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EXPERIMENTAL ARTICLES

Microbiological and Biogeochemical Processes in a Pockmark of the Gdansk Depression, Baltic Sea

N. V. Pimenov[a,1], M. O. Ul'yanova[b], T. A. Kanapatskii[a], V. V. Sivkov[b], and M. V. Ivanov[a]

[a] *Winogradsky Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117312 Russia*

[b] *Shirshov Institute of Oceanology, Russian Academy of Sciences, Atlantic Division, Kaliningrad, Russia*

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Abstract—Comprehensive microbiological and biogeochemical investigation of a pockmark within one of the sites of gas-saturated sediments in the Gdansk depression, Baltic Sea was carried out during the 87th voyage of the *Professor Shtokman* research vessel. Methane content in the near-bottom water and in the underlying sediments indicates stable methane flow from the sediment into the water. In the 10-m water layer above the pockmark, apart from methane anomalies, elevated numbers of microorganisms and enhanced rates of dark CO_2 fixation (up to 1.15 μmol C/(l day)) and methane oxidation (up to 2.14 nmol CH_4/(l day)) were revealed. Lightened isotopic composition of suspended organic matter also indicates high activity of the near-bottom microbial community. Compared to the background stations, methane content in pockmark sediments increased sharply from the surface to 40–60 ml/dm^3 in the 20–30 cm horizon. High rates of bacterial sulfate reduction (SR) were detected throughout the core (0–40 cm); the maximum of 74 μmol S/(dm^3 day) was located in subsurface horizons (15–20 cm). The highest rates of anaerobic methane oxidation (AMO), up to 80 μmol/(dm^3 day), were detected in the same horizon. Good coincidence of the AMO and SR profiles with stoichiometry close to 1 : 1 is evidence in favor of a close relation between these processes performed by a consortium of methanotrophic archaea and sulfate-reducing bacteria. Methane isotopic composition in subsurface sediments of the pockmark (from –53.0 to –56.5‰) does not rule out the presence of methane other than the biogenic methane from the deep horizons of the sedimentary cover.

Key words: pockmark, sulfate reduction, anaerobic methane oxidation, Baltic Sea.

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In the last 20 years, numerous gas seeps, with methane as the main component, have been found at the bottom of seas and continental fringes of the oceans. Along with the geophysical and geochemical anomalies of the sediment cross-section, pockmarks, characteristic features of the microrelief (craterlike depressions in the bottom surface) are good indicators of hydrocarbon seeps [1].

Gas-saturated bottom sediments and near-bottom water layers in the sites of hydrocarbon seep discharge exhibit high activity of methane-oxidizing microorganisms that utilize methane for energetic and constructive metabolism under both aerobic and anaerobic conditions [2–4]. Intense microbial CO_2 production from methane with subsequent precipitation of authigenic carbonates (methane derived carbonates) often results in massive carbonate formations in the vicinity of methane seeps [5, 6].

The anomalies in the distribution of hydrocarbon gases in the near-bottom waters of the Baltic Sea were first observed by the workers of the Shirshov Institute of Oceanology, Russian Academy of Sciences in the early 1970s [7]. Geophysical and geological investigations by a series of expeditions to the Baltic Sea revealed that elevated methane content in near-bottom waters resulted from discharge of gas-containing fluids localized at specific geomorphologic sites (craters, depressions, etc.) Methane craters with characteristic gas-saturated sediments and elevated methane content of the near-bottom water were subsequently revealed in various Baltic Sea locations within the Gdansk, Arkona, and Gotland depressions [8, 9].

Although extensive material concerning the broad distribution of methane seep in the Baltic Sea was accumulated, investigation of the activity of microbial communities of the pockmark zones and geoacoustic anomalies has not been carried out.

The goal of the present work was therefore to perform comparative microbiological and biogeochemical studies of microbial activity in the bottom sediments and near-bottom water layers inside and outside the pockmark zone in the Russian sector of the Gdansk depression.

[1] Corresponding author; e-mail: npimenov@mail.ru

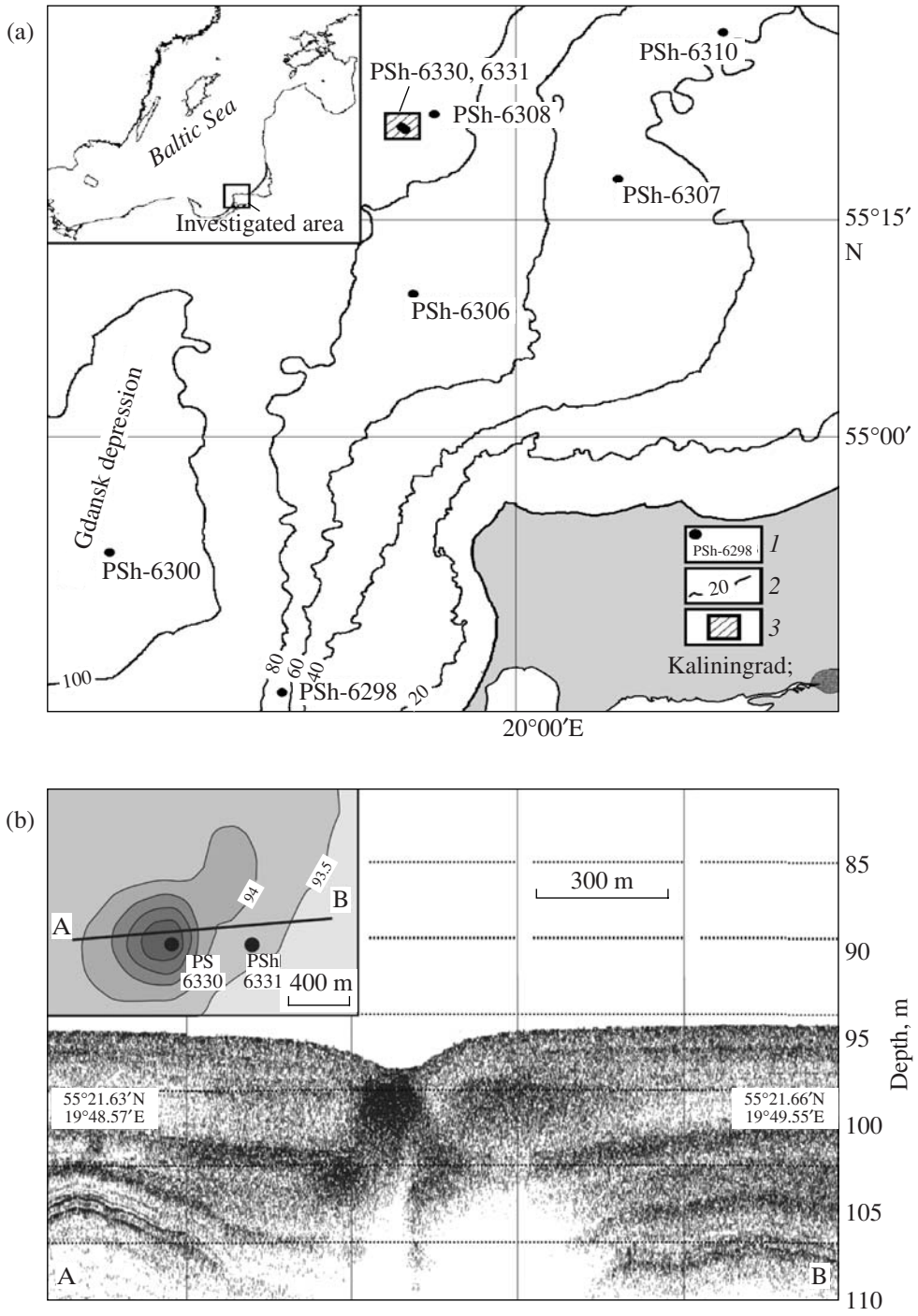

Fig. 1. a, location of the sampling stations of the 87th voyage of the *Professor Shtokman* research vessel (Gdansk basin of the Baltic Sea, Russian sector): sampling stations (*1*); isobathics, m (*2*); site of gas-saturated sediments (pockmark) (*3*); b, geoacoustic profile (A–B) of the pockmark (the depths given were not corrected for the sound speed in seawater). The insert represents the shape and horizontal size of the pockmark and the location of the geoacoustic profile (the data were obtained using a EA 400SP echo sounder during the 90th voyage of the *Professor Shtokman* research vessel in October 2007).

MATERIALS AND METHODS

Investigation of water and bottom sediments was carried out during the 87th voyage of the *Professor Shtokman* research vessel in the Russian sector of the Gdansk basin (Fig. 1a). Our choice of the pockmark for investigation was based on the data of the 44th voyage of the *Akademik Kurchatov* research vessel [8].

Water samples were collected with 1.7- and 5-l plastic Niskin bathometers positioned on a Rosetta hydrological sampling complex. Undisturbed samples of

water and sediments at the water–bottom interface were collected with a Niemiste hermetic corer. An Okean-50 grab was also used for sediment sampling.

For determination of microbial numbers, water samples were fixed with formaldehyde solution (to a final concentration of 3–4%), incubated for 5–10 min, and filtered through 0.2-μm black polycarbonate membrane filters (Osmonics, United States). The samples of bottom sediments were fixed with glutaraldehyde (0.5 ml 25% GA per 5 ml sediment) and transported to the laboratory. The cells were then desorbed from the particles by 2–10 s of sonication in an UZDN-2T device (22 kHz) in pulse mode at 0.015 A. Water solutions of DAPI or acridine orange were used as fluorochromes [10]. Bacterial cells were counted under a LUMAM-3 fluorescence microscope (LOMO, Russia).

The rates of microbial processes were determined by the radioisotope method with ^{14}C and ^{35}S substrates. Immediately after hauling on board the vessel, water samples were dispensed into 30-ml glass bottles and sealed hermetically without air bubbles using rubber stoppers. Sediment samples (3 ml) were placed into cut-off 5-ml plastic syringes; the open end was then sealed with a gas-tight butyl rubber stopper. Labeled substrates (0.2 ml) were injected through the stopper, and the samples were incubated in a refrigerator at 1–3°C for 24–48 h. The samples were then fixed with 0.5 ml of 2M KOH and transported to the laboratory, where they were treated according to a previously described procedure [11]. Methane oxidation rate was determined with ^{14}C methane dissolved in degassed distilled water. The amount of ^{14}C methane per sample was 1 μCi. Sulfate reduction rates were determined with ^{35}S sulfate (10 μCi/sample); methanogenesis rates were determined with ^{14}C bicarbonate (10 μCi/sample) and methyl-labeled ^{14}C acetate (10 μCi/sample); dark CO_2 assimilation was determined with ^{14}C bicarbonate (10 μCi/sample). The samples fixed with alkali and stored in a refrigerator for 6 h prior to injection of the labeled substrates were used as controls.

Silt water was obtained by centrifugation of the sediments. Alkalinity and sulfide content were measured with the standard reagent kits (Merck, Germany); oxygen was determined by the Winkler method. Sulfate content was determined by ion chromatography. Methane was determined with a Kristall 2000 gas chromatograph (Russia) with a flame ionization detector.

The redox potential was determined with a pH 320/Set-1 field potentiometer (WTW, Germany).

Suspended matter samples were obtained by filtering water samples (up to 5 l) through 47-mm GF/F glass fiber filters (Whatman). Filtration was carried out on board the vessel immediately after sampling. The samples for mass spectrometry were prepared according to the procedure described in [12]. In order to determine $\delta^{13}C$ of organic carbon in sediments, carbonates were removed with 8% HCl. Sediment samples were then placed in quartz ampoules with CuO and incubated in a muffler at 500°C for 18 h. Carbon dioxide was collected under vacuum and purified in liquid nitrogen. The $\delta^{13}C$ values were determined by mass spectrometry. To determine the isotopic composition of carbonate mineral carbon, silt water was acidified with 8% HCl and carbon dioxide was collected [13]. The isotopic compositions of suspended organic carbon ($\delta^{13}C$) and of carbonate mineral carbon were carried out using a Delta plus isotope mass spectrometer (Thermo Electron Corp., Germany) with laboratory standards calibrated against the PDB carbon standard.

The isotopic composition of methane ($\delta^{13}C$) was determined on a gas chromatograph (Thermo Electron Corp., Germany) coupled with a Delta plus mass spectrometer.

RESULTS

In the pockmark under investigation, the local depth increase was 1–3 m; its horizontal size was 800×400 m (Fig. 1b). The sediments consisted of dark to black aleuric–pelitic and pelitic silts.

Although free sulfide was not detected in the water column, a noticeable oxygen deficiency characteristic of summer time (Table 1) was observed below the halocline (65–75 m) of the relatively deep stations.

At stations PSh-6308, 6300, and 6298 with low O_2 content, elevated methane concentrations were found in the near-bottom water. At the gas site stations (PSh-6330, 6331), the lowest concentrations of oxygen (0.15–0.20 mg/l) and the highest concentrations of methane (8.9–10.7 μl/l) were observed.

A more detailed analysis of methane content in the near-bottom water above the pockmark (Fig. 2a) revealed that elevated methane concentrations could be traced 2–5 m from the bottom.

No oxidized layer was found in the surface (0–5 cm) layer of aleuric–pelitic sediments of stations PSh-6308, 6300, 6330, and 6331. Even in the upper 0–0.5 cm layer, Eh values were negative, from –20 to –50 mV. In more shallow sediments of stations PSh6310, 6306, and 6307, the upper 1–5 cm were oxidized with positive Eh values of 100 to 200 mV. The sediments with more reduced surface had higher methane content (Table 2).

Microbial numbers in the near-bottom water and in the upper sediment layer are presented in Tables 1 and 2. Similar to methane distribution, the highest microbial numbers were observed at stations PSh-6308 and 6300 and in the water and upper sediments of the pockmark.

For more detailed study of the rates of microbial processes at the water–bottom interface, radioisotope measurements of dark CO_2 fixation, sulfate reduction, methanogenesis, and methane oxidation were carried out at two gas site stations and at the deepest station PSh-6300 (Table 3). The rates of dark CO_2 fixation and methane oxidation in the near-bottom water of the pockmark site stations were significantly higher than at

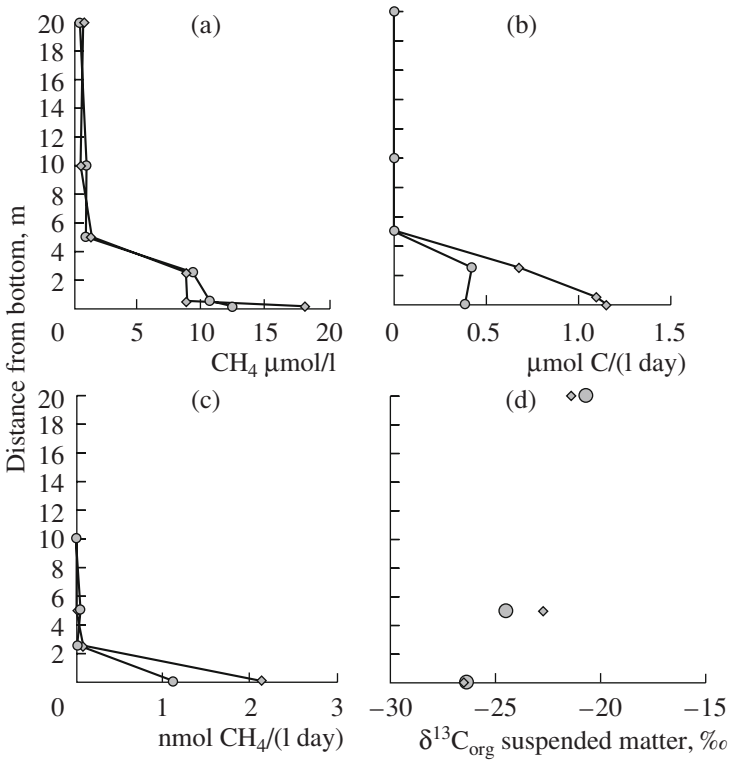

Fig. 2. Methane content (a), rate of dark CO_2 assimilation (b), methane oxidation rate (c), and carbon isotopic composition ($\delta^{13}C$) of suspended organic matter (d) in the near-bottom water above the pockmark. ● station PSh-6330; ◆, station PSh-6331.

station PSh-6300. The rates of microbial processes decreased with distance from the bottom (Figs. 2b, 2c); 10 m from the bottom they did not exceed the background values typical of the deep zone of the Gdansk depression.

Measurements of the isotopic composition of suspended organic matter ($\delta^{13}C_{org}$) are more evidence of enhanced microbial activity in the water column above the pockmark. Close to the bottom, in the zone of the highest rates of methane oxidation and dark CO_2 fixation, suspended organic carbon was enriched with the light isotope ^{12}C (Fig. 2d).

Apart from the rates of methane oxidation and dark CO_2 fixation, the rates of anaerobic processes, methanogenesis and sulfate reduction, were determined in the near-bottom water collected directly from the Niemiste core (Table 3). Anaerobic microbial processes were found to activate under conditions of low oxygen content in the near-bottom water. Only sulfate reduction was revealed at station PSh-6300, while both sulfate-reducing and methanogenic microorganisms were active in the water column above the pockmark.

The rates of microbial processes in the upper sediment layers (Table 3) were relatively high, typical of organic-rich sediments of highly productive marine environments [14, 15]. In the sediments of stations PSh-6300 and 6330, the rates of microbial processes were relatively close and varied within an order of magnitude. At station PSh-6331 located at the periphery of the pockmark, significant differences were observed. The rates of anaerobic methane oxidation in the 10–25 cm horizon were there more than an order of magnitude higher than the peak values for this process measured at other stations (Table 3). Along with methane oxidation, enhanced sulfate reduction rates were observed in the same horizons of station PSh-6331 with a peak of 74.4 μmol S/(dm^3 day) in the 15–20 cm horizon. The measured rates of microbial processes comply with the data on methane and sulfate content in the sediments. At station PSh-6331, methane content in the 10–25 cm layer varied from 863 to 2888 μmol/dm^3, while methane concentrations at stations PSh-6330 and 6300 varied from 59 to 179 μmol/dm^3. Higher sulfate reduction rate at station PSh-6331 of the gas site resulted in more rapid sulfate consumption (Table 3).

Low rates of microbial methanogenesis obtained at all stations (Table 3) suggest that high methane content in the upper sediment layers is due to methane flow from deeper sediment layers. This methane may be either of microbial origin or chemically produced in deep sediment layers via catagenesis.

Carbon isotopic composition ($\delta^{13}C$–CH_4) was determined to elucidate the origin of methane. In the upper layers of pockmark sediments at stations PSh-6330 and 6331, carbon isotopic composition of methane was similar (–53.2 and –56.6‰).

Table 1. Distribution of methane, oxygen, and total microbial numbers in the near-bottom water of the Gdansk depression

Station no.	Depth, m	Geographical coordinates, N, E	CH_4, μl/l	O_2, mg/l*	Microbial numbers, 10^4 cells/ml
PSh-6310	44	55°28.21′ 20°21.01′	0.43	8.27	30
PSh-6308	90	55°09.99′ 19°50.08′	6.87	0.31	44
PSh-6306	68	55°10.00′ 19°50.07′	0.86	8.26	19
PSh-6307	46	55°18.00′ 20°10.41′	0.41	9.36	20
PSh-6298	68	54°42.23′ 19°36.95′	2.20	3.51	–
PSh-6300	106	54°51.99′ 19°19.19′	1.46	0.51	39
PSh-6330	95	55°21.64′ 19°48.87′	10.7	0.15	70
PSh-6331	93	55°21.55′ 19°49.11′	8.9	0.20	63

* Oxygen content was determined by N.E. Trenina (Atlantic Research Institute for Fishery and Oceanography).

Table 2. Redox potential, methane content, and microbial numbers in the upper sediments (0–5 cm) of the Gdansk depression

Station no.	Depth, m	Characteristics of the sediment	Eh, mV	CH_4, μl/dm^3	Microbial numbers, 10^8 cells/ml
PSh-6310	44	Coarse brown sand with inclusions (seldom occurring quartz grains), well sorted and washed	195	30	0.5
PSh-6308	90	Hydrated pelite, dark gray to black	–10	163	2.9
PSh-6306	68	Homogeneous brownish-gray aleuropelite	140	20	0.1
PSh-6307	46	Fine sand with numerous pebbles of sedimentary (chemogenic) and igneous rocks	210	16	–
PSh-6300	106	Aleuropelite, light gray to black, smelling of hydrogen sulfide	–20	375	3.1
PSh-6330	95	Aleuropelite, light gray to black, smelling of hydrogen sulfide	–40	617	7.4
PSh-6331	93	Aleuropelite, light gray to black, smelling of hydrogen sulfide	–50	408	5.0

High rates of anaerobic methane oxidation at station PSh-6331 coincided with formation of isotopically low CO_2 and thus with enrichment of mineral carbon with light ^{12}C (Table 4). In the sediments of station PSh-6331, $\delta^{13}C$ was the lowest (–8.4‰) in the zone of the highest rates of anaerobic methane oxidation. Liberation of isotopically light CO_2 may also be due to microbial decomposition of organic matter with the isotopic composition from –24.5 to 25.7‰ (Table 4). However, since the rates of dark CO_2 fixation (a measure of the net heterotrophic activity of the microbial community in the sediments) were similar in the sediments of these stations (Table 3), high rates of anaerobic methane oxidation were probably the main reason for the lightening of mineral carbon in station PSh-6331 silts.

DISCUSSION

The results of microbiological, biogeochemical, and isotope geochemical investigation demonstrate that the near-bottom water and surface sediments of the pockmark are in some respects different from other regions of the Gdansk basin deep zone. In line with the works of Soviet oceanologists [7–9], we revealed elevated

Table 3. Sulfate and methane content, alkalinity, and the rates of microbial processes in the near-bottom water and upper sediments of the Gdansk depression

Station no./depth, m / Sample	CH_4, μmol/dm^3	SO_4^{2-}, μmol/dm^3	Alk, μmol/dm^3	Rates of microbial processes			
				Dark CO_2 assimilation, μmol C/(dm^3 day)	CH_4 oxidation, μmol C/(dm^3 day)	CH_4 formation, μmol/(dm^3 day)*	Sulfate reduction, μmol S/(dm^3 day)
Psh-6300/107 / Near-bottom water	0.01	11.46	1.8	0.11	0.12×10^{-3}	HO	3.0
Bottom sediment							
0–5 cm	30	9.56	6.0	29.1	0.02	0.012	22.0
5–10 cm	59	6.80	15	14.6	0.29	0.021	12.1
10–20 cm	135	1.46	16	41.7	0.17	0.009	19.1
Pockmark							
Psh-6330/95 / Near-bottom water	0.48	11.35	1.8	0.39	1.12×10^{-3}	4.2×10^{-3}	4.0
Bottom sediment							
0–5	47	8.77	4	39.7	0.02	0.031	16.8
5–10	87	6.81	7.5	62.8	0.57	0.024	15.1
10–15	133	4.49	10	11.7	1.62	0.042	11.9
15–20	179	3.19	15	12.5	0.39	0.028	12.7
Psh-6331/93 / Near-bottom water	0.40	11.12	1.9	1.15	2.14×10^{-3}	5.6×10^{-3}	8.2
Bottom sediment							
0–5	31	8.52	5.0	39.7	0.27	0.031	14
5–10	280	5.06	11	47.5	4.61	0.035	26.8
10–15	863	2.00	14	2.14	42.6	0.037	45.6
15–20	2083	1.16	15	5.83	80.6	0.058	74.4
20–25	2888	0.75	16.5	7.67	32.0	0.159	19.7
35–40	2872	0.68	18	12.2	7.98	0.051	18.2

* Since the contribution of aceticlastic methanogenesis was below 1% in all cases, only the rates of the autotrophic process are listed in the table.

methane concentrations in the near-bottom water above the pockmark. The concentration anomalies were traced up to 2–5 m from the bottom. However, a certain, albeit less pronounced increase was observed in July at all the stations with depth exceeding 90 m, where oxygen limitation in the near-bottom waters was pronounced (Table 1). Along with the anomalies of methane concentration in the near-bottom water above the pockmark, increased values of microbial numbers were observed, as well as of dark CO_2 assimilation rate and methanogenesis rate. Enrichment of suspended organic matter with light carbon ^{12}C revealed for the near-bottom horizons of the pockmark station confirms the anomalies of microbial activity determined by the radiocarbon method and suggests the presence, apart from methanotrophs, of a community of sulfur-oxidizing bacteria which oxidize reduced sulfur compounds delivered from highly reduced surface sediments.

Table 4. Carbon isotopic composition ($\delta^{13}C$) of organic matter, carbonates, and methane in the pockmark upper sediments

Station	Horizon, cm	Carbon isotopic composition ($\delta^{13}C$), ‰		
		$\delta^{13}C_{org}$	$\delta^{13}C_{carb}$	$\delta^{13}C$–CH_4
PSh-6330	5–10	–25.5	–3.6	–
	10–20	–25.7	–1.9	–53.2
PSh-6331	5–10	–24.5	–2.6	–
	10–20	–25.1	–8.4	–56.6

Methane content, microbial numbers, and the rates of microbial processes were higher in the pockmark sediments than at station PSh-6300 located outside the pockmark zone (Table 5). However, the calculations of integral rates of microbial processes revealed that the

Table 5. Net rates of microbial processes calculated for the upper 20 cm of the Gdansk depression bottom sediments

Station no.	Dark CO_2 fixation, μmol/(m^2 day)	CH_4 oxidation, μmol/(m^2 day)	CH_4 formation, μmol/(m^2 day)	Sulfate reduction, μmol/(m^2 day)
PSh-6300	5355	24	2.1	2660
PSh-6330	6335	139	6.3	2825
PSh-6331	5759	6404	8.1	8040

rates of sulfate reduction and anaerobic methanogenesis (compared to station PSh-6300) were 5.8 and 1.1 times higher at station PSh-6330 (central part of the pockmark); in the sediments of the pockmark periphery (station PSh-6331) the difference was 267 and 3 times, respectively. Higher rates of microbial processes at station PSh-6331 comply with methane and sulfide concentrations in silt waters. In the sediments of this station deeper than 5 cm, still higher methane concentrations were observed, as well as more rapid sulfate consumption from silt waters (Table 3). Thus, our results indicate both the heterogeneity of the pockmark surface sediments and the activation of microbial processes in gas-saturated pockmark sediments.

According to modern concepts, anaerobic methane oxidation is performed by a consortium of methanotrophic archaea and sulfate-reducing bacteria [16, 17] and follows the equation $CH_4 + SO_4^{2-} \longrightarrow HCO_3^- + HS^- + H_2O$. The 1 : 1 stoichiometry of anaerobic methane oxidation was confirmed for gas-saturated marine sediments [18, 19] and for microbial accretions on coral-like formations of the Black Sea [20]. In the sediments of the Baltic pockmark, the curves of the rates of anaerobic methane oxidation and sulfate reduction in the upper sediments of station PSh-6331 were similar not only in profile, but also in their absolute values (Fig. 3). Analysis of the data on integral rates of sulfate reduction (Table 5) revealed that the typical amount of reduced sulfur compounds formed due to oxidation of organic matter arriving via the water column slightly exceeded 2 mmol S/(m^2 day). At station PSh-6331, where significantly higher methane content was observed, anaerobic methane oxidation contributed most significantly to formation of reduced sulfur compounds. Since the rate of microbial methanogenesis in the pockmark surface sediments was several orders of magnitude lower than the rate of anaerobic methane oxidation, high methane content in the pockmark sediments resulted from its influx from lower sediment layers. The isotopic composition of methane carbon (Table 4) gives no unequivocal indication as to its origin. Our measurements of $\delta^{13}C$–CH_4 at stations PSh-6330 and 6331 in the zone of highest rates of methane oxidation yielded values of –53.2 and –56.6‰, respectively. The carbon isotopic composition of biogenic methane is generally believed to vary from –55 to –80‰ or lighter [1]. Heavier methane composition (–48.5 to –55.5‰) was revealed in earlier investigations of the sediments of the Baltic Sea pockmarks [9]. It can therefore be suggested that, apart from modern methane of microbial origin, isotopically heavier methane is accumulated, deriving from the fluid flow deeper sediment layers. Heavier isotopic methane composition may also result from high rates of anaerobic methane oxidation revealed in the pockmark zone sediments (Table 3). Thus, the issue of the origin of methane in gas-saturated sediments of Baltic Sea pockmarks still requires further investigation. Sampling and analysis of deeper sediment layers is necessary for this purpose.

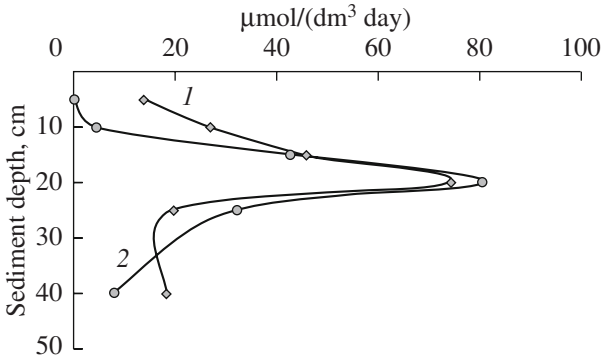

Fig. 3. Profiles of anaerobic methane oxidation (μmol CH_4/(dm^3 day)) and sulfate reduction (μmol S/(dm^3 day)): sulfate reduction (*1*); anaerobic methane oxidation (*2*).

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