
EXPERIMENTAL
ARTICLES

Biological Fractionation of Stable Carbon Isotopes at the Aerobic/Anaerobic Water Interface of Meromictic Water Bodies

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Abstract—Mass-spectrometric investigation of carbon isotope composition ($\delta^{13}\text{C}$) was carried out for suspended organic matter and dissolved mineral compounds for the water column of some meromictic water bodies differing in salinity and trophic state. As a rule, a more pronounced carbon isotope fractionation (resulting from the metabolism of phytoplankton and anoxygenic phototrophic bacteria) was revealed in the zones of enhanced oxygenic and anoxygenic photosynthesis. Carbon isotope fractionation at the border between oxidized and reduced waters depends both on the activity of microbial communities and on the dominant species of phototrophic microorganisms. Analysis of the distribution profiles of the isotopic composition of suspended organic matter and dissolved mineral carbon revealed active mineralization of the organic matter newly formed via anoxygenic photosynthesis in the monimolimnion by microbial communities, resulting in the release of isotopically light carbon dioxide. Mineral carbon in the anaerobic zones of highly productive meromictic water bodies is therefore enriched with the light ^{12}C isotope.

Key words: meromictic lakes, stable carbon isotope fractionation, anoxygenic phototrophic bacteria.

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Meromictic water bodies have a stable anaerobic zone in the monimolimnion. Anaerobic conditions usually result from active destruction processes causing consumption of dissolved oxygen. In the presence of sulfate, even at low concentrations in the monimolimnion, sulfate reduction is activated and free sulfide is released. When solar light is able to reach sulfide-containing waters, mass development of phototrophic bacteria occurs; these organisms use reduced sulfur compounds for anoxygenic photosynthesis. In a number of meromictic water bodies, mass development of anoxygenic phototrophic bacteria (APB) was reported [1, 2]. In some cases, production of anoxygenic photosynthesis in certain seasons may be comparable with or even higher than the primary production of phytoplankton [3]. Apart from anoxygenic photosynthesis, chemolithotrophic bacteria can produce organic matter (OM) at the oxidized/reduced interface; for CO_2 fixation these organisms use the energy of oxidation of reduced nitrogen and sulfur compounds from the anaerobic water column. Fractionation of carbon isotopes resulting in a certain lightening of bacterial biomass compared to the CO_2 used, has been demonstrated for pure cultures of phototrophic and chemolithotrophic bacteria [4, 5]. The

isotopic composition of suspended organic matter and dissolved inorganic carbon may therefore act as a good indicator of the zone where phototrophic and/or chemolithotrophic bacteria develop in natural communities. For instance, changes in the carbon isotopic composition of particulate organic carbon were reported in the Black Sea water column near the chemocline [6]. Significant fractionation of carbon isotopes was also detected in the meromictic relict Lake Mogil'noe at 8–9 m, i.e. in the zone of mass growth of green sulfur bacteria [3]. Changes in the carbon isotope ratio of suspended organic matter and dissolved mineral carbon were also demonstrated in the zone of mass development of purple, phototrophic bacteria in the saline, meromictic Lake Kaiike (Japan) and in the meromictic Lake Cadagno (Switzerland) [7, 8].

The goal for the present work was therefore to investigate the isotopic composition of suspended organic matter (SOM) and dissolved mineral carbon in the water column of some meromictic water bodies, differing in the species composition of phototrophs, as well as in the rates of oxygenic and anoxygenic photosynthesis, in order to reveal the factors determining the patterns of carbon isotope fractionation by microbial communities developing at the oxic/anoxic interface.

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Table 1. Carbon isotopic composition of suspended organic matter $\delta^{13}\text{C}_{\text{org}}$ and the rate of photosynthesis (PS) in the water column of the meromictic saline Lake Shira (Khakassia)

Depth, m	O ₂ , mg/l	H ₂ S, mg/l	Alkalinity, mM	$\delta^{13}\text{C}_{\text{org}}$ of suspended matter, ‰	PS, $\mu\text{g C}/(\text{l day})$
June 2002					
0	6.9	–	17.0	–26.1	26
4	7.0	–	17.2	–26.1	49
8	7.7	–	17.5	–26.5	23
10	4.5	0	17.7	–27.1	45
11.5	2.7	0.05	17.9	–26.5	9
12	0.5	0.06	18.1	–27.3	12
12.5	0.4	0.9	18.1	–27.0	8
13	0	1.4	18.2	–27.0	30
13.5	0	–	18.5	–27.1	47
14	0	2.9	18.8	–28.5	21
15	–	6.8	19.0	–27.2	16
17	–	8.2	19.0	–27.1	15
19	–	13	19.5	–26.6	5
22	–	14.8	19.7	–26.7	6
February–March 2002					
0	7.3	–	14.0	–24.7	20.9
10	6.6	–	17.0	–25.7	8.3
14	5.8	–	18.0	–25.6	3.1
14.5	5.0	0	18.5	–25.2	7.4
15	4.5	0.25	16.0	–25.4	16.1
15.5	0	2.7	9.0	–25.6	4.0
16	0	6.2	5.5	–25.9	0
16.5	–	7.5	9.5	–25.5	0
19	–	14.0	19.5	–24.9	0
21.5	–	12.5	19.5	–24.5	0

“–”, measurements were not carried out.

MATERIALS AND METHODS

The materials were collected in a series of expeditions to the meromictic, relict Lake Mogil'noe (June 1999 and September 2001), meromictic Lake Shira and Lake Shunet (Khakassia, February 2002, July 2002,

and July 2003), the high-altitude, meromictic Lake Gek-Gel (Azerbaijan, September 2003), and the meromictic, eutrophic Lake Kichier (Marii El, July 2006). Water samples were collected with a 1-l glass bathometer, a 0.75-l plastic bathometer, and a multisyringe stratification bathometer [9].

Suspended organic matter was collected by filtration of lake water samples (up to 5 l) through 47-mm GF/F glass fiber filters (Whatman). The filtrate (up to 100 ml) was collected and used for analysis of dissolved mineral carbon.

Carbon isotope composition ($\delta^{13}\text{C}$) of OM and dissolved carbonate ion was carried out on an MI-1201V mass spectrometer (Ukraine) equipped with an SNG-3 two-channel gas puffing system and a Delta plus isotope mass spectrometer (Thermo Electron Corp., Germany). Accuracy of the measurements was $\pm 0.1\text{‰}$. The samples for radioisotope analysis were prepared as described previously [10].

RESULTS

Experimental results on the isotope composition of suspended organic carbon and dissolved mineral carbon in the meromictic lakes of different salinity and trophic state are presented in Tables 1–5.

The meromictic Lake Shira (Khakassia) with a unique sulfate–chloride sodium–magnesium water composition has a stable anaerobic, sulfide-containing zone at depths over 12–13 m. Sulfide concentration in the near-bottom layer (20–23 m) is 15–20 mg/l [11]. Radioisotope and microbiological investigation demonstrated the presence of an anoxygenic, phototrophic community with *Amoebobacter purpurea* as the dominant species at the oxic/anoxic interface [14].

A certain increase in the content of the light isotope ^{12}C was recorded in Lake Shunet in the summer at the oxic/anoxic interface (12–17 m) (Table 1). At these depths, the $\delta^{13}\text{C}$ composition of suspended organic carbon differed from that of both the higher and lower horizons by 1–1.5‰. At 14 m, the lightest isotope composition of suspended organic carbon ($\delta^{13}\text{C} = -28.5\text{‰}$) was detected; we found the highest biomass of anoxygenic phototrophic bacteria in this horizon [14]. Carbon isotope fractionation in the water column was less pronounced in winter, due to decreased illumination and the consequent decrease of photosynthesis rates. The $\delta^{13}\text{C}$ value of SOM was near constant throughout the water column (Table 1).

The saline, eutrophic, meromictic Lake Shunet with the maximal depth of 6 m has a pronounced salinity gradient (from 15 g/l at the surface to 65 g/l at the bottom) and high sulfide content in the near-bottom zone (up to 500 mg/l). During the summer, mass development of the purple bacteria, phylogenetically close to the genera *Thiocapsa* and *Halochromatium* occurred at the oxic/anoxic interface; immediately below this layer,

Table 2. Carbon isotopic composition of suspended organic matter $\delta^{13}\text{C}_{\text{org}}$, dissolved mineral carbon $\delta^{13}\text{C}_{\text{min}}$, and the rate of photosynthesis (PS) in the water column of the meromictic saline Lake Shunet (Khakassia)

Depth, m	O ₂ , mg/l	H ₂ S, mg/l	Alkalinity, mM	$\delta^{13}\text{C}_{\text{org}}$ of suspended matter, ‰	$\delta^{13}\text{C}_{\text{min}}$, ‰	$\Delta = (\delta^{13}\text{C}_{\text{min}} - \delta^{13}\text{C}_{\text{org}})$, ‰	PS, $\mu\text{g C}/(\text{l day})$
July 2002							
0	5.75	–	20.5	–26.4	–1.6	24.8	3457
2	5.69	–	22.0	–26.9	–2.1	24.8	169
4	6.00	0	23.5	–27.9	–2.8	25.1	69
4.5	0.35	0.15	24.5	–28.5	–3.2	25.3	189
4.75	0	6.05	48.5	–30.8	–6.2	24.6	102
5	0	280	67.5	–25.7	–12.8	12.9	271
5.25	–	357	95.0	–26.0	–15.8	10.2	9
5.5	–	430	96.0	–25.9	–16.2	9.7	0
February–March 2002							
1	4.5	0	12.5	–24.6	–5.8	18.8	44
4	0.3	0.3	11.5	–24.5	–7.2	17.3	29.8
4.25	0	1.2	10.0	–23.7	–8.3	15.4	0
4.5	0	3.5	15.5	–23.9	–7.2	16.7	0
4.75	–	25	22.5	–23.1	–10.7	12.4	0
5.35	–	120	63.5	–23.3	–19.5	3.8	0
July 2003							
4.83	0.3	–	19.5	–26.7	–16.9	9.8	166
4.88	0.2	–	19.5	–26.7	–14.4	12.3	95
4.93	0	–	19.5	–26.6	–8.7	17.9	152
4.98	0	–	19.5	–27.3	–6.1	21.2	295
5.03	0	–	21.5	–27.5	–12.4	15.1	126
5.08	0	–	26.5	–27.3	–9.4	18.1	242
5.13	0	0	30.0	–27.5	–3.6	23.7	669
5.18	0	0	26.0	–28.1	–2.8	25.3	480
5.23	0	9	27.5	–28.4	–2.3	26.1	436
5.28	–	35	27.5	–28.0	–3.4	24.6	168
5.33	–	93	34.5	–27.8	–4.4	23.4	1678
5.38	–	146	41.0	–26.7	–11.8	14.9	557
5.43	–	220	44.5	–26.7	–14.3	12.4	14
5.48	–	286	48.0	–26.7	–14.7	12.0	42
5.53	–	362	50.0	–26.8	–16.3	10.5	6
5.58	–	327	54.0	–26.6	–16.4	10.2	40
5.63	–	385	57.5	–26.4	–17.2	9.2	2
5.68	–	–	–	–26.5	–17.4	9.1	–

“–”, measurements were not carried out.

green sulfur bacteria similar to *Prosthecochloris* prevailed [13].

The rates of oxygenic and anoxygenic photosynthesis in Lake Shunet were almost two orders of magnitude higher than in Lake Shira (Table 2). In June 2002, enrichment of suspended organic carbon with light ¹²C was detected; the $\delta^{13}\text{C}$ value of SOM decreased by

1–3‰. For instance, at the depths of 0–4 m, the $\delta^{13}\text{C}$ values varied from –26.4 to –27.9‰. In the zone of mass development of purple sulfur bacteria (4.5–4.75 m), suspended organic carbon was enriched with ¹²C; the $\delta^{13}\text{C}$ values decreased to the range from –28.5 to –30.8‰. The difference in the isotopic composition between C_{min} and C_{org} in the aerobic layer and

Table 3. Carbon isotopic composition of suspended organic matter $\delta^{13}\text{C}_{\text{org}}$, dissolved mineral carbon $\delta^{13}\text{C}_{\text{min}}$, and the rate of photosynthesis (PS) in the water column of the meromictic saline Lake Mogil'noe (Kil'din Island, Barents Sea)

Depth, m	O ₂ , mg/l	H ₂ S, mg/l	Alkalinity, mM	$\delta^{13}\text{C}_{\text{org}}$ of suspended matter, ‰	$\delta^{13}\text{C}_{\text{min}}$, ‰	$\Delta = (\delta^{13}\text{C}_{\text{min}} - \delta^{13}\text{C}_{\text{org}})$, ‰	PS, $\mu\text{g C}/(\text{l day})$
June 1999							
1	7.69	–	1.6	–30.1	–	–	43
3	7.72	–	1.9	–30.8	–	–	0.7
5	6.79	0	2.3	–31.0	–11.3	19.7	17
6	8.21	0	2.9	–31.5	–9.1	22.4	11
8	3.27	0	2.9	–29.3	–7.9	21.4	13.7
8.5	1.09	0.08	2.6	–25.3	–8.0	17.3	5.1
9	0	3.3	3.2	–24.2	–9.1	15.1	285
9.5	0	–	3.6	–23.8	–10.8	13.0	608
10	–	1.4	4.0	–23.5	–17.0	6.5	302
11	–	10.8	6.4	–27.2	–18.0	9.2	–
12	–	67.3	11.6	–27.3	–22.4	4.9	–
14	–	107.4	15.0	–25.7	–	–	–
15.5	–	102.4	12.4	–28.7	–	–	–
September 2001							
0	9.3	–	1.4	–27.4	–	–	2.2
1	8.7	–	2.0	–28.0	–	–	3.2
3	8.0	–	1.6	–27.8	–	–	9.9
5	8.0	–	1.5	–27.5	–6.1	21.4	2.8
7	7.7	0	2.2	–27.9	–	–	1.7
8	6.9	0.1	2.5	–28.6	–	–	23
9	1.2	1.25	2.7	–30.0	–6.4	23.6	51
9.5	0.5	1.32	3.3	–33.0	–	–	60
9.75	–	–	3.3	–31.0	–	–	74
10	0	2.13	2.9	–26.0	–	–	275
10.25	0	–	3.3	–26.2	–12.1	14.1	191
10.5	–	19	4.0	–26.5	–	–	25
10.75	–	–	5.1	–26.0	–	–	69
11	–	46.5	6.3	–27.0	–15.8	11.2	67
12	–	90	8.0	–26.7	–	–	33
13	–	–	13.4	–27.2	–12.2	15.0	31
14	–	146	13.5	–27.9	–21.4	6.5	18
15	–	139	13.6	–26.2	–20.0	6.2	34

“–”, measurements were not carried out.

Table 4. Carbon isotopic composition of suspended organic matter $\delta^{13}\text{C}_{\text{org}}$, dissolved mineral carbon $\delta^{13}\text{C}_{\text{min}}$, and the rate of photosynthesis (PS) in the water column of the meromictic high-altitude Lake Gek-Gel (September 2003, Azerbaijan)

Depth, m	O ₂ , mg/l	H ₂ S, mg/l	Alkalinity, mM	$\delta^{13}\text{C}_{\text{org}}$ of suspended matter, ‰	$\delta^{13}\text{C}_{\text{min}}$, ‰	$\Delta = (\delta^{13}\text{C}_{\text{min}} - \delta^{13}\text{C}_{\text{org}})$, ‰	PS, $\mu\text{g C}/(\text{l day})$
0	7.6	–	2.4	–25.3	–	–	1.23
5	8.5	–	2.5	–27.1	–7.95	19.15	1.17
10	9.6	–	2.6	–27.6	–	–	0.80
15	8.0	–	2.7	–28.2	–	–	0.50
20	4.6	–	2.8	–30.4	–	–	0.66
25	1.9	0	2.8	–29.8	–8.97	20.83	–
27	0.8	0	2.8	–36.0	–8.56	27.44	0.17
29	0.45	0.05	3.6	–33.1	–8.85	24.25	0.27
30	0.10	0.2	3.5	–37.0	–8.95	28.05	0.10
31	0	1.2	3.5	–26.5	–	–	0.0
33	0	1.4	3.4	–30.7	–9.35	21.35	0
35	–	1.5	3.5	–31.6	–	–	0
37	–	–	3.6	–29.3	–	–	–
39	–	–	3.7	–27.1	–	–	–
40	–	2.5	3.8	–29.7	–8.53	21.17	–
50	–	3.0	4.0	–30.2	–	–	–
72	–	4.2	4.2	–30.2	–8.97	21.23	–

“–”, measurements were not carried out.

in the zone of anoxygenic photosynthesis changed from 24.6 to 25.3‰. In a deeper, anaerobic horizon of the water column (5 m), where the second peak of anaerobic photosynthesis was observed, due to development of green sulfur bacteria, the difference between C_{min} and C_{org} was only 12.9‰ (Table 2). In winter, when the rate of oxygenic photosynthesis under the ice decreased and anoxygenic photosynthesis was not detected, the isotope composition of organic carbon was practically the same throughout the water column (Table 2).

The thickness of the layer for APB active development, determined with a syringe sampling with a 5-cm step, was 20–25 cm [9]. The highest rates of light CO_2 fixation were detected in the 5.18–5.38 depth interval. Mass-spectrometric determination of the isotopic composition of suspended organic carbon revealed a pronounced effect of carbon isotope fractionation by the APB community at these depths (Table 2, July 2003). The $\delta^{13}\text{C}$ value for mineral carbon in the 5.13–5.33 m depth interval showed a higher content of the heavy isotope ^{13}C than the upper water layers; it varied from –2.3 to –4.4‰ (the average of six measurements was –3.3‰). Consumption of isotopically light mineral carbon in the course of anaerobic photosynthesis resulted in the formation of organic matter enriched with ^{12}C . The $\delta^{13}\text{C}_{\text{org}}$ value of suspended organic matter at the depths of 5.13–5.33 m varied from –27.5 to –28.4‰. Carbon isotope fractionation in the zone of anoxygenic photosynthesis in Lake Shunet was 23.7–26.1‰.

Lake Mogil'noe (Kil'din Island, Barents Sea).

The relict Lake Mogil'noe is located in the southeastern part of Kil'din Island and is separated from the Barents Sea by a sandbar [3]. Seawater penetrates into the bottom layers of the lake through the bar; therefore a stable salinity gradient exists in the water column. Activity of sulfate-reducing bacteria results in sulfide accumulation at the depths exceeding 9–10 m; its concentration in the near-bottom horizons was up to 100 mg/l (Table 3). In the upper layers of the anaerobic zone (9–10 m), an increase in dark CO_2 assimilation was detected due to APB development with brown-colored, green sulfur bacteria *Chlorobium phaeovibrioides* absolutely predominating [14].

Measurements of the SOM isotopic composition in June and September revealed different tendencies. In June, almost immediately after the lake was freed from the ice cover, the zone of massive APB development contained isotopically heavier, suspended C_{org} than both the upper horizons and those deeper than 11 m. The carbon isotopic composition $\delta^{13}\text{C}_{\text{org}}$ in the 8.5–10-m layer varied from –23.5 to –25.3‰, with the average value of –24.2‰. The isotopic composition of suspended organic matter above and below this depth interval was 2–6‰ lighter. In summer, carbon isotope fractionation was most pronounced (15–17‰) in the upper part of the zone where anoxygenic phototrophs developed. In the zone with the highest rate of anaerobic photosynthesis, this value was lower (13.0‰). In autumn, the lightest isotopic composition of SOM was

Table 5. Carbon isotopic composition of suspended organic matter $\delta^{13}\text{C}_{\text{org}}$, dissolved mineral carbon $\delta^{13}\text{C}_{\text{min}}$, and the rate of photosynthesis (PS) in the water column of the meromictic freshwater Lake Kichier (July 2006, Marii El)

Depth, m	O ₂ , mg/l	H ₂ S, mg/l	Alkalinity, mM	$\delta^{13}\text{C}_{\text{org}}$ of suspended matter, ‰	$\delta^{13}\text{C}_{\text{min}}$, ‰	$\Delta = (\delta^{13}\text{C}_{\text{min}} - \delta^{13}\text{C}_{\text{org}})$, ‰	PS, $\mu\text{g C}/(\text{l day})$
Black Kichier							
0.5	9.6	–	0.5	–28.7	–10.3	18.4	283
2	7.5	–	0.7	–28.8	–9.3	19.6	716
3	5.7	–	0.6	–31.7	–13.5	18.2	42
3.5	1.6	–	0.7	–34.6	–15.3	19.3	59
3.9	0.9	0	0.75	–30.9	–14.7	16.2	–
4.1	0.2	0	–	–30.8	–15.5	15.3	10
4.3	0.1	0.1	2.1	–30.7	–14.2	16.5	97
5	0	5.0	3.7	–29.4	–13.6	15.8	4.2
6	–	–	6.7	–29.9	–15.5	14.4	3.2
7	–	–	7.0	–30.0	–20.2	9.8	1.7
Big Kichier							
0.5	9.1	–	0.5	–24.2	–5.3	18.9	195
1.5	9.0	–	0.5	–25.4	–3.2	22.2	625
3	–	–	0.5	–28.3	–6.4	21.9	–
4	1.5	0	0.7	–30.7	–6.6	24.1	7.7
5	0.3	0.2	0.8	–31.6	–6.3	27.5	–
6	0	1.0	0.9	–30.7	–6.4	26.3	4.6
8	–	2.0	1.5	–28.9	–6.8	22.1	1.1
10	–	–	–	–29.8	–9.4	20.4	0

“–”, measurements were not carried out.

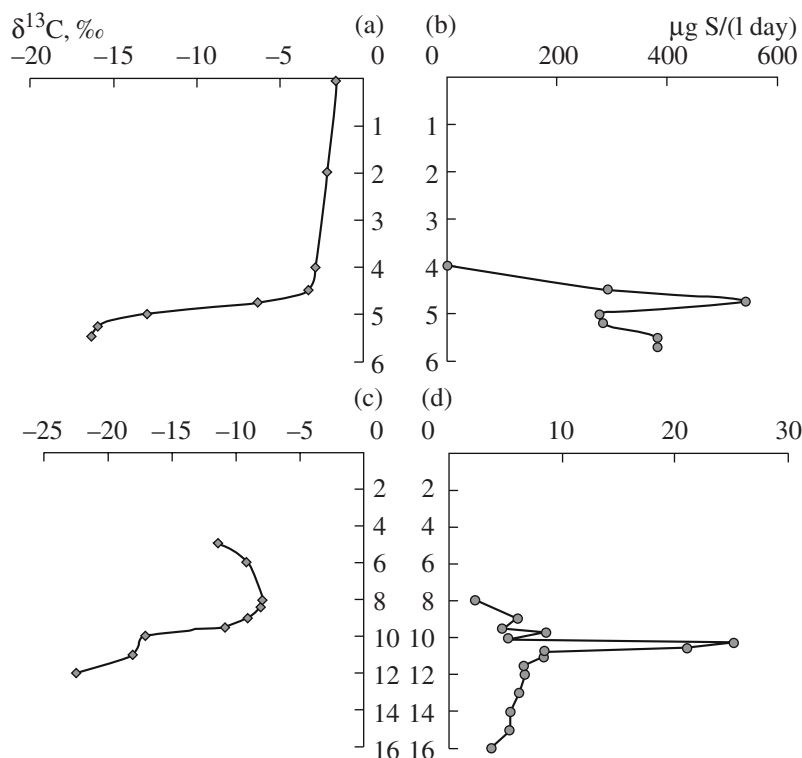
detected in the upper part of the zone of APB development, at the depth of 9–9.75 m; the $\delta^{13}\text{C}_{\text{org}}$ value of particulate carbon in this zone varied from –30 to –33‰. In the zone with the highest rate of anoxygenic photosynthesis, SOM was enriched with the heavy isotope ^{13}C ; the $\delta^{13}\text{C}_{\text{org}}$ value of particulate carbon was higher (from –26.0 to –26.2‰) (Table 3).

Lighter isotopic composition of SOM carbon in the upper part of the water column in June probably results from a significant influx of isotopically light organic matter with flood water from surrounding tundra soils. In the horizon with the highest rate of anoxygenic photosynthesis, the values of carbon isotope fractionation in different seasons were similar and varied from 13 to 14‰; in higher layers of sulfide-containing water, carbon isotope fractionation increased to 21–23‰.

High-altitude Lake Gek-Gel, Azerbaijan. Unlike other lakes considered in this work, the meromictic Lake Gek Gel has a low trophic level and a deep basin (approx. 90 m). During the sampling period (Table 4), free sulfide was detected at the depth of 29 m; its concentration increased gradually to 4.2 mg/l at the near-bottom layers [15]. Brown-colored, green phototrophic bacteria *Chlorobium phaeobacteroides*, which are able to develop at very low illumination levels, were revealed in the water column at the depths of 29–30 m [16]. The rates of anoxygenic photosynthesis in Lake Gek Gel were the lowest among the lakes investigated in this work; at the depths of 27–30 m it varied from 0.1

to 0.27 $\mu\text{g C}/(\text{l day})$. SOM carbon in the zone of photosynthesis was considerably enriched with the light ^{12}C isotope (Table 4). At these depths, the $\delta^{13}\text{C}$ value varied from –33.1 to –36.0‰ (the average of three measurements was –35.3‰). While SOM was significantly enriched with light ^{12}C , this had practically no effect on the isotopic composition of the mineral carbon; the $\delta^{13}\text{C}$ values of the latter changed very little in these horizons. The value of carbon isotope fractionation in the zone of APB development in Lake Gek Gel varied from 24.25 to 28.05‰.

Lake Kichier (Marii El). Lake Kichier is a freshwater, meromictic lake of karst origin with water of the carbonate–sulfate–calcium type; a broad natural channel (5 m deep) divides it into two parts. Since these parts differ in their hydrochemical characteristics and the rates of the processes occurring in the water column, the names Black Kichier and Big Kichier are used for the two parts of the lake [1]. Black Kichier is shallower, with somewhat higher rates of oxygenic photosynthesis and particulate matter content. Comparison of the rates of oxygenic and anoxygenic photosynthesis (Table 5) demonstrated that during the sampling time APB contributed less than 10% of the total photosynthetic production. In the zone of APB development (4–4.3 m deep), the isotopic composition of POM was almost the same as in the upper and lower water layers (Table 5). The isotopic composition of mineral carbon



Profiles of the isotopic composition ($\delta^{13}\text{C}$, ‰) of carbonates (a, c) and sulfate reduction rates, $\mu\text{g S}/(\text{l day})$ (b, d) in the water column of the meromictic lakes Shunet (a, b) and Mogil'noe (c, d).

at these depths also varied within a narrow range from -14 to -15‰.

In Big Kichier, the rate of OM production by phytoplankton was somewhat lower; the effect of carbon fractionation by APB was therefore detectable. In the zone of massive APB development (depths of 4–5 m), the isotopic composition of SOM carbon was 2.4–3.3‰ lighter than in the upper and lower water layers (Table 5). At the depths of 4–6 m, the value of carbon isotope fractionation varied from 24 to 27.5‰, i.e. it was 2.2–5.4‰ higher than in the other horizons of the water column.

DISCUSSION

Investigation of the isotopic composition of dissolved mineral carbon and of SOM carbon in the water of meromictic lakes of different trophic state, salinity, and composition of the APB population revealed that in most of the lakes APB development at the oxic/anoxic interface was accompanied by carbon isotope fractionation and production of isotopically light, organic matter. As a result of the preferential assimilation of $^{12}\text{C}-\text{CO}_2$, the residual mineral carbon in the zone of active photosynthesis becomes enriched with heavy ^{13}C . This pattern is evident in the lakes with high rates of anaerobic photosynthesis (Tables 2, 3, data of July 2003). However, no significant weighting of the isotopic composition of mineral carbon occurs in the lakes with high

phytoplankton production (Big and Black Kichier; Lake Shunet in summer 2002). Due to the active aerobic destruction of the organic carbon of phytoplankton origin, regular lightening of the mineral carbon, isotopic composition with depth was observed in the water column.

In the anaerobic zone of eutrophic lakes, production of organic matter by APB is likewise accompanied by active, destructive processes. In Lake Mogil'noe and Lake Shunet the rate of oxygenic and anoxygenic photosynthesis in summer was higher than 0.5 mg C/(l day). In these lakes, regular lightening of the isotopic composition of dissolved mineral carbon with depth, from the zone of APB development down to the bottom, was regular; in the near-bottom water, the $\delta^{13}\text{C}_{\text{min}}$ values varied from -18 to -22.4‰ (Lake Mogil'noe) and from -16.2 to -19.5‰ (Lake Shunet). Such enrichment of mineral carbon with ^{12}C results from formation of isotopically light CO_2 in the course of decomposition of organic matter with $\delta^{13}\text{C}$ from -26 to -33‰ (Tables 2, 3). High rates of OM mineralization in the anaerobic zone of Lake Mogil'noe and Lake Shunet are confirmed by our measurements of the profiles of sulfate reduction rates [3, 17]; this process is activated in the zone of anoxygenic photosynthesis and peaks immediately below the layer of APB mass development (figure). Therefore, in Lake Mogil'noe and Lake Shunet the difference between C_{min} and C_{org} isotopic composition in the upper layers of APB growth varied from 21.4 to 25.3‰. Although in

deeper layers relatively high rates of light CO₂ fixation were detected by the radioisotope method, concomitant destructive processes resulted in the enrichment of mineral carbon with ¹²C; the difference in the isotopic composition between C_{min} and C_{org} decreased therefore to 10–4.9‰.

In the eutrophic lakes Black Kichier and Big Kichier, in the zone of APB development (3.5–4.3 m for Black Kichier and 4–6 m for Big Kichier), where green sulfur bacteria *Pelodyction luteolum* predominate, suspended organic carbon is enriched with ¹²C (Table 5). However, no significant changes were detected in the isotopic composition of mineral carbon in the zones of APB development. This is probably due to the coincidence of the sampling period with the phytoplankton bloom resulting in enrichment of the water with organic matter produced in the aerobic part of the water column. Due to the low depth and the absence of sharp salinity gradients, this newly formed organic matter is decomposed both in the aerobic and anaerobic zones; isotopically light CO₂ is thus formed, and its incorporation into the pool of mineral carbon results in a less pronounced effect of carbon isotope fractionation by the APB community. In Black Kichier, where the rate of primary production was higher than in Big Kichier, the δ¹³C_{min} value varied between –9.3 and –10.3‰ even in the near-surface horizon; in deeper layers it varied from –15 to –20‰. In Big Kichier, where the water was more transparent and production processes were less intensive, mineral carbon was more enriched with ¹³C; δ¹³C varied from –3.2 to –9.4‰.

In conclusion, it should be mentioned that determination of the isotopic composition of organic and mineral carbon in meromictic lakes provides an indication of the localization and scale of OM production by APB communities. In all the meromictic lakes investigated, the layer of APB development was distinguished by a change in the isotopic composition of suspended organic matter. Heterotrophic microorganisms rapidly decompose organic matter produced by APB; OM reaching the bottom therefore has the isotopic composition close to that of OM arriving to anaerobic water from the aerobic photic zone. In the lakes with a high content of suspended organic matter (Mogil'noe, Shunet, Kichier), determination of carbon isotopic fractionation by APB communities is difficult due to the active destruction processes releasing isotopically light CO₂.

In oligotrophic Lake Gek Gel with minimal rates of anoxygenic photosynthesis, the isotopic composition of suspended C_{org} was more enriched with ¹²C than in the other lakes. In the zone of APB development where brown-colored, green sulfur bacteria dominated, the difference in the isotopic composition between C_{min} and C_{org} was 24.3–28.1‰. Laboratory cultivation of APB of the family *Chlorobiaceae* on bicarbonate usually results in fractionation not exceeding 19–22‰ [4]. More efficient carbon isotope fractionation in the APB development zone of Lake Gek Gel can possibly be

explained by enhanced carbon isotope fractionation at low growth rates; this phenomenon has been described for pure cultures of some autotrophic microorganisms [10].

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REFERENCES

1. Gorlenko, V.M., Dubinina, G.A., and Kuznetsov, S.I., *Ekologiya vodnykh mikroorganizmov* (Ecology of Aquatic Microorganisms), Moscow: Nauka, 1977.
2. Gorlenko, V.M., History of the Study of Biodiversity of Photosynthetic Bacteria, *Mikrobiologiya*, 2004, vol. 73, no. 5, pp. 633–643 [*Microbiology* (Engl. Transl.), vol. 73, no. 5, pp. 541–550].
3. Ivanov, M.V., Rusanov, I.I., Pimenov, N.V., Bairamov, I.T., Yusupov, S.K., Savvichev, A.S., Lein, A.Yu., and Sapozhnikov, V.V., Microbial Processes of the Carbon and Sulfur Cycles in Lake Mogil'noe, *Mikrobiologiya*, 2001, vol. 70, no. 5, pp. 675–686 [*Microbiology* (Engl. Transl.), vol. 70, no. 5, pp. 583–593].
4. Bondar', V.A., Gogotova, G.I., and Zyakun, A.M., On Fractioning of Carbon Isotopes by Photoautotrophic Microorganisms with Different Pathways of Carbon Assimilation, *Dokl. Akad. Nauk*, 1976, vol. 228, no. 3, pp. 720–722.
5. Ruby, E.G., Jannasch, H.W., and Deuser, W.G., Fractionation of Stable Carbon Isotopes during Chemoautotrophic Growth of Sulfur-Oxidizing Bacteria, *Appl. Environ. Microbiol.*, 1987, vol. 53, pp. 1940–1943.
6. Ivanov, M.V., Lein, A.Yu., Miller, Yu.M., Yusupov, S.K., Pimenov, N.V., Verli, B., Rusanov, I.I., and Zender, A., The Effect of Microorganisms and Seasonal Factors on the Isotopic Composition of Particulate Organic Carbon from the Black Sea, *Mikrobiologiya*, 2000, vol. 69, no. 4, pp. 541–552 [*Microbiology* (Engl. Transl.), vol. 69, no. 4, pp. 449–459].
7. Ohkouchi, N., Nakajima, Y., Matsumoto, K., Suga, H., Ogawa, N.O., Oguri, K., Koizumi, Y., Fukui, M., and Kitazato, H., Organic Geochemistry in a Saline Meromictic Lake Kaiike, Japan, *Abstr. NATO Advanced Research Workshop, Past and Present Water Column Anoxia*, 4–8 Oct. 2003, Crimea, Ukraine, pp. 68–69.
8. Camacho, A., Erez, J., Chicote, A., Florin, M., Squires, M.M., Lehmann, C., and Bachofen, R., Microbial Microstratification, Inorganic Carbon Photoassimilation and Dark Carbon Fixation at the Chemocline of the Meromictic Lake Cadagno (Switzerland) and Its Relevance To the Food Web, *Aquat. Sci.*, 2001, vol. 63, pp. 91–106.

9. Rogozin, D.Yu., Pimenov, N.V., Kosolapov, D.B., Chan'kovskaya, Yu.V., and Degermendzhi, A.G., Thin-Layer Vertical Distributions of Purple Sulfur Bacteria in Chemocline Zones of Meromictic Lakes Shira and Shunet (Khakassia), *Dokl. Akad. Nauk*, 2005, vol. 400, no. 3, pp. 426–429 [*Dokl. Biol. Sci.* (Engl. Transl.), vol. 400, no. 3, pp. 54–56].
10. Zyakun, A.M., Microbial Fractioning of Carbon Isotopes and Its Application in Biotechnological Research, *Doctoral (Biol.) Dissertation*, Pushchino, 1994.
11. Pimenov, N.V., Rusanov, I.I., Karnachuk, O.V., Rogozin, D.Yu., Bryantseva, I.A., Lunina, O.N., Yusupov, S.K., Parnachev, V.P., and Ivanov, M.V., Microbial Processes of the Carbon and Sulfur Cycles in Lake Shira (Khakassia), *Mikrobiologiya*, 2003, vol. 72, no. 2, pp. 259–267 [*Microbiology* (Engl. Transl.), vol. 72, no. 2, pp. 221–229].
12. Lunina, O.N., Bryantseva, I.A., Akimov, V.N., Rusanov, I.I., Rogozin, D.Yu., Barinova, E.S., Lysenko, A.M., and Pimenov, N.V., Seasonal Changes in the Structure of the Anoxygenic Photosynthetic Bacterial Community in Lake Shunet, Khakassia, *Mikrobiologiya*, 2007, vol. 76, no. 3, pp. 416–428 [*Microbiology* (Engl. Transl.), vol. 76, no. 3, pp. 368–379].
13. Lunina, O., Gorlenko, V.M., Popova, O.A., Akimov, V.N., Rusanov, I.I., and Pimenov, N.V., Seasonal Changes in the Structure of the Anoxygenic Phototrophic Bacterial Community in Lake Mogilnoe, a Relict Lake on Kil'din Island in the Barents Sea, *Mikrobiologiya*, 2005, vol. 74, no. 5, pp. 677–686 [*Microbiology* (Engl. Transl.), vol. 74, no. 4, pp. 588–596].
14. Lunina, O.N. and Bryantseva, I.A., Akimov, V.N., Rusanov, I.I., Barinova, E.S., Lysenko, A.M., Rogozin, D.Yu., and Pimenov N.V. Anoxygenic Phototrophic Bacterial Community of Lake Shira (Khakassia), *Mikrobiologiya*, 2007, vol. 76, no. 4, pp. 469–479 [*Microbiology* (Engl. Transl.), vol. 76, no. 4, pp. 469–479].
15. Lunina, O.N., Kevbrina, M.V., Akimov, V.N., and Pimenov, N.V., Anoxygenic Phototrophic Bacteria of the High-Altitude Meromictic Lake Gek-Gel, Azerbaijan, *Mikrobiologiya*, 2008, vol. 77, no. 5, pp. 675–682 [*Microbiology* (Engl. Transl.), vol. 77, no. 5, pp. 602–609].
16. Karnachuk, O.V., Pimenov, N.V., Yusupov, S.K., Frank, Yu.A., Pukhakka, Ya.A., and Ivanov, M.V., Distribution, Diversity, and Activity of Sulfate-Reducing Bacteria in the Water Column in Gek-Gel Lake, Azerbaijan, *Mikrobiologiya*, 2006, vol. 75, no. 1, pp. 101–109 [*Microbiology* (Engl. Transl.), vol. 75, no. 1, pp. 82–89].
17. Kallistova, A.Yu., Kevbrina, M.V., Pimenov, N.V., Rusanov, I.I., Rogozin, D.Yu., Verli, B., and Nozhzhevnikova, A.N., Sulfate Reduction and Methanogenesis in the Shira and Shunet Meromictic Lakes (Khakassia, Russia), *Mikrobiologiya*, 2006, vol. 75, no. 6, pp. 828–835 [*Microbiology* (Engl. Transl.), vol. 75, no. 6, pp. 720–726].