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EXPERIMENTAL ARTICLES

Rates of Microbial Production and Oxidation of Methane in the Bottom Sediments and Water Column of the Black Sea

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Abstract—Rates of biogeochemical (microbial) processes of methane production and methane oxidation were determined in the bottom sediments and water column of the Black Sea. Aerobic bacterial oxidation of methane was confined to the upper 20–30 cm of Holocene bottom sediments of the shelf (0.7–259 ng C/(dm^3 day)) and to oxygenated waters (0.2–45 ng C/(dm^3 day)). In reduced sediments of the deep-sea zone and in the hydrogen sulfide–containing water column, considerable rates of anaerobic methane oxidation were recorded, comparable to or exceeding the rates of methane oxidation in oxygenated layers. From one-fourth to one-half of the methane formed in bottom sediments was oxidized immediately therein. The major part of the remaining methane was oxidized in the water column, and a smaller portion arrived in the atmosphere.

Key words: methane, intensities of production and oxidation, aerobic and anaerobic processes, the Black Sea.

Recognition of the role of carbon dioxide and other minor components of the atmosphere, methane in particular, in the possible global warming of the climate has considerably increased the interest of the world's scientific community in the biogeochemical (microbial) processes of the cycles of carbon and methane. The concentrations of methane in the atmosphere and the aerobic water column of marine and freshwater reservoirs are incommensurably small compared to those in reduced waters and bottom sediments. Methane generation in sediments is quite intense, especially under conditions favoring methanogenesis. However, a considerable portion of methane is oxidized by aerobic methanotrophic bacteria in the bottom sediments and water column of marine ecosystems. Numerous field experiments point to the existence, apart from the aerobic process, of anaerobic bacterial oxidation of methane in reduced ecological niches [1–4]. It has been calculated that up to $(70–120) \times 10^{12}$ g CH_4 is annually oxidized in estuarine and shelf sediments, which makes up 10–20% of the global flux of methane from the sites of its formation and/or concealment [5]. The Black Sea, with its voluminous anoxic water column, is the most powerful anaerobic ecosystem that generates and oxidizes methane, and therefore attracts close attention from microbiologists, biogeochemists, ecologists, and climatologists.

The aim of the present work was to evaluate the rates of the processes of methane production and oxidation in the bottom sediments and water column of the Black Sea.

MATERIALS AND METHODS

Investigation geography and sampling. Materials for investigation were collected during the fifth voyage of the R/V *Professor Shtokman* in December 1980, the eighth voyage of the R/V *Vityaz'* in October–December 1984, and an expedition onboard *Bentos* submersible in December 1990 in the Black Sea (Fig. 1). Experiments were conducted onboard ship and in laboratories at the Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, and the Institute of Microbiology, Russian Academy of Sciences. Samples of bottom sediments were taken with an Okean dredger and with a straight-flow geological tube with an inner diameter of 12 cm; water samples were taken with General Oceanics bathometers (United States).

Determination of the rates of methane oxidation. The rates were determined in experiments that involved incubation of samples for 24–72 h in the presence of $^{14}CH_4$ dissolved in sterile degassed distilled water [6]. A water sample in a Hungate tube hermetically closed with a gas-tight stopper made of butyl rubber with natural caoutchouc or a sediment sample contained in a 10-cm^3 glass tube closed with an analogous stopper was supplemented with 100 μl of a $^{14}CH_4$ solution with a total activity of 3 μCi. The incubation temperature equalled the in situ temperature of water and sediments (4–8°C). At the end of incubation, the samples were fixed by introducing 1 ml of 2 N KOH [6].

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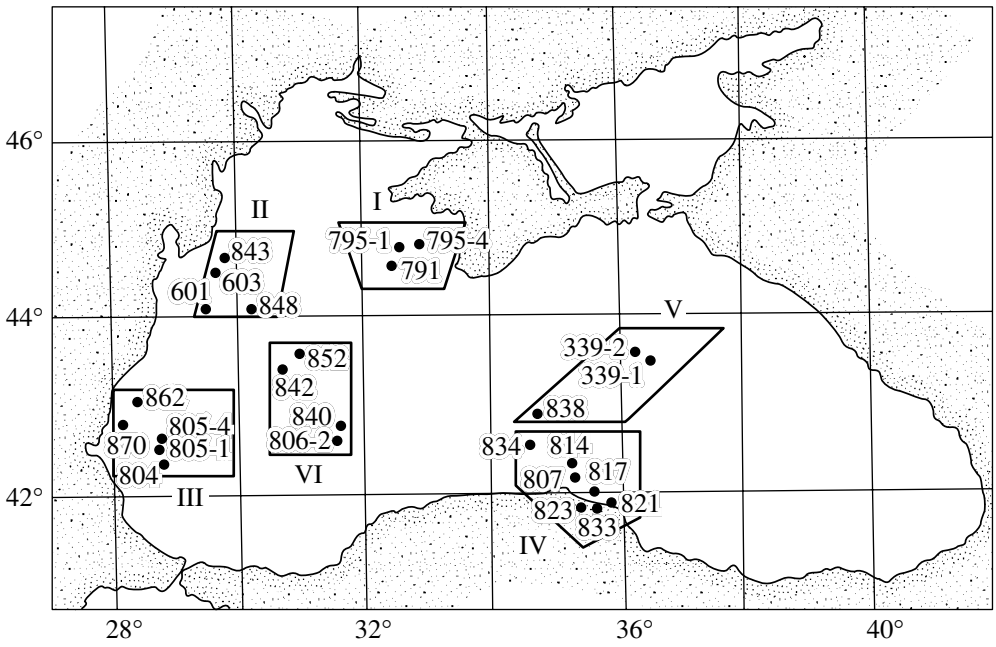

Fig. 1. Location of sampling stations in the Black Sea (the expedition in 1984; Roman numerals designate the investigation polygons (see Table 2)).

The carbon dioxide formed during bacterial methane oxidation was driven off and trapped in a distillation system with a water-cooled condenser [6]. A fixed sample was transferred to a flask and supplemented with 100 ml of tap water, and the flask was attached to the distillation system. Strong orthophosphoric acid (20 ml) was added in drops, and $^{14}CO_2$ was driven off with a flow of argon or nitrogen for 1.5 h under weak boiling and trapped with 2-phenylethylamine contained in a scintillation cocktail of the following composition: toluene, 600 ml; ethanol (96%), 200 ml; 2-phenylethylamine, 100 ml for water samples and 200 ml for sediment samples; 2,5 diphenyloxazole (PPO), 4 g; 1,4-bis-2-(5-phenyloxazolyl)benzene (POPOP), 0.2 g. After carbon dioxide distillation, the Drechsel bottles with the scintillation cocktail were replaced by new ones, and 15 g of sodium persulfate ($K_2S_3O_8$) was added to the sample flask to drive off, under boiling for 1 h, the carbon dioxide that was formed during the "wet burning" of organic exometabolites and of the biomass of methanotrophic bacteria; this carbon dioxide was trapped with 2-phenylethylamine contained in the scintillation cocktail [6].

Determination of the intensities of methanogenesis. Methanogenesis rates were determined by a method similar to the above-described method for determination of methane oxidation rates, except that $NaH^{14}CO_3$ with a total activity of 20 μCi was used instead of $^{14}CH_4$. The newly formed labeled methane was driven off with an air flow under weak boiling in a distillation system with a water-cooled condenser [6]. The methane that was driven off was burned to $^{14}CO_2$ at 800°C over silica gel impregnated with cobalt sulfate; the $^{14}CO_2$ was trapped with 2-phenylethylamine contained in the scintillation cocktail [6].

The radioactivity of $^{14}CO_2$ in the scintillation cocktail was measured in an SL-30 Intertechnique (France) or a RackBeta (Sweden) liquid scintillation counter. The rates of the process were calculated according to the formula [6]

$$I = \frac{\alpha r C}{Rt},$$

where I is the sought rate of the process (ng C/dm^3 of water or wet sediment per day); C is the concentration of methane or carbon dioxide in the sample (ng C/dm^3 of water or wet sediment); r is the radioactivity of the bacterial metabolic products ($^{14}CO_2$, $^{14}CH_4$, or ^{14}C-biomass + exometabolites, cpm); R is the radioactivity of the substrates introduced ($^{14}CH_4$ or $^{14}CO_2$, cpm); t is the time of the exposure of the samples (days); and α is the isotope fractionation coefficient (1.02 for methane oxidation [7] and 1.12 for CO_2 reduction [8]).

Determination of methane concentrations. Retrieval and determination of methane were performed by the head-space method with salting out, as described earlier [6, 9].

RESULTS AND DISCUSSION

The process of biogeochemical (microbial) oxidation of ^{14}C-methane with the formation of $^{14}CO_2$, ^{14}C-biomass of bacteria, and organic ^{14}C-exometabolites was revealed in all of the Black Sea bottom sediment samples that we investigated, both in the aerobic shelf

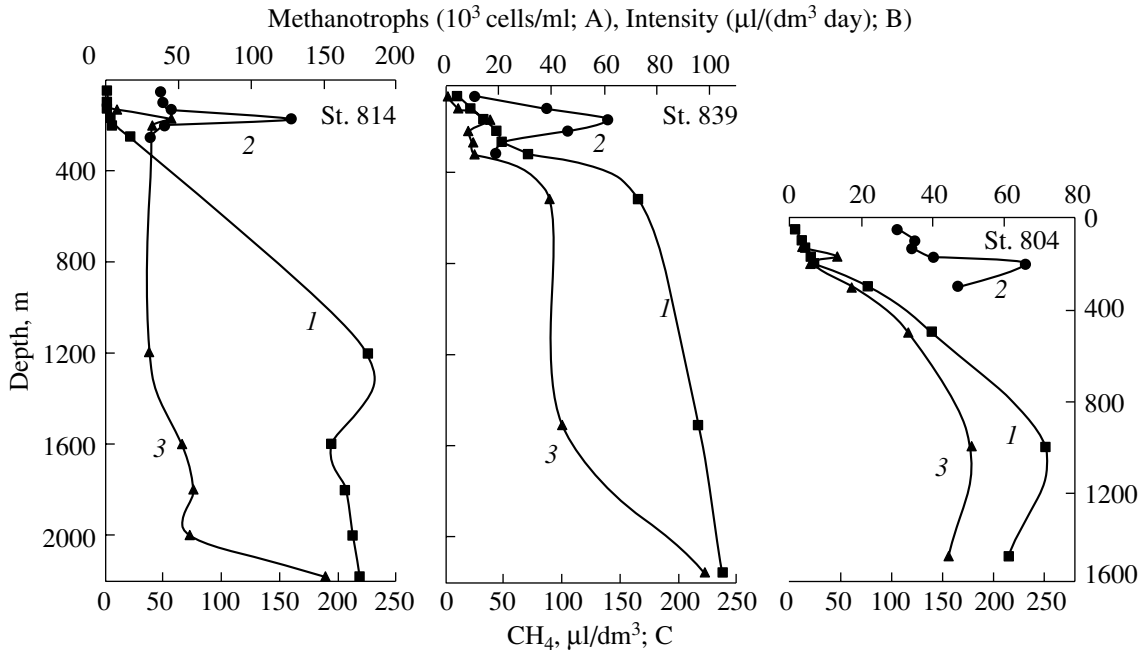

Fig. 2. Profiles of (*1*) methane concentration, (*2*) methanotroph cell number, and (*3*) methane oxidation intensity in the Black Sea water column.

and anaerobic deep-sea zones. In shelf sediments, the maximum values of methane oxidation intensity (specific rate) were recorded in the upper 0–20 cm layer, and sometimes at a depth of up to 50 cm. Deep-sea bottom sediments were characterized by a gradual increase of methane oxidation intensity to a depth of 80–100 cm; in lower layers, it increased abruptly, sometimes by 1–2 orders of magnitude (Table 1).

The determinations performed allowed us to calculate integral rates of methanogenesis and methane oxidation in bottom sediments under 1 m^2 and to a depth of 2 m for shelf sediments and 1 m for sediments of the continental slope and abyssal zone (New Euxine sediments, about 7 thousand years of age) and also to deduce the portion of newly oxidized methane that undergoes oxidation (Table 2). The ratios of average rate values in sediments of the shelf, continental slope, and abyssal zone were similar for methane production and methane oxidation (20 : 28 : 12 and 20 : 30 : 8, respectively), suggesting that these two oppositely directed processes are ecologically coupled in the Black Sea bottom sediments. Judging from the calculated values (Table 3), from one-fourth to one-half of the newly formed methane is oxidized in these sediments; in bottom sediments at stations 806-3 and 821, virtually all newly formed methane was oxidized (Table 2). Moreover, in sediments at stations of the Anatolian polygon (st. 823, 814, and 817), the amount of methane oxidized was much greater than the amount of methane produced. In this connection, it should be noted that, during the expedition in 1984, some of the bottom sediments were found to contain interlayers of gas-hydrate methane, which evidently is a source of methane (additional to that newly formed) for methanotrophic bacteria.

Earlier, we showed [2] that the portion of newly formed methane that is oxidized in the sediments of the Bulgarian shelf is much higher that in deep-sea sediments. However, after investigation of a vaster water area of the Black Sea and pulling together data for a much greater number of stations, we arrived at the conclusion that portions of newly formed methane undergoing oxidation are comparable in the shelf and deep-sea sediments (Table 3).

Considerable rates of methane oxidation were also revealed by us in all of the Black Sea water samples studied. The data obtained show that biogeochemical (microbial) process of methane oxidation occurs both in the aerobic and anaerobic hydrogen sulfide–containing water column of the Black Sea. The vertical profiles of methane oxidation intensities somewhat varied depending on the depth of the stations. Most deep-sea profiles exhibited peak values in the 150–170 m horizon of the water column (oxycline) and a considerable increase in the rate values in the anaerobic water column and near-bottom layers (Fig. 2; Table 4). At the shelf in the zone of carbonate constructions (*Bentos* expedition, December 1990), the profiles of methane oxidation values differed from the profiles recorded in the deep-sea zone. Here, changes in the methane oxidation rate correlated with the methane concentration profile and were characterized by a hyperbolic trend (Fig. 3; Table 5).

Table 1. Rates of methane oxidation and production in bottom sediments (eighth voyage of the R/V *Vityaz'*, 1984)

Station (polygon; depth)	Horizon of sampling, cm	Content of CH_4, μl/dm^3	Rate, ng C/(dm^3 day)		
			CH_4 production*	CH_4 oxidation	
				total	biomass + exometabolites, %
795-1	0–5	87	15.3	6.1	13
(I; 147 m)	10–20	82	10.6	6.3	11
	40–50	55	2.8	5.5	33
	70–80	61	–	4.3	7
	115–125	189	–	16.1	16
	150–157	205	3.4	12.5	10
795-4	0–2	120	14.9	22.3	37
(I; 150 m)	2–6	108	Ì.Ó.	9.2	14
	6–11	103	12.4	8.2	5
	11–25	59	11.1	4.6	9
791	0–4	64	2.8	4.9	4
(I; 772 m)	6–10	125	0.4	13.8	4
	11–13	100	0.6	7.2	4
	17–21	108	0.2	7.1	6
	23–26	194	1.2	14.8	6
843	0–5	48	14.1	26.2	42
(II; 52 m)	3–5	106	2.1	3.8	3
	20–25	70	17.4	2.5	4
848	0–5	50	2.8	2.7	11
(II; 118 m)	20–25	16	3.2	0.7	14
	41	14	–	1.0	10
852	0–5	53	21.8	2.7	11
(II; 1450 m)	12–20	63	3.2	2.8	4
	22–30	43	–	1.9	5
	32–38	62	4.2	3.1	3
	40–45	117	7.7	7.3	4
	62–68	4086	5.5	10.7	43
842	0–5	135	17.3	4.4	2
(II; 1458 m)	12–15	130	–	3.8	3
	25–32	93	–	2.5	4
	40–50	134	17.5	4.1	2
	50–60	119	9.5	3.4	3
	80–90	345	11.0	10.4	15
870	0–2	45	14.5	14.3	53
(III; 57 m)	8–10	48	10.2	3.2	25
	30	350	–	12.6	3
	45–55	66	1.9	2.2	5
	80–90	1943	7.0	177.6	4
	150–160	4947	64.0	197.3	3
862	0–3	20	–	1.0	10
(III; 61 m)	8–10	54	2.6	1.6	6
	28–30	189	4.4	8.0	5
	68–70	590	19.2	20.8	8
	100	ND	93.9	ND	ND

Table 1. (Contd.)

Station (polygon; depth)	Horizon of sampling, cm	Content of CH_4, μl/dm^3	Rate, ng C/(dm^3 day)		
			CH_4 production*	CH_4 oxidation	
				total	biomass + exometabolites, %
805-1	0–13	91	–	9.0	6
(III; 1605 m)	15–25	125	–	12.3	7
	35–45	108	–	7.3	7
	54–64	132	–	10.4	14
	86–92	214	–	11.4	20
	92–105	230	–	16.8	32
805-4	0–3	200	–	18.5	4
(III; 1585 m)	5–8	195	–	17.4	13
	15–18	193	–	15.0	13
823	0–5	32	7.9	16.0	1
(IV; 106 m)	15–20	62	2.0	24.7	3
	20–30	52	–	22.9	1
	30–40	54	4.5	26.5	3
	100–110	68	1.3	28.9	2
833	0–2	52	–	45.0	1
(IV; 108 m)	3–6	30	–	13.6	2
	7–10	52	–	23.1	1
	12–15	65	–	27.6	2
	20–25	49	–	24.3	1
821	0–4	250	5.7	10.5	5
(IV; 442 m)	8–12	366	–	14.2	4
	14–18	374	–	15.6	5
	20–30	211	5.0	13.5	4
	55–70	288	–	18.1	3
	90–100	514	–	35.0	4
	190–200	100	–	6.9	4
	290–300	65	23.2	4.5	9
817	0–5	248	35.2	84.5	1
(IV; 1420 m)	7–14	200	97.0	71.4	1
	15–25	268	22.0	90.7	<1
	70–80	182	–	64.9	<1
	100–110	222	13.4	81.8	1
814	0–5	141	7.5	197.6	<1
(IV; 2180 m)	10–15	160	6.3	233.2	<1
	20–25	188	13.6	258.6	<1
839	5–10	217	22.0	11.5	3
(V; 2160 m)	20–25	25	5.7	1.3	8
	40–55	356	14.0	17.7	12
	60–70	307	10.4	13.2	4
	75–85	8440	26.4	ND	ND
806-2	0–7	171	49.3	8.4	2
(VI; 2106 m)	15–20	81	23.7	3.5	3
	32–36	112	–	5.2	6
	50–60	133	64.7	5.7	7
	80–83	196	13.4	11.0	34
806-3	0–5	124	–	15.7	3
(VI; 2141 m)	20–25	108	23.7	5.9	4

* These data were obtained by S.N. Gorlatov.

Table 2. Integral values of the rates of methane production and oxidation in bottom sediments

Station	Depth, m	Rate, μg C/(m^2 day)			Oxidized portion of newly formed methane, %
		CH_4 production*	CH_4 oxidation		
			total	exo**, %	
I. Calamite polygon					
795-1	147	11	6	15	55
795-2	150	36	15	16	42
791	772	–	17	5	?
II. Danubian polygon					
843	52	18	9	16	50
848	118	6	3	12	50
603*	287	–	24	16	?
601*	1050	–	18	20	?
III. Bulgarian polygon					
555*	22	20	7	62	35
559*	26	15	6	39	40
862	61	82	19	7	23
568*	86	28	8	38	29
580*	330	66	10	20	15
546*	1240	10	1	18	10
805-1	1605	36	15	14	42
545*	1620	29	7	20	24
IV. Anatolian polygon					
823	105	6	50	2	833
833	108	–	49	1	?
821	442	39	38	5	97
817	1420	25	75	1	300
814	2180	15	54	1	367
V. Eastern gyristase					
839	2104	13	8	7	62
VI. Western gyristase					
852	1450	8	4	12	50
842	1458	15	5	5	33
806-2	2108	41	6	10	15
806-3	2141	12	11	15	92

Note: "–" stands for "not determined."

* Stations of the fifth voyage of the R/V *Professor Shtokman* (December 1980).

** Incorporation of ^{14}C into bacterial cells and organic metabolites (% of total methane oxidized).

The upper peaks of methane oxidation rate observed in the water column in the oxycline zone correlated with maximum values of the cell number of methanotrophic bacteria enumerated by the immunofluorescence method (Fig. 2) [3, 6]. In shelf sediments, overlaid by oxygen-containing waters, we revealed no correlation between the cell numbers of aerobic methanotrophs and the rates of aerobic methane oxidation. Nevertheless, on the whole, it was in the upper centimeters of shelf sediments (i.e., in the zone of occurrence of aerobic methanotrophs) that the rates of methane oxidation exhibited values highest within the depth interval of sediment sampling (0–50 cm). No such maxima were recorded in the upper horizons of sediments in the anaerobic deep-sea zone of the Black Sea.

Of particular interest is the distribution of carbon between carbon dioxide, bacterial biomass, and organic exometabolites formed during bacterial oxidation of

Table 3. Averaged values of the rates of methane oxidation and production in the Black Sea bottom sediments

	Rate*, μg C/(m^2 day)				Oxidized portion of newly formed methane, %
	CH_4 production		CH_4 oxidation		
	range	mean	range	mean	
Shelf	6–82	25	3–50	19	42
Slope	10–66	34	1–75	28	23
Abyssal zone	5–45	16	4–11	7	51

* Calculated by graphical integration.

Table 4. Content of gases and rates of methane oxidation and production in the Black Sea water column (eighth voyage of R/V *Vityaz'*, 1984)

Station (depth)	Horizon of sampling, m	Content			Rate, ng C/(dm^3 day)		
		H_2S (mg/dm^3)	O_2 (ml/dm^3)	CH_4 (μl/dm^3)	CH_4 production	CH_4 oxidation	
						total	exo*, %
804	50	–	6.32	5.5	0	1.2	75
(1485 m)	100	0	0.46	11.6	0	3.3	85
	130	0.17	0.35	15.3	19.3	3.3	73
	170	0.98	0.13	20.2	–	13.2	22
	200	2.06	0	24.0	19.3	5.6	86
	300	–	0	76.3	–	17.3	70
	500	–	–	138.8	–	33.0	84
	1000	–	–	250.5	4.5	50.7	89
	1484	–	–	215.3	6.4	44.6	83
814	50	–	–	0.5	0	0.2	50
(2180 m)	100	–	–	0.8	0	0.2	50
	130	0	0.71	0.8	31.3	7.4	23
	170	0.05	0.36	3.1	31.5	44.7	2
	200	0.56	0.22	5.2	–	31.8	4
	250	1.07	0	21.0	24.1	31.1	4
	1200	–	–	225.2	–	29.8	17
	1600	–	–	193.9	–	52.8	55
	1800	–	–	206.2	–	60.6	49
	2000	–	–	212.9	–	58.6	45
	2179	–	–	219.0	2.6	151.3	26
839	50	–	–	10.0	–	0.6	67
(2154 m)	100	0	–	22.5	–	4.6	65
	150	0.02	0.34	35.0	–	16.6	89
	200	1.17	0.06	46.9	–	8.3	83
	250	1.72	0	52.0	–	10.2	82
	300	–	0	76.5	–	10.7	83
	500	–	–	178.4	–	38.8	69
	1500	–	–	235.0	–	44.1	77
	2153	–	–	258.0	–	98.5	43

* Incorporation of ^{14}C into bacterial cells and organic metabolites (% of total methane oxidized).

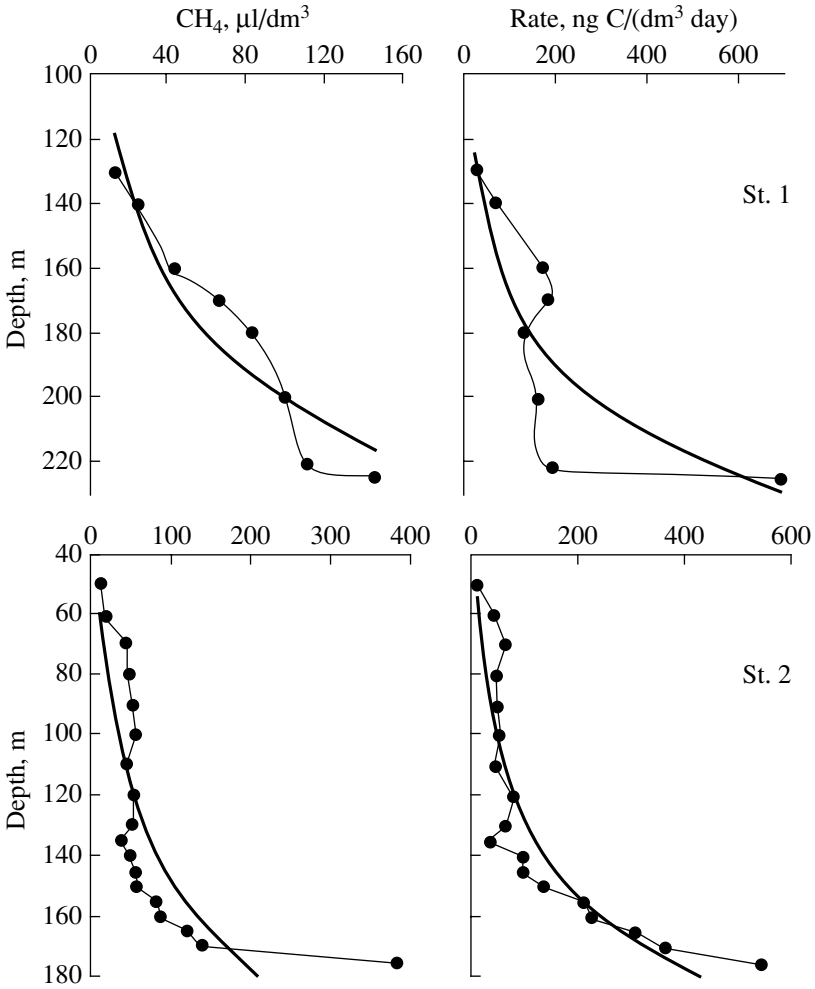

Fig. 3. Profiles of the concentrations of methane and rates of its oxidation in the water column in the region of methane seeps (*Bentos* expedition in 1990; st. 1 and st. 2 are sampling stations; smoothly bent lines are trends of the changes of values).

methane. At the beginning of our investigations (in 1980) of the biogeochemical process of methane oxidation in freshwater and marine reservoirs, we developed a method for differential determination of $^{14}CH_4$ oxidation to $^{14}CO_2$, bacterial ^{14}C-biomass, and ^{14}C-exometabolites [6, 10]. In bottom sediments of freshwater and marine ecosystems, methane is oxidized mainly to carbon dioxide. However, in a number of cases (Black Sea methane seeps and samples from certain horizons of bottom sediments), a considerable portion of the carbon of methane oxidized is incorporated into bacterial cells and organic exometabolites (Tables 1–5). In some horizons of the water column, the portion of carbon incorporated by methanotrophs into their biomass and organic exometabolites reached 70–90% of the carbon of methane oxidized (Tables 4, 5).

Rusanov and coworkers [11] showed that in the water of various marine ecosystems (the Black Sea, estuaries of the Congo, Ob, and Yenisei rivers) the methane carbon is mainly incorporated into bacterial biomass and exometabolites. It is the authors' opinion that, in the aerobic part of the Black Sea water column, the portion of carbon incorporated into biomass and exometabolites is always greater that in anaerobic waters and bottom sediments. Indeed, in the water column of two stations that we investigated in the zone of the methane seep, the maximum values of carbon incorporation into organic exometabolites reached 61 and 69% of the methane oxidized, and the trends of the values suggested a considerable decrease in the proportion of assimilated carbon from surface layers to the bottom (Table 5; Fig. 4). As far as the portion of carbon assimilated into bacterial biomass is concerned, its values varied within 2–28% at station 1 (margin of the methane seep) and 21–47% at station 2 (above the methane seep). However, these parameters showed no trend to change with depth (Fig. 4).

We performed an analysis of our own data obtained during the surface expeditions in 1980 and 1984 and the submarine expedition in 1990 in the region of the Black Sea methane seeps [12, 13], as well as of the results of studies conducted at three stations by Pimenov,

Table 5. Content of gases and rates of methane oxidation in the water column in the region of the Black Sea methane seep (*Bentos*, December 1990)

Station (depth)	Horizon of sampling, m	Content, μl/dm^3			Rate of CH_4 oxidation			Flux of CH_4***
		H_2S*	O_2*	CH_4	total	cells	exo**	
					μl/(dm^3 day)	% of total		ml/(m^2 year)
St. 1	130	0	450	14	26	19	28	–0.30
(225 m)	140	120	150	25	67	16	28	–0.26
	160	300	0	44	170	16	61	–0.62
	170	450	0	67	183	25	21	–0.43
	180	680	0	83	131	22	39	–0.23
	200	700	0	100	159	22	32	–0.14
	221	–	0	111	192	28	21	–2.35
	225	970	0	145	687	9	–	–
								Σ –4.3
St. 2	50	–	–	15	15	40	33	–0.13
(176 m)	60	–	–	20	45	21	69	–0.70
	70	0	2110	46	65	46	29	–0.06
	80	–	–	48	49	42	35	–0.13
	90	–	–	53	50	32	42	–0.11
	100	0	850	57	54	31	32	0.27
	110	–	–	47	49	33	31	–0.19
	120	0	230	54	80	26	40	0.06
	130	–	–	52	67	33	19	0.38
	135	0	120	38	41	36	21	–0.32
	140	0	200	50	99	41	32	–0.16
	145	0	120	56	99	39	32	–0.03
	150	0	150	57	138	35	21	–0.67
	155	0	140	82	211	47	23	–0.13
	160	270	110	87	226	38	26	–0.89
	165	380	0	120	304	22	27	–0.48
	170	–	–	138	362	29	36	–6.57
	176	370	0	383	1004	36	32	–
								Σ –9.8

* Concentrations of H_2S and O_2 were determined by researchers from MGI, Academy of Sciences of the Ukrainian SSR.
** Organic exometabolites.
*** Calculated by the formula $I = -D\Delta C/\Delta S$, where D is the coefficient of diffusion of CH_4 in seawater, equal to 8.5×10^{-6} cm^2/s; ΔC is the difference between CH_4 concentrations (ml/cm^2); and ΔS is the diffusion distance (cm).

Rusanov, and coworkers [4, 11]. The portion of methane carbon incorporated into bacterial biomass and exometabolites proved to vary in the very wide range of 0–98% (Fig. 5). The broadest dispersion of values was recorded in samples taken in the water layers adjoining the oxycline. However, the incorporated portion did not necessarily correlate with depth, i.e., with the redox conditions in the water column, and not in all cases did it correlate with the intensity of bacterial methane oxidation (Fig. 6).

The values of the rates of methane production and oxidation recorded in marine reservoirs vary in a rather wide range. Earlier, we made an attempt to evaluate the contribution of these processes to the methane flux to the atmosphere proceeding from data on rates of methane production and oxidation in certain seas [14]. In spite of the considerable variations in the specific rates of the two processes in marine reservoirs, their algebraic sum never exceeded 11 Tg CH_4 per year [14]. Interestingly, in the Black Sea, the integrated values of methane oxidation rate were often superior to the intensities of methanogenesis (Tables 2, 6). Thus, in the water column above the methane seep, methane fluxes

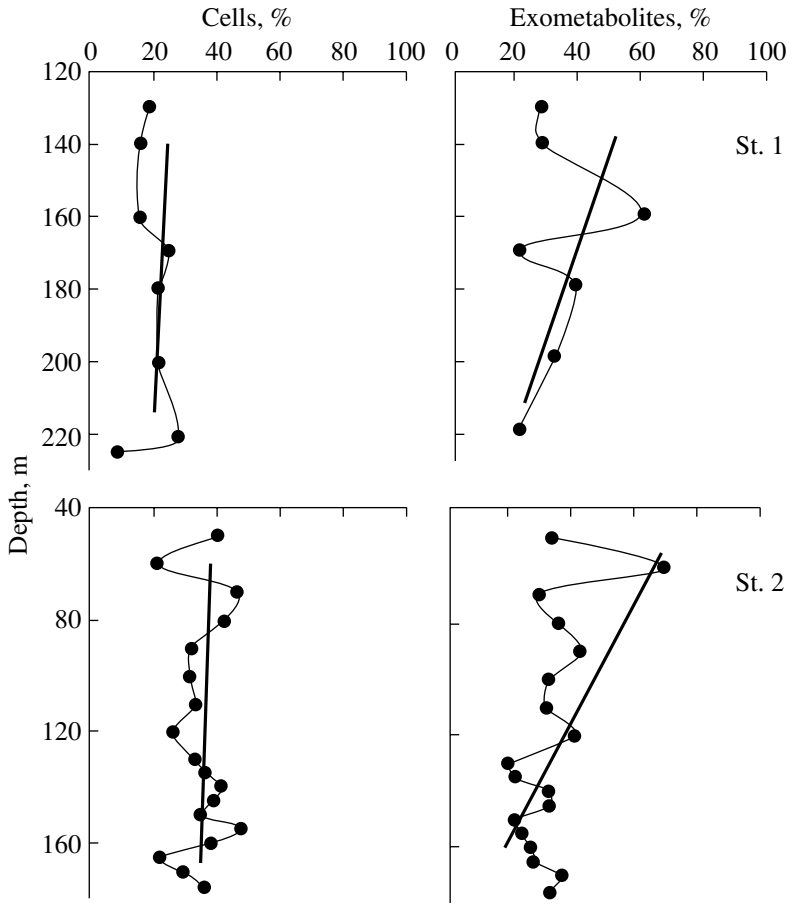

Fig. 4. Profiles of values of the portions of methane carbon incorporated into cells of methanotrophs and organic exometabolites (*Bentos* expedition in 1990; st. 1 and st. 2 are sampling stations; straight lines are trends of the changes of values).

were characterized by negative values, changing hyperbolically from bottom to surface (Table 5; Fig. 3); i.e., methane concentrations decreased (due to bacterial oxidation) most strongly immediately near the bottom. The results of our calculations cannot be considered as final estimates, because, as new information is obtained, the values calculated will need refinements. Our aim was just to consider the potentialities and limitations of various methodical procedures and the possibilities they offer for the estimation of the scale of methane fluxes in the biosphere and approximate evaluation of these fluxes.

Thus, our data suggest that in the Black Sea bottom sediments and water column, along with methanogenesis, methane oxidation also occurs, both aerobic and anaerobic. Moreover, the rate of anaerobic methane oxidation is often higher than the rate of aerobic methane oxidation. From one-fourth to one-half of the methane formed in bottom sediments was oxidized immediately therein. The major part of the remaining methane

Table 6. Integrated values of the content of methane and rates of its production and oxidation in the water column (eighth voyage of the R/V *Vityaz*', 1984)

Station (depth)	Methane content		Rate			
			CH_4 production		CH_4 oxidation	
	l/m^2	ml/m^3	mg C/(m^2 day)	μg C/(m^3 day)	mg C/(m^2 day)	μg C/(m^3 day)
804 (1485 m)	239	158	15.0	9.9	51.1	33.8
814 (2180 m)	312	143	29.4	13.5	90.9	41.7
839 (2154 m)	404	188	ND	ND	94.7	44.1

Note: ND stands for "not determined."

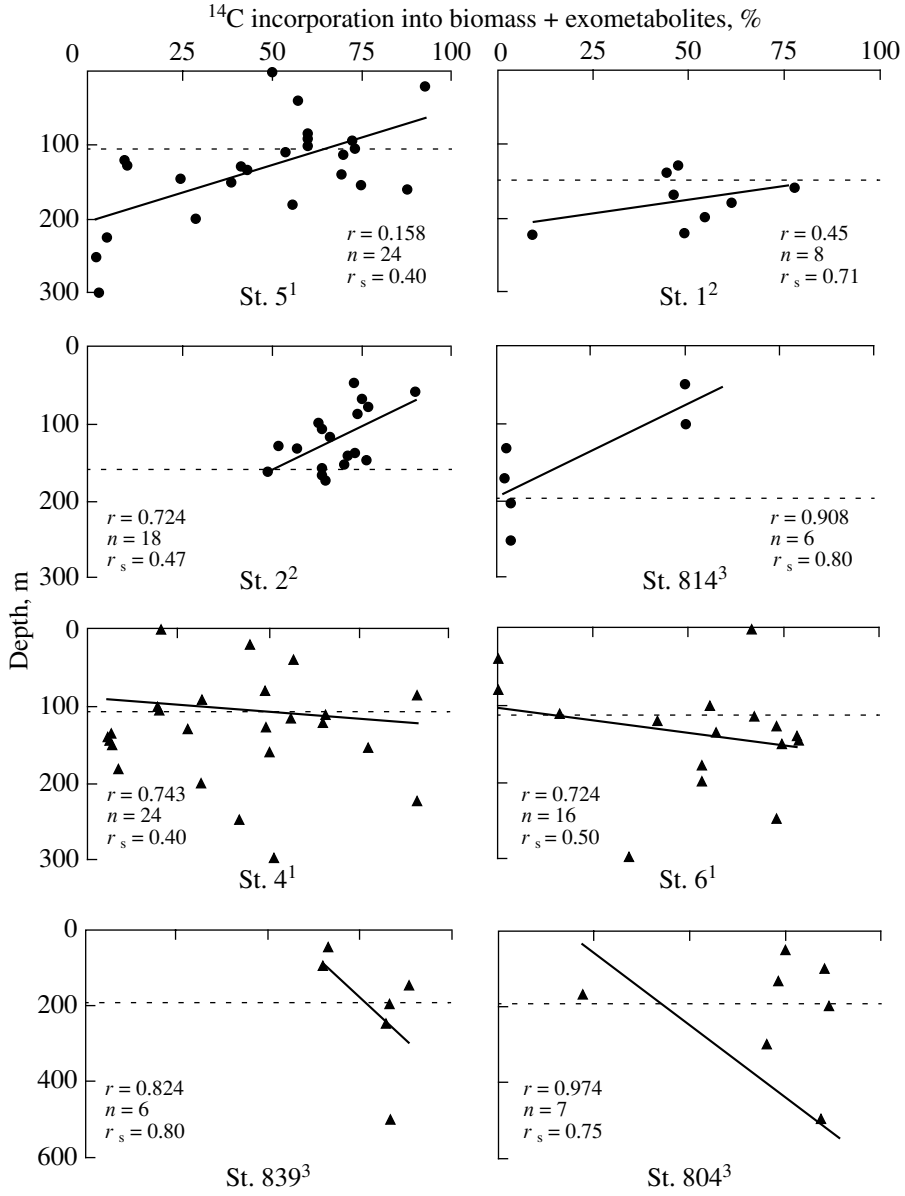

Fig. 5. Graphs showing the dependence of the portion of methane carbon incorporated into bacterial biomass and organic exometabolites on the depth of sampling. Data for st. 4^1, 5^1, and 6^1 were obtained by Pimenov, Rusanov, *et al.* [4, 14]; data for st. 1^2 and 2^2 were obtained by Rusanov, Gal'chenko, *et al.* [12, 13]; and data for st. 804^3, 814^3, and 839^3 were obtained during the expedition in 1984. Solid lines are trends of the changes of values; the dashed line shows the oxycline.

was oxidized in the water column, and a smaller portion arrived in the atmosphere.

The analysis of integrated values of the contents of methane and intensities of its production and oxidation obtained during the vertical profiling of deep-sea regions (Table 6) provided evidence of the significant predominance in the water column of methane oxidation (aerobic plus anaerobic) over methanogenesis. It should be noted that average values of methane concentrations and methane production and oxidation rates calculated per cubic meter proved to be quite close in different regions of the Black Sea.

To conclude, it is necessary to note that the data currently available, including data from inhibitory analyses performed by us [6] and other researchers [15, 16], do not provide sufficient grounds for sound judgments about the microbial agents and biochemical nature of anaerobic methane oxidation but give the impression that neither methanogenic archaea nor sulfate-reducing bacteria alone can be responsible for this process.

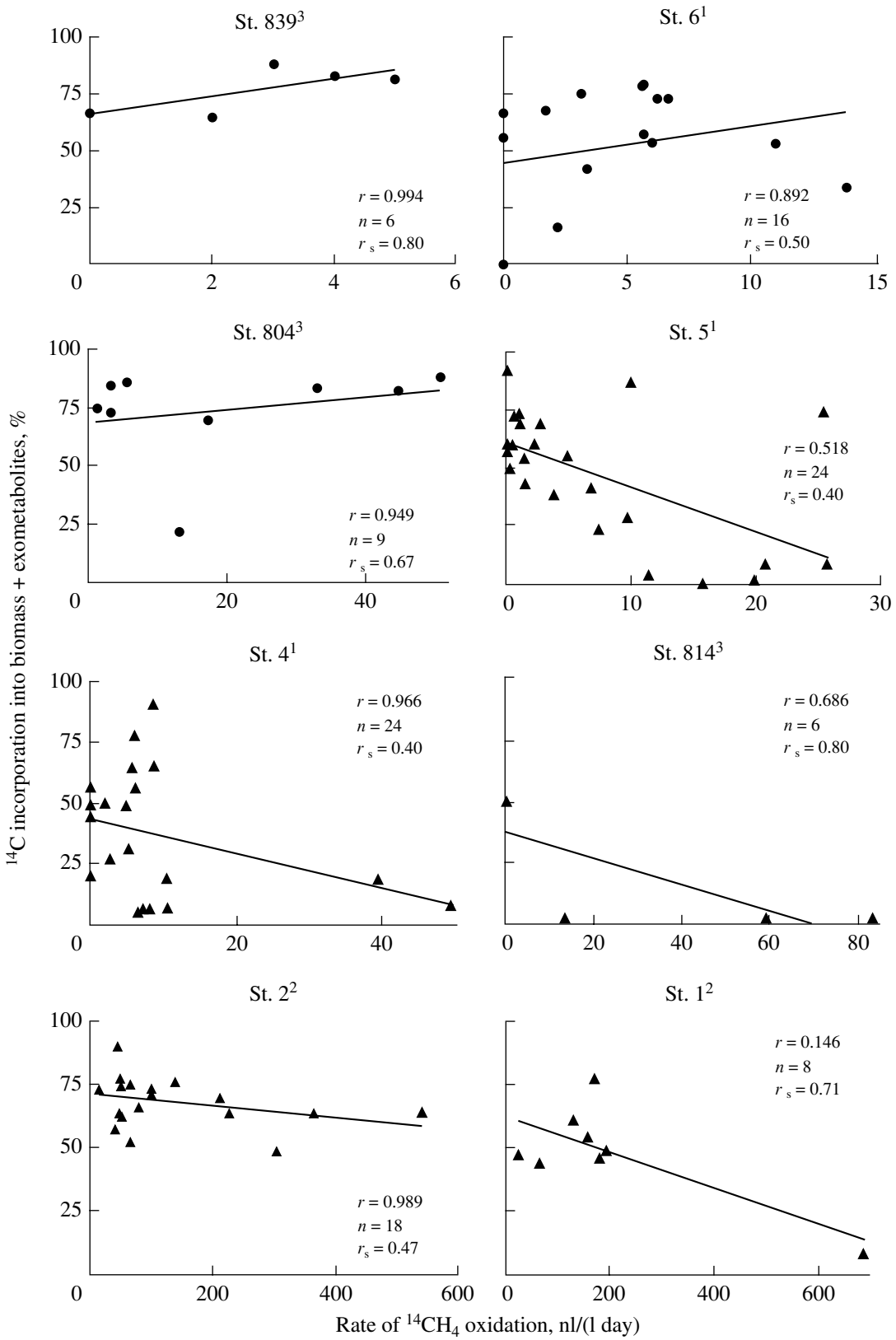

Fig. 6. Graphs showing the dependence of the portion of methane carbon incorporated into bacterial biomass and organic exometabolites on the rate of methane oxidation (the designations are as in Fig. 5).

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