



Production of Biosurfactant By *Arthrobacter* sp. P2(1) in The Carbohydrate-Containing Medium

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Abstract : Surfactants are surface active molecules which have ability to reduce the surface and interfacial tension between the two liquid phases. Biosurfactants, which is produced by living cells. Have more effective, selective, stable, and environmental- friendly than chemical synthesized surfactants. However, the producing cost of biosurfactants are tends to more expensive. Furthermore, inexpensive substrate selection and indigenous biological high-productivity producer strains is one way to reduce the production cost. The purpose of this study was to determine the ability of one of indigeneous petroleum-contaminated soil bacteria, *Arthrobacter* sp.P2(1), in producing biosurfactant in three types of carbohydrate substrates, such as High Fructose Syrup (HFS), sucrose, and molasses. Therefore, *Arthrobacter* sp. P2(1) was grown in mineral synthetic medium containing 2% carbohydrate. Cultures were incubated for 4 days and their surfactant producing ability was observed. The surface tension decrease of culture supernatant was measured using a Du Nouy tensiometer. Hydrocarbon emulsifying activity of supernatant was assayed using diesel oil and kerosene. *Arthrobacter* sp. P2(1) can grow well and produce bisurfactant in the three carbohydrate substrates and growth optimally in molasses-containing medium. *Arthrobacter* sp. P2(1) produce biosurfactant which has property as an emulsifier in the Hfs and sucrose-containing medium. Furthermore, *Arthrobacter* sp.P2(1) produce biosurfactant which has properties as surface active agent and emulsifiers in the molasses-containing medium.

Keywords: Biosurfactant, carbohydrate, surface tension, emulsification.

Introduction

Biosurfactants are surface active molecules synthesized by living cells. Compared with synthetic surfactant, biosurfactants are more effective, selective, stable, and environmental-friendly. Biosurfactants are capable to lower the surface and interfacial tension between two liquid phases, stabilizing emulsions, generally non-toxic, and biodegradable¹. Biosurfactants are needed in various industrial processes, such as food processing, pharmaceutical formulations, oil refining, and environmental bioremediation¹⁻³. Biosurfactant is also reported to have antibacterial, antifungal, antitumor, antiviral and antimycoplasma substances^{4,5}.

Biosurfactants are found in a wide range of chemical structures, include: glycolipids, lipopeptide, lipoproteins, fatty acids, neutral lipids, phospholipids, polymeric lipids, and particulate⁶. The public interest in biosurfactant is constantly increasing because of the biosurfactant's diversity and its' natural potentials to

produce in a large-scale production¹. However, biosurfactants are less able to compete with synthetic surfactants due to high production costs. Inexpensive substrate selection and biological high-productivity producer strains is one way to reduce it.

Indonesia with mega biodiversity has a great opportunity to develop biosurfactant, produced from indigenous microbes. Indigenous oil degrading bacteria isolated from petroleum-contaminated soil in Indonesia has been assayed to produce high-yield biosurfactants. One of the isolates is *Arthrobacter* sp. P2(1)^{7,8}.

Arthrobacter sp. P2(1) can grow well and produce biosurfactant on the medium contains glucose as a carbon source. Glucose is a simple carbohydrate (monosaccharide) that easily used by microbes as nutrition for their growth and substrate for biosurfactant synthesis. Biosurfactants that produced by *Arthrobacter* sp. P2(1) have characteristics as bioemulsifier and surface active agent^{9,10}.

Biosurfactant production is affected by the type and composition of the substrates in the microbes growth medium¹¹. Biosurfactants can be produced from inexpensive substrates and replace the chemically-synthesized surfactants¹². This study was aimed to find the inexpensive alternative substrates based on carbohydrate for biosurfactant production by *Arthrobacter* sp. P2(1).

Materials and Methods

Bacterial isolate

In this research, *Arthrobacter* sp. P2(1) was used as biosurfactant producing agent. It was collected from the Laboratory of Microbiology, Department of Biology, Faculty of Science and Technology, University of Airlangga, Indonesia. The pure bacterial isolate *Arthrobacter* sp. P2(1) was isolated from petroleum contaminated soil around Wonocolo petroleum drilling, Bojonegoro, East Java, Indonesia.

Bacterial culture

Arthrobacter sp. P2(1) were grown in synthetic mineral medium contained carbohydrate as a carbon source. There were 3 types of carbohydrate compounds that added into medium: High Fructose syrup, sucrose, and molasses. The growth of *Arthrobacter* sp. P2(1) of the each substrates was observed and measured using spectrophotometer Genesys 20 ($\lambda = 610$ nm).

Liquid growth medium for biosurfactant production

The liquid medium used to produce biosurfactant is a modification from medium composed by Pruthi and Cameotra¹³. This liquid medium (per litre) consisted of 3 g (NH₄)₂SO₄; 10 g NaCl; 0.2 g MgSO₄.7H₂O; 0.01 g CaCl₂; 0.001 g MnSO₄. H₂O; 0.001g H₃BO₃, 0.001 g ZnSO₄. 7H₂O; 0.001 g CuSO₄. 5H₂O; 0.005 g CoCl₂.6H₂O; and 0.001 g Na₂MoO₄. 2H₂O. Then it was dissolved with 1 L of distilled water. The solution was homogenized using a magnetic stirrer and the pH was adjusted at 7,0. Iron and phosphate solution (stock solution) were made separately: 2 g KH₂PO₄ and 5 g K₂HPO₄ dissolved in 100 ml distilled water, whereas 0.0006 g FeSO₄.7H₂O dissolved in 50 ml distilled water. All solutions were sterilized using autoclave (20 minutes, at 121 °C, 1 atm). Substrates or carbon sources used in this study were: High Fructose Syrup (HFS), sucrose, and molasses 2 % (v/v) respectively was added into the medium solution.

Suspension of microbes ($A = 0.5$, $\lambda = 610$ nm) was taken as much 2 % (v/v) then added to the medium for biosurfactant production. Bacterial cultures were incubated in rotary shaker 120 rpm, 30°C, for 4 days. Furthermore, bacterial cultures were centrifuged at 8000 rpm, for 20 minutes to separate bacterial cell from the supernatant. The presence of biosurfactant was observed by measuring the surface tension and emulsification activity of cell free supernatant.

Measuring emulsification activity

Emulsification activity was determined by measuring the emulsion index¹⁴. The supernatant was poured into a test tube contained oil (4:1, v/v), then homogenized for 2 minutes. The emulsification activity was observed for 24 h by measuring the height of the emulsified zone (cm) divided by the height of the total oil (cm) and multiplied with 100%.

Measuring surface tension

Surface tension of the samples was detected by Du Nouy tensiometer. From the bacterial suspension, 20 ml of *Arthrobacter* sp. P2(1) supernatant was poured into sterile and free of fat Petri dish. Distilled water and sterile synthetic mineral medium were used for blanko solution for comparison. Surface tension reduction unit was described with mN/m.

Results and Discussion

The growth of *Arthrobacter* sp. P2(1) on various substrates

The growth response of *Arthrobacter* sp. P2(1) in carbohydrate containing medium was shown in Figure 1. *Arthrobacter* sp. P2(1) could grow well and showed varying responses in carbohydrate-containing medium, especially on the molasses-containing medium. The optical density ($\lambda = 610$ nm) of *Arthrobacter* sp. P2(1) in the 2 % molasses-contained, sucrose, and HFS are 2.57, 1.21, and 1.16, respectively (Figure 1)

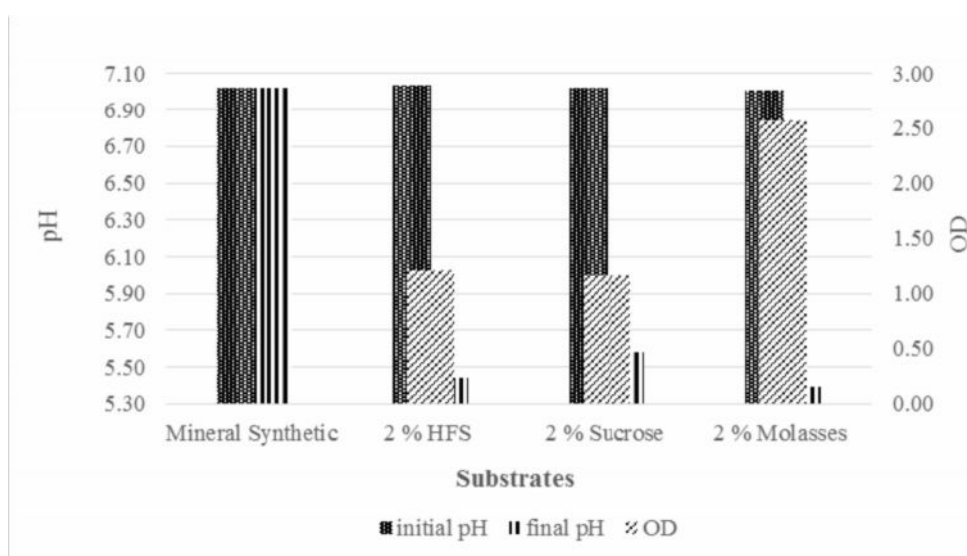


Figure 1. Growth response of *Arthrobacter* sp. P2 (1) on the carbohydrates. Synthetic Mineral is a basal medium without carbon source and was used as control

Biosurfactant producing bacteria could adapt to the environment by converting the substrates to produce varied types of biosurfactants. Several factors are known to influence the type, quantity, and quality of biosurfactants among other sources of carbon, nitrogen concentration, metal ions, and environmental conditions, including pH, temperature, and agitation speed¹⁵. The composition of medium can affect the bacterial growth. Suitable environmental condition and proper nutrition will stimulate bacteria to grow properly and increase the number of bacterial cells¹⁶. Molasses, as sugar mill waste, contains C, N, and O as beneficial substances for bacterial growth. molasses still contains considerable amounts of sucrose and another nutrition. These substance can support *Arthrobacter* sp. P2(1) to grow optimally.

Acidic condition of the medium affected bacterial growth. Therefore, adjustability of the pH for growth medium becomes very important for the determination towards bacterial growth response. In this study, the initial pH of the culture was adjusted at 7 (neutral). Decrease in pH during bacterial growth probably caused by the presence of organic acid produced in the process of cell metabolism¹⁷. Since pH is an indication of the hydrogen ions concentration, increases and decreases of the hydrogen ions concentration can cause ionization clusters in proteins that interfere with cell growth¹⁸. Nevertheless, the pH decline that occurred during the *Arthrobacter* sp. P2(1) growth period did not inhibited it's growth. This indicated *Arthrobacter* sp. P2 (1) was tolerant to acidic condition.

Biosurfactants production from *Arthrobacter* sp. P2 (1) on various substrates

The main parameters that are commonly used to measure the presence or absence of biosurfactant is a surface tension. The metabolism substance of the *Arthrobacter* sp. P2 (1) can decrease the surface tension of the supernatant bacterial culture and stimulate emulsification activity towards hydrocarbon (diesel fuel and kerosene) (Figure 2 and Figure 3).

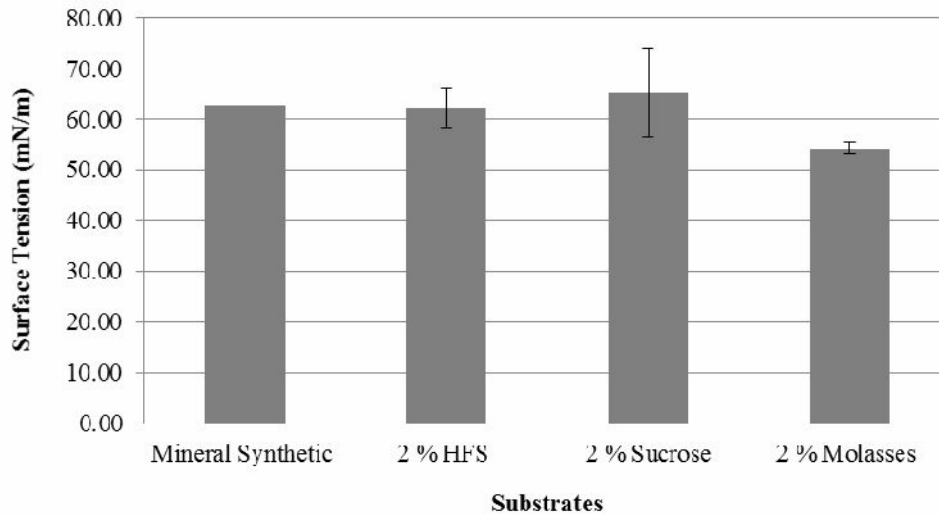


Figure 2. Surface tension of culture supernatant of *Arthrobacter* sp. P2(1) which was grown in carbohydrates-containing medium

Arthrobacter sp. P2(1) did not produce biosurfactant as surface active agent in medium contained HFS and sucrose since the surface tension decrease of the culture supernatant was undetected (Figure 2). In spite of incapacity to produce surface active agent, *Arthrobacter* sp. P2(1) could produce biosurfactant as bioemulsifier properties on both substrates. This is indicated by the presence of supernatant emulsification activity towards diesel fuel and kerosene (Figure 3). HFS or liquid sugar can be used as substrate for some biosurfactant producing bacteria¹⁹. These substrates contain monosaccharides, especially glucose in high levels which is important for bacterial metabolism. Glucose can be directly used in the trajectory of glycolysis in the metabolism of bacteria to survive²⁰.

On the contrary, the biosurfactant produced by *Arthrobacter* sp. P2(1) could decreased surface tension of culture supernatant in molasses-containing medium as much as 8.47 mN/m lower than synthetic mineral medium (Figure 2). In general, the bacteria will release surfactant compounds if the substrates in the growth medium has high hydrophobicity. Substrates with high hydrophobicity are tend to unsoluble and difficult to absorb by microbes membrane. Microbes develop a specific mechanisms to facilitate the unsoluble substrate by lowering the surface tension of growth medium. Therefore, the hydrophobic substrate (hydrocarbons) which are generally located at the top of the medium can be more easily absorbed by microbes. *Arthrobacter* sp. P2(1) was capable to produce biosurfactant in molasses substrate despite in small quantities.

Emulsification activity of *Arthrobacter* sp. P2(1) culture supernatant was assayed using diesel fuel and kerosene. This assay was performed to determine the ability of *Arthrobacter* sp. P2(1) to produce emulsifier towards hydrocarbon compounds (Figure 3).

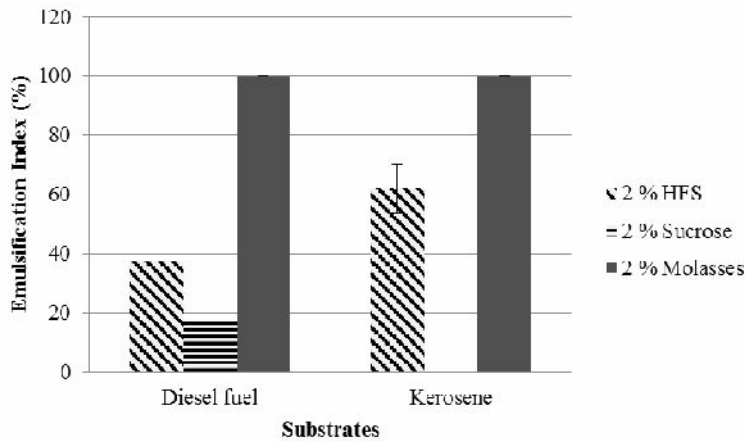


Figure 3. Emulsification index of biosurfactant from *Arthrobacter* sp. P2(1) towards diesel fuel and kerosene

Figure 3 and 4 shows that *Arthrobacter* sp. P2(1) produced emulsifier compound which were able to emulsify the oil hydrocarbons in the three different types of carbohydrates substrates. The highest emulsification activity produced by *Arthrobacter* sp. P2(1) detected in the molasses-containing medium (emulsified 100 % diesel and kerosene), then HFS-containing medium (37.5 % on diesel and 61.9 % on kerosene), and sucrose-containing medium (16.7 % on diesel and 0 % on kerosene).

Bacteria could potentially produce biosurfactant to decrease the surface tension values more than 10 dyne/cm²¹. *Arthrobacter* sp. P2 (1) did not produce biosurfactant that can

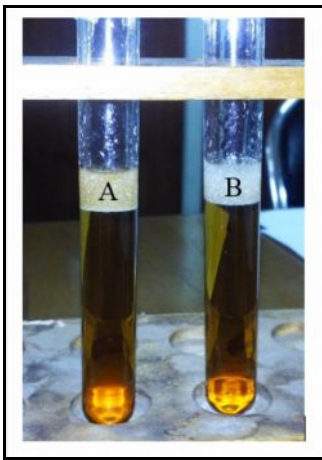


Figure 4. Water emulsion in oil (hydrocarbon) because of the presence of bioemulsifier produced by *Arthrobacter* sp. P2(1) when grown on molasses: (A) Water emulsion in diesel fuel; (B) Water emulsion in kerosene

Decrease the surface tension. However, it produced bioemulsifier in the HFS and sucrose-containing medium. Biosurfactants do not always have high emulsification activity and decrease the surface tension at once. Some biosurfactants can perform to decrease the surface tension well, but not have capability as good emulsifier agent¹⁵. Thus, some biosurfactants can perform as good bioemulsifier of hydrocarbon compounds, but have poor ability to decrease the surface tension³. From this study, *Arthrobacter* sp. P2 (1) was capable to produce bioemulsifier in carbohydrates-containing medium on each type of substrates. However, *Arthrobacter* sp. P2(1) could produce biosurfactant which could reduce the surface tension only in the molasses-containing medium.

Arthrobacter sp. P2(1) produce biosurfactant in the carbohydrates-containing medium using three different substrates: High Fructose Syrup (HFS), sucrose, and molasses. This bacteria can only produce surface active agent to reduce the surface tension on molasses-containing medium. However, this bacteria could well-

produced bioemulsifier substances in all different types substrates to emulsify insoluble hydrocarbon compounds.

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