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Enhancement of glycolipid production by *Stenotrophomonas acidaminiphila* TW3 cultivated in low cost substrate

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ABSTRACT

This study aimed to investigate potential biosurfactant production of *Stenotrophomonas acidaminiphila* TW3 isolated from sediments contaminated with palm oil soapstocks using low cost substrates. The combination of central composite rotatable design (CCRD) and response surface methodology (RSM) was used to optimize biosurfactant production. The highest biosurfactant production (2.31 g/L) was obtained under the optimal conditions of 49.96 g/L used palm oil as a carbon source, 3.00 g/L $NaNO_3$ as a nitrogen source and medium pH 6.0. RSM increased the yield of biosurfactant up to 2.1 folds however, the productivity is still low further improvement is needed. The biosurfactant obtained from the *S. acidaminiphila* TW3 was able to lower the surface tension of medium from 72 to 32 mN/m. The biosurfactant was purified by liquid column chromatography and identified as glycolipid using thin layer chromatography, Fourier transform infrared spectroscopy, Mass spectrometer and Nuclear Magnetic Resonance. The biosurfactant could reduce surface tension of pure water to 32 mN/m with a critical micelle concentration of 0.2 g/L. It is an effective biosurfactant over a wide range of temperatures, pH and salt concentrations. Moreover, the biosurfactant showed ability to enhance PAHs solubility and removed used lubricating oil contaminated in sand.

1. Introduction

Biosurfactants are microbial compounds that exhibit apparent surface and emulsifying activities. They are amphiphilic compounds contain hydrophobic and hydrophilic moieties which produced on living microbial cell surfaces, or excreted extracellular (Karanth et al., 1999). Most biosurfactants are either anionic or neutral, whereas those that contain amine groups are cationic (Santos et al., 2016). The hydrophobic moieties are based on saturated, unsaturated or hydroxylated fatty acids, whereas the hydrophilic portion can be mono-, di-, or polysaccharides, carboxylic acids, amino acids, phosphate or peptides (Lang, 2002). Biosurfactants are amphipathic molecules with both hydrophilic and hydrophobic moieties that confer the ability to accumulate between fluid phases, thus reducing surface and interfacial tension at the surface and interfaces, respectively (Santos et al., 2016). A structurally diverse group of biosurfactant compounds are mainly classified into four categories, i.e., glycolipid, fatty acid, lipopeptide and polymer based on the structure (Gudiña et al., 2013).

In recent years, biosurfactants are attracting attention because they offer several advantages over synthetic surfactant due to its lower toxicity, better biodegradability and specific activity at extreme environments (De Almeida et al., 2016). Although interest in biosurfactants is increasing, these surfactants are not economically competitive with their synthetic counterparts. One possible strategy for reducing cost is the use of low-cost renewable substrate as carbon source for cell growth and biosurfactant production, such as agricultural waste (Kumar et al., 2016), food industries waste (Kaskatepe et al., 2016), co-product from vegetable oil processing (Solaiman et al., 2004) and pollutant (Ibrahim et al., 2013). These substrates are necessary for the production of biosurfactants, which are containing high concentration of carbohydrates and lipids (Benincasa, 2007). When using low-cost renewable substrates in the production processes, production costs can be greatly reduced, and the final product generated

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Table 1
Effect of different carbon and nitrogen sources on growth and biosurfactant production by *S. acidaminiphila* TW3 grown in MSM at room temperature (30 ± 2 °C) and 150 rpm for 48 h.

	Final pH	DCW (g/l)*	ST (mN/m)*		STR(%)
			ST_i	ST_f	
C-source 1%, $NaNO_3$ 0.1%					
Glucose	3.3	0.54 ± 0.0^{d}**	60 ± 0.3	59.7 ± 1.5	10.9 ± 1.5^{c}
CS	4.2	0.75 ± 0.2^{cd}	67 ± 0.0	58.7 ± 1.2	12.4 ± 1.2^{c}
Molasses	7.6	1.25 ± 0.1^{a}	60 ± 0.0	52.2 ± 0.3	13.1 ± 0.3^{c}
UPO	6.3	1.11 ± 0.1^{ab}	52 ± 0.0	31.3 ± 0.6	39.7 ± 0.6^{a}
Crude glycerol	7.0	0.87 ± 0.2^{bc}	35 ± 0.3	32.7 ± 0.6	6.7 ± 0.6^{d}
Palm oil soapstock	6.5	0.72 ± 0.1^{cd}	50 ± 0.3	33.2 ± 0.3	33.7 ± 0.3^{b}
N-source 0.1%, UPO 1%					
$(NH_4)_2SO_4$	6.9	0.57 ± 0.0^{c}	51.7 ± 1.5	47.8 ± 0.3	8.0 ± 0.3^{d}
MSG	7.1	1.10 ± 0.1^{a}	51.8 ± 0.8	34.0 ± 0.5	33.3 ± 0.5^{bc}
Urea	8.1	1.15 ± 0.1^{a}	52.0 ± 0.0	33.8 ± 0.8	34.9 ± 0.8^{b}
Peptone	7.6	0.77 ± 0.2^{cb}	52.2 ± 0.3	36.3 ± 2.1	30.1 ± 2.1^{c}
$NaNO_3$	6.3	1.15 ± 0.1^{a}	52.2 ± 0.3	31.8 ± 0.3	38.8 ± 0.3^{a}
Yeast extract	6.1	0.85 ± 0.1^{b}	50.8 ± 1.0	33.7 ± 0.8	35.3 ± 0.8^{bc}

Abbreviations; DCW, dry cell weight; ST_i, the initial surface tension of culture broth; ST_f, the surface tension of culture broth after cultivation, STR, surface tension redudtion.** Different letters in the same column within the same parameter studied indicate significant differences ($p < 0.05$).*Values are given as mean ± SD from triplicate determinations.

Table 2
Effect of environment factors on biosurfactant production by S. *acidaminiphila* TW3 grown in MSM supplemented with used palm oil (1%) and NaNO3 (0.1%) after cultivated at room temperature (30 ± 2 °C) and 150 rpm for 48 h.

	Final pH	DCW (g/l)*	ST(mN/m) *			STR (%)***
				48h	CMD^{-1}	
			ST_i	ST_f	ST_f	
Shaking speed (rpm)						
100	6.6	0.21 ± 0.0^{c}**	52.0 ± 0.5	31.7 ± 0.3	36.3 ± 0.8	30.3 ± 0.3^{a}
150	6.8	0.79 ± 0.0^{a}	51.8 ± 0.3	31.7 ± 0.3	35.3 ± 0.6	31.8 ± 0.3^{a}
200	6.5	0.65 ± 0.1^{b}	52.2 ± 0.3	31.8 ± 0.8	38.3 ± 0.6	26.5 ± 0.8^{b}
Initial pH						
4	6.1	0.23 ± 0.0^{c}	51.2 ± 0.8	32.2 ± 0.6	41.7 ± 0.6	18.6 ± 0.6^{c}
5	6.7	0.48 ± 0.3^{bc}	51.3 ± 0.6	31.8 ± 0.6	39.9 ± 0.8	22.3 ± 0.6^{b}
6	6.6	0.64 ± 0.0^{ab}	52.2 ± 0.3	31.5 ± 0.5	36.1 ± 1.0	30.8 ± 0.5^{a}
7	6.3	0.74 ± 0.1^{a}	52.3 ± 0.3	31.7 ± 0.3	36.8 ± 0.6	29.6 ± 0.3^{a}
8	7.1	0.41 ± 0.1^{bc}	52.2 ± 0.5	31.2 ± 0.6	40.3 ± 0.3	23.2 ± 0.6^{b}
Temperature (°C)						
30	6.0	0.78 ± 0.1^{a}	51.8 ± 0.3	31.3 ± 0.3	35.5 ± 0.5	31.5 ± 0.3^{a}
37	6.2	0.78 ± 0.0^{a}	51.8 ± 0.3	31.7 ± 0.3	35.8 ± 0.3	30.9 ± 0.3^{a}
45	6.6	0.35 ± 0.1^{b}	52.2 ± 0.3	35.0 ± 0.5	37.8 ± 0.3	27.5 ± 0.5^{b}

Abbreviations; DCW, dry cell weight; ST_i, the initial surface tension of culture broth; ST_f, the surface tension of culture broth after cultivation, STR, surface tension redudtion; DCW, dry cell weight.*Values are given as mean ± SD from triplicate determinations.**Different letters in the same column within the same parameter studied indicate significant differences ($p < 0.05$).***STR (%) was calculated using ST_f of CMD^{-1}.

is of high aggregated value. Moreover, the volume of waste discharged into the environment decreased (Accorsini et al., 2012). The strategy to improve production is to optimize the growth media and cultivation conditions in order to get maximum production (Mukherjee et al., 2006). Statistical experimental strategies such as factorial design and response surface methodology (RSM) have been used to optimization of product yields. These statistical experiments can reduce the number of trials and saving time (Kiran et al., 2010).

Thus, the aims of the present study were to determine the production of biosurfactant from *Stenotrophomonas acidaminiphila* TW3 isolated from palm oil soapstocks contaminated sediments by using a response surface method. In addition, purification, characterization and its potential application were also studied.

2. Materials and methods

2.1. Materials

All chemicals and solvents used in this study were analytical grade. Anthracene, phenanthrene, naphthalene and pyrene were purchased from Fluka (Darmstadt, Germany). Sodium dodecyl sulfate (SDS) was purchased from Bio Basic (Canada). Commercial sugar (CS) was purchased from Mitrphol, Thailand and commercial monosodium glutamate (MSG) was purchased from Ajinomoto, Thailand. Palm oil soapstock was kindly provided from the Pikunthong Royal Development Study Centre, Narathiwat, Thailand. Used palm oil (UPO) was obtained from canteen in Prince of Songkla University, Thailand. Molasses was purchased from the agency in Hat Yai, Songkhla, Thailand. Crude glycerol was obtained from biodiesel production pilot plant at Faculty of Engineering, Prince of Songkla University, Songkhla, Thailand. Used lubricating oil (ULO) was obtained from motorcycle mechanical workshop around Prince of Songkla University, Songkhla, Thailand.

2.2. Bacterial strain and cultivation condition

The *S. acidaminiphila* TW3 used in the present study was previously isolated from sediments contaminated with palm oil soapstock and identified by 16S rRNA gene sequencing analysis. The sequence was deposited in the GenBank database (http://www.ncbi.nlm.nih.gov/) with accession number as AB905600. The microbial strain was kept in 30% glycerol at -20 °C. To prepare the seed culture, *S. acidaminiphila* TW3 was streaked on a nutrient agar (HiMedia, India) plate, and inclubated at 30 °C for 24 h. A single colony was transferred to 50 mL nutrient broth in the 250 mL Erlenmeyer flask. The seed culture was incubated in a rotary shaker (Orbitek, Scigenics Biotech, India) at 30 °C and 150 rpm for 24 h. This was used as inoculum at the 2% (v/v) level.

2.3. Factors affecting biosurfactant production

For biosurfactant production, a mineral salt medium (MSM) with the following composition (g/L) was used: K_2HPO_4, 0.8; KH_2PO_4, 0.2; $CaCl_2$, 0.05; $FeCl_2$, 0.01; $MgCl_2$, 0.5; $NaNO_3$, 1.0; NaCl, 5.0; and distilled water to 1000 mL (Yin et al., 2005). pH of the medium was adjusted to 7. Carbon and nitrogen sources were added separately. Cultivation was performed in 250 mL flasks containing 50 mL medium at room temperature (30 ± 2 °C), and shaken in a rotary shaker at 150 rpm for 48 h. The effect of carbon and nitrogen sources and growth conditions (temperature, agitation, and initial pH) was conducted in a series of experiments changing one variable at a time, keeping the other factors fixed at a specific set of conditions. The carbon sources used were 10 g/L of glucose, CS, molasses, UPO, palm oil soapstock, and crude glycerol with 1 g/L of $NaNO_3$ as nitrogen source. For evaluation effect of nitrogen sources for the production of biosurfactant, $(NH_4)_2SO_4$, MSG, $NaNO_3$, peptone, urea and yeast extract were employed at a concentration of 1 g/L with the optimum carbon source. The effect of environmental factors such as temperature (25–45 °C), agitation speed (50–200 rpm) and initial pH (4.0–8.0) on biosurfactant production were also investigated.

Table 3
Range of the parameters used for modeling the biosurfactant production and the specified codes for each parameter.

Variables	Coded values and the corresponding values of parameters				
	-α	-1	0	1	+ α
A: pH	5.32	6	7	8	8.69
B: Used palm oil	9.77	20	35	50	60.23
C: $NaNO_3$	0.99	1.5	2.25	3.0	3.51

Table 4
Experimental central composite design (CCD) runs in Design-Expert 7.1 and corresponding results (the response).

Run	A pH	B Used palm oil (g/L)	C $NaNO_3$ (g/L)	Response biosurfactant (g/L)*	Predicted biosurfactant (g/L)
1	6	20	1.5	1.37 ± 0.03	1.42
2	8	20	1.5	1.21 ± 0.03	1.22
3	6	50	1.5	1.12 ± 0.06	1.11
4	8	50	1.5	1.13 ± 0.02	1.16
5	6	20	3	2.11 ± 0.05	2.13
6	8	20	3	1.54 ± 0.09	1.64
7	6	50	3	2.38 ± 0.10	2.48
8	8	50	3	2.22 ± 0.11	2.24
9	5.32	35	2.25	1.74 ± 0.06	1.82
10	8.68	35	2.25	1.44 ± 0.05	1.45
11	7	9.77	2.25	1.62 ± 0.08	1.68
12	7	60.23	2.25	1.81 ± 0.10	1.93
13	7	35	0.99	0.76 ± 0.06	0.80
14	7	35	3.51	2.29 ± 0.05	2.31
15	7	35	2.25	1.46 ± 0.07	1.48
16	7	35	2.25	1.43 ± 0.08	1.48
17	7	35	2.25	1.45 ± 0.04	1.48
18	7	35	2.25	1.42 ± 0.09	1.48
19	7	35	2.25	1.46 ± 0.05	1.48
20	7	35	2.25	1.43 ± 0.01	1.48

* Values are given as mean ± SD from triplicate determinations.

2.4. Optimization and biosurfactant production using surface methodology

The critical control factors influenced the biosurfactant production by *S. acidaminiphila* TW3 were used in the response surface methodology (RSM) based experiments. Carbon source (UPO), nitrogen source ($NaNO_3$) and environment factor (initial pH) were selected as independent variables for the optimization of biosurfactant production and cell growth. Experiments were carried out in 250 mL Erlenmeyer flasks containing UPO, $NaNO_3$ and initial pH in various concentrations. Each flask containing 50 mL of sterilized MSM medium which was inoculated with 2% (v/v) microbial culture. The flasks were incubated for 48 h at 30 ˚C in a rotary shaker incubator at a speed of 150 rpm. The culture broth samples were centrifuged at 12,000 × *g* for 15 min. Biosurfactant samples were recovered by acid precipitation followed by mix solvent ($CHCl_3$:MeOH) extraction (Kiran et al., 2010). The dry weights of the biosurfactants were measured.

The central composite design (CCD) was used in the present study. It suites for fitting a quadratic surface, which usually works to process optimization. It allows estimating the second degree polynomial of relationships between the factors and the dependent variable and gives information about interaction between variables in their relation to the dependent variable. A 23-factorial CCD with six axial points ($\alpha = 0.5$) and six replications at the center points ($n_0 = 6$) leading to a total number of 20 experiments was employed to determine the selected nutrients for the maximum production of biosurfactant. The variables were coded according to the following Equation (1).

$$x_i = \frac{(x_1 - x_0)}{\Delta x},\ i = 1,\ 2,\ \ldots\ k \quad (1)$$

Where x_i is the coded value of the variable, x_1 the real value of the variable, x_0 the real value of x_1 at the centre point, k the number of factors and Δx the step change value. A quadratic model, Equation (2), which includes all interaction term, was used to evaluate the predicted response.

$$Y_i = \beta_0 + \Sigma\, \beta_i x_i + \Sigma\, \beta_{ii} x_i^2 + \Sigma\, \beta_{ij} x_i x_j \quad (2)$$

Where Y_i is the predicted response, x_i and x_j the input variables, β_0 the offset term, β_i the linear effects, β_{ii} the squared effects, and β_{ij} the interaction term.

The experiments were designed by using the Design-Expert version 7 software (Stat-Ease Inc. Minneapolis, USA) and performed in triplicate. The polynomial equations for the three responses were validated by the statistical test called ANOVA (analysis of variance), for determination of significance of each term in equation and to estimate the goodness or fit in each case. The design expert software was performed for regression and graphical analysis of data obtained. The optimum level of selected nutrients was obtained by solving the regression equation and analysis 3D response surface. F-test was employed to evaluate the statistical significance of the quadratic model; its quality was expressed by the determination coefficient of correlation R^2.

2.5. Recovery and purification

S. acidaminiphila TW3 was cultivated under the optimal condition for biosurfactant production. The supernatant was collected by centrifugation at 12,000 × *g* for 15 min. Biosurfactant was recovered by difference systems based on chloroform/methanol, 2:1 (Kim et al., 1997), salt precipitation (Satpute et al., 2010) and acid precipitation followed by solvent extraction (chloroform/methanol, 2:1)(Kiran et al., 2010). The method showing highest biosurfactant activity was selected to recover biosurfactant of *S. acidaminiphila* TW3. The crude biosurfactant was purified using reverse-phase column chromatography (Sep-Pak C8 cartridge; Waters, Massachusetts, USA) by step elution with 50% acetonitrile and 100% acetonitrile. All fractions were collected, dried and determined biosurfactant activity by oil displacement test (Morikawa et al., 2000) and checked pattern of each spot on thin layer chromatography (TLC).

2.6. Chemical analysis of biosurfactant

The obtained biosurfactant was first identified by TLC. Biosurfactant was spotted on reverse phase silica gel F_{254} TLC plates (Merck, Germany). TLC plates were developed using ethyl acetate:methanol:water (1:2:0.1, v/v/v) as mobile phase and visualized with different color developing reagents. TLC plate was sprayed with anisaldehyde reagent to detect carbohydrate (Thanomsub et al., 2004), with copper sulfate reagent for detecting lipid (Churchward et al., 2008), with ninhydrin reagent for presence of peptide (Joy et al., 2017). It was then heated at 105 ˚C for 5 min. Rf of a compound was measured which defined as the distance traveled by the compound divided by the distance traveled by the solvent (Rifai et al., 2018).

Chemical characterization of biosurfactant was further performed by fourier-transform infrared spectroscopy (FT-IR) with Thermo Niocolet, AVATAR 330. Ten milligrams of biosurfactant was milled with 100 mg of KBr to form a very fine powder. This powder was then compressed into a thin pellet which could be analyzed by FT-IR spectra measurement in wave number range of 400–4000 cm^{-1} (Najafi et al., 2010). Further characterization of the biosurfactant was performed by ^1H and ^{13}C nuclear magnetic resonance (NMR). A ^1H nuclear magnetic resonance (NMR) spectrum was recorded at 298 K on an AMX 300 NMR spectrometer (Bruker, 300 MHz). This was equipped with an Aspect 3000 computer (Bruker) locked to the deuterium resonance of solvent, $CDCl_3$ without spinning. Data were recorded at 32 K (the number of data points per parts per million of

Table 5
The analysis of variance (ANOVA) for biosurfactant production, the effect of pH (A), Used palm oil (B) and $NaNO_3$ (C). Quadratic response surface model fitting.

Source	Sum of squares	df	Mean square	F-Value	p-value* Prob > F
Model	3.320	9	0.369	209.79	<0.0001
A- pH	0.139	1	0.139	78.78	<0.0001
B– UPO	0.065	1	0.065	37.25	0.0001
C– $NaNO_3$	2.636	1	2.636	1499.37	<0.0001
AB	0.040	1	0.040	22.85	0.0007
AC	0.041	1	0.041	23.28	0.0007
BC	0.205	1	0.205	116.68	<0.0001
A^2	0.050	1	0.050	28.47	0.0003
B^2	0.152	1	0.152	86.36	<0.0001
C^2	0.019	1	0.019	10.75	0.0083
Residual	0.018	10	0.002		
Lack of Fit	0.012	5	0.002	2.10	0.2172
Pure Error	0.006	5	0.001		
Cor Total	3.337	19			

R^2 = 0.9947, Pred R^2 = 0.9673, Adj R^2 = 0.99.*Values of "prob > F" less than 0.05 indicates model terms are significant.

the plot). Final characterization of the biosurfactant was done by liquid chromatography-mass spectroscopy (LC-MS) with LCQTM quadrupole ion-trap mass spectrometer (Finnigan MAT, San Jose, CA, USA) which utilizes electrospray ionization (Thavasi et al., 2008). This utilizes ESI. The electrospray source was operated at ionization source temperature 80 °C, electrolyte voltage 200 V, and spray inlet temperature 120 °C. The equipment was run in a positive ion mode.

2.7. Study of biosurfactant stability

Experiments were carried out in glass test tubes (10 mm × 170 mm). The biosurfactant (0.8 g/L) was dissolved in 10 ml of distilled water for test biosurfactant stability. The effect of pH was varied at 2, 3, 4, 5, 6, 7, 8, 9 and 10 with 6 M HCl or NaOH solutions and allowed to stand for 24 h at 30 °C. The effect of temperature on the biosurfactant stability was studied at different temperatures (25, 30, 40, 50, 60, 70, 80 90 and 100 °C for 1 h while 110 and 121 °C for 15 min) at pH 7. The effect of NaCl concentration (0, 1, 2, 3, 4 and 5%), the effect of $MgCl_2$ and $CaCl_2$ concentration (0, 0.02, 0.04, 0.06, 0.08 and 0.1%) on activity of the biosurfactant were investigated at pH 7 and 30 °C (Silva et al., 2014). The remaining biosurfactant activity was then determined by surface tension measurement.

2.8. Application of the biosurfactant for enhancement of polycyclic aromatic hydrocarbon (PAHs) solubility

All experiments were carried out in glass test tubes (10 mm × 170 mm) with plastic screw caps. The effects of biosurfactants on the solubilization behaviors of naphthalene, phenanthrene, anthracene and pyrene were examined. For the PAHs solubility experiment, excess PAHs (dissolved in acetone) was distributed into glass test tubes and removed the acetone solvent. The biosurfactant was varied at 0–3.2 g/L (0–4 CMC) and dissolved in buffer (20 mM Tris-HCl, pH 7.0). Then, 3.0 mL of biosurfactant solutions were added in the sample tubes. The sample tubes were incubated overnight at 30 °C with shaking (150 rpm) in the dark. The suspensions were centrifuged at 13,000 × *g* for 25 min. A PAHs in the aqueous phase were extracted with dichloromethane, and their concentrations were analyzed by Ultraviolet Spectrophotometry (UV2450, Shimadzu, Japan). The UV wavelengths of naphthalene, phenanthrene, anthracene and pyrene were 228, 254, 254 and 274 nm, respectively. All the tests were conducted in triplicate (Li et al., 2015).

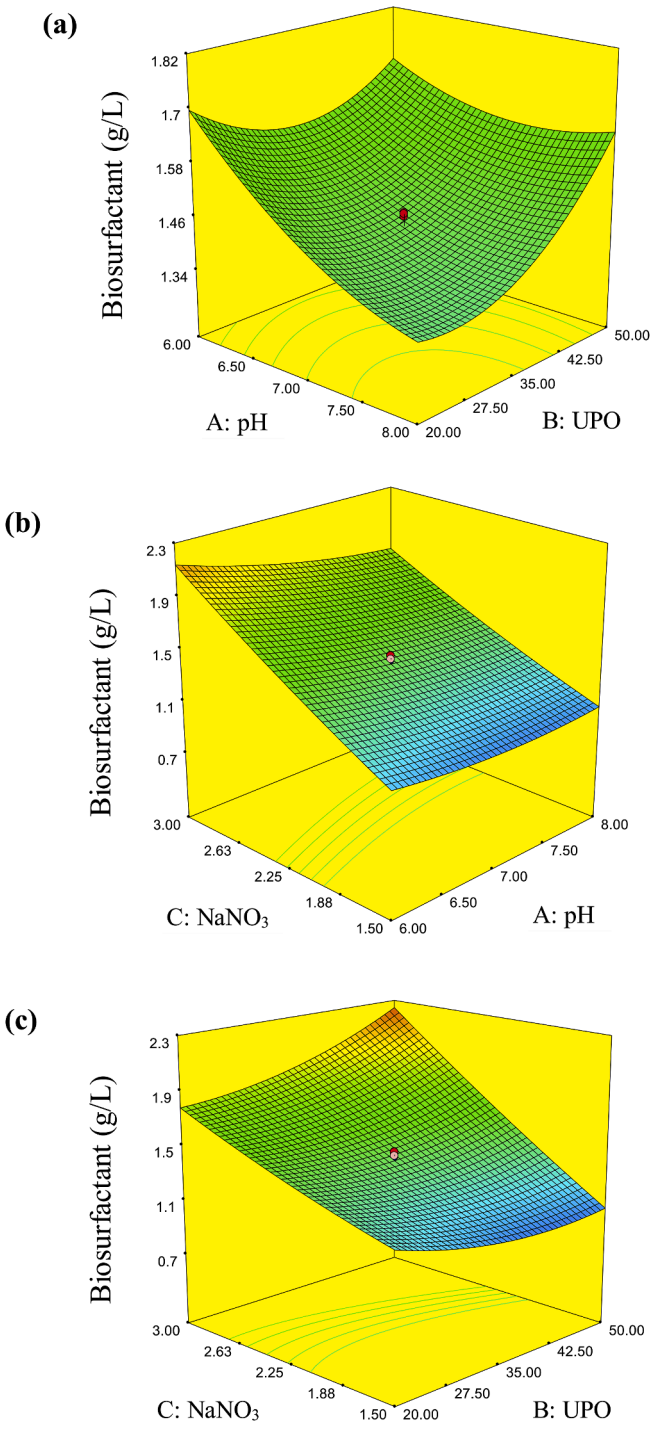


Fig. 1. Three dimensional for the maximum biosurfactant production. **a** Biosurfactant as a function of used palm oil and pH. **b** Biosurfactant as a function of $NaNO_3$ and pH. **c** Biosurfactant as a function of used palm oil and $NaNO_3$.

2.9. Application of the biosurfactant in used lubricating oil removal from contaminated sand

Biosurfactant property for enhance oil recovery was investigated using 800 g of acid washed sand (1–2 mm of diameter) impregnated with 50 mL of ULO. Among of 20 g of the contaminated sand were transferred to 250 mL flasks which were proposed to the following treatments: addition of 60 mL aqueous solutions of the biosurfactant and sodium dodecyl sulfate (SDS), and addition of 60 mL distilled water for control. These were done at the CMC of each compound (0.8

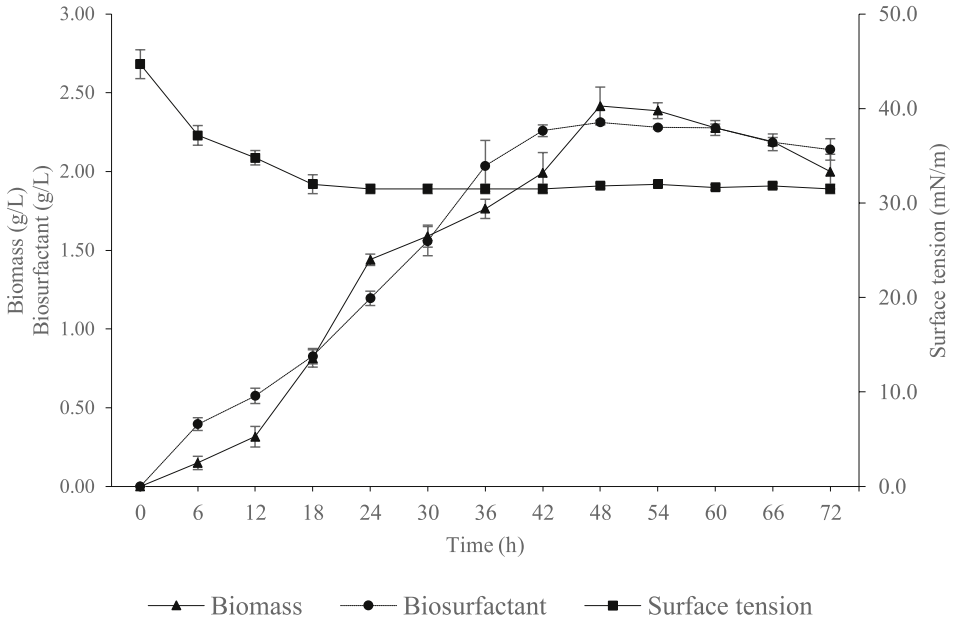


Fig. 2. Time course of growth and biosurfactant production by *S. acidaminiphila* TW3 in optimal condition (UPO (49.96 g/L), $NaNO_3$ (3.00 g/L) and pH 6 at 150 rpm for 30 °C) (Bars indicate standard derivation from triplicate determinations).

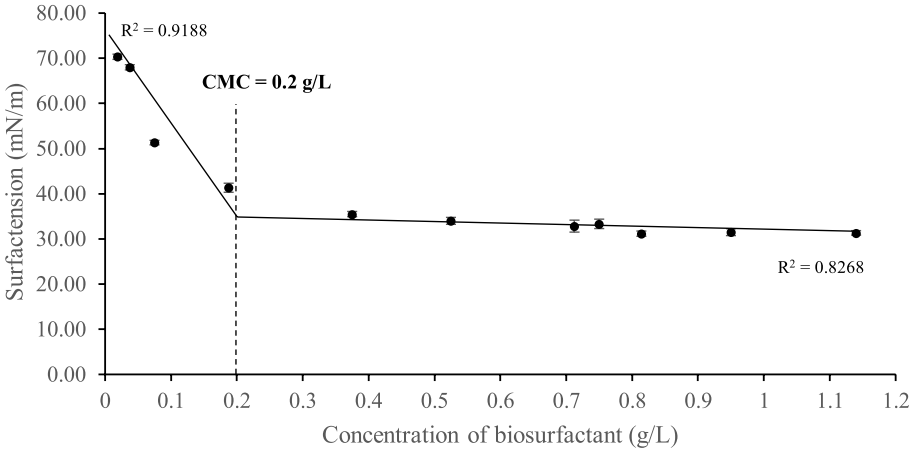


Fig. 3. Critical micelle concentration (CMC) of biosurfactant derived from *S. acidaminiphila* TW3.

and 1.12 g/L for biosurfactant and SDS, respectively). The trials were incubated on a rotary shaker (200 rpm) for 24 h at 30 °C and centrifuged at 12,000 × *g* for 20 min for the separation of the laundering solution and the sand. The amount of oil present in the sand after the impact of biosurfactant was gravimetrically determined as the amount of material extracted from the sand by hexane. The experiment was carried out at 25 °C, room temperature (30 ± 3), 45 and 65 °C to assess the influence of temperature on biosurfactant-induced oil recovery (Saimmai et al., 2012). The percentage of oil removal was calculated using the equation (Chaprão et al., 2015) as follow:

$$\text{ULO removed (\%)} = (O_i - O_r) / O_i \times 100 \quad (3)$$

Where O_i is the initial ULO in the soil (g) before washing and O_r is the ULO remaining in the sand (g) after washing.

2.10. Analytical methods

2.10.1. Biomass estimation

Biomass was determined as cell dry weight. Ten milliliters samples were centrifuged at 12,000 × *g* for 15 min. The cell pellet was washed twice with chilled distilled water to remove residue from the cultivation medium. The biomass obtained was dried overnight at 105 °C.

2.10.2. Surface tension and CMC determination

The surface tension was measured with a Model 20 Tensiometer (Fisher Science Instrument Co., PA, USA) at 25 °C. The culture broth samples were centrifuged at 12,000 × *g* for 15 min. The surface tension of supernatant was measured which compared with control (medium without inoculum). For determining the critical micelle dilutions, the cell free supernatant was diluted 10-fold (CMD^{-1}) with distilled water. Critical micelle concentration (CMC) was determined by plotting the surface tension versus concentration of biosurfactant in the solution. Different concentrations of the biosurfactant in distilled water were prepared. The CMC was determined from the intersection of regression lines that describe two parts of the curve (Saimmai et al., 2011). The validity of these measuring was confirmed by taking surface tension measuring of distilled water (72 mN/m) before each sample reading. Between each measurement, the platinum ring was rinsed three times with water, three times with acetone, and allowed to dry (Wei et al., 2005). The percentage of surface tension reduction was calculated using the equation as follow:

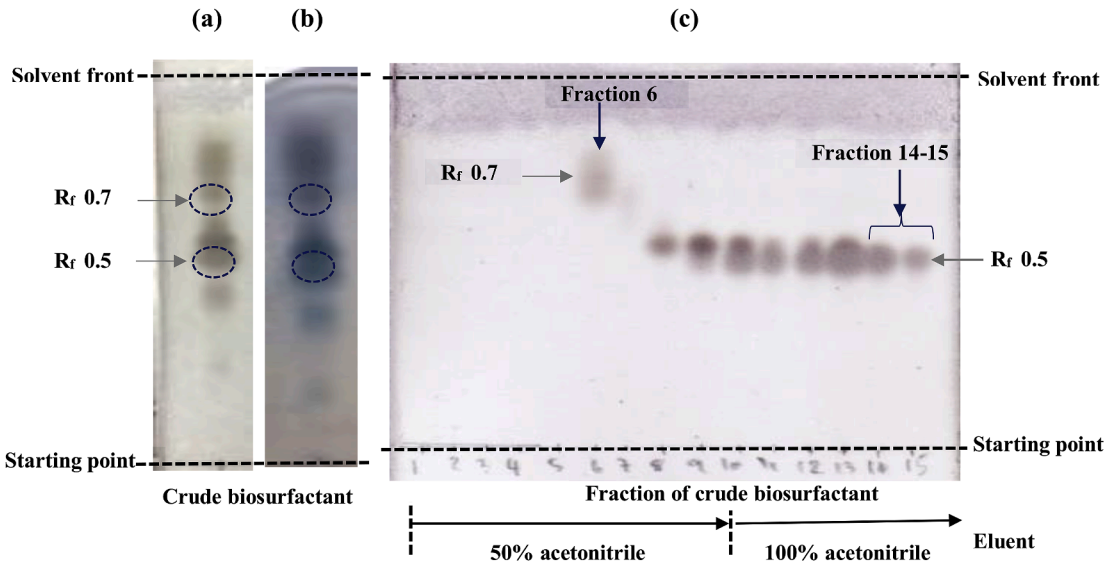


Fig. 4. Thin layer chromatography analysis of biosurfactant produced by *S. acidaminiphila* TW3, **a** using $Cu(SO_4)_2$ reagent as a spraying reagent for fatty acid detection, **b** using anisaldehyde as a spraying reagent for sugar detection and **c** TLC profiles of the fractions collected during the reverse-phase column separation procedure.

$$\text{Surface tension reduction (\%)} = (ST_i - ST_f) / ST_i \times 100 \quad (4)$$

Where ST_i is the initial surface tension of culture broth and ST_f is the surface tension of culture broth after cultivation (Chaprão et al., 2015).

2.11. Statistical analysis

The data was calculated with mean values and standard deviations (means ± SD) were determined from triplicate determinations. Statistical significance of the results were evaluated by one way ANOVA (analytical of variance) and Duncan multiple tests ($p < 0.05$) using SPSS 10.

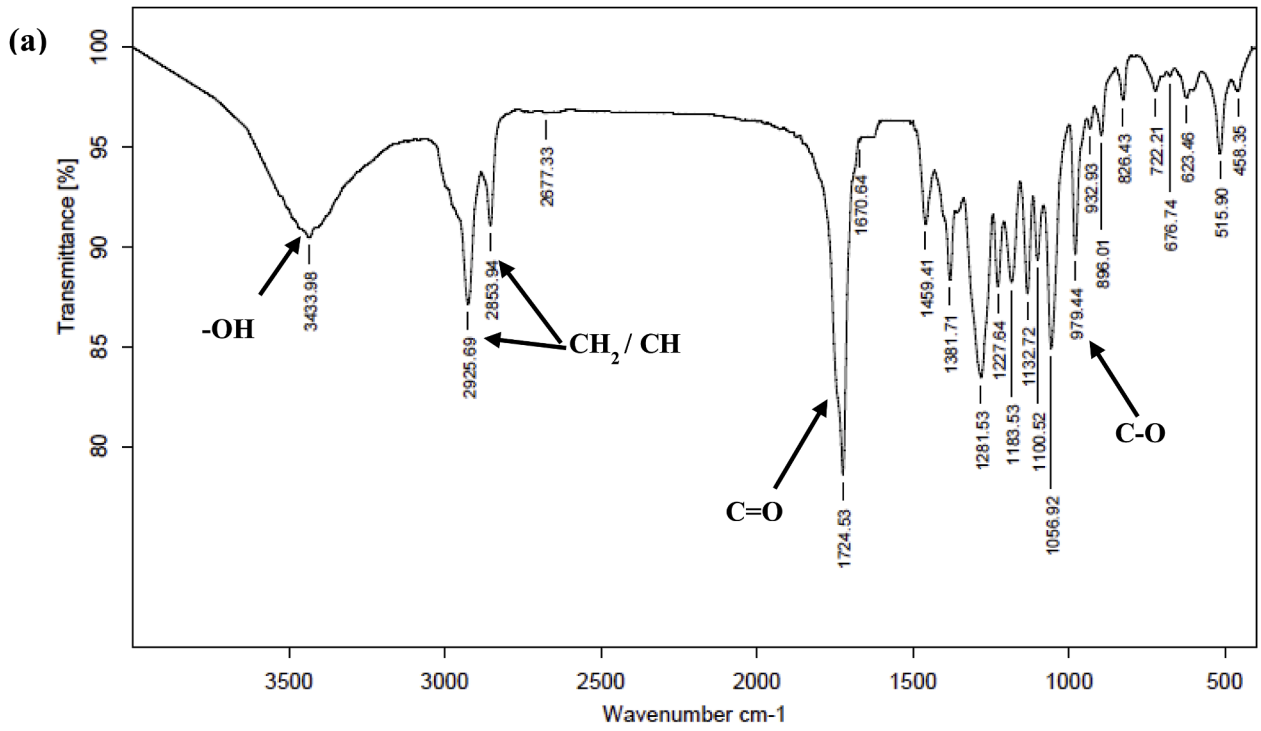
3. Results and discussion

3.1. Factors affecting biosurfactant production

Carbon sources are important factor for biosurfactant production of microbial. The type of carbon substrate used for biosurfactant production has been reported to influence both the quality and quantity of biosurfactants (Das et al., 2009; Abouseoud et al., 2008). *S. acidaminiphila* TW3 was cultivated in MSM supplemented with different carbon source: glucose, CS, molasses, UPO, crude glycerol and palm oil soapstock. The highest dry cell weight of *S. acidaminiphila* TW3 obtained were found not significantly difference when using molasses (1.25 g/L) and UPO (1.11 g/L) as the carbon source ($p > 0.05$). However, the highest surface tension reduction value was obtained when UPO (39.7%) was used as carbon substrate (Table 1). Biosurfactant production of microbes by secreted either extracellular or attached to parts of cells, predominantly during growth on water-insoluble substrates (Desai and Banat, 1997). Although sugars are easily utilized by various microbes for cell growth because the solubility of sugar in water is higher than that of other carbon sources. However, the decreased of cell growth and biosurfactant production were observed when using CS and glucose as carbon substrate due to the decrease of pH of the medium. This phenomenon probably because the microbe produced secondary acid metabolites such as uronic acid (Healy et al., 1996). In addition, the decrease of pH affected on biosurfactant causing precipitate which correlate with poor surface tension (Das et al., 2014). These results showed that UPO was the most suitable carbon source supporting biosurfactant production by *S. acidaminiphila* TW3 probably due to *S. acidaminiphila* TW3 was isolated from sediment contaminated with palm oil soapstock. *S. acidaminiphila* TW3 preferred to use water-insoluble carbon source for producing biosurfactant more than water-soluble carbon source. This obtained result was in accordance with previous result reported by Gargouri et al. (2017) who confirmed that *Stenotrophomonas* sp. B-2 was able to produce biosurfactants when growing on water-insoluble substrates. Accordingly, UPO was chosen for further studies for biosurfactant production by *S. acidaminiphila* TW3.

Nitrogen sources play an important role in the production of biosurfactant by microorganisms. In this study, *S. acidaminiphila* TW3 was cultivated in MSM containing UPO as a carbon source supplemented with different nitrogen source (1.0 g/L): $NaNO_3$, $(NH_4)_2SO_4$, urea, yeast extract, MSG and peptone. The highest dry cell weight of *S. acidaminiphila* TW3 was not difference when using MSG (1.01 g/L), urea (1.15 g/L) and $NaNO_3$ (1.15 g/L) as a nitrogen source ($p > 0.05$). However, the highest surface tension reduction (38.8%) was obtained when using $NaNO_3$ as a nitrogen source (Table 1). *S. acidaminiphila* can produce nitrate reductase for reduce nitrate to nitrogen source (Assih et al., 2002). Nitrate first undergoes dissimilatory nitrate reduction to ammonium and followed assimilation by glutamine-glutamate metabolism was occurred when using $NaNO_3$ as a nitrogen source and also stimulated biosurfactant production (Rashedi et al., 2005). Mulligan and Gibbs (1989) reported that $NaNO_3$ had an effected for glutamine synthetase activity and also enhanced biosurfactant production by *Pseudomonas aeruginosa*. For the next experiments, $NaNO_3$ was chosen as the nitrogen source to increase biosurfactant production.

Shacking speed, initial pH and incubation temperature on biosurfactant production are also important factors on cell growth and production of secondary metabolites. Our results indicated that *S. acidaminiphila* TW3 was able to grow well at shaking speed at 150 rpm and showed the highest cell dry weight of 0.79 g/L (Table 2). However, it was found that the shaking speed at 100 and 150 rpm unaffected biosurfactant production by *S. acidaminiphila* TW3 ($p > 0.05$). The shacking speed at 150 rpm was selected for biosurfactant production. Additionally, the initial pH of medium affected on cell growth and biosurfactant production. The highest dry cell weight production by *S. acidaminiphila* TW3 was obtained when cultivated at pH 7 (0.74 g/L) and 6 (0.64 g/L) ($p > 0.05$). Moreover, the highest dry cell



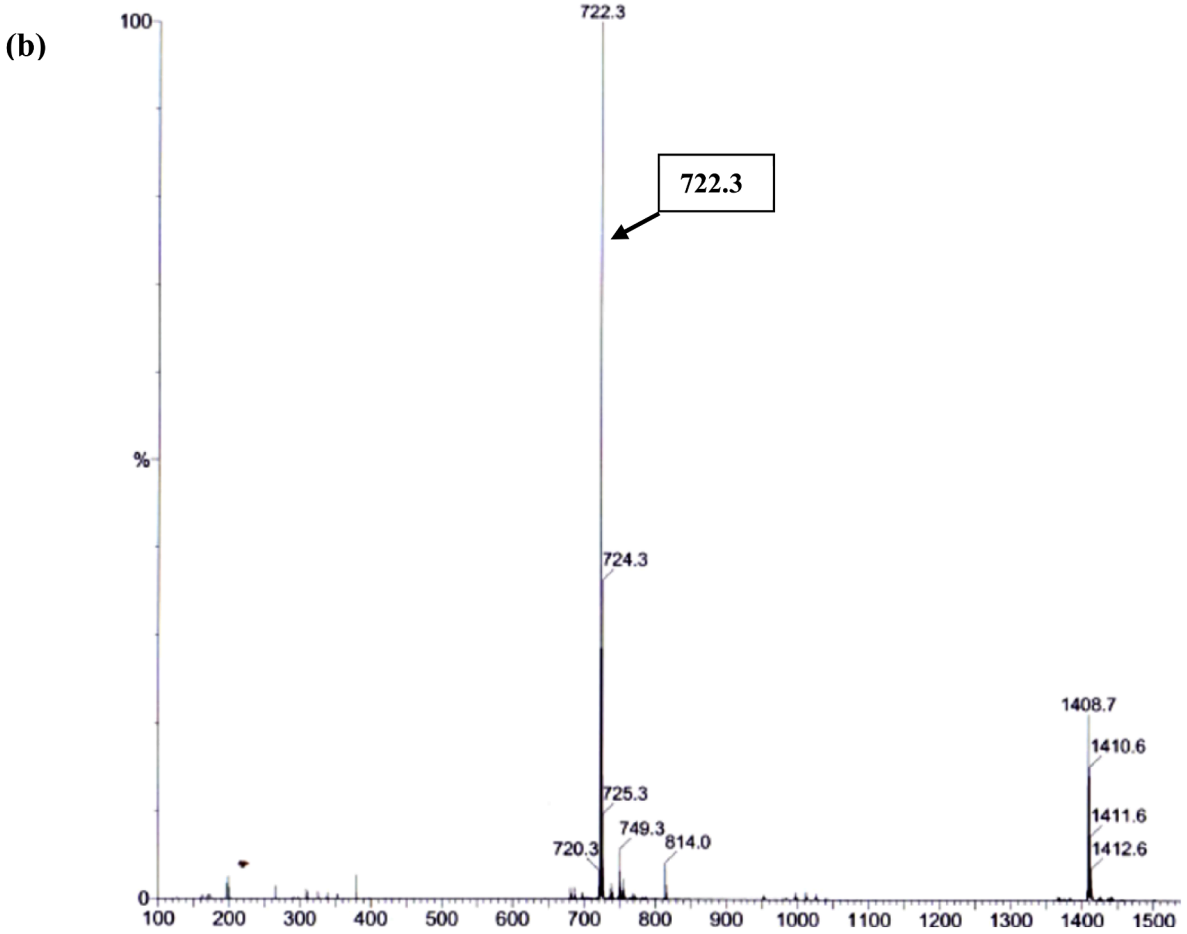


Fig. 5. The spectrum of the purified biosurfactant by *S. acidaminiphila* TW3. **a** FT-IR spectrum and **b** LC-MS spectrum.

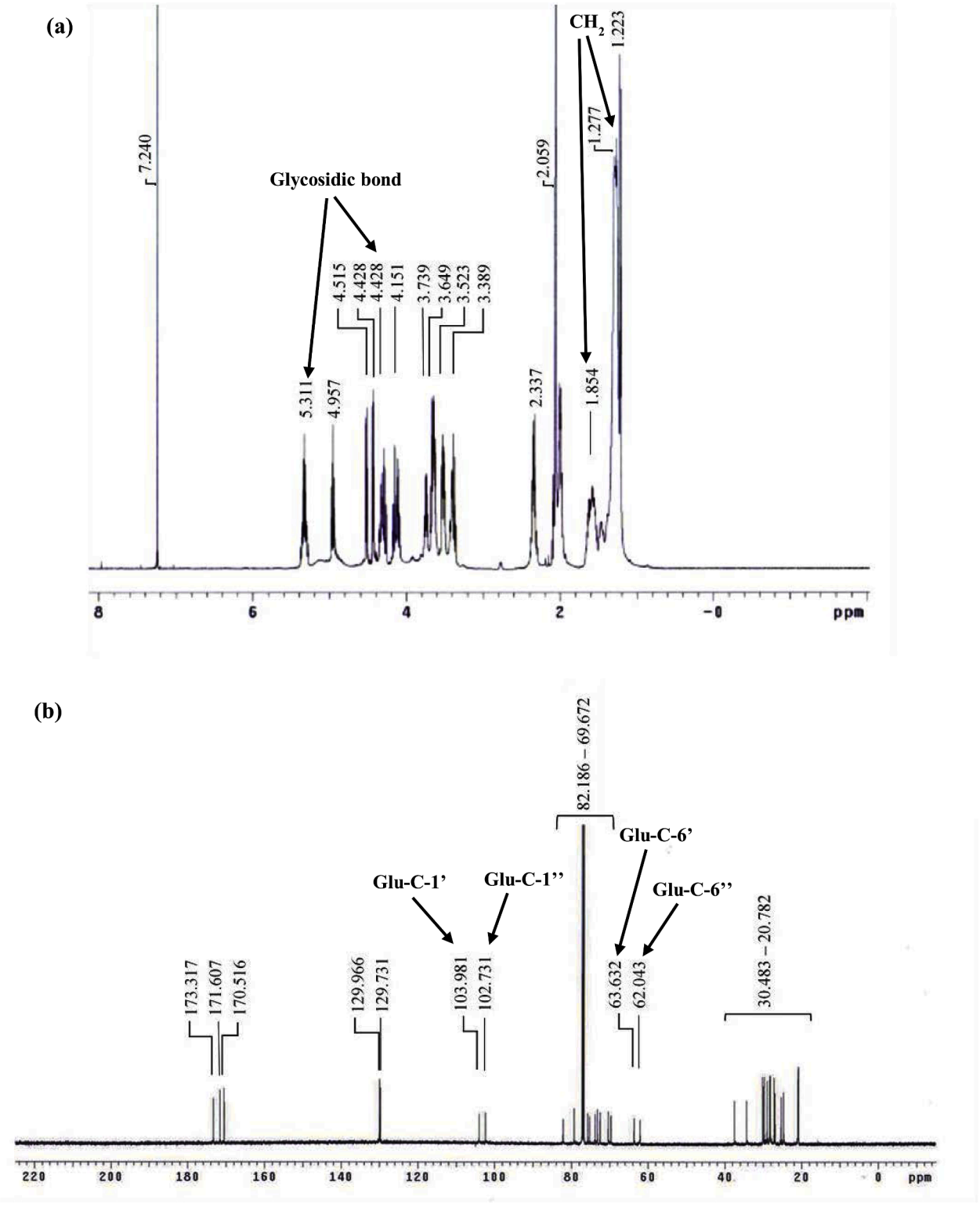


Fig. 6. NMR spectrum profile of the purified biosurfactant by *S. acidaminiphilla* TW3. **a** ^{1}H NMR, **b** ^{13}C NMR.

weight of *S. acidaminiphila* TW3 was obtained when cultivated at pH 7 (0.74 g/l) and 6 (0.64 g/l) (Table 2). This result demonstrated that *S. acidaminiphila* TW3 was able to grow well in medium initially adjusted to nearly the neutral pH. In addition, temperature is one of the critical parameters that have been controlled in bioprocess. *S. acidaminiphila* TW3 gave the highest cell dry weight of approximately 0.78 g/L and the surface tension reduction value ($p > 0.05$) at 30–37 ˚C. It was found that cell dry weight and surface tension reduction reduced when *S. acidaminiphila* TW3 was incubated at 45 ˚C. Accordingly, the incubation temperature at 30 ˚C was selected for cultivation *S. acidaminiphila* TW3.

3.2. Optimization and production of biosurfactant

Medium composition such as carbon source and nitrogen source as well as pH of medium strongly influences cell growth and the synthe-

Table 6
Effect of temperature, pH and salt concentration on surface tension (ST) of biosurfactant (BF) from *S. acidaminiphila* TW3 compared with SDS.

	ST (mN/m)***	SDS
	BF	
Temperature		
30	32.00 ± 0.0 ^{a*A**}	38.50 ± 0.5 ^{aB}
40	32.00 ± 0.0 ^{aA}	38.83 ± 0.3 ^{aB}
50	32.17 ± 0.4 ^{aA}	48.00 ± 0.5 ^{bB}
60	32.00 ± 0.0 ^{aA}	48.83 ± 0.8 ^{bcB}
70	32.33 ± 0.4 ^{aA}	48.83 ± 0.3 ^{bcB}
80	32.17 ± 0.4 ^{aA}	49.50 ± 0.5 ^{cB}
90	32.17 ± 0.4 ^{aA}	49.50 ± 0.5 ^{cB}
100	32.33 ± 0.4 ^{aA}	49.33 ± 0.6 ^{cB}
110	32.33 ± 0.4 ^{aA}	49.00 ± 0.5 ^{cB}
121	32.00 ± 0.0 ^{aA}	49.50 ± 0.5 ^{cB}
pH		
2	36.83 ± 0.3 ^{dA}	51.00 ± 1.0 ^{bB}
3	36.83 ± 0.3 ^{dA}	49.67 ± 0.3 ^{bB}
4	35.17 ± 0.3 ^{cA}	49.67 ± 0.3 ^{bB}
5	33.83 ± 0.3 ^{bA}	39.50 ± 0.5 ^{aB}
6	32.00 ± 0.0 ^{aA}	39.17 ± 0.8 ^{aB}
7	32.17 ± 0.3 ^{aA}	39.33 ± 0.6 ^{aB}
8	31.67 ± 0.6 ^{aA}	39.50 ± 0.5 ^{aB}
9	31.33 ± 0.6 ^{aA}	53.50 ± 0.5 ^{cB}
10	31.17 ± 0.6 ^{aA}	56.00 ± 0.5 ^{dB}
11	34.33 ± 0.3 ^{bcA}	60.83 ± 0.3 ^{eB}
12	37.67 ± 0.3 ^{dA}	62.67 ± 0.3 ^{fB}
	ST (mN/m)***	
	BF	SDS
NaCl (%)		
1	32.00 ± 0.0 ^{aA}	39.33 ± 0.3 ^{acB}
2	32.33 ± 0.3 ^{abA}	38.50 ± 0.9 ^{abB}
3	32.00 ± 0.0 ^{aA}	38.33 ± 0.3 ^{aB}
4	32.33 ± 0.3 ^{abA}	39.67 ± 0.6 ^{abB}
5	32.67 ± 0.3 ^{bA}	39.83 ± 0.3 ^{bB}
$MgCl_2$(%)		
0.2	32.50 ± 0.0 ^{aA}	39.17 ± 0.3 ^{abB}
0.4	32.17 ± 0.3 ^{aA}	38.33 ± 0.8 ^{aB}
0.6	32.17 ± 0.3 ^{aA}	39.00 ± 0.9 ^{abB}
0.8	32.33 ± 0.6 ^{aA}	39.83 ± 0.3 ^{aB}
1.0	32.83 ± 0.3 ^{aA}	39.33 ± 0.8 ^{abB}
$CaCl_2$(%)		
0.2	32.50 ± 0.3 ^{aA}	39.17 ± 0.8 ^{aB}
0.4	32.17 ± 0.3 ^{aA}	40.67 ± 0.6 ^{bB}
0.6	32.17 ± 0.3 ^{aA}	42.50 ± 0.5 ^{cB}
0.8	32.33 ± 0.6 ^{aA}	42.67 ± 0.3 ^{cB}
1.0	33.17 ± 0.3 ^{bA}	43.0 1.0 ^{cB}

* Different letters in the same column within the same parameter studied indicate significant differences ($p < 0.05$).** Different letters in the same row within the same parameter studied indicate significant differences ($p < 0.05$).***Values are given as mean ± SD from triplicate determinations.

sis of biosurfactants (Santos et al., 2002). Accordingly, optimization of these parameters can improve the bacterial efficiency for biosurfactant production. The RSM is generally utilized as a statistical design to model the biosurfactant production and their interaction. Range of parameters of UPO, $NaNO_3$ and pH for biosurfactant production are shown in Table 3. As a result, 20 experiments were performed in which for each experiment two additional runs were done to estimate an error for reproducibility (Table 4). The culture samples were collected at 48 h. Design-Expert version 7 suggested a quadratic equation for increasing of biosurfactant production as follow:

$$BF = 5.20545–0.87659(A) - 0.09241(B) + 0.46557 (C) + 0.00472(A)(B) - 0.09538(A)(C) + 0.014234(B) (C) + 0.058939(A2) + 0.000456(B2) + 0.064369(C2) \quad (5)$$

Where A, B and C coded values pertaining to the pH, UPO and $NaNO_3$, respectively.

ANOVA results of the quadratic model could adequately be used to describe the biosurfactant production under a wide range of operating conditions (Table 5). In this case, A, B, C, AB, AC, BC, A^2, B^2 and C^2 were identified as significant terms. For the fit model, there was expressed with R^2 value of 0.9947 and model could explain that 99.47% of the variability in the response. The model was significantly with $p < 0.0001$. The adjusted R^2 value of the model was 0.99 and predicted R^2 value was 0.9673. The adjusted R^2 value was 0.99 which supported the accuracy of the model. In biosurfactant production model, the Pred R^2 of 0.9673 is in reasonable agreement with the Adj R^2 (0.99).

Three dimensional response surface curves were plotted to study the interaction of variables on biosurfactant production. The biosurfactant production related with the factor of pH (A), UPO (B) and $NaNO_3$ (C) through the quadratic model in equation (5). Fig. 1a showed the interaction effect of pH (A) and UPO (B) on biosurfactant production at fixed $NaNO_3$ concentration of 2.25 mg/L. The biosurfactant production increased when UPO was the highest used (50 g/L) while pH was near 6. However, the biosurfactant production by the *S. acidaminiphila* TW3 decreased when alkaline condition and the lowest UPO was used. Fig. 1b showed the effect of $NaNO_3$ (C) and pH (A) on biosurfactant production on fixed UPO of 35.00 mg/L. The increasing of $NaNO_3$ concentration at low pH leads to high biosurfactant production. On the other hand, the biosurfactant deceased when $NaNO_3$ concentration was the lowest used (1.5 g/L). Fig. 1c revealed the interaction effect of UPO (B) and $NaNO_3$ (C) on biosurfactant production at fixed pH of 7. Biosurfactant production gradually increased with increasing $NaNO_3$ concentration and UPO concentration reaching a final plateau value near 3 g/L for $NaNO_3$ and 50 g/L for UPO.

The highest biosurfactant produced by *S. acidaminiphila* TW3 was obtained when using the main three factors as follow: UPO (49.96 g/L), $NaNO_3$ (3.00 g/L) and pH (6). The predicted maximum yield of the biosufactant was 2.44 g/L. The verification test was compared with the predicted values obtained from the model. In the optimum conditions, the actual yield of biosufactant (2.31 g/L) was obtained. The agreement between predicted value and verification test of biosurfactant confirms the significance of the model. In this confirmatory run, the errors between the results obtained from validation experiment was 5% for biosurfactant production. In conclusion, the biosurfactant production by *S. acidaminiphila* TW3 increased to 2.1 folds higher than that of the original (1.1 g/L). Therefore, RSM can effectively optimize the production of the biosufactant. However, the productivity of biosurfactant by *S. acidaminiphila* TW3 is still very low further improvement is needed. Few reports have been published on the use of used vegetable oil as substrates for biosurfactant production. Haba et al. (2000) studied rhamnolipid production by *Pseudomonas aeruginosa* 47T2 using waste flying oils, and a 2.7 g/L rhamnolipid could be achieved. Oliveira et al. (2009) found that *Pseudomonas alcaligenes* produced higher amount of biosurfactant using medium supplemented with 3% (v/v) palm oil as a sole source of carbon and energy.

The growth kinetics and biosurfactant production of the *S. acidaminiphila* TW3 are shown in Fig. 2. *S. acidaminiphila* TW3 started to produce biosurfactant from the beginning of the exponential growth phase and continued all over the stationary phase. During log phase, surface tension of culture medium rapidly decreased and reached a minimum surface tension (32 mN/m) as the cultivation time approached 18 h. The biosurfactant yield reached a maximum (2.31 g/L) at a cultivation time of 48 h. *S. acidaminiphila* TW3 reached stationary growth phase after 48 h of incubation. Thus, biosurfactant production by *S. acidaminiphila* TW3 is correlated with microbial growth. Growth-associated production of biosurfactant has also been described in growth kinetics of *Serratia marcescens* (Roldán-Carrillo et al., 2011),

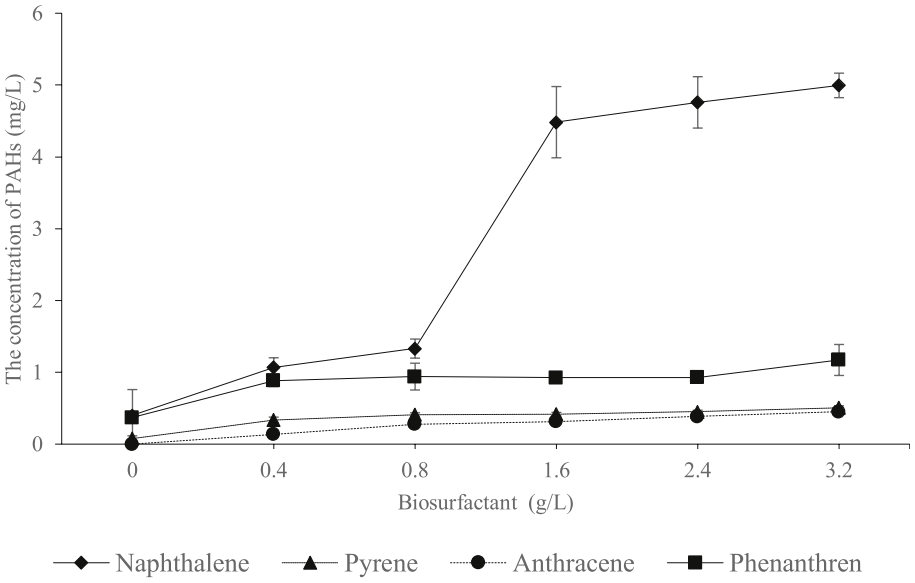


Fig. 7. Effect of biosurfactant concentration produced by *S. acidaminiphila* TW3 on solubilization of PAHs (naphthalene, pyrene, anthracene and phenanthrene) (Bars indicate standard derivation from triplicate determinations).

Bacillus sp. (Heryani and Putra, 2017) and *Pseudomonas* sp. (Arino et al., 1996).

3.3. Recovery and purification of biosurfactant

The biosurfactant was recovered from the culture supernatant using acid precipitation (pH 2–4), salt precipitation (50%–80% ammonium sulfate), solvent extraction (chloroform/methanol, 2:1) and acid precipitation (pH 2) followed by solvent extraction. Among four recovery systems, acid precipitation (pH 2) followed by solvent extraction (chloroform/methanol, 2:1) exhibited a greater of surface tension (32 mN/m) and low CMC (0.2 g/l) (Fig. 3). Biosurfactant with lower CMC value is considered to be more efficient due to a smaller amount of biosurfactant is needed to decrease the surface tension (Domingues et al., 2017). It can be concluded that the acid precipitation followed by solvent extraction was the best method for recovery of biosurfactant from supernatant of *S. acidaminiphila* TW3. The multi-step recovery for biosurfactant will be possible to obtain the product at any required degree of purity. Fig. 3 shows the surface tension decreased from 72 mN/m (for DI water) to a minimum value of 32 mN/m as the biosurfactant concentration was increased. Accordingly, the CMC of this biosurfactant was 0.2 g/l. The biosurfactant obtained from *S. acidaminiphila* TW3 showed a lower CMC value than that of the biosurfactant from *Pseudomonas aeruginosa* S6 (0.86 g/l) (Suryanti et al., 2010), *Pseudomons aeruginosa* UCP0992 (0.7 g/l) (Silva et al., 2010) and *Rhodococcus* sp. PML026 (0.25 g/l) (Wei et al., 2005).

The obtained biosurfactant was spotted on TLC plate. It was detected by anisaldehyde and copper sulfate reagent, indicating the presence of carbohydrate (Fig. 4a) and lipid (Fig. 4b) in the biosurfactant compound. Afterwards, it was purified by column chromatography using reverse phase column chromatography (C8). Fractions 1–10 and fractions 11–30 were eluted with 50% acetonitrile and 100% acetonitrile, respectively. All fractions were checked on TLC plate, the fractions containing the same spot were combined and concentrated. Two compounds (Rf 0.5, 0.7) had biosurfactant activity when tested with oil displacement test. The compound of Rf 0.7 (fraction 6) and Rf 0.5 (fraction 14–15) had the diameter of clear zone of 45 mm and 33 mm, respectively (Fig. 4c). Accordingly, compound with Rf 0.7 was chosen for further characterization.

3.4. Chemical characterization of the biosurfactant

The molecular composition of the purified biosurfactant (the compound of Rf 0.7) was first evaluated by FTIR. It showed major peak at 3433 cm^{-1} indicated the presence of O–H stretching vibrations (Fig. 5a). CH_2/C–H asymmetric vibrations were found at 2925 and 2853 cm^{-1} which confirmed the presence of alkanes (C–H). The presence of a C═O, and C–O bond was found at 1724 and 1132 cm^{-1}, respectively. CH and CH_2 deformation was found at 826 and 722 cm^{-1}. The FT-IR spectra of biosurfactant was nearly the same as those reported for other glycolipid biosurfactant produced by *Streptomyces* sp. MAB36 (Manivasagan et al., 2014) and *Bacillus* sp. Lz-2 (Li et al., 2015). The above structure of biosurfactant obtained was fully supported by its mass spectrometric analysis. Analysis of the intact molecules with LCQ-MS revealed four molecular ion peaks with molecular masses $[M^+H]^+$ of 722 (Fig. 5b). This finding was in accordance with Abdel-Mawgoud et al. (2010) who reported glycolipid has molecular mass in ranging of 302–803 Da. The differences of the composition of the glycolipid were based on the amount of unsaturated fatty acids in the substrate (Benincasa and Accorsini, 2008). To confirm the structure of the glycolipid, ^1H and ^{13}C NMR of sample allowed of assign the molecule to the sugar and fatty acid moieties. Fig. 6a shows the ^1H NMR spectrum of the biosurfactant from *S. acidaminiphila*, demonstrating two well-defined regions. The spectrum confirms the presence of a long aliphatic chain (CH_2 at 1.85–1.25 ppm). The signals of 4.95 and 5.31 ppm attributed to the glycosidic bond of the two sugar molecules. The signals at 7.2 ppm attributed to the residual signal of the solvent ($CDCl_3$). Fig. 6b is a ^{13}C NMR spectrum of the biosurfactant, which shows the presence of two =CH- groups in the fatty acid chain moiety corresponding to signals at 129.731 ppm. In addition, several –CH_2- groups in the fatty chain moiety resonated at 20.78–30.48 ppm. The spectrum also revealed signals of glucose-C-1′ at 103.98 ppm, glucose-C-1″ at 102.34 ppm, glucose-C-6′ at 63.92 ppm and glucose-C-6″ at 62.29 ppm; the other carbon atoms of glucose resonated between 69.67 and 82.67 ppm. This information from NMR confirmed the glycolipid nature of the biosurfactant. Similar characteristic spectral peaks of NMR were also observed in biosurfactant obtained from different sources (Raza et al., 2009). To our best knowledge this is the first report of production of the glycolipid from *Stenotrophomonas acidaminiphila*.

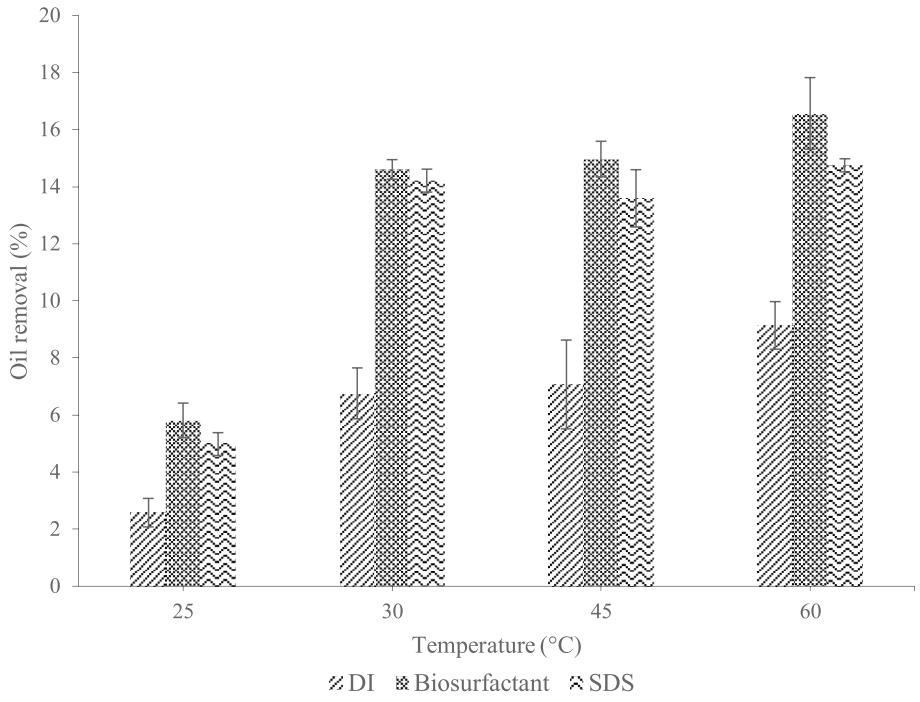


Fig. 8. Microbial enhanced used lubricating oil removal of the biosurfactant (0.8 mg/L) produced by *S. acidaminiphila* TW3 under difference temperatures (Bars indicate standard derivation from triplicate determinations).

3.5. Study of biosurfactant stability

The application of biosurfactants have potential as alternatives in a variety of applications depends on their stability at different temperature, pH, and salinity. The results of stability study between the biosurfactant from *S. acidaminiphila* TW3 in comparison with SDS are shown in Table 6. It was found that the surface tension of biosurfactant produced by *S. acidaminiphila* TW3 was thermostable at 30–121 ˚C ($p > 0.05$). The surface tension of SDS was increase at higher temperatures (50–121 ˚C). The biosurfactant was found to be more effective than SDS in decreasing the surface tension of water as well as thermal stability. Therefore, it can be concluded that this biosurfactant maintains its surface tension properties in the range of temperatures between 30 and 121 ˚C. Glycolipid biosurfactant stability at extreme temperatures was also reported by Singh and Tiwary (2016) and White et al. (2013) for *Pseudomonas otitidis* P4 and *Rhodococcus* sp. PML026, respectively.

The pH stability of biosurfactant was studied in range of pH 2–12. The surface tension of the biosurfactant remained relatively stable to pH changes between pH 6–10 (Table 6). The activity of biosurfactant decreased obviously with decreasing pH (<5). While, surface tension of SDS was stable between pH 5–8. It was found that the activity of both biosurfactant and SDS could reduce surface tension of water in alkaline condition. These results indicate that increasing pH has a positive effect on biosurfactant activity. This could be caused by a better stability of fatty acid surfactant micelles in the presence of NaOH at higher pH values (Khopade et al., 2012).

The effect of salts on the stability of biosurfactant was also studied. The surface tensions of the biosurfactant and SDS were stable in 1–4% (w/v) of NaCl ($p > 0.05$). Little changes of surface tension were observed in increased concentration of NaCl up to 5% (Table 6). The surface tension of biosurfactant and SDS were stable in 0.2–1% of $MgCl_2$ ($p > 0.05$). Additionally, the biosurfactant activity was stable in 0.2–0.8% (w/v) of $CaCl_2$, while SDS activity significantly decreased when increasing concentration of $CaCl_2$. Salt in micellar solution shows both effect of electrostatic repulsion and competitive counter ion condensation (Maiti et al., 2009). Moreover, the addition of more $CaCl_2$ have radical effect on the micellization phenomenon (Maiti et al., 2009). The similar finding report of these results indicate the good compound stability for this biosurfactant at high ionic strength environment (Ayed et al., 2014). Thus, temperature, pH and ionic strength experiments are aligned about the stability of produced biosurfactant, which suggest the potential application of the obtained biosurfactant in the extreme environment.

The biosurfactant was found to retain surface active properties under the extreme conditions. The biosurfactant exhibited excellent stability over the evaluated range of temperature, pH and salinity which indicating possibility of its usage under specific environmental conditions. The obtained results were in accordance with Sen et al. (2017) studied sophorolipid produced by *Rhodotorula babjevae* YS3. The stability of the sophorolipid was estimated over a wide range of pH (2–10), salinity (2–10% NaCl) and temperature (at 120 ˚C for time intervals of 30 up to 120 min). In addition Luna et al. (2013) reported that the properties of sophorolipid produced by *Candida sphaerica* UCP0995 from industrial wastes showed stable surface tension reduction and emulsifying activity at different pH (2-12), temperature (5–120 ˚C), and NaCl concentrations (2–10%).

3.6. Application of the biosurfacant for enhancement of polycyclic aromatic hydrocarbon (PAHs) solubility

PAHs are widespread environmental pollutants that are environmentally persistent with various structures and varied toxicity. PAHs are low solubility in water but are very soluble in most organic solvents because they are highly lipophilic (Abdel-Shafy and Mansour, 2016). In the present study, the enhancement of PAHs solubility by biosurfactant from *S. acidaminiphila* TW3 is shown in Fig. 7. The increasing biosurfactant concentration in range 0–3.2 mg/L significantly increased ($p < 0.05$) of PAHs solubility compared to the control (DI water). It was found that biosurfactant influence naphthalene solubility more than phenanthrene, anthracene and pyrene. Considering the molecular size of PAHs, they are composed of multiple aromatic ring. A naphthalene structure consists of a fused pair of benzene rings and low molecular weight. For most PAHs, an increase in molecular

weight and angularity of the PAH molecule are correlated with increased hydrophobicity and electrochemical stability. Hydrophobicity and molecular stability are factors that contribute to the persistence of PAHs in the environment (Makkar and Rockne, 2003). The concentrations of biosurfactant was 3.2 mg/L (4 times of CMC) showed the highest naphthalene solubility at 4.4 mg/L (12 times higher than control). In addition, the glycolipid was able to solubilize pyrene, phenantrene and anthracene was 6.5, 3 and 4 times higher than control treatment, respectively. The results indicate that the presence of biosurfactant has a significant impact on solubilization of PAHs. When the concentration is above the CMC, hydrophobic pollutants can readily partition into the hydrophobic core at the center of a micelle, thus increasing hydrophobic organic compounds aqueous concentration through micelle solubilization (Zhou and Zhu, 2007; Bezza and Nkhalambayausi Chirwa, 2015).

3.7. Application of the biosurfactant in used lubricating oil removal from contaminated sand

Used lubricating oils (ULOs) are classified as hazardous wastes which the ability of ULO to adsorb and entrained within the pore space surrounding the soil grains. These hinders the removal and degradation of these compounds (Guiyun et al., 1997). In the present study, the ability of biosurfactant to enhance ULO removal from contaminated sand was examined in comparison with those of synthetic surfactant (SDS). With the increasing temperatures, both SDS and biosurfactant showed a significant increase ($p < 0.05$) removal of ULO from contaminated sand. Biosurfactant was able to recovery 5.7% of ULO from contaminated sand at 25 ˚C, 15% at room temperature (30 ± 2 ˚C), 15% at 45 ˚C and 17% at 60 ˚C (Fig. 8). The synthetic surfactant (SDS) was found to be less efficient. In the case of control (DI water), the recovery rate only 5–9% could be obtained in all temperatures tested. These results have implications for the potential use of a biosurfactant produced by *S. acidaminiphila* TW3 to enhance ULO removal from environment. However, it is important not to rule out that efficiency of the removal could be vary depending on the characteristic of the contaminants and site characteristics (Silva et al., 2010). Waste ULO hydrocarbon compounds bind to soil components and are difficult to remove and degrade. Biosurfactants can emulsify hydrocarbons for enhancing their water solubility, decreasing surface tension and increase the displacement of oil substances from soil particles (Sobrinho et al., 2008). In previous studies, a biosurfactant from *Pseudomonas cepacia* CCT6659 demonstrated the ability to remove approximately 12–16% of motor oil impregnated in sand (Rocha e Silva et al., 2014). In addition, a biosurfactant produced by *P. aeruginosa* showed high percentage removals of diesel from sand samples, but lower rates (less than 20%) were found when petroleum was tested (Silva et al., 2010).

4. Conclusions

In the present study, the optimization of the biosurfactant production from *S. acidaminiphila* TW3 which was isolated from soil contaminated with soapstocks is reported. The growth characteristics were obtained and studies on the properties of the biosurfactant indicate the possibility of its environmental and industrial application. The spectra obtained from FT-IR spectroscopy, NMR, and GC-MS confirmed the present of glycolipid in the sample. The potential of obtained biosurfactant for environmental and industrial used was shown by studying its physical properties such as the surface tension and CMC as well as its stability to harsh environmental conditions like salinity, pH and temperature. The properties of the obtained biosurfactant have potential applications especially for microbial enhance oil recovery and/or reducing the intensity of environmental conditions.

CRediT authorship contribution statement

Theerawat Onlamool: Conceptualization, Methodology, Data curation, Writing - original draft. **Atipan Saimmai:** Visualization, Validation. **Naruemon Meeboon:** Investigation, Software. **Suppasil Maneerat:** Supervision, Visualization, Writing - review & editing.

Declaration of competing interest

All authors read, approved the manuscript and declare that there is no conflict of interest.

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References

Abdel-Mawgoud, A.M., Lépine, F., Déziel, E., 2010. Rhamnolipids: diversity of structures, microbial origins and roles. Appl. Microbiol. Biotechnol. 86, 1323–1336.
Abdel-Shafy, H.I., Mansour, M.S.M., 2016. A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. Egypt. J. Pet. 25, 107–123.
Abouseoud, M., Maachi, R., Amrane, A., Boudergua, S., Nabi, A., 2008. Evaluation of different carbon and nitrogen sources in production of biosurfactant by *Pseudomonas fluorescens*. Desalination 223, 143–151.
Accorsini, F.R., Mutton, M.J.R., Lemos, E.G.M., Benincasa, M., 2012. Biosurfactants production by yeasts using soybean oil and glycerol as low cost substrate. Braz. J. Microbiol. 43, 116–125.
Arino, S., Marchal, R., Vandecasteele, J.-P., 1996. Identification and production of a rhamnolipidic biosurfactant by a *Pseudomonas* species. Appl. Microbiol. Biotechnol. 45, 162–168.
Assih, E.A., Ouattara, A.S., Thierry, S., Cayol, J.-L., Labat, M., Macarie, H., 2002. *Stenotrophomonas acidaminiphila* sp. nov., a strictly aerobic bacterium isolated from an upflow anaerobic sludge blanket (UASB) reactor. Int. J. Syst. Evol. Microbiol. 52, 559–568.
Ayed, H.B., Jridi, M., Maalej, H., Nasri, M., Hmidet, N., 2014. Characterization and stability of biosurfactant produced by *Bacillus mojavensis* A21 and its application in enhancing solubility of hydrocarbon. J. Chem. Technol. Biotechnol. 89, 1007–1014.
Benincasa, M., 2007. Rhamnolipid produced from agroindustrial wastes enhances hydrocarbon biodegradation in contaminated soil. Curr. Microbiol. 54, 445–449.
Benincasa, M., Accorsini, F.R., 2008. *Pseudomonas aeruginosa* LBI production as an integrated process using the wastes from sunflower-oil refining as a substrate. Bioresour. Technol. 99, 3843–3849.
Bezza, F.A., Nkhalambayausi Chirwa, E.M., 2015. Biosurfactant from *Paenibacillus dendritiformis* and its application in assisting polycyclic aromatic hydrocarbon (PAH) and motor oil sludge removal from contaminated soil and sand media. Process Saf. Environ. Protect. 98, 354–364.
Chaprão, M.J., Ferreira, I.N.S., Correa, P.F., Rufino, R.D., Luna, J.M., Silva, E.J., Sarubbo, L.A., 2015. Application of bacterial and yeast biosurfactants for enhanced removal and biodegradation of motor oil from contaminated sand. Electron. J. Biotechnol. 18, 471–479.
Churchward, M.A., Brandman, D.M., Rogasevskaia, T., Coorssen, J.R., 2008. Copper (II) sulfate charring for high sensitivity on-plate fluorescent detection of lipids and sterols: quantitative analyses of the composition of functional secretory vesicles. J. Chem. Biol. 1, 79–87.
Das, P., Mukherjee, S., Sen, R., 2009. Substrate dependent production of extracellular biosurfactant by a marine bacterium. Bioresour. Technol. 100, 1015–1019.
Das, P., Yang, X.-P., Ma, L.Z., 2014. Analysis of biosurfactants from industrially viable *Pseudomonas* strain isolated from crude oil suggests how rhamnolipids congeners affect emulsification property and antimicrobial activity. Front. Microbiol. 5.
De Almeida, D.G., Soares Da Silva, R. de C.F., Luna, J.M., Rufino, R.D., Santos, V.A., Banat, I.M., Sarubbo, L.A., 2016. Biosurfactants: promising molecules for petroleum biotechnology advances. Front. Microbiol. 7, 1718.
Desai, J.D., Banat, I.M., 1997. Microbial production of surfactants and their commercial potential. Microbiol. Mol. Biol. Rev. 61, 47–64.
Domingues, P.M., Almeida, A., Serafim Leal, L., Gomes, N.C.M., Cunha, Â., 2017. Bacterial production of biosurfactants under microaerobic and anaerobic conditions. Rev. Environ. Sci. Biotechnol. 16, 239–272.
Gargouri, B., Contreras, M. del M., Ammar, S., Segura-Carretero, A., Bouaziz, M., 2017. Biosurfactant production by the crude oil degrading *Stenotrophomonas* sp. B-2: chemical characterization, biological activities and environmental applications. Environ. Sci. Pollut. Res. 24, 3769–3779.
Gudiña, E.J., Rangarajan, V., Sen, R., Rodrigues, L.R., 2013. Potential therapeutic applications of biosurfactants. Trends Pharmacol. Sci. 34, 667–675.

Guiyun, B., Brusseau, M.L., Miller, R.M., 1997. Biosurfactant-enhanced removal of residual hydrocarbon from soil. J. Contam. Hydrol. 25, 157–170.
Haba, E., Espuny, M.J., Busquets, M., Manresa, A., 2000. Screening and production of rhamnolipids by *Pseudomonas aeruginosa* 47T2 NCIB 40044 from waste frying oils. J. Appl. Microbiol. 88, 379–387.
Healy, M.G., Devine, C.M., Murphy, R., 1996. Microbial production of biosurfactants. Resour. Conserv. Recycl. Glob. Environ. Biotechnol. 18, 41–57.
Heryani, H., Putra, M.D., 2017. Kinetic study and modeling of biosurfactant production using *Bacillus* sp. Electron. J. Biotechnol. 27, 49–54.
Ibrahim, M.L., Ijah, U.J.J., Manga, S.B., Bilbis, L.S., Umar, S., 2013. Production and partial characterization of biosurfactant produced by crude oil degrading bacteria. Int. Biodeterior. Biodegrad. Spec. Iss. 81, 28–34 3rd International Symposium on Applied Microbiology and Molecular Biology in Oil Systems.
Joy, S., Rahman, P.K.S.M., Sharma, S., 2017. Biosurfactant production and concomitant hydrocarbon degradation potentials of bacteria isolated from extreme and hydrocarbon contaminated environments. Chem. Eng. J. 317, 232–241.
Karanth, N.G.K., Deo, P.G., Veenanadig, N.K., 1999. Microbial production of biosurfactants and their importance. Curr. Sci. 77, 116–126.
Kaskatepe, B., Yildiz, S., Kaskatepe, B., Yildiz, S., 2016. Rhamnolipid biosurfactants produced by *Pseudomonas* species. Braz. Arch. Biol. Technol. 59.
Khopade, A., Biao, R., Liu, X., Mahadik, K., Zhang, L., Kokare, C., 2012. Production and stability studies of the biosurfactant isolated from marine *Nocardiopsis* sp. B4. Desalination 285, 198–204.
Kim, H.-S., Yoon, B.-D., Lee, C.-H., Suh, H.-H., Oh, H.-M., Katsuragi, T., Tani, Y., 1997. Production and properties of a lipopeptide biosurfactant from *Bacillus subtilis* C9. J. Ferment. Bioeng. 84, 41–46 The Society for Fermentation and Bioengineering.
Kiran, G.S., Thomas, T.A., Selvin, J., 2010. Production of a new glycolipid biosurfactant from marine *Nocardiopsis lucentensis* MSA04 in solid-state cultivation. Colloids Surf. B Biointerfaces 78, 8–16.
Kumar, A.P., Janardhan, A., Viswanath, B., Monika, K., Jung, J.-Y., Narasimha, G., 2016. Evaluation of orange peel for biosurfactant production by *Bacillus licheniformis* and their ability to degrade naphthalene and crude oil. 3 Biotech 6, 43.
Lang, S., 2002. Biological amphiphiles (microbial biosurfactants). Curr. Opin. Colloid Interface Sci. 7, 12–20.
Li, S., Pi, Y., Bao, M., Zhang, C., Zhao, D., Li, Y., Sun, P., Lu, J., 2015. Effect of rhamnolipid biosurfactant on solubilization of polycyclic aromatic hydrocarbons. Mar. Pollut. Bull. 101, 219–225.
Luna, J.M., Rufino, R.D., Sarubbo, L.A., Campos-Takaki, G.M., 2013. Characterisation, surface properties and biological activity of a biosurfactant produced from industrial waste by *Candida sphaerica* UCP0995 for application in the petroleum industry. Colloids Surf. B Biointerfaces 102, 202–209.
Maiti, K., Mitra, D., Guha, S., Moulik, S.P., 2009. Salt effect on self-aggregation of sodium dodecylsulfate (SDS) and tetradecyltrimethylammonium bromide (TTAB): physicochemical correlation and assessment in the light of Hofmeister (lyotropic) effect. J. Mol. Liq. 146, 44–51.
Makkar, R.S., Rockne, K.J., 2003. Comparison of synthetic surfactants and biosurfactants in enhancing biodegradation of polycyclic aromatic hydrocarbons. Environ. Toxicol. Chem. 22, 2280–2292.
Manivasagan, P., Sivasankar, P., Venkatesan, J., Sivakumar, K., Kim, S.-K., 2014. Optimization, production and characterization of glycolipid biosurfactant from the marine actinobacterium, *Streptomyces* sp. MAB36. Bioprocess. Biosyst. Eng. 37, 783–797.
Morikawa, M., Hirata, Y., Imanaka, T., 2000. A study on the structure–function relationship of lipopeptide biosurfactants. Biochim. Biophys. Acta BBA Mol. Cell Biol. Lipids 1488, 211–218.
Mukherjee, S., Das, P., Sen, R., 2006. Towards commercial production of microbial surfactants. Trends Biotechnol. 24, 509–515.
Mulligan, C.N., Gibbs, B.F., 1989. Correlation of nitrogen metabolism with biosurfactant production by *Pseudomonas aeruginosa*. Appl. Environ. Microbiol. 55, 3016–3019.
Najafi, A.R., Rahimpour, M.R., Jahanmiri, A.H., Roostaazad, R., Arabian, D., Ghobadi, Z., 2010. Enhancing biosurfactant production from an indigenous strain of *Bacillus mycoides* by optimizing the growth conditions using a response surface methodology. Chem. Eng. J. 163, 188–194.
Oliveira, F.J.S., Vazquez, L., de Campos, N.P., de França, F.P., 2009. Production of rhamnolipids by a *Pseudomonas alcaligenes* strain. Process Biochem. 44, 383–389.
Rashedi, H., Jamshidi, E., Assadi, M.M., Bonakdarpour, B., 2005. Isolation and production of biosurfactant from *Pseudomonas aeruginosa* isolated from Iranian southern wells oil. Int. J. Environ. Sci. Technol. IJEST Heidelb. 2, 121–127.
Raza, Z.A., Khalid, Z.M., Banat, I.M., 2009. Characterization of rhamnolipids produced by a *Pseudomonas aeruginosa* mutant strain grown on waste oils. J. Environ. Sci. Health Part A Tox. Hazard. Substain. Environ. Eng. 44, 1367–1373.
Rifai, N., Horvath, A.R., Wittwer, C.T., Hoofnagle, A., 2018. Principles and Applications of Clinical Mass Spectrometry: Small Molecules, Peptides, and Pathogens. Elsevier.
Rocha e Silva, N.M.P., Rufino, R.D., Luna, J.M., Santos, V.A., Sarubbo, L.A., 2014. Screening of *Pseudomonas* species for biosurfactant production using low-cost substrates. Biocatal. Agric. Biotechnol. 3, 132–139.
Roldán-Carrillo, T., Martínez-García, X., Zapata-Peñasco, I., Castorena-Cortés, G., Reyes-Avila, J., Mayol-Castillo, M., Olguín-Lora, P., 2011. Evaluation of the effect of nutrient ratios on biosurfactant production by *Serratia marcescens* using a Box-Behnken design. Colloids Surf. B Biointerfaces 86, 384–389.
Saimmai, A., Sobhon, V., Maneerat, S., 2012. Production of biosurfactant from a new and promising strain of *Leucobacter komagatae* 183. Ann. Microbiol. 62, 391–402.
Saimmai, A., Sobhon, V., Maneerat, S., 2011. Molasses as a whole medium for biosurfactants production by *Bacillus* strains and their application. Appl. Biochem. Biotechnol. 165, 315–335.
Santos, A.S., Sampaio, A.P.W., Vasquez, G.S., Anna, L.M.S., Pereira, N., Freire, D.M.G., 2002. Evaluation of different carbon and nitrogen sources in production of rhamnolipids by a strain of *Pseudomonas aeruginosa*. Appl. Biochem. Biotechnol. 98 (100), 1025–1035.
Santos, D.K.F., Rufino, R.D., Luna, J.M., Santos, V.A., Sarubbo, L.A., 2016. Biosurfactants: multifunctional biomolecules of the 21st century. Int. J. Mol. Sci. 17, 401.
Satpute, S.K., Banpurkar, A.G., Dhakephalkar, P.K., Banat, I.M., Chopade, B.A., 2010. Methods for investigating biosurfactants and bioemulsifiers: a review. Crit. Rev. Biotechnol. 30, 127–144.
Sen, S., Borah, S.N., Bora, A., Dek, S., 2017. Production, characterization, and antifungal activity of a biosurfactant produced by *Rhodotorula babjevae* YS3. Microb. Cell Factories 16, 95.
Silva, E.J., Rocha e Silva, N.M.P., Rufino, R.D., Luna, J.M., Silva, R.O., Sarubbo, L.A., 2014. Characterization of a biosurfactant produced by *Pseudomonas cepacia* CCT6659 in the presence of industrial wastes and its application in the biodegradation of hydrophobic compounds in soil. Colloids Surf. B Biointerfaces 117, 36–41.
Silva, S.N.R.L., Farias, C.B.B., Rufino, R.D., Luna, J.M., Sarubbo, L.A., 2010. Glycerol as substrate for the production of biosurfactant by *Pseudomonas aeruginosa* UCP0992. Colloids Surf. B Biointerfaces 79, 174–183.
Singh, P., Tiwary, B.N., 2016. Isolation and characterization of glycolipid biosurfactant produced by a *Pseudomonas otitidis* strain isolated from Chirimiri coal mines, India. Bioresour. Bioprocess. 3, 42.
Sobrinho, H.B.S., Rufino, R.D., Luna, J.M., Salgueiro, A.A., Campos-Takaki, G.M., Leite, L.F.C., Sarubbo, L.A., 2008. Utilization of two agroindustrial by-products for the production of a surfactant by *Candida sphaerica* UCP0995. Process Biochem. 43, 912–917.
Solaiman, D.K.Y., Ashby, R.D., Nuñez, A., Foglia, T.A., 2004. Production of sophorolipids by *Candida bombicola* grown on soy molasses as substrate. Biotechnol. Lett. 26, 1241–1245.
Suryanti, V., Hastuti, S., Wahyuningsih, T.D., Mudasir, M., Muliawati, D.I., 2010. Biosurfactants production by *Pseudomonas aeruginosa* using soybean oil as substrate. Indones. J. Chem. 9, 107–112.
Thanomsub, B., Watcharachaipong, T., Chotelersak, K., Arunrattiyakorn, P., Nitoda, T., Kanzaki, H., 2004. Monoacylglycerols: glycolipid biosurfactants produced by a thermotolerant yeast, *Candida ishiwadae*. J. Appl. Microbiol. 96, 588–592.
Thavasi, R., Jayalakshmi, S., Balasubramanian, T., Banat, I.M., 2008. Production and characterization of a glycolipid biosurfactant from *Bacillus megaterium* using economically cheaper sources. World J. Microbiol. Biotechnol. 24, 914–925.
Wei, Y.-H., Chou, C.-L., Chang, J.-S., 2005. Rhamnolipid production by indigenous *Pseudomonas aeruginosa* J4 originating from petrochemical wastewater. Biochem. Eng. J. 27, 146–154.
White, D.A., Hird, L.C., Ali, S.T., 2013. Production and characterization of a trehalolipid biosurfactant produced by the novel marine bacterium *Rhodococcus* sp., strain PML026. J. Appl. Microbiol. 115, 744–755.
Yin, B., Gu, J.-D., Wan, N., 2005. Degradation of indole by enrichment culture and *Pseudomonas aeruginosa* Gs isolated from mangrove sediment. Int. Biodeterior. Biodegrad. 56, 243–248.
Zhou, W., Zhu, L., 2007. Efficiency of surfactant-enhanced desorption for contaminated soils depending on the component characteristics of soil-surfactant–PAHs system. Environ. Pollut. 147, 66–73.