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EXPERIMENTAL ARTICLES

Dynamics of Microbial Processes in the Stratal Waters of the Romashkinskoe Oil Field

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Abstract—The dynamics of the microbial processes developing in parallel with the exploitation of the Romashkinskoe oil field (Tatarstan) were studied in two areas differing in the degree of stratal water freshening. Flooding of the strata, in conjunction with purposeful measures on stratal microflora activation, was shown to increase the microbial population density and activate both methanogenesis and sulfate reduction; the latter process was limited by the low sulfate concentration. Development of anaerobic processes correlated with changes in acetate concentration in the stratal water. High mineralization (over 200 g/l) inhibited the stratal water microflora even if other conditions were favorable. Isotopic analysis of the carbonate carbon showed that the bicarbonate concentration increased in the stratal water due to microbial degradation of oil hydrocarbons and further participation of the biogenic carbon dioxide in dissolution of the carbonate cement of the oil-bearing strata. In strongly desalinated stratal water, the proportion of the newly formed bicarbonate was as high as 80%.

Key words: methanogenesis, sulfate reduction, acetate, oil fields, carbon isotopic composition.

Various methods of microbial enhancement of oil recovery (MEOR) mostly rely on microbial cultures grown in biotechnological installations and injected into the oil strata together with nutrient substrates [1, 2]. Other methods imply injection of microbial metabolites into the oil stratum to promote oil recovery [1, 3].

For enhancement of oil recovery, we have developed a biotechnology based on activation of indigenous microflora [4–6], which is attained by injecting water-dissolved air and phosphorus and nitrogen mineral compounds into the oil stratum. At the beginning, aerobic and facultatively anaerobic carbohydrate-oxidizing microorganisms are activated to oxidize residual oil. As a result, various metabolites are formed (alcohols, aldehydes, organic acids, biological surfactants, and CO_2) that are instrumental in oil recovery. Anaerobic microorganisms producing oil-displacing agents (CO_2, CH_4, and others) grow on these metabolites after oxygen is consumed [7].

This paper analyzes the results of our long-term study of the microbial processes in two areas of the Romashkinskoe oil field, where MEOR technology based on activation of the oil stratum microflora has been used.

MATERIALS AND METHODS

The oil stratum microflora was studied in the Severo-Aznakaevskaya and Chishminskaya areas of the Romashkinskoe oil field (Tatarstan). In both areas, the oil-bearing rock was fine-grained quartzose sandstone interstratified with argillite and loamy siltstone. The oil bed temperature varied from 30 to 35°C.

The Severo-Aznakaevskaya area included 19 injection wells and 34 production wells. The stratal water of the Devonian deposits was initially calcium chloride brine with a total mineralization of 320 g/l. From 1979, these strata were flooded with fresh water to maintain stratal pressure. Since 1996, saline wastewater has been injected, which has resulted in an increase in stratal water mineralization. In 1992–1993 and 1995, the stratal microflora was activated by the injection of air and nitrogen and phosphorus salts into the oil bed.

No water initially occurred in the Chishminskaya oil-bearing horizons. Stratal water formation occurred at the initial stage of flooding via the interaction of the injected water with the oil-bearing rock. The Chishminskaya area included 8 and 18 injection and production wells, respectively. In this area, the stratal water was strongly desalinated. Injection of fresh water was started in 1984. The stratal microflora was activated in 1993–1994, 1997, and 2000 in the same manner as at the Severo-Aznakaevskaya area.

Microbiological methods. Samples were taken from, the wells as described earlier [8]. The microorganisms' population density was determined on selective media by the method of tenfold serial dilutions. Hydrocarbon-oxidizing microorganisms were determined on modified Raymond's medium [4]. The Devonian oil from the Romashkinskoe oil field or a mixture of Parex liquid paraffin served as a substrate. Cell growth was judged from examinations under a light microscope followed by plating on agarized media.

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Table 1. Changes in the chemical composition of stratal water in the Chishminskaya and Severo-Aznakaevskaya areas over the period 1992–2000

Well no.	Year of sampling	Salinity, g/l	Content of components, mg/l					pH
			HCO_3^-	SO_4^{2-}	Ca^{2+}	NH_4^+	CH_3COO^-	
Chishminskaya area								
6694	1994	11	268	27	724	12	0	6.9
6694	2000	15	342	6	1400	3	6	7.3
6696	1993	85	293	22	6000	50	39	7
6696	1998	44	287	59	2906	21	5	7.3
3520	1993	122	146	22	8452	70	32	7.1
3520	2000	49	268	11	4100	13	7	6.8
Severo-Aznakaevskaya area								
2839	1992	236	116	102	16500	144	32	6
2839	1997	137	146	24	14800	134	10	6.8
2842	1992	302	67	174	23000	214	25	5.9
2842	1996	200	73	33	19000	100	26	5.5
2842	1998	287	79	130	21500	160	6	5.7

The population density of anaerobic microorganisms was determined in liquid media by the method of tenfold serial dilutions using the anaerobic technique. The inoculated tubes were incubated at 30°C over a month. Growth of sulfate-reducing bacteria (SRB) was judged from ferrous sulfide formation in liquid Postage B medium [9] or from an increase in the content of hydrogen sulfide in the medium [10].

Various groups of methanogens were detected according to their substrate specificity and the degree of medium mineralization. The growth of SRB was inhibited by the addition of Na_2MoO_4 (5 g/l) [11]. PPBM and GF media [12–14] were used, which contained the following energy and carbon sources: (1) $CO_2 + H_2$ (20 : 80); (2) acetate (5 g/l), $CO_2 + N_2$ (20 : 80); (3) methanol and trimethylamine (5 g/l each), $CO_2 + N_2$. Methanogen development was judged from an increase in the content of methane in the gas phase. Antibiotics (ampicillin and vancomycin) were added to the medium to inhibit acetogen growth.

Acetogens were determined on the PPBM medium [12] with $CO_2 + H_2$ as the gas phase. Bromoethanesulfonic acid was added to the medium to inhibit methanogen growth [11]. Acetogen growth was judged from an increase in the content of acetate in the medium.

Analytical methods. The chemical composition of the stratal water was analyzed using AquaMerk kits. The pH and redox potential were measured on an OP-104 Radelkis conductometer and a pH-150 millivoltmeter, respectively. Acetate and volatile fatty acids were analyzed after distillation with volatile vapor and evaporation. The content of acetate was determined by gas-liquid chromatography [5].

The rates of methanogenesis from either CO_2 or acetate, as well as the rate of sulfate reduction, were determined using $NaH^{14}CO_3$, $^{14}CH_3COONa$, and $Na_2{}^{35}SO_4$ as described previously [5, 15]. The radioactivity of newly formed hydrogen sulfide and methane was measured on a Rackbeta liquid scintillation counter.

Stable carbon isotopes in dissolved carbonates and in methane from the gas phase were determined on an MI-1201 mass spectrometer by conventional methods [16]. For this purpose, carbonates were precipitated from samples of stratal water with a saturated solution of barium hydroxide; methane was purified from homologs on a Porapak Q large-scale column and burned to CO_2.

RESULTS AND DISCUSSION

Dynamics of the Chemical Composition of Stratal Waters during Oil Field Exploitation

Flooding of the Chishminskaya area resulted in the formation of stratal liquid with water mineralization varying in a wide range—from 11 to 112 g/l (in 1992, mineralization ranged from 40 to 80 g/l in most wells, Table 1). Later on, from 1992 through 2000, freshening continued. The decrease in the salinity of highly mineralized well water was a stable tendency. Changes in the salinity of water with a mineralization lower than 30 g/l fluctuated from 10 to 25 g/l without any clear tendency.

A higher mineralization was observed in the stratal waters of the Severo-Aznakaevskaya area (Table 1). From 1992 to 1998, the stratal water salinity decreased from 196–310 to 88–214 g/l, but increased again to 112–287 g/l after the injection of wastewater.

Table 2. Rates of methanogenesis and sulfate reduction in the stratal water of the Chishminskaya and Severo-Aznakaevskaya areas over the period 1992–2000

	Rate of sulfate reduction, ng S/(l day)			Rate of methanogenesis, nl CH_4/(l day)					
				from CO_2			from CH_3COO^-		
	background values*	activation period**	stabilization period***	background values*	activation period**	stabilization period***	background values*	activation period**	stabilization period***
Chishminskaya area									
Average	2	223	10	21	922	26	45	1014	71
Range	0–10	80–1708	0–50	0–105	524–2980	0–60	0–123	306–3773	0–100
Aznakaevskaya area									
Average	81	298	89	75	284	38	26	298	89
Range	0–280	80–2100	0–202	0–114	167–1200	0–250	0–140	128–2000	0–25

* 1993.
** 1994–1997.
*** 1998–2000.

In both the Chishminskaya and Severo-Aznakaevskaya areas, changes in Ca^{2+}, Mg^{2+}, and Cl^- ion concentrations were as a rule proportional to changes in the total brine concentration. At the same time, the sulfate and acetate concentrations changed independently of the total mineralization. In the Chishminskaya area, the content of sulfate ranged from 0 to 50 mg/l except for some cases where it reached 150 mg/l. In the Severo-Aznakaevskaya area, the sulfate concentration was higher (usually 40–100 mg/l and up to 244 mg/l in some cases). In both areas, acetate, the content of which ranged from 0 to 40 mg/l, predominated over other volatile fatty acids.

The bicarbonate ion concentration, as a rule, increased with freshening of stratal water. During freshening, the HCO_3^- concentration increased from 134–256 to 213–375 mg/l in the Chishminskaya area; in the Severo-Aznakaevskaya area, it was lower (67–287 mg/l), which seems to be a result of the higher salinity. Nevertheless, in the Severo-Aznakaevskaya area, the HCO_3^- concentration was 25–120 mg/l higher than the initial value.

The pH values increased from 6.0–6.9 to 6.8–7.9 in the Chishminskaya area. Lower pH values (5.5–7.2, with fluctuations within 0.9 pH units) were determined in the Severo-Aznakaevskaya area. Water alkalization, typical of the Chishminskaya area, was observed in the Severo-Aznakaevskaya area only in the period of strong freshening, whereas acidification occurred only at the beginning of freshening of the highly mineralized water and in the period of secondary salination. Thus, a strong decrease in the total mineralization of stratal water was always accompanied by bicarbonate accumulation and an increase in pH values.

Dynamics of the Microbial Processes

The microbial processes were monitored by the microorganism population density and rates of methanogenesis and sulfate reduction, which are the terminal anaerobic processes of oil hydrocarbon degradation. The results obtained showed that anaerobic processes were substantially activated in the stratal waters of both areas during technological impact. Afterwards, the microflora activity reversed to the initial level as a rule (Tables 2, 3). Upon the activation, the highest rate of methanogenesis and the highest methanogen population density was recorded in the more desalinated stratal water of the Chishminskaya area. In the same period, the rate of sulfate reduction in the stratal water of the Severo-Aznakaevskaya area was superior to that in the Chishminskaya stratal water, although the SRB population density was higher in the Chishminskaya area. This was probably a result of the low sulfate concentration in the stratal water of the Chishminskaya area (0–50 mg/l). Thus, in five wells of the Chishminskaya area, an increase in both the SRB population density (to 10^4 cells/ml) and the rate of sulfate reduction (to 1708 ng S/(l day)) were observed simultaneously with the maximum content of sulfate and acetate in the stratal water. The subsequent decrease in the SRB population density occurred in parallel with a dramatic decrease in both the sulfate and acetate concentrations (from 158–23 to 0–4 mg/l and from 16–35 to 4–8 mg/l, respectively).

In some cases, the population density of acetogens and methylotrophic methanogens increased after technological impact (Table 3). Acetogenic bacteria were peculiar to the heavily desalinated stratal water of the Chishminskaya area. Methylamine- and methanol-utilizing methanogens were typical of stratal water with moderate salinity (40–140 g/l), where they could be detected over several years. Six isolates obtained were

Table 3. Changes in the population densities of methanogenic, sulfate-reducing, acetogenic, and oil-oxidizing bacteria in the stratal water of the Chishminskaya and Severo-Aznakaevskaya areas over the period 1992–2000

Area	Background values*	Activation period**	Stabilization period***	Background values*	Activation period**	Stabilization period***	Background values*	Activation period**	Stabilization period***
	Methanogens on $H_2 + CO_2$, cells/ml			Methanogens on acetate, cells/ml			Methanogens on methylamine, cells/ml		
Chishminskaya area	0–100	10–10^3	0–10	0–10	10–10^3	0–1	0–10	10–10^3	0–1
Severo-Aznakaevskaya area	0–100	10–100	0–1	0–1	1–10	0–1	0–1	1–10^3	0–10
	Sulfate-reducing bacteria, cells/ml			Acetogens on $H_2 + CO_2$, cells/ml			Oil-oxidizing bacteria, cells/ml		
Chishminskaya area	0–10	10^2–10^4	0–100	0–10^3	1–10^4	0–100	0–10	1–10^3	0–10^3
Severo-Aznakaevskaya area	0–10	10–100	0–10	0–1	0–10	0	0–15	1–15	0–100

* 1993.

** 1994–1997.

*** 1998–2000.

close in their morphological and physiological properties to *Methanohalophilus euhalobius*, isolated earlier from another oil field in Tatarstan [5, 14, 17, 18]. Mineralization of the original stratal water varied within a range that was substantially broader than the salinity range optimal for methanogen growth in pure culture (60–90 g/l). The optimal Ca^{2+} concentration (from 75 to 150 mM) corresponded to 40–80 g/l mineralization of the stratal water, at which the methanogen population density was the highest (10^2–10^3 cells/ml). The pH values 6.4–6.9 optimal for growth conformed to the habitat conditions. These methanogens synthesized polysaccharides, which may be important for the MEOR technology.

The population density of aerobic oil-oxidizing bacteria increased after the activation impact, although no clear-cut correlation with other microbial processes was observed. The dynamics of the density of oil-oxidizing bacteria in the stratal water was related to the activation of aerobic processes in the injection well near-bottom zones. The increased aerobic oil-oxidizing bacteria population density in stratal waters of production wells is a secondary phenomenon confirming the permeability of the oil-bearing strata to microorganisms.

Comprehensive analysis made for some wells revealed several specific features of the development of anaerobic microorganisms. In the strongly desalinated (10–20 g/l) stratal water of well 6694, the initial sulfate concentration was 27 mg/l in 1994, and virtually no acetate was detected (Fig. 1). In 1995–1996, the acetate concentration increased to 35 mg/l, and the sulfate concentration became somewhat lower. This was accompanied by an increase in the population density and activity of SRB, hydrogen- and acetate-utilizing methanogens (HMG and AMG), and acetogens (Fig. 1). In these cases, the microorganism population density changed in conformity with their activity and, therefore, is not shown in the figures. Synchronously with the rate of sulfate reduction, the SRB population density increased (from single cells per ml to 10^4 cells/ml), whereas the population densities of methanogens and acetogens increased to 10^2 cells/ml each. In 1997, the acetate concentration decreased to 5 mg/l, and the intensity of anaerobic microbial processes also decreased to background levels (Fig. 1), as did the population density of anaerobes. Thus, acetate is an important factor influencing microflora development. A similar pattern was observed in the stratal water of some other wells.

The initial acetate concentration was high in the stratal water of most production wells, probably due to their proximity to the injection wells. At the same time, the initial microbial activity was low here. Sulfate reduction and methanogenesis were activated after the biotechnological impact. The activation of the stratal water microflora was probably also due to the transfer of bacterial cells from the injection well zone, which was subjected to the biotechnological impact. It should be noted that later on the acetate concentration decreased in these stratal waters, which was followed by cessation of methanogenesis and sulfate reduction.

In some wells with a low initial sulfate concentration, it increased after the biotechnological treatment. For example, in well 6696 (Fig. 2), the content of sulfate increased from 0 to 160 mg/l after microflora activation, mineralization decreased from 85 to 40 g/l, and, simultaneously, the rate of sulfate reduction increased sharply. Afterwards, the SRB activity was reduced to zero upon the depletion of sulfate (Fig. 2). The initial content of acetate was rather high (39 mg/l), i.e., unlike the previous example (well 6694), sulfate, and not acetate, was the limiting factor for SRB development. Note

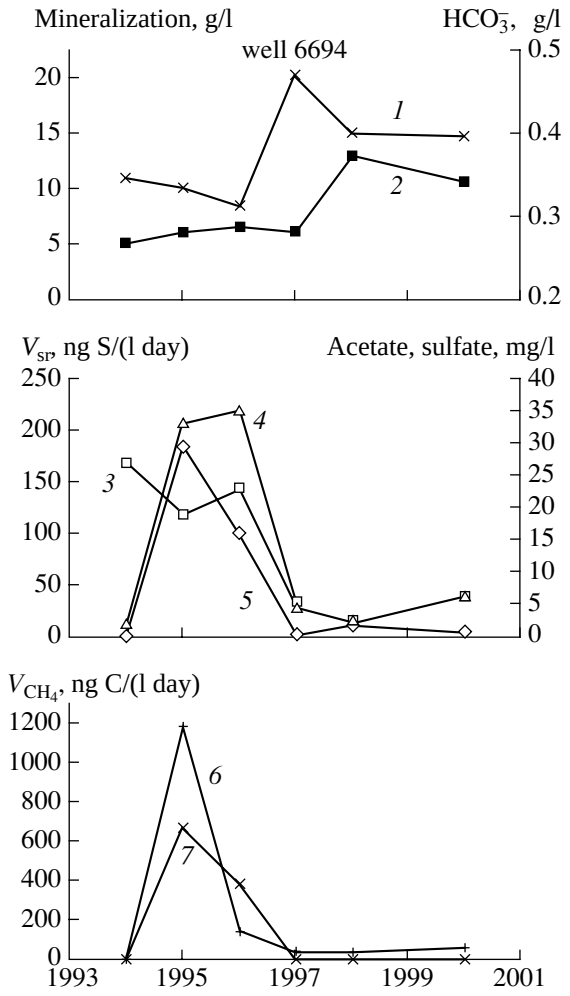

Fig. 1. Dynamics of the hydrochemical and microbial processes in the stratal water of well 6694: (*1*) mineralization; (*2*) HCO_3^-; (*3*) SO_4^{2-}; (*4*) acetate; (*5*) rate of sulfate reduction, V_{sr}; (*6*) rate of methanogenesis (V_{CH_4}) on carbonate; (*7*) rate of methanogenesis on acetate.

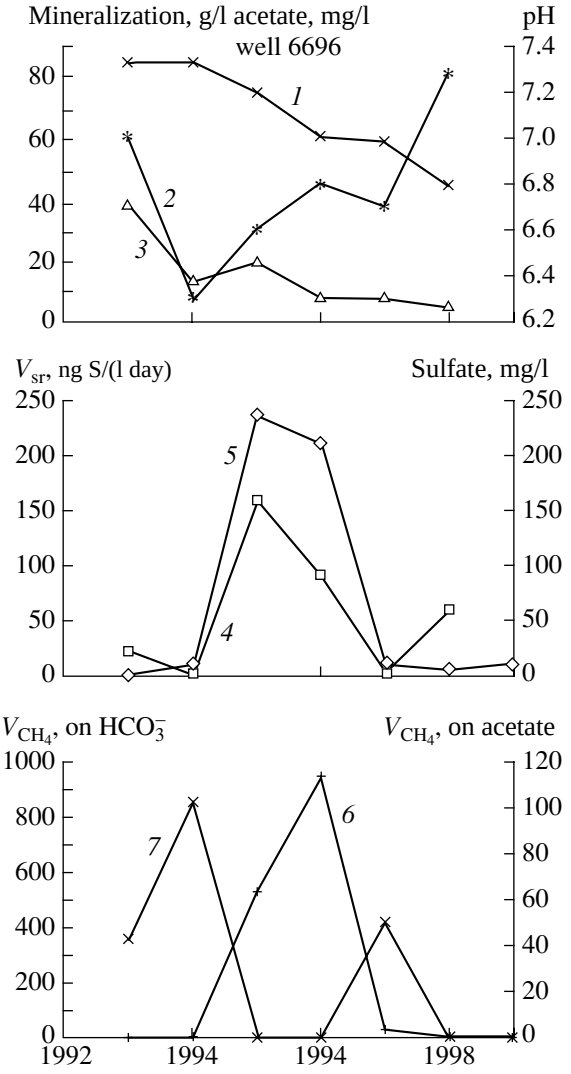

Fig. 2. Dynamics of the hydrochemical and microbial processes in the stratal water of well 6696: (*1*) mineralization; (*2*) pH; (*3*) acetate; (*4*) SO_4^{2-}; (*5*) rate of sulfate reduction, V_{sr}; (*6*) rate of methanogenesis (V_{CH_4}) on carbonate; (*7*) rate of methanogenesis on acetate.

that in 1994 an increase in methanogenesis on acetate preceded the increase in sulfate reduction (Fig. 2). Upon SRB activation, methane formation from CO_2 was observed instead of methanogenesis on acetate. After sulfate exhaustion in the stratal water, AMG developed in the stratal water instead of SRB and HMG. By this time, the acetate concentration had decreased gradually to 8 mg/l. Afterwards, no methanogenesis was observed, probably because of the low acetate concentration (4 mg/l).

Alternation of methanogenesis on either acetate or CO_2 was observed in some other wells (6697, 6730, 6732, and 6991). HMG development was mostly observed in parallel with activation of sulfate reduction, whereas AMG occurred either prior to or after the active sulfate reduction. Development of other anaerobes—acetogens and methylotrophic methanogens—was also activated in the Chishminskaya area after the technological impact, but it was not correlated with changes in acetate concentration.

Thus, low contents of sulfate and acetate (3–5 mg/l) were the limiting factors for the development of microorganisms that utilized them as substrates. The MEOR technology stimulated acetate entry into the oil bed (as the oil oxidation product), as well as the entry of sulfate, either with the injected water or as a secondary product of sulfide oxidation. In most cases, an increase in sulfate concentration coincided with a decrease in total mineralization, which confirmed the secondary origin of sulfate. Simultaneously with the acetate and sulfate entry, the anaerobic microbial processes were activated, to be then extinguished with the substrate exhaustion.

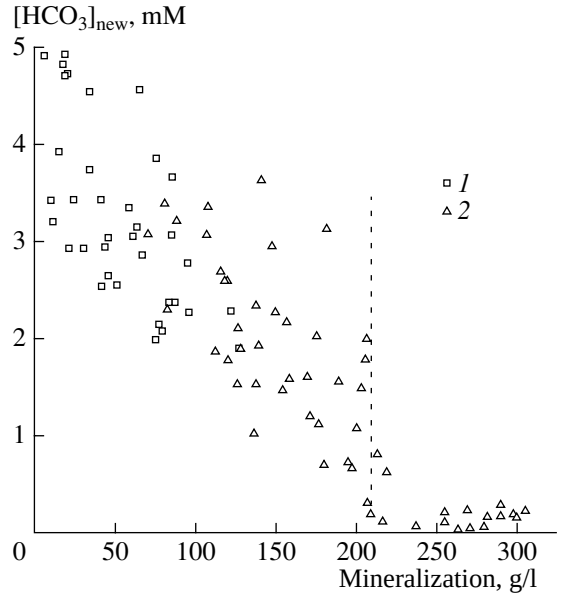

Fig. 3. Concentration of newly formed HCO_3^- versus mineralization of stratal brine. The hypothetical threshold brine concentration is indicated by the dotted line. (*1*) Samples from the Chishminskaya area; (*2*) samples from the Severo-Aznakaevskaya area.

High salinity was the major limiting factor for the development of microflora in the stratal brines of the Severo-Aznakaevskaya area. Our study showed that the microbial growth was initiated at a definite stage of freshening. For example, in the stratal water of well 2842, the rates of sulfate reduction and methanogenesis in the period from 1992 through 1995 did not exceed 24 ng S/(l day) and 100 nl CH_4/(l day), whereas total mineralization comprised 282 g/l in 1995. Despite the presence of sulfate and acetate, sulfate reduction and methanogenesis were activated only after a decrease in the salinity to 200 g/l in 1996 (Table 1). The rate of sulfate reduction increased to 350 ng S/(l day), whereas the rate of methanogenesis increased to 930 and 1400 nl CH_4/(l day) upon CO_2 and acetate consumption, respectively. A further increase in the total mineralization caused by the injection of saline water led to the inhibition of microbial activity as soon as at 238 g/l. A similar pattern was observed in some other wells of the Severo-Aznakaevskaya area, where the stratal water was desalinated below 200–220 g/l for a time.

The development of microorganisms was most active when the stratal water was desalinated and the content of bicarbonate increased. The increase in the HCO_3^- concentration could result both from biogenic CO_2 formation and from additional dissolution of the carbonate matrix. Analyses of the chemical composition of the stratal waters showed that the content of HCO_3^- usually exceeded the values calculated by the mixing equation [5].

The concentration of the newly formed "excessive" HCO_3^- can be calculated taking into account the contribution of the Devonian stratal brine and that of the injected water to the total HCO_3^- pool in a given sample. Figure 3 shows the calculated concentrations of newly formed HCO_3^- as dependent on mineralization. The calculations show that, at the initial stage of freshening, the amount of newly formed HCO_3^- in the stratal water is extremely low. However, below the salinity level of 210 g/l, the amount of newly formed HCO_3^- sharply increases. Thus, a noticeable entry of newly formed bicarbonate into the stratal brine begins at a definite level of freshening; this is probably due to the biogenic origin of this bicarbonate. Note that, in the wells with stratal water mineralization below 50 g/l, the proportion of newly formed HCO_3^- in the total soluble bicarbonate was extremely high, ranging from 69 to 79%.

Isotopic analysis of the carbon composition of the dissolved carbonate (DC C = CO_2 + HCO_3^- + CO_3^{2-}) revealed gradual carbon weighting over the period of observation (Table 4). The $\delta^{13}C$ value changed from –21.4 to –14.8‰ in the Chishminskaya area and from –19.9 to –14.8‰ in the Severo-Aznakaevskaya area. The DC $\delta^{13}C$ value of the relic Devonian brine was –18.4‰, whereas the DC $\delta^{13}C$ of the water injected into the oil bed was –14 to –16‰. The $\delta^{13}C$ of the Devonian oil was –28 to –25‰, whereas that of carbonates from the Devonian deposits was not less than +6‰. The isotopic composition of the newly formed DC can be calculated by the equation of the material-isotopic

Table 4. Isotopic composition of carbon in dissolved carbonates (DC) of stratal water and in newly formed dissolved carbonates entering the brine in different periods of observation

$\delta^{13}C$ of DC, ‰			$\delta^{13}C$ of DC (newly formed), ‰		
prior to activation, 1993	period of activation, 1994–1997	steady state, 1998	prior to activation, 1993	period of activation, 1994–1997	steady state, 1998
Chishminskaya area					
–21.4 ± 1.8	–18.7 ± 1.6	–14.8 ± 2.6	–24.1 ± 1.5	–17.4 ± 0.5	–13.0 ± 1.0
Severo-Aznakaevskaya area					
–19.9 ± 1.3	–15.7 ± 1.6	–14.8 ± 1.9	–22.0 ± 5.5	–14.8 ± 0.9	–10.5 ± 1.4

balance using $\delta^{13}C$ values and the carbonate concentrations in the stratal water, the relict Devonian brine, and the water injected into the oil bed [5].

The results of calculations, shown in Table 4, demonstrate that, prior to the biotechnological measures taken to activate microflora, light carbon entered the oil bed; it had $\delta^{13}C$ similar to that of the oil undergoing oxidation (–22.0 to –24.1‰). In particular, this is valid for the wells with mineralization of about 200 g/l. After the biotechnological impact, the proportion of heavy carbon in the dissolved carbonate increased gradually, to reach $\delta^{13}C$ values of –14.8 to –17.4‰. This isotopic composition most probably developed upon dissolution of the Devonian carbonate cement by carbon dioxide generated by oil oxidation: $Ca(Mg)CO_3 + H_2O + CO_2 = Ca^{2+}(Mg^{2+}) + 2HCO_3^-$. DC with heavier isotopic composition, $\delta^{13}C$ of –10.5 to –13.0‰, could be formed upon further dissolution of the carbonate cement by organic acids.

Thus, before the measures taken to activate stratal microflora, the content of carbonates dissolved in the stratal water increased mostly due to biogenic oil oxidation to CO_2. After the activation of the microflora, organic acids also played an important role in the carbonate cement dissolution and further increase in the carbonate concentration in the stratal water. This was followed by active utilization of organic acids by anaerobic microflora. In the opinion of specialists, both carbonate cement dissolution and increased bicarbonate concentration in stratal water (an analogue of oil bed flooding with carbon dioxide) are factors promoting enhanced oil recovery [19].

Apart from the dissolved carbonate carbon, the isotopic composition of the carbon of methane, a concomitant gas, was also studied. From 1993 to 1998, a small increase in the content of the ^{13}C carbon isotope was observed in methane. The $\delta^{13}C$ value, which had been –53.5 to –52.6, changed to –52.0 to –51.3‰. This weighting was probably a result of microbiological methane oxidation in the injection well near-bottom zones, a process demonstrated earlier [20]. However, the dynamics observed may also be attributed to the heterogeneity of the methane isotopic composition in different zones of the oil bed, differently affected by MEOR and long-term flooding.

Our study showed that biotechnological impact significantly increased the geochemical activity of the microflora in desalinated stratal waters. The oxidation of residual oil under conditions of cyclic flooding leads to formation of CO_2 and organic acids, which promote carbonate-cement dissolution and increase the permeability of the oil-bearing rock. In addition, these compounds stimulate the growth of anaerobic microflora (methanogens and sulfate-reducing bacteria) in the oxygen-free zone of the oil bed. The succession of anaerobic microorganisms evidently depends on the presence of sulfate, since sulfate-reducing bacteria compete for acetate with the acetate-utilizing methanogens. The microbial processes were extinguished with a decrease in the concentrations of necessary substrates to threshold levels (3–5 mg/l). The MEOR technology also stimulated the development of other anaerobes, which, however, also depended on the level of total mineralization. In strongly desalinated stratal water, secondary acetate formation is possible with the involvement of homoacetogens. At high mineralization of the stratal water, which prevented the development of microflora, no bicarbonate accumulation was observed.

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