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EXPERIMENTAL ARTICLES

Microbial Communities of the Stratified Soda Lake Doroninskoe (Transbaikal Region)

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Abstract—The physicochemical properties, species composition, and vertical distribution of microorganisms in the water column, shoreline microbial mat, and small shoreline mud volcanoes of the stratified soda Lake Doroninskoe were investigated in September 2007. The lake is located in the Transbaikal region, in the permafrost zone (51°25′ N; 112°28′ E). The maximal depth of the contemporary lake is about 6 m, the pH value of the water is 9.72, and the water mineralization in the near-bottom horizon is 32.3 g l^{-1}. In summer, the surface oxygen-containing horizon of the water column becomes demineralized to 26.5 g l^{-1}; at a depth of 3.5–4.0 m, an abrupt transition occurs to the aerobic zone containing hydrosulfide (up to 12.56 g l^{-1}). Hydrosulfide was also detected in trace quantities in the upper water horizons. The density stratification of the water column usually ensures stable anaerobic conditions until the freezing period (November and December). The primary production of oxygenic phototrophs reached 176–230 μg l^{-1}. High rates of dark CO_2 assimilation (61–240 μg l^{-1}) were detected in the chemocline. Within this zone, an alkaliphilic species of sulfur-oxidizing bacteria of the genus *Thioalkalivibrio* was detected (10^4 cells ml^{-1}). Lithoheterotrophic bacteria *Halomonas* spp., as well as bacteriochlorophyll *a*-containing aerobic anoxygenic phototrophic bacteria (AAP) *Roseinatronobacter* sp. capable of thiosulfate oxidation, were isolated from samples collected from the aerobic zone (0–3 m). The water transparency in September was extremely low; therefore, no visible clusters of anoxygenic phototrophic bacteria (APBs) were detected at the boundary of the hydrosulfide layer. However, purple sulfur bacteria which, according to the results of the 16S rRNA gene analysis, belong to the species *Thioalkalicoccus limnaeus, Ectothiorhodospira variabilis, "Ect. magna,"* and *Ect. shaposhnikovii*, were isolated from samples of deep silt sediments. *Ect. variabilis* and *Ect. shaposhnikovii* were the major APB species in the shoreline algo–bacterial mat. The halotolerant bacterium *Ect. shaposhnikovii*, purple nonsulfur bacteria of the genus *Rhodobacter*, and AAP of *Roseococcus* sp. were isolated from the samples collected from mud volcanoes. All these species are alkaliphiles, moderate halophiles, or halotolerant microorganisms.

Key words: meromictic soda lakes, permafrost zone, activity of microbial processes, alkaliphiles, halophiles, phototrophic bacteria, sulfur-oxidizing bacteria.

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In the steppe and forest-steppe zones of the Transbaikal region with a typical continental climate, shallow, often intermittent lakes are common, with water mineralization ranging from several grams of salts per liter to the saturation level [1, 2]. Sodium chloride and sodium sulfate prevail in the chemical composition of their waters. However, due to the presence of high concentrations of carbonates, the pH level of the water in these lakes is high. The soda Lake Doroninskoe, which is located in the eastern Transbaikal region, differs markedly from typical holomictic steppe lakes [3]. It belongs to a rare type of moderately saline alkaline soda lake with the carbonate type of mineralization and pronounced meromixis, with hydrosulfide present in the monimolimnion. There are rather few meromictic soda lakes. They are located in the United States and the African Rift Zone and are affected by postvolcanic processes. Mono Lake (California,

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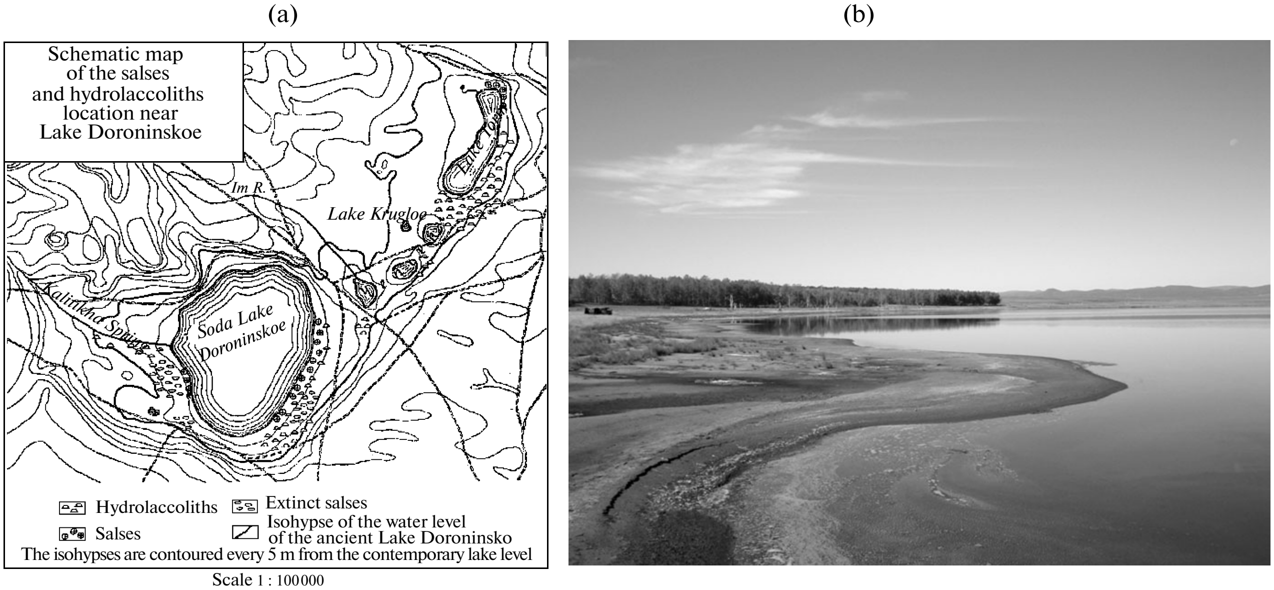

Fig. 1. Lake Doroninskoe: cartographic scheme (a) [9]; photograph of the contemporary lake with permafrost salses (b).

United States) [4, 5], Big Soda Lake [6], Soap Lake (Nevada, United States) [7], and the small Sonachi Lake (Kenya) [8] are among these lakes. The content of sulfide in the water of Soap Lake is extremely high (over 700 mg l^{-1}).

Stratified soda lakes have aroused considerable scientific interest, since it is assumed that such water bodies existed at the early stages of formation of the global ecosystem on Earth and, probably, on other planets and their satellites [7].

Lake Doroninskoe, located in the permafrost zone, is so far the only described meromictic soda lake in the Transbaikal region and all of Siberia [3, 9, 10]. Its hydrological regime, the chemical composition of its brine, and the composition of minerals precipitating as a result of brine freezing have been studied in detail [11, 12]. At the same time, available microbiological data are scarce [13].

The goal of this work was to study the microbial diversity and microbial processes of the carbon and sulfur cycles occurring in the soda Lake Doroninskoe and associated shoreline ecosystems.

MATERIALS AND METHODS

Characterization of Lake Doroninskoe. Lake Doroninskoe (previously Lake Selitryanoe) has been mentioned in the literature since 1782 [9, 10]. It belongs to the class of continental soda lakes and is located 140 km from Chita (51°25′ N; 112°28′ E), in a depression on the left bank of the Ingoda River, one of the sources of the Amur River [3]. There, the Ingoda valley runs along the southeastern border of the depression, between the Chersky and Yablonevyi ranges. Lake Doroninskoe is a relic lake formed at the site of a large ancient basin. The contours of the former lake can be discerned in a three-step terrace framing the lake. The lake is situated in the steppe zone with a typical arid climate. The lowering of the lake level due to drying was slow. The contemporary lake is ovoid and northeast–southwest oriented (Fig. 1). The surface area of the lake is approximately 5 km^2; the maximum depth is up to 7 m in high water years. The lake is closed; it is fed by the Kalitikha Spring flowing from the Yablonevyi range. The water balance of the lake largely depends on atmospheric precipitation [9, 10, 12, 14]. Pore and stratal waters of Mesozoic sediments play a significant role in feeding the lake [12]. The feed waters are hydrocarbonate, with mineralization not exceeding 1 g l^{-1}, and with calcium as the predominant cation. The waters discharge subaqueously along the shoreline and at the lake bottom [12, 15].

The brine of the contemporary lake is characterized by moderate mineralization (up to 36.0 g l^{-1}) [4]. Carbonate, bicarbonate, and sodium chloride are the main components. The mineralized water of Lake Doroninskoe is a result of the solution concentration in the course of evaporation and freezing. The lake is covered by ice almost 1.5 m thick for seven months of the year, from the end of October to the end of May. Migration of the solution through ice results in the deposition of carbonate minerals (gudzhir) on the ice surface [11]. During the ice-free season, salinity stratification of the water column occurs. At depths below 3.5–4 m, high concentrations of hydrosulfide (which in some years reached 64.6 mg l^{-1}) were observed [4].

The silt sediments of the lake are 4 to 10 m thick, finely dispersed, and black with greenish hue due to the presence of chlorophyll residues. They belong to sulfide muds containing up to 0.4% of iron sulfides [16].

Lake Doroninskoe is located in the permafrost zone. The depth of summer thaw of the soil ranges from 0.8 to 5 m [9, 16]. On the first and second terraces, hydrolaccoliths, mud volcanoes (MVs), earthflows, frost boils, and mires are numerous; these structures are various manifestations of permafrost salses [9, 14]. The swellings are filled with alkaline waters. It has been suggested that there are mud volcanoes at the lake bottom [14]. Some of them can be seen below the water surface within the shoreline zone. The mud volcanoes are 1–2 m in diameter and 45–75 cm high. The craters are filled with black or brown liquid alkaline mud. Formation of gas bubbles can be observed. According to our measurements (data not presented), the gas consists mainly of methane. The main constituents of the volcanic fluids are the following (g l^{-1}): CO_3^{2-}, 6.1; HCO_3^-, 5.6; Cl^-, 3.22; Na^+ and Ca^{2+}, 0.0013; and Mg^{2+}, 0.012 [9, 14].

Within the northeastern zone, on terraces 1 and 2 of the exposed lake depression, large travertine blocks formed mainly by the precipitation of calcium carbonate can be seen [9, 15]. The formation of tuff deposits obviously resulted from the deep water discharge into the brine of the ancient lake. Modern deposits consisting of carbonate crusts and travertine debris in the shoreline zone of the contemporary Lake Doroninskoe were detected.

Hence, in Lake Doroninskoe and its immediate surrounding areas, a system of interrelated ecological zones has developed, which differ from each other in physicochemical conditions, which determine the uniqueness of the microbial processes occurring there.

Field studies. Water samples were collected from different horizons with a Ruttner bathometer. Samples of deep silt were collected with a bottom sampler. In the shoreline zone of the lake and in the areas of mud volcanoes activity, silt and microbial mat samples were collected with a spatula into sterile plastic screw-capped Falcon tubes. The temperature was measured with an electric sensor thermometer (Amarell Electronics, Germany); pH and *Eh* were measured with portable devices (HANNA Instruments, United States). Transparency was measured with a Secchi disc. The total mineralization was determined with a Comecta refractometer (Barcelona, Spain). Sulfide concentration was determined colorimetrically, sulfate content was determined turbidimetrically, and the concentrations of carbonate and bicarbonate were determined by titration with 0.1 N HCl using phenolphthalein and methyl orange as indicators. Oxygen content was determined using the modified Winkler method allowing for the presence of sulfide [17].

The rates of photosynthetic activity and dark CO_2 assimilation were determined in separate samples using radioactive ^{14}C bicarbonate; the rate of sulfate reduction was determined using labeled sulfate [1, 2, 17]. The rate of methanogenesis was determined using ^{14}C-labeled bicarbonate as described earlier [2, 18]. The samples in 20-ml glass vials were incubated for 12 h under in situ illumination and temperature conditions. When calculating the production values according to the known formula [17], only the dissolved carbon of bicarbonate was taken into account. This assumption was made considering the high total carbonate content and the presumably slow redistribution of ^{14}C-labeled bicarbonate between natural bicarbonate and carbonate.

Laboratory analyses. Chlorophylls were determined quantitatively on an SF 56 spectrophotometer (LOMO, Russia) using acetone–methanol (7 : 2) extracts from the membrane filters used to filter 10-ml water samples [19]. Direct counting of microbial cells was carried out on membrane filters through which 2 ml of the water samples was passed [17]. The filters were dried, stained with erythrosine, and examined under an Olympus BX-41 light microscope with an ×100 objective. Tentative identification of cyanobacteria was carried out according to the cell morphology using the manual by Komarek et al. [20, 21]. DNA was extracted from the samples of cyanobacterial mats [22]. DGGE analysis of the products of the seminested PCR assay was carried out using the primer pairs CYA359F–23S30R and CYA359F–CYA781RGC(a) and (b) [23]. Enumeration of alkaliphilic microorganisms and isolation of pure cultures were carried out by inoculation of selective media with material from samples stored in a refrigerator for 2 weeks. For anaerobic phototrophic bacteria (APBs), a modified Pfennig medium was used [24, 25]. For alkaliphilic purple nonsulfur bacteria and aerobic bacteriochlorophyll *a*-containing bacteria (aerobic anoxygenic phototrophs, AAPs), the previously described media were used [26, 27]. Postgate medium was used for the enumeration of sulfate-reducing bacteria [17]. Sulfur-oxidizing bacteria were isolated under microaerobic conditions on an autotrophic thiosulfate-containing soda medium (pH 10) with 0.6 M of total sodium [4]. The total mineralization and pH of the media corresponded to those in the samples obtained. The reactions of the isolated bacteria to the levels of mineralization and pH in the medium were determined as described earlier [25, 27].

Nearly complete 16S rRNA gene sequences (1300–1500 nucleotides) of the obtained cultures of photosynthetic and sulfur-oxidizing bacteria were determined. Comparative analysis of the obtained nucleotide sequences was performed using the NCBI BLAST software package (http://www.ncbi.nih.gov). Both morphophysiological and genetic characteristics of the identified bacterial cultures were used for identification.

RESULTS

Physicochemical Conditions of Lake Doroninskoe

In early September, stratification of the water column was observed, supported by the temperature gradient and salinity stratification. The halocline (3.5 m) was slightly above the thermocline and chemocline, which occupied the same positions within the water column (4–4.25 m) (Figs. 2a and 2b). The total water mineralization increased from 26.5 to 32 g l^{-1} in the near-bottom horizon at a depth of 5 m (Table 1). The oxygen concentration was about 8.0 mg l^{-1}. At a depth of 4.0 m, oxygen disappeared completely and sulfide was detected. The highest sulfide concentration (12.56 mg l^{-1}) was recorded at a 4.5-m depth, while in the near-bottom horizon it was lower (8.4 mg l^{-1}). Reduced sulfur compounds, albeit in trace amounts, were detected in the mixolimnion as well. The water transparency determined using a Secchi disk was low (1.2 m), which limited the euphotic zone to a 2.4-m depth. The pH of the water was alkaline throughout the whole water column and varied insignificantly from 9.57 to 9.75.

Hence, the conditions in the water column of Lake Doroninskoe were favorable for the growth of alkaliphilic microorganisms. The presence of sulfur compounds both in the anaerobic zone and in the mixed upper water horizons suggests that microorganisms involved in the sulfur cycle play an important role in the lake ecosystem. During our study, the illumination intensity in the chemocline was extremely low, which promoted predominance of dark microbial processes at the interface between the sulfide and oxygen zones.

Microorganisms and Microbial Processes in Lake Doroninskoe

According to the results of a direct count performed in September 2007, the numbers of bacteria along the vertical profile of the water column were distributed in accordance with changes in the water density and redox conditions (Fig. 2c). In this respect, Lake Doroninskoe belongs to mesotrophic–eutrophic type of lakes. The first maximum of the total microbial number was detected at the surface, where, during the sampling period, the temperature was the highest (18.6°C). As the temperature decreased, the number of bacterial cells decreased as well from 4.55 to 1.97 × 10^6 cells ml^{-1} and then increased sharply at a depth of 3.5 m reaching the second maximum (6.8 × 10^6 cells ml^{-1}). This maximum coincided with the lower boundary of light penetration (triple transparency depth) and the onset of the increase in water mineralization. The next maximum (9.7 × 10^6 cells ml^{-1}) was detected in the chemocline, where oxygen disappeared and sulfide was detected. This maximum coincided with emergence of the microorganisms utilizing sulfide and other reduced compounds from the monimolimnion.

Production and Destruction Processes

Deep-water station. Aerobic zone. Conditions in the water of Lake Doroninskoe make it a favorable ecological niche for sulfur-oxidizing bacteria, since sulfide, thiosulfate, and sulfite are available all year round both in the monimolimnion and in the aerobic zone of the mixolimnion. Two strains of alkaliphilic aerobic chemoorganotrophic bacteria of the genus *Halomonas* were isolated from the surface water horizon. According to the results of 16S rDNA analysis, one strain (Fig. 3a, Table 2) was closely related to *Halomonas salina* and *Halomonas ventosae* (98% similarity) and the other belonged to the species *Halomonas campisalis* (100% similarity). Bacteria of the genus *Halomonas* are able to oxidize thiosulfate in the presence of organic substrates and grow under anaerobic conditions as denitrifiers. They are probably involved in the oxidation of reduced sulfur compounds both under aerobic conditions in the mixolimnion and under anaerobic conditions in the monimolimnion. Aerobic bacteriochlorophyll *a*-containing bacteria (AAP) of the genus *Roseinatronobacter* (Fig. 3c, Table 2) isolated from the water samples collected in the aerobic zone are also capable of chemolithoheterotrophic growth coupled to thiosulfate oxidation. Analysis of the 16S rRNA gene sequences showed a 95% similarity between the isolated strain and the known species of this genus. This isolate was tentatively classified as a new species, "*Roseinatronobacter doroninskoiensis*," the description of which is in preparation.

Filamentous cyanobacteria *Phormidium* spp, heterocystous *Nodularia* sp., and unicellular cyanobacteria *Synechococcus* sp. and *Synechocystis* sp, together with the diatoms *Nitzschia* sp. were the major representatives of oxygenic phototrophs in the planktonic community of the mixolimnion (Fig. 4a–4d). The absorption spectra of the acetone–methanol (7 : 2) extracts with a maximum absorption at 661–664 nm (Fig. 5) obtained from the concentrated water samples corresponded to those of chlorophyll *a*, indicating that cyanobacteria were the main primary producers. The first maximum of chlorophyll *a* content (15.3 μg l^{-1}) was detected in the subsurface horizon; the second one (40.5 μg l^{-1}), located below the chemocline, probably resulted from plankton sedimentation.

The highest rate of oxygenic photosynthesis was detected in the surface water layers (0.176–0.230 mg C dm^{-3} day^{-1}) was detected at the water surface (Fig. 2d). This process was quite intense down to a depth of 3 m; then, at a 3.5-m depth, the rates of light-dependent bicarbonate fixation dropped to zero.

Chemocline zone. In the chemocline (depths of 3.5 and 3.75 m), the anoxygenic photosynthetic activity with a maximum of 0.057 mg C dm^{-3} day^{-1} was recorded (Fig. 2d). At these depths, large purple spirilla identified as a new species *"Ect. magna"* (99% similarity with the type strain *Ect. shaposhnikovii*) were detected (Fig. 3f).

These results suggest that light reached the redox zone. However, in this zone, the rate of dark bicarbonate assimilation was significantly higher than that of

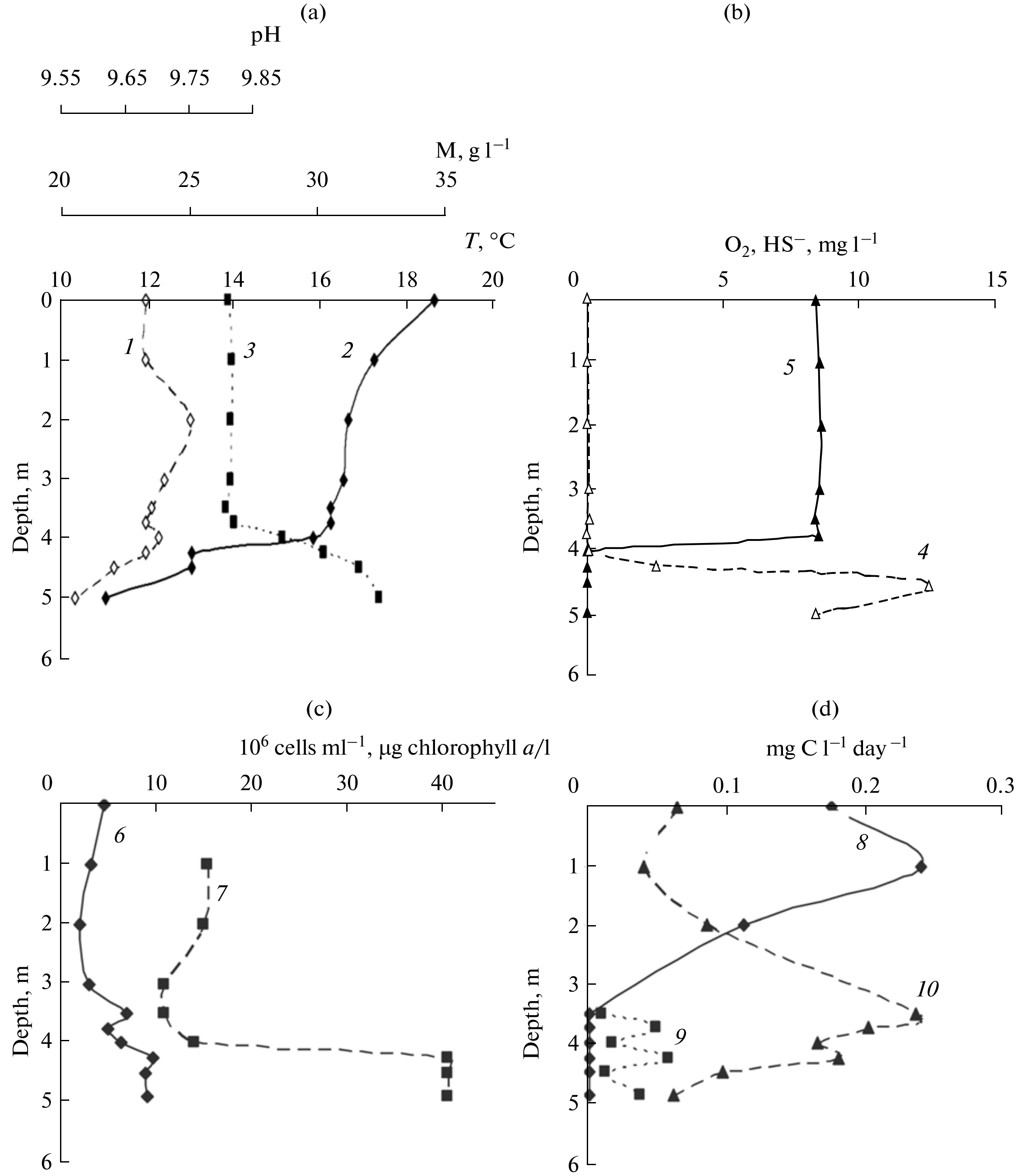

Fig. 2. Physicochemical conditions (a, b), total bacterial numbers and chlorophyll *a* content (c), photosynthetic activity, and dark bicarbonate assimilation (d) in the water column of Lake Doroninskoe (September 2007): pH (*1*); temperature (*2*); mineralization, g l^{-1} (*3*); H_2S, mg l^{-1} (*4*); O_2, mg l^{-1} (*5*); 10^6 cells ml^{-1} (*6*); μg chlorophyll *a* l^{-1} (*7*); oxygenic photosynthesis, mg C l^{-1} day^{-1} (*8*); anoxygenic photosynthesis, mg C l^{-1} day^{-1} (*9*); and dark bicarbonate assimilation, mg C l^{-1} day^{-1} (*10*).

light assimilation and was probably governed by the activity of chemoautotrophic bacteria.

According to the results of the subculturing of serial dilutions of the sample collected in the chemocline, the numbers of thiobacilli was 10^4 cells ml^{-1}. Based on the results of the 16S rRNA gene analysis, the isolated sulfur-oxidizing bacteria (Fig. 3b) were identified as a *Thioalkalivibrio* species closely related to *Thioalkalivibrio denitrificans* (98% similarity). The isolated sulfur bacteria grew on a thiosulfate-containing medium under autotrophic microaerobic conditions. They were found to be incapable of denitrification. These

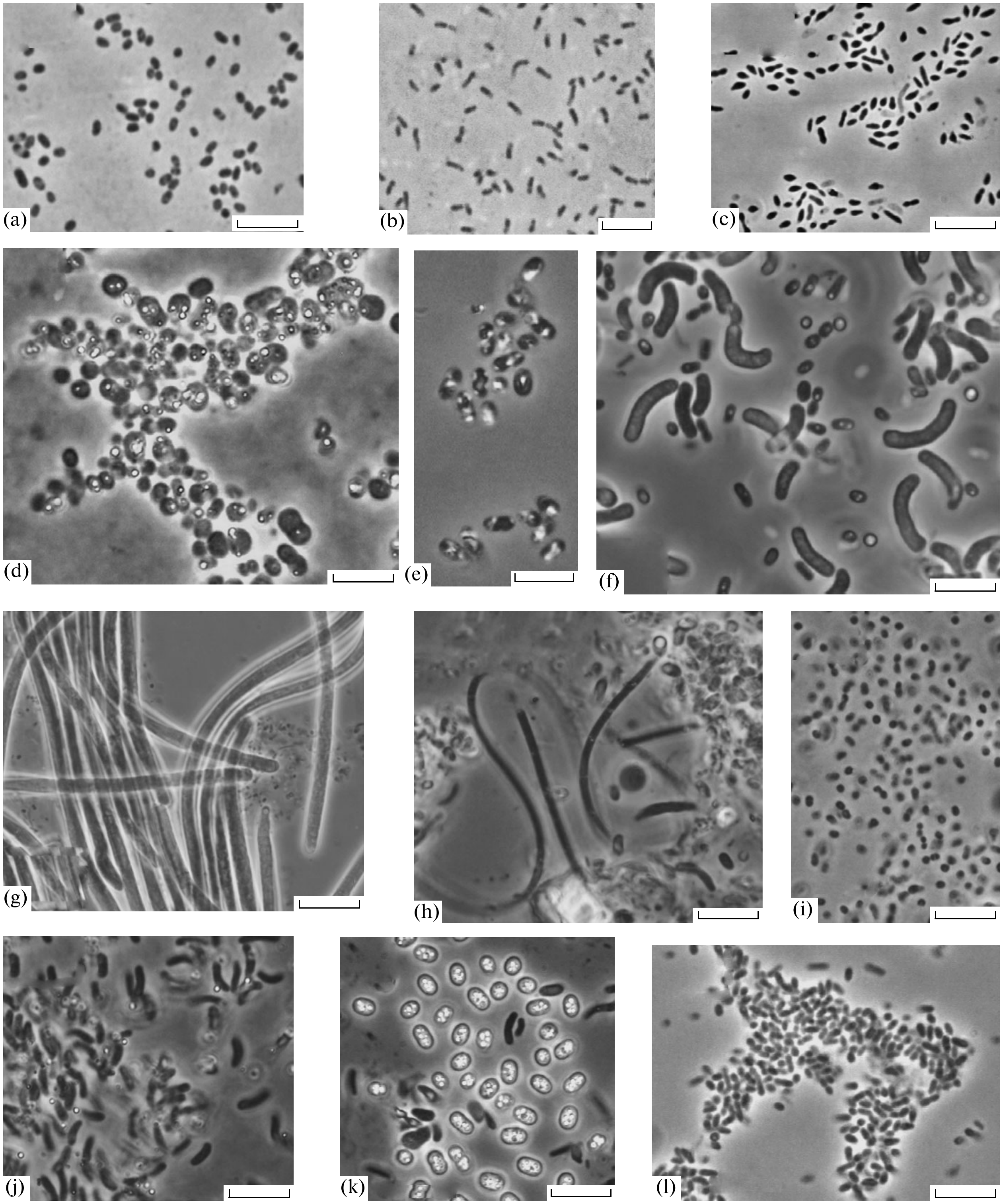

Fig. 3. Microbial cultures isolated from water samples (a–f), permafrost salses, and mud volcanoes (g–l): *Halomonas* sp. (a), *Thioalkalivibrio* sp. (b), "*Roseinatronobacter doroninskoiensis*" (c), *Thioalkalicoccus limnaeus* (d), *Ect. variabilis* (e), "*Ect. magna*" and *Ect. variabilis* (f), *Phormidium* sp. (g), *Oscillochloris* sp. (h), *Roseococcus* sp. (i), *Ect. shaposhnikovii* (j), *Chromatium* sp. (k), and *Rhodobacter* sp. (l). Bar, 5 µm.

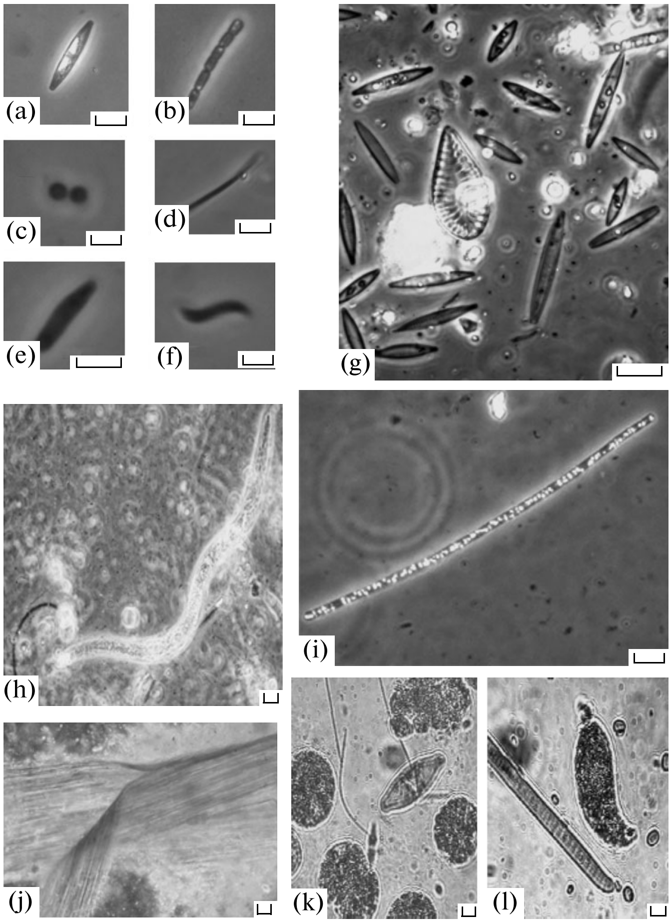

Fig. 4. Microorganisms from the water samples (3.5 m depth) (a–f) and from the shoreline mat of Lake Doroninskoe (g–l): diatoms (a and g), filamentous cyanobacteria (b and d), unicellular cyanobacteria (c), presumably purple sulfur bacteria (e and f), nematodes (h), *Beggiatoa* sp. with intracellular sulfur inclusions (i), fragment of *Cladophora* sp. tissue (j), *Chlorella* sp. and diatoms, with thin filaments presumably belonging to *Oscillochloris* sp. (k), *Phormidium* sp., *Euglena* sp (l). Bar, 5 μm.

bacteria were alkaliphiles (pH optimum is 9.5) and moderate halophiles (optimum at 20–30 g l^{-1}).

Hence, the role of anoxygenic phototrophs in organic matter production and sulfide oxidation in the chemocline was insignificant. The lack of available light at the boundary of sulfide distribution may be a possible cause of this phenomenon. At the same time, high diversity of anoxygenic phototrophic bacteria in the near-bottom silt of the deep-water station was revealed by inoculation. Among these microorganisms, purple sulfur bacteria (Fig. 3d–3f, Table 2) were predominant. This suggests that APBs initially developed in the chemocline, under more favorable light conditions. Among the APB strains isolated from silt collected from the deep part of the lake, the bacteriochlorophyll *b*-containing alkaliphilic purple sulfur bacterium *Thioalkalicoccus limnaeus* (98% similarity with the type strain) belonging to the family *Chromatiaceae* was predominant (10^3 cells ml^{-1}) (Fig. 3d) [25]. Purple sulfur bacteria of the family *Ectothiorhodospiraceae* were detected as well. These gas vacuole-containing bacteria belonged to the species *Ect. variabilis* (98% similarity with the type strain) (Fig. 3e); their number was as high as 10^3 cells ml^{-1}. The first dilutions yielded large spirilla of the species "*Ect. magna*".

Bottom sediments. Methane was the major constituent (69%) of the gas contained in the silt samples collected at the deep-water station. The nitrogen concentration in the gas was 18%. Using the radioisotope method, it was established that the rate of autotrophic methanogenesis in the bottom sediments of the central part of the lake was 0.056–0.61 mg C dm^{-3} day^{-1} (0.104–1.18 ml CH_4 dm^{-3} day^{-1}), while the sulfide production rate was 1.4–28.9 mg S dm^{-3} day^{-1}. High rates of sulfate reduction suggest that sulfidogenesis is the main process of terminal destruction of decaying organic matter. The results of hydrochemical surveys indicated the presence of iron sulfides in the bottom sediments (0.4%) [16].

Shoreline algo–bacterial mat. In the littoral zone of the lake, an algo–bacterial mat associated with *Cladophora* aquatic plant is formed (Fig. 4g–4j, Table 2). According to the results of microscopic examinations, cyanobacteria of the genera *Synechococcus, Nodularia*, and *Phormidium* were the main constituents of the shoreline mat; filamentous cyanobacteria of the genera *Oscillatoria* and *Spirulina* and green algae of the genera *Euglena* and *Chlorella*, as well as diatoms of the genera *Nitzchia* and *Surirella* (identified by N. Tashlykova), were less abundant. Nematodes were present in abundance (Fig. 4h).

The results of DGGE analysis confirmed the presence of four groups of cyanobacteria in the littoral zone—namely, close relatives of the strain *Phormidium* sp. UTCC 487 (99% similarity), several representatives of the genus *Nodularia* (98–99.7% similarity with the known strains), and close relatives of the strains *Synechococcus* PCC 8806 and *Synechococcus* MLCB (96 and 98% similarity, respectively). It is noteworthy that the latter organism was isolated from the meromictic soda Mono Lake (United States) [28]. *Phormidium* sp. UTCC 487 and *Nodularia* are typical inhabitants of waters with a mineralization level close to the marine one.

Using the radioisotope method, it was established that the rate of sulfide production in shoreline sediments was 1 mg l^{-1} day^{-1}. In the lower layers of the algo–bacterial mat, microaerobic and anaerobic conditions favorable for chemoautotrophic and APBs were created due to the influx of sulfide from the underlying silt layer. Microscopic examinations showed the presence of colorless filamentous sulfur bacteria *Beggiatoa* sp. (Fig. 4i, Table 2). The results of the bacterial cell count in selective media demonstrated that purple sulfur bacteria close to the species *Ect. variabilis* and *Ect. shaposhnikovii* (98% similarity)

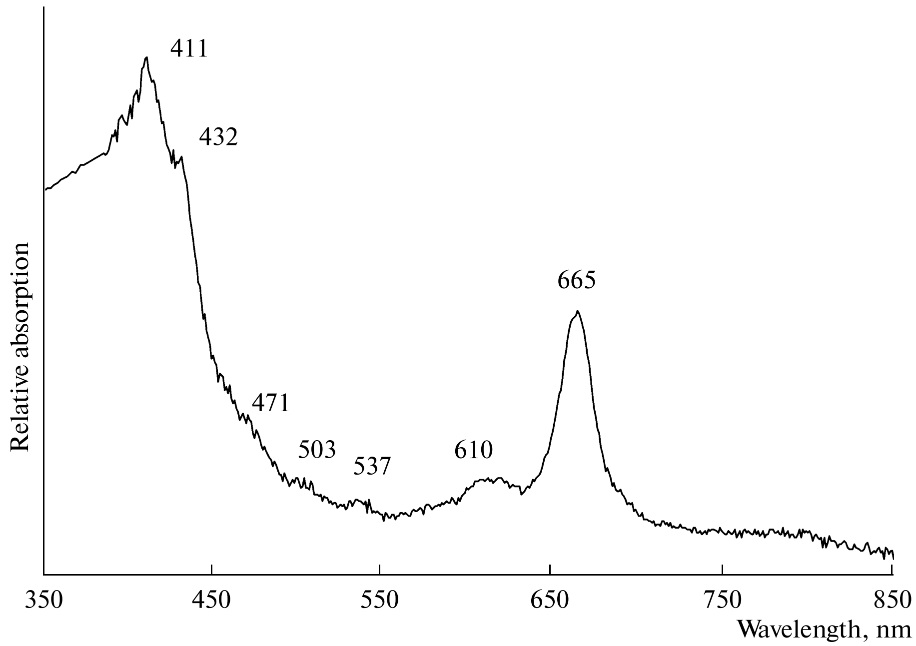

Fig. 5. Acetone-methanol extract of the water samples collected from Lake Doroninskoe at a depth of 4.75 m.

prevailed, their number ranging from 10^3 to 10^4 cells ml^{-1}. Spheroiden-containing purple nonsulfur bacteria *Rhodovulum* sp. were detected as well.

Microbial mats in the areas of mud volcanoes activity. In the areas of mud volcanoes activity, a heterogeneous sandy microbial mat has developed, in which purple sulfur bacteria, purple nonsulfur bacteria, and AAPs coexisted with diatoms and cyanobacteria (Table 2). The highest diversity of APBs was observed in the samples of "purple" sandy mat collected on the northeastern shore. Bacteria of the family *Chromatiaceae, Chromatium–Thiocystis* morphotype (10^6 cells ml^{-1}), with intracellular sulfur inclusions (Fig. 3k) were predominant. *Ect. shaposhnikovii* (98% similarity) (10^5 cells ml^{-1}) (Fig. 3j) and spheroiden-containing purple nonsulfur bacteria closely related to *Rhodobacter sphaeroides* (99% similarity) (10^4 cells ml^{-1}) (Fig. 3l) were detected. An AAP bacterium affiliated with the genus *Roseococcus* was also isolated from the algo–bacterial sandy mat. According to the results of 16S rDNA sequencing, the new isolate showed a 99% similarity with the previously described species *Rsc. suduntuensis* isolated from a low-mineralized soda lake of the Transbaikal region. The first dilutions on the media for purple sulfur bacteria yielded green filamentous bacteria with gas vacuoles, morphologically similar to *Oscillochloris* sp. (Fig. 3h).

The rate of autotrophic methanogenesis in the mud flowing from mud volcanoes was 2.42 mg C dm^{-3} day^{-1} (4.50 ml CH_4), while the rate of sulfate reduction was 38.18 mg dm^{-3} day^{-1}.

DISCUSSION

The results obtained and the literature data [29] confirm that, according to the results of the annual hydrochemical survey, Lake Doroninskoe may be considered as meromictic. Lake Doroninskoe differs from other known stratified soda lakes. These differences are determined primarily by climatic factors. The lake is located in the Transbaikal region with a typical continental climate, in the permafrost zone. The lake is covered by ice for seven months of the year. Water freezing results in concentration of the brine. In addition, the reverse temperature stratification develops, with subzero temperatures (–1.5°C) in subglacial waters. As a consequence, the density alignment and slow mixing of the water column occur after the freezing period. Occasionally, especially in recent dry years, the water of Lake Doroninskoe is mixed during the autumn period. It should be noted that occasional wind mixing was also observed in the African meromictic soda lake Sonachi (Kenya) with the same maximal depth (7 m) as Lake Doroninskoe [8].

Considerable fluctuations of the sulfide concentration (from 10 to 60 mg l^{-1}) in the near-bottom horizons during summer may be attributed to the instability of the hydrologic regime of the relatively shallow Lake Doroninskoe. It is notable that sulfide, as well as the products of its partial oxidation (thiosulfate and sulfite) are present in all parts of the water column, including the aerobic zone (0.3–3 mg l^{-1}). Under alkaline conditions, these compounds are slowly oxidized; however, they can be rapidly utilized by sulfur-oxidizing microorganisms, which are present in the lake.

Table 1. Macrocomponent composition of the water of Lake Doroninskoe, mg l^{-1} (September 5, 2007)

Depth, m	*T*, °C	*Eh*	pH	O_2	HS^-	CO_3^{2-}	HCO_3^-	SO_4^{2-}	Cl^-	Ca^{2+}	Mg^{2+}	Na^+	K^+	Total mineralization, mg l^{-1}
0.0	18.6	86	9.68	8.11	0.030	6240	7961	170	3993	12.5	27.9	7962	86.7	26465
1.0	17.2	83	9.68	8.14	0.075	6240	7961	165	4086	12.3	24.5	8030	82.4	26613
2.0	16.6	81	9.75	8.15	0.070	6300	7839	102.5	4057	12.8	22.4	7984	60.3	26552
3.0	16.5	76	9.71	8.13	0.059	6210	7961	145	4086	13.0	25.7	8030	68.9	26552
3.5	16.2	75	9.69	8.1	0.077	6240	7900	157.5	3993	12.9	22.1	7938	82.8	26357
3.75	16.2	72	9.68	8.13	0.070	6240	7900	175	4181	12.9	23.6	8093	73.4	26710
4.0	15.8	–298	9.70	0	0.168	6720	8784	172.5	4181	13.8	26.1	8591	69.0	28569
4.25	13.0	–368	9.68	0	2.54	7260	9425	112.5	4229	14.2	27.2	9030	88.5	30197
4.50	13.0	–437	9.63	0	12.56	7290	9913	180	4480	14.4	26.7	9536	85.1	31537
5.0	11.0	–432	9.57	0	8.40	7200	10462	117	4691	14.6	26.4	9710	86.7	32319

Table 2. Species composition of photosynthetic organisms and some chemotrophic bacteria in various ecological zones of Lake Doroninskoe

Microbial groups	Water, aerobic zone	Water, chemocline zone	Bottom silt	Coastal mat	Sandy mat associated with mud volcanoes
Oxygenic phototrophs	Cyanobacteria: *Phormidium* sp., *Nodularia* sp., *Synechococcus* sp., *Synechocystis* sp. Diatoms: *Nitzschia* sp.	ND	Absent	Cyanobacteria: *Synechococcus*, *Nodularia* and *Phormidium* sp., *Oscillatoria* sp. and *Spirulina* sp. Green algae: *Euglena* sp. and *Chlorella* sp. Diatoms: *Nitzschia* sp. and *Surirella* sp.	Cyanobacteria: *Phormidium* sp., *Synechococcus* sp. Diatoms: *Nitzschia* sp.
Anoxygenic phototrophs, anaerobes	Absent	"***Ect. magna***"	***Ect. variabilis, Tac. limnaeus, "Ect. magna"***	***Ect. variabilis, Ect. shaposhnikovii,*** *Rhodovulus* sp.	***Chromatium* sp.**, ***Ect. shaposhnikovii, Rba. sphaeroides,*** *Oscillochloris* sp.
Aerobic bacteriochlorophyll *a*-containing bacteria	***Roseinatronobacter* sp.**	ND	Absent	Absent	***Roseococcus* sp.**
Sulfur-oxidizing chemoautotrophs	ND	***Thioalkalivibrio* sp.**	Absent	*Beggiatoa* sp.	ND
Chemoorganotrophs	***Halomonas campisalis***, ***Halomonas* sp.**	ND	ND	ND	ND

Note: The species identified by almost complete 16S rRNA gene sequencing are indicated by boldface; ND stands for not determined.

The predominance of chlorophyll *a* in the waters of Lake Doroninskoe indicates that cyanobacteria, rather than diatoms, are principally involved in the primary production processes. The rate of photosynthetic activity in the lake water was measured for the first time. The productivity of Lake Doroninskoe and the content of chlorophyll *a* in its waters correspond to those of a mesotrophic–eutrophic lake. During our study (September 2007), the water transparency was low (1.2 m), which inhibited the growth of purple anaerobic sulfur bacteria in the chemocline. The rate of dark CO_2 assimilation was extremely high (0.240 mg dm^{-3} day^{-1}) in this zone. In the chemocline, sulfide-oxidizing chemolithoautotrophic microaerobic thiobacilli of the genus *Thioalkalivibrio* were detected, which play a key role in sulfide oxidation. Anoxygenic photosynthesis (0.057 mg dm^{-3} day^{-1}) implemented by purple sulfur bacteria was also detected in the chemocline; however, its intensity was found to be relatively low. It should be noted that no pink layers consisting of purple bacteria were detected in the chemoclines of the previously studied meromictic soda lakes Mono Lake and Soap Lake (United States), although cultures of purple sulfur bacteria of the genus *Ectothiorhodospira* have been isolated from the chemocline samples. The results of our long-term investigations indicate that, in some years, the water transparency in Lake Doroninskoe increased to 3 m (data presented by S.V. Borzenko); it may be suggested that, under these conditions, APB growth in the chemocline was more intense.

In the littoral zone of the lake, an algo–bacterial mat associated with water plants of the genus *Cladophora* is formed. *Ect. variabilis* and *Ect. shaposhnikovii* were the major APB species in this mat. The distribution of the algo–bacterial mat is uncertain; however, it may be suggested that it covers the lake bottom within the euphotic zone to a depth of 2–3 m and that the processes occurring in this mat affect the cycling of elements in the whole lake.

In the wet shoreline zone, the phototrophic communities of sandy mats associated with mud volcanoes represent a unique subsystem. The APB species isolated from these communities were alkaliphilic; however, unlike the majority of microorganisms isolated from the water column and bottom sediments, they were not halophilic, since volcanic fluids had relatively low mineralization.

Thus, there are several interrelated subsystems in the ecosystem of Lake Doroninskoe, in the water column, silt sediments, and the littoral zone.

On the whole, the process of organic matter destruction in the soda Lake Doroninskoe is similar to that in the other mixed lakes of the Transbaikal region. At the terminal stages of destruction, the bulk of organic matter is utilized for microbial reduction of sulfate; much lesser amounts of organic matter are utilized via methanogenesis. The rates of organic matter consumption in the bottom sediments of the lake and mud volcanoes via sulfate reduction were 1.05–21.69 and 28.63 mg C dm^{-3} day^{-1}, respectively. The rates of organic matter consumption in the bottom sediments and mud volcanoes via methanogenesis were as high as 2.54 and 10.24 mg C dm^{-3} day^{-1}, respectively.

The rate of sulfate reduction in the bottom sediments of Lake Doroninskoe (up 28.9 mg S dm^{-3} day^{-1}) was comparable to that in the silt sediments of the holomictic soda and saline lakes of the Transbaikal region (up 69.04 mg dm^{-3} day^{-1}) and exceeded the values obtained for the saline meromictic Lake Shira (up to 0.9 mg dm^{-3} day^{-1}) [30, 31]. However, the rate of methanogenesis in the bottom sediments of Lake Doroninskoe (up to 1.18 ml CH_4 dm^{-3} day^{-1}) is higher than the values obtained for other lakes of the Transbaikal region (up to 80.71 ml CH_4 dm^{-3} day^{-1}).

Finally, it should be noted that there is no published description of any lake similar to the stratified soda Lake Doroninskoe. The unique climatic, hydrologic, and hydrochemical conditions, as well as the long period of relatively stable existence, make it different from other lakes. The sulfur cycle, in which sulfate-reducing and various sulfur-oxidizing chemotrophic and phototrophic microorganisms are involved, as well as APBs and other thiophilic microorganisms, play a vital role in the lake ecosystem. Alkaliphilic moderately halophilic and halotolerant microorganisms are the main constituents of the microbial community of Lake Doroninskoe due to the constantly alkaline conditions and the relatively stable enhanced mineralization of water, bottom sediments, and volcanic fluids.

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