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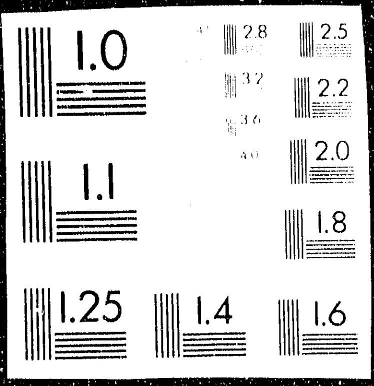

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Effects of Nonionic Surfactants on the Microbial Mineralization of Phenanthrene in Soil-Water Systems

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INTRODUCTION

Bioremediation of soils and sediments contaminated with organic compounds may be a cost effective technology for environmental clean-up and aquifer restoration. However, not much is known about the physicochemical mechanisms that may control the rate of bioremediation for environmental samples. This is particularly the case for hydrophobic organic compounds (HOCs) that may be adsorbed onto soil, or that may exist as non-aqueous phase liquid (NAPL). Understanding rate-controlling processes in bioremediation is complicated by the interplay between physicochemical phenomena governing phase-partitioning, and interfacial processes affecting both the microorganisms and the organic compounds.

Treatment under ideal conditions has indicated that a range of polycyclic aromatic hydrocarbon (PAH) compounds may biodegrade when present in aqueous solution; however, removal from a solid matrix is less predictable and, generally, much less efficient. On the basis of such data, it is hypothesized that soil-waste matrix effects prevent the release of PAHs into the aqueous phase where they may undergo biodegradation, i.e., it is mass transfer limitations associated with the release of contaminants from the soil-waste matrix that limit the rate of removal of PAHs from the soil, rather than the explicit aqueous-phase biodegradation kinetics.

It has been suggested that the application of surfactants or emulsifying agents may decrease interfacial tension and/or assist solubilization of sorbed HOCs from soils, thereby making the hydrophobic compounds more available for microbial degradation (Lee et al., 1988; Mueller et al., 1989). The experimental observations on the effect of surfactant addition on microbial degradation of HOCs are not always consistent, nor has a general explanation been advanced for their influence. In soil/sediment systems it is possible that insoluble sorbed HOCs can also be mobilized through the use of surfactants or surface-active agents (Laha and Luthy, 1991), and thereby made more available for microbial attack by decreasing the interfacial tension between the compound and water (Aronstein et al., 1991; Rittmann and Johnson, 1989). Results from previous investigation into the effects of nonionic surfactants on the biomineralization of phenanthrene in soil-water systems suggest that at surfactant concentrations resulting in the formation of aqueous-phase micelles, the microbial mineralization of phenanthrene was completely inhibited; and at sub-CMC surfactant levels the degradation of phenanthrene proceeded at best as fast as in the absence of any of the nonionic surfactants tested (Laha and Luthy, 1991). The purpose of the work reported in this paper was to determine whether the inhibitory effect on microbial degradation of phenanthrene was specific to the nonionic surfactants used previously, i.e., the alkylethoxylate and alkylphenol ethoxylate surfactants. Thus, a number of nonionic surfactants of varying structures and properties were selected for further investigation. In addition, several tests were performed to verify results from earlier experiments.

EXPERIMENTAL METHODS

The model PAH compound used in this study was phenanthrene. The surfactants used were four alkylethoxylate surfactants, two alkylphenol ethoxylates, two polyoxyethylene sorbitan ester surfactants, octylglucoside, and CHAPS, a sulfobetaine derivative of cholic acid. Both solubilization and mineralization tests employed radiotracer techniques. The systems considered were soil-water slurries with soil-to-water ratios of 1 g of soil/8 mL of water. In solubilization experiments samples of soil, water, surfactant, and ^{14}C-phenanthrene were contained in 50-mL centrifuge tubes and equilibrated for 48 hours by periodic rotation, following which they were centrifuged, and aliquots withrawn and filtered through 0.22µ Teflon filters, and counted for ^{14}C (Liu et al., 1991; Laha and Luthy, 1991). Biodegradation experiments measured the evolution of $^{14}CO_2$ captured in alkaline solution from soil-water slurries containing ^{14}C-phenanthrene, surfactant, and a PAH-degrading inoculum (Laha and Luthy, 1991). Surface tension measurements with a DuNuoy ring tensiometer were used to determine surfactant sorption onto soil.

RESULTS AND DISCUSSION

The mineralization of phenanthrene in soil-water systems reached about 50% in 4-6 weeks in the absence of surfactant. For soil-water systems receiving varying doses of $C_{12}E_4$ alkylethoxylate surfactant, inhibition of microbial mineralization was observed at surfactant doses in excess of 0.05% (v/v). Surface tension and solubilization experiments indicate that the surfactant dose at which micelles/aggregates form in the soil-water slurries are ~0.08% (v/v) for $C_{12}E_4$. A comparison of solubilization and mineralization data suggest that the inhibitory effect on microbial degradation of phenanthrene appears to be related to the CMC of the surfactant in the presence of soil, i.e., surfactant inhibition appears to be a micellar phenomenon. To determine whether or not this inhibitory effect was specific to the particular nonionic surfactants selected initially, a number of other surfactants were tested at doses below and above 0.10%, i.e., at 0.02 and 0.20% respectively.

Figures 1-3 are representative of the results obtained from biodegradation, solubilization, and surfactant sorption experiments respectively, for the polyoxyethylene sorbitan monooleate, Tween 80. This surfactant inhibits phenanthrene mineralization at a dose of 0.20% (v/v) over a period of about 45 days, and results in a lower rate of mineralization at a dose of 0.02% (Figure 1). Results from the current solubilization tests indicate that the amount of surfactant required to solubilize phenanthrene in the presence of soil is considerably greater than the corresponding clean water CMC. For example, Figure 2 shows the solubilization of phenanthrene in the soil-water system by the polyoxyethylene sorbitan monoolate surfactant, Tween 80, does not commence until a surfactant dose of ~0.20% (volume of surfactant/volume of water) is employed, which is considerably greater than the reported clean water CMC of 10^{-5} M or 1.3×10^{-3}% (v/v). Figure 3 shows a plot of surface tension against surfactant dose for a similar soil-water system with Tween 80. There is a distinct inflection point on the surface tension curve at a surfactant dose of 0.21% (v/v). This corresponds to the surfactant dose required to form micelles in the presence of soil, implying that the difference between the surfactant dose necessary to attain CMC in the soil-water system and the clean water system represents the amount of surfactant sorbed onto soil. Comparison of solubilization and surface tension results as in Figures 2 and 3 for the sorbitan monooleate Tween 80 indicate that surfactant solubilization of phenanthrene is initiated only after the formation of aqueous-phase micelles. Solubilization of soil-sorbed phenanthrene in these systems is therefore a micellar phenomenon.

The inhibition of microbial mineralization of phenanthrene observed in these soil-water systems appears also to be a micellar phenomenon. For all the surfactants used other than the high CMC, low aggregation number surfactants, i.e., octylglucoside and CHAPS, no significant phenanthrene mineralization was observed at the higher concentrations (i.e., ~0.20%), whereas at the lower surfactant dose (i.e., ~0.02%) the surfactant had little or no effect on the biodegradation of phenanthrene. The 0.02% dose is below the CMC for all these surfactants, while at ~0.2% dose the concentration is either greater than or equal to the surfactant dose required to attain CMC in the presence of soil. It is possible that the microbial inhibition observed is the result of bacterial interaction with micelles.

The likely causes for the inhibition of microbial degradation of phenanthrene at higher surfactant doses are: (i) a toxicity effect; (ii) a lower bioavailability of micellized phenanthrene, e.g., due to low exit rates from micelles; (iii) or surfactant interactions with microbial membranes and proteins. Several hydrocarbons require that bacteria adhere to the hydrocarbon-water interface prior to biodegradation. Surfactants may prevent the adherence of microorganisms and consequently may prevent biodegradation of certian oily constituents (Efroymson and Alexander, 1991), perhaps in this case, phenanthrene. Nonionic detergents are generally believed not to be excessively toxic to microorganisms and many are reported to be readily biodegradable. To examine the toxicity phenomenon, a number of dilution tests were performed where four weeks into the mineralization experiment surfactant solutions were diluted from supra-CMC levels to concentrations below those resulting in micelle formation. Such dilution was observed to result in the recovery of the phenanthrene-degrading ability of the soil-water system, suggesting that the presence of surfactant micelles did not result in cell death, and that the inhibition produced may be attributable to some reversible interaction. Furthermore, bacterial enumerations performed on soil-water suspensions at the conclusion of the mineralization tests from systems without surfactant and systems receiving 0.4% (v/v) $C_{12}E_4$ surfactant indicated the presence of comparable numbers of phenanthrene-degrading microorganisms in both systems. Thus, it appears that the presence of micellar surfactant temporarily prevents the mineralization of phenanthrene without destroying the microbial population involved in PAH degradation.

SUMMARY

The exact effects or microorganisms produced by the surfactants employed in the current study are not known. Results from mineralization experiments suggest that the inhibition of microbial degradation of phenanthrene observed at higher surfactant doses is a micellar phenomenon. Evidence from the dilution experiments and bacterial enumeration at the conclusion of biometer studies indicate that the inhibitory effect produced by these surfactants may be reversible. This suggests either that micellized PAH is not bioavailable, or that a surfactant-bacteria interaction may result in some reversible interference with cell membrane processes. There is a need to know more of the physical and biochemical means by which surfactant may affect the transport of HOCs to and across cell membranes. The effects of surfactant solubilization on biodegradation of HOCs must be better understood in order to assess whether synthetic surfactants may be used advantageously for enhancement of bioremediation of contaminated soils and sediments.

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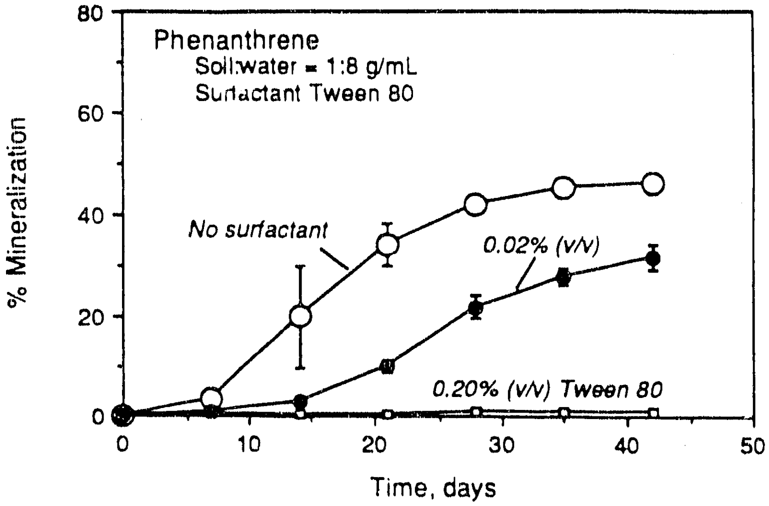

Figure 1. Mineralization of phenanthrene in soil-water systems in the presence of the sorbitan monooleate ethoxylate surfactant Tween 80.

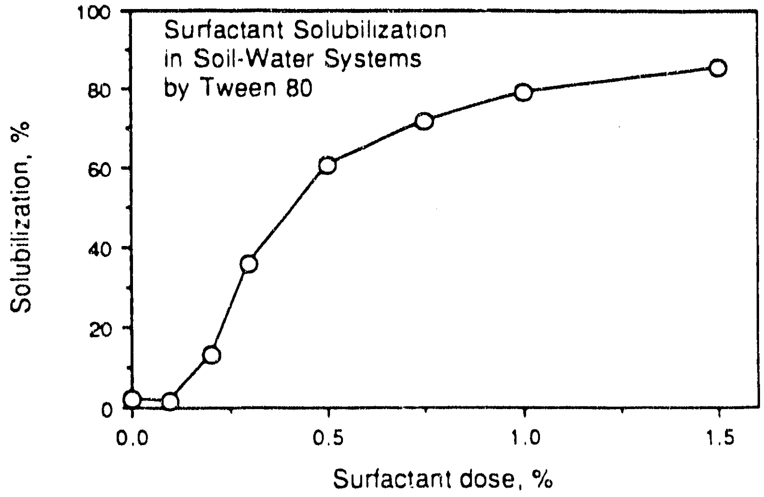

Figure 2. Phenanthrene solubilization in soil-water systems by the sorbitan monooleate ethoxylate surfactant Tween 80.

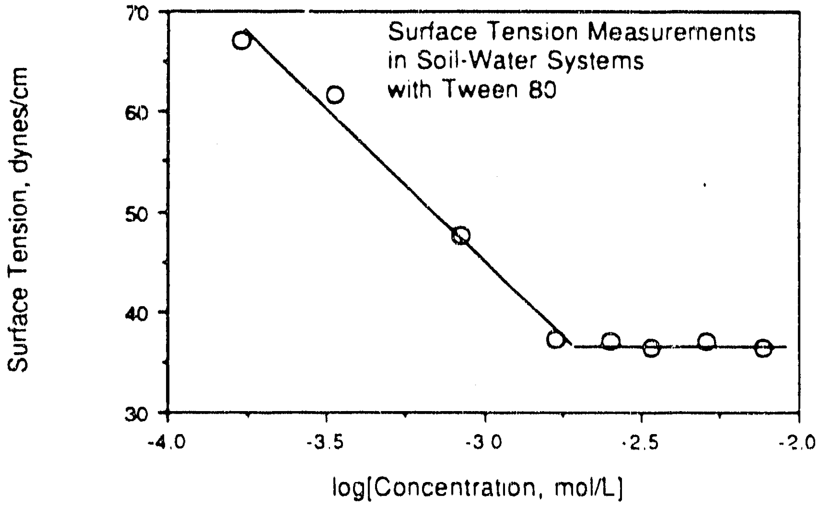

Figure 3. Determination of CMC by surface tension measurements for the sorbitan monooleate ethoxylate surfactant Tween 80 in soil-water systems.

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