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Microbial Destruction of Organic Matter in the Bottom Sediments of Lithuanian Lakes

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Abstract—The rates of the processes of bacterial sulfate reduction (SR) and total destruction of organic matter (D_{total}) were studied in the bottom sediments (BS) of 14 lakes in Lithuanian national and regional parks in the summers of 1998–2002. Anaerobic processes accounted for an average of 92% of D_{total} in the depressions of deep-water lakes; for the sediments of shallow lakes, high rates of oxygen uptake were noted. The SR rate in different lakes varied from 0.09 to 2.60 mg $\text{S}^{2-}/(\text{dm}^3 \text{ day})$. At low sulfate concentrations (13.3–70.6 mg $\text{S-SO}_4^{2-}/\text{dm}^3$), characteristic of the BS of freshwater ecosystems, the main factor that affected the SR rate in the BS of the lakes studied was the content of readily available organic matter; only in special cases, was it affected by a change in the sulfate ion concentration. In shallow lakes, temperature-dependent activation of sulfate-reducing bacteria and their inhibition by acidification of the environment were recorded. The contribution of SR to D_{total} was 0.2 to 11.0%.

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In Lithuania there are more than 6000 lakes of natural origin (glacial, karst, fluvial), and the lake area occupies about 1.5% of the whole territory. Small lakes predominate, and only in 14 of the lakes does the area exceed 1000 ha [1]. The lakes are low-eutrophic; the majority are mesotrophic, with forest or sandy, weakly cultivated basins. The depth and area of most of the lakes have considerably decreased due to sedimentation during their long period of existence (about ten thousand years); hence, the layer of bottom sediments is now 5–10 m thick, and in some lakes, up to 20 m thick [2].

Detailed ecological and microbiological investigations lasting many years were previously carried out only in extensive Lithuanian reservoirs exposed to significant anthropogenic loads (Lake Drukshyai, a basin cooler of the Ignalina Nuclear Power Plant, the Bay of Kurshsk). The data obtained allowed the assessment of the influence of economic activity on the structural and functional characteristics of planktonic communities and on the microbiological processes in the bottom sediments of these reservoirs [3, 4]. At the same time, there were virtually no studies of the rate and diversity of the microbiological processes of the carbon and sulfur cycles in the small lakes situated mainly on the territories protected by the state and subjected to low anthropogenic loads. There are also few similar studies that have been conducted in the small lakes of neighboring

Baltic countries; moreover, they were conducted more than ten years ago [5].

The aim of this work was to study the specific features of the bacterial process of sulfate reduction and total (aerobic and anaerobic) destruction of organic matter in the bottom sediments of small Lithuanian lakes not exposed to a deleterious anthropogenic effect, as well as to determine the relationship between the intensity of these processes and the environmental conditions.

MATERIALS AND METHODS

The work was carried out in the summers of 1998–2002 (July–August) in 14 lakes. The reservoirs studied are mainly of glacial origin and are typical representatives of the types of lakes in national and regional parks located in different regions of Lithuania (figure). Most of them are surrounded by a forest, and some are exposed to recreational loads (Lakes Balsys, Gulbinas, Akmyanos). The relic Lake Eserinis is situated inside a raised bog; Lake Kreivasis is near a lowland swamp. As evidenced by chemical and biological parameters, most of them can be considered mesotrophic lakes of different productivity; two (Lake Lynezeris and Lake Plaze) are eutrophic and two (Lake Kreivasis and Lake Eserinis) are dystrophic lakes [6].

The sites of water and sediment sampling were located in the deepest parts of each lake. The water for chemical analyses was sampled by the Ruttner's bottle.

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A map of Lithuania and the scheme of the location of the regions where the lakes were studied: 1, Trakai Historical National Park (Lake Akmena); 2, Verkiai Regional Park (Lake Balsys, Lake Gulbinas); 3, Labanoras Regional Park (Lake Duobulis, Lake Kreivasis); 4, Aukštaitija National Park (Lake Baluosas, Lake Baltele); 5, Zemaitija National Park (Lake Berzoras, Lake Ilgis); 6, Pajūris Regional Park (Lake Kalote, Lake Plaze); 7, Dzukija National Park (Lake Bedugnīs, Lake Lynezeris); 8, Cepkeliai Reserve (Lake Eserinis).

From the bottom sediments sampled with a box-like dredge, slabs were collected with sterile glass tubes. The upper 5-cm-thick layer of sediment was used for chemical and microbiological analyses.

Water temperature and pH were measured with electrodes of a WTW MultiLine F/Set3 portable measuring device; water clarity was measured with a Secchi disk; oxygen content was measured by Winkler's method. The organic matter of the bottom sediments, in the form of C_{org} , was determined by the bichromate oxidizability method [7]; the sulfates were analyzed in water extract colorimetrically; the hydrogen sulfide and acid-soluble sulfides, using the Volkov and Zhabina's method [8]. Volatile fatty acids (acetate, propionate, isobutyrate, butyrate) were analyzed using a Tsvet 500 M gas chromatograph with a flame ionization detector. Separation was carried out in a 1 m \times 3 mm glass column filled with Porapak Q, 100–120 mesh (Fluka). The column and detector temperatures were 180 and 210°C, respectively; argon was the carrier gas.

The rates of aerobic and the total destruction of organic matter in the bottom sediments were determined by the isolated tubes method. Glass tubes (3.5 mm in diameter) were filled with sediment (0–5 cm) by gently pressing them into a silt slab sealed at one end. Bottom water was then siphoned into slab-filled or slab-free tubes, which were then sealed at the other end; no air bubbles were left in the tubes. The samples were incubated for 24 h in the near-bottom water layer. The rates of aerobic and total destruction of organic matter were assessed by O_2 uptake of the sediments and by CO_2 emission from them [9].

The rate of sulfate reduction was determined with the radioactive tracer method, using $Na_2^{35}SO_4$ [9–11]. The bottom sediment samples were placed in two replicate glass tubes hermetically sealed with rubber caps; the water samples were placed in 60-cm³, dark, glass vials. The $Na_2^{35}SO_4$ solution, with a specific activity of $2\text{--}3 \times 10^6$ pulses/min, was injected into the samples by means of a long syringe needle. The samples with the tracer were incubated in the dark for 24 h in situ and

Table 1. General characteristic of the lakes studied

Lake	Area, ha	Maximum depth, m	Physicochemical properties of water				
			clarity, m	T, °C	pH	O ₂ , mg/l	chlorophyll a, µg/l
Akmena	276.5	30.2	6.5	23.0/9.0	8.3/7.0	11.5/4.3	2.1
Balsys	55.0	38.8	2.8	21.5/5.6	8.2/7.5	10.8/0.4	2.6–4.2
Gulbinas	47.1	13.6	1.2	24.0/7.8	8.2/7.1	10.6/0.0	4.5–6.5
Duobulis	3.8	13.5	5.0	22.7/6.8	8.3/6.6	9.1/0.0	2.2
Kreivasis	3.6	7.0	3.0	21.5/8.6	6.75/6.1	8.3/0.4	4.3
Baluosas	427.3	33.7	5.1	24.0/8.6	8.7/7.5	10.0/3.3	2.8
Baltele	–	6.5	3.9	23.8/15.0	8.3/–	10.5/4.2	–
Berzoras	52.0	6.0	2.1	21.2/17.1	8.1/7.3	9.2/4.3	8.0
Ilgis	106.2	6.1	2.2	22.5/21.2	7.9/7.8	9.8/8.8	7.1
Kalote	51.4	1.2	1.2	18.0	–	9.6	4.0
Plaze	4.9	1.0	1.2	18.0	–	11.8	30.0
Bedugnis	5.2	11.0	4.3	23.0/7.0	7.7/6.5	11.0/1.0	3.6
Lynezeris	18.5	2.0	0.4	25.0/23.0	8.5/8.5	12.0/10.3	105.3
Eserinis	14.5	4.7	4.0	23.0/23.0	3.4/3.7	9.3/9.8	2.1

Note: The numerator, near-surface values; the denominator, near-bottom values; dash (–), no data.

fixed with 2 ml of a 20 mM Na₂MoO₄ · 2H₂O solution. In the laboratory, the samples were acidified, and hydrogen sulfide was trapped in an alkaline 0.05 N KMnO₄ solution. The labeled sulfides were converted to sulfates, and the radioactivity of BaSO₄ precipitates was measured on filters using a scintillation counter (Beckman Instruments Inc.) and the scintillation cocktail Opti PhaseHiSafe 3 (Wallac Inc). Postgate's medium with lactate was used for the enumeration of sulfate-reducing bacteria (SRB) [12].

RESULTS

Physicochemical characterization of lake waters and sediments. The lakes studied differed in the area and depth (Table 1). The temperature of the bottom water layers in the deeper lakes (11–32 m) did not exceed 9°C; in shallow lakes (1.2–7 m), which were warmed up all the way to the bottom, the average temperature was 19°C. In shallow lakes (Kalote, Plaze, Lynezeris, and Eserinis), the O₂ concentration in the bottom layer of water exceeded the saturation level, due to photosynthetic activity of autotrophs; in deep lakes the O₂ concentration was 0–4.0 mg/l. The pH near the bottom slightly varied between acidic (pH 6.0) and slightly alkaline (pH 8.5). In the dystrophic Lake Eserinis, fed by the inflow of swampy waters with a high content of humic compounds, an acidic reaction (pH 3.7) was found.

The bottom sediments consisted of silts of a high water content (Table 2). The C_{org} content varied between 5 and 42.7% of the dry weight. The sediments

of the lakes located close to bogs (Lakes Eserinis and Kreivasis) had the highest C_{org} content (41–42.7%). Sulfates concentrations in the sediments were low and ranged from 13.3 to 71.4 mg S-SO₄²⁻/dm³ in different lakes.

Total destruction of organic matter in the bottom sediments. The OM decomposition rates in the bottom sediments of most of the lakes (determined by CO₂ emission) varied between 732 and 2160 mg C/(m²/day); in dystrophic lakes, the rates were significantly lower, 348–368 mg C/(m² day). In the deep-water depression zones of deeper stratified lakes (Akmena, Balsys, Baluosas, Gulbinas, Duobulis, and Bedugnis), anaerobic processes of the mineralization of organic matter predominated, accounting for an average of 92% of total mineralization of Akmena, Balsys, Baluosas, Gulbinas, and Bedugnis. In shallow lakes, a high rate of oxygen uptake by the bottom sediments, from 461 to 1268 mg O₂/(m² day), was noted. Anaerobic processes were also recorded here.

The number of sulfate-reducing bacteria and the sulfate reduction rate. In deep lakes, the SRB numbers varied from 10² to 10³ cells/cm³ of silt; in shallow lakes, it was lower, from 10 to 10² cells/cm³ of silt. SRB were not isolated from the acidic bottom sediments of Lake Eserinis.

In most of the lakes, the sulfate reduction rate, during the summer thermal stagnation, varied between 0.09 and 2.2 mg S²⁻/(dm³ day) (Table 3). The highest sulfate reduction rates were observed in the summer 2001 in Lake Balsys, one of the deepest lakes studied (Table 4). At the same time, studies conducted in this

Table 2. Physicochemical characteristics of the bottom sediments of the lakes studied in the summers of 1998–2002

Lake	Station depth	Type of sediments	Humidity, %	C _{org} , %	Fatty acids, mg C/kg*	S-SO ₄ ²⁻ , mg/dm ³	H ₂ S + HS ⁻ , mg/dm ³
Akmena	25.0	Clayey sapropel	87.0	20.2	4.2	26.0	256.0
Balsys	25.0–32.0	Limestone, dark gray silt	82.0	5.0	3.5	71.4	858.0
Gulbinas	11.5	Dark gray silt	86.0	11.0	2.3	32.0	1168.0
Duobulis	13.0	Liquid black silt	91.0	18.5	–	46.7	176.0
Kreivasis	3.0	Turfy silt	95.0	42.7	–	46.7	72.0
Baluosas	21.0	Limestone, dark gray silt	84.6	11.1	4.4	20.3	104.0
Baltele	6.5	Dark gray silt	91.2	19.4	11.0	20.3	88.0
Berzoras	5.8	Silt with an admixture of sapropel	84.0	11.1	5.8	13.3	21.6
Ilgis	4.5	Silt with an admixture of sapropel	85.0	18.9	4.8	21.3	80.0
Kalote	1.2	Black silt with detritus	83.0	16.8	8.1	29.5	128.0
Plaze	1.2	Dark gray silt covered with underwater vegetation	89.0	21.0	9.5	–	–
Bedugnis	11.0	Dark gray silt with detritus	84.0	17.2	23.5	13.3	72.0
Lynezeris	2.0	Black silt with detritus	92.0	28.0	6.3	41.3	176.0
Eserinis	4.0	Turfy silt	90.0	41.0	2.0	13.3	56.0

* The sum of volatile fatty acids (acetate, propionate, and butyrate).

Table 3. The numbers of sulfate-reducing bacteria (SRB) and the rates of the processes of organic matter destruction in the bottom sediments of Lithuanian lakes in summer (SR, D_a, and D_{total}, sulfate reduction, aerobic destruction, and total destruction, respectively)

Lake	SRB, cells/cm ³	SR, mg S ²⁻ /(dm ³ day)	OM destruction		C _{org} consumption for SR, mg/(m ² day)	SR, % of D _{total}
			D _a , mg O ₂ /(m ² day)	D _{total} , mg C/(m ² day)		
Akmena	10 ³	0.52	409.6	1920	19.5	1
Balsys	10 ³	1.42	0	1349	53.5	4
Gulbinas	10 ²	1.22	0	990	46.0	5
Duobulis	10	0.09	0	1800	3.5	0.2
Kreivasis	10	0.13	464.0	348	5.0	1
Baluosas	10 ³	0.47	480.0	720	17.5	2
Baltele	–	0.49	1248.0	2160	18.5	1
Berzoras	10 ²	0.30	892.8	1302	11.5	1
Ilgis	10 ²	0.43	1050.0	660	16.0	2
Kalote	10 ²	0.58	836.6	760	22.0	3
Plaze	–	–	644.8	284	–	–
Bedugnis	10 ²	0.67	0	1920	25.0	1
Lynezeris	10 ²	2.2	1268.0	732	82.5	11
Eserinis	0	0.13	460.8	368	5.0	1

Table 4. Physicochemical characteristics and the destruction rate of organic matter in the bottom sediments of Lake Balsys in the summers of 1998–2002

Year	Station depth, m	Bottom water layer			Bottom sediments				
		T, °C	pH	O ₂ , mg/dm ³	C _{org} , %	S-SO ₄ ²⁻ , mg/dm ³	H ₂ S + HS ⁻ , mg/dm ³	SR, mg S ²⁻ /(dm ³ day)	D _{total} , mg C/(m ² day)
1998	25.0	5.6	7.5	0.7	4.7	66.9	496	0.56	744
1999	25.0	5.8	7.5	0.6	5.7	70.6	1152	1.66	1116
2001	25.0	5.5	7.8	0.4	3.4	102.7	776	2.60	1491
2002	32.0	5.5	7.3	0.0	6.0	45.3	1008	0.85	2046

lake over several summers have demonstrated considerable year-to-year fluctuations in the rate of sulfate reduction, which were directly related to the changes in sulfate concentration. The average sulfate reduction rate in this lake was 1.42 mg S²⁻/(dm³ day).

In the forest medium-depth Lake Bedugnis, SRB were also noticeably active, in spite of their relatively low number (10² cells/cm³); sulfate reduction was detected not only in the bottom sediments (up to 0.67 mg S²⁻/(dm³ day)), but also in the near-bottom water (up to 0.08 mg S²⁻/(dm³ day)). At low sulfate concentration of 13.3 mg S-SO₄²⁻/(dm³), the SRB activity was probably sustained by a high content of low-molecular organic compounds, in particular, volatile fatty acids (23.5 mg C/kg).

With regard to shallow lakes, a relatively high sulfate reduction rate (2.2 mg S²⁻/(dm³ day) was recorded in the eutrophic Lake Lynezeris, with a high temperature of near-bottom water (23°C) and higher concentrations of sulfates (41.3 mg S-SO₄²⁻/dm³) and volatile fatty acids (6.3 mg C/kg). The rate of the process did not depend on the SRB number, which constituted not more than 10² cells/cm³, as in most shallow lakes. The lowest sulfate reduction rate (0.09–0.13 mg S²⁻/(dm³ day)) was recorded for the sediments of lakes located near swamps (Lakes Eserinis, Kreivasis, and Duobulis).

The sediment content of hydrogen sulfide and acid-soluble sulfides, the end products of sulfate reduction, was minimal in the bottom sediments of shallow lakes. The highest levels of these compounds were recorded in dug lakes, deep-water Lake Balsys (1152 mg/dm³) and the more shallow Lake Gulbinas located nearby (1168 mg/dm³). However, the concentration of the end products in lake sediments and bottom water was not always directly proportion to the rate of sulfate reduction. For example, in the bottom sediments of Lake Bedugnis, where rather intense sulfate reduction was recorded, the concentration of soluble sulfides was low (72 mg/dm³). Since the rate of light-dependent ¹⁴CO₂ assimilation in the microaerobic hypolimnion of this reservoir was significantly higher than in surface waters [6], the hydrogen sulfide that evolved during sulfate reduction was most probably rapidly oxidized by

chemo- and photoautotrophic microorganisms of the near-bottom water layers.

DISCUSSION

Due to the significant amounts of organic matter in small lakes of different depths located on the protected territories of Lithuania, the overall activity of bacteriobenthic communities, as evidenced by the evolution of CO₂ from the silt columns, was sufficiently high (except for the dystrophic lakes). The rates on CO₂ evolution from the bottom sediments determined in this work are comparable to those recorded for different types of other lakes in the neighboring Baltic states [5]. Anaerobic processes accounted for an average of 92% of D_{total} in the bottom sediments of deep lakes. In the warmed up profundal of most shallow medium-productive lakes, aerobic processes dominated among degradative processes; fluctuations of O₂ concentrations from 3 to 10 mg/l in the bottom water did not affect their rate. Despite considerable differences of the reservoir morphometric parameters and environmental conditions, the fluctuation range of the sulfate reduction rates during the summer thermal stagnation was narrow in most of the lakes. The average process rate was 0.79 mg S²⁻/(dm³ day), which is in accordance with the values obtained for other mesotrophic reservoirs of medium productivity [13, 14]. A relationship between the rate of this process and the lake depth was not revealed. The highest sulfate reduction rates, as measured in the deep Lake Balsys (2.6 mg S²⁻/(dm³ day) and in the shallow eutrophic Lake Lynezeris (2.2 mg S²⁻/(dm³ day), were similar. The stable anaerobic hypolimnion of Lake Balsys, which was 25–32 m deep, and the sulfate concentration slightly higher than in other deep-water lakes could have provided the most favorable conditions for the functioning of sulfate-reducing bacteria. Sulfate reduction was also detected in sediments of all the shallow lakes studied, despite the presence of oxygen in the bottom layers of water. The highest rate of this process was observed in the shallow-water sediments, which had a high content of detritus of plankton origin. In the sediments of the lakes located near swamps (Lakes Eserinis and Kreivasis), OM destruction was insignificant. The evolution of CO₂ by the bottom sediments of these res-

ervoirs was on average three times lower than in other shallow lakes. The sulfate reduction rate did not exceed 0.13 mg S²⁻/(dm³ day). Sulfate-reducing bacteria were not isolated from the acidic Lake Eserinis (pH 3.7). In the slightly acidic conditions of the bottom sediments of Lake Kreivasis and Lake Duobulis (pH 6.1–6.6), the scarce population of SRB (10 cells/cm³) did not exhibit metabolic diversity and physiological activity sufficient for competition with other anaerobic microorganisms. Similar results were reported when sulfate reduction was assessed in several Indiana lakes having acidic (pH 3.8) and slightly acidic (pH 6.2) reactions [15]. It is also noted [16] that the reduction of trivalent iron predominated in the upper layers of the bottom sediments with low pH values. SRB activity was not observed at a medium pH below 5.5 in laboratory experiments.

The determination of microbial process rates allows the amount of organic carbon mineralized during these processes to be assessed [17]. According to our calculations, from 3.5 to 82.5 mg C_{org}/(m² day) is mineralized via sulfate reduction in the bottom sediments of the lakes investigated, which approximately accounts for 0.2–11% of the net destruction, as determined by measuring the carbon dioxide evolution from the silts (Table 3). Thus, the contribution of sulfate reduction to the total mineralization of organic matter in the bottom sediments of most of the lakes studied was modest.

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