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EXPERIMENTAL ARTICLES

Application of ^{13}C Mineral Carbon for Assessment of the Primary Production of Organic Matter in Aquatic Environments

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Abstract—The possibility of measuring the rates of light and dark CO_2 assimilation using ^{13}C carbonate was demonstrated on Lake Kichier (Marii El). The application of methods utilizing the stable ^{13}C and the radioactive ^{14}C isotopes resulted in comparable values of the rates of light and dark CO_2 fixation. Due to its absolute environmental safety, the method with ^{13}C mineral carbon can be recommended as an alternative to radioisotope methods for qualitative measurements of CO_2 fixation rates in aquatic ecosystems.

Key words: primary production, meromictic lakes, ^{13}C stable isotope method, ^{14}C radioisotope method, anoxygenic phototrophs.

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The oxygen bottle method and the radioisotope method with ^{14}C bicarbonate are traditionally applied for the measurement of primary production in aquatic environments [1]. The oxygen method has several serious limitations. It is, for example, completely unsuitable for determination of the primary production by anoxygenic phototrophic bacterial communities; in meromictic water bodies, such communities develop in the upper part of the hydrogen sulfide zone, where oxygen is either absent or present in extremely low concentrations. In some cases anoxygenic autotrophs are known to be responsible for 30% or more of the total production of organic matter (Lake Mogil'noe [2], Mahoney Lake [3]). The radioisotope method is free from such limitations; it can be used both in the aerobic and the anaerobic zone. It, however, requires specially skilled personnel and isolated laboratory space in order to prevent radioactive contamination of the environment.

The goal of the present work was to develop a method of measuring the primary production in aquatic environments based on the stable isotope ^{13}C as an alternative to the ^{14}C radioisotope method.

MATERIALS AND METHODS

Objects of investigation. The work was performed on the meromictic Lake Kichier, in the southern part of the Marii El Republic, at the ridge of the Klenovogorskaya elevation. The lake consists of two parts connected by a broad natural channel with a depth of five meters. Lake Kichier is a eutrophic meromictic freshwater lake with elevated sulfate content and water of the carbonate–sulfate–calcium type. Since the two parts of the lake differ significantly in the hydrochemical parameters of the water and in the rates of the processes in the water column, they are usually described and studied separately and are therefore referred to as Black Kichier and Big Kichier [4].

The water samples from different depths were taken from a rubber boat with a 1-l glass limnological bathometer. Immediately after recovery, the samples were distributed into dark and transparent 30-ml glass vials and hermetically sealed with gas-tight penicillin rubber stoppers; attention was paid to avoid air bubbles. Each vial was then supplemented with 0.2 ml of ^{13}C-enriched Na_2CO_3 (Russia). The vials were incubated during daylight hours on special suspended holders at the sampling depth. Their contents were then fixed with 0.5 ml of 1 N HCl, filtered through GF/F glass fiber filters, and washed with the filtered lake water acidified to pH 2–3. The filters were dried, and the isotopic composition of the carbon of collected organic matter ($\delta^{13}C$) was determined. Water samples to which $Na_2{}^{13}CO_3$ was added after fixation with HCl were used as controls.

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The initial isotopic composition of organic matter in lake water samples was determined according to the same procedure, without $Na_2{}^{13}CO_3$ addition.

Analysis of $^{13}C/^{12}C$. The isotopic composition of mineral carbon ($\delta^{13}C$) in the samples was determined according to the standard procedure described in [5].

The isotopic composition of 85%-enriched Na_2CO_3 was calculated according to the equation:

$$\delta^{13}C_{Na_2CO_3} = (R_{Na_2CO_3}/R_{std} - 1)1000‰ = 503\,574‰, \quad (1)$$

where $R_{Na_2CO_3} = 85/15 = 5.66667$ is the $^{13}C/^{12}C$ isotope ratio in the labeled carbonate; $R_{std} = 0.0112372$ is the $^{13}C/^{12}C$ isotope ratio in the PDB international standard [6].

The isotopic composition of mineral carbon ($\delta^{13}C_\Sigma$) after the introduction of $Na_2{}^{13}CO_3$ was calculated according to the equation:

$$q_{orig}\delta^{13}C_{DIC} + q_{intr}\delta^{13}C_{Na_2CO_3} = (q_{orig} + q_{intr})\,\delta^{13}C_\Sigma, \quad (2)$$

where q_{orig} is the content of mineral carbon in the original sample; $\delta^{13}C_{DIC}$ is the isotopic composition of dissolved mineral carbon in the original sample; q_{intr} is the introduced amount of ^{13}C carbonate; $\delta^{13}C_{Na_2CO_3}$ is the isotopic composition of the introduced $Na_2{}^{13}CO_3$ calculated according to Eq. (1); and $\delta^{13}C_\Sigma$ is the isotopic composition of mineral carbon in the mixture of environmental mineral carbon and the introduced amount of $Na_2{}^{13}CO_3$.

Solving equation (2) for $\delta^{13}C_\Sigma$ resulted in the isotopic characterization of the carbon in the mixture obtained after the introduction of $Na_2{}^{13}CO_3$ according to

$$\delta^{13}C_\Sigma = (q_{orig}\delta^{13}C_{min} + q_{intr}\delta^{13}C_{Na_2CO_3})/(q_{orig} + q_{intr}). \quad (3)$$

The isotopic composition of mineral carbon after introduction of $Na_2{}^{13}CO_3$ into the water samples from other horizons differing in the content and isotopic composition of dissolved mineral carbon was calculated in a similar manner. From the measured qualitative characteristics of carbon isotopic composition in particular organic matter before the introduction of ^{13}C carbonate ($\delta^{13}C_{POC\text{-}O}$) and after exposure with labeled carbonate ($\delta^{13}C_{POC\text{-}I}$), the ratio of mineral carbon incorporated into organic matter during the exposure was calculated according to:

$$(1 - x)\delta^{13}C_{POC\text{-}O} + x\delta^{13}C_\Sigma = \delta^{13}C_{POC\text{-}I}, \quad (4)$$

where x is the ratio of mineral carbon incorporated into organic matter during the exposure (the total amount of organic carbon in the sample was assigned the unit value). The $\delta^{13}C_\Sigma$ value, i.e., the isotopic characteristics of the carbon composition in the mixture of native mineral carbon and introduced $Na_2{}^{13}CO_3$, was calculated from equation (3).

Solving Eq. (4) for x resulted in a value for the fraction of mineral carbon incorporated into organic matter during the exposure according to equation:

$$x = (\delta^{13}C_{POC\text{-}I} - \delta^{13}C_{POC\text{-}O})/(\delta^{13}C_\Sigma - \delta^{13}C_{POC\text{-}O}). \quad (5)$$

The absolute rate of mineral carbon incorporation into organic carbon of microbial biomass (I) was calculated from the equation used for the application of the radioisotope method for this process:

$$I = xC/t, \quad (6)$$

where x is the fraction of mineral carbon incorporated into organic matter during the exposure, C is the concentration of mineral carbon in the sample, and t is the exposure duration.

Since the amount of $Na_2{}^{13}CO_3$ introduced into the sample was significantly less than one third of the mineral carbon content in lake water, the proposed method for measuring the rates of CO_2 fixation in water samples can be recommended for measurements of the real rates of this process.

For comparison with ^{13}C bicarbonate, experiments with ^{14}C bicarbonate (Amersham, United States) were carried out. The experimental protocol for the radioisotope method was similar to the one described above; after filtration, the GF/F filters were dried and examined by means of a Rackbeta liquid scintillation counter (LKB, Sweden) [7].

In order to determine the zone of mass growth of anoxygenic phototrophic bacteria, bacteriochlorophyll (BChl *d*) content was measured as described previously [8].

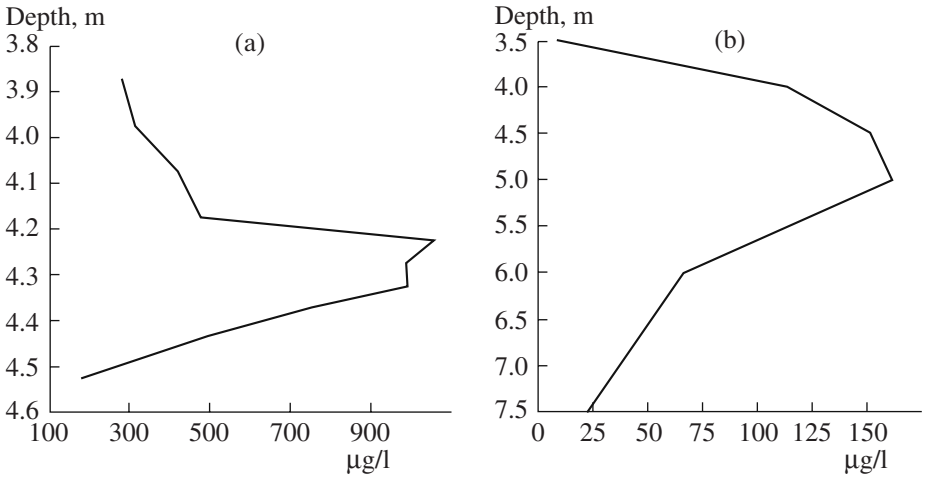
RESULTS AND DISCUSSION

Over the course of the last 30 years, the major hydrochemical parameters of the water column (pH, salinity, and concentrations of oxygen and H_2S) practically did not change [4]. During our work, sulfide in both Black and Big Kichier appeared at a depth of four m; mass growth of anoxygenic phototrophic bacteria resulted in greenish coloration of this horizon.

In Lake Black Kichier, the green coloration of water was most intensive in the upper part of the H_2S zone (3.9 m). Extensive growth of BChl *d*-containing bacteria, which had been previously reported by Gorlenko [4], occurred in this horizon. The highest BChl *d* concentration in bacterial cells (990–1058 μg/l) was detected at 4.2–4.3 m (figure a).

In Lake Big Kichier, the water of the upper part of the H_2S zone had no visible coloration. BChl *d* in bacterial cells was revealed starting from 3.5-m depth; its peak concentration (162 μg/l) was detected at a depth of 5 m; it was 6.5 times lower than the highest BChl *d* concentration in Lake Black Kichier (figure, a and b).

The two horizons for the measurements of light and dark CO_2 assimilation rates (4 and 6 m) were chosen



Profiles of BChl *d* concentration in the water of Lake Black Kichier (a) and Lake Big Kichier (b).

considering the distribution of anoxygenic phototrophic bacteria. The samples from the 4-m depth contained high numbers of viable phototrophic bacteria, evident from visual observations and BChl *d* concentrations. BChl *d* was present at the 6-m depth as well, although the number of prototrophic bacteria decreased sharply.

The data on the dark and light CO_2 assimilation obtained with ^{13}C-enriched Na_2CO_3 are presented in Table 1. In Lake Black Kichier at a depth of 4 m, the rate of light CO_2 assimilation varied from 14.77 to 16.32 μg C/(l day), the rate of dark assimilation, from 1.15 to 3.61 μg C/(l day). Above the deep-water depression of Lake Big Kichier, the rate of CO_2 fixation was 6.14–8.03 μg C/(l day) in light and 1.93–2.23 μg C/(l day) in the dark. The rate of light fixation decreased sharply at 6-m depth, probably due to the lack of light.

In order to obtain more reliable results, two repeats with different concentrations of ^{13}C carbonate were used (Table 1). The table demonstrates that the rates of microbial processes obtained with 0.12 and 0.6 mM of ^{13}C carbonate were relatively close. The differences between repeats usually did not exceed 45%; this value is similar to the error obtained by the radioisotope method. The differences were most pronounced at low rates of CO_2 assimilation.

Comparison of the results on CO_2 assimilation obtained by the stable isotope and radioisotope method (Table 2) revealed that the differences between the methods were the lowest in the case of light assimilation. In the lakes under study, the rate of CO_2 light

Table 1. The rates of light CO_2 fixation calculated after incubation with $Na_2{}^{13}CO_3$

Depth, m	q_{orig}, mM	q_{intr}, mM	$\delta^{13}C_{DIC}$, ‰	$\delta^{13}C_{Na_2CO_3}$, ‰	$\delta^{13}C_{POC\text{-}O}$, ‰	$\delta^{13}C_{POC\text{-}I\text{-}light}$, ‰	$\delta^{13}C_{POC\text{-}I\text{-}dark}$, ‰	$\delta^{13}C_{\Sigma}$, ‰	*I* total, μg C/(l day)	*I* dark, μg C/(l day)	*I* light, μg C/(l day)
Lake Black Kichier											
4	2.05	0.12	–15.4	503574	–30.8	–18.1	–28.5	27833	19.93	3.61	16.32
4	2.05	0.60	–15.4	503574	–30.8	10.7	–27.8	114005	15.92	1.15	14.77
6	4.50	0.12	–14.3	503574	–29.9	–29.0	–29.5	13066	6.60	2.93	3.67
6	4.50	0.60	–14.3	503574	–29.9	–25.8	–28.9	59231	6.64	1.62	5.02
Lake Big Kichier											
4	1.2	0.12	–6.61	503574	–30.6	–12.3	–26.7	45773	10.26	2.23	8.03
4	1.2	0.60	–6.61	503574	–30.6	22.3	–18.0	167854	8.07	1.93	6.14
6	2.3	0.12	–4.43	503574	–30.7	–28.5	–29.7	24966	4.38	2.02	2.36
6	2.3	0.60	–4.43	503574	–30.7	–16.2	–24.2	104184	6.84	3.07	3.77

Table 2. Comparison of the results obtained by the stable isotope and radioisotope methods for the rates of photosynthesis and dark CO_2 assimilation by anoxygenic phototrophic bacteria

Depth, m	Method	Photosynthesis, μg C/(day)	Dark CO_2 assimilation, μg C/(l day)
Lake Black Kichier			
4	^{14}C	9.9	1.3
	^{13}C	15.5	4.8
6	^{14}C	3.2	1.0
	^{13}C	4.3	2.3
Lake Big Kichier			
4	^{14}C	7.7	0.9
	^{13}C	7.1	2.1
6	^{14}C	4.6	0.7
	^{13}C	3.1	2.5

assimilation at depths of 4 and 6 m varied from 3.1 to 15.5 μg C/(l day). The highest values of light CO_2assimilation were revealed in Lake Black Kichier at the 4-m depth; this finding is in accord with the results of BChl *d* measurement in the water.

When dark CO_2 assimilation was measured by the stable isotope and radioisotope methods, the spread of values was 8–36% (Table 2).

The rates of dark CO_2 fixation in the water samples from Lakes Black and Big Kichier were much lower; they varied from 0.7 to 4.8 μg C/(l day). Since the rates of dark CO_2 assimilation were lower (Table 2), the spread of values obtained by different methods was relatively large (from 57 to 73%). According to our calculations, a 1.0‰ change in the isotopic composition of organic carbon after incubation with ^{13}C carbonate results in a more than 30% change in the calculated rate of CO_2 fixation when CO_2 assimilation is less than 2 μg C/(l day). Therefore, in environmental samples with a rate of CO_2 fixation below 2 μg C/(l day), the more sensitive radioisotope method yields more reliable results.

Thus, the results of measurement of photosynthetic rates with ^{13}C- and ^{14}C-labeled carbon dioxide suggest that the stable isotope method can be applied for determination of the primary production in various aquatic environments. However, the results obtained by the proposed method are comparable with those obtained by the radioisotope method only for the water samples with CO_2 fixation rates above 3–5 μg C/(l day).

In conclusion, it should be mentioned that the proposed mass-spectrometric method of measuring the rate of photosynthesis is less sensitive and requires more expensive equipment than the radioisotope method. However, present more stringent regulations concerning storage, transportation, and accountancy of radioactive materials has resulted in a worldwide tendency to decrease the scope of radioisotope works in microbial ecology and biogeochemistry. On the other hand, development of mass-spectrometric equipment has resulted in more extensive application of the isotope geochemical methods in microbiological, molecular biological, and molecular ecological research. The search for procedures enabling the substitution of environmentally safe stable isotopes for radioactive isotopes is therefore promising for development of new methods for qualitative determination of the rates of microbial processes in natural ecosystems.

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