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The Potential for Solubilizing Agents to Enhance the Remediation of Hydrophobic Organic Solutes in Soil-Water Suspensions

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ABSTRACT

This paper discusses the feasibility for use of surfactant solubilizing agents to enhance the solubility and the rate of microbial degradation of hydrophobic organic solutes in soil-water suspensions. Hydrophobic organic contaminants are strongly sorbed to soil or sediment material, and as a consequence the rate of microbial degradation may depend greatly on the desorption of the sorbed-phase contaminant and the accessibility of the contarninant to soil microorganisms. Chemical solubilizing agents may enhance the rate of hydrophobic organic solute degradation by increasing the rate of solute desorption from soil and the extent of solute partitioning to the aqueous phase.

The presentation will review on-going research on: (i) surfactant solubilization of polycyclic aromatic hydrocarbon (PAH) compounds in clean water, and in soil-water suspensions; and (ii) experiments to assess if the addition of surfactant to soil-water suspension results in faster rate of mineralization of PAH compounds in soil.

The presentation explains the methodology employed to select various surfactants for use in the experiments. Experimental results presented show the equilibrium partitioning of phenanthrene, anthracene and pyrene in soil-water suspensions. A preliminary model is shown which describes some of the features of the solubilization process.

Currently work is in progress to evaluate the rate of evolution of $^{14}CO_2$ from soil-water suspensions using ^{14}C-labeled phenanthrene and surfactants. The tests are being performed with acclimated PAH-degrading organisms. The experimental protocols for this work will be reviewed.

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Introduction

Organic compounds enter soil-groundwater systems as a result of accidental spills, improper waste disposal techniques, and through agricultural practices. Because groundwater is an important resource, and because many organic contaminants are hazardous to human health, there is considerable concern over groundwater contamination by such compounds. These concerns have prompted much research on efficient remediation techniques for contaminated soils and aquifers. In-situ bio-remediation techniques offer the advantage of on-site treatment without handling large quantities of soil. Although such techniques have been broadly discussed (Lee et al., 1989; Thomas and Ward, 1989), in general in-situ bioremediation technologies have not been widely implemented, owing in part to a lack of understanding of various mechanisms and rate-controlling processes.

Aromatic organic contaminants that have been reported to be degraded by subsurface microorganisms include polar solvents, benzene and substituted benzenes, phenols, naphthalene, phenanthrene, dibenzofuran, fluorene and benzo(a)pyrene (Zoyer, Kuhn and Schwarzenbach, 1986; Mihelcic and Luthy, 1988 a, b; Grbic-Galic and Vogel, 1986; Bauer and Capone, 1985; Battermann, 1986; Evans and Fuchs, 1988; Berry, Francis and Bollag, 1987; Major et al., 1988). Various factors may limit biodegradation of subsurface pollutants including the presence of acclimated organisms, the availability of oxygen or other electron acceptor, the concentration of the contaminant, the sorption of highly hydrophobic contaminants onto soil, and environmental factors including suitable nutrients, pH and temperature (Lee et al, 1989).

Current Research Activity

This paper outlines on-going research in our program on the subject of surfactant-enhanced solubilization of polycyclic aromatic hydrocarbon (PAH) compounds in soil-water systems. PAHs are hydrophobic compounds that tend to sorb onto soil and are not readily amenable to remediation by simple soil washing or microbial degradation. Although in some cases sorption may enhance degradation by concentrating nutrients, it has been suggested that PAH sorption may inhibit degradation by rendering the substrate unavailable to the microorganisms (Mihelcic and Luthy, 1989). Chemical solubilizing agents may enhance the rate of hydrophobic organic substrate degradation by increasing the rate of solute desorption from soil and the extent of solute partitioning to the aqueous phase. This study seeks to determine the processes whereby surfactants may solubilize PAH compounds in soil-water systems and the effect of surfactant solubilization on the biodegradation of PAH compounds such as phenanthrene, anthracene and pyrene.

The solubilization of phenanthrene, anthracene and pyrene was evaluated in soil-water suspensions with several nonionic and anionic surfactants. The following section discusses some of the results of the solubilization tests and presents a preliminary approach for describing some of the features of the solubilization process. The next section describes on-going experiments to evaluate the rate of mineralization of PAH compounds by monitoring the evolution of $^{14}CO_2$ from soil-water suspensions using ^{14}C-labeled phenanthrene and various surfactants. Several issues are being addressed in the current research:

1. How do surfactants solubilize PAH compounds in the absence of soil?
2. How are PAH compounds solubilized by surfactants in the presence of soil?
3. What is the rate of PAH compound mineralization in soil-water systems under aerobic and denitrification conditions?
4. What is the rate of PAH compound mineralization in soil-water systems in the presence of surfactants that solubilize PAH compounds from soil?

Surfactant Solubilization

A possible explanation for the persistence of many hydrophobic organic compounds in the soil environment is their tendency to sorb strongly onto soil, resulting in their non-availability to subsurface microorganisms. Stucki and Alexander (1987) report that calculations based on the rate of dissolution of phenanthrene and the rate of growth of microorganisms support the view that the rate of dissolution of the hydrophobic PAH may limit its rate of biodegradation. The addition of surfactants to such systems may assist bio-remediation by aiding desorption of the hydrophobic organic contaminant from soil. Alternatively, surfactants may also be inhibitory to microorganisms, as well as contribute to the demand for the electron acceptor; surfactants may also result in changes of the physical properties of soil.

Surfactants possess both polar and non-polar regions on the same molecule, and at solution strength greater than the critical micelle concentration (CMC) surfactant molecules aggregate to form micelles. Surfactant solutions may solubilize hydrophobic contaminants from soil by assisting contaminant desorption and incorporation of the organic compound within the aqueous phase surfactant micelle.

Several laboratory-scale investigations in the last few years have assessed the potential for surfactants to treat contaminated soils (Ellis et al., 1984; McDermott et al., 1988; Rickabaugh et al., 1988; Rajput et al., 1989; Vigon and Rubin, 1989). Generally, surfactant solutions in the range of 1 to 4% have been suggested as promising for scrubbing fuel components, PCBs and other chlorinated hydrocarbons from soil. The EPA has conducted a field test of in-situ soil washing with surfactant to evaluate removal of petroleum hydrocarbons (Nash and Traver, 1986). Potential difficulties for deployment of surfactants in soil clean-up included problems of soil clogging for in-situ use, separation and treatment of surfactant solutions, and recovery of surfactants for reuse. Rittmann and Johnson (1989) evaluated the use of dispersant to assist microbial degradation of lubricating oil in soil; the dispersant assisted the rate of microbial degradation when used in combination with slurry mixing and acclimated inoculum. The current literature contains essentially no information on the mechanism of surfactant-aided solubilization of hydrophobic organic compounds in soil-water systems.

The following summarizes experimental methodologies and some results of experiments for surfactant solubilization of PAH compounds as reported in Liu et al. (1989).

Methods: Phenanthrene, anthracene and pyrene were used in batch tests with 50 ml centrifuge tubes containing about 6.25 g soil spiked with a measured volume of PAH stock solution. The sealed centrifuge tubes were mounted on a tube rotator for an equilibration period of 24 hours or more. The soil was an undisturbed, A horizon, subhumid, grassland soil of the Barnes-Hamerly Association (Mihelcic and Luthy, 1988a), or a Hagerstown silt loam collected from the A horizon of the Agriculture Experimental Station, Pennsylvania State University. The soil was air-dried and screened to pass a US standard No. 10 mesh (2 mm) sieve. The surfactants were obtained either directly from the manufacturer or chemical distributor and were used without further purification. Unlabeled PAH compounds were obtained from Aldrich Chemical Co., Wis. (purity > 98%), and ^{14}C-labeled PAH were obtained from Amersham Corporation. The activity of ^{14}C-labeled PAH used in individual tests was about 0.2 µCi per 50 ml sample.

The PAH doses for the various tests with 6.25 g soil were designed to attain an initial equilibrium concentration of the PAH near aqueous phase saturation. This was estimated from measurement of the fraction organic carbon in the soil (f_{oc}), the mass of soil used, the volume of the aqueous phase, the PAH octanol-water partition coefficient (K_{ow}), the aqueous solubility of the PAH, and the proportionality between K_{ow} and the organic carbon-normalized sorption coefficient (K_{oc}) for PAH may be estimated by Karickhoff et al. (1979).

Prior to analysis samples were centrifuged, and aliquots withdrawn with a syringe and

expressed through a pre-conditioned 0.22 μm PTFE membrane filter. Samples were taken in triplicate and counted for ^{14}C on a Beckman LS 5000 TD Liquid Scintillation Counter to at least the 99% confidence level, using the H# quench monitoring technique with automatic quench compensation.

Table I. Structures and Properties of Selected Surfactants

Surfactant	Structure	Type (mol. wt.) [CMC, M]	Reference
Brij 30 (liquid, d = 0.95)	$C_{12}H_{25}(OCH_2CH_2)_4OH$ dodecylethoxylate with 4 ethoxylate units	Nonionic (362.5) [6.5×10^{-5}] (1)	Rubin and Vigon (1989)
Igepal CA-720 (liquid, d = 1.04)	C_8H_{17}-C_6H_4-$O(CH_2CH_2O)_{12}H$ octylphenylethoxylate with 12 ethoxylate units	Nonionic (735) [~6×10^{-4}] (2)	
Triton X-100 (liquid, d = 1.08)	C_8H_{17}-C_6H_4-$O(CH_2CH_2O)_xH$ octylphenylethoxylate with average x = 9.5	Nonionic (628) [2×10^{-4}] (3) [$(3 - 3.3) \times 10^{-4}$] (1)	McDermott et al. (1988) Kile and Chiou (1989)
Hyonic NP-90 (liquid, d = 1.06)	C_9H_{19}-C_6H_4-$O(CH_2CH_2O)_9H$ nonylphenylethoxylate with 9 ethoxylate units	Nonionic (616) [~7×10^{-5}] (2) [~5×10^{-5}] (4)	Ellis et al. (1984)
Adsee 799 (liquid, d = 1.04)	blend of polyoxyalkylated fatty acid esters	Nonionic	Ellis et al.(1984) Rajput et al.(1989)
Corexit 7664 (liquid, d = 1.02)	blend of surfactant esters	Nonionic	Rittmann & Johnson (1989)
Sodium lignin sulfonate (solid)	sulfonated polymers of complex structure containing free phenolic, primary & secondary alcoholic, and carboxylate groupings.	Anionic (1000-20,000)	Liu (1980)
Sodium dodecyl benzenesulfonate (solid)	$C_{12}H_{25}$-C_6H_4-SO_3Na	Anionic (348) [~1.5×10^{-3}] (2)	McDermott et al. Nash and Traver (1986)

(1) CMC from Rosen (1989)
(2) CMC either direct or extrapolated from Mukerjee and Mysels (1971)
(3) CMC from Kile and Chiou (1989)
(4) CMC data from Attwood and Florence (1983)

Results and Discussion: The surfactants that were used in this study are listed in Table I along with known properties. They were selected on the basis of a literature survey of surfactant-aided soil washing and biodegradation studies in order to provide a range of surfactant types for the solubilization tests. The solubilization tests involved the addition of aqueous surfactant solution to freshly-prepared PAH-dosed soil followed by an equilibration period of 24 hours during which time the samples were kept in suspension by a tube rotator. PAH pre-equilibration time prior to the addition of surfactant had no significant effect on the solubilization of PAH.

Figure 1 shows the partitioning of anthracene between soil and aqueous phase with varying concentrations of the surfactant Brij 30. The total mass of anthracene was 0.1 mg with soil mass varying from 3 to 15 g per 50 ml. The partition coefficient is defined as the ratio of the concentration of solute associated with the solid phase to its concentration in the liquid phase. In the absence of surfactant, the partition coefficient K_D is 650 ml/g_s, or log K_D = 2.8. This agrees with a value of log K_D = 2.6 as estimated from correlation with K_{ow} and f_{oc}. At a surfactant concentration of 0.1% by volume, the partition coefficient does not change appreciably (K_D = 320 ml/g_s, log K_D = 2.5), and anthracene remains predominantly in the sorbed phase. As the surfactant dose increases to 0.5% and 1%, there is marked decrease in the partition coefficient (K_D= 12.5 and 5.5 ml/g_s respectively for 0.5% and 1% v/v Brij 30). That the surfactant has little effect at concentrations less than 0.1% is in agreement with earlier studies which report < 10% solubilization for hydrophobic solutes in soil-water systems at surfactant doses of 0.1% and less.

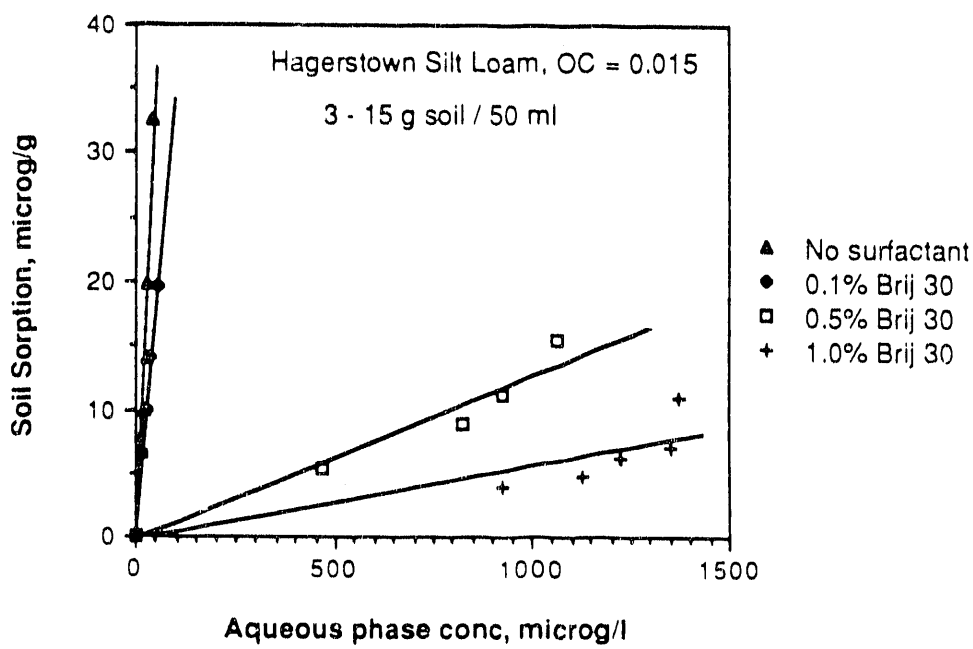

Figure 1. Partitioning of Anthracene at Various Soil-to-Water Ratios with 0 to 1% Dodecylethoxylate Surfactant Having Four Ethoxylate Units.

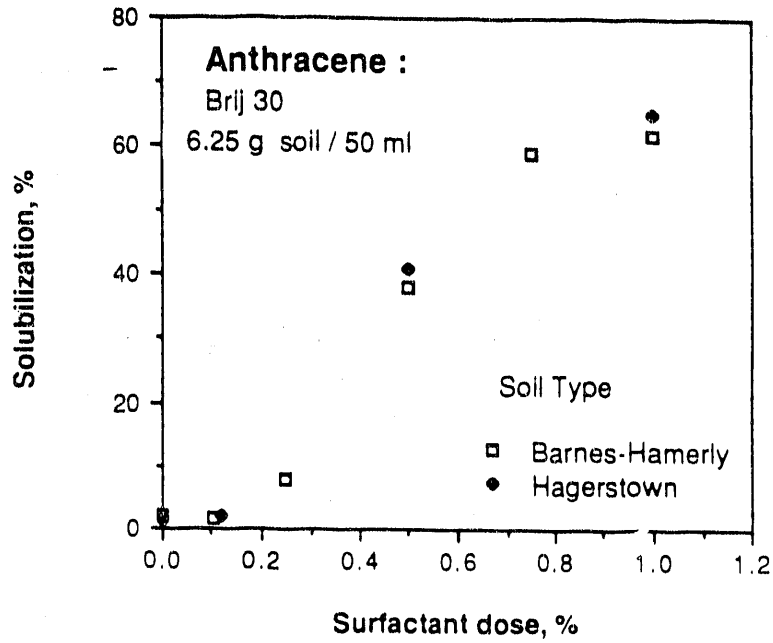

Figure 2. Solubilization of Anthracene for Two Soil Types with 0 to 1% Dodecylethoxylate Surfactant Having Four Ethoxylate Units.

The critical surfactant concentration at which solubilization is achieved is nominally the surfactant concentration at which micelles or surfactant aggregates start to form in the aqueous phase. In the soil-water systems considered, the surfactant dose required to achieve PAH solubility enhancement is considerably in excess of the reported value of the surfactant-water CMC. Figure 2 shows the solubilization of anthracene by the surfactant Brij 30. Brij 30 has a CMC of 6.4×10^{-5} M, which is equivalent to a surfactant dose of 2.5×10^{-3} % v/v; solubilization is observed only at surfactant doses > 0.1% which is 40 times greater than the CMC. The presence of soil results in a change in the solubilization pattern, presumably because the surfactant sorbs onto the soil, resulting in aqueous phase surfactant being considerably less than the total added. Figure 2 shows also that the solubilization of anthracene for both soils was similar, probably because of similar morphology and because the values for the fraction organic carbon for the soils were not very different (~1% and 1.5%). Surfactant solubilization of the PAH in the absence of soil has confirmed that the PAH solubility increases linearly with the surfactant concentration as surfactant dose is increased above the reported value of the CMC (Edwards and Luthy, 1990).

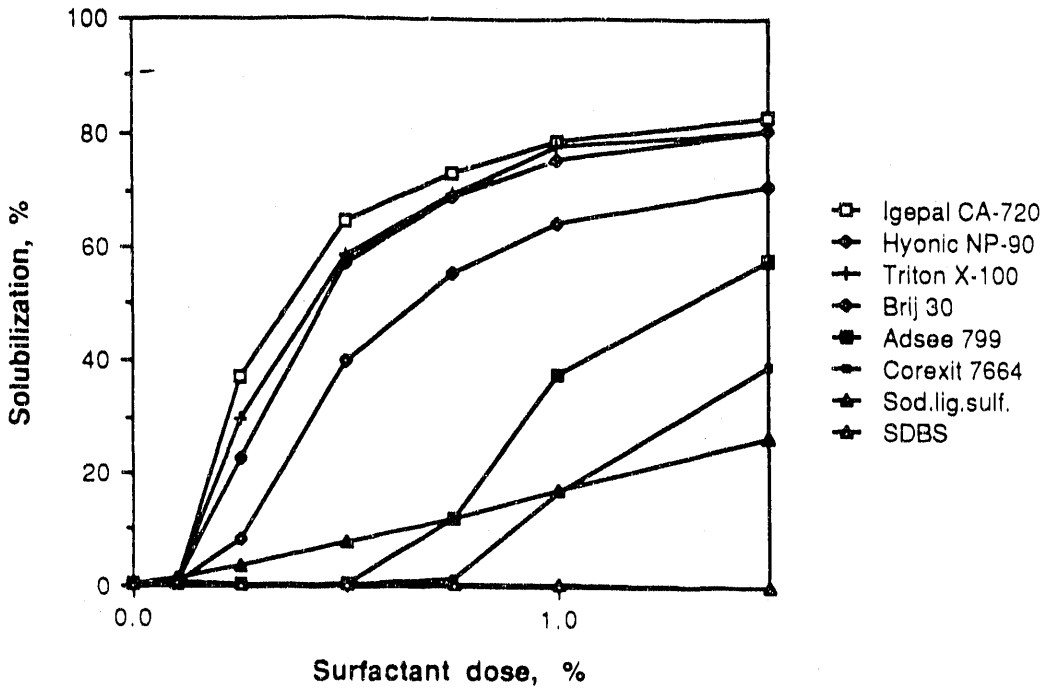

Figure 3. Solubilization of Pyrene by Various Surfactants with 6.25g soil/50 ml Suspension and Total Mass of Pyrene of 0.8 mg.

Data for surfactant solubilization of pyrene are presented in Figure 3. The solubilization curves for anthracene and phenanthrene showed similar behaviour. The nonionic surfactants Brij 30, Triton X-100, Igepal CA-720 and Hyonic NP-90 are the most effective in producing solubilization. The anionic surfactants sodium ligninsulfonate and sodium dodecylbenzenesulfonate (SDBS) have the least solubilizing ability at the doses considered. Corexit 7664 and Adsee 799, both surfactant esters, do not produce substantial solubilization until the surfactant dose applied is 1%. Additional screening tests suggest that the effect of solid-water ratio on solubilization is dependent on whether the surfactant dose is near that required to produce micelles in the soil-water suspensions.

A soil-water partition coefficient for surfactant systems may be defined as:

$$K_{D(s)} = S / C_{aq(s)} \qquad (1)$$

where S is the PAH sorbed onto the soil ($\mu g/g_s$) and $C_{aq(s)}$ refers to the PAH concentration in the liquid phase in the presence of surfactant. The reciprocal of $K_{D(s)}$, i.e. a surfactant-soil solubilization coefficient, $C_{aq(s)} / S$, was computed as a function of surfactant dose for pyrene and anthracene with the four nonionic ethoxylate surfactants. The surfactant concentration at which solubilization initiates in the presence of soil may be thought of as an "effective CMC",

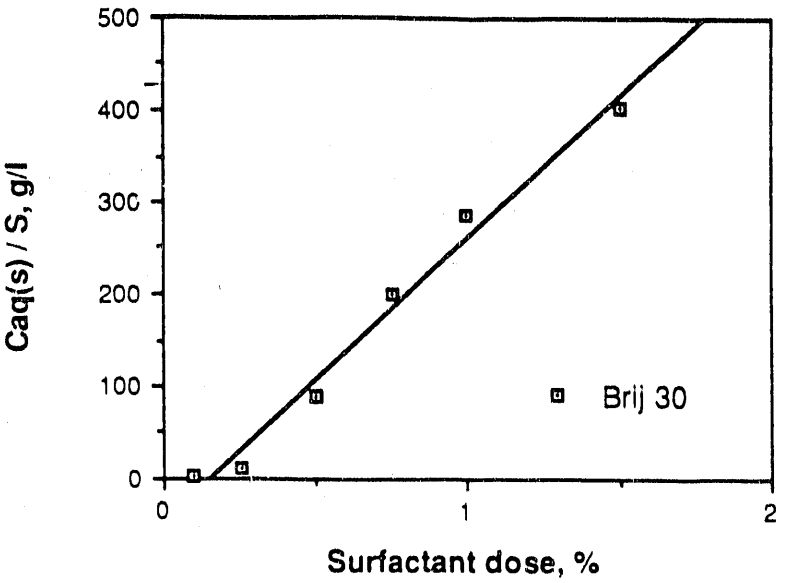

Figure 4. The Soil-Surfactant Solubilization Relationship for Anthracene with a Dodecylethoxylate Surfactant Having Four Ethoxylate Units.

[CMC_{eff}]. If one considers that portion of the $C_{aq(s)}$ / S relationship at C_{surf} greater than the "effective CMC", the $C_{aq(s)}$ / S relationship appears linear, i.e. the $C_{aq(s)}$ / S relationship may be described as:

$$C_{aq(s)} / S = k_1 (C_{surf} - CMC_{eff}) + k_2 \quad (2)$$

k_1 being the slope of the solubilization relationship with an intercept k_2 at $C_{surf} \sim CMC_{eff}$. This is shown in Figure 4 for the solubilization of anthracene by the surfactant Brij 30.

The general features of the solubilization of PAH by surfactants in soil-water suspensions may be described by considering the partitioning of PAH between two compartments: the surfactant micelles in solution and the solid phase. At surfactant concentrations less than that necessary to produce micelles in the presence of soil, the PAH is predominantly sorbed onto soil; at surfactant concentrations greater than that necessary to produce micelles in the presence of soil, the PAH is partitioned between micelle and soil. Equation (2) may be re-arranged to express PAH solubilization:

$$\text{Solubilization (\%)} = 100 \times \frac{[k_1 v_l (C_{surf} - CMC_{eff}) + k_2 v_l]}{[m_s + k_1 v_l (C_{surf} - CMC_{eff}) + k_2 v_l]} \quad (3)$$

where m_s = mass of soil and v_l = volume of liquid.

The experiments demonstrated that nonionic surfactants at doses of 0.1 to 1% by volume

may solubilize PAH from soils at solids-to-liquid mass ratios ranging from about 1:7 to 1:2. Among the nonionic surfactants considered here, the octyl- and nonyl- phenylethoxylate surfactants with 9 to 12 ethoxylate units demonstrated the best solubilizing characteristics.

Biodegradation Experiments

Tests are being performed to assess whether the addition of surfactants to soil-water suspensions can enhance the rate of microbial degradation of PAH as a result of the organic substrate being solubilized to a much greater extent in the aqueous phase of a soil-water system. As deoxygenated conditions may often prevail in contaminated soil-water systems as a consequence of microbial activity, the biodegradation of the PAH is being studied under denitrifying conditions. In these tests nitrate is present in sufficient amount to satisfy demand for PAH mineralization as well as for partial microbial degradation of surfactant.

Methods: The experimental set up for the biodegradation tests was a closed system consisting of a biometer flask fitted with a side arm containing sodium hydroxide solution. The system was sealed from the atmosphere with neoprene stoppers on both the flask and side arm. A sampling needle extended into the caustic solution in the side arm allowing for periodic extraction of NaOH. The mineralization of PAH was monitored by measuring the amount of $^{14}CO_2$ in a known volume of NaOH. Blanks were set up without soil and bacteria to assess for any potential volatilization of PAH; and controls with sterilized soil were also used to confirm results from the blanks.

The soil used in these tests was the air-dried and sieved Hagerstown silt loam having a fraction organic carbon content of 1.5%. Phenanthrene was used at a dose to approach aqueous-phase saturation. Each system generally received between 0.3 and 0.5 µCi ^{14}C-phenanthrene along with 3 mg unlabeled phenanthrene. The soil:water ratios were similar to those used in the solubilization tests with each biometer flask receiving 50 ml of BOD dilution water (APHA) for 6.25g soil. Various flasks were set up to evaluate: (i) bacterial culture inoculations, (ii) different surfactants at 1% dose, and (iii) the effects of additional salts.

The side arm of the biometer flask was filled with 20 ml of 2M sodium hydroxide. 0.5 ml NaOH was withdrawn in triplicate for each sampling interval, placed in scintillation vials containing 10 ml scintillation cocktail and counted for ^{14}C. The sodium hydroxide solution was replaced after four sampling periods. For denitrifying conditions the biometer flask was flushed with N_2 to purge oxygen, and 0.02 M calcium nitrate was added to the mineral medium. Analysis for residual nitrate was performed spectrophotometrically using a cadmium reduction method (Milton Roy SpectroKit, New York).

The side arm stopper of the biometer flask had a 1.5 inch hypodermic needle pierced through it to allow for the slow release of any N_2 gas generated by the denitrification process. The hub of the hypodermic needle was covered with parafilm to maintain anoxic conditions within the flask and to prevent atmospheric carbondioxide from entering the side arm and saturating the sodium hydroxide solution. The hub of the NaOH sampling needle was blocked

with a styrofoam plug for similar reasons. The soil was kept in suspension by mounting the biometer flasks on ganged electromagnetic stir-plates with timers to stir for 7 minutes every half an hour.

Initially, liquid surfactants have been used at 1% by volume, and solid surfactants at 1% by weight, as these doses solubilized phenanthrene in the soil-water systems studied earlier. The surfactant solutions were prepared by adding commercially available surfactant to BOD dilution water and sonicating for several hours to facilitate dissolution. The pH of the soil-water suspension was between 5 and 6 without adjustment. This pH was acceptable since it was neither too low to be harmful to microbial metabolism, nor too high to prevent the escape of any CO_2 generated. It is also representative of the pH condition which is encountered in many natural soil-water environments. Blank values for the LS counts were taken as the DPM value of 0.5 ml aliquots of pure 2M NaOH. Aqueous phase concentration of phenanthrene was measured by withdrawing 10 ml of the soil-water suspension and centrifuging for half an hour prior to sampling 0.5 ml of the supernatant in the LS counter. Mass balance on the biometer flask was performed by the extraction in hexane with an equal volume of soil-water suspension, followed by filtering and counting a 0.5 ml hexane aliquot in the LS counter. Hexane extraction involved vigorous mixing of 10 ml of hexane with 10 ml volume of suspension in a centrifuge tube, followed by sonicating for half an hour, and then centrifuging for another half an hour.

The soil used was an uncontaminated agricultural soil, and thus a lag time would be expected in uninoculated systems prior to the onset of microbial activity due to the small numbers of PAH-degrading organisms originally present in the soil (Mihelcic and Luthy, 1988a).

Experiments are in progress to determine whether acclimated populations cultured from soils exposed to phenanthrene and nitrate might reduce the lag time. Acclimated organisms were cultured in 250 ml Erlenmeyer flasks, each receiving 50 g soil, 100 ml BOD dilution water with 0.02 M $Ca(NO_3)_2$, and 22.5 mg phenanthrene. The flasks were purged with nitrogen, and sealed with rubber stoppers pierced with hypodermic needles to relieve pressure build-up due to the formation of gaseous products. The needles were covered with parafilm to exclude atmospheric oxygen. The flasks were wrapped in aluminum foil to prevent photooxidation reactions, and placed on a wrist-action shaker with a timer to shake periodically every half an hour. After nearly four months the acclimating flasks were removed, their contents centrifuged for 5 minutes at 2500 RPM, and the supernatant enumerated for phenanthrene-degrading organisms. Enumeration of PAH-degrading organisms was performed by plating 0.1 ml aliquots from appropriate dilutions of the supernatant on phenanthrene plates. The plates were prepared by spreading 0.2 ml portions of a phenanthrene-acetone mixture (5 g/l phenanthrene) on a prepared media of mineral salts and agar (Stroo, 1989; Shiaris and Cooney, 1983; Barnsley, 1975). The acetone was allowed to evaporate overnight and the inoculated plates incubated at room temperature for 5 days or longer. The PAH-degrading colonies were identified as clear circular zones against a cloudy field. A colony counter with a

UV light was employed for counting purposes.

Aerobic PAH-degrading bacteria cultures were also obtained from an engineering consulting firm with specialization in soil treatment of organics. These aerobic cultures had been isolated from various soils including contaminated sites. A culture from one sample was transferred by wire loop to an autoclaved rapid growth medium containing 100 ppm naphthalene and 1 g/l glucose. This produced the "rapid growth" culture used in later experiments. Similarly prepared media containing 100 ppm naphthalene but no glucose was used to grow the "acclimated media" culture. These cultures were grown several days, enumerated by the plate-counting technique, and refrigerated until used to inoculate biometer flasks.

Table II. Representative Results of the Anaerobic Biometers with 0.02M $Ca(NO_3)_2$

Biometer Description	DPM value of 0.5 ml NaOH samples		
	2 weeks	7 weeks	14 weeks
No phenanthrene blank	46	50	47
No soil	46	62	52
No surfactant	50	85	95
1% Adsee 799 + Hyonic NP90	45	58	49
1% Brij 30	46	64	47
1% Brij 35	45	63	55
1% Corexit 7664	46	65	54
1% Igepal CA-720	45	56	55
1% Sodium dodecylbenzene sulfonate	45	51	44
1% Sodium ligninsulfonate	47	64	60
1% Tergitol NP-10	44	75	53
1% Triton X-100	46	68	51

Each biometer flask receives:

(1) 12.5g dried and sieved soil
(2) 100 ml BOD dilution water with 0.02M $Ca(NO_3)_2$
(3) 5.72 mg phenanthrene
(4) 314800 DPM ^{14}C-phenanthrene, unless otherwise specified.

Table III. Aerobic Microbial Degradation Tests for Phenanthrene

Biometer Description	% Mineralization (DPM value of 0.5 ml NaOH)			
	1 week	2 weeks	3 weeks	5.5 weeks
6.25g soil	0.3 (91)	0.5 (127)	1.0 (215)	23 (5484)
6.25g soil + 0.02M $Ca(NO_3)_2$	0.2 (70)	0.1 (65)	0.2 (80)	0.3 (106)
2 ml "rapid growth" inoculum *(denoted by G.cult. on figure)*	1.4 (274)	26 (4556)	42 (7844)	64 (13237)
2 ml "rapid growth" inoculum + 0.02 M $Ca(NO_3)_2$	0.1 (64)	0.1 (67)	0.1 (70)	0.2 (83)
2 ml "acclimated medium" inoculum *(denoted by A.cult. on figure)*	0.1 (63)	12 (2166)	43.6 (8277)	58 (12573)
2 ml "acclimated medium" inoculum + 0.02 M $Ca(NO_3)_2$	0.1 (61)	0.1 (64)	0.1 (71)	0.1 (73)

Additionally, each biometer flask receives:
(1) 50 ml BOD dilution water,
(2) 3 mg phenanthrene, and
(3) $6.49 \times 10^{+5}$ DPM ^{14}C.

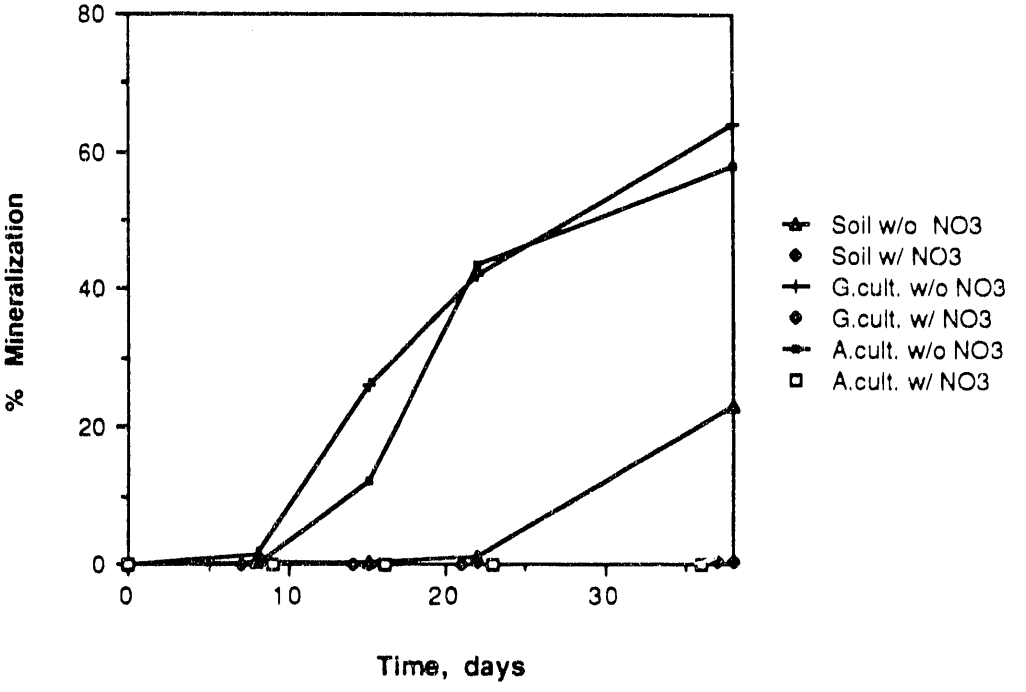

Figure 5. Aerobic Mineralization of 3 mg Phenanthrene in 50 ml BOD Dilution Water with Addition of Either Soil or Bacterial Inoculum showing 0.02M $Ca(NO_3)_2$ Inhibition.

Results and Discussion: Biometer flasks containing 0.02 M $Ca(NO_3)_2$ and phenanthrene with or without soil underwent no measureable mineralization under denitrifying conditions. Table II shows that throughout these tests the DPM values stayed close to background values even after 4 months. The presence or absence of the nine surfactants considered appeared to make no difference to the mineralization of phenanthrene, which remained insignificant. After seven weeks with no sign of phenanthrene degradation, the PAH-degrading microbial cultures were prepared and each biometer flask was inoculated with 2 ml each of both "rapid growth" culture and "acclimated media" culture. The plate count technique showed values of 6×10^8 cells/ml for the "rapid growth" media culture and about 10^7 cells/ml for the "acclimated media" culture. This inoculum produced no visible mineralization after another 7 weeks, and thus the presence of a bacterial inhibitor was suspected. A mass balance on the control systems with 0.02 M $Ca(NO_3)_2$ indicated that all the phenanthrene remained in the flask either in the aqueous phase or sorbed on the soil; there were no measureable losses. No losses through volatilization were detected in any of the biometers. DPM values for blanks of pure sodium hydroxide and pure hexane were essentially identical in value with the DPM of the blank for the scintillation cocktail alone.

Several aerobic microbial degradation tests were performed with phenanthrene and biometer flasks to examine possible causes for the failure of biological mineralization under denitrifying conditions. The aerobic tests were performed with and without surfactant. Table III outlines the various additions made to each system and the respective mineralization seen in each. The "acclimated culture" initially appears to produce slower mineralization as compared to the "rapid-growth" culture. By the third week this lag is compensated as shown for the biometer flask with 2 ml acclimated culture and no soil and no nitrate showing mineralization comparable with the similar flask receiving 2 ml of rapid growth culture (47% mineralization for the former as against 44% for the rapid growth culture recepient). This could be explained by the lesser bacterial cell density of the acclimated culture ($\sim 10^7$ cells/ml compared to $\sim 6 \times 10^8$ cells/ml for the rapid growth culture). The data indicate that the presence of soil appears to retard the mineralization process.

The conclusion from data in Figure 5 is that the presence of the $Ca(NO_3)_2$ at 0.02 M concentration definitely appears to inhibit microbial degradation of phenanthrene with organisms acclimated to phenanthrene under aerobic conditions. Another set of aerobic biometer flasks was prepared to determine whether the nitrate or the calcium or simply the high ionic strength of the mineral medium deterred mineralization. Two biometer flasks each were set up with soil, phenanthrene, BOD dilution water and one of the following: 0.02 M $CaCl_2$, 0.02 M $NaNO_3$, 0.02 M $Ca(NO_3)_2$, 1% Igepal CA-720, 1% sodium lignin sulfonate and 1% Adsee 799. Another pair of biometer flasks received no additives other than soil, phenanthrene and dilution water. All these aerobic biometer flasks received inoculations of 2 ml from either the rapid growth culture or the acclimated culture. The results from these biometer flasks are still forthcoming.

The soil-water slurries that were exposed to phenanthrene in the presence of nitrate and

maintained under anoxic environments in sealed Erlenmeyer flasks for four months showed microbial activity. The nitrate test indicated that the 0.02 M $Ca(NO_3)_2$ was consumed over the four-month acclimation period. Aliquots of the soil-water slurry were centrifuged at 2500 RPM for 5 minutes and the supernatant plated onto phenanthrene plates to enumerate the PAH-degrading organisms present. Serial dilutions were made and were plated, and the bacterial densities were greater than 10^8 cells/ml. This supernatant is being used as inoculum in on-going experiments to evaluate PAH degradation under denitrification conditions in soil-water suspensions.

Conclusions

The addition of 1% nonionic surfactant to the soil-water suspensions results in a transfer of PAH solute from the soil-sorbed phase to the aqueous phase as borne out by the solubilization tests and mass balance on the biometers. The biometer flasks used in this study allow for the measurement of mineralization product, $^{14}CO_2$, and it is possible that phenanthrene, when undergoing microbial degradation forms other soluble intermediates and end products which have not been accounted. Forthcoming tests will attempt to quantify the phenanthrene remaining in solution, and the existence of other soluble products. The soil system which was maintained under anoxic conditions in the presence of calcium nitrate and phenanthrene showed extensive microbial activity, as evidenced by the enumeration procedure and by the 0.04 M NO_3^- being utilized over the four-month acclimation period. These phenanthrene-degrading organisms acclimated to the presence of nitrate will be evaluated to determine if this culture is more effective as inoculum in future denitrifying tests. Present results indicate that a hydrophobic organic contaminant like phenanthrene shows a high potential for mineralization in aerobic, well-mixed soil-water slurries receiving bacterial inoculum.

The fact that the PAH compounds tend to persist in soil-water systems in the natural environment may be related to the potential for anoxic conditions to prevail in contaminated soils and groundwater, and because of the slow release of PAH from the soild phase to the aqueous phase. The PAH solubilization tests indicate that surfactant solubilization of polycyclic aromatic hydrocarbons may be useful in soil-washing techniques for contaminated soils. The value of these techniques for enhancing the biodegradation of PAH compounds is still to be evaluated in continuing experiments.

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