EVALUATION OF THE PURIFICATION PROCESS IN RHAMNOLIPID BIOSURFACTANT FOR APPLICATION IN MICROBIAL-ENHANCED OIL RECOVERY (MEOR)

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Received: 11.04.2019 / Revised: 03.08.2019 / Accepted: 19.08.2019 / Published on line: 08.10.2019

ABSTRACT

Biosurfactants are employed in several industrial applications which require a high purity level. However, the downstream purification processes are responsible for a large portion of the expenses incurred by biosurfactant production plants. The high costs associated with these processes turn their application on a large scale a challenge for companies. This study aimed to evaluate the influence of the purification step on the capacity of the rhamnolipid produced by a *Pseudomonas aeruginosa* strain to recover oil. Both purified and non-purified biosurfactants were efficient in their ability to recover oil. The best result, for the API gravity oil of 27.67, presented a total Recovery Factor of $47.45 \pm 1.78\%$, in which $10.2 \pm 0.85\%$ corresponds to the MEOR. However, the results show that non-purified biosurfactant was more efficient in terms of amount of oil recovered during the MEOR stage. This demonstrates that the purification step may not be necessary, reducing the production costs of the rhamnolipid.

KEYWORDS

Pseudomonas aeruginosa; biosurfactant; rhamnolipid; microbial-enhanced oil recovery; purification process

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doi:10.5419/bjpg2019-0016

1. INTRODUCTION

Typically, 35 to 55% of the oil remains trapped in the reservoir after primary and secondary recoveries (Fan et al., 2018; Safdel et al., 2017). Nevertheless, the demand for energy and the depletion of conventional oil reserves continue to increase. These factors are the main motivation for scientists to explore more efficient methods of increasing oil recovery when water injection is no longer an economically viable alternative (Geetha; Banat & Joshi, 2018; Malik et al., 2016; Zendeboudi et al., 2013).

The oil trapped must be extracted by different enhanced oil recovery techniques, such as miscible gas injection, polymer injection, and thermal methods (Zahid & Sajid, 2007). The use of chemical surfactants is the technique which is most used by the oil industry for enhanced oil recovery over the decades (Curbelo et al., 2008; Mulligan, 2005). Surfactants have the capacity to reduce oil-water interfacial tension, requiring better capillary pressure to mobilize the oil (Al-Amodi et al., 2016; Al-Wahaibi et al., 2014; Babadagli, 2005; Fernandes et al., 2016). For this effect to be efficient, high concentrations of surfactant are required, leading to high costs (Youssef et al., 2013).

Thus, the development of low-cost effective techniques that lead to improvements on enhanced oil recovery is one of the most attractive topics of recent research (Safdel et al., 2017). In this context, the Microbial Enhanced Oil Recovery (MEOR) emerges as a potential low-cost technique (Desai & Banat, 1997; Khajepour et al., 2014).

MEOR is considered a tertiary recovery technique which can extract the residual oil by using microorganisms or their products (e.g. biosurfactants) (Alvarez, Jurelevicius, and Marques, 2015; El-Sheshtawy et al., 2015; Joshi et al., 2008). Rhamnolipids are among the most well-known biosurfactants. In addition, several studies suggest promising products for use in enhanced oil recovery (Amani et al., 2010).

Rhamnolipids are produced mainly by *Pseudomonas aeruginosa* (Desai & Banat, 1997; Hassan et al., 2016). This biosurfactant has the potential to emulsify the crude oil, reducing the oil-water interfacial tension and, therefore, mobilizing the trapped oil (Mcinerney; Nagle & Knapp, 2005;

Zhao et al., 2016). However, its large-scale production is still a challenge, mainly due to downstream processing and purification issues, which can represent from 60 to 70% of the production costs (Geetha; Banat & Joshi, 2018; Joshi et al., 2008; Makkar; Cameotra, & Banat, 2011; Al-Bahry et al., 2013).

Therefore, in this article, a rhamnolipid was produced from a *Pseudomonas aeruginosa* strain isolated from an artificially contaminated soil. The biosurfactant was studied following two approaches, after a purification process and in its crude form (fermented, free of cells), to evaluate the influence of the purification step related to the features and abilities of the product. Both of them were evaluated for their ability to reduce the water surface tension and to emulsify crude oil and, consequently, their ability to increase the rates of oil produced after conventional recovery.

2. MATERIALS AND METHODS

2.1 Bacterial strain

We used a strain of *Pseudomonas aeruginosa*, sourced as reported by Câmara et al. (2019). The strain was isolated from a soil artificially contaminated, and stored at -20 °C in skim milk.

2.2 Rhamnolipid

The biosurfactant was produced in 125 mL erlenmeyers containing 50 mL of the medium, which were incubated at 30°C, under a stirring of 200 rpm, for 10 days on a rotary shaker. The medium used was optimized in prior experiments (125 g/L of glycerol, 3.86 g/L NaNO₃, 0.5 g/L MgSO₄·7H₂O, 1.0 g/L K₂HPO₄, and 0.5 g/L KH₂PO₄). As inoculum, a nutrient solution of peptone (2 g/L) was used, which remained in shaker at 200 rpm and 30 °C for 24 h (Miguez et al., 2012). After inoculation, the medium had an initial concentration of 15 mg/L of cells.

After 10 days of fermentation, the medium was centrifuged at 3500 rpm for 20 minutes. Thereafter, the cell-free medium was separated into two samples, one remained in its raw state and another went through the purification process.

To purify the biosurfactant, the cell-free

supernatant was subjected to an acid precipitation. The medium was adjusted to a 2.0 pH with a 6 mol/L solution of hydrochloric acid (HCl) and spent approximately 12 hours (overnight) at a temperature of 4 °C. Subsequently, the biosurfactant precipitate was separated by centrifugation at 3500 rpm for 20 min and washed with acidified water (2.0 pH) twice, to remove remaining impurities, such as bacterial cells. Finally, the precipitate was dissolved in distilled water and the pH was adjusted to 7.0 with a solution of sodium hydroxide (NaOH) 1 mol/L (El-Sheshtawy et al., 2015; Pereira et al., 2013).

2.3 Biosurfactant characterization

Crude and purified biosurfactant were diluted in distilled water to generate solutions ranging from 25 mg/L to 260 mg/L. They were used to determine the surface tension and emulsion rate of the crude oil.

The surface tension (ST, mN/m) was determined by a Phoenix® tensiometer at 25 °C by the pendent drop method. The results were obtained through an image processing system coupled to a computer in which the bending curvature profile of each drop contained at the tip of the needle was analyzed to determine the ST (Song & Springer, 1996). A ten-drop measurement was used to calculate the arithmetic mean and standard error. With these results, a curve relating surface tensions and their respective concentrations was projected to determine the critical micelle concentration (CMC), i.e., the concentration at which the formation of micelles starts (El-Sheshtawy et al., 2015).

The rhamnolipid solution emulsification activity was determined by the emulsion index (E_{24}). In general, 2 mL of the biosurfactant were placed in a test tube, together with 2 mL of petroleum. After vigorously stirring the solution for 2 min, the test tube was held for 24 h at room temperature and, then, the emulsion heights were measured. The emulsion index was calculated from the ratio of the emulsion height to the total height (Cooper & Goldenberg, 1987; Yeh; Wei, & Chang, 2005).

2.4 Evaluation of superficial concentration excess of the biosurfactant at the interface

In aqueous solutions, surfactants generally have two trends. The first one is its adsorption at the

interface, and the second one is its aggregation and the formation of micelles. The first option corresponds to the surfactants equilibrium activity at the interface (Li et al., 2007; Santos et al., 2009).

The adsorption extent on a liquid surface was determined by surface tension data and it is based on the Gibbs Adsorption Equation. Thus, it was possible to estimate the adsorption per unit area of surface excess concentration, Γ (Santos et al., 2009).

According to Gibbs, the surface excess concentration is given by the isotherm slope before the CMC value. This can be seen in Equation (1) for non-ionic surfactants (Castro Dantas et al., 2002; Eastoe & Dalton, 2000; Gurkov et al., 2005; Jönsson; Kronberg, & Lindman, 2003).

$$\frac{1}{\Gamma} = -RT \frac{d(\ln C)}{d\gamma} \quad (1)$$

Where:

γ = the surface tension at equilibrium (mN/m) for a surfactant solution concentration C (mol/L) R = the universal gas constant (8.314×10^{-3} kJ/mol•K)

T = the temperature (K), and

$d(\ln C)/d\gamma$ = the curve slope before the CMC value.

From the Γ value, it is possible to estimate the surface area occupied by each surfactant molecule, in m^2 , as denominated by A in Equation (2).

$$A = \frac{10^{-20}}{N_A \Gamma} \quad (2)$$

Where:

N_A = Avogadro's number.

2.5 Recovery trials

2.5.1 Preparation of the synthetic sandstone plug

The recovery trials used synthetic sandstone plugs, which were composed of 0.4 g/g clay from Lagoa dos Velhos (RN, Brazil) and 0.6 g/g of sand from Redinha beach (Natal/RN, Brazil). The mixture was hydrated with water and, then, compacted at

19.61 MPa and sintered at 850 °C during 3 h (Costa et al., 2017).

A porosity value of $20.49 \pm 0.69\%$ was obtained for the produced plug. This property was determined by the capillary rise method, in which the pore volume is obtained from the mass difference between the dry plug and the plug totally saturated with water. Thus, the porosity was obtained by the ratio between pore volume, corresponding to the volume of water absorbed, and total volume, which is calculated by the measurements made with a caliper. The result obtained is consistent with the values of natural sandstones used in enhanced recovery studies (Costa et al., 2017).

The permeability of the developed plugs presented an average value of 36.24 ± 1.88 mD. This property was determined through an experiment using the fluid injection system (Costa et al., 2017), shown in Figure 1. Thus, the pressure and data obtained, along with the water viscosity, the length and the sandstone straight section area were applied in Darcy's Law for linear flow.

2.5.2 Brine

A saline solution, composed of 2% m/v of potassium chloride (KCl) and 0.2% m/v of calcium chloride (CaCl_2), was used in the injection of fluids to simulate conventional recovery, as well as salinity of a reservoir (Câmara et al., 2019). These were chosen because they are the most typical

salts present in oil reservoirs at the Fazenda Belém Field (CE, Brazil).

2.5.3 Petroleum

The oil provided for this study by the Fazenda Belém Field (CE, Brazil) is classified as a heavy oil (API gravity of 14). Due to its high viscosity, it was not possible to use the crude oil directly in the fluid injection system. In addition, the oil did not fit into the indication range of surfactant injection as enhanced recovery method. Therefore, three solutions of 300, 400 and 500 g/L of oil were prepared, diluted in S10 diesel, presenting API gravity values of 30.34, 27.67 and 21.90, respectively. The first two oil solutions can be classified as medium, while the third is in the transition between medium and heavy. Thus, the oil samples studied were consistent with the viscosity of the petroleum present in the studied reservoirs.

2.5.4 Microbial Enhanced Oil Recovery (MEOR)

Recovery assays were performed on the same fluid injection system shown in Figure 1, according to the method described by Câmara et al. (2019). Briefly, the sandstone plug was first saturated with the saline solution at a flow rate of 1 mL/min for 100 min followed by the petroleum solution. This second step also had a duration of 100 min and occurred at a flow rate of 1 mL/min.

Figure 1. Fluid injection system: (a) mineral oil reservoir; (b) positive displacement pump; (c) injection cell; (d) and (e) pressure taps and pressure gauge; (f) source of compressed air; (g) holder manometer; (h) holder; (i) plug; (j) test tube. Source: Câmara et al. (2019) and Costa et al. (2017)

Figure 2. Surface tension versus concentration of the purified and non-purified rhamnolipid.

Subsequently, the saline solution was again injected at a flow rate of 0.7 mL/min for 85 min. This step aimed to simulate conventional recovery and 20 samples were collected, which were then analyzed to determine the amount of oil recovered. Finally, the rhamnolipid solution with a concentration of 50% above the CMC was injected following the previous step to simulate the MEOR. The ideal concentration of biosurfactant was defined in a previous work, which indicated that high concentrations of surfactant favor the formation of emulsions. It difficults the oil/water separation in treatment units (Curbelo; Garnica, & Neto, 2013). The experiments were performed in triplicate.

2.5.5 Analysis of the petroleum samples

The separation of the organic phase from the petroleum samples obtained in the conventional and enhanced recovery experiments was performed by the addition of a fixed volume of kerosene to each sample, followed by shaking and centrifugation. Subsequently, the organic phase was diluted, when necessary, and readings were taken on the UV-Visible spectrophotometer, Model 50 Conc, at 400.1 nm, to allow the calculation of the oil concentration from the previously elaborated calibration curve (Câmara et al., 2019).

3. RESULTS AND DISCUSSION

3.1 Surface activities of the biosurfactants produced

The surface tensions of both biosurfactants produced, cell-free extract and purified extract, were measured as a concentration function, as shown in Figure 2. As can it be seen in the figures, the surface tension of pure water decreases rapidly as the concentration of the biosurfactant increases. In this study, the rhamnolipid present in the cell-free extract was able to reduce the surface tension from 72 to 35.26 mN/m and the CMC was found at 127 mg/L (as shown in the left y-axis in Figure 2).

The rhamnolipid biosurfactant that underwent a purification process was able to reduce the surface tension from 72 to 40.79 mN/m at a CMC of 110 mg/L (as shown in the right y-axis in Figure 2). The highest value of CMC obtained for the cell free extract, i.e. that were not purified, can be attributed to the presence of impurities in the solution that can delay the formation of micelles, requiring a higher concentration of biosurfactant molecules in the solution (Pornsunthorntawe et al., 2008).

The lowest reduction of the surface tension to the purified biosurfactant can be attributed to the purification process itself. Since it is necessary to

Table 1. Parameters of adsorption at the water-air interface.

Biosurfactant	Γ (mol/m ²)	A (x10 ⁻²⁰ m ² /molecule)
Non-purified	7.51x10 ⁻⁶	22.10
Purified	6.90x10 ⁻⁶	24.05

acidify the solution to pH of 2.0 with hydrochloric acid (HCl), the rhamnolipid molecules can undergo protonation of the carboxylate ions (Sousa et al., 2014), modifying their structure and affecting the formation of micelles., since the micelle configuration depends on the hydrocarbon chain structure (Castro Dantas et al., 2002).

Although the free extract of cells had better properties regarding the greater reduction of surface tension, both presented values that were consistent with the literature. In general, the biosurfactant produced by *Pseudomonas aeruginosa* is able to reduce the surface tension of pure water from 72 to 30 mN/m with CMCs in the range of 5-200 mg/L, depending on the components present (Healy; Devine, & Murphy, 1997; Pornsunthorntawe et al., 2008).

3.2 Adsorption at the water-air interface

From the data obtained through the surface tension as a function of the rhamnolipid concentration, it was possible to determine the adsorption parameters at the water-air interface. These values were determined based on the adsorption isotherms using the Gibbs Equation for non-ionic surfactants. The values are summarized in Table 1.

These results indicate that, due to the greater area occupied by the purified surfactant, a smaller amount of surfactant molecules is required to saturate the interface (Γ) (Santos et al., 2009). Additionally, the structural differences in the hydrocarbon chains are responsible for the configuration of the micelles (Castro Dantas et al., 2002). These results justify the large surface area per molecule for the purified bioproduct. Besides, it is in accordance with the fact that the rhamnolipid molecules undergo protonation of the carboxylate ions during the purification process.

3.3 Emulsification rate

The ability of both rhamnolipids to stabilize an emulsion was evaluated for a concentration range from 50 mg/L to 260 mg/L. This methodology was adopted because this property is a function of the concentration of the biosurfactant in the medium (Cooper & Goldenberg, 1987; Deepika et al., 2016).

Similar to what occurred with the reduction of surface tension, the ability of the non-purified rhamnolipid to emulsify the oil was greater than that of the purified biosurfactant. The maximum emulsification rate (E_{24}) was 69% and 65%, respectively. However, both results are consistent with other studies, which obtained an emulsion rate of 68% (Amani et al., 2010) and 85% (Amani, 2015).

Therefore, as the emulsification depends on the formation of micelles, the acidification of the solution to the purification of the biosurfactant can also cause alterations in this process due to the protonation of the carboxylate ions (Sousa et al., 2014). This modification in the structure allowed the purified rhamnolipid to obtain lower emulsification rates. However, the results obtained showed that both extracts are suitable candidates for application in MEOR.

3.4 Microbial enhanced oil recovery (MEOR)

The results of the six MEOR tests developed in this work, for the three types of oil and two types of biosurfactant, were represented in a relation between the Recovery Factor (%) and the pore volume injected, in Figures 3 and 4. The first parameter is the relation between the recovered oil volume and the volume of oil present in the rock. The second parameter is the ratio between the injected fluid volume and the pore volume of the rock, which is a dimensionless measure. By

Figure 3. Recovery Factor as a function of pore volume injected to non-purified biosurfactant solution (cell-free extract).

Figure 4. Recovery Factor as a function of pore volume injected into purified biosurfactant solution.

adopting these parameters, it is possible to compare results obtained in reservoirs of different dimensions.

For the six studied situations, a stabilization of the oil production occurred after the injection of approximately six pore volumes of saline solution. It indicates that there is no longer oil production in the conventional recovery process, requiring the adoption of a tertiary recovery method. For all the considered situations, the recovery factor after the saline injection did not exceed 40%, as shown in

Table 2. This result is in accordance with what happens in oil fields, where 50 to 60% of the oil remains trapped in the reservoir after secondary recovery (Al-Wahaibi et al., 2016; Geetha, Banat & Joshi, 2018; Rudyk et al., 2017).

In all the cases studied, the largest oil removal during tertiary recovery occurred after the injection of four pore volumes. This increase in the oil production was related to the reduction of interfacial tension between water and oil due to the increase in biosurfactant molecules present in

Table 2. Summary of Recovery Factors for MEOR trials.

Rhamnolipid Solution	Petroleum	Conventional Recovery Factor	Enhanced Recovery Factor	Total Recovery Factor
Non-purified	21.90 °API	38.08±1.12%	7.67±0.77%	45.75±1.19%
	27.67 °API	37.20±1.17%	10.24±0.85%	47.45±1.78%
	30.34 °API	38.42±0.70%	5.13±0.59%	43.55±0.11%
Purified	21.90 °API	36.62±0.95%	6.89±0.33%	43.51±1.28%
	27.67 °API	31.39±1.07%	5.17±0.68%	36.56±1.01%
	30.34 °API	39.95±1.18%	3.40±0.28%	43.35±1.18%

the system. These molecules are distributed between the interface and allows the trapped oil to be extracted (Khajepour et al., 2014; Kumar & Mandal, 2017).

In general, the results presented values consistent with those obtained in previous studies. In tests performed with the rhamnolipid produced by *P. aeruginosa* SP4, a total recovery factor of 57.01±0.58% was obtained (Pornsunthorntawe et al., 2008). For the biosurfactant produced by *Enterobacter cloacae* strain, it was possible to obtain a total recovery factor of 50.5%, of which a value of 4.4% was obtained after the injection of the microbial fermentation product (Khajepour et al., 2014). The highest total recovery factor of previous studies can be attributed to the experimental methodology applied, since the study carried out with *P. aeruginosa* SP4 used a compacted sand column system and the one performed with the *Enterobacter cloacae* used a glass microstructure system. By using synthetic sandstone plugs, this study approached the conditions in studied oil reservoirs (Costa et al., 2017), both referring to the rock (Bryant et al., 1994) and to permeability (Safdel et al., 2017).

In view of the results, one can observe that the oil with the highest API grade obtained a greater factor of conventional recovery. This fact is justified by the distinct mobility between water and more viscous oils (lower API gravity), which favors the formation of preferential paths (Thomas, 2001). In addition, oil solutions with higher API gravity favor the formation of asphaltene aggregates (Mozaffari et al., 2015). They are adsorbed at the water-oil interface causing different interface behaviors and making oil recovery difficult (Liu et al., 2017; Soleymanzadeh et al., 2018).

As an exception, the recovery factor of the conventional oil of API gravity 27.67 was the smallest among all oil samples used during the experiment with the purified biosurfactant, reaching a value of approximately 31.39%. It is related to the permeability of the three used sandstones, which was an average value of 33 mD, considerably lower than the general average of 36 mD. This parameter exerts a great influence on the amount of oil extracted and has to be considered during the evaluation of the results. The reason is because the permeability is a crucial variable to evaluate the performance of the oil recovery, since it measures the ability of the fluid to flow through a porous system (Anderson, 1986; Craig Jr., 1993; Khajepour et al., 2014). Besides that, the pore morphology determines the limit of the capillary pressure required for the oil mobilization (Armstrong, Wildenschild, and Bay, 2015).

Nevertheless, the non-purified rhamnolipid obtained better results for all the studied oils, i.e., a higher recovery factor of the enhanced stage. The largest difference was obtained for the oil with API gravity of 27.67 which, using the non-purified biosurfactant, obtained an enhanced recovery rate approximately 98% higher than the purified biosurfactant. For the heavier oil this difference was 12% and for the lighter oil it was 50%. Although both were used at a concentration of 50% above CMC, this result can be justified by the presence of additional compounds in the non-purified extract, such as other metabolites produced by the bacteria and the non-metabolized substrate. These substances can act synergistically with the biosurfactant promoting greater mobilization of petroleum (Fernandes et al., 2016).

Thus, the results achieved in this study indicate that downstream purification processes may not be necessary. These processes are responsible for a significant portion of the of large-scale production costs of biosurfactant. Although, despite the differences presented, both biosurfactants studied proved to be effective in increasing the recovered oil factor after conventional recovery. They showed significant results when compared to other biosurfactants and synthetic surfactants. This can be attributed to the presence of two carbon chains in their chemical structure, which causes a greater hydrophobic nature of the compound and results in good oil solubilization and recovery (Pornsunthorntawee et al., 2008).

4. CONCLUSIONS

In this study, the biosurfactant produced by a *Pseudomonas aeruginosa* strain after undergoing a downstream purification process was able to reduce the water surface tension to 40.79 mN/m and to emulsify the oil at a rate of 65%. These characteristics demonstrate the ability to use purified rhamnolipid in enhanced oil recovery processes.

However, the cell free extract (non-purified biosurfactant) also presented evidences to be a good candidate for application in MEOR. It was able to reduce the water surface tension to 35.26 mN/m and to emulsify the crude oil at a rate of 69%.

In view of this, both purified and non-purified biosurfactants were evaluated for their ability to recover oil. In all the developed trials, for the three types of oil studied, the non-purified biosurfactant increased the recovered oil factor more efficiently after the conventional recovery. The best result obtained was for the oil with API gravity of 27.97, reaching a Total Recovery Factor of $47.45 \pm 1.78\%$, of which $10.24 \pm 0.85\%$ corresponds to the improved recovery by microorganism.

Therefore, this work demonstrates that rhamnolipids can be used efficiently in MEOR applications. In addition, it emphasizes that the application of high investment purification processes may not be required, since the presence of additional compounds into the extract can act synergistically promoting a greater oil removal.

ACKNOWLEDGMENTS

The authors thank *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) for the financial support.

NOMENCLATURE

A - surface area (m^2)
 C - surfactant solution concentration (mol/L)
 Na - Avogadro's number
 R - universal gas constant ($\text{kJ/mol}\cdot\text{K}$)
 ST - surface tension (N/m)
 T - temperature (K)
 Γ - adsorption per unit area of surface excess concentration (mol/m^2)
 γ - surface tension at equilibrium (mN/m)

5. REFERENCES

- Al-Amodi, A. O.; Al-Amodi, A. O.; Al-Mubaiyedh, U. A. Sultan, A. S.; Kamal, M. S.; Hussein, I. A. Novel fluorinated surfactants for enhanced oil recovery in carbonate reservoirs. **Canadian Journal of Chemical Engineering**, v. 94(3), p. 454–460, 2016. <https://doi.org/10.1002/cjce.22406>
- Al-Bahry, S. N.; Al-Wahaibi, Y. M.; Elshafie, A. E.; Al-Bemani, A. S.; Joshi, S. J.; Al-Makhmari, H. S.; Al-Sulaimani, H. S. Biosurfactant production by *Bacillus subtilis* B20 using date molasses and its possible application in enhanced oil recovery. **International Biodeterioration & Biodegradation**, v. 81, p. 141–146, 2013. <https://doi.org/10.1016/j.ibiod.2012.01.006>
- Al-Wahaibi, Y.; Joshi, S.; Al-Bahry, S.; Elshafie, A.; Al-Bemani, A.; Shibulal, B. Biosurfactant production by *Bacillus subtilis* B30 and its application in enhancing oil recovery. **Colloids and Surfaces B: Biointerfaces**, v. 114, p. 324–333, 2014. <https://doi.org/10.1016/j.colsurfb.2013.09.022>
- Al-Wahaibi, Y.; Al-Hadrami, H.; Al-Bahry, S.; Elshafie, A.; Al-Bemani, A.; Joshi, S. Injection of biosurfactant and chemical surfactant following hot water injection to enhance heavy oil recovery. **Petroleum Science**, v. 13(1), p. 100–109, 2016. <https://doi.org/10.1007/s12182-015-0067-0>

Alvarez, V.; Jurelevicius, D.; Marques, J. *Bacillus amyloliquefaciens* TSBSO 3.8, a biosurfactant-producing strain with biotechnological potential for microbial enhanced oil recovery. **Colloids and Surfaces B**, v. 136, p. 14-21, 2015.

<https://doi.org/10.1016/j.colsurfb.2015.08.046>

Amani, H.; Sarrafzadeh, M. H.; Haghighi, M.; Mahrnia, M. R. Comparative study of biosurfactant producing bacteria in MEOR applications. **Journal of Petroleum Science and Engineering**, v. 75(1-2), p. 209-214, 2010.

<https://doi.org/10.1016/j.petrol.2010.11.008>

Amani, H. Study of enhanced oil recovery by rhamnolipids in a homogeneous 2D micromodel. **Journal of Petroleum Science and Engineering**, v. 128, p. 212-219, 2015.

<https://doi.org/10.1016/j.petrol.2015.02.030>

Anderson, W. Wettability Literature Survey-Part 2: Wettability Measurement. **Journal of Petroleum Technology**, v. 38(11), p. 1246-1262, 1986. <https://doi.org/10.2118/13933-PA>

Armstrong, R. T.; Wildenschild, D.; Bay, B. K. The effect of pore morphology on microbial enhanced oil recovery. **Journal of Petroleum Science and Engineering**, v. 130, p. 16-25, 2015.

<https://doi.org/10.1016/j.petrol.2015.03.010>

Babadagli, T. Analysis of oil recovery by spontaneous imbibition of surfactant solution. **Oil and Gas Science and Technology**, v. 60(4), p. 697-710, 2005. <https://doi.org/10.2516/ogst:2005049>

Bryant, R. S.; Stepp, A. K.; Bertus, K. M.; Burchfield, T. E.; Dennis, M. et al. Microbial enhanced waterflooding field tests. **SPE/DOE Improved Oil Recovery Symposium**, 1994.

<https://doi.org/10.2118/27751-MS>

Câmara, J. M. D. A. et al. Application of rhamnolipid biosurfactant produced by *Pseudomonas aeruginosa* in microbial-enhanced oil recovery (MEOR). **Journal of Petroleum Exploration and Production Technology**, 25 fev. 2019. <https://doi.org/10.1007/s13202-019-0633-x>

Castro Dantas, T. N.; Moura, E. F.; Scatena Júnior, H.; Dantas Neto, A. A.; Gurgel, A. Micellization and adsorption thermodynamics of novel ionic surfactants at fluid interfaces. **Colloids and Surfaces A: Physicochemical and Engineering Aspects**, v. 207(1-3), p. 243-252, 2002. [https://doi.org/10.1016/S0927-7757\(02\)00117-6](https://doi.org/10.1016/S0927-7757(02)00117-6)

Cooper, D. G.; Goldenberg, B. G. Surface-Active Agents from Two *Bacillus* Species. **Applied and Environmental Microbiology**, v. 53(2), p. 224-229, 1987.

Costa, S. E. D.; Barros Neto, E. L.; Oliveira, M. C. A.; Santos, J. S. C. Mechanical and Petrophysical Analysis of Synthetic Sandstone for Enhanced Oil Recovery Applications. **Brazilian Journal of Petroleum and Gas**, v. 11(3), p. 131-140, 2017. <https://doi.org/10.5419/bjpg2017-0011>

Craig Jr, F. F. **The Reservoir Engineering Aspects Of Waterflooding**. Henry L. Doherty Memorial Fund of AIME, 1993. 134p.

Curbelo, F. D. S.; Garnica, A. I. C.; Dantas, T. N. C.; Barros Neto, E. L. Oil Recovery by Ionic Surfactant and Sweep Efficiency Study in Sandstones. **Brazilian Journal of Petroleum and Gas**, v. 2(1), p. 9-16, 2008.

Curbelo, F. D. S.; Garnica, A. I. C.; Neto, E. L. B. Enhanced oil recovery and adsorption of ionic surfactant. **Petroleum Science and Technology**, v. 31(7), p. 663-671, 2013.

<https://doi.org/10.1080/10916466.2010.523750>

Deepika, K. V.; Kalam, S.; Sridhar, P. R.; Podile, A. R.; Bramhachari, P. V. Optimization of rhamnolipid biosurfactant production by mangrove sediment bacterium *Pseudomonas aeruginosa* KVD-HR42 using response surface methodology. **Biocatalysis and Agricultural Biotechnology**, v. 5, p. 38-47, 2016.

<https://doi.org/10.1016/j.bcab.2015.11.006>

Desai, J. D.; Banat, I. M. Microbial production of surfactants and their commercial potential. **Microbiology and Molecular Biology Reviews**, v. 61(1), p. 47-64, 1997.

Eastoe, J.; Dalton, J. Dynamic surface tension and adsorption mechanisms of surfactants at the air-water interface. **Advances in Colloid and Interface Science**, v. 85(2), p. 103-144, 2000. [https://doi.org/10.1016/S0001-8686\(99\)00017-2](https://doi.org/10.1016/S0001-8686(99)00017-2)

El-Sheshtawy, H. S.; Aiad, I.; Osman, M. E.; Abo-Elnasr, A. A.; Kobisy, A. S. Production of biosurfactant from *Bacillus licheniformis* for microbial enhanced oil recovery and inhibition the growth of sulfate reducing bacteria. **Egyptian Journal of Petroleum**, v. 24(2), p. 155-162, 2015. <https://doi.org/10.1016/j.ejpe.2015.05.005>

Fan, Y.; Gao, K.; Chen, J.; Li, W.; Zhang, Y. Low-cost PMMA-based microfluidics for the visualization of enhanced oil recovery. **Oil and Gas Science and Technology**, v. 73, 2018. <https://doi.org/10.2516/ogst/2018026>

Fernandes, P. L.; Rodrigues, E. M.; Paiva, F. R.; Ayupe, B. A. L.; McInerney, M. J.; Tótola, M. R. Biosurfactant, solvents and polymer production by *Bacillus subtilis* RI4914 and their application for enhanced oil recovery. **Fuel**, v. 180, p. 551–557, 2016. <https://doi.org/10.1016/j.fuel.2016.04.080>

Geetha, S. J.; Banat, I. M.; Joshi, S. J. Biosurfactants: Production and potential applications in microbial enhanced oil recovery (MEOR). **Biocatalysis and Agricultural Biotechnology**, v. 14, p. 23–32, 2018. <https://doi.org/10.1016/j.bcab.2018.01.010>

Gurkov, T. D.; Dimitrova, D. T.; Marinova, K. G.; Bilke-Crause, C. Gerber, C.; Ivanov, I. B. Ionic surfactants on fluid interfaces: Determination of the adsorption; Role of the salt and the type of the hydrophobic phase. **Colloids and Surfaces A: Physicochemical and Engineering Aspects**, v. 261(1–3), p. 29–38, 2005. <https://doi.org/10.1016/j.colsurfa.2004.11.040>

Hassan, M.; Essam, T.; Yassin, A. S.; Salama, A. Optimization of rhamnolipid production by biodegrading bacterial isolates using Plackett–Burman design. **International Journal of Biological Macromolecules**, v. 82, p. 573–579, 2016. <https://doi.org/10.1016/j.ijbiomac.2015.09.057>

Healy, M. G.; Devine, C. M.; Murphy, R. Microbial production of biosurfactants. **Resources, Conservation and Recycling**, v. 18(1-4), p. 41–57, 1996. [https://doi.org/10.1016/S0921-3449\(96\)01167-6](https://doi.org/10.1016/S0921-3449(96)01167-6)

Jönsson, B.; Kronberg, B.; Lindman, B. **Surfactants and polymers in aqueous solution**. Second ed. England: John Wiley & Sons, 2003.

Joshi, S.; Bharucha, C.; Jha, S.; Yadav, S.; Nerurkar, A.; Desai, A. J. Biosurfactant production using molasses and whey under thermophilic conditions. **Bioresource Technology**, v. 99(1), p. 195–199, 2008. <https://doi.org/10.1016/j.biortech.2006.12.010>

Khajepour, H.; Mahmoodi, M.; Biria, D.; Ayatollahi, S. Investigation of wettability alteration through relative permeability measurement during MEOR process: A micromodel study. **Journal of Petroleum Science and Engineering**, v. 120, p. 10–17, 2014. <https://doi.org/10.1016/j.petrol.2014.05.022>

Kumar, A.; Mandal, A. Synthesis and physiochemical characterization of zwitterionic surfactant for application in enhanced oil recovery. **Journal of Molecular Liquids**, v. 243, p. 61–71, 2017. <https://doi.org/10.1016/j.molliq.2017.08.032>

Li, Y.; He, X.; Cao, X.; Zhao, G.; Tian, X.; Cui, X. Molecular behavior and synergistic effects between sodium dodecylbenzene sulfonate and Triton X-100 at oil/water interface. **Journal of Colloid and Interface Science**, v. 307(1), p. 215–220, 2007. <https://doi.org/10.1016/j.jcis.2006.11.026>

Liu, F.; Darjani, S.; Akhmetkhanova, N.; Maldarelli, C.; Banerjee, S.; Pauchard, V. Mixture Effect on the Dilatation Rheology of Asphaltenes-Laden Interfaces. **Langmuir**, v. 33(8), p. 1927–1942, 2017. <https://doi.org/10.1021/acs.langmuir.6b03958>

Makkar, R. S.; Cameotra, S. S.; Banat, I. M. Advances in utilization of renewable substrates for biosurfactant production. **AMB Express**, v. 1(1), p. 1–19, 2011. <https://doi.org/10.1186/2191-0855-1-5>

Malik, I. A.; Al-Mubaiyedh, U. A.; Sultan, A. S.; Kamal, M. S.; Hussein, I. A. Rheological and thermal properties of novel surfactant-polymer systems for EOR applications. **Canadian Journal of Chemical Engineering**, v. 94(9), p. 1693–1699, 2016. <https://doi.org/10.1002/cjce.22541>

McInerney, M.; Nagle, D.; Knapp, R. **Microbially Enhanced Oil Recovery: Past, Present, and Future**, p 215-238, 2005. In: Ollivier, B.; Magot, M. (ed) *Petroleum Microbiology*. ASM Press, Washington, DC. <https://doi.org/10.1128/9781555817589.ch11>

Miguez, M.; Nunes, P.; Azeredo, N.; Pedraza, S. F.; Vasconcelos, M.; Viana, O.; Coelho, M. A.; Amaral, P. Selection of yeasts for the production of L-phenyl acetyl carbinol by biotransformation in shake flasks. **Chemical Engineering Transactions**, v. 27, p. 163–168, 2012.

Mozaffari, S.; Tchoukov, P.; Atias, J.; Czarnecki, J.; Nazemifard, N. Effect of Asphaltene Aggregation on Rheological Properties of Diluted Athabasca Bitumen. **Energy and Fuels**, v. 29(9), p. 5595–5599, 2015. <https://doi.org/10.1021/acs.energyfuels.5b00918>

Mulligan, C. N. Environmental applications for biosurfactants. **Environmental Pollution**, v. 133(2), p. 183–198, 2005. <https://doi.org/10.1016/j.envpol.2004.06.009>

Pereira, J. F. B.; Gudiña, E. J.; Costa, R.; Vitorino, R.; Teixeira, J. A.; Coutinho, J. A. P.; Rodrigues, L. R. Optimization and characterization of biosurfactant production by *Bacillus subtilis* isolates towards microbial enhanced oil recovery applications. **Fuel**, v. 111, p. 259–268, 2013. <https://doi.org/10.1016/j.fuel.2013.04.040>

Pornsunthorntawee, O.; Arttaweeporn, N.; Paisanjit, S.; Sombonthanate, P.; Abe, M.; Rujiravanit, R.; Chavadej, S. Isolation and comparison of biosurfactants produced by *Bacillus subtilis* PT2 and *Pseudomonas aeruginosa* SP4 for microbial surfactant-enhanced oil recovery. **Biochemical Engineering Journal**, v. 42(2), p. 172–179, 2008. <https://doi.org/10.1016/j.bej.2008.06.016>

Rudyk, S.; Spirov, P.; Samuel, P.; Joshi, S. J. Vaporization of Crude Oil by Supercritical CO₂ at Different Temperatures and Pressures: Example from Gorm Field in the Danish North Sea. **Energy and Fuels**, v. 31(6), p. 6274–6283, 2017. <https://doi.org/10.1021/acs.energyfuels.7b00313>

Safdel, M.; Anbaz, M. A.; Daryasafar, A.; Jamialahmadi, M. Microbial enhanced oil recovery, a critical review on worldwide implemented field trials in different countries. **Renewable and Sustainable Energy Reviews**, v. 74, p. 159–172, 2017. <https://doi.org/10.1016/j.rser.2017.02.045>

Santos, F. K. G.; Barros Neto, E. L.; Moura, M. C. P. A.; Castro Dantas, T. N.; Dantas Neto, A. A. Molecular behavior of ionic and nonionic surfactants in saline medium. **Colloids and Surfaces A: Physicochemical and Engineering Aspects**, v. 333(1–3), p. 156–162, 2009. <https://doi.org/10.1016/j.colsurfa.2008.09.040>

Soleymanzadeh, A.; Yousefi, M.; Kord, S.; Mohammadzadeh, O. A review on methods of determining onset of asphaltene precipitation. **Journal of Petroleum Exploration and Production Technology**, p. 1–22, 2018. <https://doi.org/10.1007/s13202-018-0533-5>

Song, B.; Springer, J. Determination of Interfacial Tension from the Profile of a Pendant Drop using Computer-Aided Image Processing-Theoretical. **Journal of Colloid and Interface Science**, v. 184, p. 64–76, 1996. <https://doi.org/10.1006/jcis.1996.0597>

Sousa, J. R.; Correia, J. A. C.; Melo, V. M. M.; Gonçalves, L. R. B.; Cruz, A. J. G. Cinética e caracterização de ramnolípídeos produzidos por *Pseudomonas aeruginosa* MSIC02 utilizando glicerol como fonte de carbono. **Química Nova**, v. 37(3), p. 431–441, 2014. <https://doi.org/10.5935/0100-4042.20140064>

Thomas, J. E. (ORG. . **Fundamentos da engenharia de petróleo**. Rio de Janeiro: Editora Interciência, 2001.

Yeh, M. S.; Wei, Y. H.; Chang, J. S. Enhanced production of surfactin from *Bacillus subtilis* by addition of solid carriers. **Biotechnology Progress**, v. 21(4), p. 1329–1334, 2005. <https://doi.org/10.1021/bp050040c>

Youssef, N.; Randall Simpson, D.; McInerney, M. J.; Duncan, K. E. In-situ lipopeptide biosurfactant production by *Bacillus* strains correlates with improved oil recovery in two oil wells approaching their economic limit of production. **International Biodeterioration and Biodegradation**, v. 81, p. 127–132, 2013. <https://doi.org/10.1016/j.ibiod.2012.05.010>

Zahid, S.; Sajid, H. L. U. R. A Review on Microbial Enhanced Oil Recovery With Special Reference to Marginal/Uneconomical Reserves. **Society of Petroleum Engineers**, Oklahoma City, 31 March–3 April 2007. Production and Operations Symposium. <https://doi.org/10.2118/107052-MS>

Zendehboudi, S.; Ahmadi, M. A.; Rajabzadeh, A. R.; Mahinpey, N.; Chatzis, I. Experimental study on adsorption of a new surfactant onto carbonate reservoir samples-application to EOR. **Canadian Journal of Chemical Engineering**, v. 91(8), p. 1439–1449, 2013. <https://doi.org/10.1002/cjce.21806>

Zhao, F.; Zhou, J.-D.; Ma, F.; Shi, R.-J.; Han, S.-Q.; Zhang, J.; Zhang, Y. Simultaneous inhibition of sulfate-reducing bacteria, removal of H₂S and production of rhamnolipid by recombinant *Pseudomonas stutzeri* Rhl: Applications for microbial enhanced oil recovery. **Bioresource Technology**, v. 207, p. 24–30, 2016. <https://doi.org/10.1016/j.biortech.2016.01.126>