

Application of Biosurfactants Produced by *Pseudomonas putida* using Crude Palm Oil (CPO) as Substrate for Crude Oil Recovery using Batch Method

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Abstract. The application of biosurfactants which have been produced by *Pseudomonas putida* in nutrient broth medium supplemented with NaCl and crude palm oil (CPO) for oil recovery has been evaluated. The crude and purified biosurfactants have been examined for oil recovery from a laboratory oil-contaminated sand in agitated flask (batch method). Two synthetic surfactants and water as control was also performed for oil recovery as comparisons. Using batch method, the results showed that removing ability of crude oil from the oil-contaminated sand by purified and crude biosurfactants were 79.40 ± 3.10 and 46.84 ± 2.23 %, respectively. On other hand, the recoveries obtained with the SDS, Triton X-100 and water were 94.33 ± 0.47 , 74.84 ± 7.39 and 34.42 ± 1.21 % respectively.

1. Introduction

Oil recovery comprises a primary phase which uses natural stored energy and a secondary phase which includes injection of water to force oil recovery. The crude oils trapped in wells secondary phase makes up to 67% of the total reserves [1-2]. Various chemical and physical processes are applied for recovery of this residual oil which are can caused serious bad effects on the environment for long term uses. Enhanced Oil Recovery (EOR) is a tertiary oil recovery process to regain unrecoverable oil from an oil reservoir by chemicals flooding. The use of EOR methods have become an increasing important strategy. In particular, synthetic surfactants are used in chemical flooding process of EOR due to their effects on wettability alteration [3]. However, some synthetic surfactants have the potential toxicity risk or hazard to environment and human health [4].

Biosurfactants are surface-active compounds produced by microorganisms which are good candidates to replace the synthetic surfactants in EOR technology due to their low toxicity and biodegradability [5]. They are able to reduce surface and interfacial tension thus forming stable water-oil emulsions which is important for maximum oil extraction. The salt concentration and pH effect the biosurfactants solubility and activity. The effective pHs are pH ranging between 4-10 [6-7]. Many biosurfactants are precipitate at $\text{pH} \leq 4$ because their isoelectric point is near pH 4. The surfactant activities have been reported to increases 0-8% NaCl concentration due to salting-in affects [7-8].

The used of biosurfactants for oil recovery has been reported [9]. *Acinetobacter calcoaceticus* RAG-1 produces emulsan which is an extracellular protein-associated lipopolysaccharide. This biosurfactant has been used in chemical flooding process of EOR. The surfactant present in the slug reduces the interfacial tension between the oil/water and oil/rock to large extent, thereby desorbing the oil and emulsan stabilizes the desorbed oil in water, which is removed with the injection water [10]. The flooding experiment using lipopeptidic biosurfactants produced by *Bacillus mojavensis* (PTCC 1696)



showed 25% oil recovery. These biosurfactants reduce the interfacial tension from 65 to 26.7 mN/m under highly saline conditions [11]. *Rhodococcus* sp strain TA6 produces a mix of extracellular lipids and glycolipids which reduce surface tension of a hydrocarbon-based growth medium from 68 to <30 mN/m. These biosurfactants enhance the residual oil recovery of oil saturated sand packs by up to 70% [12].

On previous work, we reported the production of biosurfactants by *Pseudomonas putida* using crude palm oil (CPO) as substrate. This biosurfactants was identified as rhamnolipids [13]. As one of the world's biggest CPO producer, Indonesia exports 50% of produced CPO in an unprocessed form. Using CPO as a substrate for the production of biosurfactants is one process for diversification products of CPO in order to increase its economic value. In this work, we are reported the application of crude and purified biosurfactants produced by *P. putida* using CPO as substrate for crude oil recovery from a laboratory oil-contaminated sand in agitated flask (batch method). We compare their capacities for crude oil recovery with two synthetic surfactants (Sodium Dodecyl Sulphate and Triton X-100) and water as control.

2. Experimental

2.1. Materials

All chemical were used are analytical grade from e-Merck. The CPO was obtained from Centre for Chemical and Packaging, Jakarta, Indonesia. The crude oil was obtained from PT Pertamina Blok Cepu, Blora Indonesia. The strain used in this work, *P. putida* FNCC 0071, was purchased from IUC Food and Nutrition, Universitas Gadjah Mada, Indonesia.

2.2. Production and Purification of Biosurfactants

The production and purification of the biosurfactants have been carried following the methods of our previous work [13]. Cultures of bacteria were maintained on nutrient agar. The media were used for biosurfactants production composed of nutrient broth (8 g/L), NaCl (5 g/L) and CPO (10 % v/v). Media was sterilized prior used. The flasks containing 125 mL media were inoculated with 3 ml overnight pre-culture of the strain and incubated at room temperature on a reciprocal rotary shaker at 150 rpm.

Culture liquid of *P. putida* was filtered by Buchner filtrations using Whatman filtration paper grade 42. The supernatant was then acidified to pH 2.0 with HCl 6 N and leaved overnight at 4°C. The supernatant was extracted twice with n-hexane for removing of the remaining CPO. The aqueous solution was then extracted twice using ethyl acetate. Sodium sulphate anhydrous was then added to the organic layer. The organic layer which was free form water was then evaporated to obtain the purified biosurfactants.

2.3. Application of Biosurfactants for Oil Recovery using Batch Method

Application of biosurfactants for crude oil recovery using batch method was carried out pursuing the modified procedure reported by Suthar *et al.* (2008) [2]. Sand samples were cleaned and air dried. Sand samples of 100 Mesh particle size (15 g) were washed with NaCl 5% and contaminated with 2 g of crude oil, and then transferred to 250 mL erlenmeyer flasks. The erlenmeyer flask then was added with 30 mL of the biosurfactants and incubated under agitation of 100 rpm at room temperature for 24 h in a rotary shaker. After this period, the solution was separated with the sand. The hexane (15 mL) was added to the sand as the extracting solvent. The evaporation was performed to obtain the residual oil which was then determined by gravimetric analysis. The percentage of crude oil removal was calculated using the equation 1.

$$\text{Crude oil removed (\%)} = (O_i - O_r) / O_i \times 100\% \quad (1)$$

Where O_i is the initial oil in the sand (g) before washing and O_r is the oil remaining in the sand (g) after washing. The SDS (Sodium Dodecyl Sulphate), Triton X-100 and distilled water as a control were also analyzed at the same conditions for comparing the crude oil removal ability of biosurfactants. All experiments have been carried out in triplicate.

3. Results and Discussion

Although chemically synthesised surfactants have been used for enhanced oil recovery and oil spill clean up for over decades, biosurfactants have been studied for a possible replacement of chemical surfactants due to the toxicity and resistance to degradation of the chemical surfactants [14-15]. The contact time is an important parameter affecting efficiency of oil removal, as a sufficient contact time is required for effective oil removal. According to Lai *et al.* (2009), 24 h was sufficient contact time for solubilisation of the hydrocarbon to mobile phase, therefore, in this study, we investigated the oil removal for 24 h [16].

In order to evaluate the use of the crude biosurfactants, the removal ability of the cell-free broth was tested. Table 1 presents the results of the experiments carried out by batch method in erlenmeyer flasks for the removal of crude oil from the oil-contaminated sand by the crude and purified biosurfactants. The biosurfactants capacities for oil recovery were compared with two synthetic surfactants (SDS and Triton X-100) and water as a control. These experiments were performed at the slightly above CMC values of the biosurfactants/surfactants. Removing ability of crude oil from the oil-contaminated sand by purified and crude biosurfactants were 79.40 ± 3.10 and 46.84 ± 2.23 %, respectively. On other hand the recoveries obtained with the SDS and Triton X-100 as chemical surfactants at the same concentration were 94.33 ± 0.47 and 74.84 ± 7.39 %, respectively. Water as a control removed only 34.42 ± 1.21 % of crude oil present in sand.

Table 1. Crude oil removal ability by biosurfactants, SDS and Triton X-100 using batch method.

Samples	Crude Oil Removal (%)
Biosurfactant	79.40 ± 3.10
Crude Biosurfactants	46.84 ± 2.23
SDS	94.33 ± 0.47
Triton X-100	74.84 ± 7.39
Water	34.42 ± 1.21

The obtained results showed that the crude oil removal ability of water was about 34% which is a great economic interest. Therefore, a water washing process can be suggested applied before any other recovery process to diminish the crude oil content and consequently the consumed any surfactant quantity. As comparisons, Khalladi *et al.* (2009) report that water washing of a diesel-polluted soil remove 24% of *n*-alkanes and Chang *et al.* (2000) find that water washing remove 30-80% of PAHs. Our results are in accordance with the reported researches in the ability of positive controls of synthetic surfactants (SDS and Triton X-100) for crude oil removal [17-18]. Chang *et al.* (2000) also observe that 73.6-100% of PAHs were removed in the presence of SDS and Chaprao *et al.* (2015) report that 55-80% of motor oil pollutant was subtracted by Triton X-100 [4, 18].

From the results gained, it can be established that the purified biosurfactants are more effective in oil recovery when compared with the crude biosurfactants. The purified biosurfactants oil removal ability is slightly higher than that of the SDS, however, it is lower than that of the Triton X-100. Biosurfactants begin to form micelle in solution at the CMC value and solubilize petroleum hydrocarbons in soil-water systems at the above CMC. The biosurfactants have crude oil removing ability because they can stimulate the mobilization of oil toward aqueous phase resulting in stable emulsification and micellization, leading its removal [19]. The biosurfactants possessing a combination of surface and emulsification activity have a great ability in oil recovery.

Present studies showed that the ability of crude biosurfactants in removing crude oil were about 46%. This result indicated that the crude biosurfactants has lower ability than that of reported results. The crude biosurfactants from *Candida tropicalis* grown in waste frying oil removed 78-97% of the petroleum and motor oil from sand [20], while the crude biosurfactant from *C. guilliermondii* cultivated in industrial residues extracted about 90% of motor oil from sand [21]. Abu-Ruwaida *et al.* (1991)

reported that the crude biosurfactant produced by *Rhodococcus* sp. removed 86% of crude oil from sand [22].

Our gained results, it can be established that the crude biosurfactants are less effective in oil recovery the when compared with purified biosurfactants. Several studies report differently with our results. Some studies show that the crude biosurfactants have equal ability in removing oil with the purified biosurfactants. Other results report that the crude biosurfactants have better ability removing oil than that of the purified biosurfactants. Chaprao *et al.* (2015) report the ability of biosurfactants produced by yeast *C. sphaerica* and by bacterium *Bacillus* sp. for removing motor oil from soil [4]. The crude biosurfactants and the purified biosurfactants show almost equally effective in the removal of the motor oil pollutant under kinetic conditions (70-90%). Silva *et al.* (2010) report that the crude biosurfactants from *P. aeruginosa* was as effective as the purified biosurfactants in removing about 85% diesel oil from sand [23]. Moreover, the crude biosurfactant from *C. lipolytica* grown in medium containing animal fat and corn steep liquor was more effective in removing motor oil than that of the isolated biosurfactant [24]. Thus, the possible use of the crude biosurfactants without purification steps, which can further reduce the production cost of biosurfactants.

Other method, such as using column, need to be evaluate to for better ability in removing crude oil. Furthermore, other factor which can be affected the oil removal such as the kind of soil also need to be observe, since Silva *et al.* (2014) reported different ability of biosurfactant *P. cepacia* grown in mineral medium supplemented with corn steep liquor and soybean waste frying oil achieved for the removal of motor oil from sand and clay soil. Overall, biosurfactants produced by *P. putida* grown in media containing CPO have good prospect for crude oil removal from sand.

4. Conclusions

Biosurfactants which have been produced by *P. putida* in the nutrient broth medium containing CPO for crude oil recovery has been examined. The crude and purified biosurfactants have been investigated for crude oil recovery from a laboratory oil-contaminated sand using batch method. The purified biosurfactants were more effective than that of the crude biosurfactants in removing crude oil from sand. Furthermore, the purified biosurfactants were able to remove crude oil which were almost equally effective in the oil removal with the commercial available chemical surfactants (SDS and Triton X-100). Thus, the purified biosurfactants produced by *P. putida* could be applied in crude oil recovery by batch method.

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