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**EXPERIMENTAL ARTICLES**

# The Biogeochemical Cycle of Methane on the Northwestern Shelf of the Black Sea

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**Abstract**—Seasonal investigations of methane distribution and rates of its oxidation and generation in the water column and sediments of the Black Sea northwestern shelf were carried out within the framework of the interdisciplinary projects "European River–Ocean Systems" (EROS-2000, EROS-21) and "Biogenic Gases Exchange in the Black Sea" (BigBlack) in August 1995, May 1997, and December 1999. Experiments that involved the addition of $^{14}CH_3COONa$ and $^{14}CO_2$ to sediment samples showed the main part of methane to be formed from $CO_2$. Maximum values of methane production (up to 559 μmol/($m^2$ day)) were found in coastal sediments in summer time. In winter and spring, methane production in the same sediments did not exceed 3.6–4.2 μmol/($m^2$ day). The $\delta^{13}C$ values of methane ranged from –70.7 to –81.8‰, demonstrating its microbial origin and contradicting the concept of the migration of methane from cold seeps or from the oil fields located on the Black Sea shelf. Experiments that involved the addition of $^{14}CH_4$ to water and sediment samples showed that a considerable part of methane is oxidized in the upper horizons of bottom sediments and in the water column. Nevertheless, it was found that, in summer, part of the methane (from 6.8 to 320 μmol/($m^2$ day)) arrives in the atmosphere.

*Key words*: methane cycle, Black Sea, methanogenesis, bacterial oxidation of methane, daily methane production, seasonal dynamics, $\delta^{13}C$ of methane, methane flux to the atmosphere.

The rising level of hydrogen sulfide in the Black Sea and the anthropogenic contamination inducing asphyxiation of aquatic animals in the near-bottom water of the northwestern shelf has distracted researchers' attention from one more problem of considerable importance. Until recently, the efforts of microbiologists and biochemists were mainly aimed at the elucidation of the role of microorganisms in the sulfur cycle. However, it is well known that, under anaerobic conditions, bacteria form not only hydrogen sulfide but also other gases, first of all methane. It is formed by microbial communities that include both autotrophic and heterotrophic microorganisms. Autotrophic methanogens use molecular hydrogen to reduce $CO_2$ to methane, whereas heterotrophic methanogens produce methane from methyl groups of low-molecular-weight organic compounds [1].

As a greenhouse gas, methane ranks second only to carbon dioxide in importance. It is one of the main terminal products of the microbial transformation of organic matter under anaerobic conditions and frequently occurs in various ecosystems. It is currently considered that the main sources of atmospheric methane are swamps, rice paddies, and also freshwater and marine ecosystems. Methane emission from oceans and seas is believed to be less significant because the reduced gases formed in anaerobic sediments undergo oxidation on their way to the surface. The second part of the methane cycle, methane oxidation, also involves the activity of microorganisms, namely methanotrophic bacteria which produce carbon dioxide, cell biomass, and organic exometabolites. Recently, anaerobic oxidation of methane has been demonstrated, and the intensity of this process can exceed, under certain conditions, the intensity of aerobic oxidation of methane by methanotrophic bacteria.

In addition to contemporary methanogenesis under anaerobic conditions, there are two more potential methane sources on the northwestern shelf of the Black Sea: cold methane seeps [2] and oil fields at the Romanian coast.

The role of microorganisms in the methane cycle in seas, including the Black Sea, has as yet been poorly studied. The first data on the role of microorganisms in the methane cycle in the Black Sea were obtained in December 1980 during a voyage on board RV *Professor Shtokman.* During that expedition, distribution and geochemical activity of methanogens and methanotrophs in the sediments of the Bulgarian sector of the Black Sea were studied [3]. In 1991, we published a paper devoted to the role of methanotrophs in the oxidation of methane of methane seeps [2]. In the same year, a paper by American microbiologists was published reporting on the results of their studies of anaer-

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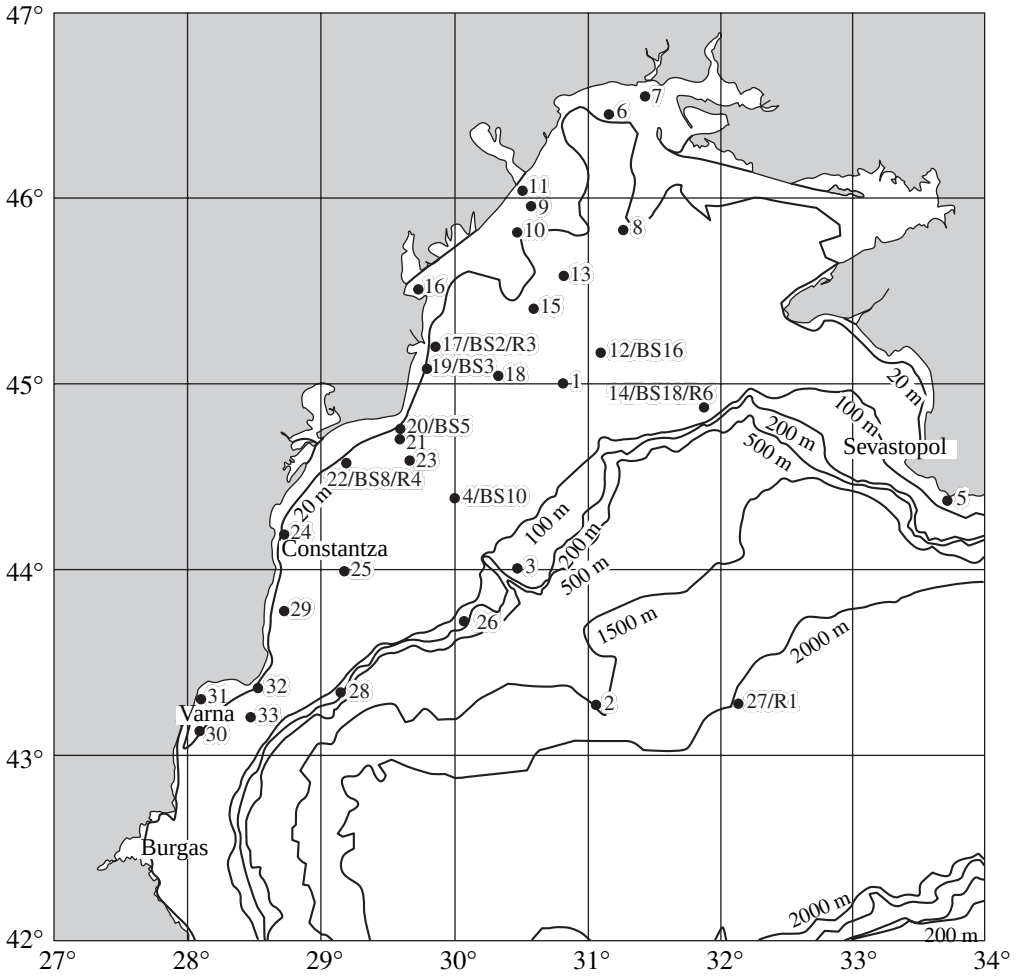


**Fig. 1.** Location of sampling stations. 1–33 are stations of the summer expedition (RV *Professor Vodyanitskii*, August 1995); BS2–BS18 are stations of the spring expedition (RV *Professor Vodyanitskii*, May 1997); R1–R6 are stations of the winter expedition (RV *Professor Vodyanitskii*, December 1999).

obic methane oxidation at one deep-sea station and one shelf station in the southern part of the Black Sea [4].

The main goal of the present work, which was carried out within the framework of the interdisciplinary projects "European River–Ocean Systems" (EROS-2000, EROS-21) and "Biogenic Gases Exchange in the Black Sea" (BigBlack), was to obtain quantitative estimates of the role of methanogens in the methane production in bottom sediments and to study the processes of aerobic and anaerobic methane oxidation in bottom sediments and the water column on the northwestern shelf of the Black Sea. The comparison of the rates of methane production and oxidation was to help elucidate the contribution of marine methane to the atmospheric pool of greenhouse gases.

The particular experimental tasks were as follows:

(1) Investigation of the distribution of methane in the water column and upper horizons of bottom sediments.

(2) Investigation of the distribution of methanotrophs and methanogens in the water column and bottom sediments.

(3) Determination of daily rates of methane production and consumption by short-term in situ incubation with radiolabeled compounds (($^{14}CO_2$, $^{14}CH_3OONa$, $^{14}CH_4$).

(4) Determination of the isotopic composition of methane carbon ($\delta^{13}C$, ‰).

(5) Calculation of the seasonal dynamics of methane production in bottom sediments.

(6) Calculation of methane fluxes from bottom sediments to the water column and atmosphere.

## MATERIALS AND METHODS

Materials for investigation were obtained in three complex international expeditions on board RV *Professor Vodyanitskii* (Institute of the Biology of Southern Seas, National Academy of Sciences of Ukraine, Sevas-

**Table 1.** Content of methane and rate of its oxidation in the water column at the northwestern shelf of the Black Sea

| Horizon, m | Methane content, μmol/l | Methane oxidation rate, nmol/(l day) | Methane portion oxidized to $CO_2$, % |
|---|---|---|---|
| **Station 12** (depth, 53 m) | | | |
| 0 | 0.022 | 0.22 | 70 |
| 30 | 0.027 | 0.13 | 70 |
| Near-bottom | 0.031 | 0.09 | 70 |
| **Station 1** (depth, 55 m) | | | |
| 0 | 0.027 | 0.045 | 40 |
| 30 | 0.036 | 0.178 | 42 |
| 50 | 0.040 | 0.223 | 38 |
| 55 | 0.045 | 0.357 | 13 |
| **Station 25** (depth, 55 m) | | | |
| 0 | 0.031 | 0.134 | 70 |
| 30 | 0.027 | 0.089 | 70 |
| 50 | 0.022 | 0.089 | 80 |
| Near-bottom | 0.045 | 1.340 | 33 |
| **Station 4** (depth, 68 m) | | | |
| 0 | 0.031 | 0.045 | 40 |
| 45 | 0.036 | 0.045 | 44 |
| 68 | 0.067 | 0.134 | 26 |
| **Station 14** (depth, 63 m) | | | |
| 10 | 0.022 | 0.357 | 70 |
| 30 | 0.134 | 0.357 | 80 |
| 40 | 0.245 | 1.116 | 70 |
| 50 | 0.156 | 0.982 | 40 |
| 60 | 0.246 | 1.339 | 60 |
| Near-bottom | 0.246 | 1.696 | 100 |

**Table 2.** Content of methane and rate of its oxidation in the coastal zone of the Black Sea

| Horizon, m | Methane content, μmol/l | Methane oxidation rate, nmol/(l day) | Methane portion oxidized to $CO_2$, % |
|---|---|---|---|
| **Station 16** (depth, 16 m) | | | |
| 0 | 0.313 | 1.786 | 50 |
| 10 | 0.335 | 2.188 | 57 |
| 12 | 0.201 | 0.357 | 100 |
| Near-bottom | 0.223 | 1.205 | 33 |
| **Station 20** (depth, 26 m) | | | |
| 0 | 0.223 | 3.214 | 40 |
| 8 | 0.134 | 2.679 | 33 |
| Near-bottom | 0.201 | 2.455 | 71 |
| **Station 22** (depth, 27 m) | | | |
| 0 | 0.054 | 0.045 | 70 |
| 10 | 0.058 | 0.134 | 75 |
| 20 | 0.170 | 0.268 | 86 |
| 25 | 0.080 | 0.179 | 81 |
| Near-bottom | 0.103 | 0.313 | 62 |
| **Station 32** (depth, 50 m) | | | |
| 0 | 0.045 | 1.429 | 48 |
| 20 | 0.045 | 1.339 | 41 |
| Near-bottom | 0.107 | 1.518 | 38 |

topol) in August 1995 (48th voyage), May 1997 (52nd voyage), and December 1999 (54th voyage). Investigations were carried out on the northwestern shelf of the Black Sea, including the open shelf, coastal zone, and the deltas of the Danube, Dnestr, and Dnepr Rivers.

Water and sediment samples were taken at 22 stations in August 1995, 16 stations in May 1997, and 3 stations in December 1999 (Fig. 1). In May 1997 and December 1999, the stations were the same as in August 1995. Sampling of bottom sediments was performed with the Boxcorer (Germany), Tripode (United States), and Multicorer (Germany) sediment samplers to a depth of 30–50 cm from the sediment surface. Water was sampled with a plastic near-bottom bathometer and a Rozett complex. Immediately after lifting, the sediments were put either in glass tubes or in 5-$cm^3$ plastic syringes, which were then sealed with butyl rubber stoppers. Water samples were poured (with 3-fold volume excess to avoid capture of air) in 30- and 100-$cm^3$ glass flasks (prewashed with the same water sample) and sealed with butyl rubber stoppers.

Analyses of the content of methane and acetate and alkalinity in the samples of water and sediments were carried out on board the ship immediately after sampling. The concentration of methane dissolved in water and bottom sediments was determined by the phase-equilibrium degassing method [5] on Khrom-5 and KhPM-4 gas chromatographs equipped with a flame ionization detector. The alkalinity reserve in water and bottom sediments was determined by titration using a standard Merck kit (Germany). Sediment waters were obtained using an INMI-2 press with pressure imposed on the membrane with compressed gas.

The cell numbers of methanogenic and methane-oxidizing bacteria were determined by the serial tenfold dilution method with inoculation of either anaerobic liquid mineral medium supplemented with $H_2$ + $CO_2$ or acetate [6] or aerobic liquid mineral medium supplemented with 5% methane added to the gas phase [7]. The growth of microorganisms was judged by chromatographically measuring the produced methane (methanogens) or carbon dioxide (methanotrophs). The total microbial number was determined on polycarbonate membrane filters (pore diameter, 0.2 μm) by the fluorescent method employing diamidino-4',6-phenyl-2-indole (DAPI) as the dye [8].

**Table 3.** Content of methane and rates of its production and oxidation in the bottom sediments of the Black Sea shelf

| Horizon, cm | Methane content, μmol/dm$^3$ | Methane production rate, nmol/(dm$^3$ day) | | Methane oxidation rate, nmol/(dm$^3$ day) | |
|---|---|---|---|---|---|
| | | total | from $CO_2$, % | total | to $CO_2$, % |
| **Station 1** (depth, 55 m) | | | | | |
| 0–3 | 0.313 | 0 | – | 7.232 | 66 |
| 4–5 | 0.402 | 11.47 | 100 | 2.143 | 100 |
| 7–20 | 0.714 | 20.45 | 95 | 0.938 | 100 |
| 20–30 | 0.670 | 10.22 | 100 | 1.696 | 100 |
| **Station 12** (depth, 53 m) | | | | | |
| 2–5 | 0.268 | 12.28 | 98 | 1.116 | 100 |
| 7–12 | 0.402 | 0.670 | 33 | 1.161 | 100 |
| 15–30 | 0.893 | 0.893 | 50 | 1.384 | 100 |
| **Station 4** (depth, 68 m) | | | | | |
| 0–3 | 0.446 | 0 | – | 1.295 | 100 |
| 3–5 | 0.580 | 2.455 | 64 | 0.580 | 100 |
| 12–17 | 0.446 | 10.05 | 79 | 0.491 | 100 |
| 24–30 | 0.536 | 20.09 | 94 | 0.714 | 100 |
| **Station 14** (depth, 63 m) | | | | | |
| 0–1 | 0.313 | 5.223 | 100 | 215.3 | 23 |
| 2–5 | 0.469 | 23.35 | 100 | 14.38 | 47 |
| 9–15 | 1.562 | – | – | 6.830 | 100 |
| 22–30 | 3.884 | 47.72 | 97 | 4.821 | 100 |

Determination of the rates of methane production and oxidation was carried out on board the ship at a temperature close to the in situ one by earlier described widely used methods [3, 6, 7, 9–12].

## RESULTS

The most detailed investigations of the distribution of methane and of the microbial processes of its production and oxidation were carried out in August 1995. Chromatographic analyses revealed methane in all samples of water (Tables 1 and 2) and bottom sediments (Tables 3 and 4). In the water column of the open shelf, the content of methane did not exceed tens of nanomoles per liter; usually, the lowest methane content was recorded in surface horizons (Table 1). A somewhat higher methane concentration was found only in the water at station 14, located in the zone of methane seeps. The methane flux in the atmosphere at station 14 could be recorded by lateral scanning, and large gas bubbles (with a high methane content) could be visually observed from a board the ship.

Higher methane concentrations (up to 335 nmol/l) were recorded in the water column at coastal stations (Table 2), especially at stations 16 and 20, located in the zone influenced by the Danube discharge (Fig. 1). At these stations, the maximum methane concentration occurred in surface water samples rather than in samples of near-bottom water. This phenomenon may be related to the arrival of a certain part of methane with the fresh Danube waters.

However, the main part of methane dissolved in seawater is formed in bottom sediments, which can be inferred from the comparison of methane content in the water column and bottom sediments both at the open shelf and coastal stations (Tables 1–4). In both ecosystems, the content of methane in the upper horizons of sediments exceeds its content in near-bottom water by at least an order of magnitude. Virtually at all stations, the methane content in bottom sediments significantly increases in the sediment thickness, reaching 1.3 mmol $CH_4$/dm$^3$ at some coastal stations. On board the ship, such sediments swelled and emitted gas bubbles (stations 19, 20, and 32, Table 4).

The results of our determinations of the methanogenesis rates were in good agreement with data on methane distribution. In the upper horizons (0–3 cm) of sediments of the open shelf, the conditions are aerobic and thus unfavorable for methanogens. Therefore, methanogenesis in such sediments was either undetectable (Table 3, st. 1, 4) or occurred at a very low rate, evidently in anaerobic microzones (Table 3, st. 14). In deeper horizons of shelf sediments, the methanogenesis rate increased (Table 3, st. 1, 4, 14).

An analogous pattern was observed in coastal sediments. However, the rate of methanogenesis here was

**Table 4.** Content of methane and rates of its production and oxidation in the coastal bottom sediments of the Black Sea

| Horizon, cm | Methane content, μmol/dm$^3$ | Methane production rate, nmol/(dm$^3$ day) | | Methane oxidation rate, nmol/(dm$^3$ day) | |
|---|---|---|---|---|---|
| | | total | from $CO_2$, % | total | to $CO_2$, % |
| **Station 7** (depth, 8 m) | | | | | |
| 0–1 | 1.518 | 12.28 | 91 | 15.80 | 91 |
| 2–5 | 3.304 | 32.81 | 97 | 27.90 | 97 |
| 6–12 | 4.732 | 89.51 | 98 | 41.52 | 100 |
| 12–30 | 12.95 | 267.9 | 100 | 144.2 | 100 |
| **Station 16** (depth, 16 m) | | | | | |
| 0–1 | 2.589 | 12.5 | 82 | 87.95 | 58 |
| 1–3 | 2.679 | 37.72 | 80 | 39.73 | 76 |
| 3–5 | 2.813 | 14.96 | 3 | 32.14 | 87 |
| 9–12 | 2.991 | 131.3 | 80 | 23.66 | 100 |
| 15–20 | 24.55 | 114.9 | 81 | 371.9 | 100 |
| 20–30 | 44.64 | 756.0 | 13 | 4017 | 100 |
| 40–55 | 89.29 | 369.9 | 48 | 7450 | 100 |
| **Station 19** (depth, 20 m) | | | | | |
| 0–2 | 23.21 | 43.75 | 34 | 290.2 | 65 |
| 3–5 | 66.96 | 652.2 | 5 | 4442 | 95 |
| 11–15 | 817.9 | 511.8 | 14 | 23000 | 100 |
| 20–30 | 1004 | 3785 | 2 | 318.9 | 100 |
| **Station 20** (depth, 25.6 m) | | | | | |
| 0–1 | 2.232 | – | – | 176.3 | 55 |
| 1–2 | 4.464 | 22.32 | 82 | 180.8 | 66 |
| 3–7 | 102.7 | 56.92 | 85 | 2825 | 100 |
| 12–17 | 848.2 | 182.6 | 69 | 178600 | 100 |
| 25–30 | 1339 | 2108 | 4 | 133900 | 100 |
| **Station 32** (depth, 50 m) | | | | | |
| 0–2 | 4.464 | 40.18 | 92 | 920.1 | 20 |
| 3–5 | 8.929 | 157.6 | 99 | 163.2 | 90 |
| 15–20 | 267.9 | 1684 | 100 | 1403 | 100 |
| 25–30 | 446.4 | 6106 | 93 | 2274 | 100 |

also rather high in the surface layer (0–1 cm) of sediments, immediately under a thin oxidized film (Table 4). In deeper horizons, the methanogenesis rate increased, reaching several μmoles per dm$^3$ in lower horizons (20–30 cm) of coastal sediments (Table 4).

In sediment samples taken at the open shelf, as well as in most samples of coastal sediments, the main part of methane was usually formed via carbon dioxide reduction (Tables 3 and 4). However, in some sediment samples from coastal stations (st. 16 and 20) and in all samples from station 19, the major part of methane was formed from acetate, whose concentration in the pore waters at station 19 reached 350 μmol/dm$^3$ (Table 4).

The most vivid demonstration of the intensity of microbial methane production in various sediments of the northwestern shelf in August 1995 is provided by

**Table 5.** Daily production of methane (μmol under 1 m$^2$ per day) and methane content (mmol under 1 m$^2$) in coastal sediments and sediments of the open shelf (0–30 cm)

| Station no. | Depth, m | Methane production | Methane content |
|---|---|---|---|
| **Coastal stations** | | | |
| 7 | 8.0 | 53.0 | 2.63 |
| 16 | 16.0 | 93.0 | 6.77 |
| 19 | 20.0 | 559.0 | 200.0 |
| 20 | 25.6 | 213.0 | 266.0 |
| 32 | 50.0 | 379.0 | 58.5 |
| **Open shelf stations** | | | |
| 1 | 55.0 | 4.3 | 0.19 |
| 12 | 53.0 | 1.0 | 0.20 |
| 4 | 68.0 | 3.4 | 0.16 |
| 14 | 63.0 | 7.2 | 0.66 |

**Table 6.** The number of methanogenic and methane-oxidizing (methanotrophic) bacteria in near-bottom water and bottom sediments at the northwestern shelf of the Black Sea

| Station no., horizon (cm) | | Methanogens, cells/cm$^3$ | | Methanotrophs, cells/cm$^3$ | |
|---|---|---|---|---|---|
| | | August | May | August | May |
| **Coastal stations** | | | | | |
| 17 | Near-bottom water | 0 | 0 | 1000 | 100 |
| | 0–1 | 10 | 0 | 1000 | 1000 |
| | 1–5 | 100 | 10 | 100 | 100 |
| | 10–15 | 100 | 100 | 0 | 0 |
| | 25–30 | 1000 | 100 | 0 | 0 |
| 19 | Near-bottom water | 0 | 0 | 10000 | 1000 |
| | 0–1 | 0 | 0 | 10000 | 10000 |
| | 1–5 | 1000 | 100 | 1000 | 1000 |
| | 10–15 | 10000 | 100 | 0 | 0 |
| | 25–30 | 10000 | 1000 | 0 | 0 |
| 20 | Near-bottom water | 0 | 0 | 1000 | 100 |
| | 0–1 | 100 | 0 | 10000 | 1000 |
| | 1–5 | 1000 | 100 | 100 | 100 |
| | 10–15 | 1000 | 1000 | 0 | 0 |
| | 25–30 | 10000 | 1000 | 0 | 0 |
| **Open shelf stations** | | | | | |
| 4 | Near-bottom water | 0 | 0 | 10 | 10 |
| | 0–1 | 0 | 0 | 100 | 100 |
| | 1–5 | 0 | 0 | 10 | 10 |
| | 10–15 | 10 | 10 | 0 | 0 |
| | 25–30 | 100 | 10 | 0 | 0 |
| 12 | Near-bottom water | 0 | 0 | 10 | 10 |
| | 0–1 | 0 | 0 | 100 | 100 |
| | 1–5 | 0 | 0 | 10 | 10 |
| | 10–15 | 10 | 10 | 0 | 0 |
| | 25–30 | 10 | 10 | 0 | 0 |
| 14 | Near-bottom water | 0 | 0 | 100 | 10 |
| | 0–1 | 0 | 0 | 1000 | 100 |
| | 1–5 | 100 | 0 | 100 | 10 |
| | 10–15 | 10 | 10 | 0 | 0 |
| | 25–30 | 10 | 10 | 0 | 0 |

values of methane production under 1 m$^2$. As can be seen from the data of Table 5, the methane production in summer was two orders of magnitude higher in coastal sediments than in sediments of the open shelf.

The fact that in summer the highest rate of methanogenesis (Table 4) and the highest methane production under 1 m$^2$ (Table 5) was observed in coastal sediments (a similar tendency was earlier observed for sulfate reduction [11]) is accounted for by the arrival of a large amount of allochthonous organic matter arriving with the Danube waters and by the extremely high primary production in the coastal zone of the northwestern shelf of the Black Sea [13].

The results of our determinations of the cell numbers of bacteria involved in the methane cycle (Table 6) are in good agreement with data on methane production in various zones of the northwestern shelf (Table 5). The highest population density of methanogens (up to 10 thousand cells/cm$^3$ sediment) was found in lower horizons of coastal sediments in summer (Table 6, st. 19 and 20). The cell number of methanogens in the sedi-

**Table 7.** Seasonal variations in near-bottom temperature (*T*, °C), methane content (*C*, mmol under 1 $m^2$ (0–30 cm)), and methane production (*P*, μmol under 1 $m^2$ per day (0–30 cm))

| Station no. | August | | | December | | | May | | |
|---|---|---|---|---|---|---|---|---|---|
| | *T* | *C* | *P* | *T* | *C* | *P* | *T* | *C* | *P* |
| **Coastal stations** | | | | | | | | | |
| 17 | 23.9 | 0.54 | 7.7 | 10 | 0.06 | 4.3 | 6.5 | 0.1 | 0.7 |
| 19 | 24.3 | 200 | 559 | – | – | – | 6.5 | 66 | 1.4 |
| 20 | 23.9 | 266 | 213 | – | – | – | 6.7 | 36 | 3.6 |
| 22 | 24.0 | 0.18 | 98 | 9.8 | 0.05 | 0.94 | 6.5 | 0.08 | 3.5 |
| **Open shelf stations** | | | | | | | | | |
| 4 | 18.6 | 0.16 | 3.4 | – | – | – | 6.2 | 0.28 | 2.3 |
| 12 | 19.4 | 0.20 | 1.0 | – | – | – | 6.6 | 0.16 | 2.0 |
| 14 | 17.1 | 0.66 | 7.2 | 8.0 | 0.21 | 0.56 | 8.0 | 0.28 | 0.11 |

ments of the open shelf even in summer usually did not exceed 100 cells/$cm^3$ sediment (Table 6, st. 4, 12, 14).

During the expeditions in May 1997 and December 1999, we repeatedly determined the cell numbers of methanogens and methanotrophs (Table 6) and also repeated the determinations of methane production rate and the content of methane in sediments (Table 7).

The cell number of methanogens and the content of methane in the upper horizons of coastal sediments decreased in the winter–spring period by an order of magnitude, and daily methane production decreased by two orders of magnitude (Tables 6 and 7). The summer and spring patterns of methane distribution along the sediment vertical profile are shown in Fig. 2.

Table 8 presents data on the isotopic composition of methane carbon in samples that we took in December 1980 [3], August 1995, and May 1997. These data unequivocally demonstrate the microbial origin of methane in the sediments of the western and northwestern shelves of the Black Sea.

**Table 8.** Isotopic composition of methane ($\delta^{13}C$, ‰) in bottom sediments of the Black Sea

| Station no. | Depth, m | Sediment horizon, cm | $\delta^{13}C$, ‰ |
|---|---|---|---|
| **December 1980*** | | | |
| 590 | 59 | 140–160 | –78.4 |
| 545 | 1620 | 100–120 | –81.8 |
| **August 1995** | | | |
| 32 | 50 | 20–30 | –75.1 |
| **May 1997** | | | |
| 3 | 20 | 30–40 | –70.7 |
| 5 | 26 | 30–40 | –77.7 |
| 13 | 11 | 20–35 | –71.7 |

* Data for bottom sediments of Bulgarian shelf [3].

The ubiquitous occurrence of methane in the water column and bottom sediments of the northwestern shelf of the Black Sea (Tables 1–4) provides the conditions for the activity of methane-oxidizing microorganisms. Under the aerobic conditions of the water column, the main factor limiting methanotroph development is most probably the concentration of dissolved methane. Therefore, the rate of methane oxidation is the lowest in the water column of the northern part of the open shelf (Table 1). As pointed out above, the methane concentration in the water at station 14 is increased due to the arrival of methane from cold seeps; therefore, the methane oxidation rate here is notably higher than at other stations of open shelf (Table 1, st. 14).

The higher methane concentrations in the water at coastal stations provide more favorable conditions for the activity of methanotrophs as compared to the conditions at the open shelf (Table 2). After short-term incubation, virtually in all of the samples studied labeled carbon of methane was found both in carbon dioxide and in the organic fraction composed of methanotroph biomass and exometabolites [12].

**Table 9.** Daily methane production (DP) and consumption in sediments ($DC_s$) and water column ($DC_w$) and minimal flux of methane from sediments to the water column (S–W) and from water column to the atmosphere (W–A), μmol/($m^2$ day)

| Station no. | Depth, m | DP | $DC_s$ | $DC_w$ | S–W | W–A |
|---|---|---|---|---|---|---|
| 22 | 27 | 98 | 1.0 | 4.5 | 97 | 92.5 |
| 25 | 55 | 90 | 1.0 | 9.0 | 89 | 80 |
| 26 | 145 | 57 | 0.5 | 19 | 56.5 | 37.5 |
| 28 | 120 | 40 | 4.0 | 17 | 36 | 19 |
| 29 | 50 | 90 | 3.5 | 10 | 86.5 | 76.5 |
| 30 | 21 | 94 | 11 | 5.0 | 83 | 78 |
| 32 | 50 | 711 | 331 | 59 | 380 | 321 |

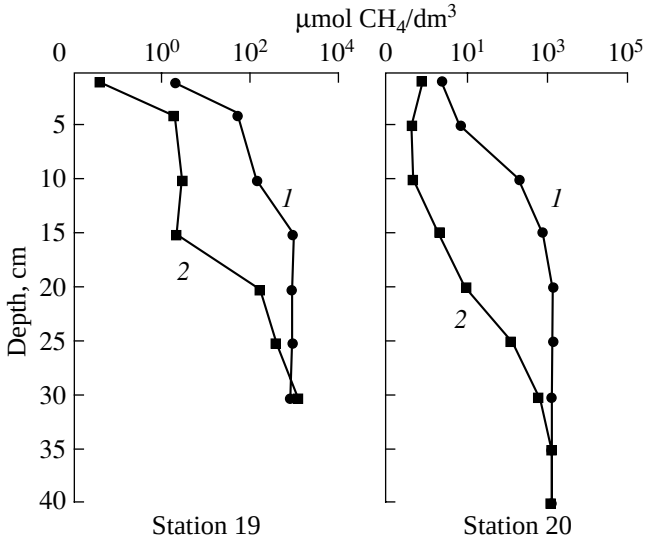


**Fig. 2.** Methane content in sediments in (*1*) August 1995 and (*2*) May 1997.

Still higher rates of methane oxidation were found in bottom sediments of the open shelf (Table 3), especially in coastal sediments rich in methane (Table 4). Methane oxidation occurred both in the upper horizons of sediments, contacting the aerobic water column, and in the strictly anaerobic sediments to the maximum depth of sampling (about 55 cm from the sediment surface) (Table 4, st. 16). A fact attracting attention is that the incorporation of labeled carbon of methane into biomass and exometabolites was found only in the uppermost horizons of coastal sediments, in which the penetration of oxygen from near-bottom water is feasible. In deeper horizons, all of the label was found in carbon dioxide, suggesting waste oxidation of methane not accompanied by an increase in the microbial biomass.

## DISCUSSION

As follows from the data of Tables 4 and 5, the highest methane concentrations in bottom sediments of the Black Sea northwestern shelf were found in the shallow-water sediments in the zone of the Danube delta and in the sediments of the Bulgarian shelf (Fig. 1, st. 32). In sediments of the near-Danube zone, industrially exploited oil fields are located. Thus, the methane found in the sediments of this zone can, theoretically, be both of a migrational and microbial diagenetic origin.

However, all our results testify to the microbial diagenetic origin of this methane. First, we revealed the wide distribution of methanogens in sediments of Black Sea shelf (Table 6). Second, experiments with short-term incubation of sediment samples with $^{14}CH_3COOH$ and $^{14}CO_2$ showed that the highest concentrations of methane occur in sediments characterized by the highest rates of methanogenesis (Tables 3 and 4). Third, the methanogenesis rate in coastal sediments exhibits seasonal dynamics, with maximum values in August and considerably lower values in May and December (Table 7). The content of methane in upper horizons of sediments shows the same seasonal dynamics (Fig. 2), and this dynamics cannot be explained within the framework of the migrational concept of methane origin. Fourth, data on the isotopic composition of the methane of bottom sediments (Table 8) also confirm the microbial origin of methane.

In addition to methanogenesis, active processes of methane oxidation occur in the studied sediments and waters (Tables 1–4). Based on data on the rates of methane production and oxidation, we calculated daily methane fluxes from sediments to the water column and from the water column to the atmosphere. It can be seen from data in Table 9 that, in spite of the active methane consumption by methanotrophic bacteria, a considerable part of the methane formed in sediments reaches the atmosphere to contribute to the atmospheric pool of greenhouse gases.

It should be noted that our estimates of methane fluxes to the atmosphere (Table 9) proved to be in a good agreement with the results of calculations performed by researchers from the Institute of Atmospheric Chemistry (Germany) [14], who worked in the same region in the summer of 1995 and determined the methane flux to the atmosphere based on the comparison of methane concentrations in the lower layer of the atmosphere and the upper horizon of the water column. The close similarity of the two estimates obtained by two very different methods demonstrates that our method based on the experimental determination of the rates of methane production and consumption can be successfully used for calculation of methane fluxes from marine ecosystems.

Thus, seasonal investigations of the activity of microorganisms involved in the methane cycle in the water column and bottom sediments of the northwestern shelf of the Black Sea revealed that in bottom sediments the major part of methane is formed from $CO_2$; the maximum rates of methane production (up to 559 μmol/(m$^2$ day)) were found in coastal sediments in summer time. In winter and spring, methane production in the same sediments did not exceed 3.6–4.2 μmol/(m$^2$ day). The $\delta^{13}C$ values of methane ranged from –70.7 to –81.8‰, demonstrating its microbial origin and contradicting the concept of the migration of methane from cold seeps or from the oil fields located on the Black Sea shelf. A considerable part of methane is oxidized in the upper horizons of bottom sediments and in the water column. Nevertheless, it was found that, in summer, part of the methane (from 19 to 320 μmol/(m$^2$ day)) arrives in the atmosphere (Table 9).

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