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Biosurfactants from *Acinetobacter calcoaceticus* BU03 enhance the solubility and biodegradation of phenanthrene

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A thermophilic bacterial strain, *Acinetobacter calcoaceticus* BU03, with a biosurfactant-producing capability, was isolated from petroleum-contaminated soil with an improved procedure which employed the solubilization of polycyclic aromatic hydrocarnons (PAHs), i.e. naphthalene in agar plate, as a selection criterion. Crude biosurfactant was recovered from the culture of BU03 by extraction with n-hexane, and its properties were investigated. Biosurfactants from *A. calcoaceticus* BU03 constitute a thermo-stable mixture, composed of different agents with surface activities. At their critical micelle concentration (CMC) of 152.4 mg L^{-1}, the crude biosurfactants produced from *A. calcoaceticus* BU03 decreased the air–water surface tension to 38.4 mN m^{-1}. In thermophilic conditions, the emulsifying activity is 2.8 times that of Tween 80. The effects of the biosurfactants produced by *A. calcoaceticus* on the solubility and biodegradation of PAHs were investigated in batch systems. Biosurfactants produced by *A. calcoaceticus* BU03 at 25 times their CMC significantly increased the apparent aqueous solubility of phenanthrene (PHE), pyrene (PYR) and benzo(a)pyrene (B[a]P) to 54.3, 6.33 and 2.08mg L^{-1}, respectively. In aqueous system, the biosurfactants at concentrations of 0.5 CMC and 1 CMC slightly enhanced the biodegradation of PHE by a consortium of PAH-degrading microrganisms. Results indicate that biosurfactants from *A. calcoaceticus* BU03 have potential to enhance the removal of PAHs from contaminated sites.

Keywords: isolation; polycyclic aromatic hydrocarbons; solubility; degradation; pyrene; benzo(a)pyrene

Introduction

Surfactants are amphiphilic molecules consisting of hydrophilic and hydrophobic domains. Biosurfactants are biologically produced from microorganisms and are grouped as glycolipids, lipopeptides, phospholipids, fatty acids and neutral lipids. The term biosurfactant has been used very loosely and refers to any usable and isolatable compound, produced from microorganisms, which affects the liquid–solid interfaces or has emulsifying ability [1]. A number of studies have shown that microbial surfactants in soil can lead to enhanced desorption of bound substrate and therefore may enhance the rates of biodegradation [2–4]. As a result, interest in biosurfactants has considerably increased in recent years. Many microorganisms were found to produce biosurfactants, e.g. *Rhodococcus* sp., *Pseudomonas* sp., *Candida* sp. and *Acinetobacter* sp. [5–7]. A wide range of *Acinetobacter* sp. can produce extracellular materials, which have surface and emulsifying activity. The most extensively studied biosurfactant from *Acinetobacter* sp. is emulsan, an amphipathic extracellular polyanionic biosurfactant produced by *Acinetobacter venetianus* RAG-1. It is a complex of an anionic heteropolysaccharide and protein [8]. The surface activity of emulsan is due to the presence of fatty acids, comprising 15% of the dry weight of emulsan, which are attached to the polysaccharide. The biosurfactant produced by *A. radioresistens*, i.e. alasan, is a complex of an anionic polysaccharide and protein with a molecular weight of 10^3 kDa [9]. Because of their potential applications in the food, paper, paint, agriculture and detergent industries and in environmental bioremediation, biosurfactants from *Acinetobacter* sp. have been extensively studied, and most of the studies focused on their chemical composition, physical properties, physiology, and fermentation along with their metabolic control [10–13]. Recently, a few studies demonstrated that biosurfactants from *Acinetobacter* sp. increased the apparent solubility of polycyclic aromatic hydrocarbons (PAHs) [14], and also increased PAH biodegradation [15]. However, all of the studies were conducted in mesophilic conditions and current information is not sufficient to verify the potential of biosurfactants from *Acinetobacter* sp. for the bioremediation of PAH-contaminated soil. More extensive studies should be conducted to elucidate the

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effects of these biosurfactants on the bioremediation, and to accelerate their practical application.

This paper presents the results on the isolation and growth characteristics of a thermophilic bacterium strain, *Acinetobacter calcoaceticus* BU03, which produces biosurfactants with new and interesting features. For example, while biosurfactants from *Acinetobacter* sp. generally show no critical micelle concentration, the CMC of the crude biosurfactants from BU03 can be assessed by measuring the surface tension of surfactant solutions. Also, the emulsifying activity of biosurfactants of *Acinetobacter calcoaceticus* BU03 significantly increases with increasing temperature. A biodegradation and solubilization experiment was also conducted to evaluate the bioremediation potential of biosurfactant produced by BU03.

Materials and methods

Isolation of microorganisms

The surfactant-producing microorganisms were isolated from PAH-contaminated soils collected from Tsing Yi, Hong Kong SAR, P.R. China and Dagang Oil Plant, China, as well as from composted soil contaminated by PAHs. Five grams of each sample were added to a flask containing 95 mL sterilized Bushnell-Haas medium (per litre: 1.00 g $Na_2HPO_4 \bullet H_2O$, 0.20 g $mgSO_4 \bullet 7H_2O$, 0.02 g $CaCl_2$, 1 g $(NH4)_2PO_4$, 1 g KNO_3 and 0.03 g $FeCl_3$) amended with 250 mg L^{-1} naphthalene or hexadecane as the carbon source. The flasks were shaken at 150 rpm at 55 °C. After growth became visible, 10 mL of the cultures were inoculated into 90 mL sterilized Bushnell-Haas medium amended with naphthalene, and then shaken again. The enrichment process was repeated four times, and then the enrichments that produced a turbid culture were selected. Serial dilutions of selected enrichments were made in sterilized 0.9% NaCl solution and then surface-plated on Bushnell-Haas agar amended with naphthalene and hexadecane. After 48 h culturing, different colonies on the agar plates were picked up, based on their microscopy and colony morphology, and then individually streaked on to nutrient agar plates to obtain pure isolates. Each isolate was tested for its ability to produce biosurfactants by measuring its emulsifying activity and surface activity. The isolates were inoculated into nutrient broth and shaken at 150 rpm at 55 °C for 24 h, and then the cell-free solutions were obtained by centrifuging the cultures at 6000 × g for 20 min. The surface tension of the cell-free cultures was determined using a Du-Nouy Tensionmeter after 12 h and 24 h. A modified emulsification assay [16] was used to measure emulsifying activity. Samples of 0.5 mL were introduced into 9.5 mL glass tubes 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 tubes were vortexed at 55 °C for 30 min. Turbidity was determined using a spectrophotometer at 600 nm. A chemical surfactant, Tween 80, was used as a comparison in this assay. One unit of emulsifying activity was defined as the emulsifying activity that yielded an A600 of 0.1 in the assay. The microorganisms showing either surface activity or emulsification activity were stored on M17 medium at 4 °C. The microorganism isolated and selected for further study was *A. calcoaceticus* BU03, which was isolated from contaminated soil collected from the Dagang oil field, P.R. China, for biosurfactant production. A PAH-degrading strain, identified as *Mycobacterium vanbaalenii* and coded as BU42 was used for the phenanthrene (PHE) biodegradation. *Mycobacterium vanbaalenii* BU42 was isolated from PAH-contaminated soil collected from Tsing Yi, Hong Kong SAR, P.R. China. The microorganisms were identified using the Midi Sherlock Automatic Microbial Identification System (MSAMIS).

Production of biosurfactants by A. calcoaceticus *BU03*

Cells of BU03 were cultured in medium (10 g glucose, 10 g peptone, 4 g NaH_2PO_4, 0.01 g $FeCl_3$ and 0.025 g $MgCl_2$ per litre, pH 6.5) on a gyratory shaker (150 rpm) at 55 °C. After 36 h, bacterial cells were removed by centrifugation at 6000 g for 20 min. The supernatant was collected and subjected to extraction by adding 100 mL n-hexane to 300 mL supernatant, and the extraction was repeated twice more. The emulsified phase was collected, washed twice with double-distilled (DD) water and rotary evaporated at 55 °C. The residues were subjected to freeze-drying and dissolved in DD water. Undissolved material was removed by filtration through a 0.45 μm cellulose filtration membrane.

Properties of biosurfactants produced by A. calcoaceticus *BU03*

Determination of the CMC of BU03 biosurfactants in thermophilic condition

Surface tension tests were performed to evaluate the CMC values of the surfactants produced by *A. calcoaceticus* BU03 at 55 °C, using a ring tensiometer (Tensiometer No. 70545, CSC Scientific Co., Fairfax, USA). Surfactant solutions at various concentrations were prepared in double distilled (DD) water with 1 mM Hg^{2+} as a microbial inhibitor, and then incubated at different temperatures for one hour. Surfactant solutions at different concentrations were tested for their

surface tension in duplicate [17]. The surface tension was plotted against the surfactant concentrations, and the CMC values of surfactant were determined as the surfactant concentration at the inflection point, if any, on the curve.

Emulsifying activity and emulsifying stability of biosurfactants produced by A. calcoaceticus *BU03*

Surfactant solutions were prepared in double distilled (DD) water for determination of the emulsifying activity with 1 mM of Hg^{2+} added as a microbial inhibitor. Surfactant solutions (0.5 mL) at various concentrations were introduced into 10 mL glass tubes containing TM buffer, and then incubated at different temperatures for one hour. The emulsifying activity was determined, and the emulsifying stability was evaluated by dividing turbidity of the emulsion after 24 h standing with its turbidity after 30 min of vortexing.

Biodegradability of biosurfactants produced by A. calcoaceticus *BU03*

The biodegradability of biosurfactants by isolated PAH-degraders, i.e. *Bacillus subtilis* B-UM, *Microbacterium vanbaalenii* BU42 and a consortium of PAH-degrading microorganisms, was evaluated in 20 mL vials. To each vial, 9.9 mL of sterilized Bushnell-Haas medium with biosurfactants at the concentration of 1 × CMC were added, and then 0.1 mL of bacteria culture, at the concentration of 10^9 colony formation units (CFU) per millilitre, was inoculated. The vials were incubated at 55 °C for 72 h. At each sampling time, duplicate samples were collected, and the growth of microorganisms was determined with the spread plate technique. Series dilutions of samples were made and 0.1 mL of selected dilutions was plated on the nutrient agar (BD Difco). Concentrations of biosurfactants were determined with the critical micelle dilution (CMD) method [17]. The bacterial cells in the samples were removed by centrifugation at 6000 × g for 20 min, and then a series of dilutions of each cell-free sample was made followed by the measurement of surface tension of the dilutions with a ring tensiometer to give the value of CMD.

Thermal stability of biosurfactants produced by A. calcoaceticus *BU03*

Surfactant solutions at the concentration of 10 × CMC were prepared in TM buffer for determination of the emulsifying activity. The pH of the surfactant solution was adjusted to 4, 7 and 10, and then 2 mM of NaN_3 was added as the microbial inhibitor. The solutions were incubated at different temperatures for 48 h; at each sampling time duplicate samples were taken for the determination of emulsifying activity.

Effect of biosurfactants on solubility of PAHs

Aqueous solubility experiments were conducted in 20 mL glass vials. Five milligrams of PAH compounds, dissolved in dichloromethane, were carefully added to the bottom of each vial. The amount of added PHE was well in excess of its aqueous saturation. After the dichloromethane was evaporated, 10 mL of Bushnell-Haas medium containing various concentrations of surfactants (0, 0.5, 1, 3, 10 and 25 × CMC) were added to the tubes. The vials were capped and shaken in a rotary shaker at 150 rpm and 55 °C for an equilibrium period of 48 h, which was determined in a preliminary study. After equilibration was reached, 2 mL of the sample were removed from each vial and filtered through a 10 mL glass syringe packed with glass wool to remove any undissolved PHE particles. The solubilized PHE in the aqueous phase was extracted three times with n-hexane. The extracts were combined and concentrated to an appropriate volume for quantification of PHE concentrations using high-performance liquid chromatography (HPLC) equipped with a fluorescence detector. Samples of 15 μl were separated on an HP Reverse phase C18 column (5 μm, 3.6 × 25 cm, ultrasphere, Beckman) with 100% acetonitrile as the mobile phase with a flow rate of 1.5 mL min^{-1}.

Effect of BU03 biosurfactants on the biodegradation of PHE

Phenanthrene biodegradation by a consortium of PAH-degrading microorganisms was carried out in 20 mL vials. To each vial, 2.5 mg of PHE dissolved in 0.2 mL dichloromethane were added. The dichloromethane was allowed to dry in a fume hood before 9.9 mL of sterilized Bushnell-Haas medium with BU03 biosurfactants at various concentrations (0, 0.5, 1 and 3 × CMC) were added to the vials. After the growth of PAH-degrading bacteria in Bushnell-Haas medium containing 250 mg L^{-1} PHE, the cells were centrifuged, washed twice by DD water, and resuspended in the Bushnell-Haas medium to give a final cell density of 1×10^9 cells mL^{-1}. The experiments were started by inoculating 0.1 mL of the cell suspension containing PAH-degrading microorganisms to the vials containing 250 mg L^{-1} of PHE in Bushnell-Haas medium. The initial concentration of degrading cells in the vials containing PAHs was 10^7 cells mL^{-1}. The vials were incubated in a shaker conditioned at 150 rpm and 55 °C. At the pre-set time intervals, duplicate vials were removed from the shaker to determine the PAH

concentration and growth of cells. Residual PAHs in the vials were extracted three times with n-hexane. The extracts were combined and concentrated to an appropriate volume to determine PHE concentration using HPLC. The growth of PAH-degrading microorganisms was evaluated as described in the previous section. Phenanthrene loss through volatilization and other abiotic processes was evaluated by the sterile control containing 1 mM $HgCl_2$.

Results and discussion

Isolation and characterization of biosurfactant-producing bacteria

Water-immiscible hydrocarbons were always used as the carbon source for the enrichment of biosurfactant-producing microorganisms, since the majority of known biosurfactants are synthesized by microorganisms grown on such materials [6]. To distinguish biosurfactant-producing microorganisms from others, haemolysis, normally performed on the blood agar plates, is generally employed as a selection criterion for isolation [7]. However, this criterion is not sufficiently sensitive and exclusive when the purpose is to obtain biosurfactants which can promote the bioremediation of soil contaminated by organic pollutants. Many biosurfactants, especially those with nonionic properties, are not likely to cause haemolysis [11]. On the other hand, haemolysis may result from other reasons, such as the pH change during the growth of microorganisms. Other criteria for screening of biosurfactant-producing microorganisms include the reduction in surface tension, reduction in interfacial tension, the presence of emulsifying activity, cell surface hydrophobicity and measurement of contact angles or the wetting of water-repelling materials. However, with these methods biosurfactant-producing microorganisms cannot be recognized on agar plates and additional tests are necessary. Hence, an improved isolation procedure was employed in the present study in which the solubilization of naphthalene served as a selection criterion. According to the microbial morphology and colony morphology such as shape, colour and texture, 19 microbial strains were isolated from PAH-contaminated soils collected from Tsing Yi, Hong Kong, while five strains were isolated from the soils collected from Dagang Oil Plant, P.R. China. All of the isolated strains were evaluated for their ability to produce biosurfactants by measuring emulsifying activity and surface activity, and the results are shown in Table 1. To evaluate the biosurfactant-producing activity of isolated bacteria, *Pseudomonas* ATCC9027 was used as a comparison. Among the 24 isolated bacteria, strain BU03 showed the highest emulsifying activity, which indicated a relatively high biosurfactant-producing activity. This microbial strain was identified as *Acinetobacter calcoaceticus* by MSAMSI.

Table 1. Emulsifying and surface activity of isolated microbial strains.

Isolate	Source	Surface tension (mN m^{-1})	Emulsifying activity (Unit)
ATCC9027	–	36.1±1.2*	343.5±2.3
BU01	DG	47.7±1.5	121.3±0.9
BU02	DG	67.2±3.3	11.7±0.3
BU03	DG	45.1±2.1	778.2±3.2
BU04	DG	55.3±2.0	111.4±1.2
BU05	DG	58.5±6.5	117±1.3
BU11	TY	58.2±3.2	75.6±7.0
BU12	TY	69.0±3.5	62.6±10.8
BU13	TY	61.0±3.5	43.0±10.9
BU21	TY	62.4±1.2	155.2±21.2
BU22	TY	42.2±1.4	136.3±11.0
BU31	TY	49.2±1.8	142.0±21.1
BU32	TY	48.6±2.5	45.8±6.2
BU33	TY	53.2±3.7	25.6±2.9
BU34	TY	59.3±2.9	32.3±5.6
BU35	TY	54.7±1.0	121.0±21.0
BU41	TY	56.9±1.3	125.2±12.6
BU42	TY	61.6±5.6	121.0±12.8
BU43	TY	58.7±5.8	115.6±15.1
BU44	TY	52.0±2.5	112.0±11.4
BU45	TY	56.5±2.6	118.4±11.9
BU46	TY	49.2±4.1	88.5±10.9
BU47	TY	47.5±4.3	35.7±2.9
BU48	TY	48.3±4.1	61.2±10.3
BU49	TY	55.8±3.7	56.3±10.9

DG: Strains isolated from soil collected from Dagang oil field, P.R. China.
TY: Strains isolated from soil collected from Tsing Yi, Hong Kong SAR, P.R. China.
*Mean ± standard deviation (n = 2)

Production and properties of biosurfactants from A. calcoaceticus *BU03*

Production of biosurfactants from A. calcoaceticus *BU03*

The results on the production and recovery of biosurfactants from *A. calcoaceticus* BU03 are shown in Table 2. When grown on glucose as the main carbon source, A. *calcoaceticus* BU03 yielded an emulsifying activity of 1038.6 U mL^{-1}; however, the removal of cells from culture decreased the emulsifying activity to 703.7 U mL^{-1}, while the stable emulsifying activity, which was measured after 24 h of vortexing, was not significantly decreased after the removal of cells. This indicated that most of the stable emulsifying activity was provided by

Table 2. Changes in emulsifying activity and emulsifying stability of biosurfactants produced by *A. calcoaceticus* BU03 during recovery process.

Test material	Emulsifying activity		Loss of emulsifying activity (%)
	Emulsifying ability (U ml^{-1})	Emulsifying stability (%)	
Crude culture	1038.6	63.3	0
Cell-free culture	703.7	85.7	32.2
Recovered biosurfactants*	389.3	84.6	62.5

*The recovered biosurfactants were dissolved in DD water (to the same volume as that of the crude culture).

the cell-free materials. The total remaining emulsifying activity in the recovery process was 37.5%.

The CMC of biosurfactants from A. calcoaceticus *BU03*

Interestingly, the CMC of biosurfactants from *A. calcoaceticus* BU03 can be determined by measuring the surface tension. Figure 1 shows the relationship between surface tension and the log of surfactant concentrations. The CMC values of surfactants were determined as the surfactant concentration at the intersection of the two linear portions of the curve, i.e. 152.4 mg L^{-1} for the crude biosurfactants. The emulsifying materials produced by *Acinetobacter* sp. generally show no CMC since their micelles are mainly inter-molecular types [1]. The presence of CMC in our study indicated that the biosurfactant from *A. calcoaceticus* BU03 may form extra-molecular micelles, which are formed by a group of molecules with hydrophilic and hydrophobic parts. The surface tension of the biosurfactants was decreased only when the concentration of surfactant molecules reached a certain value at which the micelles can be formed.

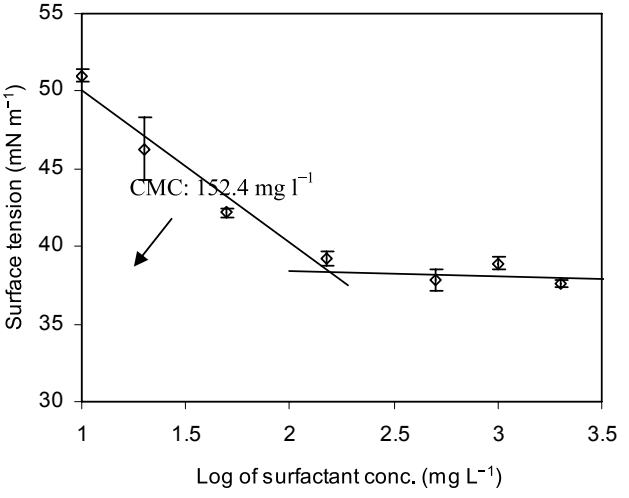

Figure 1. Reduction of surface tension by biosurfactants produced by *A. calcoaceticus* BU03.

The emulsifying activity and emulsifying stability of biosurfactants from A. calcoaceticus *BU03*

Two chemical surfactants, Tween 80 and Triton X100, as well as two biosurfactants, from *A. calcoaceticus* BU03 and from ATCC9027, were tested in the experiment. The emulsifying activities were plotted against concentrations of surfactants (Figure 2). At the same concentrations, the biosurfactants produced by BU03 have the highest emulsifying activity. Furthermore, the emulsifying activity of BU03 was increased by 38.3% with the increasing of temperature from 30 °C to 55 °C, while no significant effect of temperature was found on the other three surfactants. Compared with chemical surfactants, biosurfactants were found to provide a higher emulsifying stability. The elevated temperature decreased the emulsifying stability of the chemical surfactants and the biosurfactant from ATCC 9027 while slightly enhancing the emulsifying stability of BU03 biosurfactants. The observations indicated that biosurfactants from BU03 are an appropriate candidate for application under thermophilic conditions.

Biodegradability of biosurfactants from A. calcoaceticus *BU03*

Figure 3 illustrates the biodegradation of biosurfactants from BU03 by isolated and mixed microorganisms. The biosurfactants were totally degraded within 72 h by mixed microorganisms enriched from PAH-contaminated soil, while the isolated microorganisms, i.e. *B. subtilis* B-UM and *M. vanbaalenii* BU42, cannot utilize biosurfactants, as evidenced from the lack of growth for up to 72 h.

Effect of biosurfactants produced by A. calcoaceticus *BU03 on the solubility of PAHs*

In Figure 4, the solubility of PAHs is plotted as a function of surfactant aqueous concentration up to 25 × CMC. The aqueous equilibrium concentration of PHE and PYR without surfactants was measured to be 1.82 and 0.012 mg L^{-1} at 55 °C in DD water, while the

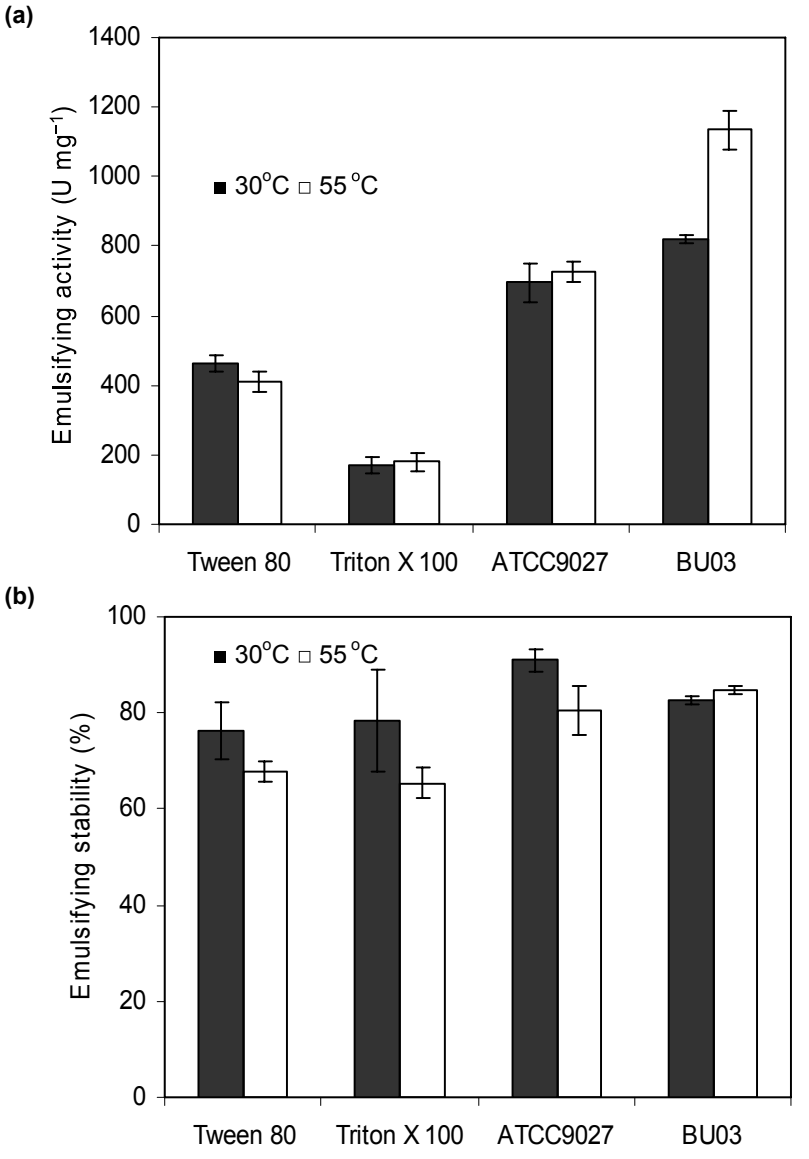

Figure 2. (a) Emulsifying activity and (b) emulsifying stability of chemical surfactants and biosurfactants produced by *P. aeruginosa* ATCC9027 (ATCC9027) and *A. calcoaceticus* BU03 (BU03).

solubility of benzo(a)pyrene (B[a]P) in the absence of surfactant was undetectable. Below their CMCs, the surfactants mainly existed as monomers and did not enhance the solubility of PAHs, while at concentrations above their CMCs, all of the surfactants enhanced the solubility of PAHs because of the formation of micelles, which allows the PHE to partition in. At the same concentrations with respect to CMC, biosurfactants from BU03 was the most effective in enhancing the solubility of PAHs. In the presence of biosurfactants from BU03 at 25 CMC, the aqueous solubility of PHE, pyrene (PYR) and B[a]P was increased to 54.3, 6.33 and 2.08 mg L^{-1}, respectively.

Effect of biosurfactant from BU03 on the biodegradation of PHE in aqueous phase

The growth of a consortium of PAH-degrading microorganisms on PHE in the absence and presence of surfactants is shown in Figure 5(a). The concentration of PAH-degrading cells was 10^7 CFU mL^{-1} initially, and increased four- to five-fold in the first six days because of the utilization of PHE as substrate for growth. After the 12th day, the PAH-degrading populations decreased slightly due to the decreased PHE concentrations (Figure 5(b)). The addition of biosurfactants from BU03 slightly promoted the growth of PAH-degrading cells,

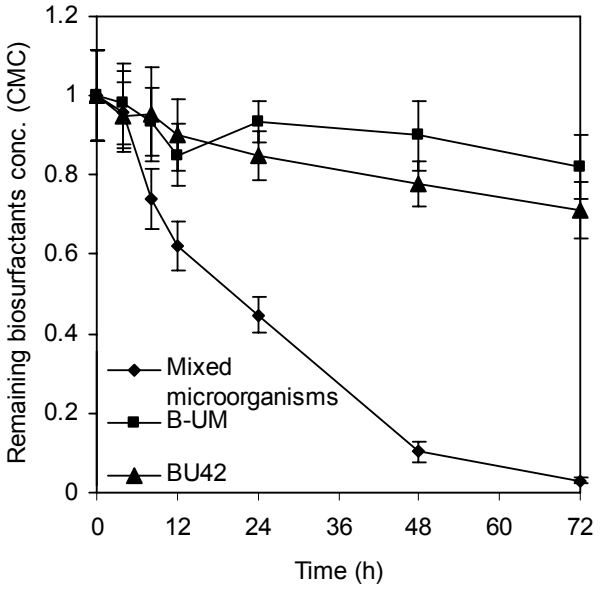

Figure 3. Biodegradation of biosurfactants produced by *A. calcoaceticus* BU03 by isolated and mixed microorganisms.

which may be caused by either accelerated utilization of PHE in the presence of biosurfactants or direct utilization of biosurfactants as a substrate. As shown in Figure 5(b), there were no significant losses of PHE through volatilization and other abiotic processes, as indicated by only 9.5% decrease in the concentration of PHE during the experimental period in the control. Hence, abiotic processes should be playing only a minor role in the disappearance of PHE. In the absence of surfactants, 84.8% of PHE was removed by the consortium of PAH-degrading microorganisms in 30 days. Addition of biosurfactants from *A. calcoaceticus* BU03 at 0.5 and 1 × CMC slightly increased the extent of PHE biodegradation to 94.5 and 91.8%, respectively. However, biosurfactants at 3 × CMC had no significant effect on the biodegradation of PHE. Recently, biosurfactants from *Acinetobacter* spp. were investigated in limited studies for their potential effects on the biodegradation of organic pollutants such as PAHs. Biosurfactants from strain *A. calcoaceticus* RAG-1, i.e. emulsan, reduced the biodegradation of linear alkanes and aromatic compounds by isolated and mixed strains [18], while alasan, a biosurfactant from *Acinetobacter radioresistens* KA53, enhanced the aqueous solubility and biodegradation rates of PAHs [15]. At 500 mg L^{-1}, alasan increased the solubility of PHE and PYR to 7.6 and 2.7 mg L^{-1}, respectively [15]. In the present study, the biosurfactants produced by *A. calcoaceticus* BU03 at 3 × CMC (457 mg L^{-1}) increased the aqueous solubility of PHE and PYR to 9.22 and 2.63 mg L^{-1}, respectively. Furthermore, the biodegradation of PHE by a consortium of PAH-degrading microorganisms was increased

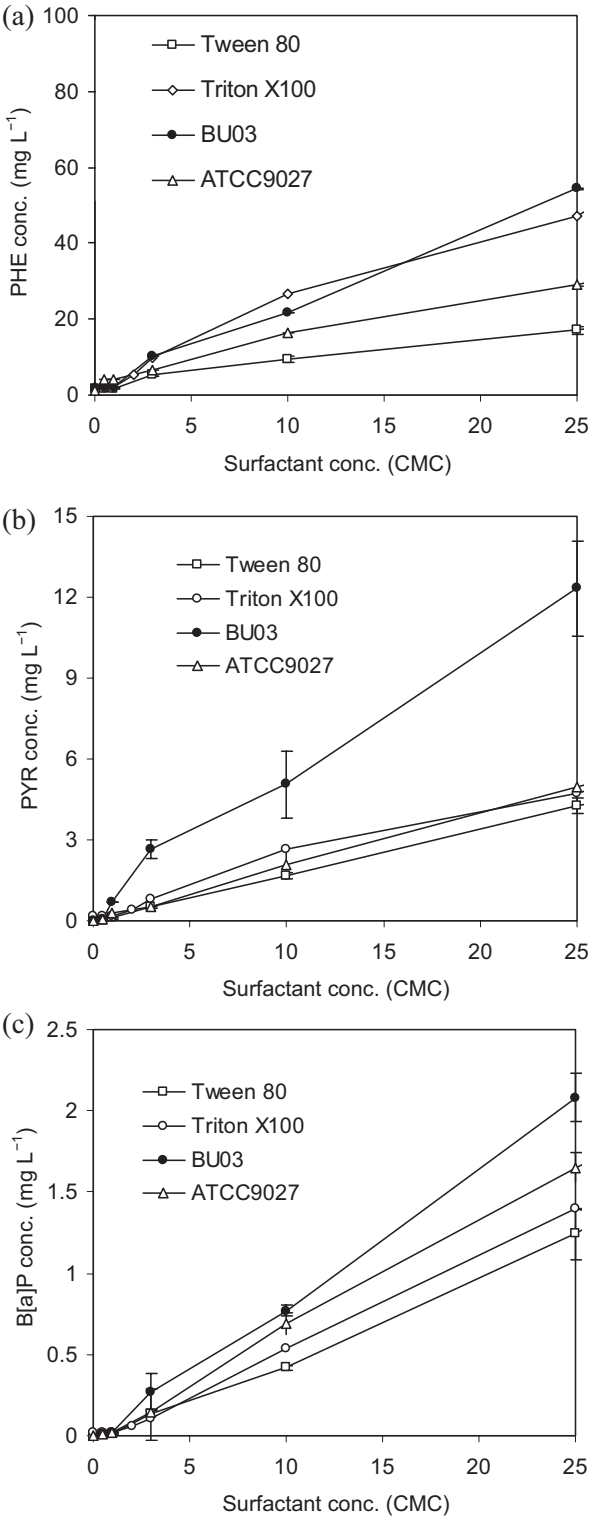

Figure 4. Effects of chemical surfactants and biosurfactants produced by *P. aeruginosa* ATCC9027 (ATCC9027) and *A. calcoaceticus* BU03 (BU03) on the solubility of (a) phenanthrene (PHE), (b) pyrene (PYR) and (c) benzo(a)pyrene (B[a]P).

in the presence of biosurfactants at low concentrations, i.e. 0.5 and 1 × CMC, compared with those without addition of surfactants. In contrast to other studies [15], biosurfactants at lower concentrations were more effective in enhancing the biodegradation of PHE. Our other studies (data not shown) indicated that biosurfactants may promote the biodegradation of PHE by enhancing the mass transfer rate from the solid to the aqueous phase. Simultaneously, they may inhibit the biodegradation by obstructing the contact between dissolved phenanthrene in the aqueous phase and microorganisms. At lower biosurfactant concentrations, e.g. 0.5 CMC, the first effect may play a main role and so the biosurfactant enhanced the biodegradation, while at higher concentrations, e.g. 3 × CMC, the positive effect may be offset and even surpassed by an increasing inhibition effect, thus the biosurfactants may not benefit biodegradation.

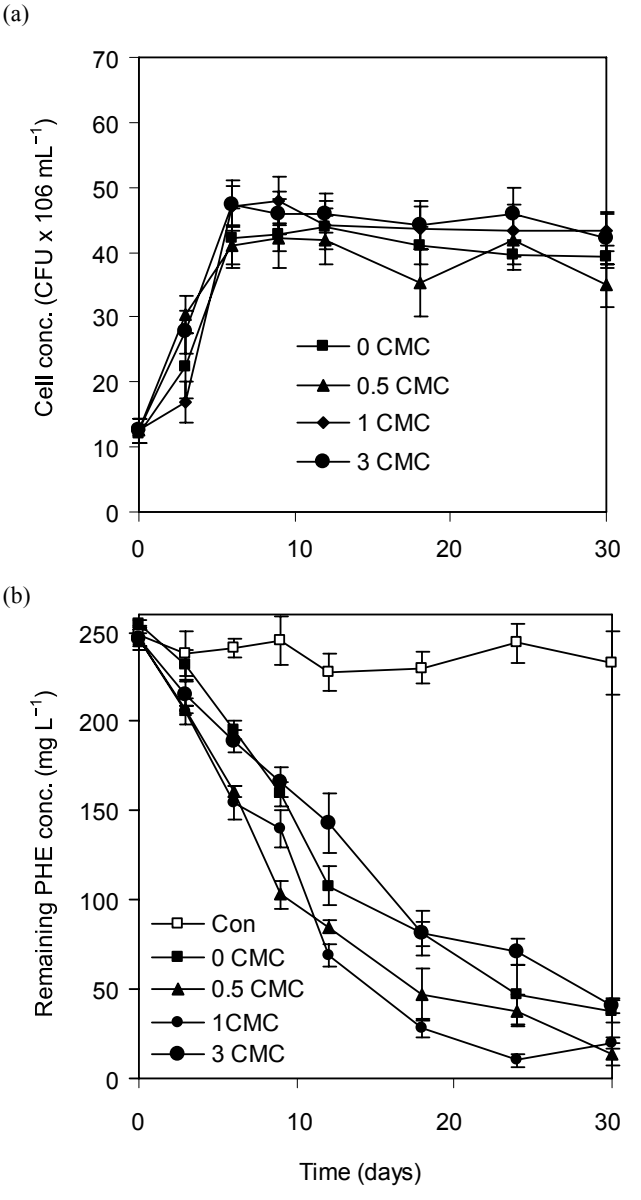

Figure 5. (a) Growth of PAH-degrading microorganisms and (b) biodegradation of phenanthrene (PHE) in the presence of biosurfactants produced by *A. calcoaceticus* BU03.

Conclusions

The present paper reported on the isolation of the thermophilic bacteria *A. calcoaceticus* BU03 for the production of biosurfactants, and the unique properties of the biosurfactants produced by this strain. The emulsifying activity of these biosurfactants was significantly increased by elevated temperature, and the stability of emulsification was not affected by the thermophilic conditions. Compared with chemical and other biological surfactants, biosurfactants produced by *A. calcoaceticus* BU03 are the most effective in enhancing the solubility and biodegradation of PAHs. These properties indicated the potential application of such biosurfactants in the removal of persistent pollutants, e.g. PAHs, under thermophilic conditions.

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