ISSN 0026-2617, Microbiology, 2013, Vol. 82, No. 4, pp. 499–504. © Pleiades Publishing, Ltd., 2013.
Original Russian Text © A.N. Dzyuban, 2013, published in Mikrobiologiya, 2013, Vol. 82, No. 4, pp. 483–488.

EXPERIMENTAL ARTICLES

Methane Cycle in Different Types of Water Bodies during the Ice Period

A. N. Dzyuban[1]

Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences, Borok, Russia

Received September 28, 2012

Abstract—Studies conducted on different types of water bodies from January to March showed that microbial processes of the CH_4 cycle during the ice period are significant for degradation of organic carbon and trophic webs of the ecosystems. In productive polluted lakes, CH_4 formation occurs in sediments and in the water column, with its total output reaching the high summer level of 300–680 mL $CH_4/(m^2$ day).

Keywords: methane cycle, water bodies, ice period

DOI: 10.1134/S0026261713040024

In freshwater ecosystems, methane is the main terminal link of anaerobic decomposition of organic matter, thus causing interest in the studies of the methane cycle. While the important ecological role of methane transformation was mentioned in many papers [1–3], the data on the methane cycle in temperate and northern waters were collected during the vegetation period. The publications on this process in winter are rare [4].

The goal of the present work was to evaluate the rates of microbial methane formation and oxidation in the bottom sediments and the water column during the ice period and to reveal the characteristic features of these processes, depending on the ecological conditions in the water bodies of different types.

MATERIALS AND METHODS

The work was carried out in 1987–2007, in several lakes of different morphometry, trophicity, and pollution grade located in the of Yaroslavl, Vologda, and Tver oblasts, as well as in Latvia and at the sites of the Rybinsk Reservoir (Table 1).

Investigations were conducted from January to early March from the ice surface of the central or of the deepest parts of the reservoir. At Lake Plescheevo and the Rybinsk Reservoir, littoral sites with depth of ~1 m were also studied. In summer this area of the lake is covered by aquatic macrophyte vegetation; in the Rybinsk Reservoir, this area is separated from the open waters by islands and is filled with submerged vegetation.

Water for analysis was collected with a 1-L plastic bathometer. The bottom sediments were collected with a small dredge, which preserved the sediment structure. The vessels (stratimetric vials [5] and bottles) were filled with water and sludge [5] immediately after sampling. Some samples were preserved for chemical and gas chromatographic analysis. The redox potential of the medium (Eh) was measured with a Radelkis ionomer, the concentration of dissolved O_2 was measured with a KL-115 oxygen meter, and the content of organic carbon (C_{org}) in the sediments was accessed using a CNH-1 gas chromatographic analyzer, with the labile fraction (C_{lab}) separated by treating the sample with 5% H_2SO_4. Methane concentration in the water and sediments was determined by the phase equilibrium method [6] on a Chrom-5 gas chromatograph (Czech Rebuplic) with a flame ionization detector, a 2.4 m column with Porapack-N sorbent, and helium as a carrier gas at 36°C.

The rates of microbial methanogenesis (MG) and methane oxidation (MO) in the water and sediment was determined from the differences in the gas content of in situ incubated experimental vials compared to the controls [7, 8]. Since methanogenesis was often recorded in microaerobic areas of productive and polluted water bodies [9], allylthiourea, a methane oxidation inhibitor, was applied for the samples from these environments [10].

All water samples were carefully (avoiding air bubbles) dispensed from the bathometer into 60-mL vials in three replicates. The vials were sealed with silicone stoppers; the excess of water was removed with an injection needle. The control samples were immediately fixed with 0.1 mL of mercury chloride, Hg_2Cl_2, saturated solution; the second and third series, where allylthiourea solution was added to concentration of 1 mg/L, were incubated in dark bags for 12–24 h, depending on the sampling site. After incubation, the

[1] Corresponding author; e-mail: microb@ibiw.yaroslavl.ru

Table 1. General characteristics of water bodies studied

Water reservoir	Location	Area, km^2	h, maximum depth, m	Trophic level	Pollution at the sampling sites
Rybinsk Reservoir	Yaroslavl oblast	4550	30 (8–16)	Mesotrophic	Low to moderate
Lake Pleshcheyevo	"	51.4	25	Mesotrophic	"
Park pond, town of Borok	"	<0.01	2.5	Oligo-mesotrophic	Moderate
Lake Ferapontov	Vologda oblast	1.5	15	Chtonio-eutrophic	Low
Lake Siverskoe	"	9.6	18	Oligo-mesotrophic	Moderate
Lake Dotkas	Latvia	0.2	3.5	Eutrophic	High
Lake Vidogoshch	Tver oblast	0.21	16	Hyper-eutrophic	High

Note: Depths in the central part of the reservoir are given in parentheses.

samples were fixed and the vials were stored in inverted position prior to processing in the laboratory.

The experiments with the sediments were carried out according to the scheme described in [3] in graduated bottles, which made it possible to cut the sediment column of the required thickness and volume from the dredge, on top of which near-bottom water was carefully poured. A total of three pairs of bottles were filled, two for the experiments and one for the control. The third series was immediately fixed with 0.5 mL of mercury chloride saturated solution. The methane oxidation inhibitor was added to a pair of experimental vials according to [10] (under aeration of the sediments), and it was incubated in dark bags for 8–24 h together with the vials with no additives. After incubation, the samples were fixed and stirred.

The samples for laboratory analysis were injected with inert gas and the bottles were incubated for 2 to 3 h to equalize CH_4 partial pressure; 0.5 mL of the headspace gas was then collected for gas chromatography.

The CH_4 concentration, as well as its formation and oxidation rate considering the volumes of the sediment, water, and gas phase, were calculated as follows [3, 6, 11]:

MG (anaerobic conditions) = Experiment (no additives) – Control,

MG (aerobic conditions) = Experiment (with MO inhibitor) – Control,

MO = Experiment (with MO inhibitor) – Experiment (no additives).

RESULTS AND DISCUSSION

The reservoirs studied varied significantly in their typological and ecological characteristics, including oxygen concentration, productivity and pollution level (see Table 1). These characteristics play a key role in forming the ecological conditions which affect the microbial community, specifically that of the sediment.

During the research period, the water column in most of the water bodies was stratified, as was evidenced by the vertical distribution of oxygen and methane. While oxygen concentration close to the surface was generally 6.1–10.3 mg/L, it was lower close to the bottom, even at the sites of the stream of the Rybinsk Reservoir. In most of the lakes the anaerobic hypolimnion was formed by the end of winter (Table 2). This resulted in low redox potential of the sediments, particularly in the eutrophic and polluted lakes rich in labile organic matter. The redox potential of the bottom sediments decreased over the winter period, as was determined by repeated studies of the Lake Dotkas and of the littoral zone of the reservoir (Table 2). Negative Eh values were, however, registered only in subsurface (1 to 3 cm) layers of the latter.

Another important factor determining bacterial activity is the total organic matter stock and its availability. The total C_{org} content of all the water bodies studied did not vary significantly. C_{org} concentration was 16–28 mg C/cm^3 in deep-water zones, with a maximum in-peat bottom sediments, and 2–3 times lower in the littoral zone. However, the C_{lab} concentration was much higher in lake sediments than in the sediments of the reservoir, reaching 28% of C_{org} in highly productive and polluted lakes (Table 2). The reservoir sediment, rich in natural and anthropogenic allochthonous compounds, contained only 10–18% C_{lab} of the total [12].

Winter studies showed that, similar to the open water period, the rates and the direction of microbial processes of the methane cycle in the sediments depended primarily on the redox conditions and the availability of labile organic matter.

Methanogenesis was detected in the sediments of all the water bodies, but its level varied by 2–3 orders of magnitude (Table 3). In the weakly oxidized and mineralized pond and reservoir sediments, methanogenesis rate was only 0.02–0.35 mL CH_4/(dm^3 day). In reduced sediments of highly eutrophic or polluted lakes it reached 6–16 mL CH_4/(dm^3 day). Experiments on Lake Dotkas and at the closed reservoir lit-

Table 2. Physicochemical characteristics of water and upper sediment layer

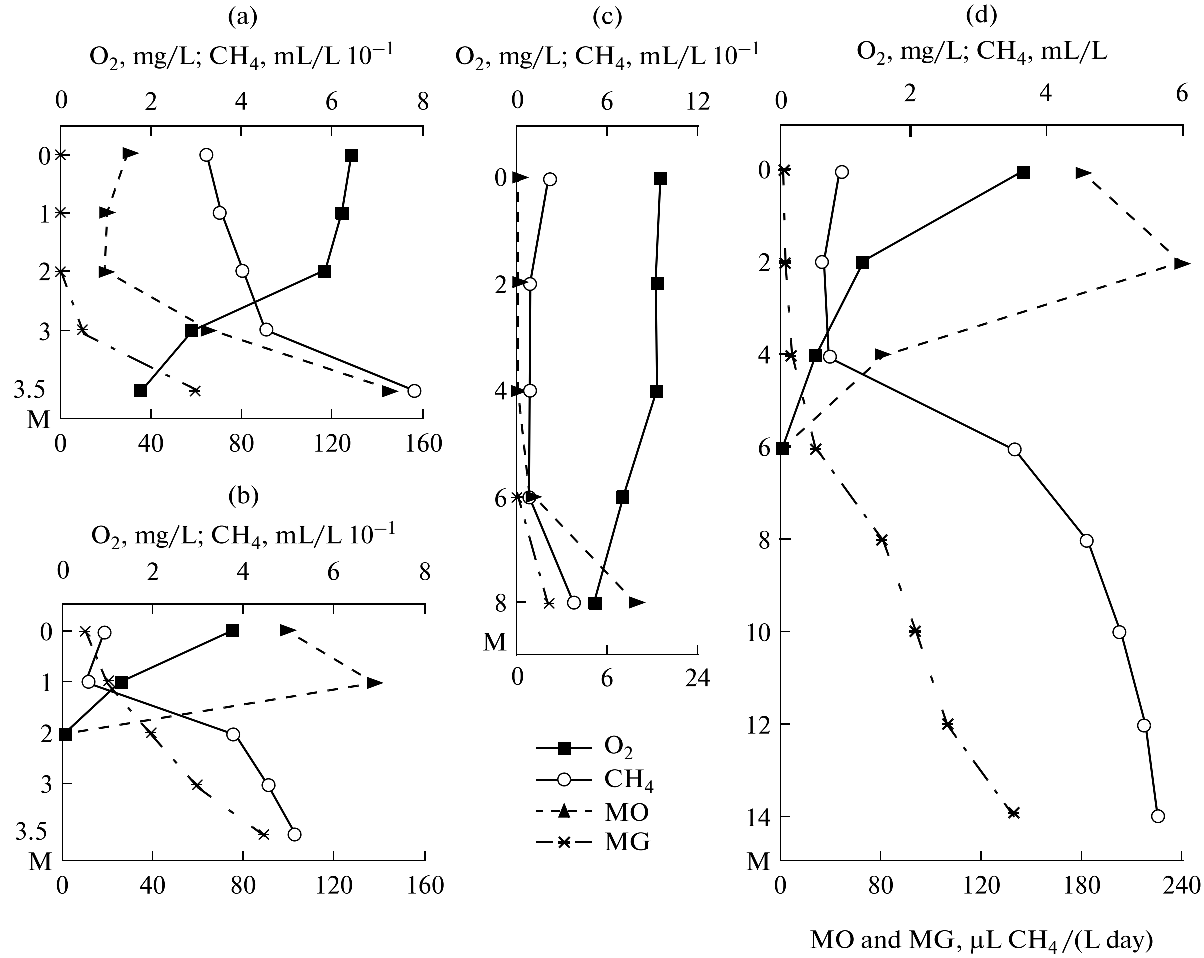
Water bodies and sampling sites	Sampling period	Water		Bottom sediments (0–5 cm layer)				
		O_2, mg/L	CH_4, mL/L	Appearance	Eh, mV, 0.5/1–5 cm	C_{org}, mg/cm^3	C_{lab}, % C_{org}	CH_4, mL/dm^3
Water reservoir								
Main river reach, dw	02.91	10.3/7.6*	0.012/0.12	Peat sediment	60/15	24	10	1.2
Reach of the Volga river, dw	02.91	7.8/5.2	0.022/0.28	Black silt	30/–5	16	18	2.4
The same, lit	01.07	6.1/5.8	–/0.045	Silty sand	65/15	8.2	12	0.7
The same, lit	03.07	2.6/2.3	–/0.32	Black silty sand	20/–5	8.0	14	4.8
Lakes								
Lake Pleshcheyevo, dw	03.87	10.2/0	0.018/1.87	Black silt	–90/–	23	20	10.7
The same, lit	03.87	2.8/2.2	–/0.54	Rough detrital sediment	15/–20	9.3	24	5.2
Lake Ferapontov, dw	02.93	7.8/0	0.047/5.49	Peat sediment	–	28	–	
Lake Siverskoe lake, dw	02.93	11.2/1.4	0.007/0.11	Black silt	–	17	22	
Lake Dotkas, site 1, center	01.87	6.4/1.8	0.32/0.85	"	10/–80	20	26	8.3
Lake Dotkas, site 2	02.87	3.8/0	0.85/4.85	"	–40/–100	22	24	12.6
Lake Vidogoshch	02.97	3.6/0	0.87/5.68	Rough detrital silt	–80/–100	21	28	24.2
Pond, center	02.07	6.6/4.9	–/0.09	Sandy silt	60/10	16	–	0.8

Note: Here and in Table 3 dw designates deep-water zone, lit is the littoral zone, dash indicates lack of analysis.
* Values for the surface and near-bottom waters are given above and below the dash, respectively.

Table 3. Rates of microbial processes of methane production and oxidation in water and sediments, and consumption of C_{org} for CH_4 production

Water bodies and sampling sites	Water		Bottom sediments		C_{org} consumption for total methane production, mg C/(m^2 day)
	MG	OM	MG (0–5 cm)	OM (0–0.5 cm)	
	μL CH_4/(L day)		mL CH_4/(dm^3 day)		
Water reservoir					
Main river reach, dw	0/0	0.1/2.1	0.06–0.1*	0.04	7.2 (0)**
Reach of the Volga river, dw	0/0.01	0.1/16	0.12–0.3	0.11	22 (<0.1)
The same, litoral site 1	–/0	–/1.8	0.02–0.1	0.04	5.1 (0)
The same, litoral site 2	–/0.2	–/23	0.12–0.35	0.24	27 (0.5)
Lakes					
Lake Pleshcheyevo, dw	0/10	6.5/0	0.38–4.3	0	230 (12)
The same, lit	–/10	–/60	0.2–0.8	0.8	75 (3)
Lake Ferapontov, dw	0/12	–	0.41–1.4	0	115 (–)
Lake Siverskoe, dw	0/7.1	–	0.21–1.1	–	81 (–)
Lake Dotkas site 1, center	0.01/60	30/140	0.88–6.4	1.1	245 (9)
Lake Dotkas site 2	20/90	100/0	1.8–8.1	0	540 (16)
Lake Vidogoshch	0.01/140	210/0	2.7–16.3	0	1220 (31)
Pond, center	0/0	0.1/2.2	0.02–0.17	0.08	9.1 (0)

* Fluctuation limits in layer. ** Methane production in the water, %, is given in parentheses.



Vertical distribution of oxygen, CH_4, and rates of methanogenesis (MG) and methane oxidation (MO) in waters of Lake Datkas in January (a) and February (b), Lake Vidogoshch (d) and Rybinsk Reservoir, the Volga reach (c).

toral, in spite of the ecological differences between these objects, revealed a common tendency: in the conditions of increasing anaerobiosis and reduced state of the sediments the methane generation rate increased. The potential capacity for such an increase probably depends on the C_{lab} reserve and biochemical pathways of methanogenesis. Generally, the methanogenesis rate in the sediment during ice period was comparable to that for the same water bodies in summer [12, 13].

Methane oxidation in the silts occurred even at low oxygen concentrations in the near-bottom waters (Table 3), although availability of oxygen was not the only factor determining its rate. For example, in the oxidized sediments of the pond and the reservoir, methane oxidation was 0.04–0.24 mL CH_4/(dm^3 day), which is close to the figures of the vegetation season [12]. In the sediments of Lake Dotkas, station 1 (January) and of the littoral of Lake Plescheevo, where oxygen was still present in the bottom water at the time of sampling, methane oxidation was significantly lower than in summer [13]. At the low redox potential values, which were registered in the uppermost subsurface layers of the lake sediment, the methanotrophic community probably experienced severe depression and could only function at the sediment–water boundary.

While the methane cycle processes were also detected in the water column, their rate and distribution varied significantly, depending on the oxygen regime, rates of CH_4 flux from the sediments, and other ecological conditions.

In highly productive lakes, which exhibited active methanogenesis and no methane oxidation, the major part of methane entered water, and in the layers containing dissolved oxygen, methane oxidation reached 140–240 μL CH_4/ (L day) (Table 3, figure). The vertical dynamics of methane oxidation was uneven, and its character was changing during the ice period. At the initial stage, when dissolved oxygen was still penetrating down to the bottom in some reservoirs, the maximum CH_4 oxidation rate was observed in the near-bottom water layers (figure, a). After formation of the anaerobic, methane-saturated hypolimnion, the zone of the most intensive methane oxidation was detected in the microaerobic metalimnion layers (figure, b, d).

Methane oxidation rates in the pond and reservoir waters in January–February were low (Table 3), increasing only in deep waters immediately above the bottom (figure, b). The rate of methane oxidation in the bottom waters of the coastal zone increased by the end of the ice period with the activation of the silt methanogenic community (Table 3), but even then the rate observed in the shallow waters did not exceed 23 μL CH_4/(L day).

Methanogenesis was registered in the bottom waters of most of the reservoirs during winter stratification, as a result of the lack of oxygen arriving into the water column from the atmosphere (Table 3). Methanogenesis was detected in just two layers of the reservoir immediately above the bottom, where the MG rate did not exceed 0.01–0.2 μL CH_4/(L day), whereas in lake waters it was 2–3 orders of magnitude higher (Table 3). In highly productive and polluted lakes, active methanogenesis was registered in the entire column of the methane-saturated hypolimnion, and in the shallow Lake Dotkas it reached the ice horizon by the end of winter (figure, b, d).

Production of CH_4 under 1 m^2 and C_{org} consumption for its formation were measured in different reservoirs during the ice period of for better understanding of the ecological significance of methanogenesis [14]. The results made it possible to perform calculations for both bottom sediments and the water column. We found that in the pond and reservoir, methanogenesis was responsible for the degradation of only up to 5.1–27 mg C_{org}/(m^2 day), and in most of the sites this happened exclusively due to the sediment processes. In the lakes, organic matter consumption was much more pronounced. In less productive and weakly polluted waters, 75–115 mg C_{org}/(m^2 day) was degraded, while 540–1220 mg C_{org}/(m^2 day) was degraded in highly trophic lakes (Table 3). In the highly trophic water systems, 9–31% of organic carbon consumption for methanogenesis was consumed in the water column.

We were also interested in comparing the calculated data with the similar characteristics of the relevant water bodies during the summer period. Methanogenesis rate in the sediments in winter was found to be slightly lower than during the summer maximum of phytoplankton development and detritus precipitation to the bottom. Nevertheless, the total geochemical activity of methanogens in these periods was comparable [12, 13]. At the same time, the role of microbial communities of the sediments and the water column in CH_4 formation and organic carbon consumption varied significantly in different seasons. During the ice period, the significance of methanogenesis in the water column, especially in highly polluted lakes, was 2–3 times higher than in summer [13].

The study of the methane cycle in diverse water bodies showed that during the ice period, the microbial processes of CH_4 transformation are ubiquitous, but their direction, rate, and environmental significance differ strongly, depending on the environmental conditions, namely the oxygen regime, C_{org} availability and the redox potential. Methane formation was registered in all the bottom sediments with oxygen stratification, but the maximum of methanogenic activity was detected in highly reduced and heavily polluted lake sediments, rich in C_{lab}. In these lakes intense methanogenesis occurred also in the near-bottom waters. By the end of winter, it was detected throughout almost the entire water column. As a result, the total CH_4 production reached the summer level, the gas formed was actively consumed by meta- and epilimnion methane-oxidizing bacteria and was included into the food web via protozoa and zooplankton [15]. Thus, in the absence of photosynthesis, methane formation is an important source of energy-rich organic matter, and, in general, the microbial processes of the CH_4 cycle play a very important role in the degradation of organic carbon and functioning of the ecosystems food web during the ice period.

REFERENCES

1. Gal'chenko, V.F., Dulov, L.E., Kramer, B., Konova, N.I., and Barysheva, S.V., Biogeochemical processes of methane cycle in the soils, bogs, and lakes of Western Siberia, *Microbiology*, 2001, vol. 70, no. 2, pp. 175–185.
2. Thebrath, B., Rothfuss, F., Whiticar, M.J., and Conrad, R., Methane production in littoral sediment of lake Constance, *FEMS Microbiol. Lett.*, 1993, vol. 102, pp. 279–289.
3. Dzyuban, A.N., *Destruktsiya organicheskogo veshchestva i tsikl metana v donnykh otlozheniyakh vnutrennikh vodoemov* (Organic Matter Degradation and Methane Cycle in the Bottom Sediments of Inland Water Bodies), Yaroslavl: Printkhaus, 2010.
4. Dzyuban, A.N., Seasonal dynamics of the methane cycle in the bottom sediments of Lake Pleshcheevo, *Gidrobiol. Zh.*, 2010, vol. 46, no. 4, pp. 41–48.
5. Kuznetsova, I.A. and Dzyuban, A.N., Determination of net degradation of organic matter in the bottom sediments, *Gidrobiol. Zh.*, 2002, vol. 38, no. 5, pp. 94–98.
6. Naguib, M., A rapid method for the quantitative estimation of dissolved methane and its application in ecological research, *Arch. Hydrobiol.*, 1978, vol. 82, pp. 66–73.
7. Saralov, A.I., Gas chromatographic method for determination of methane oxidation rates in aquatic environments, *Mikrobiologiya*, 1979, vol. 48, no. 1, pp. 125–128.
8. Sorrell, B.K. and Boon, P.J., Biogeochemistry of billabong sediments. 2. Seasonal variations in methane production, *Freshwater Biol.*, 1992, vol. 27, no. 3, pp. 435–445.

9. Dzyuban, A.N., Methane and the microbiological processes of its transformations in the water of the Upper Volga reservoirs, *Water Res.*, 2002, vol. 29, no. 1, pp. 61–71.

10. Bange, H.W., Dahlke, S., Ramesh, R., Meyer-Reil, L., Rapsomanikis, S., and Andreeae, M.O., Seasonal study of methane and nitrous oxide in the coastal waters of the southern Baltic Sea, *Estuar. Coast. Shelf Sci.*, 1998, vol. 47, no. 6, pp. 807–817.

11. Kuznetsov, S.I. and Dubinina, G.A., *Metody izucheniya vodnykh mikroorganizmov* (Methods for Investigation of Aquatic Microorganisms), Moscow: Nauka, 1989.

12. Dzyuban, A.N., Microbiological processes of organic matter turnover in deposits of the Volga–Kama chain of reservoirs, *Water Res.*, 1999, vol. 26, no. 4, pp. 411–420.

13. Dzyuban, A.N., Intensity of the microbiological processes of the methane cycle in different types of Baltic lakes, *Microbiology*, 2002, vol. 71, no. 1, pp. 98–104.

14. Adams, D.D. and van Eck, G.Th., Biogeochemical cycling of organic carbon in the sediments of the Grote Rug reservoir, *Arch. F. Hydrobiol. Ergebn. Limnol.*, 1988, vol. 31, pp. 319–330.

15. Dzyuban, A.N., Georgiev, A.N., Krylov, A.V., and Kuznetsova, I.A., Bacterioplankton and zooplankton it three lakes of different types during the ice period, *Biol. Vnutr. Vod*, 1998, no. 2, pp. 44–51.

Translated by M. Sokolov