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EXPERIMENTAL ARTICLES

Microbial Processes at the Aerobic–Anaerobic Interface in the Deep-Water Zone of the Black Sea

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Abstract—Chemical and key microbiological processes (assimilation of carbon dioxide, oxidation and formation of methane, and sulfate reduction) occurring at the aerobic–anaerobic interface in the deep-water zone of the Black Sea were investigated. Measurements were taken at depths from 90 to 300 m at intervals of 5–10 m. The integral rate of the dark assimilation of carbon dioxide varied from 120 to 207 mg C/(m^2 day) with a maximum at the boundary of cyclonic currents. The organic matter (OM) formed from methane comprised less than 5% of the OM formed from carbon dioxide. A comparison between the rates of methane oxidation and methane production suggests that methane that is oxidized at depths from 100 to 300 m was formed in deeper water horizons. The maximum rate of sulfate reduction (1230 mg S/(m^2 day)) was observed in the western halistatic region, and the minimum rate (490 mg S/(m^2 day)), in the eastern halistatic region. The average rate of hydrogen sulfide production measured at three deep-sea stations amounted to 755 mg S/(m^2 day), or 276 g S/(m^2 year).

Key words: carbon dioxide assimilation, sulfate reduction, methane oxidation, methane formation, chemocline, the Black Sea.

Since N.I. Andrusov's expedition [1], it has been known that the Black Sea is a meromictic water body. In the central part of the sea, aerobic waters spread to depths of about 90–100 m, while they spread to depths of 130–160 m in the zone of the continental slope. Below the aerobic layer, waters are saturated with hydrogen sulfide, whose concentration reaches 335–370 μM at depths of 1500–2000 m. A.A. Lebedintsev suggested that hydrogen sulfide in the Black Sea resulted from the reduction of sulfates during the decomposition of organic matter (OM) [3]. Later, B.L. Issatchenko [4] and other investigators isolated sulfate-reducing bacteria from samples of water and bottom sediments. Based on the experiments with ^{35}S-sulfate, Yu.I. Sorokin assumed that hydrogen sulfide in the Black Sea mainly results from bacterial sulfate reduction in bottom sediments [5]. However, the use of more sensitive radioisotopic methods allowed M.V. Ivanov, A.Yu. Lein, and M.B. Gulin [6–8] to obtain strong evidence that hydrogen sulfide in the Black Sea is primarily due to bacterial sulfate reduction in the water column. This finding is supported by the results of investigation of the stable sulfur isotope composition of hydrogen sulfide: the $\delta^{34}S$ of hydrogen sulfide in waters at depths below 300 m was found to range from –38.5 to – 40.9‰ [6, 7, 9, 10], while the $\delta^{34}S$ of reduced sulfur compounds in deep bottom sediments ranges from –24.0 to –36.3‰ with a mean value of –31.5‰, as derived from the results of analysis of 38 pyrite samples [6].

The conclusion about the intense geochemical activity of sulfate-reducing bacteria concerns not only the sulfur cycle but also the carbon cycle. Indeed, it is known that these microorganisms are involved in the terminal anaerobic stages of OM decomposition, utilizing low-molecular-weight organic compounds and hydrogen for sulfate reduction.

Microbiological processes at the interface between aerobic and anaerobic waters of the Black Sea are the subject of particular interest and extensive research. Investigations indicate an increase in the bacterial population at this interface and in the rates of carbon dioxide assimilation and of the oxidation and reduction of sulfur compounds [11–13]. The considerable variety of data reported by different and sometimes even by the same authors may result from the complexity and variability of microbial communities in the chemocline.

In this work, we performed detailed studies of some chemical and microbiological processes in the zone of interaction of aerobic and anaerobic deep waters of the Black Sea. Along with the estimation of the bacterial population and the rates of dark CO_2 assimilation and sulfate reduction, we measured the rates of the poorly studied processes of bacterial oxidation and production of methane.

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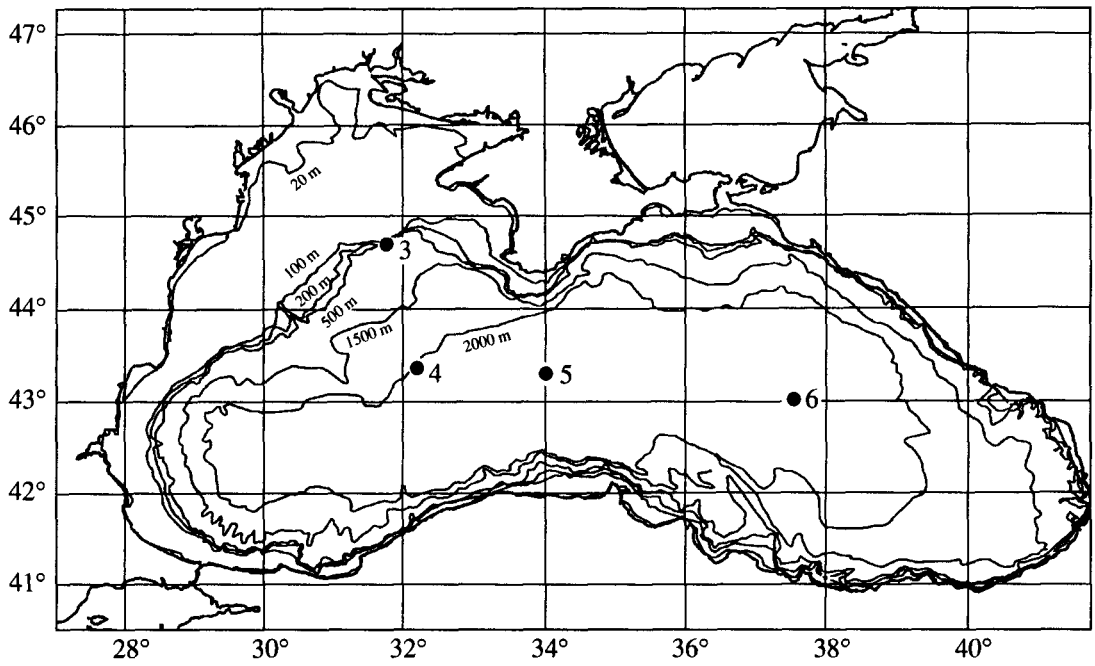

Fig. 1. Scheme showing the location of bathymetry stations during the May 1998 expedition in the Black Sea aboard the research vessel *Professor Vodyanitskii.* Figures indicate station numbers.

MATERIALS AND METHODS

Water samples were taken during the Russian–Swiss expedition in early May 1998 aboard the research vessel *Professor Vodyanitskii.* Bathymetry was performed at stations located in the western (st. 4) and eastern (st. 6) halistatic regions and at the boundary of cyclonic currents (st. 5), as well as at station 3 located at the northwestern continental slope (Fig. 1).

Water samples were collected using 12-l plastic bottles combined with a Mark-III sampling complex. In the zone of interaction of aerobic and anaerobic waters, samples were taken at depth intervals of 5–10 m.

Chemical analysis of seawater. Ammonium and nitrates were measured aboard the vessel using a flow–injection analyzer. Methane was assayed by the phase–equilibrium degassing method on a Chrom-5 gas chromatograph equipped with a flame ionization detector [14]. Hydrogen sulfide was measured by iodometric titration. The concentration of dissolved oxygen was determined by the Winkler method. Taking into account that this method underestimates the oxygen content in media with low concentrations of oxygen and hydrogen sulfide and based on the data available in the literature, we took the water horizon with an estimated H_2S concentration of 8.6–8.9 μM to be the lower boundary of waters containing both H_2S and O_2 (the so-called C-layer).

Enumeration of microorganisms and estimation of the ratio between archaea and other prokaryotes. The total microbial population was estimated by the direct epifluorescent filter technique using 0.2-μm-pore-size polycarbonate membrane filters and the fluorescent dye 4',6-diamidino-2-phenylindole [15].

Bacteria and archaea were counted by the whole-cell fluorescent in situ hybridization (FISH) method using labeled Cy3 oligonucleotides [16]. EUB 338 (5'-GCTGCCTCCGTAGGAGT-3') was used as the probe for bacteria, and ARCH 915 (5'-GTGCTC-CCCCGCCAATCCCT-3'), as the probe for archaea [17]. The probes were purchased from MWG-Biotech GmBH. Microscopic analysis was performed using a Zeiss Axiolab epifluorescence microscope.

Radioisotopic measurements. The rates of dark CO_2 assimilation, sulfate reduction, and of the oxidation and production of methane were determined by the radioisotopic method using labeled compounds listed in Table 1. Measurements were carried out in 25-ml vials completely filled with sampled water and sealed with rubber stoppers without leaving air bubbles. Then, each vial was supplemented, using a syringe, with the respective labeled substrate in a volume of 0.1 ml (Table 1). Prior to the substrate injection, control vials were supplemented with 0.2 ml of a 25% solution of glutaraldehyde.

The conditions of incubation of water samples with radioactive compounds were determined experimentally. Three replicated measurements of the carbon dioxide assimilation rate in samples collected from a depth of 135 m (st. 4) showed that the time dependence of this rate was linear at an incubation time of 6 to 24 h (Fig. 2). Similar results were obtained when the rates of methane oxidation and sulfate reduction were measured in water samples taken from a depth of 160 m.

Table 1. Radioactive compounds and conditions used for evaluation of the rates of microbial processes

Process	Substrate	Amount of the radioactivity introduced, μCi	Reagent used to stop the reaction
1. Dark CO_2 assimilation	$NaH^{14}CO_3$	9	1% orthophosphoric acid (+ filtration)
2. CH_4 oxidation	$^{14}CH_4$	2	2 N KOH
3. CH_4 formation	$NaH^{14}CO_3$	9	2 N KOH
	$^{14}CH_3COONa$	10	2 N KOH
4. Sulfate reduction	$Na_2{}^{35}SO_4$	10	10% Cd acetate

Based on these data, the rates of carbon dioxide assimilation, methane oxidation, and sulfate reduction were estimated by incubating water samples at 7–8°C for 24 h. However, during the estimation of the methane production rate, the incubation time was increased to 2–3 days, since lesser incubation times were insufficient to reveal methanogenesis.

After incubation, the samples were fixed as described in Table 1, treated as described earlier [7, 18, 19], and their radioactivity was quantified in a Rackbeta liquid scintillation counter.

RESULTS

Microbial count and the ratio between archaea and bacteria. The vertical profiles of the microbial populations in seawater at four stations are presented in Fig. 3. The samples studied contained from 1×10^5 to 3.4×10^5 microbial cells/ml. The microbial population exhibited two maxima, which corresponded to the photic zone at depths from 20 to 60 m and to the boundary between aerobic and anaerobic waters. A distinct minimum in the microbial population corresponded to cold waters at depths from 70 to 100 m.

The distribution profiles of archaea and bacteria at deep-sea stations 5 and 6 (Fig. 4) were found to be similar in shape: both profiles exhibited several peaks at depths from 100 to 160 m. However, the population of archaea was almost two orders of magnitude lower than that of other bacteria. Unexpectedly, archaea were detected in both anaerobic and aerobic water layers.

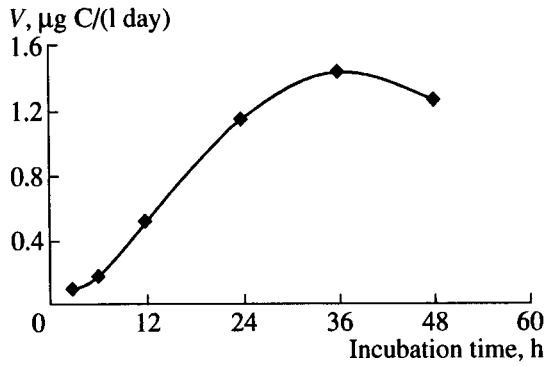

Fig. 2. Dependence of the $^{14}CO_2$ assimilation rate on the incubation time.

Dark CO_2 assimilation. Table 2 presents the results of determination of the dark CO_2 assimilation rates. The intensification of this process in the chemocline was observed at all four stations. At depths below 300 m, the CO_2 assimilation rate decreased virtually to zero.

Figure 5 presents the profiles of CH_4 assimilation at depths from 170 to 400 m in the zone of the continental slope (st. 3) and at depths from 90 to 300 m at three deep-sea stations. The dotted line indicates the boundary of the hydrogen sulfide–containing waters. The boundary waters and waters immediately above them were characterized by low rates of CO_2 assimilation (no more than 0.2 μg C/(l day)). In deeper hydrogen sulfide–containing waters, the CO_2 fixation rate drastically increased. At the deep-sea stations, the first maximum of the CO_2 fixation rate (1.7–2.1 μg C/(l day)) was observed at depths of 125 to 140 m, where the concentration of hydrogen sulfide was 8.9 μM and the concentration of oxygen was less than 0.01 mg/l. The second maximum of the CO_2 assimilation rate was observed at depths of 180 to 200 m (strictly anaerobic zone), where the concentration of hydrogen sulfide was 35 μM. At station 3 (the zone of the continental slope), the first, less pronounced, maximum of the CO_2 fixation rate also occurred in the zone of H_2S appearance (at a depth of 190–200 m); the second maximum with a CO_2 fixation rate of 1.45 μg C/(l day) was observed at a depth of 220 m.

Sulfate reduction. The rate of this process was determined at depths from 90 to 300 m (deep-sea stations) and from 170 to 600 m (st. 3 in the zone of the continental slope) (Table 2). Above the continental slope, the bacterial reduction of sulfates was detected at depths below 180 m, where hydrogen sulfide was present in trace amounts; the sulfate reduction rate reached maximum values (11 μg S/(l day)) at depths of 205 and 235 m (Fig. 6). At the deep-sea stations, the first maximum of sulfate reduction, which was observed at depths of 120–140 m, corresponded to the lower boundary of the C-layer; the second maximum corresponded to depths from 150 to 200 m. At depths below 200 m, the rate of sulfate reduction drastically decreased, making up no more than 1 μg S/(l day) at a 250-m depth.

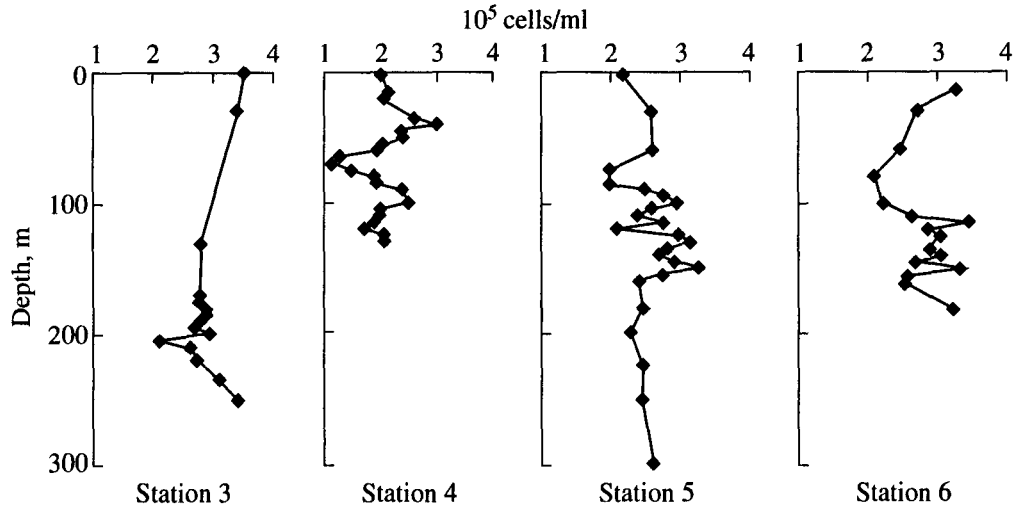

Fig. 3. Total microbial population profiles in the water column of the Black Sea.

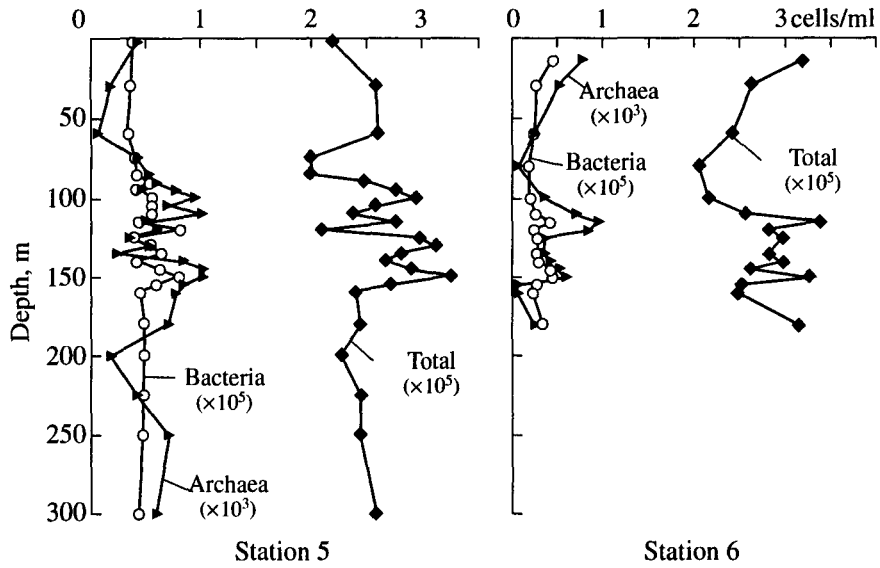

Fig. 4. Number of bacteria and archaea in the water column of the Black Sea.

Methane concentration and the rate of methane oxidation. Data on the depth distribution of methane are presented in Table 2 and Fig. 7. It can be seen that methane is present in seawater in noticeable amounts only at depths deeper than 100 m, i.e., below the upper boundary of the zone of interaction of aerobic and anaerobic waters. The methane content of aerobic waters was very low (less than 1 μl/l).

Table 3 summarizes data on the content of methane and the rate of its oxidation at depths from 2 to 300 m at the three deep-sea stations. In general, the vertical profiles of the methane oxidation rate and the methane concentration are similar. In the aerobic layer to depths of 80–100 m, the methane oxidation rate did not exceed 1 nl/(l day). At the boundary between aerobic and anaerobic waters, which occurred at a depth of 100–105 m, the intensity of methane oxidation considerably increased (Fig. 7).

The profile of methane oxidation rates in the western halistatic region (station 4) substantially differed from those in the eastern halistatic region (station 6) and in the zone of interaction of cyclonic currents (station 5). In general, the methane oxidation rates at station 4 were greater than those at stations 5 and 6. Moreover, the maximum rate of methane oxidation at station 4 observed at a depth of 300 m was an order of magnitude higher than the maximum rates of methane oxidation observed at the other deep-sea stations. Similarly, the concentration of methane at station 4 at depths between

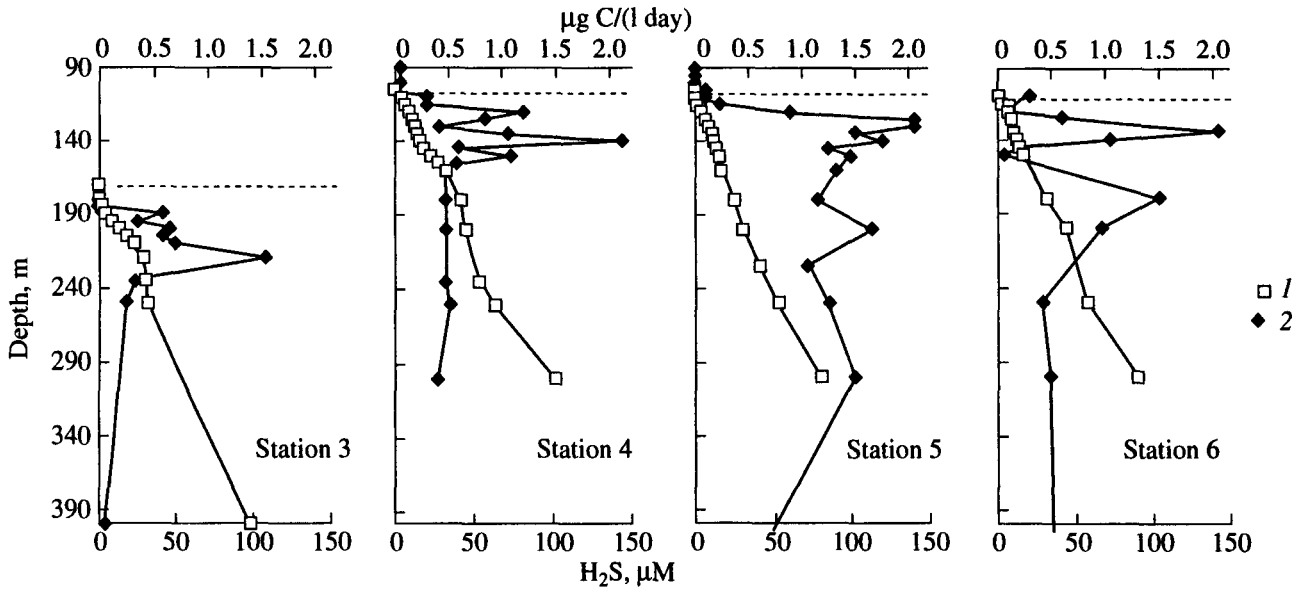

Fig. 5. Rates of dark CO_2 assimilation in the Black Sea water column at depths between 90 and 400 m: *1*, H_2S concentration, μM, and 2, CO_2 assimilation rate, μg C/(l day). Dotted lines show the aerobic zone boundary.

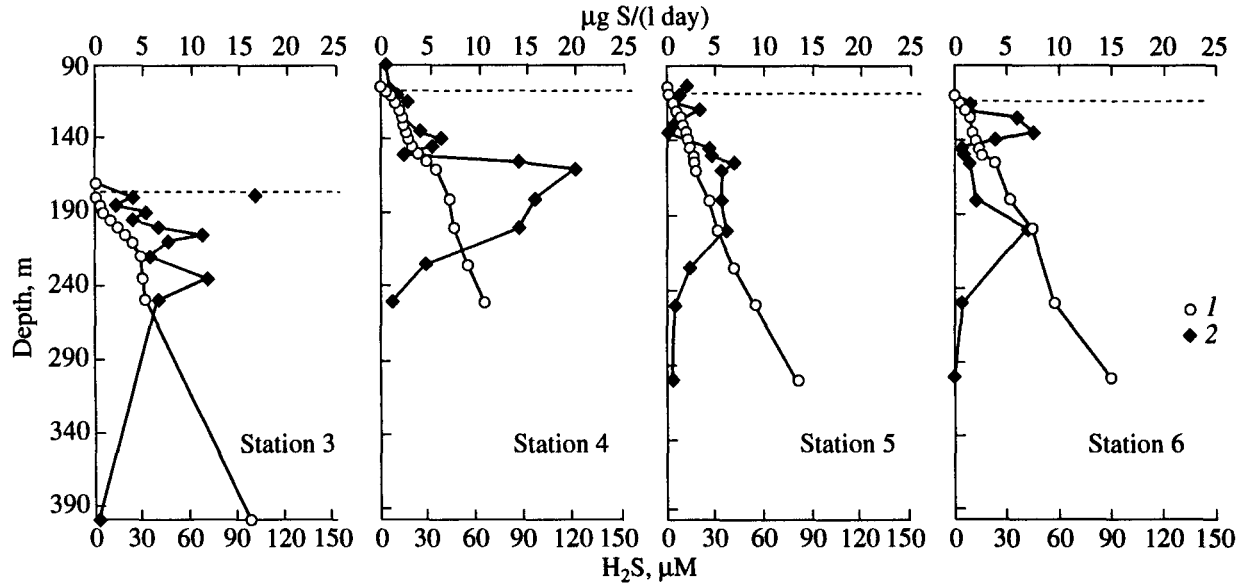

Fig. 6. Sulfate reduction rates in the Black Sea water column at depths between 100 and 400 m: *1*, H_2S concentration, μM, and *2*, sulfate reduction rate, μg S/(l day). Dotted lines show the aerobic zone boundary.

90 and 300 m was almost twofold higher than that at stations 5 and 6.

Methane production. Figure 8 presents the methane production profiles at depths between 100 and 250 m. As opposed to other microbiological processes, intense methanogenesis in the upper layer of the anaerobic zone was detected only in some water horizons (horizons 120–130 and 150–155 m at stations 4, 5, and 6). At depths below 160 m, methane production was observed only at stations 4 and 5. It should be noted that the rate of methanogenesis from acetate was very low (less than 10 nl/(l day)). For this reason, only data on the methane production from carbon dioxide and hydrogen are presented (Fig. 8).

DISCUSSION

It is known that the microbial communities of natural ecosystems grow well in the zones of stable gradients of compounds used by microorganisms as sources of carbon and energy. This may explain why the zone of interaction of aerobic and anaerobic waters in the Black Sea is the habitat of many physiological groups

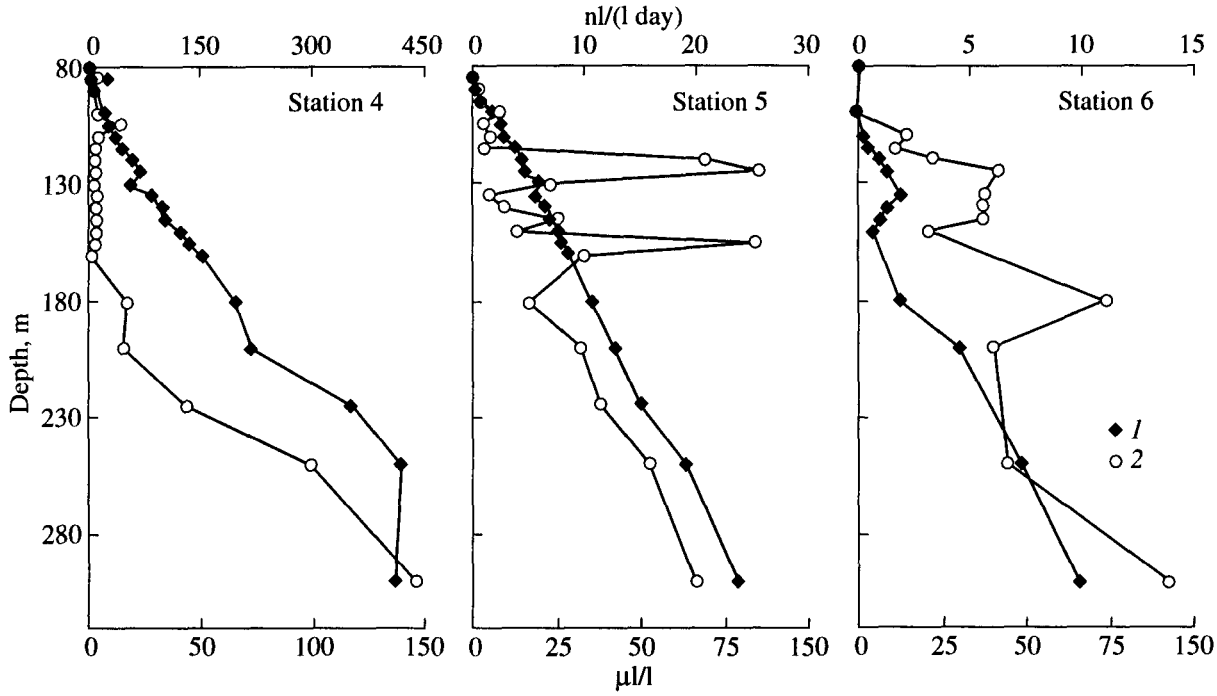

Fig. 7. Concentration of methane and rates of its oxidation in the Black Sea water column at depths between 80 and 300 m: *1*, methane concentration, and *2*, methane oxidation rate.

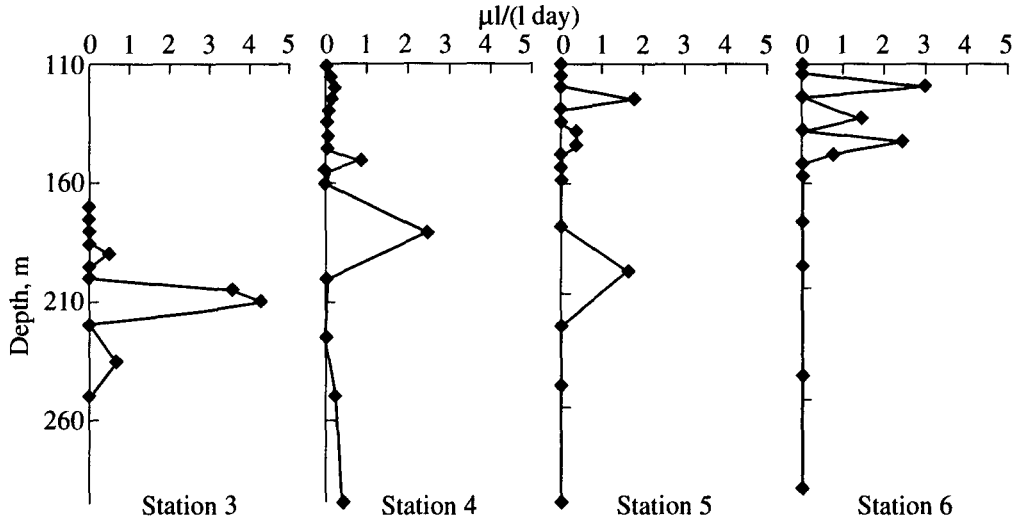

Fig. 8. Methane formation rates in the Black Sea water column at depths between 100 and 300 m.

of bacteria. According to some estimations, the bacterial population in the redox zone is 1.5 to 10 times greater than in the overlying water horizons [11]. Our investigations also indicated the rise in the population of microorganisms, including bacteria and archaea, at depths between 90 and 300 m (Figs. 3 and 4). The increase in the population of archaea in the hydrogen sulfide–containing waters may be due to the growth of methanogens. The presence of archaea in amounts of up to 10^3 cells/ml in aerobic waters (Fig. 4) can be explained by the fact that archaea (probably methanogens) are excreted from the intestines of zooplanktonic animals.

Dark CO_2 assimilation is a good indicator of the total activity of microbial communities. The intensification of this process in the chemocline was reported in a number of publications [11, 13, 20]. The detailed studies of Gulin [8] showed the presence of at least three maxima in the CO_2 fixation rate at depths from 90 to 300 m. Our data confirmed a saw-tooth pattern of the vertical profile of the CO_2 assimilation rates in the zone of interaction of aerobic and anaerobic seawaters (Fig. 5).

Table 2. Concentrations of some chemical compounds and rates of microbial process in the Black Sea water column

Hydrochemical characteristic of the zone	Depth, m	O_2^*, μM	H_2S^*, μM	CH_4, μl/l	NH_4^{+*}, μM	NO_3^{-*}, μM	CO_2 assimilation rate, μg C/(l day)	Sulfate reduction rate, μg S/(l day)
Station 3, depth 620 m, 44°39.49 N; 31°45.84 E								
Aerobic zone with the O_2 > 200 μM (I)	2	–	–	0.27	ND	0.004	–	–
	30	284	–	0.15	ND	0.015	–	–
	50	–	–	0.23	–	–	–	–
Oxycline zone with the O_2 from 0 to 174 μM (II)	130	174	–	0.38	ND	3.381	–	–
	150	–	–	0.11	ND		–	–
	170	18	–	0.08	ND	3.315	<0.05	ND
	175	11	ND	1.31	ND	1.156	<0.05	ND
Upper part of the C-layer with the H_2S content < 0.2 μM (III)	180	–	Traces (<1)	2.2	1.44	0.450	<0.05	3.9
Lower part of the C-layer with the H_2S content > 6.7 μM (IV)	185	Traces (<1)	2.2	6.2	7.39	0.004	<0.05	2.1
	190	Traces	4.5	11.1	31.19	ND	0.55	5.2
Anaerobic zone with the H_2S content > 8.9 μM (V)	195	ND	8.9	12.8	40.83	ND	0.35	3.9
	200	ND	13.4	15.5	44.80	ND	0.62	6.51
	205	–	–	18.7	61.79	ND	0.56	11.1
	210	–	22.3	19.8	83.04	ND	0.66	7.56
	220	–	29.0	22.2	84.74	ND	1.45	5.73
	235	–	–	25.2	105.43	ND	0.32	11.7
	250		31.2	31.3		–	0.24	6.51
	400		98.2	77.8	424.89	–	0.05	0.5
	600		138.4	153	613.47	–	<0.05	0.5
Station 4, depth 1998 m, 43°20.23 N; 32°09.54 E								
(I)	2	–	–	0.043	ND	ND	–	–
	35	238	–	0.085	ND	0.068	–	–
(II)	50	140	–	0.090	ND	1.988	–	–
	80	11	–	0.144	ND	0.768	–	ND
	90	Traces	–	2.327	ND	0.004	0.05	0.25
	100	Traces	ND	6.880	9.287	0.004	0.05	–
(III)	105	ND	Traces (<1)	8.73	28.768	0.004	<0.05	0.76
(IV)	110	ND	4.5	11.9	47.851	ND	0.37	1.52
	115	ND	6.6	14.5	79.657	ND	0.37	2.59
(V)	120	ND	8.9	19.4	82.671	ND	1.54	1.83
	125	ND	11.1	22.4	96.354	ND	0.94	2.13
	130	–	–	19.0	80.452	–	0.61	2.59
	135	–	–	28.0	121.18	–	1.12	3.80
	140	–	–	32.5	–	–	2.11	5.94
	145	–	–	33.8	–	–	0.56	5.18
	150	–	–	40.8	–	–	1.58	2.28
	155	–	–	44.7	–	–	0.58	14.2
	160	–	–	50.6	–	–	0.58	20
	180	–	–	65.4	–	–	0.50	15.8
	200	–	44.6	72.1	–	–	1.70	14.2
	225	–	–	117	–	–	0.35	4.5
	250	–	–	140	–	–	0.55	1.05
	300	–	–	137	–	–	0.35	0.5
	500	–	156.2	274	–	–	<0.05	–
	1000	–	–	314	–	–	<0.05	–
	1992	–	334.8	263	–	–	<0.05	–

Table 2. (Contd.)

Hydrochemical characteristic of the zone	Depth, m	O_2*, μM	H_2S*, μM	CH_4, μl/l	NH_4^+*, μM	NO_3^-,* μM	CO_2 assimilation rate, μg C/(l day)	Sulfate reduction rate, μg S/(l day)
Station 5, depth 2172 m, 43°14.92 N; 34°00.11 E								
(I)	2	–	–	0.226	–	0.085	–	–
	40	268	–	0.024	–	0.789	–	–
(II)	60	24	–	0.028	–	3.265	–	–
	85	1	–	0.305	–	0.177	<0.05	–
	90	1	–	1.11	21.206	0.096	<0.05	–
	95	–	–	2.68	18.934	0.096	<0.05	ND
	100	Traces	ND	6.00	39.005	0.096	<0.05	ND
(III)	105	ND	Traces (<1)	8.20	55.667	0.085	0.07	1.76
	110	ND	0.4	9.20	70.057	0.108	0.05	1.22
(IV)	115	ND	2.2	12.6	81.418	0.073	0.22	0.76
	120	ND	4.5	14.7	92.021	0.073	0.73	3.35
	125	–	6.7	15.5	95.051	0.073	1.70	1.52
(V)	130	–	8.9	19.5	131.78	0.073	1.70	0.76
	135	–	11.2	18.7	121.18	0.085	1.25	<0.5
	140	–	–	21.4	152.61	0.096	1.51	1.83
	145	–	–	22.7	164.35	0.096	1.03	4.41
	150	–	15.6	25.2	171.17	0.073	1.25	4.57
	155	–	–	26.3	179.88	0.073	–	6.86
	160	–	–	28.4	192.75	0.062	1.05	5.63
	180	–	–	35.4	228.72	0.062	1.00	5.48
	200	–	31.2	42.4	248.80	0.062	1.31	6.09
	225	–	–	49.8	289.70	0.062	0.86	2.28
	250	–	53.5	63.3	299.16	–	1.03	0.75
	300	–	80.3	78.8	345.36	–	1.25	0.5
	500	–	174.1	129	605.14	–	<0.05	–
	1000	–	276.8	159	742.99	–	<0.05	–
	2171	–	321.4	141	791.46	–	<0.05	–
Station 6, depth 2126 m, 43°00.20 N; 37°30.25 E								
(I)	2	–	–	0.024	–	–	–	–
	30	308	–	0.030	ND	0.103	–	–
	60	306	–	0.030	ND	3.086	–	–
(II)	80	29	–	0.026	ND	3.571	–	–
	100	11	–	0.032	ND	1.954	<0.05	–
(III)	110	9	Traces (<1)	1.8	ND	0.103	0.25	ND
(IV)	115	ND	2.2	3.1	ND	0.127	0.07	1.53
	120	ND	6.6	6.5	–	–	0.12	1.37
(V)	125	ND	–	9.1	61.758	–	0.52	5.93
	135	ND	–	12.8	93.854	–	1.80	7.46
	140	–	–	8.8	–	–	0.92	3.83
	145	–	–	6.9	81.550	–	0.16	0.7
	150	–	15.6	4.2	99.738	–	0.07	0.9
	180	–	–	12.5	109.61	–	1.30	2.05
	200	–	31.2	30.3	260.46	–	0.89	6.84
	250	–	–	48.4	379.21	–	0.34	0.68
	300	–	89.3	65.7	465.87	–	0.38	0.5
	500	–	156.2	118	525.43	–	0.05	–
	1000	–	290.2	137	1263.6	–	<0.05	–
	2125	–	334.8	112	1480.3	–	<0.05	–

Note: "–" stands for "not determined"; "ND" stands for "not detected".

Table 3. Concentrations of methane and rates of its oxidation in the upper 300 m of the Black Sea waters

Station 4					Station 5					Station 6				
Depth, m	Methane concentration, µl/l	CH_4 oxidation rate, nl/(l day)			Depth, m	Methane concentration, µl/l	CH_4 oxidation rate, nl/(l day)			Depth, m	Methane concentration, µl/l	CH_4 oxidation rate, nl/(l day)		
		to CO_2	to biomass + exometabolites	total			to CO_2	to biomass + exometabolites	total			to CO_2	to biomass + exometabolites	total
2	0.043	0.004	0.001	0.005	2	0.226	0.09	0.09	0.18	2	0.024	0.002	0.004	0.006
20	0.026	0.021	0.017	0.038	20	0.031	0.004	0.074	0.08	40	0.026	0.005	0	0.005
40	0.127	0.02	0.027	0.048	40	0.024	0.006	0.008	0.014	80	0.026	0.006	0	0.006
80	0.144	0.047	0.045	0.092	85	0.305	0.035	0.048	0.08	100	0.032	0.004	0.005	0.009
85	0.767	0.772	7.734	8.506	90	1.11	0.199	0.306	0.51	110	1.8	1.832	0.36	2.19
90	2.327	3.653	1.67	5.323	95	2.68	0.176	0.456	0.63	115	3.1	0.56	1.16	1.72
100	6.88	8.48	2.02	10.5	100	6.01	0.88	1.313	2.19	120	6.5	1.95	1.4	3.35
105	8.73	31.7	7.67	39.37	105	8.2	0.273	0.747	1.02	125	9.1	1.68	4.5	6.18
110	11.9	3.05	5.71	8.76	110	9.2	0.675	0.787	1.46	135	12.8	2.42	3.27	5.69
115	14.5	2.72	3.45	6.17	115	12.6	0.336	0.756	1.09	140	8.8	1.21	4.36	5.57
120	19.4	1.98	3.65	5.63	120	14.7	18.898	1.895	20.79	145	6.9	1.18	4.45	5.63
125	22.4	2.51	2.41	4.92	125	15.5	23.181	2.428	25.61	150	4.2	0.79	2.32	3.11
130	19	1.98	0.74	2.72	130	19.5	3.987	2.817	6.81	180	12.5	3.75	7.19	10.94
135	28	7.72	0.5	8.22	135	18.7	0.877	0.665	1.54	200	30.3	2.79	3.23	6.02
140	32.5	6.32	0.36	6.68	140	21.4	0.832	1.878	2.71	250	48.4	1.78	4.84	6.62
145	33.8	6.84	0.45	7.29	145	22.7	5.65	1.79	7.44	300	65.7	8.98	4.75	13.73
150	40.8	9.84	0.73	10.57	150	25.2	2.35	1.48	3.83					
155	44.7	1.39	4.72	6.11	155	26.3	6.4	18.79	25.19					
160	50.6	1.07	1.07	2.14	160	28.4	1.26	8.65	9.91					
180	65.4	45.2	4.22	49.42	180	35.4	2.16	2.71	4.87					
200	72.1	30.44	13.7	44.14	200	42.4	6.93	2.78	9.71					
225	116.8	11.55	117.71	129.26	225	49.8	11.01	0.44	11.45					
250	139.5	178.1	116.56	294.65	250	63.3	15.61	0.14	15.75					
300	136.6	213.86	223.42	437.28	300	78.8	19.53	0.35	19.88					

The problem of accurately distinguishing the autotrophic and heterotrophic fixation of CO_2 remains to be solved. The method of inhibition analysis suggested in our earlier work [21] has a number of limitations, especially when applied to the zone of transition from aerobic to anaerobic conditions. The rates of the heterotrophic assimilation of CO_2 calculated by Yu.I. Sorokin [13] from the results of measurement of the consumption of ^{14}C-labeled protein hydrolysate in the aerobic zone, where CO_2 is mainly fixed by heterotrophic microorganisms, are also fairly approximate. In our experiments, we determined the conditions of measurements (the amount of the ^{14}C-bicarbonate added and the incubation time) under which the dark assimilation of CO_2 was detected neither in the aerobic zone nor in the anaerobic zone at depths below 300 m (Table 2). Taking into account that the number of microorganisms in the chemocline increases no more than twofold and the rate of CO_2 assimilation more than tenfold, the estimated values of the dark assimilation of CO_2 in the upper layer of the anaerobic zone can be considered to indicate bacterial chemosynthesis. In this case, the rate of chemosynthesis may be overestimated due to the presence in the redox zone of not only chemoautotrophic thionic bacteria [20] but also litho-heterotrophic thiosulfate-oxidizing bacteria [22] (in these bacteria, CO_2 assimilation can substantially contribute to the biomass synthesis due to the oxidation of thiosulfate in the energy-dependent reaction of pyruvate carboxylation). Hence, CO_2 assimilation in these bacteria can be referred to as chemosynthesis, and the increase in the rate of CO_2 assimilation at depths of 120–140 m is undoubtedly associated with the activity of thionic bacteria, which can use not only oxygen but also other oxidized compounds, such as NO_3^- and Fe^{3+}, as electron acceptors. It is unlikely that chemoautotrophic nitrifying bacteria can substantially contribute to the production of organic matter due to the oxidation of ammonium or nitrites, since these bacteria are strict aerobes whose activity should be expected above the hydrogen sulfide–containing waters (at depths of 90–100 m), where the concentration of ammonium is low and that of nitrates is high (Table 2). However, just in these water horizons, CO_2 assimilation was very low (Table 2).

The intense assimilation of CO_2 at depths of 180–200 m, where the presence of oxygen is unlikely and the concentration of alternative oxidants is very small (Table 2), is unclear. It is known that methanogenic and sulfate-reducing microorganisms can implement anaerobic chemosynthesis using hydrogen as an electron donor. Analysis of the rates of sulfate reduction showed that their maximum values are at depths between 160 and 200 m (Table 2 and Fig. 6). At station 4, methanogenesis is also maximum at a depth of 180 m (Fig. 8). Hydrogen necessary for anaerobic chemosynthesis can be produced from degrading organic matter, whose presence at the depths mentioned was reported by some authors [23].

At the boundary between aerobic and anaerobic waters, organic matter can also be produced by bacterial photosynthesis [24, 25]. Using samples taken in the zone of interaction of oxygenated and hydrogen sulfide–containing waters, V.M. Gorlenko [26] isolated two strains of the brown sulfur bacterium *Chlorobium phaeovibrioides* which were capable of low-light anoxygenic photosynthesis. However, we failed to obtain convincing evidence for the important role of these bacteria in the sulfur and carbon cycles in the chemocline. Therefore, this problem needs further investigation. We may suggest that anoxygenic photosynthesis in deep waters of the Black Sea is characterized by pronounced seasonal activity.

Much attention in our investigations was given to the microbial oxidation of methane. As is evident from the data presented in Table 2, the methane concentration in the Black Sea waters begins to increase from depths of 90–100 m. Taking into account that methane can be oxidized only by microorganisms, the microbial population at the boundary between aerobic and anaerobic waters may play an important role in the oxidation of methane. Unfortunately, there is only limited information on the rates of methane oxidation in the chemocline [27, 28]. The data presented in this paper suggest that methane can be oxidized by different groups of microorganisms at depths of 90 to 300 m. The upper maximum of methane oxidation observed at depths between 100 and 140 m is probably due to the activity of the aerobic bacteria that are able to oxidize methane under anoxic conditions. At depths below 150 m, methane is oxidized anaerobically by a mechanism which is still far from being well understood. Presumably, this process can be implemented by methanogens via the inverse reaction of methanogenesis [29] or by the syntrophic associations of methanogenic and sulfate-reducing bacteria [30].

The integral rate of the dark assimilation of carbon dioxide at the boundary between aerobic and anaerobic waters at depths of 100–300 m (Table 4) was found to range from 115 to 207 mg C/(m^2 day) with the maximum at the boundary of cyclonic currents. According to the estimations of V.I. Vedernikov [31], the average monthly production of photosynthesis from April to October is 200–400 mg C/(m^2 day). Assuming that the dark assimilation of carbon dioxide is mainly due to the activity of lithotrophic microorganisms, the chemosynthetic bacterial production of OM in the zone of interaction of aerobic and anaerobic waters must comprise about 50% of the production of OM in the photic zone. The rates of the chemosynthetic production of OM estimated by us agree well with the data of Yu.I. Sorokin [11, 13] but are slightly less than the estimations of M.B. Gulin, who considered the dark assimilation of CO_2 as bacterial chemosynthesis and did not distin-

Table 4. Integral rates of microbial processes in the zone of interaction of aerobic and anaerobic waters (depths from 100 to 300 m) in the deep regions of the Black Sea

Station location	CO_2 assimilation rate, mg C/(m^2 day)	Sulfate reduction rate, mg S/(m^2 day)	Methane oxidation rate*, ml CH_4/(m^2 day)	Methane formation rate, ml CH_4/(m^2 day)
St. 4 (western halistatic region)	120	1230	25.8 (16.5)	0.80
St. 5 (between western and eastern halistatic regions)	207	545	2.3 (8.5)	0.52
St. 6 (eastern halistatic region)	115	490	1.4 (5.8)	0.23

* Parenthesized is the total methane content of waters (l/m^2) at depths between 100 and 300 m.

guish between the autotrophic and heterotrophic types of carbon dioxide fixation [8].

At the boundary between aerobic and anaerobic waters, OM can result from the fixation of not only carbon dioxide but also methane. As is evident from Table 3, up to 85% (40% on average) of the carbon atoms of the methane oxidized by methane-oxidizing bacteria are accumulated in their biomass or exometabolites. At station 4, where the rate of methane oxidation is maximum, about 10 ml CH_4/(m^2 day) must be spent on the production of OM (calculated for the 100–300 m depth interval, see Table 3). If we recalculate with respect to carbon, this value corresponds to 5.4 mg C/(m^2 day). Therefore, the OM produced from methane comprises less than 5% of the OM produced from carbon dioxide. In other words, the microbial oxidation of methane comprises an insignificant part of the productive processes at the boundary between aerobic and anaerobic waters.

Comparing the rates of methane oxidation and methane production suggests that methane that is oxidized at depths from 100 to 300 m was formed in deeper water horizons (Table 3).

The maximum rate of sulfate reduction (1230 mg S/(m^2 day)) was observed in the western halistatic region (station 4), and the minimum rate (490 mg S/(m^2 day)), in the eastern halistatic region (station 6). The average rate of hydrogen sulfide production amounted to 755 mg S/(m^2 day), or 276 g S/(m^2 year), showing a close agreement with the earlier data [7, 11, 13]. Relevant estimations indicate that sulfate reduction at depths between 100 and 300 m plays a decisive role in the production of hydrogen sulfide in the Black Sea.

A comparison of the rates of microbiological processes in the western and eastern halistatic regions at depths between 100 and 300 m showed that the rates of CO_2 fixation in these regions are close; however, the processes of sulfate reduction, methane formation, and methane oxidation are more intense in the western halistatic region (st. 4). This can be accounted for by the effect of intense riverine inflow in the western part of the Black Sea, which enhances primary productive processes in this part of the sea [32].

At depths between 100 and 300 m, the total methane content in the western halistatic region is almost threefold higher than in the eastern halistatic region (Table 3); this can be explained not only by the higher rate of methanogenesis here but also by the existence of a great number of bottom methane seeps on the northwestern shelf of the Black Sea [33]. The high rate of methane oxidation in the western halistatic region may be due to the higher methane content of waters in this region.

The data presented in this paper can be summarized as follows.

(1) At depths from 90 to 300 m in the deep-sea zone, the total population of archaea and other bacteria is maximum; archaea are present in both aerobic and anaerobic waters.

(2) The distribution of the dark CO_2 assimilation rate in the chemocline is described by a saw-tooth curve; the upper maximum of this rate corresponds to the lower boundary of the C-layer at depths of 120–140 m; its lower maximum, together with the maximum of the sulfate reduction rate, corresponds to a depth of 180–200 m; the integral rate of CO_2 assimilation at depths between 90 and 300 m ranges from 115 to 207 mg C/(m^2 day) and is maximum in the central part of the sea at the boundary of cyclonic currents.

(3) The distribution pattern of the methane oxidation rates at depths between 90 and 300 m suggests that this process occurs in both aerobic and anaerobic waters; the OM synthesized from methane at the boundary between aerobic and anaerobic waters comprises less than 5% of the OM synthesized from carbon dioxide.

(4) The integral rate of methane oxidation in the chemocline at depths from 90 to 300 m is an order of magnitude higher than that of methane formation, indicating the oxidation of methane formed in deeper water horizons.

(5) The distribution of sulfate reduction rates at depths from 90 to 300 m has two maxima, one of which corresponds to depths of 130–140 m (the lower boundary of the C-layer), and the other corresponds to depths between 160 and 200 m (the hydrogen sulfide–containing zone); the maximum rate of sulfate reduction (1230 mg S/(m^2 day)) is observed in the western halistatic region, and the minimum rate (490 mg S/(m^2 day)), in the eastern halistatic region; the average rate of hydrogen sulfide production is 755 mg S/(m^2 day) or 276 g S/(m^2 year).

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