

Optimization Production of Biosurfactant by *Pseudomonas putida* Using Crude Palm Oil (CPO) as Substrate

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Abstract. The production of biosurfactant by *Pseudomonas putida* has been studied. *P. putida* FNCC 0071 was grown in the nutrient broth medium supplemented with NaCl and crude palm oil (CPO). The effect of CPO concentration and fermentation time on the biosurfactant production were evaluated. The biosurfactant production was evaluated every 24 h for 10 days by optical density, surface tension and emulsification index. The best culture medium was found to be medium containing 5% v/v of CPO with 5 days of incubation time. The biosurfactant was identified as rhamnolipids.

Keywords: biosurfactants; *Pseudomonas putida*; palm oil

1. Introduction

Surfactants are surface active molecules which are usually derivatization products of petroleum oil that are non-renewable and non-degradable. Therefore, there is a high demand in replacing chemically surfactants with biosurfactants. Biosurfactants are naturally occurring amphiphilic molecules produced by a variety of microorganism. They have better environmental compatibility; e.g higher biodegradable, lower toxicity, higher selectivity and specific activity; than that of the chemical surfactants. Biosurfactants have potential application in various fields such as in food and pharmaceuticals industries, oil recovery, bioremediation and biodegradation [1-4]. Nevertheless, due to their high production cost, they have not being widely used yet [5].

It is important to improve the method of biosurfactant production and characterizing the major properties of the biosurfactant. Chemical structures of biosurfactants and their physicochemical properties depends on the physical and chemical conditions under which they are grown [6]. Generally, the structure of biosurfactant relates with the structure of the substrate provided in the media and modify the substrate commonly modifies the structure and hence properties of the biosurfactants.

In recent years, research in optimizing factors for biosurfactant production has considerably increased, where numerous renewable substrates have been used, such as carbohydrates, lipids and proteins. Vegetable oils, such as soybean oil, olive oil, corn oil, castor and sunflower oil, have been used as substrates in the production of biosurfactants [7-14]. The production of sophorolipids by *Candida bombicola* have been reported using glucose and/or palm as substrates [15].

Indonesia is one of the world's biggest producer in crude palm oil (CPO) production. It is reported that 50% of produced CPO is exported in an unprocessed form, while the remaining is subject to processing into essential ingredient for food industries, washing powder, soap and biodiesel. Therefore, diversification products of CPO still need to be produced to increase the economic value of CPO. It was interesting to use CPO as a renewable substrate for the production of biosurfactants in our research.

Pseudomonas aeruginosa is considered as potentially the main producer of rhamnolipids [16]. Yet, *P. aeruginosa* species is pathogenic. Thus, our research was focused on using *Pseudomonas putida* which is generally considered non-pathogenic as producer of rhamnolipids biosurfactants. In this report, the production of biosurfactant by *P. putida* using CPO as substrate are discussed.



2. Materials and Methods

2.1. Materials

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

2.2. Media Used and Growth Condition

Cultures of bacteria were maintained on nutrient agar. The media were used for optimization of biosurfactants production composed of nutrient broth (8 g/L), NaCl (5 g/L) and CPO (0, 5, 10 and 20 % 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.

The microbial growth, surface tension and emulsification index (E24) were observed every day for 10 days of incubation. Microbial growth was evaluated by measuring the absorbance of the culture at 600 nm. The capillary rise method was used for measuring the surface tension. The surface tension was calculated using equation (1), where γ_a is aquadest surface tension (72.13 mN/m), γ_x is surface tension of x (dyne/cm), h_a is aquadest height, h_x is height of x, d_a is aquadest density (0.997 gr/cm³) and d_x is density of x (gr/cm³).

$$\gamma_x = \frac{h_x \rho_x}{h_a \rho_a} \gamma_a \dots\dots\dots (1)$$

The E24 was determined by adding 1 mL of vegetable oil to the same amount of aqueous culture containing biosurfactants, mixing with a vortex for 2 minutes, and leaving to stand for 24 h. The E24 index is given as the height of the emulsion layer, divided by the total height, multiplied by 100.

2.3. Biosurfactants isolation and FT-IR analysis

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. The sample was then analysed by FT-IR Spectroscopy. The spectra of the sample was recorded at room temperature as KBr pellets on Shimadzu type FT-IR 8201 PC spectrophotometer. The light source of transmittance was in the range of 4000-500 cm⁻¹.

3. Results and Discussion

The effect of addition of CPO and fermentation time on the biosurfactant production were examined. Incubation was carried for 10 days where the optical density, surface tension and E24 were noted every day (Fig. 1). Biosurfactant production optimization was carried out in media containing variation amount of CPO (5, 10 and 20 %). The media with no addition of CPO was also performed as a negative control. The optimum condition for the biosurfactant production was explored for the highest emulsification index and the lowest surface tension.

Figure 1(a) presents growth optimization of biosurfactants production using CPO as substrate. The results showed that the addition of CPO in all concentration enhanced the growth of *P. putida* culture significantly. The highest emulsification index was achieved for the media containing 5% v/v of CPO on 5 days of fermentation (Fig. 1(b)). It is noted that the lowest surface tension was obtained for the media containing 5, 10 and 20% v/v of CPO on 5 days of fermentation. These three media have similar value of surface tension (Fig. 1(c)). Base on the Duncan statistical analysis for the emulsification index and surface tension data, the media containing 5% v/v of CPO on 5 days of fermentation was selected as the optimum condition for the biosurfactants production.

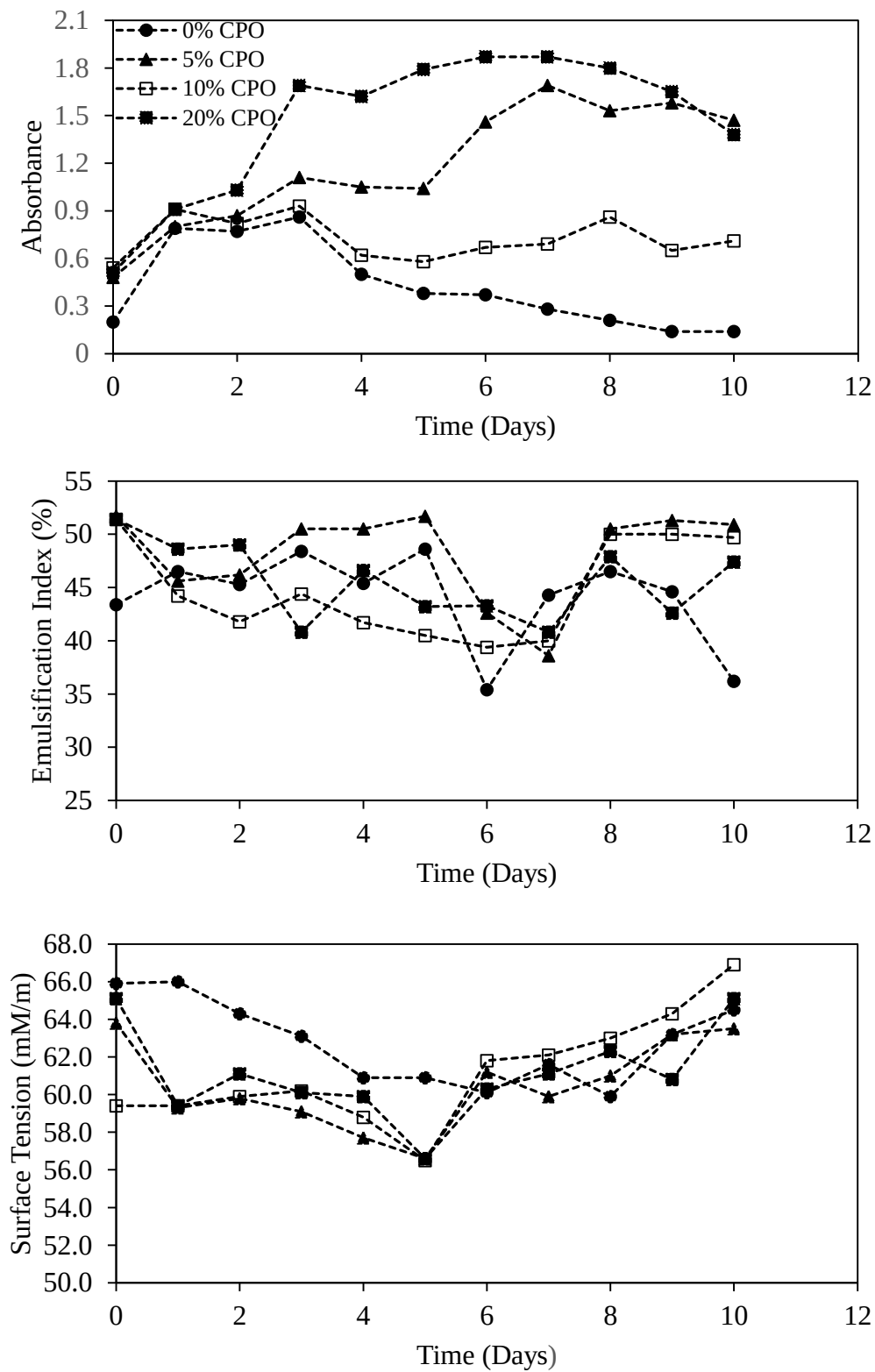


Figure 1. Biosurfactants production by *P. putida* grown on CPO was monitored for: (a) optical density, (b) emulsification index and (c). surface tension.

The production of the biosurfactants was applied for 125 mL media containing 5% v/v of CPO in 250 mL flask for 5 days of fermentation and resulted 0.503 g of biosurfactants for 250 mL media. The FT-IR analysis was performed to identify the functional groups present in the biosurfactants (Fig. 2). The O-H stretching vibration has been observed by a strong and broad band at 3316 cm^{-1} due. The band at 2955 and 2925 cm^{-1} confirmed the presence of the $-\text{CH}$ stretch of methylene. The asymmetric stretching vibration of $\text{C}=\text{O}$ was characterised by the band at 1740 cm^{-1} for the carboxylate and 1660 cm^{-1} for the ketone. The presence of alkyl chains in the compounds was confirmed by the band at 1433 cm^{-1} as a result of deformation and bending vibrations of aliphatic chains ($-\text{CH}_3-$ and CH_2-). The stretching of $\text{C}-\text{O}$ ester was suggested by the presence of the intense and strong band at 1166 cm^{-1} . The rhamnolipids biosurfactants were produced by *P. putida* which was confirmed from the obtained peaks of biosurfactants shown in FT-IR spectrum (Fig. 2). This results was supported by earlier studies reported by other researchers that glycolipids usually produced by *Pseudomonas* spp [17-20].

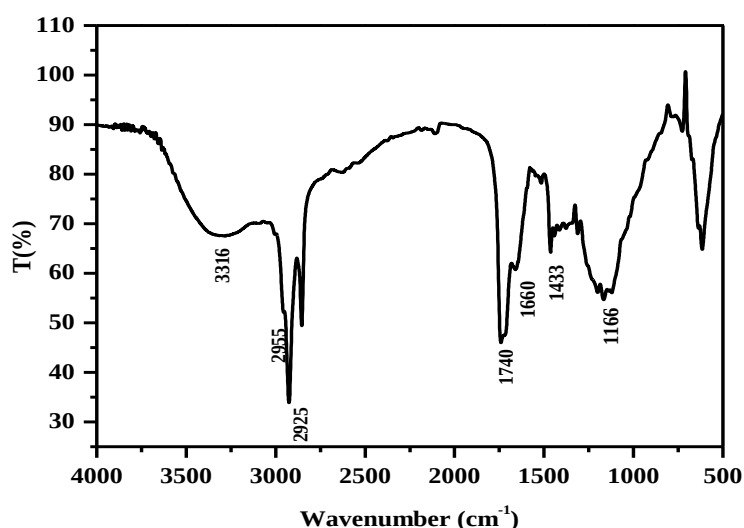


Figure 2. FT-IR spectrum of the produced biosurfactant.

4. Conclusions

Biosurfactant production by *P. putida* in crude palm oil was developed, where the effect of medium composition and fermentation time were evaluated. The optimum condition for biosurfactant production was achieved when a medium containing of 8 g/L nutrient broth, 5 g/L NaCl and 5% v/v crude palm oil was used as media with 5 days of fermentation. Biosurfactant was identified as glycolipids type biosurfactant, which was rhamnolipid.

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