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Possible Use of Biosurfactant Produced by Microbial Consortium from Contaminated Soil for Microbially Enhanced Oil Recovery

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Despite recent efforts to introduce renewable energy sources to the market, the world still relies heavily on crude oil and petroleum-based products. It is estimated that over two-thirds of the crude in an oil reservoir remains untouched. Primary oil recovery, the process through which simple drilling and pressure differences allows gushing oil to be captured, harvests only 5–10% of the original oil in place. Several enhanced oil recovery processes are currently employed worldwide: thermal, chemical, physical, etc. However, these processes are very expensive as well as environmentally harmful. Thus, the search for alternative, cost-effective, eco-friendly alternatives to the chemical and thermal enhanced oil recovery methods is necessary. MEOR consists of the tertiary recovery of oil in which microorganisms or their metabolic products are used to recover residual oil. Microorganisms produce polymers and biosurfactants, which reduce oil-rock surface tension by diminishing the capillary forces that impede the movement of oil through the pores of rock.
Since long term exposure to petroleum hydrocarbons would be expected to select for the development of biosurfactant-producing bacteria via horizontal gene transfer and metabolic switching, chronically contaminated sites should contain bacteria that produce effective surfactants that can be used by many different petroleum-degrading species that are indigenous to petroleum-dominated habitats. Microbial consortium isolated from creosote tar contaminated soil revealed a significant production of biosurfactant with important emulsification and surface tension reduction activities during growth on sunflower oil (2%, v/v). After, 6 days of growth of the microbial consortium on oil supplemented with mineral salt medium the biosurfactant produced was recovered. The biosurfactant produced by the consortium showed enhanced oil recovery performance compared to distilled water control. The biosurfactant (cell free supernatant) showed an 88% oil recovery from spiked sand in 48 hours compared to 26% with distilled water. The results suggested the potential application of the biosurfactant produced by the microbial consortium for enhanced oil recovery and other related applications in petroleum industry.

1. Introduction

Increasing energy demands due to global population growth and the difficulty in discovering new oil fields as an alternative to the exploited oil fields urged to find out alternative technologies to increase oil recovery from existing oil fields around the world. It is a fact that fossil fuels will remain the key source of energy, regardless of the gross investments in other energy sources such as biofuels, solar energy, and wind energy (Shibulal et al., 2014). During the first stage of oil production, natural reservoir pressure declines and considerable amount of the original oil in place is left behind depending on the characteristics of the rocks and the fluids in place. Water flooding has been used widely to maintain the reservoir pressure, pushing more oil out of the rock toward the producing wells. After all, more than half of the oil is trapped in the reservoir rock after primary oil recovery and secondary (water flooding) process because of pore structure heterogeneities, fluids/ fluids and rock/fluids surface forces. Geological structural and physicochemical characteristics of the carbonate reservoir turn oil extraction into a complex process (Sarafzadeh et al., 2013).
In recent years, a new set of oil recovery methods has been introduced. In a process known as enhanced oil recovery (EOR), chemicals such as surfactants, emulsifiers, polymers, acids, dispersants, and solvents have

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been used in conjunction with the aforementioned secondary recovery techniques in abandoned oil fields to improve crude oil yield, as well as for bioremediation efforts after oil spills (Patel et al., 2015). However, most of the currently used EOR techniques are expensive, not environmentally friendly, and technically hard to perform in undeveloped parts of the world. Considering these facts and limitations, microbial enhanced oil recovery (MEOR) in different modes including in situ and ex situ has recently gained increasing attention by oil companies and research canters (Sarafzadeh et al., 2014).

MEOR technology consists of injecting or stimulating microorganisms present in the reservoir that are able to produce CO_2, CH_4, H_2, acids, solvents and biosurfactants. Gas production increases reservoir pressure and reduces oil viscosity (Youssef et al., 2009). Acids, such as acetic acid, can modify rock permeability and facilitates oil mobility. Furthermore, biosurfactants reduce the superficial and interfacial tension between oil and water and these mechanisms enhance the recovery of oil (Castorena-Cortés et al., 2012).

Biosurfactants, known as one of the most important bioproducts reduce the capillary forces in the reservoir rock by the reduction of oil/water interfacial tension and wettability alteration. Previous studies have shown that several effective mechanisms including interfacial tension reduction, wettability alteration, flow pattern variation, gas production and selective plugging control MEOR oil recovery efficiency (Sarafzadeh et al., 2013). Much attention has been directed towards biosurfactants owing to their different advantages such as lower toxicity, higher biodegradability, better environmental capability, higher foaming, high selectivity, specific activity at extreme temperatures, pH and salinity, and the ability to be synthesized from renewable feed stocks (Chaprão et al., 2015).

In the current study, we investigated the biosurfactant enhanced oil recovery by consortium isolated from wood treatment plant soil (Bezza and Chirwa, 2016)

2 Materials and methods

2.1. Crude oil and sand

The crude oil used in this study is supplied by a coal gasification plant in South Africa. Sand of maximum particle size 18 mm was obtained from local market.

2.2. Microorganisms and preparation of seed cultures

Pseudomonas aeruginosa CB1 and *Bacillus subtilis* CN1 isolated from wood treatment plant soil (Bezza and Chirwa, 2016) were maintained on nutrient agar slants at 4°C. For pre-culture, the strains were transferred to 50 mL of nutrient broth to prepare the seed culture. The cultivation conditions for the seed culture were 28°C, 150 rpm for 24 h of incubation.

2.3. Production of biosurfactant

The biosurfactant was produced in mineral salt medium (MSM) composed of 6.0 g $(NH_4)_2SO_4$; 0.4 g $MgSO_4.7H_2O$; 0.4 g $CaCl_2.2H_2O$; 7.59 g $Na_2HPO_4.2H_2O$; 4.43 g KH_2PO_4; and 2 mL of trace element solution (Trummler et al., 2003). The trace element solution consisted of (in 1 L distilled water: 20.1 g EDTA (disodium salt); 16 g $FeCl_3.6H_2O$; 0.18 g $CoCl_2.6H_2O$; 0.18 g $ZnSO_4.7H_2O$; 0.16 g $CuSO_4.5H_2O$, and 0.10 g $MnSO_4.H_2O$. Two percent sunflower oil was added to the medium as a source of carbon. Two percent aliquots (v/v) of the cell suspension (0.7 optical density at 600 nm), corresponding to an inoculum of 10^7 CFU/mL, were used to inoculate the sterile production medium. Fermentation was carried out in 500 mL Erlenmeyer flasks containing 200 mL MSM at 28°C and 150 rpm for 144 h.

2.4. Crude biosurfactant solution preparation for oil recovery experiments

The crude biosurfactant solution (cell-free supernatant) was obtained by centrifuging the prepared bacterial solution at the 25 °C for 25 min at a rate of 4000 rpm.

2.5. Extraction and Critical Micelle Concentration (CMC) determination of the biosurfactant

The cell free supernatant was extracted with chloroform: methanol (2:1) solvent as previously described by Sharma et al. (2015). The concentration at which micelles begin to form is called the Critical Micelle Concentration (CMC). The critical micelle concentration (CMC) was determined by measuring the surface tensions of dilutions of extracted biosurfactant in distilled water using a DuNouy Tensiometer (KrüssTensiometer, K11 model—Germany) at room temperature as previously described by Rodrigues et al. (2006).

2.6 Application of the biosurfactant in motor oil removal from contaminated sand

Biosurfactant suitability for enhanced oil recovery was carried using 60g of beach sand impregnated with 5 mL of motor oil. Fractions of 20g of the contaminated sand were transferred to 250mL Erlenmeyer flasks, which were submitted to the following treatments: addition of 60mL distilled water (control) and addition of 60 mL of aqueous solution of biosurfactant at 5CMC and at the 10CMC. The samples were incubated on a rotary shaker (150 rpm) for 48 h at 27°C and then were centrifuged at 5000 rpm for 20 minutes for separation of the laundering solution and the sand. The amount of oil residing in the sand after the impact of biosurfactant was gravimetrically determined as the amount of material extracted from the sand by hexane (Nistchke and Pastore, 2002; Luna et al., 2009).

3 Results and Discussion

3.1 Surface tension and critical micellar concentration (CMC) of the biosurfactant

The critical micelle concentration (CMC) is the minimal concentration of surfactant needed to reduce surface tension to the maximal degree after which additional surfactants have no further effect. The CMC is the most frequently used measure for the assessment of surfactant activity (Campos et al., 2013). As shown in Fig. 1, the CMC of the biosurfactant produced by *Pseudomonas aeruginosa CB1 and Bacillus subtilis CN1* was of approximately 180 mg/L and the surface tension at that point was of 31 mN/m. The CMC value is comparable to other potent biosurfactants CMC values produced by *Pseudomonas and Bacillus strains* (Gudiña et al., 2015a and 2015b; Bezza and Chirwa., 2015).

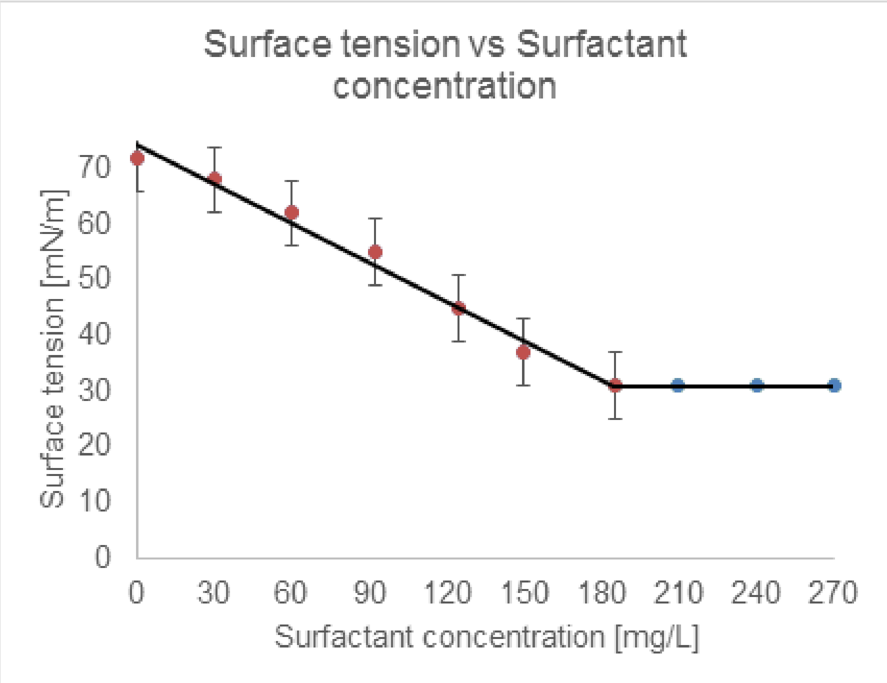

Figure 1 Critical Micelle Concentration (CMC) of the biosurfactant from Pseudomonas aeruginosa CB1 and Bacillus subtilis CN1 grown in mineral medium supplemented with 2%(v/v) sunflower oil for 144 h at 200 rpm and 30 ºC.

3.2 Biosurfactant Enhanced oil recovery

The *Pseudomonas aeruginosa* CB1 and *Bacillus subtilis* CN1 biosurfactant solution at 5CMC concentration (half distilled water diluted cell free supernatant) and 10 CMC concentration (cell free supernatant) recovered 88% and 67% of the oil adsorbed in the sand, respectively, while the distilled water (control) recovered 26% oil (Table 1).

Table 1: Recovery of crude oil adsorbed in sand by different concentrations of biosurfactant from Pseudomonas aeruginosa CB, Bacillus subtilis CN1 and distilled water (control)

Biosurfactant Concentration	Oil Recovery Percentage
Control (Distilled water)	26
5 CMC (Half diluted cell free supernatant)	67
10 CMC (Cell free supernatant)	88

Similar results were obtained by Abu-Ruwaida et al., (1991) for the cell-free broth containing a biosurfactant produced by Rhodococcus cells, which was able to recover 86% of crude residual oil adsorbed in the sand, while distilled water removed about 65% of the oil. Al-Wahaibi et al. (2014) reported that the crude biosurfactant from *Bacillus subtilis* B30 strain enhanced light oil recovery by 17–26% and heavy oil recovery by 31% in core-flood studies. Apart from some EOR activities during in-situ experiments, no oil mobilization with the addition of purified biosurfactants in sandstone cores were observed by Yakimov et al. (1997). The authors reported that this failure of ex-situ EOR might be due to complete adsorption of biosurfactant applied in insufficient amounts to the surfaces of core material. Therefore, loss of their interfacial activity and/or a necessity for co-surfactants, such as short chain alcohols and acids was concluded. This is in line with the finding reported by Fernandes et al. (2016) that other compounds in culture fluids such as 2,3-butanediol act synergistically with the biosurfactant to promote oil mobilization. McInerney et al. (2005) showed that tertiary oil recovery experiments were more effective when 2,3-butanediol was used in presence of biosurfactants. Although it alone did not mobilize oil, when applied as co-surfactant this effect can be related to reduction of IFTs between crude oil and water.

Three main strategies have been developed for use of biosurfactants in MEOR viz. (1) injection of biosurfactant producing microbes along with nutrients into the oil reservoir through the reservoir rocks facilitating multiplication of microorganisms in situ, (2) biostimulation of indigenous biosurfactant producing microorganisms by injection of selected nutrient into oil reservoir to stimulate the microbial growth and (3) injection of ex situ produced biosurfactants into reservoir (Pathak and Keharia, 2014). The mechanism behind biosurfactant-enhanced removal and recovery of oil has been proposed to take place through solubilization, mobilization, or emulsification, increasing the area of contact of hydrocarbons (Santos et al., 2016). Solubilisation capacity measures a surfactant's ability to increase the solubility of hydrophobic components in an aqueous phase. A significant increase in this capacity occurs when micelles are formed as a result of the partitioning of the hydrocarbon in the hydrophobic part of the micelles (De Almeida et al., 2016). All the above strategies increase petroleum yields from a depleted reservoir by decreasing oil-rock surface and interfacial tension and reducing the capillary forces which may impede oil movement through the rock pores. Biosurfactants also enhances the formation of stable water-oil emulsions and the breakdown of the oil film in the rocks which is important for a maximizing oil extraction ultimately extending the reservoir life time (Bachmann et al., 2014; De Almeida et al., 2016).

Real potential of microbial products (biosurfactants) in MEOR applications can only be fully assessed in field-scale applications. The real impact of biosurfactant-based MEOR trials however has rarely been tested at a larger-scale,because of lack of both quantitative information regarding micro-bial growth and metabolism in situ and insufficient data collection and processing (Al-Wahaibi et al., 2014). Even though biosurfactants are green alternatives for chemical counterparts, as it can be produced from renewable sustainable resources. Production economy is still considered as bottleneck for widespread biosurfactants applications, which hinders the field-scale applications. The cost of production can be drastically reduced when agro-industrial waste products (like molasses) or used waste frying oil (as fatty acid supplement) be used as raw material for its production at large scale fermentation (Elshafie et al., 2015). Besides reducing interfacial and surface tensions biosurfactants can enhance oil recovery through altering wettability, aiding in the degrading of long alkyl chains, and cleaning up contaminated soil (Patel et al., 2015).

4 Conclusion

The favorable properties of the biosurfactants make them good candidates for application in microbial enhanced oil recovery process. Because of their general nontoxicity to humans, biodegradability, and the wide applicability of biosurfactant properties, these products have a promising future in the developing green economy. While there are some concerns with the cost of biosurfactants, using effective crude biosurfactant products instead of the expensive pure forms derived for medical purposes is one viable way to make this method economically competitive with current surfactants. MEOR is well-proven technology to enhance oil recovery from oil wells with high water cuts and also to improve it in mature oil wells, but still in order for

MEOR processes to be well accepted and successful, extensive and cost-effective laboratory and field scale tests of biosurfacatnt production and application need to be conducted.

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