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Study of enhanced oil recovery by rhamnolipids in a homogeneous 2D micromodel

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ABSTRACT

In this study, homogeneous glass micromodel is used for investigation of oil recovery by rhamnolipid. The intention of this study was to investigate whether the rhamnolipid mixture could be produced in commercial quantities for enhanced oil recovery (EOR) projects in bioreactor and prove of its potential use as an effective material for field application. In this work, the ability of *Pseudomonas aeruginosa* HATH to grow and produce rhamnolipid on sunflower as a sole carbon source under nitrogen limitation was shown. The production of Rha-C_{10}-C_{10} and Rha_2-C_{10}-C_{10} was confirmed by thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC) analysis. The yield of rhamnolipid per biomass ($Y_{RL/x}$), rhamnolipid per sunflower oil ($Y_{RL/s}$), and the biomass per sunflower oil ($Y_{x/s}$) for bioreactor were obtained about 0.54 g g^{-1}, 0.059 g g^{-1}, and 0.11 g g^{-1}, respectively. The rhamnolipid mixture obtained was able to reduce the surface and interfacial tension of water to 26 and 2 mN/m, respectively. Produced rhamnolipid is an effective surfactant at very low concentrations over a wide range of temperatures, pHs and salt concentrations and also has the ability to emulsify oil, which is essential for enhanced oil recovery. With a critical micelle concentration, 5% of original oil in place was recovered after water flooding from a micromodel. This result suggests rhamnolipids as appropriate model biosurfactants for microbial enhanced oil recovery.

1. Introduction

Microbial enhanced oil recovery (MEOR) is the use of microbes in petroleum reservoirs to enhance the amount of oil that can be produced (Bordoloi and Konwar, 2008; Sen, 2008; Amani et al., 2010a,b; Al-Bahry et al., 2013; Youssef et al., 2013). The microbes use hydrocarbons as a food for their metabolic processes and excrete natural and non-toxic bio-products such as biosurfactants and biopolymers. Some of the advantages of biosurfactants over conventional surfactants include lower toxicity, high biodegradability, tolerance to at extreme temperatures, pH and salinity and their potential applications in environmental protection and management (Joshi et al., 2008; Amani et al., 2010a,b; Chrzanowski et al., 2012; Ismail et al., 2012; Ławniczak et al., 2013; Youssef et al., 2013). Rhamnolipids are among the best known biosurfactants and have been proven to be very promising in enhanced oil recovery (Banat, 1995; Wang et al., 2007; Sen, 2008; Amani et al., 2010a,b; Hörmann et al., 2010; Müller et al., 2010; Youssef et al., 2013). The rhamnolipids can change the physical and chemical properties of the crude oil and stimulate oil–water–rock interactions that improve oil recovery. They are the most effective biosurfactants with the ability to reduce the water surface tension from 72 to 30 mN/m as well as interfacial tension reduction in water/oil system from 43 to below 1 mN/m at trace concentrations (Amani et al., 2010a,b; Hörmann et al., 2010). The following positive effects in a reservoir have been documented: (i) biosurfactants reduce the interfacial tensions between oil/rock and oil/water, improving oil flow, (ii) if reservoir is oil wet, biosurfactants increase the wettability of the rock toward water, causing the rock to be preferentially wet by water, dislodging the oil, again helping it flow more freely, and (iii) biosurfactants reduce the interfacial tension between water and oil, and therefore a lower hydrostatic pressure is required to move the liquid entrapped in the pores to overcome the capillary effect. Rhamnolipid always consists of one or two units of rhamnose linked to one or two fatty acid chains with C8–C14 carbon atoms, which may or may not be saturated (Hörmann et al., 2010; Müller et al., 2010). Four different rhamnolipid homologs, produced by *Pseudomonas aeruginosa*, have been identified and characterized (Hörmann et al., 2010; Müller et al., 2010).

The focus of the proposed study is production of rhamnolipids, purification and identification of rhamnolipid, and a visual study of enhanced oil recovery by rhamnolipid flooding in a homogeneous 2D micromodel.

In recent years, micromodels have been served as an excellent laboratory instrument to investigate and understand the mechanisms of biosurfactant flooding in removing the residual oil. Micromodels provide the opportunity to observe fluid flow within reservoirs

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(Stewart and Kim, 2004; Soudmand-asali et al., 2007; Sayegh and Fisher, 2009). Several investigations have been carried out on the *in situ* bacteria flooding but the *ex situ* rhamnolipid production and addition to the micromodel as agents for MEOR has little been studied. In order to develop a suitable technology for *ex situ* MEOR processes, it is essential to carry out tests about *ex situ* enhanced oil recovery by rhamnolipids. Therefore this work tries to fill the gap. Another intention of this study was to investigate whether the rhamnolipid mixture could be produced in commercial quantities for EOR processes in the bioreactor and to prove its potential use as an effective material for reservoirs in Iran.

2. Materials and methods

2.1. Microorganism

The biosurfactant producing strains were isolated from petroleum contaminated garage site using oil spreading method as described by Youssef et al. (2007). The isolation and screening of the biosurfactant producing bacteria were performed in the Tehran University Institute of Biotechnology. Those isolates which showed bigger zone of clearance on oil layer and reduced surface tension (ST) below 30 mN/m were selected for further experiments. One strain (HATH) was identified as *P. aeruginosa* according to its 16SrRNA sequences by Pasteur Institute (Paris, France). The strain was kept in laboratory of Industrial Microbiology at Tehran University (Tehran, Iran) and nominated as *P. aeruginosa* HATA.

2.2. Media and culture conditions

Lysogeny broth (LB) was used for pre-cultivation step one. For the culture, a nitrogen-limited production medium consisting of 250 g/L sunflower oil (Bellasan, Aldi Süd, Rastatt, Germany) and a Ca-free mineral salt solution with 1.5 g/L $NaNO_3$, 0.05 g/L $MgSO_4 \times 7H_2O$, 0.1 g/L KCl, containing a 0.1 M sodium phosphate buffer at pH 6.5 was used. A total of 1 mL/L of trace element solution was added. The trace element solution contained 2.0 g/L sodium citrate $\times 2H_2O$, 0.28 g/L $FeCl_3 \times 6H_2O$, 1.4 g/L $ZnSO_4 \times 7H_2O$, 1.2 g/L $CoCl_2 \times 6H_2O$, 1.2 g/L $CuSO_4 \times 5H_2O$, and 0.8 g/L $MnSO_4 \times H_2O$. Trace elements were filter-sterilized through a 0.22 μm membrane filter (Carl Roth GmbH, Karlsruhe, Germany). Production medium was adjusted to pH 6.5 with HCl (6 N) and NaOH (1 N). Mineral salt solutions, phosphate sources, and sunflower oil were autoclaved separately for all experiments (Müller et al., 2010). All shake flask cultures were incubated in a shake incubator chamber (Multitron II, HT Infors, Bottmingen, Switzerland). First 25 mL of LB in a 100-mL baffled shake flask was inoculated with a total volume of 100 μL from the glycerol stock solution of *P. aeruginosa* HATH and, incubated for 24 h at 37 °C and 120 rpm. The second pre-cultures also containing 25 mL LB were cultivated in a 100-mL baffled shake flask and inoculated using a total of 0.5 mL from the 24-h LB culture. This culture was incubated for 24 h at 37 °C, 120 rpm. The production was started after inoculation with the second LB pre-culture resulting in OD_{580} of 0.05 in the aqueous phase at the start of the bioreactor cultivation.

2.3. Bioreactor experiment

Fermentation was carried out in an integrated stirred tank bioreactor with accessories and automatic systems for dissolved oxygen (DO), pH, impeller speed, aeration rate, and temperature (Infors AG, Headofice, Switzerland). The reactor used was a 2.5-L batch stirred bioreactor with three baffle plates provided to reduce vertex effects and enhance the mixing. Two Rushton impellers attached to the same shaft were used for agitation. Air was sparged through a pipe sparger placed below the bottom impeller. A foam collector was connected to the top of the bioreactor to recover the biosurfactant by withdrawing the foam produced during the process. Stirrer speed was set fix at 600 rpm, temperature at 37 °C and the aeration rates at 2 vvm. For the cultivation, 0.55 L of the production medium was used. The trace element solution was added at cultivation times of 0, 20, 40, 70, and 120 h, as described before (Müller et al., 2010). The production was carried out for 9 days. According to the analysis purposes, sampling was undertaken on a daily basis.

2.4. Analysis of sunflower oil, rhamnolipid and biomass

Culture suspension was mixed vigorously with n-hexane 1:1 (v/v) and centrifuged (4600 g, 4 °C, 30 min) for separation of cells, aqueous and n-hexane phase. The n-hexane phase was used for gravimetric determination of sunflower oil concentrations, after evaporation of n-hexane. We can only analyze the extractable portion of the sunflower oil and at $t=0$ h, it is only the theoretical value of 250 g/L. For rhamnolipd measurement, an aliquot of the aqueous phase was acidified with 85% phosphoric acid 1:100 (v/v) to adjust a pH of about 2–3, leading to precipitation of the rhamnolipids. Rhamnolipids were extracted twice with ethyl acetate 1:1.25 (v/v). A 10 μL of ethyl acetate extracts was used for rhamnolipid identification by TLC. The TLC was performed according to Müller et al. (2010). A rhamnolipid standard was prepared from Jeneil JBR425 (Jeneil Biosurfactants Company, Saukville, United States). Appropriate amounts of ethyl acetate extracts were evaporated and used for rhamnolipid quantification by HPLC (Hörmann et al., 2010; Müller et al., 2010). For biomass measurement, the conventional cell dry weight measurement method was used to determine biomass dry weight. Following the centrifugation of sample and separation of hexane phase and aqueous phase from the biomass, the biomass was washed once in 0.9% NaCl solution (4600 g, 4 °C, 30 min) in distillated water and then it was transferred into a pre-tared vial. The bacterial dry weight was determined after drying at 105° C for 24 h. A complete working scheme for analytics is shown in Fig. 1.

The surface tension (ST), interfacial tension (IFT) and critical micelle concentration measurement (CMC) were measured at 25° C by a digital tensiometer (Kruss, K10ST, Germany) using the ring method. IFT measurements were carried out against crude oil (API=34°, acid number=0.2 mg KOH/g oil, base number=0.05 mg KOH/g oil). The CMC was determined by measuring surface tension at different concentrations of rhamnolipid in distillated water up to a constant value of surface tension saturated (Amani et al., 2010a,b; Hörmann et al., 2010).

2.5. Stability studies

Rhamnolipid produced by *P. aeruginosa* HATH was used for stability studies. Samples of rhamnolipid at CMC concentration (5 cc of the produced rhamnolipid was poured into a test tube containing 5 mL of crude oil (water oil ratio=1)) were checked for stability of surface activity (ST) under different environmental conditions: High temperatures (40, 60, 70, 80, 90 and 100 °C for 60 min and at 120 °C for 20 min), different salt concentrations (NaCl: 0, 1, 2, 4, 6, 8, 10, 15 and 20%, w/v) and a wide range of pH (2, 4, 6, 7, 8, 10 and 12). The pH was adjusted with HCl (6 N) and NaOH (1 N).

2.6. Emulsification and oil spreading studies

Emulsification activity of the rhamnolipid solutions at CMC concentration was determined by measuring the emulsion index (E_{24}) at 25 °C. In brief, 4 ml of crude oil was poured separately into