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EXPERIMENTAL ARTICLES

Microbiological Processes in a High-Temperature Oil Field

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Abstract—Thermophilic sulfate-reducing bacteria (SRB) oxidizing lactate, butyrate, and C_{12}–C_{16} *n*-alkanes of oil at a temperature of 90°C were isolated from samples of water and oil originating from oil reservoirs of the *White Tiger* high-temperature oil field (Vietnam). At the same time, no thermophiles were detected in the injected seawater, which contained mesophilic microorganisms and was the site of low-temperature processes of sulfate reduction and methanogenesis. Thermophilic SRB were also found in samples of liquid taken from various engineering reservoirs used for oil storage, treatment, and transportation. These samples also contained mesophilic SRB, methanogens, aerobic oil-oxidizing bacteria, and heterotrophs. Rates of bacterial production of hydrogen sulfide varied from 0.11 to 2069.63 at 30°C and from 1.18 to 173.86 at 70°C μg S/(l day); and those of methane production, varied from 58.4 to 100 629.8 nl CH_4/(l day) (at 30°C). The sulfur isotopic compositions of sulfates contained in reservoir waters and of hydrogen sulfide of the accompanying gas indicate that bacterial sulfate reduction might be effective in the depth of the oil field.

Key words: high-temperature oil reservoir, thermophilic sulfate-reducing bacteria, oxidation of crude oil alkanes.

Investigation of anaerobic microbiological processes in oil fields is motivated by the hope to isolate new anaerobic microorganisms possessing new features and by the need to find solutions to some topical issues in the oil industry. Microbiological degradation of oil hydrocarbons in anaerobic conditions is by itself an important problem both for theoretical and applied research. It is widely accepted that sulfate reduction and methanogenesis in water-flooded oil fields are associated with the two-stage process of oil oxidation. In the first stage, hydrocarbons are oxidized by aerobic bacteria to acids and other organic compounds. In the second stage, these compounds are utilized by anaerobic sulfate-reducing bacteria (SRB), methanogens, and other bacteria [1, 2]. Over the last few years, SRB able to directly oxidize aromatic and paraffin hydrocarbons were isolated, characterized, and argued to have played a role in altering the composition of oil in oil fields [3, 4]. No data, however, are available on the distribution and geochemical activities of such bacteria in oil reservoirs.

Microbiological processes in high-temperature oil reservoirs are of special interest. These processes were partly studied in the oil fields of the North Sea, Africa, western Siberia, and some other parts of the world [5–7]. The main emphasis in these studies was on the isolation and investigation of microorganisms. Only limited evidence is available on the kinetics of anaerobic microbiological processes, particularly sulfate reduction, in hot oil reservoir waters and on their influence upon the composition of sulfur compounds in oil field liquids and accompanying gas. Investigations of this kind were undertaken in western Siberian oil fields, where the original oil reservoir temperature of 62–80°C dropped at some places to 18–40°C as a result of flooding with surficial water [6]. At these locations, mesophilic microorganisms were predominant. No oil fields with temperatures higher than this one have so far been studied in detail.

The purpose of this work was to explore the microbiological processes effective in the *White Tiger* oil field (Vietnam), which is characterized by extremely high temperatures and exploited by seawater flooding. The specific goals of the study were (1) to determine the environmental conditions in the oil field; (2) to study the distribution of thermophilic SRB of different physiological types (including those utilizing *n*-alkanes) in reservoir waters, in injected water, which is a possible source of microorganisms, and in technological reservoirs used for oil storage, treatment, and transportation, where the water phase is represented by reservoir water; (3) to determine rates of microbiological processes; and (4) to determine the isotopic composition of sulfur in sulfates and hydrogen sulfide.

MATERIALS AND METHODS

The *White Tiger* oil field is located in the middle part of the Central Rise of the Kyulong depression, the stratigraphic section of which is represented by a Pre-

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cenozoic crystalline basement underlying terrigenous deposits of the Oligocene, Miocene, and Pliocene (Quaternary Period). On top of the basement, the southern, central, and northern roof deposits were identified [8]. Oil is extracted from sea platforms from several Miocene and Oligocene horizons and also from fractured and cavernous basement granitoids at a depth of 4000 m. The oils recovered are sweet, highly paraffinic, and crude, with a setting point of 29.5–33.0°C. The oil density (ρ_4^{20}) is 0.833–0.863, and the paraffin and tar (including asphaltenes) contents of the oil vary from 18.7 to 24.0 and from 3.3 to 11.8%, respectively. According to NIPI Morneftegas SP Vietsovpetro, as a result of cooling with superficial seawater, the temperature in the near-bottom zones in injection wells is only 55–100°C, whereas the reservoir temperatures are as high as 120–160°C.

Samples were taken from wells located in different parts of the oil field. The northern roof (basement) is exploited using wells on platforms MSP-6 and MSP-4. Parts of the central roof are opened by wells on platforms MSP-1, MSP-2, MSP-9, BK-3, and BK-4 (basement and Miocene). The Early Miocene oil reservoir is opened with wells on platform MSP-5.

To maintain the pressure in the oil reservoir, seawater is injected. Prior to injection, seawater is treated to remove mechanical particles, organic matter, and dissolved oxygen. The water in the oil reservoirs contains chloride and calcium and a salinity is 2–7 g/l (according to SP Vietsovpetro). It also has a low sulfate content.

The design of an injection well is schematically shown in Fig. 1. The liquid driven into the annular space under the pressure of the injected seawater is mostly stagnant with small mass exchange at the near-bottom level. When the well is switched into the backflow mode (Fig. 1b), samples can be taken from different horizons of the water column in the annular space and from the near-bottom zone.

In this study, we analyzed samples of water from the top, middle, and lower (near-bottom) zones of the water column in the annular space; samples of water and oil taken directly from oil-bearing beds; samples of injected seawater; and samples of water from technological reservoirs used for oil storage, treatment, and distribution (buffer reservoirs and tanks), which contain water extracted together with oil.

The number of SRB utilizing oxidized organic compounds was determined by the method of serial dilutions, using the seawater II Widdel's liquid medium [9]. Different variants of the medium contained sodium lactate (3 g/l), sodium butyrate (1 g/l), or sodium acetate (1 g/l) as organic substrates. In a number of cases, crude oil was used as the inoculum. To reveal the oil-oxidizing SRB, the medium with butyrate (1 g/l) was inoculated with crude oil from the oil field studied (1 ml per 10 ml of the medium). The growth of microorganisms

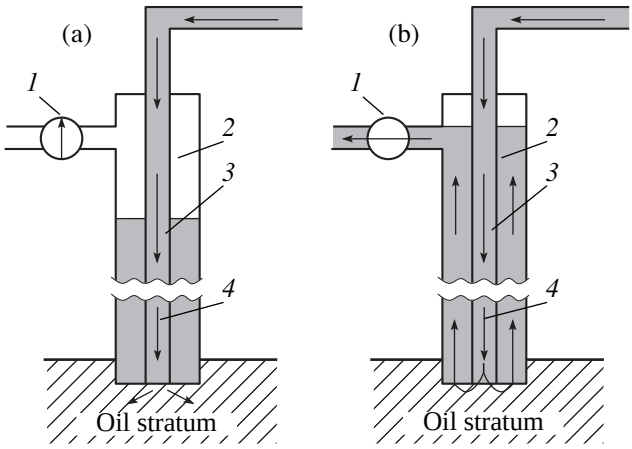

Fig. 1. Diagram of the sampling from the annular space of an injection well: (a) the water injection mode; (b) the backflow mode used for sampling. (*1*) Stopcock; (*2*) water column in the annular space of the injection well; (*3*) tube; (*4*) injected water flow.

was detected from the increased content of hydrogen sulfide in the culture [10] and from the decreased concentration of C_{12}–C_{16} *n*-alkanes in the oil, determined by gas–liquid chromatography [11].

Methane- and acetate-producing bacteria were enumerated by the method of serial tenfold dilutions on a sulfate-free liquid medium containing (g/l): NaCl, 20; $MgCl_2 \cdot 6H_2O$, 2.0; $CaCl_2 \cdot 2H_2O$, 0.5; NH_4Cl, 0.5; $CH_3COONa \cdot 3H_2O$, 0.5; MES, 3.0; Tris, 2.0; EDTA, 0.5; KCl, 1.0; K_2HPO_4, 0.04; yeast extract (Difco), 1.0; resazurin, 0.001; $NaHCO_3$, 5; Na_2S and cysteine-HCl, 0.2; and vitamin and trace element solutions, according to Balch [12]. The pH was 7.0–7.3. To suppress the growth of SRB, Na_2MoO_4 (5 g/l) was used [13].

For enumerating methanogens, a mixture of trimethylamine, methanol, and sodium acetate (3 g/l each) or a gas mixture of H_2 and CO_2 (80 : 20) was added to the medium as a growth substrate. To enumerate the acetogens, a medium with the same gas phase (H_2 + CO_2) was supplemented with 70 mM sodium bromoethanesulfonate, which is known to specifically inhibit methanogenesis [13]. The development of methanogens and acetogens was monitored by the formation of methane and acetate, respectively, which were determined chromatographically [14]. All inoculated samples of anaerobic bacteria were incubated for 30 days at 30 and 70°C.

Aerobic oil-oxidizing and heterotrophic bacteria were enumerated using a medium similar in its mineral composition to the seawater II Widdel's medium but free of Na_2SO_4. Sterile oil from the basement was used as the organic substrate. Bacterial growth was determined from an increase in the number of cells in the aqueous and oil phases with a light microscope with phase contrast at 1000× magnification. Heterotrophs were enumerated by plating aliquots from the corresponding dilution onto the surface of the plate count

Table 1. Variation range of different characteristics for stagnant water from the annular space of injection wells of the *White Tiger* oil field and in seawater used for its flooding

Water characteristics	Ranges	
	injected water	water in annular space
SO_4^{2-}, mg/l	2573–2648	1177–2671
H_2S, mg/l	0	0–1.2
HCO_3^-, mg/l	140–174	18–415
Acetate, mg/l	1.4–3.9	0.9–95.7
pH	7.6–8.1	5.6–8.9
Redox potential, mV	from +220 to +235	from –145 to +105

agar (Difco) and incubating at 30°C and by introducing the inoculum into a semiliquid medium of the same composition and incubating at 70°C. Trace elements were added to all media [9].

Rates of bacterial sulfate reduction and methanogenesis (from carbonate and acetate) were determined radioisotopically [15, 16]. Samples were incubated for 1 day at 30 or 70°C.

The isotopic composition of sulfate sulfur was determined on an MI-1201 mass spectrometer using the procedure described in [16]. Sulfates and carbonates in water were determined by standard techniques [17] with the use of Aqua Merck kits (Merck).

RESULTS

Distribution of Microorganisms and Microbiological Processes in Injected Seawater and in Water Samples from the Annular Space of Injection Wells

As mentioned above, the injected water is plain seawater that has been given special treatment. It contains sulfates, bicarbonates, small amounts of acetate, and is mildly alkaline (Table 1).

There are noticeable changes in the chemical composition of water in the annular space of the injection wells associated with microbiological processes that develop there. The concentration of SO_4^{2-} declines and the range of concentrations of HCO_3^- and acetate increases (Table 1). The samples of injected water given different treatments were found to contain only mesophilic aerobic and anaerobic microorganisms (Table 2). No thermophilic forms were detected. In several samples, sulfate reduction was active and proceeded at a rate of 0.40–1.05 μg S/(l day) at 30°C (Table 2). The concentration of sulfates in the samples studied was 2573–2648 mg/l, and $\delta^{34}S_{sulfates}$ varied between +19.1 and +20.1‰ (Table 2). Although no methanogens were found, the radioisotopic method showed that, at 30°C, methane was formed at a rate of 1.5–91.4 nl CH_4/(l day) (Table 2). In three out of four samples, methane was predominantly produced from acetate. The injected seawater, therefore, does not become sterile during treatment, and since it is injected in huge amounts, microorganisms from different physiological groups must enter the oil reservoir in considerable numbers.

The analysis of water samples obtained from different horizons of the water column filling the annular space of an injection well gave the following results. The activities of mesophilic microbiological processes (numbers of aerobic and anaerobic microorganisms, rates of sulfate reduction and methane formation) in the top and middle water column horizons were higher than in the injected water (samples 1 and 2 in Table 3). The rate of sulfate reduction varied from 1.41 to 76.17 μg S/(l day), and the rate of methanogenesis ranged from 12.6 to 591.1 nl CH_4/(l day). In most of the samples, methane was formed from CO_2 rather than from acetate, which was present in the samples in low concentrations.

The value of $\delta^{34}S_{sulfates}$ showed considerable variation. In five samples, it ranged from +18.7 to +19.9‰, and in another five samples, it ranged from +20.5 to +35.6‰ (Table 3). In several cases, therefore, sulfate sulfur became heavier than the sulfur of seawater sulfates. The highest rate of sulfate reduction and the largest concentration of hydrogen sulfide were observed in a sample from the top horizon in well 37 (MSP-1), which also turned out to contain the isotopically heaviest sulfates, $\delta^{34}S_{sulfate}$ = +35.6‰ (Table 3).

Neither thermophilic bacteria nor high-temperature microbiological processes were detected in the injection wells.

Distribution of Microorganisms and Microbiological Processes in Water Samples from Near-Bottom Zones of Injection Wells and in Reservoir Liquids from Producing Wells

Thermophilic lactate-oxidizing SRB numbering 1–10 cells/ml were found in the water samples from the near-bottom zones of two injection wells (Table 4). No thermophilic microorganisms of other physiological groups were present in these samples. The rate of thermophilic sulfate reduction in one of the samples was 12.94 μg S/(l day). The concentrations of sulfates in these samples were somewhat lower (2567–2387 mg/l). The values of $\delta^{34}S_{sulfate}$ were +19.9 and +20.4‰ (Table 4). In addition to thermophilic sulfate-reducing bacteria, the samples from the near-bottom zones of injection wells contained mesophilic bacteria from various physiological groups. A likely explanation for this is that when a well is operated in the back-flow mode, some water from higher horizons of the water column filling

Table 2. Number of mesophilic microorganisms and rates of anaerobic microbial processes in injected water

Microorganisms and other parameters	Platforms			
	MSP-9	BK-3	MSP-6	MSP-5
Aerobic microorganisms, cells/ml				
Oil-oxidizing	$>10^2$	10^2	$>10^2$	$>10^2$
Heterotrophs	2320	650	14000	8400
Anaerobic microorganisms, cells/ml				
Sulfate-reducing, lactate-oxidizing*	0	few	10^2	few
Methanogens on medium with TMA + Ac + MeOH	0	0	0	0
Methanogens on medium with $H_2 + CO_2$	0	0	0	0
Acetogens	0	0	few	few
Microbiological processes (30°C)				
Rate of sulfate reduction, μg S/(l day)	0	n. d.	1.05	0.40
Rate of methane production, nl CH_4/(l day)	10.8	1.5	91.4	35.2
CH_4 from acetate, %	100	100	86.9	100
SO_4^{2-}, mg/l	2643	2600	2573	2648
Isotopic composition of sulfate sulfur				
$\delta^{34}S_{sulfates}$, ‰	+20.1	+19.3	+19.9	+19.1

Note: Here and in Tables 3–7, TMA is trimethylamine; Ac is acetate; MeOH is methanol; and n. d. means "no data available."
* No bacteria developing on medium with butyrate.

the annular space might enter and mix with the near-bottom water.

An enrichment culture of sulfate-reducing bacteria, which forms hydrogen sulfide in the temperature range of 46–90°C, but fails to develop at 100°C, was isolated from the near-bottom zone of injection well 100 (Fig. 2).

Most of the samples of water and oil were obtained from production wells located in oil field patches affected by secondary flooding. The two exceptions were samples from wells 110 and 806, which pierce the basement and the Early Miocene bed, respectively (Table 5). Untouched by the injected seawater, these two wells can be treated as controls. The oil reservoir waters contained sulfates (Table 5), HCO_3^{2-} (73–421 mg/l), and low amounts of acetate (2.1–8.4 mg/l). The acidity of water (pH 6.5–7.5) was favorable for the development of microorganisms. The numbers of microorgan-

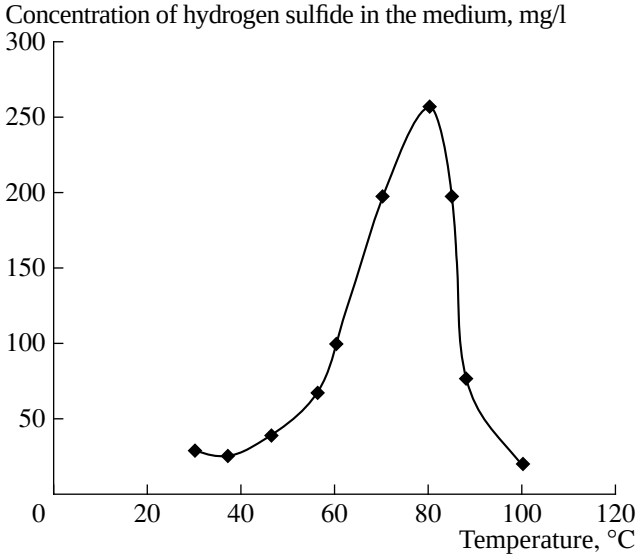

Fig. 2. Accumulation of hydrogen sulfide over a period of five days in a culture of the sulfate-reducing bacterium 100-3 at different cultivation temperatures.

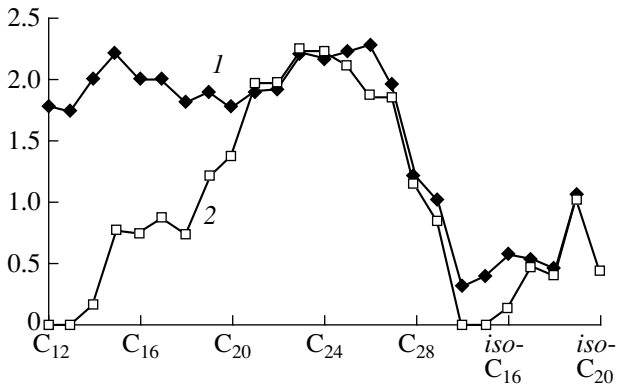

Fig. 3. Changes in the spectrum of alkanes in an oil sample from a Miocene bed caused by the development of an SRB culture isolated from well 98 (incubation at 70°C): (*1*) original oil; (*2*) the same oil oxidized by SRB. The *n*- and *iso*-alkanes with a given chain length are indicated on the *x*-axis in the order of their appearance on the chromatogram; the *y*-axis is the relative concentration of *n*-alkane in the oil sample.

Table 3. Number of mesophilic microorganisms and rates of anaerobic microbial processes in the water column filling the annular space of injection wells

Parameters	Sampling horizon*	Platforms and well nos.				
		MSP-9, 905	MSP-6, 100	MSP-5, 116	MSP-1, 37	MSP-4, 202
Aerobic microorganisms, cells/ml						
Oil-oxidizing	1	$>10^2$	$>10^2$	$>10^2$	0	$>10^2$
	2	$>10^2$	$>10^2$	$>10^2$	0	0
Heterotrophs	1	11770	112000	12810	5790	60
	2	10500	15200	98700	2450	41000
Anaerobic microorganisms, cells/ml						
Sulfate-reducing, lactate-oxidizing	1	$>10^3$	$>10^3$	0	$>10^3$	$>10^3$
	2	$>10^3$	$>10^3$	0	$>10^3$	$>10^3$
Sulfate-reducing, butyrate-oxidizing	1	$>10^3$	$>10^3$	0	$>10^3$	few
	2	$>10^2$	$>10^2$	few	$>10^1$	0
Methanogens on medium with $H_2 + CO_2$	1	0	0	0	few	few
	2	0	0	0	few	0
Methanogens on medium with TMA + Ac + MeOH	1	few	10^2	0	few	10
	2	few	few	0	few	0
Acetogens on medium with $H_2 + CO_2$	1	10^2	10^2	0	10^2	10
	2	10	10^2	10	10^2	0
Microbiological processes (30°C)						
Rate of sulfate reduction, μg S/(l day)	1	18.73	11.54	0	76.17	1.41
	2	22.54	3.03	n. d.	n. d.	n. d.
Rate of methane production, nl CH_4/(l day)	1	40.9	12.6	591.1	19.9	97.0
	2	21.3	18.3	20.4	17.6	51.0
CH_4 from acetate, %	1	74.5	12.3	99.4	11.7	3.5
	2	21.1	20.0	46.9	39.0	29.5
SO_4^{2-}, mg/l	1	2671	2220	1177	1374	2502
	2	2608	2620	2637	2589	2646
H_2S, mg/l (dissolved)	1	n. d.	0.06	0.02	1.2	0.08
	2	n. d.	0.02	0	0.04	0.02
Isotopic composition of sulfate sulfur (30°C)						
$\delta^{34}S_{sulfates}$, ‰	1	+19.9	+20.8	+19.8	+35.6	+19.2
	2	+20.8	+20.5	+18.7	+19.2	+20.6

* 1 relates to the top part of water column and 2 to its middle part.

isms were determined in 16 samples of reservoir liquid from the basement, 4 samples from the Early Oligocene bed, and 2 samples from the Early Miocene bed. Eleven of these samples contained thermophilic sulfate-reducing bacteria (Table 6), which oxidized lactate, butyrate, and certain oil paraffins. The latter organisms were found in 8 of 11 flooded oil samples (Table 6). No thermophilic bacteria of other physiological groups were detected, and mesophilic heterotrophs were found only in a water sample from well 110 (5400 cells/ml). In the same sample, at a temperature of 30°C, bacterial methanogenesis was observed to proceed at a rate of 33.2 nl CH_4/(l day).

Enrichment cultures of sulfate-reducing bacteria decomposed C_{12}–C_{16} *n*-alkanes of oil and, in some cases, C_{14}–C_{16} *iso*-alkanes. The change in the spectrum of oil alkanes caused by culturing SRB isolated from well 98 (Late Miocene bed) is shown in Fig. 3. In addition to alkanes, microorganisms of enrichment culture 98 utilized butyrate and sodium caproate at 70–80°C.

The data on the sulfur isotopic composition of sulfates in the reservoir water and hydrogen sulfide of the accompanying gas are given in Table 5. The concentrations of sulfates in several samples of seawater or its mixtures with reservoir water were lower, and these

sulfates had a heavier isotopic composition of sulfur ($\delta^{34}S_{sulfates}$ from +21.8‰ to +31.5‰) than those of injected water. At the same time, as suggested by the value of $\delta^{34}S_{sulfates}$ equal to +15‰, the sulfur of sulfates in native reservoir waters from the basement and the Miocene bed (wells 110 and 806) is much lighter than sulfate sulfur of injected seawater. Sulfur of the hydrogen sulfide of the accompanying gas from wells 110 and 457 ($\delta^{34}S_{sulfide}$ = +3.3 and +5.9‰, respectively) is much lighter than that of sulfates. It should be recalled that thermophilic sulfate-reducing bacteria were found in water samples from these wells. A particularly low value of $\delta^{34}S_{sulfates}$ equal to –0.2‰ was obtained for hydrogen sulfide in the gas from well 417.

Distribution of Microorganisms and Microbiological Processes in Water Samples from Technological Tanks Used for Oil Storage, Treatment, and Distribution

Mesophilic and thermophilic sulfate-reducing bacteria oxidizing organic acids were found in five out of six water samples from the bottom of the buffer tanks. The number of mesophiles ranged from several cells per ml to 10^4 cells/ml. The number of thermophilic sulfate-reducing bacteria never exceeded 10^2 cells/ml, and the predominant organisms were those oxidizing lactate (Table 7). Mesophilic and thermophilic sulfate-reducing bacteria were also present in samples taken from the bottom of two distribution tanks. Hydrogen sulfide was present in water of most of the tanks studied and in the gas phase of all tanks. The rate of mesophilic sulfate reduction in all kinds of tanks varied widely from 0.0002 to 2069.63 μg S/(l day) (Table 7). Incubation of samples taken from such tanks at 70°C gave rise to high-temperature reduction of sulfates at a rate of 1.18–173.86 μg S/(l day). In addition to sulfate reduction, mesophilic methanogenesis was also found to take place in such tanks at different rates, the highest one being 100.6 ml CH_4/(l day). Methane was predominantly produced from acetate. Mesophilic methanogens, acetogens, and heterotrophs were present in the water samples studied (Table 7).

Assays of sulfates and measurements of the isotopic composition of sulfur of sulfates and sulfides in the water from technological reservoirs used for oil storage, treatment, and distribution, confirmed that sulfate reduction was effective in such reservoirs. In some samples, low concentrations of sulfates (9–21 mg/l) were noted (Table 7). Sulfur of sulfates was isotopically heavier than sulfur in seawater. In six samples, $\delta^{34}S_{sulfates}$ ranged between +20.6 and +29.2‰. Sulfide sulfur (water samples) was much lighter than the sulfate sulfur, with $\delta^{34}S_{sulfide}$ varying between +0.5 and +11.2‰ (Table 7). High concentrations of acetate, up to 350 mg/l, were found in several samples.

The obtained data suggest that the conditions in the technological reservoirs are favorable for the development of mesophilic microflora.

Table 4. Number of microorganisms and rates of anaerobic microbial processes in water in the near-bottom zones of injection wells drilled to the basement

Parameters	Platforms and well nos.	
	MSP-9, 905	MSP-6, 100
Aerobic mesophilic microorganisms, cells/ml		
Oil-oxidizing, 30°C	$>10^2$	10^2
Heterotrophs, 30°C	65700	17000*
Anaerobic microorganisms, cells/ml		
Sulfate-reducing, oxidizing		
lactate, 30°C	$>10^3$	$>10^3$
butyrate, 30°C	0	10^2
lactate, 70°C	few	10^1
butyrate, 70°C	0	0
Microbiological processes		
Rate of sulfate reduction, μg S/(l day), at 70°C	12.94	n. d.
Rate of methane production, nl CH_4/(l day), at 30°C	36.6	21.4
CH_4 from acetate, %	2.6	14.6
Isotopic composition of sulfate sulfur		
$\delta^{34}S_{sulfates}$, ‰	+19.9	+20.4

* Thermophilic heterotrophs were also found in this sample.

DISCUSSION

Thermophilic sulfate reducers developing at temperatures up to 90°C were found in the samples of liquids (oil and water) from reservoirs of the *White Tiger* water-flooded high-temperature oil field. Temperatures propitious for the development of such organisms occur in the near-bottom zones of injection wells and probably in some parts of the water-flooded reservoirs. The rate of sulfate reduction obtained in culturing a water sample from the near-bottom zone of injection well 905, equal to 12.94 μg S/(l day), is comparable to the rate of sulfate reduction in reservoir waters of western Siberian oil fields [6].

Thermophilic representatives of SRB were previously found in marine sediments [18] and isolated from an oil field [19]. At the same time, no thermophilic SRB were detected in water injected into the *White Tiger* oil field and in the depth of the annular space of injection wells. Therefore, the origin of microorganisms found in reservoir liquids deserves consideration. The fact that thermophilic SRB occurred in a water sample from the basement oil stratum in well 110 (the near-bottom temperature as high 130°C) can be taken as evidence of their indigenous origin. However, both the isolation of mesophilic aerobic bacteria from the same sample and the observed mesophilic methanogenesis indicate that some water could find its way into well 110 from the overlying oil field hozizons. Transportation of the bac-

Table 5. Isotopic composition of sulfur in sulfates of reservoir waters and in hydrogen sulfide of accompanying gas

Horizon	Well	SO_4^{2-}, mg/l	Seawater content, %	$\delta^{34}S_{sulfates}$, ‰	$\delta^{34}S_{sulfides}$, ‰
Basement	447	2614	100	+19.6	n. d.
	804	2592	100	+23.7	n. d.
	802	1444	100	+31.5	n. d.
	90	350	100	+21.8	n. d.
	110	97.5	0*	+15.2	+3.3
	457	n. d.	n. d.	n. d.	+5.9
	417	n. d.	n. d.	n. d.	–0.2
Early Oligocene	83	416	55	+20.4	n. d.
Early Miocene	98	923	65	+26.1	n. d.
	46	315	77	+26.4	n. d.
	806	25	0*	+15.2	n. d.

* 100% reservoir water.

Table 6. Distribution of thermophilic SRB in fluids* as revealed by increased production of H_2S** and decreased *n*-alkanes content of oil***

Microorganisms (cells/ml) and other parameters	Platforms and well nos.										
	Basement							Early Oligocene		Early Miocene	
	MSP-9		MSP-3		BK-4	TsTP-2	MSP-6	MSP-5		MSP-1	MSP-4
	901_o	90_o	413_o	404_o	457_o	2_o	110_w	503_{o+w}	510_{o+w}	46_{o+w}	98_{o+w}
Sulfate reducers											
oxidizing lactate	0	0	0	30	0	0	170	0	68	0	106
oxidizing butyrate	0	0	0	0	0	0	0	0	30	120	30
oxidizing oil	*n*-C_{12}–C_{14}	*n*-C_{12}–C_{13} *iso*-C_{14}	*n*-C_{12}	0	*n*-C_{12}–C_{13}	*n*-C_{12}	n. d.	*n*-C_{12}	*n*-C_{12}	0	*n*-C_{12}–C_{16} *iso*-C_{14}–C_{16}
H_2S in reservoir gas, cm^3/m^3	4.5	130.0	3.0	7.5	5.0	5.0	130.0	4.5	7.5	7	2

* Sample designation: o is flooded oil and w is water.

** The figures relate to hydrogen sulfide formed (mg/l) in cultures grown from 1 ml of reservoir fluid inoculum.

*** Vanishing or declining alkanes with the indicated chain length.

teria from one horizon to another along rock fissures would be impossible without the existence of a chain of colder environmental zones that are in contact with the producing horizon. Recent investigations of different natural high-temperature environments exposed the existence of hyperthermophilic microorganisms that develop at temperatures above 100°C. One example is *Pyrolobus fumarius*, a bacterium isolated from a hydrothermal black smoker, which develops optimally at 113°C and is able to sustain temperatures as high as 120°C [20]. Sulfate reduction proceeding at temperatures close to 100°C was shown to occur in oceanic rift zones [21]. In this study, no attempt was made to isolate bacteria at temperatures above 100°C, and the question of whether or not such forms did occur in the oil field studied, where temperatures could be as high as 130 to 150°C, remains on answered.

Butyrate-oxidizing SRB could possibly utilize oil paraffins in the conditions of the oil reservoir. This conclusion is confirmed by studies of an oil-oxidizing SRB isolated from fluids of well 98 and by data from the literature [3, 4]. No physiological groups of thermophilic microorganisms other than SRB were found in the *White Tiger* oil field.

The presence of paraffin-oxidizing sulfate-reducing bacteria in the obtained oil samples indicates that such bacteria might participate in hydrogen sulfide generation in oil reservoirs. Developing on the water–oil interface, these microorganisms are able to accumulate in

Table 7. Number of microorganisms and microbiological activities in water samples from buffer reservoirs and tanks used for the treatment and distribution of oil

Parameters	Platforms, buffer reservoirs						Tanks	
	MSP-9	MSP-6	MSP-5	MSP-4	TsTP-2		4c	5s
					C2-2	E-3		
Aerobic microorganisms, cells/m								
Oil-oxidizing*, 30°C	$>10^3$	$>10^2$	$>10^2$	$>10^2$	$>10^2$	0	0	0
Heterotrophs, 30°C	44300	300	9080	10500	2800	9920	0	0
Anaerobic microorganisms, cells/ml								
Sulfate reducers								
oxidizing lactate, 30°C	$>10^4$	10^2	$>10^3$	0	$>10^3$	0	0	10^2
oxidizing lactate, 70°C	10^2	10^1	0	$>10^2$	10^2	0	few	$>10^2$
oxidizing butyrate, 30°C	$>10^4$	10^1	$>10^3$	few	0	0	0	$>10^2$
oxidizing butyrate, 70°C	0	10^1	0	0	few	0	0	0
Methanogenic								
on medium with $H_2 + CO_2$, 30°C	10^2	0	few	0	10^2	0	0	few
on medium with TMA + Ac + MeOH, 30°C	10^3	10^1	10^1	0	10^2	0	0	10^2
Acetogens, 30°C	10^2	0	10^3	few	10^1	0	0	few
Microbiological processes								
Rate of sulfate reduction, μg S/(l day)								
at 30°C	2069.63	44.62	80.77	0.19	n. d.	0.0002	0.11	21.36
at 70°C	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	0.18	173.86
Rate of methane production, nl CH_4/(l day)** at 30°C	271.5	211.3	210.6	456.5	100629.8***	58.4***	763.3	73.2
CH_4 from acetate, %	67.0	57.0	98.5	73.6	n. d.	n. d.	100.0	100.0
SO_4^{2-}, mg/l	1297	140	27	639	n. d.	9	143	71
Isotopic composition of sulfur in sulfates and sulfide								
$\delta^{34}S_{sulfates}$, ‰	+20.7	+20.6	n. d.	+25.2	n. d.	n. d.	+29.2	+14.5
$\delta^{34}S_{sulfide}$, ‰ (gas phase)	n. d.	n. d.	+10.6	+0.5	+3.1	+3.2	+11.2	n. d.
Hydrogen sulfide in water, mg/l	n. d.	n. d.	0.24	0.13	0****	0.03	0****	3.2

* No thermophilic oil-oxidizing bacteria found.

** From $H^{14}CO_3^-$ and $^{14}CH_3COO^-$.

*** From $^{14}CH_3COO^-$.

**** Hydrogen sulfide present in the gas.

the oil phase. Hydrogen sulfide is readily soluble in oil and can evolve when oil is degassed. Given that the water in the oil reservoir contains up to 50 mg/l iron, dissolved hydrogen sulfide will not appear in the water phase until all iron ions are precipitated as sulfide.

The occurrence of microbiological processes in high-temperature oil fields is noted by a decrease in the sulfate content of water in seawater-flooded reservoirs, by an increase in the isotopic weight of sulfur in sulfates, and by enrichment of the hydrogen sulfide in the accompanying gas with lighter sulfur.

It can be concluded from our investigation that the anaerobic bacterial oxidation of oil associated with the reduction of sulfates might be effective in the *White Tiger* oil field despite the fact that no changes in the composition of oil were so far recorded.

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