ISSN 0026-2617, Microbiology, 2014, Vol. 83, No. 1–2, pp. 56–66. © Pleiades Publishing, Ltd., 2014.
Original Russian Text © A.S. Savvichev, O.N. Lunina, I.I. Rusanov, E.E. Zakharova, E.F. Veslopolova, M.V. Ivanov, 2014, published in Mikrobiologiya, 2014, Vol. 83, No. 2, pp. 191–203.

EXPERIMENTAL ARTICLES

Microbiological and Isotopic Geochemical Investigation of Lake Kislo-Sladkoe, a Meromictic Water Body at the Kandalaksha Bay Shore (White Sea)

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Received April 11, 2013

Abstract—Microbiological, biogeochemical, and isotopic geochemical investigation of Lake Kislo-Sladkoe (Polusolenoe in early publications) at the Kandalaksha Bay shore (White Sea) was carried out in September 2010. Lake Kislo-Sladkoe was formed in the mid-1900s out of a sea gulf due to a coastal heave. At the time of investigation, the surface layer was saturated with oxygen, while near-bottom water contained sulfide (up to 32 mg/L). Total number of microorganisms was high (12.3×10^6 cells/mL on average). Light CO_2 fixation exhibited two pronounced peaks. In the oxic zone, the highest rates of photosynthesis were detected at 1.0 and 2.0 m. The second, more pronounced peak of light CO_2 fixation was associated with activity of anoxygenic phototrophic bacteria in the anoxic layer at the depth of 2.9 m (413 μg C L^{-1} day^{-1}). Green-colored green sulfur bacteria (GSB) predominated in the upper anoxic layer (2.7–2.9 m), their numbers being as high as 1.12×10^4 cells/mL, while brown-colored GSB predominated in the lower horizons. The rates of both sulfate reduction and methanogenesis peaked in the 2.9 m horizon (1690 μg S L^{-1} day^{-1} and 2.9 μL CH_4 L^{-1} day^{-1}). The isotopic composition of dissolved methane from the near-bottom water layer ($\delta^{13}C$ (CH_4) = −87.76‰) was significantly lighter than in the upper horizons ($\delta^{13}C$ (CH_4) = −77.95‰). The most isotopically heavy methane ($\delta^{13}C$ (CH_4) = −72.61‰) was retrieved from the depth of 2.9 m. The rate of methane oxidation peaked in the same horizon. As a result of these reactions, organic matter (OM) carbon of the 2.9 m horizon became lighter (−36.36‰), while carbonate carbon became heavier (−7.56‰). Thus, our results demonstrated that Lake Kislo-Sladkoe is a stratified meromictic lake with active microbial cycles of carbon and sulfur. Suspended matter in the water column was mostly of autochthonous origin. Anoxygenic photosynthesis coupled to utilization of reduced sulfur compounds contributed significantly to OM production.

Keywords: meromictic lakes, carbon isotope fractionation, anoxygenic phototrophic bacteria

DOI: 10.1134/S0026261714010111

Meromictic lakes with vertical thermal and saline stratification are the classical objects of research for limnologists, biogeochemists, and microbiologists [1]. The most interesting biological peculiarity of such lakes is intense sulfate reduction in the hypolimnion and involvement of photo- and chemoautotrophic bacteria in production of organic matter with unusual carbon isotopic composition [2]. The specificity of microbial processes in meromictic lakes is to a considerable degree associated with their geographical position and genesis. The composition and functional activity of microbial communities are determined by temperature, salt composition, trophicity, depth of light penetration, and some other parameters. The marked seasonality of the rates of microbial processes has been shown for the closed lakes Shira and Shunet in the Khakassia steppe [3].

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Substantial changes in the carbon isotopic composition of suspended organic matter, associated with microbial processes implemented by anaerobic photosynthetic bacteria *Chromatium* sp. and *Chlorobium* sp., have been shown in the Framvaren Fjord (Norway) [4]. The modified $\delta^{13}C$ carbon composition in organic and inorganic matter is typical of the zone of contact between oxygen and sulfide-containing waters of the Black Sea, the largest meromictic basin [5].

Lake Kislo-Sladkoe (in earlier publications, Lake Polusolenoye [6]) is a peculiar-type meromictic basin. This small (60 m wide and 100 m long) lake with the maximum depth of 4.2 m is located 2 km to the east of the Moscow State University White Sea Biological Research Station on the Rugozerskaya Gulf of the Kandalaksha Bay, which is on the White Sea at the latitude of the Arctic Circle. Lake Kislo-Sladkoe is an example of a basin that has only recently lost its connection with the sea. The lake was formed in the mid-20th century due to seashore rising. Previously, a nar-

row strait existed between the continent and a small island, with inlet and outlet sills. The transformation of a strait into a gulf and then into an isolated lake was caused by the Karelia shore rising by 4 mm per year on average [7]. At present, the lake is replenished with fresh water mainly from melting snow, while the freshwater brook summer flow does not exceed 1.5 m^3 day^{-1}. Seawater penetrates the lake episodically during big (spring) tides and storm surges.

It is known (according to A.N. Pantyulin's data [8]) that the lake water column becomes pronouncedly stratified by the end of the summer season: the subsurface layer is supersaturated with oxygen due to the activity of algae and cyanobacteria, while the cooled bottom layer is saturated with sulfide. Our early works showed that the rates of microbial processes in the carbon and sulfur cycles in shallow coastal waters of Lake Kislo-Sladkoe (down to 20 cm deep) were exceptionally high [6, 9].

The relevance of investigation of the water bodies separated from the main sea basin is associated, first and foremost, with the necessity of predicting the negative consequences of sulfide contamination in artificially closed seawater areas that have appeared as a result of construction of weirs, dams, tidal power plants, etc.

Hence, the main goal of the present work was to study the conditions of existence and to determine the scales of biogeochemical activity of microbial communities in the meromictic Lake Kislo-Sladkoe (the total number of microorganisms, the rates of autotrophic and heterotrophic CO_2 fixation, methane production and oxidation, sulfate reduction, as well as counting and isolation of the key representatives of anaerobic phototrophic bacteria), as well as to perform isotopic geochemical studies (to determine the carbon isotopic composition of suspended organic matter, dissolved methane, and bicarbonate carbon) in the water column and bottom sediments of the lake.

MATERIALS AND METHODS

The works at Lake Kislo-Sladkoe (66°32.87′ N, 33°08.14′ E; Fig. 1) were carried out in the early September 2010. Water and bottom sediment samples were taken at the point with the maximum depth (4.2 m). The vials with the samples were exposed in situ on a fastened nylon halyard.

Water samples were taken using a silicon tube fixed on a calibrated cable and a Whale Premium Submersible Pump GP1352 (Ireland). The sampled water was poured into 30-mL glass vials, sealed with a gas-tight rubber stopper without an air bubble, and closed with a perforated aluminum cup. Bottom sediments were sampled using a limnological stratometer with a glass tube 6 cm in diameter. From the stratometer tube, sediment samples were transferred into cut-off 5-mL plastic syringes with a rubber plunger. After filling the syringe with a sample of structurally undisturbed sediment, it was closed with a gas-tight rubber stopper without access to air.

The oxygen and sulfide content and the alkalinity value were measured immediately after sampling with standard Aquamerck reagent kits (Germany).

The total number of microorganisms (TNM) and microbial biomass were assayed as follows. Water samples were fixed with glutaraldehyde solution at a 2% final concentration in the sample. The fixed sample (1–5 mL) was filtered through black polycarbonate filters (Millipore) with a pore diameter of 0.2 μm. The cells on the filters were stained with acridine orange solution [10]. The preparations were examined under a LUMAM I-2 fluorescence microscope with an Image Scope Color (M) visualization system (magnification ×1000). The cells were counted on the monitor screen in 20 fields of vision. The volume of bacterial cells was calculated by measuring their length and width; the volumes of cocci and rods were calculated using the formulas for the volumes of a sphere and a cylinder, respectively.

The carbon isotopic composition of suspended organic matter ($\delta^{13}C–C_{org}$) was determined as follows: water samples were filtered through precalcined 47-mm GF/F glass-fiber filters and dried at 60°C. The filtrate was used to determine the carbon isotopic composition of dissolved bicarbonate. The $\delta^{13}C$ value was measured in a Delta Plus mass spectrometer (Germany). The accuracy of measurements was 0.1‰.

The rates of microbial processes of light and dark CO_2 assimilation (LCA and DCA, respectively), sulfate reduction (SR), methanogenesis (MG) and methane oxidation (MO) were determined by the radioisotope method with the following labeled compounds: $NaH^{14}CO_3$, $^{14}CH_4$, and $Na_2{}^{35}SO_4$. The LCA and DCA rates were determined at each sampling layer using 2 light and 1 dark glass vials, with 0.2 mL (20 μCi) $NaH^{14}CO_3$ solution added into each of them. Diuron at a final concentration of 10^{-7} mM was used as a selective inhibitor of oxygenic photosynthesis [11]. Glass vials were suspended on a nylon halyard and exposed at a buoy station for 24 h in the respective layers in situ. After the exposure, the contents of the vials were fixed with 1 mL of diluted HCl and filtered through nylon membrane filters with a pore size of 0.2 μm. Photosynthetic production was calculated by the difference between the rates measured in the light and dark bottles. The production of oxygenic photosynthesis was calculated by the difference between total and anoxygenic (the light bottle with diuron) photosynthesis.

To determine the rates of other processes, the water and sediment samples were also incubated in situ. After the incubation, the samples were fixed with 1 mL of 0.1 M KOH solution. Further laboratory treatment of the samples was performed by the methods

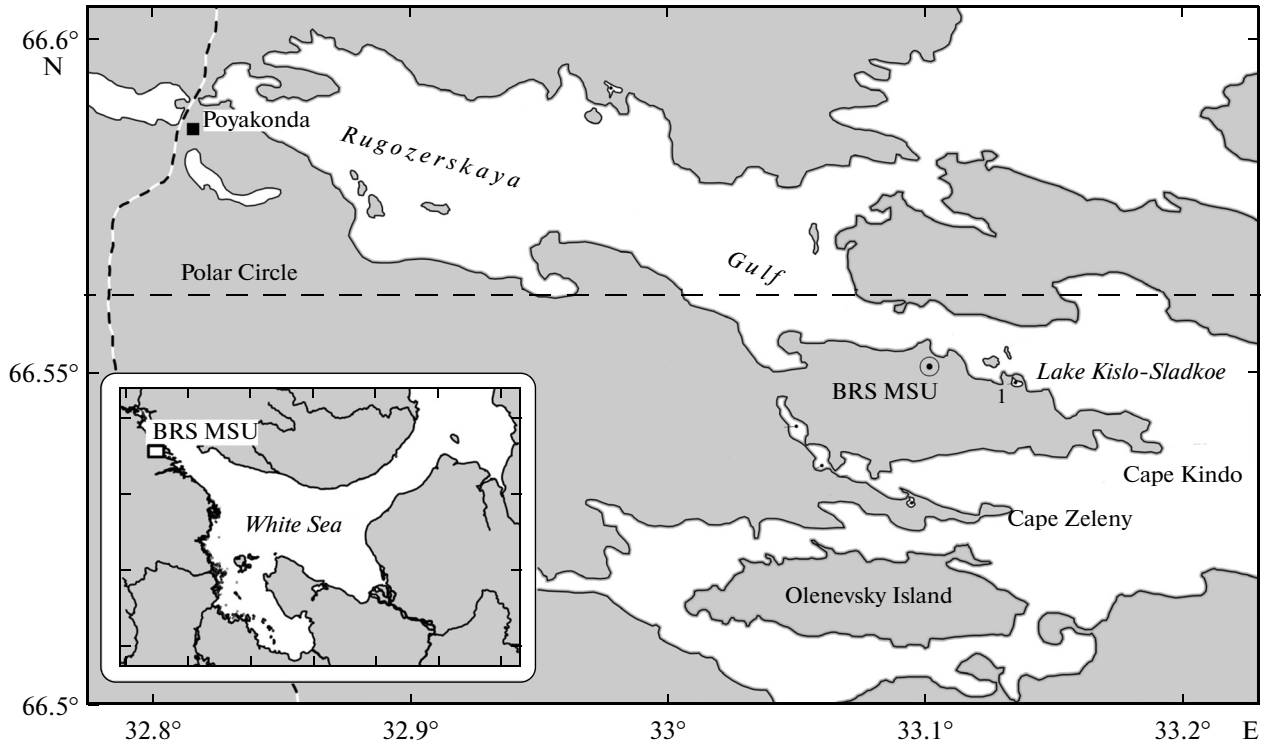

Fig. 1. The meromictic Lake Kislo-Sladkoe (Rugozerskaya Gulf coast, the Kandalaksha Bay of the White Sea).

described previously [12]. Radioactivity of the final products was measured with a Rack-Betta 1219 scintillation counter (LKB, Sweden). DCA and LCA rates were calculated with due account for $^{14}C–CO_2$, both within bacterial cells and within extracellular dissolved organic matter. The confidence interval for the numerical indices of LCA, DCA, MG, MO and SR varied from 10 to 40%.

The number of photosynthetic bacteria was determined by inoculation of the terminal dilutions of freshly collected water samples into semisolid (0.8% agar) nutrient medium containing the following (g/L distilled water): KH_2PO_4, 0.7; NaCl 5, 10, 15, 20, or 25 (variants); NH_4Cl, 0.7; $MgSO_4 \cdot 7H_2O$, 0.5; KCl, 0.33; $NaHCO_3$, 0.15; $CaCl_2$, 0.01; $Na_2S_2O_3 \cdot 5H_2O$, 0.1; $Na_2S \cdot 9H_2O$, 0.05; Na-acetate, 0.05; Na-pyruvate, 0.05; yeast extract, 0.01; trace element solution, 1 mL; vitamin B_{12}, 20 μg; pH 7.5–8.0. Sulfate-reducing microorganisms were grown in the Widdel marine medium [13].

The content of methane was determined using the head-space method of water and sediment sampling. Methane content in the samples was determined by the method of phase-equilibrium degassing [14]. Methane concentration was measured in a Kristall-2000-M gas chromatograph with a flame ionization detector. The sulfate and chloride contents (with preliminary distillation and concentration) were determined in a Biotronik ion chromatograph (Germany). The results were statistically processed using Excel 2000.

RESULTS

Lake Kislo-Sladkoe. Hydrological characteristics. The thermohaline structure of Lake Kislo-Sladkoe is determined by both the season and specific weather conditions. In the period of our research (early autumn), the temperature of the surface convective layer varied from 11 to 14°C throughout a day (in August, the surface water layer was heated to 20–22°C; A.N. Pantyulin's data), while the lower layer maintained the temperature conditions of the summer season (8–10°C) (Fig. 2). The increase in salinity with depth was insignificant: 18.0 g L^{-1} in the surface layer and 22.5 g L^{-1} in the bottom layer (Fig. 2). The weakly desalinated water layer reached a depth of 1.0 m. Thus, the thermohaline structure of the basin remained essentially double-layered.

From the surface down to the depth of 2.7 m, the redox potential had a positive value. A sharp decrease in Eh was revealed within a depth interval of 2.8–2.9 m. The oxygen and sulfide concentration measurements showed the presence of a narrow redox zone at a depth of 2.7–2.9 m, which was characterized by complete disappearance of oxygen and appearance of sulfide (Fig. 3). The minimum oxygen concentration was found together with the traces of sulfide in the 2.7-m layer. The maximum oxygen and sulfide content

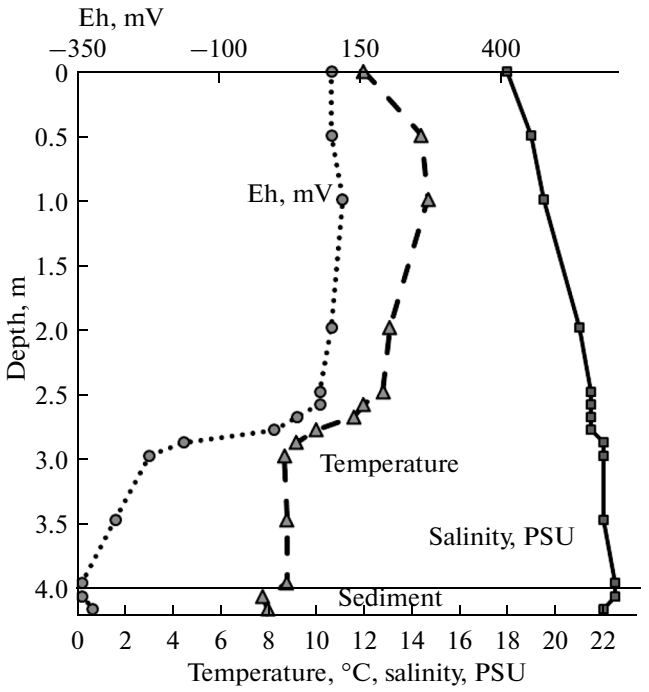

Fig. 2. Temperature, salinity, and redox potential of the water column and the upper sediment layer of Lake Kislo-Sladkoe in September 2010.

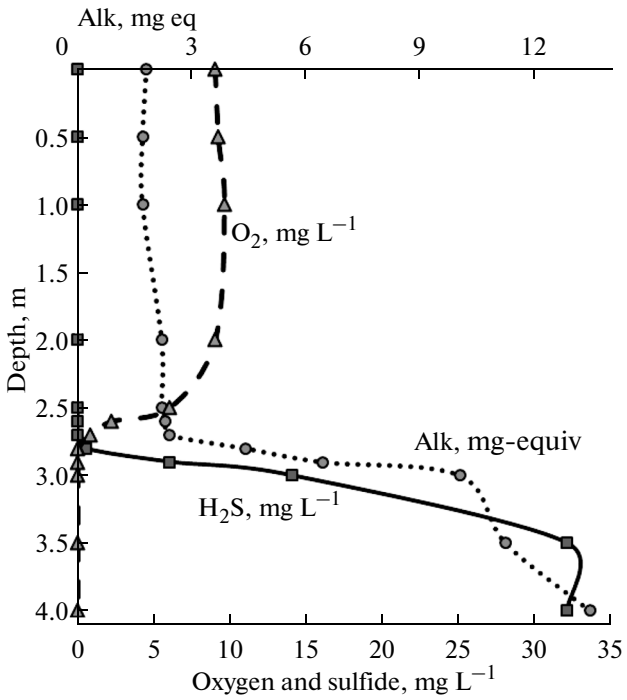

Fig. 3. Concentrations of oxygen and sulfide content and alkalinity in the Lake Kislo-Sladkoe water column.

was recorded in the subsurface water layer (9.6 mg L^{-1}) and in the bottom layer (32 mg L^{-1}, Fig. 3), respectively. The alkalinity (Alk) slightly grew from 1.7 to 2.4 mg-equiv/L from the surface layer to the 2.8-m layer and then abruptly increased in the redox layer to 10.0 mg-equiv/L at a depth of 3.0 m with further increase to 13.4 mg-equiv/L in the bottom layer (Fig. 3).

The bottom sediments (fluid, pelitic, black-colored, reduced, with a strong hydrogen sulfide flavor in the surface layer) included plant debris. Below 2–5 cm, the sediment became slightly compacted and acquired the aleurite and sandy fraction, with the alkalinity of the silt water decreasing from 13 to 8 mg-equiv/L. Total salinity (22.5‰) in the pore water squeezed out of the sediment sample corresponded to the values typical of the bottom water (Fig. 2).

Total number and biomass of microorganisms, rate of CO_2 assimilation in lake water column. The results of determination of the total number of microorganisms (TNM) and the rates of light and dark CO_2 assimilation are given in Fig. 4. The TNM value was very high (12.3×10^6 cells mL^{-1} on average), indicating the mesotrophic/eutrophic state of the basin. The minimum TNM value (8×10^6 cells mL^{-1}) corresponded to the surface layer and the maximum value ($15–17 \times 10^6$ cells mL^{-1}) was found in the 2- and 3-m layers. The average volume of bacterial cells was 1.1 μm^3 in the oxic water column, 1.4 μm^3 at the depths of 2.6 and 2.7 m, and up to 2.0 μm^3 in the "purple" layer. Accordingly, the value of microbial biomass was very high (9–17 mg L^{-1} in the oxic layer and 26 mg L^{-1} in the 2.9-m layer, Fig. 4).

Dark carbon dioxide fixation (DCF) is a cumulative index including the rates of both heterotrophic carboxylation and autotrophic carbon dioxide uptake during chemosynthesis. From the water surface to the depth of 2.6 m, the DCF value characterized the activity of heterotrophic bacterioplankton, since chemoautotrophic processes were weak because of the absence (shortage) of inorganic reducers. The observed gradual increase in DCF rate from the surface water layer to the upper boundary of the redox zone (from 9.3 to 18.1 μg C L^{-1} day^{-1}) is the evidence of active decomposition of fresh autochthonous organic matter formed by phytoplankton mainly in subsurface layers (Fig. 5). Beginning from 2.7 m, the DCF value increased sharply to 145 μg C L^{-1} day^{-1}. Since this layer is characterized by the co-existence of oxygen and sulfide, it would be logical to explain the DCF peak by the activity of aerobic autotrophic sulfide-oxidizing microorganisms. While DCF increased drastically in the 2.7-m layer, TNM remained nearly the same (10×10^6 cells mL^{-1}). This may be explained by a considerable difference in carbon dioxide uptake by heterotrophic and chemoautotrophic microorganisms. In the underlying water layers, DCF value decreased gradually from the redox zone to the bottom

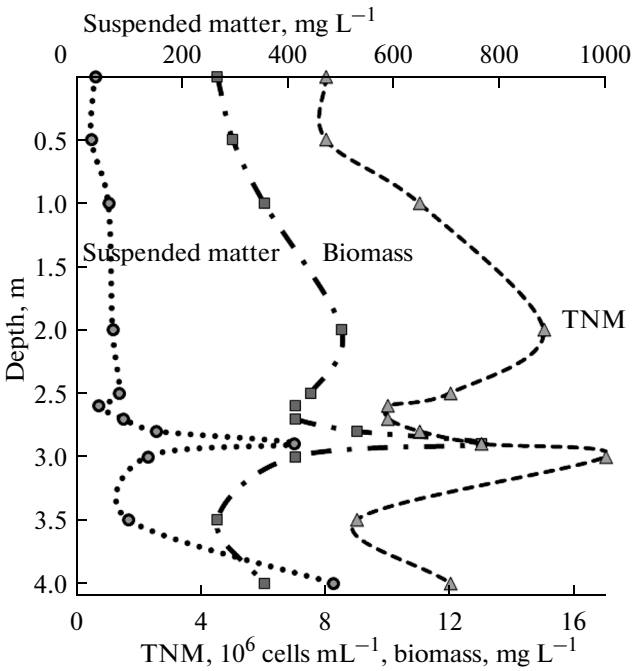

Fig. 4. Total number of microorganisms (TNM), microbial biomass (MB), and suspended matter content in the water column of Lake Kislo-Sladkoe.

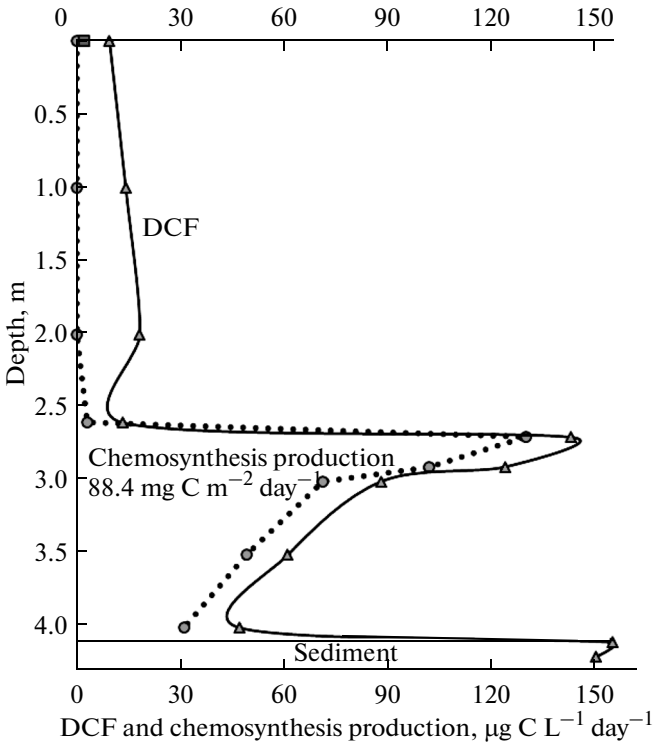

Fig. 5. Rates of dark CO_2 fixation (DCF) and chemosynthesis (CS) in the water column of Lake Kislo-Sladkoe. Chemosynthesis production in the water column is 88.4 mg C m^{-2} day^{-1}.

layer (from 143 to 47 μg C L^{-1} day^{-1}). Carbon dioxide assimilation was implemented due to both anaerobic heterotrophic microorganisms and anaerobic chemoautotrophs: hydrogenotrophic sulfate reducers and methanogens. In the upper layer of bottom sediments, DCF value increased to 455 μg C L^{-1} day^{-1}.

Tentative quantification of chemoautotrophic CO_2 fixation in the dark process was performed by the method proposed by Ivanov for Lake Mogilnoye [2]. The mean specific DCF (per 10^6 cells of TNM) for the lower part of the oxic zone (the calculation was made for the 2.6-m layer), where chemoautotrophs do not develop because of the absence of reduced substrates, was 1.32 μg C day^{-1}. We assume that the same specific heterotrophic assimilation activity corresponds to the underlying layers, where, apart from heterotrophic microorganisms, chemoautotrophs actively function. Multiplication of the averaged specific DCF by TNM of the respective layer will give the values of the heterotrophic component of DCF. The difference between these values and total DCF is considered as a quantitative estimate of microbial chemosynthesis (Fig. 5). The peak of chemosynthetic activity was observed in the 2.7-m water layer (130 μg C day^{-1}); the chemosynthetic activity decreased to 102 μg C day^{-1} in the 2.9-m layer and down to 31–71 μg C day^{-1} in the anoxic layer.

The light CO_2 fixation value had two distinct peaks (Fig. 6). The maximum value of phytoplankton photosynthesis in the oxic zone was recorded at the depths of 1.0 and 2.0 m. At the lower boundary of the oxic zone (2.6 m), the rate of photosynthesis decreased twofold. The second peak of light CO_2 fixation, which was more pronounced than the first one, was observed in the anoxic layer, beginning from the depth of 2.9 m (413 μg C L^{-1} day^{-1}). With further submersion, the rate of photosynthesis dropped, while still remaining high enough even in the bottom layer (76 μg C L^{-1} day^{-1}). Application of diuron as a specific inhibitor of oxygenic photosynthesis made it possible to obtain the values of CO_2 assimilation rate for anoxygenic phototrophic bacteria (APB) (Fig. 6). Active anoxygenic photosynthesis became noticeable at a depth of 2.7 m (45 μg C L^{-1} day^{-1}) and reached the maximum value (411 μg C L^{-1} day^{-1}) in the 2.9-m layer.

Anoxygenic phototrophic bacteria in Lake Kislo-Sladkoe. The natural color of water is the primary indicator of active development of phototrophic microorganisms, both algae and bacteria. In our studies, the water of the upper layers (up to 2.5 m) had no pronounced color. The water was light lettuce green in the redox zone (2.6–2.7 m) and light green at a depth of 2.8 m due to the development of green-colored APB. A layer of pink-colored water was clearly seen immediately under the redox zone at a depth of 2.9 m. Microscopic studies revealed that the pink layer contained numerous unicellular algae: cryptophytic

phytoflagellates (*Cryptophyta*; *Cryptomonadaceae*). At a depth of 3 m and below, the water was brown-green due to intensive development of green- and brown-colored green sulfur bacteria (GSB).

Spectral analysis of the pigments from suspended matter collected at different depths (Table 1) and quantification of APB colonies formed in semiliquid medium inoculated with freshly sampled water showed predominance of green-colored GSB in the upper part of the anoxic layer (2.7–2.9 m), with the maximum quantity of 1.12×10^4 cells mL^{-1}, according to the inoculation data; the single cells of purple nonsulfur bacteria (PNB) were present as a minor component. Brown-colored GSBs prevailed in the lower part of the anoxic zone. Their quantity (also according to the inoculation data) noticeably increased beginning from the depth of 2.9 (6.75×10^3 cells/mL) and down to the bottom (5.06×10^4 cells/mL). Purple sulfur bacteria (PSB) were isolated from all layers of the anoxic water column but showed no visible zonality (Table 1). The following organisms were isolated from enrichment cultures obtained from different layers of the Lake Kislo-Sladkoe water column: unusual green-colored GSB resembling *Chlorobium chlorovibrioides* (phylogenetically very close to *Chlorobium phaeovibrioides*), brown-colored GSB *Chlorobium phaeovibrioides*, purple sulfur bacteria *Thiocapsa rosea* and *Thiorhodococcus kakinadensis*, and the purple nonsulfur bacterium *Rhodovulum sulfidophilum* [15].

Bacterial sulfate reduction. Radioisotope experiments showed that the process of sulfate reduction became noticeable in the 2.7-m layer corresponding to the upper layer of the redox zone (Fig. 7). Below, sulfate reduction rate increased, reaching the maximum values (1690 μg S L^{-1} day^{-1}) in the "purple" water layer of 2.9 m. In this layer, the production of new organic matter was most active due to anoxygenic photosynthesis. Below 2.9 m, sulfate reduction rate decreased considerably: 2-fold in the 3.0-m layer and 6-fold in the 3.5-m layer. It is notable that sulfate reduction rate was twice higher in the 2.9-m water layer than in the most active upper layer of the sediment (0–2 cm, 890 μg S L^{-1} day^{-1}). The number of viable cells of sulfate reducers was 10^4 cells per mL in the "purple" layer (according to the colony count) and 10^3 cells per mL in the bottom layer with the maximum sulfide content.

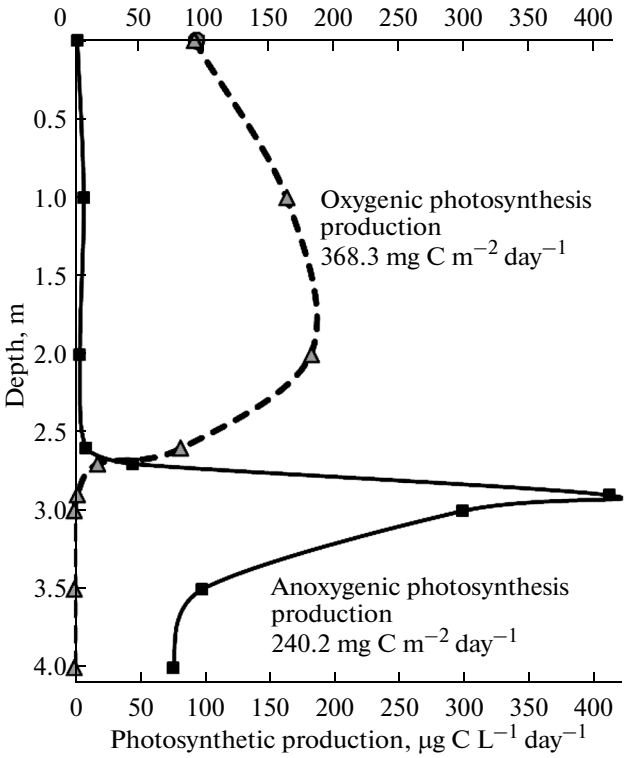

Fig. 6. Rates of oxygenic (OP) and anoxygenic (AP) photosynthesis in Lake Kislo-Sladkoe in September, 2010.

The change in sulfate concentration provided the possibility for indirect estimation of the rates of both oxidative and reductive microbial processes of the sulfur cycle. Absolute values of sulfate concentration may be used for marine basins with a constant ratio of basic salts. For stratified basins with variable salinity within the water column, it is appropriate to use the value of the fractional content of sulfate expressed as the sul-

Table 1. Content of photosynthetic pigments in the water horizons and the number of anoxygenic photosynthetic bacteria counted on agarized nutrient media (Lake Kislo-Sladkoe, September 2010)

Depth, m	Content of photosynthetic pigments, μg L^{-1}		Viable cell number (10^3 cells mL^{-1})		
	chlorophyll *a*	bacteriochlorophyll (*d* + *e*)	green-colored GSB	brown-colored GSB	PSB
2.0	8.2	0	0	0	0
2.7	0	120	11.2	0.5–0.8	3.4
2.9	690	66	11.3	6.8	3.4
3.0	0	133	3.5	3–9	4.5
4.0	0	160	99	50	3.4

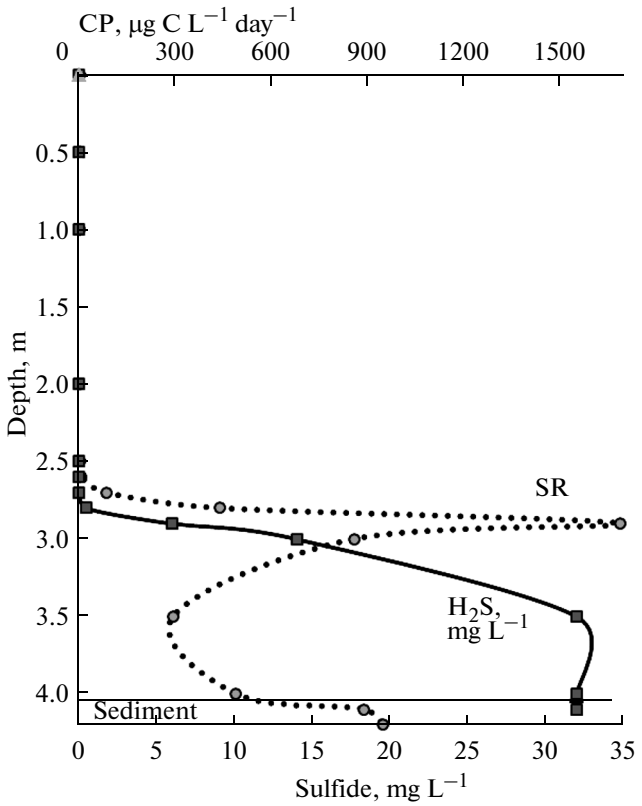

Fig. 7. Sulfate reduction (SR) rate and sulfide [H_2S] content in the water column and in the upper sediment layer of Lake Kislo-Sladkoe.

fate/chloride coefficient (SO_4^{2-}/Cl^-). The data from Fig. 8 show that the value of this coefficient in the oxic zone of Lake Kislo-Sladkoe (down to the 2.6-m layer) varied from 0.130 to 0.141, which corresponds to the value for the open White Sea. At the depths of 2.7–2.8 m, the fractional content of sulfate was higher than in the surface layer (0.153–0.162). These data showed that sulfate production is due to oxidative processes associated with the activity of sulfur-oxidizing bacteria, and purple and green sulfur bacteria exceeded its uptake by sulfate-reducing bacteria. The 2.9-m layer, which exhibited the highest rates of both photosynthesis and sulfate reduction, had a sulfate/chloride coefficient of 0.131. Thus, an approximate equilibrium of sulfur balance occurred in this horizon, which was slightly shifted towards reductive processes leading to sulfate uptake. In the underlying layers, beginning from 3.0 m, the content of sulfate was evidently lower (to 0.092 in the bottom layer), which was a consequence of active sulfate reduction.

Methane content, its production and oxidation. Figure 9 shows the data on vertical distribution of methane in the water column and upper sediment and the rates of its production and oxidation. Methane was present throughout the water column. The concentration of CH_4 in the 1-m surface layer was 1.3–2.6 μL L^{-1}, while at a depth of 2 m its content rose to 28 μL L^{-1} and then gradually increased to the maximum value of 112 μL CH_4 L^{-1} in the near-bottom layer.

Methanogenesis (MG) was abruptly detected in the "purple" layer of 2.9 m. In the lower layers (beginning from 3.0 m), MG decreased, although it still remained high in the near-bottom water layer. In the bottom sediments, the maximum methanogenesis rate was observed in the uppermost (0–2 cm) layer (Fig. 9). The absolute values of methane production rates in the bottom sediments varied from 2.1 to 4.85 μL CH_4 dm^{-3} day^{-1}. Microbial methane oxidation (MO) occurred both in the oxic and anoxic parts of the water column. The maximum MO rate was found in the "purple" layer (1630 nL L^{-1} day^{-1}), where it constituted 57% of MG rate. In the overlying layers of the water column, MO rate decreased considerably, although its absolute values exceeded those of MG. In the uppermost 2-m layer, both MO rate and methane content decreased (Fig. 9).

The carbon isotopic composition of dissolved methane from the near-bottom water layer ($\delta^{13}C$ (CH_4) = –87.76‰) was considerably lighter than in the overlying horizons ($\delta^{13}C$ (CH_4) = –77.95‰, Fig. 10). Heavier methane carbon isotopic composition indicated fractionation resulting from the preferential uptake of the light carbon isotope by microorganisms. The most isotopically heavy methane was found in the "purple" layer of 2.9 m ($\delta^{13}C$ (CH_4) = –72.61‰), where the process of methane oxidation was most active. In the overlying oxic layers of the water column, methane became slightly lighter due to its additional production in situ. The substrates for methanogenic archaea in this layer were probably reduced compounds from the underlying anoxic sulfide-containing water layer.

Carbon isotopic composition of suspended organic matter and isotopic composition of dissolved mineral carbon. The data on the carbon isotopic composition of suspended organic matter and the isotopic composition of dissolved mineral carbon in Lake Kislo-Sladkoe water (Fig. 10) were in good agreement with the results of determination of photosynthetic activity (Fig. 6). The main peak of oxygenic photosynthesis production at a depth of 2.0 m coincided with the local maximum of carbon isotope fractionation (Δ = 17.03‰). In the overlying layers (the surface and 1.0 m), where the amount of suspended matter was less, both photosynthetic production and carbon isotope fractionation were lower (Δ = 8.32 and 12.29‰). The carbon isotopic composition of suspended organic matter in the uppermost two-meter layer was little different from the respective values typical of marine suspended matter, indicating that the share of allochthonous suspended matter from the freshwater brook was negligible. The 2.6-m layer was characterized by a local minimum of photosynthetic activity (Fig. 6), which resulted in the relative decrease of car-

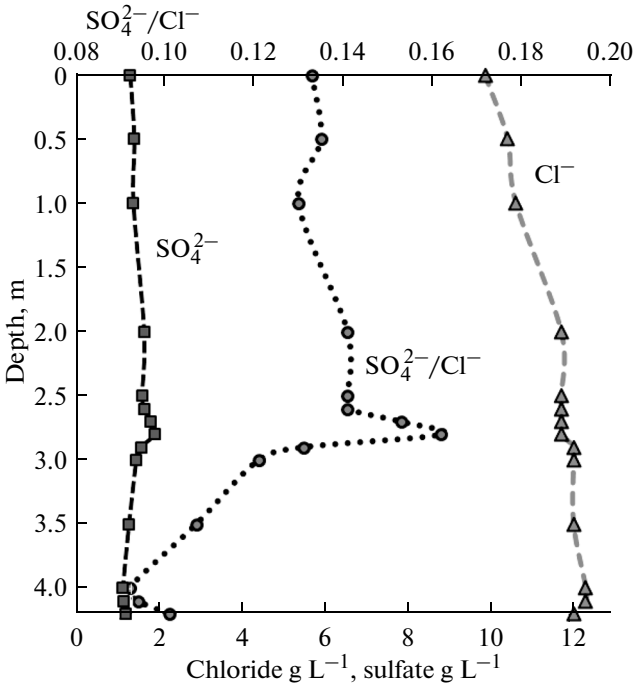

Fig. 8. Chloride and sulfate ion concentrations, their ratio in the water and pore water of sediments in Lake Kislo-Sladkoe.

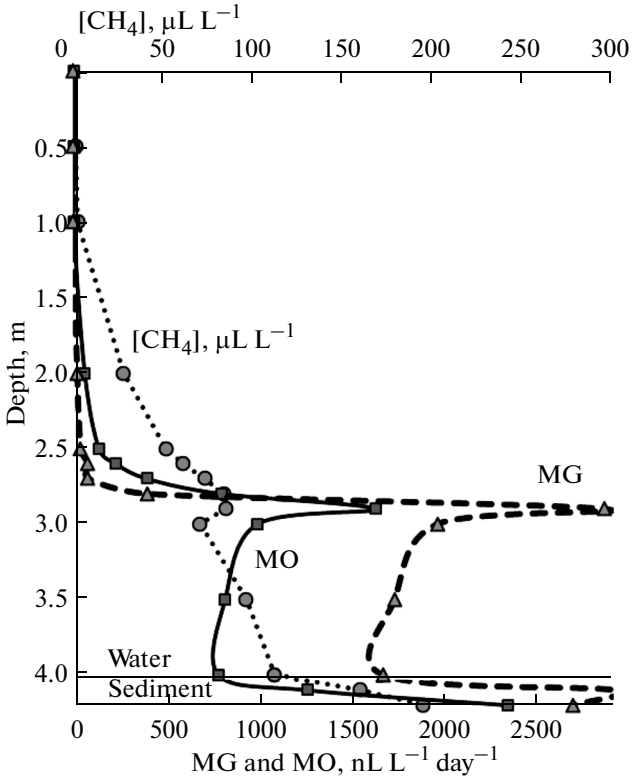

Fig. 9. Methane $[CH_4]$ content, rates of methanogenesis (MG) and methane oxidation (MO) in the water column and upper sediments of the Lake Kislo-Sladkoe.

bon isotope fractionation ($\Delta = 11.94‰$). In the narrow "purple" water layer at 2.9 m, the rates of both autotrophic and heterotrophic processes (anoxygenic photosynthesis, MG, MO, and SR) were maximal. As a result of these reactions, organic matter carbon became lighter to –36.36‰, while the mineral carbon became heavier to –7.56‰, respectively, with the difference between the values of 28.8‰. In the bottom layer, the carbon isotopic composition of organic matter became heavier, reaching –22.42‰, while the fractionation value dropped again to $\Delta = 11.84‰$.

DISCUSSION

During the period of our research (early September 2010), the temperature of the surface water layer in Lake Kislo-Sladkoe decreased to 11–14°C and salinity increased to 18‰ (in late July 2010, the surface temperature was 22°C and salinity was 12‰; A.N. Pantyulin, personal communication). Thus, thermohaline stratification was less pronounced than in the summer season. However, the water column maintained a distinct thermocline and distinct vertical zonality of biogeochemically significant microbial processes. The upper oxic zone from the surface to the depth of 2.5 m was characterized by relatively high photosynthetic production: 368.3 mg C m^{-2} day^{-1} (Fig. 6). Analysis of the carbon isotopic composition of suspended organic matter ($\delta^{13}C$ C_{org} from –21.7 to –22.9‰) in the uppermost two-meter layer revealed the autochthonous origin of this suspended matter (Fig. 10). The total number of microorganisms and the rate of dark CO_2 assimilation (Figs. 4, 5) obviously exceeded the values typical of mesotrophic lakes. Attention should be also drawn to the considerable average volume of bacterioplankton cells (0.8–2.0 μm^3); the average volume of bacterial cells in the White Sea is only 0.28 μm^3 [15].

A consolidated decrease of all the parameters characterizing microbial activity (TNM, DCF, photosynthetic activity) was observed at a depth of 2.6 m (the lower boundary of the oxic layer, Figs. 4–6). Substantial changes in microbial activity indices began at a depth of 2.7 m, where trace amounts of dissolved oxygen were yet detected and where sulfide flow penetrated; in this layer, active chemosynthesis was associated with the upper boundary of sulfate reduction (Figs. 5, 7). The "purple" water layer localized at a depth of 2.9 m was characterized not only by intense color but also by the maximum rates of microbial activity (both autotrophic and heterotrophic). In this local layer, the production of anoxygenic photosynthesis was up to 411 μg C L^{-1} day^{-1} and sulfate reduction was up to 1.7 mg S L^{-1} day^{-1} (Figs. 6, 7). Active anoxygenic photosynthesis continued below the "purple" layer. The total production of bacterial photosynthesis was 240 mg C m^{-2} day^{-1}, only 1.5-fold lower

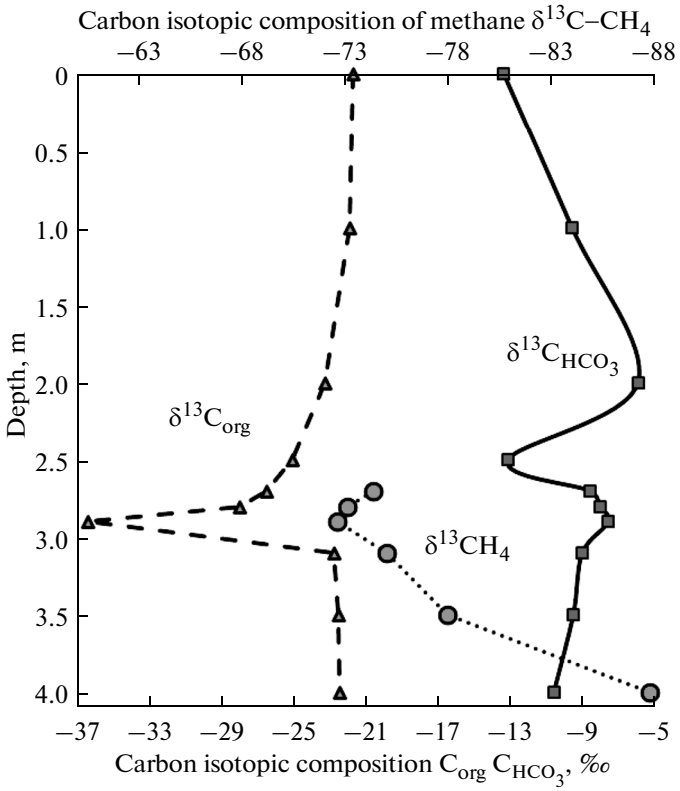

Fig. 10. Carbon isotopic composition of suspended organic matter ($\delta^{13}C_{org}$), the carbon of bicarbonate ($\delta^{13}C–HCO_3^-$) and methane ($\delta^{13}C–CH_4$) in the water column of Lake Kislo-Sladkoe.

than the production of oxygenic photosynthesis. It should be noted that the well-illuminated layer of oxygenic photosynthesis was 2.7 m thick, while the "darkened" layer of bacterial photosynthesis was only 1.3 m thick. Intense production of organic matter in this zone was confirmed by a substantial increase in the amount of suspended matter (up to 410 mg L^{-1} in the 2.9-m layer, Fig. 4). In the 2.9-m layer, the carbon of suspended organic matter had the most lightweight composition ($\delta^{13}C_{org} = -36.36‰$, Fig. 10). The community of anoxygenic phototrophic bacteria was represented by both green-colored (*Chlorobium* sp.) and brown-colored green sulfur bacteria (*Chl. phaeovibrioides*).

It is known that the bloom of anoxygenic phototrophic bacteria at the interface between oxic and sulfide-containing waters in meromictic lakes of different types leads to a noticeably lighter carbon isotopic composition of suspended organic matter [17]. The 9.5-m water layer of Lake Mogilnoye in September 2001 had the value of $\delta^{13}C_{org} = -33.0‰$, and the brown-colored green sulfur bacterium *Chl. phaeovibrioides* was predominant in the community of anoxygenic phototrophic bacteria [17]. In September 2003, the 30-m water layer of the alpine meromictic Lake Gek-Gel had the value of $\delta^{13}C_{org} = -37.0‰$ directly in the area of contact between oxic and sulfide-containing waters, while the fractionation value was 28.05‰ [18]. The brown-colored green sulfur bacterium *Chl. phaeobacteroides* was predominant in the chemocline of Lake Gek-Gel.

Below 3.0 m, there was the anoxic zone proper, where the content of both sulfide (32 mg L^{-1}) and methane (70–112 μL L^{-1}) was maximal; at the same time, DCF rate and the rates of anoxygenic photosynthesis and chemosynthesis abruptly decreased (Figs. 5, 6). The rates of sulfate reduction and methanogenesis also decreased and became stable (Figs. 7, 9).

The rates of microbial processes in the uppermost layer of the bottom sediments was not significantly different from the respective values in the bottom water layer, which is typical of meromictic basins with indistinct water–sediment interfaces. Organic matter that is difficult to degrade under anaerobic conditions is probably concentrated here. The calculation showed that the daily sulfide production in the 20-cm layer of the sediment was 470 μM S m^{-2}, while the daily sulfide production in the water column was 2200 μM S m^{-2}. By using the generalized equation for sulfate reduction $2[CH_2O] + SO_4^{2-} > H_2S + 2HCO_3^-$ and knowing the production of reduced sulfur, we have calculated organic matter consumption for sulfate reduction. During the period of our research, the daily C_{org} uptake coupled to sulfate reduction in the water column and in the upper sediment layer was estimated to be 65 mg C m^{-2}. It is known that, along with sulfate reduction, the terminal phase of anaerobic degradation of organic matter is the process of bicarbonate reduction and methanogenesis occurring mainly with the involvement of hydrogen: $4[CH_2O] > 4H_2 + CO_2 > CH_4 + 2H_2O$. The calculation showed that daily methane production was 2.2 μM m^{-2} in the sediment layer and 10 μM m^{-2} in the water column. Taking into account that all hydrogen is of biogenic (microbial) origin, it may be derived that daily consumption of organic matter for methane production was 0.6 mg C m^{-2}. Thus, sulfate reduction in Lake Kislo-Sladkoe was a much more significant (×110) biogeochemical process than methanogenesis.

Comparison between the biogeochemical processes in Lake Kislo-Sladkoe and its known analogs seems relevant for assessment of their significance and scale. At present, the best studied meromictic basin of marine genesis is the relict Lake Mogilnoye [2, 17]. The most significant parameters of microbial processes in the water columns of these lakes are presented in Table 2. The principal characteristics confirm the fundamental similarity of these two meromictic basins. Integral values of production of oxygenic and anoxygenic photosynthesis are close. However, Lake Kislo-Sladkoe is four times shallower than Lake Mogilnoye and, hence, its vertical biogeochemical

Table 2. Comparison of the biogeochemical microbial activity in the hypolimnion of meromictic basins: Lake Mogilnoye (the Barents Sea) and Lake Kislo-Sladkoe (the White Sea)

Parameter		Lake Mogilnoye	Lake Kislo-Sladkoe		
		1973 Aug.–Sept.	1999 June	2001 Sept.	2010 Sept.
OM production by oxygenic photosynthesis, mg C m^{-2} day^{-1}		295	280	110	370
OM production by anoxygenic photosynthesis, mg C m^{-2} day^{-1}		330	620	180	240
Maximum number of microorganisms, $\times 10^6$ cells mL^{-1}		0.6	2.6	1.8	17
Maximum H_2S content, mL L^{-1}		180	102	107	20
SR rate, μg S L^{-1} day^{-1}	Interval	8–150	2.5–33	3.0–37	8–1700
	Average	40	8	10	320
Methane content, μL L^{-1}	Interval	10–410	5–51	0.8–115	60–112
	Average	156	21	60	80
CIC SOM (carbon isotopic composition of suspended organic matter), $\delta^{13}C_{org}$	Max		–23.5	–26.0	–21.69
	Min		–31.5	–33.0	–36.36

zonality is more contrasted. The total number of microorganisms in Lake Kislo-Sladkoe was substantially higher than the TNM of Lake Mogilnoye.

The value of photosynthetic production in Lake Kislo-Sladkoe is higher; therefore, more fresh organic matter arrives into the underlying layers. The maximum production of anoxygenic photosynthesis in the Lake Kislo-Sladkoe (411 μg C L^{-1} day^{-1}) slightly exceeds the respective values for Lake Mogilnoye obtained in September (274 μg C L^{-1} day^{-1} [16]) but is lower than the July values (608 μg C L^{-1} day^{-1}). The adequacy of such comparison implies that the summer values of anoxygenic photosynthesis in the Lake Kislo-Sladkoe should be higher than the September values. Sulfate reduction rate in the near-bottom water layer was very high (320 μg S L^{-1} day^{-1} on average) due to consumption of organic matter formed via both anoxygenic and oxygenic photosynthesis; however, sulfide did not accumulate at extremely high concentrations but was consumed by phototrophic bacteria.

Thus, the studies performed in September 2010 revealed that Lake Kislo-Sladkoe is a pronounced stratified meromictic basin with actively functioning microbial carbon and sulfur cycles. Suspended matter of the water column is mainly of autochthonous genesis. Substantial contribution to organic matter production is made by the process of anoxygenic photosynthesis, which involves reduced sulfur compounds. In contrast to Lake Mogilnoye, where the period of active anthropogenic activity was accompanied by increased organic matter flow [2], Lake Kislo-Sladkoe has not yet experienced any anthropogenic loads. We assume that the medium-term changes in the general pattern of microbial processes will be determined by the rise of the sea bridge separating the lake from the Rugozerskaya Gulf, which will result in a gradual weakening of the influence of the sea basin. However, in the foreseeable future critical tides and storm surges will continue to replenish the reservoir with seawater.

ACKNOWLEDGMENTS

The authors are sincerely grateful to the personnel of the Pertsov White Sea Biological Research Station, Moscow State University: A.B. Tseitlin and E.D. Krasnova, and to the colleagues A.N. Pantyulin, N.A. Demidenko and N.A. Belyaev for their practical assistance in field research.

The work was supported by the Russian Foundation for Basic Research, project no. 11-04-00175a, and the basic research programs of the Presidium of the Russian Academy of Sciences: "Molecular and Cell Biology" and "Biosphere Origin and Evolution".

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Translated by E. Makeeva