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Bacterial Response of Oil-Degrading Bacteria Consortia to Heavy Oil upon Its Transition to Easy-Flowing Hydrocarbons.

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ABSTRACT

This paper deals with the effect of hydrocarbon degrading bacteria of the re-formed consortia on biodegradation of different heavy oils, their impact on the vital activity of the association (the survival and the preservation of the species composition at an elevated concentration of the initial substrate and its long-term exposure), and the clarification of optimal conditions for their development ensuring the completeness and intensity of the oxidation of viscous and extra-viscous fluids under controlled cultivation mode. We found that the microorganisms being combined into consortia subject to their compatibility with each other could split aromatics and polycyclic hydrocarbons in addition to long-chain aliphatic ones, including asphaltenes and resins. We observed changes in the qualitative composition of microflora resulted from the effect of high doses of viscous oil: from rod-like and coccoid Chromobacterium, Flavobacterium, Brevibacterium, and Micrococcus to Pseudomonas, with the genus Bacillus dominating. These changes are largely due the composition and type of fluids from different viscous oil deposits used in the experiments. Aqueous medium with high salinity up to 248 g/dm^3 does not inhibit growth and developments of the consortia od hydrocarbon degrading bacteria. The biogenic elements in the form of $(NH_4)_2NO_3$, NH_4 NO_3 added into the medium in the amount of up to 30 mg/l, as well as glutamic, succinic, oxaloacetic, and pyruvic acid, valine, alanine, glucose and maltose in the ratio of 1:1:1:1:1:1:1, and at a concentration of $35 \cdot 10^{-6}$M intensify the destruction of heavy oil with its further transition to more movable hydrocarbons.

Keywords: heavy viscous and highly-viscous oil, consortium, hydrocarbon degrading bacteria, optimization, biodestruction.

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INTRODUCTION

The major component of the raw material base of the Russian oil industry and other oil-producing countries is now heavy, highly viscous oil. Domestic reserves of heavy fluids are about 13.1% of the total proven oil reserves in Russia [1]. They are concentrated in three major regions such as Volga-Ural, Western Siberian and Timano-Pechora. The proportion of Volga-Ural province exceeds 71% of the Russian reserves. Among the presented provinces, the Republic of Tatarstan is of great interest having proven reserves of more than 1.1 billion ton heavy oil [Table 1].

Table 1: Distribution of major heavy oil reserves in the Volga-Ural basin [2, 3].

Deposits	Reserves (billion ton)	Proportion in total Russian reserves (%)
Republic of Tatarstan	1.163	18.7
Republic of Udmurtia	0.285	4.6
Samara region	0.273	4.6
Perm region	0.273	3.8
Republic of Bashkortostan	0.151	2.4
Total:	4.45	71.4

The proportion of the developed initial recoverable oil reserves in the Republic of Tatarstan is more than 80%. Residual hydrocarbons are mainly sulfur, high-sulfur (99.9%) and highly viscose ones (68%). Due to the decline in proportion of high-gravity oil, the production of highly viscous oil is curbed by many reasons, both at home and abroad [3]:

- The available technologies require huge investments, creation of new processing technologies;
- Ineffectiveness (according to The Boston Consulting Group, the average internal return rate of heavy oil production is 16%, while the traditional one is 28%) [4];
- Significant amount of waste and resulted environmental pollution and disturbance of the environmental condition of entire regions involved in mining and processing of these types of oil [5].

The pressing economic need for development of viscous oil deposits poses the problem of finding ways to turn them into movable light-end hydrocarbons. The main objective of these studies is to enhance oil recovery rather by applying cost-effective, practical and promising ways than by all manner of means. Today, a special place among the technologies, including hydrodynamic, physical, chemical, and thermal [6] belongs to the microbiological one based on the widespread use of activity of various heterotrophic microorganisms, primarily hydrocarbon degrading bacteria providing a wide range of biodegradation of petroleum and petroleum products.

Microbiological method for enhanced oil recovery becomes more recognized every day in the world as a cost-effective in the investment needs and safe for the environment.

It is known[7] that many taxonomic groups of heterotrophic microorganisms can metabolize a variety of hydrocarbons, producing alcohols, aldehydes, surfactant fatty acids, a number of biopolymers, and related gases: H_2, CO_2, CH_4, C_2H_6, generally promoting the modification of solid surfaces, increasing the porosity of rocks and fluids, changing the nature of the wettability and the interfacial tension of oil. This increases oil mobility and recoverability.

Enhancement of heavy oil recovery is possible only by using highly active but selective hydrocarbon degrading bacteria (HDB) modifying the rheological properties of oil [8].

Analysis of current and previously published domestic [9,10,11] and foreign [12] works dealing with the study of enhanced oil recovery by the example of a particular well, the use of monoculture (in most cases) of hydrocarbon degrading microorganisms, the use of a certain product for the activation of HDB vital activity (molasses technology, approbated in 1992-1994 at the well 302 of Romashkinskoe oil field (Russia) shows that the absence of detailed studies regarding the use of the HDB associations in relation to the developed technologies has impeded to complete such developments, therefore, there is no such technologies today.

The development of appropriate technologies in this field requires:

- Creating consortia of hydrocarbon degrading bacteria with a wide degradation range of various fractions of light, viscous, and heavy oil;
- Studying the relationships of hydrocarbon degrading bacteria of the created consortia that ensure tolerance, preservation of interspecies relations and microflora composition in the same state for a long period of storage or use;
- Investigating the living conditions of bacteria in the variable environmental parameters (pH, dissolved oxygen, temperature, pressure, water chemistry, including varying degrees of salinity, etc.), and finally, analyzing the resistance of the strains being a part of the consortium to the presence of extra-viscous fluids from different fields differing in their composition, origin, and other reasons. Crude oil, moreover, is a complex mixture of aliphatic, aromatic and other hydrocarbons, which have toxic and carcinogenic effects on the natural inhabitants of natural ecosystems [13]. Using widely the selected HDB for enhancing oil recovery one cannot exclude the direct toxic effect of oil on the vital activity of these bacteria subject to the conditions of their occurrence in the reservoir, the formation of quality indicators and other environmental factors.

Based on the above, the objective of the present study is to investigate the role of the newly created consortia of hydrocarbon degrading bacteria (HDB) in the biotransformation of viscous oils of various districts of Tatarstan, to find out the effect of the fluids on the preservation of their species composition, to select environmental conditions upon their long-term operation, and to provide the transition of heavy oils into movable hydrocarbons.

MATERIALS AND METHODS

Object of these studies was consortia of hydrocarbon degrading bacteria, including:

1. Nine HDB species of genera Alcaligenes (1 species), Bacillus (2 species), Brevibacterium (2 species), Flavobacterium (1 species), Clostridium (1 species), Micrococcus (1 species) and Pseudomonas (2 species);
2. Three strains of the genera Micrococcus, Pseudomonas and Rhodococcus (one species each).

To compare the activity of hydrocarbon degrading activity of microbial consortia the latter were tested in various media (tap water, water from the river Volga, highly mineralized stratal produced water[a)], the Muntz medium[b)]) with viscous oil of Vishnevo-Polianskoe field of JSC "Nurlatneft" at a concentration of 100 mg to 30 g.

The response of HDB consortia to the effect of fluids (conservation or variation of species composition in the course of the experiment) was determined by applying MALDI TOF (method of matrix laser desorption ionization time-of-flight) method with the use of mass spectrometer MALDI Biotyper CA [14]. The Bruker MALDI Biotyper CA system allows us to study the molecular organization of bacteria and identify them in the FDA library.

We revealed the ability to intensify the processes of oil biodegradation in HDB consortia by adding various nutrients (NH_4NO_3, KNO_3, KH_2PO_4 – 20, 30, 50, 100 and 150 mg/cm^3), organic acids (acetic, butyric, formic - 2.0; 4.0 and 6.0 mg/cm), reservoir produced water[c)] at a dilution of 1:1, 1:2, 1:3, 1:5), and calcium chloride not diluted with tap water (for determination of stability) at 3:5.7 to 30%, and others into the cultivated media. The assessment was carried out by standardized bacteriological and chemical methods [15, 16]. Oil defrostation degree was determined according to the residual oil content using the Fourier spectrometer “Infralium FT-08”. The duration of each series of experiments (three replicates) was equal to 16 days at a cultivation temperature of 23- 25°C.

RESULTS

It is known [8] that oil is a high-component system that includes hundreds of individual substances; therefore, its biodegradation process is complex and depends on the composition, properties and its constituent elements.

A series of experiment has shown that the metabolism of oils with low specific density involves various groups of microorganisms: Acetobacter, Micrococcus, Corinebacterium, Achromobacter, Bacillus, Actinomyces [17]. Most authors agree that the bacteria Mycobacterium, Pseudomonas, Rhodococcus, Arthrobacterium, Brevibacterium, Nocardia etc. show the highest oxidation activity in this oil category among all heterotrophic microorganisms [18, 19, 20]. Our perennial investigations have established [21, 22, 23] that the dominating bacteria in the biodegradation of aliphatic long-chain hydrocarbons, aromatic, naphthenic acids, and, partially, asphaltenes are Arthrobacter, Pseudomonas, Chromobacterium, Micrococcus, Planacarcina, Bacillus and others:

a) highly mineralized stratal water (mg/cm^3): Ca^{2+}- 10861.7 ; Mg^{2+}-28893; Na^+ +K^+ - 63568.8; SO_4^- HCO_3^- - 2050.0; Cl- -124838.0; Fe(tot) - traces; Sr^{2+} - 100,9; H_2S - 103.9; total dissolved solids – 230280.0.

b) the Muntz medium (g/l): $(NH_{4)}$ HPO_4 – 2.0; K_2 HPO_4 1.0; $MgSO_4$ - 0.2; KNO_3 -1.0; $FeCl_2$ – traces, tap water 100cm^3, distilled water 900cm^3, pH-7.2

There is also an important role played by the association of oil-oxidizing bacteria, which includes microorganisms of such genera as Alcaligenes, Bacillus, Brevibacterium, Flavobacterium, Micrococcus, Pseudomonas, etc. [22, 23]. The advantage of the consortium in degrading certain hydrocarbons that combines interspecies compatibility is a high efficiency. As compared to a single species of bacteria (monoculture), the microorganisms of different taxonomic groups ranging from 1 to 3, from 1 to 9 or 10 species, combined into a community, are capable of decomposing oil into simple compounds for a short period of contact. The other authors have ascertained the same [24, 25]. Microorganisms enter into complex relationships during their development in the association. The most active bacteria decompose hydrocarbons that are more complex, the others continue to use stepwise the oxidation intermediates formed in the process of biodegradation and eventually they ensure the full biooxidation up to final products such as CO_2, and H_2O.
We conducted a series of experiments and traced a biodegradation process of viscous oil induced by hydrocarbon degrading bacteria (HDB) combined into individual consortia (see above). In these experiments, we used oil with the qualitative composition represented by liquid ($C_{10}H_{122}$) and solid ($C_{17}H_{36}$ – $C_{34}H_{70}$) fractions with the dominance of heavy fractions. Based on a boiling temperature (200-390^0 C), the latter accounts for more than 30% of the initial weight [c)].

In the course of observations, we found that the active biodegradation of viscous oil in all variants starts on the 4-10 day of contact. It is visually expressed in a gradual increase of turbidity of the medium. Quantitative determination of the dynamics of HDB population growth showed that their number grows slowly while adapting to the original substrate and reaches a maximum on the 8 - 13 day (Figure 1). At the same time, the number of microorganisms increases in the range of 2.5 to 3 times depending on the oil properties and cultivation environment.

The content of oil fractions based on the boiling temperature (the weight of 178, 9262): 53-75 ^{o} C - 2.26; 75-100 ^{o} C - 1.39; 100-125 ^{o} C - 1.42; 125-150 ^{o} C - 3.64; 150-175 ^{o} C - 2.99; 175-200 ^{o} C - 4.31; 200-225 ^{o}C - 5.63; 225-250 ^{o} C - 6.12; 250-300 ^{o} C – 12.61; 300-350 ^{o} C – 10.2; 350- 400 ^{o} C - 18.48. Residue 108.87; losses 1.74

Experiments with low doses of the crude oil (0.5-1% by volume) show an easier adaptation of microorganisms and maximum growth that can be observed on 2-4 day, and further there is a rapid decrease in the number of cells caused by a reduced content of light fractions.

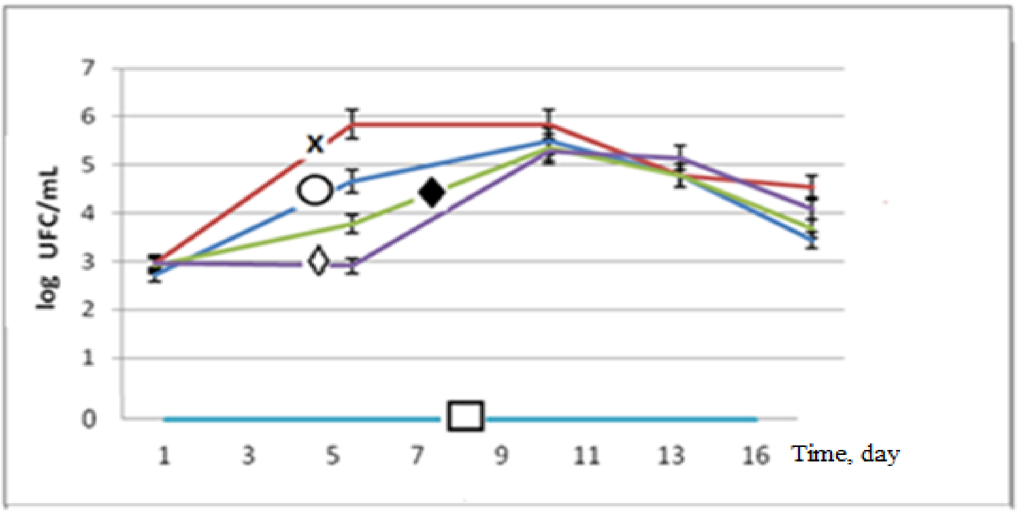

Figure 1: Bacterial growth of two HDB consortia in the Muntz medium

X - Consortium 1 (nine HDB strains) + 0.5% of oil;
O - Consortium 2 (three HDB strains) + 0.5% of oil;
♦- Control 1 (Consortium 1, oil-free);
◊ - Control 2 (Consortium 2, oil-free);
□ - Control 3 (0.5% of germless oil)

At high doses of oil (2%, 5% or more) there is an inhibition of HDB growth and a very low number of microorganisms even in the period of their maximum buildup (Fig. 2).

Analysis of species composition of microbial consortia, being conducted in the course of the experiment showed that the composition remains the same under the influence of small oil doses (0.5-1.0%). When the concentration of oil is two or more percent with a specific gravity higher than 0.9%, the consortia loss the species of genera Micrococcus, Chromobacterium, Brevibacterium and Alcaligenes. Species of Pseudomonas and especially Bacillus - dominant spore forms - remain unchanged. Conservation of bacteria of the genus Pseudomonas in the environment heavily polluted with viscous oil can be explained by:

- These bacteria assimilate a wide range of hydrocarbons, including aliphatic mono- and poly-aromatic, heterocyclic, heavy fractions such as asphaltenes and resins, as well as the halogenated and methylated organic compounds [26, 27];
- Release polysaccharide mucus acting as an oil dispersant into the environment [28, 29, 30];
- Synthesize the extracellular emulsifiers - peptidoglycolipids that promote liquefaction of fluids, both viscous and highly-viscous [31, 32];
- Produce special mechanisms of adaptive resistance to hydrocarbons through the cell wall thickening, increased development of the membrane system, the formation of capsules, the changing of the metabolic activity of assimilating different hydrocarbons, etc.

The genus Bacillus, represented in a consortium by three types, remains unchanged upon a long-term cultivation with oil up to 10% by volume.Sustainable conservation is probably due to the ability f the latter to withstand critical concentration of fluids. This is indicated by their presence in tar, oil wells with pH 1-1.5, and the soil and water mudded with oil and petroleum products. It is known that the spore microorganisms of the genus Bacillus account for 90-93%, coccoid - within 2%, and the others - about 0.5-1.5% in areas of emergency or other accumulation of crude oil (in surface water of constantly oiled rivers) [20]. We found that the development of spore forms of hydrocarbon degrading microorganisms is in direct proportion to the increasing salinity of water.

The laboratory experiments revealed that the development of HDB population (quantitative increase) is due to the presence of mineral salts in the environment. The selective Muntz medium with balanced amount of mineral salts, shows an increase of CFU of the first consortium – $2.4 \cdot 10^6$, of the tap water - $5 \cdot 10^5$, of Volga water - 9,8·10^5. CFU of the second consortia was 1.2·10^6, 5.9·10^5 and 8.26·10^5, respectively, during the same period and under the same conditions.

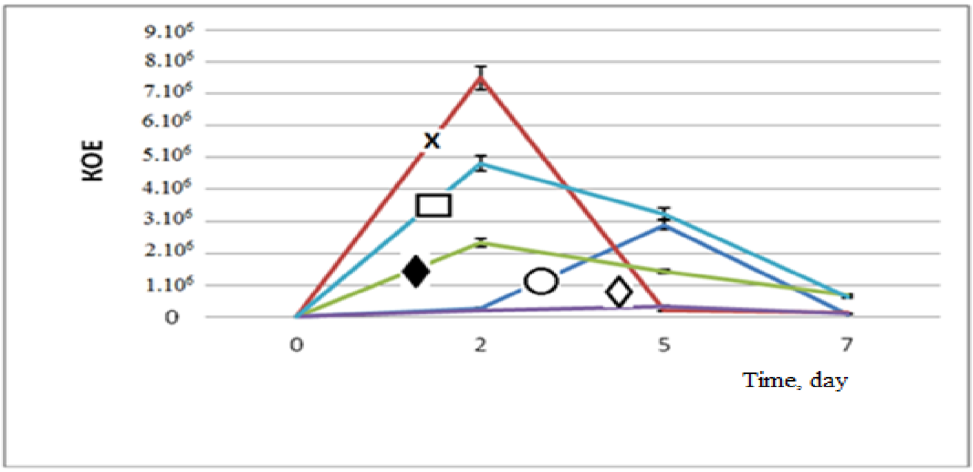

Figure 2: Bacterial growth of the first consortium (9 strains) for 7 days in water of the river Volga containing various oil concentrations

□ - 0.5% ; **X** - 1%; ♦ - 2%; ◊ - 5%

Highly mineralized produced water affects with different intense the vital activity of hydrocarbon degrading bacteria in the oiled medium. The higher the water dilution, the higher the HDB number. The number of microorganisms in the viscous oil of the Nurladskoe field (specific gravity about 0.9) at a ratio of 1:5 is more than 20000 cells/cm^3 in the period of their maximum growth, at a ratio of 1:3 is 1200-15500 cell/cm^3, and 1500 - 2000 cells/cm^3 in the non-diluted oil. The intensity of oil biodegradation in this variants of experiments also varies and is 36-24 and 3%, respectively. Modeling of the medium salinity with addition of $CaCl_2$ in the amount of 3, 5, 7, 10 and 30% also showed a reverse dependence of HDB growth intensity on the content of calcium chloride in the culture medium. These experiments showed that the presence of HDB in the amount of 1000-1500 cells /ml in the medium ensures 4 to 10% oil biodegradation by the hydrocarbon-oxidizing microflora. This is probably due to the significant amount of potassium and magnesium in the produced water, which stimulate the development of consortia of oxidizing microorganisms. Some authors also point to this possibility including the intensification of the microbial oil decomposition [33]. There is an attractive fact that the degree of biodegradation is tied in with a high ambient temperature (Fig. 3, 4).

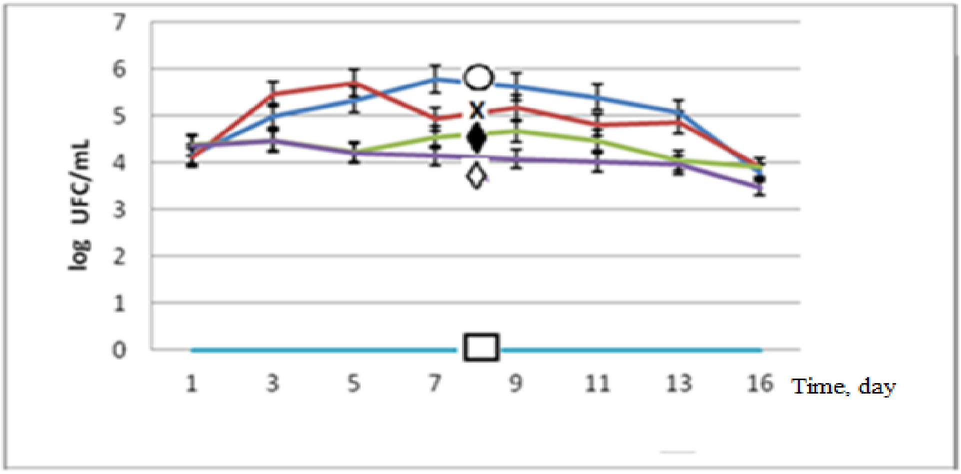

Figure 3: Bacterial growth of two HDB consortia and 0.5% oil in the tap water

X - Consortium 1 (nine HDB strains); **O** - Consortium 2 (three HDB strains); □ - Control 3 (germless); ♦ - Control 1 (Consortium 1, oil-free); **◊**- Control 2 (Consortium 2, oil-free);

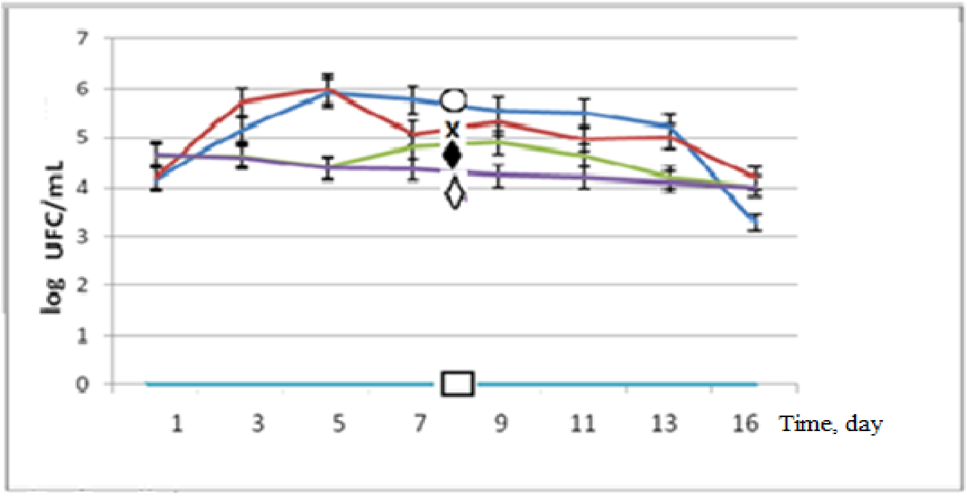

Figure 4: Bacterial growth of two HDB consortia and 0.5% oil in the water of the river Volga

X - Consortium 1 (nine HDB strains);
O - Consortium 2 (three HDB strains);□ - Control 3 (germless);
♦- Control 1 (Consortium 1, oil-free);
◊- Control 2 (Consortium 2, oil-free);

In case of diluting the highly mineralized stratal water at 400C with the river water in the ratio of 1:1, 1:2, 1:5 and when non-diluted the rate of oil degradation caused by HDB was 35.18; 30.24; 39.85 and 29.34; and at 300C - 15.46; 16.37; 32.0 and 20.42%, respectively. This is achieved at oil dose of 1% or more by volume, and certainly at increased concentration of salts in the produced water (total salinity of stratal water without its dilution is equal to 230.3 mg/dm^3). The observed results are associated with active enzyme reaction typical of hydrocarbon-degrading bacteria in the temperature range of 30-400^{0}C and the related acceleration of biodegrading processes. It was also promoted by the adaptability of the formed oil-oxidizing microflora to a high optimum temperature of growth (28-300^{0}C). Balance calculations (0.5-1% concentration) performed in the experiments with tap water, Volga water and the Muntz medium also confirm this fact. A passage temperature of HDB consortia in these experiments was 22-25 ^{0}C, and the concentration of used oil on the 16 day of contact by variants of the experiments was: 13; 21.7 and 19.8 % with the firs consortia, and 10.17 and 9%, respectively, with the second consortia. This is nearly 2 times lower than the results of biodegradation at 40^{0}C. The degree and rate of oil oxidation is generally higher when the consortium is formed from numerous HDB species and lower when a limited amount is involved in this process. According to previously obtained data [34], the effectiveness of oil bio-oxidation by oxidizing microorganisms did not decrease below 9.12% even at a temperature range of 6-8°C. The values of viscous oil biodegradation induced by the consortium including nine isolates of hydrocarbon degrading bacteria are high enough (29.34 - 39.85%) for 14-16 days of contact, and point to the possibility of their further increase under controlled regulation of the medium components.

We demonstrated [34, 35, 36] that easily oxidizable organic substances such as peptone, amino acids, carbohydrates, organic acids as well as lactate and calcium nitrate at a concentration of 1 to 5 mg/l accelerate the logarithmic phase of oxidizing bacteria growth in the process of oil biodegradation, which in a few hours changes to the stationary phase. These are glutamine, α-ketoglutaric, succinic acid, etc. Their influence on the oil oxidizing microflora at a dose of $5 \cdot 10^{-6}$ up to $7.5 \cdot 10^{-6}$ M for 3-18 h of continuous contact causes biodegradation of 86-98.2% of oil (initial concentration of 200±6 mg/l) and just 42.3% of the control (the Muntz medium with pH 7.0-7.2). In an alkaline medium with maximum pH 9 the biodegradation drops sharply and ranges from 34 to 40% in the experiments, and dos not exceed 26% in control.

There were no visible effect observed in the biostimulation of HDB consortium activity (in the growth of the consortium and its role in oil biodegradation) in the water of the river Volga with addition of valeric, isovaleric, butyric, acetic, and formic and isoformic acid at a concentration of 2, 4 and 6 mg/l.

We ascertained the same results in tap water and the Muntz medium. Checking the effect of organic acids of butyric fermentation, considered in terms of the use of alcoholic fermentation micro-filtrate (liquid phase of stillage) as a composite product for intensification of microbiological transfer of viscous oil into easy-flowing hydrocarbons is still under discussion. While the amination products of primary amino acids such as glutamic, α-ketoglutaric, oxaloacetic, and pyruvic acid, and their derivatives and compounds formed in the process of reductive amination such as alanine, valine, etc., being a part of the complex of inducing substances (IS) at a concentration of $7.5 \cdot 10^6$ to $35 \cdot 10^6$ M can be used to enhance oil recovery of heavy oils (microbiological method). The latter always cause intensification of HDB growth resulting in the accelerated biodegradation of oil of different nature [23, 25].

Further experiments with tap water and water from the river Volga with 0.5-5% adjustment of oil by volume, nutrients adjustment by nitrogen from 20 to 150, and phosphorus within 7-50 mg/l, showed that the effect of inducing (bio-catalyzing) compounds in the amount of $35 \cdot 10^{-6}$ M against nutrients are important for the process of microbial degradation of oil impurities. Experiments with oil dose of 0.5% by volume showed that the percentage loss of oil on 12-16 days exceeded 96.7%, while the same remained 47-51% in control. In case of using ammonium salts ($(NH)_2HPO_4$, NH_4NO_3 - 30 mg/l) as a nitrogen source without adding any inducing substances, the oil consumption was about 75% for equal time interval, and did not exceed 60% with nitrogen nitrate KNO_3.

Another experiment with viscous oil of Nurlatskii field (1% by volume) in the Muntz medium showed the best bacterial growth of HDB consortium with concentrations of KNO_3 and NH_4NO_3 in 150 mg/mL (CFU $6.7 \cdot 10^6$ and $4.92 \cdot 10^6$, respectively). When reducing the amount of nutrients up to 50 mg/l, CFU was $1.93 \cdot 10^6$, while the same was $1,0 \cdot 10^6 – 3,0 \cdot 10^6$ cells/ml in the control at the same period of contact (7 days).

The effectiveness of bio-oxidation of viscous oil by these variants remained low and was 12.0-16.5%. The salts of phosphorus having favorable effect on the processes of biodegradation of viscous oil were one- and diammonium phosphate salt at a concentration of 10-30 mg/l.

To achieve a significant effect in the biodegradation of oil of various nature by HDB consortia we require managing constantly both mineral forms of nitrogen and phosphorus and the inducing substances. Special pilot-plant tests conducted on the basis of the package pilot unit newly designed for high-level biodegradation of oil impurities (in soil, water, oil-containing process drain system) have determined all necessary parameters [34].

These investigations have provided the basis for comprehensive work on the selection and creation of industrial consortia of hydrocarbon degrading bacteria for further development of biotechnology for enhancing oil recovery of heavy oil.

CONCLUSION

Domestic and foreign research, as well as our laboratory and natural experiments have shown that viscous oil from different fields within the same region, are subjected to biodegradation by the selected hydrocarbon degrading microorganisms almost in the same sequence. When HDB are exposed to oil, there occurs an adaptation to the initial substrate for the initial few days, further - the transition from exponential to stationary phase, and then - a rapid drop upon oxidation of available factions. The biodegradation degree varies in time, depending on the composition and concentration of fluids in the environment. Lower specific density ensures a faster biodegradation of viscous oil, while increased specific density up to 0.9 leads to its 23-fold reduction. We found that the intensity of biodegradation affects the diversity of species and the number of hydrocarbon-degrading bacteria involved in this process. Monocultures and a limited number of HDB species (three or less) in the community causes almost 3-fold reduction of oil consumption (from 39 to 18%). Association causes almost 3-fold reduction of oil consumption (from 39 to 18%). The association, including 9 and more isolates, uses a wide range of hydrocarbons from $C_{17}H_{36}$ to $C_{34}H_{70}$ with a specific gravity of oil equal to 0.9 or higher. The higher the concentration of viscous oil ranging from 2, 5, or 10 g/dm^3 or more, the greater the reduction in the number of HDB taxonomic groups. The genera Pseudomonas and Bacillus remain dominant. On the one hand, the result observed is associated with the possibility of some groups of bacteria to participate in the breakdown of a wide range of hydrocarbons (genus Pseudomonas uses aliphatic, mono- and poly-aromatic, and heterocyclic hydrocarbons, etc.), while on the other hand, with the resistant impact of high concentrations of oil and petroleum products in the environment. The fact is that the spore heterotrophic microorganisms, including oil-oxidizing ones dominate in areas with heavy accumulation of oil impurities (emergency or other oil spill on the surface water, basins, production facilities, washers, tanks, etc.) As a rule, there is a highly mineralized water in the places of oil and dissolved hydrocarbons concentration. It is obviously that hydrocarbon-degrading bacteria of genera Pseudomonas and Bacillus live in wastewater with a high salt formation and, although this water is not characterized as truly halophilic, it is, however, close to these conditions.

Laboratory experiments with produced water with salt content of more than 270 g/dm^3 and artificial medium with the content of $CaCl_2$ - 3, 5, 7, 10 and 30% showed a resistant growth of hydrocarbon degrading bacteria and a relatively high degree of viscous oil consumption excessing 10%. It is notable that this occurs at a temperature range of 20-40^{o}C. The formation of most viscous oil fields has a temperature ranging from 21 to 26 ^{o}C (the Bashkirian stage), and, consequently, a positive activity of hydrocarbon degrading bacteria in the biodegradation of these fluids is quite acceptable. Our observations imply that biooxidation of viscous oil by HDB can be intensified by adding nutrients (KNO_3, NH_4NO_3, superphosphate), inducing compounds (amino acids, organic acids, n-alkane sugars), and other easily oxidizable substances into the medium. The hydrocarbon degrading bacteria use the latter as easily oxidizable substrate to produce adaptive enzymes, and further degrade more difficult mono- and polycyclic hydrocarbons through the energy obtained and stimulating factor. This ensures the transition of viscous fluids into easy-flowing fractions. Special experiments on the biodegradation of viscous oil from various fields prove the possibility of this type of regulation through the co-metabolic or co-degrading processes on an industrial scale.

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Conflict of Interest

The author declares that the provided information has no conflicts of interest.

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