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# Enhanced *In-situ* Mobilization and Biodegradation of Phenanthrene from Soil by a Solvent/Surfactant System

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**Abstract** The mobilization and biodegradation of phenanthrene in soil was enhanced by using paraffin oil, which was stabilized by the addition of a surfactant (Brij 30). The ratio of paraffin oil/Brij 30 was determined by measuring the change in the critical micelle concentration. When only surfactant was used, the stabilized paraffin oil emulsion could dissolve more phenanthrene in the water phase. Column experiment showed increased phenanthrene mobilization from the contaminated soil. The phenanthrene mobilized in the paraffin oil/Brij 30 emulsion was biodegraded faster than that in water phase or surfactant solution. This result indicates that a paraffin oil/surfactant system can be effectively used for the removal of PAH from contaminated soil.

**Key words:** Paraffin oil, surfactant, phenanthrene, mobilization, biodegradation

Organic solvents, such as alcohols or alkanes, can dissolve high concentrations of PAH (poly aromatic hydrocarbon) and wash PAH-contaminated soil very efficiently [6, 9, 10, 13]. The affinity of a solvent for a given PAH is typically quantified by a distribution coefficient. Among the available solvents, paraffin oil (mineral oil) is safe for human consumption and is used as an oral laxative [2, 5]. The addition of this solvent to water has been shown to increase the biodegradation of PAH [8]. However, paraffin oil is not miscible with water. Therefore, the remediation of PAH contaminated soils by augmentation with paraffin oil is presently limited to *ex-situ* treatment processes. In order to use *in-situ* treatment, paraffin oil must be stabilized by some method, such as the addition of a surfactant. Surfactant is a molecule containing hydrophobic and hydrophilic moieties in the same molecule. By increasing the concentration of surfactant in the water phase, this molecule begins to form a sphere, called a micelle, at certain concentration of surfactant. This concentration is known as the critical micelle concentration (CMC). The presence of surfactant can stabilize the emulsion of paraffin oil. In this work, we used a nonionic surfactant paraffin oil emulsion to increase the aqueous concentration of test PAH (phenanthrene) and to enhance the mobilization of phenanthrene from test soil.

Polyoxyethylene-4-lauryl ether (Brij 30), purchased from Sigma, was used as the nonionic surfactant. The paraffin oil was purchased from a local drug store. The [$^{14}$C]-labeled phenanthrene was purchased from Sigma. A bacterial consortium, a gift from Dr. M. Alexander (Cornell University, U.S.A.), was used which had been obtained from soil. The surface tension of the solution was measured by using a Fisher Tensiomet 20 (Fisher Scientific, New York, U.S.A.). The paraffin oil and surfactant mixture was emulsified using an ultrasonicator for 1 min (Fisher Scientific, New York, U.S.A.). Phenanthrene solubility in the water phase was determined as follows. In 20-ml vials with screw caps, 5 ml of a mixture of [$^{14}$C]-phenanthrene and unlabeled phenanthrene dissolved in methanol was added. After the methanol was allowed to evaporate, 10 ml of sodium azide solution (0.02% with 5 mM $CaSO_4$) was added. The total amount of phenanthrene was 10 ppm, which was about 8 times of the solubility (1.29 ppm) [8]. The vials were placed in a shaker at 30°C for 4 days. Duplicate samples were taken and passed through 0.1 µm PTFE syringe filters to remove any undissolved phenanthrene. A predetermined amount of the sample (4 ml each) was passed through filters before collecting the filtrate to minimize the adsorption during sampling. The filtrate samples were analyzed for radioactivity in a liquid scintillation counter for the amount of phenanthrene.

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Extraction of phenanthrene from the soil was investigated in a chromatographic experiment: 10 ml of a saturated phenanthrene solution containing 5 mM $CaSO_4$ and 0.005% sodium azide were added to 5 g of noncontaminated soil. The soil contained 2.4% organic matter, and the size of the soil ranged between 0.0116 and 0.0087 inches. After equilibrating for 4 days, the soil slurry was slowly poured onto a glass column to pack upto 10 cm (1 cm diameter). The effluent from the bottom was collected until one pore volume of the solution was left in the column. The total radioactivity in the collected effluent was measured to calculate the amount of phenanthrene in the soil column. Then, the washing solution containing surfactant/paraffin oil was pumped into the column at a 10 cm/h pore velocity. The porosity of the column was determined as 42%. The effluent was collected using a fraction collector, and the radioactivity of each fraction was measured for the amount of eluted phenanthrene.

The biodegradation experiments were conducted in 50-ml Erlenmeyer flasks. During the mineralization, the $CO_2$ evolved was absorbed into 1 ml of 1 N NaOH solution in the vial located in the head space of the flask. A silicone rubber stopper wrapped with aluminum foil was used to minimize the loss due to sorption. Fifty µl of a mixture of [$^{14}$C]-phenanthrene and unlabeled phenanthrene dissolved in methanol was added. After the methanol was allowed to evaporate, 10 ml of a minimal medium was added. The total final concentration of phenanthrene was 10 ppm. The minimal medium contained (per liter) 1 g $(NH_4)_2SO_4$, 3.8 g $KH_2PO_4$, 0.95 g KOH, 0.1 g NaCl, 0.2 g $MgSO_4$, and 1 mg $FeSO_4 \cdot 7H_2O$. Phenanthrene-degrading bacterial cells, grown for 3 days with unlabeled phenanthrene (1 g/l), were filtered using sterile coarse filter-paper (#1 Whattman paper) to remove any granular phenanthrene and then innoculated with an initial cell density of 0.005 $OD_{600}$. During the cultivation at 30°C in a shaking incubator, one ml of the NaOH solution was removed by syringe intermittently and the radioactivity was measured to determine the mass of $CO_2$ and mineralization ratio.

## Ratio of Surfactant/Paraffin Oil and Increase in Phenanthrene Solubility

An oil and surfactant mixture can be stabilized if the surface of the oil drop is surrounded by the surfactant molecules. Since the surfactant will locate on the water/air surface after surrounding the oil drop, more surfactant is required to form micelle [15]. Therefore, by measuring the difference in the CMCs between the surfactant-water system and the surfactant-oil-water system, the ratio of oil and surfactant can be determined. As shown in Fig. 1, in the presence of paraffin oil, more surfactant was needed to achieve the minimum surface tension for micelle formation. One hundred and forty ppm (v/v) more surfactant was required for 5,000 ppm paraffin oil (0.5% v/v). Therefore,

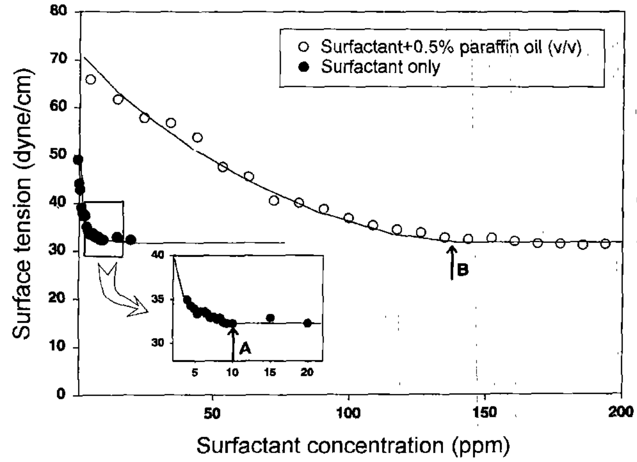


**Fig. 1.** Determination of the ratio between oil and surfactant. The oil in water was emulsified by a sonicator and a surfactant added. A and B indicate the critical micelle concentration of the surfactant-water system and surfactant-oil-water system, respectively

2.8 ppm (v/v) of surfactant was required to surround a 100 ppm (v/v) oil drop. After determining the ratio of surfactant and paraffin oil, the solubility of the phenanthrene was determined at various concentrations of surfactant and surfactant/oil. As shown in Fig. 2, the surfactant/oil mixture solubilize more phenanthrene than the surfactant alone. This was attributed to the presence of oil drops in the surfactant/oil mixture.

## Phenanethrene Mobilization in the Soil Column

In order to compare the effectiveness of the phenanthrene mobilization by the surfactant/oil mixture with the surfactant-only system, a column test was performed. As shown in Fig. 3, the addition of just the surfactant resulted in a poor mobilization of the phenanthrene from the soil. The concentration of surfactant, 16 ppm, was too low to extract

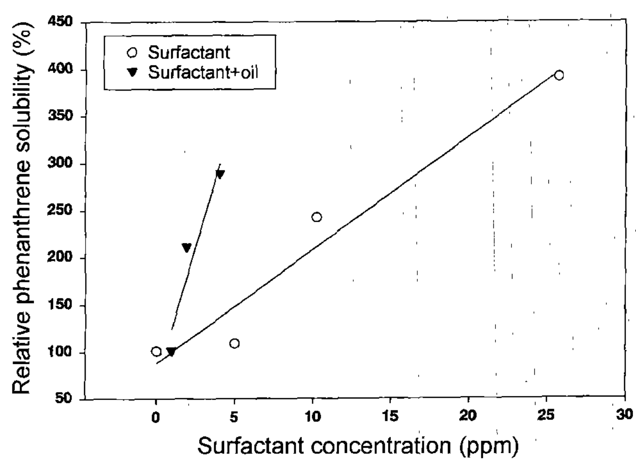


**Fig. 2.** Increased solubility with the surfactant-solvent system.

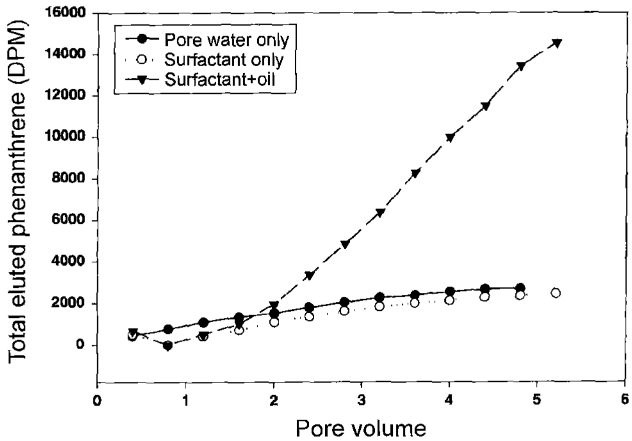


**Fig. 3.** Effects of surfactant and oil in soil washing in column.
Paraffin oil was used in an optimized ratio with the surfactant (oil: surfactant=570 (ppm): 16 (ppm) (v/v)).

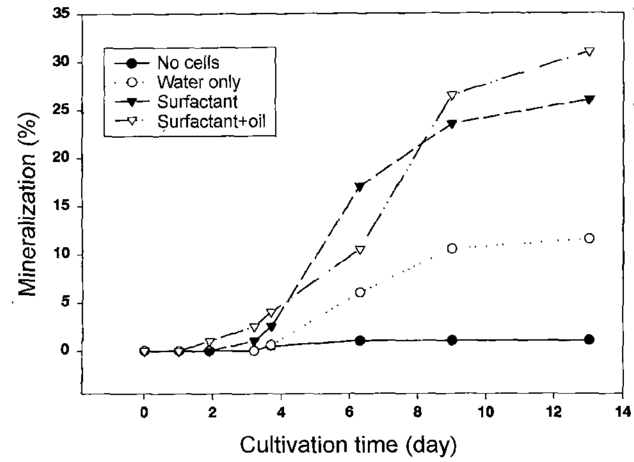


**Fig. 4.** Mineralization of phenanthrene with surfactant and paraffin oil.
"No cell" indicates a sample without the addition of cells. The cells were previously grown in a medium with the surfactant (16 ppm).

phenanthrene compared to 1% used in the actual field. Therefore, most of the surfactant could be absorbed to the soil. However, when the same amount of surfactant was mixed with paraffin oil, the surfactant/oil mixture was able to extract phenanthrene significantly more than other solutions. Extraction of soil with paraffin oil only was unsuccessful, since paraffin oil was not miscible with water. Comparison of the extraction ability of the surfactant/oil system was more clear in the column experiment than in the batch experiment where extracting agents such as surfactant or paraffin oil were mixed with contaminated soil for a determined time and extracted pollutant was determined. In that case, only equilibrium data were available.

**Biodegradation of Phenanthrene in the Surfactant/Oil**
Phenanthrene-washed soil by a surfactant/oil should be mineralized by microorganisms and the surfactant/oil recycled to lower the cost of remediation [1, 14, 17]. Accordingly, to investigate the bioavailability of the phenanthrene dissolved in the oil drops coated by the surfactant, a mineralization experimentation was performed to measure the $^{14}CO_2$ produced during the phenanthrene mineralization. As shown in Fig. 4, the phenanthrene in the surfactant/oil system was mineralized faster than that in the water. The rate of mineralization in the surfactant/oil was similar to that in the surfactant, thereby, indicating that phenanthrene in the paraffin oil is easily accessible to microbes.

For the mineralization, the adaptation of the cells to the surfactants shortened the lag time for the initiation of biodegradation as shown in Fig. 5. Modifications in the cell-membrane structure during the hydrocarbon degradation have already been reported by other investigators [12]. Similar modification may also occur during the adaptation period. However, further study is required to elucidate these modifications. This step of adaptation is not required in a continuous bioreactor, once the cells are adapted to the surfactant.

The increased rate of PAH-degradation by augmentation of either a solvent or a surfactant alone has already been reported by many workers [3, 4, 6, 9, 10, 13]. However, the simultaneous use of both surfactant and solvent in the soil washing and biodegradation, as shown in the present work, can effectively increase the mobilization and biodegradation of PAH. This result indicates that a surfactant/oil can be successfully used to enhance the *in-situ* mobilization of phenanthrene in soil and its subsequent biodegradation.

In this work, we showed that simultaneous use of paraffin oil and a surfactant can increase the mobilization and subsequent biodegradation of phenanthrene from contaminated

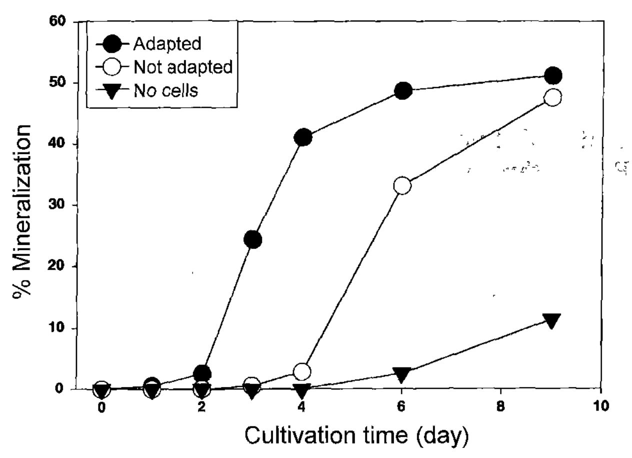


**Fig. 5.** Effects of cell adaptation to surfactant.
Cells grown with the surfactant (16 ppm) or without the surfactant were respectively inoculated into the medium including the surfactant. The $^{14}CO_2$ was measured intermittently.

soil. An emulsion of an environmentally-safe solvent, paraffin oil, was stabilized by surfactant augmentation. The exact amount of surfactant required was determined by simply measuring the surface tension. The phenanthrene entrapped in an oil/surfactant emulsion drop degraded faster than that in water. Although this experiment is preliminary and limited in the laboratory, it is anticipated that the technique can be used in *in-situ* extraction and the biodegradation of PAH from contaminated soil.

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