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Estimation of direct-contact fraction for phenanthrene in surfactant solutions by toxicity measurement

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Abstract

The toxicity of solutions containing nonionic surfactants Tween 80, Brij 35 and/or phenanthrene to *Pseudomonas putida* ATCC 17484 was investigated. The fraction of direct contact between micellar-phase phenanthrene and bacterial cell surface was estimated by using the toxicity data and a mathematical model. The mathematical model was used to calculate phenanthrene concentration in the micellar phase and aqueous pseudophase separately. The first-order death rate constant increased from 0.088 ± 0.016 to $0.25 \pm 0.067\,h^{-1}$ when the phenanthrene concentration was increased from 0 to 5.17×10^{-6} M (equals water solubility). The intrinsic toxicity of surfactant was higher in Brij 35 than in Tween 80. When phenanthrene concentration was increased to 9.7×10^{-5} M in surfactant solutions, the death rate constant increased to 1.8 ± 0.024 and $0.41 \pm 0.088\,h^{-1}$ for 8.4×10^{-4} M Brij 35 and 7.6×10^{-4} M Tween 80. The direct-contact fraction was 0.083 and 0.044 for Brij 35 and Tween 80, respectively, under these conditions using exponential model. The toxicity increased with increasing phenanthrene concentration at a fixed surfactant concentration. The toxicity decreased with increasing the surfactant concentration at a fixed phenanthrene concentration due to decreased contact of bacteria with phenanthrene present in the interior of surfactant micelles.

Keywords: Bioavailability; Direct contact; PAH; Surfactant; Toxicity

1. Introduction

Bioremediation of hazardous hydrophobic organic compounds (HOCs), such as polycyclic aromatic hydrocarbons (PAHs), is a major environmental concern due to their toxicity and carcinogenic properties (Keith and Telliard, 1979; Cerniglia, 1992; Woo et al., 2001; Bamforth and Singleton, 2005). These compounds are released to the environment as a result of incomplete combustion of fossil fuels or by accidental discharge during the transport, use, and disposal of petroleum products (Cerniglia, 1992). Due to hydrophobicity of HOCs, surfactants are often used for soil bioremediation in order to increase bioavailability of HOCs by enhancing their solubilization (Scheibenbogen et al., 1994; Tiehm, 1994; Volkering et al., 1998).

A number of studies have been performed on the effectiveness of surfactants on biodegradation of PAHs, but the results have been inconclusive. Some reports have demonstrated that nonionic surfactants stimulate the biodegradation of PAH in liquid or soil systems (Guerin and Jones, 1988; Tiehm, 1994; Liu et al., 1995; Sartoros et al., 2005). However, in other cases the presence of surfactants resulted in little or no PAH degradation (Laha and Luthy, 1991; Tiehm, 1994; Volkering et al., 1995; Zhang et al., 1997; Cort et al., 2002; Lei et al., 2005; Li and Bai, 2005; Sartoros et al., 2005). The negative observations can be explained by one or more of the following effects: (a) toxicity of surfactants due to surfactant-induced permeabilization or lysis of the bacterial cell membrane (Helenius and Simons, 1975; Cserháti et al., 1991); (b) toxicity of surfactant-enhanced aqueous PAH concentrations (Bramwell and Laha, 2000); (c) prevention of bacterial adhesion to the hydrophobic

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substrate (Neu, 1996; Chen et al., 2000; Stelmack et al., 1999); (d) decreased availability of the surfactant-micelle-solubilized PAH (Volkering et al., 1995; Guha and Jaffé, 1996a; Zhang et al., 1997; Cort et al., 2002; Li and Bai, 2005); or by (e) competitive substrate utilization (Tiehm, 1994).

Among the above negative effects, reduced bioavailability of PAHs in micelles is an inevitable property for micelle-forming nonionic surfactants. It is known that reduced bioavailability is attributed to a role of surfactant as a barrier to hinder direct contact of bacterial cells to micellar-phase PAHs (Guha and Jaffé, 1996b; Willumsen and Arvin, 1999). Bioavailability is closely related to the fraction of direct contact between the cell surface and the compounds present in micelles (Guha and Jaffé, 1996a; Guha et al., 1998). If the direct-contact fraction increases, the toxicity of micellar-phase PAHs to bacteria having no adaptation will increase (Willumsen et al., 1998). In our previous study, a method to assess the direct contact of phenanthrene dissolved in surfactant solutions with microorganisms was proposed by measuring toxicity instead of biodegradation experiments requiring several days (Jang et al., 2007). In order to analyze the direct-contact fraction of a surfactant system, the concentrations of PAH in micellar phase and aqueous pseudophase must be separately established. The concentrations cannot be measured experimentally, but can be estimated by using a mathematical partitioning model (Edwards et al., 1991, 1994). In the present study, a modified mathematical model was developed and used to obtain the fraction of direct contact quantitatively with the toxicity data.

2. Materials and methods

2.1. Materials

Phenanthrene with purity greater than 98% and Triton X-100 was purchased from Aldrich (USA). Two commercially available nonionic surfactants, Tween 80 and Brij 35 (Aldrich, USA) were used for the solubilization of phenanthrene and toxicity tests. The chemical properties of the surfactants are listed in Table 1.

2.2. Microorganism

Pseudomonas putida (ATCC 17484) was purchased from Korean Culture Type Collection (KCTC). This strain is able to degrade naphthalene and phenanthrene. The culture was preserved in cryostat vials with 50% glycerol at −70 °C. To recover the frozen culture, a sterilized toothpick was stabbed into the cryostat vial, inoculated into 5 ml Nutrient Broth (NB, Difco, USA), and cultivated for 24 h. One milliliter of the culture was inoculated into 20 ml NB and grown for 24 h once again. The cultures were harvested by centrifugation and washed twice with phosphate buffer solution (PBS, 8.5 g/l NaCl, 0.6 g/l KH_2PO_4, 0.3 g/l Na_2HPO_4). This was resuspended in PBS and the optical density at 600 nm was adjusted to 1.0. The solution was diluted to 1/1000 with PBS and 20 μl was used as inoculum for all toxicity experiments.

2.3. Solubility measurements

The solubility of phenanthrene was measured for two nonionic surfactants having a range of concentrations (0–1 g/l). An individual 20-ml glass vial containing 10-ml solution in a given concentration of surfactant received excess phenanthrene crystals. The vials were autoclaved at 121 °C for 20 min for sufficient solubilization and cooled to room temperature. The dissolved phenanthrene concentration was analyzed by HPLC after removal of excess crystals by filtration (0.2 μm PTFE filter, Whatman, USA).

2.4. Toxicity experiments

The toxicity experiments were performed for three different kinds of components: (i) dissolved phenanthrene-only solution, (ii) Dissolved surfactant-only solution, and (iii) mixed solution with phenanthrene and surfactant. Dissolved phenanthrene-only solution was prepared by dissolving phenanthrene crystals into 100 ml PBS solution and autoclaving at 121 °C for 20 min. The excess crystals were removed by filtration (0.2 μm PTFE filter, Whatman, USA). The phenanthrene concentration of the saturated solution was measured as 5.17×10^{-6} M by HPLC analysis. The saturated solution was diluted with PBS and used for the preparation of the different concentrations of phenanthrene solution. The total volume of the solution was identical for all conditions. The tests used 2 ml of liquid in a 5-ml borosilicate glass vial sealed with Teflon-lined septa. Prior to use in experiments, the vials were conditioned with 4 ml of the solution containing the same concentration of phenanthrene for 24 h. Phenanthrene loss by volatilization or adsorption onto the wall of the glass vial was negligible within 10%, which was confirmed by control tests without the addition of surfactant and microorganisms.

Surfactant-only solution was prepared by dissolving surfactant into 100 ml PBS solution at the concentration of 1 g/l as a concentrated solution. The various concentrations were prepared

Table 1
Selected properties of nonionic surfactants used in this study

Surfactant	Average molecular formula	Average MW[a]	HLB[a]	CMC (M)[b]	MSR[b]	log K_m[a]
Tween 80	$C_{18}H_{34}O_2C_6H_{10}O_4(OCH_2CH_2)_{20}$	1310	15.0	7.6×10^{-6}	0.21	6.3
Brij 35	$C_{12}H_{25}(OCH_2CH_2)_{23}$	1198	16.9	7.6×10^{-5}	0.14	6.1

[a] Prak and Pritchard (2002).
[b] Results in this study.