Screening and Optimization of Carbon Source to Increase High Thermostable Biosurfactant Production for Microbial Enhanced Oil Recovery (MEOR) Application

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Abstract. Petroleum, is by far, one of the world's major energy sources. However, its production continues to decline every year. One of the effort developed to increase crude oil production is biosurfactant addition. This research aimed to optimize carbon source as substrate used to produce bacterial biosurfactant. The first stage of the study was carbon source screening with based on biosurfactant's dry weight, IFT value, and emulsification index parameters. The carbon source candidates were crude oil, glucose, molasses, and palm oil mill effluent (POME). Screening stage of crude oil, glucose, molasses, and POME resulted in (a) biosurfactant yield of 0.58, 3.18, 1.47, and 1.38 g/L; (b) IFT decrease of 6.9, 7.7, 4.6, and 6.1 dyne/cm; and (c) emulsification index of 62.5, 72.5, 47.5, and 58.75% for respective treatments. POME was chosen as the best carbon source in consideration that it was readily available in large quantities and lower cost compared to crude oil, glucose, and molasses. Treatments of 1, 3, 5, and 7% POME treatment yielded biosurfactant in the concentrations of 1.08, 1.28, 0.7, and 0.98% g/L respectively. Treatment of 3% POME was chosen as the best concentration to stimulate biosurfactant production. Biosurfactant produced was subsequently applied to sand-pack column simulation and additional oil recovery (AOR) of 20-40% was obtained. Functional group characterization using FTIR showed that biosurfactant produced in this study belonged to lipopeptide group.

1. Introduction
To date, petroleum has been one of the main energy source in many countries. World oil demand will also increase by 1.1 bph hence it is also predicted that crude oil consumption will increase to 19 bph by 2035 [1]. However, this is not followed by an increase in oil production and supply. According to ESDM ministry's statistics (2010) [2], oil production in 2000-2004 decreased by 100 barrels the four-year period. Whereas from 2004 to 2010, oil production continued to decrease until the production was just 350 million barrels a year. This problem is partly attributed to the lack of effective technology to recover all the remaining oil that is trapped inside reservoir rock's pores.

One of the emerging technology to increase oil production is Microbial Enhanced Oil Recovery (MEOR). MEOR is a method to increase oil production by utilizing microorganism or microbial products. MEOR can be implemented through several mechanisms. One of them is injection of biosurfactants produced by microorganism. Biosurfactants are molecule that is composed of hydrophilic and hydrophobic part such that it can interact with both water and crude oil. This
characteristic can decrease oil's interfacial tension (IFT) and emulsify oil. Hence, Biosurfactants can increase oil recovery while sweeping the oil well with water [3] [4].

MEOR research by SITH has successfully isolated indigenous bacteria from oil well which can produce biosurfactant. Biosurfactant produced was stable at the 25-121°C, pH of 2-10, and 2-10% salinity [5]. Therefore, this biosurfactant compound has the potential to be applied to oil well with extreme condition. However, biosurfactant production is still experiencing many obstacles such as the high operational cost [6]. Therefore, this study aimed to increase microbial biosurfactant production through optimization of low cost carbon source.

2. Materials and methods

2.1. Materials

Biosurfactant was produced by bacteria isolated from one of South Sumatran oil well. SMSS (Stone Mineral Salt Solution) medium was used as the medium to support biosurfactant production. The composition of SMSS medium per liter is NH$_4$NO$_3$ (2.5 g); MgSO$_4$.7H$_2$O (0.5 g); MnCl$_2$.4H$_2$O (0.2 g); CaCO$_3$ (0.5 g); Na$_2$HPO$_4$.7H$_2$O (1 g); and KH$_2$PO$_4$ (0.5 g), supplemented with 0.1% (w/v) NH$_4$NO$_3$. In screening process, 3% (v/v) crude oil, 4% (v/v) glucose, 1% (v/v) molasses, and 3% (v/v) palm oil mill effluent (POME) was added as carbon source.

2.2. Bacterial growth curve

Culture stock was inoculated to 100 ml of nutrient broth (NB) medium and incubated at 50°C for 24 hours. NB inoculum was inoculated into treatment medium at 10% of the volume for adaptation. After adaptation stage, the culture was treated with carbon source for 6 days. Every 12 hours, culture was sampled and its pH and cell count were measured. Cell count was measured using plate count method. The sample for cell count measurement was serial diluted with 0.85% NaCl solution to obtain cell density of 10$^6$-10$^7$ cell/ml. Bacterial cell was grown on nutrient agar (NA) medium using pour plate method and incubated at 50°C for 24 hours.

2.3. Biosurfactant production curve

Biosurfactant production curve was made by plotting biosurfactant dry weight data on treatment duration. Biosurfactant extraction was performed every 24 hours for 6 days. Culture from each treatment was sampled 10 ml, and centrifuged in sterile falcon tube at 7000 rpm for 30 minutes to obtain the supernatant. Supernatant was separated from the pellet, adjusted with concentrated HCl to obtain pH 2, and incubated at 4°C for 12 hours. The supernatant was again centrifuged at 7000 rpm for 30 minutes to obtain crude biosurfactant pellet. The remaining biosurfactant in supernatant was re-extracted with chloroform-methanol solution. Biosurfactant was dried at 50°C and the dry weight was measured.

2.4. Emulsification index measurement

Maximum emulsification index was measured to determine surfactant's ability to emulsify oil. Oil was added to deionized water mixed with crude biosurfactant in 1:1 ratio. The mixture was homogenized using vortex for 2 minutes, and let to settle for 24 hours. The emulsion formed after 24 hours of silence was measured in height [7]. Emulsification index was calculated using the following equation:

$$E_{24} = \left( \frac{H_{\text{emulsion}}}{H_{\text{total}}} \right) \times 100\%$$

(1)

2.5. Interfacial tension measurement

IFT was measured using Du Nuoy tensiometer. This measurement was repeated three times and the value measured was stated in dyne/cm [8].

2.6. Biosurfactant characterization
To characterize the functional groups in biosurfactant, dried biosurfactant obtained from extraction and purification was analyzed using FTIR with infra-red spectrum at 450-4000 cm\(^{-1}\) wavelength [9].

2.7. Sand-pack column simulation
Sand-pack column simulation was performed according to Gudina [10] with some modification. Acrylic tube with ±250 ml volume was filled with quartz sand (30 mesh). The column was closed with a small opening as the inlet and outlet of the flow. The column was then saturated with sand, formation water, and crude oil to obtain pore volume (PV) parameter from the water volume in the column. Then, original oil in place (OOIP) parameter was determined from the oil volume in the column. From this two parameter, another two parameter, Soi (oil initial saturation) and porosity, can be calculated with the following equations [11]:

\[
S_{oi} (%) = \frac{OOIP}{PV} \times 100\%
\]

\[
\text{Porosity} (%) = \frac{PV}{BV} \times 100\% 
\]

\[
BV = \text{column volume}
\]

Then, water was flooded to the column to sweep crude oil. Oil recovered after water flooding is called Sorwf while the remaining oil trapped in the column is called Sor (oil residual saturation) [11]. Sor was calculated with the following equation:

\[
S_{or} (%) = \frac{(OOIP - S_{orwf})}{OOIP} \times 100\%
\]

Next, biosurfactant flooding was performed to recover the remaining residual oil. The volume of oil recovered through this process is called Sorbf (saturation of oil recovered after biosurfactant flooding). The percentage of crude oil recovered by biosurfactant was called AOR (additional oil recovery). AOR volume was calculated as follows:

\[
AOR(\%) = \frac{S_{orbf}}{(OOIP - S_{orwf})} \times 100\%
\]

3. Results and Discussion
Screening stage was conducted using glucose, crude oil, molasses, and POME as carbon sources that can best improve biosurfactant production. The concentration used for each carbon source was equivalent to 4% glucose as standard carbon source. The concentration of 4% for glucose has been demonstrated as the optimum concentration for biosurfactant production [12]. The equivalent concentration for crude oil, molasses, and POME were 3%, 1% and 3%, respectively.

![Figure 1](image)

**Figure 1.** Bacterial growth curve and biosurfactant production curve on SMSS medium added with carbon source variation and 0.1% NH\(_4\)NO\(_3\).
Fig. 1 showed that biosurfactant production peaked at the beginning of bacterial growth. This might be due to the characteristic of biosurfactant which can emulsify oil. Therefore, it was produce in high quantity to aid crude oil uptake as substrate. After 24 hours, biosurfactant production decreased. This might be because bacteria has adapted to utilize crude oil as substrate and biosurfactant was no longer needed to aid uptake.

In glucose treatment, biosurfactant was produced when cell count began to decrease. This might be because biosurfactant production was a form of adaptation in carbon-starvation. Thus, the consumed glucose was not only utilized as energy source, but also for lipid synthesis. Lipid accumulation can lead to biosurfactant production [13].

In molasses treatment, the highest biosurfactant concentration was produced when cell count decreased. This might be because biosurfactant was produced to adapt with catabolite repression. Molasses was composed of several types of sugars i.e. 32% sucrose, 14% glucose, 16% fructose, 1% invert sugar, and 1% raffinose [14]. Utilizing different types of sugar might trigger the occurrence of catabolite repression to produce enzymes.

In POME treatment, biosurfactant was produced at exponential growth phase. This might be due to the use of lipid as carbon source in exponential phase. POME is composed of water, carbohydrate, crude lipid, crude protein, nitrogen free extract, and ash [15].

In addition to biosurfactant dry weight, IFT decrease and emulsification index were also measured to investigate biosurfactant activities. Fig. 2 showed that glucose had the highest value of dry weight (3.18 g/l), IFT decrease (7.7 dyne/cm), as well as emulsification index (72.40%). Crude oil treatment yielded the second highest result for emulsification index and IFT decrease. However, the resulting dry weight was the lowest (0.58%). The third best carbon source was POME, and the fourth was molasses.

![Figure 2](image-url)

**Figure 2.** Biosurfactant characteristic parameters for carbon source screening on SMSS medium added with varied carbon source and 0.1% NH₄NO₃.

In screening stage, glucose and crude oil was used as comparison as the commonly used carbon source for biosurfactant production. Fig 2 showed that POME treatment resulted in higher parameter values compared with molasses. In addition, the rate of biosurfactant production after 48 hours of treatment in POME treatment (0.036 g/hours) was also higher than molasses (0.033 g/hours). As a comparison, biosurfactant production rate for crude oil and glucose treatment were 0.015 and 0.075 g/hours respectively. Therefore, from this screening, POME was chosen as the most optimal carbon source for biosurfactant production and will be used for further carbon source concentration optimization stage.
Figure 3. Indigenous bacteria growth curve and biosurfactant production curve on SMSS medium added with 0.1% NH$_4$NO and 1% (a), 3% (b), 5% (c), and 7% (d) POME.

POME concentration of 1, 3, 5, and 7% were chosen as optimization variation. These variations were chosen because preliminary study showed that 8% POME inhibited bacterial growth. Fig. 3a and 3b showed that the highest concentration of biosurfactant produced in 1% and 3% POME treatment was obtained in exponential phase. The highest biosurfactant yield for 1% POME treatment was 1.01 g/l with the highest production rate of 0.036 g/hours at 24 hours of incubation. At 3% POME treatment, the highest biosurfactant yield was 1.38 g/l with production rate of 0.036 g/hours at the 48 hour incubation. High biosurfactant production during exponential phase might be due to need for high concentration of carbon for cell growth. Carbon source in POME consists of lipid (oil and grease), lignin, cellulose, and a small number of reducing sugar [16]. Reducing sugar present in solution might not be sufficient to meet the carbon requirement while utilizing lignin and cellulose require the production of particular enzymes. Therefore, bacteria might also utilize lipid as carbon source as nutrient to support growth. This lipid utilization could initiate biosurfactant production because biosurfactant aids the uptake of hydrophobic nutrition.

At 5% POME treatment, biosurfactant production tended to occur in stationary phase. Fig. 3c showed that the highest biosurfactant yield (0.86 g/l) was obtained at 96 hours incubation at with production rate of 0.023 g/hours. Biosurfactant production which occurred in stationary phase in this treatment might be due to higher concentration of reducing sugar content in 5% POME, compared with 1% and 3% POME. Therefore, bacterial carbon demand to support growth and prepare for enzymatic digestion of lignin and cellulose might have been fulfilled. After glucose, lignin and cellulose has been used up in stationary phase, lipid was finally used as carbon source, which initiated biosurfactant production.

At 7% POME treatment, biosurfactant was produced at exponential phase. Fig. 3d showed that the highest biosurfactant yield (0.98 g/l) was obtained after 48 hours of incubation with the highest
production rate of 0.018 g/hours. This could be caused by the decrease in bacterial cell count due to catabolite repression. POME is a complex substrate which contains various compounds of carbohydrate. Aside from reducing sugars, POME also contains other complex carbon source such as lignin, cellulose, and hemicellulose [16]. In this condition, biosurfactant was produced to support bacterial growth in the process of re-adaptation.

Infrared spectrum result showed detected the presence of medium stretching aliphatic primary amine and strong broad stretching amine salt (N-H bond), strong broad stretching carboxylic acid (O-H bond), medium bending alkane (C-H bond), medium stretching alkene (C=O bond) and medium bending amine (N-H). In finger-print region, strong stretching primary and secondary alcohol (C-O bond) were also detected. These functional groups showed a similarity with groups found in amino acid and lipid, indicating that biosurfactant in this study belong to lipopeptide family [17].

Simulation was carried out in two duplication. Pore volume (PV) formed in the first and second simulation were 62 and 63.5 ml respectively. Difference in pore volume will cause initial differences in the percentage of water (Swi) and oil (Soi) in the column. Water flooding was performed to recover oil in column (Sorwf) which yielded 18.3 ml oil in simulation I and 13.8 ml in simulation II. The percentage of remaining oil in column (Sor) was 40% in simulation I and 55.48% in simulation II. Then, biosurfactant flooding was performed to sweep the remaining residual oil (Sorbf) which yielded 4.7 ml in simulation I and 3.8 ml in simulation II. AOR value was calculated from the obtained parameters.

Table 1. Parameters calculated from sand pack column simulation results.

| Parameter | Simulation I | Simulation II |
|-----------|--------------|---------------|
| PV (ml)   | 62.00        | 63.50         |
| OOIP (ml) | 30.50        | 31.00         |
| Porosity (%) | 24.80        | 22.40         |
| Soi (%)   | 49.19        | 48.10         |
| Swi (%)   | 50.80        | 51.18         |
| Sorwf (ml) | 18.30        | 13.80         |
| Sor (%)   | 40.00        | 55.48         |
| Sorbf (ml) | 4.70         | 3.80          |
| AOR (%)   | 38.52        | 22.09         |

Table 1 showed that AOR in simulation I (38.52%) was higher than in simulation II (22.09%). This might be due to the difference in pore volume formed by sand grains. Thus, the data could not be calculated as duplication. AOR value obtained from this sand-pack simulation was stated as a range of 20-40%. This range was close to average AOR obtained from similar study using other species of indigenous bacteria from oil well. Biosurfactant from B. subtilis 20B produced 30.22% AOR [18] and B. mojavensis JF2 produced 29.4% AOR [19].

4. Conclusions

The screening of thermostable biosurfactant found that 3% (v/v) of palm oil mill effluent (POME) was the optimum carbon source for biosurfactant production by indigenous bacteria from oil well. Carbon source and optimization result showed that 3% POME produced the highest biosurfactant dry weight after 48 hours of incubation e.g. 1.38 g/l. IFT decrease and emulsification index resulted from this biosurfactant activity were 6.1 dyne/cm and 58.75% respectively. Simulation on sand pack columns yielded additional oil recovery (AOR) of 20-40%. These results showed the thermostable biosurfactant was promised to be applied for MEOR application.

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