ISSN 0026-2617, Microbiology, 2007, Vol. 76, No. 3, pp. 287–296. © Pleiades Publishing, Ltd., 2007.
Original Russian Text © T.N. Nazina, A.A. Grigor'yan, N.M. Shestakova, T.L. Babich, V.S. Ivoilov, Qingxian Feng, Fangtian Ni, Jianqiang Wang, Yuehui She, Tingsheng Xiang, Zhibin Luo, S.S. Belyaev, M.V. Ivanov, 2007, published in Mikrobiologiya, 2007, Vol. 76, No. 3, pp. 329–339.

EXPERIMENTAL ARTICLES

Microbiological Investigations of High-Temperature Horizons of the Kongdian Petroleum Reservoir in Connection with Field Trial of a Biotechnology for Enhancement of Oil Recovery

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Received July 10, 2006

Abstract—The physicochemical conditions and microbiological characteristics of the formation waters of the Kongdian oilfield of the Dagang oilfield (China) were studied. It was demonstrated that this oilfield is a high-temperature ecosystem with formation waters characterized by low mineralization. The concentrations of nitrogen and phosphorus compounds, as well as of electron acceptors, are low. Oil and oil gas are the main organic matter sources. The oilfield is exploited with water-flooding. The oil stratum was inhabited mostly by anaerobic thermophilic microorganisms, including fermentative (10^2–10^5 cells/ml), sulfate-reducing (0–10^2 cells/ml), and methanogenic (0–10^3 cells/ml) microorganisms. Aerobic bacteria were detected mainly in the near-bottom zone of injection wells. The rate of sulfate reduction varied from 0.002 to 18.940 μg S^{2-} l^{-1} day^{-1} and the rate of methanogenesis from 0.012 to 16.235 μg CH_4 l^{-1} day^{-1}. Microorganisms with great biotechnological potential inhabited the oilfield. Aerobic thermophilic bacteria were capable of oxidizing oil with formation of biomass, the products of partial oxidation of oil (volatile acids), and surfactants. During growth on the culture liquid of oil-oxidizing bacteria, methanogenic communities produced methane and carbon dioxide, which also had oil-releasing capabilities. Using various labeled tracers, the primary filtration flows of injected solutions at the test site were studied. Our comprehensive investigations allowed us to conclude that the method for microbial enhancement of oil recovery based on the activation of the stratal microflora can be applied in the Kongdian oilfield.

Keywords: oilfields, thermophiles, sulfate reduction, methanogenesis, oil oxidation, MEOR.

DOI: 10.1134/S0026261707030058

In the middle decades of the 20th century, the American scientist Claude ZoBell conceived the idea of using microorganisms to enhance oil recovery (cited in [1]). Later, the effect of microbial metabolites on oil release was repeatedly confirmed using laboratory models, as well as during pilot tests of the methods for microbial enhancement of oil recovery (MEOR) [2–5]. Gases, acids, solvents, polymers, and surfactants of microbial origin displayed oil-releasing properties, like their chemically synthesized analogues. Microbial biopolymers and biosurfactants obtained on the surface are used to enhance oil recovery [1–4]. Xanthan is the best known biopolymer of microbial origin used in the oil industry. There are a number of biotechnologies based on the application of flooding with the introduction of nutrient solutions which stimulate the development of the oil stratum microbial community or its individual components (oil-oxidizing, denitrifying, fermentative, and methanogenic microorganisms) [2–8]. In a number of cases, nutrients are introduced along with microorganisms in order to produce oil-releasing metabolites directly in the oil stratum [5–8]. Biotechnologies contributing to an increase in the oil inflow from production wells are in most common use; for this purpose, easily utilizable substrates (e.g., molasses) and fermentative bacteria are injected, and the well is

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closed for some period. As a result of anaerobic microbial processes, gases, organic acids, and solvents are produced, which promote the oil influx into the well. Experiments aimed at the application of such a biotechnology were most successful in carbonate oil collectors, where the use of microbial metabolites resulted in the dissolution of the carbonate matrix [5]. Biotechnologies for decontamination of the near-bottom zone of production wells of asphaltic–resinous–paraffin deposits through the introduction of oil-oxidizing microorganisms are of frequent use as well. These biotechnologies are usually applied at oilfields, which have been exploited for prolonged periods of time with the use of water-flooding, when the water content in the produced liquid is as high as 70–90% and oil production is thus unprofitable. The choice between microbiological biotechnologies is an important issue which determines the strategy of further development of an oil stratum. In spite of the numerous positive results of the pilot tests of MEOR technologies in many countries, these biotechnologies still have not found wide application [5].

In water-flooded oilfields of Russia and the former Soviet Union, a method based on the activation of the oil stratum microbial community, including hydrocarbon-oxidizing and methanogenic microorganisms, has the widest application. The injection of an aerated aqueous solution of nitrogen and phosphorus mineral salts exerts an essential stimulatory effect on the whole subsurface microbial community. The main purpose of this biotechnology is the activation of microbial metabolism aimed at the biosynthesis of oil-releasing metabolites produced by stratal microflora as a result of the partial oxidation of residual oil. This is the main difference from other MEOR biotechnologies based on the injection of organic substrates and/or microorganisms grown on the surface. A series of pilot tests of the biotechnology at a number of oilfields in Tatarstan, Bashkortostan, Western Siberia, and Azerbaijan resulted in additional recovery of more than 600000 tons of oil [2].

This biotechnology has been applied mostly in the oilfields with a temperature ranging from 20 to 45°C. It was necessary to explore the potential of its application in high-temperature oilfields. The Kongdian oilfield of the Dagang oilfield was selected as an object of our study. Stratal microflora exerts a considerable influence on the transformation of the substrates injected into the oil stratum, as well as on the production of various oil-releasing compounds.

The goal of the present work was to study the physicochemical conditions in the oilfields, to elucidate the composition of the microbial community, to explore the possibility of production of oil-releasing metabolites by this community, and to evaluate the tates of contemporary microbial processes occurring in the Kongdian oilfield. Moreover, it was essential to study the direction of the hydrodynamic flows of injected water and compare the results of microbiological investigations with the geological, hydrochemical, and production characteristics of the studied oil stratum.

MATERIALS AND METHODS

Characteristics of the Kongdian oilfield. The Kongdian oilfield of the Dagang oilfield is situated in Hebei Province (China). The studied sandstone oil-bearing horizons are located at depths of 1206–1435 m below sea level and have a temperature of 59°C; the average porosity is 33%, and the stratum permeability is 1.878 μm^2. Dagang oil had a density of about 0.900 g/cm^3 and contained 53% saturated hydrocarbons, 20% aromatic compounds, and 21.15% resins and asphaltenes. The initial formation water of sodium hydrocarbonate type was characterized by low mineralization (5612 mg/l). The initial gas content was 26.5 m^3/ton oil. The oil gas contained methane (95–98%), higher C_2–C_5 homologues (0.8–1.8%), N_2 (0.5–3.3%), and CO_2 (0.06–0.77%). The oilfield has been exploited for about 30 years with water-flooding in order to maintain the stratal pressure. In some wells, the produced oil contained 95% of water. The co-produced formation water of the Kongdian oilfield (40–60°C) was used for flooding.

The pilot area on the North block of the Kongdian oilfield was hydrodynamically isolated from the other zones of the stratum. A total of 22 production and 11 injection wells were contained at this pilot area (Fig. 1).

Sampling procedure. In June and December 2000, water samples were taken from 22 production wells and two injection stations, as well as from a number of closed injection wells operated on a regime of back flow. The samples were collected into sterile bottles, hermetically sealed, and sent to the laboratory. Within 4–6 h after sampling, bacterial numbers were determined by inoculation of appropriate media. The separate water samples were supplemented with isotope solution to assess the rates of microbial processes. The samples were stored at 6°C until all the chemical analyses had been performed.

Medium composition and methods used for enumeration of bacteria. The number of microorganisms belonging to the major metabolic groups was determined by inoculating tenfold dilutions of formation water samples into liquid media (in duplicates). The results were calculated using the McCready tables of the most probable number. For enumeration of aerobic organotrophs, the medium was used containing bacto tryptone (0.5 g/l), yeast extract (2.5 g/l), and glucose (1.0 g/l); pH 7.0. The number of hydrocarbon-oxidizing bacteria was determined using a mineral medium supplemented with a mixture of C_{10}–C_{22} n-alkanes (2% vol/vol). Anaerobic organotrophic bacteria with fermentative metabolism were enumerated on the medium supplemented with peptone (4 g/l) and glucose (10 g/l). The numbers of sulfate-reducing bacteria were determined by the increase in hydrogen sulfide in the

dilution series in Postgate B medium with sodium lactate (4 g/l) supplemented with microelements and reduced with 200 mg/l $Na_2S \cdot 9H_2O$. The numbers of methanogens were assayed by the methane increase in the dilution series in the Zeikus media with acetate (2 g/l) and $H_2 + CO_2$ (4 : 1), supplemented with microelements and yeast extract (1 g/l) (references to the composition of the media were cited in a preceding publication [13]). The inoculated media were incubated for 30 days. Thermophilic bacteria were enumerated at 60°C and mesophilic bacteria at 30°C. All cultures were then examined using an Olympus microscope with a phase contrast device.

Analytical methods. The contents of methane, hydrogen, and carbon dioxide were analyzed by gas chromatography. Hydrogen sulfide was determined by the colorimetric method of Pachmayr with dimethyl-p-phenylenediamine [14]. The chemical composition of the formation waters was determined by the standard methods [15]. In KOH-fixed samples, we determined the content of short-chain fatty acids and lower alcohols. Before the analysis was started, the samples were acidified with 25% HCl and analyzed in a Chrom-41 chromatograph equipped with a flame ionization detector and a glass column (2.0 m × 0.3 cm) filled with Porapak Q (100–120 mesh). The evaporation temperature was 180°C. A mixture of N_2 (95%) and CO_2 (5%), at a flow rate of 70 ml/min, was used as the carrier gas.

The emulsifying activity was estimated using a modified method [16]. The C_{16}–C_{22} hydrocarbon mixture (4 ml) was supplemented with 4 ml of formation water (or of the grown microbial culture). The mixture was then agitated on a Vortex mixer for 3 min. After 24 h, the volume and stability of the formed emulsion was recorded. The emulsifying activity was expressed as the percent ratio of the emulsion volume to the total volume of the mixture. The viscosity of the formation waters was determined with a viscosimeter (Programmable Rheometer Model DV-III Brookfield), equipped with a Brookfield TC-500 water bath, at 60°C. The surface tension was measured at the liquid/air interface by the ring-tearing-off method on a Krüss K10 ST tensiometer at 60°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 using labeled compounds ($Na_2^{35}SO_4$, $NaH^{14}CO_3$, and $^{14}CH_3COONa$) [17-19].

The stable isotope composition of mineral carbonates dissolved in the formation waters and of methane in the co-produced gas was analyzed by Craig's method [20] in a MI-12-12V mass spectrometer with an accuracy of ±0.5‰[per mille].

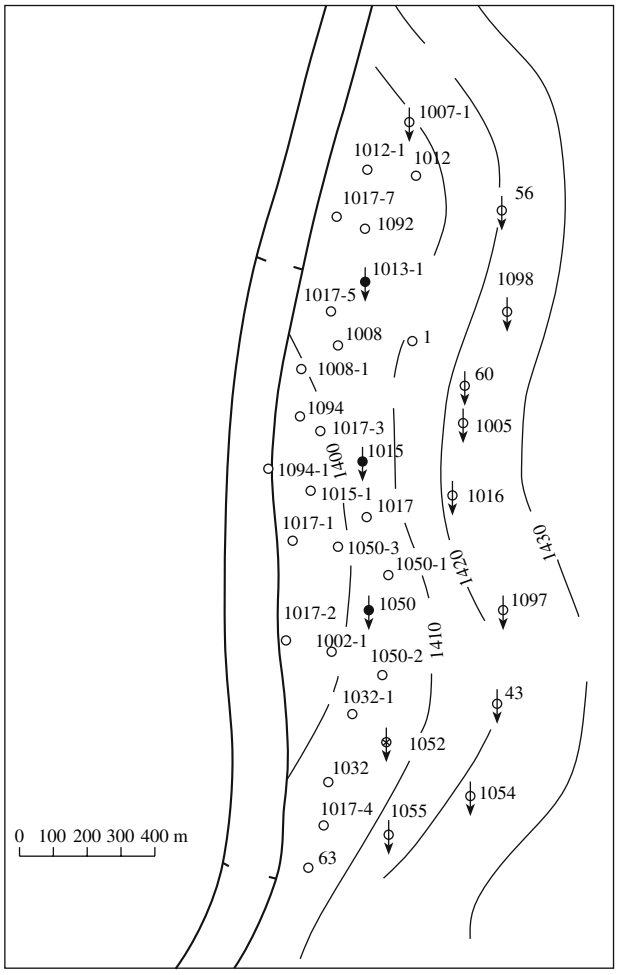

Figure. The layout of the injection (○) and production (●) wells at the test site of the Kongdian oilfield of the Dagang oilfield.

RESULTS AND DISCUSSION

Physicochemical conditions and microbial processes in the Kongdian oilfield

The physicochemical characteristics and the composition of the microbial community of the oil stratum were investigated in order to explore the potential of the method of microbial enhancement of oil recovery, as well as to predict the consequences of the application of biotechnology. The major physicochemical and microbiological parameters of the formation waters of the Kongdian oilfield are shown in Tables 1 and 2.

The oil-bearing horizons of the North block of the Kongdian oilfield had the temperature of about 60°C. The co-produced formation waters (separated from oil) of this oilfield were used for injection in order to maintain the stratal pressure. As a result, the chemical composition of the injection water was similar to that of the formation water. The process of oil and water separation usually takes two or three hours. The water becomes enriched with dissolved oxygen (0.8–2.6 mg

Table 1. Chemical composition of formation water, stable carbon isotope composition of mineral carbonates and methane, and the rates of thermophilic sulfate reduction and methanogenesis in the formation water of the Kongdian oilfield (June–December 2000)

Well number, volume of back-flushed water, m^3	Salinity, mg/l	Concentrations, mg/l					$\delta^{13}C/\Sigma CO_2 + HCO_3^- + CO_3^{2-}$, ‰	$\delta^{13}C/CH_4$, ‰	Rate of methanogenesis, μg CH_4 l^{-1} day^{-1}			Rate of sulfate reduction, μg S^{2-} l^{-1} day^{-1}
		$K^+ + Na^+$	Mg^{2+}	Ca^{2+}	Cl^-	HCO_3^-			From $NaH^{14}CO_3$	From $^{14}CH_3$-COONa	Total	
Injection water	6304	2283	31	59	3414	517	1.1	N/D	N/D	N/D	N/D	N/D
1098* –18 m^3	6256	2272	33	52	3396	503	4.2	N/D	1.505	14.73	16.235	2.850
1098* –27 m^3	6282	2272	34	59	3414	503	3.6	N/D	0.935	13.165	14.100	0.360
Production wells												
63	6456	2324	51	44	3493	544	N/D	–41.9	0.011	0.003	0.014	18.940
1002-1	5876	2160	30	34	3233	419	N/D	–42.2	0.05	0.073	0.123	0.164
1008	6663	2433	37	30	3563	600	N/D	–43.1	0**	0.012	0.012	0.024
1012	6459	2335	30	59	3449	596	N/D	–43.1	0.070	0	0.070	2.820
1012-1	6309	2290	31	52	3405	531	N/D	–42.7	0.017	0.093	0.110	0.715
1015-1	6333	2296	39	44	3423	531	–1.5	N/D	0	0.021	0.021	0.298
1017	6314	2275	42	59	3449	489	5.4	–42.4	0.123	0	0.123	2.76
1032	6477	2326	42	69	3537	503	3.3	–42.7	1.595	0.001	1.596	0.029
1032-1	6295	2274	45	32	3344	600	N/D	–41.6	0.063	5.610	5.673	4.200
1050-1	6208	2242	40	62	3431	433	4.8	–42.8	1.470	0.001	1.471	16.04
1050-2	6372	2290	33	81	3493	475	N/D	N/D	0.295	0	0.295	0.002
1050-3	6265	2290	33	49	3460	433	3.5	N/D	0.05	0.011	0.061	0.063
1092	6455	2346	37	37	3449	586	5.3	N/D	0.171	0.003	0.174	0.402
1094	6590	2384	36	44	3484	642	5.1	–42.5	0.064	0	0.064	1.860

Notes: * Back-flushed water from injection well 1098.
** Corresponds to 0.000; N/D—no data.

Table 2. Numbers of thermophilic and mesophilic microorganisms (cells/ml) in the injection and formation water of the Kongdian oilfield (June–December 2000)

Well number, volume of the back-flushed water, m^3	Aerobic organotrophs	Hydrocarbon-oxidizing bacteria	Fermentative bacteria		Sulfate-reducing bacteria	Methanogens	
			1**	2**			
	Glucose + bacto-tryptone + yeast extract + O_2	C_{10}-C_{22} + O_2	Peptone + glucose		Lactate + SO_4^{2-}	H_2 + CO_2	Acetate
Thermophilic microorganisms (60°C)							
First injection station	10^2	10^3	10^2	10^4	10^4	10^3	Single cells
1098* – 18 m^3	10^2	10^2	2.0×10^3	10^5	2.5×10^4	6.0×10^2	Single cells
1098* – 27 m^3	10	10	1.3×10^4	10^5	6.0×10^4	5.0×10^2	Single cells
Production wells:							
63	0	0	10	10	2.5×10^2	0	Single cells
1002-1	10	0	10^4	$\geq 10^4$	25	10^2	10
1008	0	Single cells	10	5.0×10^4	0	10	0
1012	0	0	10	1.3×10^3	Single cells	60	0
1012-1	10	6.0×10^2	25	1.3×10^2	60	6.0×10^3	0
1015-1	0	0	10^3	10^3	25	60	Single cells
1017	0	0	60	10^5	13	6.0×10^3	0
1032	0	0	Single cells	2.0×10^4	10^2	25	0
1032-1	Single cells	0	10^2	6.0×10^4	60	2.5×10^3	0
1050-1	Single cells	10^2	Single cells	2.0×10^2	25	60	0
1050-2	0	0	50	10^5	10^2	10^3	0
1050-3	10	10	10^2	10^5	10	10^3	10
1092	N/D	10^3	Single cells	1.2×10^2	Single cells	0	0
1094	0	0	10	5.0×10^4	10	10^2	0
Mesophilic microorganisms (30°C)							
First injection station	10^2	10^4	10^2	$\geq 10^4$	10	10	Single cells
1098* – 18 m^3	$\geq 10^4$	$\geq 10^4$	6.0×10^4	$\geq 10^5$	2.5×10^2	Single cells	10
1098* – 27 m^3	2.5×10^2	6.0×10^2	50	$\geq 10^5$	Single cells	25	Single cells
Production wells:							
1032-1	10^5	2.5×10^2	Single cells	N/D	Single cells	Single cells	Single cells
1050-3	10^3	10	Single cells	$\geq 10^2$	0	0	0
1092	10	$\geq 10^4$	0	N/D	0	0	0
1094	$\geq 10^4$	0	0	$\geq 10^4$	0	0	0

Notes: * Back-flushed water from injection well 1098.

** Number of fermentative bacteria according to the H_2 content in the gas phase (1) and microscopic analyses (2); N/D—no data.

O_2 per liter); its temperature drops to 30–50°C. Then it is injected into the oil stratum through the injection wells. The presence of oxygen in the oil separation system results in the development of an aerobic bacteria in the formation waters; the viability of anaerobic microorganisms does not change over this short period.

The studied formation waters of the sodium hydrocarbonate type were slightly alkaline (pH 7.1–7.6) and had low mineralization (5.8–6.6 g/l). The bicarbonate concentration varied from 419 to 642 mg/l; the anions SO_4^{2-} and CO_3^{2-} were not detected (Table 1). Hydrogen sulfide (less than 2 mg/l) was detected in the water from the near-bottom zone of the injection wells and was not found in the injection waters and in the waters from the production wells. The acetate concentration in most samples was less than 5 mg/l; only in the water from well 1032-1 was it as high as 60 mg/l. Other volatile acids and lower alcohols were not detected. The con-

tents of PO_4^{3-} and ammonium ions in the formation waters were 0–0.8 and 2.3–7.1 mg/l, respectively.

Mesophilic and thermophilic bacteria contained in the injection waters were represented by aerobic organotrophs and anaerobic fermentative, sulfate-reducing, and methanogenic microorganisms (Table 2). These microorganisms were also present in the near-bottom zone of the injection wells operated in a regime of back flow. The presence of dissolved oxygen in the injection water promoted the growth of aerobic bacteria involved in oil biodegradation and, consequently, of anaerobic microorganisms utilizing the products of oil oxidation. In the water from the injection zone, the numbers of microorganisms belonging to the studied physiological groups were considerably higher than in the waters from production wells (Table 2).

Aerobic and anaerobic thermophilic bacteria were also found in the water of production wells. The number of aerobic bacteria was low, or they were not detected at all. The oil stratum was inhabited, for the most part, by thermophilic anaerobic fermentative (10^2–10^5 cells/ml), sulfate-reducing (0–10^2 cells/ml), and methanogenic (0–10^3 cells/ml) microorganisms. Microorganisms able to grow at 30°C were also present in the water of production wells; however, their numbers were much lower than those of thermophilic bacteria (Table 2). It is possible that incubation at 30°C revealed some thermophilic bacteria with wide temperature growth ranges.

The rate of thermophilic sulfate reduction ranged from 0.002 to 18.940 μg S^{2-} l^{-1} day^{-1}. These values are rather high considering the fact that sulfates have not been detected in the formation waters. The rates of thermophilic methanogenesis from $NaH^{14}CO_3$ and $^{14}CH_3$–COONa in the water of the production wells did not exceed 1.595 and 5.610 μg CH_4 l^{-1} day^{-1}, respectively, reaching 1.505 and 14.730 μg CH_4 l^{-1} day^{-1}, respectively, in the near-bottom zone of the injection well (Table 1). The rates of these thermophilic processes are comparable to those recorded in the Mykhpayskoe, Talinskoe, and Samotrlor high-temperature oilfields of Western Siberia, as well as in the high-temperature Liaohe oilfield (China), which are also exploited with water-flooding [13, 21, 22].

Mesophilic methanogenesis was not detected by the radioisotope method in the formation liquid from the production wells tested (63 and 1032-1). In the near-bottom zone of injection well 1098, the rates of methanogenesis from labeled bicarbonate and acetate were 0.192 and 19.56 μg CH_4 l^{-1} day^{-1}, respectively (Table 1).

The stable carbon isotope composition of mineral carbonates ($\delta\,^{13}C/\Sigma CO_2 + HCO_3^- + CO_3^{2-}$) dissolved in the formation waters of the Kongdian oilfield, varied from –1.5 to 6.4‰ (Table 1). The $\delta\,^{13}C$ values of the carbonates dissolved in injected water sampled at the first and sixth injection stations were similar (1.1 and 2.1‰). The value $\delta\,^{13}C$ of the methane carbon contained in coproduced gas varied within a narrow range (from –41.6 to –43.1‰).

Oil gas contained methane (94.7–96.7%), its higher C_2–C_5 homologues (1.51–2.18%), N_2 (0.51–1.8%), and CO_2 (0.96–1.8%).

The rheological properties of the formation waters were studied. The emulsifying activity was not detected in the formation waters of the production wells. The surface tension of the formation waters against air ranged from 43.4 to 55.2 mN/m; the interfacial tension against the mixture of liquid paraffins ranged from 19 to 30.2 mN/m. The viscosity of the formation waters from the near-bottom zone of the injection well and from the production wells was low (0.67–0.74 and 0.67–0.72 mPa s, respectively).

Biotechnological potential of the stratal microflora

The formation waters of the Kongdian oilfield have been studied in order to reveal the presence of biotechnologically useful bacteria. The main emphasis was placed on aerobic oil-oxidizing bacteria and methanogens. A total of 22 pure cultures of thermophilic and mesophilic aerobic organotrophic bacteria were isolated from the oil stratum. The isolated thermophilic bacteria belonged to the genera *Geobacillus* and *Thermoactinomyces* and the mesophilic bacteria belonged to the genera *Bacillus, Micrococcus, Mycobacterium, Cellulomonas, Pseudomonas, Acinetobacter*, and to novel γ-Proteobacteria. Members of the genus *Geobacillus* (*G. subterraneus*, *G. stearothermophilus*, *G. thermoglucosidasius*, and *Geobacillus jurassicus* sp. nov.) were dominant in the stratal oil-oxidizing microflora [23, 24].

The capacity of the microbial population inhabiting the near-bottom zone of the injection well to produce biosurfactants during aerobic growth on crude oil and other substrates was studied (Table 3). The nutrient media supplemented with various substrates were inoculated (10% vol/vol) with the formation water from the near-bottom zone of injection well 1098 (sample 25 m^3). For comparison, the growth of the oil-oxidizing bacteria *G. jurassicus* $DS1^T$, also isolated from the Kongdian oilfield, and *Bacillus licheniformis* strain 421 isolated from the Daqing oilfield were studied. It is known that bacteria of the species *B. licheniformis* are often used in various model and field tests of the methods for microbial enhancement of oil recovery [5]. Inoculated media were incubated without agitation at 60°C for five days (Table 3).

Strains $DS1^T$ and 421 were able to synthesize biosurfactants and to induce oil emulsification. Strain $DS1^T$ produced biosurfactants on all the substrates used, such as sucrose, acetate, butyrate, liquid paraffins, and crude oil. The most pronounced decrease of the interfacial tension by this strain (down to 12 mN/m)

Table 3. Growth on various substrates of thermophilic hydrocarbon-oxidizing bacteria and of the microbial community from the near-bottom zone of the injection well (1098, 25 m^3) of the Kongdian oilfield; influence exerted by microorganisms on the rheological characteristics of the culture media

Sample, strain, substrate	OD_{600}	Emulsifying activity, %	Surface tension, mN/m	Interfacial tension against paraffins, mN/m	Viscosity*, mPa s
1098*– 25 m^3					
Sucrose	0.46	50	53.1	19.3	0.68
Acetate	N/D	30	51.3	19.0	0.66
Ethanol	0.95	30	54.9	22.9	0.64
Paraffins	0.48	40	51.5	25.1	0.62
Crude oil	0.39	0	41.9	11.8	0.63
G. jurassicus DS1^T					
Sucrose	0.64	30	45.3	15.5	0.64
Acetate	0.61	30	47.3	15.3	0.61
Butyrate	0.51	50	47.9	15.4	0.63
Paraffins	0.40	5	51.3	22.7	0.64
Crude oil		30	41.9	12.5	0.60
Bacillus licheniformis 421					
Sucrose	1.35	50	41.0	17.9	0.72
Acetate	0.55	20	56.6	24.1	0.68
Butyrate	0.45	50	46.7	17.4	0.67
Paraffins	0.27	20	54.8	21.0	0.70
Crude oil	0.22	N/D	50.7	19.0	0.695
Controls: sterile medium					
Sucrose	0.05	0	51.0	19.0	N/D
Acetate	0.05	0	50.0	20.0	N/D
Butyrate	0.05	0	56.0	22.0	N/D
Ethanol	0.05	0	55.0	22.0	N/D
Without substrate	0.05	0	58.0	29.0	N/D
H_2O	0.04	0	72.0	46.0	N/D

was observed in the medium supplemented with the crude oil from the Kongdian oilfield. The microorganisms isolated from the near-bottom zone of injection well 1098 also decreased the interfacial and surface tensions the most in the medium supplemented with crude oil. Bacteria of strain 421 from the Daqing oilfield produced surfactants less actively than those of the strain DS1^T and of the microbial population of the Kongdian oilfield (Table 3).

The results obtained suggest that the Kongdian oilfield was inhabited by aerobic thermophilic, biosurfactant-producing microorganisms. The effect of the surfactants both released into the medium and contained in the microbial biomass may be one of the possible mechanisms involved in the enhancement of oil recovery.

Methanogenic microorganisms are involved in the terminal stages of oil biodegradation. The methanogens from the Kongdian oilfield were grown in media with acetate and $H_2 + CO_2$, as well as in the medium with peptone and glucose together with fermentative bacteria. The numbers of methanogens grown in the hydrogen-containing medium, as a rule, were greater than those of the methanogens grown in the acetate-containing medium. Methane production from both substrates, $NaH^{14}CO_3$ and $^{14}CH_3$-COONa, was detected by radioisotope methods (Tables 1, 2).

To elucidate the phylogenic diversity of the microorganisms involved in the terminal processes of oil biodegradation, the methanogenic community of the Kongdian oilfield was analyzed using molecular techniques [25]. In the enrichment cultures, as well as in the natural microbial community of the formation waters, the phylotypes of the species *Methanothermobacter thermautotrophicus* of the order *Methanobacteriales* prevailed. This led us to conclude that thermophilic H_2-utilizing methanogens dominated in the subsurface ecosystem of the Kongdian oilfield [25]. We did not

Table 4. The rate of injection water migration and of the tracer appearance in the formation waters of production wells situated around the injection wells

Well number	Tracer	Distance from the injection well, m	Tracer concentration, BQ/l	Time of the tracer appearance, days	Rate of water migration, m/day
1050*	^{3}H				
1050-1		120	89.0	40	3.0
1002-1		130	58.9	60	2.2
1050-2		180	36.0	80	2.2
1032-1		360	Tracer not detected for 110 days		
1017		280	″		
1015*	^{3}H				
1094		221	64.2	35	6.3
1094-1		246	418.3	11	22.4
1015-1		172	115.8	7	24.6
1017		147	Tracer not detected for 80 days		
1050-3		233	″		
1013-1*	^{35}S				
1		194	1.96	18	10.7
1008		180	1.44	14	12.8
1008-1		290	13.78	50	5.8
1017-5		180	1.89	30	6.0
1092		145	1.76	38	3.8
1017-7		242	Tracer not detected for 125 days		

Note: * Injection wells; other, production wells.

detect any phylotypes of acetate-utilizing methanogens. Bacterial clones belonged to the orders *Thermoanaerobacteriales* (genera *Thermoanaerobacter, Thermovenabulum, Coprothermobacter*, and *Thermacetogenium), Thermotogales, Nitrospirales* (genus *Thermodesulfovibrio*), and *Planctomycetales.* A bacterium related to *Thermacetogenium phaeum* was identified for the first time in the enrichment cultures isolated from the high-temperature oilfields. The bacterium *T. phaeum* is known to oxidize acetate during syntrophic growth together with H_2-utilizing methanogens [26]. We believe that *T. phaeum* and microorganisms with a similar type of metabolism (e.g., bacteria of the genus *Desulfotomaculum*), which utilize acetate or other fatty acids in the course of sulfate reduction, can switch to syntrophic growth in the absence of sulfate and use H_2-utilizing methanogens as biological electron acceptors.

The pure cultures of methanogens isolated previously from various geographically distant oilfields were represented by H_2-utilizing members of the genera *Methanothermobacter*, *Methanococcus*, and *Methanoculleus* [27]. The results of studies of methanogenic microorganisms obtained by us and other authors [27] allowed us to suggest that acetate contained in the high-temperature oil stratum is decomposed by syntrophic associations of methanogens with acetate-oxidizing bacteria, rater than by aceticlastic methanogens alone.

The methanogenic enrichment cultures isolated from the Kongdian oilfield produced methane during growth in the culture liquid of aerobic oil-oxidizing bacteria reduced with 500 mg $Na_2S \cdot 9H_2O$, as was demonstrated earlier for the mesophilic microbial communities of oilfields [28]. Carbon dioxide is a common product of the metabolism of aerobic and anaerobic microorganisms. Hence, methane and carbon dioxide can be effective oil-releasing agents produced by microorganisms in the Kongdian oilfield.

Hydrodynamic links between the injection and production wells of the Kongdian oilfield

Before the pilot trial, a number of experiments with various indicators (tritium and ^{35}S) were carried out at the test site of the Kongdian oilfield. These experiments were aimed at the elucidation of the hydrodynamic links between the injection and production wells, and well as of the direction of the hydrodynamic flows of injection water.

Tritium is the isotope most extensively employed in such investigations due to its inertness to formation fluids and collector rocks. Tritium water (THO, 10 Ci) was injected through injection well 1050. The presence of

tritium in formation water was detected with a scintillation counter (model 2550, Packard Co., United States) and analyzed for more than 110 days. The tritium water flowed through injection well 1050 into production wells 1050-1, 1002-1, 1050-2, and 1050-3 at a speed ranging from 2.2 to 3.0 m/day and was registered in the zone of the production wells after 40–88 days, depending on the distance from the zone of the injection well (Table 4).

Tritium water (THO, 5 Ci) and butyl alcohol [$CH_3(CH_2)_2CH_2OH$, 10 Ci], in which one or several ^1H atoms were substituted for ^3H (tritium), flowed through injection well 1015 to the west and was registered in production wells 1015-1, 1094-1, and 1094 after 7, 11, and 35 days, respectively (Table 4). Tritium migrated throughout the stratum at a maximum speed of up to 22.4 m/day (well 1094-1) and 24.6 m/day (well 1015-1). The radioactivity of formation water from well 1094-1 was maximal. In wells 1017 and 1050-3, this indicator was not detected.

In the zone where injection well 1013-1 is located, the indicator (labeled sulfur ^{35}S; 0.5 Ci) migrated to the production wells at a speed ranging from 3.8 to 12.8 m/day; its maximum concentration was detected by the 50th day in well 1008-1, situated to the southwest of the injection well (Table 4).

The results obtained demonstrated the close hydrodynamic connection between the injection and production wells, as well as the fact that, at the testing site, injected water migrates predominantly from the northwest to the west and southwest. These studies allow us to predict the direction of the microorganism distribution and the minimum time it will take to detect microbial activity in the Kongdian oilfield during the trial of the biotechnology for microbial enhancement of oil recovery.

This study indicates that the physicochemical conditions of the Kongdian oilfield are suitable for the growth of thermophilic bacteria belonging to various physiological groups; in the near-bottom zone of injection wells, conditions are created favoring the development of mesophilic species as well. Low concentrations of nitrogen and phosphorus salts, as well as a lack of electron acceptors, inhibit microbial growth in the oil stratum. Oil components, gas, and organic matter dissolved in the formation waters can be the nutrient substrates for the microorganisms inhabiting the oil stratum. In the absence of chemical electron acceptors (SO_4^{2-}, Fe^{3+}, and NO_3^-), electron transfer to a biological electron acceptor (syntrophic growth) becomes especially essential. Many sulfate-reducing bacteria can grow together with H_2-utilizing methanogens; owing to this capacity, both groups of microorganisms gain a significant ecological advantage over other microorganisms inhabiting sulfate-free environments [27]. Capacity for syntrophic growth ensures survival not only of sulfate-reducing bacteria, but also of other microorganisms inhabiting ecological niches with a low content of electron acceptors. Sulfate-reducing bacteria have usually been present in the enrichment cultures of methanogens obtained from the Kongdian oilfield, growing on sulfate-free media with such substrates as benzoate, acetate, or propionate. Therefore, the input of sulfates should be controlled, since it may stimulate the undesirable sulfate reduction in the stratum.

Thus, it has been shown that the Kongdian oilfield of the Dagang oilfield is inhabited by aerobic bacteria which are able to produce biosurfactants when grown on oil, as well as by methanogens producing methane and carbon dioxide when grown on the products of oil biodegradation. Since fermentative bacteria did not grow directly on crude oil, their ecological role probably lies in the deeper transformation of the products formed during oil oxidation by aerobic bacteria; these products are the substrates for secondary anaerobes (sulfate-reducing and methanogenic microorganisms). Analysis of the results of our microbiological investigations, as well as of the geological, hydrodynamic, and productive characteristics of the oil stratum, allow us to conclude that the method for enhancement of oil recovery based on the stratal microflora activation is appropriate for use at the Kongdian oilfield.

ACKNOWLEDGMENTS

This work was supported by the Chinese National Petroleum Corporation and the Dagang Oilfield Company (China) (contract no. DFT04-122-IM-18-20RU), as well as the Russian Foundation for Basic Research (projects no. 05-04-39029 and 06-04-49128), Ministry of Education and Science of the Russian Federation (Leading Scientific Schools, grant no. 02.445.11.7409), and the Presidium of the Russian Academy of Sciences (Basic Research Program "Molecular and Cellular Biology"). The authors thank Mr. Yao Heqing, President of the Dagang Oilfield Co. (Tianjin, China), and the staff members of this company, Mr. Zhou Jiaxi and Mr. Zhou Lihong, as well as Mr. Yuang Zhihua from Yangtze University, for their help in organizing the field investigations at the Dagang oilfield.

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