

Experimental Transformation of Organic Matter by the Microbial Community from the Bottom Sediments of Akademicheskoy Ridge (Lake Baikal)¹

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Abstract— We discuss the results of a model experiment on cultivation of the microbial community from the area of the Khoboy mud volcano (Akademicheskoy Ridge) conducted under conditions typical of the hydrocarbon generation zone (80 °C, 5 MPa). The cultivation under conditions of biomass enrichment with the Baikal diatom *Synedra acus* changed the composition of organic matter. The transformation degree of organic matter in the sediment after the experiment was 16%, whereas the concentration of phenanthrenes relative to methyl-substituted homologues, including retene, decreased, and the concentration of dibenzothiophenes relative to normal alkanes increased. We have identified tri- and monoaromatic steroids, including 17-dimethyl-, 23-methyl monoaromatic steroids C₂₇. An increase in the concentration of tri- and monoaromatic steroids in the sediments after the experiment might indicate that the biomass of the Baikal diatom *S. acus* was destructed, which led to an increase in the steroid concentration. In the control (sterile) sediment, we detected no changes in the composition of organic matter. Representatives of various taxa capable of surviving in anaerobic thermophilic conditions have been identified in the microbial community by molecular genetic methods. Their presence in the surface sediments might be due to the inflow of deep-seated mineralized fluids and breccia from deep-seated sedimentary rocks.

Keywords: deep biosphere, transformation of organic matter, microbial community, temperature and pressure conditions, Lake Baikal

INTRODUCTION

Bottom sediments of marine and freshwater bodies represent the largest reservoir of organic carbon on Earth and, at the same time, a unique habitat for microbial communities. According to some estimates, the total biomass of microorganisms in marine sediments is 2.9×10^{29} cells, and it exceeds the number of prokaryotes in seawater (1.2×10^{29}) and soil (2.6×10^{29}) (Kallmeyer et al., 2012). The activity of microorganisms determines almost all bottom sediment processes in diagenesis and early catagenesis. The characteristics of deep bottom sediments are lack of oxygen, high hydrostatic pressure and a shortage of electron donors and acceptors, as well as an absence of readily available organic sources of carbon due to active processes of organic matter

destruction in the water column and the surface sediment layer (Parkes et al., 2014). With the sediment depth, the number of microorganisms decreases significantly (Kallmeyer et al., 2012). However, in areas with geological anomalies, where concentrations of organic matter (OM) and/or inorganic electron donors and acceptors are high, the number and activity of microbial populations are much higher (Parkes et al., 2000; Bonch-Osmolovskaya et al., 2003; Horsfield et al., 2006; Bennett et al., 2013; Anderson et al., 2014; Parkes et al., 2014; Ruff et al., 2015).

Baikal, the world's deepest lake dating back to 25–30 million years, is one of the promising sites for studying microbial communities associated with geological anomalies (Biddle et al., 2011). Lake Baikal, occupying the Baikal rift depression in the central part of the Baikal Rift Zone, has a long history of sedimentation (Grachev et al., 1998). The OM transformation in the Baikal sediments as the temperature and pressure conditions changed in the depths of the Cenozoic strata contributed to the generation of hydrocar-

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bons and the emergence of their accumulations of various types: oil, gas and gas-hydrate ones (Ryabukhin, 1934; Kuzmin et al., 2001; Kontorovich et al., 2007).

Early diagenesis in bottom sediments of Lake Baikal is the subject of numerous studies (Mizandriontsev, 1978; Granina, 2008; Och et al., 2012), whereas the processes of OM transformation by microbial communities from deep bottom sediments and a possibility of their entry into the near-surface sediments of Lake Baikal have been studied sporadically. Biomarker values of oil catagenesis and carbon isotopic composition of methane and ethane indicate that in some areas of Lake Baikal oil and gases migrate from depths of at least 2–3 km characterized by temperature and pressure conditions to the bottom surface, where the temperature is close to 4 °C (Khlystov et al., 2007; Kontorovich et al., 2007). In pore waters of these areas, there are abnormal concentrations of some ions due to heat fluxes of different intensity, the presence of gas hydrates and the type of geological structures (Lomonosov, 1974; Granina et al., 2001; Kuzmin et al., 2001; Kontorovich et al., 2007; Khlystov et al., 2013, 2017). Obviously, ascending fluids of various nature and origin can capture microorganisms from the deep layers and carry them into the surface sediments.

The deep microorganisms coming from the zone of hydrocarbon generation into the surface sediments of Lake Baikal were identified near the Posol'sk Bank and Goloustnoe methane seeps. Their role in the destruction of OM under temperature and pressure conditions was shown in (Vaneste et al., 2002; Klerkx et al., 2006; Bukin et al., 2016; Chernitsyna et al., 2016; Pavlova et al., 2016; Khanaeva et al., 2017). Reproduction of the conditions characteristic of the tectonically active zone of Lake Baikal (temperature 80 °C and pressure 50 atm) became possible after the development of special high-pressure chambers (HPCs) (Nikolaev Institute of Inorganic Chemistry SB RAS). In HPCs, model experiments were carried out under the conditions of temperature and pressure in a CH₄ : H₂ : CO₂ atmosphere. The first experiments have shown that in the bottom sediments of methane seeps enriched with detritus of the Baikal diatom *Synedra acus*, microbial communities are involved in the destruction of algae biomass to form hydrocarbon biomarkers, such as retene or gammacerene (Bukin et al., 2016; Pavlova et al., 2016).

In fact, these studies are insufficient to understand the processes occurring in other zones of deep hydrocarbon gas discharge in Lake Baikal, where other types of geological structures exist, with various temperature conditions, flow intensity and composition of deep fluids.

The study of OM transformation by microbial communities in bottom sediments of the Akademicheskoy Ridge is of particular importance. The OM of Akademicheskoy Ridge bottom sediments is a mixture of organic carbon and biogenic silica formed mainly by diatoms (Bezrukova et al., 1991; Granina, 1992; Granina et al., 1992). The tectonic uplift in the central part of Baikal separated from the coast by deep basins has always been of interest to studies focused on

the paleoclimatic reconstructions (Kuzmin et al., 1995, 2001; Zonenshain et al., 1995; Grachev et al., 1997; Moore et al., 1997; Khlystov et al., 2000, 2001; Mats et al., 2000; Vologina et al., 2003; Vologina and Fedotov, 2013). Currently, a new mud volcanic and hydrate-containing region of Lake Baikal has been described in an area of Akademicheskoy Ridge with no previous records of mud volcanoes or gas hydrates. A multibeam sonar survey has shown a group of mud volcanoes on the slope of Akademicheskoy Ridge. Biostratigraphic and seismostratigraphic correlation has indicated that the material constituting the mud breccia of the studied mud volcanoes has an age interval from the late Miocene to the early Pliocene (from 5.6 to 4.6 Ma) and could rise from a depth of less than 310 m below the bottom. The crest in the central part of the Ridge has been called “AkademKhrebet”, and that near Ol'khon Island—“Khuboy” (Khlystov et al., 2017). There has been no previous microbiological research in the area of these structures.

The aim of this work is to study the processes of the OM transformation influenced by the bottom sediments microbial communities from the Khuboy mud volcano under the conditions of temperature and pressure.

MATERIALS AND METHODS

Core St. 3. GC-8 (length 200 cm) was sampled from Akademicheskoy Ridge in 2015. The coordinates of the point were 53°41,528' N, 107°87,549' E (Fig. 1). The sediments were sampled with a large gravity corer from the board of the LIN SB RAS research vessel “G.Yu. Vereshchagin”. Samples were taken in compliance with the sterility rules

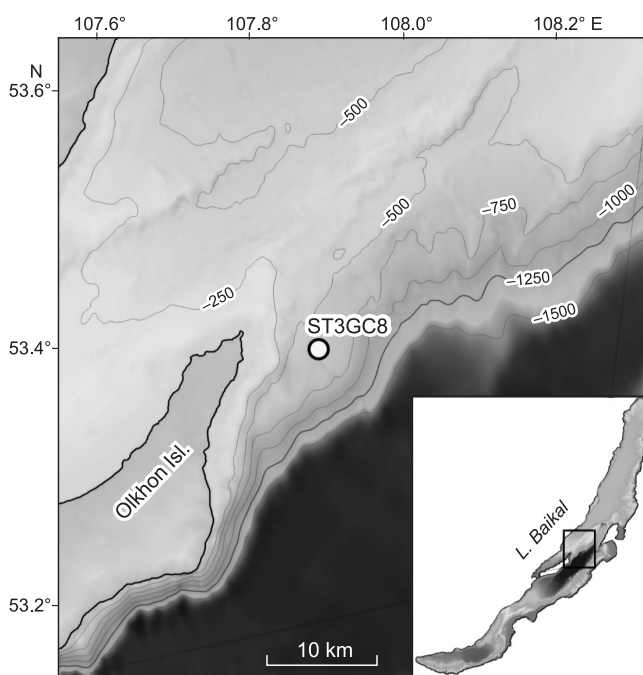


Fig. 1. Sampling map. Bathymetric data—INTAS Project 99-1669 Team, 2002.

and placed into liquid nitrogen, where they were stored until the experimental cultivation.

Experimental cultivation was carried out during seven months at 80 °C and methane pressure of 50 atm in two special HPCs developed and assembled in Nikolaev Institute of Inorganic Chemistry SB RAS according to the method of (Bukin et al., 2016; Pavlova et al., 2016). The sediments were enriched with the biomass of the Baikal diatom *Synedra acus* from the axenic culture provided by the Department of Cell Ultrastructure at LIN SB RAS (Shishlyanikov et al., 2011). HPC No. 1 contained natural sediments; the sediments in HPC No. 2 were sterilized by autoclaving and served as a negative control.

The content and distribution of hydrocarbons of maltene part in the preliminarily obtained chloroform extract from sediment samples before the beginning (considering the additional organic substrates) and at the end of the experiment was investigated by the chromatography-mass spectrometry method according to (Kashirtsev et al., 2001).

To analyze changes in the structure and composition of microbial communities from the samples of the natural sediments, total DNA preparations were isolated after cultivation using an enzymatic lysis method followed by phenol-chloroform extraction (Sambrook et al., 1989). DNA of the loci subject to analysis were obtained using the polymerase chain reaction (PCR), from the products of which the libraries were prepared for massively parallel sequencing on the Illumina MiSeq platform (SB RAS Genomics Core Facility, Novosibirsk). Massively parallel sequencing and phylogenetic analysis were performed as previously described in (Bukin et al., 2016).

RESULTS AND DISCUSSION

Lithological characteristics of sediments used in the experiment. The sediment layers used in the experiment (150–185 cm) have a reduced loose gray aleuopelitic silt with numerous degassing cracks adjoining the gas-hydrate layer. The sediments are oxidized from the surface to a depth of 0.5 cm. From the surface to a depth of 20 cm, the sediments contain diatom silts with hydrotroilite interlayers. At a depth of 20–182 cm, there is an aleuopelitic clay containing mud-volcanic breccia with hydrotroilite inclusions. At a depth of 182–200 cm, there are layers of massive gas hydrates.

Transformation of OM in the experiment. The control sample from HPC No. 2 did not show geochemical changes in the OM composition because of small quantity of chloroform extract. The OM composition of bottom sediments enriched with detritus of the *S. acus* diatom (HPC No. 1) showed the processes of its transformation.

The content of organic carbon in the samples after the experiment (0.79%) almost did not change ($\pm 0.09\%$ for each rock) relative to the initial one (0.88%); chloroform bitumoids—from 0.02 to 0.05%.

Small samples of bitumoids (7 mg) did not allow isolation of asphaltenes and division of maltenes into saturated and aromatic hydrocarbons. Before the experiment, the total amount of hydrocarbons and asphalt-resinous components in bitumoid from a sample was 22.8% and 77.2%, respectively. After the experiment, the hydrocarbon concentration increased to 29.3%; the asphalt-resinous components decreased to 70.7%.

The distribution curve of normal alkanes was sawtooth-like dominated by odd hydrocarbons (C_{23} , C_{25} , C_{27} , C_{29} , C_{31} , and C_{33}). At the same time, as their molecular mass grew in the sample before the experiment, the concentration increased, reaching the maximum at $n-C_{31}$ up to 17% of the total amount of n -alkanes (Fig. 2).

The content of $n-C_{33}$ is comparable with $n-C_{23}$ (6.4–6.8% of the total amount of n -alkanes) in the samples before the experiment and with $n-C_{24}$ (4.8% of the total amount of n -alkanes) in the sample after the experiment, respectively.

Carbon preference index (CPI) for high molecular weight hydrocarbons calculated by the formula (Peters et al., 2007) decreased insignificantly from 4.3 to 3.6.

$$\text{CPI} = \left(\frac{C_{25} + C_{27} + C_{29} + C_{31} + C_{33}}{C_{24} + C_{26} + C_{28} + C_{30} + C_{32}} + \frac{C_{25} + C_{27} + C_{29} + C_{31} + C_{33}}{C_{26} + C_{28} + C_{30} + C_{32} + C_{34}} \right) / 2.$$

After the experiment, the ratio of the concentration of normal alkanes to acyclic alkanes in the sample almost twice decreased (32 vs. 58). The value of the $n-C_{27}$ to $n-C_{17}$ ratio decreased fivefold after the experiment (6 vs. 31). Before and after the experiment, the composition of hydrocarbons showed 1- and 3-alkenes, which confirms the low degree of the OM transformation (Fig. 2).

Before the experiment, the composition of hydrocarbons (HC) included alkylcyclohexanes (AC) at m/z 83 dominated by even HCs (AC_{20} , AC_{22} and AC_{24}) in the samples (Fig. 2). After the experiment, the maximum of ACs in the samples shifted to odd HCs (AC_{19} , AC_{21} , AC_{23} , and AC_{25}) (Fig. 2). Chromatography mass fragmentograms at m/z 127 indicated 2,7-dimethylalkanes with a maximum concentration on even HCs, which decreased compared to the content of normal alkanes after the experiment (Fig. 2). Different dominance of even and odd ACs, lower concentrations of 3-alkenes and 2,7-dimethylalkanes were due to the greater OM transformation in the sample after the experiment. Among isoprenoids (C_{13} – C_{25}), phytane had the maximum concentration. The pristane to phytane ratio was 0.5 before and after the experiment.

Ethyl cholestanes dominated the composition of cyclic HC biomarkers of sterane series, i.e., 39–48% of the amount of C_{27} – C_{30} steranes (Fig. 2). The ratios of C_{29} to C_{27} steranes in the sediments before and after the experiment were 1.2–1.4. After the experiment, the sample had a decreased con-

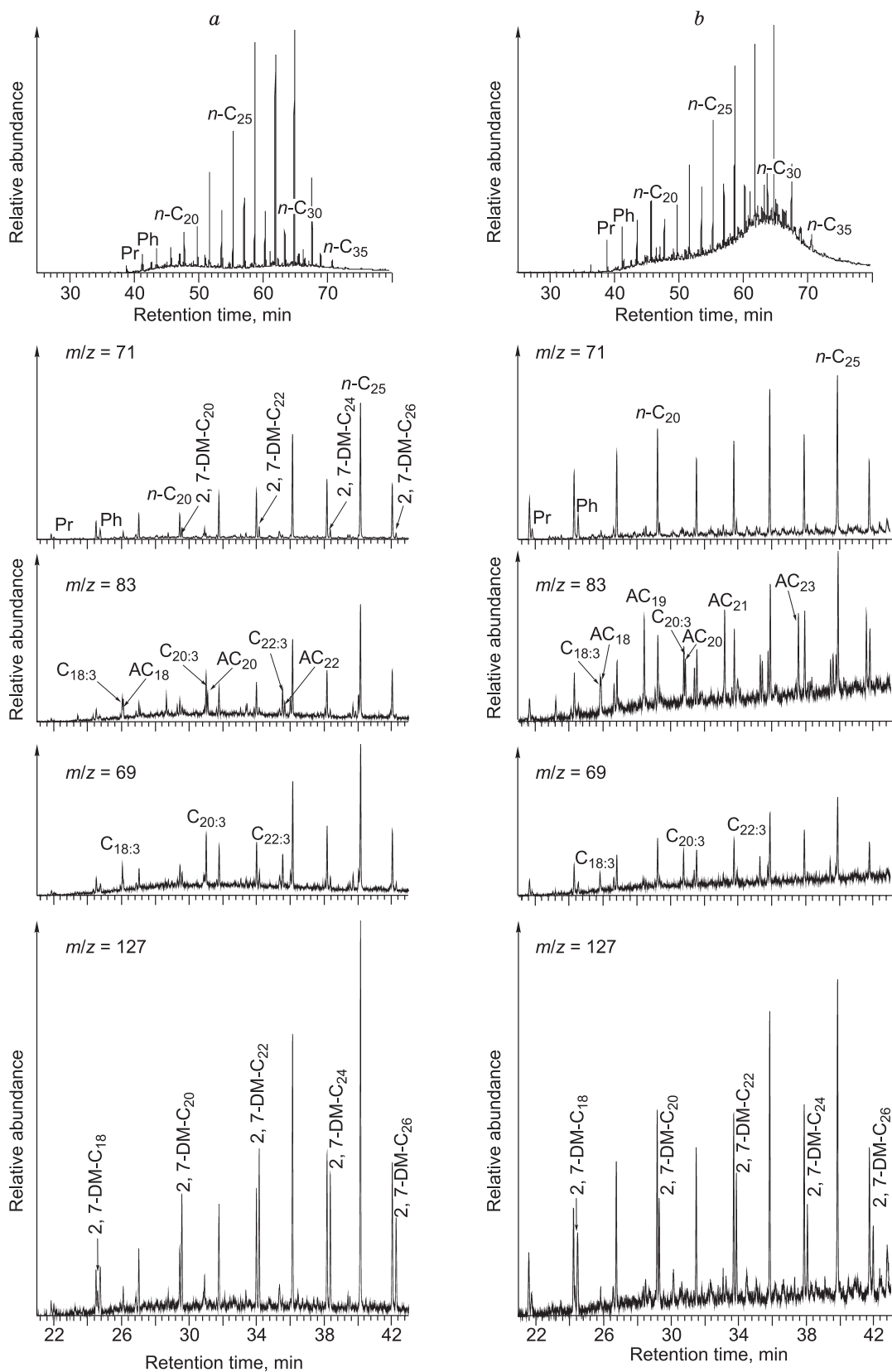


Fig. 2. Chromatograms and chromatography mass fragmentograms of n -alkanes (m/z 71), alkylcyclohexanes (m/z 83) and 3-alkenes (m/z 69) as well as 2,7-dimethylalkanes (m/z 127) in bitumoids from the samples before (a) and after (b) the experiment. n -C_{*n*}, normal alkanes, AC_{*n*}, alkylcyclohexanes, C_{*n*}:3, 3-alkenes, 2,7-DM-C_{*n*}, 2,7-dimethylalkanes, where n is the number of carbon atoms; Pr, prytane, Ph, phenanthrene.

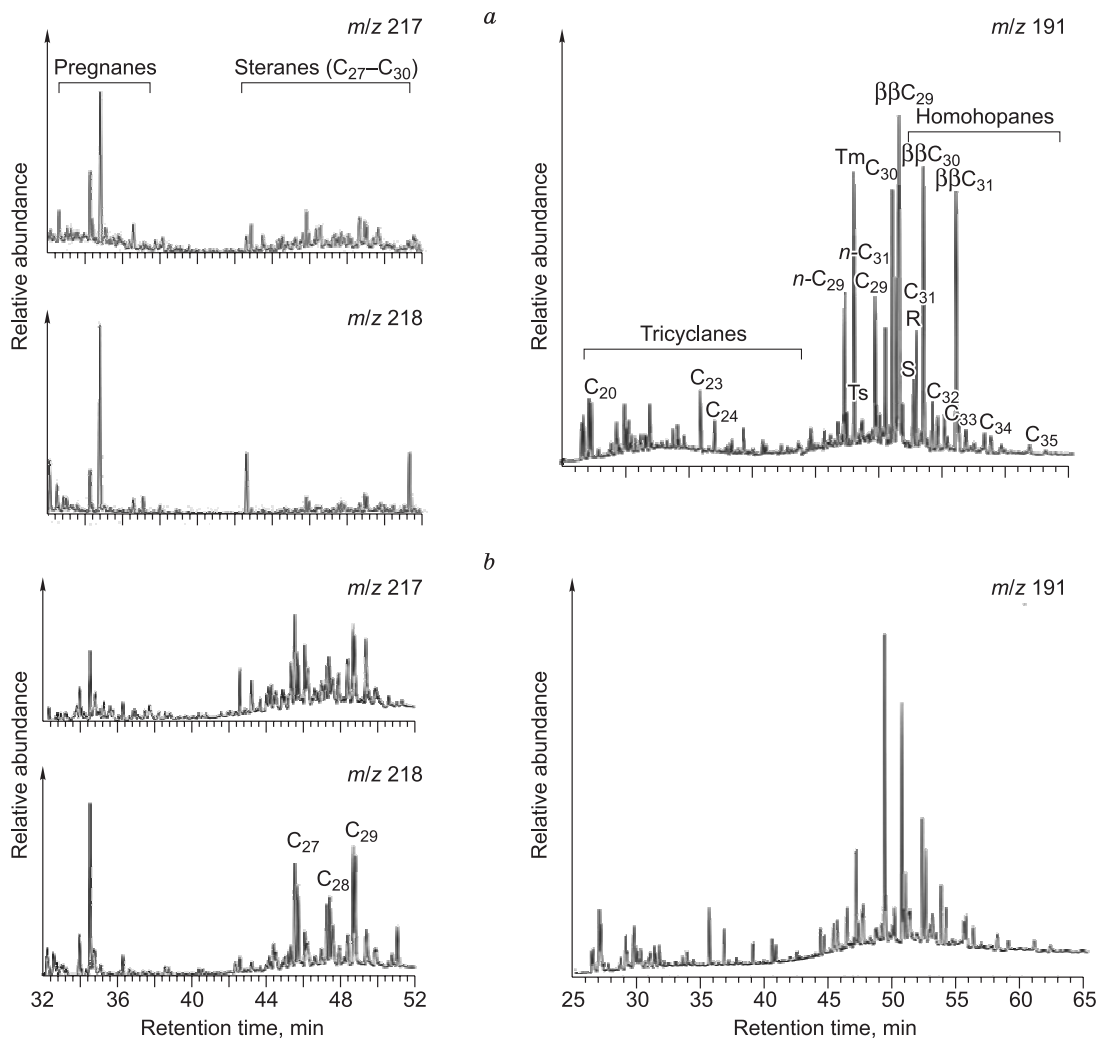


Fig. 3. Chromatography mass fragmentograms of steranes (m/z 217 and 218) and terpanes (m/z 191) in bitumoids from the samples before (a) and after (b) the experiment.

centration of low molecular weight steranes, i.e., pregnanes (the ratio (steranes + pregnanes) to pregnanes was 12.7 vs. 3.6), which was obvious from the chromatography mass fragmentograms at m/z 217 and 218. Hopanes (C_{27} – C_{35}) dominated terpanes (Fig. 3).

HCs with an average chain length of C_{23} – C_{26} dominated tricyclanes (C_{19} – C_{31}) (37.8% of the total amount of tricyclanes). The tricyclane index ($2(C_{19} + C_{20})/\Sigma C_{23-26}$) was 0.6. Before the experiment, the samples showed biohopanes ($\beta\beta$ -hopanes), and the concentrations of R isomers were higher than S isomers in homohopanes (C_{31} – C_{35}). After the experiment, the chromatography mass fragmentogram of the sample at m/z 191 showed trace amounts of biohopanes; S isomers were greater than R isomers in homohopanes, which confirms an increase in the degree of OM transformation (Fig. 3). The ratio of Ts to Tm hopanes remained low after the experiment ($Ts/Tm \leq 0.5$).

The maltene fraction of bitumoids also showed aromatic compounds (Fig. 4). Phenanthrenes showed an increase in

methyl-substituted homologues after the experiment. The value of the methyl phenanthrene index ($MPI-I = (1.5 \times (2MP + 3MP))/(P + 1MP + 9MP)$) changed insignificantly (≤ 0.8). The calculation of the maturity parameter (PP-1 modified = $(1MP + 9MP)/(2MP + 3MP)$) indicated a larger variation (Peters et al., 2007). Phenanthrenes also included retene, which is formed in coniferous plants (Kashirtsev et al., 2018). After the experiment, there was an increase in methyl-substituted homologues in the composition of dibenzothiophenes. At the same time, the maturity parameter 1-MDBT/4-MDBT did not change and was 2.0. There were monoaromatic steroids in maltenes after the experiment at m/z 253, and the ion at m/z 366 showed four diastereomers, 17-dimethyl, 23-methyl monoaromatic steroids C_{27} , which were previously detected in the Carboniferous, Permian and Cretaceous deposits (Kashirtsev et al., 2016; Kashirtsev, 2018). Figure 4 indicates that after the experiment the concentration of aromatic compounds increases in comparison with normal alkanes. Changes in the composition of aro-

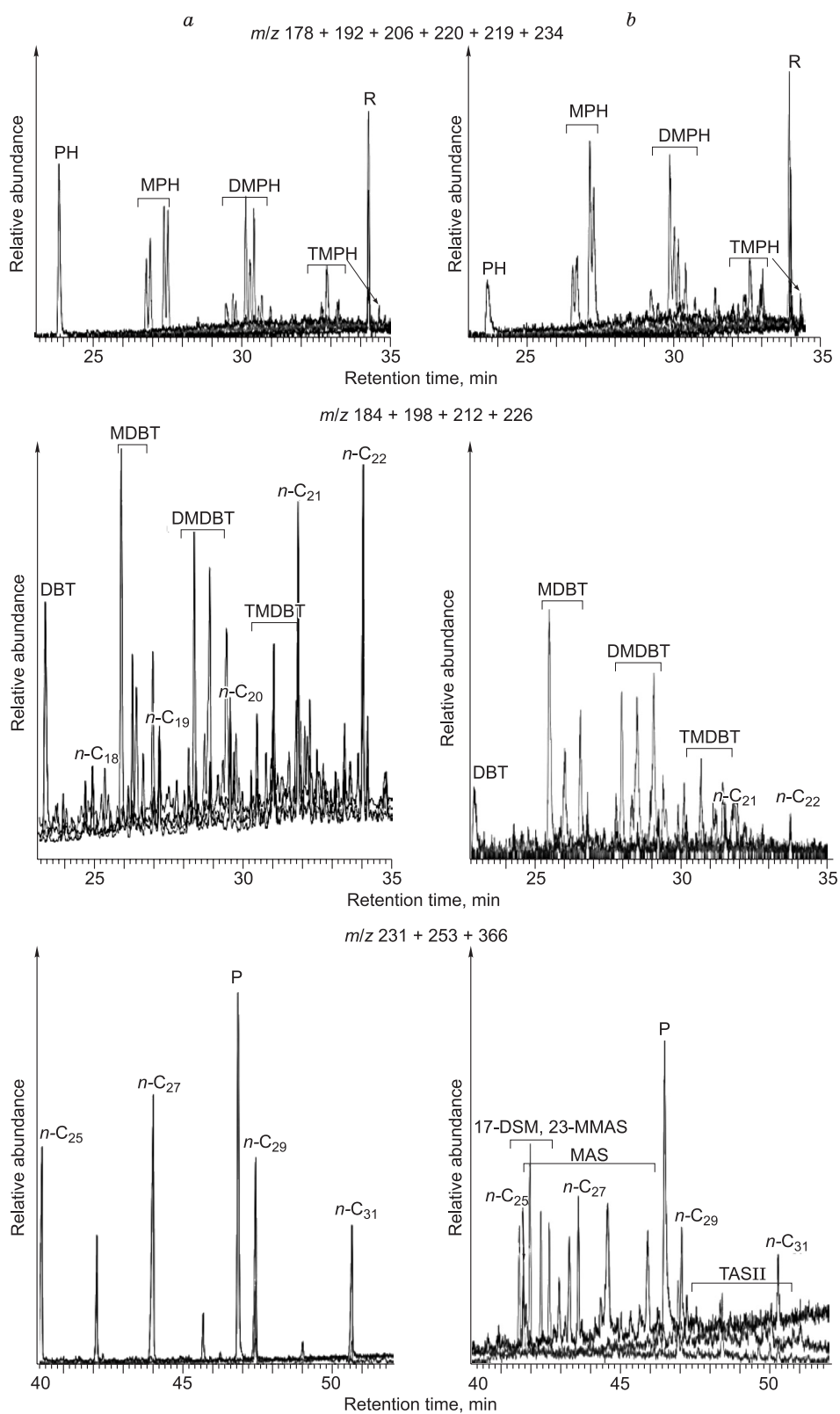


Fig. 4. Chromatography mass fragmentograms of phenanthrenes (m/z 178, 192, 206, 219, 220, and 234), dibenzothiophenes (m/z 184, 198, 212, and 226) and aromatic steroids (m/z 253, 231 and 366) in bitumoid maltenes from the samples before (a) and after (b) the experiment. PH, phenanthrenes; MPH, methyl-phenanthrenes; DMPH, dimethyl-phenanthrenes; TMPH, trimethyl-phenanthrenes; R, retene (1-methyl, 7-propylphenanthrene); DBT, dibenzothiophenes; MDBT, methyl-dibenzothiophenes; DMDBT, dimethyl-dibenzothiophenes; TMDBT, trimethyl-dibenzothiophenes; steroids: TAS, triaromatic steroids; MAS, monoaromatic steroids and 17-DSM, 23MMAS, 17-dismethyl, 23-methyl monoaromatic steroids C_{27} ; P, perylene and $n-C_n$, normal alkanes, where n is the number of carbon atoms.

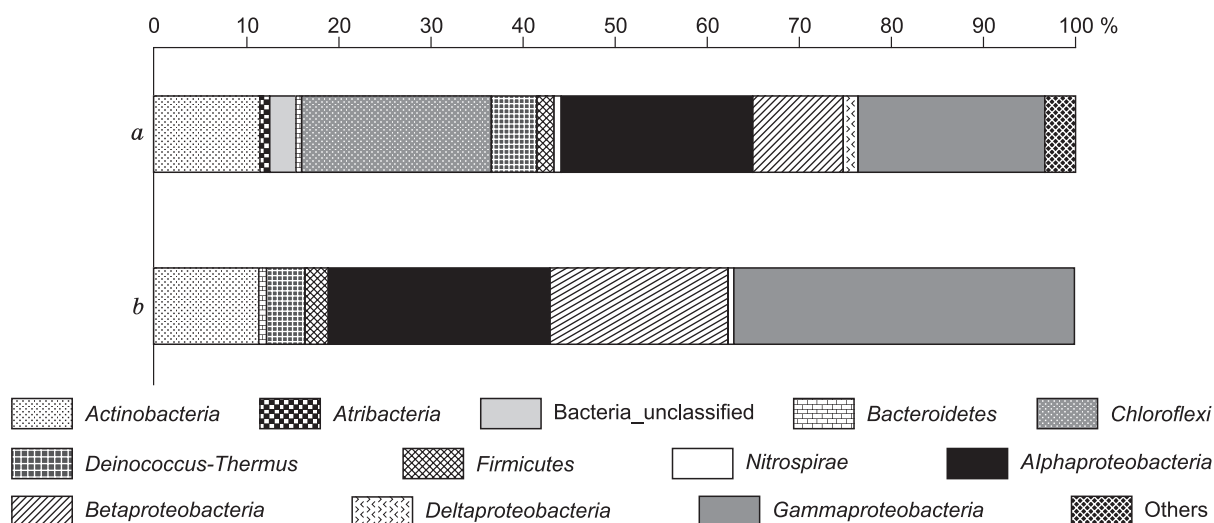


Fig. 5. Composition of the bottom sediments bacterial community from the Khoboy mud volcano before (a) and after (b) the temperature-pressure cultivation.

matic compounds also confirm the greater OM transformation after the experiment. HC fraction contained perylene, i.e., polyaromatic HC. Its concentration in the sediment sample was higher after the experiment. It is considered to have both aquagenic and terrigenous source.

Phylogenetic composition of the microbial community in the bottom sediments samples before the experiment.

The results of massively parallel sequencing of 16S rRNA gene amplicon libraries of the natural sample before the experiment have indicated that the sequences of the members of the phyla *Chloroflexi* (20.7%), *Actinobacteria* (11%), α - (20.7%), β - (9.7%) and γ -*Proteobacteria* (20.5%) classes, and *Deinococcus-Thermus* (5%) are the most numerous among *Bacteria*. The 16S rRNA gene sequences of *Atribacteria* (OP9), *Bacteroidetes*, *Firmicutes*, *Nitrospirae*, δ -*Proteobacteria* class, etc. are minor (less than 3%) (Fig. 5a).

In the 16S rRNA gene library of archaea, there were sequences of the phyla *Bathyarchaeota* (96.2%) and *Diapherotrites* (0.14%) as well as *Thermoplasmata* (3.2%) and *Methanomicrobia* (0.1%) classes representing the phylum *Euryarchaeota* (data not shown).

The dominance of the members of the phyla *Proteobacteria* (α -, β -, γ -) and *Actinobacteria* in the composition of the BS microbial communities is characteristic of most sediment samples from other areas of Lake Baikal associated with the HC discharge. Previously, the molecular biological analysis of the composition of microbial communities from the areas characterized by the discharges of oil- and gas-bearing fluids showed a significant share of 16S rRNA gene sequences of the phyla *Proteobacteria*, *Actinobacteria* and *Cyanobacteria* (Zemskaya et al., 2015; Chernitsyna et al., 2016), as in the sediments of many other freshwater lakes (Winters et al., 2014; Zhang et al., 2014; Ding et al., 2015).

The 16S rRNA gene library of the microbial community had a high content of α -*Proteobacteria* (20.7%). The order

Shpingomonadales represented this class, which members are found in various habitats (freshwater and marine ecosystems, soils, plant root systems, clinical specimens, etc.). The widespread occurrence in the environment is due to the ability to use a wide range of organic compounds, including toxic ones, as well as grow and survive under low levels of nutrients.

Sequences of β -*Proteobacteria* comprised 9.7% of the total number with the dominance of the sequences representing the order *Burkholderiales*, which can anaerobically oxidize acetate with perchlorate as electron acceptors in surface sediments (Yoshida et al., 2005) or oxidize hydrogen in deep sediments (Orcutt et al., 2011). Before the experiment, the DNA library of the sample had the sequences of the genera *Pelomonas* and *Methylophilus*. The latter ones oxidize methanol and use nitrate salts and ammonium salts as a nitrogen source.

The class γ -*Proteobacteria* represented the sequences of the orders *Oceanospirillales*, *Alteromonadales* and *Pseudomonadales*, which members, except for *Pseudomonadales*, are mainly halophilic inhabitants of marine ecosystems.

Chloroflexi sequences comprised 20.7% of the total number. The share of the sequences of this phylum was comparable with that (21.8%) identified in the microbiomes from the deep bottom sediments of the methane seep Posolsk Bank (Chernitsyna et al., 2016). There were mainly sequences of the uncultivated group MSB-5B2, class *Dehalococcoidia* and family *Anaerolineaceae*. The sequences of the genus *Pelolinea*, which members were isolated from the bottom sediments methanogenic community in Japan (Imachi et al., 2014), were the most representative among the sequences of the family *Anaerolineaceae*. At the same time, the members of the class *Dehalococcoidia* use hydrogen as an electron donor and obtain the energy through organolipid respiration (by reducing dehalogenated organic compounds) (Major et al., 2002).

The sequences of the orders *Micrococcales*, *Corynebacteriales* and *Propionibacteriales* represent the phylum *Actinobacteria*, which usually dominates the bottom sediments of Lake Baikal. *Propionibacteriales* comprised 95% in the 16S rRNA gene library. Bacteria of the genus *Propionibacterium* are the anaerobic microorganisms with a special metabolism, which are capable of synthesizing propionic acid, using the unusual enzymes transcarboxylases (Cheung et al., 1975).

In the 16S rRNA gene library of the microorganisms detected in the sediments prior to cultivation, the bacterial sequences of the phylum *Deinococcus-Thermus* (5%) were also numerous; though they are usually minor in other Baikal areas, except for deep bottom sediments of the methane seep Posol'sk Bank (Chernitsyna et al., 2016). Among the sequences of the phylum *Deinococcus-Thermus*, the operational taxonomic units of bacteria of the genus *Thermus* were the most representative. Their obligate aerobic, chemorganoheterotrophic, extremely thermophilic members inhabit hot springs, deep marine springs and geysers of Yellowstone National Park (Garrity et al., 2001).

Therefore, the results of the comparative analysis of nucleotide sequences of the 16S rRNA gene libraries of bacteria and archaea indicated that before the experiment the natural microbial community of the studied bottom sediments sample contained phyla with numerous metabolic capabilities, which are widely distributed in freshwater environments (Newton et al., 2011). Along with the typical mesophilic microorganisms, the microbial community contained members of thermophilic taxa (*Deinococcus-Thermus*, *Firmicutes*, etc.), whose dominance is characteristic of the communities from various marine deep ecosystems, including seeps and gas-hydrate sites (Orcutt et al., 2011; Parkes et al., 2014; Ruff et al., 2015).

In the 16S rRNA gene libraries of the domain *Archaea*, the sequences of the members of the phylum *Bathyarchaeota* dominated. It contains many various phylogenetic lineages, which are ubiquitous (Inagaki et al., 2006; Webster et al., 2006; Yanagawa et al., 2014; Parkes et al., 2014). The members of *Bathyarchaeota* are likely to have an enzymatic type of metabolism and/or ability to methylotrophic methanogenesis (Evans et al., 2015; Lazar et al., 2016; Kallistova et al., 2017). Another widely represented archaeal group was *Thermoplasmata*. Although most cultured strains of this class were isolated from aerobic/thermophilic habitats, sequences of uncultivated members (uncultivated *Thermoplasmatales* in our case) are numerous in cold bottom sediments of marine and freshwater environments (Lloyd et al., 2013). The members of other detected taxa can also perform the functions of primary and secondary anaerobes in the microbial community of the studied bottom sediments. These are *Deinococcus-Thermus*, *Firmicutes*, *Atribacteria* (OP9), *Bacteroidetes*, *Nitrospirae*, and δ -*Proteobacteria*.

After seven months of cultivation under the conditions of temperature and pressure in HPC No. 1 containing bottom

sediments enriched with the biomass of *S. acus* diatom, there were changes in both, the OM composition and the composition of the microbial community.

In the 16S rRNA gene libraries, there were no sequences of the members of the domain *Archaea*. Among the sequences of the domain *Bacteria*, there were no members of the phyla *Atribacteria*, *Nitrospirae*, *Chloroflexi* and class *Deltaproteobacteria*. The percentage of the sequences of the phyla *Actinobacteria*, *Deinococcus-Thermus* and *Firmicutes* remained the same. After cultivation, the sequences of the phylum *Proteobacteria* (α -, β -, γ -) dominated the microbial community (Fig. 5b). The lack of significant changes in the composition of the microbial community is likely due to the dominance of microorganisms in the natural sediments, which are capable of surviving under anaerobic thermophilic conditions. These include the members of the phyla *Actinobacteria*, *Deinococcus-Thermus* and *Firmicutes*, which can come with mineralized fluids from deep sediments.

Our studies showed that during the cultivation the initially immature OM from bottom sediments of a mixed type (terrigenous-aquagenic) became more mature in the experiment despite the slight decrease in the CPI coefficient.

Since HC molecules with an odd number of carbon atoms dominate OM of sediments, which did not have diagenetic and catagenetic transformations, the odd/even ratio (the CPI coefficient in various modifications) is equal to 5–6. During the thermal OM transformation, the number of even and odd HC molecules equalizes. The value of the CPI coefficient becomes close to one, and it does not change with the subsequent late catagenetic transformations (after passing through the main stage of oil formation) (Mukhopadhyay et al., 1979). In nature, temperature conditions of the thermal OM decomposition (catagenesis) vary from 85 to hundreds of degrees Celsius (Schobert, 2013). However, in our case (cultivation at 80 °C), the recorded changes are a consequence of the processes of biological destruction, since these values approach the threshold for living organisms; on the other hand, they are the lower limit of the values characteristic of the mesocatagenesis, preventing from the influence of the physical factors on the OM transformation.

The recorded changes in the OM composition of bottom sediments from the mud volcano Khoboy are rather insignificant (16%) in comparison with the data obtained in the experiment with bottom sediments of the methane seep Posol'sk Bank, where this indicator is 41% (Bukin et al., 2016). During even a sufficiently short exposition period (seven months), the cultivation of the sediments from the Khoboy mud volcano indicated a decrease in the concentration of phenanthrenes relative to its methyl-substituted homologues (including retene) and an increase in sulfur-containing compounds (dibenzothiophene and its methyl-substituted homologues) relative to normal alkanes. We also recorded the formation of monoaromatic steroids, including four diastereomers, 17-dimethyl, 23-methyl monoaromatic steroids C_{27} , and triaromatic steroids. The dominance of

S-isomers over R-isomers in homohopanes and the traces of biohopanes also indicate a greater OM transformation in the sample after the experiment.

The decrease in the concentration of phenanthrenes can be due to the activity of the members of the genera *Pseudomonas*, *Burkholderia*, the phylum *Actinobacteria*, and *Firmicutes*, whose 16S rRNA gene sequences were significant in the composition of the microbial community in the bottom sediments samples before and after the experiment. The ability of the members of these taxa to degrade the polycyclic aromatic HCs is generally recognized. Previously, bacteria of the genera *Pseudomonas* and *Bacillus* isolated from Lake Baikal showed selective biodegradation of fluoranthene, phenanthrene and pyrene. In a model experiment for 30 days, the conversion degree of polycyclic aromatic HCs was 18–30% (Pavlova et al., 2005).

Steroids are widespread in the biosphere. They mainly include cholesterol (C_{27}), campe- and crinosterols (C_{28}), sito- and stigmasterols (C_{29}), lanosterol and cycloartenol (C_{30}). Algae have the widest range of steroids, including derivatives of all groups (Volkman, 1986; Kodner et al., 2008). The increase in the concentration of tri- and monoaromatic steroids in the sediment sample after the experiment can indicate the biodegradation of the biomass of the Baikal diatom *S. acus*. As previously shown, diatoms produce various sterols, which can have 3–10 compounds in the isolated sterol fractions (Kalinovsky et al., 2010). Moreover, the Baikal diatom *Stephanodiscus meyerii* Genkal and Popovskaya has two main sterols in the composition: cholesterol and 24-methylenecholesterine, as well as phytol (Ponomarenko et al., 2004).

Our results correlate with the data obtained in (Serebrennikova et al., 2014). The researchers showed that C_{29} -steroids dominate the peat samples from lowland swamps of West Siberia. This indicated that the microalgae *Botryococcus braunii* were the main peat-forming plants (Metzger et al., 1990). In these microalgae containing C_{27} -, C_{28} - and C_{29} -sterols, high molecular weight homologues of *n*-alkanes dominate.

Dibenzothiophenes (DBT) normally accumulate during the diagenetic transformations of the aquagenic OM in the sediments of marine water bodies with hydrosulphuric contamination or oxygen deficiency in the near-bottom waters. In fresh or even salty but well-aerated water bodies, the formation of DBT in the significant concentration does not occur. They do not form in bitumoids of well-aerated freshwater bodies as well as during the burial of the remnants of the higher terrestrial vegetation (Kontorovich et al., 2004). In case of our temperature and pressure experiment, the microbial community was cultivated under anaerobic conditions with the atmospheric methane. These conditions could contribute to the formation of DBT in the freshwater sediment samples.

The transformation of the aquagenic-terrigenous OM of bottom sediments resulted in the formation of mono- and triaromatic steroids and sulfur-containing compounds, DBT. The obtained results correlated with the data shown by

A.E. Kontorovich with co-authors. The study of bitumoids from different-facies Jurassic rocks of the West Siberian basin indicated that in the terrigenous OM phenanthrene and its methyl derivatives are mainly formed; whereas in the aquagenic OM, along with phenanthrenes, many aromatic steroids are formed. Steroids of the buried aquagenic OM are mostly transformed into triaromatic ones, but steroids of the terrigenous OM—into monoaromatic ones (Kontorovich et al., 2004).

CONCLUSIONS

The experiment performed under the conditions characteristic of the zone of HC generation (80 °C, 5 MPa) has shown that the cultivation of the microbial community of bottom sediments from the area of the Khoboy mud volcano (Akademicheskoy Ridge) enriched with the biomass of the Baikal diatom *Synedra acus* changes the OM composition. The degree of the OM transformation in the sediments after the experiment was 16%. At the same time, the concentration of phenanthrenes relative to methyl-substituted homologues, including, retene, decreased. The concentration of DBT relative to normal alkanes increased. We have detected tri- and monoaromatic steroids, including 17-dimethyl-, 23-methyl monoaromatic steroids C_{27} .

Diatoms produce a variety of sterols; sterol fractions can have 3–10 compounds. An increase in the concentration of tri- and monoaromatic steroids in the sediment sample after the experiment can indicate that the biomass of the Baikal diatom *S. acus* was destroyed, which increased the concentration of sterols. The dominance of S-isomers over R-isomers in homohopanes and trace concentrations of biohopanes also indicate a greater OM transformation in the sample after the cultivation. The control (sterile) sediment did not show the changes in the OM composition. The lack of significant changes in the composition and diversity of the bottom sediments microbial community after the exposure under the experimental conditions is likely due to the microorganisms capable of surviving in the anaerobic thermophilic conditions (the members of the phyla *Proteobacteria* (α -, β -, γ -), *Actinobacteria*, *Deinococcus-Thermus*, and *Firmicutes*), which dominate the natural sediments. They can come along with mudflows as a part of the deep-seated sedimentary rocks.

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