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EXPERIMENTAL ARTICLES

Detection of Methane in the Water Column at Gas and Oil Seep Sites in Central and Southern Lake Baikal

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Abstract—Microbiological and biogeochemical investigation of the water column of oligotrophic Lake Baikal at the sites of the K2 and Bolshoi mud volcanoes and the Gorevoy Utes oil seep was carried out in July 2013. Total microbial numbers (TMN), cell numbers of type I and type II methanotrophs, and methane concentrations were measured; the rate of methane oxidation was determined. Methane concentrations in Lake Baikal water column varied from 0.09 to 1 μL/L, while methane oxidation rates varied from 0.007 to 0.9 nL/(L day). The highest rates of methane oxidation were revealed in the near-bottom water horizons at the sites of the Bolshoy mud volcano and the Gorevoy Utes oil seep. These were the sites where the most pronounced anomalies in methane concentration were also detected. TMN varied from 0.123×10^6 to 1.64×10^6 cells/mL. Methanotrophic bacteria were revealed in the water column at all sites, their abundance did not always correlate with methane concentrations and the rates of methane oxidation. Methanotrophs constituted not more than 1.63% of the total microbial number, with their highest abundance in the upper 200 m of the water column.

Keywords: methanotrophic bacteria, methane oxidation, methane concentration, Lake Baikal

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Lake Baikal is a unique freshwater basin, with methane and oil seeps, as well as mud volcanoes and subsurface gas hydrate (GH) deposits present at its bottom [1]. Elevated methane concentrations up to hundreds nL CH_4/L (0.01–0.11 μL/L according to Granin and 0.01–0.3 μL/L according to Egorov) have been reported for the sediments and water column of the lake [2, 3]. Far from being systematic, investigation of methane levels in Lake Baikal water has been carried out occasionally using a variety of methods and equipment [3–5]. Global warming may result in GH decomposition and therefore in anomalously high methane concentrations in deep water horizons. Elevated methane content in Lake Baikal water and its possible reasons were discussed in some recent publications [6, 7]. The possibility of massive Proterozoic methane emission from gas hydrate reservoirs [8] or of a smaller scale Quaternary emission [9] has been previously discussed in the literature.

In all ecosystems, including aquatic ones, microorganisms play the major role in the methane cycle, carrying out both methanogenesis and methane oxidation. Prokaryotes are known to oxidize methane under both oxic and anoxic conditions. Under oxic conditions, methane oxidation by methylobacteria depends on methane monooxygenase, a unique enzyme responsible for the first stage of methane oxidation with oxygen (to methanol) [10]. Methane biogeochemical cycles have been studied in many lakes [11–13] and seas, including the areas of methane seeps and deep-water high-temperature hydrotherms [14].

Occurrence of methanotrophic bacteria in the water column of Lake Baikal has not been studied previously. Available data are restricted to upper sediment layers, where type I and type II methane-oxidizing bacteria were identified by various methods [15]. The work by Namsaraev et al. [16] is the only one dealing with direct radioisotope measurements of methane oxidation rates in the Lake Baikal water column [16], which were on average ~0.5 μL/(L day). Since the typical concentrations of dissolved methane are several times lower than its daily consumption by the methylotrophic bacterial community, this value looks somewhat doubtful. The rates of methane oxidation calculated based on the Michaelis–Menten equation were lower (0.004 to 0.021 nL/(L day) [2].

The goal of the present work was to carry out detailed investigation on the rates of methane oxidation and the numbers of methanotrophic bacteria in the areas of methane and oil discharge using fluorescent in situ hybridization (FISH).

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Table 1. Characterization of the central and southern Baikal sampling stations

Station	No.	Depth, m	Coordinates	Temperature, °C	Mineralization, mg/L
K-2 mud volcano, Kukui canyon (central Baikal)	R-1	890	52.5925000° N 106.7722670° E	3.3–7.5	96.12–97.61
Gorevoy Utes oil seep (central Baikal)	R-6	855	53.3045170° N 108.3919330° E	2.8–3.2	96.72–97.58
Bolshoy mud volcano (southern Baikal)	R-9	1370	51.8779000° N 105.5505170° E	3.3–5.0	96.48–97.48

Table 2. Probes used for fluorescent in situ hybridization (FISH)

Probe	Stain	Sequence	Target organism	Reference
M-450	Cy3	ATCCAGGTACCGTCATTATC	Type I	19
M-84	Cy3	CCACTCGTCAGCGCCCGA	Type II	19
M-669	Tamra	CTCACCTTAAAGTCCACATCG	*Methylomonas* and *Methylobacter*	19
MA-221	Tamra	GGACGCGGGCCGATCTTTCG	All *Methylosinus* spp. and some *Methylocystis* spp.	20

MATERIALS AND METHODS

The research was carried out in early July 2013 at three stations of central and southern Baikal at the sites of discharge of hydrocarbon-containing fluids (Table 1). The samples were collected from R/V *G.Yu. Vereshchagin* using an SBE 32 Carousel Water Sampler.

The samples for determination of methane concentrations in the upper 25-m layer and in the 50-m layer of near-bottom water were taken every 5 m, while a 100-m step was used for the rest of the water column. Temperature and salinity were determined at the same depths using an SBE 19 Plus probe. Methane concentrations were determined using the headspace method [17] on an Ekho-EW gas chromatograph with a flame ionization detector (Novosibirsk, Russia). Total microbial numbers (TMN) were determined in the water samples collected at the following depths: 0, 50, 100, 200, 300, 500, 700 m, and near-bottom water. The samples were fixed with 4% formalin, stained with DAPI (4,6-diamino-2-phenylindole) [18], and examined under an AxioImager.M1 epifluorescence microscope (Carl Zeiss, Germany).

The structure of methanotrophic communities was determined by fluorescent in situ hybridization (FISH) with Cy3- and Tamra-labeled oligonucleotide probes. The structure of the probes used in the present work (Biosintez, Novosibirsk, Russia) is listed in Table 2 [19, 20].

Microorganisms were fixed as described previously [21]. Water samples (100 to 250 mL) were filtered through white polycarbonate membranes (25 mm; 0.2 μm pore size; Millipore, Germany) using a peristaltic pump, and the filters were treated with 3 mL of cold 4% paraformaldehyde (PFA, Sigma, Germany) solution in phosphate buffer (PBS, pH 7.2) for 30 min at room temperature. The fixing solution was then removed by filtration, and the filter was washed three times with 3-mL portions of PBS buffer and with distilled water. Air-dried filters were stored at –20°C. The cells were counted under an AxioImager.M1 epifluorescence microscope using the ImageTest software package.

The rate of methane oxidation in the water column was measured by the radioisotope method with ^{14}C-methane. Immediately after hauling on board, water samples from the bathometers were dispensed into 30-mL penicillin vials and sealed with rubber stoppers. Solution of ^{14}C-methane in distilled water (0.2 mL, 1 μCi) was injected using a sterile syringe. The vials were incubated for 24–36 h in a refrigerator at 3–4°C. The samples were then fixed with 1 mL of 2 N NaOH. The vials with the water from the same horizons supplemented with 1 mL of 2 N NaOH prior to addition of ^{14}C-methane were used as controls. The samples were then treated as described previously [22]. Both oxidation of ^{14}C-methane to CO_2 and ^{14}C incorporation into organic matter (the fraction not volatile under acidification) were considered in the calculation of methane oxidation rates.

RESULTS

Profiles of temperature and salinity in the water column varied from station to station (Fig. 1). At station R-6, they were similar to those obtained during the period of spring homothermy, while at stations R-1 and R-9 the profiles resembled those of the period of direct stratification. Methane profiles at stations R-1 and R-9 were similar, with elevated concen-

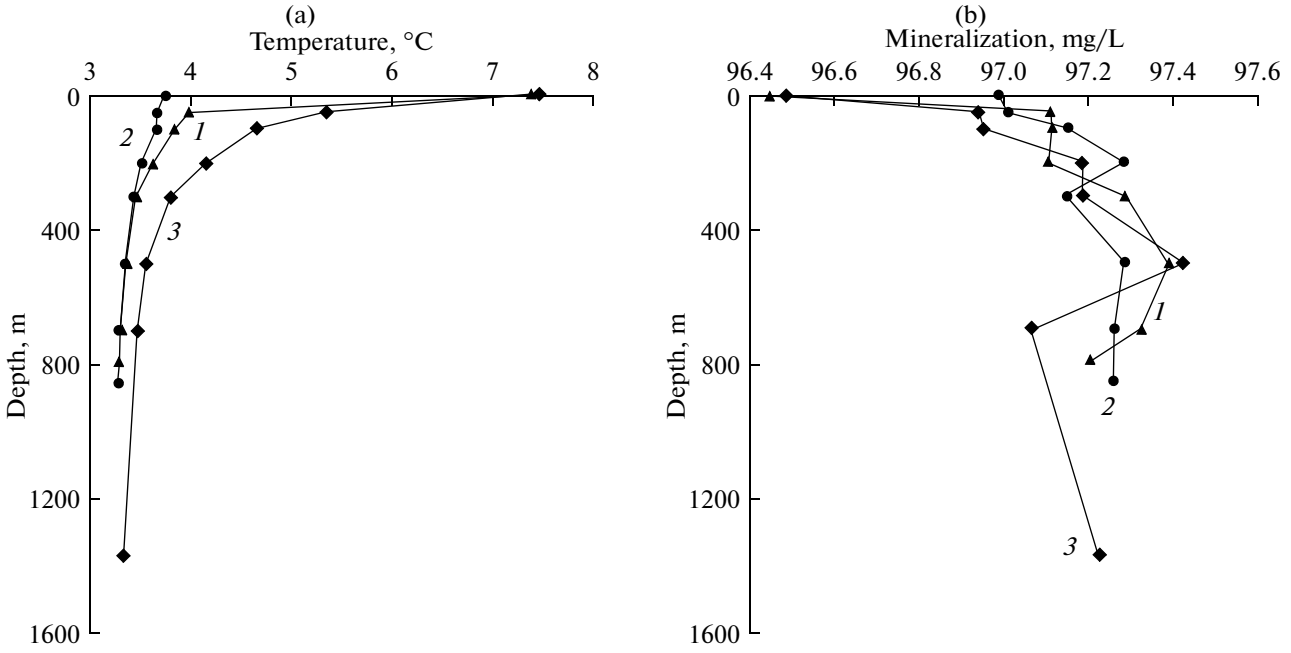

Fig. 1. Profiles of temperature (a) and mineralization (b) in Lake Baikal water column at the sites of hydrocarbon discharge. *1*—station R-1; *2*—station R-6; *3*—station R-9.

trations in the surface horizons (to the depth of 50–200 m) and intermediate maxima at 300 and 400 m (0.56 and 0.33 μL/L, respectively) (Figs. 2a, 4a). Methane concentration at station R-6 was almost constant from the surface to 600-m depth, probably due to vertical water exchange during spring homothermy (Fig. 3a). At all stations, higher methane concentrations were revealed in near-bottom horizons. Thus, at station R-1 CH_4 concentrations at 860–890 m varied from 0.49 to 0.66 μL/L. At station R-6, methane concentrations were 1 μL/L at 830 m and 0.63 μL/L at 850–855. At station R-9 methane concentration if the 1300–1370 m horizon varied from 0.19 to 0.36 μL/L. Interestingly, elevated methane concentration was observed in 5-m horizons of station R-1 (up to 0.36 μL/L) and R-9 (up to 0.26 μL/L) (Figs. 2a, 4a), where high biomass of diatoms was found during this period. Methanogenesis in the zones of high activity of phytoplankton has been reported for an oligotrophic Lake Stechlin (Germany) [23]. The authors attributed it to activity of methanogenic archaea in anaerobic microniches associated with green algae.

TMN profiles were similar at all stations, with microbial numbers decreasing with depth (Figs. 2b–4b). At every station, the highest TMN values were observed in the surface horizons. Such distribution is typical of deep oligotrophic lakes, including Lake Baikal [24].

At station R-1, the highest and lowest TMN values were revealed in the surface layer ($1.64 \pm 0.016 \times 10^6$ cells/mL) and at 800 m ($0.12 \pm 0.001 \times 10^6$ cells/mL), respectively. At this site, the values were somewhat higher ($0.18 \pm 0.002 \times 10^6$ cells/mL) in the near-bottom horizon (890 m).

At station R-6, TMN varied from 0.13 ± 0.002 to $0.96 \pm 0.005 \times 10^6$ cells/mL, decreasing considerably from 200 to 700 m. It increased in the near-bottom zone (855 m) to $0.78 \pm 0.008 \times 10^6$ cells/mL.

At station R-9, TMN varied from 0.19 ± 0.002 to $1.32 \pm 0.004 \times 10^6$ cells/mL. Cell numbers increased from the surface to 100-m depth and then decreased with depth.

Methanotrophic bacteria were revealed at all stations, albeit not in all horizons. At station R-1 (Fig. 2b), high numbers of type I methanotrophs ($26.7 \pm 1.01 \times 10^3$ cells/mL or 1.63% of TMN) were revealed with the M669 probe labeled with xanthene dye Tamra, while the number of type II methanotrophs revealed with the probe MA-221 was lower ($0.5 \pm 0.38 \times 10^3$ cells/mL or 0.03% of TMN). Methanotrophic bacteria of types I and II were not detected below 200 m. Insignificant numbers of methanotrophs (within the experimental error) were detected in near-bottom water (890 m) with all probes.

At station R-6 methanotrophic bacteria were revealed in the photic layer using the probes labeled with the cyanine dye Cy3 (M84 and M450). Type I methanotrophs ($2.28 \pm 0.26 \times 10^3$ cells/mL or 0.23% of TMN) were detected at 50 m, while type II methanotrophs ($4.81 \pm 0.11 \times 10^3$ cells/mL or 0.5% of TMN) were found in the surface water layer. At 700 m, methane concentration increased to 0.56 μL/L, while

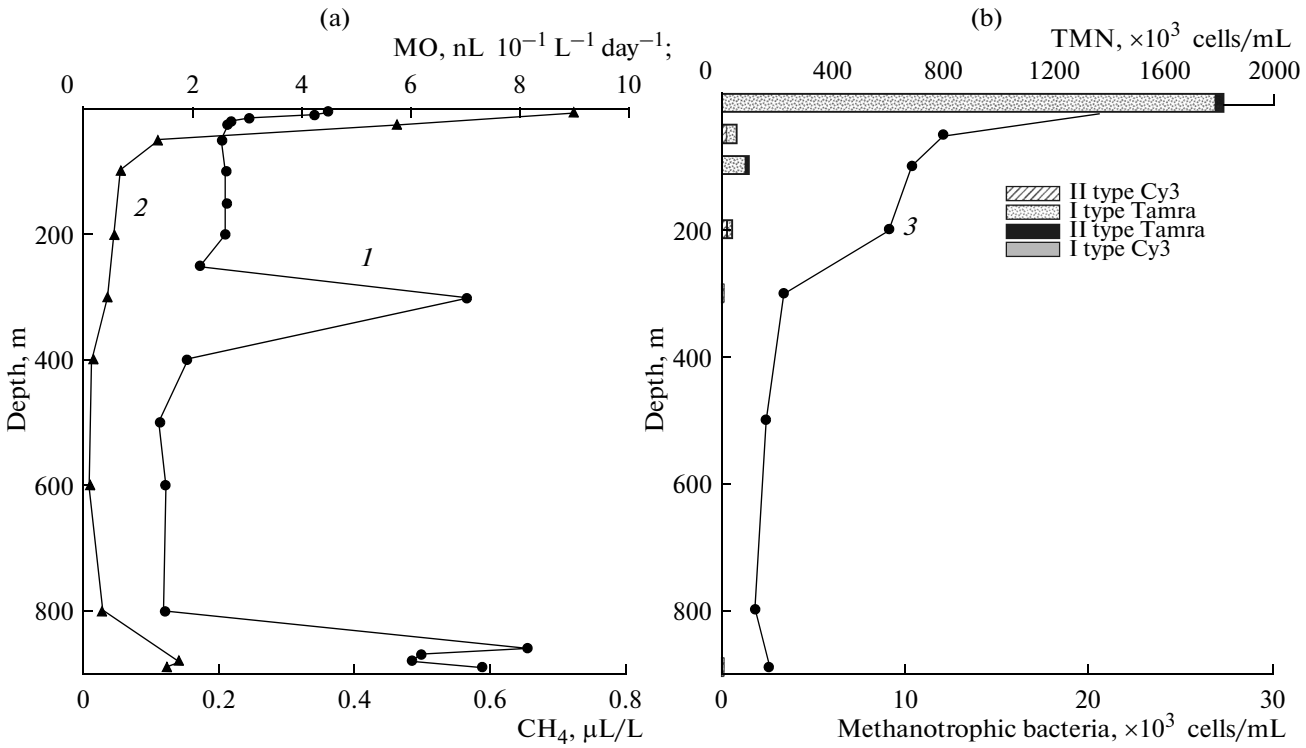

Fig. 2. Profiles of methane concentrations (*1*) and the rates of bacterial methane oxidation (*2*) at station R-1 (a). Profiles of TMN (*3*) and numbers of type I and type II methanotrophic bacteria determined with Cy3- and Tamra-labeled probes (b).

the number of type II methanotrophs revealed with the probe M450 was $0.61 \pm 0.054 \times 10^3$ cells/mL (0.45% of TMN). At the depths of 100 and 300 m, methanotrophic bacteria were not detected by any of the four probes used (Fig. 3b).

At station R-9, only type II methanotrophs (M450) were found in the surface horizon and at 50 m. The highest number of type II methanotrophs (M450) revealed at 100 m was $13.63 \pm 0.48 \times 10^3$ cells/mL (1% of TMN). In the same horizon, the probe M84 revealed elevated numbers of type I methanotrophs, $10.15 \pm 0.16 \times 10^3$ cells/mL (0.8% of TMN). Very few cells of methanotrophic bacteria were detected at 300, 500, and 700 m, while none were found at 1370 m (Fig. 4b).

Radioisotope studies revealed several layers in the water column of Lake Baikal, in which the rates of methane oxidation (MO) varied considerably (Figs. 2a–4a).

The upper photic layer (to 50-m depth) was characterized by high MO activity. The highest MO rate of 9×10^{-1} nL L^{-1} day^{-1} was detected at station R-1, where high methane concentrations (up to 370 nL/L) and a peak of type I methanotrophs ($26.7 \pm 1.01 \times 10^3$ cells/mL or 1.63% of TMN) were found. In general, MO rates in the upper 50 m at the studied stations varied within the range of $0.57–9.0 \times 10^{-1}$ nL L^{-1} day^{-1}.

In the zone from 50 m nearly to the bottom, methane oxidation rates decreased drastically. In deep water layers, MO rates varied from 0.064 to 0.69×10^{-1} nL L^{-1} day^{-1}. Similar to the near-surface layers, the highest methane concentrations were found in deep water of station R-1 (Fig. 2a).

Methane concentrations and methane oxidation rates varied greatly in near-bottom water layers (100 m from the bottom and deeper), probably due to position of the stations relative to the active zones of methane discharge. The highest MO rates in near-bottom water were found at stations R-6 and R-9, where anomalies in methane concentration were most pronounced. The highest MO rate and the highest methane concentration (7.87×10^{-1} nL L^{-1} day^{-1} and up to 1 μL/L, respectively) were found at station R-6. The peaks of MO and methane concentration were found at 850 m (5 m above the bottom), rather than directly at the bottom. Similar results were obtained for station R-9. This was probably due to the patterns of near-bottom currents and to the horizontal and vertical transfer of the water layers enriched with methane and methane-oxidizing bacteria from the zone of methane seeps. MO rates in the near-bottom horizons (100 m from the bottom and deeper) varied from 0.36 to 7.86×10^{-1} nL L^{-1} day^{-1}.

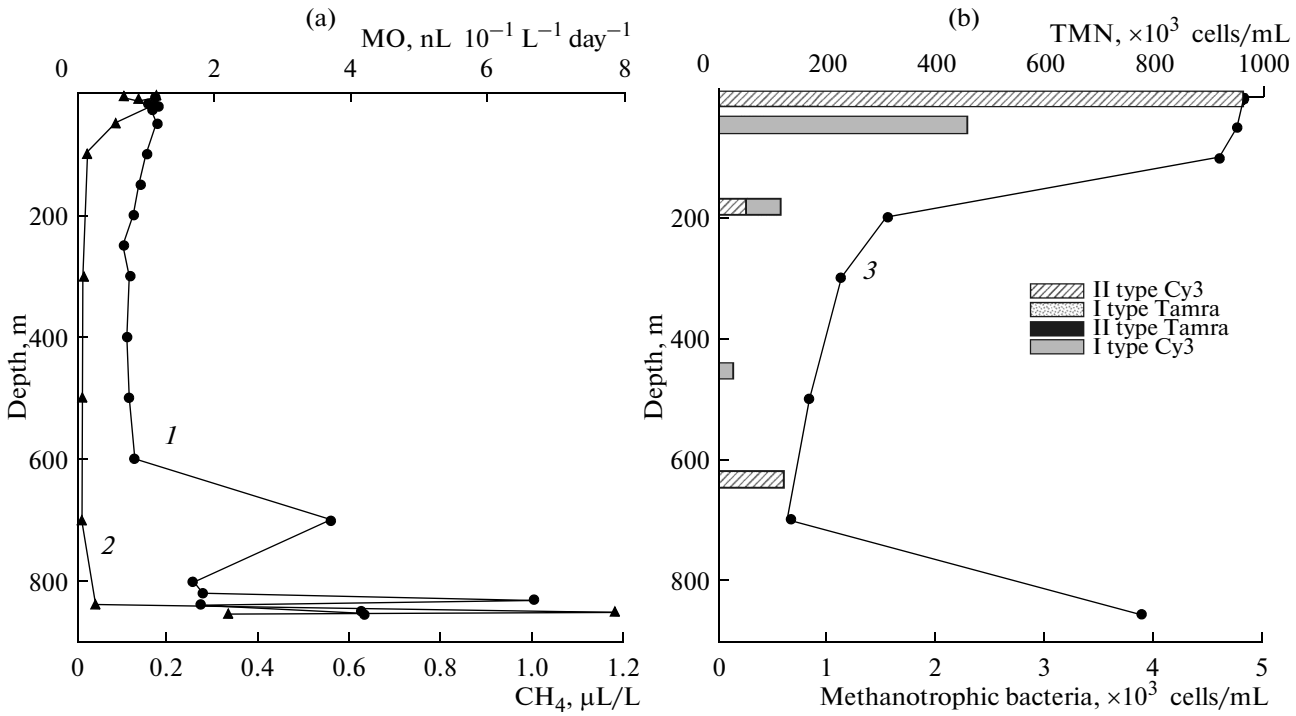

Fig. 3. Profiles of methane concentrations (*1*) and the rates of bacterial methane oxidation (*2*) at station R-6 (a). Profiles of TMN (*3*) and numbers of type I and type II methanotrophic bacteria determined with Cy3- and Tamra-labeled probes (b).

DISCUSSION

At the stations studied, methane concentrations in the water column varied from 0.09 to 1 μL/L, which exceeded the values reported for the regions of Lake Baikal in the areas remote from discharge of gas-bearing fluids, but were lower than the concentrations found in the near-bottom zone of the deep part of the lake in the area of gas-bearing sediments (0.2–14.0 μL/L) [5]. The lower methane concentrations reported in the present work are most probably caused by hydrological factors, such as enhanced convection and water exchange during transition from homothermy to temperature stratification, which cause intense water mixing in the studied areas. Moreover, uneven distribution of methane in near-bottom water and in the water column could result from sampling bias (drift of the ship and Rosette sampling device) or from dissolution of small methane hydrate crystals, similar to those observed from the *Mir* submersible in the near-bottom zone [7]. Methane peaks at the depths of 300–400 m were probably caused by activity of *Macrohectopus*, the only planktonic amphipode known to reside below 200 m in daytime [25]. Development of anaerobic microniches in the intestine and pellets, where conditions may be favorable for methanogenic archaea, may result in local methane maxima in deep layers of the water column.

Comparison of the data on MO obtained by the radioisotope method with the results of the calculations based on the averaged profiles of tritium–helium age of the water masses and of dissolved methane showed good correlation for Lake Baikal deep water horizons. According to Granin et al. [2], MO rates varied from 0.3 to 2.1 × 10^{-2} nL L^{-1} day^{-1} in Lake Baikal southern and central regions and from 1.0 to 2.8 × 10^{-2} nL L^{-1} day^{-1} in its northern regions. Our direct measurements with ^{14}C-CH_4 revealed MO rates in the deep-water zone from 0.7 to 6.3 × 10^{-2} nL L^{-1} day^{-1}.

The highest MO rates found in the surface and near-bottom horizons were much higher than those calculated by Granin et al. [2]. This may be easily explained by the fact that direct measurement of MO rates provides a real-time characterization of the process, while the distribution of methane concentrations and the averaged profiles of the tritium–helium age of the water masses provide information on the average values of methane oxidation rates and on the general patterns of MO along the depth profile.

Methane oxidation rates in Lake Baikal (Table 3) were comparable to the rates of this process in the White Sea [14] and lower than in meromictic lakes: Gek-Gel' (oligotrophic) [11], Mogil'noe (mesotrophic) [12], Mono (eutrophic) [13], and in the Kara Sea [14].

We found predominance of type I methanotrophs in the water column of station R-1, while at stations R-6 and R-9 equal numbers of type I and type II methanotrophs were revealed. Their distribution did

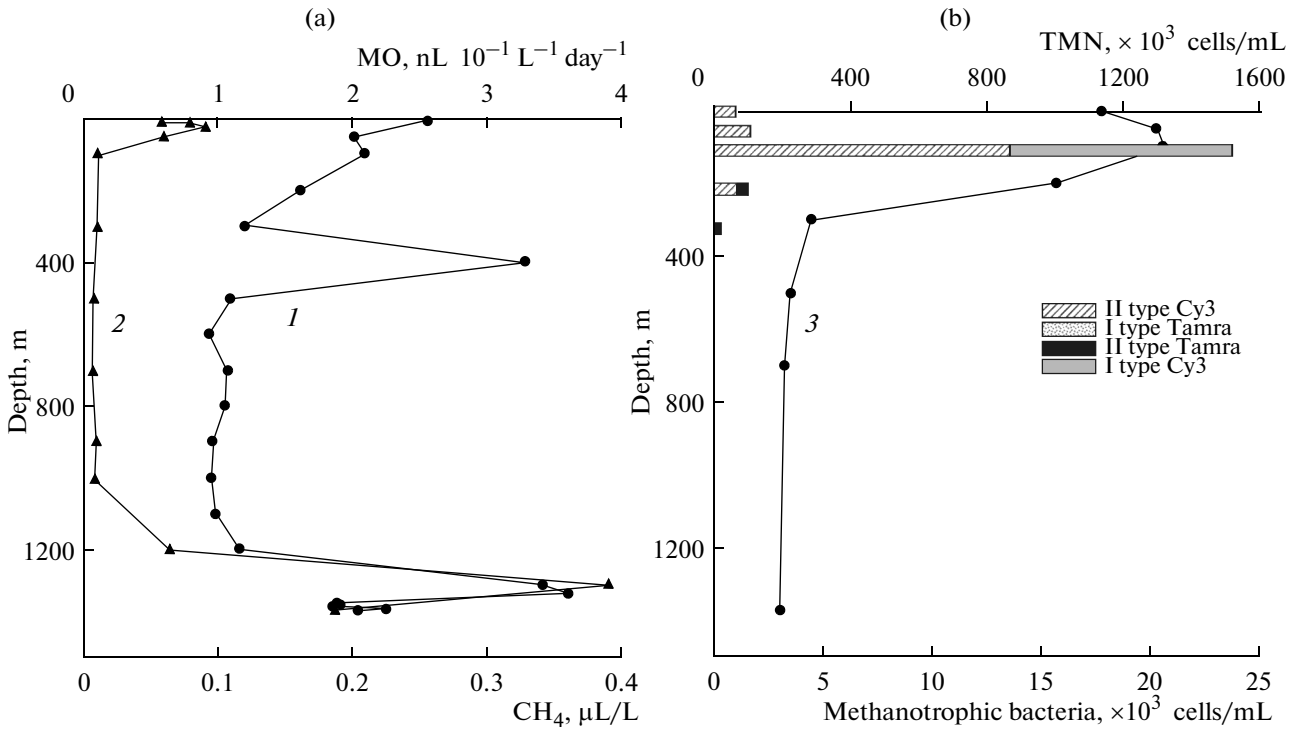

Fig. 4. Profiles of methane concentrations (*1*) and the rates of bacterial methane oxidation (*2*) at station R-9 (a). Profiles of TMN (*3*) and numbers of type I and type II methanotrophic bacteria determined with Cy3- and Tamra-labeled probes (b).

Table 3. Methane concentrations, rates of methane oxidation, and numbers of methanotrophic bacteria in the water column of some basins

Basin	Depth, m	Methane concentration, μL/L	Methane oxidation, nL L^{-1} day^{-1}	Methanotrophic bacteria, cells/mL	Reference
Lake Mogil'noe	0–15.5	0.127–51.1	0.64–1862	(6.4–14.4) × 10^3	12
Lake Gek-Gel'	Up to 70	Up to 1456	Up to 560	Type II methanotrophs	11
Lake Mono	Up to 23	Up to 1344	38.08–150.08—February, 0–2757.4—April, 0–1776.3—June	Type I 4.1–9.3 × 10^5, Type II 1.4–3.4 × 10^5	13
Lake Stechlin	Up to 69.5	On average up to 33.6	–	Type I (99% similarity to *Methylobacter tundripaludum*)	23
Lake Gril'	Up to 4	–	–	5 × 10^4	22
Lake Belykh Dymov	Up to 82.5	–	–	2 × 10^3 cells/mL	22
White Sea	Up to 200	0.04–0.42	0.08–0.3	–	14
Kara Sea	Up to 52	0.049–0.096	0.4–8.8	0.12–7.9 × 10^3 cells/mL	14
Southern Baikal	Up to 1600	0.01–0.11 (averaged data)	0.004–0.021 (averaged data)	–	3
Lake Baikal (southern and central)	Up to 1370	0.09–1.0	0.007–0.9	Type I—up to 26.7 × 10^3 Type II—up to 13.6 × 10^3	Our data

not always correlate with methane concentration and methane oxidation rates.

The numbers of methanotrophic bacteria determined by FISH in Lake Baikal water were considerably lower than their numbers in Lake Mono (California) [13], but were close to those found in the Kara Sea [14], Lake Mogil'noe [12], and Antarctic lakes [22]. Their share in the total bacterioplankton (1.63%) was about half of that in Antarctic lakes (up to 4.8%) [22]. Low numbers of methanotrophs revealed resulted probably from a bias in the FISH procedure applied. While the probes used in the present work were developed for detection of methanotrophic bacteria in cold aquatic ecosystems, they probably did not hybridize with the DNA of all methanotrophs present in this environment [19, 20]. This was probably the reason for the absence of methanotrophic bacteria in near-bottom water, where elevated CH_4 concentrations and MO rates were observed. Isolation of pure cultures of methanotrophic bacteria from the Lake Baikal water column and investigation of their metabolism in order to construct specific probes, as well as detection of methanotrophic bacteria in suspension DNA using the 16S rRNA and particulate methane monooxygenase (*pmoA*) genes, are therefore to be carried out in near future.

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