ISSN 0026-2617, Microbiology, 2007, Vol. 76, No. 4, pp. 453–460. © Pleiades Publishing, Ltd., 2007.
Original Russian Text © A.E. Ivanova, I.A. Borzenkov, A.L. Tarasov, E.I. Milekhina, S.S. Belyaev, 2007, published in Mikrobiologiya, 2007, Vol. 76, No. 4, pp. 515–523.

EXPERIMENTAL ARTICLES

A Microbiological Study of an Underground Gas Storage in the Process of Gas Injection

A. E. Ivanova[1], 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, 2006; in final form, December 12, 2006

Abstract—The liquid phase of different units of an underground gas storage (UGS) in the period of gas injection was studied with respect to its hydrochemical composition and characterized microbiologically. The presence of viable aerobic and anaerobic bacteria was revealed in the UGS stratal and associated waters. An important source of microorganisms and biogenic elements in the ecosystem studied is water and various technogenic admixtures contained in trace amounts in the gas entering from the gas main in the period of gas injection into the storage. Owing to this fact, the bacterial functional diversity, number, and activity are maximal in the system of gas treatment and purification and considerably lower in the observation well zone. At the terminal stages, the anaerobic transformation of organic matter in the UGS aqueous media occurs via sulfate reduction and methanogenesis; exceptionally high rates of these processes (up to 4.9×10^5 ng S^{2-} l^{-1} day^{-1} and 2.8×10^6 nl CH_4 l^{-1} day^{-1}, respectively) were recorded for above-ground technological equipment.

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

DOI: 10.1134/S002626170704011X

To date, oilfields have been rather well studied by microbiologists. It was shown that when oil fields are exploited using flooding, dissolved oxygen, biogenic elements, and microorganisms enter the stratum along with the water injected, and the microbial processes of oil destruction are activated [1, 2]. Aerobic organotrophs, including hydrocarbon- and oil-oxidizing bacteria, as well as various anaerobic microorganisms (sulfate-, thiosulfate-, and elemental sulfur-reducing microorganisms, fermenting bacteria, methanogens, acetogens, nitrate and iron reducers), were revealed in the composition of the microbial community of stratal waters [3–6]. The number of these bacteria peaks in the near-bottom zones of injection wells, i.e., in the oil oxidation zones. Beyond these zones, primarily anaerobic microorganisms occur, and their number decreases with the distance from the injection zone [7].

The microflora of gas and gas-condensate fields is poorly studied. The sporadic data available in the literature [8, 9] suggest the complete absence of viable microorganisms in these specific subterranean ecosystems or their insignificant number and biodiversity.

An increase in gas consumption in regions remote from gas production areas, and the necessity of reliable permanent gas supply stimulates the creation of gas reserves, especially in the winter period, in the form of a well-developed UGS system. Due to the great economic significance and the potential danger of these facilities to the environment, they are the subjects of intense attention of gas specialists and ecologists. However, to our knowledge, no complex microbiological studies of UGS have been conducted to date, although the products of the activity of the bacterial community may unfavorably influence the gas-bearing stratum and the UGS equipment. In particular, sulfide poses a serious problem for the gas industry owing to its toxicity, corrosive activity, and the capacity for the formation of insoluble ferric (ferrous) sulfide sediments, causing collector rock clogging and the formation of stable water–oil emulsions. The organic solvents and surface-active substances formed in the course of aerobic decomposition of organic matter and the anaerobic bacterial processes of fermentation, sulfate reduction, and acetogenesis (carbonic acid, fatty acids, and lower alcohols) may be involved in carbonate cement dissolution. The joint activity of sulfate- and iron-reducing bacteria may lead to significant changes in the behavior of sulfur and iron compounds in the ecosystem in question, including the metamorphosis of the argillaceous iron-containing materials surrounding the gas reservoir. As a result of these processes, the strength of the collector rocks decreases to the point of their destruction, which causes an impairment in the permeability of the productive stratum.

UGSs are often built at the sites of exhausted gas fields. Therefore, in terms of geological, physicochemical, and some other ecologically important stratum

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

Table 1. Composition of the gas entering the UGS in the injection period

Component	vol %	Component	vol %
Methane	94.86	*iso*-Pentane	0.01
Ethane	2.2	*n*-Pentane	0.02
Propane	0.42	CO_2	0.61
iso-Butane	0.04	Nitrogen	1.78
n-Butane	0.06	Oxygen	0

Note: Hydrogen sulfide and mercaptans were present in the gas in small amounts.

characteristics, they are similar to oil-and-gas fields. At the same time, the technology of UGS operation differs significantly from that used at natural oil-and-gas fields.

The aim of this work was to explore the hydrochemical and microbiological situation in a UGS in the period of gas injection.

MATERIALS AND METHODS

The object of study. The UGS studied was created on the basis of an exhausted gas field. Two strata are used as reservoirs for gas storage.

The embedding rock is represented by terrigenous rocks including mainly aleurolites, aleurites, and clay varieties. Among the rocks constituting the collector, glauconite is widespread. The bedding depth is 800–1000 m on average; the initial pressure and temperature were 7.37 MPa and 55–65°C, respectively. The gas-bearing strata are covered by 500-m-thick clay masses. The gas field is of a combined-stratum type. The initial formation waters were of the sodium hydrocarbonate genetic type and had a mineralization of 6.5–11.0 g/l and a sulfate content ranging from trace amounts to 220 mg/l. The hydrocarbonate ion content reached 1.5 g/l.

In the process of exploitation, condensation water with a mineral content of less than 1 g/l enters the UGS along with the main gas. As a result of pumping of the gas, whose temperature does not exceed 20–23°C, the stratum temperature decreased considerably. This phenomenon was observed in all of the operation and injection wells, whereas for the observation wells, this tendency was less markedly pronounced. An increase in the stratum temperature in the process of gas extraction and its decrease in the injection period are observed. These temperature changes occur within a relatively insignificant range, from 30 to 38–40°C. This temperature interval is favorable for the activity of mesophilic microflora.

The UGS is operated in a cyclic mode: the gas injection period lasts from May to October, and the gas extraction period is from November to April (180 days). The average composition of the raw gas introduced into the UGS is shown in Table 1.

Sampling. Water samples from the observation wells were taken by means of a sludge pump. Water-phase sampling directly from the operating wells is technically unfeasible. Therefore, the liquids entering the stratum with the main gas were sampled as the gas moved along at the gas compression station (GCS) and the final gas compression station (FGCS) connected to the specific gas distribution sites (GDS), as well as at the GDS proper from separators or, if the separator appeared to be dry, from the subsequent links of the technological chain of gas treatment and purification (oil traps and sumps). On the sampling day, the samples were poured into sterile flasks and test tubes to capacity, without access to air, and hermetically closed with gas-tight butyl rubber stoppers fixed with metal caps. Further work was performed at a stationary laboratory.

The medium composition and methods for bacterial enumeration. As a rule, the composition of the microbial community of associated waters was analyzed by inoculating liquid selective media intended for revealing specific physiological groups of microorganisms with serial 10-fold sample dilutions. Further incubation was at 30–37°C for 7–50 days depending on the growth rate of the microorganisms.

Various organotrophic aerobic bacteria were enumerated in aggregate in the rich ready-for-use commercial Brain Heart Infusion Agar (BHIA, Sigma, B7278, United States) medium by inoculating Petri dishes, with subsequent counting of the colonies grown. The number of aerobic hydrocarbon- and oil-oxidizing bacteria was determined in modified Raymond medium with hexadecane or oil as substrates [10].

To enumerate and enrich for sulfate-reducing bacteria, anaerobic mineral medium for freshwater bacteria [11], supplemented with sodium lactate (20 mM), was used. A mixture of fatty acids (acetate, propionate, and butyrate, 5 mM each) was also used for studying the metabolic capacities of this physiological group. The development of sulfate-reducing bacteria was judged from the increment in the hydrogen-sulfide content in the final dilutions. The unified mineral base of Zeikus medium [12] with one of the following carbon sources—sodium acetate (30 mM), methanol and trimethylamine (30 mM each), or an $H_2 + CO_2$ (4 : 1) gas mixture—was used for enumeration. The methane increment in the gas phase served as a criterion to assess the development of methanogens. Chemolithotrophic acetogeinc bacteria were identified in modified Brown's medium [13] with an $H_2 + CO_2$ gas mixture used as substrate by acetate accumulation in the culture liquid. The number of denitrifying microorganisms was determined in the medium close in composition to that of the medium for sulfate-reducing bacteria, where sulfate was, however, replaced with nitrate (20 mM) and acetate was the only source of carbon and energy. In the process of preparation, the medium was

freed from dissolved oxygen, but no additional chemical reductants were used. Bacterial growth was accompanied by nitrate reduction, via a number of intermediates, to molecular nitrogen, which was determined visually by the appearance of gas bubbles in the medium. The formation of gaseous nitrogen was verified by chromatographic analysis of the denitrification products. To reveal microorganisms with the fermentative type of metabolism, we used the mineral base of the medium for sulfate-reducing bacteria without sulfate. Ethanol (10 mM) served as the substrate. The fermentation products (acetate, propionate, hydrogen) were analyzed chromatographically.

The enumeration of iron-reducing bacteria was performed in a medium close in its mineral base to that used for sulfate-reducing bacteria in which sulfate was replaced with 50 mM amorphous ferric oxide-hydroxide (ferrihydrite), which we obtained according to the method described in [14]. Dithionite was not used as an additional strong reductant; the medium content of hydrogen sulfide was decreased to 0.4 mM. Acetate (5 mM) was the only source of carbon and energy. To clarify whether iron-reducing bacteria are capable of utilizing other substrates for growth, an aliquot (1 ml) from the final dilutions in which the microorganisms in question were revealed was subcultured in a medium of the same composition where an alternative source of carbon and energy (hydrogen, lactate, propionate, or ethanol) was substituted for acetate. The growth of iron-reducing bacteria was judged from microscopic examinations and from changes in Fe^{3+} and Fe^{2+} contents in the medium, measured using a standard Merck kit.

Radioisotopic studies. The rates of microbial processes in stratal and associated waters were determined with the radioisotopic method using ^{14}C-acetate, ^{14}C-bicarbonate, and ^{35}S-sulfate. The activity of the label introduced into the sample constituted 0.8 MBq for $NaH^{14}CO_3^-$, 1.2 MBq for $Na^{14}CH_3COO$, and 2 MBq for $Na_2^{35}SO_4$. The samples were incubated at a temperature close to that of the stratum (35°C) for two days. After the completion of incubation, the samples were fixed with 0.5 ml of a saturated KOH solution. The samples into which the fixative was introduced before the addition of the labeled substrate served as controls. Further treatment of the samples was carried out according to the methods described in detail earlier [15, 16].

Analytical methods. The chemical composition of stratal and associated waters was determined using a Biotronic ion chromatograph. The total alkalinity and the iron content in the samples were analyzed using standard Merck kits (Aquamerck, FRG, 1.11109 and 11 136/8023, respectively). When determining alkalinity by the titration method, the ionic forms of acetic and carbonic acids are determined in aggregate. Therefore, the hydrocarbonate and carbonate content was calculated using the equilibrium constants for the reactions:

1. $CH_3COOH \rightleftharpoons CH_3COO^- + H^+$ (pK = 4.75)

2. $HCO_3^- \rightleftharpoons CO_3^{2-} + H^+$ (pK = 10.33).

The acetate ion $[CH_3COO^-]$ content was calculated according to the formula $[CH_3COO^-] = \Sigma\,\text{Acetate}/(1 + (10^{-pH}/10^{-4.75}))$, where Σ Acetate is the total content of all the forms of acetic acid in the sample.

The hydrocarbonate content was calculated according to the formula $[HCO_3^-] = \text{Alkalinity} - [CH_3COO^-]$. The carbonate ion $[CO_3^{2-}]$ content was calculated according to the formula $[CO_3^{2-}] = (\text{Alkalinity } [CH_3COO^-])/\ 1 + (10^{-pH}/10^{-10.33})$.

The dissolved sulfide and free hydrogen sulfide contents were determined with the colorimetric method using N,N-dimethyl-1,4-phenylenediamine [17].

Methane, hydrogen, and carbon dioxide in the gas phase of bacterial cultures, as well as C_2–C_4 carboxylic acids were analyzed using gas chromatography as described earlier [18]. Monocarboxylic acids and lower alcohols were determined in samples fixed after sampling with concentrated orthophosphoric acid. The samples were analyzed on a model 3700 chromatograph (Russia) with a flame-ionization detector. Porapak Q (80–120 mesh) pretreated with 5% H_3PO_4 was used as the sorbent. The column length was 2 m; the inner diameter was 3 mm; argon was the carrier gas; the gas flow rate was 30 ml/min; the injector, column, and detector temperatures were 130, 180, and 180°C, respectively.

RESULTS AND DISCUSSION

Observation wells. In terms of their chemical composition, the waters of the observation wells examined were attributed to the sodium hydrocarbonate type and were mainly neutral or slightly alkaline (Table 2). The sample mineralization varied between 1.4 and 24.2 g/l. All the samples contained the sulfate ion at a concentration of 1 to 130 mg/l. The high ammonium content, attaining 37.3–52.0 mg/l, is noteworthy. Clarification of its origin needs further study. The iron content in the samples seems to have depended on the amount of the solid phase incorporated into the sample during the sampling procedure, because no preliminary anaerobic filtration of the samples before their stabilization with acid was carried out. The iron content usually did not exceed several tens of milligrams per liter. An exception was the sample from observation well 134, in which Fe_{tot} was present in a very high concentration (2000 mg/l).

A considerable bicarbonate (156–2651 mg/l) and volatile monocarboxylic acid content was noted in all the samples (Table 2). Apart from acetate, whose concentration in the waters attained 390 mg/l, a number of

Table 2. Chemical composition of the UGS water sampled at the gas injection stage

Well no., sample	pH	Mineralization*, g/l	Content of mineral components, mg/l					Fatty acid content, mg/l				
			SO_4^{2-}	$\Sigma HCO_3^- + CO_3^{2-}$	NH_4^+	Fe_{tot}	ΣH_2S, HS^-, S^{2-}	Formate	Acetate	Propionate	Butyrate	*iso*-Butyrate
Lower productive horizon: observation wells (80–163) and the system of gas purification												
80	7.1	1.4	33	619	7.7	50	2	0	80	10	10	0
163	7.5	4.5	130	1528	14	10	2	0	3	0	0	0
GCS	5.8	1.3	773	5	16	500	2	25	1290	10	0	0
GDS-2**	6.3	0.8	10	23	ND	50	20	26	220	215	10	0
Upper productive horizon: observation wells (22–116) and the system of gas purification												
22	9.3	23.3	1	485	33.2	10	15	0	3	0	0	0
143	8.2	6.1	11	2651	12.5	25	2	0	390	496	15	15
115	7.7	3.8	4	1222	52	10	2	0	3	0	0	0
802	8.1	7.9	24	1470	19.6	10	15	6	96	30	0	0
147	7.4	2.1	38	597	ND	25	ND	0	30	0	0	0
134	7.1	2.3	32	1099	37.3	2000	2	0	5	0	0	0
116	6.3	24.2	5	156	33.8	10	18.5	0	3	0	0	0
FGCS-2	5.2	5.5	292	6	ND	5000	62	39	14000	150	1000	500
GDS-2**	5.4	3.5	24	6	78	1000	2	7	1540	20	40	0
GDS-12**	6.5	0.6	83	27	ND	10000	ND	6	3300	100	1200	0

Notes: ND stands for "no data."

* Mineralization was calculated as the sum of the following components: Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, HCO_3^-, and CO_3^{2-}.

** Ethanol (100 mg/l) was detected in the lower-horizon GDS-2 sample; methanol (3000 and 1000 mg/l, respectively), in the upper-horizon GDS-2 and GDS-12 samples.

Table 3. Number of aerobic bacteria in the UGS water samples in the period of gas injection

Well no.	Number of organotrophs, cells/ml	
	nonspecific	oil-oxidizing
Lower productive horizon		
80	1.0×10^5	0
163	8.0×10^4	10^4
GCS	2.2×10^4	10^3
GDS-2	7.2×10^4	10^3
Upper productive horizon		
22	5.6×10^4	10^3
143	2.9×10^4	10
115	2.4×10^4	10^4
802	7.2×10^4	10^4
147	7.0×10^4	10^4
134	7.0×10^3	10
116	2.1×10^3	0
FGCS-2	3.2×10^3	10^2
GDS-2	5.2×10^4	10
GDS-12	2.3×10^3	single cells

the samples contained formate, propionate, butyrate, and *iso*-butyrate. Lower alcohols were not detected (with a sensitivity of the method of 1 mg/l).

The samples of liquid from the observation wells were analyzed for the presence of aerobic and anaerobic microorganisms of different physiological groups. As seen from Table 3, the number of nonspecific organotrophic aerobic bacteria enumerated on the BHIA medium was steadily high, attaining 8×10^4–1.0×10^5 cells/ml. In most wells, aerobic oil-oxidizing bacteria were found at a concentration of 10 to 10^4 cells/ml.

The observation well waters were inhabited by diverse anaerobic microflora (Table 4). The sulfate-reducing bacteria enumerated on lactate medium dominated in terms of the occurrence frequency and number, which equaled 10^4–10^5 cells/ml in the upper productive horizon wells 802, 147, and 134. In wells 115 and 116, no sulfate-reducing bacteria were revealed.

The metabolic capacities of the sulfate reducers were not limited to the use of lactate as the substrate. Inoculations of selective media revealed in the observation well samples the presence of other representatives of this physiological group, including those decomposing monocarboxylic acids (acetate, propionate, and butyrate). Their number determined in some water sam-

Table 4. Number of anaerobic microorganisms in the UGS water samples in the period of gas injection

Well no., sample	Number of microorganisms, cells/ml							
	iron-reducing	sulfate-reducing	acetogens	methanogens			fermenters	denitrifiers
	Cultivation substrate							
	acetate	lactate	$H_2 + CO_2$	$H_2 + CO_2$	acetate	methanol, trimethylamine	ethanol	acetate
Lower productive horizon: observation wells (80, 163) and the system of gas purification								
80	10^2	10^2	10	0	0	0	single cells	0
163	0	single cells	10^2	0	0	0	single cells	0
GCS	10^4	10^4	10^4	0	0	0	10^4	10^3
GDS-2	10^6	10^5	10^3	10	10	10	10^2	0
Upper productive horizon: observation wells (22–116) and the system of gas purification								
22	0	10	0	0	0	10^2	0	0
143	10^2	10^3	10	0	0	0	10	10
115	0	0	0	0	0	0	0	0
802	0	10^4	0	0	0	0	0	0
147	10^5	10^5	10	0	single cells	0	10	10^3
134	10^6	10^4	10	10^2	10	10	10^2	10
116	0	0	10	0	0	0	0	0
FGCS-2	10^2	10^2	10	0	0	0	10^2	0
GDS-2	10^5	10	10^4	10	single cells	10	10^5	10^3
GDS-12	10^6	10^6	10^4	10^2	10	10^2	10^6	10^3

ples (wells 80, 134, 143, and 147) was comparable to that determined on lactate. Note that on medium with lactate, significant H_2S formation was observed as early as on the second or third day of incubation, whereas on medium with a mixture of monocarboxylic acids, sulfidogenesis was preceded by a rather long lag phase (no less than 10 days). The addition of these substrates directly to the stratal water samples also led to the accumulation of considerable amounts of hydrogen sulfide.

In addition to sulfate-reducing microorganisms, chemolithotrophic homoacetogenic bacteria, ethanol-fermenting microflora (up to 10^2 cells/ml), and iron-reducing microorganisms (up to 10^5–10^6 cells/ml) were revealed in samples from the observation wells (Table 4). Except for well 802, the greatest number of iron reducers was typical of the wells where the sulfate reducer content was high (wells 147 and 134). Examination of the catabolic capacities of the iron-reducing microorganisms demonstrated that, in addition to acetate, these bacteria were capable of utilizing hydrogen, formate, ethanol, and lactate as growth substrates.

Methanogenic bacteria (autotrophic, aceticlastic, and methylotrophic) were revealed sporadically (Table 4), and their number did not exceed 10–10^2 cells/ml. Denitrifying microorganisms were not revealed in most of the wells explored; however, in some of the wells, their number attained 10^3 cells/ml.

In low-sulfate waters from the UGS observation wells, methanogenesis was the main terminal process of organic matter decomposition. The mesophilic methanogenesis rate varied between from 11 to 480 nl of methane per 1 l of water daily (Table 5). The sources of the newly formed methane were $H_2 + CO_2$ (up to 50.5%) and acetate (up to 45.5%). One of the samples (well 802) did not exhibit methane formation. The rate of the mesophilic process of bacterial sulfate reduction was significantly lower and constituted 0.1–6.7 ng S^{2-} per 1 l daily. In wells 163, 115, 22, and 802, we did not succeed in revealing sulfate reduction by means of labeled sulfate.

The technological system of gas purification. The samples from the UGS technological units were represented by slightly acid waters with a mineral content of 0.6 to 5.5 g/l. Hydrogen sulfide and hydrosulfides (2 to 62 mg/l) were identified; sediments containing carbonates and iron sulfide were present in varying amounts (41–1990 mg/l according to data from the gas production department) (Table 2). The sulfate ion content varied within a wide range: from trace amounts to 773.0 mg/l. The bicarbonate content was insignificant. Ethanol was found in the sample from GDS-2 of the

Table 5. Rates of the microbial processes of methanogenesis and sulfate reduction in the waters from the observation wells and the UGS technological units in the period of gas injection

Well no., sample	Methanogenesis, nl CH_4 l^{-1} day^{-1}			Acetate contribution, %	Sulfate reduction, ng S^{2-} l^{-1} day^{-1}
	from carbonate	from acetate	Σ		
Lower productive horizon					
80	79.6	35.0	114.7	30.5	1.9
163	135.1	1.8	137.0	1.3	0.0
GCS	1.7	946.8	948.5	99.8	163.6
GDS-2	2879.2	1254036.8	1256916.0	99.8	216576.5
Upper productive horizon					
115	39.3	2.2	41.5	5.4	0.0
116	8.8	1.9	10.6	17.5	0.1
134	8.8	8.7	17.5	49.5	0.7
143	347.4	136.4	483.8	28.2	6.7
147	31.2	24.7	55.9	44.2	1.9
22	58.5	0.7	59.2	1.2	0.0
802	47.3	0.0	47.3	0.0	0.0
FGCS-2	5.5	15093.5	15099.0	100.0	2511.7
GDS-2	18.3	2993.8	3012.1	99.4	4260.1
GDS-12	10060.6	2837624.7	2847685.3	99.6	489457.5

lower productive horizon; methanol was found in the samples from GDS-2 and GDS-12 of the upper productive horizon. Among the monocarboxylic acids, acetate, whose content reached extremely high values (up to 14 g/l), dominated. It is essential that, along with acetate, propionate and butyrate were present in the samples at high concentrations (up to 212 and 1200 mg/l, respectively); formate and *iso*-butyrate were also identified. The presence of salts of various monocarboxylic acids (i.e., of a whole spectrum of compounds) in the deep hydrocarbon-containing sites is usually treated as a result of the microbial destruction of organic matter of oil genesis. The high methanol concentrations revealed by us in some samples from the system of gas treatment can be explained by the large-scale use of this compound for the prevention of hydrate formation during gas extraction.

Microbiological analysis of the water samples from individual units of the treatment system (GCS, FGCS, and GDS) of the gas introduced into the UGS testifies to the presence of an active bacterial community in which all of the ecologically significant groups of aerobic and anaerobic microorganisms studied in this work are represented (Tables 3, 4). We discovered aerobic nonspecific organotrophic and hydrocarbon-oxidizing bacteria (up to 7.2×10^4 cells/ml and 10^3 cells/ml, respectively), as well as numerous (up to 10^3–10^6 cells/ml) anaerobic microorganisms: sulfate-reducing, acetogenic, iron-reducing, and fermenting. Along with these groups, which predominated quantitatively, denitrifying and methanogenic microorganisms were revealed at a concentration of 10^2–10^3 cells/ml in three out of five samples.

In these samples, active processes of methanogenesis and sulfate reduction were recorded (Table 5). While the sulfate reduction rate in the samples from the observation wells did not exceed several nanograms of sulfur per 1 l of water daily, in the technological reservoirs it increased by two to five orders of magnitude, constituting 164–4.9×10^5 ng S^{2-}/(l day). Methanogenesis remained the predominant process. The methane production rate was 2.8×10^6 nl CH_4 per 1 l daily; virtually all biogenic methane (99.4–100%) was produced from acetate. Modern microbial methanogenesis may be regarded as a positive contribution of microbial processes to the technological UGS system.

DISCUSSION

The data presented in this work indicate that natural gas injected in the storage that contains a certain amount of water and technogenic admixtures (methanol, pipeline corrosion products, etc.) may be an important source of microflora and biogenic elements for the ecosystem in question. Along with the introduction of microorganisms during the injection process, we cannot rule out the existence of indigenous microflora in the productive strata long before the beginning of exploitation, or the introduction of bacteria in the process of opening and exploitation of the gas-bearing horizons with the use of clay solutions and industrial water.

The presence of sulfate-reducing bacteria of different metabolic types was established in different links of the technological chain of the UGS. A number of them are characterized by conditions amenable to the development of sulfate-reducing bacteria that oxidize monocarboxylic acids. Acetate and other short-chain compound of this class were invariably revealed in the aqueous phase of the facilities explored and seem to be the in situ substrates for this group of secondary anaerobes.

The stimulation of sulfate and iron reduction in the samples by the addition of organic substrates may indicate that the development of these bacteria is limited by the available sources of carbon and energy in associated and stratal waters, despite the abundance of organic matter of hydrocarbon nature. At the same time, the ecological conditions in the UGS theoretically allow the development of sulfate and iron reducers to occur. In the presence of sulfates in the waters and ferric oxide in the terrigenous rocks of the collector, these microorganisms are able to accomplish the processes of sulfate and iron reduction. The fact that sulfate- and iron-reducing bacteria utilize a number of common substrates leads us to suggest the existence of competition for available organic matter between these groups of strict anaerobes under the UGS conditions.

Only sporadic data are available on the distribution of iron-reducing bacteria in underground waters, especially in carbohydrate fields [19]. At the same time, it has been established that bacterial iron reduction exerts a significant influence on the geochemistry of nonsulfidogenic anaerobic ecotopes, in which iron is among the most abundant elements with varying valence [20]. Some of the units of the technological UGS system studied by us seem to be examples of such ecotopes. The activity of iron-reducing bacteria under stratal conditions may be sustained by the processes of oxidation of organic matter dissolved in associated and stratal waters and of aromatic and other components of oil and gas condensate with the transfer of electrons to ferric oxide, which is contained in the embedding terrigenous rocks (in particular, in the form of the argillaceous iron-containing mineral glauconite). Iron at different concentrations was also invariably revealed in the liquid media of the technological UGS chain. In order to assess the real possibilities of this group of bacteria in this specific ecotope, it is necessary to look into the variety of the iron species and their availability to microorganisms and to consider the possibilities of interaction between iron and other oxidizing and reducing (primarily sulfide) agents that are able to effectively remove this metal from the turnover.

We did not determine directly the acetogenesis rate in different units of the technological UGS system in this work, but, comparing the results on the chemical composition of the waters and the number of microorganisms, we may suggest that at least part of the acetate of the stratal and associated waters is of biogenic origin. Along with the homoacetogenic microorganisms, the bacteria of other physiological groups for which CH_3COOH is the end metabolic product could made a certain contribution to the process of acetate accumulation in associated waters.

The enumeration of acetogenic bacteria, which are a permanent component of the biocenosis of the ecosystem studied, was carried out on a medium with an $H_2 + CO_2$ mixture, because most of the homoacetogens are capable of growing chemolithoautotrophically with this gas mixture as a source of carbon and energy. On the other hand, this physiological group of microorganisms is by far the most multipotent among strict anaerobes in relation to their substrate spectrum [21], i.e., it has a high metabolic potential. Further work should be aimed at determining the key substrates maintaining acetogenesis in situ. Methanol, which is widespread in the aqueous phase of the UGS owing to the specific features of the operation of this system, may be a possible candidate.

The exceptionally high activity of the anaerobic bacterial processes recorded by us in the UGS seems to be determined by the specific features of its operation. In the process of the main gas treatment before its injection into the stratum, part of the moisture contained in it is removed by separation, and microorganisms are concentrated in it. Since the total gas volumes passed through the technological equipment are measured in billions of cubic meters a year, the bacterial number and activity in the aqueous phase may attain extremely high values.

Underground gas storages set up in exhausted hydrocarbon fields are specific objects that differ significantly from exploited natural gas accumulations both in the conditions and rate of formation and in the mode of operation. Due to the high operation rate of gas storages, active cyclic processes occur in them, including the formation of artificial gas pools, changes in stratal temperature, underground water motion, etc., closely connected with the injection–extraction gas cycles. In terms of their rates, these processes significantly surpass similar natural changes [22]. Therefore, their influence on the stability of the UGS system should not be underestimated.

Our study showed that the injected gas carries viable microorganisms and nutrients necessary for them. As the number of the gas injection–extraction cycles increases, the microbial colonization of the stratum increases, as well as the possibility of its contamination due to microbial activity. The insufficiently studied rapid biotechnogenic processes occurring in UGSs require further thorough and regular studies, since they may negatively affect the environment and industrial equipment.

REFERENCES

1. Kuznetsova, V.A. and Li, A.D., Regularities of the Development of Sulfate-Reducing Bacteria in the D1 Oil-Bearing Strata of the Romashkinskoe Oil Field upon Flooding, *Mikrobiologiya*, 1964, vol. 33, no. 2, pp. 314–320.
2. Belyaev, S.S. and Ivanov, M.V., Contemporary Geochemical Activity of Microorganisms in Exploited Oil Fields and the Possibility of Its Regulation, *Geokhimiya*, 1990, no. 11, pp. 1618–1625.
3. Magot, M., Ollivier, B., and Patel, B.K.C., Microbiology of Petroleum Reservoirs, *Antonie van Leeuwenhoek*, 2000, vol. 77, pp. 103–116.
4. Ravot, G., Magot, M., Ollivier, B., Patel, B.K.C., Ageron, E., Grimont, P.A.D., Thomas, P., and Garcia, J.L., *Haloanaerobium congolense* sp. nov., an Anaerobic, Moderately Halophilic, Thiosulfate- and Sulfur-Reducing Bacterium from an African Oil Field, *FEMS Microbiol. Lett.*, 1997, vol. 147, pp. 81–88.
5. Eckford, R.E. and Fedorak, P.M., Planktonic Nitrate-Reducing Bacteria and Sulfate-Reducing Bacteria in Some Western Canadian Oil Field Waters, *J. Ind. Microbiol. Biotechnol*, 2002, vol. 29, pp. 83–92.
6. Grabowski, A., Nercessian, O., Fayolle, F., Blanchet, D., and Jeanthon, C., Microbial Diversity in Production Waters of a Low-Temperature Biodegraded Oil Reservoir, *FEMS Microbiol. Ecol.*, 2005, vol. 54, pp. 427–443.
7. 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. 181–192.
8. Rozanova, E.P. and Nazina, T.N., Distribution of Thermophilic Sulfate-Reducing Bacteria in Oil-Bearing Strata of Apsheron Peninsula and Western Siberia, *Mikrobiologiya*, 1979, vol. 48, no. 6, pp. 1113–1117.
9. 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.
10. Rozanova, E.P. and Nazina, T.N., Hydrocarbon-Oxidizing Bacteria and Their Activity in Oil-Bearing Strata, *Mikrobiologiya*, 1982, vol. 51, no. 2, pp. 342–348.
11. Widdel, F. and Bak, F., *Gram-Negative Mesophilic Sulfate-Reducing Bacteria, The Prokaryotes*, 2nd ed., Balows A. et al., Eds., Berlin: Springer, 1992, vol. 3, pp. 3352–3378.
12. Zeikus, J.G., The Biology of Methanogenic Bacteria, *Bacteriol. Rev.*, 1977, vol. 41, pp. 514–541.
13. Davydova-Charakhch'yan, I.A., Kuznetsova, V.G., Mityushina, L.L., and Belyaev, S.S., Rod-Shaped Methanogenic Bacteria from Oil-Bearing Strata, *Mikrobiologiya*, 1992, vol. 61, no. 2, pp. 299–315.
14. Schwertmann, U. and Cornell, R.M., *Iron Oxides in the Laboratory: Preparation and Characterization*, New York: Weinheim, 1991.
15. Laurinavichus, K.S. and Belyaev, S.S., Determination of the Intensity of Microbial Methanogenesis by Radioisotopic Method, *Mikrobiologiya*, 1978, vol. 47, no. 6, pp. 1115–1117.
16. Belyaev, S.S., Borzenkov, I.A., Milekhina, E.I., Charakhch'yan, I.A., and Ivanov, M.V., Development of Microbial Processes in Exploited Strata of the Romashkinskoe Oil Field, *Mikrobiologiya*, 1990, vol. 59, no. 6, pp. 1118–1126.
17. Truper, H.G. and Schlegel, H.G., Sulphur Metabolism in *Thiorhodaceae.* Quantitative Measurement of Growing Cells of *Chromatium okenii, Antonie van Leeuwenhoek*, 1964, vol. 30, pp. 225–238.
18. Nazina, T.N., Rozanova, E.P., Belyaev, S.S., and Ivanov, M.V., Chemical and Microbiological Methods for Investigation of Stratal Fluids and Cores from Oil Fields, *Preprint*, Pushchino, 1988.
19. Nazina, T.N., Ivanova, A.E., Golubeva, O.V., Ibatullin, R.R., Belyaev, S.S., and Ivanov, M.V., Occurrence of Sulfate- and Iron-Reducing Bacteria in Stratal Waters of the Romashkinskoe Oil Field, *Mikrobiologiya*, 1995, vol. 64, no. 2, pp. 245–251 [*Microbiology* (Engl. Transl.), vol. 64, no. 2, p. 203].
20. Straub, K., Benz, M., and Schink, B., Iron Metabolism in Anoxic Environments at Near Neutral pH, *FEMS Microbiol. Ecol.*, 2001, vol. 34, pp. 181–186.
21. Diekert, G. and Wohlfarth, G., Metabolism of Homoacetogens, *Antonie van Leeuwenhoek,* 1994, vol. 66, pp. 209–221.
22. Petrenko, V.I., Zinov'ev, V.V., Aksyutin, O.E., and Petrenko, N.V., Comparison of the Rates of Technogenic and Natural Processes Occurring during the Exploitation of Natural and Industrial Gas Reservoirs in Connection with Possible Impact on the Natural Environment, *Mater. Mezhd. konf. "Fundamental'nye problemy razrabotki neftegazovykh mestorozhdenii, dobychi i transportirovki uglevodorodnogo syr'ya," 24–26 noyabrya 2004 g.* (Fundamental Problems of Exploitation of Oil and Gas Fields and Extraction and Transportation of Raw Hydrocarbons, Nov. 24–26, 2004), Moscow, pp. 279–280.