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Investigation of Biosurfactant-Producing Indigenous Microorganisms that Enhance Residue Oil Recovery in an Oil Reservoir After Polymer Flooding

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Abstract Three biosurfactant-producing indigenous microorganisms (XDS1, XDS2, XDS3) were isolated from a petroleum reservoir in the Daqing Oilfield (China) after polymer flooding. Their metabolic, biochemical, and oil-degradation characteristics, as well as their oil displacement in the core were studied. These indigenous microorganisms were identified as short rod bacillus bacteria with white color, round shape, a protruding structure, and a rough surface. Strains have peritrichous flagella, are able to produce endospores, are sporangia, and are clearly swollen and terminal. Bacterial cultures show that the oil-spreading values of the fermentation fluid containing all three strains are more than 4.5 cm (diameter) with an approximate 25 mN/m surface tension. The hydrocarbon degradation rates of each of the three strains exceeded 50%, with the highest achieving 84%. Several oil recovery agents were produced following degradation. At the same time, the heavy components of crude oil were degraded into light components, and their flow characteristics were also improved. The surface tension and viscosity of the crude oil decreased after being treated by the three strains of microorganisms. The core-flooding tests showed that the incremental oil recoveries were 4.89–6.96%. Thus, XDS123 treatment may represent a viable method for microbial-enhanced oil recovery.

Keywords Polymer flooding · Indigenous microorganisms · Biosurfactant · Crude oil degradation · Microbial-enhanced oil recovery

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Introduction

Presently, polymer (polyacrylamide (PAM)) flooding technology is an important tertiary oil recovery technique and has been widely applied in the Daqing Oilfield (China). However, the unexploited crude oil occupies 50% of the reservoirs after polymer flooding. It is very difficult to mine this segment of oil using conventional technology. Therefore, alternative recovery methods need to be explored. One such method, as presented in this paper, involves conducting exploratory research into activating indigenous microorganisms to enhance oil recovery (AIMEOR) [1, 2].

During the process of water injection, the quantity and species of indigenous microorganisms remains stable for a certain period of time. The microorganisms survive in the oil reservoirs, being either inherent microorganisms existing in the reservoirs prior to production or being inoculating microorganisms entering the reservoirs with the injection water. The distribution of indigenous microorganisms in an oil reservoir ecosystem is mainly dependent on water injection. Due to higher temperatures and higher pressure, the structure of indigenous microorganisms, in the fairly deep oil reservoirs, is relatively simple. On the other hand, due to lower temperatures and pressure, the microbial species are complex in relatively shallow depths. In microbial-enhanced oil recovery (MEOR), existing indigenous microorganisms in the reservoir are utilized to increase oil recovery by identifying strains with a strong capacity to produce substances (agents) that will enhance the oil's flow characteristics and otherwise improve its ability to be recovered. This is accomplished by adding several neurotrophic agents, such as ammonium dibasic phosphate, sodium nitrate, urea, molasses, starch etc., to activate indigenous microorganisms such as hydrocarbon-degrading bacteria, denitrifying bacteria, and methanogens that have been identified as having the potential to enhance recovery in a particular reservoir [3–6]. AIMEOR is environmentally suitable in a reservoir setting, is economical, and strengthens reservoir preservation and production. This technology has been applied in more than 20 oil field tests and blocks with cumulative incremental oil of over 600,000 t [1, 7, 8].

There are many types of indigenous microorganisms in the Daqing Oilfield after polymer flooding, in particular, *Pseudomonas* and *Bacillus* [9]. Many of these are capable of metabolizing and degrading petroleum hydrocarbon and producing active material such as biosurfactant for oil displacement, which is useful for enhancing oil recovery [10–13]. In this paper, biosurfactant-producing indigenous microorganisms were isolated from the petroleum reservoir after polymer flooding in the Daqing Oilfield. Their metabolic, biochemical, oil-degradation, viscosity-reducing characteristics, and crude oil displacement in the core (model of sandstone oil reservoir) were studied in order to evaluate their MEOR application potential.

Materials and Methods

Materials

Strains and Crude Oil Source

Oil and formation water samples were collected from the Daqing Oilfield. Sample numbers 306–310 are oil samples from several oil wells (6-30-B620, 6-30-B617, 6-40-621, S6-20-B618, S6-40-B619) for which results are presented here.

Hydrocarbon-Degrading Bacterial Medium or Enrichment Culture

The hydrocarbon-degrading bacterial medium was (grams per liter): liquid paraffin 20, NH_4Cl 2, NaCl 1, KH_2PO_4 2, $MgSO_4$ 0.2, K_2HPO_4 0.8, peptone 1, yeast extract 1, pH 7.0–7.2.

The beef extract peptone medium used was (grams per liter): beef extract 5, peptone 10, NaCl 5, agar 14, pH 7.0–7.2.

Fermentation Medium

The fermentation medium was (grams per liter): liquid paraffin or crude oil 20, $MgSO_4$ 0.2, $CaCl_2$ 0.02, KH_2PO_4 1.0, K_2HPO_4 1.0, NH_4NO_3 1.0, $FeCl_3$ 0.05, yeast extract 1.0, cholesterol 0.3, pH 7.0–7.2.

Methods

Isolation and Screening of Strains

Oil and water samples from the Daqing Oilfield were cultured in order to collect hydrocarbon-degrading indigenous microorganisms using an enrichment medium. Then, the samples were cultured a second time using an enrichment medium at 45°C with shaking for 4 days. The beef extract peptone medium was then inoculated with the enrichment culture solution using the plate streaking method at 45°C for 1 day. Single colonies were selected, and pure strains were isolated using the plate streaking method a second time. The isolated pure strains were used to inoculate the fermentation medium at 45°C with shaking for 4 days. Changes in the fermentation liquid were observed. The better strains were selected by comparing the oil displacement activity, emulsifying property, and surface tension of the fermentation liquid, as described below.

Oil Displacement Activity of the Fermentation Liquid was Measured

A total of 20 ml of water and 10 ml of liquid paraffin were added to a 60-mm culture dish. The centrifuged fermentation liquid was added into the center of the oil film after the oil film was formed. Then, the diameter of the oil spread was measured [14].

Emulsifying Property of the Fermentation Liquid

The emulsifying effect was observed by inoculating 0.2% crude oil with 5% fermentation liquid and incubating with shaking at 45°C for 4 days. Finally, the emulsifying effect was observed [15].

Identification via Electron Microscope and Physiological and Biochemical Analysis of the Strains

In accordance with Bergey's Manual of Determinative Bacteriology, species strains are identified using relative biochemistry experiments and a dyeing experiment.

Degradation Properties of Crude Oil

Samples were cultured with shaking and isolated into four components using a chromatographic method. The composition of saturated hydrocarbon was measured by employing gas chromatography-mass spectrometry (Agilent 5975C GC/MSD)

Viscosity-Reducing Characteristics, Surface Tension, and pH

The viscosity (digital viscometer, BROOKFIELD, DV-II+Pro, USA), surface tension (tensiometer, Cahn, Model DCA-322, USA), and pH of the fermentation liquid were measured before and after fermentation.

The fermentative fluid removed cells by centrifugation.

Core-Flooding Test

The potential application of the indigenous bacteria and biosurfactant-producing strains XDS123 for MEOR was evaluated using the sandpack core-flooding technique described by Sugihardjo and Fang et al. [16–18]. The test temperature was 45°C. The heterogeneous core models had a length of 7.02–7.55 cm, a diameter of 2.5 cm, a permeability of $134.94–175.13\times10^{-3}$ μm^2 (man-made sandstone core), a porosity of 28.7–31%, and a crude oil viscosity (45°C) of 30 mPas. Displacement is effect as follows: first, oil-bearing core with oil saturation of 70% was water-flooded until no further oil was observed in the outlet of the core. Second, 0.5 pore volume of indigenous bacteria or XDS123 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°C. Finally, water flooding was again performed until no further oil was observed in the outlet of the core.

Results and Discussion

Screening Hydrocarbon-Degrading Bacteria

Three biosurfactant-producing indigenous strains were screened using enrichment cultures, oil spreading, blood plates, slant cultures, re-screening, and shaking cultures. The isolated pure strains were inoculated on the fermentation medium a second time and cultured with shaking at 167 rpm at 45°C for 4 days. The characteristics of biosurfactant-producing strains were measured using an oil-spreading method. The strains with larger oil-spreading values were fermented and measured again. Finally, three strains, named XDS1, XDS2, and XDS3, were identified as having an oil spread of over 4.5 cm in diameter and a longer stability. In addition, the surface tension decreased to approximately 25 mN/m. Results are summarized in Table 1.

Table 1 Oil-spreading and surface tension values of cultures of the three indigenous strains.

Strain no.	Hemolytic zone	Oil-spreading value (cm)	Surface tension (mN/m)
XDS1	+	4.5	30.5
XDS2	+	5.8	28.6
XDS3	+	5.2	25.3

The surface tension of a blank culture medium at 62.3 mN/m

Electron Microscope and Physiological and Biochemical Analyses

The electron microscope (TEM, JEM-1400) and physiological and biochemical analyses of three indigenous microorganisms are presented in Fig. 1 and Table 2.

The three strains were able to utilize various saccharides as their sole carbon source and nitrate as their sole nitrogen source. They were able to decompose tryptophan in peptone using tryptophanase to produce indole, and they were also able to utilize organic sulfur compounds in the culture media to produce hydrogen sulfide gas. They could ferment glucose to produce acid and were able to further convert the produced acid into a neutral compound. They were also able to decompose citric acid into carbon dioxide. In addition, starch was hydrolyzed into smaller molecules such as dextrin via amylase. The starch–iodine complex has a blue-violet color, and the starch–dextrin complex is colorless. The growing temperature range is 15°C to 65°C, and the range of growing salinity is 1% to 10%.

Biochemical testing indicated a single colony of three strains, identified as *Bacillus*, with the following properties: short rods, white color, round shape, a protruding structure, and a rough surface. Strains with peritrichous flagella were able to produce endospores, and the sporangia were swollen and terminal.

The Molecular Identification Using 16S rRNA Gene for the Three Strains

The results of molecular identification of three strains are as follows: XDS1 (HM592996) was identified as *Bacillus licheniformis* strain (GQ375246) with a similarity of 100%, XDS2 (HM592995) was identified as *B. licheniformis*, (GU945233) with a similarity of 100%, and XDS3 (HM592994) was identified as *Bacillus cereus* (HM003210) with a similarity of 100%.

Applicability of the Three Strains Under Reservoir Conditions

The culture conditions for XDS1, XDS2, and XDS3 indicate that the cultures were aerobic. The optimal carbon source was liquid wax at an optimum content of 1% (tetradecane ranked second), and yeast extract at 1 g/l was the optimal nitrogen source with peptone as the next best source. Optimum culture temperature was 45°C. Figure 2 shows the growth curve under optimal culture conditions.

The experimental results demonstrate that the three strains grew well and reached their peak volume within 6 h, which is applicable for microbial-enhanced oil recovery. Many oil

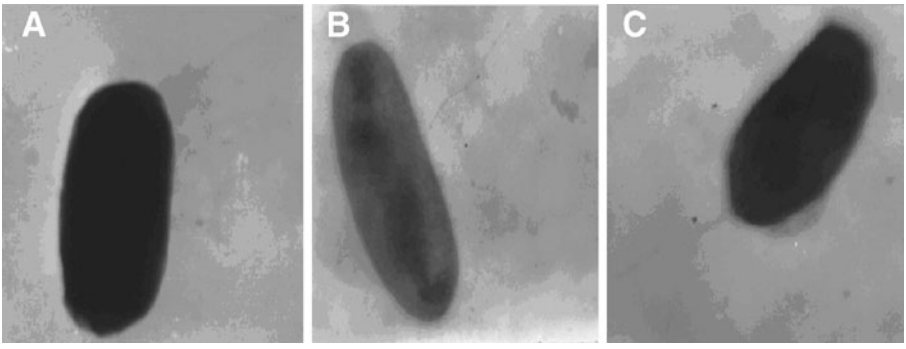

Fig. 1 Electron microscope photos of XDS1 (**a**), XDS2 (**b**), and XDS3 (**c**) (×10,000) (TEM, JEM-1400)

Table 2 Physical and biochemical characteristics of the three strains.

Test name	XDS1	XDS2	XDS3	
Only carbon resource	Glucose	+	+	+
	Maltose	+	+	+
	Lactose	+	+	+
	Galactose	+	+	+
	Rhamnose	+	+	+
	D-Raffinose	+	+	+
	Sorbitolum	+	+	+
	Sucrose	+	+	+
Only nitrogen resource	Ammonium Chloride	+	−	+
	Sodium nitrate	+	+	+
	Sodium nitrite	+	−	−
Indole test		+	+	+
H_2S producing test		+	+	+
M.R. test		+	+	+
V.P. test		+	+	+
Hydrolysis of starch test		+	+	+
Citrate test		+	+	+
Temperature range		15–65°C		
Salinity range		1–10% NaCl		

recovery agents were producible by continuously adding nutrients to hasten the microorganism propagation and the degradation of petroleum hydrocarbon.

Three strains were cultured in the produced fluid containing 500 mg/l PAM from Daqing Oilfield. Figure 3 shows their growth curves and organic quantity. The results indicate the three strains grew well in the produced fluid with a PAM concentration of 500 mg/l and reached their maximum volume within 8 h. The maximum organic quantity of XDS1 reached 3.5×10^7 CFU/ml, XDS2 reached 2.3×10^8 CFU/ml, and XDS3 approached 1.1×10^9 CFU/ml.

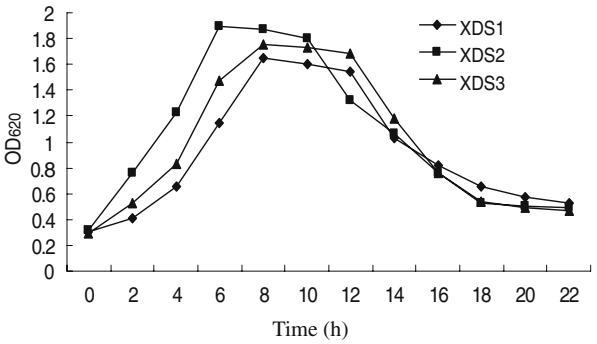

Fig. 2 Growth curve of the three strains in fermentation medium

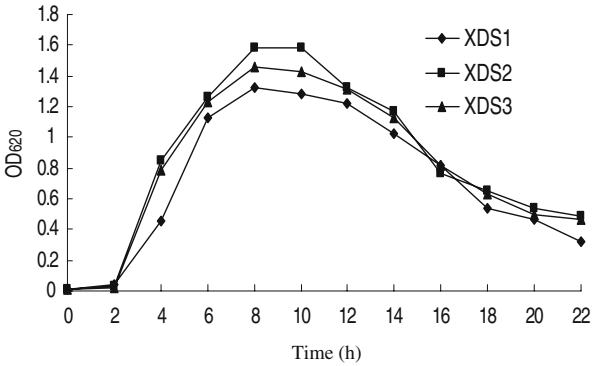

Fig. 3 Growth curve of the three strains in the produced fluid containing 500 mg/l polyacrylamide from Daqing Oilfield in fermentation medium

Degrading Effect on Crude Oil

Figure 4 and Table 3 presents the alkane component change of the crude oil along the distribution curve of crude oil saturated hydrocarbon and a ratio change of pristane/C17, phytane/C18, and (C21+C22)/(C28+C29).

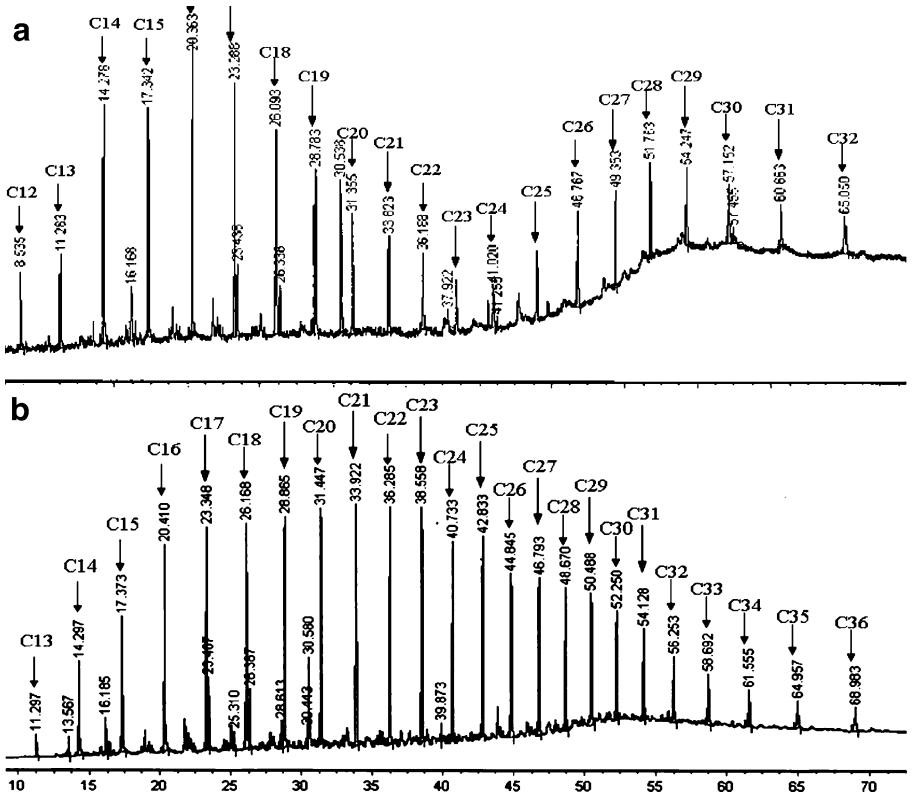

Fig. 4 GC/MS analysis of biodegradation of 307# crude oil with XDS123. **a** Control sample with 307# crude oil. **b** Sample after 3 days incubation with XDS123

Table 3 The changes of saturated hydrocarbon in crude oil samples.

Crude oil	Treatment	C21−/C22+	(C21+C22)/(C28+C29)	Pr (pristane)/Ph (phytane)	Pr/C17	Ph/C18
306	Before	1.13	1.46	0.37	0.63	1.47
	After	1.40	1.56	0.39	0.61	1.59
307	Before	1.25	1.67	0.35	0.74	1.46
	After	1.31	1.72	0.43	0.69	1.48
308	Before	2.18	1.13	0.42	0.54	1.70
	After	2.23	1.17	0.4	0.55	1.67
309	Before	0.96	2.56	0.46	0.75	1.43
	After	1.14	2.84	0.48	0.77	1.46
310	Before	0.85	3.64	0.52	0.69	1.68
	After	1.12	3.72	0.45	0.66	1.71

As shown in Figure 4 and Table 3, in crude oil samples 306, 309, and 310, components lower than C_{22} significantly increased, while components greater than C_{22} decreased after being treated with a mixture of strains of XDS123. Components lower than C_{14} increased, components between C_{14} and C_{26} decreased, and components greater than C_{26} remained stable after XDS123 was introduced. Before and after XDS123 was introduced, crude oil components were tested using gas chromatography, which indicated that XDS123 had a beneficial degrading effect on crude oil samples 306, 309, and 310.

Table 4 The changes of viscosity of crude oil samples.

Sample no.	Before biotreatment (mPas)	Strain	After biotreatment (mPas)	Ration (%)
306#	42.36	XDS1	32.35	23.6
		XDS2	36.47	13.9
		XDS3	35.85	15.4
		XDS123	34.86	17.7
307#	32.68	XDS1	30.45	6.8
		XDS2	31.47	3.7
		XDS3	29.25	10.5
		XDS123	25.34	22.4
308#	36.47	XDS1	32.48	10.9
		XDS2	35.94	1.5
		XDS3	30.85	15.4
		XDS123	30.21	17.2
309#	19.68	XDS1	15.36	22
		XDS2	16.24	17.5
		XDS3	15.47	21.3
		XDS123	15.23	22.6
310#	23.57	XDS1	20.31	13.8
		XDS2	22.36	5.1
		XDS3	19.65	16.7
		XDS123	20.47	13.2

Table 5 The changes of surface tension and pH of the fermentation liquid of oil samples after treating with the three strains.

Sample no.	Strain	Surface tension (mN/m)	Ration (%)	pH
306#	XDS1	42.36	41.68	6.9
	XDS2	45.32	37.61	6.7
	XDS3	36.54	49.69	6.2
	XDS123	38.76	46.64	6.1
307#	XDS1	45.63	37.18	6.8
	XDS2	47.36	34.8	5.8
	XDS3	42.16	41.96	6.3
	XDS123	35.29	51.42	6.8
308#	XDS1	52.31	27.98	6.4
	XDS2	54.76	24.61	6.8
	XDS3	50.34	30.69	6.9
	XDS123	40.67	44.01	6.7
309#	XDS1	54.31	25.23	6.4
	XDS2	57.64	20.65	6.9
	XDS3	36.49	49.77	6.7
	XDS123	39.45	44.47	6.1
310#	XDS1	46.32	36.32	5.6
	XDS2	44.98	38.08	6.7
	XDS3	41.58	42.76	6.3
	XDS123	42.68	41.24	6.2

The measured value of surface tension and pH of the fermentation liquid of oil samples before biotreatment was 72.6 mN/m and 7.1. The temperature for surface tension and pH determination is 25°C

The ratio of $C_{21}-/C_{22}+$ increased. Also, light components relatively increased and heavy components relatively decreased. At the same time, components with high molecular weights were degraded into components with low molecular weights in the crude oil by the microbes.

The results indicate that microbes preferentially degraded the saturated hydrocarbon. The screened indigenous microorganism degradation rates in the Daqing Oilfield all exceeded 50%, with the highest achieving 84%. The microorganism culture XDS123 consists of three strains at a ratio of 1:1:1. Its degradation rate achieved 90%. Many oil recovery agents were produced after crude oil degradation. At the same time, the heavy components of crude oil were degraded into light components, and the flow properties of the oil were improved.

Viscosity Change and Emulsification Effect of Crude Oil, Surface Tension, and pH Change

Tables 4 and 5 present the changes in crude oil viscosity, surface tension of the fermentation liquid, and changes in pH before and after the three strains produced effects on the crude oil.

The surface tension and viscosity decreased after the three strains were introduced to the crude oil. The viscosity decreased by 20% to 30%, and the surface tension decreased to less than 30 mN/m. Among them, the surface tension of crude oil sample 307 decreased by

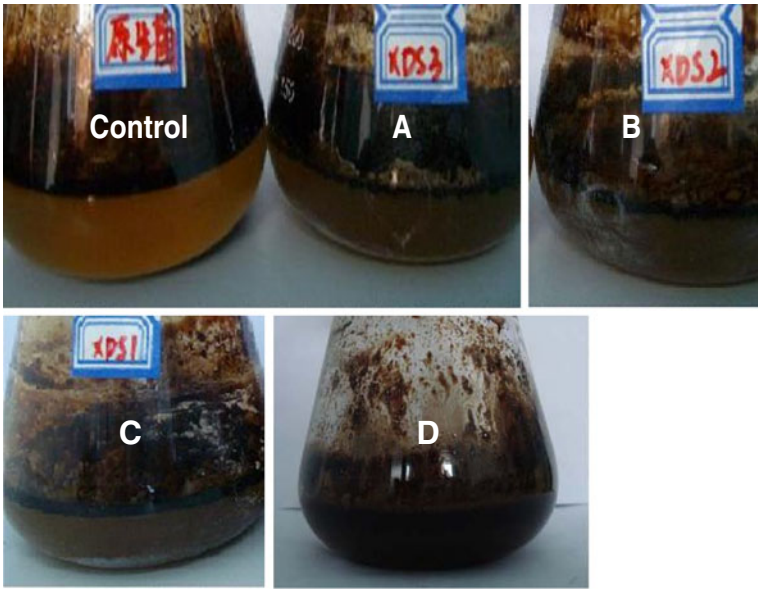

Fig. 5 Photos for emulsification effect of the three strains of XDS1, XDS2, XDS3 grown on 20 mg/ml of 307# crude oil in fermentation medium at 45°C. **a** XDS3 culture after 4 days. **b** XDS2 culture after 4 days. **c** XDS1 culture after 4 days. **d** XDS123 culture after 4 days. Control sample with 307# crude oil at 20 mg/ml but no microbials

35.29 mN/m following the addition of XDS3, the viscosity of 306 decreased by 23.6% following the addition of XDS1, and the fermentation fluid became acidic, indicating that an organic acid was produced. The emulsification effect (Fig. 5) was sufficient after the three strains and their metabolites were allowed sufficient time to act on the crude oil samples from the Daqing Oilfield.

The Results of Core-Flooding Tests

The core-flooding test results (Table 6) show that the incremental oil recoveries with indigenous bacteria and XDS123 suspensions are 4.89–6.96% original oil in place (OOIP) with different permeability cores in the pressure of 0.1 and 15 MPa.

The results show that the indigenous bacteria and XDS123 are able to increase residue crude oil recovery. This indicates that the indigenous strains are able to live in high pressure and positively impact the crude oil by decreasing its viscosity and reducing the interfacial

Table 6 The results of core-flooding test.

Core samples	Porosity (%)	Water Permeability (10^{-3} μm^2)	Bacteria/ pressure MPa	EOR after water flooding (%)	EOR after bacteria flooding (%)	MEOR (%)
D1	28.7	159.56	IM/0.1	68.42	74.50	6.08
D2	30.0	134.94	XD123/0.1	58.56	65.52	6.96
D3	31.0	175.13	IM/15	53.30	58.19	4.89
D4	29.3	137.73	XD123/15	62.94	68.70	5.76

tension between water and oil, hence improving oil mobility. The indigenous bacteria used in MEOR application usually are piezophile bacteria, which can survive and grow well in high pressure conditions [19, 20].

Conclusions

Three biosurfactant-producing indigenous strains were screened. Their oil-spreading values were more than 4.5 cm, and the hemolytic zone was found to be visible. The results of GC/MS analysis indicated that those microbes preferentially degrade saturated hydrocarbon, and the degradation rates of crude oil exceeded 50%, with the highest rate to 84%. In addition, the heavy components of crude oil were degraded and the flow properties of the remaining oil were improved. Hence, the viscosity of cure oil decreased by 20% to 30%, and the surface tension of culture batch decreased to less than 30 mN/m. The fermentation fluid was acidic with an average pH of less than 7. Many oil recovery agents can be produced after the degradation of crude oil, which emulsified crude oil into one phase. Therefore, the results of core-flooding test show that the incremental oil recoveries with indigenous strains were approximately 5–7% OOIP both in atmospheric pressure and reservoir high pressure. Thus, XDS1, XDS2, and XDS3 provide a potential application of microbial-enhanced oil recovery (MEOR).

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