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EXPERIMENTAL ARTICLES

Regulation of Geochemical Activity of Microorganisms in a Petroleum Reservoir by Injection of H_2O_2 or Water–Air Mixture

T. N. Nazina[a,1], N. K. Pavlova[a], Fangtian Ni[b], N. M. Shestakova[a], V. S. Ivoilov[a], Qingxian Feng[b], Zhao Dongyun[b], T. S. Prusakova[a], S. S. Belyaev[a], and M. V. Ivanov[a]

[a] *Winogradsky Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117312 Russia*

[b] *Dagang Oil Field Co., Tianjin, China*

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Abstract—In the course of pilot trials of biotechnologies for the enhancement of oil recovery in formation waters of the Gangxi bed of the Dagang oil field (China), microbiological processes were investigated. The biotechnologies are based on injection into the petroleum reservoir of different oxygen sources (H_2O_2 solution or a water–air mixture) with nitrogen and phosphorus salts. The injection of water–air mixture with nitrogen and phosphorus salts resulted in an increase in the number of aerobic and anaerobic organotrophic bacteria, rates of sulfate reduction and methanogenesis in formation water and also the content of CO_2 (from 4.8–12 to 15–23.2%) and methane (from 86–88 to 91.8%) in the gas. The preferential consumption of isotopically light bicarbonate by methanogens resulted in a higher content of the light ^{12}C in methane; the $\delta^{13}C/CH_4$ value changed from –45.1…–48.3 to −50.7…–59.3‰. At the same time, mineral carbonates of the formation water became isotopically heavier; the $\delta^{13}C/\Sigma$carbonates value increased from 3.4…4.0 to 5.4…9.6‰. Growth of hydrocarbon-oxidizing bacteria was accompanied by production of biosurfactants and decreased interfacial tension of formation water. Injection of H_2O_2 solution resulted in the activation of aerobic processes and in suppression of both sulfate reduction and methanogenesis. Methane content in the gas decreased from 86–88 to 75.7–79.8%, probably due to its consumption by methanotrophs. Due to consumption of isotopically light methane, the residual methane carbon became heavier, with the $\delta^{13}C/CH_4$ values from –39.0 to –44.3‰. At the same time, mineral carbonates of the formation water became isotopically considerably lighter; the $\delta^{13}C/\Sigma$carbonates value decreased from 5.4… 9.6 to –1.4…2.7‰. The additional amount of oil recovered during the trial of both variants of biotechnological treatment was 3819 t.

Key words: petroleum reservoir, sulfate reduction, methanogenesis, petroleum oxidation, biosurfactants, MEOR.

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Technologies for microbial enhancement of oil recovery (MEOR) are under development in many of oil-producing countries [1–5]. A method for the enhancement of oil recovery based on the activation of the microflora of the petroleum reservoir by the injection of oxygen (as an water–air mixture) and mineral nitrogen and phosphorus salts was developed in Russia over 20 years ago [6–8]. The purposeful regulation of microbial activity by this technology results in the production of efficient oil-releasing agents (biosurfactants, organic acids, gases, and biopolymers) directly in the pores of an oil-bearing matrix. Aerobic petroleum-oxidizing bacteria are primarily activated; their metabolism results in formation of the products of incomplete petroleum oxidation (alcohols and fatty acids), biosurfactants, CO_2, etc. [6–8]. These metabolites, and microbial biomass, are used as substrates by methanogenic and fermentative microorganisms; thus, other compounds facilitating oil mobility, such as volatile fatty acids, alcohols, and gases are formed in the anaerobic zone of the oil field.

The method has been successfully tested in petroleum deposits with reservoir temperature form 25 to 60°C and salinity of formation water up to 150 g/l [9–11]. Application of this method on petroleum reservoirs of Russia (Tatarstan, Bashkortostan, and Western Siberia), Azerbaijan, and China has resulted in the additional recovery of over 650000 t of oil [9–11].

The effect of injection of an aerated aqueous solution of nitrogen and phosphorus mineral salts on microbial processes in an oil reservoir has been studied in detail on the Bondyuzhskoe petroleum reservoir (Republic of Tatarstan) [8, 15, 16]. Both aerobic and

[1] Corresponding author; e-mail: Nazina@inmi.host.ru

anaerobic processes of the methane cycle were revealed in the zone of injection wells of a water-flooded oil field. The technological treatment results in the intensification of microbial methanogenesis in the zone of penetration of the water–air mixture with mineral salts [8], as well as changes in the carbon isotopic composition of methane in gas and of the mineral carbonates dissolved in formation water. Formation of isotopically light microbial methane resulted in a noticeable lightening of the isotopic composition of methane; since methanogenesis was carried out by autotrophic methanogens, residual dissolved mineral carbon was enriched with the heavy isotope ^{13}C [17, 18].

Injection of an aerated aqueous solution of nitrogen and phosphorus mineral salts into the petroleum reservoir creates conditions favorable for petroleum oxidation only in the near-bottom zone of injection wells. Air solubility in oil and in water is determined by diffusion processes and decreases markedly at elevated temperatures. The possibility of substitution of oxygen of air by peroxide as the sole oxygen source for aerobic bacteria has been considered in the literature [12, 13]. In the present work, the effect of hydrogen peroxide on microbial processes directly within a petroleum reservoir was studied for the first time. Hydrogen peroxide solution can penetrate deeply within the reservoir together with injected water; it can therefore facilitate wider spreading of oil biodegradation within the reservoir.

Since additional production of microbial methane has a positive effect on oil recovery, for two variants of biotechnology for the enhancement of oil recovery, the methane content and stable carbon isotopic composition of methane in the gas were systematically monitored, as well as the isotopic composition of mineral carbonates in formation water.

The object of the present work was to determine the effect of oxygen injection as a water–air mixture or H_2O_2, as well as an aqueous solution of nitrogen and phosphorus mineral salts on microbial numbers, biogeochemical processes, and oil replacement in the Gangxi bed of the Dagang oil field (PRC).

MATERIALS AND METHODS

Characterization of the Gangxi bed. The work was carried out on the Gangxi bed (block no. 3) of the Dagang oil field (Hebei Province, PRC). The sandstone oil-bearing horizons were located on an incline in a wide interval of depths, from 602 to 1359 m; the temperature therefore varied from 35 to 54°C (Table 1). The average porosity of oil-bearing rocks was 31%. The oil contained saturated hydrocarbons (60%), resins and asphaltenes (18.1%), and bitumen (9.6%). The original formation water belonged to the hydrocarbonate–sodium type with mineralization of 8662 mg/l. In order to increase the pressure, the reservoir was flooded with coproduced formation water. In 2004, average

Table 1. Geological, geochemical, production, and microbiological parameters of the Gangxi oil bed (block no. 3) prior to the biotechnological trials for the enhancement of oil recovery

Parameters	Value
Geological characteristics of the stratum	
Lithology	Sandstone
Depth of the layer below the sea level, m	810–879
Productive area, km^2	0.9
Average porosity, %	31
Average permeability, μm^2	1.878
Average effective thickness of the stratum, m	19.5
Stratal temperature, °C	35–54
Fluid characteristics	
Oil density, g/cm^3 (25°C)	0.925
Oil viscosity, mPa · s (in situ)	17.8
Initial gas content in oil, m^3/t oil	26.5
Formation water salinity, g/l	5.2–8.6
Acetate content, mg/l	0.5–15
Surface tension of formation water, mN/m	43.4–65.6
Interfacial tension of formation water, mN/m	19.0–30.2
Emulsifying activity of formation water, %	0
$\delta^{13}C/CH_4$, ‰	–45.1…–51.7
$\delta^{13}C/\Sigma CO_2 + HCO_3^- + CO_3^{2-}$, ‰	3.4…4.0
Production characteristics	
Average daily oil production from one well, t^3/day/well	6.2
Average daily water production from one well, t^3/day/well	68
Water cut, %	44.9–83
Microbiological characteristics	
Aerobic organotrophic bacteria, cells/ml	Single cells – 10^4
Aerobic hydrocarbon-oxidizing bacteria, cells/ml	0–10^2
Fermentative bacteria, cells/ml	10–10^6
Sulfate-reducing bacteria, cells/ml	0–10^4
Methanogens, cells/ml	
growing on $H_2 + CO_2$	0–10
growing on acetate	Single cells – 10^2
Sulfate reduction rate, $\mu g\ S^{2-}\ l^{-1}\ day^{-1}$	3.32–7.88
Methanogenesis rate, $\mu g\ CH_4\ l^{-1}\ day^{-1}$	
from $NaH^{14}CO_3$	0
from $^{14}CH_3$–COONa	0.02–0.36

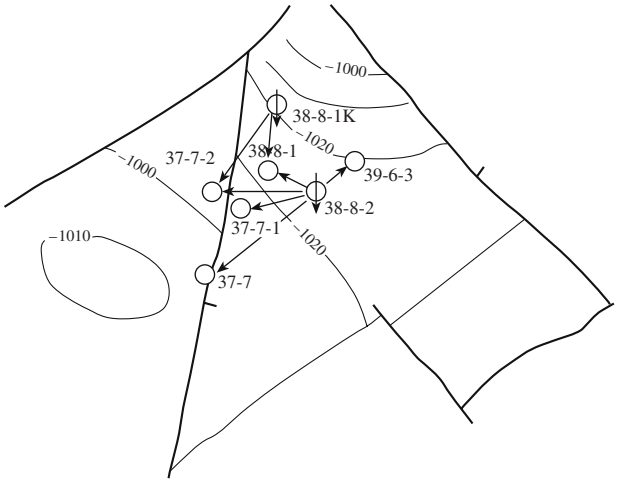

Fig. 1. Layout of injection (⊕) and production wells (○) and direction of injected water flows on the experimental site of the Gangxi oil bed.

water content in the production of the experimental site was 72.8%. Figure 1 illustrates the location of two injection and five production wells, as well as the direction of the flows of injected water on the experimental site, as determined by tracer studies. The production wells delivered oil from a depth of 810 to 879 m with a temperature of 45°C.

Sampling. Injection water, the fluid from the near-bottom zone of injection well, and formation water and gas from production wells were analyzed. The fluid was collected into sterile bottles flushed with formation gas and sealed hermetically. Formation water samples were used as inoculating material for determination of microbial numbers and for assessment of the rates of microbial processes. These analyses were carried out in the laboratory within four to six hours after sampling. The remaining samples were stored at 6°C prior to chemical analysis.

Media composition and enumeration of bacteria. The number of microorganisms of the main metabolic groups was determined in liquid media by inoculating tenfold dilutions in two repeats; the results were calculated by the most probable number method using McCredy's table. The number of hydrocarbon-oxidizing bacteria was determined in mineral media with the mixture of C_{10}–C_{22} n-alkanes (2% vol/vol). Anaerobic organotrophs with the fermentative type of metabolism were enumerated in medium supplemented with peptone and glucose (4 and 10 g/l, respectively). The number of sulfate-reducing bacteria was determined by the production of hydrogen sulfide in the dilution series in Postgate B medium with sodium lactate (4 g/l) supplemented with trace elements and reduced with $Na_2S \cdot 9H_2O$ (200 mg/l). The number of methanogens was assayed by the methane increase in the dilution series in the Zeikus media with acetate (2 g/l) or $H_2 + CO_2$ (4 : 1), supplemented with trace elements and yeast extract (1 g/l). References to the publications citing the media composition were given in our previous article [19].

Microbial numbers in Gangxi formation water were determined at 45°C, the temperature enabling growth of both mesophilic and thermophilic microorganisms. Growth was ascertained after 14–30 days of incubation; the absence of growth was confirmed after 40 days. All cultures were then examined using an Olympus microscope with a phase contrast device.

Analytical techniques. Gaseous hydrocarbons (C_1–C_5), H_2, CO_2, N_2, and O_2 were determined by gas chromatography. Sulfide was determined colorimetrically by the Pachmayr dimethyl-*p*-phenylenediamine method [20]. The content of Ca^{2+}, Mg^{2+}, $K^+ + Na^+$, Cl^-, HCO_3^-, and SO_4^{2-} in formation water was determined by the standard methods [21]. Volatile fatty acids were determined in formation water samples fixed with KOH as described previously [19].

Production of biosurfactants was assessed by the changes in the rheological characteristics of formation water. The surface tension was measured at the liquid/air interface by the ring-tearing-off method on a Krüss K10 ST tensiometer at 45°C. The interfacial tension was measured at the interface between the studied liquid and the C_{16}–C_{22} hydrocarbon mixture.

The rates of sulfate reduction and methanogenesis were determined by radioisotope methods with the following labeled compounds: $Na_2{}^{35}SO_4$, $NaH^{14}CO_3$, and $^{14}CH_3COONa$ [22–24]. The stable isotope composition of carbon in mineral carbonates dissolved in formation water ($\delta^{13}C/\Sigma CO_2 + HCO_3^- + CO_3^{2-}$), and in methane of accompanying gas ($\delta^{13}C/CH_4$) was determined by the Craig method [25] on a Delta Advantage mass spectrometer (Finnigan) with an accuracy of ±0.01‰.

RESULTS AND DISCUSSION

In 2004 and 2005, pilot trials of two variants of biotechnology for the enhancement of oil recovery based on the activation of formation microflora were carried out in the Gangxi bed of the Dagang oil field (PRC). The basic variant of the biotechnology included injection of nitrogen and phosphorus mineral salts (as diammonium phosphate) and an oxidant (as a water–air mixture) through the injection wells into the oil field [6–8]. From September through November 2004, five cycles of injection of an water–air mixture with diammonium phosphate were carried out on the Gangxi bed. During each cycle, the mineral solutions were injected through two injection wells for two to three days; the wells were then closed for 24 h, and subsequently water injection was carried out in a regular regime.

In 2005, two cycles of injection of diammonium phosphate with a water–air mixture (August through

September) and three cycles of injection of dimmonium phosphate with hydrogen peroxide (October through December) were carried out. The physicochemical, microbiological, geochemical, and production characteristics of the oil stratum were monitored from September 2003 until May 2006.

Physicochemical Conditions and Microbiological Processes in Formation Waters of the Gangxi Bed Prior to Biotechnology Trials

Mineralization of injection and formation waters varied from 5.4 to 6.3 g/l; hydrocarbonate content was 1.9–3.3 g/l; sulfate content varied 44–61 mg/l; pH of formation water was 6.5–7.2. Formation water from production wells contained 0.5 to 15 mg/l of acetate (Table 1). Aerobic organotrophs (up to 10^7 cells/ml), hydrocarbon-oxidizing bacteria (10^5 cells/ml), anaerobic fermentative bacteria (10^8 cells/ml), sulfate-reducing (10^4 cells/ml), and methanogenic microorganisms (singles cells/ml) were delivered to the oil stratum with injection water (Fig. 2).

The number of aerobic organotrophs in formation water of production wells was lower and varied from 10 to 10^3 cells/ml (Table 1, Fig. 2). The population density of anaerobes with fermentative metabolism was high (10^4–10^7 cells/ml). The number of methanogens as determined on media with $H_2 + CO_2$ gas mixture or with acetate did not exceed 10^3 cells/ml; methanogens remained viable even in injection water. Although lithoautotrophic methanogens were present in formation water, methanogenesis from $NaH^{14}CO_3$ was not detected; the rates of methanogenesis from $^{14}CH_3COONa$ varied from 0 to 0.228 μg CH_4 l^{-1} day^{-1}.

Thus, a mature microbial community was formed in the Gangxi oil bed during four years exploitation with water flooding (1999–2004); this community included the major components of the microbial trophic chain capable of growth on crude oil. Aerobic hydrocarbon-oxidizing bacteria, anaerobic fermentative, sulfate-reducing, and methanogenic microorganisms were present in formation water. Analysis of 16S rRNA genes isolated from formation water revealed phylogenetic diversity of the microorganisms of the oil stratum. Aerobic organotrophic and oil-oxidizing bacteria belonged to the genera *Bacillus*, *Pseudomonas*, *Sphingomonas*, and *Stenotrophomonas*; fermentative bacteria belonged to the genera *Anaerobaculum, Bacteroides, Cellulomonas, Coprothermobacter, Dysgonomonas, Exiguobacterium, Moorella, Pannonibacter, Proteiniphilum, Ruminobacillus, Soehngenia, Thermoanaerovibrio*, and *Thermoanaerobacterium.* Sulfate reducers belonged to the genera *Desulfocaldus, Desulfomicrobium*, and *Thermodesulfovibrio.* Archaeal phylotypes belonged to methanogens of the genera *Methanobacterium, Methanococcus, Methanomethylovorans*, and *Methanothermobacter* [26].

Microbiological Processes in the Gangxi Oil Bed in the Course of Biotechnological Treatment

Injection of the water–air mixture with phosphorus and nitrogen salts into the oil stratum resulted in the changes of microbial numbers and activity, as well as of the composition of formation water and gas. During the experiment, the number of aerobic and anaerobic organotrophic bacteria increased in all the five production wells of the experimental site (Fig. 2a–2f). The number of aerobic organotrophs immediately after the treatment was 10^4–10^6 cells/ml; it decreased slightly after seven months (May 2005, Fig. 2). The increase in the number of hydrocarbon-oxidizing bacteria was most pronounced (up to 10^5 cells/ml) in the water from wells 37-7-1 and 37-7-2. The number of methanogens and methanogenesis rate also increased (Table 2). In wells 37-7 and 38-8-1, local increase in sulfate reduction rates was revealed, from 3.3–7.88 to 61.26–494.8 μg S^{2-} l^{-1} day^{-1}, although the number of sulfate-reducing bacteria in formation water remained practically the same.

Injection of hydrogen peroxide into the stratum resulted in the increase of the number of aerobic microorganisms. The rates of sulfate reduction and methanogenesis decreased; however, the number of viable methanogens in formation water increased from single cells–ten cells/ml (May 2005) to 10^4–10^5 cells/ml (December 2005). This phenomenon can be explained by microzonal growth of anaerobic microorganisms on products of oil oxidation and by supply of formation fluids from the layers not affected by peroxide.

Thus, the application of different oxygen sources for oil oxidation resulted in the activation of microorganisms of different metabolic groups in the oil stratum.

Carbon Stable Isotope Composition in the Methane of Accompanying Gas and in the Mineral Carbonates of Formation Water

Prior to biotechnological trials, the content of methane and CO_2 in the accompanying gas from production wells was found to vary within the range of 86–89 and 4.8–12.4%, respectively. The $\delta^{13}C$ value for mineral carbonates ($\delta^{13}C/\Sigma CO_2 + HCO_3^- + CO_3^{2-}$), dissolved in formation water varied from 3.4 to 4.0‰. For nine producing wells of the block no. 3, the $\delta^{13}C$ value for methane carbon varied from –45.1 to –51.7‰.

Changes in the gas chemical composition were detected in the course of the biotechnological treatment. Injection of the water–air mixture with mineral salts resulted in the activation of both aerobic and anaerobic components of the microbial trophic chain. Oil biodegradation was accompanied by an increase in CO_2 content in the gas of wells 39-6-3, 37-7-2, and 37-7 from 4.8–12.4 to 15–23.2% (Fig. 3). Methane content in the gas and the stable isotope composition of the car-

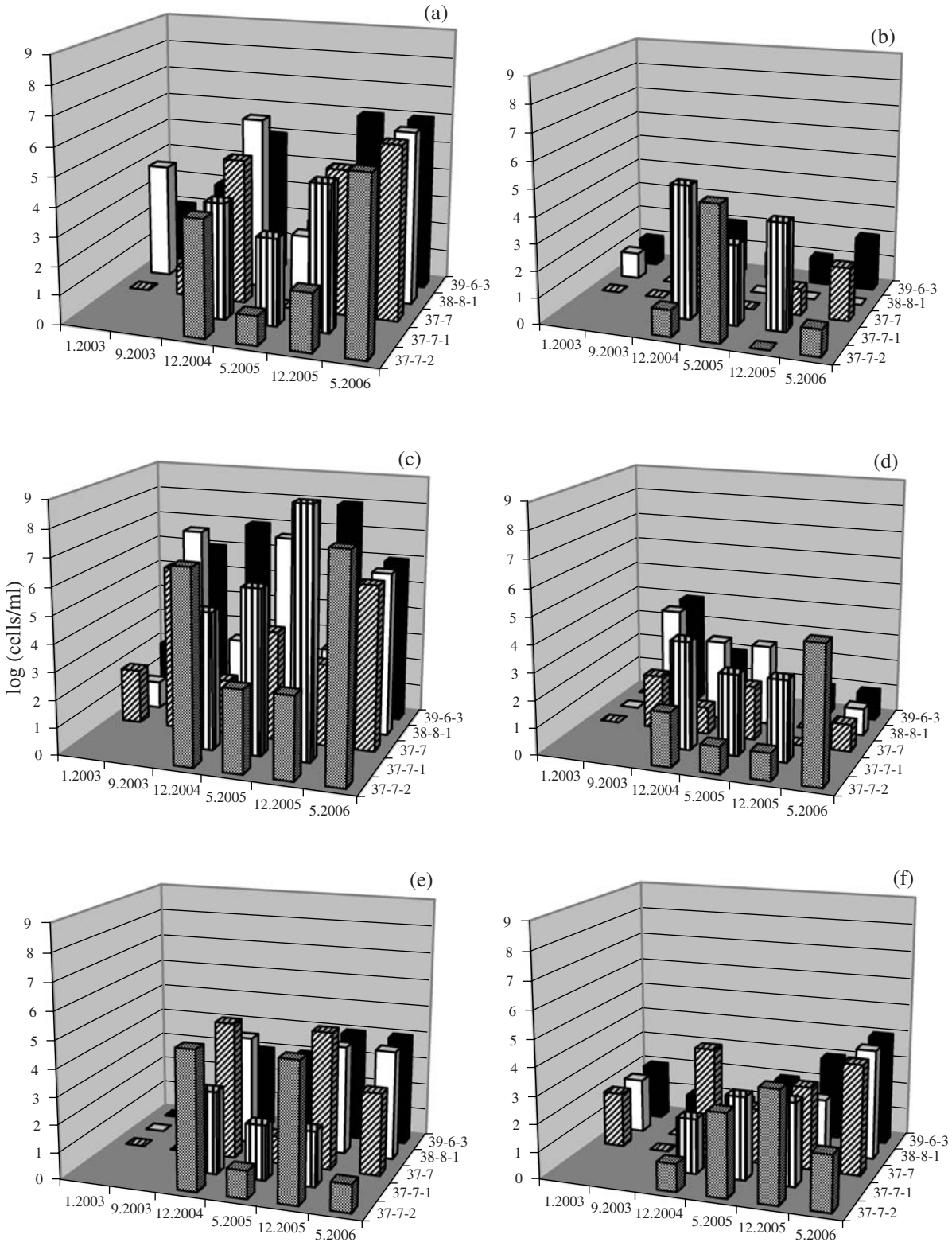

Fig. 2. Number of microorganisms in formation water from production wells of the Gangxi oil bed in the course of biotechnology trial: aerobic organotrophic bacteria (a); aerobic hydrocarbon oxidizing (b); anaerobic organotrophs with fermentative metabolism (c); sulfate-reducing (d); methanogens growing in medium with $H_2 + CO_2$ (e); methanogens growing in medium with acetate (f).

bon of mineral carbonates ($\delta^{13}C/\Sigma CO_2 + HCO_3^- + CO_3^{2-}$) of formation water exhibited a complex dynamic. In well 38-8-1, methane content increased from 86.7–89 to 91.8% due to the growth of methanogens. In three wells (39-6-3, 37-7-2, and 37-7-1), the methane carbon became isotopically lighter than before the experiment; the $\delta^{13}C/CH_4$ value changed from –45.1…–51.7 to –53.7…–59.3‰. Methanogens are known to consume preferentially the isotopically light carbonates. Utilization of ^{12}C carbonates by autotrophic sulfate reducers of formation water is possible. Due to the biogenic processes, the mineral carbonates of formation water became enriched with ^{13}C, so that the $\delta^{13}C/\Sigma$carbonates increased from 3.4–4.0 to 5.4–9.6‰.

Three months after the injection of water–air mixtures (March 2005), methane content in the gas decreased from 86.7–89.1 to 75.7–83.2% (wells 37-7-2, 39-6-3, and 37-7), probably due to microbial oxidation. Both aerobic and anaerobic microorganisms capable of microzonal growth (which is usually observed in the near-bottom zone of injection wells) can consume methane. In the Gangxi bed, methane oxidation coincided with the highest rate of sulfate reduction in the stratum (December 2004–May 2005). The relation between sulfate reduction and anaerobic methane oxidation in marine sediments was previously reported [27]. However, the role of sulfate-reducing bacteria in methane oxidation is hardly probable due to their small number and low sulfate content in formation water. Consumption by methanotrophs of isotopically light methane resulted in enrichment of residual methane with heavy ^{13}C (as compared to 2004); the $\delta^{13}C/CH_4$ value increased to –39…–44.3‰ (wells 37-7 and 39-6-3). Due to the preferential oxidation of isotopically light methane, the total carbon of dissolved carbonates was enriched with ^{12}C; the $\delta^{13}C/\Sigma$ carbonates value decreased from 5.4…9.6 to –1.4…2.7‰. In the course of our experiments, aerobic methane-oxidizing bacteria were not enumerated; however, both the active cultures of aerobic methanotrophs obtained from the reservoir and the data on stable carbon isotope composition in methane and mineral carbonates indicate the presence of microorganisms of the methane cycle and considerable geochemical changes in the Gangxi bed in the course of the biotechnological treatment.

Rheological Characteristics of Formation Water

The surface active characteristics of microorganisms are usually registered as their emulsifying activity (the ability of biosurfactants to form emulsions when culture media or microbial cells are shaken with hydrocarbons or oil) and by direct measurement of surface tension or interfacial tension against paraffin. Production of biosurfactants by microorganisms from petro-

Table 2. Rates of sulfate reduction and methanogenesis in formation waters of the Gangxi oil bed in the course of the biotechnological treatment

Well no., volume of back-flushed water	IX.2003	XII.2004	V.2005	XII. 2005	V.2006
Sulfate reduction, μg S^{2-} l^{-1} day^{-1}					
38-8-2* – 8 m^3	1561.9				
38-8-2* – 23 m^3	1716.0				
37-7	7.88	0.093	61.26		0
37-7-1	ND	ND	ND	1.90	ND
37-7-2	ND	0.044	0.66	1.13	0
38-8-1	3.317	415.33	494.8	0.13	0
39-6-3	ND	ND	0.058	ND	ND
Methanogenesis from $NaH^{14}CO_3$, μg CH_4 l^{-1} day^{-1}					
38-8-2* – 8 m^3	35.57				
38-8-2* – 23 m^3	24.98				
37-7	0**	0.608	0.66	0	0
37-7-1	ND	ND	ND	0.109	ND
37-7-2	ND	0	0.31	0.502	0
38-8-1	ND	0.125	0.49	0.024	0
39-6-3	0	0.456	0	ND	ND
Methanogenesis from $^{14}CH_3COONa$, μg CH_4 l^{-1} day^{-1}					
38-8-2* – 8 m^3	0.747				
38-8-2* – 23 m^3	110.220				
37-7	0.017	0	0	0.034	0
37-7-1	ND	ND	ND	0.001	ND
37-7-2	ND	0.001	0.002	0.008	0
38-8-1	0.365	0.280	0.228	0.037	0
39-6-3	0.283	0.934	0	ND	ND

Note: ND stands for no data.

* Back-flushed water from the near-bottom zone of injection well 38-8-2.

** Corresponds to a value of 0.000. In the absence of sulfate in formation water, sulfate reduction rates were calculated from the quantity of introduced label sulfate (20 μg/l). In the absence of acetate in formation water, methanogenesis rate was calculated from the quantity of introduced label acetate (70 μg/l).

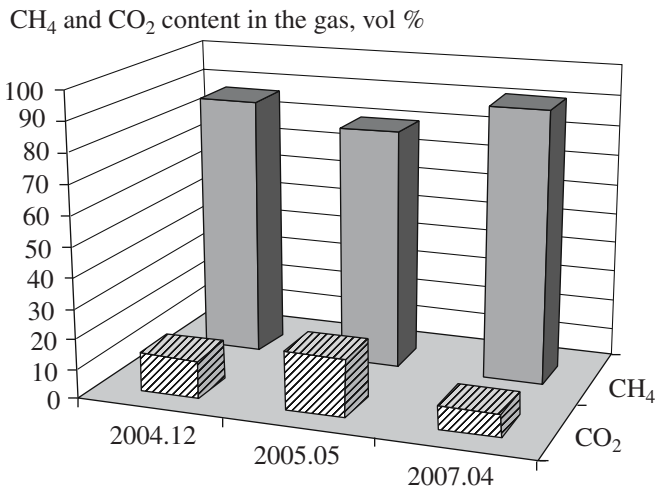

Fig. 3. Methane and CO_2 content in the gas from production well 37-7 in the course of biotechnology trial.

leum reservoirs is still insufficiently understood [5, 28–30].

Prior to the biotechnology trials, the surface tension of formation water of the Gangxi bed varied from 43.1 to 65.6 mN/m; the interfacial tension against the mixture of C_{10}–C_{20} paraffin was 18.5–19.7 mN/m. In the course of the biotechnological treatment, the surface tension of formation water remained within the 48.3–61.8 mN/m range, while the interfacial tension of formation water against the mixture of C_{10}–C_{20} paraffins decreased to 4.7–10.6 mN/m in all five wells of the experimental site. The decrease in the interfacial tension was most pronounced in formation water from wells 38-8-1 and 37-7-2 (4.7 and 6.7 mN/m, respectively); these wells have close hydrodynamic connection with injection wells. Considerable emulsification of oil observed during the trial is probably the result of biosurfactant production.

Oil Recovery in the Experimental Site of the Gangxi Bed

Apart from the changes in the microbiological, geochemical, and rheological characteristics of the oil stratum, biotechnological treatment of the experimental site of the Gangxi bed affected oil recovery from four of five production wells. Since well 37-7 was the most remote from the injection zone, no changes in oil recovery occurred there, although microbiological monitoring revealed active microbial processes in the zone of this well also. Injection of the water–air mixture with mineral salts resulted in an increase of oil recovery (for all five wells) from 19.74 to 28.9 t/day; the additional amount of recovered oil was 2022 t. Injection of H_2O_2 solution with mineral salts for the same period of time resulted in a 1294 t increase in oil recovery; however, one efficiently operating well (37-7-1) was excluded from the experiment for technical reasons. Dynamics of production characteristics of two production wells are illustrated by Fig. 4. During two years of biotechnological trials involving the activation of microbial processes in the stratum caused by the introduction of different oxygen sources, the additional amount of oil recovered from the Gangxi oil bed was 3316 t. A slight increase in oil recovery was observed as late as June 2007; the overall amount of additional oil from the site was 3819 t.

Thus, injection of different oxygen sources for oil oxidation into the stratum resulted in the activation of different groups of microorganisms. A number of oil-releasing agents (microbial biomass, biosurfactants, CO_2, and methane) was formed in the course of oil biodegradation by the stratal microflora. Microorganisms

Table 3. Carbon isotope composition of mineral carbonates ($\delta^{13}C/\Sigma CO_2 + HCO_3^- + CO_3^{2-}$, ‰ PDB) dissolved in formation water and of methane ($\delta^{13}C/CH_4$,‰) in the Gangxi oil bed in the course of the biotechnological treatment

Well no., sample	$\delta^{13}C/\Sigma CO_2 + HCO_3^- + CO_3^{2-}$, ‰				$\delta^{13}C/CH_4$, ‰		
	IX 2003	XII 2004	V 2005	XII 2005	XII 2004	V 2005	XII 2005
37-7	4.0	ND	9.7	2.0	–50.7	–52.1	–39.0
37-7-1	ND	5.4	ND	–1.4	–53.7	–49.5	–49.0
37-7-2	ND	9.6	7.5	ND	–55.9	–51.9	–46.4
38-8-1	3.4	7.6	8.8	2.7	–51.4	–52.2	–50.0
39-6-3	ND	ND	8.7	ND	–59.3	–52.8	–44.3

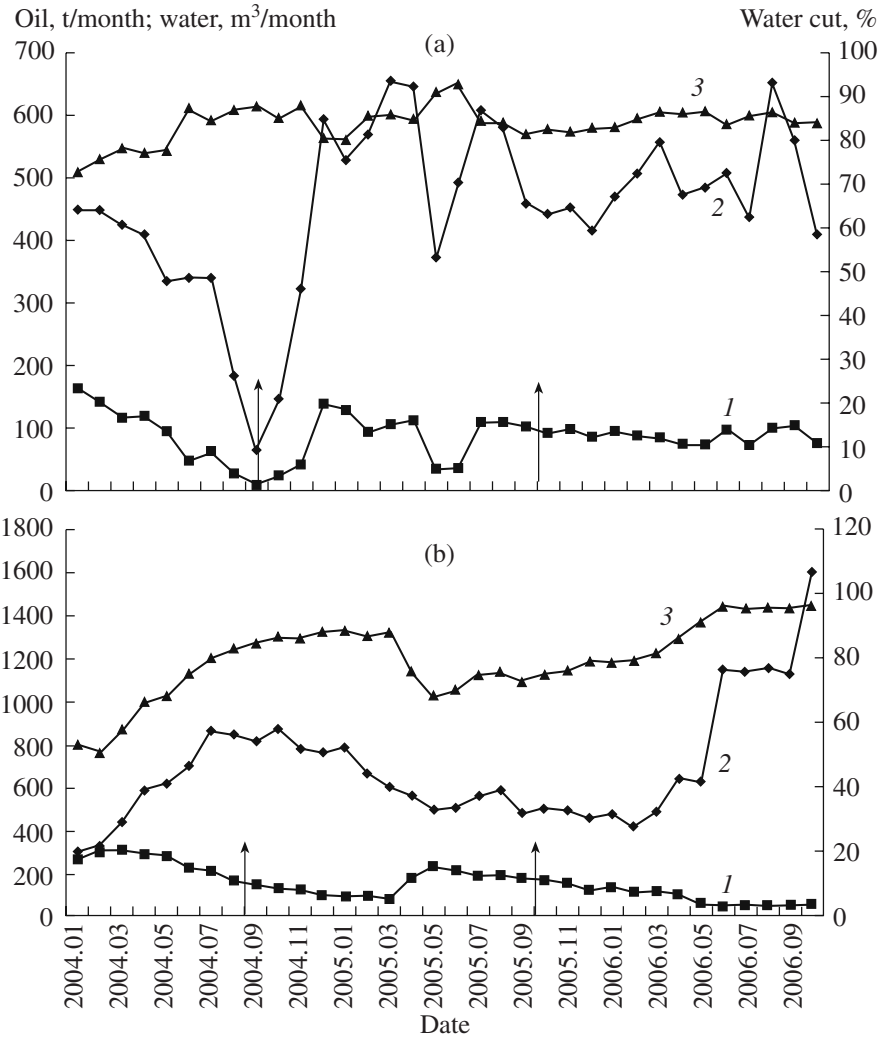

Fig. 4. Oil production and water cut of production from wells 38-8-1 (a) and 37-7-2 (b) in the course of biotechnology trial. Arrows indicate the onset of injection of the water–air mixture or H_2O_2 solution. Designations: oil production, t/month (*1*); water production, m^3/month (*2*); water cut (water content in the produced liquid), % (*3*).

and their metabolites were delivered by injection and formation water into the zones with the closest hydrodynamic connection with injection wells (wells 38-8-1 and 39-6-3); these results correlate with the data obtained by tracer studies. Oil emulsification by biosurfactants and a local increase in the reservoir pressure are probably the major mechanisms of the enhancement of oil recovery from the experimental site treated with the water–air mixture. Injection of H_2O_2 solution promoted biosurfactants formation and methane oxidation in the reservoir but inhibited methane production; these events affected the dynamics of oil recovery. Changes in the rheological characteristics of formation water due to biosurfactant production have been previously reported in the experiments with injection of molasses and fermentative bacteria into carbonate collectors [5, 28]. Production of biosurfactants by the stratal microflora due to oil oxidation has been demonstrated on the high-temperature horizons of the Kongdian bed of the Dagang oil field [11, 19] and was confirmed by the present investigation of the Gangxi oil bed. The overall amount of additional oil recovered from the experimental site by two variants of the biotechnological treatment was 3819 t.

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