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**Effect of surfactant addition on phenanthrene biodegradation in
sediments**

Tsomides, Harry James, M.S.

Rice University, 1994

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300 N. Zeeb Rd.
Ann Arbor, MI 48106

RICE UNIVERSITY

EFFECT OF SURFACTANT ADDITION ON PHENANTHRENE
BIODEGRADATION IN SEDIMENTS

by

HARRY JAMES TSOMIDES

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE

MASTER OF SCIENCE

APPROVED, THESIS COMMITTEE

C.H. Ward, Foyt Family
Chair of Engineering

J.B. Hughes, Assistant Professor,
Environmental Science
and Engineering

C.A. Miller, Professor and Chairman,
Chemical Engineering

Houston, Texas

April 1994

Abstract

Effect of Surfactant Addition on Phenanthrene Biodegradation in Sediments

Harry James Tsomides

A literature search and laboratory treatability study were conducted to determine the potential of commercial surfactants to enhance the bioremediation of PAH-contaminated sediments. Phenanthrene was used as a model PAH and an inoculum of PAH-degrading microorganisms enriched from an aquatic sediment was used in sediment-water slurry microcosm biodegradation experiments.

The abilities of nonionic surfactants to reduce surface tension and enhance phenanthrene solubility, the effect of sediment on the physical behavior of surfactants, and the effects of surfactants on phenanthrene biodegradation were examined. Of the surfactants tested, all except Triton X-100 severely inhibited phenanthrene mineralization. Adding Triton X-100 to microcosms with or without sediment significantly enhanced the extent of phenanthrene mineralization after 1 week.

These experimental results suggest that this surfactant may be useful in the bioremediation of PAH-contaminated sediments and that further studies using pilot-scale systems or a field demonstration are warranted.

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1.0 Introduction

1.1 PAHs in the Environment

Polynuclear aromatic hydrocarbons (PAHs) are widespread in the environment due to natural and anthropogenic sources. Natural sources include oil seeps and terrestrial fires. The major anthropogenic source of PAHs is fossil fuel combustion (Heitkamp and Cerniglia, 1987). Additional sources of PAH's include wastewater discharges from industrial processes (Herbes and Schwall, 1978), oil spills, and wood treating. Sediments and soils become contaminated with PAHs because of their hydrophobic nature. As the number of aromatic rings increases, a PAH's water solubility decreases and its octanol-water partition coefficient (K_{ow}) increases (Means et al., 1980). This leads to a greater extent of partitioning into soils and sediments.

The environmental fate of PAHs can be greatly influenced when aquatic sediment-bound PAHs are resuspended by dredging. Public health is threatened by introduction of PAHs into the food chain. In biological systems, PAHs accumulate in fatty biological tissues due to their hydrophobic nature. The genotoxic and carcinogenic potential of these compounds increases as ring number and molecular weight increase (Verschuren, K.; World Health Organization).

1.2 PAH Bioremediation

Sediments, which act as a sink for PAH in aquatic environments, are resuspended during dredging operations. PAHs are released, posing a threat to surrounding ecosystems. The fate and effects of contaminated dredged material have become a subject of increasing research in recent years. The Marine Protection, Research and Sanctuaries Act mandated the EPA and U. S. Army

Corps of Engineers (COE) to develop methods aimed at evaluation of dredge spoils and fill material (Burton, 1991).

A number of technologies have been tested for bioremediation of PAH-contaminated soils and sediments. In-situ biological treatments such as liquid delivery systems, bioventing, air sparging, and biofiltration have all been examined for their potential usefulness. Above-ground liquid-solids contact bioreactors can also be used to treat soils, sediments or dredged material contaminated with hydrophobic organic pollutants.

Treating contaminated material in liquid-solids reactors holds several advantages. Bioreactors accommodate rapid on-site treatment of waste sludges and contaminated solids and minimize the risk of off-site contamination. Parameters such as temperature, inorganic nutrients and aeration can be carefully controlled. As mass transfer rates of PAHs from a sorbed phase to the aqueous phase can limit the rate of PAH biodegradation, surfactants can be added to solubilize a PAH and increase its availability for microbial utilization if the surfactant is not excessively inhibitory to the PAH-degrading microbial population. This can result in enhanced rates of PAH biodegradation. Finally, liquid-solids treatment in bioreactors can be used in conjunction with other biological, chemical, or physical treatments to achieve the desired level of site remediation.

1.3 Objectives of This Study

The following experiments are intended to assess the effectiveness of commercial surfactants in the bioremediation of PAH-contaminated sediments from the Houston Ship Channel. They are part of a comprehensive study aimed at evaluating the potential of bioremediation processes for treatment of contaminated sediments and dredged materials. It is hoped that this bench-scale

study using microcosms will answer questions regarding whether or not surfactants may be used advantageously in pilot-scale bioreactors or in field demonstrations of bioremediation of PAH-contaminated ship channel sediments.

Phenanthrene is a polynuclear aromatic hydrocarbon (PAH) consisting of three fused benzene rings, and was the target compound used in these studies. It is an intermediate molecular weight PAH (mw=178), and shows solubility, physical behavioral characteristics and toxic effects as intermediate within the PAH class.

The objectives of this research were to

- 1) collect and characterize sediments from the Houston Ship Channel (HSC)
- 2) characterize the physical behavior of phenanthrene in HSC sediment systems
- 3) conduct physical behavioral characterization of surfactants through measurements of surface tension and enhanced solubilization of phenanthrene, and determine the effects of sediments on these parameters
- 4) confirm phenanthrene biodegradation by microbial populations from a variety of sediments in and around the HSC
- 5) evaluate several nonionic surfactants for their potential to enhance phenanthrene biodegradation by an enrichment culture in sediment-free systems
- 6) select a potentially effective surfactant and evaluate its effect on phenanthrene mineralization to determine if surfactants may be useful in larger sediment bioremediation systems
- 7) use two different analytical methods to compare the extent of phenanthrene mineralization to the biotransformation of phenanthrene from its parent form.

2.0 Literature Review

2.1 PAH Biodegradation

Certain environmental conditions are necessary for PAH biodegradation to occur, such as appropriate temperature, salinity, pH, redox potential, availability of nutrients, and accessibility to the PAH substrate. Rates of PAH biodegradation in the laboratory and in the environment can be affected by these conditions. Aromatic ring reduction and hydrolytic cleavage have been reported for monoaromatic benzoates under anaerobic conditions, but similar reductive catabolism of PAHs is not thought to occur anaerobically. PAH ring cleavage is catalyzed by oxygenase enzymes which incorporate molecular oxygen into an aromatic ring of the PAH. Ring cleavage products are then converted to substrates which are used for energy production and biosynthesis in complex metabolic pathways. Common aromatic ring cleavage substrates are catechol, protocatechuic acid, and gentisic acid. Pure and mixed cultures have been shown to be capable of utilizing, as sole carbon sources, PAHs containing up to four rings. PAHs may be mineralized to harmless endproducts, carbon dioxide and water, or may undergo biotransformation without being mineralized.

Prolonged exposure to PAHs in natural waters and sediments can promote adaptations in microbial populations, resulting in greater resistance to their toxic effects or a greater ability to utilize the PAHs as substrates for metabolism. Exposure to PAHs can cause increases in total microbial populations, selective increases in populations of microorganisms with PAH-degrading enzymes, and induction of PAH-degrading enzymes in some indigenous microorganisms (Cerniglia and Heitkamp, 1989). PAH biodegradation rate increases are probably related to the presence and expression of plasmid-encoded catabolic enzymes. Microbial adaptation and increased biodegradation rates of PAHs have been observed in laboratory

studies. Bauer and Capone (1985) found that sediment microbial populations which had been pre-exposed to anthracene and naphthalene degraded these compounds at higher rates than populations which were not pre-exposed. Higher concentrations of added PAHs resulted in a greater percentage of the PAH being mineralized. There appeared to be a minimum threshold naphthalene concentration below which adaptation did not occur. Herbes and Schwall (1978) observed substantially greater PAH transformation rates by microbes from sediments with a history of exposure to PAHs when compared with microbes from pristine sediment samples.

Biodegradation rates of PAHs are often limited by their low water solubilities and dissolution rates (Stucki and Alexander, 1987; Thomas et al., 1986; Wodzinski and Coyle, 1974). The hydrophobic nature of PAHs commonly results in their association with organic matter-rich soils and sediments (Karickhoff, 1980). Sparingly soluble organic substrates, such as PAHs, are generally considered unavailable for microbial uptake when in a sorbed state. Surfactants have been examined for their potential to increase PAH biodegradation rates by influencing the equilibrium partitioning of PAH among phases in soil-water systems. By enhancing PAH solubility, surfactants can increase the bulk solution fraction of the PAH and increase the mass of PAH available for microbial uptake (Laha and Luthy, 1991; Aronstein et al., 1991; Bury and Miller, 1993).

Some researchers have observed accumulation of intermediate degradation products of PAHs or incorporation of degradation products into cellular biomass. Herbes and Schwall (1978) observed incorporation of large fractions of ^{14}C from anthracene and naphthalene into microbial cellular material. Heitkamp et al. (1988) suggest that the rate-limiting step for PAH biodegradation may be the initial ring oxidation and that following this event, PAH degradation by

mixed bacterial cultures proceeds quickly without the accumulation of intermediates. They observed that a pure culture (*Mycobacterium*), however, degraded pyrene, and significant accumulation of 4-phenanthroic acid occurred over a 96-hour period. Guerin and Jones (1988), reported that 1-hydroxy-2-naphthoic acid (1H2NA) accumulates as a polar phenanthrene degradation intermediate in a two-step mineralization event, and that PAH degradation can result in the formation of aromatic acid and phenolic products which could interact with humic substances, effectively detoxifying the PAH. They found that the fraction of 1H2NA accumulated is dependent on initial phenanthrene concentration. Bauer and Capone (1985) found that partial naphthalene mineralization accompanies complete parent compound disappearance in oxic sediments, resulting in 58% of ^{14}C -labeled naphthalene accumulating into cellular pools or as intermediate metabolites.

2.2 PAH Sorption to Sediments

Sorption is an important factor governing the fate of hydrophobic molecules in soil/sediment systems (Means et al., 1980). Karickhoff (1980) proposed a two-compartment model of PAH sorption in soils and sediments:

Here, a rapid equilibration process describes equilibrium partitioning of the pollutant P between an unsorbed and a rapidly sorbed state (S_1), and between S_1 and a state of more permanent sorption, S_2 . K_d is a first-order partitioning rate constant between the sorbed states, and t_1 is the time required for sorptive equilibration of S_1 and P. X_1 denotes the fraction of total sorptive capacity associated with S_1 and $1-X_1$ is the fraction of total sorptive capacity associated

with S_2 . Karickhoff reports that about one-half of the equilibrium sorption of polynuclear aromatic hydrocarbons to natural sediments occurs within minutes, and that equilibrium partitioning into the more highly sorbed phase S_2 may take days or weeks to complete. The extent and strength of sorption of a hydrophobic organic molecule such as a PAH can strongly influence its rate of biodegradation since compounds in a sorbed state are generally considered unavailable for microbial utilization.

There are two main driving forces for adsorption, the process of becoming sorbed (Weber, 1972). First, if the solute is lyophobic, or 'solvent-disliking', it will tend towards a sorbed state. A hydrophobic compound in water might be influenced in this manner. Second, adsorption will occur if the solute has a high affinity for a solid. This second driving force for adsorption can be characterized by 1) electrical attraction between oppositely charged ions, 2) 'physical' Van der Waals forces which promote adsorption but allow movement of adsorbed solute along the interface, and 3) 'chemical' sorption whereby specific bonding prevents movement of solute molecules between sorption sites. Non-specific Van der Waals interactions of nonionic, low-solubility organic compounds with the organic fraction of the sediment are the dominant predictor of the sorption partition coefficient, K_p (Podoll and Mabey, 1984). In many instances, adsorption is influenced by combinations of these three mechanisms (Weber, 1972).

Soil- or sediment-associated organic content in aqueous systems is the main factor determining the extent of binding of hydrophobic compounds such as PAH. Karickhoff (1980) suggests that the sediment-water partitioning of hydrophobic pollutants, including phenanthrene and other PAHs, is strongly dependent on the organic carbon content of the sediment. Means et al. (1980) conducted sorption studies with the PAHs pyrene, dimethylbenz(a)anthracene, methylcholanthrene and dibenzanthracene and found the linear partition

coefficient K_p , which can be determined by conducting an adsorption isotherm, was dependent on the organic carbon content of a wide range of soil/sediment types. Sediments were collected from rivers proximal to energy-generating facilities. Isotherm linearity is expected for this compound in sediment systems when the aqueous concentration of phenanthrene is below one-half its aqueous solubility (Karickhoff et al., 1979).

The theoretical value for K_p (ml/g) is related to the organic carbon content of the soil/sediment by the following equation (Karickhoff, 1980):

$$K_p = (f_{oc})(K_{oc}),$$

where f_{oc} is the fraction of organic carbon in the sediment and K_{oc} is the organic carbon sorption coefficient for the PAH. Since K_{oc} is estimated by the octanol-water partition coefficient (K_{ow}) by the expression

$$K_{oc} = (0.63)(K_{ow}),$$

an expected value for K_p of phenanthrene can be determined based on the f_{oc} of the soil/sediment used in experiments and the K_{ow} for phenanthrene, through the equation

$$K_p = (f_{oc})(0.63)(K_{ow}).$$

Longer equilibration times for sorption of nonionic organic compounds may decrease rate or altogether inhibit PAH desorption from soils and sediments (Karickhoff, 1980; Steinberg et al., 1987). Goring and Hamaker (1972) observed a correlation between the degree to which pesticides bind irreversibly to soil and

the time over which exposure has occurred. The highly volatile 1,2-dibromoethane (EDB) was found 19 years after its application to agricultural topsoil (Steinberg et al., 1987). Other observations that desorption of pollutants from soils becomes more difficult as residence times increase have been reported (Pavlostathis and Jaglal, 1991; Pignatello, 1990). Kan et al. (1994) observed naphthalene desorption rates to be one to three orders of magnitude smaller than predicted by modeling. Similar results were obtained using phenanthrene. They suggest that this slower-than-expected release of these PAHs from a sorbed state supports desorption hysteresis as a possible explanation. They also found that about 50% of naphthalene initially present in the sediment at a concentration of 1-2 mg/kg, became irreversibly bound to or trapped within the soil.

While sediments rich in organic matter act as strong adsorbents for nonionic hydrophobic compounds such as PAHs, high mineral content surfaces are polar or charged, rendering the surfaces susceptible to hydration and thus potentially disabling their capacity to sorb hydrophobic organic compounds (Guerin and Boyd, 1992). Dissolved humic substances also serve as strong adsorbents for some PAHs, potentially altering their fate and transport in aquatic sediment systems (McCarthy and Jimenez, 1985).

2.3 Biodegradation of PAH in Different Physical States

Contact between microbial cells and the hydrocarbon is necessary for biodegradation to occur. Hydrocarbon biodegradation may occur by interaction of cells with (i) dissolved hydrocarbon, (ii) large drops of hydrocarbon and (iii) fine droplets of hydrocarbon which are much smaller than the cells themselves (Singer and Finnerty, 1984). The rate of utilization of liquid hydrocarbons such as straight-chain alkanes, which are too insoluble in water to support growth by

only dissolution, is often governed by the above mechanisms (ii) and/or (iii), in which the limiting factor for substrate uptake is the contact surface area between cell and substrate (Goswami and Singh, 1991). Biodegradation rates of polynuclear aromatic hydrocarbons, which exist as solids in their pure form, are limited by rates of PAH dissolution (Wodzinski and Coyle, 1974), supporting mechanism (i) as a factor controlling the microbial utilization of PAHs. PAH mixtures in the environment, meanwhile, may be transported to cells by one or more of these mechanisms.

Numerous reports have indicated that solid PAHs suspended in liquid medium cannot be utilized by bacteria. The resistance of undissolved crystalline hydrocarbons to microbial degradation may be due in part to the energy requirements needed to disperse this form of the hydrocarbon (Zilber et al., 1980). Wodzinski and Johnson (1968) showed a positive correlation between bacterial growth rates on naphthalene, phenanthrene, anthracene and naphthacene and the respective water solubilities of these compounds when using them as sole carbon sources. Thomas et al. (1986) found that dissolution rate is one of the factors which affects the rate of hydrocarbon biodegradation. Their studies involved naphthalene, 4-chlorobiphenyl, and other organic compounds. Wodzinski and Coyle (1974) showed that phenanthrene is metabolized only in its dissolved state, when present with undissolved particles in liquid suspension. The microbial inoculum was a pure culture (*Pseudomonas* sp.) obtained from raw sewage and grown on phenanthrene under enrichment culture conditions. They had previously observed similar results when naphthalene and biphenyl were used as carbon sources (Wodzinski and Bertolini, 1972). Stucki and Alexander (1987) suggest that biphenyl, phenanthrene, and anthracene degradation by microbes isolated from sewage settling tanks is limited by dissolution rate, based on bacterial growth rates and

compound dissolution rates. *Flavobacterium* grew faster on phenanthrene than on the more soluble biphenyl, indicating that substrate solubility does not always parallel biodegradation rate. They concluded that while this experiment showed that rates of dissolution of highly insoluble hydrocarbons limit biodegradation rates, extrapolation of this phenomenon to field conditions is not always appropriate. *In situ* studies were cited as necessary in further relating the dissolution rate of highly insoluble compounds to biodegradation.

While it is generally accepted that solid PAHs are not available for microbial utilization and that the rate-limiting step in their biodegradation is dissolution, less is known about the availability and utilization of sorbed PAH. Many factors can affect the bioavailability of sorbed compounds, including the chemical properties of the sorbate, the nature of the sorbent, the mechanism of sorption, the time allowed for the sorbate-sorbent equilibrium, and the substrate-degrading abilities of the microbes capable of pollutant degradation (Guerin and Boyd, 1992).

Guerin and Boyd (1992) observed that sorption of naphthalene to soil limits both the rate and extent of naphthalene mineralization by a pure culture; the rate and extent of naphthalene mineralization by another strain, however, exceeds the values predicted by a model. They state that organism-specific properties, such as bacterial motility and attachment, may affect naphthalene bioavailability and may account for different results in experiments performed with different organisms. These organism-specific properties add to the uncertainty in making generalizations regarding the bioavailability of sorbed substrates. Subba-Rao and Alexander (1982) reported both inhibitory and stimulatory effects of clay-associated organic matter on the mineralization of benzylamine. Rijnaarts et al. (1990) conclude that intraparticle mass transfer processes control the rate of desorption and the bioconversion of hexachlorocyclohexane and other xenobiotic compounds in soils and sediments.

Sorption can limit biodegradation by preventing an aqueous substrate concentration, $S_{\min}$, adequate for microbial growth (Alexander, 1985). In desorption-controlled biodegradation, low desorption rates limit otherwise potentially high degradation rates (Subba-Rao and Alexander, 1982). Ogram et al. (1985) showed that (2,4-dichlorophenoxy)acetic acid in a sorbed state is protected from bacterial degradation. Mihelcic and Luthy (1991), however, found that the naphthalene sorption-desorption process is reversible and rapid compared to the microbial degradation of naphthalene in soil-water suspensions. Numerous other studies have investigated the bioavailability of a variety of types of sorbed compounds and have arrived at different conclusions (Gordon and Millero, 1985; Shimp and Young, 1988; Steinberg et al., 1987). In some cases where the substrate or its metabolites are toxic to microbial populations, sorption can stimulate biodegradation by lowering the free concentration of the toxic compound (Ehrhardt and Rehm, 1985; Subba-Rao and Alexander, 1982).

While most studies have shown sorption and biodegradation to be competing processes, the varied results observed using different chemical classes, sediment types and microbial populations do not allow generalization regarding the bioavailability of sorbed organic substrates. The complex nature of sorbent-sorbate dynamics, particularly in biological systems, and the different physical, chemical and biological properties of the many experimental systems studied have led researchers to a variety of findings which have prohibited consensus conclusions. The sensitive nature of experiments such as these also contributes to inconsistencies in experimental findings. Kan et al. (1993) studied naphthalene and phenanthrene desorption and pointed to laboratory artifacts such as volatilization, fines removal, biodegradation, and chemical reactions with the organic matter or mineral phases as potentially confounding factors.

2.4 Surfactants

2.4.1 Surfactant Classification

Surfactants, or surface-active agents, are complex molecules which exhibit behavioral characteristics in liquid media including surface tension reduction and micelle formation. Commercial surfactants are intended to solubilize or mobilize fatty, hydrophobic materials. Naturally synthesized surfactants, or biosurfactants, can be produced by some microorganisms as a response to the presence of water-insoluble organic substrates or for no apparent reason. While surfactants vary widely in molecular nature and surface-active properties, most surfactants of any kind operate via similar principles involving hydrophobicity and hydrophilicity.

Surfactants are classified most commonly on the basis of (i) the charge and/or the nature of the hydrophilic group and (ii) the flexibility and/or the chemical nature of the hydrophobic 'tail' section (Neugebauer, 1988). Functional groups at the hydrophilic end can impart a charge to the molecule. Carboxylate, sulfate or sulfonate groups will render the molecule anionic, or negatively charged. Cationic surfactants are positively charged, usually by the presence of a quarternary ammonium in halide salt form, such as cetyl trimethylammonium bromide (CTAB). Betaines and sulfobetaines are examples of zwitterionic surfactants possessing both a positive and a negative charge. Many of the surfactants which have been subjects of study involving hydrocarbon and PAH solubilization and biodegradation are nonionic and possess no charge.

Nonionic surfactants are also classified on the basis of the degree of their hydrophilic character. A larger hydrophile-lipophile balance (HLB) number indicates a greater degree of hydrophilicity and thus water solubility, and is an indicator of longer chain length for nonionic surfactants of a particular class. This arbitrary classification scheme of numbers from zero to twenty (Attwood and

Florence, 1983) has been useful in choosing surfactants with different HLB numbers for biochemical research. Surfactants with different HLB values solubilize membrane structures to different degrees. HLB number can also be used to determine the potential for recovery of the surfactant from solution.

2.4.2 Surfactant Chemistry and Physical Behavior

Surfactants can mobilize trapped contaminants in a soil matrix by lowering the capillary forces in the matrix (Bury and Miller, 1993), thus facilitating recovery for above-ground treatment. Surfactants can also affect the fate of hydrophobic organic pollutants, such as PAHs, by enhancing their solubility (Edwards et al., 1991; Jafvert and Heath, 1991). Contaminant solubilization and mobilization are two phenomena that may influence the success of bioremediation. Since the physical state of a hydrocarbon can determine its rate of biodegradation, surfactants which solubilize or mobilize a PAH may transfer it from an unavailable to an available phase, facilitating increased microbial utilization rates.

Mobilization is a surfactant-mediated process dependent on creating an extremely low (less than 0.001 dynes/cm) interfacial tension condition between a non-aqueous phase liquid (NAPL) and the surfactant (West and Harwell, 1992). Capillarity, an adhesive-cohesive force which traps NAPL, is proportional to the interfacial tension of the NAPL-groundwater interface. Reducing interfacial tension with a surfactant lowers the energy requirement necessary to push residual NAPL out of saturated pore spaces in the soil matrix, thus mobilizing the contaminant. The nature of the contamination determines the appropriateness of a particular surfactant in designing a remedial system. Surfactants must be appropriately matched with the contaminant of interest in order to increase solubility in bioreactors or enhance mobilization and/or solubilization in the subsurface.

Solubilization of a contaminant by a surfactant in soil-water systems is dependent on attaining minimum surface tension. Surface tension reduction is an adsorption-mediated process. Adsorption is an interfacial phenomenon describing the accumulation of a substance at an interface between phases in a system. Sediment-organic matter typically acts as a strong adsorbent for an adsorbate, such as a nonionic surfactant molecule. If a surface active or adsorbing molecule, such as a surfactant, is added to water, surfactant molecules will accumulate at interfaces in the system. In a sediment-water slurry, molecules adsorb mostly at the sediment-water interface and at the air-water interface, with the hydrophobic 'tail' portion of each molecule being attracted to the sediment or the air phase. The hydrophilic heads will be excluded from relatively hydrophobic phases such as sediment-organic-carbon and air. Surfactant molecules arrange themselves at the air-water adsorptive interface, disrupting cohesion among water molecules and lowering free energy along that boundary and reducing surface tension until a maximum number of molecules exist in a packed arrangement. A further increase in surfactant concentration leads to aggregations of surfactant molecules, or micelles. The concentration at which a surface tension minimum is reached and micelles begin to form in an aqueous system is called the critical micelle concentration (CMC), beyond which the concentration of surfactant in monomer form usually stays constant. Micelle formation occurs because it reduces free energy of the system (Weber, 1972).

As surface tension is a direct measure of adsorption of surfactant molecules at the water-air interface, comparing surface tension in a non-sediment system to surface tension in sediment systems can determine the degree of adsorption of the surfactant to the sediment. Adding sediment to a system is equivalent to adding favorable adsorption sites for surfactant

molecules, increasing the surfactant concentration necessary to cause an equivalent degree of adsorption at the air-water interface. A greater concentration of surfactant is therefore necessary to achieve minimum surface tension conditions, micelle formation, and enhanced PAH solubilization when sediment solids are present. Surface tension, measured with a ring tensiometer, indicates the degree of adsorption of surfactant molecules at the air-water interface. The tension at the air-water interface is measured by the force necessary to pull a platinum-iridium ring through that interface.

Micelles are the surfactant structures which directly influence hydrocarbon solubility. Solubilization by a surfactant is defined as "the spontaneous dissolving of a substance by reversible interaction with the micelles of a surfactant in a solvent to form a thermodynamically stable isotropic solution" (Rosen, 1989). The interior hydrophobic core of the micelle, being a less polar environment than water, provides a favorable partitioning site for a hydrocarbon. This results in an increase in the bulk solution fraction of the total hydrocarbon mass. When solids are present in surfactant systems, micelle interiors compete with sediment-associated partitioning sites for hydrophobic organic compounds (Laha and Luthy, 1991) and can increase the amount of hydrocarbon available to a microbial population for utilization. Factors affecting critical micelle concentration under experimental conditions are temperature, pH, ionic strength, and the presence of additives such as multivalent ions or impurities such as metal ions, pigments, synthesis byproducts or degradation products (Neugebauer, 1988).

Some nonionic surfactants must exist below a certain temperature for spherical micelle formation to occur. Nonionic ethoxylated alcohol surfactants with a low number of ethoxyl groups form two liquid phases at room temperature, one rich in surfactant and one with less surfactant (Bury and Miller, 1993). A greater number of ethoxyl groups necessitates a higher temperature to impose

this effect. Above cloud point temperatures, nonionic surfactants can also form lamellar crystalline bilayers. Most nonionic surfactants with 6 or more ethoxyl groups form spherical micelles, 2-4 nm diameter, at room temperature. This is a thermodynamically favorable shape which is formed by the surfactant as soon as the CMC is reached.

2.5 PAH Solubilization by Surfactants

Four factors can affect the degree of PAH solubilization by a surfactant in a soil-water system (Figure 1) (Liu et al., 1991):

- 1) interaction of PAH with surfactant monomers
- 2) PAH sorption to soil
- 3) surfactant sorption to soil
- 4) aqueous PAH-micelle partitioning

Mechanism 1 may be insignificant, however, as Liu et al. (1991) showed that anthracene, phenanthrene and pyrene interact weakly with nonionic surfactant monomers. Mechanisms 2, 3 and 4 are all important to consider in sediment-water-surfactant systems (mechanism 3 is discussed below).

A number of studies have indicated the effectiveness of various surfactants in solubilizing PAHs and other hydrophobic organic compounds. Abdul et al. (1990) studied the solubilization of transmission fluid in soils and found that nonionic ethoxylated and phenylethoxylated alcohols are effective in removing automatic transmission fluid from soil. One of the effective surfactants had a specific gravity of 0.98 and a relatively low molecular weight. These properties would allow it to remain in the water table region where petroleum pollutants might be found and would afford it enhanced mobility through an

Figure 1. PAH partitioning affecting bioavailability in sediment-surfactant systems

aquifer. Rittmann and Johnson (1989) suggest that nonionic surfactants, including alkylphenolethoxylates, alkylethoxylates, esters of fatty acids and polyhydric alcohols, are effective oil dispersants because they promote reductions in interfacial tension between water and the hydrocarbon. Liu et al (1991) examined the enhanced solubilization of phenanthrene, anthracene and pyrene by anionic and nonionic surfactants in soil-water suspensions. Nonionic alkyl- and phenylethoxylates with an average of 9-12 ethoxyl groups proved to be the most effective surfactants in solubilizing these PAHs. These surfactant types would be logical choices for related studies. The extent of solubilization of an aliphatic hydrocarbon by nonionic polyoxyethylenes increases as the number of oxyethylene units decreases and as the length of the hydrophobic group increases (Rosen, 1989). Liu et al. point to a need for further research to elucidate the characteristics of surfactant-soil sorption events in order to fully describe PAH solubilization using surfactants. Surfactant sorption is discussed further below.

2.6 Effects of Surfactants on Hydrocarbon Biodegradation in Laboratory Experiments

A number of studies have examined surfactant effects on biodegradation in systems without solids. Bury and Miller (1993) found that *n*-decane and *n*-tetradecane degradation rates are greatly increased through enhanced solubilization by nonionic ethoxylated alcohol surfactants. Efroymson and Alexander (1991) observed increased extents of mineralization of naphthalene and *n*-hexadecane by an *Arthrobacter* strain with increasing volumes of an organic solvent. In the same set of experiments, Triton X-100 increased the rate and extent of mineralization of naphthalene initially dissolved in an organic solvent. Enhanced phenanthrene degradation was observed by Guerin and

Jones (1988) in the presence of several nonionic Tween surfactants. They attributed this observation to increased bioavailability resulting from solubilization of phenanthrene by the surfactants. Enhancement of phenanthrene degradation by each surfactant did not correlate with the degree of solubilization imparted by the respective surfactant, leading the researchers to cite differing physiological as well as physical-chemical effects of each surfactant.

Studies have also documented the beneficial effects of surfactants on hydrocarbon biodegradation in laboratory experiments involving soil or sediment solids. Cultured oil-degrading bacteria and surfactants added to lubricating oil-contaminated soils (Rittmann and Johnson, 1989) greatly increased initial oil degradation rates and extent of removal achieved. Rates decreased over time as the more readily degradable components were used. An enrichment culture of oil-degraders was grown in liquid medium using lubricating oil as the sole carbon source, along with a dispersant to maintain an oil-in-water emulsion. The primary reason for the degradation enhancement in the surfactant system was attributed to the increased interfacial area which made the substrate more bioavailable.

In addition to synthetic surfactants, biosurfactants have been observed to enhance hydrocarbon degradation. A hydrocarbon mixture of alkanes and naphthalene was suspended in a 10% solids slurry. Addition of a sophorose lipid biosurfactant doubled hydrocarbon degradation rate (Oberbremer et al., 1990). Reduced interfacial tensions accompanied addition of the biosurfactant, and it was determined that the added biosurfactants were the primary reason for this rather than the production of new biosurfactant during the incubation.

Surfactant concentration can be an important factor controlling the biodegradation of organic compounds in soil. Aquatic humic and fulvic acids (HA/FA) at normal environmental concentrations (< 100 mg/l), are comparable in molecular weights and polar group contents to many surfactants. HA/FA exist in

an unassociated state and do not form micelles, and there is a linear dependence of solute solubility with HA/FA concentration (Kile and Chiou, 1989). Since solubility enhancement of some water-insoluble organics can be enhanced by low concentrations of HA/FA, Kile and Chiou (1989) suggest examination of solubility enhancement effects of some surfactants below their CMC on low solubility compounds. Aronstein et al. (1991) examined the effects of low concentrations of nonionic surfactants on the degradation of soil-sorbed aromatic compounds. Enhanced biodegradation of phenanthrene occurred in the presence of two nonionic surfactants. Surfactant use at low concentrations stimulated mineralization of the sorbed aromatics phenanthrene and biphenyl despite a lack of surfactant-induced desorption. The surfactants had positive effects on bioavailability without enhancing solubilization. It was hypothesized that the surfactants may somehow affect the strength of sorption of the compound at concentrations below their CMC.

Aronstein and Alexander (1993) added a nonionic surfactant to the surface of a soil comprised of 7.6% organic matter. The surfactant at a concentration below its CMC increased the extent of mineralization of phenanthrene and biphenyl and the rate of phenanthrene mineralization. A ten-fold higher surfactant concentration did not exhibit such a stimulatory effect. Their study concluded that application of low concentrations of nonionic surfactants to soil surfaces may be an effective way of enhancing *in situ* biotreatment without causing migration of the pollutant to groundwater. The authors indicated that further research is needed to determine properties of chemicals, soils and surfactants and their interactions.

Neutral or negative effects by surfactants on mineralization have also been observed. Laha and Luthy (1991) found that nonionic alkylethoxylate and alkylphenylethoxylate surfactants are inhibitory to phenanthrene degradation at surfactant concentrations higher than critical micelle concentration. They found

that without sediments, (i) Triton X-100 at 0.2% completely inhibited phenanthrene mineralization in an 8-week experiment, and (ii) Triton X-100 at a concentration of 0.072% prevented phenanthrene mineralization, and upon dilution to sub-CMC levels after 5 weeks, phenanthrene mineralization increased gradually to reach 30% after 12 weeks. They suggest that Triton X-100 exhibits some reversible, membrane-associated inhibitory effect by surfactant micelles on phenanthrene utilization which does not result in cell death. Based on chemical modeling, they rule out the possibility that partitioning of phenanthrene into micelles, and thus a decrease in the aqueous free concentration of phenanthrene, causes phenanthrene concentration to fall below the threshold level that might be expected for biodegradation, as phenanthrene biodegradation in natural systems has been observed at ng- and µg/L concentrations. Laha and Luthy determined in the study that Triton X-100 was not used as a preferential substrate over phenanthrene. Triton X-100 surfactant concentrations below critical micelle concentration had no effect on mineralization.

Aronstein et al. (1991) observed suppressed mineralization of phenanthrene (compared to a non-surfactant control) by two nonionic surfactants at concentrations above CMC in soil water slurries. Triton X-100 completely prevented mineralization of hexadecane dissolved in an organic solvent (Efroymson and Alexander, 1991). They suggest that the surfactant prevents bacterial adherence to the solvent-water interface or exhibits some effect(s) on the cellular membrane. Tiehm (1993) found that several alkylethoxylate and alkylphenolethoxylate surfactants are toxic to a PAH-degrading *Mycobacterium* sp. and to several PAH-degrading mixed cultures. Sodium dodecyl sulfate inhibited PAH degradation by serving as a preferential growth substrate. Mulkins-Phillips and Stewart (1974) found that some non-toxic surfactants are

preferentially used as a substrate over crude oil, resulting in slower rates of crude oil biodegradation.

The inhibitory effects of many surfactants have been attributed to their ability to disrupt cellular membrane structure (Womack et al., 1983; Attwood and Florence, 1983; Elworthy and Treon, 1966). The outer membrane of Gram-negative bacteria, not found in Gram-positive microorganisms, has been cited as a reason for variations in observed toxicity. Berg and Zimmerer (1987) observed bacteriostatic effects of anionic surfactants only to Gram-positive organisms. Negative charges of Gram-negative outer membranes have been hypothesized as the reason for repulsion and exclusion of negatively charged anionic surfactants (Inouye, 1974). The complex nature of the Gram-negative outer membrane may afford it increased protection from membrane-altering surfactants. Gram-negative outer membranes contain porins which are trans-membrane protein channels that serve to control passage of low-molecular-weight substances across the membrane. Antibiotic resistance has been related to porin structure, and it is thought that porins can be opened or closed in response to chemical conditions (Brock and Madigan, 1988).

Application of surfactants to enhance the availability of sparingly soluble substrates for microbial utilization has been extensively studied. The variety of experimental conditions such as physical-chemical soil characteristics, organism-specific properties associated with microbial populations, molecular nature of the surfactant and the various ratios at which the components are mixed may be a reason for the variety and conflicting nature of results obtained. Nevertheless, as more studies are conducted, we will see better-defined trends which will enable us to design better remedial systems, and hopefully we will be able to determine the precise mechanisms which control interactions among sediments, pollutants, microbes and surfactants so that emerging technologies can be used effectively.

2.7 Biodegradation of Surfactants

While some microbes are not equipped to resist the inhibitory or toxic effects of surfactants, other microbes utilize surfactants as a carbon and energy source. Since biosurfactants are known to be produced in response to naturally-occurring substances, it is not surprising that some microorganisms possess enzyme systems capable of biodegrading commercial surfactants which are often similar in structure and physical-chemical property to biosurfactants.

Anionic alkylbenzenesulfonates (ABS) dominated the cleaning industry following the post-World War II expansion of the petrochemical industry. Their mass production was a result of heavy demand during this time, and the invention of a one-step condensation process lowered their production cost significantly. In the 1950's, however, undesirable properties of ABS such as visible foaming and tendency to deplete dissolved oxygen resulted following their discharge from sewage treatment plants, where incomplete biodegradation took place (Cain, 1981). In the late 1960's, a more biodegradable linear alkylbenzenesulfonate (LABS) came into production, lacking the branched structure of the alkyl chain portion of the molecule characteristic of the originally synthesized ABS. The unbranched and biodegradable 'soft' form of the surfactant is susceptible to beta-oxidation as well as enzymatic oxidation of terminal methyl groups. A recent monitoring study concerned with the environmental fate of LABS showed the extent of LABS removal in various aerobic environmental compartments to be substantial (McAvoy et al., 1993). Fifty wastewater treatment plants in eleven states across the country were selected as sites for evaluation. They reported 99.3% LABS removal during activated sludge treatment, 98.5% removal during lagoon treatment, 98.0 % removal in oxidation ditches, 96.2% in rotating biological contact, and 77.4% at trickling filter facilities. LABS concentrations in anaerobically digested sludge

were one to two orders of magnitude greater than concentrations found following aerobic sludge digestion.

Microbes can degrade surfactants to different degrees (Stiff, 1978). A minimal degree of biodegradation confers some minor change in the molecule, not necessarily affecting its properties. Further biodegradation, or primary biodegradation, results in loss of characteristic surfactant properties. Finally, the surfactant may become mineralized to carbon dioxide, water, and if applicable, inorganic endproducts. Surfactant biodegradability is thought of as the removal which might be expected during normal wastewater treatment conditions, and is often assessed in standard tests which attempt to predict surfactant fate under applied conditions (Stiff, 1978).

Most nonionic surfactants are considered to be relatively biodegradable. For linear ethoxylated alcohols, the two surfactant properties influencing biodegradation are the number of ethoxylate groups and the structure of the hydrophobic group. A large number of ethoxylate groups and branching of the hydrophobic 'tail' portion both result in slow rates of biodegradation (Swisher, 1987). Alkylphenylethoxylates as a group are less degradable than linear ethoxylated alcohols due to the presence of a phenyl group. Branching of the carbon chain also results in reduced biodegradability of alkylphenylethoxylates. Nonionic surfactants in general compare in biodegradability to linear anionic surfactants, such as LABS. Primary biodegradation of linear alcohol ethoxylates is usually rapid under favorable conditions and is most often measured either by analytical methods or by surfactancy. Complete biodegradation to inorganic endproducts, of which oxygen uptake is one measure, usually is extensive (Swisher, 1987).

2.8 Environmental Fate and Effects of Surfactants

Surfactants may become ecological or public health threats directly by imposing toxic effects on biological systems, or indirectly by mobilizing and spreading hazardous compounds from sites. They can also create significant biochemical oxygen demand (BOD) in wastewater discharge areas. Surfactants can be assessed for their inhibitory effects to hydrocarbon degraders in laboratory experiments, whereas toxicity testing and risk assessment of surfactants for ecological or public health concerns is more difficult. Bioassays which assess not only acute but sub-acute and chronic toxicity to physiological systems may be necessary to gain a more thorough understanding of risk. Laboratory testing using microorganisms, invertebrates, fish or small mammals in assessing risk to ecosystems or humans involves a complex set of variables and its value is debated. A battery of tests may be necessary using different species and evaluating different levels of physiological function impairment; chemical mixtures may behave differently than single compounds; and chemical analysis and in-situ ecological studies may be valuable in conjunction with bioassays in providing information about ecological and public health risk (Chapman, 1989).

Consideration of the potential fate and effects of surfactants in subsurface systems has been necessitated not only by their common household use but also by the increasing use of surfactants in environmental applications, such as enhanced oil recovery, in-situ bioremediation, and pesticide delivery to crops. The behavior and fate of surfactants in soils is governed by many factors including soil physics, soil chemistry and soil biology (Kuhnt, 1993). The type of surfactant also determines its own fate in soil systems. Anionic, nonionic and cationic surfactants all show different reactivities, distributions and behavior patterns in the presence of soils (Kuhnt, 1993). For instance, the effects of surfactants on soil conditions depends largely on soil composition (Mustafa and

Letey, 1971). If surfactant use is limited to closed systems, such as in bioreactors, then proper disposal, detoxification or surfactant recovery from solution can be implemented to minimize the threat of environmental release. When considering surfactants for *in situ* use, however, the potential for off-site migration of surfactants or surfactant-mobilized or solubilized contaminants necessitates a detailed evaluation of the remedial system for its ability to operate as a closed-loop system.

Low surfactant concentrations can cause surface tension changes in soil water (Law et al., 1966). Modified capillary pressure in a soil matrix changes the distribution of water and air in differently-sized soil pores, thus altering infiltration capacity, reaeration, and redox potential. Depending on the surfactant type, soil physical parameters, such as permeability and capillarity, dispersion, water capacity, infiltration and percolation, porosity and aggregation, may all lead to changes in water mobility, soil erosion potential, wetting and drying, soil structure collapse and nutrient or pollutant washout (Kuhnt, 1993). Altered redox potential and the creation of hydrophobic surfaces are two major chemical changes that can be caused by surfactants.

Sorption processes can play a large role in determining the fate of surfactants in the subsurface (DiToro et al., 1990). Sorption is controlled by surfactant properties, molecular structure, critical micelle concentration and water solubility, and by soil properties (Kuhnt, 1993). The degree of sorption of nonionic surfactants to soils is controlled largely by water solubility and by soil organic matter content. Cationic surfactants can sorb to negatively charged surfaces of clays, minerals or humic substances, changing the surficial character of clay from hydrophilic to hydrophobic. This leads to water displacement and higher soil aggregate stability (Kuhnt, 1993), and might provide a more favorable partitioning site for a hydrophobic organic compound. This could exacerbate

cleanup efforts. A positively charged surfactant ion can migrate among layers of expandable clay components where negative electrostatic binding forces dominate (Kuhnt, 1993). Clays have also been found to be significant adsorption sites for anionic surfactants in oil recovery research. Amorphous iron hydroxides can impart positive charges to surfaces which attract anionic surfactant molecules under some conditions.

Laha and Luthy (1991) note that surfactant sorption to soils causes a change in the surfactant micellization pattern such that aqueous phase surfactant is substantially less than the total added. They confirmed with surface tension experiments that with soils present, a higher surfactant concentration is necessary to begin micelle formation than in non-soil systems. Some of the properties which control sorption of nonionic alkylethoxylate and alkylphenylethoxylate surfactants are carbon chain length and the number of ethoxyl groups. A larger number of ethoxyl groups in nonionic ethoxylated alcohols, for instance, results in a more hydrophilic character (larger HLB number), an increase in CMC, and a reduced extent of sorption.

Surfactants can affect soil biology. Laboratory evidence of inhibitory effects of surfactants on PAH-degrading microorganisms is well documented. In a soil matrix, this effect may be paralleled. Soil microorganism activity and vitality has been negatively influenced by some types and concentrations of surfactants (Litz et al., 1987). Microbial population diversity is often reduced as a result of surfactant exposure, possibly upsetting the ecological balance and affecting other organisms. Soil quality can deteriorate under such conditions and deleterious effects to plant nutrition can result. Direct toxic effects to plants may also occur. Soil bacteria seem to be less resistant than aquatic bacteria to surfactant exposure. Nitrification bacteria, which play a vital role in soil fertility, are sensitive to surfactant exposure (Kuhnt, 1993).

3.0 Materials and Methods

3.1 Materials

3.1.1 Chemicals

Non-radiolabeled phenanthrene (purity > 99.5%), naphthalene, anthracene, chrysene, phenanthrene, acenaphthene, and fluorene were obtained from Aldrich Chemical Co. (Milwaukee, WI) and ^{14}C -labeled phenanthrene (specific activity 8.3 mCi/mmol, purity > 98%) was obtained from Sigma Chemical Co. (St. Louis, MO). Phenanthrene standards in methylene chloride (purity $\geq$ 99%) were obtained from Supelco, Inc. (Bellefonte, PA). Ethanolamine and HPLC-grade methanol, dichloromethane, and ethylene glycol monomethyl ether (EGME) were obtained from Aldrich Chemical Co. Ready-Gel (Beckman Instruments Inc., Fullerton, CA) was used in the scintillation counting procedure. Acridine orange dye was manufactured by Sigma Chemical Co.

3.1.2 Media

The mineral salts medium consisted, on a per liter basis, of 20 g NaCl, 1.0 g K_2HPO_4 , 1.0 g KH_2PO_4 , 1.0 g NH_4NO_3 , 0.41 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.03 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, and 0.002 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$. Mineral salts were dissolved in Milli-Q (Millipore corp., Bedford, MA) filtered water. After adjusting the pH to 7.0 with sodium hydroxide, sterilization was carried out by passing the liquid medium through 0.2- μm filter units. In the present study, 1.5% sodium chloride was added to Bushnell-Haas (BH) medium (Bushnell and Haas, 1941), a decision based on the field sampling salinity conditions. Salinity values of laboratory media were confirmed using a hydrometer, as density and salinity are linearly dependent (CRC, 1972). This formula was used throughout all experiments. Nutrient Agar and Marine Agar 2216 (Difco Labs, Detroit, MI) were used for the plating of microbes.

3.1.3 Sediments

Sediment sampling of the Houston Ship Channel was conducted with assistance from the Texas Water Commission (TWC) (which recently merged with the Texas Air Control Board and is now collectively called the Texas Natural Resource and Conservation Commission). Sampling was conducted from a small twin-engine boat owned by TWC during one of their periodic water and sediment sampling trips. Table 1 shows three sample locations and details the sampling site conditions. A site in Vince Bayou at the Houston Ship Channel was also sampled.

Table 1. Physical conditions of water adjacent to collected sediments.

Condition	Sample Location		
	Turning Basin	Monument	Morgan's Point
Sample Depth (ft)	30	40	40
Salinity (%)	1.5	1.7	2.0
D.O. (mg/L)	0.3	4.7	4.3
pH	6.8	7.6	7.9
Temperature (°C)	29.3	28.5	28.2

Ship channel sampling was performed with an Ekman dredge. The top 3-4 cm of the six-inch-by-six-inch dredged square was scraped gently off with the scoop. A site along Dickinson Bayou was also chosen for sampling. This location is proximal to and empties directly into Galveston Bay. This site was sampled by wading into the bayou and manually scooping mud. The dredged or scooped material was placed into clean, sterile glass jars with Teflon scoops. Jars were filled to zero headspace and sealed with Teflon-lined screw caps. Samples were placed in a cooler for transport to the laboratory, where they were maintained at 4°C.

3.1.4 Surfactants

Nonionic surfactants Alfonic 810-60 and Novel II 1412-70 were obtained from Vista Chemical Company (Austin, TX). Neodol 25-7 and Neodol 25-9 were manufactured by Shell Chemical Company (Houston, TX) and donated by Dr. Clarence Miller, Department of Chemical Engineering, Rice University. Triton X-100, Triton X-114 and Tween 60 were obtained from Aldrich Chemical Company. These seven surfactants represent three different chemical structure types for nonionic surfactants (Figure 2).

3.2 Analysis

3.2.1 Extractions for Sediment Characterization

EPA method 3050 (Acid Digestion of Sediments, Sludges and Soils) was used to prepare sediment samples for heavy metal content (Zn, Cr, Pb, Mn, Cu, As, Ba) analysis.

Sediment samples were extracted with methylene chloride by soxhlet extraction (EPA method 3540) and analyzed chromatographically for background PAHs.

3.2.2 Analytical Methods

Extracts from acid digestions were analyzed for heavy metal content analysis with a Perkin-Elmer (Norwalk, CT) Model 400 ICP Emission Spectrometer. Some heavy metals were present (Table 2), and samples contained elevated zinc levels and slightly high barium levels, but most levels were around the same order of magnitude as average levels found in Texas rivers, streams and estuaries. River and estuarine sediment data from the entire state of Texas, acquired by the Texas Water Commission (TWC) from 1978 to

General structure	Class	Examples tested
$C_nH_{(2n+1)}(OC_2H_4)_xOH$	Alkylethoxylates	Alfonic 810-60 Neodol 25-7 Neodol 25-9 Novel II 1412-70
Alkylphenyl-ethoxylates	Triton X-100 Triton X-114
Ethoxylated sorbitan esters	Tween 60

Figure 2. Various nonionic surfactant types used in experiments.

present, were analyzed using a mainframe statistical package (SPSS-X) on a Unisys 1100/72. These data were made available to us by the TWC, and provided a reference from which to assess the metal content of our sediments relative to other sites in Texas.

Table 2. Heavy metal content of Houston Ship Channel sediments (mg/kg dry sediment).

Metal	Sample Location			
	Turning Basin	Monument	Morgan's Point	Vince Bayou
Zn	124.6	167.2	111.3	142.8
Cr	13.9	18.2	8.9	19.5
Pb	45.9	22.6	9.1	53.9
Mn	44.7	45.1	21.7	61.2
Cu	12.4	6.8	0.1	18.5
As	0.1	0.1	0.1	0.1
Ba	144.8	119.2	64.7	96.3

Analysis of soxhlet-extracted sediments for background PAHs was performed with a Hewlett-Packard HP 5890 gas chromatograph (Hewlett-Packard, Wilmington, DE) (GC), an HP Ultra-2 column (crosslinked 5% phenyl methyl silicone gum phase, 0.25 mm i. d., 25 m length), and an HP 5970 B MSD detector, following the protocol for EPA method 8270 (Gas Chromatography/Mass Spectrometry for Semivolatile Organics: Capillary Column Technique). An HP RTE-A software system (Hewlett-Packard) reduced the data.

Analysis of factory standards, prepared standards, and extracts from experimental microcosms for phenanthrene was performed with a Hewlett-Packard HP 5890 gas chromatograph equipped with a flame ionization detector (FID), an HP-1 column (crosslinked methyl silicone gum phase, 0.2 mm i. d., 0.5 µm film thickness, 25 m length), and an HP 3392A integrator. Injection temperature was 250°C and the detector temperature was maintained at 275°C. This program used two temperature ramps at different speeds and holding times in order to allow proper elution of the compound from the column to the detector,

and involved an initial temperature of 100°C with a two minute holding time, a ramp speed of 15°C/min to 200°C with a one minute holding time, a 5°C/min ramp to a final temperature of 230°C, with a final holding time of two minutes. Total run time per injection was 17.7 minutes. Routine GC maintenance, such as capillary inlet cleaning, replacing inserts and septa, ferrule replacing and column-fitting, was periodically necessary. Multiple injections (8-12) of a reagent-grade phenanthrene standard of a known concentration established an average peak area and a consistent retention time. Standardization was repeated every two weeks to account for subtle changes in GC performance over time. The integrator was programmed to compare peak areas of sample injections to those from standard injections in order to minimize cumbersome manual data reductions which would otherwise be necessary.

Radioactivity was counted in a Beckman LS 3801 scintillation counter, using the H# quench monitoring technique (Beckman Instruments) with automatic quench compensation.

Organic carbon content of the dried Turning Basin sediment was 2.1% as analyzed by a commercial laboratory (NDRC Laboratories, Inc., Houston, Texas).

3.2.3 Microbial Counts

Total heterotrophic counts of sediment microbes were conducted by plating onto Marine Agar 2216 (Difco). 10 g wet sediment were placed into 95 ml sterile seawater and shaken vigorously for 30 seconds. A dilution series was prepared over a range from 10^{-2} to 10^{-5} . Replicate platings of 0.1 ml of each dilution showed 10-12 different colony types of varying morphologies. Total heterotrophic plate counts for all sediments after 3 days incubation revealed approximately 10,000 cfu/gram wet sediment. Microbial morphology descriptions and plate counts of samples were made as soon as possible after collection, and

individual colony types were stored on plates or agar slants for possible future pure culture work.

Microbial numbers were assessed by acridine orange direct counting (AODC) of samples (Hobbie et. al, 1977). Aliquots (1.0 ml) from non-sediment microcosms were diluted as necessary, stained with orange dye, filtered through a Nuclepore (Costar Corp, Cambridge, MA) polycarbonate filter and the cells were counted by fluorescence microscopy.

3.2.4 Surfactant Measurements

Surface tension measurements of surfactant solutions were made with a duNouy interfacial tensiometer (CSC Scientific Company, Inc., Fairfax, VA) in 50-ml beakers. The beakers had been soaked overnight in concentrated sulfuric acid containing Nochromix oxidant (Godax Labs, New York, NY) and thoroughly rinsed with deionized water. The critical micelle concentration (CMC) for each surfactant was determined by equilibrating acid-washed test tubes amended with a range of surfactant concentrations and then analyzing the liquid for surface tension. In microcosms with sediments, the sediments were allowed to settle for 6-8 hours before withdrawing 10-ml aliquots for surface tension analysis.

Surfactants were tested for their capacity to enhance the solubility of phenanthrene. The procedure was similar to the protocol used by Liu et al. (1991) to investigate surfactant solubilization of PAH compounds in soil-water slurries. Known amounts of ^{14}C -labeled and non-labeled phenanthrene were amended with different concentrations of surfactant solutions, sealed with Teflon septa and equilibrated for 3-4 days. An aliquot of the liquid was filtered through 0.22- μm Teflon filters and the radioactivity was counted by liquid scintillation. The counts in disintegrations per minute (DPM), as a proportion of the total

radiolabel added, were related to the total mass of phenanthrene initially added to the sample.

3.2.5 Statistics

Sample standard deviations were calculated for 3-5 sample replicates. An $F_{\max}$ -test was performed to determine homogeneity of sample variances (Sokal and Rohlf, 1969) for some studies. This test determined the validity of a two-sample t -test for the difference between the two sample means. The t -test was performed using the SYSTAT (SYSTAT Inc., Evanston, IL) statistical analysis program. A t -test was performed for all data which qualified on the basis of the $F_{\max}$ -test.

3.3 Cultures

3.3.1 Extraction of Microbes from Sediments

Houston Ship Channel sediment microbes were separated from sediments by taking 150-200 ml wet sediment from each site and placing it into beakers containing 1 liter of mineral salts solution. Scoops, beakers and spatulas were sterile, and all manipulations were carried out in a sterile transfer chamber to minimize the possibility of introducing contaminants. Vigorous mixing for 30 minutes with a magnetic stir bar was performed to separate microorganisms from sediment particles. Sediments were allowed to settle and overlying liquid was used as inoculum.

3.3.2 Enrichment Culture

An enrichment culture was inoculated with Dickinson Bayou sediment. PAH compounds used for the enrichment were naphthalene, anthracene, chrysene, phenanthrene, acenaphthene, and fluorene, and the PAH stock feed

solution was supersaturated with these PAHs in artificial seawater (Table 3).

Fresh PAH stock solution replaced an equivalent volume of overlying liquid on a daily basis.

Table 3. Composition of artificial seawater used in enrichment culture.

Compound	Concentration, g/L
NaCl	19.45
MgCl	5.90
NaSO ₄	3.24
CuCl ₂	1.80
KCl	0.55
NaHCO ₃	0.16
KBr	0.08
Strontium Chloride	0.034
Boric Acid	0.022
Sodium Silicate	0.004
Sodium Fluoride	0.0024
Ammonium Nitrate	0.0016
Disodium Phosphate	0.0008

3.4 Microcosm Studies

3.4.1 Microcosm Design

Microcosms consisted of 60-ml test tubes. Before use, the test tubes were soaked overnight in Alconox (Alconox Inc., New York, NY) detergent and scrubbed thoroughly. Tubes were rinsed thoroughly with distilled water, dried, rinsed with methanol, and sterilized by autoclaving. All additions to microcosms were made in aseptic transfer chambers to avoid the introduction of contaminants. Tubes were sealed tightly with Teflon-lined screw caps following liquid mineral salts/sediment/surfactant addition. 3-5 replicate microcosms for each treatment/analysis time were equilibrated in a prone position on an Eberbach Model 6000 reciprocating shaker (Eberbach Corp., Ann Arbor, MI) at a speed of 100 epm for 3-4 days, inoculated with 2.0 ml enrichment culture in a sterile transfer chamber, incubated for the desired time, removed from the shaker

for analysis, and then discarded. During equilibration it was ensured that the liquid covered the entire area where the hydrocarbon had been applied (i. e., the bottom of the tube). Equilibration and incubation were performed at 22-25 °C.

A procedure was developed in an effort to inoculate each microcosm with an equal number of microbes. The inoculation procedure involved withdrawing liquid from the enrichment culture and placing it into a 250-ml sterile glass beaker with a stir bar. The liquid was stirred to achieve a vortex of about 50% of the depth of the liquid volume. Two milliliters of the liquid inoculum was pipetted during stirring for inoculation into each tube.

Microcosm size was determined by calculating the amount of oxygen necessary for conversion of phenanthrene to carbon dioxide at phenanthrene saturation conditions in 25 ml water. However this did not account for potential oxygen demand by the surfactant or by sediment-associated organic material. Therefore dissolved oxygen levels were monitored periodically throughout experiments.

Phenanthrene addition to empty microcosms was performed by making stock solutions of ^{14}C -labeled and non-labeled phenanthrene in dichloromethane or methanol. The tubes were left uncapped for 24 hours in the aseptic transfer chambers to allow volatilization of the solvent. Volatilization of phenanthrene is not expected during solvent volatilization (Mihelcic and Luthy, 1988). In order to quantify the precise mass of phenanthrene added to each microcosm, the concentrations of the stock solutions were confirmed by (i) counting replicate injections of ^{14}C -labeled phenanthrene in vials of scintillation fluid and (ii) GC/FID analysis of non-labeled phenanthrene compared to phenanthrene standards of known densities. It was not necessary to add ^{14}C -labeled phenanthrene to microcosms designated for analysis for phenanthrene disappearance. For each experiment, an equal mass of total phenanthrene, 15 μg , was added to each

microcosm, and additions were equivalent to around one-half of the previously reported aqueous solubility of phenanthrene of 1.3 mg/L (Walters and Luthy, 1984), except in experiments examining phenanthrene disappearance, where the amount of phenanthrene added to each microcosm was just less than the calculated amount which would not exceed the combined sorptive capacity of the sediment and the aqueous solubility of phenanthrene (Laha et al., 1991). Following phenanthrene addition, Houston Ship Channel Turning Basin sediments and 25 ml liquid mineral salts or 25 ml surfactant solution made up directly in liquid mineral salts were added.

3.4.2 Sampling of Microcosms for $^{14}\text{CO}_2$

Microcosm tubes for mineralization experiments were sealed with open-faced screw caps fitted with Tuf-Bond (Pierce, Rockford, IL) Teflon/Silicone septa (100 mils thick, 22 mm diameter). Figure 3 shows the method used to sample microcosm headspace following acidification of the soil-water slurry with 10 drops of concentrated sulfuric acid. The septa were pierced by a needle for headspace sampling of $^{14}\text{CO}_2$. The purging method was based on the carbon dioxide trapping apparatus used by Marinucci and Bartha (1979) and the trapping solutions used by Abbott et al. (1992). Scintillation vials (Fisher Scientific) containing 10 ml trapping solution were arranged in tandem on the purging apparatus. A vacuum pump drew air through a 10% potassium hydroxide solution, through the sediment-water slurry and into the microcosm headspace, through the solutions in the 'downstream' vials, and finally through an activated carbon filter attached to minimize solvent vapor passage through the vacuum pump. The trapping solutions (10 ml in each vial) consisted of two ethylene glycol monomethyl ether (E) traps followed in airflow sequence by two (S) traps, which consisted of a 5:4:1 mixed ratio of scintillation fluid: methanol:

Figure 3. Purging apparatus for headspace sampling of carbon dioxide from microcosms.

monoethanolamine. E vials were designed to allow passage of CO₂ while acting as traps for any volatilized phenanthrene. S vials, meanwhile, were designed to trap CO₂. Purging was conducted for 15 minutes per sample at an intensity which provided a substantial airflow but which did not result in excessive bubbling. The entire apparatus was run in a fume hood approved for radioactive material use, which minimized the possibility of respiratory exposure to solvent vapors and radioactivity in the form of carbon dioxide. After purging for 15 minutes, the vials were removed and 5 ml scintillation fluid were added to each E vial. The vials were then capped, shaken and the radioactivity was counted in the scintillation counter. Radioactivity recovered as carbon dioxide in S traps, as a proportion of the labeled phenanthrene originally added, was taken to represent the proportion of total phenanthrene mineralized based on the total amount of phenanthrene initially added to each microcosm. An efficiency trial conducted with this particular laboratory set-up showed that 97% of ¹⁴C-labeled sodium bicarbonate added to sediment-water microcosms was recovered as carbon dioxide in S vial traps.

3.4.3 Analyzing Microcosms for Remaining Phenanthrene

The procedure used to extract and analyze remaining phenanthrene from each microcosm after a period of incubation is outlined in Figure 4. Sediment-liquid slurries were poured into 30-ml Teflon centrifuge tubes and spun for 10 minutes at a speed of 7000 rpm in a Sorvall model RC2-B automatic refrigerated centrifuge (Sorvall Inc., Norwalk, CT). The liquid portion was expressed through a pre-wet carbon-18 (C-18) Sep-pak (Millipore Corp., Bedford, MA) cartridge which had been fitted to a DCM-rinsed 30-ml glass syringe. The centrifuged sediments remaining at the tube's bottom were mixed with 5 grams sodium sulfate per gram of wet sediment in order to attain the flowing consistency

Figure 4. Method for extraction of phenanthrene from microcosms for chromatographic analysis.

described in EPA Method 3550 (Sonication Extraction), and then extracted by EPA Method 3550 with a model W185 Ultrasonic Processor (Heat System-Ultrasonics, Inc., Farmingdale, NY), equipped with standard disruptor horn and fitted with a 0.5-inch flat tip. Emptied microcosms were filled with 20 ml DCM and refitted with their screw caps. This was done to extract any remaining phenanthrene from the walls of the tube and from any material which was not removed. This 20 ml of DCM was used in the first of three sonications of each sample. Samples were concentrated to exactly 10 ml, crimp-sealed, and vortexed for 15-20 seconds prior to GC/FID analysis.

Recovery efficiency was tested before implementation of the procedure and it was found that virtually all phenanthrene added to abiotic sediment-water slurries, which had been allowed to equilibrate for 3-4 days, was recovered. Analysis of extracts from all abiotic microcosms throughout the experiments yielded close to 100% of the amount of phenanthrene initially added, when compared to GC analyses of commercially-prepared phenanthrene standards.

3.5 Experimental Approach

It was expected that sediment collection from the Houston Ship Channel would yield PAH-contaminated sediments. Analysis by GC/MS determined, however, that while traces of phthalate esters were detected in some samples, all collected samples were free of PAH contamination, and sediments from the Houston Ship Channel Turning Basin were contaminated with phenanthrene in the laboratory. Turning Basin sediments were used in all sediment experiments. Sediments were dried at room temperature, sieved to remove large particles and debris, autoclave-sterilized, and equilibrated with added phenanthrene as described above. The particle size range used for all experiments was from 90 μm to 500 μm in diameter. Sediments were sterilized by autoclaving three times

at 120°C for 45 minutes at 15 psi. Sterilization was confirmed by plating dilutions of sediment suspensions onto 1.5% Nutrient Agar plates and observing no colony forming units after 7 days of incubation at 22-25 °C.

4.0 Results

4.1 Sorption and Surfactant Characterization

4.1.1 Phenanthrene Sorption to HSC Sediment

In order to describe the partitioning behavior of phenanthrene in experimental sediment systems, an adsorption isotherm was performed with phenanthrene and Houston Ship Channel Turning Basin sediments, and an experimental value for the sediment-dependent equilibrium partition coefficient (K_p) was obtained. The mass of phenanthrene adsorbed per gram of Turning Basin sediment increased in direct proportion with increasing sediment concentration (Figure 5). K_p (ml/g), is determined as the slope of the line of linear regression analysis. K_p determined by this method was 800.

4.1.2 Surfactant Characterization and Effect of HSC Sediment

The presence of sediments usually results in surfactant sorption and requires a higher surfactant concentration to minimize solution surface tension, initiate micelle formation, and enhance solute solubility. A variety of nonionic surfactants were tested for their CMCs and for their abilities to enhance phenanthrene solubility. The effect of HSC Turning Basin sediments on surface tension reduction and on phenanthrene solubility enhancement were tested for different surfactants to estimate the degree of surfactant sorption to such sediments and to evaluate how sediments affected surfactant micelle formation and surfactant-enhanced phenanthrene solubility. The intent was not to relate surfactant properties to physical behavior but rather to find a single surfactant which had a relatively low CMC, which could enhance phenanthrene solubility both with and without sediments present, and for which sorption to sediments was low relative to other surfactants tested.

Figure 5. Adsorption isotherm for phenanthrene (phenanthrene partition coefficient K_p and correlation coefficient R in inset).

The effect that various surfactants had on solution surface tension and also the effect of sediments on surface tension reduction is shown in Figures 6-9. CMC is taken as the best estimate of the inflection point of a curve describing the relationship between surfactant concentration and surface tension, i. e., the surfactant concentration at which surface tension first reaches a minimum value. Surfactant sorption to sediments reduces the free aqueous surfactant concentration and consequently affects micelle formation. The presence of 4% sediments necessitated at least a one order of magnitude greater surfactant concentration to achieve a surface tension minimum and begin micelle formation for all surfactants tested. Figures 6-9 show how sediments affected surface tension reduction for four of the surfactants tested.

Surfactants were tested for their ability to enhance phenanthrene solubility with phenanthrene present in excess of its aqueous solubility. Solubilization of the entire mass of phenanthrene added would have yielded an aqueous concentration of 18 mg/L (phenanthrene aqueous solubility is around 1.3 mg/L). Sediment solids reduced the degree to which phenanthrene was solubilized by Triton X-100 (Figure 10). Triton X-100 showed the greatest enhancement of phenanthrene solubilization out of three surfactants tested in the presence of 4% sediment (Figure 11). Phenanthrene solubility enhancement began at about the same surfactant concentration that initiated micelle formation when no solids were present (Figure 12). The same effect was observed for Triton X-100 when 4% sediments were present (Figure 13); however, the surfactant concentration at which micelle formation and solubility enhancement began was substantially higher (1000-2000 mg/L) with 4% solids present than with no sediments present (100-200 mg/L).

Figure 6. Effect of 4% (w/v) sediment on surface tension with Triton X-100 surfactant (bars represent standard deviation).

Figure 7. Effect of 4% (w/v) sediment on surface tension with Triton X-114 surfactant.

Figure 8. Effect of 4% (w/v) sediment on surface tension with Alfonic 810-60 surfactant.

Figure 9. Effect of 4% (w/v) sediment on surface tension with Novel II 1412-70 surfactant.

Figure 10. Effect of 4% (w/v) sediment on enhanced solubilization of 18 $\mu\text{g/ml}$ phenanthrene by Triton X-100 (bars represent standard deviation).

Figure 11. Enhanced solubilization of 18 $\mu\text{g/ml}$ phenanthrene by three nonionic surfactants in the presence of 4% (w/v) sediment.

Figure 12. Relationship between surface tension and phenanthrene solubilization in non-sediment systems amended with Triton X-100 surfactant.

Figure 13. Relationship between surface tension and phenanthrene solubilization in 4% sediment systems amended with Triton X-100 surfactant.

4.1.3 Equilibration of Phenanthrene with 1% Triton X-100

An experiment was conducted to determine if amendment with 1% Triton X-100 would affect the fraction of initially added phenanthrene that partitioned into the liquid phase during the 4-day equilibration time applied to all experiments. As phenanthrene was added to microcosms in a solvent (which evaporated, leaving solid phenanthrene), equilibration of phenanthrene systems involved transfer of added phenanthrene from a solid phase to a liquid phase, potentially affecting the bulk solution fraction of phenanthrene present prior to inoculation. In sediment-free systems, around 90% of initially added phenanthrene was found in the liquid phase after a 4-day equilibration period in both unamended and 1% Triton X-100 amended systems (Figure 14). Equilibration appeared complete after 1 day. Extraction of glassware with dichloromethane failed to recover more than 2-3% of the remaining 10% initially added.

4.2 Phenanthrene Biodegradation Screening Studies

4.2.1 Phenanthrene Mineralization by Microbes from Different Sediments

An experiment was conducted to determine the presence of phenanthrene degraders in a variety of sediments in and around the Houston Ship Channel (HSC). Microcosm studies were conducted using mixed culture inocula from three sediment sampling locations along the HSC, from Dickinson Bayou, and from the laboratory enrichment culture. Most inocula mineralized at least 40% of added phenanthrene after 10 days of incubation (Figure 15). Patrick Bayou and Dickinson Bayou sediment microbes degraded phenanthrene to nearly 60% after 21 days. Phenanthrene mineralization rate appeared to be increasing slowly for all of the inocula after 21 days. HSC sediments appeared to contain microbes capable of phenanthrene degradation without prior laboratory acclimation to the

Figure 14. Percent of initially added phenanthrene which was recovered in liquid phase during equilibration with and without 1% Triton X-100 (bars represent standard deviation).

Figure 15. Mineralization of 0.5 $\mu\text{g/ml}$ phenanthrene by microbes from different sediments.

compound. The enrichment culture was chosen as the inoculum for subsequent microcosm experiments.

4.2.2 Effects of Different Surfactants on Phenanthrene Mineralization by the Enrichment Culture

Surfactant addition at concentrations above surfactant CMC can be detrimental to hydrocarbon biodegradation. To determine the potential for surfactants in further laboratory biodegradation experiments, surfactants were screened for their effect on the 6-day extent of phenanthrene mineralization by the enrichment culture. All surfactants other than Triton X-100 substantially inhibited the extent of phenanthrene mineralization after 6 days at surfactant concentrations of 1000 mg/L or higher (Figure 16). Microcosms amended with up to 5400 mg/L Triton X-100, meanwhile, either had no effect or increased the 6-day extent of phenanthrene mineralization from 40% with no surfactant to 52 % with 5400 mg/L surfactant. The inhibition of 6-day phenanthrene mineralization by surfactants other than Triton X-100 does not preclude the possibility that these effects were not reversible and would not have been overcome after further incubation. Nevertheless, it was determined that surfactants other than Triton X-100 would be unlikely to have a greater potential to enhance phenanthrene mineralization rate in sediment systems, and Triton X-100 was chosen as the surfactant for further studies.

4.3 Cell Density Studies With Triton X-100

As Triton X-100 was chosen as the only surfactant for further biodegradation studies, its effect on microbial growth was determined with phenanthrene present. There were fewer cells present in microcosms amended with 1% Triton X-100 than in non-surfactant microcosms after 4 days (Figure 17),

Figure 16. Effects of various nonionic surfactants on mineralization of 0.5 µg/ml phenanthrene in aqueous solution after 6 days of incubation.

Figure 17. Effect of 1% (v/v) Triton X-100 on cell density in non-sediment microcosms with $0.5 \mu\text{g/ml}$ aqueous phenanthrene, as measured by acridine orange direct counting (AODC) (bars represent standard deviation).

and cell densities were equal in surfactant and non-surfactant systems after 7 days, with the stationary growth phase apparently having reached completion by this time.

4.4 Biodegradation Studies With Triton X-100

4.4.1 Phenanthrene Mineralization Without Sediments

An experiment was conducted to determine the effect of surfactant addition on the rate and extent of phenanthrene mineralization with no sediments present. Amendment of non-sediment microcosms with 1% Triton X-100 resulted in initial inhibition of phenanthrene mineralization at the 2.5-day sampling time, a recovery after 4.5 days to roughly equal the extent of mineralization in non-surfactant microcosms, and an enhancement of phenanthrene mineralization after 1 week (Figure 18). After 8.5 days, the extent of phenanthrene mineralization in surfactant-amended microcosms was close to 60%, whereas without surfactant present, mineralization was around 50%. Cell density studies without sediments (Figure 17) revealed that after 4 days, microbial numbers were greater in the absence of surfactant compared to 1% Triton X-100-amended systems. These data suggest that the surfactant was toxic or inhibitory to the phenanthrene-degrading microorganisms.

4.4.2 Phenanthrene Mineralization With Sediments Present

To determine the potential effectiveness of Triton X-100 in sediment microcosms and the effects of sediments on phenanthrene mineralization in surfactant microcosms, mineralization experiments were conducted at different sediment and Triton X-100 surfactant concentrations. Amendment of sediment-free microcosms with 2700 mg/L, or 0.25% Triton X-100, resulted in a reduced extent of 4-day phenanthrene mineralization (Figure 19). This finding indicates

Figure 18. Effect of 1% (v/v) Triton X-100 surfactant on mineralization of 0.5 $\mu\text{g/ml}$ phenanthrene in systems with no sediment (bars represent standard deviation).

Figure 19. Effects of sediment concentration on mineralization of 0.5 $\mu\text{g/ml}$ phenanthrene after 4 days in systems with different concentrations of Triton X-100 surfactant (bars represent standard deviation in one direction).

that there may be some inhibitory effect by the surfactant which is apparent after 4 days but which is overcome after 6 days, since surfactant screening results (Figure 16) indicate a roughly equal extent of phenanthrene mineralization after 6 days with 2700 mg/L Triton X-100, compared to non-surfactant-amended microcosms.

Microcosms amended with 1% Triton X-100 showed an increased degree of phenanthrene mineralization as sediment concentration increased (Figure 19). With 4% sediments present, the 4-day extent of phenanthrene mineralization nearly doubled as a result of amendment with 1.0% Triton X-100, or approximately 10 times the concentration at which micelle formation begins at this sediment concentration. Meanwhile, 2700 mg/L Triton X-100, a concentration at which micelle formation is just beginning in 4% sediment systems (Figure 6), was not enough surfactant to enhance mineralization at this sediment concentration, supporting micelle formation as a necessary event for enhanced phenanthrene mineralization in sediment systems.

Amendment of 2% and 9% sediment microcosms with 1% Triton X-100 resulted in an enhanced extent of phenanthrene mineralization after 1 week without an initial inhibitory effect by the surfactant (Figures 20 and 21). A two-sample *t*-test determined that phenanthrene mineralization was significantly higher ($p < 0.03$) after 6.5 and 8.5 days in surfactant-amended microcosms at all sediment concentrations tested (Table 4).

4.4.3 Phenanthrene Disappearance and Mineralization Comparison Studies

Experiments were conducted to estimate the fraction of phenanthrene which was degraded from its parent compound state but remained unmineralized. In these experiments, the amount of phenanthrene added to each microcosm was just less than the calculated amount which would not

Figure 20. Effect of 1% (v/v) Triton X-100 surfactant on mineralization of 0.5 $\mu\text{g/ml}$ phenanthrene in systems with 2% (w/v) sediment (bars represent standard deviation).

Figure 21. Effect of 1% (v/v) Triton X-100 surfactant on mineralization of 0.5 $\mu\text{g/ml}$ phenanthrene in systems with 9% (w/v) sediment (bars represent standard deviation).

Table 4. Summary of data and two-sample *t*-test statistics at 6.5-day and 8.5-day sampling times from experiment examining temporal effect of 1% (v/v) Triton X-100 on phenanthrene mineralization.

% Sediment	1 % Surfactant (No/Yes)	Time, days	% Minerali- zation (mean of 4 samples)	Stan- dard devia- tion	p (significance level of surfactant amendment)
0	No	6.5	49.6	1.1	.022
0	Yes	6.5	54.3	3.5	
0	No	8.5	50.2	2.1	.003
0	Yes	8.5	56.9	2.5	
2	No	6.5	49.4	1.1	.007
2	Yes	6.5	54.3	2.6	
2	No	8.5	50.3	2.0	.001
2	Yes	8.5	57.2	2.4	
9	No	6.5	51.2	2.1	.008
9	Yes	6.5	58.7	4.0	
9	No	8.5	52.7	2.8	.008
9	Yes	8.5	58.0	1.4	

exceed the combined sorptive capacity of the sediment and the aqueous solubility of phenanthrene (Laha et al., 1991). Comparison with other mineralization experiments, therefore, may not be appropriate, since initial substrate concentration can affect biodegradation. The surfactant did not appear to have any effect on the rate or extent of phenanthrene biodegradation in sediment systems. In microcosms with 4% sediment, phenanthrene disappeared completely within 4 days both with and without 1% Triton X-100 present (Figure 22). Phenanthrene mineralization, meanwhile, reached an apparent maximum of 65% after 7 days in both unamended and 1% Triton X-100-amended sediment systems, with mineralization rates within 7 days appearing roughly equal (Figure 22). While phenanthrene disappearance in sediment-free microcosms was complete after 9 days in 1% Triton X-100 surfactant and non surfactant systems, the rate of disappearance was lower with the surfactant amendment (Figure 23). Amendment of microcosms with 1% Triton X-100 inhibited phenanthrene mineralization in sediment-free systems within 3 days but increased the extents of mineralization after 7 and 12 days (Figure 23). Roughly 35% of the phenanthrene added was biotransformed but remained unmineralized after 21 days in all systems.

Phenanthrene disappearance was nearly complete after 4 days in all systems amended with sediment and surfactant with the exception of the sediment-free surfactant systems (Table 5). This reflects the same trend observed in phenanthrene mineralization experiments with Triton X-100 in sediment-free systems (Figure 18), whereby 1% Triton X-100 imposed an initial inhibitory effect on phenanthrene biodegradation which was overcome with further incubation, and which was not observed with sediments present. The surfactant amendment had no beneficial effect on phenanthrene disappearance

Figure 22. Disappearance and mineralization of 17 $\mu\text{g/ml}$ phenanthrene in 4% (w/v) sediment systems with or without 1% (v/v) Triton X-100 surfactant (bars represent standard deviation).

Figure 23. Disappearance and mineralization of 1 $\mu\text{g/ml}$ phenanthrene in sediment-free systems with or without 1% (v/v) Triton X-100 surfactant (bars represent standard deviation).

in sediment microcosms. Phenanthrene disappearance rates may have been different prior to 4 days, the first sampling time.

Table 5. Disappearance of phenanthrene (1 $\mu\text{g/ml}$ in non-sediment microcosms and 17 $\mu\text{g/ml}$ in sediment microcosms) in unamended and Triton X-100-amended microcosms (bars represent standard deviation).

Treatment*	Phenanthrene, % remaining			
	0 days	4 days	9 days	18 days
No sed't / No surfactant	100	7	1	0
No sed't / 1% surfactant	100	59	0	0
4% sed't / No surfactant	100	4	5	1
4% sed't / 1% surfactant	100	0	1	0
abiotic	100	103	97	94

* sediment concentration as wt/vol, surfactant concentration as vol/vol

5.0 Discussion

These experiments provided information which helped to determine whether or not surfactants might be used advantageously to treat PAH-contaminated sediments. Several nonionic surfactants were evaluated for their abilities to enhance phenanthrene solubility in sediment systems. Sediments were collected from a variety of sites in and around the HSC, and all sediments contained microbes capable of phenanthrene mineralization. Surfactants were screened for their effects on the short-term extent of phenanthrene mineralization by an enrichment culture of phenanthrene-degraders, and Triton X-100 was the only surfactant which did not inhibit phenanthrene mineralization. This surfactant, which enhanced the solubility of phenanthrene in sediment systems, increased the extent of phenanthrene mineralization in sediment and sediment-free systems at a concentration in excess of its CMC. The enhancement of PAH biodegradation in soil-water slurries by a nonionic surfactant at a concentration in excess of its CMC does not seem to be a previously published finding. Initial phenanthrene mineralization rates were not enhanced, however, and the extent of enhancement was slight. In the absence of sediment, this surfactant exhibited an inhibitory effect on phenanthrene mineralization which was overcome with further incubation. A cost analysis would have to be performed to determine if the benefit afforded by the enhancement, as assessed by the goals of site remediation, would be worth the cost. A significant fraction of phenanthrene which was biotransformed from its parent form remained unmineralized.

Phenanthrene desorption rate did not appear to be limiting to biodegradation in these experiments. A phenanthrene adsorption showed isotherm linearity, indicating that increasing percent sediments resulted in a higher percentage of total phenanthrene found in the sorbed state. In the absence of surfactant, the 4-day extent of phenanthrene mineralization was not

significantly different in non-sediment compared to 1%, 4% or 15% sediment systems (Figure 19). In a temporal experiment, phenanthrene mineralization in the absence of surfactant was essentially the same at all three sediment concentrations (Figures 18, 20 and 21). These data suggest that phenanthrene mineralization rates were unaffected by the fraction of initially sorbed phenanthrene. The 3-4 day period used to equilibrate phenanthrene with sediments may not be an accurate indicator of the sorptive behavior of PAHs in chronically exposed sediments. Longer equilibration times for sorption of nonionic organic compounds may decrease rate or altogether inhibit PAH desorption from soils and sediments (Karickhoff, 1980; Steinberg et.al., 1987; Pavlostathis and Jaglal, 1991; Pignatello, 1990; Kan et al., 1994), possibly involving entrapment into soil/sediment micropores (Wu and Gschwend, 1986). Karickhoff reports that about one-half of the equilibrium sorption of polynuclear aromatic hydrocarbons to natural sediments occurs within minutes, and that equilibrium partitioning into a more highly sorbed phase S_2 may take days or weeks to complete. The extent and strength of sorption of a hydrophobic organic molecule such as a PAH can strongly influence its rate of biodegradation since compounds in a sorbed state are generally considered unavailable for microbial utilization. While this short-term equilibration approach has often been used in biodegradation studies, extrapolation of laboratory findings using this approach may not be accurate as an indicator of the biotreatability of sediments which have been chronically exposed to PAHs.

A theoretical partition coefficient K_p for phenanthrene can be determined based on the fraction of organic carbon (f_{oc}) of sediments used in experiments, the octanol-water partition coefficient (K_{ow}) for phenanthrene, and a proportionality constant which can be used to estimate K_{oc} , the organic carbon sorption coefficient, from K_{ow} :

$$K_p \text{ (ml/g)} = (f_{oc})(0.63)(K_{ow}) = (0.63)(.021)(33884) = 450.$$

The difference between the expected value for K_p (450 ml/g) and the experimental value (800 ml/g) (Figure 5) may be due to an experimental factor such as sorption of phenanthrene to the walls of the glass microcosm. This event would result in an effective loss of phenanthrene from the sediment-water system. Since the sediment phenanthrene concentrations in the experimental isotherm were calculated based on the difference between the total added phenanthrene and that found in solution, the result of 'lost' phenanthrene would be a decreased sediment concentration for each corresponding aqueous concentration, and a smaller slope for the linear regression. Assumed loss of 10% of added phenanthrene, for example, would result in an experimental K_p of around 650 ml/g. In addition, there is a degree of uncertainty in determining theoretical K_p which is based on an estimate for K_{oc} .

Sorption processes are important in determining the fate of surfactants in the subsurface (DiToro et al., 1990). Surfactant sorption is controlled by surfactant properties, molecular structure, critical micelle concentration and water solubility, and by soil properties (Kuhnt, 1993). The degree of sorption of nonionic surfactants to soils and sediments is controlled largely by water solubility and by soil/sediment organic matter content. Surfactant sorption to some sediments can change the surficial character of sediment from hydrophilic to hydrophobic. This leads to water displacement and higher soil aggregate stability (Kuhnt, 1993), and might provide a more favorable partitioning site for a hydrophobic organic compound, exacerbating cleanup efforts.

Surfactant sorption to soils causes a change in the surfactant micellization pattern such that aqueous phase surfactant is substantially less than the total

added (Laha and Luthy, 1991). They confirmed with surface tension experiments that with soils present, a higher surfactant concentration is necessary to begin micelle formation than in non-soil systems. Sorption of Triton X-100 to 4% sediments was evident in these experiments, and necessitated an approximately one order of magnitude greater surfactant concentration in order for micelle formation to begin (Figure 6). Some of the properties which control sorption of nonionic alkylethoxylate and alkylphenylethoxylate surfactants are carbon chain length and the number of ethoxyl groups. A larger number of ethoxyl groups in nonionic ethoxylated alcohols, for instance, results in a more hydrophilic character (larger HLB number), an increase in CMC, and a reduced extent of sorption.

If enhanced solubilization of a hydrophobic organic contaminant is desired, information concerning surfactant sorption is necessary so that surfactants can be added to sediment-water slurries at concentrations adequate for the onset of micelle formation and subsequent contaminant solubilization. An estimate of the degree of sorption of a surfactant to sediment can be made by determining both the CMC and the surfactant concentration at which micelles form with sediments present. The degree of surfactant sorption to sediment can be used to predict how sediments may affect the performance of surfactants in larger systems. Choosing a surfactant type which sorbs less to sediments might reduce the concentration necessary to solubilize the PAH with sediments present, and operating costs and waste volume could be minimized. Surfactant concentration in sediments can be estimated by the equation

$$[S] = (CMC_m - CMC)(V)/m,$$

where $[S]$ is sorbed surfactant concentration (mg/g sediment), CMC is the clean water CMC (mg/L), CMC_m is the surfactant concentration at which micelles begin to form with mass m (g) of sediment present, and V is the liquid volume (L) of each microcosm. Of these surfactants, the extent of sorption of Triton X-100 to sediments appeared to be the lowest, based on CMC estimates (Table 6). Figure 6 shows that the clean water (with no sediment) CMC of Triton X-100 is 200 mg/L and that micelle formation begins at 2000 mg/L with 4% sediment present. The degree of sorption of Triton X-100 to collected HSC Turning Basin sediment is estimated using the previous equation:

$$[\text{Triton X-100}] = (2000 \text{ mg/L} - 200 \text{ mg/L})(.027 \text{ L}) / (1.0 \text{ g}) = 48.6 \text{ mg/g sediment.}$$

Studying the effects of several sediment concentrations on surface tension in a surfactant system would provide greater accuracy in determining the degree of surfactant sorption. Laha and Luthy (1991) found the degree of sorption of Triton X-100 to a soil of 1.5% organic carbon content to be 11.9 mg/g soil, and the higher organic carbon content of HSC sediment (2.1%) may explain the greater degree of sorption of this surfactant to HSC sediment. CMC values are estimated on the basis of surface tension reaching a minimum, adding to the uncertainty in this equation, but in engineering larger sediment treatment systems this approach can give a rough indication of the degree to which the surfactant will adsorb to sediments. CMC values obtained for surfactants compared reasonably well with previously reported values (Table 6).

Table 6. Estimates of critical micelle concentration (CMC) for various surfactants without sediment and in the presence of 4% sediment, and comparison with previously reported values.

Surfactant	CMC, mg/L		
	No Sediment	4% Sediment	No sediment, previously reported (reference)
Triton X-100	200	2000	188-207 (Rosen, 1989)
Triton X-114	100	3000	110 (Kile and Chiou, 1989)
Alfonic 810-60	300	3000	275 (Aronstein et al., 1991)
Novel II 1412-70	40	4000	41-43* (Rosen, 1982)

*surfactant tested was generic and had the same molecular structure as Novel II 1412-70.

Microbes from sediments in and around the HSC mineralized phenanthrene when provided with oxygen and inorganic nutrients, without prior laboratory acclimation to this compound. While this confirmation does not assure that PAH-degrading microbes are present in all HSC sediments, it is likely that PAH-degrading microbes are present at other sites in and around the HSC. Prolonged exposure to PAHs in natural waters and sediments can cause increases in total microbial populations, selective increases in populations of microorganisms with PAH-degrading enzymes, and induction of PAH-degrading enzymes in some indigenous microorganisms (Cerniglia and Heitkamp, 1989). Sediment microbial populations which had been pre-exposed to anthracene and naphthalene degraded these compounds at higher rates than populations which were not pre-exposed (Bauer and Capone, 1985). Another study (Herbes and Schwall, 1978) found substantially greater PAH transformation rates by microbes from sediments with a history of exposure to PAHs when compared with microbes from pristine sediment samples. PAH biodegradation rate increases are probably related to the presence and expression of plasmid-encoded

catabolic enzymes (Cerniglia and Heitkamp, 1989). Successful bioremediation of PAH-contaminated HSC sediments may be dependent on the presence of such microbes.

The observed inhibitory effects on 6-day phenanthrene mineralization by surfactants other than Triton X-100 were likely due to a surfactant-microbial interaction rather than an inhibitory effect by phenanthrene. While different micellar forms of various surfactants may change the way in which phenanthrene-microbial contact is made, the suspected toxicity of many surfactants and the high concentrations relative to the spare amounts of phenanthrene, along with demonstrated biodegradability of phenanthrene by the enrichment culture, makes inhibition by surfactants a more likely explanation. The inhibition of 6-day phenanthrene mineralization by surfactants other than Triton X-100 does not preclude the possibility that these effects were not reversible and would not have been overcome after further incubation.

Surfactants which are similar in structure may have different biological effects, and each surfactant must be evaluated individually to determine its potential effect on PAH biodegradation. Triton X-114, which differs from Triton X-100 only by containing two fewer ethoxyl groups, inhibited phenanthrene mineralization while Triton X-100 slightly enhanced mineralization within the same 6-day incubation period (Figure 16). While relationships between surfactant property and toxicity to pure and mixed cultures have been observed, for example the decreased toxicity of nonionic surfactants with a greater number of ethoxyl groups (Tiehm, 1994), generalizations relating surfactant properties to toxicity must be made with caution.

Surfactant biodegradation is an important consideration. Inhibition of phenanthrene mineralization by surfactants other than Triton X-100 may have been due to the preferential microbial utilization of the surfactant over

phenanthrene. Alkylphenylethoxylates such as Triton X-100 and Triton X-114 are generally less biodegradable than linear alkylethoxylates due to the presence of a phenyl group and branching of the carbon chain. Triton X-114, though, is shorter and might be more degradable than Triton X-100. Cell growth studies (Figure 17) support the likelihood that Triton X-100 was not used preferentially over phenanthrene as a growth substrate. While resistance to biodegradability may prevent a surfactant from being used as a preferential substrate by microbes in sediment bioremediation, this characteristic may make surfactant-laden wastewater treatment more difficult.

A surfactant-phenanthrene partitioning interaction may explain the inhibition of phenanthrene mineralization by Triton X-100 in sediment-free systems prior to 1 week (Figure 18). A decreased phenanthrene mineralization rate may have been the result of a decreased fraction of the total amount of phenanthrene in non-micellar, molecular solution. Without sediments present, phenanthrene at a concentration below its aqueous solubility should be (i) completely in molecular solution without surfactant present, and (ii) either in molecular solution or in surfactant micelle interiors when micellar surfactant is present. Thus, partitioning of phenanthrene into micelles may have decreased the molecular solute phenanthrene concentration and consequently reduced the rate of phenanthrene mineralization. With sediments present (Figures 20 and 21), a reduced micelle concentration due to surfactant sorption to sediments may have resulted in a greater fraction of phenanthrene in molecular solution, increasing the amount available for microbial uptake. Surfactant sorption to sediments offers another explanation. The presence of as few as 2% sediment solids may have prevented the lag in phenanthrene mineralization by serving as an adsorbent for surfactant molecules, thereby reducing the free aqueous

surfactant micelle concentration and thus the degree of inhibitory contact between cells and surfactant.

Resistance to toxic or inhibitory effects of surfactants may depend on the nature of inoculum of phenanthrene degraders. The known ability of nonionic surfactants to solubilize cellular enzymes, receptors and proteins, and the observed toxicity of nonionic surfactants in this and other laboratory biodegradation experiments points to a surfactant-microbial interaction as a cause for inhibition of phenanthrene mineralization by most surfactants in these experiments. The presence of microbes which are able to resist such adverse effects and still degrade phenanthrene quickly and extensively may be a reflection of the environment from which they were obtained. Triton X-100 at a concentration around fifty times its CMC imposed an inhibitory effect only in the absence of sediment, and phenanthrene degraders overcame the effect in a matter of days. Chronic exposure of HSC sediments to petrogenic chemicals may have resulted in the selection of a microbial population with the ability to resist adverse effects of chemical exposure. This adapted population may be naturally more resistant to toxic or inhibitory effects of surfactants.

An increased extent of phenanthrene mineralization (increase of 5-7% in sediment and sediment-free systems) was observed in the presence of 1% Triton X-100 (Figures 18, 20 and 21). This appears to be due to an effect by the surfactant rather than a factor due to the combined effect of the sediment and surfactant, since the surfactant had a roughly equivalent enhancement effect in non-sediment compared with sediment microcosms. If several species of phenanthrene degraders were present in the enrichment culture inoculum, the surfactant may have imposed a selective pressure which resulted in a populational shift and an increased extent of phenanthrene mineralization by microbes which survived the surfactant's initial inhibitory effect. The surfactant

may also have increased the availability of an intermediate which remained unavailable and unmineralized in the absence of surfactant.

It was suspected that the enhancement of the extent of phenanthrene mineralization might have been due to the surfactant solubilizing a portion of phenanthrene which remained sorbed to the walls of the glassware in the absence of surfactant. In sediment-free systems, however, around 90% of initially added phenanthrene was found in the liquid phase after a 4-day equilibration period in both unamended and 1% Triton X-100 amended systems, indicating an equivalent bulk solution fraction of phenanthrene potentially available for microbial utilization. The 10% of phenanthrene which was not recovered, either in surfactant or non-surfactant systems, may have been lost to volatilization or may have remained sorbed. Extraction of glassware with dichloromethane failed to recover more than 2-3% of the remaining 10% initially added.

Triton X-100 did not have an effect on the disappearance of phenanthrene in more highly contaminated sediment systems. Phenanthrene disappearance was nearly complete after 4 days in all systems amended with sediment and surfactant with the exception of the sediment-free surfactant systems (Table 2). A similar phenomenon was seen in phenanthrene mineralization experiments with Triton X-100 in sediment-free systems (Figure 18), whereby 1% Triton X-100 imposed an initial inhibitory effect on phenanthrene mineralization which was overcome with further incubation. The surfactant amendment had no beneficial effect on phenanthrene disappearance in 4% sediment microcosms. A significant fraction of phenanthrene (35-40%) was biotransformed but remained unmineralized in 4% sediment systems, both in the presence and absence of 1% Triton X-100 (Figure 22). In sediment-free systems, 1% Triton X-100 affected the rates of phenanthrene disappearance prior to 4 days and mineralization prior to

21 days, but a similar percentage of phenanthrene, 35-40%, remained unmineralized after 21 days (Figure 23). A number of studies have found accumulation of intermediate degradation products of PAHs or incorporation of degradation products into cellular biomass (Herbes and Schwall, 1978; Heitkamp et al., 1988; Guerin and Jones, 1988; Bauer and Capone, 1985).

A difference between two experiments which should theoretically have produced the same result could have been due to the subtle changes associated with conducting experiments at different times. In a temporal experiment, phenanthrene mineralization in sediment-free systems was substantial after 4.5 days (Figure 18) whereas in another experiment, phenanthrene mineralization was not evident after 4 days at the same Triton X-100 concentration, 1% (Figure 19). While the enrichment culture is useful for studying effects by various amendments on pollutant biodegradation in a given experiment, there may be (i) slight microbial populational changes in the enrichment culture, or (ii) subtle environmental changes such as room temperature which might affect microbial activity. The lag and log phases for microbial growth appear to be occurring within 4-5 days (Figure 17), and a minor difference in the duration of lag phase, for instance, might cause such variation.

The rate of phenanthrene disappearance from sediment-water systems may be the primary concern to site remediation. If this is the case, and if surfactants have no effect on the rate or extent of compound disappearance, their application may not be warranted. Compound disappearance often does not parallel compound mineralization. Detoxification of contaminated sediments could also be a concern to site remediation. If surfactants had no effect on compound disappearance but enhanced the rate or extent of mineralization and thereby acted to reduce sediment toxicity (for example, by enhancing mineralization of a toxic metabolite or degradation intermediate), surfactant

application could be useful; however, the surfactants themselves might render the sediments more toxic.

The known toxicity of many nonionic surfactants creates a great deal of concern when considering their potential to enhance PAH bioremediation. Surfactants must be applied with caution in remedial systems, and screening for adverse effects of surfactants on contaminant biodegradation should be conducted prior to their implementation. Nevertheless, surfactants in some instances have enhanced biodegradation rates. While most surfactants tested were inhibitory to phenanthrene mineralization by an enrichment culture, Triton X-100 showed promise as a surfactant potentially capable of enhancing phenanthrene mineralization by the enrichment culture.

Consideration of the potential fate and effects of surfactants in subsurface systems has been necessitated not only by their common household use but also by the increasing use of surfactants in environmental applications, such as enhanced oil recovery, in-situ bioremediation, and pesticide delivery to crops. The behavior and fate of surfactants in soils is governed by many factors including soil physics, soil chemistry and soil biology (Kuhnt, 1993). If surfactant use is limited to closed systems, such as in bioreactors, then proper disposal, detoxification or surfactant recovery from solution can be implemented to minimize the threat of environmental release. When considering surfactants for *in situ* use, however, the potential for off-site migration of surfactants or surfactant-mobilized or solubilized contaminants necessitates a detailed evaluation of the remedial system for its ability to operate as a closed-loop system.

Application of surfactants to enhance the availability of sparingly soluble substrates for microbial utilization has been extensively studied. The variety of experimental conditions such as physical-chemical soil characteristics, organism-

specific properties associated with microbial populations, molecular nature of the surfactant and the various ratios at which the components are mixed may be a reason for the variety and conflicting nature of results obtained. As more studies are conducted, we will see better-defined trends which will enable us to design better remedial systems, and hopefully we will be able to determine the precise mechanisms which control interactions among sediments, pollutants, microbes and surfactants so that emerging technologies can be used effectively.

6.0 Conclusions

Houston Ship Channel sediments are strong adsorbents for nonionic surfactants as well as for phenanthrene. Sorption of surfactant molecules to sediment affected surfactant performance as a solubility enhancer by necessitating greater surfactant concentrations in order to achieve surface tension minima and micelle formation. While there has been some evidence for enhanced solubility of hydrophobic organic compounds by surfactants in the absence of micelles, enhanced solubility is most often associated with the presence of micelles. When considering the use of surfactants in soil or sediment systems, a detailed consideration of surfactant sorption is necessary to evaluate the potential performance of the surfactant in enhancing contaminant solubilization and/or mobilization.

Sediments from the Houston Ship Channel contain microbes capable of phenanthrene biodegradation under aerobic conditions. Microbes were obtained from several ship channel sites and did not require laboratory acclimation to mineralize phenanthrene extensively within 1 week's time. Sediments from other sites in and around the ship channel may also contain phenanthrene degraders. A laboratory enrichment culture seeded with sediment and grown on a variety of PAHs as the only added carbon sources served as a dependable inoculum in laboratory experiments designed to test various amendments for their effects on biodegradation.

Sorption of low (less than 0.5 mg/L) phenanthrene concentrations to sediments may not limit the rate of phenanthrene biodegradation. The apparent lack of rate-limiting effect of desorption on biodegradation may be due to the relatively short (4 days) equilibration time used, since longer equilibration times have been implicated in increased degrees of sorption.

All but one of the nonionic surfactants tested were inhibitory to the short-term (less than 6 days) extent of phenanthrene mineralization at concentrations in excess of their CMC. As many nonionic surfactants are noted for their cellular membrane solubilizing characteristics, disruption of membrane-mediated cellular events or destruction of membrane integrity are likely explanations for inhibition of phenanthrene mineralization by nonionic surfactants. This phenomenon may involve an initial inhibitory effect that can be overcome with further incubation rather than an irreversible toxic effect.

Nonionic surfactants at concentrations greater than their CMC, if not toxic or irreversibly inhibitory to the microbes capable of phenanthrene degradation, can enhance the extent of phenanthrene mineralization within short time periods when supplied with inorganic nutrients and oxygen. In the absence of sediment, addition of a nonionic surfactant in excess of its CMC reduced the initial rate of phenanthrene mineralization but enhanced the extent of mineralization after 1 week. The presence of as little as 2% sediment prevented this initial inhibitory effect, perhaps by acting as an adsorbent for surfactant molecules, reducing the free concentration of surfactant and thus its possible inhibitory effect on phenanthrene-degrading microorganisms. A greater concentration of micelles in the absence of sediment may have reduced the fraction of phenanthrene in molecular solution, thereby rendering the phenanthrene more difficult to degrade. This is a possibility since phenanthrene was at a concentration below its aqueous solubility. A sediment-specific property not relating to sorptive capacity for the surfactant may also be a factor in eliminating the initial inhibitory effect by the surfactant in the absence of sediment.

While recent studies have documented the potential usefulness for nonionic surfactants at concentrations below their CMC on PAH biodegradation in soil-water slurries, or have found that supra-CMC concentrations of nonionic

surfactants altogether prevent PAH mineralization, this study indicates a potential for this nonionic surfactant in enhancing phenanthrene mineralization when used at a concentration in excess of its CMC. Triton X-114, a surfactant structurally similar to Triton X-100, inhibited short-term phenanthrene mineralization, suggesting that subtle structural differences among surfactants within the same class may result in dramatically different biological effects and that any surfactant considered for use must be evaluated individually.

7.0 Future Studies

The findings of this research provide a foundation from which to make decisions about the utility of this surfactant in larger systems, such as in pilot-scale studies or in a field demonstration. Information from a laboratory-scale treatability study such as this can be useful in engineering larger systems. Such information includes determining the degree of phenanthrene sorption to sediments, surfactant sorption to sediments, and the effect of surfactants on microbes capable of PAH degradation. The enhancement of phenanthrene mineralization by Triton X-100 in laboratory experiments indicates a potential role for this surfactant in the bioremediation of PAH-contaminated sediments from the Houston Ship Channel.

Higher molecular-weight PAHs such as pyrene, fluorene or benzo(a)pyrene could be target compounds in further studies. Sediment concentration as well as contaminant concentration could both be increased and studied in larger bioreactors. Rather than monitoring only levels of compound biodegradation, one could periodically sample the water phase for toxicity reduction, as measured by Microtox, and sample sediments and water for PAH extraction and subsequent analysis by gas chromatography. The toxicity assay may allow conclusions regarding risk reduction. Triton X-100 would be the surfactant, as it was not detrimental to phenanthrene degradation by HSC sediment microbes in sediment systems, and may play a significant role in enhancing mass transfer with more hydrophobic compounds such as these. Three bioreactors therefore would be a non-surfactant system, a Triton X-100 surfactant-amended system, and an abiotic system. All three would contain a high sediment concentration, highly contaminated with one or several PAHs, but loaded such that crystalline PAH would not be present.

Alternatively, sediment-free microcosm studies could be conducted with micellar Triton X-100 and a variety of higher molecular weight PAH at concentrations in excess of their aqueous solubility, similar to a study by Bury and Miller (1993). Solid PAH would serve as the hydrocarbon, however, and a PAH enrichment culture could serve as the inoculum in mineralization studies. Monitoring toxicity reduction via Microtox (aqueous test) would be possible here, as adaptation of sediment microcosms for the Microtox Solid-Phase test is difficult due to the large sediment sample required for each assay. Cell growth could be monitored by measuring solution optical density.

Experimental approach could be changed to reflect longer-term exposure of sediments to PAH contamination. Increasing PAH-sediment equilibration time may reveal information on how exposure time of sediments to PAH affects PAH biodegradability. Here, a single, high sediment concentration might be used with a PAH contamination level whereby the combined sorptive capacity of the sediment for the PAH and the PAH aqueous solubility would nearly saturate the sediment water system (based on Liu et. al., 1991). A surfactant could be incorporated as a treatment.

For any biodegradation experiments, frequent sampling immediately following inoculation is strongly recommended, particularly if the inoculum is known to contain PAH degraders.

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9.0 Appendix

Figure 5 data

1,353,998 dpm/microcosm

% sediment (w/v)	Cw (dpm/ml)	stdev	Cw (µg/ml)	% aqueous	Cs (µg/g)	% sorbed
0.25	6074	250	0.06074	12.1	47.93	87.9
0.5	3567	184	0.03567	7.1	25.22	92.9
1	1901	125	0.01901	3.8	13.02	96.2
2	1006	39	0.01006	2.0	6.62	98.0
4	584	15	0.00584	1.2	3.34	98.8

Figure 6 data

NO SEDIMENT:

Triton X-100 conc (mg/l)	54	108	216	540	1080	2160	5400	10800	54000
st1	71.3	61.6	54.0	43.6	39.0	37.2	35.8	35.7	35.8
st2	66.6	58.9	52.9	43.5	39.8	37.3	35.8	35.8	35.9
st3	68.6	58.8	52.7	42.8	40.0	37.0	35.6	35.8	35.9
average surface tension	68.6	59.8	53.2	43.3	39.6	37.2	35.7	35.8	35.9
s. t. correction factor	0.9336	0.9248	0.9182	0.9083	0.9046	0.9022	0.9007	0.9008	0.9009
corrected avg. surface tension	64.1	55.3	48.8	39.3	35.8	33.6	32.2	32.2	32.3
standard deviation	2.2	1.5	0.6	0.4	0.5	0.1	0.1	0.1	0.05

4% SEDIMENT:

Triton X-100 conc (mg/l)	54	108	216	540	5400
st1	44.0	38.7	35.5	35.3	35.4
st2	43.6	37.3	35.2	35.4	35.7
st3	43.2	37.4	35.2	35.2	35.7
average surface tension	43.6	37.8	35.3	35.3	35.6
correction factor	0.9086	0.9028	0.9003	0.9003	0.9006
corrected avg. surface tension	39.6	34.1	31.8	31.8	32.1
standard deviation	0.4	0.7	0.2	0.1	0.2

Figure 7 data

NO SEDIMENT:

Triton X-114 conc (mg/l)	9.5	48	95.4	477	4770	19080	
st1	52.8	46.1	35.4	33.7	33.4	33.4	
st2	53.8	45.2	34.7	33.9	33.5	33.6	
average surface tension	53.3	45.7	35.1	33.8	33.5	33.5	
correction factor	0.9183	0.9107	0.9001	0.8988	0.8985	0.8985	
corrected avg. surface tension	48.9	41.6	31.6	30.4	30.1	30.1	
standard deviation	0.6	0.6	0.4	0.1	0.1	0.1	
4% SEDIMENT:							
Triton X-114 conc (mg/l)	48	95	191	477	954	1908	47700
st1	68.6	59.6	50.2	42.0	38.2	36.4	34.0
st2	67.7	60.7	49.1	41.8	38.1	36.5	34.0
st3	69.0	61.9	49.7	41.0	38.4	36.5	34.0
average surface tension	68.4	60.7	49.7	41.6	38.2	36.5	34.0
correction factor	0.9334	0.9257	0.9147	0.9066	0.9032	0.9015	0.8991
corrected avg. surface tension	63.8	56.2	45.5	37.7	34.5	32.9	30.6
standard deviation	0.6	1.1	0.5	0.5	0.1	0.1	0.1

Figure 8 data

NO SEDIMENT:

Alfonic 810-60 conc (mg/l)	18.9	28.4	56.7	123	236	472	7087
st1	60.6	55.6	49	42.3	33.2	30.5	28.8
st2	60.8	56.1	49	42.1	33.3	30.4	28.6
st3	60.5	55.5	49.7	42.2	33.4	30.5	27.8
st4	60.6	55.4	49.6	42.1	33.7	30.7	
average surface tension	60.6	55.7	49.3	42.2	33.4	30.5	28.4
correction factor	0.9256	0.9207	0.9143	0.9072	0.8984	0.8945	0.8924
corrected avg. surface tension	56.1	51.2	45.1	38.3	30.0	27.3	25.3
standard deviation	0.1	0.3	0.3	0.1	0.2	0.1	0.5

Figure 8 data, cont.

4% SEDIMENT:									
Alfonic 810-60 conc (mg/l)									
st1	94.5	473	945	1890	3780	7560	11340		
st2	54.0	37.9	34.1	33.0	31.2	31.0	31.0		
st3	53.9	38.4	34.4	33.0	31.5	30.9	31.0		
average surface tension	52.9	38.4	34.3	33.0	31.4	31.1	31.1		
correction factor	53.6	38.2	34.3	33.0	31.4	31.0	31.0		
corrected avg. surface tension	0.9186	0.9032	0.8993	0.898	0.8954	0.895	0.895		
standard deviation	49.2	34.5	30.8	29.6	28.1	27.7	27.8		
	0.6	0.3	0.1	0.0	0.1	0.1	0.1		

Figure 9 data

NO SEDIMENT:									
Novel II 1412-70 conc (mg/l)									
st1	4.8	9.5	48	95	475	4750			
st2	54.0	52.0	41.5	41.5	40.6	40.7			
average surface tension	55.0	51.8	42.1	41.0	40.9	40.3			
correction factor	54.5	51.9	41.8	41.3	40.8	40.5			
corrected avg. surface tension	0.9195	0.9169	0.9068	0.9063	0.9058	0.9055			
standard deviation	50.1	47.6	37.9	37.4	37.0	36.7			
	0.7	0.1	0.4	0.3	0.2	0.3			
4% SEDIMENT:									
Novel II 1412-70 conc (mg/l)									
corrected avg. surface tension	95	950	1900	4750	9500	47500			
	72.6	66.3	42.6	37.4	37.1	36.7			

Figure 10 data

NO SEDIMENT:
Triton X-100 conc (mg/L)

1 (dpm/ml)	0	540	1080	2160	3780	5400	10800	21600
2 (dpm/ml)	69.3	1161.3	1396.6	1498.1	1526.2	1528.4	1590.1	1664.7
3 (dpm/ml)	64.1	1183.4	1416.0	1486.8	1608.4	1553.9	1635.3	1668.3
avg. dpm/ml	71.1	1134.6	1429.3	1490.7	1555.1	1586.6	1640.8	1661.5
stdev	68.2	1159.8	1414.0	1491.9	1563.2	1556.3	1622.1	1664.8
mg/l aqueous	3.6	24.4	16.4	5.7	41.7	29.2	27.8	3.4
stdev	0.7	11.4	13.9	14.6	15.3	15.3	15.9	16.3
	0.0	0.2	0.2	0.1	0.4	0.3	0.3	0.0

4% SEDIMENT:
Triton X-100 conc (mg/L)

1 (dpm/ml)	0	540	1080	2160	3780	5400	10800	21600
2 (dpm/ml)	66.2	52.0	41.0	408.6	864.1	1050.3	1294.4	1486.0
3 (dpm/ml)	70.0	47.1	43.2	406.0	842.2	1084.1	1303.9	1510.7
4 (dpm/ml)	54.1	57.9	50.5	415.9	860.8	1058.9	1339.4	1553.5
avg. dpm/ml	54.0	60.8	51.4	421.3	896.9	1052.8	1387.6	1541.2
stdev	61.1	54.5	46.5	413.0	866.0	1061.5	1331.3	1522.9
mg/l aqueous	8.3	6.1	5.2	7.0	22.7	15.5	42.2	30.5
stdev	0.6	0.5	0.5	4.1	8.5	10.4	13.1	14.9
	0.1	0.1	0.1	0.1	0.2	0.2	0.4	0.3

Figure 11 data

Alfonic 810-60 conc (mg/L)									
1 (dpm/ml)	0	945	1890	3780	7560	15120			
2	36.8	33.1	30.7	65.4	231.6	370.4			
3	46.2	32.3	32.5	68.5	251.4	369.5			
4	42.3	34.6	36.6	63.4	250.0	362.3			
5	37.4	33.6	37.1	57.3	233.2	360.6			
6	44.7	30.2	33.6	57.7	239.0	375.9			
avg. dpm/ml	41.2	30.1	37.8	56.3	239.9	367.7			
stdev	41.4	32.3	34.7	61.4	240.9	367.7			
mg/l aqueous	3.8	1.8	2.9	5.0	8.3	5.6			
stdev	1.0	0.8	0.8	1.5	5.8	8.8			
	0.1	0.0	0.1	0.1	0.2	0.1			
Triton X-100 conc (mg/L)									
1 (dpm/ml)	0	540	1080	2160	3780	5400	10800	15120	21600
2	66.2	52.0	41.0	408.6	864.1	1050.3	1294.4	1486.0	1486.0
3	70.0	47.1	43.2	406.0	842.2	1084.1	1303.9	1510.7	1510.7
4	54.1	57.9	50.5	415.9	860.8	1058.9	1339.4	1553.5	1553.5
avg. dpm/ml	54.0	60.8	51.4	421.3	896.9	1052.8	1387.6	1541.2	1541.2
stdev	61.1	54.5	46.5	413.0	866.0	1061.5	1331.3	1522.9	1522.9
mg/l aqueous	8.3	6.1	5.2	7.0	22.7	15.5	42.2	30.5	30.5
stdev	0.6	0.5	0.5	4.1	8.5	10.4	13.1	14.9	14.9
	0.1	0.1	0.1	0.1	0.2	0.2	0.4	0.3	0.3
Neodol 25-7 conc (mg/L)									
1 (dpm/ml)	0	334	738	1475	2950	5901	11802	2397	2397
2	46.3	40.9	43.6	41.3	176.7	356.5	385.0	491.1	491.1
3	46.5	39.0	40.6	41.2	179.1	320.2	379.3	474.8	474.8
avg. dpm/ml	43.6	42.6	46.9	38.1	171.4	347.8	384.3	501.6	501.6
stdev	45.5	40.8	43.7	40.2	175.7	341.5	382.9	489.2	489.2
mg/l aqueous	1.6	1.8	3.2	1.8	3.9	19.0	3.1	13.5	13.5
stdev	1.1	1.0	1.1	1.0	4.3	8.3	9.3	11.8	11.8
	0.0	0.0	0.1	0.0	0.1	0.5	0.1	0.3	0.3

Figures 12,13 data
data above

Figure 14 data

Time, days	1	2	4
No surfactant	87.4	86.6	85.6
st. dev.	0.8	1.3	1.1
Surfactant	88.7	91	88.7
st. dev.	1.5	2.3	1.1

Figure 15 data

% Mineralization

Time, days	0	6.0	11.0	21.0
Turning Basin	0	43.4	45.8	50.5
Patrick Bayou	0	32.4	50.3	57.3
Vince Bayou	0	1.0	32.6	42.5
Dickinson Bayou	0	11.0	52.3	59.8
Enrichment Culture	0	47.8	51.1	47.9

Figure 16 data

Neodol 25-9									
Surfactant concentration, mg/L	0	86	857	2121	4333				
% Mineralization	40	32	14	5	4				
Triton X-100									
Surfactant concentration, mg/L	0	100	1004	2484	5076				
% Mineralization	40	45	48	51	52				
Neodol 25-7									
Surfactant concentration, mg/L	0	86	857	2121	4333				
% Mineralization	40	44	4	3	2				
Tween 60									
Surfactant concentration, mg/L	0	97	971	2401					
% Mineralization	38	38	30	4					
Novel II 1412-70									
Surfactant concentration, mg/L	0	88	883	2185					
% Mineralization	38	35	3	1					
Triton X-114									
Surfactant concentration, mg/L	0	89	887	2194					
% Mineralization	38	44	30	8					
Alfonic 810-60									
Surfactant concentration, mg/L	0	88	879	2174					
% Mineralization	46	40	1	0					

Figure 17 data

# Cells/ml					
Time, days	0	1	2	4	7
No surfactant	1.0E+5	8.9E+4	1.6E+6	5.3E+7	2.5E+6
st. dev.	2.3E+4	2.9E+4	4.6E+5	1.0E+7	3.5E+5
Surfactant	1.0E+5	1.0E+5	7.1E+5	4.5E+6	2.4E+6
st. dev.	2.2E+4	2.4E+4	1.6E+5	6.6E+5	2.6E+5

Figure 19 data

% Mineralization					
Surfactant concentration, mg/L	0	2700	10800		
No sediment	28	4	1		
st. dev.	2	3	0		
1% (w/v) sediment	32	26	14		
st. dev.	4	4	5		
4% (w/v) sediment	34	30	47		
st. dev.	9	9	10		
15% (w/v) sediment	34	33	49		
st. dev.	3	7	15		

Figures 18, 20, 21 data

% Sediment	1% Surfactant (n/y)	Time, days	% Mineralization	standard deviation	p value (from 2-sample t-test)
0	N	2.5	40.6	2.7	
	Y	2.5	11.5	0.5	0.000
	N	4.5	48.5	1.8	
	Y	4.5	51.6	3.0	0.090
	N	6.5	49.6	1.1	
	Y	6.5	54.3	3.5	0.022
	N	8.5	50.2	2.1	
	Y	8.5	56.9	2.5	0.003
	N	2.5	41.9	9.0	
	Y	2.5	50.1	3.0	0.068
2	N	4.5	47.7	2.3	
	Y	4.5	54.0	2.2	0.004
	N	6.5	49.4	1.1	
	Y	6.5	54.3	2.6	0.007
	N	8.5	50.3	2.0	
	Y	8.5	57.2	2.4	0.002
	N	2.5	43.1	2.9	
	Y	2.5	43.9	4.3	0.392
	N	4.5	49.2	2.4	
	Y	4.5	54.3	2.7	0.015
9	N	6.5	51.2	2.1	
	Y	6.5	58.7	4.0	0.008
	N	8.5	52.7	2.8	
	Y	8.5	58.0	1.4	0.008

Figures 22,23 data

Phenanthrene, % remaining (standard deviation in parentheses):

Treatment	4% Sediment (N/Y)	Time, days	9	18
1% Surfactant (N/Y)		0		
N	N	100	1 (1)	0 (1)
Y	N	100	0 (0)	0 (0)
N	Y	100	5 (7)	1 (1)
Y	Y	100	1 (1)	0 (0)
N (abiotic)	N	100	97 (9)	94 (12)

Phenanthrene, % mineralized (standard deviation in parentheses):

Treatment	4% Sediment (N/Y)	Time, days	7	12	21
1% Surfactant (N/Y)		0			
N	N	0	37 (9)	52 (10)	64 (4)
Y	N	0	63 (4)	66 (1)	68 (3)
N	Y	0	62 (2)	62 (6)	67 (2)
Y	Y	0	67 (2)	66 (2)	68 (2)
N (abiotic)	N	0	0 (0)	0 (0)	1 (1)