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Biosurfactant-enhanced removal of total petroleum hydrocarbons from contaminated soil

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ABSTRACT

A screening method was developed to evaluate the oil removal capability of biosurfactants for oil-contaminated soils collected from a heavy oil-polluted site. The ability of removing total petroleum hydrocarbon (TPH) from soil by two biosurfactants was identified and compared with that of synthetic surfactants. The results show that biosurfactants exhibited much higher TPH removal efficiency than the synthetic ones examined. By using 0.2 mass% of rhamnolipids, surfactin, Tween 80, and Triton X-100, the TPH removal for the soil contaminated with ca. 3,000 mg TPH/kg dry soil was 23%, 14%, 6%, and 4%, respectively, while removal efficiency increased to 63%, 62%, 40%, and 35%, respectively, for the soil contaminated with ca. 9000 mg TPH/kg dry soil. The TPH removal efficiency also increased with an increase in biosurfactant concentration (from 0 to 0.2 mass%) but it did not vary significantly for the contact time of 1 and 7 days.

1. Introduction

Soil pollution by petroleum hydrocarbons brings up critical issues regarding world-wide environmental and health concerns. This leads to further attention with respect to investigation of innovative and environmental-compatible technologies for its remediation. The major problems of environmental damage arise from accidental spillages and discharge of oil or oily waste intentionally [1]. Consequently, the US EPA has proposed various technologies for treating soil contaminated by petroleum hydrocarbons, including chemical, physical, biological means [2]. One of the feasible ways is bioremediation, which utilizes the natural degradative ability of plants or microorganisms, usually fungi and bacteria, to convert contaminants into less toxic compounds, or ideally carbon dioxide and water. Bioremediation is effective and environmental friendly but it often takes time and not cost-effective on treating large volumes of polluted materials. However, some methods, such as using soil washing to separate the contaminants from soil without causing chemical damage to the soil, may markedly enhance the biodegradation rate [1,3]. The soil washing method is cost-effective and relatively fast, thereby having potential to be applied in treating and removing a large amount of pollutant [4].

The major difficulty in bioremediation of oil-contaminated soil is the bioavailability or mass transfer limitation of the oil pollutants in the soil, causing poor food-microorganism contact and thus poor biodegradation efficiency [5,6]. Oil penetration through soil is an extremely complex process related to physical, chemical, and biological factors [7]. Petroleum hydrocarbons are highly hydrophobic material with low water solubility and those components attach to soil particles, reducing the bioavailability of oil compounds to microorganisms, thereby limiting the rate of mass transfer for biodegradation [5,6]. The possible physical forms for oil contaminants in soil can be dissolved in pore water, adsorbed onto soil particles, absorbed into soil particles, or be present as a separate phase, which can be a liquid or a solid phase [5]. The key process to enhance the bioavailability of the oil contaminant is to transport the pollutant to the aqueous bulk phase [8].

One of the effective ways to increase the bioavailability (or solubility) of petroleum hydrocarbon pollutants in soil is using surfactants to enhance the desorption and solubilization of petroleum hydrocarbons, thereby facilitating their assimilation by microorganisms [5,7,9–13]. In particular, recent studies showed that biosurfactant (a more environmental friendly type of surfactant) has the ability to effectively solubilize and mobilize organic compounds adsorbed on soil constituents [10,13–17]. On the other hand, some synthetic surfactants, such as Triton X-100, Tween 80, Afonic 1412-7, are also shown to be able to enhance the concentration of nonpolar compounds in the aqueous phase [13,18–20]. However the problems with using synthetic surfactants are associated with their

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toxicity and resistance to biodegradation [12,13,21]. When compared with synthetic surfactant, biosurfactants in general exhibit greater environmental compatibility, better surface activity, lower toxicity, and higher biodegradability [22–24]. Therefore, biosurfactants seem to be better candidates for the use in bioremediation of contaminated soil and subsurface environments [13]. In addition, biosurfactants could easily be produced from renewable resources via microbial fermentation, making it an additional advantage over chemically synthetic surfactant.

In this work, two biosurfactants, i.e., rhamnolipids produced by *Pseudomonas aeruginosa* [25] and surfactin produced by *Bacillus subtilis* [26], were used to remove total petroleum hydrocarbons (TPH) from real oil-contaminated soils collected from a polluted site in an oil refinery plant located in southern Taiwan. The TPH removal efficiency was examined for slightly and severely petroleum hydrocarbons contaminated soil (containing TPH concentration of ca. 3000 and. 9000 mg/kg dry soil, respectively) using the two biosurfactants (rhamnolipids and surfactin) as well as two commonly used synthetic surfactants (Tween 80, and Triton X-100) for comparison. The effect of biosurfactant type and concentration, and the contact time on TPH removal efficiency was also investigated. The outcome of this work is expected to provide a useful tool for screening the effective surfactants used in bioremediation of oil-polluted environments.

2. Materials and methods

2.1. TPH-contaminated soil

The contaminated soil used for this study was obtained from an oil-contaminated site in an oil refinery plant located in southern Taiwan. The soil was contaminated with petroleum hydrocarbon (mainly fuel oil) and weathered for years. The soil properties are shown in Table 1. The soil was grouped into low TPH-contaminated soil (LTC soil; containing ca. 3000 mg/kg dry soil of TPH) and high TPH-contaminated soil (HTC soil; containing ca. 9000 mg/kg dry soil of TPH). The basic characteristics of the two types of soils were similar except for a significant difference in their TPH concentration.

2.2. Biosurfactants production

The rhamnolipids applied in this study was produced by *P. aeruginosa* S2, an indigenous bacteria isolated from a diesel-contaminated soil site located in southern Taiwan. The *P. aeruginosa* S2 strain was cultivated at 37 °C using MSI medium. The details of rhamnolipids production were described by Chen et al. [25]. Quantification of total rhamnolipids concentration in the sample was determined by measuring the concentration of hydrolysis-released rhamnose by the modified orcinol method [27]. *B. subtilis* ATCC 21332 grown on an iron-enriched mineral salt medium was used for surfactin production. The procedures of surfactin production can be found in Wei et al. [28]. The surfactin concentration was determined by high performance liquid chromatography (HPLC) before being used in biosurfactant-enhanced TPH removal experiments. Serrawettin, a cyclic lipopeptide biosurfactant, was produced from *Serratia marcescens* [29]. The fermentation broth containing serrawettin was directly used in this work without purification. In addition, a novel bioemulsifier (not purified) produced from *Agrobacterium* sp. QS-6 was also used in the TPH removal experiments. The concentration of serrawettin was determined according to our previous work [29]. The quantification of the novel bioemulsifier was determined by measuring the total carbohydrate concentration using phenol-sulfuric acid method [30] since the bioemulsifier is a polysaccharide-type compound.

2.3. Synthetic surfactants used

Two chemically synthesized surfactants (namely, Tween 80 and Triton X-100) were also used for TPH removal from contaminated soil to compare their performance with that from biosurfactants. Tween 80 (purchased from Mallinckrodt Baker Chemical Inc., USA) is a nonionic surfactant and an oil-in-water emulsifier. Triton X-100, also obtained from Mallinckrodt Baker Chemical Inc., USA, is a nonionic surfactant possessing a hydrophilic polyethylene oxide group and a hydrocarbon lipophilic or hydrophobic group.

2.4. Analytical methods

Emulsification activity of the biosurfactant solutions was determined by measuring the emulsion index (E_{24}) [31]. In general, 6 mL of test oil substrates (kerosene and diesel were used in this work) was added into a test tube containing 4 mL of the biosurfactant solution. After being vigorously vortexed for 2 min, the test tube was kept still for 24 h and the height of oil, emulsion and aqueous zones were measured. As indicated in Eq. (1), the emulsion index (E_{24}) was then calculated from the ratio of the height of the emulsion zone to the total height of the oil, emulsion and aqueous zones.

$$\text{Emulsion index } (E_{24};\%) = \frac{\text{Height of the emulsion layer}}{\text{Total height of the liquid zones}} \times 100\% \quad (1)$$

In addition, surface and interface tension of the sample were also determined with a FACE Surface Tensiometer (Model CBVP-3, Tokyo, Japan) following the method described in Wei et al. [32]. The

Table 1
Soil properties for low contaminated (LTC) and high contaminated (HTC) soils used in this work.

Item		LTC soil[a]	HTC soil[b]
Soil texture		Sandy loam	Sandy loam
Soil size fraction (%)	Sand (0.05–2 mm)	95.16	89.4
	Slit (0.002–0.05 mm)	4.84	45.0
	Clay (<0.002 mm)	–	6.1
pH		6.95	7.13
Organic mater (%)		1.3	1.34
Total nitrogen (N, %)		0.03	0.062
Total Phosphorus (P, %)		0.046	0.046
Exchangeable Potassium (K) (mg/kg)		34.1	36.7
Cation exchange capacity (cmol/kg)		3.7	4.37
Original total petroleum hydrocarbon (TPH) (mg/kg dry soil)	Weathered	ca. 3000	ca. 9000
Component of total petroleum hydrocarbon		C_{10}–C_{40}	C_{10}–C_{40}

[a] Low TPH-contaminated soil (TPH concentration = ca. 3000 mg/kg dry soil).
[b] High TPH-contaminated soil (TPH concentration = ca. 9000 mg/kg dry soil).

Table 2
Properties of biosurfactants and synthetic surfactants.

(Bio)surfactants	Producing strain	CMC (mass%)	E_{24} (%)	Surface tension (mN/m)
Rhamnolipids	*Pseudomonas aeruginosa*	0.0031	71–74	29.5
Surfactin	*Bacillus subtilis*	0.002	70–74	27.2
Serrawettin	*Serratia marcescens*	0.0033	61–70	25.1
Novel bioemulsifier	*Agrobacterium* sp.	nd	69–71	51.0
Tween 80	Chemical synthesis	0.0124	70–74	43.7
Triton X-100	Chemical synthesis	0.0183	69–71	32.7

nd: not determined.

critical micelle concentration (CMC) of biosurfactant was detected by observing the biosurfactant concentrations at which the surface tension first became minimum [24].

2.5. The procedures of batch experiments

Contaminated soil samples (50 g) were measured and poured into 500 mL flask. A known volume (100 mL) and concentration of each surfactant solution was added to the contaminated soil in the flask. Water-carried effect, referring to the extent of oil removal contributed by flushing surfactant-free water, is considered as "blank effects" in comparison with using surfactant solution to remove the oil. The flask was shaken in a temperature regulated shaker (25 °C) at a fixed agitation speed of 50 rpm for 24 h or 168 h. After treatment, the soil and solution were separated for the following extraction and analysis as described in Section 2.6. The TPH removal efficiency was estimated as below:

$$\text{TPH removal efficiency} = \frac{\text{TPH from liquid extraction}}{\text{TPH from liquid extraction} + \text{TPH from soil extraction}} \times 100\%$$

2.6. Determination of total petroleum hydrocarbons (TPH)

Total petroleum hydrocarbons (TPH) were extracted with 30% of dichloromethane as extraction solvent, following the procedure recommended in U.S. EPA test methods 8015B [33]. In general, the quantity of TPH in extract was determined using a gas chromatograph with a flame ionization detector (GC-FID, 7890A, Agilent, USA) equipped with a 30 m capillary column (DB-1HT, 0.32 mm I.D., 0.1 μm film thickness). The temperature conditions of GC-FID were operated at 300 °C for injection port, 350 °C for detector, and an oven temperature program of 50 °C (held for 5 min) to 350 °C (held for 25 min) at a rate of 10 °C/min. Nitrogen was used as the carrier gas at a flow rate of 30 mL/min [17].

3. Results and discussion

3.1. Surface and emulsification properties of biosurfactants and synthetic surfactants

The basic characteristics of tested biosurfactants are shown in Table 2. The rhamnolipids and surfactin was able to decrease the surface tension of water from 72.5 mN/m to 29.5 and 27.2 mN/m, respectively. The critical micelle concentration (CMC) of surfactin and rhamnolipids was about 0.002 mass% and 0.0031 mass%, respectively. Both rhamnolipids and surfactin have excellent emulsification ability able to achieve an emulsion index of 70% for diesel and kerosene. Furthermore, the other biosurfactant, serrawettin, produced from *S. marcescens* showed good surface activity (reducing surface tension of water to 25.1 mN/m) with an emulsion index of 61–70% and a CMC of 0.0033 mass%. On the other hand, the bioemulsifier produced from *Agrobacterium* sp. QS-6 can slightly decrease the surface tension to 51 mN/m but exhibited a high emulsion activity of 69–71% for diesel and kerosene.

The property of synthetic surfactants is also shown in Table 2. The synthetic surfactant-Tween 80 is a nonionic surfactant and emulsifier. The CMC of Tween 80 was about 0.0124 mass% and the surface tension was able to be reduced to 43.7 mN/m. For another chemical surfactant-Triton X-100, the surface tension can be decreased from 72.5 to 32.7 mN/m as the concentration increased to about 0.0183 mass%. Both Tween 80 and Triton X-100 are also good bioemulsifiers able to emulsify diesel and kerosene with an emulsion index of over 70%.

3.2. Determination of shaking speed for TPH removal tests

A mild shaking was applied in the TPH removal experiments to ensure efficient contact between the added surfactants and the TPH content in soil. However, to avoid significant TPH removal due to purely mechanical detachment arising from vigorous shaking, the shaking condition was determined by observing the effect of shaking speed on TPH removal from the contaminated soil by adding water (instead of (bio)surfactant). For these experiments, the contaminated soil used contained about 3000 mg TPH per kg of dry soil. After adding 100 mL of water into a flask containing 50 mg of contaminated soil, the flask was shaken under different speed (0–200 rpm) at room temperature (ca. 25 °C). After shaking for 24 h, the TPH released from soil was measured to determine the TPH removal efficiency. The results (Fig. 1) show that the TPH removal efficiency was around 5% for 0 and 50 rpm shaking. When the shaking speed increased to 100 and 200 rpm, the water-carried TPH removal reached 8% and 16%, respectively. These results suggest that significant TPH removal due to shaking occurred only when

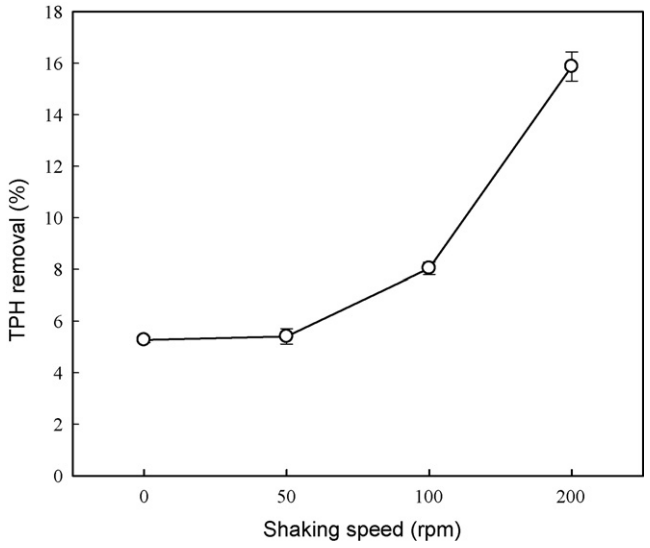

Fig. 1. Control experiment: Water-carried TPH release from contaminated soil under different agitation speed (under low TPH-contaminated soil).

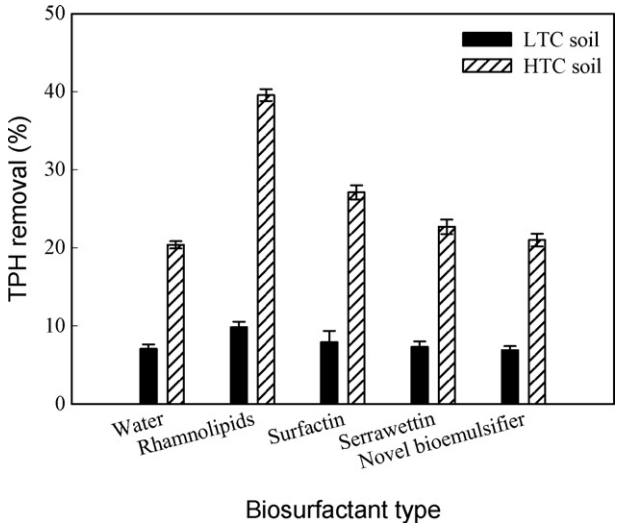

Fig. 2. Effect of biosurfactant type on TPH removal efficiency (added biosurfactant concentration = 0.05 mass%).

the shaking speed was higher than 100 rpm, whereas 50 rpm seems to be the best shaking speed for our purpose as it gave a similar TPH removal result to that from static incubation. At this shaking speed, the soil and washing solution could be mixed well, whereas the interference on oil removal due to vigorous physical mixing can be avoided. Therefore, the following surfactant-enhanced TPH removal experiments were carried out using 50 rpm shaking.

3.3. Effect of biosurfactant type on TPH removal efficiency

Four biosurfactants were examined for their efficiencies on TPH removal from low TPH-contaminated soil (LTC soil; containing ca. 3000 mg/kg dry soil of TPH) and high TPH-contaminated soil (HTC soil; containing ca. 9000 mg/kg dry soil of TPH) (Fig. 2). The control experiment (water only) showed a TPH removal of about 5.4% from LTC soil and about 20.4% for HTC soil. With addition of rhamnolipid solution (0.05 mass%), TPH removal was enhanced to 9.8% and 39.6% for LTC and HTC soil, respectively. The surfactin (0.05 mass%) treatment also exhibited good TPH removal of 7.9% and 27.1% from LTC and HTC soil, respectively. However, addition of 0.05 mass% of serrawettin and the novel bioemulsifier did not lead to significant improvement in the oil removal efficiency for both LTC and HTC soil, when compared to water-carried control test. From results indicated in Fig. 2, the two more effective biosurfactants (i.e., rhamnolipid and surfactin) were chosen for the following experiments.

3.4. Effect of biosurfactant concentration on TPH removal efficiency

Biosurfactant concentration is usually a critical factor for the removal of oil compounds from soil. To evaluate the performance of rhamnolipids and surfactin in removing TPH from the contaminated soil, various biosurfactant concentration were applied to wash LTC and HTC soil. It was observed that increasing the concentration of biosurfactants (both rhamnolipids and surfactin) appeared to enhance TPH removal from soil regardless of the soil used (Fig. 3a and b). For LTC soil, the maximum oil removal efficiency of rhamnolipid and surfactin both occurred at 0.2 mass%, giving a removal percentage of 23.4 and 14.0, respectively (Fig. 3a). In HTC soil, both rhamnolipids and surfactin showed superior efficiency and the maximum TPH removal were over 62% when biosurfactant concentration increased from 0 to 0.2 mass% (Fig. 3b). Regardless of the TPH concentration, both biosurfactants showed

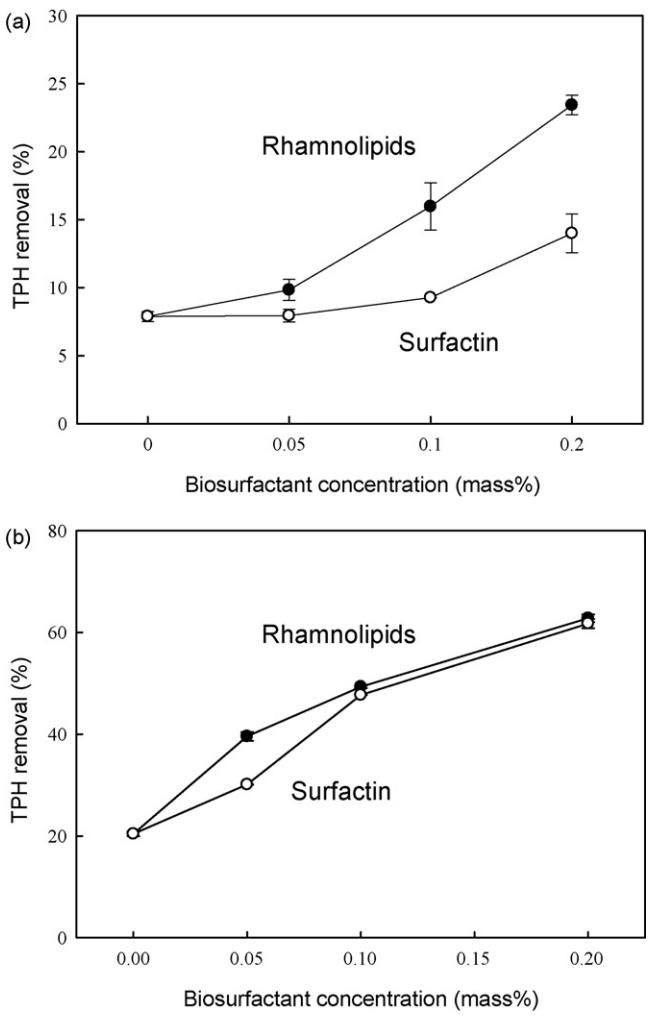

Fig. 3. Effect of rhamnolipids and surfactin concentration on TPH removal from (a) low TPH-contaminated soil (LTC soil) and (b) high TPH-contaminated soil (HTC soil).

excellent effectiveness on TPH removal from contaminated soil, thereby being suitable for future application for biostimulation of oil bioremediation in soil.

Usually, the oil mobilization or solubilization ability of biosurfactant is highly dependent on its concentration [7]. Certain biosurfactants, such as aescin, lecithin, tannin could not enhance the solubilization of crude oil in soil at concentrations greater than their CMC values [3]. However, when rhamnolipids was used, the solubility of crude oil seemed to increase with an increase in rhamnolipids concentration [3], which is consistent with our results (Fig. 3). Also, the toxic effect of some biosurfactants needs to be considered when the biosurfactant was used to facilitate biodegradation of oil pollutants with the indigenous microbial population in the soil [13,34]. Literature showed that a rhamnolipids concentration of 0.004–0.5 mass% was used for the purpose of enhancing bioremediation of oil contaminated soils [1,4]. The oil removal activity of surfactin had been evaluated by sand pack test with fresh kerosene contaminated soil [35–37], showing a 34–62% oil recovery by flushing with 0.1 mass% surfactin solution. Based on its high surface activity, surfactin seems to have the potential for the use in mobilizing crude oil in biostimulation processes. However, for the purpose of washing the oil-contaminated soil to remove the oil pollutants to mobile phase for oil recovery or further ex-situ treatment, the amount of biosurfactant used could be much higher [3]. In this work, we observed that the TPH removal efficiency is positively correlated with the concentration of rhamnolipids and surfactin for

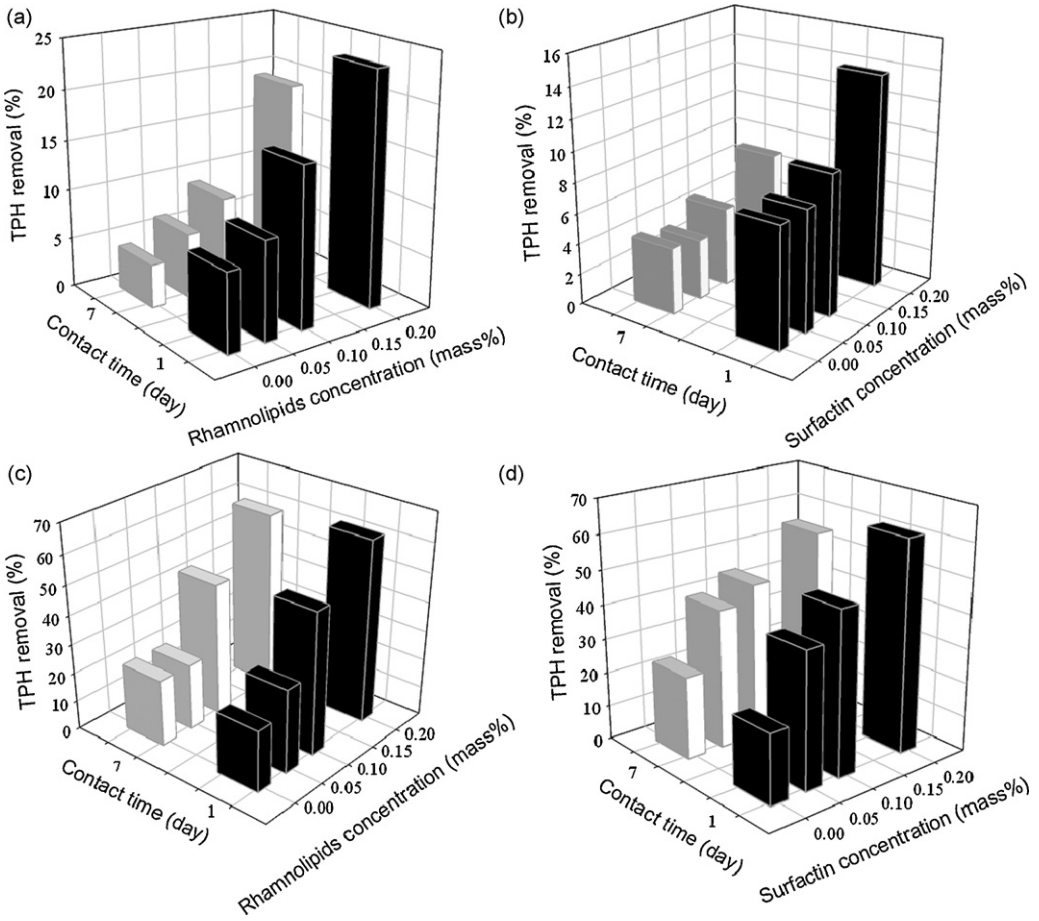

Fig. 4. Effect of biosurfactant concentration and contact time on TPH removal efficiency. (a) Rhamnolipids on LTC soil, (b) surfactin on LTC soil, (c) rhamnolipids on HTC soil, and (d) surfactin on HTC soil.

the concentration range of 0–0.2 mass%. In practical applications, the dosage of biosurfactant should be determined based upon the consideration of the purpose of usage, the cost, the toxic effect, as well as the efficiency of oil removal.

3.5. Effect of contact time on TPH removal efficiency

The contact time is also an important parameter affecting the efficiency of oil removal, as a sufficient contact time is required for effective oil removal. In this study, we investigated the effectiveness of oil removal at two contact time, namely, 1 day and 7 days. As indicated in Fig. 4, irrespective of the biosurfactant type, biosurfactant concentration, and TPH concentration in soil, an increase in contact time from 1 day to 7 days in general led to either a similar TPH removal efficiency or a slightly decrease in TPH removal performance. These results indicate that a contact time of 1 day seemed to be enough for TPH removal with the biosurfactant applied. As for the cases, in which the TPH removal efficiency decreased when more contact time (i.e. 7 days) was applied, since the soils were not sterilized before use, it is likely that biodegradation activity in the soil could occur during 7-day incubation, resulting in the decrease in TPH concentration in the mobile phase.

3.6. Comparison of TPH removal efficiency between biosurfactants and synthetic surfactants

For practical application of rhamnolipids and surfactin on oil removal from soil, it is of great interest to compare the performance of the two biosurfactants with that of two commonly used synthetic surfactants (i.e., Tween 80 and Triton X-100). After adding 0.2 mass% of (bio)surfactants on LTC and HTC soils for 1 day, the TPH removal efficiency appeared to decrease in the order of rhamnolipids > surfactin > Triton X-100 > Tween 80, regardless of the type of soil used (Fig. 5). For LTC soil, addition of 0.2 mass% of rhamnolipids, surfactin, Triton X-100 and Tween 80 resulted in a TPH removal of 23,

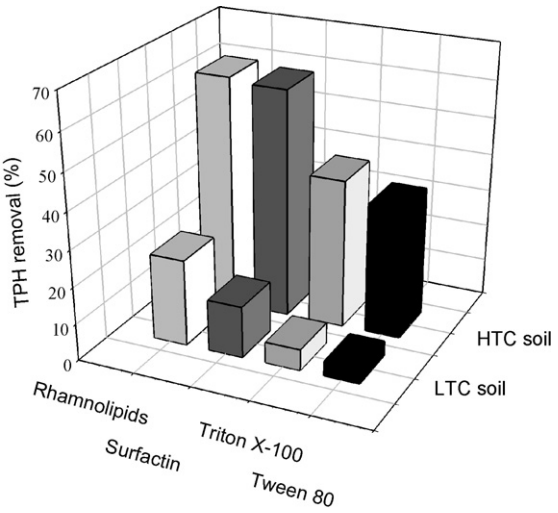

Fig. 5. Comparison of TPH removal efficiency of biosurfactants and synthetic surfactants with low contaminated (LTC) and high contaminated (HTC) soils (added (bio)surfactant concentration = 0.2 mass%).

14, 6 and 4, respectively, while for HTC soil a significantly higher TPH removal efficiency of 63%, 62%, 40% and 35%, respectively, was obtained (Fig. 5). These results indicate the superior performance of biosurfactants over synthetic surfactants in terms of mobilization of oil pollutants from the contaminated soil and thus the two biosurfactants (especially, rhamnolipids) examined in this work have the potential to be used as biostimulation agents for bioremediation of oil-polluted soils.

4. Conclusions

This work demonstrated a screening protocol for determination of oil removal efficiency from contaminated soils. The optimal conditions (i.e., shaking speed, contact time, etc.) were rationally determined. Among four biosurfactants tested, rhamnolipids and surfactin showed superior performance on TPH removal from both slightly and high TPH-contaminated soils. Moreover, the effectiveness of biosurfactant-stimulated mobilization of petroleum hydrocarbons from contaminated soil was better than synthetic ones. In particular, rhamnolipids possessed the highest TPH removal efficiency of 23% and 63% for LTC and HTC soil, respectively, and is considered as a good candidate for assisting oil pollutant remediation in practice. In addition, the results from this work also provide a useful assessment tool for rapid selection of surfactants for their effectiveness of removing petroleum hydrocarbons from contaminated soil.

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