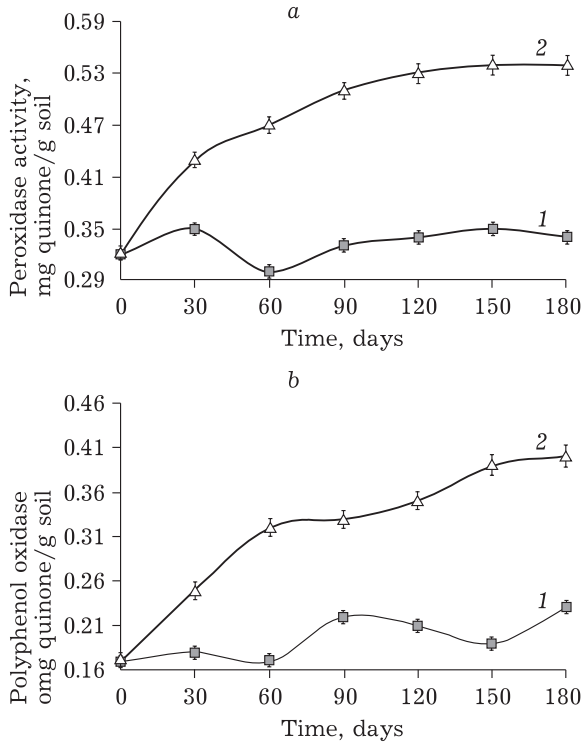

Fig. 3. Change of peroxidase (*a*) and polyphenoloxidase (*b*) activity in pure (*1*) and polluted (*2*) soils.

alcohols, aldehydes, and ketones, saturated and aromatic carboxylic acids and further to CO_2 and H_2O.

Rheological measurements of samples of tar and its biodegradation products were carried out in dynamic mode (*i.e.* at oscillating shear stress); the data are presented in Table 1. The findings clearly evidence to significant increasing elastic properties (G', δ) and rising product viscosity (η) of sludge biodegradation products (after degradation in soil). The viscosity of the biodegraded sample is by 2–3 orders of magnitude higher than the corresponding value of the initial tar.

In the biodegraded sample, its viscosity significantly changes with rising temperature, to 100 °C at heating: G' decrease by 4 orders of magnitude, η – by 3, in initial tar – in 16 and 40. Deformation outpaces behind the shear stress by 90° during dynamic measurements of Newtonian liquid, which is expressed *via* the phase shift angle δ = 90°. In the studied temperature range, δ ≈ 89–88° in the initial tar, therefore, it is arguable that it is a liquid and elastic properties are completely absent in it. The phase shift angle (δ = 65.8°) in tar that is oxidized during biodegradation significantly decreases (see Table 1); notable elasticity appears; the course of the temperature dependence of the phase shift angle also changes, *i.e.* there is a transition of a system from liquid condition into a state of an almost rigid body.

Apparently, this is related to a relative increase in the content of resin-asphaltene

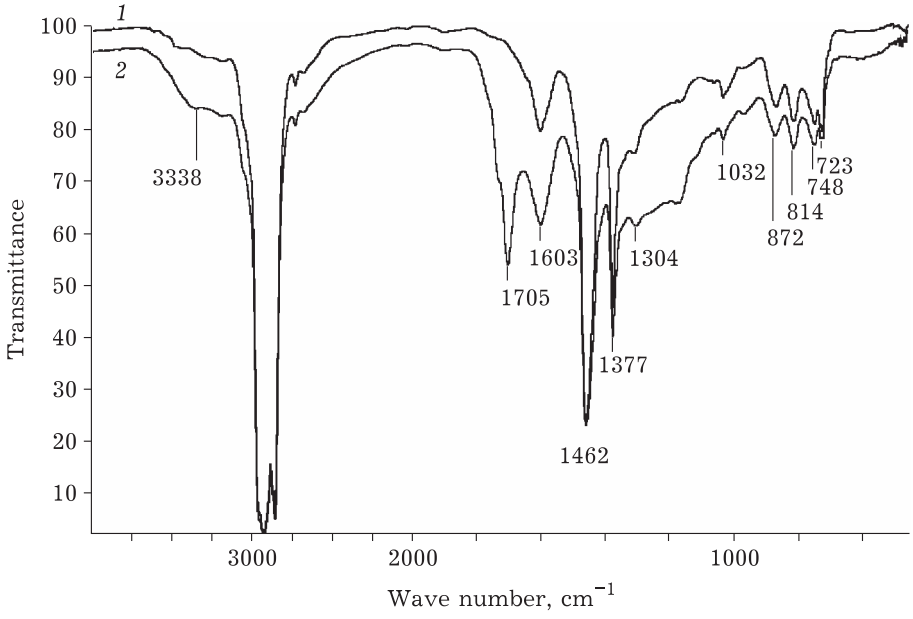

Fig. 4. Infrared spectra of initial (*1*) and biodegraded (*2*) tar.

TABLE 1

Reological properties of Novokuibyshevsk refinery sludge before and after destruction

T, °C	*G′*, Pa		Viscosity η, Pa·s		Phase-shift angle δ, degr	
	Initial	After degradation	Initial	After degradation	Initial	After degradation
50	2.33	28870.80	22.51	10 443. 66	89.05	65.84
60	0.82	6615.20	9.55	3293.95	89.21	72.41
70	0.13	1088.80	5.24	923.61	89.69	79.37
80	0.17	186.60	1.83	279.61	89.16	83.93
90	0.16	34.89	0.97	100.47	88.51	86.73
100	0.15	4.48	0.57	35.48	87.63	88.86

substances (RAS) in the biodegraded tar sample, where RAS constitute more than a half of its composition (Table 2). Supermolecular structures based on the asphaltene nucleus and the solvate shell consisting of resin-asphaltene component species are probably formed during biodegradation. Tar shows viscoelastic properties due to a large content of high-molecular-mass-components (resins and asphaltenes). There was no similar phenomenon in the whole studied temperature range. It is worth noting that the initial tar spreads out much better than biodegraded one, *i.e.* tar degradation changes its surface tension.

All HC that are a part of tar underwent biodegradation, as demonstrated by chromatography-mass spectrometry analysis (Table 3). Total degradation of saturated alkanes was 58.6 %, however, solid high-molecular-mass paraffins remained unoxidized. Although aromatic hydrocarbons are much more stable to microbiological oxidation than paraffins and naphthenes, degradation of holonuclear naphthalene proceed by 60.3 %, herewith, destruction of its alkyl-substituted homologs is from 77.8 to 100.0 %. Degradation of mononuclear phenanthrene and fluoronaphthalene + pyrene is 84.9 and 75.2 %, respectively, and biodestruction of their methyl-substituted homologs – 91.4–96.3 %. Apparently, biodegradation of polar aromatic compounds proceeds much easier; oxidation mainly occurs at the addition site of the side chain. Microbiological oxidation of heteroatom compounds (dibenzothiophenes) proceeds by 88.1–97.1 %, herewith, methyl-substituted derivatives show comparative oxidation easiness than holonuclear species, as in the case with aromatic hydrocarbons. Aromatic hydrocarbons decompose to final aliphatic acids under the action of microorganisms. The final products enter into microbial cell metabolism [6].

TABLE 2

Tar material composition before and after biodegradation

Components	Content, mass %	
	Initial tar	Tar after degradation
Oils	60.70	48.82
Resins	33.60	40.31
Asphaltenes	5.70	10.86

Steranes are among the representatives of tetracyclic saturated hydrocarbons, while hopanes are pentacyclic isoprenoid HC. These compounds are considered to be most stable ones among oil components and serve as markers for geochemical purposes. However, as we demonstrate in the present work, they undergo rather intense oxidation under aerobic conditions. Total destruction of steranes and hopanes was 71.6 and 65.3 %, respectively.

So, as a result of biochemical oxidation of tar, microorganisms destroy not only *n*-alkanes, which are most available for microbiological oxidation, but also aromatic, polycyclic and heteroatomic compounds (see Table 3). This may be due to the diversity of soil microorganisms that are able to oxidize all the components of oil products introduced.

Detailed analysis of biodestruction of high-molecular hetero-organic compounds (resins and asphaltenes) showed that microbiological oxidation causes deep changes of the structural group characteristics of resins and asphaltenes. Biodegradation of tar involves noticeable

TABLE 3
GC/MS analysis of initial and biodegraded tar of Novokuibyshevsk refinery

Hydrocarbons	Concentration in tar, μg/g		Destruction, %
	Initial tar	Tar after degradation	
Alkanes	1053.788	435.999	58.62
Naphthalene	0.877	0.348	60.31
Methylnaphthalene	0.394	0.087	77.87
Dimethylnaphthalene	1.591	0.005	99.66
Trimethylnaphthalene	2.420	0.000	100.00
Tetramethylnaphthalene	6.229	0.000	100.00
Phenanthrene	3.121	0.468	84.98
Methylphenanthrene	13.364	0.492	96.31
Dimethylphenanthrene	43.038	3.694	91.41
Trimetylphenanthrene	24.654	2.498	89.86
Dibenzothiophene	3.252	0.384	88.18
Methyldibenzothiophene	13.420	0.382	97.15
Dimethyldibenzothiophene	34.904	2.566	92.64
Fluoranthene + pyrene	0.945	0.233	75.29
Methylfluoranthene + pyrene	3.141	0.259	91.76
Dimethyfluoranthene + pyrene	6.103	0.283	95.36
Sum of arenes	157.449	10.986	93.02
Steranes	48.849	13.860	71.62
Hopanes	262.062	90.838	65.34

changes of the material composition, which is connected first of all with biodestruction of petroleum oils; their content decreases from 60.70 to 48.82 mass % (see Table 2).

An increase in the fraction of resins from 33.60 to 40.31 mass % and asphaltenes from 5.70 to 10.86 mass % is due both to a decrease in the fraction of petroleum oils and to the transformation of the oxidized components of oils into resins and asphaltenes. This is confirmed by the data obtained by means of SGA (Table 4), according to which the molecular mass and the amount of heteroaromatic atoms (especially oxygen and sulphur) increase in an average asphaltene molecule.

Averaged asphaltene species of the initial tar has M of 718 amu, consists of two units that are mainly composed of aromatic structures (aromaticity factor f_a = 46.5), H/C ratio is 1.03.

The composition of asphaltenes changes not only quantitatively but also qualitatively during biodegradation. The average M of asphaltenes increases from 718 to 1390 amu; the number of units ma in average species increases from 2.0 to 3.1; the average number of carbon atoms increases almost twice from 49.6 to 90.7; the amount of oxygen atoms increases in 5.5 times. These changes may be explained by the inclusion of oxidized components of resins and oils into the composition of asphaltenes. The transformation chain of the components may look as follows: oils → resins → asphaltenes. Herewith, the aromaticity factor (f_a) reduces from 46.5 to 46.2; the H/C atomic ratio increases from 1.03 to 1.10. Therefore, this also indicates the inclusion of more saturated components of resins and oils into the composition of asphaltenes.

The averaged species of the initial tar has M = 600 amu. The average size of ma units in species is 1.5. The initial resins have a low aromaticity factor (4.5) and mainly comprise of naphthene rings of the total number of rings (K_t) of 6.3, 3.3 falls on naphthene ones (K_n). The H/C ratio is relatively low that is 1.41, which may be explained by the high cyclicity of heteroatoms in the structure.

Biodegradation significantly affects

TABLE 4

The average structural parameters of the molecules of resins and asphaltenes of the investigated tar and products of its biodegradation

Parameters	Asphaltenes		Resins	
	Initial	After degradation	Initial	After degradation
		Average molecular mass, amu		
	718	1390	600	844
		Elemental composition, mass %		
C	82.9	78.3	81.7	80.3
H	7.1	7.2	9.6	7.8
N	1.7	1.1	1.3	1.1
S	4.9	3.8	4.1	4.4
O	3.3	9.6	3.3	6.3
		Number of atoms in average species		
C	49.6	90.7	40.8	56.5
H	50.9	99.4	57.4	65.3
N	0.9	1.1	0.6	0.7
S	1.1	1.6	0.8	1.2
O	1.5	8.3	1.2	3.3
H/C	1.03	1.10	1.41	1.16
		Ring composition		
K_t	13.4	18.6	6.3	16.0
K_a	5.6	10.6	3.1	4.0
K_n	7.8	7.9	3.3	12.0
		Aromaticity factor, %		
f_a	46.5	46.2	14.5	28.1
		Average number of units in species		
m_a	2.0	3.1	1.5	1.7

structure-group parameters of tar resin species: M increases from 600 to 844 amu. The average number of blocks (m_a) – from 1.5 to 1.7. The average amount of carbon atoms rises from 40.8 to 56.5; sulfur – 1.5 times, from 0.8 to 1.2; oxygen in 2.75 times, from 1.2 to 3.3. Herewith, the aromaticity factor increases from 14.5 to 28 and the H/S ratio decreases from 1.41 to 1.16. Increasing the aromaticity factor and the number of oxygen atoms in average species is due to the inclusion in the composition of the polyaromatic resins oxidized components of oils.

The findings of SGA analysis of high-molecular-mass heteroatom tar components allow concluding that the structure of these species of soil microflora is undergoing profound changes during degradation. At the same time, it cannot be claimed that tar oxidation in soil proceeds only under the action of microbiological processes. Abiotic medium factors are likely to also play a certain part in OP degradation.

The transformation degree and the rate of soil self-purification from OP are not constant values and depend not only on oil product composition and concentration in soils. The ability of ecosystems to self-recovery upon ingress of oil or OP components therein depends on soil type, the geological-geographical landscape, climatic conditions, species composition of vegetation cover, and soil microorganisms.

CONCLUSION

The findings attest to native soil microflora adaptation to a relatively low concentration of the oil product under study. The microorganism population increases by 1.5–2.0 orders of

magnitude by the experiment end, the activity of enzymes under study in 2–4 times. Processes of microbiological tar transformation in soil proceed intensively, as demonstrated by the studies carried out. Total biodegradation of tar was 50.0 mass % of initial pollution for 180 days, moreover, all hydrocarbons underwent oxidation by 58.6–100.0 %.

Tar oxidation process was accompanied by a change in the group and individual hydrocarbon composition. Herewith, accumulation of resins and asphaltenes occurs in biodegradation products due to both a decrease in the fraction of hydrocarbon components and the formation of various cyclic oxygen compounds that are a part of the composition of resins and asphaltenes.

Thus, the findings lead to the conclusion that bioremediation processes of soils polluted with various organic compounds of oil series may be intensified by the stimulation of natural microflora without using commercial microbial drugs. The use of oil-oxidizing strains is justified only at high levels of hydrocarbon pollution of soils or when natural microflora is poorly developed, for example, in poor soils.

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