Microbial Processes of the Carbon and Sulfur Cycles in Lake Mogil'noe

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Received April 18, 2001

Abstract—In the beginning of the summer of 1999, complex microbiological and biogeochemical investigations of meromictic Lake Mogil'noe (Kil'din Island, Barents Sea) were carried out. The analysis of the results shows a clearly pronounced vertical zonality of the microbial processes occurring in the water column of the lake. To a depth of 8 m, the total number and activity of microorganisms was limited by the relatively low content of organic matter (OM). In the upper part of the hydrogen-sulfide zone of the lake (beginning at a depth of 8.25 m), the content of particulate OM and the microbial number sharply increased. In this zone, the daily production of OM during anaerobic photosynthesis at the expense of massive development of colored sulfur bacteria reached 620 mg C/m^2, which was twofold greater than the daily production of phytoplankton photosynthesis and led to a considerable change in the isotopic composition ($\delta^{13}C$) of the particulate OM. In the same intermediate layer, the highest rates of sulfate reduction were recorded, and fractionation of stable sulfur isotopes occurred. Below 10 m was the third hydrochemical zone, characterized by maximum concentrations of H_2S and CH_4 and by a relatively high rate of autotrophic methanogenesis. The comparison of the results obtained with the results of investigations of previous years, performed in the end of summer, shows a decrease in the intensity of all microbial processes inspected. An exception was anoxygenic photosynthesis, which can utilize not only the de novo formed H_2S but also the H_2S accumulated in the lake during the winter period.

Key words: meromictic Lake Mogil'noe, anaerobic photosynthesis, sulfate reduction, methane oxidation, methane production, fractionation of carbon and sulfur isotopes.

The mesotrophic meromictic Lake Mogil'noe is located in the southwestern part of Kil'din Island in the Barents Sea. The lake is separated from the sea by a narrow barrier of rolled rock waste and gravel, through which seawater penetrates the lake. From the opposite northeastern coast, the lake receives fresh surface and ground water, which drain the swamped part of the island. Mixing of these two types of waters produces a pronounced salinity gradient in the deep part of Lake Mogil'noe, from virtually fresh water on the surface to weakly diluted seawater in the lake monnimolimnion.

The first mention of the unusual hydrochemical regime of Lake Mogil'noe belongs to Knipowitsch [1]. Seven years later, Palmkwist found hydrogen sulfide in the deep part of the lake. In 1906, B. Issatchenko performed the first microbiological investigations of Lake Mogil'noe [2] and showed that at the boundary of the hydrogen-sulfide zone there occurs a layer of water having a pink color determined by massive development of the purple bacterium *Chromatium*. Issatchenko believed that the enrichment of the near-bottom water with hydrogen sulfide occurred due to the activity of sulfate-reducing bacteria and that this hydrogen sulfide was oxidized by purple bacteria in the process of anoxygenic photosynthesis.

New data on the microflora of Lake Mogil'noe and on the peculiarities of the microbial processes therein were obtained in the beginning of the 1970s [3–5]. By using ^{14}C-bicarbonate, Gorlenko *et al.* [3, 4] demonstrated that the neogenesis of organic matter in Lake Mogil'noe mainly occurs at the expense of the activity of a community of various photosynthetic sulfur bacteria. Another supposition of Issatchenko (that photosynthetic sulfur bacteria play the key role in the oxidation of hydrogen sulfide formed by sulfate-reducing bacteria in deep waters and bottom sediments of Lake Mogil'noe) was also confirmed [5, 6].

Long-term hydrochemical observations (1901–1973) performed at Lake Mogil'noe show gradual lift of the upper boundary of the hydrogen-sulfide zone and an increase in the H_2S content in the near-bottom water (Table 1). An analogous phenomenon was recorded in the meromictic Lake Belovod' (Vladimir oblast), where the H_2S content in near-bottom water increased from 4.5 mg/l in 1928 to 95–100 mg/l in the 1960s [10]. In this connection, one of the goals of the present work

Table 1. Changes in the depth of location of the upper boundary of the hydrogen-sulfide zone and in the content of hydrogen sulfide in the near-bottom waters of Lake Mogil'noe over the 20th century

Year of observation	Upper boundary of the H_2S zone, m	H_2S content, ml/l	Reference
1901	–	22.9	[7]
1909	12.5	17.7	[8]
1921	12.0	25.7	[8]
1969	10.0	127.0	[9]
1973	9.5	175.4	[4]

was to perform a complex of microbiological and biogeochemical investigations aimed at quantitative assessment of the rates of anoxygenic photosynthesis and sulfate reduction in Lake Mogil'noe and to compare the results obtained with data of the previous investigations.

Besides, although the microbiological investigations of Lake Mogil'noe have been rather profound, no data on the activity of the methane cycle microorganisms are available in the literature. At the same time, according to Gurevich [11], the content of methane in the water column of the lake was as high as hundreds of μl/l in 1968–1969. Therefore, another task of the present work was to study the distribution of methane in the water column and bottom sediments of the lake and to determine the rates of microbial production and oxidation of methane.

MATERIALS AND METHODS

The investigations at Lake Mogil'noe were performed from June 19 through June 25, 1999 in the course of a complex hydrological–hydrochemical expedition guided by V.V. Sapozhnikov (All-Russia Research Institute of Fishery and Oceanography). Samples of water and bottom sediments were taken in the deep (15.5 m) part of the lake from a boat.

Bottom sediments were sampled with a limnological stratometer with transparent changeable tubes made of organic glass (diameter, 5 cm). This device allows upper sediment horizons (to a depth of 20–40 cm) to be sampled without impairing their structure. Water was sampled with 0.75-l horizontal plastic Niskin bathometer; the sampling depth interval was 25 cm.

Immediately after lifting, the sediment samples were placed in 5-ml plastic syringes with an edge cut off and closed with a gas-tight rubber stopper. The water from bathometer was dispensed into 30-ml glass flasks which were closed (without leaving an air bubble) with gas-tight rubber stoppers and aluminum caps with a perforation for the introduction of required reagents.

The rates of microbial processes of glucose consumption, light and dark assimilation of carbon dioxide, sulfate reduction, methane oxidation, and methane production were determined radioisotopically using uniformly labeled ^{14}C-glucose, $NaH^{14}CO_3$, $^{14}CH_4$, $Na_2{}^{35}SO_4$, and $^{14}CH_3COONa$. The syringes with sediment samples and glass flasks with water samples were supplemented, using a syringe, with 0.1–0.2 μl of a solution of the necessary labeled compound and sunk on a capron puller to the horizons of sampling to be incubated under the in situ conditions. The flasks where dark assimilation of carbon dioxide was to be determined were wrapped with foil before the introduction of the samples. The incubation time was 6–12 h, after which the samples were fixed with 0.5–1 ml of a concentrated KOH solution and transported to the Institute of Microbiology, where the rates of microbial processes were determined as described earlier [12, 13].

The content of methane in samples was determined by the phase-equilibrium degassing method [14]. The contents of sulfates and chlorides were determined on a Biotronik liquid ionic chromatograph (Germany).

The total number of microorganisms was determined on 0.2-μm-pore-size polycarbonate membrane filters by the fluorescent method using 4',6-diamidino-2-phenylindole (DAPI) to stain the cells [15], which were then counted under a LUMAM-3 luminescent microscope.

The species composition of the aerobic methanotrophic microflora was quantitatively studied by indirect immunofluorescence method as described by Gal'chenko *et al.* [16]

The isotopic composition of the carbon of the particulate organic matter and that of sulfate and hydrogen-sulfide sulfur was determined on an MI-1201 V mass spectrometer (Ukraine) as described earlier [17].

RESULTS

Physicochemical Characteristics of the Lake Mogil'noe Water Column

The chemical composition of the Lake Mogil'noe water is determined by the two components of the water balance: the arrival of surface fresh water and the filtration of seawater through the barrier separating the lake from the Barents Sea. The upper 3 m of the water column are slightly mineralized (about 10% seawater) and saturated with oxygen (Table 2). At a depth of 3 to 5 m, the total salinity increases from 3.1 to 20.7‰, and the concentration of dissolved oxygen considerably decreases, apparently due to active processes of oxidation of particulate organic matter, whose sedimentation decelerates in the halocline zone.

At a depth of 6 m, there is the absolute maximum of dissolved oxygen, related to the activity of marine phytoplankton. Beginning from a depth of 7 m, the concentration of oxygen decreases to 1.09 and 0.47 ml/l at the

Table 2. Physicochemical characterization of the water and of the surface layer of bottom sediments of Lake Mogil'noe

Depth	Alkalinity, mg-equiv/l	Content of:			
		O_2, ml/l	H_2S, ml/l	S, ‰	SO_4^{2-}, mg/l
Water column, m					
0	1.6			–	–
1	1.6	7.69	0	3	267
3	1.9	7.72	0	3.1	536
3.5	2	–	–	–	–
4	2.1	–	–	–	–
4.5	2.2	–	–	–	–
5	2.3	6.79	0	20.7	1550
6	2.9	8.21	0	23.4	1750
7	2.9	5.44	0	25.9	–
8	2.9	3.27	0	27.8	2100
8.25	2.7	2.18	0	–	–
8.5	2.6	1.09	0.08	–	2150
8.75	3	0.47	0.16	–	–
9	3.2	0	3.3	29.4	2194
9.25	3.4	0	–	–	–
9.5	3.6	0	–	–	2194
9.75	3.8	0	–	–	–
10	4	0	1.4	29.9	2065
10.5	5	0	–	–	–
11	6.4	0	10.8	30.1	2194
12	11.6	0	67.3	30.2	1935
14	15	0	107.4	30.9	1613
15.5	12.4	0	102.4	31.2	1677
Bottom sediments (horizon, cm; content of components per dm^3 of wet sediment)					
0–5	5.0	–	–	–	1605
10–15	5.0	–	–	–	1690
20–25	6.0	–	–	–	1230

depths of 8.50 and 8.75 m, where the low amounts of O_2 occur together with traces of hydrogen sulfide (Table 2).

Low concentrations of hydrogen sulfide (0.08–3.3 ml/l) occur at the depths of 8.5 to 10.0 m. Beginning with the depth of 11 m, the concentration of H_2S sharply increases, reaching 107.4 and 102.4 ml/l in near-bottom water. The total alkalinity of water also increases with the depth of the anaerobic zone (Table 2).

*The Total Number and Activity
of Microorganisms in the Lake Water*

The results of the determination of the total number of microorganisms and of the activity of dark and light assimilation of CO_2 are presented in Table 3. The results of the determination of the heterotrophic poten-

tial in experiments with radiolabeled glucose are shown in the figure.

In the aerobic part of the lake, rather active (although not very abundant) heterotrophic microflora occurred to a depth of 4 m: the specific rate of dark CO_2 fixation varied from 0.66 to 3.11 µg per 10^8 cells. In the depth interval of 4.5–7.0 m, the number of microorganisms and the value of the heterotrophic potential (figure) gradually increased, but the specific rate of dark assimilation of CO_2 decreased significantly as compared with the above water column, apparently due to the decrease in the content of readily available organic matter, the main part of which is utilized in the surface waters.

The increase in the total number of microorganisms and in the value of the heterotrophic potential, against

μg C/(l day)
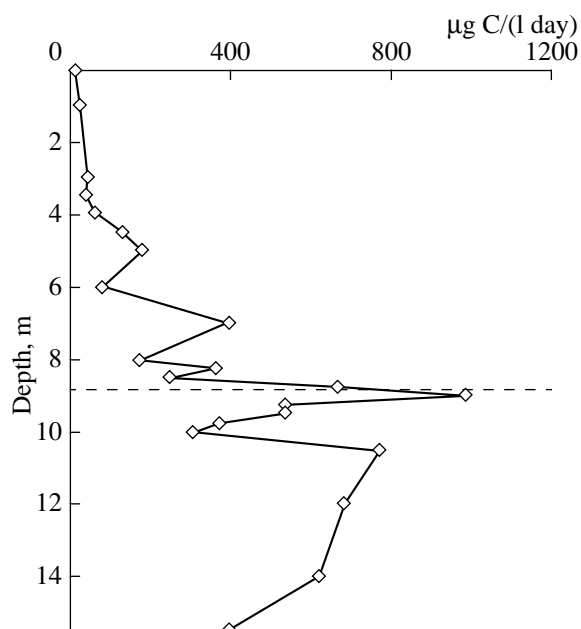
Rate of glucose consumption in the water column of Lake Mogil'noe (dashed line shows the lower boundary of the oxygenated zone).

the background of the very weak dark CO_2 fixation, continued to a depth of 8.5 m. Below this depth, the rate of dark CO_2 assimilation increased abruptly to 60.7 μg/(l day) in the 8.75-m horizon and even to 145 μg/(l day) at a depth of 9.0 m (Table 3). It was in these two horizons that the highest values of specific rate of dark CO_2 fixation were recorded (up to 9.06 μg per 10^8 cells).

Analysis of the hydrochemical data (Table 2) shows that it is in these two horizons that the most favorable conditions for the massive development of aerobic chemolithotrophic thionic bacteria occur: dissolved oxygen and considerable concentrations of hydrogen sulfide were recorded here. In addition, in the water of this zone (the depths of 8.75 and 9 m), the highest rate of aerobic methane oxidation was recorded, which was an order of magnitude higher than in the above water column (Table 4).

In the anaerobic part of the lake (below 9.0 m), the absolute and specific rates of dark CO_2 fixation considerably decreased (Table 3). However, the chemosynthetic processes did occur here, apparently due to the development of methanogens and sulfate reducers (Tables 4, 5).

A tentative estimate of the contribution of chemosynthesis to dark CO_2 fixation, presented in Table 3, was obtained by the following calculations. The average specific value of dark CO_2 fixation in the lower part of the anaerobic zone (from 4.0 to 8.5 m), in which chemoautotrophs cannot develop because of the lack of reduced substrates, was 0.33 μg per 10^8 bacterial cells. Dark heterotrophic fixation of CO_2 in the anaerobic

zone was calculated by multiplying this value by the total bacterial number in each horizon. The values obtained were subtracted from the total value of dark CO_2 fixation in each horizon. The difference was considered as the quantitative estimate of bacterial chemosynthesis (Table 3).

In the last column of Table 3, the data on CO_2 fixation in the light are presented. In the aerobic zone, the maximum rate of phytoplanktonic photosynthesis was recorded at a depth of 3 m, i.e., at the upper boundary of picnocline. A considerable increase in the rate of CO_2 fixation in the light began in the anaerobic zone from a depth of 8.75 m, and its maximum value was recorded at a 9.25-horizon, where the production of organic matter reached 0.8 mg C/(l day) (Table 3).

It was in these horizons that the maximum numbers of bacterial cells were recorded and large (8–10 μm long) cells were revealed, which most probably represented photosynthetic bacteria. According to data of V.M. Gorlenko, in August and September 1973, the green bacteria *Chlorobium phaeovibrioides* prevailed; the species *Pelodictyon phaeum* and *Prosthecochloris phaeoasteroides* were more rare; in the chemocline, the purple sulfur bacteria *Thiocystis violaceae* and *Thiocapsa roseopersicina* were found.

Thus, at the boundary of the aerobic and anaerobic waters of Lake Mogil'noe, intense production of organic matter takes place, due to massive development of aerobic and anaerobic chemosynthetic and anaerobic photosynthetic bacteria of the sulfur cycle. Parallel to the increase in the hydrogen sulfide content, the share of chemosynthesis in the total organic matter production decreases notably (Table 3).

The data on the determination of particulate organic matter and the isotopic composition of its carbon (Table 6) correlated with the data on the abundance and geochemical activity of phytoplankton and microorganisms in the water column of Lake Mogil'noe (Table 3). The upper peak of particulate organic matter at a depth of 3 m coincided with the maximum of photosynthetic activity of phytoplankton. The greatest amount of particulate organic matter occurred in the 9.0-m horizon (Table 6), i.e., in the immediate vicinity of the zone of maximum microbial number and highest rate of CO_2 fixation (9.25-m horizon, Table 3).

Active production of organic matter by chemoautotrophic and photoautotrophic bacteria in the upper part of the hydrogen-sulfide zone resulted in considerable changes in the carbon composition of particulate organic matter. As can be seen from Table 6, in the aerobic part of the water column, the $\delta^{13}C$ value of particulate organic matter varied within the range of –30.1 to –31.5‰, which is typical of the organic matter of swamp origin. However, in the horizons where active photo- and chemosynthesis occurred, the organic carbon was considerably heavier (the $\delta^{13}C$ value reached –23.5‰ at a depth of 10 m, Table 4). A notable concomitant increase in the amount of particulate organic

Table 3. Total number of microorganisms and the rates of dark and light CO_2 fixation

Horizon, m	Total number of micro-organisms, 10^5 cells/ml	Dark CO_2 fixation, µg C/(l day)	Specific rate of CO_2 fixation, µg/10^8 cells	Heterotrophic fixation of CO^2, µg C/(l day)	Chemo-synthesis, µg C/(l day)	CO_2 fixation in the light, µg C/(l day)	Total autotrophic fixation of CO_2, µg C/(l day)
0	0.9	2.3	2.55	2.3	0	33.8	0
1	5	3.3	0.66	3.3	0	42.8	0
3	0.9	2.8	3.11	2.8	0	0.7	0
3.5	1.7	1.8	1.06	1.8	0	2.8	0
4	0.6	0.7	1.16	0.7	0	11.3	0
4.5	1.7	0.4	0.33	0.4	0	6.8	0
5	2.6	0.5	0.19	0.5	0	17.0	0
6	2.0	0.9	0.45	0.9	0	10.8	0
7	5.0	2.3	0.46	2.3	0	9.0	0
8	18	2.9	0.16	2.9		13.7	0
8.25	20	10.1	0.50	10.1	0	4.8	0
8.5	18	2.4	0.13	2.4	0	5.1	0
8.75	26	60.7	2.33	8.32	52.38	217	270 (19.6)
9	16	145	9.06	5.12	139.88	285	425 (32.9)
9.25	26	24	0.92	8.12	15.98	812	828 (1.9)
9.5	18	32	1.77	5.76	26.24	481	507 (5.1)
9.75	15	49	3.26	4.80	43.20	531	574 (7.5)
10	17	27	1.59	5.44	21.56	302	324 (6.7)
10.5	19	–	–	–	–	–	–
12	9	–	–	–	–	–	–
14	7	–	–	–	–	–	–
15.5	13	–	–	–	–	–	–

Note: Parenthesized is the percent of chemoautotrophic production.

matter (Table 6) and bacterial biomass (Table 3) suggests a considerable increase in the isotopically heavy biomass of photo- and chemosynthetic bacteria. The enrichment of the residual carbon dioxide with the ^{13}C isotope at a depth of 8.5 m (Table 6) also testifies to active biological CO_2 consumption, accompanied by the weighting of residual mineral carbon.

The organic matter produced by photo- and chemosynthetic bacteria in the upper part of the hydrogen-sulfide zone activates the development of heterotrophic bacteria oxidizing organic matter to CO_2. This process is manifest in the increase in the total alkalinity of water, which is observed already at a depth of 9.0 m (Table 2). Beginning with the 9.5-m horizon, gra dual enrichment of inorganic carbon with the light isotope occurs (Table 6) along with the continuing increase in total alkalinity, which is due to the oxidation of the newly formed organic matter. From the depth of 11 m, an analogous change in the isotopic composition of particulate organic matter becomes apparent; this is most probably due to the fact that not-readily-available autochthonous compounds become prevalent. There-

fore, the δ^{13}C value of the particulate organic matter in the near-bottom water (δ^{13}C = –28.7‰) is close to the δ^{13}C value of the particulate organic matter in the surface waters of the lake (Table 6). The virtually complete consumption of easily available organic matter produced in the zone of photo- and chemosynthesis is confirmed by the fact that the δ^{13}C value of inorganic carbon (–22.4‰) is very close to the δ^{13}C value of organic carbon in the zone of its maximum production at the depth of 9.5–10 m (–23.5 and –23.8‰).

The Processes of Methane Production and Methane Oxidation

Table 4 presents data on the vertical distribution of methane and the rates of its production and oxidation in the water column and bottom sediments of the lake. The zone of the maximum rates of methanogenesis in the water column (10.5–14.0 m) coincides with the zone of maximum methane content (Table 4). Near the boundary with the aerobic zone, the rate of methanogenesis was an order of magnitude lower. However, a small

Table 4. Content of methane and the rates of its production and oxidation in the water column of Lake Mogil'noe

Horizon	CH$_4$ content, μl/l	Methane production, nl/(l day)*	Methane oxidation, nl/(l day)**
Water column			
0	0.127	–	13.38 (86)
1	0.145	–	0.64 (64)
3	0.127	–	1.95 (20)
3.5	0.138	–	1.07 (36)
4	0.215	–	3.25 (40)
4.5	0.321	–	1.77 (26)
5	0.705	–	2.79 (33)
6	0.459	–	3.47 (10)
7	0.350	–	3.35 (14)
8	0.917	–	7.33 (14)
8.25	0.847	26 (19)	2.70 (50)
8.5	1.31	–	29.9 (55)
8.75	5.08	23 (0)	90.9 (69)
9	9.53	66 (55)	39.7 (69)
9.25	8.26	19 (0)	14.5 (35)
9.5	7.30	219 (18)	28.2 (34)
9.75	8.57	74 (45)	30.4 (84)
10	8.89	315 (10)	39.4 (82)
10.5	31.1	434 (5)	270 (67)
12	35.6	459 (12)	218 (79)
14	51.1	655 (5)	276 (69)
15.5	47.9	433 (7)	1862 (38)
Bottom sediments (the rates of processes were calculated per dm^3 of wet sediment per day)			
0–5	21.0	409 (2)	748 (65)
10–15	17.5	28 (21)	135 (88)
20–25	17.5	430 (1)	984 (96)

 * Parenthesized is the percent ratio of aceticlastic methanogenesis to total methanogenesis.
** Parenthesized is the percent of carbon dioxide formed from methane.

amount of methane (26–29 nl/(l day)) was also produced in the zone of concomitant occurrence of methane, oxygen, and hydrogen sulfide, in the horizons 8.25 and 8.75 m. It can be assumed that anaerobic methanogens function in this zone inside particles suspended in the water.

Of interest was one more phenomenon atypical of meromictic bodies of water, namely a decrease in the methanogenesis rate in the near-bottom water and bottom sediments (Table 4). We believe that these data are not erroneous, because a notable decrease in the methane content was also recorded in these horizons (Table 5). Most probably, the decrease in the methanogenesis rate

is due to the exhaustion of readily available organic matter, which is actively consumed in the upper parts of the anaerobic water column.

Another reason for the decrease in the contents of both methane (Table 4) and hydrogen sulfide (Table 2) in the near-bottom water of the deep part of Lake Mogil'noe may be the constant inflow of seawater, which has a higher density than the water of the lake. The feasibility of this process is suggested by the data on total mineralization, obtained at Lake Mogil'noe by the researchers from the Institute of Fishery and Oceanography.

Virtually in all horizons of the water column and bottom sediments, the major part of methane (from 55 to 100%) originates from autotrophic methanogenesis. The very low rate of aceticlastic methanogenesis, even in the presence of notable acetate concentrations (Table 4), is most probably due to acetate consumption by sulfate reducers, which are most abundant in the depth interval of 8.25–9.25 m (Table 5); their enzymatic systems have much higher affinity to acetate. A considerable increase in the rate of autotrophic ($H_2 + CO_2$) methanogenesis, beginning from the depth of 9.5 m seems to be related to gradual anaerobic decomposition of isotopically light autochthonous organic matter by anaerobic cellulolytics and fermenters, producing hydrogen as one of metabolic products.

Microbial oxidation of methane occurs both in aerobic and anaerobic parts of Lake Mogil'noe. The content of methane in the aerobic zone is very low (in no sample was its higher than 1 μl/l), therefore the rate of its oxidation is also low. Beginning with a depth of 8.5 m, the methane concentration considerably increased. It should be noted that, to a depth of 8.75 m, the water still contained traces of dissolved oxygen. It was in this horizon of the water column that the first activity peak of methane-oxidizing microorganism was recorded (Table 4).

A second, even more pronounced peak of methane oxidation occurred between the depths of 10.5 and 14 m. In this layer, the concentrations of dissolved methane were also maximum.

The near-bottom waters and the uppermost horizon of bottom sediments were also characterized by a high rate of anaerobic methane oxidation. The absolute rate of methane oxidation here was considerably higher than the rate of methane production; this fact may be one more reason for the decrease in the methane concentration in the near-bottom water and upper horizons of bottom sediments (Table 4).

On the whole, the balance of the microbial processes of methane production and methane oxidation in Lake Mogil'noe is positive. In the anaerobic part of the water column and in bottom sediments, 3.25 ml of methane is formed as calculated for a water column with a surface of 1 m^2 and for the 30-cm bottom sediment layer of the same surface. Simultaneously, 2.60 ml of CH$_4$ per m^2 is consumed in the course of microbial

Table 5. Rate of sulfate reduction (SR) and the isotopic composition of sulfur compounds in the water column and surface layer of bottom sediments of Lake Mogil'noe

Horizon, m	1973*			1999**		
	SR rate, μg S/(l day)	δ^{34}S[H_2S], ‰	δ^{34}S[SO_4^{2-}], ‰	SR rate, μg S/(l day)	δ^{34}S[H_2S], ‰	δ^{34}S[SO_4^{2-}], ‰
			Water column			
7	0			0		21.4
8	0			7.9		20.5
8.25	–			261.6		21.0
8.5	–			265.5		20.9
8.75	–			434.0	−41.5	20.9
9	27			382.0		21.5
9.5	25			33.3		24.4
9.75	23			6.5		24.7
10	29	−11.0	21.0	4.1	−31.0	26.6
10.5	8			4.7		25.7
12	34	−20.4	22.7	2.7		29.7
14	37	−22.3	24.4	2.2	−27.0	30.0
15.5	150	−18.0	27.8	2.2	−25.6	29.9

  * Data of the expedition in 1973 [4].
** Our data.

oxidation (0.05 ml in the aerobic part of the water column and 2.55 ml in the anaerobic zone of the lake).

Table 7 presents data on the distribution of certain species of methanotrophic bacteria. It can be seen that the population density of methanotrophs varied within the range of $(6.4–14.4) \times 10^3$ cells/ml of water.

The Process of Bacterial Sulfate Reduction

Data presented in Table 5 demonstrate that the maximum rate of sulfate reduction occurred in the uppermost horizons of the hydrogen-sulfide zone of the lake, in the zone where the production of organic matter in the course of photo- and chemosynthesis takes place (Tables 3, 6). Below 9.75 m, the sulfate reduction rate decreased by two orders of magnitude to be about 10 times lower than the values recorded by Gorlenko *et al.* in August–September 1973 [4].

Unfortunately, the upper layers of water of the hydrogen-sulfide zone, characterized by the most active sulfate reduction, were not studied during the work performed in 1973.

A considerable decrease in the rate of sulfate reduction in the hypolimnion produced an effect on the isotopic composition of hydrogen sulfide. It is known [18] that the value of the isotopic effect in the course of sulfate reduction is inversely related to the rate of this process. The progressive enrichment of residual sulfates with heavy isotopes of sulfur in the deep part of the lake is

related to the utilization of a part of sulfates and to restricted mixing of the deep waters of the lake (Table 5).

DISCUSSION

Summing up the results obtained in this work, we would like first of all to emphasize the well-pronounced

Table 6. Amount and isotopic composition of particulate organic carbon in the water column of Lake Mogil'noe and isotopic composition of the carbon of dissolved bicarbonate

Horizon, m	Amount of particulate matter, mg/l		δ^{13}C of HCO_3^-, ‰
	mg/l	$\delta^{13}C_{org}$, ‰	
1	1.6	−30.1	
3	3.4	−30.8	
5	1.4	−31.0	−11.3
6	1.1	−31.5	−9.1
8	2.0	−29.3	−7.9
8.5	2.9	−25.3	
9	13.4	−24.2	−9.1
9.5	4.2	−23.8	−10.8
10	2.4	−23.5	−17.0
11	3.4	−27.2	−18.0
12	4.4	−27.3	−22.4
14	6.7	−25.7	
15.5	7.5	−28.7	

Table 7. Population density of certain species of methanotrophic bacteria in the water column of Lake Mogil'noe as determined with specific immune antiserums

Species	Cells/ml, $\times 10^3$					
	1 m	3 m	5 m	6 m	8 m	9.5 m
Methylocystis echinoides	2	2.5	2.2	NF*	NF	NF
Methylocystis methanolicus	NF	NF	NF	NF	NF	NF
Methylomonas methanica	NF	NF	NF	NF	NF	NF
Methylocystis pyriformis	NF	NF	NF	NF	NF	NF
Methylosinus sporium	NF	NF	NF	NF	NF	NF
Methylomonas methanica	3.8	2.6	7.2	6	NF	NF
Methylosinus trichosporium	2.3	2.7	3.6	2	NF	NF
Methylobacter bovis	NF	NF	NF	NF	NF	NF
Methylobacter chroococcum	NF	NF	NF	NF	NF	NF
Methylocystis parvus	NF	NF	NF	NF	NF	NF
Methylococcus capsulatus	1.2	5.4	1.4	4.2	NF	NF
Total	9.3	13.2	14.4	6.4	NF	NF

* "NF" means "not found."

Table 8. Changes in some microbiological and hydrochemical parameters in the hypolimnion of Lake Mogil'noe over the period from August–September 1973 through June 1999

Parameter		1973 (August–September)*	1999 (June)**
Primary production of phytoplankton, mg C/(m² day)		295	280
Organic matter production in anaerobic photosynthesis, mg C/(m² day)		330	620
Maximum microbial number, cells/ml		60×10^6	26×10^6
Maximum content of H_2S in near-bottom water, ml/l		180	102
Sulfate reduction rate, m S/(l day)	limits	8.0–150.0	2.2–33.3
	average	40.5	8.1
Sulfur isotope fractionation during sulfate reduction, ‰	limits	32.0–46.7	55.5–62.4
	average	41.9	58.1
Methane content, ml/l;	limits	10.0–410.0	5.0–51.0
	average	156.0	21.4

 * Data of 1973 [4].
** Our data.

vertical zonality of the microbial processes in the water column of Lake Mogil'noe.

The upper aerobic zone, to a depth of 8.0 m, is characterized by a rather low level of the production of organic matter, which is formed in the course of the photosynthesis of phytoplankton (about 280 mg C/(m² day)), and by a low amount of particulate organic matter (1.1–3.4 mg C/l, Tables 6, 8). The analysis of the $\delta^{13}C$ values of the particulate organic matter (from –30.1 to –31.5‰, Table 6) suggests allochthonous origin of its major part. The total number of microorganisms and the absolute rate of dark fixation of carbon dioxide (Table 3) are not high and are rather typical of mesotrophic lakes.

Considerable changes in the microbial abundance and activity begin at a depth of 8.0–8.25 m, in a horizon penetrated by a diffusive flow of hydrogen sulfide and containing trace amounts of dissolved oxygen. The total number of microorganisms and the level of dark CO_2 fixation increase abruptly (Table 3), the sulfate reduction process begins (Table 5), and the amount of particulate organic matter increases. All three of the above values continue increasing to a depth of 9.0–9.25 m: the maximum number of microorganisms occurs in the depth interval of 8.75–9.25 m (Table 3); the maximum level of dark CO_2 fixation and chemosynthesis is in the depth interval of 8.75–9.0 m; and the sulfate reduction rate is maximum in the 8.75–9.0-m layer (Table 5).

One of the major peculiarities of the upper horizons of the hydrogen-sulfide zone of the lake is powerful anaerobic photosynthesis at the expense of massive development of colored sulfur bacteria, whose activity was the highest in the 9.25-m horizon (Table 3). The total production of bacterial photosynthesis in the 8.5- to 10.0-m water layer reached 620 mg C per m^2 per day; in the period of our investigations, it was more than two times higher than the photosynthetic production of phytoplankton in the 8-m aerobic water column (Table 8).

The fact of the intense autotrophic production of organic matter in this zone is confirmed by a considerable increase in the amount of particulate organic matter (up to 13.4 mg/l in the 9.0-m horizon, Table 6), by notable change in the $\delta^{13}C$ value of particulate organic matter (to –23.5 and –23.8‰), and by somewhat less drastic changes in the $\delta^{13}C$ of mineral carbon (Table 6), indicating consumption of isotopically light CO_2 in the processes of photo- and chemosynthesis.

Of particular interest is the consideration of the interaction of the processes of sulfate reduction and autotrophic organic matter production in the upper part of the hydrogen-sulfide zone (8.25–10.0 m). The total daily production of organic matter (photosynthesis + chemosynthesis = 620 + 66 = 686 mg C/(m^2 day)), calculated from data of Table 3, can with an excess provide for the daily production of H_2S in the same water layer (417 mg S/(m^2 day) as calculated from data of Table 5). On the other hand, this production of H_2S is insufficient to sustain the process of autotrophic CO_2 fixation at the level of 686 mg/(m^2 day). According to the balance equation $2H_2S + CO_2 = (CH_2O) + 2S + H_2O$, the daily expenditure of H_2S should be at least 3950 mg, a value almost 10 times higher than the daily production of H_2S due to sulfate reduction. It evident that the high rate of autotrophic CO_2 fixation can be provided for only at the expense of hydrogen sulfide previously formed in the water column.

Our work at Lake Mogil'noe was conducted in the very beginning of the Arctic summer; we believe that in winter, when the penetration of light into the upper part of the hydrogen-sulfide zone is limited by the ice cover of the lake and the processes of photosynthesis are retarded, sulfate reduction processes result in the accumulation of a large reserve of H_2S. After the ice breaks up, active processes of bacterial photosynthesis begin, resulting in an abrupt decrease in the H_2S content in the upper part of the anaerobic zone. The latter inference is experimentally supported by the data on the distribution of H_2S: in the 8.25–10.0 m layer, its concentration did not exceed 3.3 ml/l; from the depth of 11.0 m, a significant increase in the H_2S content began (10.5 ml/l) and, at depth of 12.0 m, its concentration was as large as 67.3 ml/l (Table 2). Such a distribution of H_2S is absolutely atypical of the summer period, when the content of H_2S usually gradually increases from the surface of the hydrogen-sulfide zone to the bottom of a lake [4, 7].

Below 10 m, there was a third hydrochemical zone, whose characteristic features were maximum concentrations of hydrogen sulfide and methane (Tables 2, 4) and very low rates of both sulfate reduction and methanogenesis (Tables 4, 5). An increase in the content of particulate organic matter (Table 6) against the background of a decreasing microbial number (Table 3) suggests that the major part of this organic matter is represented by not-readily-available allochthonous substances entering the lake with spring flood waters and sedimented, by the moment of our investigations, to the deep part of the lake.

The allochthonous origin of this organic matter is confirmed by data on the isotopic analysis of carbon: in the near-bottom water, the $\delta^{13}C$ values of the particulate organic matter are close to those of the particulate organic matter in the surface waters (Table 6). A slight shift toward heavier values can easily be explained by an admixture of newly formed organic matter entering deep waters from the zone of autotrophic $\delta^{13}C$ fixation (Table 6). As already mentioned above, the observations of the hydrogen-sulfide regime of Lake Mogil'noe have been carried out for almost a hundred years, and, until 1973, a notable increase in the H_2S concentration in the near-bottom water was recorded (Table 1). Our data, obtained in June 1999, showed however a considerable (almost twofold) decrease in the H_2S concentration as compared to the latest determinations of August–September 1973 (Table 8). These data are in good agreement with a considerable (almost fivefold) decrease in the rate of sulfate reduction in the monnimolimnion of the lake and with the increase in the fractionation of stable sulfur isotopes in the process of sulfate reduction (Tables 5, 8).

These results, obtained by various methods, unequivocally demonstrate the decrease in the rate of sulfate reduction in the monnimolimnion of Lake Mogil'noe. Moreover, there are other indications of a decrease in the microbial activities in this body of water: the maximum total density of microorganisms decreased more than two times, and the methane content decreased more than sevenfold, both in terms of maximum and minimum concentrations and in terms of average values (Table 8).

In an attempt to understand the reasons for the changes in the hydrochemical regime of Lake Mogil'noe over the last 100 years, two hypotheses should be considered. We think that the increase in the H_2S content, especially pronounced from 1921 through 1969 (Table 1), was related to intense economical development of the Kil'din Island in the Soviet period. Several large military objects were built on the island at that time; a big fishing collective farm "Staryi Kil'din" was functioning in the vicinity of the lake. The anthropogenic pollution of the island resulted in an increase in the discharge of allochthonous organic matter into the lake, and this lead to activation of anaerobic sulfate-reducing and methanogenic bacteria. Over the last

decade, the anthropogenic load on the Kil'din Island and Lake Mogil'noe considerably decreased, and this resulted in a decrease in the contamination of the lake.

A second possible reason for the significant distinctions in our hydrochemical and microbiological data from the data of 1969 and 1973 (Tables 1, 8) may be that our work was performed in the beginning of summer, immediately after the break up of ice, whereas our predecessors worked in the end of summer and in the beginning of autumn.

The most significant distinctions are listed below:

(1) the production of phytoplanktonic photosynthesis in June was comparable to that in August: 280 and 295 mg C/(m^2 day) (Table 8);

(2) the H$_2$S content in the zone of active anaerobic fixation of CO$_2$ in June was considerably lower than in August: 1.4–3.3 versus 29.7–45.2 ml/l;

(3) the rate of sulfate reduction in the hypolimnion in June was an order of magnitude lower than in August (Table 8);

(4) the content of H$_2$S in the near-bottom water in June was almost two times lower than in August: 102 versus 180 ml/l;

(5) the production of organic matter in the anaerobic zone in June was however notably higher than in August: 620 versus 330 mg C/(m^2 day).

Based on the data available, the scenario of the development of the processes of hydrogen sulfide formation and oxidation in the period between June and August can be hypothesized to be as follows. In winter, the processes of anaerobic photosynthesis utilizing H$_2$S are strongly retarded because of the deficiency of light. After the melting of ice, vigorous development of photoautotrophic microorganisms begins, due to which the anaerobic production of organic matter reaches considerable values. However, this process results in almost complete depletion of hydrogen sulfide in the water layer where massive development of phototrophs occurs (8.25 to 10 m, Table 2).

The depletion of the reserve of hydrogen sulfide, which is not compensated for by sulfate reduction, should result in rapid dying off of a part of the population of photosynthetics. This, in turn, should increase the migration of a part of organic matter to the anaerobic part of the lake and enhance the rates of sulfate reduction and methanogenesis with a consequent increase in the contents of H$_2$S and CH$_4$ in the monnimolimnion (probably, up to values recorded in August–September 1973).

One more source of additional organic matter for the anaerobic zone may be the summer production of the organic matter of phytoplankton and the inflow of allochthonous organic matter.

Thus, to elucidate the reasons of the dynamics of hydrochemical and microbiological processes in Lake Mogil'noe, additional investigations in different seasons are necessary.

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