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Sulfate Reduction and Microbial Processes of the Methane Cycle in the Sediments of the Sevastopol Bay

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Abstract—The rates of microbial processes of sulfate reduction and of the methane cycle were measured in the bottom sediments of the Sevastopol basin, where seeps of gaseous methane have been previously found. Typically for marine environments, sulfate reduction played the major role in the terminal phase of decomposition of organic matter (OM) in reduced sediments of this area. The rate of this process depended on the amount of available OM. The rate of methanogenesis in the sediments increased with depth, peaking in the subsurface horizons, where decreased sulfate concentration was detected in the pore water. The highest rates of sulfate-dependent anaerobic methane oxidation were found close to the methane–sulfate transition zone as is typical of most investigated marine sediments. The data on the carbon isotopic composition of gaseous methane from the seeps and dissolved CH_4 from the bottom sediments, as well as on the rates of microbial methanogenesis and methane oxidation indicate that the activity of the methane seeps results from accumulation of biogenic methane in the cavities of the underlying geological structures with subsequent periodic release of methane bubbles into the water column.

Keywords: sulfate reduction, methane oxidation, methanogenesis, gas emission, Sevastopol basin, Black Sea

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For decades, microbiological investigation of the Crimean coastal waters dealt mainly with bacterioplankton abundance and detection of pathogenic microorganisms in the sediments and water column [1]. Considerable contamination of the Black Sea coastal waters with industrial and municipal wastes affects the normal course of the biogeochemical cycles of organic matter (OM) and biogenic elements. In some cases, the shallow and most productive areas of the sea become zones of environmental disaster. Thus, since mid-1970s, activity of bacterioplankton degrading autochthonous and allochthonous OM resulted in oxygen deficiency in the estuarine regions of the Black Sea northwestern shelf in summer. Increased influx of autochthonous OM resulted from higher primary production due to greater inflow of nitrogen and phosphorus compounds with river flow, while disposal of industrial and municipal wastes enriched the coastal ecosystems with dissolved and suspended allochthonous OM of anthropogenic origin [2]. Oxidation of a broad range of organic compounds by the aerobic microbial community of the water column results in rapid consumption of oxygen with development of reduced conditions favorable for anaerobic prokaryotes (fermenting, sulfate-reducing, methanogenic, etc.) in near-bottom waters and upper sediment horizons. The terminal phase of OM mineralization in reduced sediments in the presence of sulfates was shown to be carried out mainly by sulfate-reducing bacteria, which utilize low-molecular organic compounds or H_2 formed at the initial stages of OM decomposition and reduce sulfate to sulfide [3]. The anaerobic zone of the sediment becomes a source of free H_2S, which during the periods of high rates of sulfate reduction (SR) may penetrate into the water column, causing mass suffocation of the near-bottom fauna. Investigation of the ecosystem of the Black Sea northwestern shelf by the team of the Winogradsky Institute of Microbiology, Russian Academy of Sciences in the course of a number of Russian and international expeditions provided abundant microbiological and biogeochemical evidence of the activation of bacterial SR due to increased anthropogenic load in the region being the main factor responsible for the seasonal contamination of the Black Sea northwestern shelf [4, 5]. Methanogenesis, together with sulfate reduction, is another important process of OM decomposition. The scale of microbial methanogenesis depends primarily on OM flow into the anoxic zone. Some methane synthesized in the anoxic zone is oxidized by aerobic methanotrophic microorganisms inhabiting the upper, oxidized sediment layer and the water column [5]. A consortium of sulfate-reducing

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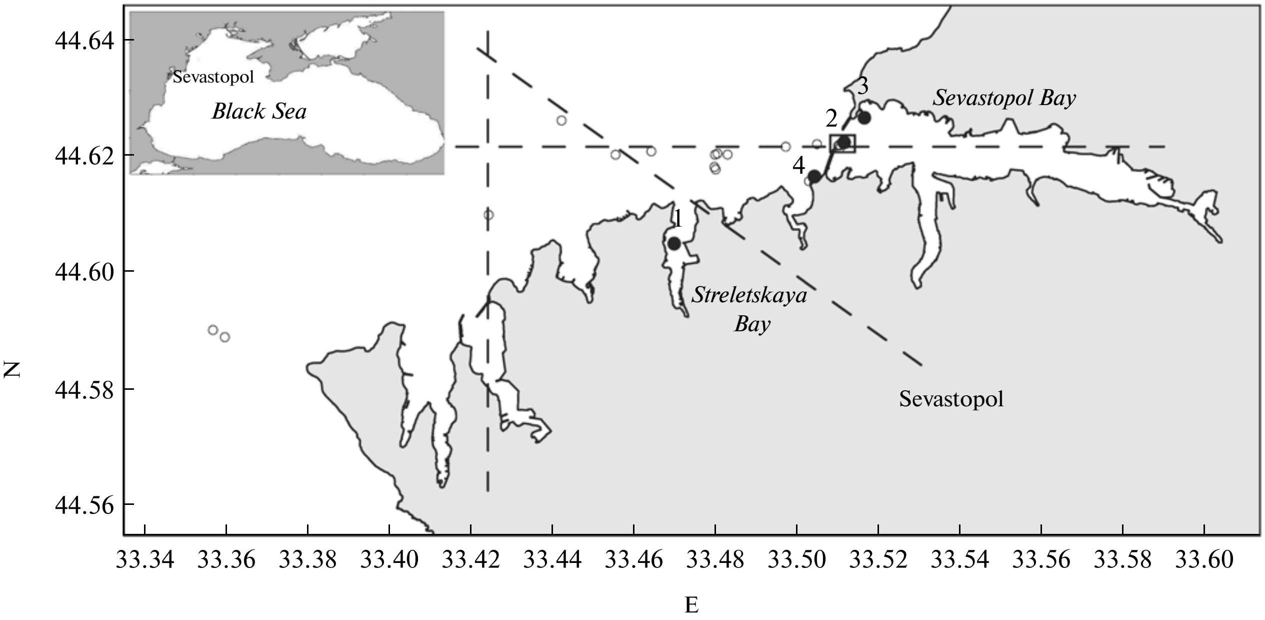

Fig. 1. Locations of methane seeps (○) in 1989–2007 and sampling stations in June 2012 (●). The rectangle indicates the area of bubble gas seepages observed in October 2011; geodynamic faults are marked by the dotted line.

bacteria and anaerobic methanotrophic archaea phylogenetically related to methanogens is responsible for methane oxidation (MO) in the anoxic zone of the sediment [6, 7]. Significant amounts of methane produced in the shallow sediments of the northwestern shelf were, however, shown to be released into the atmosphere, where they join the pool of greenhouse gases [5, 8].

Apart from anthropogenic flows, methane flares, which belong to cold methane seeps according to the geological classification, are also numerous at the northwestern shelf of the Black Sea. They occur at depths from several meters to 500 m and have a significant effect on the biogeochemical processes in the region [9]. Acoustic anomalies identified as gas seepage from the seafloor were found in the coastal zone of Sevastopol [10]. The team of the Kovalevsky Institute of Biology of the Southern Seas, NAS Ukraine detected 18 gas flare sites in the Sevastopol water area [11], both along the lines of geodynamic faults and outside them. Investigation of one of the sites of gas discharge (2011) revealed over 20 individual gas flares on a 500 m^2 area [12]. Radiocarbon dating of the methane initially yielded the age not exceeding 150 years, suggesting its biogenic origin. Recent microbiological and biogeochemical investigation of an area of flare gas seepage in the Sevastopol Bay showed that the anomalies in methane content in near-bottom water and upper sediments, together with enhanced activity of methanotrophic microorganisms, may indicate the zones of bottom discharge [12].

The goal of the present work was to investigate the microbial processes of methane oxidation and methanogenesis, as well as of sulfate reduction at the site of shallow-water methane seeps in the Sevastopol area and in the strongly contaminated Streletskaya Bay.

MATERIALS AND METHODS

Field research was carried out in early June 2012 from the *Antares* launch equipped with a mobile acoustic complex including a SeaCharter 480 DF echosounder with a GPS receiver, a hydroacoustic antenna, and a universal antenna mounting. The map of the sampling region is shown on Fig. 1. Results of the hydroacoustic measurements were analyzed using the SonarViewer V2.1.2 WaveLens software packages [13].

At stations 1 and 3 the samples were collected with a geological tubular corer, making it possible to retrieve up to 50 cm of the sediment core without disturbing its structure. At stations 2 and 4, the sediment samples were collected by divers. The hydrological parameters of the water (temperature, density, and salinity) were measured in situ using an SD204 CTD probe (SAIV A/S, Norway). Methane content in the near-bottom water and bottom sediments was determined by headspace analysis [14]. Methane concentrations in the gas phase were determined using a Kristall 2000 gas chromatograph (Russia) with a flame ionization detector. The experimental error did not exceed 5%.

Pore water was obtained by centrifugation of the sediments (8000 *g*, 10 min) on a TsUM-1 centrifuge

(Russia). Total alkalinity of pore water was determined by titration with the standard reagent kit (Merck, Germany). Sulfate in pore water was determined on a Staier ion chromatograph (Russia).

C_{org} content in the sediments was determined on a TOC-Vcph analyzer (Shimadzu, Japan) with an SSM-5000A add-on.

The rates of microbial processes were determined by the radioisotope method. Immediately after heaving the geological corer, the sediment (3 mL) from a relevant horizon was transferred into a cut-off plastic syringe (5 mL) and sealed with a gas-tight butyl rubber stopper. The labeled substrate (0.2 mL) was injected through the stopper, and the sample was incubated for 24–48 h at 12°C. The incubation temperature was the same as the temperature of near-bottom water at the time of sampling measured by the CTD probe. After incubation, the samples were fixed with 1 mL of 2 N KOH and transported to the laboratory. Subsequent treatment of the samples was carried out as described in [15]. The rate of methane oxidation (MO) was determined with ^{14}C (1 μCi per sample) methane dissolved in degassed distilled water. The rate of sulfate reduction (SR) was determined with ^{35}S sulfate (10 μCi per sample). The rates of methanogenesis were determined with ^{14}C bicarbonate or methyl-labeled (10 μCi per sample each). The alkali-fixed samples incubated in a refrigerator for 6 h prior to the injection of the labeled substrate were used as the controls.

For mass spectral carbon analysis ($\delta^{13}C$), methane was collected as follows. A glass vial (250 mL) was half-filled with the sediment, filled with saturated salt solution 230 mL, sealed hermetically with a rubber stopper, and shaken vigorously. In the laboratory, the gas phase was collected with a syringe (by substitution with the salt solution) and stored in a salt trap. The $\delta^{13}C$ value of methane was measured on a TRACE GC gas chromatograph (Germany) coupled with a Delta plus mass spectrometer (Germany).

Mass spectral determination of $\delta^{13}C$ in pore water carbonates was carried out with carbon dioxide (CO_2) as the working gas as described earlier [16]. The error of $\delta^{13}C$ measurements did not exceed ±0.1‰.

RESULTS AND DISCUSSION

Hydroacoustic mapping of the bottom of the Sevastopol Bay in October 2011 revealed over 20 gas flares on the area of ~600 m^2 (Fig. 1) [12]. No gas seepages were revealed in June 2012 (station 2, Fig. 1), although the temperature and salinity of the water were almost the same as in 2011 [12]. No bubble discharge of the gas was observed at station 1 in the strongly contaminated Streletskaya Bay and at the background station 3. In the Martynov Bay (station 4), gas bubbles rising from the bottom sediment to the surface were observed near the radiobiological building of the Institute of Biology of the Southern Seas.

Coordinates and depths of the sampling stations, as well as the physicochemical parameters of the sediments, are listed in Table 1. At station 2, the upper 2 cm of the core consisted of brownish aleuro-pelitic oxidized or weakly reduced silt. Reduced aleuro-pelitic silts with a strong smell of hydrogen sulfide were located below. Mollusk shell fragments were present from 10 cm on, their number increasing with depth. The absence of shell fragments in the upper sediment horizons indicated a significantly increased anthropogenic load in this region which prevented the normal development of benthic communities. Since the sedimentation rate in the Streletskaya Bay is about 3.5 mm per year [17], the mollusks disappeared from the surface of the sediment 30–35 years ago.

The profiles of methane and sulfate, as well as of the total alkalinity in the pore water of station 1, were typical of the organic-rich coastal silts (Table 1). The highest content of dissolved methane (1640–1840 μmol/dm^3) was revealed in the 6–16 cm layer. The sulfate–methane transition zone (SMTZ) was located at 4–5 cm (Fig. 2a). While methane content in the sediments in June 2012 was considerably higher than in October 2011, the SMTZ was located in the same sediment horizon [12].

The physicochemical and lithological characteristics of the sediments at station 2, where the fields of gas seepages were previously observed, exhibited no anomalies indicating methane seeps in the vicinity of the station at the time of sampling. The upper 4 cm of the sediments were weakly oxidized yellowish–brown clayey aleurites with an admixture of sand. Below 4 cm, grayish–black reduced aleuric sediments with sandy and shell rock fractions were located. Methane content in the sediments increased with depth from 10–12 cm on, although it was significantly lower than in the Streletskaya Bay. Methane concentration in the sediments of this station was almost two times higher than in 2011, varying from 0.98 to 12.5 μmol/dm^3. Sulfate content in pore waters did not change significantly in the upper 10 cm and then decreased to 5.1 mM in the 36–40 cm horizon. In the sediments of this station, SMTZ was located at the depth of 36–40 cm, i.e., significantly lower than in the Streletskaya Bay (Fig. 2b). Unlike the 2011 results [12], no subsurface peaks of methane concentration and methane oxidation rate, indicative of gas seepages, were found Figs. 3a, 3b).

The sediments of the background station 3, located outside the methane seep field, contained more sand than those of station 2. The watered layer of silty sand at station 3 had positive Eh and the levels of methane and sulfates close to those of the near-bottom water.

The whole core (10 cm) of the sediments from the shallow station 4, located at the gas seep site in the Martynov Bay, consisted of reduced black, sulfide-rich

Table 1. Physicochemical characterization of the bottom sediments of the Sevastopol basin

Station no/depth, m	Horizon, cm	Eh, mV	CH_4, µmol/dm^3 dry silt	C_{org} content, %	Content in poer waster	
					Alk, mM	SO_4^{2-}, mM
St. 1, Streletskaya Bay/17 44°36.166 N 33°28.120 E	0–2	60	7.89	5.46	5.5	16.4
	2–4	–160	46.9	4.97	14.0	13.1
	4–6	–161	1041	4.45	20.0	3.06
	6–8	–167	1840	4.02	22.5	1.01
	8–12	–136	1640	4.24	26.5	0.86
	12–16	–73	1650	4.13	31.0	0.75
	16–20	30	1270	3.90	30.0	0.25
St. 2, gas seepage area in the Sevastopol Bay/18 44°37.335 N 33°30.666 E	0–2	23	1.41	2.15	5.0	19.0
	2–4	30	0.98	1.77	7.5	18.9
	4–8	–114	1.24	2.35	6.0	16.5
	8–10	–64	1.14	1.88	6.0	17.3
	10–12	–98	1.53	1.96	7.0	13.9
	13–17	–130	2.23	2.03	8.5	13.3
	25–27	–134	5.68	1.42	16.0	9.46
	36–40	–158	12.5	1.69	29.0	5.10
St. 3, background station in the Sevastopol Bay/7 44°37.530 N 33°31.137 E	0–5	11	17.2	2.17	5.5	20.0
	6–12	60	3.30	1.59	5.0	19.8
St. 4, Martynov Bay, zone of gas seepage/4 44°36.944 N 33°30.180 E	0–5	–158	520	7.53	27.0	8.20
	5–10	–255	234	4.89	25.5	5.65

pelitic silts with a noticeable admixture of terrigenous material. Methane content was high, reaching 520 µmol/dm^3 in the uppermost horizon (0–5 cm). Decreased sulfate concentration indicated active SR in these sediments.

The rates of microbial processes are presented in Table 2. The highest SR rates (>100 µmol/(dm^3 day)) were found at station 4, as well as the highest MO rates (>100 µmol/(dm^3 day)).

Significant differences between the profiles of MO and SR rates (Figs. 3, 4) in the sediments of the Streletskaya (station 1) and Sevastopol (station 2) bays agreed with the results of gas–geochemical analysis of these silts. Higher C_{org} and CH_4 levels in the sediments of station 1 correlated with higher MO and SR rates than at station 2. In the sediments of Streletskaya Bay, the peak of SR rates was in the subsurface horizon close to SMTZ. The layer with the highest rate of anaerobic MO was somewhat deeper (6–8 cm), in reduced silts. The rates of both SR and MO decreased drastically below 14 cm. In the Sevastopol Bay sediments (station 2), where SMTZ was in the 35–40 cm horizon, the profile of SR rates also exhibited a maximum in the subsurface horizon (2–4 cm), while anaerobic MO, which is coupled to sulfate reduction, was detected much deeper, in the in SMTZ (35–40 cm) (Fig. 3, Table 2).

The measured rates of hydrogenotrophic and aceticlastic methanogenesis (MG) (Table 2, Fig. 5) indicate predominance of methanogenesis form acetate in most of the Sevastopol Bay sediments studied. Predominance of organotrophic (aceticlastic or methylotrophic) methanogenesis is relatively rare in marine sediments, occurring mainly in the highly productive coastal ecosystems subject to pronounced anthropogenic contamination or in the regions affected by upwelling and run-off of large rivers [5, 18]. Similar to SR and MO rates, the rate of methanogenesis was significantly higher in the sediments of Streletskaya Bay than in the silts of the Sevastopol Bay (station 2). At station 1, MG rate increased sharply in SMTZ, so that

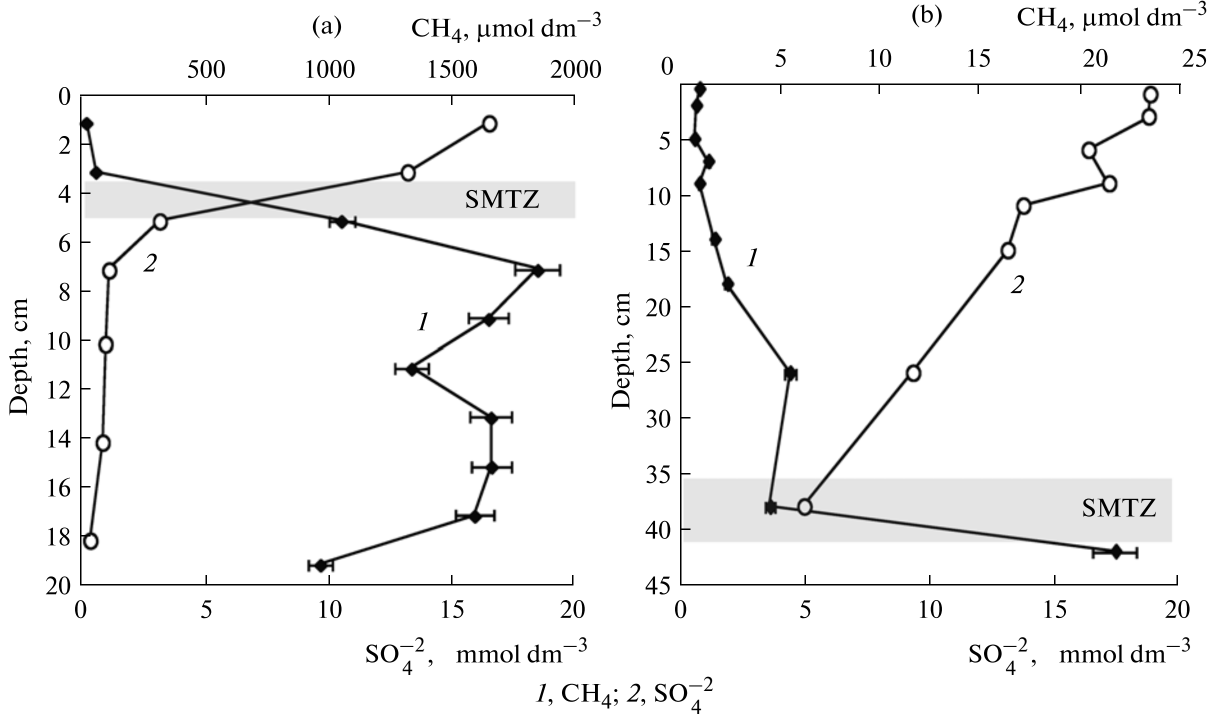

Fig. 2. Profiles of methane (*1*) and sulfate (*2*) content in the sediments of Streletskaya ((a), st. 1) and Sevastopol ((b), st. 2) bays. SMTZ is the sulfate–methane transition zone.

it varied from 311 to 413 nmol/(dm^3 day) at the depth of 6 cm. In the Sevastopol Bay sediments, MG increased gradually to 51 nmol/(dm^3 day) in SMTZ (36–40 cm horizon).

Comparison of integral values of methane concentrations and the rates of microbial processes in 2011 and 2012 (Table 3) showed that the rates of microbial processes in coastal silts depended mainly on their anthropogenic contamination. OM-enriched sediments of the Streletskaya and Martynov bays had higher levels of methane and high rates of microbial processes. Integral rates of SR and MO in the silts of Streletskaya Bay were considerably higher in early summer 2012 than in autumn 2011. This may be due to decreased activity of the sediment microbial community in the cold winter period, resulting in a more pronounced oxic zone in the sediments and in penetration of sulfate ions (a prerequisite for SR) into deeper silt horizons. Warming up of the water in spring and additional OM inflow during the spring “bloom” of the phytoplankton provided for the activation of the microbial community of the sediments (both the primary degraders and sulfate-reducing bacteria responsible for the terminal phase of OM decomposition). We have previously reported a similar increase in microbial activity in spring for OM-enriched coastal sediments of the Danube and Dnieper estuaries [19]. The integral MG rate in autumn 2011 was, however, more than two times higher than in early June 2012 (Table 3). Sulfate reduction in the sediments during spring and summer results in active sulfate consumption, which may cause a decrease in SR rates due to sulfate depletion in pore waters. Decreased SR rates are accompanied by the activation of methanogenic archaea. These organisms, similar to sulfate-reducing bacteria, carry out the terminal phase of OM decomposition, although they compete for the substrate less efficiently. In the Sevastopol Bay sediments, where sulfate concentration did not decrease drastically with depth, the rate of methanogenesis was considerably lower, while integral SR rates for 2011 and 2012 were almost the same.

Time scale of the biogeochemical processes is one of the important characteristics of diagenesis of the sediments. Methane content in a given sediment horizon at a given time is the result of the equilibrium between methane inflow and consumption. Therefore the ratio between methane content in the sediments and the rates of its inflow or consumption characterizes the time scale of methane turnover. The calculated ratios of methane concentrations to the rates of its oxidation in the sediment cores of the Sevastopol Bay, characterizing the duration of this process, are presented on Fig. 6. It can be seen that the seasonal (3 months) scale of methane oxidation was observed in the upper sediment horizons of all three stations. This scale increased to two years in the 10–25 cm layers corresponding to 30–100 years of the sediment age [20], while in 150-year old horizon of station 2 (40 cm)

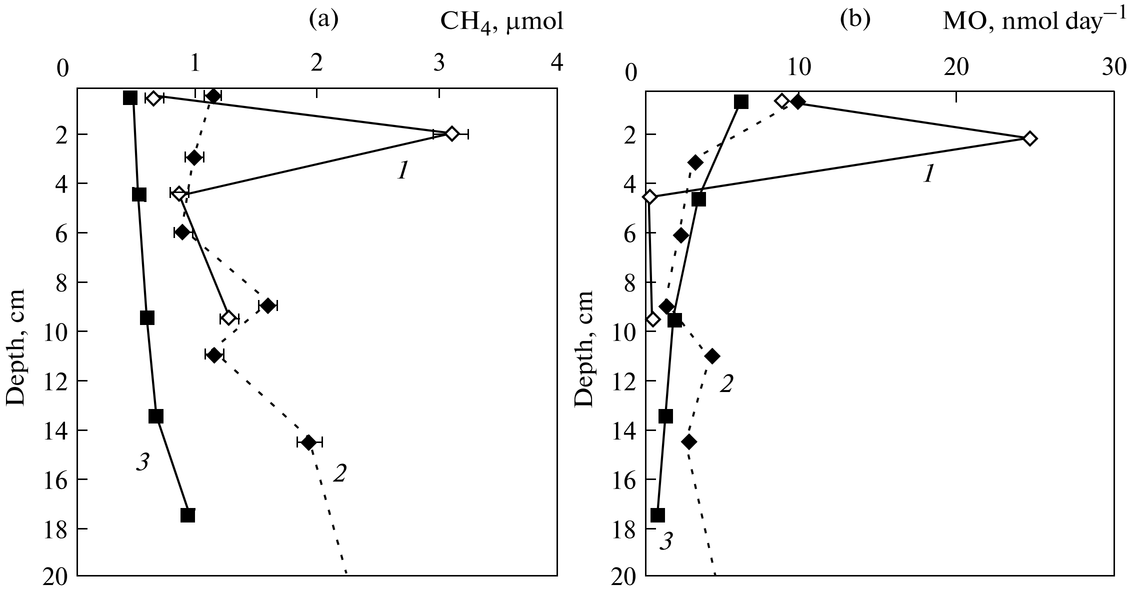

Fig. 3. Profiles of methane content (a) and methane oxidation rates (b) in the sediments of the seep area during the active (October 2011) (*1*) and inactive phase (June 2012) (*2*) and at the background station (October 2011) (*3*) located less than 1 km from the seepage area.

at the site of the methane seeps active in 2011, the rates of biogeochemical processes increased relatively to the seasonal time scale. These data indicate higher biogeochemical activity of methane oxidation in the surface layers of the Sevastopol Bay sediments than in the lower layers.

Activity of hydrogenotrophic methanogenic archaea in reduced sediments is known to result in enrichment of the pore water carbonates with the heavy ^{13}C isotope, while the light isotope ^{12}C is preferably used for methanogenesis [16, 21, 22]. Although aceticlastic methanogenesis prevailed in the sediments

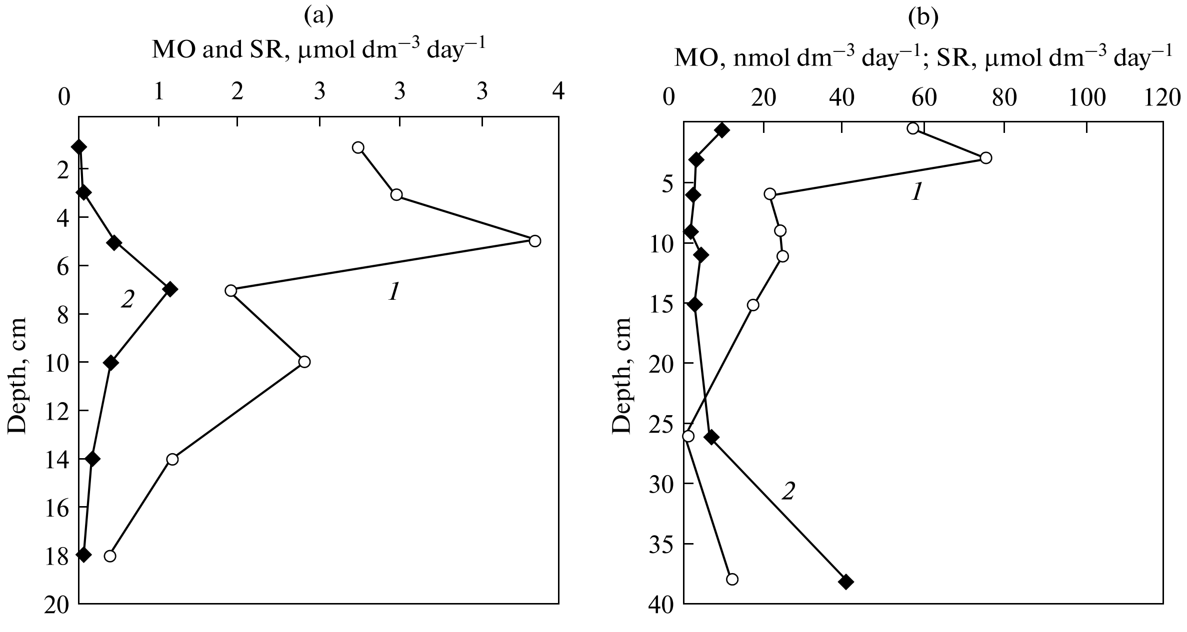

Fig. 4. Profiles of the rates of sulfate reduction (*1*) and methane oxidation (*2*) in the sediments of Streletskaya ((a), st. 1) and Sevastopol ((b), st. 2) bays.

Table 2. Rates of microbial methane oxidation (MO), methanogenesis (MG), and sulfate reduction (SR) in the bottom sediments of the Sevastopol basin

Station no.	Horizon, cm	Rates of microbial processes		
		MO, nmol/(dm^3 day)	MG*, nmol/(dm^3 day)	SR, μmol/(dm^3 day)
St. 1	0–2	81.7	6.42(1.85)	68.7
	2–4	984	12.8(16.2)	78.6
	4–6	8670	13.0(186)	113
	6–8	22690	104(303)	37.4
	8–12	7937	95.3(318)	56.2
	12–16	3228	43.1(268)	23.2
	16–20	904	55.8(352)	7.6
St. 2	0–1	9.87	0.67(0)	56.4
	2–4	3.40	3.33(0.59)	75.2
	4–8	2.47	3.17(4.70)	21.5
	8–10	1.60	2.00(15.9)	23.9
	10–12	4.37	0(10.3)	24.5
	13–17	2.86	1.13(26.6)	17.5
	25–27	6.29	0.44(46.8)	0.58
	36–40	40.1	0.32(51.0)	11.8
St. 3	0–5	13.3	3.38	20.1
	6–12	2.10	0.05(0)	0
St. 4	0–5	154120	86.3(0.92)	417
	5–10	101460	52.4(665)	146

* Rates of methanogenesis by hydrogenotrophic and aceticlastic (in parentheses) methanogens; 0 indicates that the rate was not reliably determined (<0.1 μmol/(dm^3 day) for sulfate reduction and <0.01 nmol/(dm^3 day) for methanogenesis).

Table 3. Integral rates of microbial processes calculated for the upper 15 cm of the bottom sediments of the Sevastopol basin

Station no. and location	CH_4 content, mmol/dm^3	CH_4-oxidation, μmol/(m^2 day)	CH_4-production, μmol/(m^2 day)	Sulfate reduction, μmol/(m^2 day)
St. 1, Streletskaya Bay	167 (88*)	1062 (721*)	38.5 (78.9)	8901 (6220)
St. 2, gas seepage area in the Sevastopol Bay	0.20 (0.21)	0.54 (0.65)	1.89 (0.03)	4460 (4074)
St. 3, background station in the Sevastopol Bay	0.37 (0.1)	0.49 (0.45)	0.02 (0.08)	0.230 (2400)
St. 4, Martynov Bay, zone of gas seepage	38.7**	205	33.2	137200

* The rates of microbial processes measured at the same sites in October 2011 are given in parentheses.
** In Martynov Bay, integral values were calculated for the upper 10 cm of the sediment.

of the Sevastopol bays, the carbon isotopic composition of pore water carbonates became heavier with depth (Table 4), reaching 1.8‰ at station 1 in the 16–20 cm horizon, while at station 2 the $\delta^{13}C$ value of the carbonates in the lower part of the core (36–40 cm horizon) was 2.64‰.

The isotopic composition ($\delta^{13}C$) of methane in the sediments varied from –71.5 to –75.8‰ (Table 4), confirming its biogenic origin by microbial methanogenesis. These results are in agreement with the date obtained previously for this region [23]. The subsurface horizon of station 1, where the isotopic composi-

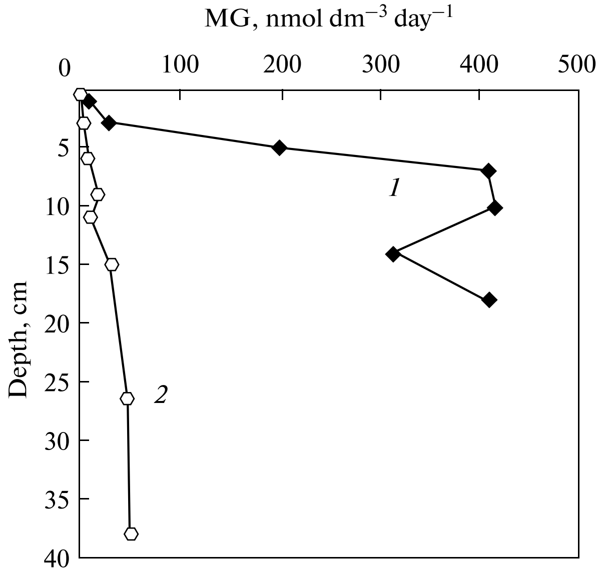

Fig. 5. Profiles of the rates of methanogenesis in the sediments of Streletskaya (*1*, st. 1) and Sevastopol (*2*, st. 2) bays.

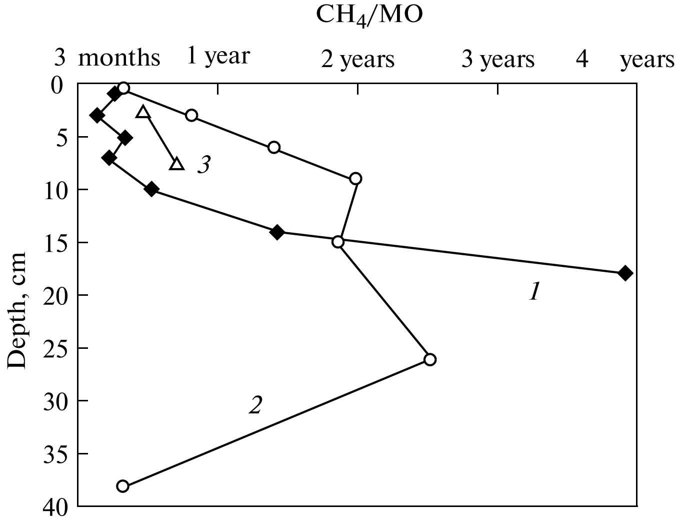

Fig. 6. Time scale for methane oxidation in the bottom sediment cores from the Sevastopol basin: st. 1 (*1*), st. 2 (*2*), and st. 4 (*3*).

tion of methane was –59.9‰, was exceptional. Such heavier methane isotopic composition in subsurface horizons near SMTZ has been described in the literature and results probably from the activity of methanotrophic archaea, which carry out anaerobic methane oxidation accompanied by considerable fractioning of CH_4 carbon isotopes [22, 24].

The isotopic composition of bubble methane collected from the seep in Martynov Bay in 2011 and 2012 was unexpected (Table 4). Compared to the freshly formed methane from the surface sediments, the bubble methane carbon was noticeably enriched with the heavy isotope ^{13}C. The values of –60.8 and –63.8‰ were close to the isotopic composition of CH_4 from the methane seeps of the Black Sea northwestern shelf [25]. Most researchers consider the Black Sea methane to originate by microbial methanogenesis in the 3500–7000 years old clay–sapropelite sediments [19].

Table 4. Carbon isotopic composition (δ^{13}C) for the methane of the bubble gas from the seeps, dissolved CH_4 of the bottom sediments, and pore water carbonates (C_{carb}) in the Sevastopol basin

Station no. and location	Horizon, cm	(δ^{13}C), ‰		
		CH_4, bubbles	CH_4, bottom sediments	C_{carb}
St. 1, Streletskaya Bay	0–4			–10.3
	4–10		–59.9	–8.80
	10–15		–75.8	–0.53
	16–20			1.81
St. 2, gas seepage area in the Sevastopol Bay	0–2			–7.45
	2–10			–5.85
	10–12			–3.96
	13–27		–73.2	0.28
	36–40			2.64
St. 4, Martynov Bay, zone of gas seepage	0–10	–63.,8; –60.8*	–71.5	–12.4

* Gas collected June 6, 2011.

Thus, association between methane seeps in some Sevastopol bays and methane migration from deep geological structures cannot be ruled out. The variability of gas phenomena observed in the Martynov and Sevastopol bays and especially of the geological structure of the sea floor may indicate the presence of gas-filled cavities within the sediments of these regions. Increased pressure in these cavities may result in emission of gas flares to the surface. This suggestion is supported by the radiocarbon dating of methane from the seeps of the Sevastopol bays, which does not exceed 100–150 years [11].

Summarizing the results of two-year investigation of microbial processes in the sediments of the Sevastopol Black Sea area, it may be concluded that gas seepages have an insignificant effect on the rates of the key microbial processes in the upper sediment layers. Similar to most marine environments, SR is the major terminal phase of OM decomposition in reduced sediments. The rate of this process depends on the amount of OM available. The rate of methanogenesis increases with depth and peaks in the subsurface horizons, where decreased sulfate concentration in pore waters was observed. The highest rates of sulfate-dependent anaerobic methane oxidation were found close to SMTZ, which was also typical of most studied marine sediments [26]. Unlike 2011, no gas seepages were found in the same region of the Sevastopol Bay, indicating the periodicity of this phenomenon. Complete absence of the anomalies in methane distribution and the rates of methane oxidation revealed in 2012, in contrast to 2011 data [12] suggests that these parameters may be used for the search of active underwater gas phenomena. Comparison of the results on the isotopic composition of bubble methane from the seep and dissolved CH_4 from the bottom sediments, as well as of the rates of microbial methanogenesis and methane oxidation, indicates that the functioning of methane seeps is probably associated with accumulation of methane of microbial origin within the cavities of the underlying structures and its periodical release into the water column as bubble seepages.

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