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EXPERIMENTAL ARTICLES

# Microbial Oxidation of Methane in the Sediments of Central and Southern Baikal

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**Abstract**—Methane levels and methane oxidation rates were determined in the sediments from geochemically different areas in the central and southern parts of the low-mineral Lake Baikal. At different stations, integral rates of methane oxidation varied from 60 to 6592 μmol $m^{-2}$ $day^{-1}$. Typically, two distinct peaks of methane oxidation rates were revealed, located in the oxic and anoxic sediment horizons. In most cases, the rates of aerobic and anaerobic methane oxidation were comparable. Due to low sulfate concentration in pore water (<0.15 mM), a mechanism different from reverse methanogenesis, which involves methanotrophic archaea and sulfate-reducing bacteria and is common in marine sediments, was probably responsible for this process in Lake Baikal reduced sediments. The possible alternative mechanisms and electron acceptors are discussed.

*Keywords*: methane oxidation rate, aerobic methanotrophs, anaerobic oxidation of methane, gas-bearing sediments, Lake Baikal

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Recent studies revealed gas-saturated and gas-hydrate-bearing sediments covering considerable areas of Lake Baikal bottom [1, 2]. At the same time, methane concentrations in the water column are very low, usually not exceeding 200–300 nL/L, except for the areas of discharge of methane-enriched hydrothermal fluids and of mud volcanoes. In these cases, gas hydrate layers occur in subsurface sediment horizons, and methane anomalies are observed in the near-bottom water layers. Methane profiles in most studied Lake Baikal sediments and limited methane occurrence in the water column may be explained as a result of high activity of a methanotrophic bacterial community responsible for aerobic methane oxidation in the water column and in the upper sediment layers. The first quantitative results on the rates of methane oxidation (MO) in the water and bottom sediments were obtained during RV *G.Yu. Vereshchagin* expeditions in 1990–2000. The highest MO rates were detected in the upper sediment layer of the discharge area of low-temperature hydrothermal fluids in Frolikha Bay (up to 53 μmol $dm^{-3}$ $day^{-1}$) and in the water above the southern Baikal gas hydrate-bearing sediments (up to 79 nmol $dm^{-3}$ $day^{-1}$) [3]. Species-specific sera were used to reveal methanotrophic bacteria *Methylosinus trichosporium*, *Methylobacter capsulatus*, *Methylosinus echinoides*, *Methylomonas methanica*, and *Methylobacter bovis* in Baikal sediments; in Frolikha Bay sediments, the numbers of *M. trichosporium* were as high as $1.3 \times 10^5$ cells $cm^{-3}$. Wide occurrence of methanotrophic bacteria in the upper sediment layers of a number of Baikal sites was subsequently confirmed by 16S rRNA gene sequencing [4], pyrosequencing of the 16S rRNA genes [5], and sequencing of the amplified fragments of the *pmoA* gene encoding particulate methane monooxygenase, the key enzyme of methane oxidation by methanotrophic bacteria [6].

In spite of considerable progress in investigation of MO in Lake Baikal sediments, quantitative data on MO rates in gas-saturated and gas hydrate-bearing sediments are scarce, especially in the case of subsurface horizons, where, apart from aerobic MO, the anaerobic process may occur. Since numerous publications report anaerobic methane oxidation (AOM) in anoxic horizons of soils and freshwater basins with compounds other than sulfate ($NO_3^-$, $NO_2^-$, Fe(III), Mn) used as terminal electron acceptors [7–9], this area of research gains special importance.

Quantitative measurements of MO rates in the sediments at different sites of Lake Baikal characterized by elevated methane content were carried out during RV *G.Yu. Vereshchagin* expeditions in 2012–2013.

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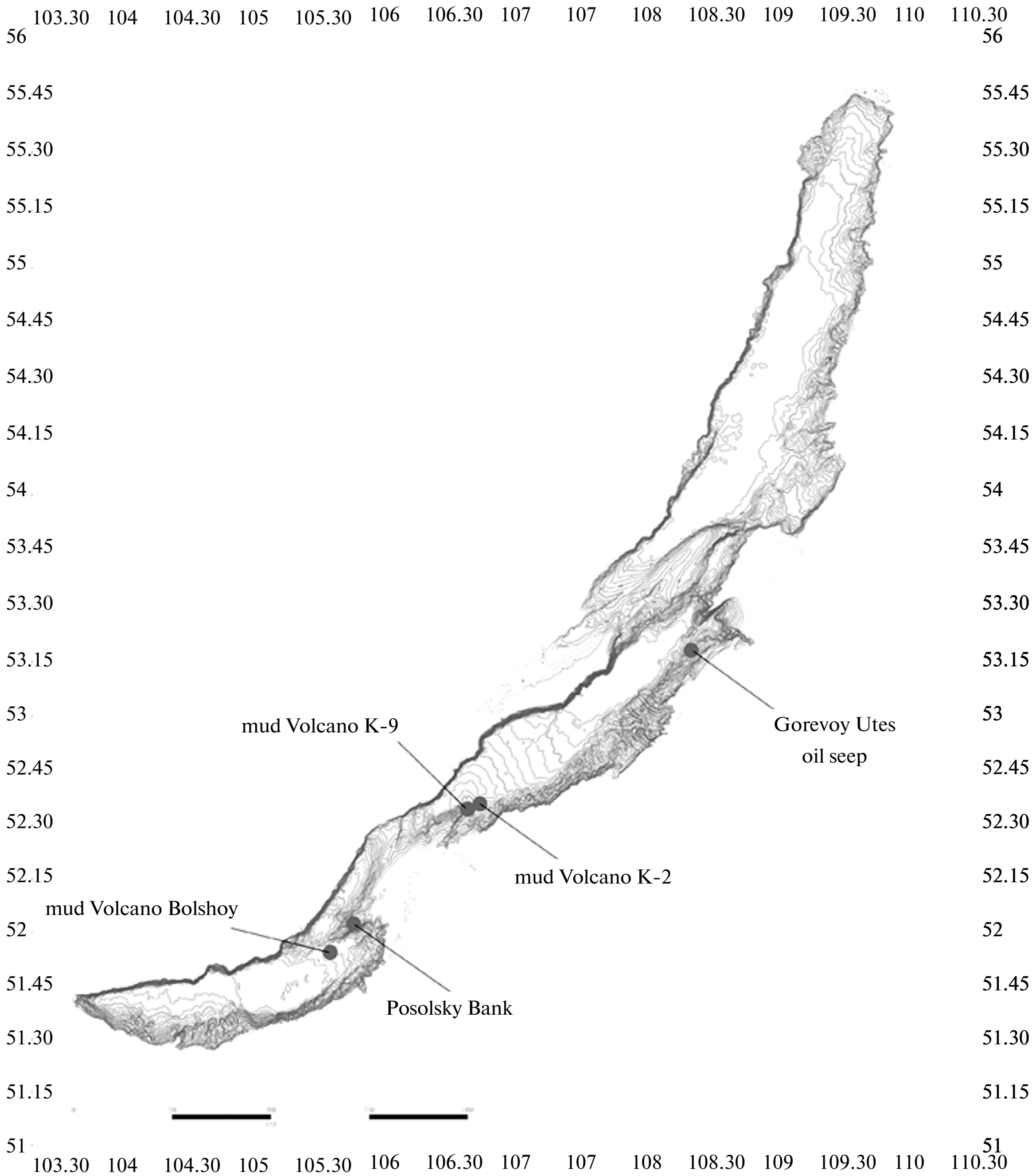


**Fig. 1.** Sampling stations, RV *Professor Vereshchagin*, July 2012 and 2013.

## MATERIALS AND METHODS

Bottom sediment samples were collected from board RV *G.Yu. Vereshchagin* in July 2012 and 2013. The sampling sites included gas-saturated and gas hydrate-bearing sediments of the Posolsky Bank area, mud volcanoes of the Kukui Canyon, oil seepage areas in central Baikal (Gorevoy Utes), and the Bolshoy volcano in southern Baikal (Fig. 1). Coordinates of the sampling stations and the sampler types are listed in Table 1.

Methane content in the sediments was measured on board by headspace analysis [9] on an ECHO-PID chromatograph equipped with a flame ionization detector (2 m × 2 mm packed column, Porapak sorbent, isothermic mode at 100°C).

For mass spectral analysis of carbon ($\delta^{13}C$), methane was collected from the sediments as follows: a 250-mL glass vial was half-filled with the sediment, filled with concentrated NaCl solution to 230 mL, sealed with a rubber stopper, and stirred vigorously. In the laboratory, the gas phase was collected with a

**Table 1.** Characterization of the sampling stations

| Station no., sampling year/depth, m | Sampler | Coordinates | Characterization of the sediment |
|---|---|---|---|
| Posolsky Bank, southern Baikal | | | |
| 3-2012/51 | BT, GC | 52°04.7965 N 105°54.2216 E | 0–2 cm, oxidized brownish-yellow clayey aleurite; deeper, dark gray and gray aleuro-pelitic silt with dark layers |
| 4-2012/508 | GR, GC | 52°03.2460 N 105°54.2681 E | 0–1 cm, oxidized brownish-yellow clayey aleurite; deeper to 50 cm, dark gray aleuro-pelitic silt with dark layers; below 50 cm, lamellar plastic gray silt with dark layers |
| 5-2012/500 | BT, GC | 52°02.1705 N 105°50.6025 E | 0–1 cm, oxidized brownish-yellow clayey aleurite; deeper, gray aleuro-pelitic silt with dark strands; below 20 cm, gas-saturated with gas caverns; below 75 cm, GH |
| 10-2012/850 | BT, GC | 52°10.4786 N 105°48.5499 E | 0–1 cm, oxidized brownish-yellow clayey aleurite; 1–90 cm, dark gray and gray aleuro-pelitic silt with indication of gas saturation below 40–50 cm; below 90 cm, gray clayey silt with numerous GH layers |
| Kukui Canyon, K-2 mud volcano, central Baikal | | | |
| 1-2013/925 | GC, GR | 52°35.4618 N 105°46.2670 E | 0–10 cm, oxidized brownish-yellow clayey aleurite; 10–90 cm, dark gray and gray aleuro-pelitic silt with indication of gas saturation below 40–50 cm; below 90 cm, gray clayey silt with numerous GH layers |
| Oil seep, Gorevoy Utes, southern Baikal | | | |
| 6-2013/890 | BT, GC | 52°18.2656 N 105°23.4670 E | 0–0.5 cm, oxidized yellow-brown warp; 0.5–80 cm, reduced dark gray aleuro-pelitic silt, darker at the upper 10–15 cm and gradually lightening. Below 18–20 cm, oil layers and indication of gas saturation; below 55–60 cm, small spicular GH |
| Bolshoi mud volcano, southern Baikal | | | |
| 9-2013/1410 | BT, GC | 52°53.5449 N 105°32.9739 E | 0–1 cm, oxidized yellow-brown warp; below, gray silts with hydrotroilite strands at 10–20 cm; indication of gas saturation below 50–60 cm; GH layer at 2.5 m |

* BT, benthic tube, GC, gravity corer, GR, grab.

syringe by replacing the volume with salt solution, and the sample was stored above salt solution. The $\delta^{13}C$ of methane was measured on a TRACE GC gas chromatograph (Germany) coupled to a Delta plus mass spectrometer (Germany). The error of $\delta^{13}C$ measurements did not exceed ±0.1‰.

A ProfiLine pH 3210 field millivoltmeter (WTW, Germany) was used to measure Eh in the sediments.

Methane oxidation rates in the sediments were measured by the radioisotope method with $^{14}CH_4$. Immediately after being brought on board, the sediment from the relevant horizon (3 mL) was collected into cut-off 5-mL plastic syringes, which were then sealed with butyl rubber stoppers. The radioactive substrate (0.2 mL of $^{14}C$-methane dissolved in degassed distilled water, 1 μCi) was injected through the stopper, and the samples were incubated in a refrigerator (3–5°C) for 24–48 h. After incubation, the samples were fixed with 1 mL of 2 N KOH and transported to a stationary laboratory. The samples were treated as described previously [10]. The samples fixed with alkali 1–2 h prior to $^{14}C$-$CH_4$ injection were used as the control.

## RESULTS

**Methane isotopic composition.** At most central and southern Baikal stations, methane content in the sediments increased drastically with depth. The isotopic

**Table 2.** Range of methane concentrations and methane oxidation rates in the upper 10 cm of Lake Baikal sediments

| Station no./depth, m | $CH_4$, μmol $dm^{-3}$ | MO rate, μmol $dm^{-3}$ $day^{-1}$ |
|---|---|---|
| Posolsky Bank, 3-2012/51 | 0.018–5.58 | 0.02–0.13 |
| Posolsky Bank, 4-2012/508 | 17.8–208 | 0.71–3.89 |
| Posolsky Bank, 5-2012/500 | 11.0–19.0 | 0.41–1.42 |
| Posolsky Bank, 10-2012/850 | 1.1–597 | 0.60–60 |
| Kukui Canyon, 1-2013/925 | 0.41–0.67 | 0.31–0.37 |
| Oil seep, 6-2013/890 | 1432–4230 | 5.52–40 |
| Bolshoy volcano, 9-2013/1410 | 0.43–54.0 | 0.08–0.74 |

composition ($\delta^{13}C$) of methane carbon indicated differences in its origin at different sites. In the sediments of the Bolshoi mud volcano (st. 9-2013) in the southern basin of Lake Baikal, microbial methane was detected ($\delta^{13}C$-$CH_4$ from –64.4 to –62.58‰). Methane of thermogenic genesis was detected in the sediments of the Gorevoy Utes oil seep (st. 6-2013, $\delta^{13}C$-$CH_4$ from –44.5 to –37.9‰) and within the Posolsky Bank polygon (st. 5-2012, $\delta^{13}C$-$CH_4$ from –48.5 to –40.6‰). The sediments of other stations of the Posolsky Bank polygon (st. 3-2012, 4-2012, and 10-2012) and of the K-2 mud volcano in Kukui Canyon (st. 1-2013) contained methane of mixed genesis with $\delta^{13}C$-$CH_4$ varying from –55.3 to –66.4‰, indicating considerable contribution of microbial methane to the overall pool of this gas. These results are in agreement with the previously obtained data on Baikal methane [2, 11].

**Methane content and methane oxidation rates.** The profiles of methane oxidation rates usually exhibited two maxima (Fig. 2). The first maximum was located in the upper oxidized (Eh from 180 to 250 mV) or weakly reduced sediments (to the depth of 10–15 cm; Eh from –20 to 100 mV) and was probably due to the activity of methanotrophic bacteria (MOB). Methanotrophic bacterial communities have been previously revealed in the upper sediment layers of many Lake Baikal areas by various approaches [3–6]. Activity of the MOB community depended on methane concentrations in the upper 10 cm of the sediment. Methane oxidation rates at the stations with methane content in the upper sediment horizons below 1 μmol $dm^{-3}$ did not exceed 1 μmol $dm^{-3}$ $day^{-1}$ (Table 2). In the sediments with elevated methane content, higher activity of the MOB community was observed in the upper sediment horizons. In the case of Posolsky Bank gas hydrate-bearing sediments (st. 10-2012), methane concentration in the upper 10 cm of the sediment increased drastically from 1.1 to 597 μmol $dm^{-3}$, while the rate of methane oxidation in the 1–5 cm horizon was as high as 60 μmol $dm^{-3}$ $day^{-1}$ (Fig. 2d). High rates of methane oxidation were revealed in the oil seep area, where methane concentration was 1.4 μmol $dm^{-3}$ even in the 0–1 cm horizon (Fig. 2f).

The second maximum of methane oxidation rates was found in deeper sediment horizons, where the redox potential (Eh) varied from –150 to –50 mV and where the presence of free oxygen was highly improbable, even assuming the possible sediment bioturbation by benthic fauna. Spatial separation between the zones of elevated MO rates in the uppermost and deeper (usually 20–60 cm) horizons was observed in the sediments from most stations (Fig. 2). This may be an indirect indication that activity of the microorganisms carrying out anaerobic oxidation of methane (AOM) is probably responsible for the second, deeper maximum. In most of the sediments studied, methane concentration increased sharply in this layer (Fig. 2). In gas hydrate-bearing sediments this layer was several decimeters above the gas hydrate zone. Accurate measurement of methane oxidation rates in still deeper horizons of gas-saturated and gas hydrate-bearing sediments, where methane concentrations exceed 4–5 mmol $dm^{-3}$, proved possible only for the oil seep area (Fig. 2f). At this site, methane concentration of 14 mmol $dm^{-3}$ and high AOM rate (~40 μmol $dm^{-3}$ $day^{-1}$) were revealed.

Comparison of the peak rates of aerobic and anaerobic methane oxidation (Fig. 3) revealed that they were generally comparable, although at some stations the maximum rate of methane oxidation was considerably higher in the upper sediment layers than in deeper horizons. However, in some silts of the Posolsky Bank area (st. 3-2012 and 5-2012) and of the Bolshoy volcano (st. 9-2013), AOM rates were considerably higher than the rates of methane oxidation in the oxidized sediment horizons.

Since no methane-oxidizing activity was revealed in the sediments containing gas hydrate layers, the data shown on Fig. 2 present the results for the upper 1 m.

## DISCUSSION

Measurement of methane oxidation rates in the sediments of various Lake Baikal sites reliable revealed MO in the upper meter of the sediments. Below 70–80 cm MO rates decreased to the threshold values of the sensitivity of the radioisotope method. High gas saturation of the sediments presented another problem, decreasing the sensitivity of

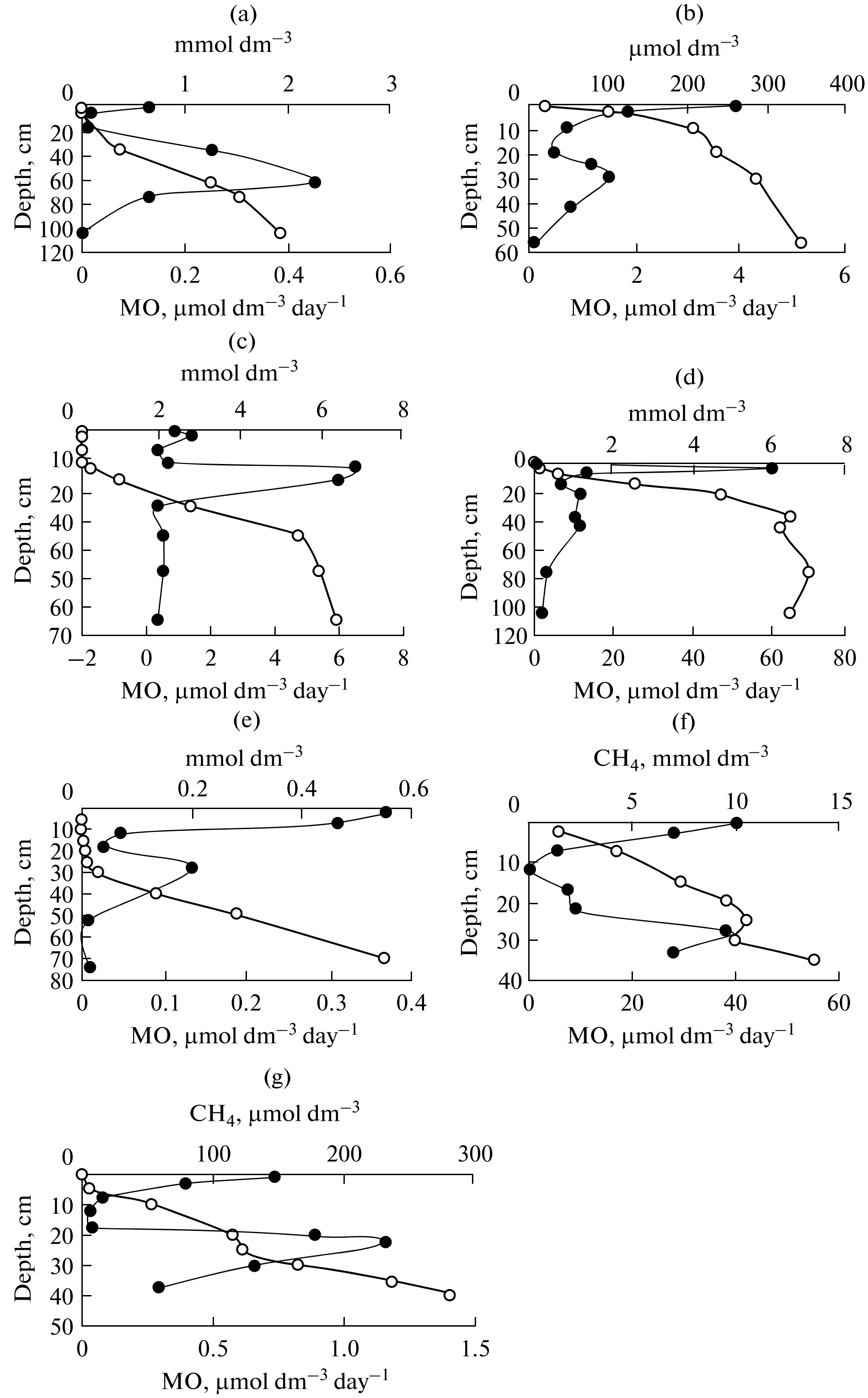


**Fig. 2.** Profiles of methane (○) and methane oxidation rates (●) in Lake Baikal sediments. Posolsky Bank, stations 3-2012 (a); 4-2012 (b); 5-2012 (c); 10-2012 (d); K2 volcano, Kukui Canyon, station1-2013 (e); Gorevoy Utes oil seep, station 6-2013 (f); and Bolshoy volcano, station 9-2013 (g).

the method considerably, so that incubation of gas hydrate-containing layers with $^{14}C$-$CH_4$ was possible only after gas hydrate removal and a significant decrease in methane content. Our data therefore reflect the potential activity of methanotrophic microflora, rather than MO rates in situ.

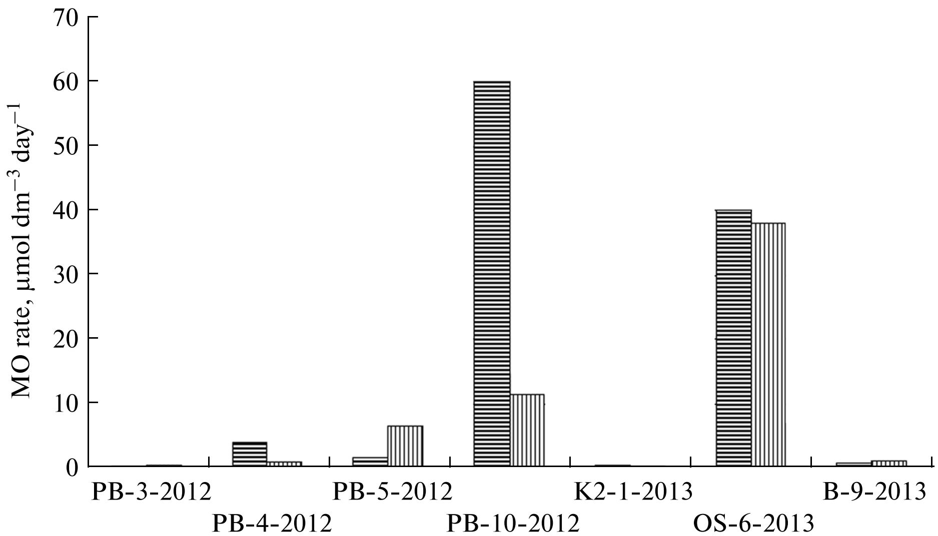


**Fig. 3.** Maximal rates of aerobic (horizontal hatching) and anaerobic (vertical hatching) methane oxidation, μmol dm$^{-3}$ day$^{-1}$ in Lake Baikal sediments: Posolsky Bank (PB), K2 mud volcano, Kukui Canyon (K2), oil seep area, Gorevoy Utes (OS), and Bolshoy volcano (B).

The calculated integral rates of methane oxidation, as well as the values for aerobic and anaerobic MO, are presented in Table 3. According to our calculations, integral rates of aerobic and anaerobic methane oxidation in Lake Baikal sediments were comparable. At some Posolsky Bank stations (st. 3-2012, 5-2012), the oil seep, and the Bolshoy volcano, anaerobic methane oxidation was considerably higher than MO in the aerobic zone. Thus, similar to marine environments, AOM is a geochemically important process in Lake Baikal subsurface sediments, contributing significantly to decreasing the methane flow into the water column. Our findings agree with the results of [12]. Based on the measurements of carbon isotopic composition of methane ($\delta^{13}C$) in subsurface, gas hydrate-bearing sediments of mud volcanoes—in which $^{13}C$-enriched methane was found in the 5–100 cm column at some stations—the authors concluded that this change in methane isotopic composition was probably a result of microbial methane oxidation accompanied by isotope fractionation due to selective oxidation of isotopically light $^{12}CH_4$ by microorganisms.

It is still unclear what microorganisms are responsible for AOM in reduced sediments of the low-mineral Lake Baikal. AOM has long been known in Lake Tanganyika, a large, deep rift lake somewhat resembling Lake Baikal [13]. The relatively high sulfate concentration in the water of this lake is considered sufficient for the functioning of the well-known AOM mechanism, reverse methanogenesis coupled to sulfate reduction (SR) [14]. Our previous measurements of SR rates in Posolsky Bank sediments with different methane levels [15] revealed SR in reduced silt of all stations, immediately below the layer of oxidized sediments. Comparison of MO and SR integral rates (Table 3) shows, however, that methane oxidation

**Table 3.** Integral rates of methane oxidation in Lake Baikal sediments

| Station no. | Aerobic methane oxidation, μmol m$^{-2}$ day$^{-1}$ | Anaerobic methane oxidation, μmol m$^{-2}$ day$^{-1}$ | Total methane oxidation, μmol m$^{-2}$ day$^{-1}$ |
|---|---|---|---|
| Posolsky Bank, 3-2012 | 9.0 | 154 | 163 (87)* |
| Posolsky Bank, 4-2012 | 184 | 324 | 507 (278) |
| Posolsky Bank, 5-2012 | 90 | 667 | 757 (169) |
| Posolsky Bank, 10-2012 | 3485 | 3107 | 6592 (130) |
| Kukui Canyon, 1-2013 | 36 | 24 | 60 |
| Oil seep, 6-2013 | 1810 | 4125 | 5935 |
| Bolshoy volcano, 9-2013/1410 | 26 | 150 | 176 |

* In parentheses, integral rates of sulfate reduction measured in 2012 at the same horizons (Pimenov et al. 2014).

rates exceeded those of SR at all studied Posolsky Bank stations. An AOM mechanism in Lake Baikal reduced sediments is therefore different from reverse methanogenesis described for marine sediments. The latter involves sulfate-reducing bacteria and methanotrophic archaea phylogenetically related to methanogens [16–18]. Low sulfate concentration (<0.15 mM) in pore waters of most stations prevents sulfate-dependent AOM [12, 15]. Areas of discharge of sulfate- and sulfide-containing deep fluids and of sulfate-containing wastewater are exceptional in this respect. Thus, at some stations in the area of the Malenky mud volcano [19] sulfate content in pore water was high enough to support AOM (up to 15 mM). Detection of archaeal 16S rRNA gene fragments phylogenetically related (up to 98% similarity) to methanogens of the order *Methanosaeta* [20] in subsurface horizons of the Malenky mud volcano gas hydrate-containing sediments was therefore not surprising. Apart from methanogens, this order comprises methanotrophic archaea of the ANME I and ANME II groups, which, in consortium with sulfate-reducing bacteria, are responsible for anaerobic methane oxidation in marine environments [20].

The possible mechanisms of anaerobic methane oxidation in low-salinity soil and aquatic environments, where sulfate concentrations are insufficient for sulfate reduction coupled to AOM, have recently been discussed [7]. Anaerobic methane oxidation in the absence of sulfate by a methanotrophic archaeon phylogenetically related to ANME and an uncultured denitrifying bacterium was reported to occur according to the following equation: $5CH_4 + 8NO_3^- + 8H^+ \rightarrow 5CO_2 + 4N_2 + 14H_2O$ [21]. It was subsequently determined that AOM coupled to denitrification could be carried out by both bacteria [22] and archaea phylogenetically related to the ANME-2d group [23]. The name *Candidatus* 'Methanoperedens nitroreducens' was proposed for the latter organism. A bacterium oxidizing methane under anaerobic conditions, *Candidatus* 'Methylomirabilis oxyfera,' formed a new taxonomic lineage phylogenetically related to aerobic methanotrophic bacteria. For methane oxidation, this organism uses the oxygen produced intracellularly in the course of nitrite reduction to dinitrogen [24]. Methane oxidation coupled to denitrification was revealed in nitrate-enriched river sediments [25]. In our opinion, however, occurrence of denitrification-coupled AOM in Lake Baikal sediments is limited by low levels of nitrate in this environment. Similar to sulfate, locally increased nitrate concentrations are possible in Lake Baikal sediments, although their effect on the biogeochemical cycle of methane in the lake is most probably quantitatively insignificant.

Other electron acceptors probably involved in AOM may include Fe(III) and Mn. Data on Fe and Mn occurrence in the upper sediment layers indicate significantly higher Fe content (compared to Mn) for the upper sediments of central and southern Baikal [26]. Low rates of sulfate reduction in the sediments imply low sulfide content and therefore low degree of reduction, with Eh values seldom lower than –100 mV. Thus, Fe and Mn in Lake Baikal sediments may be present in the oxidized form. Microbial oxidation of methane by ferrihydrite- and birnessite-utilizing microorganisms was recently revealed in marine sediments affected by river flow [27]. AOM in gas-saturated, sulfide- and sulfate-depleted sediments of the Argentine Basin is probably coupled to iron reduction [28]. While no data are available concerning occurrence of this process in freshwater, low-mineral ecosystems, contribution of Fe- or Mn-dependent methane oxidation to AOM in Lake Baikal sediments cannot be ruled out.

Pyrosequencing of the 16S rRNA genes and analysis of the sequences of the *mcrA* gene encoding methyl-coenzyme M reductase (the key enzyme of methanogenic and methanotrophic archaea) were used to investigate the archaeal component of the methane-poor sediments at the Sankt Peterburg gas hydrate field, Lake Baikal [5]. Predominance of methanogenic archaea of the orders *Methanomicrobiales* and *Methanosarcinales* was reported, while no ANME-related organisms were detected, unlike the sulfate-rich sediments of the Malenky mud volcano. These findings indicate that AOM is associated with the activity of the methanogenic archaeal community; wide occurrence of these organisms in Lake Baikal sediments is beyond doubt. Long ago, radioisotope studies revealed that under certain conditions methanogenic archaea were able to oxidize methane by the reaction reverse to methanogenesis [29]. Ability of archaea to oxidize methane was recently confirmed by cultivation of *Methanobacterium thermoautotrophicum*, *Methanosarcina barkeri*, and *Methanosarcina acetivorans* on various substrates, including methanol and trimethylamines [30], as well as by experiments with Black Sea microbial mats [31]. Correlation between methane formation and oxidation in marine sediments of a methane seep area was shown [32]. The authors suggest that at high methane concentrations methanogenesis may be an AOM side reaction required by archaea to obtain additional energy. Available data on the rates of methanogenesis in Lake Baikal sediments [3, 4] are insufficient for comparison of the rates of methanogenesis and methane oxidation. In many cases, however, the rate of methane oxidation in subsurface sediments was found to considerably exceed the rate of methane formation. High AOM rates in gas-saturated Lake Baikal sediments are probably associated with activity of methanogenic archaea, which increase methane oxidation in the presence of high methane concentrations.

The results of our research suggest that methane oxidation in Lake Baikal sediments depends on the geochemical activity of aerobic and anaerobic microbial communities located at different depths and separated by a zone of decreased MO rates. Our data, together with the results of earlier measurements, make it possible to determine “hot spots” where MO rates in some horizons exceed 10 μmol $dm^{-3}$ $day^{-1}$. The highest MO rates were revealed in the Posolsky Bank sediments above the gas hydrate layer (st. 5-2012) and in the oil seep area (st. 6-2013). Methane oxidation rates up to 12 μmol $dm^{-3}$ $day^{-1}$ were previously reported for the Malenky volcano sediments [4] and at the hydrothermal spring discharge area in Frolikha Bay (up to 53 μmol $dm^{-3}$ $day^{-1}$) [3]. Similar MO rates (1–50 μmol $dm^{-3}$ $day^{-1}$) were found in marine coastal silts, with the highest rate of sulfate-coupled AOM 20 with the sulfate–methane transition zone [20]. Thus, the existence of both aerobic and anaerobic methane oxidation in gas-saturated and gas hydrate-bearing sediments of Lake Baikal, which decrease considerably the methane flow into the water column, is beyond doubt. The mechanisms of AOM in low-mineral Lake Baikal sediments, the organisms responsible for this process, and the enzymatic systems and electron acceptors involved remain, however, unknown.

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