

Biodestruction of Petroleum Hydrocarbons

L.I. Svarovskaya, L.K. Altunina*

Institute of Petroleum Chemistry, Russian Academy of Sciences
3, Academichesky Ave., Tomsk, Russia

Abstract

Biodegradation of light and high-viscosity oils by hydrocarbon-oxidizing microflora has been studied. Microflora was isolated from the formation waters recovered from West Siberian and "White Tiger" (Vietnam) oil fields. To activate microorganisms one used the solution of IKhN-KA system containing a multi-component nitrogenous nutrient substrate. Under the condition of active development of microorganisms during 5 days of biodegradation the concentration of n-alkanes C_{10} - C_{32} in light oils decreased by 70-85 % and that of viscous oil – by 86-93 %. Destruction of mono-aromatic compounds accounted for 55-65 % and that of aromatic compounds of naphthalene series – 70-90 %. The content of methyl- and trimethyl phenanthrenes decreased 3 times.

Therefore a degree of oil destruction depends on nutrient substrates, which stimulate biochemical processes of vital activity.

Introduction

The ability of microorganisms for hydrocarbon biodegradation is used to develop commercial biotechnologies, including biotechnologies for enhanced oil recovery.

Spontaneous biodegradation of hydrocarbons is constantly proceeding in nature and depends on many factors. Under the formation conditions at deficit of oxygen dissolved in water and nutrient substrates, especially nitrogenous, the processes of oil oxidation proceed very slowly.

Destruction of oil hydrocarbons by microorganisms is defined by their fermentative activity and depends on growth and reproduction rates. One can regulate these processes by means of nutrient substrates. Deficit of nitrous compounds in the formation waters of oil fields determined the choice of IKhN-KA system containing nitrogenous compounds as a substrate to stimulate microflora.

At stimulation by nutrient substrates the dynamics of bacterial cell reproduction sharply increases [1]. Fermentative processes of hydrocarbon oxidation take an active course [2]. Generation and accumulation of metabolism products are increased to promote oil displacement [3].

A series of the experiments aimed to simulate the

*corresponding authors. E-mail: alk@ipc.tsc.ru

growth of the formation microflora allowed one to conclude about maximal activity of ferment system of the cell during the exponential growth [4]. This phase is characterised by a rapid accumulation of biomass and active work of catalytic sites in a microbial cell. Therefore the process of hydrocarbon biodegradation depends on the injected nutrient substrates, which stimulate the reproduction of microbial cells.

The aim of the present work – to study biodegradation of light oils recovered from West Siberian oil fields and that of viscous and highly paraffinic oils recovered from White Tiger oil field (Vietnam) under stimulation with nitrogenous substrates.

Experimental

Oil biodegradation was carried out by natural slimes of hydrocarbon-oxidizing bacteria separated from the formation water and oil of oil fields under study, i.e. West Siberian oil fields and White Tiger oil field (Vietnam). The contact of oil with microorganisms continued for 5-10 days. Sterile formation water was used as a cultural medium.

Biodegradation of oil with microorganisms was carried out via periodic cultivation in flasks of volume 1000 ml, containing 300 ml of the sterile formation water, microbial suspension, oil 0.5-0.25 % of the volume of cultural medium and 0.1 % of nitrog-

enous substrate. Then the flasks were placed into thermostat on a magnetic stirrer at a temperature close to the formation one.

To stimulate the growth and fermentative activity of microflora we used 0.1 % solution of oil-displacing IKhN-KA system as a nitrogenous substrate. Ammonia buffer system constitutes the base of IKhN-KA system. Its components are a part of nitrogen geochemical cycle. They are used in trophic chains of microbial biocenosis. Under the temperature influence the system components are hydrolysed to form nitrogenous compounds and are easily utilized by microflora. Under the experiment conditions the hydrolysis of IKhN-KA system was carried out in autoclave at a pressure of 1 atm during 2 hours. Applying the solutions of IKhN-KA system to stimulate the formation microflora we sought to use its oil-displacing ability at the development of biotechnology intended to enhance oil recovery.

One injected nitrogenous substrate once on the 1-st day of microorganism cultivation at the contact with light oils recovered from West Siberian oil fields or repeated the injection 3 times on the 1-st, 3-rd and 5-th days at biodegradation of viscous oils recovered from "White Tiger" oil field. Test flasks with oil, formation water and nitrogenous substrate, were subjected to thermostatic control under the conditions identical to experimental ones.

One determined the dependence of dynamics of microorganisms growth on the concentration of the injected stimulating substrates via sampling and inoculation of the samples of cultural medium into beef-extract agar on the 1-st, 3-rd, 5-th and 10-th days of the cultivation.

At the end of the experiment the residual oil occurring in the experimental and test samples was trice extracted with chloroform. Chloroform extracts of oil from the cultural medium were combined and released from chloroform on a rotary evaporator. The changes in the concentration of n-alkanes in oil samples were studied by gas-liquid chromatography after biodegradation in crude oil samples. Gas-chromatographic analysis of n-alkanes was carried out on chromatograph "Modul 3700" equipped with flame-ionization detector on a capillary quartz column 25 m×0.22 mm with a fixed phase SF-52. Temperature varied from 50 to 300°C, Heating rate was 4 °C/min. A degree of saturated hydrocarbon utilization was estimated by a coefficient of biodegradation.

One determined a coefficient of biodegradation by the ratio of the sum of peak height for normal alkanes

$nC_{17}+nC_{18}$ to the sum of iso-alkanes $iC_{19}+iC_{20}$ (pristane + phytane). The residual oil content was quantitatively determined by the ratio of the sum of peak areas to the area of internal standard (dioctylphthalate).

Concentrates of aromatic hydrocarbons were separated from oil samples by liquid-sorption chromatography on Al_2O_3 and concentrated by thin-layer chromatography on silica gel.

Results and Discussion

Overflooded oil fields of Cretaceous and Jurassic deposits of West Siberia with temperature 45-95°C - Samotlorskoye, Sovetskoye, Strezhevskoye, Vakhskoye and others contain rich and various hydrocarbon-oxidizing microflora. The separated microorganisms are referred to Pseudomonas, Rhodococcus, Arthrobacter, Mycobacterium, Micrococcus, Nocardia, Bacillus. The total amount of the formation microflora reaches 10 mln cell/mL.

Oils recovered from West Siberian oil fields are light and low viscous under the formation conditions. The content of paraffins is 1.9-2.5 % and asphaltenes - 0.8-1.9 %. At 50°C oil viscosity varies from 2.3 to 4.1 mPa·s and density - from 0.840 to 0.891 g/cm³.

Under the formation simulated conditions the destruction of n-alkanes with a chain length from C_{10} to C_{32} has been revealed for most hydrocarbon-oxidizing microorganisms. The rate of oil destruction was 3-5 mg/L in a day at 45°C. At the same time biodegradation of n-alkanes $C_{13} - C_{32}$ of oils recovered from Sovetskoye and Samotlorskoye oil fields did not exceed 10 % during 30 days.

With the injection of 0.1 % solution of IKhN-KA system, which was preliminary autoclaved, into a cultural medium the number of microflora increased by 3-6 orders and the rate of oil hydrocarbon destruction increased up to 35 mg/L in a day. During 5 days of biodegradation the concentration of n-alkanes $C_{10} - C_{32}$ of oils recovered from Sovetskoye, Samotlorskoye and Vakhskoye oil fields decreased by 70-85 %.

Figure 1 presents chromatograms of saturated hydrocarbons (n-alkanes) of oil recovered from Samotlorskoye oil field after biodegradation by activated hydrocarbon-oxidizing microflora (a) and of crude oil (b). One determined the height of n-alkane peaks by their concentration in oil. It is seen from the figure that in biodegraded oil the concentration of all n-alkanes significantly decreased. Relative concentration of pristane and phytane iso-alkanes

remained unchanged and these two peaks raised above the rest ones (Fig 1a). Pristane and phytane peaks serve as if markers at determining relative concentrations of saturated hydrocarbons before and after oil biodegradation. Destruction coefficient, determined by the ratio of the sum of n-alkanes $C_{17} + C_{18}$ to the sum pristane + phytan, was 0.18. For native oils of West Siberian oil field this ratio was determined in the range of 1.26-1.58.

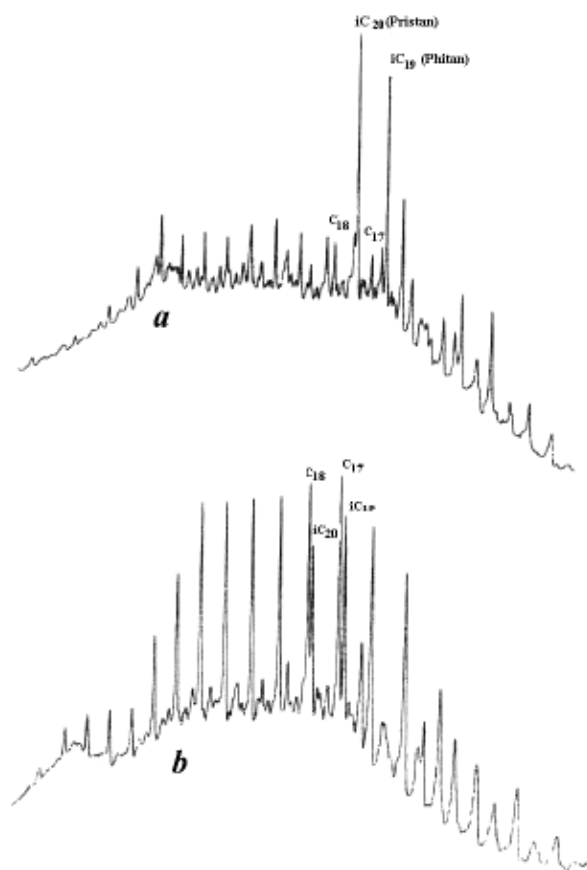


Fig. 1. Chromatogram of n-alkanes for biodegraded (a) and native (b) oils recovered from Samotlorskoye oil field.

Biodegradation of n-alkanes in light oils recovered from West Siberian oils were accompanied with the destruction of mono-aromatic compounds, the concentration of which decreased by 50 %. Destruction of aromatic compounds of naphthalene series was 70 %. The compounds of dimethyl naphthalene group degraded almost completely. Microorganisms – destructors of oil aromatics of naphthalene and phenanthrene series were identified as the representatives of numerous and various *Pseudomonas* genus.

Therefore biodestruction of light oils recovered from West Siberian oil fields by the formation microflora in an aqueous medium was characterized by a

high percent (70-85 %) of n-alkane and aromatic hydrocarbon oxidation at a single injection of 0.1 % solution of IKhN-KA system, containing nitrogenous components. Utilization of light oil hydrocarbons was visible on the 3-rd day with contact with microorganisms. By that time a continuous oil film destroyed to convert into the suspension of small particles. On the 5-th day of the contact of microflora with oil cultural medium became of saturated milk colour. Visually one determined no oil hydrocarbons.

Microbial processes, proceeding in White Tiger oil field (Vietnam), depend on the development features, physico-chemical and geological properties. The oil fields are located on a continental shelf of South China Sea. They are developed by flooding with sea water. Annual temperature of sea water is +26°C, total amount of microflora reaches 8 mln cell/mL.

White Tiger oil field includes three production horizons, i.e. n-Miocene, n-Oligocene and foundation at 110-170°C. Viscosity of oil recovered from Miocene horizon is 20.250 cSt at 50°C and that of oils recovered n-Oligocene and foundation reaches up to 22.440 cSt.

The number of microflora in the samples of the formation water and oils recovered from White Tiger oil field varies from 500 thou. cell/mL to 2.6 mln cell/mL. Spore forms of *Bacillus* predominate in microflora composition. The representatives of *Flavobacterium*, *Micrococcus*, *Pseudomonas*, *Arthrobacter*, *Actinomyces*, fungic and yeast cultures have been determined among other microorganisms.

It has been experimentally determined that maximal growth and reproduction of the formation microflora in White Tiger oil field is also achieved at the injection of 0.1-0.2 % solution of IKhN-KA system, containing nitrogenous substrate (Fig. 2). Total destruction of oils recovered from the production horizons of White Tiger oil field was 56.5-64 % (Table 1).

Destruction of viscous paraffinic oils requires for special intensity of fermentative processes of microflora vital activity, selection of the methods intended to inject nutrient substrates and the duration of exponential growth maintaining a high number of microorganisms [5].

A series of the experiments allowed one to come to a conclusion about maximal activation of fermentative processes for oxidation of viscous oils recovered from White Tiger oil field at triple injection of 0.1 % solution of IKhN-KA system (Table 2). Total destruction of oils under study was 76-81 %. The number of

microflora increased by 4-5 orders, duration of exponential growth of microorganism reproduction was 7 days. In the control variant due to chemical reaction

and activity of spontaneous microflora, the number of which did not exceed 32 thou. cell/mL, the destruction of the total amount of oil was 9-10 %.

Table 1

Oil biodegradation by microflora occurring in production horizons of White Tiger oil field at single injection of the solution of IKhN-KA system

Production Horizons	Well number	Amount of oil, g		Oil destruction, %
		At the beginning of the experiment	At the end of the experiment	
n- Miocene	917	0.222	0.080	64
n- Oligocene	702	0.205	0.078	62
Foundation	503	0.237	0.103	56.5
Control	917	0.209	0.188	10

Table 2

Biodegradation of oil occurring in White Tiger oil field at trice-repeated injection of the solution of IKhN-KA system

Production Horizons	Well number	Amount of oil, g		Oil destruction, %
		At the beginning of the experiment	At the end of the experiment	
n- Miocene	917	0.230	0.044	81
n- Oligocene	702	0.218	0.024	89
Foundation	503	0.195	0.047	76
Control	917	0.212	0.193	9

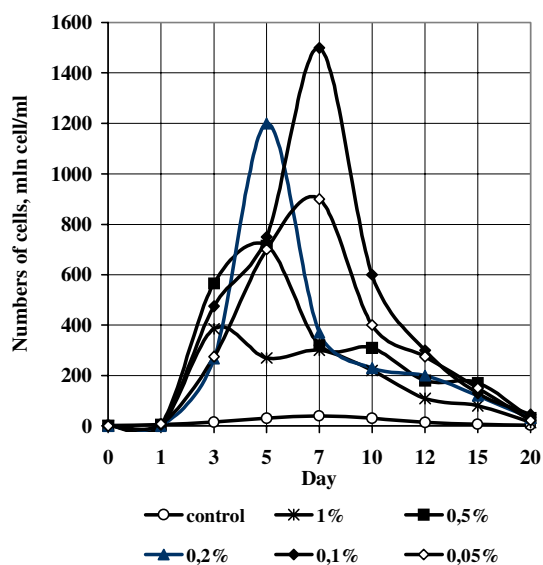


Fig. 2. The effect of the solutions of IKhN-KA systems on growth dynamics of the formation microflora occurring in White Tiger oil field. Miocene

Figure 3 presents the change in the concentration of n-alkanes of viscous oil recovered from White Tiger oil field. The change was observed at biodegradation by activated microflora. Along x axis – C number in a molecule of each hydrocarbon from C₉ to C₃₄. Along y axis – concentration of each hydrocarbon under study in relative percents. The legends indicate the following: grey columns denote hydrocarbon concentration before biodegradation, solid line denotes the change in the concentration of hydrocarbons after oil biodegradation. Biodestruction of heavy n-alkanes C₂₁-C₃₄ accounted for 100 % and they are absent in the chromatogram. The results obtained corroborate E. Birschteher conclusions (1957), that the ability of various bacteria to oxidize saturated hydrocarbons increases with the increase in chain length of these hydrocarbons [6]. In the process of destruction at the multiple injection of a stimulating substrate the concentration of n-alkanes C₁₀-C₂₀ of

oil recovered from Miocene decreased by 92-98 % and that of heavy alkanes C_{21} - C_{44} —by 100 % (Fig. 3). An average percent of utilization of n-alkanes recovered from foundation and Oligocene was 86-93 %. Destruction factor for hydrocarbons occurring in viscous oils was 0.06.

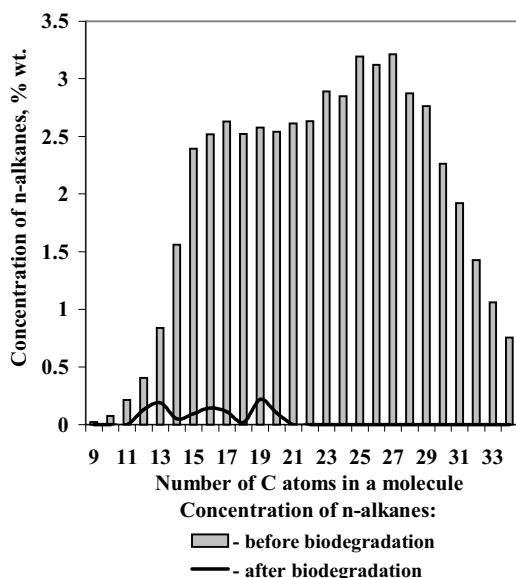


Fig. 3. Biodegradation of n-alkanes occurring in White Tiger oil field at thrice-repeated stimulation of the formation microflora

Conclusions

Diluted solution of IKhN-KA system (0.1 %) is a multi-component nutrient nitrogenous substrate prolonging the duration of exponential growth of reproduction up to 7 days and increasing the number of microflora by 4-6 orders at the simulation of the formation conditions.

Single injection of the solution of IKhN-KA system in the formation water increased fermentative activity of microflora in biodegradation of light oils recovered from West Siberian oil fields by 8-10 times. Destruction of n-alkanes C_{10} - C_{32} was 70-85 %, monoaromatic compounds – 50 %, aromatic compounds of naphthalene series – 70 % and that of the compounds of dimethyl naphthalene series – 100 %. Total coefficient of hydrocarbon destruction was equal to 0.18.

Biodestruction of n-alkanes in viscous oils recovered from production horizons of White Tiger oil field was carried out at thrice stimulation by the solution of IKhN-KA It accounted for 86-93 % with

a predominant oxidation of heavy alkanes C_{35} - C_{44} . Total coefficient of hydrocarbon destruction was equal to 0.06.

Therefore at the development of biotechnology intended to enhance oil recovery one can regulate the processes of oil destruction and growth dynamics of the formation microflora by means of multi-component nitrogenous solution of IKhN-KA system as well as by the methods of its injection into the formation.

References

1. Svarovskaya L.I., Altunina L.K., Kuvshinov V.A., Rozhenkova Z.A., Buldakova E.P. Microbiological aspects of enhanced oil recovery employing IKhN composition in oil fields of West Siberia // Proceeding of the Conference on Microbiology in the Oil Industry and Lubrication. Hungary, Sopron. 1991. September 10-12. P. 246-251.
2. Svarovskaya L.I., Altunina L.K., Rozhenkova Z.A., Nazina I.V. The effect of microorganism reproduction rate on biodegradation of oil n-alkanes recovered from West Siberian oil field // Theoretical and practical bases for physico-chemical control of the properties of oil dispersed systems. Collected papers – Russia. Tomsk. 1997, P.66-71.
3. Svarovskaya L.I., Altunina L.K., Rozhenkova Z.A., Buldakova E.P. Use of microorganisms for EOR. // Proceedings of the 4-th Symposium on Mining Chemistry. The Ukraine. Kiev. 1992. October 6-9. P. 25-32.
4. Pheoktistova V.S., Svarovskaya L.I., Cherkasov S.V. Fermentative and sorption activities of soil microflora. // Proceedings of the IV-th International conference «Oil and Gas Chemistry» – Russia. Tomsk. 2000. V.2.P. 35-38.
5. Vinh T.D., Svarovskaya L.I., Altunina L.K., Doan F.V., Shon Ch.T., Hyong M.X. Degradation of high viscosity oils recovered from White Tiger oil field by the formation microflora. // Proceedings of International Congress «New Ideas in Ecology». – Russia. St.-Peterburg. 2000. June 14-16. V. 2. P. 147-150.
6. Birshtecher E. Petroleum microbiology. Translated from English. Leningrad. 1957. P. 165.

Received 11 April 2001.