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Production of lipopeptide biosurfactant by *Bacillus subtilis* GY19 and its application as oil-contaminated surface cleaning agent

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ABSTRACT: The present study investigated the effect of nutrient composition and pH on the lipopeptide production by *Bacillus subtilis* GY19. The maximum lipopeptide production (2.2 g/l) was achieved when the strain was grown in productive medium containing glycerol (4 g/l) and palm oil (0.75%, v/v) as substrates, sodium nitrate (0.5%, w/v) as nitrogen source, and glucose (1 g/l) and beef extract (0.5 g/l) as co-substrates with pH 7.5. In addition, the lipopeptide of *B. subtilis* GY19 could be applied for removal of slideway oil covered on metallic surface. Taguchi method was employed to evaluate the factors affecting the cleaning process. The results indicated that the presence of high levels of crude lipopeptide concentration positively affected surface washing efficacy. Further removal of slideway oil from the washing water could be achieved by the addition of immobilized oil-degrading bacterium, *Acinetobacter* sp. R2. The presence of lipopeptide increased the removal efficiency of slideway oil from 70% to 82% and did not show toxic effect on bacterial cells. This study shows promising ability of the lipopeptide from *B. subtilis* GY19 as a cleaning agent for oil-contaminated surface. In addition, it could subsequently enhance biodegradation of residual oil in the washing water.

KEYWORDS: biosurfactant, surface washing, oil removal, lipopeptide, *Bacillus*

INTRODUCTION

Biosurfactants are surface-active substances synthesized by bacteria, yeast, and filamentous fungi. They are amphipathic molecules with both hydrophilic and hydrophobic fragments preferentially partitioned at the interfaces between phases, which have different degrees of polarity and hydrogen bonding such as oil-water or air-water interfaces [1]. The biosurfactants produced by bacteria are classified into 4 types based on their chemical composition: glycolipids, lipopeptides, phospholipids, and polymeric surfactants [2]. Lipopeptides are the most known types of biosurfactants which are normally produced by members of *Bacillus* species and possess good surface-active characteristics [3].

Biosurfactants have increasingly attracted attention due to their safe and environmentally sustainable properties [4]. Biosurfactants have shown numerous environmental applications such as enhancing oil recovery and remediation of oil-contaminated environments [5, 6]. Based on the properties of biosurfactants, their applications as alternative petroleum oil-contaminated surface cleaning agents are interesting [7, 8]. The biosurfactants are expected to enhance oil removal from surface and will not cause further hazardous waste as using chemical solvent. In addition, biosurfactants might also facilitate biodegradation of residue petroleum oil in subsequence treatment system. However, the use of biosurfactants is limited by the low biosurfactant yield. The optimization of production medium and conditions for maximizing the yield of biosurfactant could contribute to expanding the biosurfactant applications [9]. A variety of factors was reported to influence the biosurfactant production such as carbon and nitrogen sources, pH, temperature, time of cultivation, and agitation speed [10–12].

Bacillus subtilis GY19 was previously isolated from soil samples in Thailand. This strain was immobilized on chitosan to produce biosurfactant, and it has been demonstrated to be an efficient lipopeptide biosurfactant producer using waste glycerol and palm oil as

substrates. The components of lipopeptide from GY19 were previously investigated, and the major lipopeptide in this bacterium is the surfactin isoform [13]. However, the scale-up production of biosurfactants by immobilized cells is quite complicated and difficult. In the present study, the production of lipopeptides by free cells of *B. subtilis* GY19 was optimized to be more practical in the future scale-up production. The objectives of this study were as follows: (1) to investigate the effect of nutrient composition and pH on the lipopeptide production from *B. subtilis* GY19; (2) to apply the produced lipopeptide for washing an oil-contaminated surface and to investigate the effects of heating temperature, heating time, shaking time, shaking speed, and crude lipopeptide concentration on washing efficiency; and (3) to determine the influence of lipopeptide and several chemosynthetic surfactants on oil biodegradation.

MATERIALS AND METHODS

Lipopeptide-producing bacteria and culture condition

The lipopeptide-producing strain *Bacillus subtilis* GY19 previously isolated from soil samples in Thailand was used in the present study [13]. The productive medium used for biosurfactant production was modified from Nawawi et al [14], which contains (g/l): glucose (1.0); beef extract (0.5); K_2HPO_4 (3.3); KH_2PO_4 (0.14); $NaNO_3$ (0.2); NH_4NO_3 (3.3); $CaCl_2$ (0.04); NaCl (0.04); $MgSO_4 \cdot 7H_2O$ (0.3); $FeSO_4 \cdot 8H_2O$ (0.1); and waste glycerol (4.0), and the initial pH of the medium was 6.3. Waste glycerol was obtained from Thai Oleochemicals Co., Ltd. (Thailand). It contained (g/l): glycerol (190); sodium (17.5); potassium (43.8); sulfate (22.7); phosphate (0.2); total nitrogen (0.1); and COD (1069) [13]. *B. subtilis* GY19 was cultivated on a rotary shaker at 200 rpm, room temperature for 5 days as mentioned in our previous study [13].

Effects of nitrogen sources, co-substrates, pH, and lipophilic substrates on lipopeptide production

Inoculum of GY19 was prepared in 100 ml of Luria-Bertani (LB) broth [15], and the culture was incubated at room temperature and shaken at 200 rpm for 24 h. Bacterial cells were harvested by centrifugation at 8000 rpm at 4 °C for 20 min, and the bacterial pellet was suspended in 0.85% (w/v) NaCl solution. The optical density at 600 nm was adjusted to 1.0. The inoculum (3% v/v) was added to the productive medium and incubated as described above. The effects of media components and pH on optimization of lipopeptide production were examined. In this study, glycerol (4 g/l) was used as a hydrophilic substrate. The effect of nitrogen source was studied by comparing $(NH_4)_2SO_4$, $NaNO_3$, and NH_4NO_3 as sole nitrogen source. The nitrogen sources were added to the culture medium at 0.1, 0.35, and 0.5% (w/v). The effect of co-substrate was evaluated by supplementing glucose and/or beef extract to the medium. The pH of the medium was varied from 6.5 to 7.5. The influence of type and concentration of lipophilic substrates including commercial soybean oil, rice bran oil, and palm oil for biosurfactant production was investigated at 0.25, 0.5, and 0.75% (v/v). After incubation, the total amount of biomass was measured in terms of dry cell weight. Biosurfactant production was analyzed in terms of surface tension reduction and biosurfactant yield. Surface tension activity of cell free supernatant was measured by using Tensiometer (Dataphysics, DCAT 11EC, Germany). The biosurfactant yield was calculated from the amount of crude lipopeptide per liter of the production medium.

Table 1 Controllable factors and their levels in the Taguchi method.

Factor	Unit	Level			
		1	2	3	4
Lipopeptide concentration	g/l	0	0.7	1.4	5.6
Heating temperature	°C	80	100	120	140
Heating time	min	60	90	120	150
Shaking time	min	60	80	100	120
Shaking speed	rpm	75	100	125	150

Extraction of crude lipopeptide

To extract the produced lipopeptide from culture medium, the bacterial cells were separated by centrifugation at 8000 rpm for 15 min. The residual vegetable oil in supernatant was removed by hexane extraction. The obtained supernatant was then acidified to pH 2 with 6 M HCl and incubated overnight at 4 °C. Supernatant containing biosurfactant was extracted 3 times with equal volume of chloroform-methanol (2:1). The lower solvent phase was collected and evaporated by a rotary evaporator to recover the crude lipopeptide. The amount of crude lipopeptide was measured by using an analytical balance. In this study, quantification of biosurfactant after solvent extraction from culture supernatant was used to preliminarily determine the biosurfactant concentration. The lipopeptide purification process was not conducted since the crude lipopeptide extract was used for preparing washing solutions in the following experiment.

Surface washing with produced lipopeptide and optimization of washing conditions

The application of the produced lipopeptide for cleaning a contaminated surface with a layer of oil was evaluated. The contamination of slideway oil (PTT slideway oil 68; PTT Public Co. Ltd., Thailand) on hard surface was carried out by blotting 0.02 g of slideway oil on the 5×5 cm^2 stainless steel for 24 h and dried in oven at 70 °C. Slideway oil is a lubricating oil usually

used for machine tool slides and tables in factory. Posteriorly, the contaminated metal was immersed in 300 ml of crude lipopeptide solution. To assess the effect of heating temperature, heating time, shaking time, shaking speed, and crude lipopeptide concentration on oil-contaminated surface cleaning efficiency, the washing condition was designed by using Taguchi method. The parameters were selected based on the cleaning operation conditions in factories. For example, machines in petroleum refining were operated under high temperature ranging from 200 to 600 °C [16]. Although oil contaminated machines were cleaned after cooling down, the temperature would be higher than 100 °C. In addition, the high temperature can affect the oil transport such as reducing viscosity and decreasing the persistence of the stranded oil.

Table 1 shows the ranges and levels of different independent variables of cleaning conditions, which were designed for Taguchi method. After washing, the amount of removed oil was determined by Horiba OCMA-310 oil content analyzer (Horiba, Japan). The removal percentage of oil from stainless steel surface was calculated for each experiment by following equation (Eq. (1)):

$$\text{Oil removal efficiency}\,(\%) = \frac{M_i - M_r}{M_i} \times 100 \qquad (1)$$

where M_i and M_r are initial and residual oil on stainless steel (mg), respectively.

The Taguchi method utilizes an orthogonal array (OA) for experimental design and applies the signal-to-noise ratio (S/N) for quality evaluation [17]. As the purpose of this study was to remove maximum oil from stainless steel surface, the S/N ratio with the larger-the-better characteristic was needed. Therefore, the S/N calculation conforming to the larger-the-better was determined by applying following equation (Eq. (2)):

$$S/N = -10 \log 10\left[1/\left(n\sum(1/PRE)\right)\right] \qquad (2)$$

where n is the number of experiments under similar experimental condition, and PRE is the results of measurements.

Oil biodegradation in the presence of produced lipopeptide

To compare the enhancing oil removal efficiencies between biosurfactants and synthetic surfactants, crude lipopeptide, tween 80, sodium dodecyl sulfate (SDS), and cetyltrimethylammonium bromide (CTAB) were supplemented in media containing slideway oil- and immobilized oil-degrading bacteria. The petroleum oil-degrading strain *Acinetobacter* sp. R2 (MSCU 0467) obtained from the culture collection of the Department of Microbiology, Faculty of Science, Chulalongkorn University, Thailand was used in this experiment. The bacterium was immobilized on plastic pellets (2H GmbH, Germany) to increase cell density and activity. Inoculum of R2 was added to 50 ml of 2-fold-diluted LB medium supplemented with 20 µl of slideway oil and 5 g of sterilized plastic pellets and incubated for 24 h. The number of R2 cells immobilized on the carrier material were approximately 10^9 CFU/g. The degradation experiments were performed by adding 5 g of immobilized cells into 50 ml carbon free mineral medium (CFMM) [18] containing 300 mg/l of slideway oil and shaken at 200 rpm at room temperature for 5 days. A crude lipopeptide and synthetic surfactants were added to the samples at $1 \times$ CMC and $5 \times$ CMC. The selected surfactant concentrations allowed the micelle formation, which could promote petroleum solubilization in the system. After incubation, the residual oil was quantified using thin-layer chromatography with flame ionization detection (TLC-FID) (Iatron Labs, Tokyo, Japan) as described by Nopcharoenkul et al [19]. The study in the absence of biosurfactant was carried out as control. All experiments were carried out in triplicate. The inhibitory effect of the surfactant towards bacterial growth was studied using viable plate count technique on LB agar.

RESULTS AND DISCUSSION

Influence of nutrient composition and pH on the production of lipopeptide

The production of biosurfactant in microorganisms is strongly influenced by medium composition and other physical parameters, hence optimization of culture conditions can be used to maximize the biosurfactant yield [20]. The effects of varying nitrogen sources, co-substrates, pH, and lipophilic substrates on lipopeptide production by *Bacillus subtilis* GY19 are indicated in Table 2. Nitrogen source is one of the important factors to influence the biosurfactant production [21]. Table 2 shows that sodium nitrate at 0.5% (w/v) was the best in producing the lipopeptide with a yield of 1.7 g/l (and surface tension 28.4 mN/m); therefore, 0.5% (w/v) sodium nitrate was noted as the suitable nitrogen source and used for further experiments. Several studies have shown the influence of nitrogen source on the production of biosurfactant. Sodium nitrate is frequently used and found to give the high biosurfactant production yield [22, 23].

In the next optimization, effect of co-substrate on the production of lipopeptide was studied. Data in Table 2 revealed that the highest lipopeptide production was achieved when adding both glucose and beef extract as co-substrates. From our data, it was noted that glucose (1 g/l) and beef extract (0.5 g/l) were the potential co-substrates for the lipopeptide production. These conditions were chosen for further experiments. Raza et al [24] demonstrated that the production of rhamnolipids by *Pseudomonas putida* 33 using waste frying oil as carbon source increased when adding glu-

Table 2 Effects of nitrogen, co-substrates, pH, and lipophilic substrates on lipopeptide production by *Bacillus subtilis* GY19.

Experiment			Surface tension (mN/m)	Dried cell weight (g/l)	Crude lipopeptide (g/l)
Effect of nitrogen	$(NH_4)_2SO_4$	0.10%	39.0 ± 3.0	2.2	0.6
		0.35%	39.0 ± 3.0	2.2	1.6
		0.50%	38.0 ± 1.0	1.9	1.6
	$NaNO_3$	0.10%	29.7 ± 0.4	2.3	0.9
		0.35%	28.8 ± 0.1	2.3	1.2
		0.50%	28.4 ± 0.4	2.2	1.7
	NH_4NO_3	0.10%	29.3 ± 0.3	1.9	0.7
		0.35%	30.8 ± 0.2	1.9	0.9
		0.50%	29.3 ± 0.3	1.8	1.3
Effect of co-substrate	Both glucose and beef extract		28.4 ± 0.4	2.2	1.7
	Only glucose		29.9 ± 0.4	1.2	1.1
	Only beef extract		34.3 ± 0.9	0.8	0.5
	None		34.3 ± 0.6	1.0	0.7
Effect of pH medium	pH 6.5		28.6 ± 0.6	2.1	1.6
	pH 7.0		28.5 ± 0.5	2.2	1.7
	pH 7.5		28.2 ± 0.4	2.0	1.8
Effect of lipophilic substrate	Soybean oil	0.25%	34.5 ± 0.6	1.8	ND
		0.50%	34.4 ± 0.2	1.7	ND
		0.75%	30.5 ± 1.5	1.9	1.5
	Palm oil	0.25%	33.4 ± 0.2	1.9	ND
		0.50%	30.2 ± 0.9	1.8	ND
		0.75%	29.7 ± 0.5	2.0	2.2
	Rice bran oil	0.25%	35.4 ± 0.2	1.7	ND
		0.50%	31.6 ± 0.5	1.8	ND
		0.75%	29.3 ± 0.4	2.0	1.9

ND, not determined.

cose as co-substrate. In addition, Kiran et al [25] found that the beef extract showed significant increase in the production of glycolipid by *Nocardiopsis lucentensis* MSA04.

Change in pH is one of the environmental factors to influence the biosurfactant production [26]. The effects of pH on the production of lipopeptide by *B. subtilis* GY19 were investigated by varying the pH of the culture medium from 6.5 to 7.5 (Table 2). In the present study, the production of lipopeptide was not different under different pH values. The maximum yield recorded at pH 7.5 was 1.8 g/l. Hence, the optimum pH was noted as 7.5 and used for further experiments.

To enhance lipopeptide production, soybean, rice bran, and palm oil were used as lipophilic substrates for lipopeptide production in this study in the optimal nutrient and pH obtained above. Different vegetable oils enhanced lipopeptide production differentially; maximum production occurred in the presence of palm oil followed by rice bran oil and soybean oil, respectively (Table 2). The use of vegetable oil as the lipophilic substrate was found to enhance the production of a biosurfactant. Qazi et al [27] observed that the addition of 2% olive oil could enhance biosurfactant production by *Pseudomonas putida* SOL-10.

In this study, the maximum lipopeptide yield at 2.2 g/l and surface tension of 29.7 ± 0.5 mN/m were obtained by using productive medium consisting of glycerol (4 g/l) as hydrophilic substrate, palm oil (0.75%, v/v) as lipophilic substrate, sodium nitrate (0.5%, w/v) as nitrogen source, glucose (1 g/l) and beef extract (0.5 g/l) as co-substrates with pH 7.5. The yield of lipopeptides obtained in the present study was found either higher than or comparable with that from other reports at shake flask level. For example, Vigneshwaran et al [28] showed that the highest lipopeptide yield of 1.29 g/l was achieved under the optimized conditions with the addition of 0.9% (v/v) used engine oil and 0.53% (w/v) potassium nitrate as carbon source and nitrogen source, respectively, at pH 7.1. Another result was observed by Sharma et al [29] who optimized the culture conditions for maximum lipopeptide yield by *Bacillus amyloliquefaciens* SAS-1 and *B. subtilis* BR-15 and found that lipopeptide yield was increased from 1.13 to 2.08 g/l for strain SAS-1 and 1.72 to 2.40 g/l for strain BR-15. Nevertheless, lipopeptide biosurfactants by GY19 may be produced with the higher yield. Other process parameters such as temperature, inoculum size, and incubation time

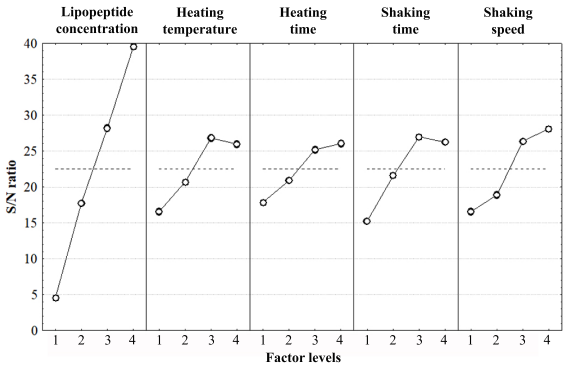

Fig. 1 Main effect plots (S/N ratio) for oil removal efficiency. Signal-to-Noise Ratio: The larger-The better.

should be further evaluated as suggested by several studies [30, 31].

Oil-contaminated metallic surface washing by produced lipopeptide and factors affecting washing efficacy

The application of crude lipopeptide for cleaning oil-contaminated metallic surface was evaluated. To minimize the number of tests required and maximize the effectiveness, some efficient experimental designs, i.e., orthogonal design and Taguchi method can be applied to address multifactor experiments, and the screening of optimum levels by using an orthogonal design table can be used [32]. In this study, effects of heating temperature, heating time, shaking time, shaking speed, and crude lipopeptide concentration on slideway-contaminated surface washing efficiency were studied by Taguchi method, and the results of the 16 experiments are summarized in Table 3. Taguchi method was particularly designed by using a numerical value called signal-to-noise (S/N) ratio to evaluate all the experiments. Moreover, this ratio is very much helpful for estimation of best combination of factors [33]. As a result, lipopeptide concentration had the highest S/N ratio, which indicated the greatest effect on oil cleaning efficiency followed by shaking speed, shaking time, heating temperature, and heating time, respectively (Fig. 1). The optimum level of each factor was determined from the highest value of S/N ratio. The optimum conditions to achieve the maximum oil removal were found to be lipopeptide concentration of 5.6 g/l, shaking speed of 150 rpm, shaking time of 100 min, heating temperature of 120 °C, and heating time of 150 min (Table 4). Effect size helps understand the magnitude of differences found, whereas statistical significance examines whether the findings are likely to be due to chance [34]. Thus, concentration of crude lipopeptide was the crucial factor. The optimal concentration of crude lipopeptide was higher than its critical micelle concentration (CMC) of 1.4 g/l [35]. The high oil cleaning efficiency at above CMC of lipopeptide demonstrated that the solubilization of oil into lipopeptide micelle was the main cleaning mechanism. Micelles are important in cleaning because they can solubilize insoluble oils by incorporating and trapping them within the micellar structure [36]. The mechanisms were expected to involve (1) adsorption of micelles on stainless steel surface; (2) solubilization of oils into micelles; and (3) desorption of oil-containing micelles. Similarly, Zheng et al [37] observed that oil recovery from oil sludge by a biosurfactant formula was higher with the increase of biosurfactant concentration. This study showed that biosurfactants displayed oil removal properties from the solid surfaces and was in accordance with the studies summarized by previous reviews [7, 8]. For instance, Silva et al [38] reported that the biosurfactant produced by *Pseudomonas cepacian* CCT6659 could be applied for cleaning beaker walls contaminated with an oil layer.

Effects of produced lipopeptide and chemical surfactant addition on oil removal

To degrade slideway oil in the washing water, crude lipopeptide from *B. subtilis* GY19 and other chemical surfactants were added to an artificial wastewater treatment system containing the immobilized oil-degrading *Acinetobacter* sp. R2 cells. The oil removal efficiencies in the presence of crude lipopeptide from GY19 compared with chemical surfactants (tween 80, SDS, and CTAB) are shown in Fig. 2a. The results demonstrated that the addition of lipopeptide at 1 × CMC (1.4 g/l) and 5 × CMC (7.0 g/l) increased the slideway oil removal percentage from 70.04% in the treatment without surfactant to 77.29 and 82.10%, respectively. The addition of the tween 80 and SDS at 1 × CMC also increased the slideway oil removal efficiencies; however, the removal efficiencies decreased with increasing concentrations of these synthetic surfactants. In the case of CTAB, the oil removal performance obtained using this surfactant was low.

Furthermore, the effect of surfactant solution on the survival of strain R2 was investigated. As shown in Fig. 2b, tween 80 and SDS at 1 × CMC had no negative effect on immobilized R2 cells. The number of bacteria on the plastic pellets were not different from a control treatment (no addition of surfactant). When the concentration of tween 80 and SDS increased to 5 × CMC, the number of R2 cells decreased resulting in a decrease in the removal efficiency of immobilized R2 cells. For CTAB, it showed negative effect on immobilized R2 cells at both concentrations (1 × CMC and 5 × CMC). Bucci et al [39] suggested that CTAB has good properties for the antimicrobial activity. Moreover, CTAB is a known chemical used for microbial cell lysis [40]. In the treatment containing the biosurfactant, the number of R2 cells at both lipopeptide con-

Table 3 Taguchi design for optimization of cleaning conditions, oil cleaning efficiency, and S/N ratio.

Run No.	Independent variable					Dependent variable	S/N ratio
	Lipopeptide concentration (g/l)	Heating temperature (°C)	Heating time (min)	Shaking time (min)	Shaking speed (rpm)	Oil removal efficiency (%)	
1	0.0	80	60	60	75	0.11	−19.1721
2	0.0	100	90	80	100	0.68	−3.3498
3	0.0	120	120	100	125	9.95	19.9565
4	0.0	140	150	120	150	11.10	20.9065
5	0.7	80	90	120	125	10.34	20.2904
6	0.7	100	60	100	150	8.75	18.8402
7	0.7	120	150	80	75	5.46	14.7439
8	0.7	140	120	60	100	7.10	17.0252
9	1.4	80	120	80	150	18.00	25.1055
10	1.4	100	150	60	125	26.43	28.4419
11	1.4	120	60	120	100	42.48	32.5637
12	1.4	140	90	100	75	21.56	26.6730
13	5.6	80	150	100	100	100.00	40.0000
14	5.6	100	120	120	75	86.85	38.7754
15	5.6	120	90	60	150	100.00	40.0000
16	5.6	140	60	80	125	91.17	39.1970

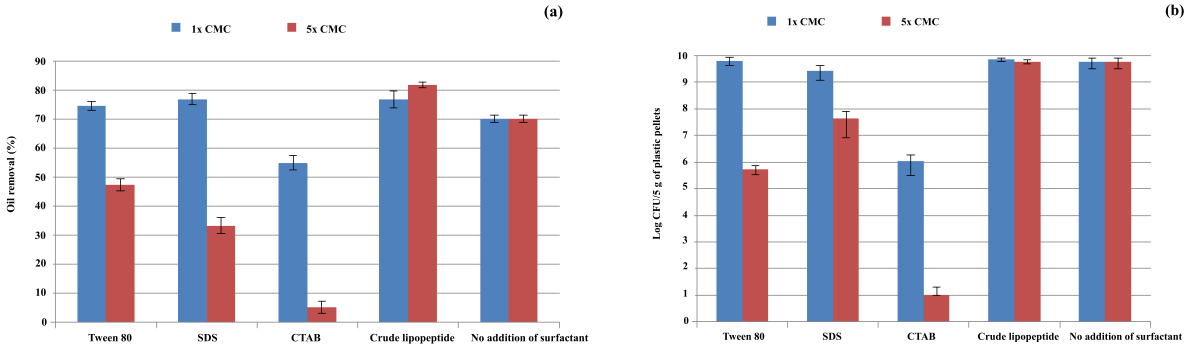

Fig. 2 Effects of addition of surfactant solution on (a) slideway oil removal by immobilized *Acinetobacter* sp. R2 and (b) the number of *Acinetobacter* sp. R2 on carrier materials. The critical micellar concentration (CMC) of tween 80, SDS, and CTAB in water is estimated to be around 0.01, 8 and, 0.9 mM, respectively.

Table 4 The optimum factors for slideway oil cleaning from stainless steel surface.

Factor	Unit	Level description	Effect size
Lipopeptide concentration	g/l	$(5.6)_4$	16.9933
Heating temperature	°C	$(120)_3$	4.3162
Heating time	min	$(150)_4$	3.5232
Shaking time	min	$(100)_3$	4.4716
Shaking speed	rpm	$(150)_4$	5.6342

Expected S/N = 57.4384. Signal-to-Noise Ratio: The larger-The better.

centrations were not different from a control treatment (Fig. 2b). The oil removal efficiencies in the presence of crude lipopeptide from GY19 compared with tween 80 and SDS at 1 × CMC were not significantly different and did not affect the survival of strain R2. However, when the surfactant concentration was higher than the CMC which was recommended for enhancing hydrocarbon degradation, it was found that tween 80 and SDS had a negative effect on the R2 cells. On the other hand, the highest oil removal was achieved when adding crude lipopeptide from GY19 at 5 × CMC without any negative effect on the R2 cells. These results demonstrated that lipopeptide from *B. subtilis* GY19 could be applied to promote biodegradation of oil in a subsequent wastewater treatment system.

CONCLUSION

In this study, the effects of culture media components and pH on the lipopeptide production by *Bacillus subtilis* GY19 were investigated. The maximum lipopeptide yield of 2.2 g/l was obtained from the optimized production medium, which contained 4 g/l glycerol, 0.75% (v/v) palm oil, 0.5% (w/v) sodium nitrate, 1 g/l glucose, and 0.5 g/l beef extract at pH 7.5.

The produced lipopeptide showed the effective action in the cleaning of slideway oil-contaminated metallic surface. The concentration of crude lipopeptide was a variable that most influenced oil washing efficiency. Furthermore, the presence of the produced lipopeptide caused a positive effect on slideway oil degradation by the immobilized R2 cells and did not exhibit any toxic effect to bacterial cells. These results indicated that *B. subtilis* GY19 lipopeptides could be used as an alternative oil-contaminated surface cleaning agent and could promote biodegradation of oil in a subsequent wastewater treatment system.

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