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Investigation of Indigenous Microbial Enhanced Oil Recovery in a Middle Salinity Petroleum Reservoir

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Abstract. Culture-based techniques were applied to analyze the diversity of indigenous microbial communities in the Qinghai middle salinity petroleum reservoir (QH-MSPR). The results of the most probable number (MPN) method indicated there was an abundance of indigenous microbes (10^5-10^6MPN/ml). Two isolations (BIOS682) from the QH-MSPR were identified as Brevibacillus agri and Brevibacillus levickii. The study showed that BIOS682 enhanced the degradation rate of Huatugou crude oil. The viscosity and freezing point of crude oil after treatment by BIOS682 were all decreased. The results of TLC and FTIR spectrum analysis of the biosurfactant produced by BIOS682 indicated that it was identical to that of lipopeptide. The core-flooding tests showed that the incremental oil recoveries were 7.05-10.15%. Thus, BIOS682 may provide a viable application of microbial enhanced oil recovery (MEOR).

Introduction

Microorganism research has revealed the diversity of microbial communities in petroleum reservoirs. A variety of bacteria, such as sulfate reducing bacteria, sulfur producing bacteria, fermentative bacteria, manganese-reducing bacteria, iron-reducing bacteria, methanogenic bacteria, and acetic acid bacteria, have been isolated from these communities[1,2,10].

Microbial enhanced oil recovery (MEOR) is an important tertiary recovery technology utilizing microorganisms and/or their metabolites to recover incremental oil from a petroleum reservoir that has been produced beyond primary and secondary recovery techniques[3,4]. Youssef et al. [13]reported that in situ biosurfactant production by bacillus strains injected into a limestone petroleum reservoir. A push-pull test was conducted on five oil production wells in the Bebee field (Section 19, T5N, R5E; Pontotoc City, OK) that produce from the same formation (a Viola limestone). Two halotolerant biosurfactant producers, Bacillus strain RS-1 and Bacillus subtilis subsp. spizizenii strain NRRL B-23049, were used as the inoculum. Their results demonstrate the technical feasibility of microbial processes for oil recovery.

This work studied the indigenous microbial diversity, biosurfactant-producing bacterials identification, and their potential application of microbial enhanced oil recovery in the Qinghai petroleum reservoir (China). The indigenous microorganisms of four blocks (Huatugou,Youshashan, Gasi, Qigequan) within the Qinghai middle salinity petroleum reservoir (QH-MSPR) were cultured using a traditional culture method and counted using the most probable number method (MPN). Two strains of both hydrocarbon-degrading and biosurfactant-producing bacteria were isolated from samples of the QH-MSPR, and identification and crude oil degradation and biosurfactant-producing studies were conducted in order to identify the potential application in MEOR.

Materials and Methods

A. Sampling

The Qinghai Oilfield is located northwest of Qaidamu Basin in Qinghai Province (China). Four different brine samples were collected from a high salinity petroleum reservoir with a temperature range between 45-60℃ and a salinity of formation brine range between 50-150 g/l. Each sample was collected in a 2-liter glass bottle that was allowed to overflow to minimize contact with air. Samples were collected and were immediately sent to the laboratory and then stored at a temperature of 4℃ until analyzed.

B.Culture medium

The culture medium of hydrocarbon-degrading bacteria (HDB)[8] is as follows (g/l): NH_4Cl 20.0, NaCl 70.0, KH_2PO_4 2.0, $MgSO_4 \cdot 7H_2O$ 0.2, K_2HPO_4 0.8, peptone 1.0, pH 7.0-7.2. The culture medium of fermentative bacteria (FMB) (g/l): peptone 4.0, glucose 10.0, Na_2SO_4 2.0, $MgSO_4 \cdot 7H_2O$ 1.0, NaCl 70.0, $(NH_4)_2Fe(SO_4)_2$ 0.5, pH 7.0; culture medium of saprophytic bacteria (TGB) (g/l): beef extract 5.0, peptone 10.0, glucose 10.0, NaCl 70.0, pH 7.0; culture medium of sulfate-reducing bacteria (SRB): K_2HPO_4 0.5, yeast extract 1.0, NH_4Cl 1.0, $CaSO_4$ 10.0, $MgSO_4 \cdot 7H_2O$ 2.0, sodium lactate 3.5, $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ 0.5, vitamin C (1%), thioglycolic acid 10.0, NaCl 70.0, pH 7.1-7.5; and culture medium of iron-reducing bacteria (IRB) (g/l): $MgSO_4 \cdot 7H_2O$ 0.5, $(NH_4)_2SO_4$ 0.5, KH_2PO_4 0.5, $CaCl_2$ 0.5, $NaNO_3$ 0.5, sodium citrate 5.0, ammonium ferric sulfate 5.0, NaCl 70.0, pH 6.6－6.8.

C.Enrichment culture medium

The enrichment culture medium is as follows[8] (g/l): $NaNO_3$ 2.0, $Na_2HPO_4 \cdot 12H_2O$ 2.0, KH_2PO_4 0.6, $MgSO_4 \cdot 7H_2O$ 0.25, yeast extract 2.0, $CaCl_2 \cdot 2H_2O$ 0.06，pH 7.5. The slant medium is (g/l): beef extract 5.0, peptone 10.0, NaCl 70.0, pH 7.0.

D.MPN count

A 1 ml sample of liquid was diluted by adding saline, after sterilization, to different concentrations from 10^{-1} to 10^{-9}. The liquids, with different concentrations, were respectively added into culture mediums of HDB, FMB, IRB, SRB, and TGB. Samples were cultured for seven days in a quiet atmosphere at a temperature of 45℃ (Gasi sample at 60℃). Their numbers were counted after seven days.

E.HDB strain screening

According to the culture results of indigenous bacteria, samples with a high content of HDB were chosen for further culturing. The samples of Huatugou blocks in HSPR, with large indigenous microbial content, were respectively inoculated in an enrichment medium with a dose of 5% of the sample and then cultured by means of a continuous rotary shaking of 167 rpm at 45℃ for seven days. After ten times of enrichment, screened and purified strains were streaked on the slant and incubated at 45℃. Single loop bacteria were placed in the axenic water and was mixed by shaking. A dilution method (Nitu and Banwari 2008) was used to form a single colony on the culture dish, and the single colony was screened two to three times using the above mentioned methods.

F.Strain identification and phylogenetic analysis

Pure cultures were identified by molecular approach using the 16S rDNA. DNA extraction and PCR amplification were following Mulligan et al.[11]. The productions were ligated to PGEM-T easy vector (Promega) and transferred into E. coli DH5a using the hot-shock method. Positive clones were selected, based on ampicillin resistance (100 μg/ml) and blue-white colour screening and sequencing. The results were compared with the GenBank database. A phylogenetic tree was constructed using the DNAMAN software.

G.Degradation of isolated HDB in crude oil of Huatugou block in QH- HSPR

Saturate, aromatics, resin, and asphaltene (SARA) fractions in a crude oil were analyzed using the methods of Fan and Mansfield[5]before and after being treated with a mixture strains of BIOS682. BIOS682 culture liquid was cultured at a reservoir temperature of 45℃ by taking 2% of the crude oil of the Huatugou block in QH－HSPR as the sole carbon source. Duplicate samples and one control sample were collected every seven days. The effects of bacterial consortium of BIOS682 culture time on the SARA contents of crude oil were investigated.

H.Properties of fermentative fluid

In the identification of surface tension of fermentative fluid, indigenous microorganisms BIOS682 were cultured for seven days using crude oil of four blocks in QH-MSPR as the sole carbon source. The fermentative fluid removed cells by centrifugation. A surface tension of supernatant fluid was measured using a tensiometer (Cahn, Model DCA-322, USA).

Crude oil viscosity changes in samples both before and after treatment with indigenous microorganisms BIOS682 for seven days were measured using a digital viscometer (BROOKFIELD, DV-II+Pro, USA). The crude oil sample in the flasks was collected using centrifugal and electrically dehydrated in a pressurized tank. Freezing point changes were also measured using NGB-01 (Tianjin, China).

I.Analytical method of bio-surfactant

Biosurfactants isolated by acid precipitation method at pH 2 using HCl. Preliminary characterization of the biosurfactant was done by Thin Layer Chromatography (TLC) method. A portion of the crude biosurfactant was separated on a silica gel plate using n-butanol:ethanol:water (40:10:10, v/v/v) as a developing solvent system with different colour developing reagents. Ninhydrin reagent (0.5 g ninhydrin in 100 ml ethanol) was used to detect lipopeptide biosurfactant as red spots.

To use Fourier Transform Infrared Spectroscopy (FTIR) analysis, IR absorption spectra were obtained with an EQUINOX 55 (Bruker, Germany) in a dry atmosphere. IR spectra were collected from 400-4000 wavenumbers per wavenumber. Samples were prepared by dispersing the solid uniformly in a matrix of potassium bromide.

J.Core flooding test

The potential application of the indigenous BIOS682 in MEOR was evaluated using the sandpack core flooding technique described by Sugihardjo and Fang et al. [6]. The test temperature was 45℃. The heterogeneous core models had a length of 9.0－10.0 cm, diameter of 2.5 cm, permeability of 299－301 × 10^{-3} μm^2 (man-made) and 36－45 × $10^{-3}\mu m^2$ (natural cores from QH-MSPR), a porosity of 19－32%, and a crude oil viscosity (45℃) of 15.2 mpa.s (Huatugou) and 11.9 mpa.s (Youshashan). The major procedures in the displacement are as follows: First, water-flooding of oil-bearing core with oil saturation of 65% was performed until no further oil was observed in the outlet of the core. Second, 0.3－0.6 pore volume (PV) of BIOS682 suspension with a density of 2×10^7 cells/ml was injected into the water-flooded core, followed by a 7－day shut-in period at 45℃. Finally, water-flooding was again performed until no further oil was observed in the outlet of the core. The control experiment was run in the same conditions but without injection of BIOS682 suspensions into the cores.

III. Results

A.Microbial abundance

MPN test results indicated the numbers of beneficial microbes (effective to MEOR) such as HDB, TGB, and FMB were high (10^5 to 10^6 MPN/ml), while the numbers of harmful microbes (causing corrosion and scaling) such as SRB and IRB were low (10^2 to 10^3 CFU/ml). Therefore, the formation water of the QH-MSPR showed a richness of beneficial indigenous microorganisms that are well adapted to a petroleum reservoir environment.

B.Identification of HDB strains

Two strains were isolated from Huatugou HSPR samples and marked as BIOS682-1 and BIOS682-2, with the diameter of the hemolytic zone being 6.0 cm and the diameter of oil spread being 4.5 cm and 4.8 cm, respectively. Both strains were identified as soil *Brevibacillus agri*. The similarity of BIOS682-1 and *Brevibacillus agri* in their phylogenetic trees was 99%. The similarity of BIOS682-2 and its nearest neighbour, *Brevibacillus levickii*, was 95%. Thus, BIOS682-2 might be a new strain in *Brevibacillus levickii*.

C.Analysis results of four components of crude oil before and after degradation

The results of SARA analysis indicate that components less than C_{22} were significantly increased in crude oil removed from the Huatugou MSPR after degradation using mixed strains of BIOS682, while components greater than C_{22} were greatly decreased. Therefore, BIOS682 enhanced the degradation rate of crude oil in Huatugou MSPR. The content of light chain components increased while the content of heavy long chain components (resins and asphaltenes) decreased after degradation by BIOS682. Results indicated that BIOS682 enhanced the degradation rate of crude oil by degrading the components from a high molecular weight to a low molecular weight.

D.Results of surface tension of fermentative fluid, viscosity, and freezing point of crude oil

The measured value of surface tension of tag water was 72.64 mN/m. The surface tension between fermentative fluid and air and the viscosity of crude oil were decreased by 30-40% and 11-30%, respectively. The surface tension between fermentative fluid and air, the viscosity and freezing point of crude oil, and the pH of fermentative fluid from two blocks of the Huatugou and the Youshashan Oilfields were all decreased. The changes indicate that some biosurfactants were produced when indigenous microorganisms degraded the crude oil. The biosurfactants were able to decrease the viscosity and freezing point of crude oil.

E.Analysis of biosurfactant from BIOS682 fermentative fluid

Biosurfactants isolated by acid precipitation method at pH 2 were identified by TLC method. Surfactin from Bacillus subtilis was identified by red colour spot. Biosurfactant was partially purified and subjected to FTIR analysis. The IR spectrum in KBr showed bands characteristic of peptides at 3405.67cm^{-1}, 3393.46 cm^{-1} (NH stretching mode of molecule hydrogen-bondin and intramolecule hydrogen-bondin), and 1653.66 cm^{-1}, 1540.85 cm^{-1} (stretching mode of the I and II CO-N bond). The bands at 2959.27～2923.56 cm^{-1} reflect aliphatic chains (-C-H) of the fraction, whilst a strong band observed at 1734.66 cm^{-1} was due to a carbonyl group. The results infer that the biosurfactant produced contains aliphatic hydrocarbons as well as a peptide-like moiety, which is similar to the biosurfactant produced by thermotolerant and halotolerant bacteria.

F.The results of core-flooding tests

The core-flooding test results show that the incremental oil recoveries with BIOS682 suspensions are 7.03-10.15% OOIP (original oil in place) with different permeability cores, compared with the control core flooding results.

Discussion

According to the MPN results, an abundance of cultured indigenous microorganisms were virtually similar, as were their orders of magnitude in the four different blocks in QH-MSPR. They are composed of hydrocarbon-degrading bacteria (HDB), fermentative bacteria (FMB), sulfate-reducing bacteria (SRB), saprophytic bacteria (TGB), and iron-reducing bacteria (IRB). The results were similar to isolations made by Nazina[8].

HDB was assembled in the formation with hydrocarbon and degradate hydrocarbons. Two strains of BIOS682, isolated from Huatugou blocks, enhanced the degradation rate of crude oil. The SARA analysis of four fractions of crude oil after degradation demonstrated the mixed strains of BIOS682 could effectively degrade the heavy, long chain components of crude oil. Then, the viscosity and the freezing point of the crude oil were decreased. The results of TLC and FTIR spectrum of the purified bio-surfactant indicate that its chemical structure is identical to that of lipopeptide, whose hydrophobic end is composed of an ester group and aliphatic chain. Lipopeptide is one of chemical structures such as glycolipid that are widely found in previous studies of bio-surfactants[7,12].

A lipopeptide surfactant by thermotolerant and halotolerant *B.Licheniformis* BAS50 has been characterized, and the purified biosurfactant decreased the surface tension of water to 28 mN/m. Physical and chemical characteristics of an oil reservoir are definitely important and put considerable restrictions on the performance of the indigenous microbes. Temperature and salinity

are among the most influential parameters. Researchers have found that there is a positive relationship between the ability of the organisms to grow at high salt concentration and their growth ability at high temperatures. In the present work, the QH-MSPR reservoir has a temperature of 45–60℃ and salinity of 5–15% (w/v). BIOS682 were moderately halophilic bacteria. The sand-pack core-flooding tests show that the incremental oil recoveries with BIOS682 are approximately 7-10%. Thus, the mixed strains of BIOS682 may provide an application of microbial enhanced oil recovery in the QH-MSPR.

In this study, MPN test results indicated the numbers of beneficial microbes were high (10^5 to 10^6 CFU/ml), while the numbers of harmful microbes were low (10^2 to10^3 CFU/ml). Two lipopeptide biosurfactant producing strains, BIOS682, were isolated, and they were able to decrease the surface tension of fermentative fluid and the viscosity and the freezing point of crude oil. Therefore, BIOS682 could potentially provide an application of microbial enhanced oil recovery.

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