

A Pore Scale Evaluation of Produced Biosurfactants for Ex-situ Enhanced Oil Recovery

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Abstract

Microbial enhanced oil recovery (MEOR) is an economical method used to improve the oil recovery from reservoirs. In the MEOR techniques, by applying different microorganisms, a variety of products such as bioacid, biogas, biosurfactant, and biopolymer are generated, among which biosurfactant, one of the important metabolites, is produced by bacteria. It is worthy to note that bacteria are suitable candidates to enhance oil recovery due to their small size, rapid growth, capability of tolerating reservoir conditions, and production of different metabolites. Therefore, in this research, two bacteria, namely *Enterobacter cloacae* subsp with PTCC: 1798 isolated from oil-contaminated soil in south of Iran and *Acinetobacter Calcoaceticus* with PTCC: 1318, are used to produce biosurfactants.

In order to evaluate the performance of generated biosurfactants, ex-situ flooding tests were performed in a glass micromodel to visualize the oil displacement and fluid front flow. In addition, water flooding is performed as a common EOR method for the better investigation of the produced biosurfactants. The results represented that injecting *Enterobacter* with a salinity concentration of 6% and *Acinetobacter* with a salinity concentration of 3% respectively increases the oil recovery factor by 27 and 35% compared to water flooding. In other words, the highest reduction in interfacial tension is achieved by the biosurfactant produced from *Enterobacter* and *Acinetobacter* at 6% and 3% salinity respectively, and the sequent changes in the interfacial tension are from 45 to 7 and 45 to 4 mN/m.

Keywords: Biosurfactant, Microbial Enhanced Oil Recovery, Micromodel, Ex-Situ, Interfacial Tension

1. Introduction

There are three main stages by which oil is recovered from reservoirs. The first stage is the primary recovery stage, where oil is recovered due to natural energy inherent in the reservoir. When the inherent pressure of a reservoir tends to fall, secondary recovery methods are applied. In this method, external fluid or gases are injected to maintain the reservoir pressure (Sen, 2008). The third stage of oil recovery called tertiary recovery or the enhanced oil recovery involving chemical flooding,

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thermal recovery, and miscible displacement using carbon dioxide (CO₂), hydrocarbon, or nitrogen injection. However, most of enhanced oil recovery methods are not cost effective (Al-Sulaimani et al., 2011).

One of the applications of biotechnology is the use of microorganisms in the oil industry, which is widely based on the productions obtained from bacteria to increase the efficiency of the oil recovery (Desai et al., 2004). Studies have demonstrated that microbial enhanced oil recovery (MEOR) is one of the most promising novel approaches that can potentially be implemented at an exceptionally low operating cost (Lazar et al., 2007). In addition, there exist some reports about the field application of MEOR illustrating the applicability of this method in the production of original oil in place (OOIP) (Qingxin Li et al., 2002; Liu jinfeng et al., 2005; Al-Sulaimani et al., 2010). It is worthy to note that temperature is one of the most significant factors in MEOR since it is a characteristic of oil fields. In other words, 93 °C is usually the maximum temperature, at which MEOR can be effective (Saikrishna et al., 2007; Abtahi et al., 2003). Also, pressure changes can affect the performance of bacteria and their production (Halim et al., 2009; Abe et al., 1999). Usually, depending upon the functional characteristics of the reservoirs or the oil fields, one of the MEOR methods is used. MEOR procedures can be divided into two categories: in-situ and ex-situ. In the in-situ method, the residing bacteria of the reservoirs become stimulated by consuming the injected nutrition and produce some substances such as biogas, bioacid, biopolymer, biosurfactant, and solvent, which leads to an enhancement in the oil recovery by itself. On the other hand, the biologic products in the ex-situ method are obtained in the industrial fermenter under controlled conditions, and they will be injected into reservoirs when purification and analyses are performed (Ghazali et al., 2001; Youssef et al., 2013).

The recovery of oil reservoirs induced by the application of microorganisms has been justified by a plethora of mechanisms such as acid generation (Bryant, 1987), gas production (Volk et al., 2010), interfacial tension (IFT) reduction (Deng, 1999; Crecente et al., 2005; Hiorth, 2007; Lazar et al., 2007; Gray et al., 2008; Sen, 2008), oil viscosity reduction, the modification of the fluids mobility ratio in the reservoir, relative permeability improvement, enhancing the sweep efficiency of water flooding process (Raiders, 1986; Bryant et al., 1989; Li et al., 2011), and wettability alteration (Kowalewski et al., 2005; Gautam, 2006; Heidari et al., 2011; Aparna et al., 2012; Vaz et al., 2012). Among the studied mechanisms, the IFT reduction and wettability alteration, which are related to the capability of biosurfactants, have been considered as the most effective ones, especially in the oil wet carbonate reservoirs (Crescente et al., 2006; Gandler et al., 2006; Biria et al., 2007; Afrapolis et al., 2009; Amani et al., 2010; Zargari et al., 2010).

Biosurfactants are a heterogeneous group of surface-active molecules synthesized by microorganisms with both hydrophilic and hydrophobic domains which allow them to partition at the interface between fluid phases with different degrees of polarity such as oil–water or air–water interfaces, thereby reducing surface and interfacial tensions (Jamaloei, 2009; Ghazali et al., 2001). Biosurfactants are the main products of microorganisms which are able to alter the surface tension, interfacial tension, and wettability. These functions, in some cases, may increase surface activities, while they simultaneously are able to decrease critical micelle concentrations (CMC). Therefore, compared to the most of traditional chemical surfactants, they have a great role in improving the oil recovery factor (Li et al., 2002; Ben Ayed et al., 2013; She et al., 2011). The lower toxicity and higher biodegradability of biosurfactants, compared to the synthetic chemical surfactants, make them an attractive alternative (Mohan et al., 2006).

Therefore, in the present study, for the first time, the performance of two biosurfactants extracted from *Enterobacter cloacae* subsp with PTCC: 1798 isolated from oil-contaminated soil in the south of Iran and *Acinetobacter Calcoaceticus* with PTCC: 1318 is compared. Furthermore, the effect of salt concentration on the performance of the biosurfactants in decreasing the interfacial tension and surface tension, as well as the application of each biosurfactant in enhancing oil recovery using the glass micromodel, is investigated.

2. Materials and methods

2.1. Glass micromodel

The glass micromodel was made up of a glass with a thickness of 4 mm. The flow pattern was designed using Corel Draw graphic software, and it was then implemented by means of a laser device on the glass. A polisher was used to clean the waste materials from the glass surface to obtain an optimum depth. Then, the directing plate was placed on a smooth glass plate. The plates were then fused together at a temperature of 700 °C for 4 hours. The physical aspects of the designed micromodel are indicated in Table 1. In the present study, a dolomite rock type pattern was selected as the micromodel pattern to perform the MEOR injection tests. Since the fluid analysis in micromodel experiments was carried out through the image analysis, methylene blue was added into the injected deionized water. The increased cost of natural oil caused by the global high-demanding approach toward the natural oil and a decrease in the natural production of oil has resulted in a further attention to the novel procedures for the extraction of heavy oil. Therefore, in this study, a heavy oil sample from one of the Iranian oil fields with 17.5° API and a viscosity of 340 cP (measured in ambient conditions) was used to completely saturate the porous medium. Viscosity was measured using a Brookfield viscometer (NDJ-4).

Table 1
Physical properties of the micromodel.

Size (cm)	Pore volume (cc)	Thickness (µm)	Porosity (%)	Injection pattern	Permeability (mD)
6×9	0.12	60	38	Linear	240

2.2. Bacterial culture and evaluation of decreased surface and interfacial tension

In this study, two bacteria called *Enterobacter cloacae* subsp with PTCC: 1798 and *Acinetobacter Calcoaceticus* with PTCC: 1318 were employed. The reason for choosing these species is their ability to produce biosurfactants and to reduce the surface and interfacial tension. Then, they were transferred to the growth medium to grow. The properties of this medium are listed in Table 2. In the growth medium, the bacteria were inseminated and were then put in a shaker incubator (Wise, 20R; Korea) at a temperature of 30 °C and at a rotational speed of 135 rpm for 72 hours. The biosurfactants produced by bacteria were extracted and purified after the required time (5 days) to reach the optimum concentration of microorganisms. First, the bacteria were added to a medium of 2 liters in order to isolate the pure biosurfactant. After the time required for bacteria's growth (72 hours) and after the production of biosurfactant, the microbial mass (biomass) was centrifuged (Vision, VS-550; Korea). The solution containing biosurfactant (pH=2) was kept at a temperature of 4 °C overnight until the obtained biosurfactants completely precipitated in the medium. The white biosurfactant resulted by the precipitation was centrifuged and separated from the solution. The biosurfactant was then dissolved in deionized water (pH=7), and the obtained material was finally dried by a freeze dryer (Christ, Alpha 1-2 ld; Germany). The surfactant derived from this phase was called the acid

sediment of the biosurfactant. The acid sediment of the biosurfactant was later dissolved in the methanol-chloroform solvent (volume ratio: 2/1) and was dried by rotating the vacuum evaporator (Wise, WB-11; Korea) when filtrations was performed. The obtained biosurfactant at this stage was called the crude biosurfactant. It should be pointed out that the pH adjustment throughout the whole process mentioned above was carried out by HCl and NaOH.

Table 2
Properties of the growth medium.

Meat extract (g/l)	Pepton (g/l)	MnSO ₄ H ₂ O (g/l)
3	5	0.01

The IFT and surface tension (ST) in the prepared sample at different concentrations of biosurfactants were measured in order to investigate the ability of the produced biosurfactants to reduce the surface and interfacial tension. The surface tension was measured by a tensiometer (Kruss, DSA100; Germany). Since the salinity concentration is a key parameter in the biosurfactant function and in order to study its effect on the MEOR process, the decreased interfacial tension between heavy oil with 17.5° API and the injected fluid (water containing various percentages of biosurfactants at different concentrations of salinity) was investigated in the present study.

2.3. Experimental setup for flooding

The schematic of the experimental setup is illustrated in Figure 1. Before each test, the glass micromodel was washed up with toluene solution, the air inside the micromodel was then discharged using a vacuum pump, and the micromodel was filled up with the heavy oil. In this study, the effect of irreducible water saturation on oil recovery was neglected due to the lack of control on the place where water settles in the micromodel. In other words, different places of water in each injection affect the flow behavior, and this matter finally influences the oil recovery. Hence, irreducible water saturation was ignored and the porous medium was completely saturated with oil. As shown in Figure 1, an infusion pump (FNM; Iran) was used to inject the fluids and to saturate the micromodel glass. A vacuum pump was also used to evacuate the micromodel system from air and washing fluids. In all the steps, to satisfy a laminar flow regime, the tests were performed at a very low injection rate of 0.0008 ml/min using the infusion pump, and photographs were automatically taken (every 2 minutes) by a camera (Canon, 7D; Japan). The camera was placed on top of the micromodel glass to capture photos of the injection process, and the captured photographs were analyzed with Photoshop software to determine the oil recovery.

3. Results and discussion

The obtained results indicate that the surface and interfacial tension of the injected fluid and oil are reduced in the presence of the biosurfactants. In fact, in the present study, the reduced ST and IFT are found as one of the most effective mechanisms in oil recovery. The surface and interfacial tensions were also investigated in the presence of different concentrations of the biosurfactant extracted from various bacteria, and the results are presented in Figures 2-3. As these figures depict, the biosurfactants produced by *Enterobacter cloacae* subsp with PTCC: 1798 and *Acinetobacter Calcoaceticus* with PTCC: 1318 can reduce the surface tension from 72 to 29 mN/m and from 72 to 24 mN/m respectively. Furthermore, the biosurfactants from *Enterobacter* and *Acinetobacter* can cause the interfacial tension to decline from 45 to 9 mN/m and from 45 to 6 mN/m respectively.

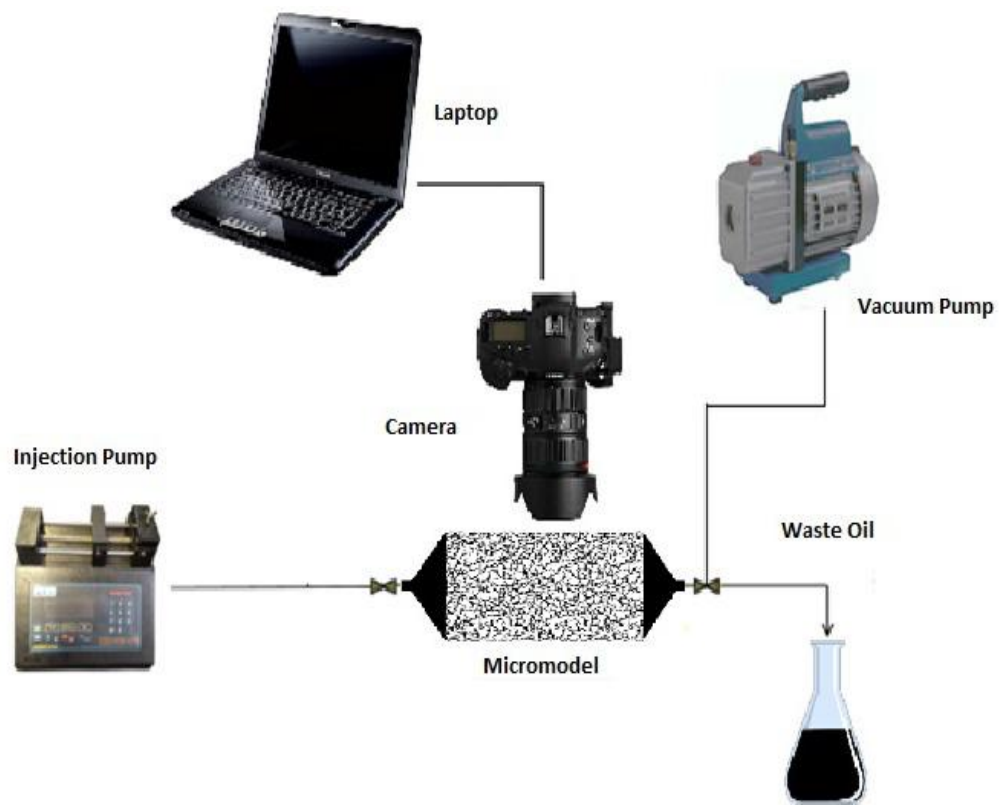
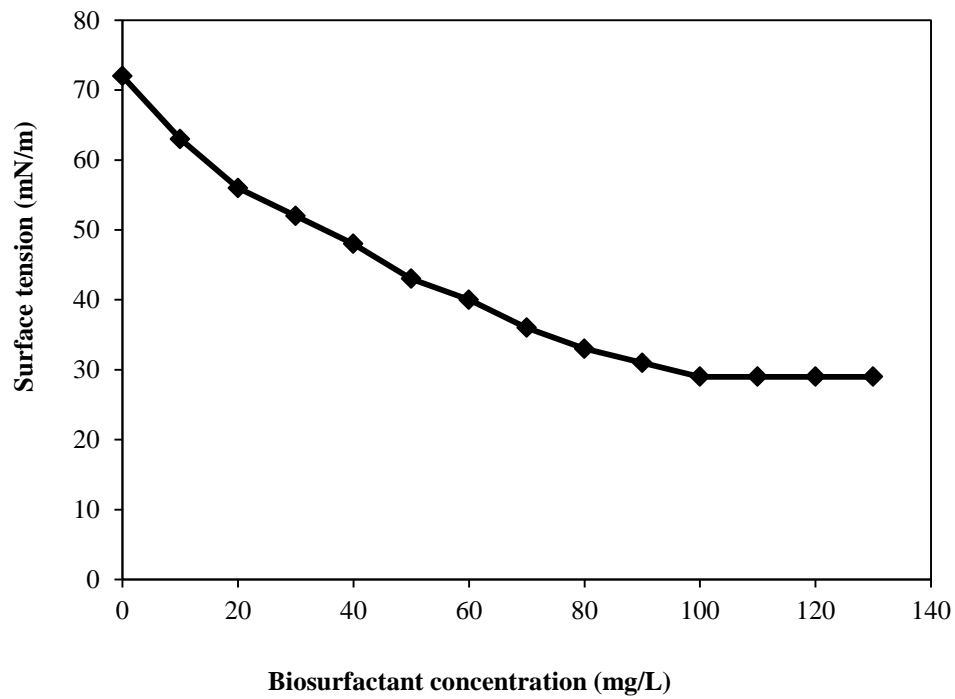
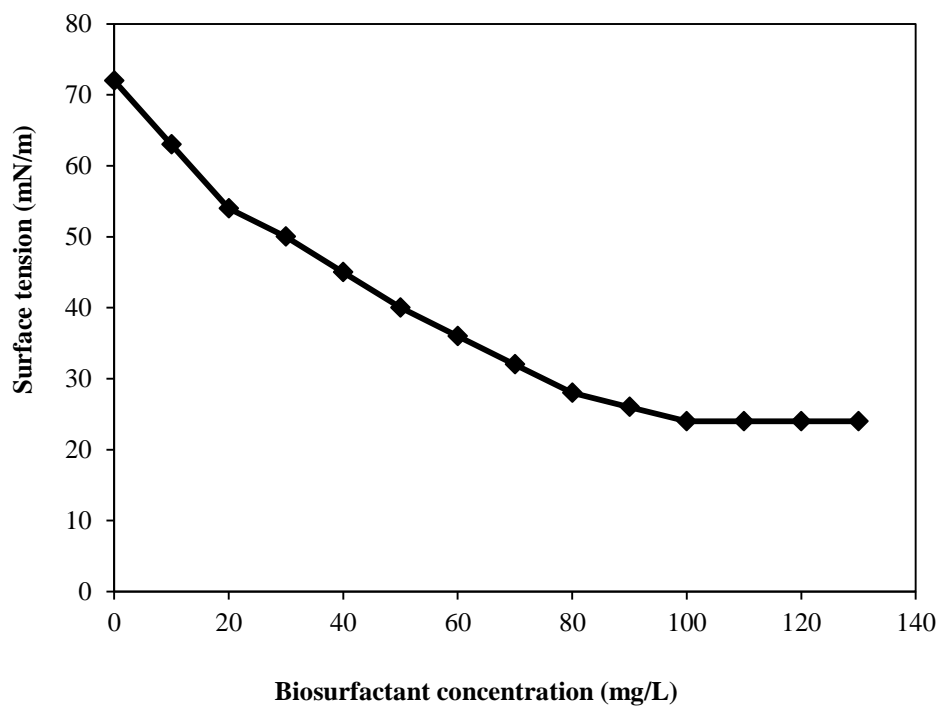


Figure 1
A schematic of the experimental setup.

a)

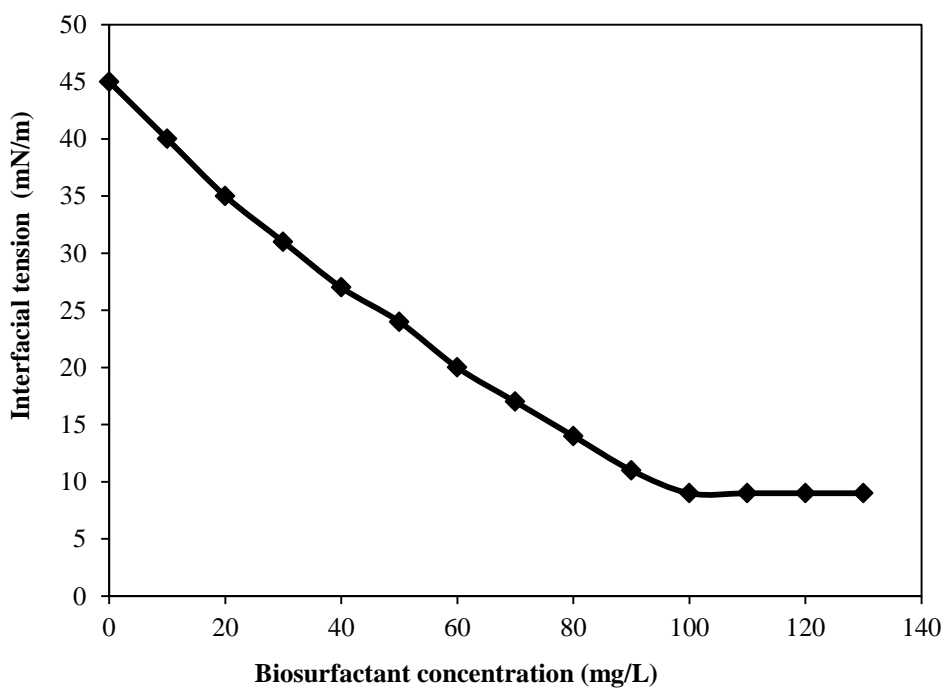


b)

**Figure 2**

Surface tension reduction by using the biosurfactant produced from a) *Enterobacter cloacae* and b) *Acinetobacter Calcoaceticus*.

a)



b)

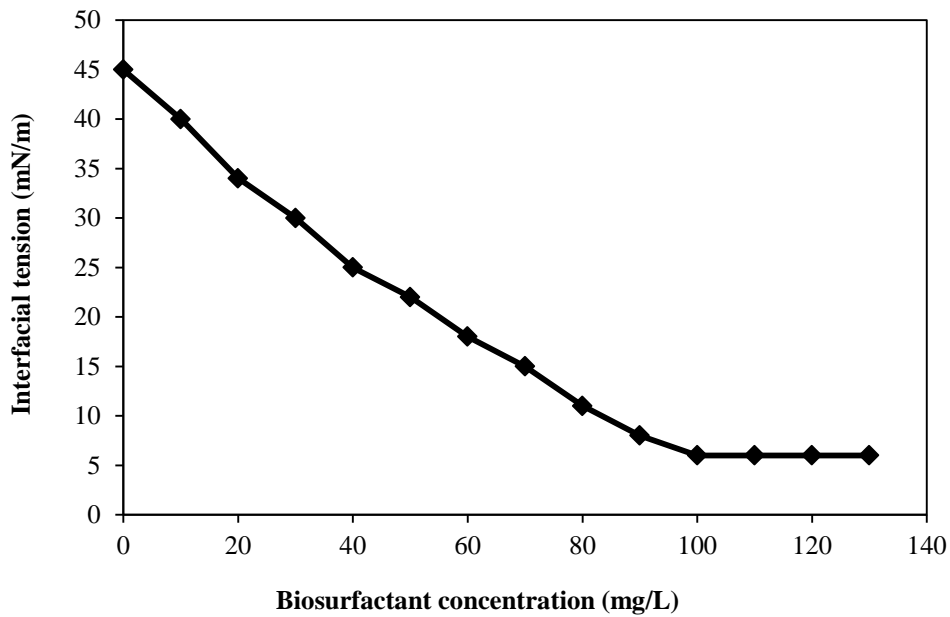
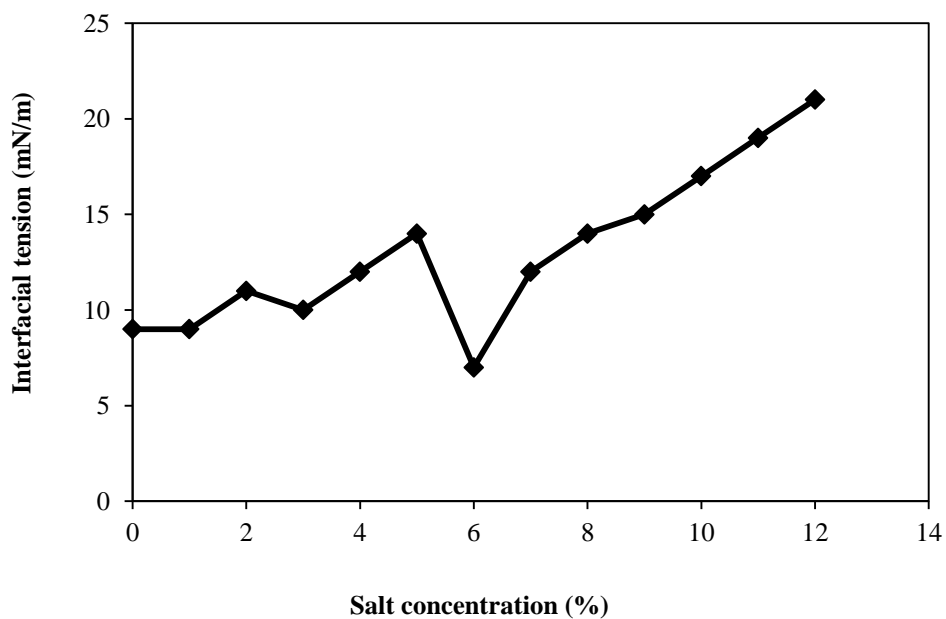


Figure 3

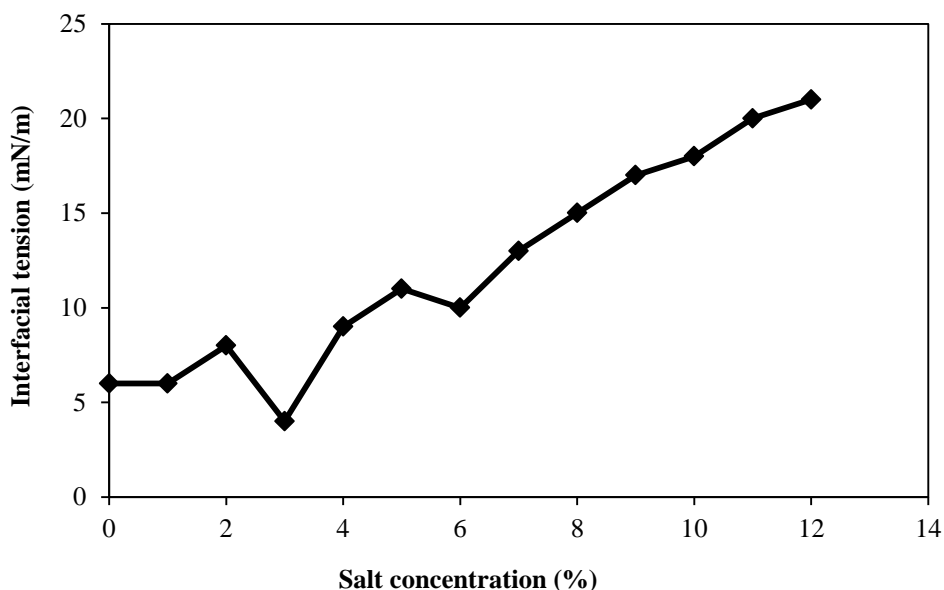
Interfacial tension reduction by using the biosurfactant produced from a) *Enterobacter cloacae* and b) *Acinetobacter Calcoaceticus*.

The salinity concentration is one of the most effective parameters in biosurfactant's functionality. As Figure 4 illustrates, both biosurfactants obtained from *Enterobacter cloacae* (at a salinity concentration of 6%) and *Acinetobacter Calcoaceticus* (at a salinity concentration of 3%) perform well in reducing the interfacial tension from 9 to 7 mN/m and from 6 to 4 mN/m respectively.

a)



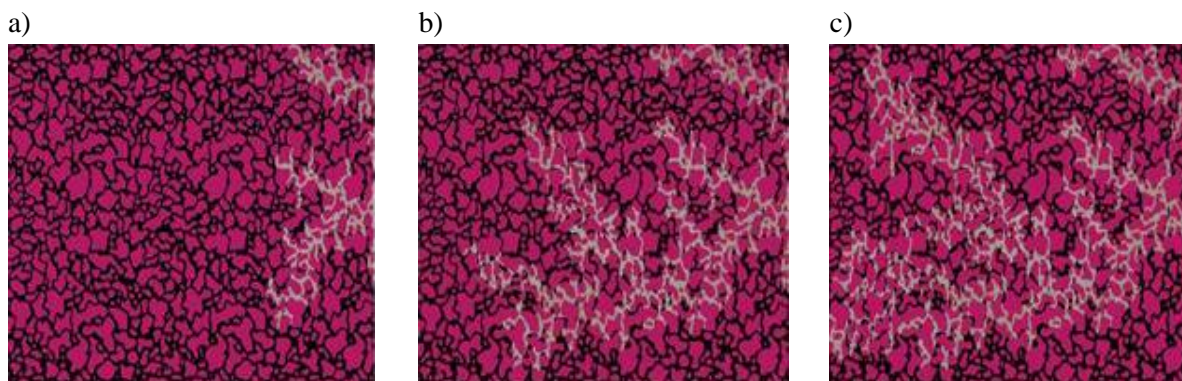
b)

**Figure 4**

The effect of salinity concentration on the interfacial tension: a) *Enterobacter cloacae* and b) *Acinetobacter*.

Furthermore, to evaluate the effectiveness of the obtained biosurfactants in the MEOR method, the findings of ex-situ injection are compared at 4 levels of salinity, namely 0, 3, 6, and 12%, by employing a conventional method known as “water flooding.” As demonstrated in the previous diagrams, both produced biosurfactants (extracted from *Acinetobacter* and *Enterobacter*) at a concentration of 100 mg/L have the greatest effect on reducing the interfacial tension. Therefore, all of the injections of the fluid containing biosurfactants have been performed at this concentration.

The results of deionized water injection into the micromodel as a common EOR method are studied, and it has been found that the oil recovery is 32%. As shown in Figure 5, the breakthrough happened quickly and a remarkable amount of oil remained intact in place in the porous medium. In addition, the high IFT between water and oil, and consequently the low capillary number, can potentially cause a reduced oil recovery during water injection.

**Figure 5**

Oil displacement during water injection after a) 30 minutes, b) 50 minutes, and c) one pore volume of the injected fluid.

In addition, the obtained data indicated that the injection of water containing the biosurfactant extracted from *Enterobacter cloacae* subsp with PTCC: 1798 at a concentration of 100 mg/L and salinity concentrations of 0, 3, 6, and 12% can result in oil recovery at the levels of 44, 50, 59, and 39% respectively (Figure 6). Therefore, it has been understood that the application of biosurfactants in the injected fluid could reduce fingering, which in turn expands the movement of the injected fluid vertically. As shown in Figure 6c, the injected fluid moved on with lower fingering. This process of changes along with the vertical development of the movement can increase the oil recovery compared to the alternative salinity concentrations.

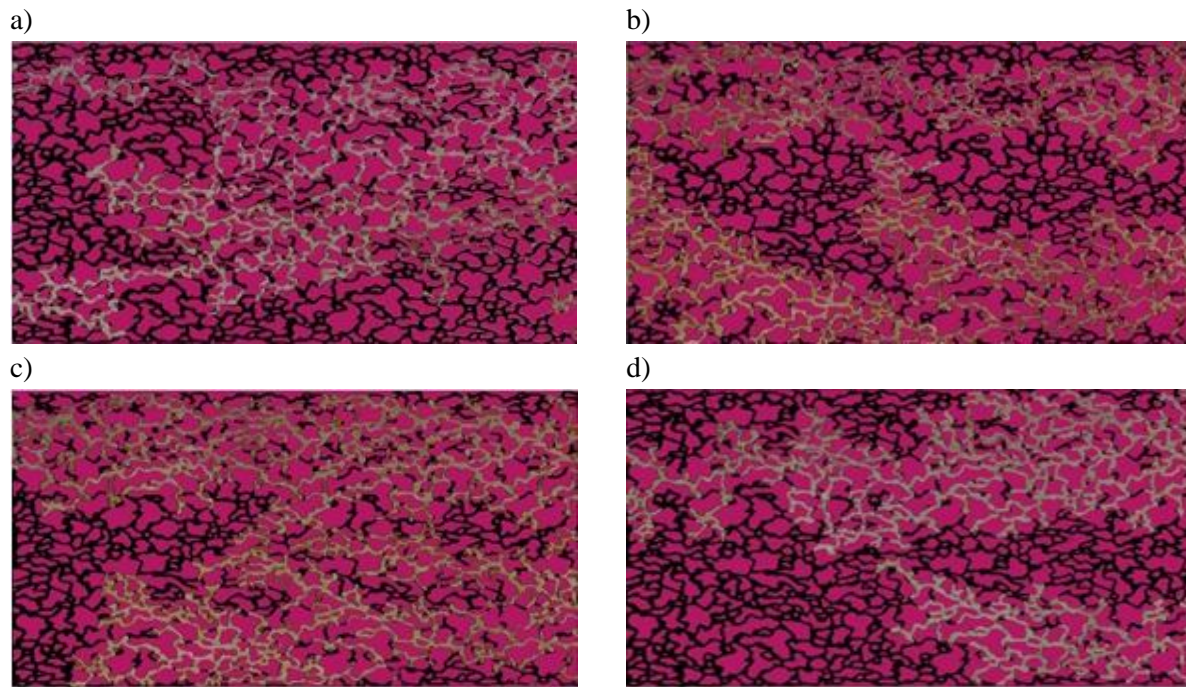


Figure 6

Oil displacement during water/biosurfactant (extracted from *Enterobacter cloacae*)/salinity injection at a salinity concentration of a) 0%, b) 3 wt.%, c) 6 wt.%, and d) 12 wt.%.

Compared to water flooding, the injection of water containing biosurfactant (12% salinity) could increase oil recovery. Thus, the observed reduced IFT in this study can justify the higher efficiency of water/biosurfactant mixture, as can be seen in Figure 6d.

Furthermore, as shown in Figure 7 by the injection of water containing biosurfactant obtained from *Acinetobacter Calcoaceticus* with PTCC: 1318 at a concentration of 100 mg/L and a salinity of 0, 3, 6, and 12%, the oil recovery was enhanced by 57, 67, 61, and 40% respectively.

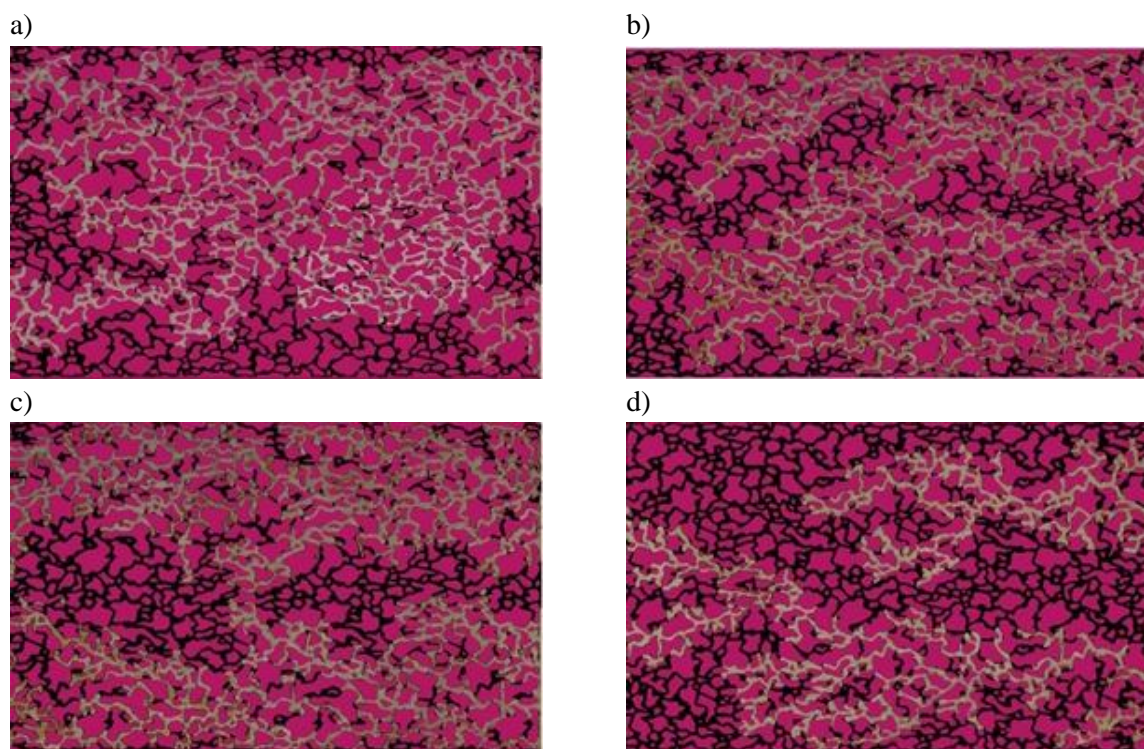


Figure 7

Oil displacement during water/biosurfactant (extracted from *Acinetobacter Calcoaceticus*)/salinity injection at a salinity concentration of a) 0%, b) 3 wt.%, c) 6 wt.%, and d) 12 wt.%.

As shown in Figure 7b, compared to the other salt concentrations, the water containing biosurfactant (3% salinity) induced more oil recovery (69%). This can be caused by a reduced IFT along with a high capillary number and a low capillary pressure. Figures 8-9 show the oil recovery ratio to the volume of the injected fluid. The oil remained in the ruck closed pores cannot be moved by water injection because the oil is trapped in the porous medium. The existence of biosurfactant molecules between the oil and water can cause the drag forces, which in turn can pull forward the oil drops remained in the corners of the porous medium (pulling mechanism), as illustrated in Figure 10.

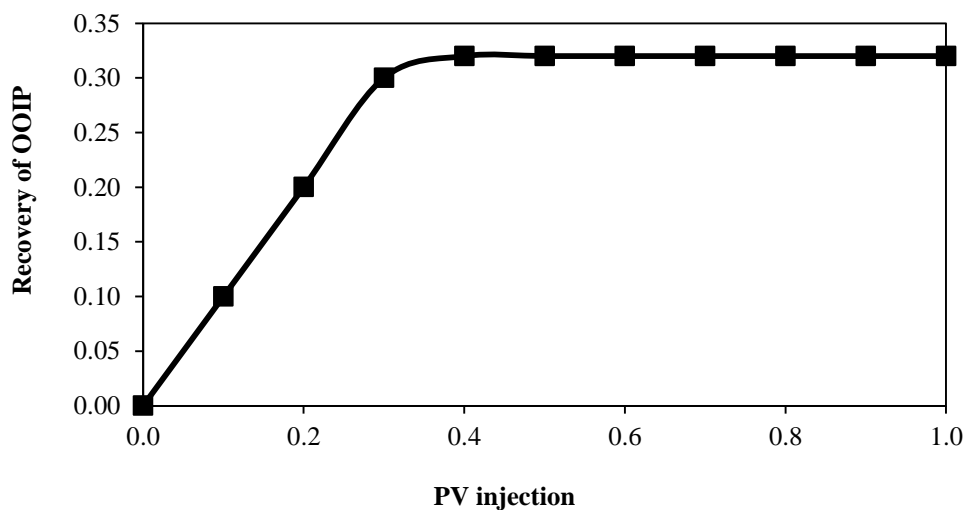
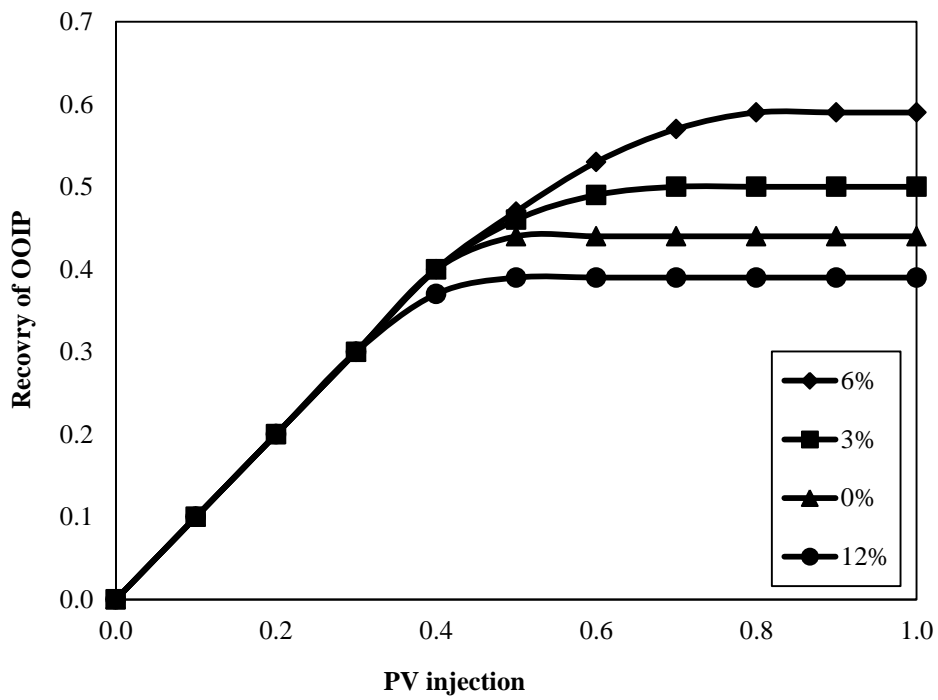


Figure 8

The oil recovery versus pore volume of the injected water.

a)



b)

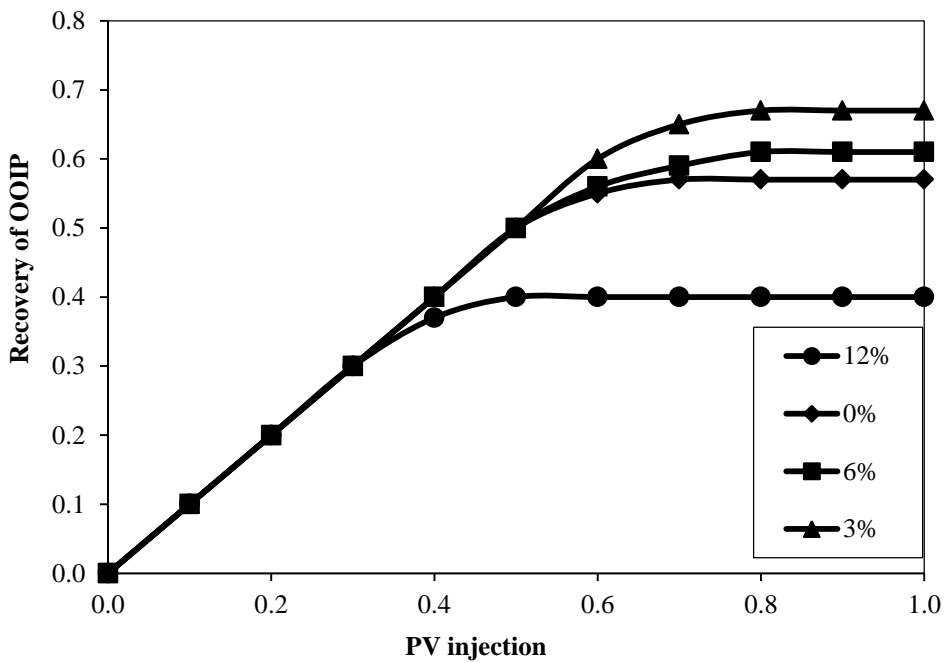


Figure 9

The oil recovery versus pore volume of the injected water containing biosurfactant extracted from a) *Enterobacter cloacae* and b) *Acinetobacter Calcoaceticus* at different salinity concentrations.

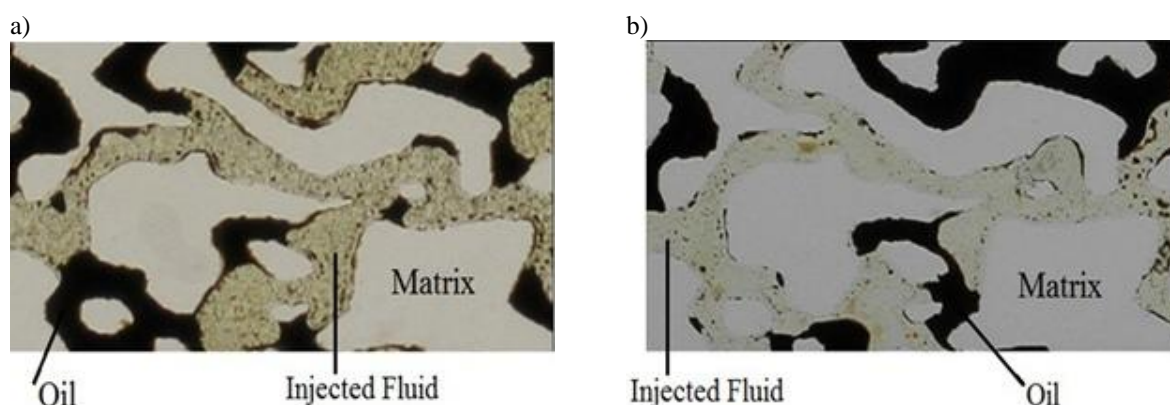


Figure 10

Effect of the pulling mechanism on the oil recovery a) water injection and b) water/biosurfactant injection.

4. Conclusions

In this research, the function of the biosurfactants produced from *Enterobacter cloacae* subsp with PTCC: 1798 and *Acinetobacter Calcoaceticus* with PTCC: 1318, with an emphasis on salinity concentration as one of the main parameters affecting biosurfactant function, was studied in a MEOR method.

Both types of the used biosurfactants have the potential to reduce the interfacial tension between the injected fluid and in-situ oil, leading to a greater capillary number, which is the most important mechanism in increasing oil recovery. As the results show, the fluid containing biosurfactant of *Enterobacter cloacae* has the greatest efficiency on oil recovery at a salt concentration of 6%. The injected fluid has a quite piston-like flow, and, as a result, the areal sweep efficiency of the porous medium increases compared to when only water is injected. Therefore, a more amount of oil is in contact with the injected fluid, and the system consequently has lower residual oil saturation on a macroscopic scale. The results also show that the fluid containing the biosurfactant of *Acinetobacter Calcoaceticus* perform best at a salt concentration of 3%. Finally, fingering phenomenon related to the injected fluid is remarkably reduced in the presents of these two types of biosurfactants, which causes a delayed breakthrough of the injected fluid, thereby enhancing the economy of this process.

5. Acknowledgements

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Nomenclature

CMC	: Critical micelle concentrations
EOR	: Enhanced oil recovery
IFT	: Interfacial tension
MEOR	: Microbial enhanced oil recovery
OOIP	: Original oil in place
PTCC	: Persian Type Culture Collection
ST	: Surface tension

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