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Biosurfactant Production from *Pseudomonas aeruginosa* ATCC27853 with Carbon Source from Crude Palm Oil for Oil Recovery

Said Zul Amraini[1]*, Sri Rezeki Muria[1], Bahruddin[1], Irdoni HS[1], Ulfa Dwi Artha[1], Reno Susanto[2]

[1]Chemical Engineering, Faculty of Engineering, University of Riau, Bina Widya Km 12,5, Pekanbaru, Indonesia
[2]Chemical Engineering, Faculty of Industrial Engineering, Bandung Institute of Technology, Bandung, Indonesia

Corresponding Author: saidzulamraini@eng.unri.ac.id

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Abstract

Biosurfactants are surfactants that are synthesized by microorganisms using organic materials and have biodegradable properties, making them environmentally friendly. One of the applications of biosurfactants in the recovery of petroleum. This study aims to determine the type of biosurfactant produced by *Pseudomonas aeruginosa* bacteria using crude palm oil as the main carbon source, to determine the effect of variations in pH and CPO concentration on surface tension reduction and emulsification, and compare the best biosurfactant with surfactant synthesis. The production of biosurfactants has 3 stages, namely the bacterial preparation, the biosurfactant production, and the analysis in the form of surface tension, emulsification, crude oil removal, and FTIR. The best biosurfactant was obtained at pH 7 and a carbon source concentration of 3% v/v with surface tension and emulsification values of 42.49 mN/m and 58%, respectively. The pH value and CPO concentration can affect the growth in the biosurfactant production process, thus also affecting the surface tension and emulsification values. The biosurfactants obtained were rhamnolipid biosurfactants. The biosurfactants produced in this study have lower crude oil recovery capabilities than synthetic surfactants with crude oil removal values of 57.78% and 79.34%, respectively.

Keywords: Biosurfactant, CPO, pH, Pseudomonas aeruginosa, FTIR.

INTRODUCTION

The need for fuel is increasing as the population increases and technology develops (Rezki et al., 2017). In recent years, oil production in Indonesia has decreased (Sutapa et al., 2013; Bandjar et al., 2014). Oil production in Indonesia from 2016 to 2018 decreased by 4%. In 2016, 2017, and 2018, oil production was 875 thousand, 837 thousand, and 808 thousand barrels/year, respectively. One of the reasons is the condition of the oil field which is old. As much as 72% of crude oil production comes from old fields that have been producing for more than 30 years, thus experiencing a decline in field production of 29% per year (Habibullah, 2018).

Enhanced oil recovery (EOR) technology is one of the technologies that can increase petroleum recovery by injecting a material into the reservoir (Gozan et al., 2014). One of the EOR techniques that can increase oil production by 30% from the reservoir that has been extracted from the oil is the surfactant injection technique (surfactant flooding) (Gudina et al., 2012). In general, the surfactants used in EOR technology are surfactants synthesized from petroleum such as petroleum sulfonate. However, the use of synthetic surfactants is not environmentally friendly because it contains petrochemicals (Ginting et al., 2017; Nugroho & Buchori, 2019). Currently, the development of EOR technology is being carried out towards more environmentally friendly biotechnology or known as microbial enhanced oil recovery (MEOR) technology. MEOR technology is a technology that is carried out by injecting a bioproduct in the form of a biosurfactant. The manufacture of biosurfactants focuses more on the use of raw materials and microbes to be used. The raw materials used are usually vegetable and animal oils which are cheaper and environmentally friendly (Kandasamy et al., 2019).

Several previous studies have been conducted on the Production of biosurfactants using *Pseudomonas aeruginosa* ATCC 9027 and varying temperatures of 37 ^{o}C and 55 ^{o}C and pH 5, 6, 7, and 8. The optimum conditions for reducing surface tension and interfacial tension were at pH 8 and 37 ^{o}C with a surface tension value of 71 mN/ m to 34 mN/m and the interfacial tension from 41 mN/m to 8 mN/m (Kanna, 2017). The production of biosurfactants using the bacteria *Pseudomonas putida* MTCC 2467 and crude palm oil as a carbon source obtained consists of two types of purity levels, namely low purity (raw) and high purity (pure). The efficiency of pure biosurfactant, crude biosurfactant, SDS, and Tween-80 at the same concentration was 79.40%; 46.84%; 64.10%; and 36.91% (Kanna, 2018).

This study used the bacteria *Pseudomonas aeruginosa* and crude palm oil (CPO) or better known as crude palm oil as a carbon source by varying the concentration of the carbon source and the pH of the fermentation medium. The use of crude palm oil (CPO) as a carbon source is due to the production of CPO in Riau Province in 2006 as many as 7.43 million tons or about 23.58 percent of Indonesia's total production (Habibullah, 2018). Palm oil can be chosen as a raw material for biosurfactant production because of its fatty acid components, which make up triglycerides. Fatty acids with C16-C18 carbon chains can be applied because they have detergency properties and are able to play a good role in hard water, while fatty acids with C12-C14 carbon chains have a role in the foaming effect (Yuliasari et al, 2014). The use of *Pseudomonas aeruginosa* bacteria are bacteria that have the ability to synthesize several compounds by biotransformation and degrade various types of hydrocarbons (Rengga et al., 2018).

This study aims to determine the type of biosurfactant produced by the bacterium *Pseudomonas aeruginosa* using crude palm oil as the main carbon source, to determine the effect of variations in pH and CPO concentration on the reduction of surface tension and emulsification, and to compare the best biosurfactant with synthetic surfactant SDS in recovering petroleum.

METHODOLOGY

Materials and Instrumentals

The raw materials used in this study were *Pseudomonas aeruginosa* ATCC 27853 bacteria, Nutrient Agar, and Nutrient Broth from the Microbial Laboratory, University of North Sumatra, crude palm oil (SMART TBK), distilled water (Brataco).

Methods

Bacterial Preparation

This bacterial preparation process consists of bacterial rejuvenation and bacterial cultivation. The bacterial rejuvenation process was carried out in a petri dish containing an agar plate. The agar plate media was made by dissolving 0.28 g of NA into 10 mL of distilled water. The solution is to be stirred continuously while heated until it boils. Next, the agar solution was transferred into sterile Petri dishes and autoclaved at 121 °C for 15 minutes, then cooled to become gelatinous. A pure culture of *P. aeruginosa* ATCC 2785 was taken as much as 1 ose needle and inoculated on the agar plate aseptically, then incubated for 24 hours at 37 °C.

After completion of the bacterial rejuvenation process, the next process is the cultivation process which is carried out in a 250 mL Erlenmeyer containing liquid media. Liquid media was prepared by dissolving 1.3 gr of NB into 100 mL of distilled water. The solution was stirred until homogeneous and autoclaved at 121 °C for 15 minutes. Bacteria that have been rejuvenated are taken as many as 2 to 3 ose needles and inoculated on liquid media aseptically, then incubated in a shaker incubator at a speed of 120 rpm, for 24 hours at 37 °C (Nurani & Marsudi, 2013; Gozan et al., 2014))

Biosurfactant Production

The biosurfactant production process used a batch fermentation method in a fermenter with a capacity of 250 mL. The fermentation process was carried out by combining 100 mL of active bacterial culture and concentrations of crude palm oil (1, 3, and 5% v/v). Fermentation was carried out for 72 hours (3 days) and was shaken in an incubator shaker at 120 rpm at 37 °C. For 72 hours, the pH of the growth medium was kept constant from 6, 7, and 8. After the fermentation was complete, the biomass was separated by centrifugation at 3300 rpm for 30 minutes and filtered using filter paper. The solids that remain on the filter paper in the form of biomass are discarded, while the liquid that is filtered on the filter paper is a biosurfactant (Kanna, 2017; Gozan et al., 2014).

Analytical measurement

Surface tension analysis is one of the test parameters for the ability of biosurfactants to reduce surface tension. This analysis was carried out using a tensiometer.

Emulsification is one of the test parameters for the ability of biosurfactants to emulsify the oil. This analysis starts by mixing cooking oil, water, and biosurfactant with as much as 5 mL each in a test tube. The mixture was stirred with the highest speed vortex for 5 minutes. The mixture was allowed to stand for 24 hours and measure the height of the emulsion layer and the total height of the solution. Calculation of % emulsification (IE24) can use the following Equation 1.

$$IE_{24} = \frac{\text{High emulsion layer}}{\text{Total height of solution}} \times 100\% \quad (1)$$

Crude oil removal analysis was carried out to see the efficiency of biosurfactants in EOR technology. This analysis starts with sand sieving with a sieve size of 50 mesh. After that, 40 grams of sand was put into a 250 mL Erlenmeyer and 10 mL of kerosene was added. The mixture of sand and kerosene is allowed to stand for ± 7 days. After 7 days, sand and kerosene were weighed as initial weight (Oi). Next, add 10 mL of biosurfactant into a 250 mL Erlenmeyer containing a mixture of sand and kerosene. The mixture of sand, kerosene, and biosurfactant was stirred with a rotary shaker with a rotation speed of 200 rpm for 20 minutes and allowed to stand for 24 hours. After settling, the mixture was washed with dichloromethane twice and heated at 50 ^oC, and weighed as the final weight (O_r) (Costa et al.,2010). Calculation of % crude oil removal can use the following Equation 2.

$$\%\ Crude\ Oil\ Removal = \frac{O_i - O_r}{O_i} \times 100\% \quad (2)$$

Where:
O_i = weight of sand and kerosene (gr).
O_r = weight of sand after washing with dichloromethane (gr).

FT-IR analysis was also carried out for biosurfactants which aim to determine the functional groups of the biosurfactants produced.

RESULTS AND DISCUSSION

Surface Tension and Emulsification

The analysis of surface tension and emulsification aims to determine the best biosurfactant with the lowest surface tension value and the highest emulsification percentage value. The surface tension analysis used distilled water as a positive control and the emulsification analysis used a mixture of water and cooking oil. Figure 1 and Figure 2 show the effect of increasing pH and concentration of carbon source on the surface tension and emulsification of a biosurfactant. The biosurfactant obtained at pH 7 and the carbon source concentration of 3% v/v had the lowest surface tension value of 42.49 mN/m and the highest emulsification value of 58%. The surface tension value of distilled water as positive control is 73.05 mN/m (Ikhwan, 2017)

Based on the calculation, the decrease in surface tension of biosurfactants at pH 7 treatment and carbon source concentration of 3% v/v was 30.6 mN/m. pH and concentration of CPO can affect the growth of bacteria in the biosurfactant production process so it also affects the surface tension and emulsification. The low acidity (pH) of the growth media will result in bacteria not being able to efficiently produce biosurfactants and the pH of the media will always increase during the bacterial growth period (Saikia et al, 2012). In addition, the optimum pH for the growth of *Pseudomonas aeruginosa* bacteria is 6.6 to 7.0 (Badal, 2018). The concentration of CPO is a nutrient source of bacterial carbon where excess nutrients can lead to the accumulation of toxic materials and the limited availability of dissolved oxygen can inhibit bacterial growth. However, a lack of nutrients can cause bacteria to compete for nutrients, resulting in inhibition of bacterial growth (Radzuan et al., 2016).

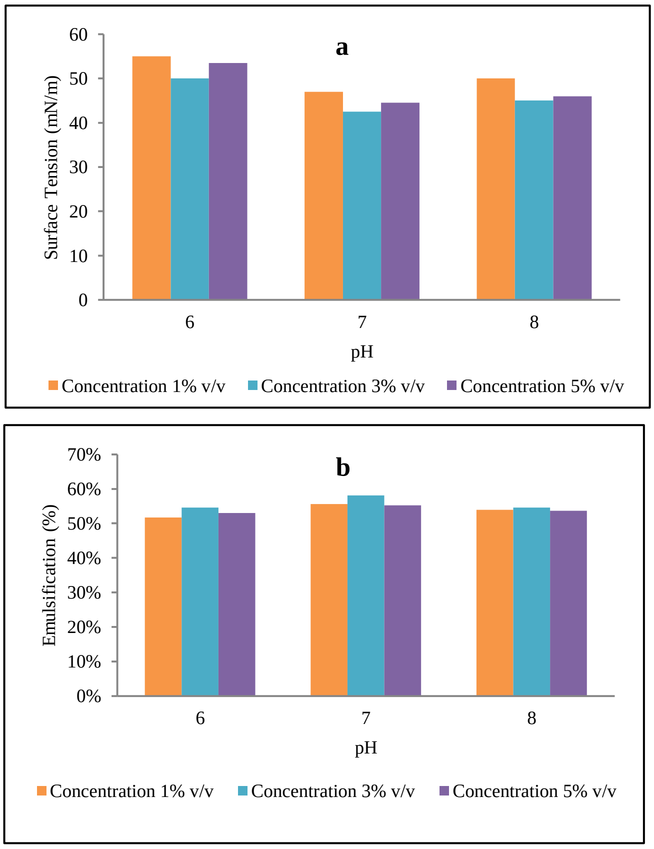

Figure 1. Effect of pH and concentration carbon source against (a) Surface tension; (b) Emulsification

Fourier Transform Infra-Red (FTIR)

In the Fourier transform infrared (FTIR) analysis, the sample used was the best biosurfactant obtained at pH 7 treatment with a carbon source concentration of 3% v/v. Figure 2 shows the absorption band at a wavelength of 3347.60 cm^{-1} indicating the presence of O-H bonds in the rhamnopiranosil compound. The presence of rhamnopiranosil compounds in the resulting biosurfactant indicates that the biosurfactant is a rhamnolipid type (Bordoloi & Kowar, 2009). In addition, in Figure 2, there is an absorption band at a wavelength of 1616.42 cm^{-1} which indicates the presence of -C=C bonds in carboxylate anion compounds (Saikia et al, 2012). In Figure 2, there is also an absorption band at a wavelength of 1634.74 cm^{-1} which indicates the presence of C=O bonds in carbonyl compounds (Suryanti et al, 2014).

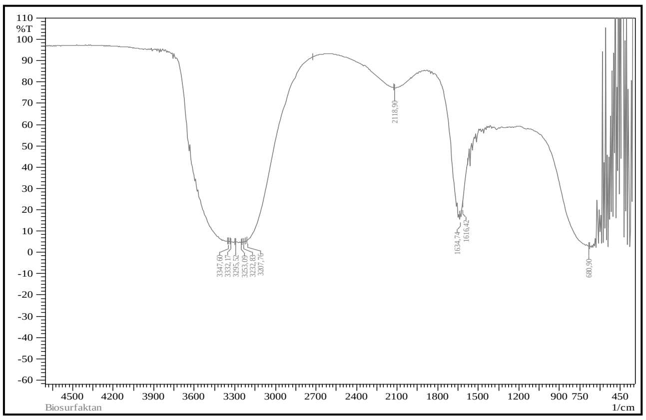

Figure 2. FTIR Spectrum of Biosurfactants at pH 7 and Carbon Source Concentration 3% v/v

***Crude Oil Removal* (COR)**

In crude oil removal (COR) analysis, the biosurfactant used was the best biosurfactant obtained at pH 7 treatment with a carbon source concentration of 3% v/v. Figure 3 shows the comparison of crude oil removal values between biosurfactants and synthetic surfactants in the form of SDS (sodium dedocyl sulfate). The percentage value of crude oil removal on biosurfactants obtained from this study was 57.78%, while the percentage value of crude oil removal for synthetic surfactants in the form of SDS was 79.34%. The biosurfactant from this study had a lower %COR than the synthetic biosurfactant (SDS) because this biosurfactan is an impure product, while the synthetic surfactant in the form of SDS is a pure product (Ikhwan, 2017). Unpurified biosurfactant contains mostly water, dissolved biomass, and biosurfactants rhamnolipids (Rahayu, 2015). In addition, Kanna (2018) conducted research by producing biosurfactants from the bacterium *Pseudomonas putida* MTCC 2467 with crude palm oil as a carbon source. The percentage value of crude oil removal in crude biosurfactant is 46.84%, while the percentage value of crude oil removal in pure biosurfactants by 79.40%.

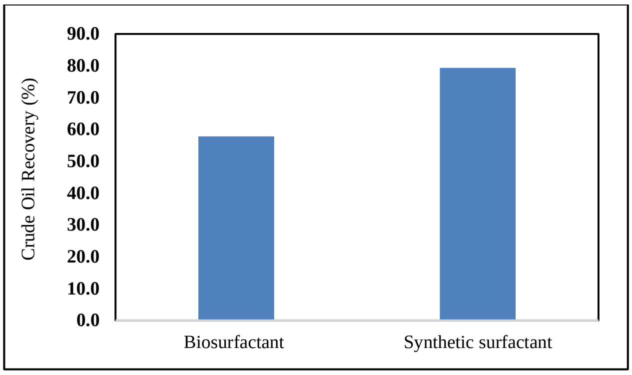

Figure 3. Crude Oil Value Comparison Removal Between Biosurfactants and Synthetic Surfactants

CONCLUSION

The type of biosurfactant produced by *Pseudomonas aeruginosa* bacteria using crude palm oil as the main carbon source is rhamnolipid biosurfactant. The best results were obtained at pH 7 with a carbon source concentration of 3% v/v, a surface tension value of 42.49 mN/m, and emulsification of 58%. The biosurfactant produced in this study had a lower crude oil recovery ability than the synthetic surfactant (sodium dodecyl sulfate/SDS) with crude oil removal values of 57.78% and 79.34%, respectively.

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REFERENCES

Ali, N., Wang, F., Xu, B., Safdar, B., Ullah, A., Naveed, M., Ce, W., Rashid, M. T.. (2019). Production and Application of Biosurfactant Produced by Bacillus licheniformis Ali5 in Enhanced Oil Recovery and Motor Oil Removal from Contaminated Sand. *Molecules*, *24*, 4448

Badal, M. S (2018). Uji Aktivitas Antibakteri Getah Batang Kamboja Putih (Plumeria Acuminata WT Ait) Terhadap Pertumbuhan Bakteri *Pseudomonas aeruginosa*. *Doctoral Dissertation*. Poltekkes Kemenkes Kupang.

Bandjar, A., Sutapa, I. W., Rosmawaty, R., & Mahulau, N. (2014). The Utilitasion of Beef Tallow Into Biodiesel With Heterogenous

Catalyst. *Indonesian Journal Of Chemical Research, 2*(1), 166-170.

Bordoloi, N., & Konwar, B. (2009). Bacterial Biosurfactant in Enchacing Solubility and Metabolism of Petroleum Hydrocarbons. *J. Hazard Mater*, 170(1), 495-505

Costa, S. G., Nitschke, M., Lepine, F., Deziel, E., & Contiero, J. (2010). Stucture,Properties and Applications of Rhamnolipids Produced by *Pseudomonas aeruginosa* L2-1 from Cassava Wastewater. *Process Biochemistry, 45*, 1511-1516.

Ginting, H. A., Masyithah, Z., Herawan, T., & Silaen, D. S. (2017). Optimasi Sintesis Biosurfaktan Karbohidrat Ester Dari Asam Palmitat Dan Fruktosa Menggunakan Enzim Lipase Terimobilisasi. *Jurnal Teknik Kimia USU, 6*(2), 48-54.

Gozan, M., Fatimah, I. N., Nanda, C., & Haris, A. (2014). Produksi biosurfaktan oleh Pseudomonas aeruginosa dengan substrat limbah biodiesel terozonasi untuk peningkatan. *Warta Industri Hasil Pertanian, 31*(02), 39-44.

Gudina, E. J., Pereira, J. F., Rodrigues, L. R., Coutinho, J. A., & Teixeira , J. A. (2012). Isolation and Study of Microorganisms From Oil Samples For Application in Microbial Enhanced Oil Recovery. *International Biodeterioration & Biodegradation*, 68, 56-64.

Habibullah, M. (2018). *Indonesian Palm Oil Statistics 2017.* (S. S. Plantation, Ed.) Jakatra, DKI Jakarta, Indonesia: Central Bureau of Statistics.

Ikhwani, A. Z. (2017). Optimization of Biosurfactant Production from Pseudomonas aeruginosa with Differences in Media pH and Crude Oil Carbon Sources. *Tesis*. Institut Pertanian Bogor, Bogor.

Kandasamy, R., Rajasekaran, M., Venkatesan, S. K., & Uddin, M. (2019). New trends in the biomanufacturing of green surfactants: biobased surfactants and biosurfactants. In *Next Generation Biomanufacturing Technologies* American Chemical Society, 243-260.

Kanna, R. (2017). Biological Surfactant Production by Pseudomonas aeruginosa ATCC 9027 and Probable Application in Microbial Enhanced Oil Recovery (MEOR). *International Journal of Civil Engineering and Technology*, 8(10), 619-629.

Kanna, R. (2018). Production of Biosurfactant Using Crude Palm Oil by Bacteria Pseudomonas putida MTCC 2467 and Its Application in Improved Oil Recovery. *International Journal of Civil Engineering and Technology, 9*,2132-2138.

Nugroho, A., & Buchori, L. (2019). Sintesa Metil Ester Sulfonat dari Minyak Jarak Pagar (Jathropa Curcas Oil) dan Aplikasinya pada Proses Enhanced Oil Recovery (EOR). *Metana*, 15(1), 19-24.

Nurani, D., & Marsudi, S. (2013). Produksi Biosurfaktan Ramnolipid oleh Pseudomonas aeruginosa IFO 3924 dengan Teknik Kultivasi Umpan Curah dan Sumber Karbon Minyak Sawit. In *Seminar Nasional Matematika, Sains dan Teknologi Universitas Terbuka.*, 4, 130-142

Radzuan, M. N., Banat, I., & Winterburn, J. (2016). Production and Characterization of Rhamnolipid Using Palm Oil Agricultural Refinery Waste. *Bioresource Technology*, 225, 99-105

Rahayu, S. (2015). Pengaruh Sumber Karbon dan Nitrogen pada Produksi Biosurfaktan Oleh Bakteri *Pseudomonas aeruginosa* Biopa 2411. *Undergraduated Thesis*. Institut Teknologi Sepuluh November, Surabaya.

Rengga, W. D., Riyadi, D. H., Bintang, A., & Kuntoro. (2018). Study of Production and Process of Biosurfactants from Palm Oil Industrial Waste and Its Derivatives Using *Pseudomonas aeruginosa*. *National Seminar on Energy and technology*, 84-94.

Rezki, R., Musta, R., & Haetami, A. (2017). Minyak Biji Nyamplung (Calophyllum inophyllum) Dengan Etanol. *Indonesian Journal of Chemical Research*, *4*(2), 406-412.

Saikia, R. R., Deka, S., & Deka, M. (2012). Isolation of Biosurfactant Producing Pseudomonas aeruginosa RS29 from Oil Contaminated Soil and Evalution of Different Nitrogen Sources in Biosurfactant Production. *Annals Microbiogy*, 62(2),753-763.

Sari, E. A. (2019). Analisis Bakteri Penghasil Metabolit Sekunder Ekstraseluler dari Pemandian Air Bersih di Desa Ulak Bandung Kecamatan Muara Sahung Kabupaten Kaur Provinsi Bengkulu. *Undergraduated Thesis*, Universitas Islam Negeri Raden

Suryanti, V., Hastuti, S., Handayani, D. S., & Windrawati. (2014). Biosurfactant Biosynthesis by Pseudomonas aeruginosa Using Tapioca Industrial Liquid Waste as Media. *ALCHEMY Journal of Chemical Research*, 10(1), 22-30.

Sutapa, I. W., Rosmawaty, R., & Samual, I. (2013). Biodiesel Production From Bintanggur Oil (Callophyllum inophyllum L.) Using Calsium Oxide (CaO) Catalyst. *Indonesian Journal of Chemical Research*, 1(1), 53-60.

Yuliasari, S., Fardiaz, D., Andarwulan, N., & Yuliani, S. (2014). Characteristics of Enriched Red Palm Oil Nanoemulsion. *Littri*, 20(3), 111-121.