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Synergistic effect of thermophilic temperature and biosurfactant produced by *Acinetobacter calcoaceticus* BU03 on the biodegradation of phenanthrene in bioslurry system

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ABSTRACT

This study aimed at investigating the synergistic effect of temperature and biosurfactant on the biodegradation of phenanthrene in bioslurry. Bench-scale bioslurry experiments were conducted at 25 and 55 °C. The desorption rate coefficients of phenanthrene (K_{des}) obtained using the pseudo-first order model were 0.0026 and 0.0035 kg mg^{-1} h^{-1} at 25 and 55 °C, respectively. Addition of 1500 mg L^{-1} biosurfactant, produced by *Acinetobacter calcoaceticus* BU03, marginally increased the K_{des} at 25 °C since most of biosurfactant was sorbed onto soil; however, significantly increased the K_{des} to 0.0087 kg mg^{-1} h^{-1} at 55 °C as the thermophilic temperature reduced the adsorption of the biosurfactant onto soil and subsequently enhanced the desorption of phenanthrene. The biodegradation of phenanthrene well fitted pseudo-first order kinetics based on the assumption that biodegradation was limited by the desorption. About 78.7% of phenanthrene was degraded in 30 days at 25 °C; and addition of biosurfactant did not affect the biodegradation. However, addition of the biosurfactant or inoculation of *A. calcoaceticus* BU03 at 55 °C significantly enhanced the biodegradation by increasing the K_{des}. Results indicate that synergistic application of thermophilic temperature and biosurfactant or inoculation of biosurfactant producing microorganisms is an effective and innovative method to enhance the efficiency of PAH degradation in bioslurry system.

1. Introduction

The remediation of soils contaminated with polycyclic aromatic hydrocarbons (PAHs) is of major concern because many PAHs are known to be carcinogens and mutagens [1,2]. As an *ex situ* bioremediation technology, bioslurry has been widely employed for cleanup of sites contaminated with PAHs [3,4]. Compared with other bioremediation processes, bioslurry process can promote the biodegradation of PAHs by increasing the mass transfer rate of PAHs from solid to aqueous phase and stimulating microbial activity through mixing of degradative microorganisms, PAHs and essential nutrients [5]. However, the effectiveness of bioslurry process is deterred by the low bioavailability of PAHs due to their high sorption tendency [6,7]. As desorption of PAHs from soil to aqueous phase is often a prerequisite for biodegradation, biodegradation capacity of the microorganisms exceeds the capacity of the bioslurry system to deliver the PAHs to aqueous phase [8,9] thus limiting the degradation, owing to the low desorption rate [10,11]. To alleviate this situation, addition of synthetic surfactants to bioslurry process to enhance the desorption and biodegradation of PAHs was demonstrated [12–14]. Compared to synthetic surfactants, biosurfactants can be preferred as they are more biodegradable and less toxic, thus environmental friendly [15–17]. Biosurfactants produced by microorganisms enhance the solubilization and desorption of PAHs similar to synthetic surfactants. Two kinds of rhamnolipids (dirhamnolipid and monorhamnolipid) were reported to enhance the biodegradation of phenanthrene in aqueous system [18]; and a biosurfactant from *Acinetobacter* sp. increased the apparent solubility and biodegradation of phenanthrene, fluoranthene and pyrene [19]. In our previous study, a thermophilic bacterium, *Acinetobacter calcoaceticus* BU03 isolated from petroleum contaminated soil, was found to produce anionic heteropolysaccharides biosurfactant under thermophilic condition [17].

Previous studies on bioslurry treatment of soil contaminated by PAHs were conducted under mesophilic condition. But thermophilic condition may be more effective for the bioremediation since elevated temperature is expected to increase the solubility and mass transfer rates of PAHs [20]. Besides, the substrate utilization rates of thermophilic bacteria were 3–10 times greater than the mesophiles. Under thermophilic condition (55 °C), a high desorption rate of phenanthrene and pyrene from contaminated soil was achieved [21]. Therefore, in this study, we investigated the synergistic effect of biosurfactant produced by *A. calcoaceticus* BU03 and

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thermophilic temperature on the biodegradation of phenanthrene in a bench-scale bioslurry system. Additionally, inoculation of biosurfactant producing *A. calcoaceticus* BU03 instead of biosurfactant addition was also evaluated.

2. Materials and methods

2.1. PAHs spiked soil

Sandy-loamy soil (56.4% sand; 22.2% slit; and 21.4% clay) collected from abandoned shipyards at Northern Tsing Yi, Hong Kong was air-dried at room temperature; sieved to <2 mm and then spiked with phenanthrene (96% purity, Sigma–Aldrich, St. Louis, MO) dissolved in dichloromethane (DCM, 99.5% purity, Labscan, Stillorgan, Ireland) to a final concentration of 250 mg kg^{-1}. The DCM in soil was allowed to evaporate in a fume hood and the spiked soil was stored at ambient condition for 12 months for aging.

2.2. Production of biosurfactant by A. calcoaceticus BU03

Acinetobacter calcoaceticus BU03 was cultured in Bushnell-Haas medium (Difco, Detroit, MI) amended with 10 g L^{-1} of glucose (Sigma–Aldrich) and 10 g L^{-1} Bacto peptone (Difco) on a gyratory shaker (150 rpm) at 55 °C. After 36 h, bacterial cells were removed by centrifugation at 5000 × *g* for 20 min. The biosurfactant was recovered from the supernatant as described in our previous study [17].

2.3. Equilibrium sorption of surfactants on soil

One gram of soil was added to 20-mL glass vial, followed by the addition of 10 mL of Bushnell-Haas medium containing biosurfactant produced by *A. calcoaceticus* BU03 at various concentrations (from 75 to 1800 mg L^{-1}). 1 mM Hg^{2+} (as $HgCl_2$) was added to each vial to inhibit the microbial activity and the vials were shaken at 250 rpm on a rotary shaker under mesophilic (25 °C) or thermophilic (55 °C) condition. At an equilibrium time of 12 h, as determined from preliminary experiment, triplicate samples were centrifuged at 5000 × *g* for 10 min and the concentrations of biosurfactants in the supernatants were quantitatively determined based on the surface tension measurement of the samples, or different dilutions of samples to concentrations where the surface tension was linearly correlated with the logarithm of the aqueous concentration [17]. Surface tension was measured in triplicate with K11 tensiometer employing the Du-Nouy ring method. The surface tension of the supernatants or their dilutions was then calibrated with the standard curve relating surface tension to the logarithm of rhamnolipids concentration prepared in Bushnell-Haas medium.

2.4. Desorption kinetics of phenanthrene in soil slurry

Desorption kinetics of phenanthrene were evaluated in a bioslurry system where the ratio of soil to aqueous phase was 1:10 (w/v on dry weight basis). To each 2000-mL glass tank, 800 mL of sterilized Bushnell-Haas medium and 80 g of soil spiked with phenanthrene at the concentration of 250 mg L^{-1} were added. To inhibit the microbial activity, 1 mM Hg^{2+} (as $HgCl_2$) was added. The soil slurry in the tank was mixed continuously with a magnetic stirrer conditioned at about 300 rpm to keep the solids in suspension. Four different conditions were evaluated in triplicate: The effects of temperatures (25 and 55 °C), and biosurfactant produced from *A. calcoaceticus* BU03 (0 and 1500 mg L^{-1}) on the desorption kinetics of phenanthrene were investigated in the bioslurry system. At set time intervals, a 2-mL sample was collected for determination of phenanthrene concentration in aqueous phase. Samples were centrifuged at 5000 × *g* for 10 min to remove any suspended soil particles. The supernatant was carefully collected and extracted three times with n-hexane. The extracts were combined and concentrated to appropriate volume for the determination of phenanthrene concentrations using high-performance liquid chromatography equipped with a fluorescence detector (HPLC-FLD). A 10-μL sample was separated using Ultrasphere C18 column (5 μm × 4.6 mm × 25 cm, Beckman Coulter) with 100% acetonitrile as mobile phase and the flow rate was 1.5 mL min^{-1} [16].

2.5. Biodegradation of phenanthrene in bioslurry system

Phenanthrene biodegradation experiment was performed in the same bioslurry system as mentioned in Section 2.4. To each glass tank, 800 mL of sterilized Bushnell-Haas medium and 80 g of spiked soil were added. The tank was then capped with glass lids and soil slurry in the tank was mixed continuously with a magnetic stirrer conditioned at approximately 300 rpm for keeping the solid in suspension. Seven different conditions were evaluated in duplicate in the bioslurry system: The tanks were incubated at different temperatures, i.e., 25 °C for treatments 1, 2 and 6; and 55 °C for treatments 3, 4, 5 and 7. Biosurfactant produced from *A. calcoaceticus* BU03 at the concentration of 1500 mg L^{-1} was introduced to the tanks for treatments 1 and 3 while biosurfactant producing *A. calcoaceticus* BU03 was inoculated to the tanks at the concentration of 1×10^7 colony forming unit (CFU) mL^{-1} for treatment 5. For treatments 2 and 4, no biosurfactant or biosurfactant producing microorganisms was added. Phenanthrene loss through volatilization and other abiotic process under mesophilic and thermophilic conditions was evaluated with treatments 6 and 7, respectively, by the addition of 1 mM $HgCl_2$. At set intervals, samples were taken from the tanks and populations of PAHs degradative microorganisms were enumerated on agar plates containing phenanthrene as sole carbon source as described elsewhere [16], while emulsifying activity of the soil slurry was determined as described by Zhao and Wong [17]. Briefly, soil slurry was centrifuged at 5000 × *g* for 10 min to remove any suspended soil particles. About 0.5 mL of supernatant was introduced into a 10-mL glass tube containing TM buffer (20 mM Tris–HCl buffer, pH 7.0 and 10 mM $MgSO_4$) to a final volume of 1.5 mL, and then 0.02 mL of a 1:1 (v/v) mixture of hexadecane and 2-methylnaphthalene was added. The tube was vortexed for 30 min and the turbidity was determined using a spectrophotometer at 600 nm. One unit of emulsifying activity (EU) was defined as the emulsifying activity that yielded an absorbance of 0.1 at 600 nm. To determine the phenanthrene residue, a 10-mL sample of the soil slurry was freeze dried and phenanthrene in the sample was extracted with Soxhlet extraction (USEPA method 3540) and quantified with HPLC-FLD.

2.6. Statistical analyses

Data analyses were performed for triplicate samples and the mean values with standard error were presented. The data was subjected to one way analysis of variance (ANOVA) and Duncan's multiple range test using SPSS ver.11.5 software.

3. Results and discussion

3.1. Surfactants partition in soil slurry

Fig. 1 illustrates the partition isotherms of biosurfactant produced by *A. calcoaceticus* BU03 in soil slurry at different temperatures. At the aqueous concentration below the critical micelle concentration (CMC), i.e., 163 mg L^{-1} at 25 °C and 152 mg L^{-1} at 55 °C [17], the adsorption of biosurfactant onto soil increased with increasing aqueous concentrations. However, further increase of

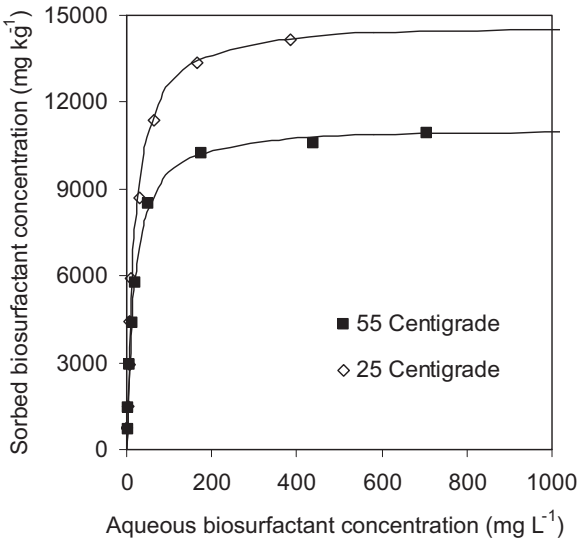

Fig. 1. Equilibrium adsorption isotherms of biosurfactant produced by *Acinetobacter calcoaceticus* BU03 and fitting curves by Langmuir equation (r^2 = 0.98 and 0.97 for 25 and 55 °C).

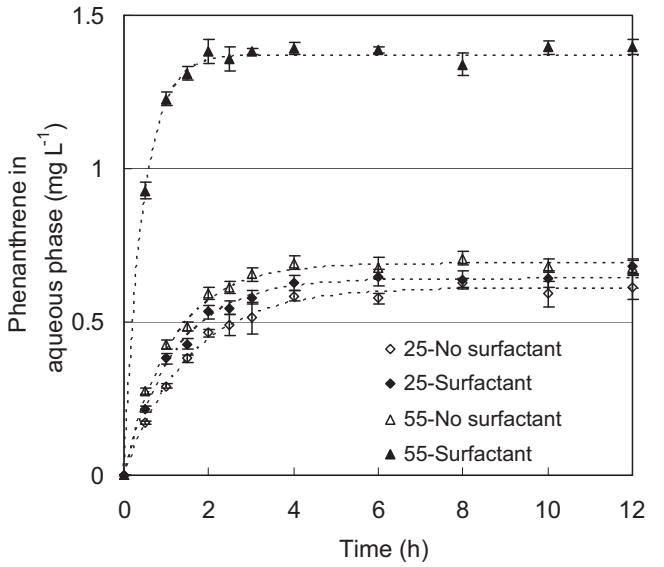

Fig. 2. The desorption kinetics of phenanthrene in soil slurry and fitting curves by pseudo-first order.

biosurfactant after its aqueous concentration greater than the CMC did not result in any significant increase in sorption, which agreed with previous studies [22,23]. It is obvious that the adsorption isotherms are of the typical Langmuir type. The maximum adsorption of biosurfactant can be obtained by fitting the experimental data to the Langmuir equation:

$$\frac{C_{ad}}{C_{aq}} = \frac{1}{Q_{max} b} + \frac{C_{ad}}{Q_{max}} \quad (1)$$

where C_{aq} and C_{ad} are the aqueous concentration in mg L^{-1} and absorbed concentration in mg kg^{-1} at equilibrium, respectively; b is the Langmuir constant, Q_{max} is the maximum adsorption capacity of biosurfactant on soil in mg kg^{-1}.

As shown in Table 1, the maximum adsorption of biosurfactant in soil slurry was 14,855 mg kg^{-1} at 25 °C. At a higher temperature, i.e., 55 °C, the maximum adsorption was only 11,219 mg kg^{-1} indicating that the adsorption of biosurfactant in soil slurry was reduced by elevated temperature. It has been reported that increase in temperature led to remarkable decrease in the sorption of anionic surfactants, which was possibly due to a decrease of aggregate organization of surfactant on the surface of the adsorbent [24].

3.2. Desorption kinetics of phenanthrene

The desorption kinetics of phenanthrene in soil slurry are shown in Fig. 2.

The desorption equilibrium was achieved within 6 h. At 25 °C, the equilibrium concentration of phenanthrene in aqueous phase, i.e., C_{eq} was 0.61 mg L^{-1} (Table 1) while addition of biosurfactant at the concentration of 1500 mg L^{-1} only marginally increased the aqueous concentration of phenanthrene to 0.64 mg L^{-1}. At 55 °C, however, addition of biosurfactant significantly enhanced the C_{eq} to 1.37 mg L^{-1}.

The desorption kinetics of phenanthrene from soil to aqueous phase can be described with pseudo-first order equation [25]:

$$\frac{dC_{aq}}{dt} = K_{des} C_{sorb} (C_{eq} - C_{aq}) \quad (2)$$

$(C_{eq} - C_{aq})$ is the concentration difference between the equilibrium and aqueous concentration of phenanthrene as driving force of desorption. K_{des} is the desorption rate coefficient of phenanthrene in kg mg^{-1} h^{-1}. C_{sorb} is the concentration of phenanthrene sorbed on soil in mg kg^{-1}.

The desorption curve is given by Eq. (3), the integral form of Eq. (2):

$$C_{aq} = C_{eq} (1 - e^{-(C_{sorb} K_{des} t)}) \quad (3)$$

The values of K_{des} were obtained by fitting the experimental data in Fig. 2 to Eq. (3) and are provided in Table 1. The conformity between experimental data and the model-predicted values was evaluated with the coefficient of determination (r^2). It is obvious that the experimental data is well represented by the pseudo-first order kinetics as indicated by the high values of r^2. As shown in Table 1, the value of K_{des} at 25 °C was 0.0026 kg mg^{-1} h^{-1}, while addition of biosurfactant at 1500 mg L^{-1} caused a marginal increase in the value to 0.0033 kg mg^{-1} h^{-1} indicating that addition of biosurfactant at the tested concentration had no significant effect on the desorption rate of phenanthrene under mesophilic condition. Under thermophilic condition, the elevated temperature facilitated

Table 1
Maximum adsorption capacity of biosurfactant produced by *Acinetobacter calcoaceticus* BU03 on soil and its effect on the desorption of phenanthrene in bioslurry system.

Temperature (°C)	Q_{max} (mg kg^{-1})[a]	Addition of biosurfactant (mg L^{-1})	Biosurfactant concentration in aqueous phase (mg L^{-1})	C_{eq} (mg L^{-1})[b]	K_{des} (kg mg^{-1} h^{-1})[c]
25	14,855	0	0	0.61	0.0026 (0.98)
		1500	177	0.64	0.0033 (0.98)
55	11,219	0	0	0.69	0.0035 (0.99)
		1500	451	1.37	0.0087 (0.98)

[a] Q_{max} is the maximum adsorption capacity.
[b] C_{eq} is the equilibrium concentration of phenanthrene in aqueous phase.
[c] K_{des} is the desorption rate coefficient of phenanthrene.

the desorption of phenanthrene, as the value of K_{des} increased to 0.0035 kg mg^{-1} h^{-1}. In the presence of biosurfactant, the value of K_{des} dramatically increased to 0.0087 kg mg^{-1} h^{-1} indicating that addition of biosurfactant under thermophilic condition significantly promoted the desorption of phenanthrene in soil slurry system.

The influence of temperature on the desorption kinetics of hydrophobic organic pollutants has been evaluated in previous studies [26–30]. The temperature could potentially affect the desorption rate in two ways. Firstly, the increased equilibrium desorption of PAHs was generally observed at higher temperatures [27–29] and therefore the difference between C_{eq} and C_{aq} was steeper than that under mesophilic condition. Secondly, the desorption rate constant is temperature dependent. Piatt et al. [28] reported that the desorption rate constants in batch experiment with phenanthrene and pyrene sorbed to sediment increased by 1.2–1.5 times, respectively, with an increase in temperature from 4 to 26 °C. The increased desorption rate constants under elevated temperature were possibly due to the increased diffusivities of PAHs in bulk solution caused by the decreased solution density and viscosity at elevated temperature [28,30].

It is evident that PAHs desorption is positively related to the surfactant concentration in aqueous phase [31,32]. The adsorption of surfactant onto soil may result in the decrease of surfactant concentration in aqueous phase and therefore, reduce the effectiveness of surfactant on the desorption of PAHs [33]. In the present study, more than 88% of the biosurfactant at 1500 mg L^{-1} was sorbed onto soil at 25 °C and the aqueous concentration was about 177 mg L^{-1} merely above its CMC. The positive effect of this small amount of biosurfactant in aqueous phase on desorption of phenanthrene was counteracted by the adsorption of biosurfactant onto soil which led to the increase in the sorption site. Therefore under mesophilic condition, addition of biosurfactant has no significant effect on the desorption of PAHs at 25 °C. Under thermophilic condition, however, the adsorption amount of biosurfactant onto soil decreased significantly and its aqueous concentration remarkably increased to 451 mg L^{-1} equivalent to about 3 times of its CMC. The desorption of phenanthrene dramatically increased due to the partitioning of phenanthrene to the micelle pseudophase. Therefore in bioslurry system, the elevated temperature enhanced the positive effect of biosurfactant on the desorption of phenanthrene by reducing the loss of biosurfactant through adsorption onto soil.

3.3. Biodegradation of phenanthrene in bioslurry system

As shown in Fig. 3, the growth of PAHs degradative microorganisms under mesophilic condition in soil slurry exhibited a growth curve characterized by an initial exponential growth, and a short stable phase followed by a dramatic decrease in population. Under thermophilic condition, the PAHs degradative microorganisms dramatically increased after an initial lag phase in the first three days suggesting that PAHs degradative microorganisms derived from contaminated soil were well adapted to the thermophilic condition. It is obvious that the thermophilic temperature benefited the growth of PAHs degradative microorganisms. At 55 °C, the maximum population increased by 38% compared to that at 25 °C. Under both thermophilic and mesophilic conditions, addition of biosurfactant to the soil slurry caused a slightly higher maximum population of PAHs degradative microorganisms observed on day 6 but had no significant effect during the overall treatment period. Inoculation of *A. calcoaceticus* BU03 to the soil slurry under thermophilic condition significantly promoted the growth of PAHs degradative microorganisms in the first 12 days but the effect was not significant after day 18.

The removal of phenanthrene at two different temperatures in soil slurry system is plotted with time in Fig. 4. Within the

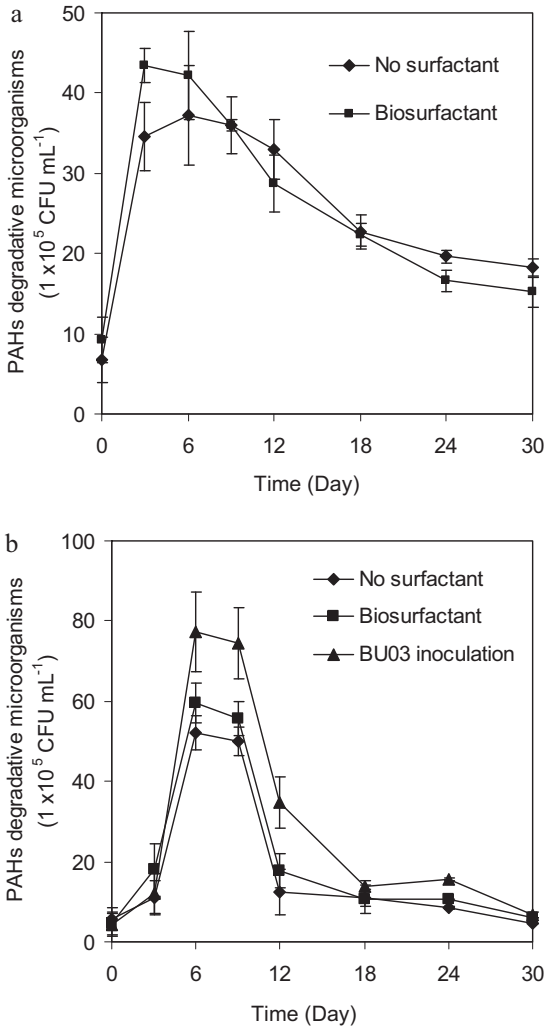

Fig. 3. Growth of PAHs degradative microorganism in lab-scale bioslurry system at (a) 25 °C and (b) 55 °C.

experimental period of 30 days, the loss of phenanthrene through volatilization and other abiotic processes was about 2.5 and 11.1% respectively, at 25 and 55 °C. Under mesophilic condition, about 72% of phenanthrene was degraded and addition of biosurfactant did not result in any significant difference. At 55 °C, the biodegradation of phenanthrene was more rapid and about 86.7% of phenanthrene was removed. The combined application of thermophilic condition and biosurfactant produced by *A. calcoaceticus* BU03 or inoculation of BU03 enhanced the removal of phenanthrene and the complete depletion of phenanthrene was achieved within 24 and 18 days, respectively.

During the biodegradation of phenanthrene from day 0 to day 30 at 25 °C or day 3 to day 30 at 55 °C, the concentration of phenanthrene in the soil–free aqueous phase was always below 0.01 mg L^{-1} indicating the consumption of dissolved phenanthrene by microorganisms. It is therefore the biodegradation of phenanthrene was limited by its mass transfer rate in bioslurry system and the biodegradation can be described as:

$$\frac{dC_{deg}}{dt} = \frac{dC_{aq}}{dt} = K_{des}(C_{sorb} - C_{de})(C_{eq} - C_{aq}) \quad (4)$$

The C_{deg} is the concentration of phenanthrene degraded by microorganisms. C_{aq} is negligible compared to C_{eq} and therefore the degradation curve of phenanthrene can be described with the

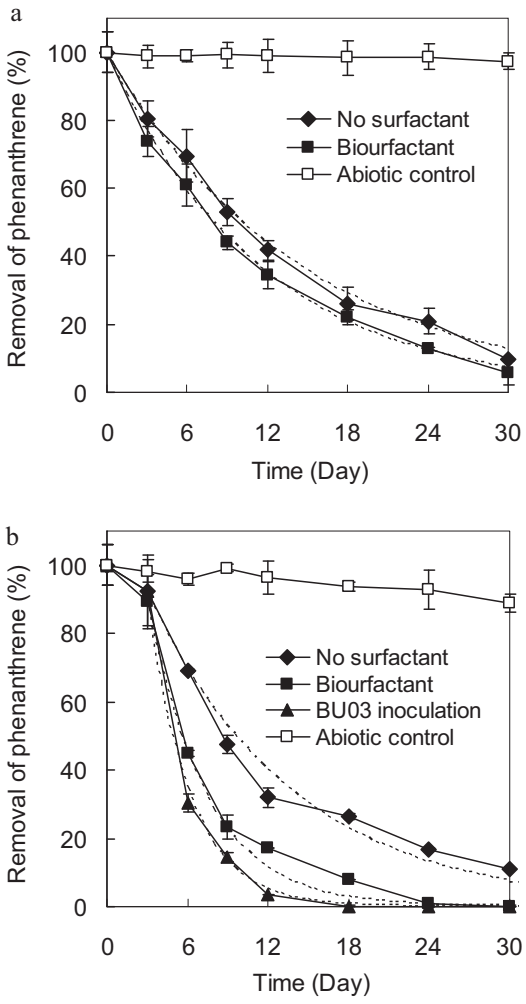

Fig. 4. Degradation of phenanthrene in lab-scale bioslurry system at (a) 25 °C and (b) 55 °C. The dotted lines show the fitting of biodegradation to pseudo-first order.

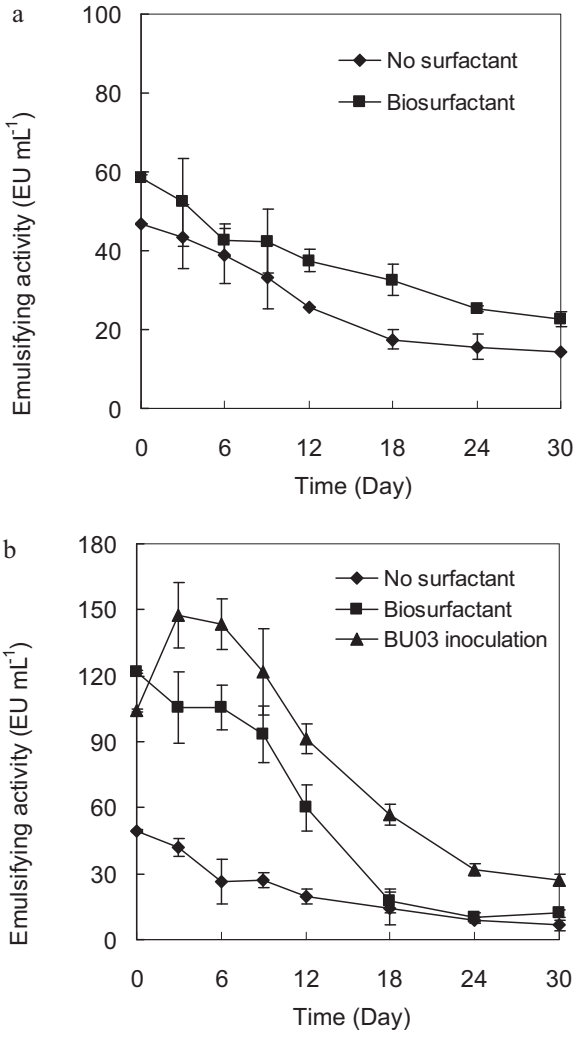

Fig. 5. Emulsifying activity in lab-scale bioslurry system at (a) 25 °C and (b) 55 °C.

integral form of Eq. (4):

$$C_{deg} = C_{sorb}\,(1 - e^{-(C_{eq}\,K_{des}\,t)}) \quad (5)$$

The model describes the close relationship between desorption and biodegradation of phenanthrene. The slow desorption of phenanthrene restricted its biodegradation. On the other hand, the biodegradation in aqueous phase promoted the desorption of phenanthrene by increasing the difference between C_{eq} and C_{aq}.

The dashed curves in Fig. 4 show the simulation of phenanthrene biodegradation with Eq. (5). For the biodegradation under thermophilic condition, the experimental data in the lag phase (Day 0–3) was excluded from fitting since mass transfer may not be a rate-limiting factor of biodegradation when the concentration of degrading cells was low. Generally, the experimental data fitted well with the Eq. (5). However, under thermophilic condition and in the presence of biosurfactant the degradation of phenanthrene after day 9 was obviously less than that obtained by data fitting, which was possibly due to that the desorption rate of phenanthrene decreased with time as a result of the loss of biosurfactant.

In the presence study, the kinetics of phenanthrene biodegradation were well described by the pseudo-first order model indicating that the biodegradation was controlled by the rate of desorption process. It agreed with a previous study [9], in which the biodegradation rate of anthracene was mainly determined by the factors controlling the mass transfer rate such as the diffusive exchange constant and maximum aqueous solubility whereas the biological factors such as uptake rate and maximal specific degradation rate of substrate by microorganisms had minor impact.

To elucidate the mechanism responsible for the positive effect of biosurfactant and biosurfactant producing microorganisms on the biodegradation of phenanthrene under thermophilic condition, emulsifying activity of soil slurry was analyzed and presented in Fig. 5. In our previous study, the emulsifying activity, which increased with increasing concentration of biosurfactants in aqueous phase, was positively related to the desorption and biodegradation rate of PAHs [34]. The emulsifying activity of soil slurry was about 47 and 49 EU mL^{-1} respectively, under mesophilic and thermophilic condition, and decreased continuously during the experimental period due to the degradation of the emulsifying agents by microorganisms. Under mesophilic condition, addition of the biosurfactant did not show any significant effect on the emulsifying activity since most of biosurfactant was sorbed onto soil. Under thermophilic condition, however, both the addition of the biosurfactant or inoculation of *A. calcoaceticus* BU03 significantly increased the emulsifying activity. The emulsifying activity of the treatment with biosurfactant addition was 121 EU mL^{-1}, and decreased continuously during the bioslurry treatment possibly due to the loss of biosurfactant through bioassimilation. On the other hand, the emulsifying activity of the inoculated treatment increased initially indicating the production of biosurfactant by *A. calcoaceticus* BU03 in the bioslurry system, and decreased slightly

during day 3 to day 30. Compared to addition of biosurfactant, treatment with inoculation of biosurfactant producing microorganisms demonstrated higher emulsifying activity throughout the experimental period. Therefore the possible mechanism responsible for the positive effect of *A. calcoaceticus* BU03 on biodegradation of phenanthrene might be due to the production of biosurfactant following the inoculation of BU03, as supported by the increase in emulsifying activity. The presence of biosurfactant in aqueous phase may increase the desorption rate of phenanthrene and subsequently, the biodegradation rate. It has to be highlighted that direct addition of biosurfactants may not be a practical approach for large scale application, since the production and recovery of biosurfactants are expensive. On the other hand, the inoculation of biosurfactant producing microorganisms into the soil slurry system would result in a rapid depletion of phenanthrene, which is likely a more practical and cheaper alternative for remediation of PAHs contaminated soils.

4. Conclusions

A novel synergistic effect of biosurfactant produced by *A. calcoaceticus* BU03 and elevated temperature on the biodegradation of phenanthrene in bioslurry system was demonstrated. The elevated temperature mitigated the sorption of biosurfactant resulting in higher aqueous concentration, and therefore enhanced the positive effect of biosurfactant on the desorption rate of phenanthrene. As a result, the biodegradation of phenanthrene, which was limited by the desorption rate, was significantly increased. Inoculation of biosurfactant producing *A. calcoaceticus* BU03 as an alternative to direct addition of biosurfactant further intensified the advantage of using biosurfactant, by enhancing and extending the emulsifying activity in soil slurry. This study indicates that combinations of thermophilic temperature and biosurfactant or inoculation of biosurfactant producing microorganisms are effective methods to enhance the bioremediation of PAHs contaminated soil.

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