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A Technical Feasibility Analysis to Apply *Pseudomonas aeroginosa* MR01 Biosurfactant in Microbial Enhanced Oil Recovery of Low-Permeability Carbonate Reservoirs of Iran

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Abstract. *The effect of an efficient biosurfactant produced from Pseudomonas aeroginosa MR01, a bacterial strain isolated from oil excavation areas in southern Iran, on the recovery of residual oil trapped within carbonate rocks, was investigated. In a core holder set-up, bearing a number of limestone- and dolomite-containing core samples, biosurfactant flooding resulted in oil recoveries as large as 20% to 28% Residual Oil (RO). Biosurfactant injection in less permeable rocks in a range of 0.5 to 32 md was more successful, in terms of oil production. In the case of the least oil recovery via biosurfactant flooding, incubation of the core with a biosurfactant solution at reservoir conditions, increased the recovery from 13% residual oil at zero resting time to 15% after a resting time of one week and to about 30% after a resting time of about two weeks. Based on interfacial tension measurements, salinity and, to a larger extent, biosurfactant reduced interfacial tension. When salinity increased from 170000 to 200000 ppm, the fraction of residual oil production increased to about 20%.*

Keywords: *Oil recovery; Biosurfactant; Pseudomonas aeroginosa; Interfacial tension; Biosurfactant flooding; Water flooding.*

INTRODUCTION

Approximately half of the world's known oil reserves are in carbonate reservoirs [1]. Usually, a small portion of these reserves is produced in primary recovery and as much as 50% of the oil is trapped in the reservoir even after water flooding. Due to a number of factors, including positive zeta potential on the rock surface at a typical pH of about 7 and the presence of sufficient numbers of negatively charged asphaltenic molecules in the oil [2], as many as 80% of these reservoirs are reported to be either mixed-wet or oil-wet [3]. Therefore, a high capillary pressure occurs in the rock and water flooding does not lead to a significant amount of oil recovery, because water cannot penetrate easily into the matrix. The use of Enhanced Oil Recovery (EOR) methods to recover as much of this residual oil as possible has become an increasingly important strategy worldwide. In particular, surfactant flooding may be an effective EOR method, largely due to its effect on wettability alteration.

Successful surfactant flooding methods have been developed for sandstone reservoirs [4-8]. However, carbonate reservoirs are different from sandstone because of heterogeneity and low permeability. The recovery with anionic surfactants was quite slow, as compared to that with cationic surfactants [9-12]. Several commercial cationic surfactants were able to recover 50-90% of oil [13-15]. However, the high cost of cationic surfactants necessitates evaluating other surfactants.

Chen et al. have performed dilute surfactant imbibition tests for carbonate cores with a nonionic and an anionic surfactant [16]. Computerized Tomography (CT) scans indicated that enhanced imbibition is made possible by counter-current flow at the beginning and gravity-driven flow during the later

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stages [17]. Spinler et al. conducted spontaneous imbibition and adsorption tests with a surfactant that was an ammonium salt of ethoxylated and sulfated alcohols at very low concentrations and high reservoir temperatures. It was found to be effective in improving imbibition in North Sea chalk cores and adsorption was low [18]. Hirasaki and Zhang have evaluated several ethoxylated and propoxylated sulfates in the presence of low concentration alkali. They found that wettability can be changed to intermediate wettability and imbibition can be improved by more than 35% OOIP by using very dilute anionic surfactant/alkali solutions [19].

At present, the majority of useful surfactants are chemically synthesized. These surfactants are costly and are usually harmful to the environment. Moreover, most previous studies using these surfactants have concentrated on rather low concentrations of NaCl with little or no divalent ions present [20-23].

Under certain conditions, many microbes can be induced to produce extracellular biosurfactants. Bacteria that are able to grow under saline and anaerobic conditions could produce these biosurfactants within oil reservoirs directly. Microbially generated biosurfactant could be adsorbed on the rock surface and change the reservoir to nearly water-wet [24]. These molecules are usually composed of lipids, phospholipids, polysaccharides, proteins and other biological macromolecules and contain various functional groups including carboxyl, amino and phosphate groups [25,26]. To date, successful applications of biosurfactants have been reported in food, cosmetics, pharmaceutical, agricultural and petrochemical industries and a notable potential exists in oil recovery applications [27-29]. Based on this, Microbial Enhanced Oil Recovery (MEOR) using biosurfactants can provide a low cost and simple tertiary oil recovery method.

Surfactant flooding of Iranian carbonate reservoirs is still a difficult proposition due to the harsh brine conditions and the structure of carbonate reservoirs. In this work, a biosurfactant produced from the MR01 bacterial strain from the Pseudomonas family, isolated from oil excavation areas in southern Iran, was applied to improve the wettability in Iranian carbonate reservoir rocks and its influence on oil recovery was studied. This biosurfactant has been reported to have characteristic features, such as low toxicity, proper biodegradability, great selectivity and, in particular, appropriate specific activity at extreme temperatures and salinity [29]. This study is concerned with the feasibility of application of the biosurfactant in conditions analogous to that of prevailing carbonate reservoirs. In particular, the effects of parameters, such as the resting time of the biosurfactant and brine salinity on the performance of the biosurfactant, are analyzed.

MATERIALS AND METHODS

Materials

Properties of Brines

Two types of brine were used in the experiments. Brine 1 had 170000 ppm and brine 2 had 200000 ppm salinity. Characteristics of the two brines are shown in Table 1. The salinities of the brine used correspond to the salinity of Iranian reservoirs, and experiments were carried out under conditions analogous to those of prevailing oil formations.

Properties of Crude Oil

Properties of crude oil used in this study are shown in Table 2.

Compositional analysis revealed that the crude oil was composed of high percentages of Saturates (55.96%, w/w), Aromatics (27.94%, w/w) and Resins (11.597%, w/w) in addition to a minor fraction of Asphalthenes (4.39%, w/w).

Biosurfactant

From several bacterial strains, ultimately, one efficient bacterial strain that had the highest biosurfactant production and activity was selected and purified by plating on nutrient agar. The isolate formed uneven, convex and wet colonies, which were rather circular, with diameter 1-2 mm within 24 h growth on nutrient agar at 37°C. It was positive for gelatin liquefaction, casein, lysine and arginine hydrolysis. Further identification was performed, based on the 16S rRNA gene sequence analysis. Examination with two sets of primer for almost full and partial 16S rRNA sequence alignment revealed that strain MR01 was closely related to the species in genus Pseudomonas. Strain MR01 also

Table 1. Characteristics of the two brines .

Brine Sample	Temperature °C	Salinity ppm	Density g/cm^3	Viscosity cP
1	25	170000	1.133	−−
1	77	170000	1.116	ND
2	25	200000	1.145	1.4004
2	77	200000	1.131	0.7013

ND: Not Determined

Table 2. Characteristics of crude oil.

Temperature °C	IFT (Brine 2/Fluid) mN/m	Density gr/cm^3	Viscosity cP
25	28	0.8686	ND
77	-	0.8563	6.0055

Table 3. Characteristics of core plugs.

Sample No.	Orientation	Length (cm)	Diameter (cm)	ϕ (%)	K (md)	PV (cc)
1	Horizontal	4.893	3.853	13.63	0.584	9.4138
2	Vertical	5.163	3.848	18.59	2.962	12.060
3	Horizontal	5.190	3.846	17.33	7.452	10.192
4	Horizontal	5.196	3.846	19.64	32.097	13.42

exhibited the highest similarity (99%) to Pseudomonas aeroginosa [29].

Cores

Details of the core plugs cut from limestone-dolomite rock samples collected from different depths of the Bibihakimeh oil well no.122, Gachsaran, Iran, are given in Table 3.

Methods

Interfacial Measurements

Interfacial tension between the immiscible phases was measured by an interface tension instrument (Model IFT 700, VINCI, FRANCE). Basically, a pendant drop or standing bubble/drop (drop fluid) may be generated in a second immiscible fluid. The drop shape image is computed, then, the interfacial tension is computed from solving the algorithm of the Laplace equation. If an equilibrium state is reached, the energy required to increase the droplet volume is positive. In other words, this state matches the minimum energy level.

Experimental Setup

All plugs were cleaned by toluene via a distillation/extraction method in a Soxhlet or Dean-Stark apparatus [30,31]. All cleaned samples were placed in an oven at a temperature of 400°C for 48 h and dried to constant weight.

Helium porosimeter and an air permeameter were used for porosity and absolute permeability measurements.

The following steps were used to restore all clean samples to reservoir conditions and perform the intended experiments thereafter:

1. The selected core plugs were initially evacuated using a vacuum pump for 4 h.
2. Samples were saturated with formation water and pressure saturation at 13790 kPa for 48 h.

   To determine complete saturation, the pore volume of each sample was ascertained by mass balance and compared with that determined by porosimetry. In all cases, saturations were deemed suitable to proceed with the test schedule.
3. The core holder was connected to the main core flooding apparatus. One hour was sufficient to stabilize core temperature.

   Figure 1 shows the details of the stainless steel core flooding system used in this study. It consists of a core-holder, three reservoirs (brine, biosurfactant solution and crude oil), a pressurizing system using a hydraulic pump, and proper tubing. The pressurized tank and the pump served as the pressure source to force fluids through the core-holder containing the plug sample.
4. Core plugs were then flooded with a minimum of 5 pore volumes of brine in the core holder. After differential pressures were stabilized, pressures at several different flow rates were recorded and absolute water permeability (k_{w1}) for all plugs was calculated through knowledge of the upstream pressure, oil viscosity, sample dimensions and flow rate:

$$k_w = \frac{L}{A} \times \mu_w \times \frac{\Delta V}{\Delta T \times \Delta p}, \tag{1}$$

$$q = \frac{\Delta V}{\Delta T}. \tag{2}$$

5. The 100% brine saturated samples were then flooded with the reservoir oil until approximately zero water cut in effluent was obtained. At this time, the lowest water saturation in any core plug and the irreducible water saturation (S_{wirr}) were determined.
6. After differential pressures were stabilized (minimum of 3 pore volumes eluted through the core flooding), oil permeability (k_o) at residual water saturation for all plugs was calculated:

$$k_o = \frac{L}{A} \times \mu_o \times \frac{\Delta V}{\Delta T \times \Delta p}. \tag{3}$$

7. At this stage, all samples were allowed to age with oil for 1000 h ($\sim$ 41 days) at 77°C, in order to restore reservoir equilibrium conditions. The experiments could be operated at any temperature. By using some heating elements and sensors in the line of injection, the temperature under reservoir condition was thermostatically controlled until stabilized.

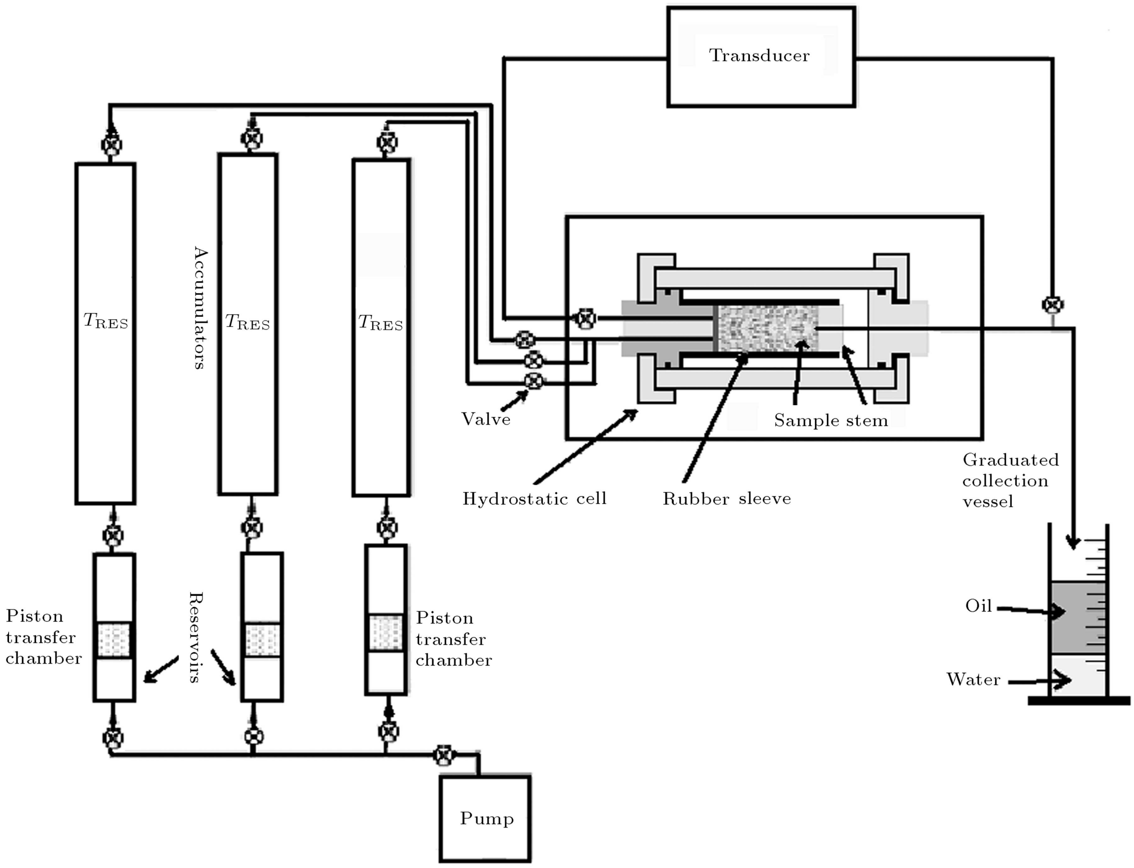

Figure 1. A schematic drawing of the experimental set-up used for the displacement experiments.

8. Injecting brine into the cores commenced. The water volumes and time at breakthrough were recorded. After breakthrough, the produced oil and brine volumes as well as the pressure drop across the core, were recorded with time until initial residual oil saturation (S_{or}) was reached. Oil and water relative permeability was calculated using graphical techniques presented by Jones and Roszelle [32].

   After approximately 100% water cut, pressures and flow rate recordings and k_{w2} calculations continued. The total volume of recovered oil as well as the recovery factor was carefully measured.

Biosurfactant Flooding

In biosurfactant flooding experiments, 20 mg of the biosurfactant dry powder [29] was dissolved in 950 mL of brine. The isolated bacterial strain of *Pseudomonas aeroginosa* MR01 used in this study was maintained on a nutrient agar medium at 4°C [29]. It exhibited high levels of secretion of biosurfactants and could reduce interfacial tension down to 28 mN/m.

RESULTS AND DISCUSSION

Water Flooding Experiments

As seen in Figure 2 for horizontal samples, after a 5 pore volume of water injection, the oil production was about 55, 42 and 36 percent of the pore volume. The spent time for this injection was 457, 82 and 65 minutes, respectively, and corresponds to the pressure drop across the core holder (6142, 367 and 154.4 kPa).

The average recovery by the water flooding

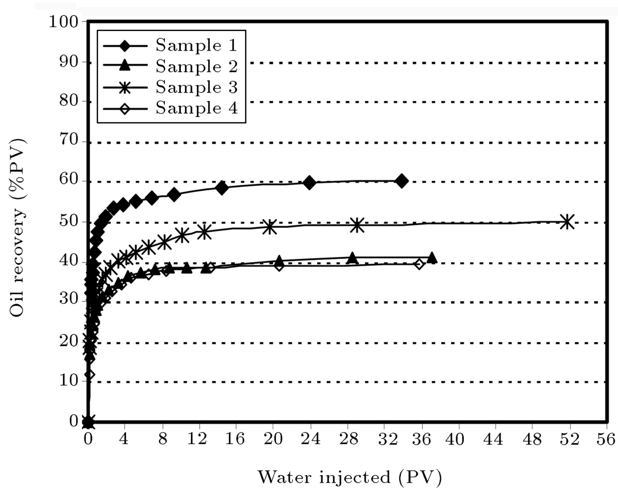

Figure 2. Oil recovery for water injection experiments with plug samples no. 1-4.

method was found to be 47%, although it varied depending on the rock permeability and properties.

All core samples showed a high oil recovery in water flooding, which is not common in real field cases. The high recovery as compared to the field practice could be attributed to the high sweep efficiency in the laboratory-scale test experiments in which a very well-defined low diameter and short core is used. Of particular interest is the shape of the curve, illustrating data collected from sample 1. From Figure 3 (data of the first sample has been truncated in Figure 3), it may be observed that oil recovery is greater and happens after a noticeably longer injection time of about 1700 min in core sample 1. This is apparently due to the very low absolute permeability (0.584 md as compared to 2.962, 7.452 and 32.097 md for the other 3 samples) of this sample. Also, because of the high pressure drop across the core for the lower permeability sample (ΔP = 6140 kPa), the rate of injection was low and spending time was high. Therefore, it had a higher oil recovery with time.

Biosurfactant Flooding Experiments

To evaluate the potential application of the biosurfactant in enhanced oil recovery, after the water flooding experiments, the core plugs were again flooded to saturation with the biosurfactant solution (200000 ppm Salinity), under reservoir conditions, until no more oil was produced. Biosurfactant injection was initially performed with a constant rate delivery of 1 cc/min, but oil production was low. Lowering the injection rate to 0.1-0.08 cc/min based on sample permeabilities, showed an incredible increase of oil production and was used in the biosurfactant flooding experiments (data not shown). A high injection rate does not allow for a better sweep of oil; this can be because of water breakthrough occurring in the core.

The final residual oil saturated (S_{orc}) and the percentage recovered after biosurfactant flooding is presented in Table 4. The core experiments reported in this table show that the less permeable the sample, the greater the residual oil production due to biosurfactant injection. The least permeable sample, sample 1, was totally depleted at the end. This indicates that biosurfactant effectiveness was more significant in the cores with lower permeability.

Figure 4 shows oil recovery and the influence of rock permeability on the oil production of the four plugs. There is an obvious effect of permeability convoluted with the effect of the biosurfactant. This phenomenon may be due to a decrease of biosurfactant adsorption on the rock at larger permeabilities and should be investigated separately.

The extra amount of oil produced via flooding the core with biosurfactant is obtained. Utilization of the biosurfactant was indeed effective in mobilizing oil in

Table 4. Comparison of oil recovery obtained from water flooding and residual oil recovery by biosurfactant flooding afterwards.

Sample No.	Water Injected (PV)	Previous Oil Recovery (%PV)	Residual Oil Recovery Due to Biosurfactant Injection (%PV)
1	43.844	60.145	27.8
2	37.172	41.211	21.3
3	35.70	49.882	19.6
4	28.79	39.419	14.3

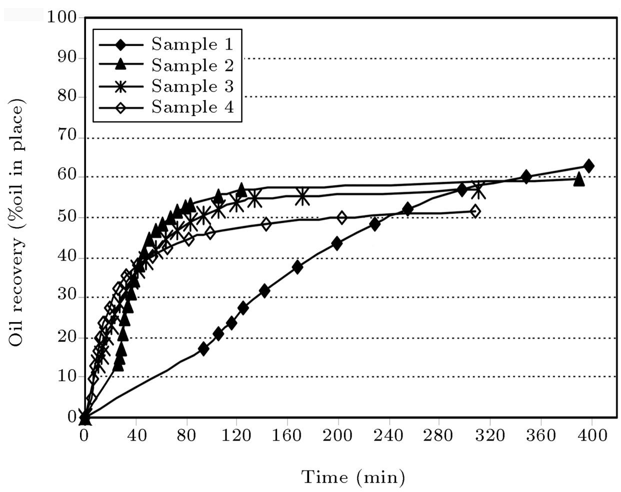

Figure 3. Oil recovery due to water flooding (tests performed on plug samples no. 1-4).

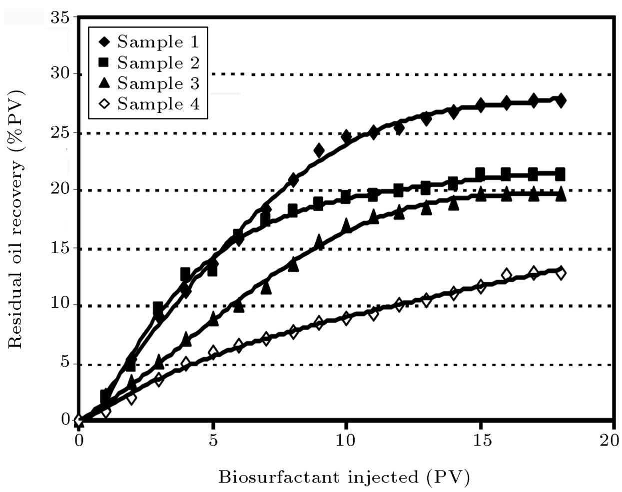

Figure 4. Residual oil recovery due to biosurfactant flooding after water flooding (200000 ppm solution).

the core holder model, and more oil was recovered in a range of 13% to 28% of the residual oil, respectively, for samples 4 to 1.

Effect of Resting Time of Biosurfactant

Due to the non-equilibrium conditions prevailing inside the core, it may be expected that providing more time for the biosurfactant to treat the core will result in larger recovery. To examine this possibility, the entire flooding protocol was repeated on sample no. 4, which had lower oil recovery compared to other samples. Therefore, the core was washed, dried and treated with brine, crude and brine, consecutively, to achieve the simulated S_{or} condition. The core was then flooded with about 5 pore volumes of surfactant to assure good contact between biosurfactant and the rock. Then, the process was stopped to age the system at 77°C in two time intervals, for one and two weeks, as detailed in Figure 5. It is interesting to note that incubation of the core with biosurfactant solution under reservoir conditions had a pronounced effect on oil production. While the recovery for zero resting time in Figure 4 was lower than 13% of the residual oil for sample 4 after 20 days of injection, it was higher than 15% after a resting time of one week, and about 30% after a resting time of about two weeks. This is in favor of our initial expectations and verifies that increasing the resting time provides more time for treating the system. This effect is probably due to mechanisms such as adsorption of biosurfactant on the rock, reduction of surface tension and wettability alterations towards stronger water-wet conditions.

Effect of Salinity

Salinity has been reported in the literature to affect bio-

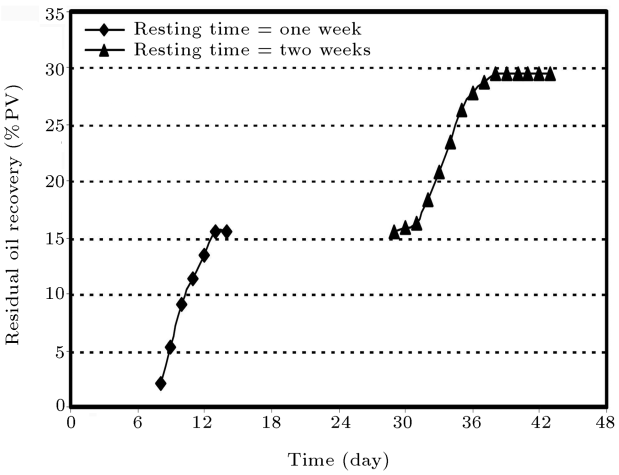

Figure 5. Residual oil recovery due to biosurfactant resting time (sample no. 4).

surfactant flooding via its adsorption on the rock [33]. To explore this idea again in this study, the previous study on core samples no. 2 and 3 were repeated with a biosurfactant solution with 170000 ppm salinity, as compared to the previous salinity of 200000 ppm. Figure 6 shows that when salinity increased from 170000 to 200000 ppm, the fraction of oil production for sample no. 2 also increased. A similar trend was observed for sample no. 3. This may be because of increasing ion concentration. The increase in ion concentration is more likely to alter the dissociation of the ionic species, which will alter their propensity to adsorb. The decrease in IFT between the crude and biosurfactant solution at higher salinity indicates that such species are present in the oil. This matter was investigated by measuring interfacial tension between the crude oil and biosurfactant solutions at the two salinity contents of 170000 and 200000 ppm. The results of these measurements are presented in Table 5. As seen in Table 5, a moderate but noticeable reduction of interfacial tension was achieved. These measurements imply that both salinity and biosurfactant reduce interfacial tension, though the effect of biosurfactant seems to be more pronounced.

Figure 6 illustrates the extra amount of oil produced via biosurfactant flooding with a change in the

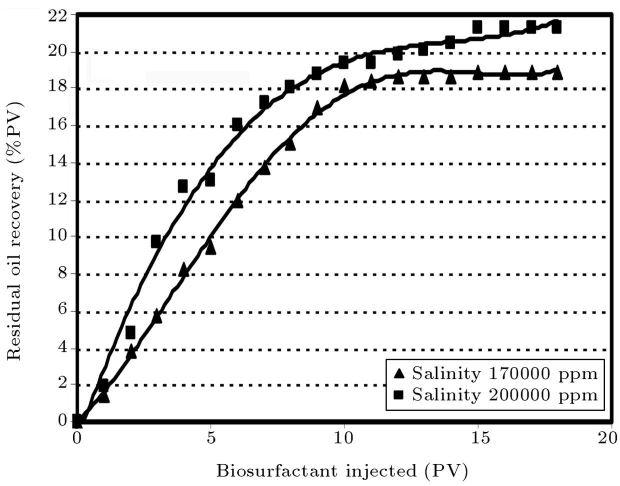

Figure 6. Residual oil recovery due to biosurfactant flooding with different salinity (sample no. 2).

Table 5. Interfacial tension at reservoir temperature.

Solution	Biosurfactant	IFT (mN/m)
Brine 170000 ppm/Oil	-	21.221
Brine 170000 ppm/Oil	+	7.368
Brine 200000 ppm/Oil	-	18.382
Brine 200000 ppm/Oil	+	5.646

brine salinity. When salinity increased from 170000 to 200000 ppm, the fraction of oil production increased from 18.9 to 21.3 percent in sample no. 2 and a similar trend was observed for sample no.3 (from 17.3 to 19.6 percent). This is equivalent to up to a 20% increase in oil recovery, due to the increased salinity, and is in favor of more saline oil formations as carbonate reservoirs in the Middle East.

CONCLUSIONS

Analyses of the data collected in this study reveal a positive outlook as to the applicability of the nominated biosurfactant in MEOR operations in targeted oil fields. Moreover, it sheds more light on the details of these processes. For example, absolute permeability has a pronounced effect on the ability to achieve successful results in this practice such that in a low-permeability rock with a permeability of 0.584 md, oil recovery was greater as compared to more permeable rocks with permeabilities of 2.962, 7.452 and 32.097 md. Moreover, a 90% reduction in injection rate from 1 to about 0.1 cc/min showed an incredible increase of oil production.

In biosurfactant flooding experiments similar to those of reference water flooding, the lower the sample permeability, the greater the oil production due to biosurfactant injection. Therefore, an obvious effect of permeability convoluted with the effect of the biosurfactant may be seen in these studies, probably due to a decrease of biosurfactant adsorption on the rock at larger permeabilities. The rock with a low absolute permeability of 0.584 md was totally depleted at the end of biosurfactant flooding experiments.

Incubation of the core with biosurfactant under reservoir conditions had a pronounced effect on oil production. While the recovery, in terms of RO% for zero resting time, was lower than 13%, it was higher than 15% after a resting time of one week, and about 30% after a resting time of about two weeks. This may be due to mechanisms such as adsorption of biosurfactant on the rock, reduction of surface tension and wettability alterations towards stronger water-wet conditions.

Interfacial tension measurements imply that salinity and, to a larger extent, biosurfactant reduce interfacial tension. When salinity increased from 170000 to 200000 ppm, an increase of up to 20% in oil recovery was observed. This may be due to the improved adsorption of biosurfactant on a solid-fluid interface because of increasing ion concentration and altering the dissociation of ionic species, which will alter their propensity to adsorb, and smaller interfacial tension between the crude and biosurfactant solution at higher salinity.

Noting that the core holder system used in this study was a reasonable simulation of the real targeted oil formations in terms of rock and oil conditions, these results support the possibility of a successful application of biosurfactant in the field.

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NOMENCLATURE

cp	centi poise
f_w	water cut fraction
k	permeability
ppm	parts per million
PV	pore volume
S	saturation
RO	residual oil

Greek Letters

μ	viscosity
ρ	density
σ	interfacial tension, IFT
ϕ	porosity

Subscripts

f	final
i	initial
ir	irreducible
o	oil
r	residual
w	water
c	connate

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