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REVIEWS

Use of Microorganisms in the Biotechnology for the Enhancement of Oil Recovery

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The existing methods of oil field exploitation give the opportunity to extract no more than half of the geological resources of oil; from carbonate oil collectors, only 20% of oil is extracted [1]. Furthermore, the portion of recovered oil is tending to decrease due to the development of deposits of viscous oils under difficult geological and physical conditions. Thus, new methods for enhancing oil recovery are needed to at least maintain oil extraction at a constant level.

There are various chemical and physicochemical methods for enhancing oil recovery. The method of secondary flooding, developed by the Soviet academician A.P. Krylov in the 1930s, is widely used in many oil-producing countries. Surface water is pumped into the oil stratum through a system of water-injection wells, which results in an increase in the stratal pressure. This technology is highly efficient. Chemical methods, used in combination with secondary flooding, belong to the so-called tertiary methods of oil recovery enhancement. These include immiscible flooding (alkaline or polymeric) and miscible flooding with solvents and acids, as well as flooding with the use of surface-active compounds (surfactant flooding, or microemulsion flooding). The surfactants widely used in Europe and North America are chemically synthesized oil sulfonates. Gas injection is also applied, with CO_2, N_2, and hydrocarbon gases being mainly used. Thermal methods include injection of a heat carrier (hot water or vapor) or intrastratal combustion. Selective plugging of highly permeable layers for the improvement of flooding is little used in Europe and Siberia but is widely applied in North America, Canada in particular [2].

The development of methods for microbial enhancement of oil recovery started in the middle of the 20th century. They are highly efficient and diverse, environmentally safe, and relatively cheap, although science-intensive. Three substantial prerequisites exist that allow us to believe in the prospects for the development of new efficient biotechnologies for enhancing oil recovery based on the geochemical activity of microorganisms. First, oil fields exploited with water flooding are widely inhabited by aerobic and anaerobic microorganisms belonging to various physiological groups, which retain viability and biochemical activity in oil strata. Second, microorganisms are able to degrade oil hydrocarbons to smaller and more labile organic molecules and to synthesize such oil-releasing agents as CO_2, CH_4, fatty acids, alcohols, polysaccharides, surfactants, and other technologically active substances. Third, microorganisms are capable of in situ production of oil-releasing substances directly in zones and microzones containing residual oil.

Present research that aims at the development of biotechnologies for oil recovery enhancement should be concerned with study of the regularities of microbial distribution and geochemical activity in oil fields under different geophysical conditions, as well as with advanced investigation of physiological and biochemical characteristics of microorganisms and development of methods for regulation of microbial processes in oil fields [3].

BRIEF HISTORICAL REVIEW OF MICROBIOLOGICAL METHODS

The first works on oil microbiology that gained publicity were carried out in 1926 by Russian and American researchers simultaneously. T.L. Ginzburg-Karagicheva [4, 5] studied formation waters of the Apsheron oil fields, paying particular attention to microbial formation of hydrogen sulfide. E.S. Bastin [6] detected sulfate-reducing bacteria in the waters of oil fields of California and Illinois, United States. At the same time, the first works of V.O. Tauson [7, 8] and J.W. Beckmann [9] on microbial oxidation of oils and individual oil components were published. Beckmann proposed the use of bacterial degradation of heavy oil fractions remaining in oil-bearing rock. However, this idea was not developed until the 1940s, when the American microbiologist C.E. Zobell [10–12] studied *Desulfovibrio* sp. isolated from oil-bearing shale. He found this species to be capable of utilizing oil as the source of carbon and assumed that treatment of the oil stratum

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with a suspension of this organism could result in the production of gases (N_2, CO_2, CH_4), which would enhance intrastratal pressure and oil mobility; acids (carbonic acid in particular), which would cause the enlargement of pores in limestone oil collectors and thus enhancement of oil mobility; and surfactants; in addition, degradation of high-molecular-weight hydrocarbons was expected to occur, with the formation of low-molecular-weight compounds, which would decrease oil viscosity.

Other researchers recommended flooding strata with suspensions of *Bacillus, Desulfovibrio, Clostridium, Micrococcus, Pseudomonas, Arthrobacter, Peptococcus, Microbacterium*, and other microorganisms of different taxonomic groups.

However, the oil-releasing properties of most of these microorganisms were predicted based only on their ability to produce certain technologically useful metabolites under laboratory conditions. The process of oil displacement in most cases was studied with laboratory models of oil strata. In situ testing was carried out only by J. Karaskiewicz [15] and J.B. Davis [14], who flooded oil strata with *Clostridium* sp. suspensions and molasses solutions [13–17].

The method described in [18] was based on intrastratal injection of a so-called geobioreagent composed of lake sediments and peat chips rich in microflora. Later, the geobioreagent was replaced with activated sludge from plants treating wastewater of the biochemical industry and containing populations of various microorganisms [19]. Unfortunately, the mechanisms of the enhancement of oil recovery by geobioreagents have not been adequately studied.

An interesting method for the microbial enhancement of oil recovery was developed in laboratory studies by I.L. Andreevskii [20, 21]. He proposed the activation of aboriginal microflora by injection of a mineral nutrient solution containing sulfates and phosphates. Nitrogen compounds were not recommended as microorganisms were supposed to utilize oil nitrogen and to transform oil into more labile organic components.

Methods of a third group are based on improving oil-releasing properties of injected water by using microorganisms and their metabolites. M.A. Messineva [22] developed a method for obtaining alkaline water by using urea degradation by urobacteria [22]. Heteropolysaccharides produced by *Xanthomonas* spp. grown on starch and sugar were recommended for the thickening of injected water; injected water can also be enriched with culture liquids containing surfactants, enzymes, and other microbial metabolites [23].

An important place in the research on oil field microflora is occupied by the works of S.I. Kuznetsov, who worked at the Institute of Microbiology, Russian Academy of Sciences, for about 45 years, and by the works of his disciples M.V. Ivanov, E.P. Rozanova, and N.N. Lyalikova. These works are summarized in two monographs, *An Introduction to Geological Microbiology* [13] and *Microflora of Oil Fields* [24], which describe the regularities of microbial distribution as dependent on the type of water regime and the hydrodynamic regime of the oil-bearing structures; different taxonomic and physiological groups of microorganisms inhabiting oil fields; and modern processes of methane formation in oil-and-gas-bearing facies of the Middle Volga region. Kuznetsov together with G.A. Vorob'eva [13, 25] carried out the first in situ experiment in the USSR at well no. 2 of the Sernye Vody oil field in Kuibyshev (Samara) oblast, which contained highly viscous oil. The stratum was deposited at a depth of 1300 m and contained highly mineralized chlorine–calcium brine. A mixed microbial culture grown on molasses (3 tons of molasses, 100 kg of superphosphate, 7 m^3 of fresh water) was injected into the well. The enrichment culture was obtained from formation waters of the Sernovodskoe and Berezovskoe oil fields and contained *Pseudomonas* sp. and plectridial rods. Bacteria fermented molasses with the formation of CO_2, H_2, and N_2. After the injection, the well was closed for six months. When the well was opened, the daily oil output increased from 37 to 40 tons and the pressure at the top of the well increased by 1.5 atm. The proportion of CO_2, N_2, and higher hydrocarbons ($>C_5$) in the gas mixture increased. However, the oil viscosity did not decrease, and the daily output dropped to 36.4 t in four months. The introduced microorganisms, along with sulfate-reducing, saprophytic, and clostridium-type bacteria, could be detected in the formation waters.

Analyzing this industrial trial from the present-day competence, we can say that better results could not have been achieved because the culture was injected into an oil stratum with a high sulfate ion content. The main part of molasses was utilized by sulfate-reducing microorganisms, which resulted in the formation of hydrogen sulfide, which produces an adverse impact on the oil properties and causes corrosion of oil-extracting equipment but not enhancement of oil recovery. Moreover, microbial production of CO_2 in chloride–calcium solutions may lead to the formation of secondary calcites, which results in clogging of the pores of the collector.

The studies of sulfate-reducing bacteria in oil fields of various types played a significant role in theoretical and applied geomicrobiology. In connection with this problem, another disciple of Kuznetsov, V.A. Kuznetsova, should be mentioned. Kuznetsova worked in close cooperation with the oil industry workers of the Giprovostokneft' Institute under the direction of K.B. Ashirov [26–29]. It was Kuznetsova who first applied the backflow method for the study of the microbial population and geochemical characteristics of injection well near-bottom zones.

It was shown that the most part of the microflora developing in exploited oil fields is injected there with water used for secondary flooding [26]. The highest

Table 1. Microbial products influencing the release of oil

Products	Impact
Organic acids	Change in the collector properties of rocks: enhancement of porosity and permeability and carbonate dissolution with CO_2 formation
Biomass	Selective or nonselective plugging; oil emulsification or de-emulsification, as dependent on the capacity for adhesion to hydrocarbons; change in the rock wettability
Gases (CO_2, CH_4, N_2)	Local stabilization of stratal pressure; oil swelling; oil viscosity decrease; permeability increase due to dissolution of carbonate rocks in the reaction with CO_2
Solvents (alcohols, etc.)	Oil dissolution
Biosurfactants	Reduction of interfacial tension; emulsification
Biopolymers	Effect on mobility of stratal fluids; selective or nonselective plugging

Table 2. Basic factors controlling microbial activity in flooded oil fields

Natural	Technological
Stratum temperature	Duration of the flooding period
Mineralization and chemical composition of stratal water	Tertiary methods for enhancement of oil recovery earlier applied at the site
Collector type	Temperature of injected water
Bacterial density	Type of injected water (fresh, salt) and its chemical composition
Stratum permeability	Injection regime
Saturation with oil	Introduced (or activated) microorganisms
Stratum porosity	Injected nutrients
Component composition of oil	

density of both aerobic and anaerobic microorganisms was found in injection well near-bottom zones, where surface microflora and the oxygen dissolved in water were introduced. This oxygen is used by aerobic microorganisms for the oxidation of some part of residual oil with the formation of fatty acids and other water-soluble organic compounds, which, in their turn, are utilized by sulfate-reducing bacteria in the anaerobic zone of the stratum [28, 29].

It should be stressed that these works by Kuznetsova and V.M. Gorlenko [28, 29] initiated studies of the microbial processes in the injection well near-bottom zones, which led to the development of a series of technologies for enhancing oil recovery.

PRESENT DEVELOPMENT AND APPLICATION OF MICROBIAL TECHNOLOGIES FOR ENHANCING OIL RECOVERY

The new stage of development of microbial technologies in Russia started in the 1970s and was concentrated mainly in two laboratories: the Department of Geological Microbiology at the Institute of Microbiology, Academy of Sciences of the USSR, headed by Kuznetsov, and the Laboratory of Microbial Biogeochemistry at the Institute of Biochemistry and Physiology of Microorganisms, Academy of Sciences of the USSR (in Pushchino), which was organized by Ivanov in 1969. In 1986, these research groups were united on the basis of the Institute of Microbiology, Russian Academy of Sciences. However, Kuznetsov's group investigated oil fields in Azerbaijan, whereas Ivanov's group investigated oil fields in Tatarstan and Siberia.

Present-day technologies for microbial enhancement of oil recovery (MEOR) can be divided into two groups. The first group of technologies involves the use of microbial metabolites produced in industrial fermentors. These methods are close to chemical or physicochemical ones and are based on the improvement of the oil-releasing properties of injected water with biosurfactants, biopolymers, solvents, emulsifying substances, etc. These studies, mainly by foreign microbiologists, are reviewed in [17, 23, 40, 42].

Methods of the second group, which are actively developed by our research group, are based on in situ activation of microbial processes and intrastratal metabolite formation by aboriginal [30–38] and introduced microflora [39–41]. Microorganisms produce a wide range of metabolites with oil-releasing properties. Metabolites influencing oil displacement from oil strata are listed in Table 1.

Aerobic and anaerobic microorganisms that grow in flooded strata utilize oil hydrocarbons remaining in the injection well near-bottom zones [32, 34, 35, 37] or different organic compounds, mainly beet molasses [14, 15, 17, 39, 40], introduced through injection wells.

MEOR efficacy depends on the intensity and scale of the geochemical activity of microorganisms. One of the most important factors providing for MEOR efficacy is creation of ecological conditions optimal for microorganisms or rapid adaptation of microorganisms to the existing conditions. This makes impossible the development of a MEOR technology without detailed study of the physicochemical and microbiological parameters of the oil stratum, which are connected by complex interrelations and closely interdependent (Table 2).

Due account of the natural and technological factors listed in Table 2 promotes the choice of oil fields suitable for application of a MEOR technology. However, certain microbiological characteristics of the oil stratum and injection well near-bottom zone need additional study. Methods preceding experimental injection during both variants of intrastratal activation of microbial processes [30, 32, 34, 36–38] are presented in Table 3. Samples of formation fluids are taken from production wells and injection wells operated in the backflow regime.

Among the various MEOR technologies, the technology based on injection of saccharolytic microorganisms of the genera *Clostridium* or *Bacillus* with mineral additives and molasses containing no less than 40% sugar is widely used in oil-producing countries [14, 15, 39, 40, 42]. Adaptation and field testing of this biotechnology in Russia were carried out by our group in cooperation with German researchers in Tatarstan in 1992–1995 at oil bed no. 302 of the Romashkinskoe oil field (Bashkir formations, Carboniferous Period).

The thickness of the oil-saturated stratum at this site is 8.3 m; the average porosity, 0.110; and the initial oil saturation, 0.79. Oils of Bashkir formations are heavy, highly viscous, and rich in sulfur (viscosity at 20°C is 99.8 mPas; sulfur content, 3.5 wt %; paraffin content, 3.0 wt %; density, 902 kg/m^3). Formation waters were of two types, sodium–sulfate (predominant) and calcium–chloride.

Preliminary studies of formation waters showed that the microbial community was represented by hydrocarbon-oxidizing, fermenting, sulfate-reducing, and methanogenic prokaryotes. Fermenting and sulfate-reducing bacteria were found to be closely interrelated during the process of molasses fermentation. *Clostridium* isolates were shown to actively ferment molasses with the formation of CO_2 and lower fatty acids; their activity was comparable to that of the best industrial cultures used in molasses biotechnologies for the enhancement of oil recovery.

The microbiological, hydrochemical, and physicochemical parameters of the test site of oil bed no. 302 of the Romashkinskoe oil field were found to be suitable for a semiindustrial trial of the molasses biotechnology for the enhancement of oil recovery.

Injection of molasses and microbial cultures was started in 1992 at a test site with one injection well and was extended to involve five injection wells in 1993. The results of the experiment were monitored at 24 production wells.

On the whole, 1052.3 tons of molasses were injected during 1992–1994. Molasses was fermented with the formation of large quantities of metabolites (CO_2, lower fatty acids, alcohols, etc.) that changed the properties of the formation water, oil, gas mixture, and carbonate matrix and thus played a significant role in the recovery of residual oil. During the experiment, the microbial community was significantly enriched with fermenting and methane-producing prokaryotes and their metabolic activity increased. The activity of acid-forming bacteria resulted in a decrease in the formation water pH. The composition of the gas mixture changed.

Table 3. Complex of physicochemical, microbial, and molecular biological methods used to study the geochemical activity of microorganisms in oil fields

Methods	Aim
Physicochemical methods	Determination of key parameters of the microbial habitat: temperature, total mineralization, pH, Eh, contents of HCO_3^- + CO_3^{2-}, SO_4^{2-}, CH_4, H_2S, and low-molecular-weight organic acids
Microbiological methods	Enumeration of microorganisms of major physiological groups: hydrocarbon oxidizers, organotrophs, aceticlastic and auto-trophic methanogens, and sulfate reducers
Radioisotopic methods	Determination of in situ activity of major microbial groups: sulfate reducers (with $^{35}SO_4^{2-}$), aceticlastic and autotrophic methanogens (with $^{14}CH_3COONa$ and $^{14}CO_2$, respectively), and methanotrophs (with $^{14}CH_4$)
Stable isotopic methods	Analysis of proportion of stable isotopes (^{32}S and ^{34}S, ^{12}C and ^{13}C) in substrates and products of in situ microbial activity: SO_4^{2-}; H_2S; CO_2; HCO_3^-; CO_3^{2-}; CH_4
Molecular biological methods	Determination of systematic position of pure cultures of microorganisms isolated from stratal waters and rocks of oil fields

Molasses fermentation was shown to result in significant changes in the isotopic composition of the methane carbon and soluble mineral carbon compounds. As a result of microbial activity, the formation water was enriched with light CO_2 and bicarbonate formed during fermentation of molasses, whose $\delta^{13}C$ value was from –27.9 to –28.5‰. The $\delta^{13}C$ value of dissolved carbonates decreased from –12.2 to from –20 to – 28.3‰. The reactions of microbial metabolites (carbon dioxide, volatile acids) with the carbonate matrix (a $\delta^{13}C$ value of +0.4 to –4.0‰) resulted in the matrix dissolution and enrichment of the solution with heavy carbonates, which correlated with the increase in calcium concentration in the formation water. Consumption of light carbon dioxide by chemolithotrophic methanogens (whose activity increased significantly during the experiment) resulted in methane enrichment in the ^{12}C isotope and in a decrease in the $\delta^{13}C$ value of CH_4 from –42.3 to –54.8‰.

The rate of sulfate reduction and the cell number of sulfate-reducing bacteria decreased at certain periods of time but remained high on the whole.

As a result of the biotechnology application, additional oil recovery by January 1, 1996, comprised 4806 tons, or 30.6% of the total oil recovery from the beginning of the experiment. In addition to the increase in the production well output (Table 4), the water content of the

Table 4. Dynamics of oil recovery and water content in the product at the test site of oil bed 302 during molasses flooding in 1992–1994

No. of production well	Oil output, tons/day		Water content, %	
	Sept. 1, 1992	Jan. 1, 1994	Sept. 1, 1992	Jan. 1, 1994
414	0.5	1.7	67.8	40.9
416	0.5	0.8–1.0	83.7	66.8
422	1.0	2.6	61.0	30.6
432	0.1	1.0	99.7	73.5
440	1.7	2.5	16.7	7.9
509	0.1	2.0	99.6	42.0

product extracted decreased significantly. The technological efficacy of the method comprised 4.58 tons of additionally recovered oil per ton of injected molasses.

In parallel, we developed another biotechnology for enhancing oil recovery for oil fields with terrigenous collectors flooded with fresh water or solutions of low mineralization: a biotechnology based on the activation of geochemical activity of stratal microflora [32, 38].

During laboratory experiments with stratal microflora, we deduced trophic schemes that were based on aerobic oxidation of oil hydrocarbons and involved different physiological groups of microorganisms.

In our experiments with flow-type porous models of an oil stratum, which contained a water phase, residual hydrocarbons (oil or hexadecane), and hydrocarbon-oxidizing bacteria, we demonstrated the formation of a range of fatty acids with chain lengths of 2–16 carbon atoms and oleic acid [43].

Products of the activity of hydrocarbon-oxidizing bacteria were used as substrates by a methanogenic community isolated from an oil field. The community was dominated by an acetate-oxidizing *Methanosarcina* sp. [44]. We also monitored the development of methanogens using models of residual hydrocarbons. The water phase contained acetate. Methanogens were found to develop mainly at the entry zone of the model. Gas moved along near-wall zones that imitated permeable layers and filled a collecting flask at the end zone of the model. The gas mixture contained methane and CO_2. Oil migration due to microbial methane production was visible in the pores of plane models. Thus, our model experiments demonstrated the possibility of development of methanogens in stratal pores and methane migration through the porous matrix.

Experiments with excess pressure employed flow-type models containing core sandstone, a water phase, and residual hexadecane. The model installation consisted of a pressurizing unit filled with mineral medium enriched with nitrogen and phosphorus salts, a horizontal core (the model per se), and a collector. The water phase in the pressurizing unit was saturated with atmospheric oxygen under excess pressure. The microbial community consisted of rhodococci and pseudomonads. The development of the community was accompanied by the accumulation of fatty acids and an increase in the number of bacterial cells. The rate of acid accumulation was higher than that in models without pressure. The content of CO_2 increased in the gas mixture. Bacterial development led to displacement of some part of the hexadecane [45].

Base biotechnology for enhancement of oil recovery (BTEOR) is applied at oil fields (or their sites) exploited for prolonged periods of time (no less than one year) with the use of flooding with fresh or low-mineralization water, which results in the formation in the injection well near-bottom zones of a microbial community (biocenosis) primarily consisting of aerobic hydrocarbon-oxidizing bacteria and anaerobic methanogenic bacteria [32, 36–38].

The technological scheme for the enhancement of microbial activity was developed based on the results of a complex of studies of the microflora of the stratum and injection well near-bottom zones (Table 3).

The technology is cyclic and consists of two subsequent stages. At the first stage, aerobic microflora in the injection well near-bottom zone is activated through the injection of an aerated solution of nitrogen and phosphorus mineral salts (inorganic nutrition). This results in the formation of biodegradation products of residual oil, such as fatty acids, alcohols, surfactants, biopolymers, CO_2, and other compounds with oil-releasing properties. At the second stage, in compliance with the scheme of the development of the site, usual flooding is applied, which results in the migration of the oil-releasing compounds that accumulated during the first stage to production wells. In addition, with the biological and chemical consumption of oxygen and creation of anaerobic conditions in the stratum, a part of the low-molecular-weight organic products of aerobic oxidation is utilized by methanogenic bacteria with the formation of methane, one more well-known oil-releasing agent.

The factual material collected during the trials shows a significant increase in the number of aerobic organotrophic and hydrocarbon-oxidizing bacteria during the first stage (activation of microflora of the injection well near-bottom zones). Oxidation of a part of the residual oil results in a pH decrease and an increase in the levels of acetate and other soluble organic compounds, CO_2, and bicarbonate ion. The isotopic composition of the mineral carbon forms lightens. At the same time, there occurs an increase in the number of anaerobic (mainly methanogenic) bacteria in the anaerobic zone of the stratum, as well as a considerable increase in the methanogenesis rate. Newly formed isotopically light methane appears. The rate of sulfate reduction usually does not change significantly as sandstone collectors contain little SO_4^{2-} [30, 32, 33, 46–48].

Table 5. Additional oil recovery due to the application of various modifications of the biotechnology for enhancement of oil recovery at oil fields of Russia (1983–2002)

Modification	Region, oil field	Period of application	Number of injection and production wells	Additional oil recovery, t
1. Basic: activation of microflora of the injection well near-bottom zone	1. Bashkortostan:			
	1.1 Sergievskoe	1988–1990	3–5	2300
	2. Western Siberia:			
	2.1. Bystrinskoe	1991–1992	1–8	10564
	2.2. Solkinskoe	1991–1992	1–7	5779
	3. Tatarstan:			
	3.1. Bondyuzhskoe	1983–1988	2–6	47000
	3.2. Romashkinskoe	1987–1992	6–15	40800
	3.3. Pervomaiskoe	2000–2002	3–10	34342
	Total:	1983–2002	16–51	140785
2. Activation of microflora + hydrodynamics	1. Tatarstan:			
	1.1. Romashkinskoe	1992–2002	59–118	324497
3. Activation of microflora + hydrocarbon nutrition (crude oil)	1. Tatarstan:			
	1.1. Romashkinskoe	1991–2002	13–33	30288
	1.2. Novo-Elhovskoe	1996–1999	5–9	4170
	1.3. Pervomaiskoe	1995–2002	9–27	57833
	Total:	1991–2002	27–69	86291
4. Activation of microflora + biopreparation	1. Tatarstan:			
	1.1. Romashkinskoe	1994–2001	26–72	38305
		1999–2002	3–4	2379
		2000–2002	3–11	5337
	Total	1994–2002	32–87	46021
Total		1983–2002	134–325	597594

Analogous changes occur in the microflora composition and hydrochemistry in the production wells that are hydrodynamically connected with the impacted injection wells. In three to six months from the beginning of injection of aerated water, oil recovery is enhanced and water content of the stratal fluids in production wells decreases [32, 34, 36–38, 41].

Semiindustrial trials of the base BTEOR were carried out in 1983–1992 at a number of Tatarstan, Bashkortostan, and Western Siberia oil fields (Table 5). The experiments demonstrated high efficacy of the BTEOR developed. After the implementation of this technology at the Romashkinskoe oil field, the work was continued at the Pervomaiskoe oil field in Tatarstan, where the technology allowed recovery of an additional 140785 tons of oil from 1983 to 2002 [46–48]. The technological expenses did not exceed $5 per additional ton of oil, whereas the best American technologies require about $14 [49].

The technology was fully compatible with the scheme of oil field exploitation by using flooding and did not require significant additional investments.

An industrial experiment on the activation of stratal microflora was also carried out at the Lokbatanskoe oil field in Azerbaijan. The Lokbatanskoe oil field was distinguished by the injection of seawater with a high sulfate content. The experiment was carried out at one injection and two production wells. The biotechnological treatment resulted in additional recovery of 521 tons of oil, or 33% of the total amount extracted. The effect lasted for three and a half months [45]. After that, monitoring was stopped.

In order to extend BTEOR application to oil fields flooded with fresh or low-mineralization (up to 30 g/l) waters, two modifications of the technology were developed. The first modification was developed for the sites exploited with cyclic flooding. It consists in the activation of stratal microflora combined with cyclic hydrodynamic treatment.

The method developed by us is based on the injection of an aerated water solution of nutrient compounds (nitrogen and phosphorus salts), so that the end of the injection process is coordinated with the end of the flooding cycle, which is carried out in accordance with the program of flooding. In this case, the pause in the operation of the injection well or group of wells during which the microbial population in the injection well near-bottom zone develops is contemporized with the pause specified by the plan of flooding. The period before the next flooding cycle is long enough for maximal development of microorganisms. The combination of hydrodynamic and microbiological methods does not require changing the system of exploitation of the oil field (or its site) and allows significant enhancement of oil recovery due to the synergistic effect of the two technologies. From the standpoint of site coverage and convenient management, the most effective way of BTEOR application is together with hydrodynamic impact through cluster injection stations.

The above-described technology was tested and is currently successfully applied at the Romashkinskoe oil field. In 1992–2002, this technology yielded more than 320000 additional tons of oil (Table 5).

The second modification of BTEOR was developed for the sites where injection wells have been working for prolonged periods of time (more than three to five years). The residual oil in the near-bottom zones of such injection wells is mostly oxidized. This modification combines basic activation of stratal microflora with the injection of additional hydrocarbons (crude oil) for microorganism nutrition. It was widely tested and is currently applied at the Romashkinskoe, Novo-Elkhovskoe, and Pervomaiskoe oil fields in Tatarstan. It allowed additional recovery of about 86000 tons of oil (Table 5).

Finally, one more BTEOR modification should be considered. In Tatarstan and many other regions of Russia, flooding is often performed with highly mineralized solutions or so-called wastewaters. These waters, as a rule, are formed in the process of separation of oil products. The necessity of utilization of these wastewaters demands their involvement in the technological cycle, i.e., their intrastratal injection to increase the intrastratal pressure. In Tatarstan, only highly mineralized waters (wastewaters) are used for flooding in most oil fields, and the development of a BTEOR modification for such oil fields is highly pertinent.

Analysis of the results of preliminary studies of the distribution, geochemical activity, and physiological peculiarities of microorganisms of such oil fields shows that the sites with highly mineralized stratal waters are characterized by a low microbial density and weakly developed biogeochemical processes. At the same time, such oil strata are inhabited by specialized halotolerant and halophilic microorganisms, which retain metabolic activity under the conditions of high salinity provided that the necessary nutrients are available.

During microbiological researching of Tatarstan oil fields, we isolated and studied more than 100 strains of different microorganisms capable of hydrocarbon oxidation. On the basis of this collection, we developed the microbial preparation Devoroil [41, 50], consisting of one yeast and several bacterial strains. These strains are capable of efficient oxidation of aliphatic (C_9–C_{30}) and aromatic hydrocarbons with the formation of CO_2, fatty acids, alcohols, surfactants, and other organic compounds with oil-releasing properties. The microorganisms included in the preparation are active at temperatures of 10–50°C and in a wide pH range (4.5–9.5). Among the bacterial species, there are organisms with hydrophilic and lipophilic cell walls, which makes possible oil oxidation both at the water/oil interface and in the hydrocarbon phase. The microorganisms of the preparation actively oxidize oil components in water solutions containing nitrogen and phosphorus mineral salts; the concentration of NaCl may be up to 150 g/l. Studies conducted at institutes of the Russian Ministry of Health and the Sanitary and Epidemiologic Service showed that the preparation is not pathogenic and not toxic. The microbial preparation Devoroil has been recommended for wide use in industrial technologies implying the oxidation of oil products. The production of the preparation with its subsequent lyophilization or freezing from a pasty consistence is carried out on the basis of biotechnological equipment [51].

Semiindustrial trials of the new variant of the biotechnology for the enhancement of oil recovery were carried out at a number of sites of the Romashkinskoe oil field flooded with salt wastewaters. During the trials, 46000 additional tons of oil were recovered (Table 5). The enhancement was not so impressive compared with other modifications of BTEOR, but it is quite promising given that this BTEOR modification can find most wide application.

CONCLUSIONS

Analysis of the history of the elaboration and practical use of methods for microbial enhancement of oil recovery allows us to formulate the following points concerning further development of this field of applied microbiology and biotechnology.

First, it is hardly expedient to use oil-releasing components (organic acids, solvents, surfactants, etc.) and polymers produced by biotechnological methods for the substitution of chemically synthesized analogues. Any products of microbial biosynthesis injected into a stratum will most probably be easily consumed by the aerobic and anaerobic microorganisms inhabiting exploited oil strata.

Second, injection of suspensions of any aerobic microorganisms, including hydrocarbon-oxidizing ones (which is recommended in many patents), cannot be efficient without water aeration or addition of other oxidants.

Third, injection of anaerobic microorganisms without provision of utilizable organic substances is also inefficient as very few species are capable of anaerobic degradation of oil hydrocarbons.

Fourth, for the molasses flooding in combination with the introduction of fermenting microbial cultures, the use of cultures isolated from the production wells of the same oil field is preferable.

Collection cultures of fermenting species, as well as cultures isolated from other oil fields, may turn out to be incapable of retaining metabolic activity in oil fields with different ecological characteristics (temperature, salt content, oil composition, etc.).

Fifth, among the present-day methods, our method of the activation of the aerobic–anaerobic microbial community of the near-bottom zones of injection wells [32, 34, 36–38] is the method that is the most substantiated theoretically and was successfully tested at dozens of sites of oil fields (Table 5). The method is easily compatible with the usual technology for oil recovery by secondary flooding, is not capital-intense, and is environmentally friendly.

However, even this method has certain limitations at terrigenous collectors (high temperature, immature microbial biocenosis of the injection well near-bottom zone, high salinity, etc.); these limitations may produce an adverse effect on the results of field trials. Therefore, every new object requires thorough analysis of the natural and technological parameters of the selected site (Table 2), as well as preliminary studies of the composition and activity of the microflora of the injection well near-bottom zones.

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