BIOTROPIA Vol. 25 No. 2, 2018: 130 - 139 DOI: 10.11598/btb.2018.25.2.808

PRODUCTION AND CHARACTERIZATION OF BIOSURFACTANTS PRODUCED BY *Pseudomonas aeruginosa* B031 ISOLATED FROM A HYDROCARBON PHYTOREMEDIATION FIELD

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Received 31 January 2017 / Accepted 29 January 2018

ABSTRACT

The biosurfactants are used by several industrial sectors such as petroleum, agriculture, food production, chemistry, cosmetics, and pharmaceuticals. Because of their hydrophobic and hydrophilic moieties, they have potency to reduce surface tension, interfacial tension between water-hydrocarbon systems, and low micelle concentration. Their characteristics strongly depend on the producer strain as well as on the medium composition, such as carbon and nitrogen sources. This study was conducted to investigate the influence of different sources of carbon (n-hexadecane, glycerol and glucose) and nitrogen (urea, NH_4Cl and $NaNO_3$) for the production of biosurfactants by a new strain of *Pseudomonas aeruginosa* B031 isolated from a rhizosphere of *Paraserianthes falcataria* L. Nielsen, a hardwood plant species at a phytoremediation field. The biosurfactant characteristics of the strain were evaluated, particularly its surface-active properties and potential to remove hydrocarbon. Glycerol was found to be the optimum carbon source, with rhamnose concentration, emulsification index, and critical micelle concentration (CMC) of 718 mg/L, 37%, and 35 mN/m, respectively. Sodium nitrate ($NaNO_3$) was observed as the optimum nitrogen source, with rhamnose concentration, emulsification index, and CMC of 290 mg/L, 30%, and 24 mN/m, respectively. These biosurfactants efficiently reduced surface tension of culture broth from 42 mN/m to 31 mN/m for the glycerol treatment and from 37 mN/m to 24 mN/m for the sodium nitrate treatment. The crude biosurfactants from the glycerol and sodium nitrate treatments also removed 87.5% and 84%, respectively, of crude oil from sand. These rates were higher than those of the chemical surfactants (SDS and Triton X-100). These findings indicate that the biosurfactants produced by the strain from both glycerol and $NaNO_3$ treatments can efficiently decrease the interfacial tension of culture broth dilution and have a high emulsion index, thus hold promise in hydrocarbon bioremediation application.

Keywords: bioremediation, biosurfactant, glycerol, $NaNO_3$, optimum

INTRODUCTION

Biosurfactants are amphiphatic compounds produced by a wide variety of microorganisms that either adhere to cell surface or are excreted extracellular in the growth medium (Al-Bahry *et al.* 2013; Geetha *et al.* 2018). They contain hydrophobic and hydrophilic moieties that reduce surface tension, interfacial tension between water-hydrocarbon systems, and low micelle concentration (Zdziennicka & Jańczuk 2018). As such, biosurfactants are used by several industrial sectors such as petroleum, agriculture, food production, chemistry, cosmetics, and pharmaceuticals (Lai *et al.* 2009; Pacwa-Plociniczak *et al.* 2011). They could be used also to remove hydrocarbon contamination (Mnif & Ghribi 2015; Ma *et al.* 2018).

Biosurfactants are composed of lipopeptides, glycolipids, phospholipids, fatty acids, neutral lipids, and polymeric biosurfactants (Moya *et al.* 2015). Often produced during the stationary phase of bacterial growth, they exhibit considerable substrate specificity (Calvo *et al.* 2009). Their characteristics strongly depend on the producer strain as well as on the medium

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composition, such as carbon and nitrogen sources (Janek *et al.* 2010; Liu *et al.* 2015).

Microbial strains belonging to species *Bacillus* sp. (Barakat *et al.* 2017), *Candida lipolytica* (Rufino *et al.* 2011), *Rhodococcus* sp. (Shavandi *et al.* 2011), *Pseudomas* sp. (Thavasi *et al.* 2011; Sakthipriya *et al.* 2015) and *Sphingobacterium detergens* (Burgos-Diaz *et al.* 2013) have been reported to produce biosurfactants. Of these, the rhamnolipids biosurfactants produced by *Pseudomonas* sp. is of interest in that it could remove hydrocarbon contamination (Deepika *et al.* 2016; Ma *et al.* 2016; Ma *et al.* 2018). Due to economic considerations in the industry, biosurfactants are commonly applied as whole-cells culture or crude biosurfactant. As reported by Patowary *et al.* (2018), purification process of biosurfactants consumed almost 60% of the total production costs.

A phytoremediation field in Balikpapan, East Kalimantan, Indonesia, uses *Paraserianthes falcataria* L. Nielsen, a hardwood plant species, for hydrocarbon removal. Previous studies show that plants for phytoremediation could remove hydrocarbon pollutants through mechanisms such as biodegradation, phytovolatilization, accumulation, and transformation (Kong *et al.* 2016; Guo *et al.* 2017). When soil hydrocarbon concentration in the phytoremediation field was measured in May 2014, it was found that the plants removed 50% of the hydrocarbon pollution within 3 months. In comparison, other hardwood plants, such as *Tectona grandis* and *Gmelina arborea*, are reported to remove only 10% and 15%, respectively (Agbogidi *et al.* 2007; Yenn *et al.* 2014). The differences in the hydrocarbon removal ability are influenced by the plant species, which create unique habitats for the soil microbial population (Cristaldi *et al.* 2017). That is, the soil microbial population is strongly influenced by the plant species used for phytoremediation.

The process of removing hydrocarbon in a phytoremediation field results from the complex interaction between roots, rhizosphere and soil microorganisms (Khan *et al.* 2013; Fatima *et al.* 2018). These association induce the particular physiology and biochemistry of plant roots, resulting in the process of hydrocarbons removal. As such, phytoremediation processes can make nutrients available, such as carbon and nitrogen (Hou *et al.* 2015). Glucose, a carbon source, is produced during photosynthesis, and is exuded into the soil by plant roots (Khan *et al.* 2013). On other hand, the degradation process of hydrocarbon contamination in the soil environment induces n-hexadecane and glycerol, which are carbon sources also (Varjani 2017). During the phytoremediation process, adding N fertilizer (e.g., urea, NH_4Cl, $NaNO_3$) enhances hydrocarbon degradation because nitrogen becomes available to both plant and soil microorganism.

A strain of *Pseudomonas aeruginosa* B031, which was isolated from the rhizosphere of *Paraserianthes falcataria* L. Nielsen, probably has unique characters, as shown by Thavasi *et al.* (2011) and Sakthipriya *et al.* (2015), especially in terms of biosurfactant production. In this regard, it is important to investigate the medium compositions involving variations in carbon sources (n-hexadecane, glycerol, and glucose) and nitrogen sources (urea, NH_4Cl and $NaNO_3$) for the production of biosurfactants by *Pseudomonas aeruginosa* B031. The characteristics of the strain were evaluated, particularly its surface-active properties and potential to remove hydrocarbons.

MATERIALS AND METHODS

Biosurfactant-producing Bacterial Strain

A biosurfactant-producing bacterial strain belonging to *Pseudomonas aeruginosa* B031 was isolated from a hydrocarbon phytoremediation field in Balikpapan, East Kalimantan, Indonesia. The strain was isolated and screened using methods described by Hassanshahian and Emitazi (2008). It was identified following the methods described in Bergey's Manual Systematic of Bacteriology (Brenner *et al.* 2005). This bacterial isolate was then sub-cultured on Luria Bertani medium agar tubes as a stock culture before use as inoculum.

Inoculum Propagation

The strain stock culture of *P. aeruginosa* B031 was inoculated into a plate containing Luria Bertani and incubated at 37ºC. After 24 h, one loop of culture was inoculated to 50 mL of Stone Mineral Salt Solution (SMSS) medium in a 250 mL Erlenmeyer flask. The culture was then incubated at 37ºC for 18 h and agitated at 200 rpm in a shaking incubator (Thorsen *et al.* 2013). At the end of the incubation period, a culture sample

was taken and adjusted with sterile SMSS medium in order to obtain a cell suspension of 0.7 OD (optical density), determined by a spectrophotometer. The number of bacterial cells corresponded to an inoculum of 10^7 CFU/ml. The SMSS medium used for these experiments consisted of the following (g L^{-1}): 0.05% KH_2PO_4, 0.1% K_2HPO_4, 0.05%, $MgSO_4.7H_2O$, 0.01% KCl, and 0.001% $FeSO_4.7H_2O$; the pH was adjusted to 7.0 by 1.0 M HCl (pH 7.2).

Optimization of Medium and Cultivation Condition

The influence of carbon and nitrogen sources on the biosurfactant yield and its properties was studied in the SMSS medium supplemented with 2% (v/v) of their sources. The carbon sources were n-hexadecane, glycerol, and glucose. The nitrogen sources were urea, NH_4Cl and $NaNO_3$. A 2% cell suspension of 0.7 OD at 600 nm was inoculated into a 500-ml flask containing 100 mL SMSS medium. The culture was then incubated at 37ºC under agitation of 200 rpm. After 96 hours of incubation, culture samples were collected and further evaluated for bacterial biomass and biosurfactant characteristics.

Biomass and Biosurfactant Characteristics

The culture samples collected previously were centrifuged at 36,000 g, temperature of 4ºC for 1 h. The pellet cells were dried overnight at 105ºC and then weighed to measure the *Pseudomonas aeruginosa* B031 biomass. The crude biosurfactants in the cell-free culture medium obtained were characterized in terms of rhamnose concentration and surface-active properties, including emulsification index (E24), surface tension, and critical micelle concentration (CMC).

Rhamnose Concentration

Rhamnose concentration in the cell-free culture medium was measured using the phenol-sulphuric method (Seedevi *et al.* 2018). One mL of the cell-free culture broth was mixed with 0.5 mL of 80% phenol and 2.5 ml of concentrated sulphuric acid. After the mixture was incubated for 10 min at room temperature, the absorbance was measured at 490 nm using a spectrophotometer. The rhamnose concentration was then calculated using a standard curve prepared using different concentrations of rhamnose (Vinogradov *et al.* 2016).

Emulsification Index (E24)

The emulsification index of the culture samples was determined using the method described by Panjiar *et al.* (2015). Two ml of hexadecane and 2 ml of the cell free supernatant were mixed in a test tube and homogenized in a vortex at 3,500 rpm high speed for 2 min. The emulsification stability was measured after 24 h. The emulsification index was calculated as follows:

$$E24 = \frac{\text{Height of emulsion formed (cm)}}{\text{Total height of solution (cm)}} \times 100$$

Surface Tension Measurement

The surface tension of the cell-free supernatant was measured in a K6 tensiometer (Shimadzu), using the du Nouy ring method. The values reported are the mean of three measurements (Joshi & Shekhawat 2014).

Critical Micelle Concentration (CMC)

Cell-free culture media of different carbon and nitrogen sources were diluted into various extents with sterile medium to analyze for surface tension (1/128, 1/64, 1/32, 1/16, 1/8, ¼, ½, and 1). The dilutions at which a surfactant begins to aggregate and had no further significant reduction in surface tension were assessed as CMCs. These measurements were estimated graphically by plotting the surface tension values versus the culture broth dilution values (Cheng 2013).

Hydrocarbon Removal Ability

The potential of the crude biosurfactant to remove hydrocarbons from contaminated sand was evaluated using the methodology described by Aparna *et al.* (2012). Sandy soil samples were collected from the Parangtritis Beach, Indonesia; the samples were air dried and then sieved using a 2-mm mesh. Furthermore, samples were mixed with tap water up to the moisture content 19 % (v/w). Soil samples (20 g each) were polluted by 2 g of crude oil (Pertamina, Balongan, Cilacap, Indonesia), then transferred to 250-mL Erlenmeyer flasks. The flask was added with 40 mL of the cell-free fermented broth and then

incubated at 37°C under agitation of 200 rpm in a shaking incubator. After 18 h incubation, the sample was added with 120 mL of dichloromethane as the extracting solvent, then centrifuged at 10,000×g for 15 min. The amount of residual hydrocarbon in the sample was determined by gravimetric analysis (Villalobos *et al.* 2008). For comparison, the hydrocarbon removal ability of SDS, Triton X-100 and distilled water (control) was also analyzed under the same conditions.

Statistical Analysis

All statistical analyses were performed using Stat View for Windows (SAS Institute, Cary, NC, USA) based on $P<0.05$. The effects of the treatments and their differences were assessed using analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT) method, respectively.

RESULTS AND DISCUSSION

Bacterial Strain Identification

Table 1 lists the morphological, cultural, and biochemical characteristics of the bacterial strain selected for this study. This isolate developed pale green pigmentation on asparagine medium and released a sweet grape-like odor. Its colony was small, rough, and convex. The isolate was gram negative and showed motility under microscope investigation. The biochemical analysis showed that it had abilities on denitrification, gelatin liquefaction, and starch hydrolysis. It showed positive results in the oxidase and catalase tests. It could use glucose, fructose, and mannitol as carbon source to ferment, resulting in acid production. In addition, the isolate could grow in a temperature range of 30°C to 42°C and in a pH range of 5-9. According to Bergey's Manual of Systematic Bacteriology, along with the results of the analyses, the isolate was identified as *Pseudomonas aeruginosa* (Brenner *et al.* 2005).

Table 1 Morphological, biochemical and cultural characteristics of the selected strain B031

Characteristic	Observation*
Morphology	
Colony color	Pale green
Colony type	Small, rough, convex
Cell shape	Rods
Gram staining	Negative
Motility	+
Biochemistry	
Denitrification	+
Gelatin liquefaction	+
Starch hydrolysis	+
Oxidase test	+
Catalase test	+
Carbon utilization	
Glucose	+
Fructose	+
Mannitol	+
Culture	
Temperature	
30°C	+
37°C	+
42°C	+
pH	
5	+
7	+
8	+
9	+

Note: * + indicates growth of strain, - indicates no growth of strain

Effect of Carbon on Strain Biomass and Biosurfactant Properties

Table 2 presented the bacterial biomass and characteristics of the biosurfactant produced by *Pseudomonas aeruginosa* B031 using different carbon sources, such as glycerol, glucose and n-

Table 2 Effect of carbon source on cell growth, rhamnolipid concentration, emulsion index, surface tension and final pH during biosurfactant production by *Pseudomonas aeruginosa* B031

Carbon source	Biomass (g/L)*	pH*	Rhamnose (mg/L)*	Emulsion index (%)	Surface tension (mN/m)*
n-hexadecane	0.3 ± 0.01^{b}	5.1 ± 0.03^{a}	449 ± 19^{c}	$20\pm0.10.3^{b}$	37 ± 0.3^{c}
Glycerol	0.5 ± 0.02^{a}	7.0 ± 0.02^{c}	718 ± 15^{d}	37 ± 0.2^{a}	31 ± 0.2^{b}
Glucose	0.1 ± 0.01^{c}	3.4 ± 0.01^{b}	180 ± 11^{a}	10 ± 0.01^{c}	44 ± 0.1^{a}
Control	0 ± 0^{d}	7.2 ± 0.01^{d}	0 ± 0^{b}	0 ± 0^{d}	63 ± 0^{d}

Note: *Data in this table are presented as mean ± standard deviation. Their values with different superscripts in the same column are significantly different ($P < 0.05$).

hexadecane. As observed, the strain was able to use all the evaluated carbon sources to grow and produce biosurfactants. All the carbon sources were found to have significantly influenced bacterial biomass, pH, rhamnose concentration, emulsion index and surface tension.

Among the tested carbon sources, glycerol was found to be the optimum for biosurfactant production of the strain. Its yield was higher than those reported by previous researchers who also used *Pseudomonas* sp and obtained rhamnolipids (Amani *et al.* 2013; Sodagari *et al.* 2018). The highest cell biomass and rhamnose concentration among the observations were 0.5 g/L and 718 mg/L, respectively. These results were expected since glycerol as a carbon source is taken up more easily than the others. On other hand, the use of n-hexadecane, which is a very complex and heterogeneous carbon source, resulted in the lowest biomass and biosurfactant yield. Moreover, the biosurfactant yield was lower than the results obtained by Varjani and Upasani (2016) who reported that the *Pseudomonas aeruginosa* NCIM 5514 strain produced 1.450 mg/L of rhamnolipid when glycerol was added at 3% w/v as carbon and energy source.

Further, the biosurfactant produced by the strain using glycerol had the highest significant reduction in surface tension and formed a stable emulsion on cultivation media compared with n-hexadecane and glucose ($P<0.05$). The surface tension and emulsion index values were 31 mN/m and 37 %, respectively. The use of glycerol also stabilized the pH condition, whereas the use of glucose and n-hexadecane resulted in a decreased pH. This lower pH value was probably due to the production of secondary metabolic acid (Müller *et al.* 2012).

Critical micelle concentration (CMC), which indicates the efficiency of a surfactant, is another important characteristic to consider. To measure the CMCs of the biosurfactant under study, culture broths of three treatments (n-hexadecane, glycerol, glucose) were diluted to various extents with a sterile medium and analyzed for surface tensions (Fig. 1). The point of inflection of the curve, which was obtained by measuring the surface tensions of the various dilutions, corresponds to the surfactant concentration equal to CMC. Analysis of the CMCs values of the glycerol, glucose, and n–hexadecane treatments were 35 mN/m, 44 mN/m, and 41 mN/m, respectively ($P<0.05$). The lower CMC value of glycerol treatment indicates that glycerol is the most efficient carbon source among the three sources tested (Kłosowska-Chomiczewska *et al.* 2017). Monteiro *et al.* (2018) reported that glycerol could be obtained abundantly and cheaply as a by-product of biodiesel from animal fats and vegetable oil production. Glycerol could be obtained also from trans-esterification of vegetable oils and fossil sources (petroleum, natural gas, coal). Therefore, glycerol is a promising and abundant substrate for biosurfactant production.

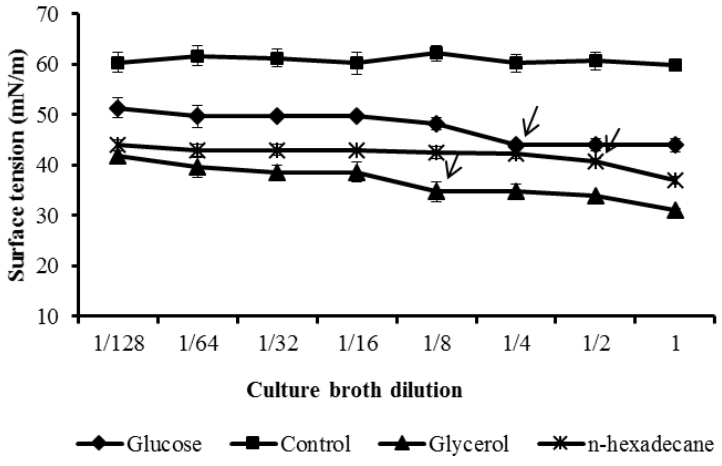

Figure 1 Changes in surface tension in culture broths of *Pseudomonas aeruginosa* B031 at various dilutions using three carbon sources. Arrows correspond to the dilution values that are equal to CMCs. Vertical bars indicate ± standard deviation of means (n=3).

Effect of Nitrogen Sources on Strain Biomass and Biosurfactant Properties

Sodium nitrate was found to be significantly more effective than urea and ammonium chloride ($P<0.05$) (Table 3). The data obtained imply that the use of sodium nitrate as nitrogen sources is better for supporting the growth of the strain and stabilizing the pH of the cultivation medium. These conditions induced a significantly higher surface tension reduction and the formation of a stable emulsion on the cultivation media using sodium nitrate treatment compared with the urea and ammonium chloride treatments ($P<0.05$). The results were in accordance with previous studies (Ma *et al.* 2016).

According to Kryachko *et al.* (2016), the bacteria cell uses nitrates, ammonia, and amino acids as nitrogen sources to produce biosurfactant. During these processes, the NO_3^- have to be reduced to NO_2^- and then to NH_3. The NH_3 can be assimilated either by glutamate dehydrogenase to produce glutamate or, with glutamine, by glutamine synthetase to produce glutamine. Glutamine and α-ketoglutarate are then transformed to glutamine by l-glutamine 2-oxoglutarate aminotransferase. Therefore, in comparison with NH_3, the assimilation of NO_3^- as a nitrogen source is slower, simulating a nitrogen-limiting condition that is favorable to biosurfactant production (Rizzo *et al.* 2017).

The evaluation of the CMCs of the culture broths of three treatments (sodium nitrate, urea, and ammonium chloride) shows significant differences among the treatments ($P<0.05$) (Fig. 2). The CMC values of the treatments were 24 mN/m for sodium nitrate, 35 mN/m for urea, and 43 mN/m for ammonium chloride.

The reduction in surface tension and CMC of the biosurfactants is used as primary criterion for selection of biosurfactant-producing microorganisms (Thavasi 2011). This research demonstrated that the crude biosurfactant produced by the strain using glycerol and sodium nitrate as substrates decreased surface tension of the cultivation media by more than10 mN/m, thus they can be considered good surfactants (Vijayakumar & Saravanan 2015). As presented in Fig. 1 and 2, surface tension decreased from 42 mN/m to a minimum value of 31 mN/m for the glycerol treatment and from 37 mN/m to 24

Table 3 Effect of nitrogen source on cell growth, rhamnolipid concentration, emulsion index, surface tension and final pH during biosurfactant production by *Pseudomonas aeruginosa* B031

Nitrogen source	Biomass (g/L)*	pH*	Rhamnose (mg/L)*	Emulsion index (%)*	Surface tension (mN/m)*
Urea	1.8 ± 0.01^a	6.8 ± 0.01^a	220 ± 19^c	28 ± 0.06^a	35 ± 0.04^a
NH_4Cl	1.0 ± 0.01^b	4.8 ± 0.02^b	90 ± 15^d	17 ± 0.2^b	42 ± 0.5^b
$NaNO_3$	2.5 ± 0.02^c	7.3 ± 0.01^c	290 ± 11^a	30 ± 0.1^c	24 ± 0.1^c
Control	0 ± 0^d	7.2 ± 0.01^c	0 ± 0^b	0 ± 0^d	63 ± 0^d

Note: *Data in this table are presented as mean ± standard deviation. Their values with different superscripts in the same column are significantly different ($P< 0.05$).

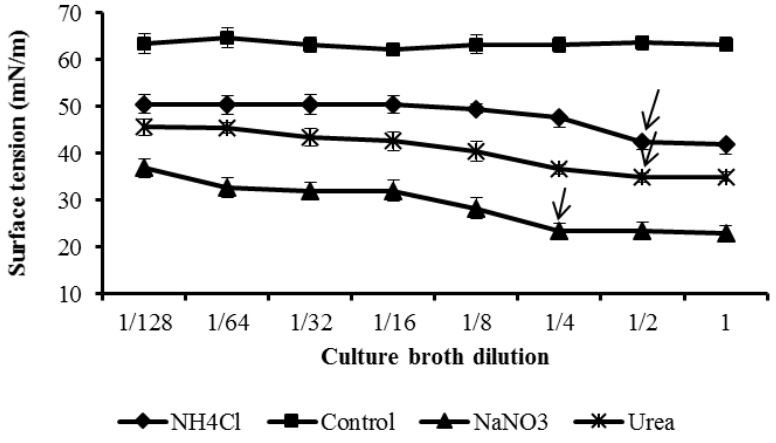

Figure 2 Changes in the surface tension of culture broths of *Pseudomonas aruginosa* B031 at various dilutions using three nitrogen sources. Arrows correspond to the dilution values that are equal to CMCs. Vertical bars indicate ± standard deviation of means (n=3).

mN/m for the sodium nitrate treatment. These capacities are comparable to the outcomes of similar previous research wherein surface tension was reduced to a minimal value of 27-28 mN/m (Petrikov *et al.* 2013). Other studies had been carried out also on biosurfactants by *P. fluorescens*, for which the minimal value of surface tension was 27.5 mN/m (İkizler *et al.* 2017). Therefore, this study showed that both glycerol and sodium nitrate are efficient and promising substrates for biosurfactant production from *Pseudomonas aruginosa* B031.

Application of Biosurfactants in Hydrocarbon Removal

An increasing number of sites have been polluted by hydrocarbons become a seriously effect to ecosystem and human health critical. For removing these pollutions, environmentally and low-cost technology was highly required. Because oil has low water solubility, which increase its sorption by soil particles, researchers have been exploring the use of biosurfactants to accelerate hydrocarbon removal from the contaminated sites (Arslan *et al.* 2017).

The use of crude biosurfactant to remove hydrocarbon contaminants is common in bioremediation technologies. Therefore, this study investigated the application of the biosurfactant from *P. aeruginosa* B031 in its crude form, without prior costly extraction or purification steps. The experiment used cell-free supernatant containing the crude biosurfactant and chemical surfactants (SDS and Triton X-100) to verify the former's capacity to remove crude oil from sand samples.

The biosurfactant produced *P. aeruginosa* B031, SDS, Triton X-100, and distilled water (control) showed significantly different ($P<0.05$) capabilities in removing crude oil from contaminated sand (Table 4). The crude biosurfactants from both glycerol and sodium nitrate treatments yielded higher values (87.5% and 84%, respectively), but no significant difference ($P<0.05$) was observed between them. The removals obtained by SDS and Triton X-100 as synthetic surfactants at the same concentration were lower (73% and 71.6%, respectively), which that of distilled water (control) was only 30.2%. The results imply that the cell-free supernatant containing the crude biosurfactant was practically as effective as the isolated biosurfactant in removing crude oil, indicating the possible use of the unpurified biosurfactant to minimize production costs.

The ability of biosurfactants to remove crude oil can be explained as follows: biosurfactants promote the transport of hydrophobic contaminants toward an aqueous phase through some particular interactions, resulting in emulsification and micellization, thus leading to their removal (Costa *et al.* 2010). The results of the experiments indicate that these biosurfactants are more effective in oil recovery than the chemical surfactants (SDS or Triton X-100). These results were in accordance with the results of Lai *et al.* (2009) who observed that biosurfactants such as rhamnolipids and surfactin are more efficient in removing hydrocarbon than Tween-800 and Triton X-100. Therefore, these biosurfactants indicate good prospects for applications, especially for bioremediation.

CONCLUSION

The biosurfactant produced by *Pseudomonas aeruginosa* B031 was found to produce the highest biomass and the best surface-activities and emulsification properties when glycerol and sodium nitrate were used as carbon and nitrogen sources, respectively. The crude biosurfactants

Table 4 Comparison of the hydrocarbon removal ability of the crude bacterial biosurfactant, SDS, Triton X-100 and distilled water (control)

Sample	Crude oil removal (%)
Crude bacterial biosurfactant from glycerol treatment	87.5 ± 7^{d}
Crude bacterial biosurfactant from $NaNO_3$ treatment	$84.\pm^{a}\ 6^{d}$
SDS	73.0 ± 3^{b}
Triton X-100	71.6 ± 2^{c}
Distilled water (control)	30.2 ± 2^{a}

Note: * Data in this table are presented as mean ± standard deviation. Their values with different superscripts in the same column are significantly different ($P < 0.05$).

produced by the strain were able to efficiently decrease the interfacial tension of culture broth dilution. These biosurfactants were capable of efficiently removing crude oil from sand samples, performing better than chemical surfactants, thus hold promise for hydrocarbon bioremediation application.

ACKNOWLEDGEMENTS

We acknowledge financial support from BOPTN 2015 Research Grant of Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia (Contract Number: UGM/BI/2460/UM/03/02). Authors also thank Ms Sofianingtyas for her help in preparing the strain.

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