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Solubilization and mineralization of polycyclic aromatic hydrocarbons by *Pseudomonas putida* in the presence of surfactant

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Abstract

The solubilization and mineralization of polycyclic aromatic hydrocarbons (PAHs) in a soil system amended with different surfactants was examined. Mineralization experiments were conducted with the addition of [^{14}C]pyrene. An inoculum of the PAH-degrading microorganism, *Pseudomonas putida*, was investigated for its sensitivity towards four non-ionic and one anionic surfactants with different polyoxyethylene (POE) chain lengths. The addition of surfactant was found to enhance the bioavailability of naphthalene, phenanthrene and pyrene with efficiencies ranging from 21.1 to 60.6%, 33.3 to 62.8% and 26.8 to 70.9%, respectively. The enhanced efficiency followed the order of Brij 30, Triton X-100, Tween 80, and Brij 35, which is correlated with the polyoxyethylene chain of the surfactants. Brij 35 and Tween 80 inhibited the growth of *P. putida*. However, microorganisms can utilize Triton X-100 and Brij 30 as the sole carbon and energy sources at concentrations above CMC values. In the aqueous system without the addition of surfactants, microorganisms could mineralize [^{14}C]pyrene to $^{14}CO_2$ which corresponds to 28% of mineralization. The addition of surfactants decreased the mineralization rate of pyrene. Also, the fraction of the micellar-phase pyrene that can be directly biodegraded decreased as the concentration of micelle increases. However, the mineralization rate can be enhanced by the amendment of Brij 30 when soil was applied to the cultures. This suggests that biodegradable surfactants can be applicable for increasing the bioavailability and mineralization of PAHs in soil systems.

Keywords: Polycyclic aromatic hydrocarbons (PAHs); Solubilization; Mineralization; Surfactants; Critical micelle concentration (CMC)

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants and primarily occur as a result of incomplete combustion processes. Owing to their high partition coefficients, these compounds can be strongly sorbed onto the surface of particles and be deposited to the soil environments. With respect to their suspected carcinogenicities and mutagenicities, PAHs pose threats to aquatic organisms and human health. Therefore, an understanding of the fate of these compounds in soil environments is necessary so that suitable remedial techniques can be applied.

Mineralization of low-molecular weight PAHs, such as naphthalene and phenanthrene under various electron acceptor conditions has been well documented [1,2]. Several bacterial species, such as *Mycobacterium* sp., *Pseudomonas* sp., *Stenotrophomonas maltophilia*, and *Rhodococcus* sp. have been reported to be pyrene degraders [3–6]. Evidence has recently accumulated that the bioavailability of the hydrophobic xenobiotics could be diminished due to sorption, volatilization or abiotic decomposition [7]. Consequently, it has been concluded that mass transfer processes associated with contaminants released into the water phase limited the removal rate of PAHs rather than the explicit aqueous phase biodegradation kinetics [8].

The application of surfactants to soil environments contaminated with PAHs has become a possible means to increase the bioavailability of these hydrophobic compounds and to facilitate their biodegradation [9–14]. It has been demonstrated that addition of surfactants can enhance the biodegradation efficiency of contaminants when the surfactant concentration is above the critical micelle concentration (CMC). Solubilization and lowering of the surface and interfacial tension are thought to be the main reasons for facilitating the transport of pollutants adsorbed on solid phases to the surfactant-containing aqueous phases. However, the inhibition of non-ionic surfactants on biodegradation at concentrations above CMC has also been reported [15,16]. This discrepancy may be attributed to the different physicochemical properties of surfactants and pollutants. The biological compatibility of non-ionic surfactants, even those from the same homologous series, appears to be system-specific. It was reported that non-ionic surfactants with a high hydrophobicity obtained by a long ethoxylate chain were non-toxic to PAH-degrading bacteria [17,18]. More recently, a contrasting effect on PAHs oxidation by different bacteria under the same experimental conditions was also demonstrated [13,19]. These results show that the application of surfactants to the PAH-contaminated sites for bioremediation has yielded inconclusive results and needs further investigation.

In this study, *Pseudomonas putida*, a PAH-degrading microorganism, was used to elucidate the surfactant effect on the desorption and mineralization of PAHs in soil. Naphthalene, phenanthrene and pyrene, the two-, three- and four-ring PAHs, were selected as the model compounds. Also, [^{14}C]pyrene was used for evaluating the mineralization efficiency of PAHs in the presence of surfactant. Four non-ionic surfactants with different polyoxyethylene (POE) chain lengths on the solubilization and one anionic surfactant were selected. Moreover, the biodegradability of surfactant was evaluated for the applicability of these surfactants to the in situ bioremediation of contaminated soil.

Table 1
The characteristics of surfactants used in this study

Surfactant	Molecular formula	Molecular weight(g/mol)	HLB	CMC (M)	Type
SDS	Sodium dodecyl sulfonate	288	40	8×10^{-3}	A
Triton X-100	POE(10)octylphenol	625	13.5	1.7×10^{-4}	N
Brij 35	POE(23)laurylether	1198	16.9	9.2×10^{-5}	N
Brij 30	POE(4)laurylether	363	9.7	5.5×10^{-5}	N
Tween 80	POE(20)sorbitan monooleate	1310	15.0	1.2×10^{-5}	N

A: anionic surfactant; N: non-ionic surfactant, POE: polyoxyethylene, HLB: hydrophilic–lipophilic balance.

2. Materials and methods

2.1. Chemicals and soils

Naphthalene (99%) and phenanthrene (98%) were obtained from Jassen (Belgium). Pyrene (96%) was purchased from Riedel-de Haen (Seelze, Germany). The radiochemical, [9,^{14}C]pyrene (58.7 mCi/mmol) was obtained from Sigma (St. Louis, MO). The surfactants, Triton X-100 (TX100), sodium dodecyl sulfonate (SDS) and Tween 80 were obtained from Merck (Darmstadt, Germany). Brij 30 and 35 were obtained from Aldrich (Milwaukee, MI, USA). The surfactants were used as received from the supplier without further purification. The characteristics of the surfactants are described in Table 1.

An uncontaminated top soil was collected from Hsueh-chia, Tainan County, Taiwan. The air-dried bulk soil was homogenized and screened with 20 mesh sieve to remove large particles and debris. The characteristics of soil are illustrated in Table 2. PAH mixtures containing naphthalene, phenanthrene and pyrene were added to the 500 g soil through a 5 ml methanol carrier. One hundred millilitre deionized water were then added to soils and agitated at 50 rpm for 24 h to well distribute PAHs and evaporate methanol. After evaporation, soils were air-dried and homogenized again and stored in a glass jar before use.

2.2. Organism and growth conditions

P. putida (NCIMB 9816), purchased from the Food Industrial and Development Institute (Hsinchu, Taiwan), was cultured in a 250 ml conical flask containing 50 ml nutrient

Table 2
Characteristics of Hsueh-chia soil used in this study

Parameter	Value
Texture	Sandy soil
Sand	85%
Silt	13%
Clay	2%
Bulk density	1.54 g/cm^3
pH	7.1
Organic carbon	0.8%
CEC	15.9 meq./100 g

broth, with monthly subculturing and incubating on an orbital shaker at 125 rpm and at 25 °C. Purity of the microorganism was checked by microscopy every 2 weeks. For experiments, inoculum was transferred from the nutrient broth to the growth medium with 50 mg/l glucose as the sole carbon and energy sources. The compositions of growth medium contained (per liter): 1.36 g KH_2PO_4, 1.74 g K_2HPO_4, 0.8 g NaCl, 1 g NH_4Cl, 0.1 g KCl, 0.2 g $MgSO_4{\cdot}7H_2O$, 40 mg $CaCl_2{\cdot}2H_2O$, and 5 ml of trace element containing (per liter): 0.2 g $CoCl_2{\cdot}6H_2O$, 0.2 g $ZnSO_4{\cdot}7H_2O$, 0.02 g $CuCl_2{\cdot}2H_2O$, 0.02 g $NiCl_2{\cdot}6H_2O$, 0.02 g $Na_2MoO_4{\cdot}2H_2O$, 0.02 g Na_2SeO_4, and 0.02 g Na_2WO_4. The pH of the final medium was adjusted to 7.1 ± 0.1.

2.3. The solubilization assay

The solubilization of PAHs from the soil to liquid phase was determined by the difference between systems in the presence and the absence of surfactants. For each experiment, 4 g of fresh PAH-contained soil was mixed with 40 ml deionized water and placed into a 50 ml reaction vessel. The soil–water suspensions were agitated on an orbital shaker at 125 rpm and at 25 °C for 24 h. The glass centrifuge tubes were covered with aluminum foil to protect the samples from direct photolysis. At the end of experiments, the tubes were centrifuged at $4000 \times g$ for 20 min to separate the aqueous phase from the solid phase.

2.4. Mineralization assay

The mineralization experiment was conducted in 125 ml reaction vessels that contain in their headspace a cup in which the $^{14}CO_2$ evolving during the biodegradation can be absorbed into a KOH solution. [^{14}C]pyrene (specific radioactivity of 185,000 Bq) and unlabelled pyrene (50 mg/g of soil) were added to the sterilized growth medium in flask. One milliliter of inoculum (about 10^9 cells/ml) was added into the system and then sealed with a rubber stopper. Stoppers were lined with Teflon to minimize losses due to the sorption from headspace. A total reactant volume of 50 ml containing pyrene, seeding inoculum and surfactant was placed in the reaction vessel. A volume of 0.25 ml of 1 N KOH was placed in the headspace cup. Reaction vessels were then placed on a magnetic stirrer to mix the reactor continuously and incubated in the dark at 25 °C. At the end of the desired reaction time, concentrated sulfuric acid was introduced into the vessels to stop the reaction and to release any dissolved carbon dioxide from the aqueous solution. Samples from the KOH cup and reaction chamber were then mixed with 10 ml of scintillation cocktail and analyzed for radioactivity by liquid scintillation counter (LSC) to determine the mass carbon dioxide produced and the loss of the pyrene, respectively.

2.5. Analytical methods

Microwave-assisted solvent extraction technique was applied for the extraction of PAHs from 4 g soils. Soil samples were placed into medium-pressure PTFE vessels and 20 ml 1:1 dichloromethane:hexane were used as the extraction solvent. The temperature increased from room temperature to 120 °C at 130 psig and maintained for 10 min. The extracts were cooled down in the vessels to room temperature and then centrifuged at $5000 \times g$ for

10 min, preconcentrated to 2–3 ml with a rotary evaporator, and then replaced the solvent by approximately 1 ml of hexane. A 1 g Alumina-N SPE cartridge was used for the cleanup of the extracts. The cartridge was washed with 5 ml hexane at a rate of 5 ml/min and then the PAHs were eluted with 10 ml dichloromethane at a rate of 2 ml/min. The elutes were concentrated to about 1 ml on a rotary evaporator and then quantified to 2 ml with small amounts of dichloromethane. The solubilized PAHs in aqueous solution were obtained from the difference between the total amounts spiked and the sorbed PAHs onto soil.

Analysis of PAHs was performed using a Hewlett-Packard 6890 gas chromatograph (GC) equipped with a 5973 mass spectrometer (MS) and a split/splitless injection port (splitless mode). The column used was a 30 m HP-5 (5% phenyl-95% methylpolysiloxane) (0.25 mm i.d., 0.25 μm film thickness). One microlitre of each sample was injected into the GC system for separation and determination of PAHs. The column temperature was increased from 40 to 230 °C at a rate of 20 °C/min, held for 3 min, then programmed to 240 °C at a rate of 6 °C/min, held for 3 min and finally heated to 270 °C at a rate of 3 °C/min, held for 1 min. Helium was used as the carrier gas, and the flow rate was maintained at 3 ml/min (linear velocity 60 cm/s). The ionization was carried out in the electron impact mode (70 eV). The electron multiplier voltage and automatic gain control target were set automatically. The transfer line and ion trap mainfold were set at 280 and 230 °C, respectively. The mass range scanned was from 50 to 550 amu under full scan acquisition mode. The MS was tuned to m/z 69, 219 and 502 for EI corresponding to perfluorobutylamine (PFTBA). The relative standard deviations of PAHs were within 5%. The detection limits were 0.35, 0.38 and 0.35 μg/g, respectively. Also, the recoveries of PAHs were 75.4% for naphthalene, 78.0% for phenanthrene and 92.1% for pyrene.

3. Results and discussion

3.1. Desorption of PAHs

To assess whether the observed desorbed concentrations of PAHs in soil environments were consistent with the concept that PAHs was available for equilibrium partitioning, the equilibrium time of desorption was determined. Fig. 1 illustrates the desorption behaviors of naphthalene, phenanthrene and pyrene in soil environment without the addition of surfactant. The desorption rate of PAHs decreased with time and reached a plateau after 24 h. The initial desorption rate constants were 54, 8.6 and 8.0 μg/g-h, respectively. Also, the desorption efficiencies for naphthalene, phenanthrene and pyrene were 82.8, 76.4 and 77.2%, respectively.

3.2. Enhancement of desorption efficiency and bioavailability

The bioavailability of PAHs can be decreased due to the sorption onto soil. An addition of surfactant can enhance the apparent solubility of PAHs, subsequently increasing the bioavailability of PAHs. Fig. 2 illustrates the enhanced bioavailability of pyrene in the presence of different surfactants. A rapid desorption of pyrene from the soil into the surfactant-containing medium was observed. For the anionic surfactant, the bioavailability

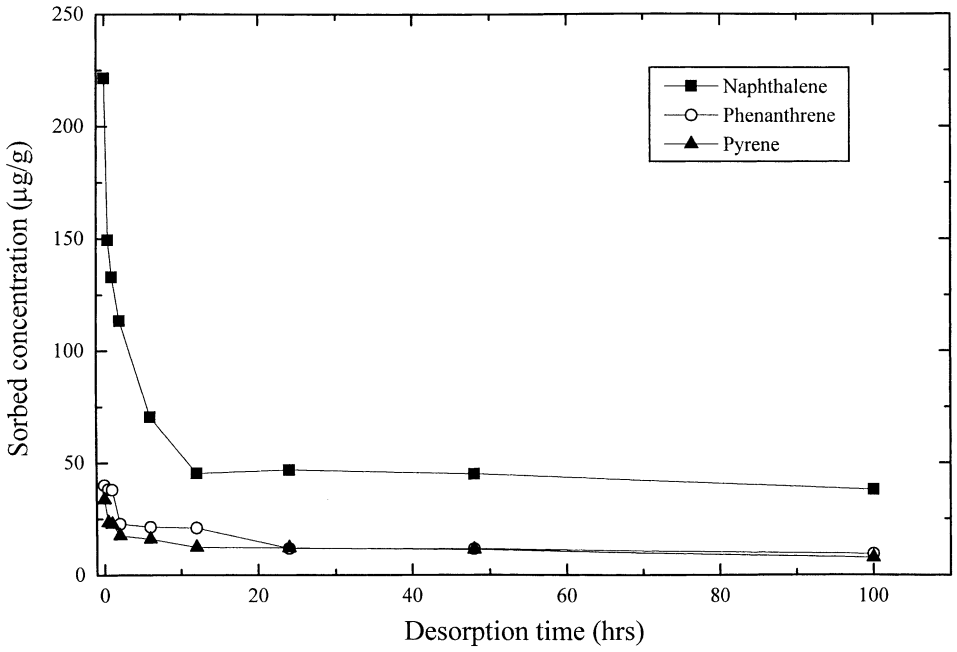


Fig. 1. The desorption of naphthalene, phenanthrene and pyrene in soil environment without the addition of surfactant.

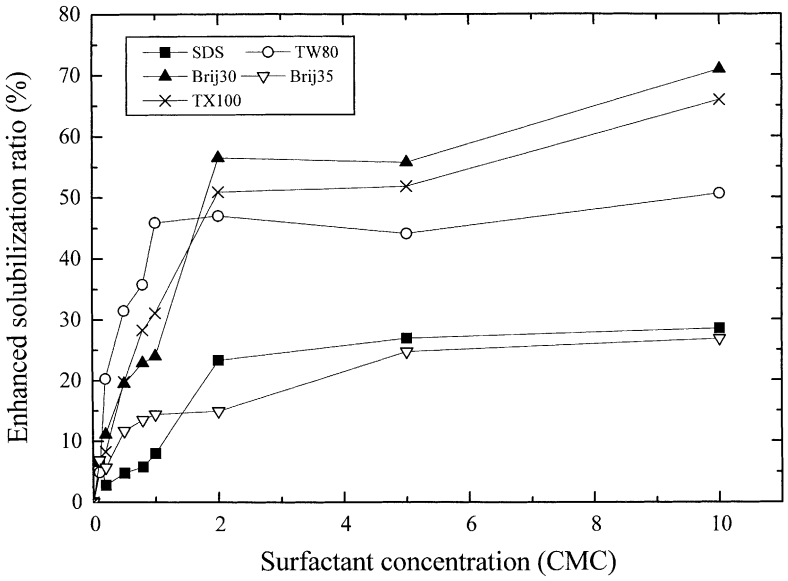


Fig. 2. The enhanced bioavailability of pyrene in the presence of five different types and concentrations of surfactants.

Table 3
The sorbed amounts and enhanced solubilization ratios of PAHs with the amendment of five different surfactants

Surfactant	Naphthalene		Phenanthrene		Pyrene	
	Sorbed amount (μg/g)	Enhanced ratio (%)	Sorbed amounts (μg/g)	Enhanced ratio (%)	Sorbed amounts (μg/g)	Enhanced ratio (%)
No addition	78.4	–	50.7	–	42.6	–
Brij 30	30.9	60.6	18.8	62.8	12.4	70.9
Triton X-100	33.1	57.9	20.2	60.1	14.5	65.9
Tween 80	47.6	39.3	30.1	40.6	21.1	50.6
Brij 35	62.1	21.1	37.5	33.3	31.2	26.8
SDS	52.5	33.1	32.1	36.6	32.7	23.3

of pyrene increased when the concentration of SDS increased from 0 to 2 CMC. Further increasing the SDS concentration to 10 CMC did not have much effect in enhancing the desorption efficiency of pyrene. Similar desorption behaviors were observed when non-ionic surfactants were amended. The sorbed amounts of pyrene decreased from 42.6 μg/g of soil in the absence of surfactant to 12.4–31.2 μg/g of soil when non-ionic surfactants were amended. The enhanced effect followed the order Brij 30, Triton X-100, Tween 80, and Brij 35.

The enhanced solubilization ratios of PAHs in the presence of surfactant can be calculated as follows:

$$R_{es} = \frac{C_p - C_{ps}}{C_p} \times 100\% \quad (1)$$

where R_{es} is the enhanced solubilization ratio of PAHs, C_p is the sorbed PAH concentration onto soil in the absence of surfactant, and C_{ps} is the sorbed PAH concentration onto soil in the presence of surfactant. Table 3 illustrates the enhanced solubilization ratios of PAHs with the amendment of five different surfactants. The enhanced solubilization ratios of pyrene in the presence of surfactants were 70.9% for Brij 30, 65.9% for TX-100, 50.6% for Tween 80, and 26.8% for Brij 35. Similar patterns but less extent of enhancement were observed for naphthalene- and phenanthrene-amended systems. The sorbed amounts of naphthalene decreased from 78.4 to 33.1 μg/g when the concentration of Triton X-100 increased from 0 to 2 CMC. The enhanced solubilization ratios of naphthalene and phenanthrene in the presence of non-ionic surfactants ranged from 21.1 to 60.6% and 33.3 to 62.8%, respectively. Also, the enhanced solubilization ratios followed the order pyrene, phenanthrene, and naphthalene, which is consistent with the hydrophobicities of PAHs.

The addition of surfactant can significantly enhance the solubilization of PAHs. However, the efficiency was surfactant-dependent. The enhanced solubilization ratio of PAHs was in the order Brij 30, Triton X-100, Tween 80, and Brij 35. This may be attributed to the different numbers of polyoxyethylene of the surfactants. Guha and Jaffe [11] reported that phenanthrene could not partition into the micelle of Brij 35, while the result for Brij 30, a surfactant of the same alkyl group, showed that phenanthrene that had partitioned into its micellar phase was directly bioavailable. Brij 30 and 35 have the same alkyl chain, but the length of their polyoxyethylene chain differs significantly, 4 and 23, respectively. Since the

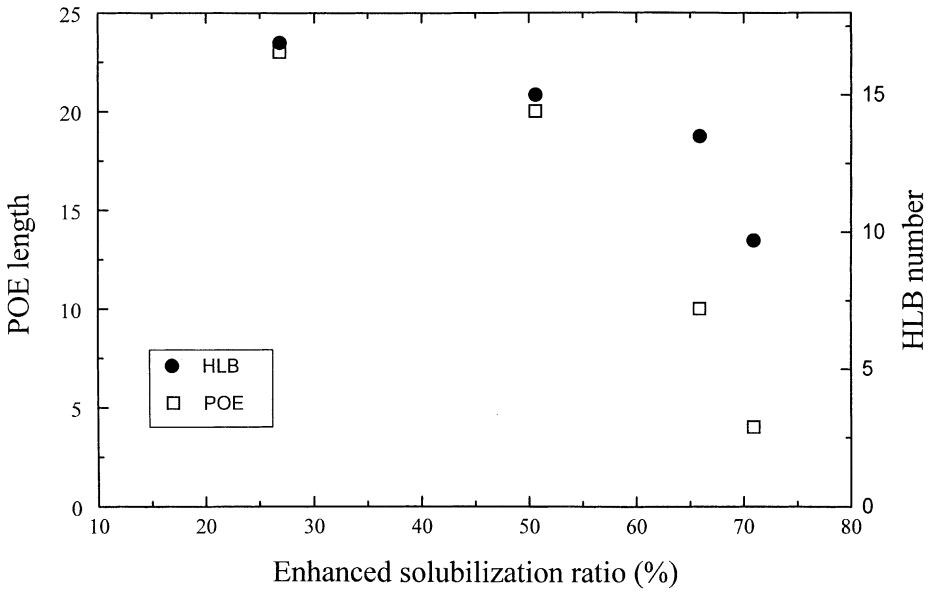


Fig. 3. The relationship between POE length and enhanced solubilization ratios of non-ionic surfactants.

sorption of hydrophilic polyoxyethylene chains decrease with a increasing chain length, the longer hydrophilic chain of Brij 35 may hinder the interaction between the micelles and soil surface. Fig. 3 illustrates the relationship between POE length and enhanced solubilization ratios of non-ionic surfactants. Surfactants with a short POE chain have a high capacity for enhancing the solubilization of PAHs. This suggests that surfactant with high hydrophobic property (low POE chain or HLB number) is suitable for the enhancement of PAH solubility.

Only 23.3–33.1% of the apparent solubility was enhanced when SDS was amended into the system. This may be attributed to the high value of hydrophilic–lipophilic balance (HLB) number of SDS. The HLB scale is designed for matching surfactant structure to an organic chemical to be emulsified, and a higher HLB number represents a higher water solubility of surfactant. The HLB number of SDS is 40, which is higher than those of non-ionic surfactants. The high CMC value also caused some dispersion of soil colloids in the system [5]. The soil colloids can also sorb the PAHs, thereby decreasing the enhanced efficiency of PAHs from soil to water. Deschenes et al. [20] reported that the addition of high concentration of SDS decreased the biodegradation rate of four-ring PAHs, presumably because that the preferential utilization of surfactants by PAH degraders was responsible for the inhibition observed in the biodegradation of pyrene.

3.3. Biodegradation of surfactants

The degradation of surfactants by *P. putida* was carried out by adding 50 mg/l of non-ionic surfactants (Triton X-100, Brij 30, Brij 35, Tween 80) and 50 mg/l of pyrene in 50 ml of mineral growth medium containing 1 ml of inoculum (10^9 cells/ml). Growth of *P. putida*

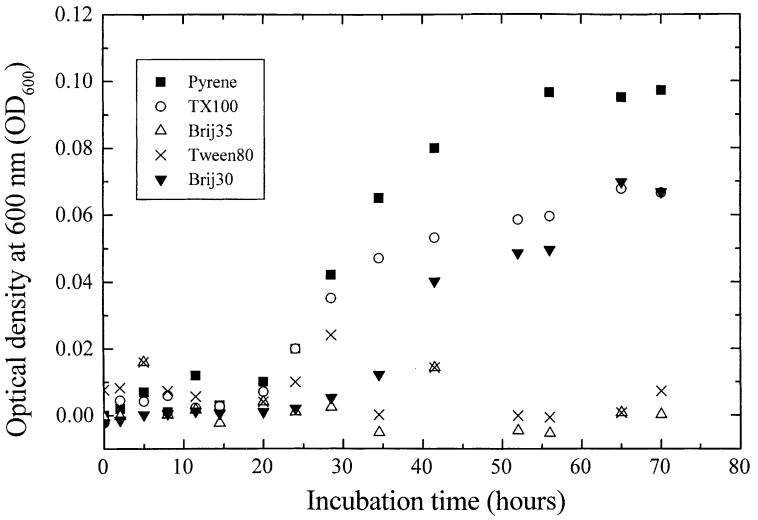


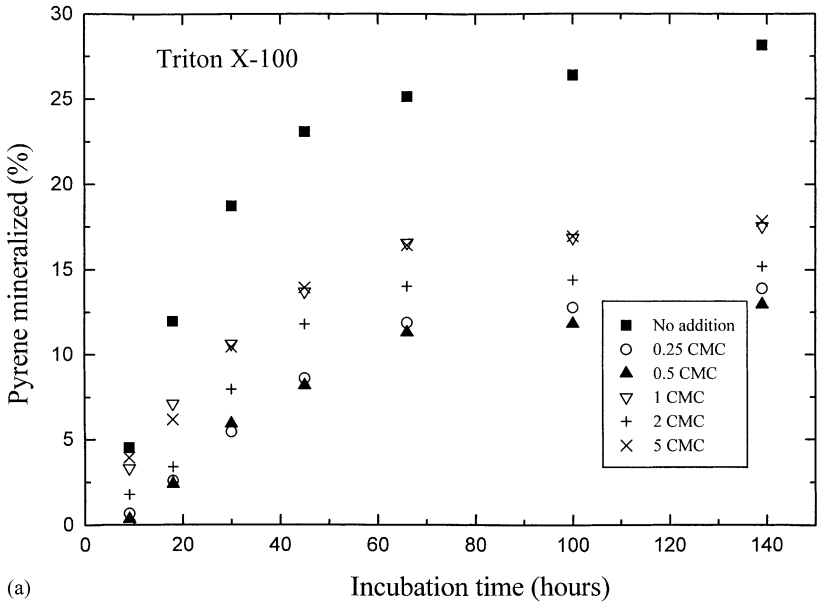
Fig. 4. The growth curves of *Pseudomonas putida* in the presence of different surfactants.

using surfactant or pyrene as sole carbon and energy sources was followed by measuring the optical density at 600 nm (OD_{600}) with a spectrophotometer in a 1 ml cuvette with a 1 cm light path. Fig. 4 illustrates the growth curves of *P. putida* in the presence of different surfactants. No growth of microorganism was observed within 70 h in Brij 35- and Tween 80-amended systems, indicating that *P. putida* could not use these two non-ionic surfactants as sole carbon and energy sources. However, microorganisms can utilize Triton X-100 and Brij 30 as sole carbon and energy sources and obvious growth curves were demonstrated within 70 h. The cell density of *P. putida* in Triton X-100-amended system increased after 15 h of acclimation and reached the stationary phase at 42 h. Longer acclimation was needed for Brij 30 and the cell density of *P. putida* increased after 24 h. Therefore, Triton X-100 and Brij 30 were further selected as the main surfactant systems for the mineralization of PAHs.

P. putida can also use pyrene as its sole carbon and energy sources (Fig. 4). The cell density increased after 20 h of acclimation and reached the stationary phase at 60 h. Also, the OD_{600} at the stationary phase in the pyrene system was higher than those in the presence of surfactant, indicating that the addition of non-ionic surfactants may inhibit the growth of *P. putida*.

3.4. Mineralization of [^{14}C] pyrene

Fig. 5 illustrates the mineralization of [^{14}C]pyrene in an aqueous system in the presence of different concentrations of surfactants. The concentrations of surfactants were 0.25, 0.5, 1, 2, 5 and 10× CMC values. In the aqueous system without the addition of Triton X-100, the microorganisms began to mineralize [^{14}C]pyrene to $^{14}CO_2$ after 9 h of acclimation and the production of $^{14}CO_2$ reached the maximum concentration after incubation of 66 h, which corresponds to 28% of mineralization. However, the addition of Triton X-100 decreased the mineralization rate of pyrene, depicting that the fraction of the micellar-phase pyrene that



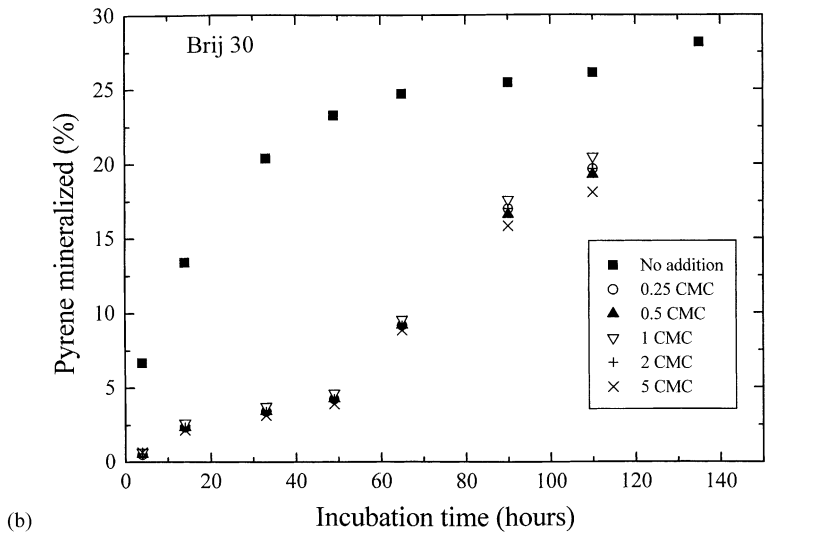


Fig. 5. The mineralization of [^{14}C]pyrene in an aqueous system in the presence of different concentrations of Triton X-100 and Brij 30. The concentrations of surfactants were 0.25, 0.5, 1, 2, 5 and 10× CMC values.

can be directly biodegraded decreases as the concentration of micelle increases. Guha and Jaffe [11] used 4 surfactants to enhance the bioavailability of phenanthrene and found that the bioavailability of PAH decreased with increasing surfactant concentrations. This may be due to the limitation of mass transfer from micelle to *P. putida*. Since PAHs always partition into

a surfactant's micellar phase to some degree, there is a direct mass transfer limitation from the micelle to microorganisms. Mass transfer processes are proportional to a concentration gradient, which depends on the concentration of PAHs in the micellar phase. Therefore, as the PAHs are diluted in a large micellar mass, the transfer of PAHs to the cells decreased. However, Auger et al. [21] showed that the addition of non-ionic surfactant could increase solubilization and mass transfer to the extent of naphthalene degradation by *Pseudomonas*. Another possibility for lowering the mineralization efficiency with the addition of surfactant is the catabolite repression [22,23]. Dual substrates, pyrene and Triton X-100 were existed when surfactant was added into the system. Since pyrene was partitioned into the interior of the micelle, *P. putida* would preferentially utilize Triton X-100 as the carbon and energy sources over pyrene. Therefore, the mineralization rate of pyrene decreased when surfactant was added into the system.

A similar mineralization pattern of pyrene in the presence of Brij 30 was observed (Fig. 5b). The addition of surfactants decreased the mineralization efficiency of pyrene. No significant difference in mineralization efficiency was observed when different surfactants were applied. However, different mineralization behaviors of pyrene were observed when systems contained 4 g soil. As depicted in Fig. 6, the $^{14}CO_2$ was detected at 15 h and maintained a plateau until 50 h. The evolved CO_2 was again increase and reached a plateau after 80 h. This behavior is similar to the growth curve of *P. putida* (Fig. 4), showing that microorganisms can utilize pyrene as their carbon and energy sources and convert to

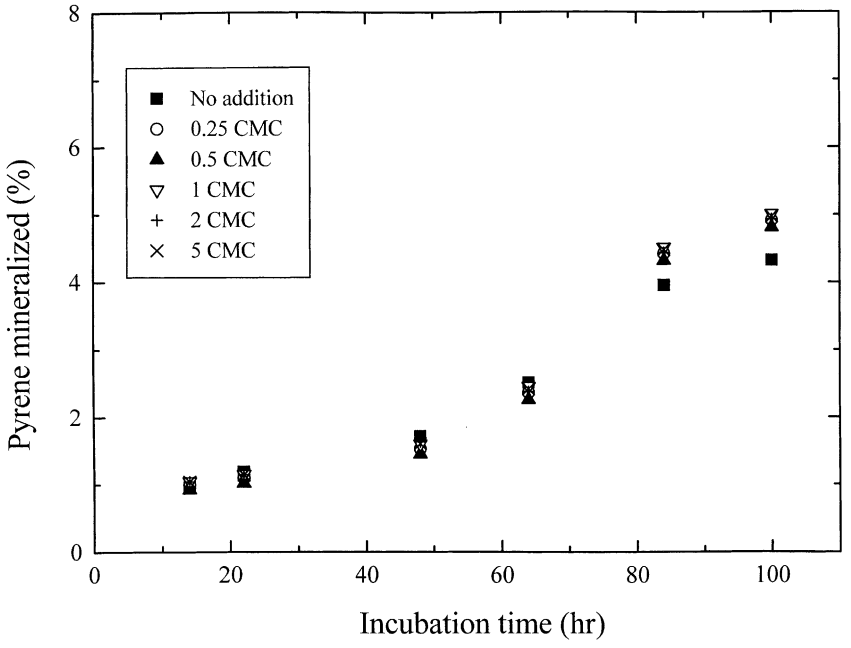


Fig. 6. The mineralization of [^{14}C]pyrene in soil-amended system in the presence of different concentrations of Brij 30. The concentrations of surfactants were 0.25, 0.5, 1, 2, 5 and 10× CMC values.

CO_2. Moreover, the addition of Brij 30 can enhance the bioavailability of pyrene and the cumulative $^{14}CO_2$ increased with the increasing surfactant concentrations. These results clearly indicate that Brij 30 is a promising surfactant to enhance the solubilization and bioavailability of PAHs.

The micellar-enhanced bioavailability means that the contaminants partitioned into the micellar-phase are biodegradable without having to transfer to the dissolved phase first [21]. This bioavailability of the micellar-phase contaminant depends on the surfactant type, surface characteristics of the biomass, and surfactant concentration. In this study, the bioavailability of pyrene decreased with the addition of surfactants in aqueous solution, whereas the micellar-enhanced bioavailability of pyrene increased with time as soils were amended. This may be attributed to that Triton X-100 and Brij 30 can be utilized by *P. putida*. In the aqueous solution, microorganisms utilized surfactants prior to pyrene because pyrene was partitioned into the interior of the micelles. In soil system, however, pyrene is primarily sorbed on the soil. The addition of surfactants can enhance the solubility and bioavailability of pyrene, subsequently increasing the mineralization ration of pyrene. Jaffe and co-workers [11,24] proposed a bioavailability factor to indicate the degree of direct micellar-enhanced bioavailability for a given surfactant concentration and showed that a PAH such as phenanthrene partitioned into the micellar-phase of some non-ionic surfactants is, to some extent, directly bioavailable. However, the micellar-enhanced bioavailability of PAHs is inversely related to the surfactant concentration. This is in good accordance with our results on the enhancement of bioavailability of pyrene with the addition of surfactants.

Since biodegradation of the surfactant is the probable hypothesis, there is a contradiction concerning the biodegradability of the surfactants. The surfactant must be biodegradable in order to avoid contamination of the soil. However, there is also evidence that the biodegradability of the surfactants can decrease the effectiveness of PAHs biodegradation [15,20]. In this study, the biodegradation of surfactants decreased the mineralization rate of pyrene in aqueous solution. However, the mineralization rate of pyrene in the presence of presence of soil can be enhanced by the amendment of biodegradable surfactants. The decrease in mineralization rate in the aqueous phase with the amendment of surfactants may be attributed to the toxicity of surfactants. The surfactant could have altered the physiological characteristic of the degrading microbial population by altering the integrity of the cell member. In this study, the OD_{600} decreased from 0.097 without the addition of surfactant to 0.067 with 50 mg/l of Triton X-100, showing that the microbial activity can be inhibited by the addition of a surfactant. Several researches also demonstrated the inhibition of microbial activities by the addition of a surfactant to a level above the CMC [5]. However, surfactant can sorb onto the surface of soil particle, subsequently decreasing the toxicity of surfactant to microorganism. Therefore, an addition of biodegradable surfactant can enhance the bioavailability and mineralization efficiency of high-ring PAHs in soil environments.

4. Conclusions

The results obtained in this study show that the addition of surfactant has different effects on the solubilization and mineralization of two- to four-ring PAHs. The surfactants with high hydrophobic property (low POE chain or HLB number), such as Triton X-100 and Brij 30

are suitable for the enhancement the apparent solubility of PAHs. The enhanced efficiency followed the order of Brij 30, Triton X-100, Tween 80, SDS and Brij 35. Brij 35 and Tween 80 inhibited the growth of *P. putida*. However, microorganisms can utilize Triton X-100 and Brij 30 as the sole carbon and energy sources at concentrations above CMC values. In the aqueous system without the addition of surfactants, the microorganisms could mineralize 28% of [^{14}C]pyrene to $^{14}CO_2$. The addition of surfactants decreased the mineralization rate of PAHs. Also, the fraction of the micellar-phase PAHs that can be directly biodegraded decreases as the concentration of micelle increases. However, the mineralization rate of pyrene in the presence of soil can be enhanced by the amendment of Brij 30.

Acknowledgements

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