## **EXPERIMENTAL** = ARTICLES

# A Microbiological Study of an Underground Gas Storage in the Process of Gas Extraction

A. E. Ivanova<sup>1</sup>, I. A. Borzenkov, A. L. Tarasov, E. I. Milekhina, and S. S. Belyaev

Winogradsky Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117312 Russia Received June 5, 2007

Abstract—The numbers of microorganisms belonging to ecologically significant groups and the rates of terminal microbial processes of sulfate reduction and methanogenesis were determined in the liquid phase of an underground gas storage (UGS) in the period of gas extraction. The total number of microorganisms in water samples from the operation and injection wells reached  $2.1 \times 10^6$  cells/ml. Aerobic organotrophs (including hydrocarbon- and oil-oxidizing ones) and various anaerobic microorganisms (fermenting bacteria, methanogens, acetogens, sulfate-, nitrate-, and iron-reducing bacteria) were constituent parts of the community. The radioisotopic method showed that, in all the UGS units, the terminal stages of organic matter decomposition included sulfate reduction and methanogenesis, with the maximal rate of these processes recorded in the aqueous phase of above-ground technological equipment which the gas enters from the operation wells. A comparative analysis by these parameters of different anaerobic ecotopes, including natural hydrocarbon fields, allows us to assess the rate of these processes in the UGS as high throughout the annual cycle of its operation. The data obtained indicate the existence in the UGS of a bacterial community that is unique in its diversity and metabolic capacities and able to make a certain contribution to the geochemistry of organic and inorganic compounds in the natural and technogenic ecosystem of the UGS and thus influence the industrial gas composition.

Key words: underground gas storage, microflora, anaerobic, sulfate reduction, methanogenesis.

**DOI:** 10.1134/S0026261707040121

Until now, no due attention has been given to the study of microflora in underground gas storages. At the same time, many problems arising in the process of their operation (stratal sealing, corrosion of equipment, degraded quality of hydrocarbon products, environmental pollution) may, to a certain degree, be connected with the vital activity of microorganisms. In the framework of the solution of the general task of studying the possible influence of the activity of microorganisms on the productive stratum and UGS equipment, we were the first in this country to conduct a complex study of a UGS in the period of gas injection [1]. That study showed a significant population density of viable microorganisms of different physiological groups in stratal and associated waters, as well as a high rate of modernprocesses of sulfate reduction and methanogenesis in the liquid phase of the individual units of the UGS technological system.

The present study was designed to study the distribution and geochemical activity of microorganisms in the period of gas extraction from the storage.

### MATERIALS AND METHODS

A UGS set up on the basis of an exhausted gas field was studied. A general characterization of this facility was given earlier [1].

Samples of the liquids carried away from the stratum with the gas represented a mixture of waters from all the operation wells of the corresponding gas distribution site (GDS). The total numbers of microorganisms in the samples obtained from the UGS were determined by counting the erythrosin-stained cells on filters according to the technique proposed by Romanenko and Kuznetsov [2]. The medium composition, the methods for enumerating microorganisms of separate physiological groups and for determining the rates of microbial processes, as well as the analytical methods used in this research, were described in detail earlier [1].

#### RESULTS AND DISCUSSION

The waters from the GDS explored had a low mineral content (360–3400 mg/l) and a near-neutral pH (6.25–6.98). All the samples contained sulfate ions, from several milligrams to 65–100 mg/l. Noteworthy features include the presence of ammonium and nitrate

<sup>&</sup>lt;sup>1</sup> Corresponding author; e-mail: aivan@newmail.ru.

**Table 1.** Number of aerobic bacteria in the water samples from the GDS in the period of gas extraction

no.	Sampling site	Organotroph number, cells/ml					
	Sampling site	nonspe- cific	hydrocarbon- oxidizing	oil- oxidizing			
	Upper p	productive	horizon				
1	GDS 1, sump	$9.9 \times 10^{4}$	$10^{3}$	$10^{3}$			
2	GDS 2, oil trap	$1.5 \times 10^{4}$	$10^{3}$	$10^{3}$			
3	GDS 3, separator	$2.4 \times 10^{4}$	$10^{2}$	$10^{3}$			
4	GDS 4, sump	$1.6 \times 10^{3}$	$10^{3}$	10			
5	GDS 5, separator	$1.1 \times 10^{4}$	$10^{3}$	$10^{3}$			
6	GDS 6, separator	$1.6 \times 10^{3}$	$10^{3}$	$10^{3}$			
7	GDS 7, separator	$2.6 \times 10^{4}$	$10^{2}$	$10^{2}$			
8	GDS 8, sump	$7.8 \times 10^{3}$	$10^{3}$	$10^{3}$			
9	GDS 9, sump	$4.0 \times 10^{2}$	$10^{3}$	$10^{3}$			
10	GDS 10, separator	20	$10^{2}$	$10^{3}$			
11	GDS 11, separator	$9.4 \times 10^{2}$	$10^{3}$	10			
12	GDS 12, separator	$4.0 \times 10$	$10^{2}$	$10^{3}$			
13	GDS 13, sump	$7.2 \times 10^{4}$	$10^{3}$	ND			
Lower productive horizon							
14	GDS 1, separator	20	10	$10^{3}$			
15	GDS 2, separator	$5.3 \times 10^4$	$10^{2}$	ND			
16	GDS 3, sump	$1.6 \times 10^{4}$	$10^{2}$	ND			

Note: ND stands for "no data."

ions, attaining a concentration of 10.0–12.0 and 29.0–49.0 mg/l, respectively, as well as the very low hydrocarbonate content (it was absent in most samples, and its maximum content did not exceed 162 mg/l). Along with this, acetate, whose amount varied between 125 and 1250 mg/l, was revealed in all the samples, and divalent iron (50–500 mg/l) was present in most of the samples. In a number of cases, these two components taken together made a more tangible contribution to the mineral content of the samples than all the rest taken together. In addition, the samples from all the GDSs exhibited a high content of methanol (from 600 mg/l in sample 16 to 12000 mg/l in samples 8 and 13, and even 14500 mg/l in sample 11), which is used for well treatment to prevent gas hydrate formation.

The discovery of a high number of aerobic microorganisms—nonspecific organotrophs (up to  $9.9 \times 10^4$  cells/ml) and hydrocarbon- and oil-oxidizing bacteria (up to  $10^3$  cells/ml)—was unexpected in the operation well zone, which is actually a strictly anaerobic ecotope (Table 1). The question of their functioning, role in the community, and mechanisms of survival under conditions of oxygen deficiency needs additional consideration. It may be suggested that, under the UGS

conditions, aerobic bacteria maintain their vital activity and a high level of population density owing to their flexible metabolism and the capacity of using alternative electron acceptors. This may be evidenced by the fact that most (85–97%) aerobic heterotrophs grown in BHIA medium and Raymond medium with oil respired anaerobically with nitrate and/or Fe<sup>3+</sup>.

The analysis of the liquids carried out by the operation wells (averaged samples from GDSs) showed the presence of diverse anaerobic microflora in them. Sulfate-reducing, acetogenic, and denitrifying bacteria appeared to be the groups that were the most wide-spread and numerically dominant (Table 2).

Sulfate-reducing bacteria were revealed in all the samples; their number determined on medium with lactate varied within a wide range, from single cells to 10<sup>5</sup> cells/ml. Representatives of various metabolic types of this group of microorganisms, including those decomposing fatty acids, lower alcohols (methanol, ethanol), and aromatic substrates (phenol, benzoate, cyclohexane carboxylate), were prevalent in the operation well waters. Hydrogen sulfide formation on most of these substrates, as well as on medium with lactate, began as early as after two or three days of cultivation. H<sub>2</sub>S accumulation also occurred in samples of stratal water supplemented with sulfate but not with a carbon source, which is due to the fact that the waters obtained from the GDSs contained organic compounds supporting the process of biogenic sulfate reduction. Nevertheless, despite the abundance of organic matter of a hydrocarbon nature, sulfate reduction was in most cases stimulated (to different degrees) by the introduction of additional growth substrates.

The number of homoacetogenic bacteria, enumerated on medium with a hydrogen–carbon dioxide mixture, ranged from single cells to tens of thousands of cells/ml (Table 2). In the GDS 1 and GDS 13 samples, representing the upper productive horizon, these microorganisms were absent. Wide distribution of methanolutilizing acetogens was also found in the stratal waters. It may be suggested that methanol, widely employed at the UGS as a hydrate formation inhibitor, is an important substrate sustaining the activity of acetogenic microflora in situ.

As seen from Table 2, denitrifying microorganisms were abundant in water samples obtained from the operation wells. Their number reached 10³–10⁴ cells/ml. Acetate, constantly revealed in stratal waters and likely to be the end or an intermediate metabolic product in many physiological groups of microorganisms, and succinate, known as one of the main fermentation intermediates, are equally favorable substrates for denitrifiers. We believe that the denitrifiers in the UGS are facultatively anaerobic bacteria, since the process of nitrate reduction occurred both in a prereduced medium and in a medium containing oxygen as a constituent part of the gas phase.

Table 2. Number of anaerobic microorganisms (cells/ml) in the GDS waters in the period of gas extraction

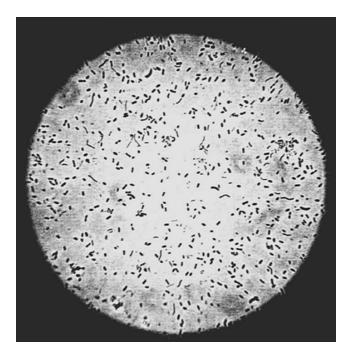
			U	,		1	C					
	Microorganism number, cells/ml											
no.	iron- reducing	sulfate- reducing	acetogens	methanogens			fermenters	denitrifiers				
	Cultivation substrate											
	acetate	lactate	$H_2 + CO_2$	$H_2 + CO_2$	acetate	methanol	ethanol	acetate	succinate			
	Upper productive horizon											
1	Single cells	$10^{2}$	0	0	Single cells	0	Single cells	$10^{2}$	$10^{2}$			
2	0	Single cells	$10^{4}$	Single cells	Single cells	0	$10^{2}$	$10^{4}$	$10^{3}$			
3	0	$10^{3}$	$10^{2}$	Single cells	Single cells	Single cells	$10^{2}$	$10^{2}$	$10^{3}$			
4	0	$10^{3}$	Single cells	0	Single cells	0	$10^{4}$	10	10			
5	0	$10^{5}$	$10^{4}$	Single cells	0	Single cells	ND	$10^{3}$	$10^{4}$			
6	Single cells	$10^{4}$	$10^{3}$	0	Single cells	0	10	$10^{2}$	$10^{4}$			
7	0	$10^{5}$	$10^{4}$	Single cells	0	Single cells	$10^{3}$	10	$10^{3}$			
8	0	10	$10^{2}$	0	Single cells	Single cells	10	$10^{4}$	$10^{4}$			
9	10	$10^{4}$	Single cells	$10^{2}$	Single cells	Single cells	Single cells	$10^{2}$	$10^{2}$			
10	Single cells	$10^{3}$	10	$10^{3}$	Single cells	0	Single cells	10	$10^{2}$			
11	0	$10^{5}$	10	0	0	0	10	$10^{3}$	$10^{3}$			
12	0	$10^{4}$	$10^{3}$	Single cells	Single cells	Single cells	0	$10^{2}$	$10^{2}$			
13	0	$10^{4}$	0	0	0	0	$10^{3}$	$10^{3}$	$10^{3}$			
Lower productive horizon												
14	0	$10^{2}$	$10^{2}$	Single cells	Single cells	Single cells	10	$10^{2}$	ND			
15	0	$10^{2}$	$10^{2}$	0	Single cells	0	ND	Single cells	ND			
16	$10^{2}$	$10^{2}$	$10^{4}$	Single cells	Single cells	Single cells	ND	$10^{3}$	ND			

Table 3. Total count of bacterial cells in the samples from GDS in the period of gas extraction from UGS

Sample no.	Sampling site	Cell number, thousand/ml	Sample no.	Sampling site	Cell number, thousand/ml
1	Sump	47	9	Sump	396
2	Oil trap	28	10	Separator	162
3	Separator	114	11	Separator	49
4	Sump	59	12	Separator	2097
5	Separator	370	13	Sump	63
6	Sump	459	14	Separator	48
7	Separator	305	15	Separator	8
8	Sump	357	16	Sump	600

Methanogenic bacteria were widespread across the stratum and were revealed in the samples obtained from all GDSs, except GDS 11 and GDS 13, representing the upper productive horizon. Along with the autotrophic bacteria of this physiological group, aceticlastic and methylotrophic methanogenic archaea were revealed. The number of methanogens varied from single cells to hundreds and thousands of cells per 1 ml of associated waters, which is typical of well-developed stratal biocenoses of natural hydrocarbon fields.

Direct count provides a general idea of the population density of microorganisms in an ecological niche. Judging from the results of the microscopic count of bacteria in averaged samples from GDSs, the number of microorganisms varied between  $8 \times 10^3$  (sample 5) and  $2.1 \times 10^6$  cells/ml (sample 12); no correlation was observed between the microbial number and the nature of the sampling site (separator, oil trap, or sump) (Table 3).



Micrograph of the surface of a membrane filter after filtration of 1 ml of water from sample no. 12.

The specimens stained with erythrosin revealed microorganism cells of different shapes and sizes. The results of inoculation of selective nutrient media enabled us to preliminarily identify some of the morphotypes. Thus, large bean-shaped cells dominated the enrichment cultures of acetogens. Thin, long, slightly curved rods predominated in the enrichment cultures of methanogens. Vibrioid shape was considered indicative of a sulfate-reducer phenotype.

The figure shows a micrograph of the surface of the filter through which an aliquot of sample no. 12 with the maximal number of microorganisms was filtered. The typical morphological types of the bacterial cells inhabiting the GDS 12 liquid phase and resembling the bacteria of different physiological groups can be seen. All these morphotypes are seen to be present in the sample in considerable amounts.

The direct count of microorganisms on filters (Table 3) and the enumeration of the total number of cultivated bacteria in selective nutrient media (Tables 1, 2) yielded, in a number of cases, values of the same order of magnitude (samples nos. 1, 2, 4, 5, 7, 13). This, together with the morphological patterns observed, allows us to believe that the spectrum of the microorganisms studied by us mainly corresponds to the general biodiversity in the associated waters of GDSs at the stage of gas extraction.

Along with the high population density of sulfatereducing and methanogenic microorganisms, modern processes of sulfate reduction and methane formation were recorded by the radioisotopic method in the averaged samples of the GDS waters. The sulfate reduction rate was 0.0– $67.5 \,\mu g \, S^2$ –per  $1 \, l$  of water daily (Table 4). The rate of mesophilic methanogenesis varied from  $0.1 \, to \, 17.6 \, \mu l$  of methane per  $1 \, l$  daily. In most samples, the source of the newly formed methane was acetate (Table 5). Thus, at the stage of gas extraction, methanogenesis and sulfate reduction, which were virtually equal in rate, were the terminal processes of biodegradation of organic matter in stratal waters sampled from the operation wells.

Theoretically speaking, in most of the water samples studied, the sulfate content (Table 4) may have limited microbial sulfate reduction: a concentration of less than 3 mM is conventionally considered as limiting [3]. To reveal the factors influencing sulfate reduction, we studied the effect of exogenous sulfate on the rate of the process in question in isolated samples of associated waters from all the GDSs of the UGS. The results are shown in Table 4. In most samples (11 of 16), the sulfate reduction rate increased (the maximal and minimal positive effects were 11.7- and 1.5-fold) after the introduction of 3 mM  $SO_4^{2-}$ . However, no stimulation occurred in the remaining samples; on the contrary, a slight inhibitory effect was noted. This was observed in samples with different initial sulfate contents, which is indicative of the more complex dependence of the process rate on several factors simultaneously. In particular, one cannot rule out the presence in the UGS stratal waters of other sulfur compounds and elemental sulfur, which, along with sulfate, could sustain the sulfidogenic activity of the microbial group in question. In addition, the deficiency of available electron donors may exert a negative effect on the development of sulfate reduction in the operation wells, as evidenced by the stimulation of the process by various organic substrates.

Another unique feature of the hydrochemical composition of the GDS waters, already noted above, is the extremely low content of carbonate, which was revealed only in two of the water samples examined (samples nos. 2 and 12) at concentrations of 21 and 162 mg/l, respectively. Acetate was invariably present at significant concentrations in the waters examined (Table 5). Carbon dioxide and acetate are known to be the most important substrates for the microbial process of methanogenesis. That is why we thought it necessary to clarify the possibility of stimulating microbial methanogenesis by the addition of hydrocarbonate to the samples.

According to the results obtained, the virtually complete absence of substrate for autotrophic methanogenesis is an important (but apparently not the only) factor limiting the development of the anaerobic process in question in the UGS operation well zone: the additional introduction of 2 mM HCO<sub>3</sub><sup>-</sup> appreciably stimulated methane formation (Table 5). In several samples, the positive effect was as great as 6000-fold; however, the scatter of data over different UGS samples was very

Table 4. Sulfate reduction rate in samples of the UGS associated and stratal waters in the period of gas extraction

	Predicted stimulation	ng agent (sulfate), mg/l	Sulfate reductio							
Sample no.	initial content in sample	concentration after introducing 3 mM SO <sub>4</sub> <sup>2-</sup>	with no additions	after introduction of $SO_4^{2-}$	Stimulatory effect					
Upper productive horizon										
1	3	99	5.06	37.71	7.45					
2	10	106	0.01	0.07	6.06					
3	12	108	3.75	2.51	0.67					
4	14	110	67.48	97.75	1.45					
5	65	161	0.20	2.29	11.32					
6	100	196	29.11	7.48	0.26					
7	2	98	28.82	273.65	9.49					
8	18	114	6.62	1.80	0.27					
9	2	98	14.81	173.47	11.71					
10	4	100	44.44	89.16	2.01					
11	15	111	4.03	24.56	6.10					
12	7	103	52.14	385.84	7.40					
13	13 21		0.03	0.10	3.62					
Lower productive horizon										
14	4	100	7.69	6.01	0.78					
15	3	99	12.56	3.79	0.30					
16	3	99	66.67	141.78	2.13					

Note: The effect of stimulation of sulfate reduction was assessed as the factor of the increase in the rate of the process after the addition of 3 mM sulfate.

large (the stimulation factor varied from 1.3 to 6391.7). Absence of stimulatory effect was noted only for two samples—5 and 13—in which the only precursor of the newly formed methane was acetate.

#### DISCUSSION

When considering the hydrochemical and microbiological situation in the UGS at the gas injection stage, we showed that microflora and biogenic elements, as well as admixtures of technogenic origin (methanol, pipeline corrosion products, etc.), may be introduced into the gas storage from the gas main along with natural gas [1].

The present study established that the processes occurring in the near-to-well zone when a well is operated in the gas extraction mode lead to a substantial change in the chemical composition of the associated waters. The content of short-chain monocarboxylic acids, primarily acetate, decreases. The bicarbonate and ammonium contents also decrease. The sulfate and iron contents vary within wide ranges, but, on the whole, tend to decrease.

The studies conducted by us using radioactive carbon and sulfur isotopes unambiguously testify to the fact that modern anaerobic bacterial processes ([1] and

this study) occur at a high rate throughout the operation cycle in various UGS units. The functional diversity, number, and activity of bacteria peak in the operation well zone (averaged GDS samples were studied) and the technological equipment, and are significantly lower in the observation well zone. However, it is difficult to say at which stage the microbial activity significantly increases (either in the production wells proper as a result of accumulation of liquid from several wells of a GDS or in the above-ground equipment as such due to the activation of microbial processes directly therein).

According to the data of radioisotopic analysis, in most samples of stratal water from the operation wells and technological reservoirs (averaged samples obtained from GDS, gas compression station, and final gas compression station), aceticlastic methanogenesis prevailed over lithoautotrophic (that hydrogen and carbon dioxide) ([1] and this study). The acetate content in associated water remained at a sufficiently high level throughout the operation cycle and could not limit the process of methane formation. On the other hand, the experiments on stimulation of autotrophic methanogenesis with carbonate convincingly demonstrated that, at a certain stage, the latter could actually be the factor limiting the rate of the process in question. A decrease

Table 5. Methanogenesis rate (nl CH<sub>4</sub>/(l day) in the GDS waters in the period of gas extraction

Sample no.	Sampling site	Content, mg/l		Methanogenesis rate			% from	Methano- genesis	Ctimulatamy
		HCO <sub>3</sub>	CH <sub>3</sub> COO	HCO <sub>3</sub>	CH <sub>3</sub> COO	total	acetate	after stimulation	Stimulatory effect
			Up	per produc	tive horizon				
1	Sump	NF	450	1.64	2837	2839	99.9	6018.8	3670.7
2	Oil trap	162	200	81.06	67	148	45.2	104.5	1.3
3	Separator	NF	375	0.96	260	261	99.6	36.9	38.2
4	Sump	NF	475	0.13	173	173	99.9	817.3	6391.7
5	Separator	NF	1250	0.37	832	833	100.0	0.0	0.0
6	Sump	NF	625	2.81	295	297	99.1	450.0	160.1
7	Separator	NF	325	34.05	1478	1512	97.7	2572.7	75.6
8	Sump	NF	225	0.04	109	109	100.0	15.0	341.0
9	Sump	NF	875	1023.29	9763	10786	90.5	246701.2	241.1
10	Separator	NF	450	115.65	4190	4305	97.3	53508.7	462.7
11	Separator	NF	425	20.93	1431	1452	98.6	488.7	23.4
12	Separator	21	425	2630.19	3218	5848	55.0	75233.2	28.6
13	Sump	NF	475	0.00	289	289	100.0	0.0	0.0
	Lower productive horizon								
14	Separator	NF	750	17.07	2970	2988	99.4	3015.5	176.7
15	Separator	NF	125	55.85	180	236	76.3	252.8	4.5
16	Sump	NF	300	102.00	17470	17572	99.4	18411.0	180.5

Notes: The effect of stimulation of methanogenesis is determined as a factor of the methanogenesis rate increase after the addition of  $HCO_3^-$  at a concentration of 120 mg/l. NF stands for "not found."

was observed in the total rate of methanogenesis throughout the annual cycle of the UGS operation: the maximal value of the process rate in the extraction period virtually equaled the minimal value in the injection period (17.6 and 15.1 µl of methane per 1 l daily, respectively) and was two orders lower than the maximal value in the injection period (17.6 and 2847.7 µl of methane per 1 l of water daily). Additional investigations are required to clear up the causes of such dynamics of the process. Bicarbonate is known to influence the pathways of conversion of the main methanogenic substrates (acetate, H<sub>2</sub>/CO<sub>2</sub>, and methanol) in the community and to influence significantly the total rate of methanogenesis [4–6]. Moreover, if the system is depleted of bicarbonate, this should inevitably affect the rates of other anaerobic processes proceeding with its involvement, including the direct utilization of methanol by acetogens, syntrophic utilization of methanol and acetate, acetate formation from C<sub>1</sub> units, and sulfate reduction.

Theoretically, the hydrogen sulfide arriving from the UGS in the extraction period may be introduced during gas injection from the gas main, or may be of biogenic origin. This work shows that biogenic hydrogen sulfide

formation does take place. The high number and the versatile catabolic capacities of sulfate-reducing bacteria in UGS allow us to suggest that they actively participate in the carbon cycle in this ecosystem. The labile metabolism of these organisms may contribute to their spreading in the liquid media of the UGS units with different physicochemical conditions. Despite the ambiguous results of the experiment with the sulfate introduction into stratal water samples, the role of sulfate should not be underrated. The initial  $SO_4^{2-}$  content is known to influence considerably the growth of sulfate-reducing bacteria and the kinetic parameters of anaerobic sulfate reduction [7] and to favor the realization of the metabolic capacities of these bacteria [8]. Therefore, under the UGS conditions, strict control should be exercised over the sulfate content, because, in the presence of active microflora, the introduction of SO<sub>4</sub><sup>2-</sup> may dramatically stimulate the process of bacterial sulfate reduction.

The data presented show that the microflora density of the UGS liquid phase may be assessed as high. The absence of injected oxygen-containing fluids is a specific feature of the ecosystem. The transformation of

**Table 6.** Comparison of the rates of the microbial processes of methanogenesis and sulfate reduction in different deep anaer-obic ecotopes

Content, mg/l Methanogenesis, µl CH <sub>4</sub> /(l day)								
Ecotope					Sulfate reduction,			
	carbonate	acetate	sulfate	from carbonate	from acetate	$\mu g S^{2-}/(1 \text{ day})$		
Oil-and-gas fields with sandy collectors								
Mykhpaiskoe [9]	153–341	18–277	0–1	0.56-49.19	0.00-4.72	0.000-1.218		
Talinskoe [9]	73–597	2–4	1–17	0.00-0.255	0.000-0.595	0.001-1.830		
Lok-Batan [10]			5-675			3–24		
Binagady [10]			12-2110			72.00–3680.00		
	nate collectors	ı	I					
Bavly [11]	183–488	0–11.9	19.5-50.9	0.000-2.755	0.00-0.23	0.66-237.50		
Romashkinskoe, pool 302 [12]	100–600	0–74	500-2500	0.00-38.25	0.00-65.31	0.00-171.37		
	Hydrog	en sulfide-co	ntaining gas	condensate fields	' 	,		
Orenburgskoe [13]				2.	.80*			
	ı	Undergi	round gas sto	rage		'		
UGS [1; present work]	0-162	125-14000	2–773	0.000-10.061**	0.067-2837.625**	0.01-489.46**		
Anaerobi	c marine sec	diments conta	ining natural	gas in the form o	f gas hydrates	'		
Pacific Ocean, Cascadia [14]				0.028-4.352		0.035-219.200		
Atlantic Ocean, Blake Ridge [15]		41.3–885.0	480–1632	0.16–43.68	932.80–19843.20	28.16–12809.60		
Anaerobic water column and the surface layers (1-30 cm) of bottom sediments								
Lake Mogil'noe [16]			1230-2194	0.019-0.622	0.000-0.055	8.00-150.00		
Pacific Ocean, Peru Margin [15]				0.736–59.040		0.051–279.680		
Japanese Sea [15]				0.48-10.24		0.058–792.640		

Notes: \* Total methanogenesis rate (averaged over 52 days of cultivation) measured with a modified gas chromatographic method is given; in the remaining cases, the rates of the processes of sulfate reduction and methanogenesis were assessed using sulfur and carbon radioactive isotopes, respectively.

the organic matter available to microorganisms mainly occurs under strictly anaerobic conditions. The nature of the substrates providing for the functioning of the bacterial community, including the key substrates and the pathways of substrate metabolism, require further investigations.

The methanogenesis rate in the UGS recorded by us in the gas injection period [1] was higher than in any of the exploited hydrocarbon fields studied in this respect (Table 6) [9–13]. Despite the insignificant sulfate content in the UGS-associated waters, sulfate reduction also occurred at high rates. The maximal rate of the process was one to three orders of magnitude higher than the corresponding values recorded for the low-sulfate injected and stratal waters of Western Siberian oil fields (Mykhpaiskoe, Talinskoe) [9] and exceeded the sulfate reduction rate in the Apsheron (Lok-Batan) and Tatarstan (Bavly, Romashkinskoe) oil fields, which are enriched with  $SO_4^{2-}$  due to the injection of sea water in the former case and sulfate leaching from the collector rocks in the latter [10–12]. An exception was the long-

exploited oil field Binagady (Apsheron), in which the sulfate reduction rate was still higher [10]. Sulfate reduction rates comparable to those revealed by us in the UGS and even exceeding them were also noted for some bottom sediments [14–16], including the sub-seafloor sediments of the Blake Ridge zone, containing natural gas in the form of gas hydrates [15]. Interestingly, the bulk of methane in the latter case also originated from acetate (Table 6).

The high rates of terminal anaerobic processes, together with the broad distribution of microorganisms over the UGS, their great number, and unique catabolic potential, gives evidence of the significant contribution of the biogenic component to the geochemistry of the ecosystem in question and, in the final analysis, to the industrial gas composition. The contribution of microorganisms of a number of physiological groups (such as sulfate-reducing, acetogenic, and methanogenic) to the aggregate activity of the biocenosis is clear enough, whereas the role of some other community members (e.g., denitrifying bacteria) remains to be clarified.

<sup>\*\*</sup> The rates of biogenic processes for the operation and injection wells and technological reservoirs is given.

The comparison of the rates of natural and technogenic processes occurring in exploited oil-and-gas fields and in UGS shows that the specific features of the operation of the latter contribute to the intensification of microbial activity. The activation of the activity of microorganisms in the UGS ecosystem becomes even more obvious when the rates of microbial processes in the UGS are compared with those observed in gas or gas-condensate fields (Table 6). In this connection, it is necessary to include the microbiological aspects into the complex ecological monitoring of UGSs. Such monitoring will make it possible to opportunely reveal the zones of maximal technogenic activity, obtain data on modern geodynamic and bacterial processes, minimize losses and degradation of gas, and enhance the safety of UGS operation.

#### REFERENCES

- Ivanova, A.E., Borzenkov, I.A., Tarasov, A.L., Milekhina, E.I., and Belyaev, S.S., A Microbiological Study of an Underground Gas Storage in the Process of Gas Injection, *Mikrobiologiya*, 2007, vol. 76, no. 4 [*Microbiology* (Engl. Transl.), vol. 76, no. 4].
- Romanenko, V.I. and Kuznetsov, S.I., Ekologiya mikroorganizmov presnykh vodoemov. Laboratornoe rukovodstvo (Ecology of the Microorganisms of Fresh Water Bodies), Leningrad: Nauka, 1974.
- Smith, D.W., Ecological Actions of Sulfate-Reducing Bacteria, The Sulfate-Reducing Bacteria: Contemporary Perspectives, New York: Springer, 1993, pp. 161–187.
- 4. Florencio, L., Field, J.A., and Lettinga, G., Substrate Competition between Methanogens and Acetogens during the Degradation of Methanol in UASB Reactors, *Water. Res.*, 1995, vol. 29, pp. 915–922.
- Paulo, P.J., Jiang, B., Rebac, S., Hulshoff-Pol, L., and Lettinga, G., Thermophilic Anaerobic Digestion of Methanol in UASB Reactor, *Water. Sci. Technol.*, 2001, vol. 44, pp. 129–136.
- Paulo, P.L., Villa, G., van Lier, J.B., and Lettinga, G., The Anaerobic Conversion of Methanol under Thermophilic Conditions: pH and Bicarbonate Dependence, J. Biosci. Bioeng., 2003, vol. 96, pp. 213–218.
- 7. Moosa, S., Nemati, N., and Harrison, S.T.L., A Kinetic Study on Anaerobic Reduction of Sulfate. Part I: Effect

- of Sulfate Concentration, Chem. Engin. Sci., 2002, vol. 57, pp. 2773–2780.
- 8. Scholten, J.C.M. and Stams, A.J.M., The Effect of Sulfate and Nitrate on Methane Formation in a Freshwater Sediment, *Antonie van Leeuwenhoek*, 1995, vol. 68, pp. 309–315.
- 9. Nazina, T.N., Ivanova, A.E., Borzenkov, I.A., Belyaev, S.S., and Ivanov, M.V., Occurrence and Geochemical Activity of Microorganisms in High-Temperature, Water-Flooded Oil Fields of Kazakhstan and Western Siberia, *Geomicrobiol. J.*, 1995, vol. 13, pp. 403–408.
- 10. Galushko, A.S., Sulfate-Reducing Bacteria of Water-Flooded Oil Fields of the Apsheron Peninsula, *Cand. Sci. (Biol.) Dissertation*, Moscow, 1990.
- Nazina, T.N., Ivanova, A.E., Ivoilov, V.S., Miller, Yu.M., Ibatullin, R.R., Belyaev, S.S., and Ivanov, M.V., Microbiological and Geochemical Characterization of Carbonate Oil Collectors of Tatarstan, *Mikrobiologiya*, 1998, vol. 67, no. 5, pp. 694–700 [*Microbiology* (Engl. Transl.), vol. 67, no. 5, pp. 575-581].
- Nazina, T.N., Ivanova, A.E., Kandaurova, G.F., Ibatullin, R.R., Belyaev, S.S., and Ivanov, M.V., Microbiological Investigation of the Carbonate Collector of the Romashkinskoe Oil Field: Background Study before Testing a Biotechnology for the Enhancement of Oil Recovery, *Mikrobiologiya*, 1998, vol. 67, no. 5, pp. 701–709 [*Microbiology* (Engl. Transl.), vol. 67, no. 5, pp. 582–589].
- 13. Ivanovskaya, I.B., Tsinberg, M.B., and Belyaev, S.S., The Use of Gas Chromatography for Determination of Bacterial Methane Production, *Mikrobiologiya*, 1991, vol. 61, no. 2, pp. 383–385.
- Gragg, B.A., Parkes, R.J., Fry, J.C., Weightman, A.J., Rochelle, P.A., and Maxwell, J.R., Bacterial Populations and Processes in Sediments Containing Gas Hydrates (ODP Leg 146: Cascadia Margin), *Earth Planet. Sci. Lett.*, 1996, vol. 139, pp. 497–507.
- 15. Parkes, R.J., Cragg, B.A., and Wellsbury, P., Recent Studies on Bacterial Populations and Processes in Subseafloor Sediments: A Review, *Hydrogeol. J.*, 2000, vol. 8, pp. 11–28.
- Ivanov, M.V., Rusanov, I.I., Pimenov, N.V., Bairamov, I.T., Yusupov, S.K., Savvichev, A.S., Lein, A.Yu., and Sapozhnikov, V.V., Microbial Processes of the Carbon and Sulfur Cycles in Lake Mogil'noe, *Mikrobiologiya*, 2001, vol. 70, no. 5, pp. 675–686 [*Microbiology* (Engl. Transl.), vol. 70, no. 5, pp. 583–593].