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SLURRY-PHASE BIOREMEDIATION OF CONTAMINATED SOIL FROM A FORMER MANUFACTURED-GAS PLANT SITE

By

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ABSTRACT

Contaminated soil from a former manufactured-gas plant (MGP) site was treated in bench-scale, slurry-phase biological reactors, in which the biodegradation of seven polycyclic aromatic hydrocarbons (PAH) – anthracene, benz[*a*]anthracene, benzo[*a*]pyrene, chrysene, fluoranthene, phenanthrene, and pyrene - was followed. In the first phase of the project, no difference in PAH removal was observed between reactors operated at solids residence times (SRT) of 18 and 35 d. A modified reactor with an 18 d SRT was operated during the second phase of the project. From 42% to > 87% of the PAH of interest were removed over a three-month operating period except for benzo[*a*]pyrene, which was not removed at all. Slurry from the reactor was able to mineralize phenanthrene, pyrene, chrysene, and benz[*a*]anthracene. Benzo[*a*]pyrene was not mineralized significantly, consistent with its lack of removal in the slurry-phase reactor. Liquid-phase concentrations of benzo[*a*]pyrene in effluent from the reactor were near the aqueous solubility of benzo[*a*]pyrene, indicating that its lack of removal probably was not due to limitations in mass transfer rate. These results suggest that the soil slurry either contained fewer microorganisms capable of degrading benzo[*a*]pyrene, or that rates of benzo[*a*]pyrene degradation were too slow to be observed over the time scale of the mineralization experiment (96 hr) or the SRT of the reactor.

Experiments were conducted to determine the effects of a surfactant (Brij 30) on the solubilization of PAH in untreated soil and in slurry removed from the reactor. Sorption of this hydrophobic surfactant was approximately 25 g/kg soil solids for the untreated soil and 47 g/kg soil solids for the treated soil. Above these doses, the surfactant was able to solubilize up to 40% of most of the PAH and up to 80% of the chrysene. Rates of PAH solubilization were rapid, with approximately 80% of the saturation (equilibrium) concentration reached within three to four hours for both the untreated and treated soils.

Addition of the surfactant to treated soil from the reactor did not enhance PAH degradation over a five-day period relative to killed controls in which sodium azide was added. In the absence of azide it appeared that the tar and other nonaqueous phases were emulsified rather than solubilized, so that mass transfer rates were probably different than in the experiments used to quantify the rates and extents of PAH solubilization. The addition of salicylate and phthalate as substrates that could potentially stimulate PAH metabolism also did not influence PAH degradation relative to killed controls, either in the presence or absence of the surfactant. The causes of incomplete PAH removal during slurry-phase treatment therefore remain unknown at this time.

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SUMMARY AND CONCLUSIONS

Until the mid-1960s combustible gas for heating, lighting and cooking was manufactured from coal, coke and oil feedstocks at local "town gas" plants or gasworks. Following a transition to the use of natural gas, many of the manufactured-gas plants were decommissioned by removing above-ground structures. Below-ground tanks, pipelines and other structures at the decommissioned plants were typically not removed and still contained liquids (e.g., tars) when they were filled with soil and demolition debris. There are an estimated 1,000 to 2,000 former manufactured-gas plant (MGP) sites in the United States, many of which still contribute to local contamination of soil, groundwater and surface water. More than 30 MGP sites have been identified in North Carolina since the late 1980s.

The high concentrations of PAH in tars represent a long-term source of these compounds in contaminated soils. The U.S. Environmental Protection Agency (EPA) regulates 16 PAH as indicators of contamination in soil and groundwater, and seven of the PAH are designated as class B human carcinogens. Remedial actions for PAH-contaminated soils are often based on numerical limits on the sum of the concentrations of the 16 total PAH (tPAH) and/or the sum of the concentrations of the seven carcinogenic PAH (cPAH). The 16 regulated PAH range in size from two rings to six rings. All of the cPAH are four-, five-, or six-ring compounds.

Both low and high molecular weight PAH have been observed to be degradable by indigenous microorganisms in soil and sediments. As a result, bioremediation is considered to be a potential remediation option at PAH-contaminated sites. In fact, as of 1995 PAH represented the largest class of contaminants for which bioremediation was selected as a treatment action at Superfund sites in the United States. One potential limitation of bioremediation, however, is that contaminant concentrations often decline to an asymptotic value which may still be above the required level of treatment. For PAH-contaminated soil or sediment, this concern may be more significant for the high-molecular weight (HMW) compounds, which are often degraded more slowly or less extensively than the low-molecular weight (LMW) PAH. Several explanations have been offered for the relative recalcitrance of the HMW PAH, including limited bioavailability, the absence of significant numbers of degraders for one or more PAH compound, or complex interactions among substrates leading to inhibition of the degradation of one PAH in the presence of other PAH.

The objectives of the research described in this report were to evaluate the biological treatment of contaminated soil from an MGP site using bench-scale slurry-phase reactors, and to evaluate three strategies to enhance PAH degradation in the contaminated soil. The effect of increased solids residence time (SRT) in the slurry-phase reactor was evaluated by comparing two residence times over a period of five months. We then evaluated the effects of a nonionic surfactant on PAH solubilization and degradation in treated soil from the slurry-phase reactor. In principle, surfactants can improve the rate of PAH dissolution into water and thus potentially increase the bioavailability of the PAH. We also studied the effects of adding carbon sources that could serve as water-soluble growth substrates for PAH-degrading bacteria and potentially stimulate their ability to degrade HMW PAH. The effects of supplemental carbon sources on PAH degradation in treated soil from the slurry-phase reactor were evaluated alone and in conjunction with the addition of the surfactant. It should be noted that although we conducted these experiments

under conditions in which limitations in dissolved oxygen did not occur, the addition of readily degraded carbon sources in the field will lead to an increase in the demand for oxygen or other electron acceptors that must be taken into account.

Operation of the slurry-phase reactors was conducted in two phases. In Phase I, two reactors were operated in parallel, one at an SRT of 18 d and the second at an SRT of 35 d. We also evaluated whether the biomass in the reactors was capable of mineralizing phenanthrene, pyrene, benz[*a*]anthracene, chrysene, and benzo[*a*]pyrene. Overall, the findings from Phase I were that (1) the reactors contained active PAH-degrading biomass capable of mineralizing all of the compounds tested except benzo[*a*]pyrene; (2) increased solids residence time (beyond 18 d) in the reactor did not significantly increase PAH removal; (3) mixing in the reactors was inadequate and led to aggregation of tar or some other separate phase containing the majority of the PAH; (4) removal of benzo[*a*]pyrene probably was limited by biological activity, whereas removal of the other six PAH that were followed probably was limited by mass transfer.

In Phase II, a new reactor with an 18 d SRT was operated with substantially greater mixing than during Phase I. Phase separation of the reactor contents was not observed during Phase II, most likely as a result of the higher mixing speed. Approximately one month after routine sampling began we noticed a substantial decrease in the concentrations of fluoranthene, pyrene, benz[*a*]anthracene and chrysene in the treated soil, and the lower concentrations were sustained through the remainder of Phase II operation. Reactor performance during the last three months of operation during Phase II is summarized in Table S1. No net removal of benzo[*a*]pyrene was observed during the entire operating period of Phase II.

Table S1. Performance of the slurry-phase bioreactor during the last three months of Phase II.

Compound	Feed Soil, mg/kg [a]	Treated Soil, mg/kg [b]	% Reduction
phenanthrene	16 ± 9.0	2.5 ± 2.5	84
anthracene	3.8 ± 3.1	2.2 ± 1.0	42
fluoranthene	38 ± 6.6	< 5.0 ± 4.8 [c]	> 87
pyrene	89 ± 13	19 ± 5.6	79
benz[*a*]anthracene	16 ± 1.6	6.3 ± 1.5	61
chrysene	7.5 ± 1.3	< 1.6 ± 1.4 [d]	> 79
benzo[*a*]pyrene	22 ± 1.7	22 ± 3.9	0

[a] Mean and standard deviation of six samples.

[b] Mean and standard deviation of 17 samples.

[c] Six samples were below the detection limit of 1.0 mg/kg.

[d] Fourteen samples were below the detection limit of 1.0 mg/kg.

Remediation standards applied to the treatment of PAH-contaminated soil are generally site-specific. However, the lack of benzo[*a*]pyrene degradation during Phase II suggests that the removal of benzo[*a*]pyrene and, perhaps, other high molecular weight PAH that were not analyzed might govern the technical feasibility of bioremediation at a given site. Thus, strategies to enhance PAH degradation during slurry-phase or other forms of biological treatment may need to be developed.

The surfactant Brij 30 was able to solubilize a substantial fraction of the PAH initially present in the soil, both for the untreated soil and the treated soil. However, relatively large quantities of the surfactant sorbed to the soil (25 g surfactant/kg soil solids for the untreated soil and approximately 47 g surfactant/kg soil solids for the treated soil) before appreciable concentrations were observed in the liquid phase. At doses exceeding those required to account for sorption to the soil, Brij 30 resulted in rapid solubilization of the PAH of interest, reaching approximately 80% of equilibrium in the liquid phase within three to four hours.

The experiments conducted with surfactants alone were performed under conditions intended to preclude confounding effects of biological activity, particularly biodegradation of the PAH, the surfactant, or both. Sodium azide at a concentration of 1% (wt:wt) was used for this purpose, but in a subsequent experiment there were significant differences in liquid-phase characteristics between systems amended with azide and those that were not. In the presence of azide, both the apparent surfactant concentrations and the PAH concentrations in the liquid phase were lower than in systems to which azide was not added. In the absence of azide it appeared that emulsification rather than solubilization was the primary mechanism by which the PAH were transferred into the liquid phase. It is important to understand the distinctions between these mechanisms of mass transfer, as they may have very different impacts on PAH bioavailability. Many laboratory experiments involving the addition of surfactants to soil systems are performed with either sodium azide or mercuric chloride as inhibitors of biological activity, and our results suggest that changes in ionic strength associated with the use of these agents may influence the results of the experiments.

A single experiment was conducted to test the effects of surfactants and supplemental carbon sources on PAH degradation in treated soil removed from the slurry-phase reactor. Neither the surfactant, the carbon sources, nor the combined additives stimulated PAH biodegradation over a five-day period. After five days there appeared to be saturating concentrations of the PAH in the liquid phases of the flasks containing surfactant; because of the emulsified and particulate nature of the contaminants in the liquid phase, however, it is possible that dissolution into the aqueous phase was still rate-limiting in these flasks. We do not know if the surfactant itself had a negative effect on the biomass in the system during this experiment.

There were various indications that benzo[*a*]pyrene was the least degradable of the PAH investigated. A final experiment to evaluate whether the slurry biomass was capable of mineralizing benzo[*a*]pyrene indicated that mineralization might be possible but at rates much slower than for the other PAH tested. We do not yet know if such slow degradation rates are caused by inherently slow rates of metabolism by PAH-degrading organisms, or whether benzo[*a*]pyrene mineralization is carried out by fewer organisms than is the degradation of other PAH. Our research on pure cultures of PAH-degrading bacteria isolated from contaminated soils

does not indicate that benzo[*a*]pyrene mineralization is less frequent than the mineralization of other high molecular weight compounds, but instead may be slower in those organisms capable of mineralizing the high molecular weight compounds (unpublished data).

RECOMMENDATIONS

Results obtained from the operation of bench-scale slurry-phase reactors were consistent with findings by others: that PAH biodegradation is achievable but the extent of degradation appears to be limited by as-yet unknown factors. The addition of a surfactant or supplemental carbon sources did not enhance the degradation of the PAH we followed beyond that achieved during slurry-phase treatment, although the scope of the experiments was limited.

There are at least five factors that may lead to incomplete removal of PAH from contaminated soil during biological treatment: (1) slow rates of mass transfer from the soil solid phase or from organic liquid or solid phases; (2) an insufficient number of organisms capable of degrading a particular compound; (3) competitive inhibition of less-readily degradable PAH by the more-readily degradable PAH; (4) insufficient growth substrates to sustain populations of PAH-degrading microorganisms at a level required to achieve acceptable rates of degradation; and (5) accumulation of metabolites that inhibit subsequent PAH degradation. Except for benzo[*a*]pyrene, we can rule out the possibility that there were insufficient numbers of PAH degraders in the soil. Since the concentrations of the low molecular weight PAH were low relative to the higher molecular weight compounds, it is also not likely that competitive inhibition was responsible for incomplete removal of most of the PAH. For benzo[*a*]pyrene, however, it is possible that its rate of degradation is so inherently slow that the mere presence of other degradable PAH reduces the degradation rate to undetectable levels. This may also be true for other regulated five- and six-ring PAH that we did not follow. Alternatively, the data obtained in this project do not *yet al*low us to rule out the possibility that benzo[*a*]pyrene removal was limited by the number of benzo[*a*]pyrene degraders in the soil. As of now, however, we have no information on inherent rates of degradation of benzo[*a*]pyrene and other high molecular weight PAH by any microorganism. This information must be obtained if we are to understand and eventually overcome those factors responsible for limitations in PAH degradation in soil.

The lack of effect of salicylate and, to a lesser extent, phthalate on PAH degradation suggests that depletion of growth substrates alone does not explain the incomplete removal of PAH. Salicylate in particular is a key intermediate in the metabolism of low molecular weight PAH, and from unpublished work we know that it may be important in the metabolism of high molecular weight PAH as well. The lack of effect of growth substrates alone was not surprising to us, but we had hoped that stimulating growth and improving bioavailability simultaneously would enhance PAH degradation. Improving bioavailability through the use of surfactants may still be promising, but we have much to learn about the application and behavior of surfactants in soil systems before we can draw conclusions on their efficacy in remediation of PAH-contaminated soils. Experiments designed to evaluate the effects of a surfactant on biological activity simultaneously with its effect on PAH mass transfer would help elucidate whether factors other than bioavailability govern the biodegradation of PAH in the presence (or even absence) of the surfactant.

The surfactant used in this study, Brij 30, was probably too hydrophobic to be used to enhance biodegradation in the field (and in any event it was unable to enhance biodegradation in this study); the amount of the surfactant consumed by sorption to the soil alone probably would represent an uncompetitive cost. A less hydrophobic surfactant would sorb less to the soil but

also would solubilize less hydrocarbon per unit surfactant in the liquid phase. Thus, selection of a surfactant of the appropriate hydrophobicity depends on the specific application.

For an application such as soil flushing or soil washing, in which the surfactant would not be reused, the optimum surfactant would be that which minimizes total surfactant requirements (cost) in both the solid and liquid phases while removing the desired quantity of contaminant. For the application envisioned in conjunction with biodegradation of the contaminant (i.e., the surfactant effectively is reused until the desired reduction in contaminant concentration is achieved), the effect of the surfactant on rates of contaminant mass transfer into the liquid phase must be considered as well. We observed that Brij 30 resulted in rapid solubilization of the PAH of interest, reaching approximately 80% of equilibrium in the liquid phase within three to four hours. This rate of increase in liquid phase concentrations is far higher than would be required to stimulate biodegradation, for which a residence time or reaction period of several days would be adequate. Thus, it probably would be acceptable to use a surfactant resulting in slower rates of mass transfer for a given liquid-phase concentration if that surfactant sorbed to the soil much less significantly than did Brij 30. In addition to hydrophobicity, the other key property of a surfactant intended to be used to enhance bioavailability is that it should have minimal impact on the biological activity in the system. There is currently too little information in the literature to evaluate surfactants on this basis, however. Obtaining such information should be a priority in further research on the effects of surfactants on the biodegradation of hydrophobic contaminants in soil.

The possibility that biological activity in PAH-contaminated soil leads to the accumulation of inhibitory metabolites has not been explored. Much of the published research on PAH degradation, particularly for the high molecular weight compounds, suggests that incomplete metabolism (i.e., lack of complete metabolism to CO_2 and water) is relatively common among organisms that grow on the lower molecular weight substrates. In some cases the metabolites resulting from partial transformation of a parent compound have been identified, but characteristics such as their impacts on subsequent degradation have not been evaluated. This is also an important area for further research.

Above-ground processes such as slurry-phase bioremediation are most appropriate for treating excavated soils at intermediate levels of contamination. Over the past several years, however, the use of thermal desorption has emerged as a highly cost-competitive option for the remediation of excavated soils from MGP sites. Economic comparisons of thermal desorption or bioremediation still must be done on a case-by-case basis, but current methods of bioremediation, such as slurry-phase treatment, may not be competitive for treatment of excavated soils based on performance (technical feasibility) alone. The most significant limitation of biological processes may be their ability to reduce the concentrations of the carcinogenic PAH to required or acceptable levels. However, it is important to continue studying the biodegradation of PAH and pursuing methods to optimize biodegradation in soil. To date there are few options to treat contaminated soils at MGP sites *in situ*, and in many cases the off-site migration of contaminants is assumed to be mitigated by natural attenuation. An improved understanding of mechanisms and rates of PAH biodegradation would permit the development of better predictive tools to evaluate the adequacy of *in situ* bioremediation or natural attenuation, or the development of low-impact and economical methods of stimulating biodegradation in the subsurface.

1. INTRODUCTION

Combustible gas has been used for heating, lighting and cooking in the United States since the early part of the 19th century. Until the mid-1960s gas was manufactured from coal, coke and oil feedstocks at local "town gas" plants or gasworks. The peak period of manufactured-gas production was from approximately 1920 through the mid-1950s, after which a sharp decline in production corresponded to a rapid increase in the use of natural gas (Harkins *et al.*, 1988). Following this transition to the use of natural gas, many of the manufactured-gas plants were decommissioned by removing above-ground structures. Below-ground tanks, pipelines and other structures at the decommissioned plants were typically not removed and still contained liquids (e.g., tars) when they were filled with soil and demolition debris (Harkins *et al.*, 1988; Luthy *et al.*, 1994). There are an estimated 1,000 to 2,000 former manufactured-gas plant (MGP) sites in the United States, many of which still contribute to local contamination of soil, groundwater and surface water (Harkins *et al.*, 1988; Luthy *et al.*, 1994). More than 30 MGP sites have been identified in North Carolina since the late 1980s (personal communication: B. Nicholson, N.C. Dept. of Environment and Natural Resources, Superfund Section,1995).

The composition of tars and wastes generated at gas plants depended on the raw materials (e.g., coal vs. oil) and manufacturing processes used. In the Southeast, gas was made primarily from coke or coal in a process referred to as the carbureted water process (Harkins *et al.*, 1988). Tars from this process comprised primarily aromatic hydrocarbons, and contained little of the cyanide and ammonia that were characteristic of the coal carbonization process (Harkins *et al.*, 1988; Luthy *et al.*, 1994).

Tars are more dense than water and hence form dense nonaqueous-phase liquids (DNAPLs) when released in substantial quantities into the subsurface. The density of tars is a result of the predominance of polycyclic aromatic hydrocarbons (PAH), but they also contain benzene and other monoaromatic hydrocarbons (Luthy *et al.*, 1994). Contact of groundwater or surface water with tars results in the dissolution of the tar constituents, particularly the relatively water-soluble compounds such as benzene and naphthalene (Luthy *et al.*, 1994). Migration of contaminated groundwater and free liquid tar has led to extensive subsurface contamination at several well-characterized MGP sites (Harkins *et al.*, 1988). Subsequent construction activities near or at MGP sites have also resulted in the release of contaminants that had been confined prior to the construction (Harkins *et al.*, 1988).

In addition to dissolution and migration of the water-soluble components of tars, the high concentration of PAH represents a long-term source of these compounds in contaminated soils. There are many individual PAH compounds in tars, more than can be identified by conventional analytical techniques (Luthy *et al.*, 1994). The U.S. Environmental Protection Agency (EPA) regulates 16 PAH as priority pollutants in wastewaters (Table 1.1), but also uses these 16 compounds as indicators of contamination in soil and groundwater. Among the 16 priority pollutant PAH, EPA identifies seven as class B human carcinogens (U.S. EPA, 1992). Remedial actions for PAH-contaminated soils are often based on numerical limits on the sum of the concentrations of the 16 total PAH (tPAH) and/or the sum of the concentrations of the seven carcinogenic PAH (cPAH). It is important to note that the regulated PAH range in size from 2 aromatic rings (e.g., naphthalene) to six rings (e.g., indeno[*1,2,3-cd*]pyrene).

Table 1.1. Priority pollutant PAH.

Compound [a]	Number of Rings	Carcinogen Class [b]
naphthalene	2	D
acenaphthylene	3	D
acenaphthene	3	not classified
fluorene	3	D
phenanthrene	3	D
anthracene	3	D
fluoranthene	4	D
pyrene	4	D
benz[*a*]anthracene	4	B2
chrysene	4	B2
benzo[*b*]fluoranthene	5	B2
benzo[*k*]fluoranthene	5	B2
benzo[*a*]pyrene	5	B2
dibenz[*a,h*]anthracene	5	B2
indeno[*1,2,3-c,d*]pyrene	6	B2
benzo[*g,h,i*]perylene	6	D

[a] Carcinogenic PAH are in bold.

[b] U.S. EPA, 1992. The carcinogenic potential of chemicals is classified on a scale of A through E, with A corresponding to the highest probability of human carcinogenicity and E corresponding to evidence of non-carcinogenicity in humans (U.S. EPA, 1996b).

Many bacterial species can biodegrade the lower-molecular weight PAH (those with two or three rings) and use these compounds as sole sources of carbon and energy for growth (Cerniglia and Heitkamp, 1989; Sutherland *et al.*, 1995). It is only relatively recently, however, that bacteria able to grow on the four-ring compounds chrysene, fluoranthene and pyrene have been isolated (Heitkamp *et al.*, 1988; Mueller *et al.*, 1990; Weissenfels *et al.*, 1990; Walter *et al.*, 1991; Boldrin *et al.*, 1993; Kästner *et al.*, 1994; Bouchez *et al.*, 1995; Caldini *et al.*, 1995; Burd and Ward, 1996; Dean-Ross and Cerniglia, 1996). To date, no organism has been isolated that can use any PAH with five or more rings as a sole carbon and energy source for growth; instead, degradation of these compounds by pure cultures of bacteria usually is observed after growth on another PAH substrate. In general the range of PAH compounds degraded by a given PAH-degrading organism is broad, but the exact range seems to be unique to the organism (Kiyohara *et al.*, 1983; Foght and Westlake, 1988; Mahaffey *et al.*, 1988; Heitkamp *et al.*, 1988; Mueller *et al.*, 1990; Walter *et al.*, 1991; Weissenfels *et al.*, 1991; Boldrin *et al.*, 1993; Grifoll *et al.*, 1995; Ye *et al.*, 1996; Aitken *et al.*, 1996; Dagher *et al.*, 1997).

Both low and high molecular weight PAH have been observed to be degradable by indigenous microorganisms in soil and sediments (Sims and Overcash, 1983; Cerniglia and Heitkamp, 1989; Cerniglia, 1992; Shuttleworth and Cerniglia, 1995). As a result, bioremediation is considered to

be a potential remediation option at PAH-contaminated sites. In fact, as of 1995 PAH represented the largest class of contaminants for which bioremediation was selected as a treatment action at Superfund sites in the United States (U.S. EPA, 1996a). One potential limitation of bioremediation, however, is that contaminant concentrations often decline to an asymptotic value which may still be above the required level of treatment (U.S. EPA, 1993; Huesemann, 1997). For PAH-contaminated soil or sediment, this concern may be more significant for the high molecular weight (HMW) compounds, which are often degraded more slowly or less extensively than the low molecular weight (LMW) PAH (Herbes and Schwall, 1978; Bossert and Bartha, 1986; Shiaris, 1989; Grosser *et al.*, 1991; Mueller *et al.*, 1991a; Mueller *et al.*, 1991b; Lauch *et al.*, 1992; Wild and Jones, 1993; Jerger *et al.*, 1994; Kästner *et al.*, 1994; Brown *et al.*, 1995; Grosser *et al.*, 1995; Carmichael and Pfaender, 1997; O'Reilly *et al.*, 1997). Several explanations have been offered for the relative recalcitrance of the HMW PAH, including limited bioavailability (Weissenfels *et al.*, 1992; Erickson *et al.*, 1993), the absence of significant numbers of degraders for one or more PAH compound (Kästner *et al.*, 1994), or complex interactions among substrates leading to inhibition of the degradation of one PAH in the presence of other PAH (Bouchez *et al.*, 1995; Kelley and Cerniglia, 1995; Millette *et al.*, 1995; Stringfellow and Aitken, 1995). It is important to consider the biodegradation of HMW PAH compounds during bioremediation because remedial actions at many MGP sites are likely to be governed in part by the removal of the carcinogenic PAH, all of which are HMW species.

Objectives and Scope of Research

The objectives of the research described in this report were to evaluate the biological treatment of contaminated soil from an MGP site using bench-scale slurry-phase reactors, and to evaluate the effects of strategies to enhance PAH degradation in the contaminated soil.

Three strategies to increase PAH degradation were considered. First, the effect of increased solids residence time in the slurry-phase reactor was evaluated by comparing two residence times over a period of five months. We then evaluated the effects of a nonionic surfactant on PAH solubilization and degradation in treated soil from the slurry-phase reactor. In principle, surfactants can improve the rate of PAH dissolution into water and thus potentially increase the bioavailability of the PAH (Grimberg *et al.*, 1996). We also studied the effects of adding carbon sources that could serve as water-soluble growth substrates for PAH-degrading bacteria and potentially stimulate their ability to degrade HMW PAH. The effects of supplemental carbon sources on PAH degradation in treated soil from the slurry-phase reactor were evaluated alone and in conjunction with the addition of the surfactant.

2. BACKGROUND

Characteristics of PAH

Selected properties of the 16 priority pollutant PAH are listed in Table 2.1. These compounds can be described as hydrophobic, as evidenced by their low aqueous solubilities and their high octanol-water partition coefficients (K_{ow}). As a result, PAH in soils are likely to be found associated with nonaqueous phases such as tar or partitioned into soil organic matter. In either case the limited availability of the PAH in the aqueous phase can influence the rate and extent of biodegradation. The hydrophobicity of PAH generally increases with increasing molecular weight.

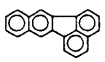
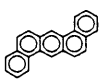
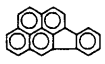
Treatment of Contaminated Soil from MGP Sites

Remediation of MGP sites usually involves management of both groundwater and soil, but this report focuses on the soil phase only. There is relatively little documented literature on full-scale remediation of MGP soils, most of which has focused on excavation to remove sources of groundwater contamination (Luthy *et al.*, 1994). Excavated soils can be managed in several ways, including incineration, combustion in utility boilers (Luthy *et al.*, 1994), or thermal desorption. Thermal desorption seems to provide acceptable removal of both PAH and BTEX (the monoaromatic hydrocarbons benzene, toluene, ethylbenzene and xylenes) (Lewis, 1993; O'Shaughnessy and Nardini, 1997) at a lower cost than incineration (U.S. EPA and U.S. Air Force, 1993; Umfleet, 1997). Extraction of MGP soil with solvents (Luthy *et al.*, 1994; Hayden and Van der Hoven, 1996) or surfactants (Yeom *et al.*, 1995; Kayser *et al.*, 1997) has also been evaluated in the laboratory.

There is relatively little information on the field-scale application of biological processes to treat MGP soil. In a recent report on the use of innovative technologies at Superfund sites, EPA indicated that bioremediation was used at five sites classified as "coke manufacturing/coal gasification" (U.S. EPA, 1996a). The use of *in situ* biosparging and bioventing was evaluated in a recent pilot study (Leahy *et al.*, 1997). In this technique, air is passed through the vadose zone by forced aeration into wells (biosparge wells) and air is simultaneously withdrawn from other wells (bioventing wells); biological activity was documented by monitoring carbon dioxide production and oxygen uptake. Full-scale, *in situ* sparging with ozone has also been evaluated (Nelson, 1997; Leahy *et al.*, 1997), although there was no discussion of the economic feasibility of this treatment method; biodegradation was also assumed to occur in this case by observing elevated CO_2 in the headspace of monitoring wells. Natural attenuation was selected recently as the remediation strategy at an MGP site (Gromicko *et al.*, 1997).

Although there is a limited database on full-scale bioremediation of MGP soils, there is continued interest in the development of biological processes for this purpose. An entire session was devoted to the bioremediation of MGP sites at the recent International *In situ* and On-site Bioremediation Symposium (New Orleans, Louisiana, April 1997).

Table 2.1. Properties of the 16 priority pollutant PAH [a]

Compound	Structure	MW (g/mol)	Vapor Pressure (Pa)	Log K_{ow}	Aq. Solubility (mg/L)
naphthalene		128.2	36.8	3.37	31.0
acenaphthylene		150.2	4.14	4.0	3.93
acenaphthene		154.2	1.52	3.92	3.47
fluorene		166.2	0.715	4.18	1.90
phenanthrene		178.2	0.113	4.57	1.10
anthracene		178.2	0.078	4.54	0.07
fluoranthene		202.3	8.7×10^{-3}	5.22	0.26
pyrene		202.3	0.012	5.18	0.13
benz[*a*]anthracene		228.3	6.0×10^{-4}	5.91	0.011
chrysene		228.3	1.1×10^{-4}	5.65	~ 0.004
benzo[*b*]fluoranthene		252.3	NA	5.80	0.00115
benzo[*k*]fluoranthene		252.3	4.1×10^{-6}	6.0	0.0008
benzo[*a*]pyrene		252.3	2.1×10^{-5}	6.04	0.0038
benzo[*g,h,i*]perylene		268.4	2.3×10^{-8}	6.50	0.00026
dibenz[*a,h*]anthracene		278.3	9.2×10^{-8}	6.75	0.0006
indeno(1,2,3-*c,d*)pyrene		276.3	NA	7.66	0.062

[a] Data from Mackay *et al.*, 1992. NA, not available.

Biological Treatability of MGP Soils

PAH-degrading bacteria have been isolated from PAH-contaminated soils in a number of studies (Foght and Westlake, 1991; Lauch *et al.*, 1992; Mueller *et al.*, 1994; Kästner *et al.*, 1994; Aitken *et al.*, 1996), but these bacteria do not necessarily represent the range of PAH-degrading activity in any given soil. Attempts to quantify or broadly characterize the indigenous microbial community in MGP and other PAH-contaminated soils have focused on activity against the low molecular weight PAH, such as naphthalene and phenanthrene (Bogardt and Hemmingsen, 1992; Sanseverino *et al.*, 1993; Durant *et al.*, 1995; Ginn *et al.*, 1995; Madsen *et al.*, 1996). This is appropriate when considering the influence of microbial activity on the transport of contaminants in groundwater, because the two- and three-ring compounds are the most mobile of the PAH contaminants (Durant *et al.*, 1995). However, it must be kept in mind that there are 16 regulated PAH compounds, and acceptable soil remediation at any site may require extensive removal of a majority of them.

No single organism can be expected to degrade all 16 of the regulated PAH compounds, so that degradation of all 16 would require the presence of a diverse (relative to PAH-degrading phenotypes) microbial community. Kästner *et al.* (1994) clearly demonstrated that the range of PAH which could be degraded by a given bacterium depended on the compound used to enrich for that organism from the soil. For example, organisms isolated on naphthalene as sole carbon source were unable to grow on anthracene, phenanthrene, fluoranthene, or pyrene, whereas organisms isolated on any of the other four compounds were able to grow on at least one of the other substrates. Bouchez *et al.* (1995) reported similar findings on the specificity of the PAH substrate range for individual bacterial strains. In general, organisms enriched or enumerated with phenanthrene as sole carbon source are more representative of PAH-degrading bacteria than are bacteria enriched or enumerated with naphthalene as sole carbon source (Foght *et al.*, 1990; Foght and Westlake, 1991). In the absence of molecular probes suitable for detecting organisms able to degrade a broad range of PAH, particularly the HMW PAH, enumeration methods that employ a mixture of PAH substrates (Wrenn and Venosa, 1996) probably offer the best chance of quantifying PAH-degrading bacterial phenotypes in soil.

Several studies have illustrated that PAH degradation can be achieved by indigenous bacteria in soils from MGP sites, both in slurry reactors (Srivastava *et al.*, 1990; Vernieri *et al.*, 1992) and in saturated soil columns (Tiehm *et al.*, 1997). Trends observed in these studies were were similar to those observed in other studies on PAH degradation in contaminated soils: PAH degradation appeared to reach a "plateau," and the HMW PAH were more resistant to biodegradation than were the two- or three-ring compounds. Contrary to these findings, Erickson *et al.* (1993) did not observe any significant PAH biodegradation in contaminated soil from an MGP site that was incubated in microcosms at moisture contents from 60 - 80% of field capacity. Based on the ability of the indigenous microbial community to degrade freshly added naphthalene or phenanthrene, these investigators concluded that the lack of native PAH degradation was due to limited bioavailability.

In general, it appears that most MGP site soils contain significant quantities of PAH-degrading bacteria. Although this is only one factor required to achieve successful bioremediation, it does indicate that the potential for bioremediation exists at most sites.

Slurry-phase Biological Treatment

Slurry-phase biological treatment can be defined as the treatment of soil as a suspension in an aqueous medium. Typical solids contents in slurry-phase reactors are from 10 - 40% (wt:wt), and it has been suggested that organic contaminant concentrations as high as 25% (wt:wt) can be treated with slurry-phase processes (U.S. EPA, 1990). Although the details differ among various designs, all slurry systems require mixing. Mixing is usually continuous, but it has been suggested that substantial power savings could be achieved by operating mixers intermittently (Stormo and Deobold, 1995). The majority of systems used in practice are aerobic (U.S. EPA, 1990) and thus require aeration either with diffused air, pure oxygen, or liquid oxygen. Slurry-phase bioreactors have been used to treat PAH-contaminated soils at scales ranging from small laboratory systems to full-scale units, as summarized in Table 2.2. At larger scales, slurry-phase treatment often follows a soil washing or fractionation process which removes the larger (e.g., sand), relatively uncontaminated particles (Berg *et al.*, 1994; Castaldi, 1994) (Jerger *et al.*, 1994; Glaser *et al.*, 1995; Jerger and Woodhull, 1995; Geerdink *et al.*, 1996).

Relatively little effort has been devoted to optimizing the design of the slurry-phase process (Glaser *et al.*, 1995). As shown in Table 2.2, slurry-phase bioreactors are most often operated as batch processes. Semi-continuous operation involves the periodic removal of a fraction of the reactor contents and replacement with the same amount of untreated soil and liquid medium. Daily removal and replacement have been used (Simpkin and Giesbrecht, 1994; Brown *et al.*, 1995), but other intervals can also be selected. A variation of semi-continuous operation involves periodic settling of the solids and replacement of the liquid phase only (Blackburn *et al.*, 1995), but this clearly would lead to accumulation of solids in the reactor. Other operating modes for slurry reactors include continuous flow (Geerdink *et al.*, 1996) and fed-batch (or semi-batch) operation, in which the soil and liquid medium are fed continuously to the reactor without any discharge (Otte *et al.*, 1994).

Although batch processes are often the simplest to implement in practice, from a reaction engineering perspective they do not seem to be the best choice for slurry-phase biological treatment of soil. The function of a bioreactor is to provide conditions that support a high concentration of bacteria capable of degrading the contaminants in the soil, generally a much higher concentration than would be found in the untreated soil. In a batch reactor the initial stage of the treatment period is devoted to increasing the bacterial concentration, so that corresponding rates of degradation during this stage of operation would not be as high as at the maximum bacterial concentration. To compensate for this effect, the batch process can be started by inoculating the reactor with a microbial community grown in another reactor (Lauch *et al.*, 1992; Castaldi, 1994) or with treated slurry from a previous batch (Gray *et al.*, 1994; Banerjee *et al.*, 1997). Semi-continuous or continuous processes preclude the need to inoculate, since the majority of the treated soil remains in the reactor as untreated soil is

Table 2.2. Summary of case studies on slurry-phase biological treatment of PAH-contaminated soils.

Reference(s)	Contamination Source	Scale (Reactor Size)	Solids Content (%)	Operating Mode	Residence Time (d)
Srivastava *et al.*, 1990	MGP	bench	unspecified	batch	35
Mueller *et al.*, 1991b	wood treatment	bench (1.5 L)	2.1 - 2.7	batch	30
Lauch *et al.*, 1992; Lewis, 1993	wood treatment	pilot (64 L)	30	batch	84
Vernieri *et al.*, 1992	MGP	bench (20 L)	25	batch	36 - 43
Castaldi, 1994	unspecified	pilot (62 L)	40	batch	60
Berg *et al.*, 1994	wood treatment	bench (2 L)	20	batch	21
		pilot (454 L)	14	batch	8
Jerger *et al*, 1994	wood treatment	full (680,000 L)	20 - 25	batch	20 -30
Simpkin and Giesbrecht, 1994	unspecified	pilot (60 L)	25	semi-continuous	3 - 6
Otte *et al.*, 1994	wood treatment	bench (15 L)	5	fed-batch	35
Brown *et al.*, 1995	wood treatment	pilot (60 L)	30 -40	semi-continuous	10
O'Reilly *et al.*, 1997	refinery	bench (20 L)	50	batch	120
		pilot (25,000 L)	50	batch	35
Banerjee *et al.*, 1997	creosote, petroleum	bench (2 L)	50	batch	84

added. These modes of operation are analogous to the activated sludge process, which has been used to treat municipal and industrial wastewaters for nearly a century. In cases in which only one or a few batches of soil require treatment (e.g., *in situ* treatment of contaminated soil or sediment in a lagoon modified to provide slurry-phase treatment), batch treatment may be the only or the most feasible alternative despite its potential kinetic limitations.

Costs. The cost of slurry-phase bioremediation has been evaluated in two studies (Salameh *et al.*, 1994; Jerger and Woodhull, 1995). In a detailed study that considered various treatment options and endpoints (Salameh *et al.*, 1994), the cost of slurry-phase treatment was estimated to be $85 - $275 per cubic yard of soil (1994 dollars) for a process train that includes preliminary screening and soil washing, slurry-phase treatment of the soil fines, and dewatering of the slurry. Jerger and Woodhull (1995) reported an actual cost of $110 - $145 per ton of soil for a case in which the same process train was used. Costs increase with increasing residence time in the reactor (Jerger and Woodhull, 1995; Geerdink *et al.*, 1996); the cost increases essentially linearly with residence time above a baseline cost associated with excavation, soil preparation and subsequent dewatering (Jerger and Woodhull, 1995). Costs also decrease with increasing solids content in the reactor (Jerger and Woodhull, 1995), presumably due to lower capital costs associated with smaller reactors. For comparison, limited data on the cost of thermal desorption range from $82 - $178 per ton (Umfleet, 1997).

The cost of slurry-phase treatment is likely to be higher than landfarming or other above-ground, "solid-phase" biological processes, but may achieve more rapid or more extensive (or both) removal of contaminants than the solid-phase processes (Salameh *et al.*, 1994). The unit cost of slurry-phase treatment is expected, however, to be significantly lower than that for incineration (U.S. EPA and U.S. Air Force, 1993; Salameh *et al.*, 1994; Jerger and Woodhull, 1995).

Role of Bioavailability in PAH Biodegradation

Depending on the extent of soil contamination, PAH will be associated primarily with soil organic matter (SOM), with residual nonaqueous phase liquids (NAPL) such as tars that may exist within the soil pores in heavily contaminated areas, or both. However, biodegradation requires that the PAH either be in the aqueous phase or at an interface between aqueous and nonaqueous phases. Whether PAH are associated with residual NAPL or soil organic matter, the porous structure of soil creates pathways that impede the mass transfer of PAH to the aqueous phase. In highly contaminated soil, desorption of PAH from NAPL is believed to be the major rate-limiting factor governing the availability of PAH in the aqueous phase (Luthy *et al.*, 1994; Ghoshal and Luthy, 1996; Nelson *et al.*, 1996; Rutherford *et al.*, 1997). For less contaminated soil, sorption in the soil organic matter matrix may be a more relevant rate-limiting mechanism.

The sorptive (partitioning) behavior of hydrophobic contaminants in soils and sediments has been a subject of considerable study (DiToro *et al.*, 1991), and principles of *equilibrium* distributions of neutral organic contaminants in soil and sediment have become well established (Schwarzenbach *et al.*, 1993). The *kinetics* of sorption and desorption of contaminants in actual contaminated materials have received less attention, but it is generally recognized that desorption rates decrease as the time period over which sorption occurred increases (Pignatello *et al.*, 1993; Pignatello and Xing, 1996). The release of contaminants from "aged" materials (those that have

been contaminated for many years) into a bulk aqueous phase has been shown to involve a relatively rapid rate of mass transfer followed by a considerably slower rate of mass transfer, presumably due to diffusion of the desorbing solute through a network of micropores (Pignatello and Xing, 1996; Aochi and Farmer, 1997); this phenomenon was documented recently for the release of naphthalene and phenanthrene from MGP soils (Williamson *et al.*, 1997). Less is known about rates of desorption from NAPL in the soil matrix; however, in experiments designed to distinguish between desorption from NAPL and from SOM in two creosote-contaminated soils, Rutherford *et al.* (1997) recently demonstrated that the NAPL dominated the sorptive behavior of naphthalene. Regardless of the mechanism by which PAH are isolated from the aqueous phase, it has been shown that aged contaminants are considerably less available for biodegradation than are freshly added compounds (Weissenfels *et al.*, 1992; Erickson *et al.*, 1993; Hatzinger and Alexander, 1995; Carmichael and Pfaender, 1997). The conditions associated with aged materials are expected to predominate at MGP sites.

Effects of Surfactants on PAH Biodegradation

One strategy that has been proposed to increase rates of mass transfer for hydrophobic chemicals in soil is the addition of surfactants. Surfactants can solubilize (incorporate into micelles) or emulsify hydrophobic chemicals, increasing the apparent liquid phase solubility (in the case of solubilization) or the surface area over which dissolution can occur (in the case of emulsification). Surfactants have been proposed for *in situ* soil flushing and *ex situ* soil washing, but also have been proposed to be coupled to biodegradation processes (reviewed in (Rouse *et al.*, 1994)). Unfortunately much of the research on the effects of surfactants on biodegradation has been empirical, and has involved contrived experimental systems in which contaminants were spiked into soil. Such systems are not expected to be representative of contaminated soils, particularly with respect to mechanisms governing mass transfer. In some cases radiolabeled chemicals have been spiked into contaminated soil (e.g., (Tsomides *et al.*, 1995; Thibault *et al.*, 1996)), but in these cases the spiked chemical is also not representative of the chemical originally present in the contaminant matrix. Since surfactants are intended exclusively to affect mass transfer processes, it is important to examine such effects in actual contaminated materials if the efficacy of surfactant addition is to be evaluated properly. Once quantitative relationships between surfactant addition and rates of mass transfer are developed, it becomes possible to develop quantitative models to evaluate the consequent effects of surfactants on biodegradation of the target hydrophobic chemicals (Grimberg *et al.*, 1996).

The interactions among surfactant, soil and hydrophobic chemicals can be complex (Liu *et al.*, 1992; Edwards *et al.*, 1994a; Edwards *et al.*, 1994b; Jafvert *et al.*, 1995; Yeom *et al.*, 1995; Yeom *et al.*, 1996). In the presence of surfactants at concentrations high enough to achieve liquid-phase concentrations above the critical micelle concentration (CMC), hydrophobic chemicals can partition among five phases - soil, NAPL, micelles (sometimes referred to as a pseudophase), emulsified hydrocarbon, and the aqueous phase - as well as a sixth phase (vapor) for volatile contaminants. The surfactant itself is susceptible to sorption to SOM or to NAPL, and in some cases may precipitate (Shiau *et al.*, 1995). In addition, biodegradation of both the surfactant and the hydrophobic chemicals must be considered. In ideal situations, the time scale over which the hydrophobic chemicals are degraded will be considerably shorter than the time scale over which biodegradation of the surfactant will occur. The time scale over which

biodegradation of the surfactant occurs can be maximized by not exposing the soil microbial community to the surfactant continuously. For example, the surfactant could be supplied intermittently in a single semi-continuous reactor or in the second stage of a two-stage process.

With respect to quantifying and predicting the effects of surfactants on mass transfer of PAH in soil, distinctions must be made between desorption from soil organic matter and desorption or dissolution from NAPL. We recently quantified the effects of surfactants on dissolution of pure, crystalline phenanthrene (Grimberg *et al.*, 1995; Grimberg *et al.*, 1996), in which the major mechanism by which surfactants enhanced dissolution was concluded to be the transport of micelles away from the hydrocarbon/water interface. Although the dissolution of a NAPL containing a wide mixture of compounds is much more complex, the influence of surfactants in such systems is likely to be governed by the same mechanism. Yeom *et al.* (1996) recently offered a different explanation for the effects of surfactants on PAH mass transfer in tar-contaminated soil, in which they suggested that surfactants enhanced desorption of the PAH by increasing the diffusivity of contaminants within the organic matrix. However, in a more thorough examination of mechanisms of PAH mass transfer from NAPL, Kraatz *et al.* (1997) concluded that the aqueous-side mass transfer dominated the kinetics of PAH desorption (or dissolution) over a wide range of NAPL viscosity. The effects of surfactants on desorption of hydrophobic compounds in contaminated soil in the absence of a significant NAPL phase have not been well studied. DiCesare and Smith (1994) suggested that surfactants can increase desorption rates by increasing the driving force for desorption (by increasing saturation concentrations of the contaminant in the liquid phase) or by interacting with SOM in such a way that the SOM would expand to permit much greater contaminant diffusivity in the SOM matrix.

Regardless of the specific mechanism by which mass transfer of hydrophobic solutes is limited, we expect that surfactants can increase the bioavailability of these chemicals by increasing the rates of mass transfer (Volkering *et al.*, 1995; Grimberg *et al.*, 1996). However, it is possible to overdose surfactant in some systems. For example, it may be possible to add surfactant at a concentration that exceeds the concentration required to solubilize all of the target contaminant(s) in a given system. We have shown that in such cases it is possible to *decrease* biodegradation rates because contaminant concentrations in the aqueous phase are depressed by partitioning into surfactant micelles (Grimberg and Aitken, 1995). The direct microbial uptake of micellized PAH is possible in certain situations (Liu *et al.*, 1992; Guha and Jaffé, 1996a; Guha and Jaffé, 1996b), but a conservative assumption is that only the aqueous phase material is directly available for biodegradation. Thus, there is likely to be a window of surfactant dosages for treating contaminated soil. This window may be bounded on the low side by the amount of surfactant required to reach the CMC in the liquid phase (i.e., after accounting for surfactant sorption) and on the high side by the amount of surfactant required to completely solubilize all of the contaminant(s) of interest.

There have been only two previous studies on the effects of surfactants on PAH biodegradation in contaminated soils, both involving MGP soils (Ghosh and Yeom, 1997; Tiehm *et al.*, 1997). Results in both studies suggested that improved PAH degradation occurred in the presence of surfactant. In the more comprehensive study (Tiehm *et al.*, 1997), the addition of a surfactant to water recirculating over a column containing a bed of contaminated soil resulted in an immediate increase in both the liquid phase PAH concentrations and CO_2 production. Since the

concentrations of surfactant-degrading bacteria were low shortly after surfactant addition, the authors concluded that the increase in CO_2 production was due to increased biodegradation of PAH.

Effects of Supplemental Carbon Sources on PAH Biodegradation

Since the high molecular weight PAH are not growth substrates for many PAH-degrading bacteria, these organisms must grow on other substrates if they are to metabolize the HMW compounds. Presumably they grow on the low molecular weight PAH, but it is not known whether the depletion of the LMW compounds over time results in decreased activity toward the HMW PAH. In fact, the extent to which the metabolism of HMW PAH is related to the metabolism of LMW PAH has not been well established for any organism.

Bauer and Capone (1988) observed that enrichment of marine sediment microcosms with one PAH substrate resulted in an enhanced degradation of other PAH substrates in the sediment. They suggested that PAH-degrading bacteria may have metabolic pathways that overlap for various PAH substrates since these compounds are, in general, structural analogues. It was not possible, however, for them to evaluate their hypothesis with sediment systems because mixed cultures of bacteria were involved.

To understand the concept of metabolic overlap for different PAH substrates, it is necessary to understand the series of reactions, or pathways, that lead to the complete metabolism of a given PAH. The complete metabolic pathway has been identified for only a few PAH, most notably naphthalene and phenanthrene. The naphthalene degradation pathway in aerobic bacteria is illustrated in Figure 2.1.

In three recent studies in which molecular biology techniques were used (Denome *et al.*, 1993; Takizawa *et al.*, 1994; Yang *et al.*, 1994), it was demonstrated that the same enzymes responsible for catalyzing the transformation of naphthalene to salicylate (enzymes for the upper pathway in Figure 2.1) catalyze the transformation of phenanthrene to 1-hydroxy-2-naphthoate (1H2N; Figure 2.2). With the addition of an enzyme to convert 1H2N to dihydroxynaphthalene (Figure 2.2), an organism can degrade both naphthalene and phenanthrene with virtually the same set of enzymes. In addition to their ability to transform phenanthrene, the upper pathway enzymes for naphthalene metabolism were also shown to transform dibenzothiophene in *Pseudomonas* strain C18 (Denome *et al.*, 1993), fluorene in *Pseudomonas putida* NCIB 9816 (Yang *et al.*, 1994), and anthracene in *Pseudomonas putida* G7 (Sanseverino *et al.*, 1993). The first enzyme in the naphthalene degradation pathway, naphthalene dioxygenase, has also been shown to catalyze the oxidation of a wide range of LMW PAH compounds (Foght and Westlake, 1996; Goyal and Zylstra, 1996; Resnick and Gibson, 1996; Selifonov *et al.*, 1996). For some of these compounds, no further transformation takes place beyond this initial oxidation (see, for example, Foght and Westlake, 1996). It appears, then, that the enzymes required for complete PAH metabolism are more specific for certain compounds than for others. We do not yet know whether such specificity is unique to the particular bacterial strain, but this might be one reason that individual strains appear to have a unique range of PAH substrates they can metabolize.

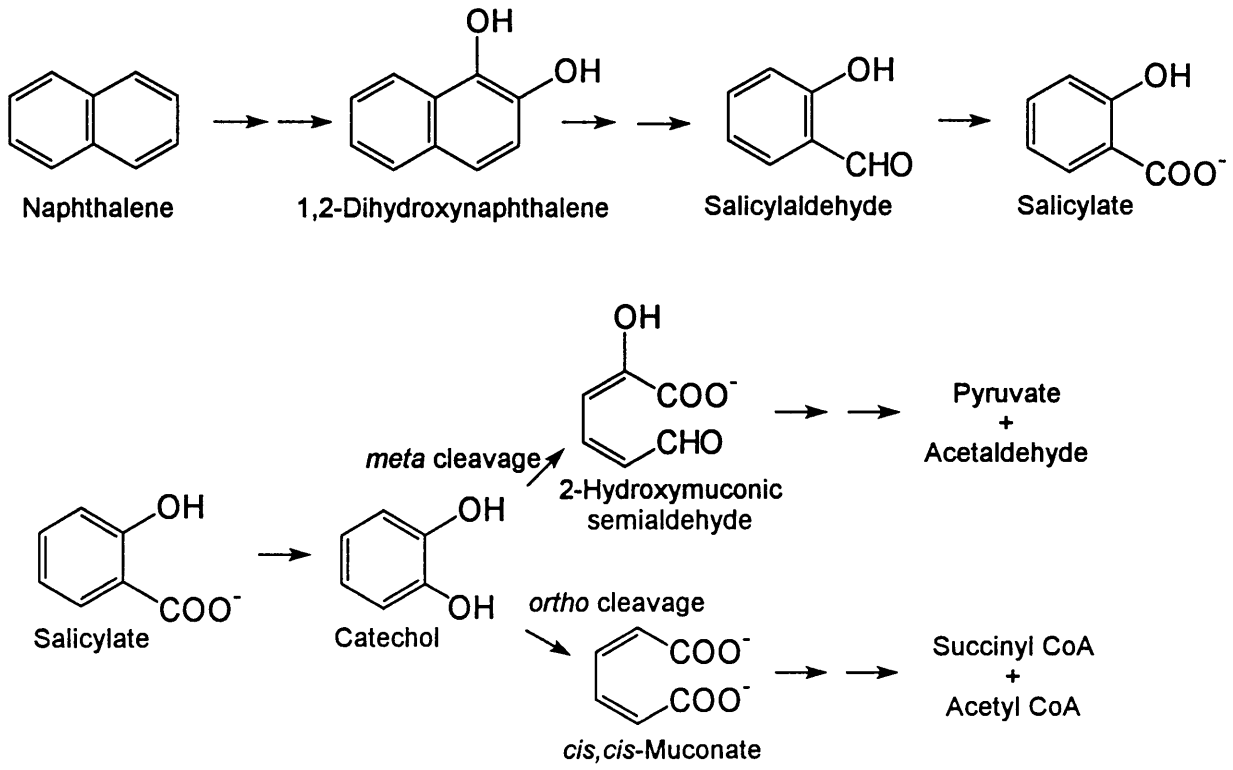
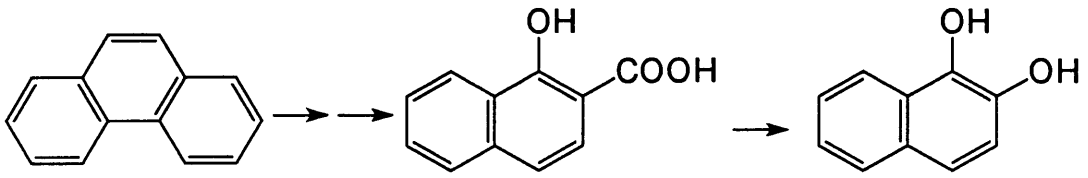


Figure 2.1. Some of the reactions in the principal pathway for aerobic metabolism of naphthalene by bacteria. The *upper pathway* is the series of reactions leading to salicylate and the *lower pathway* is the series of reactions leading to catechol and subsequent ring cleavage products. Ring cleavage of catechol by either the *meta* or *ortho* route depends on the individual strain.

Bacteria often do not synthesize, or express, high concentrations of the enzymes they need to degrade a particular chemical if that chemical is not present. Instead, they may express high enzyme concentrations only after the chemical is introduced into the medium. The process by which enzyme levels are increased well beyond background levels is referred to as *induction*.

The inducibility of PAH metabolism has not been well studied. In fact, the details of induction have been worked out for only one compound, naphthalene. It is now well established that the intermediate salicylate (Figure 2.1) is the chemical that induces naphthalene metabolism in bacteria (Yen and Serdar, 1988). When naphthalene is introduced to a culture capable of degrading naphthalene, the background levels of the naphthalene metabolic enzymes generate low concentrations of salicylate, which then induces the synthesis of higher enzyme concentrations. Salicylate induces both the upper and lower pathways of naphthalene metabolism shown in Figure 2.1.

If many of the same enzymes are involved in the degradation of multiple PAH compounds, it follows that a single inducer such as salicylate may induce the metabolism of a range of PAH. Salicylate induces the degradation of benz[*a*]anthracene (Mahaffey *et al.*, 1988) by an organism recently classified as *Sphingomonas yanoikuyae* (Khan *et al.*, 1996), as well as the degradation of



Phenanthrene 1-Hydroxy-2-naphthoate 1,2-Dihydroxynaphthalene

Figure 2.2. Selected steps in one of the known pathways for the aerobic bacterial metabolism of phenanthrene. Once dihydroxynaphthalene is formed, its subsequent metabolism involves the same enzymes required for naphthalene metabolism. In some bacteria, the enzymes required to convert phenanthrene to 1H2N are the same as those used to convert naphthalene to salicylate (Figure 2.1).

phenanthrene, pyrene and fluoranthene by *Pseudomonas saccharophila* P16 (Stringfellow *et al.*, 1995). Salicylate cannot be the only inducer of PAH metabolism, however, since there are known pathways for phenanthrene and pyrene metabolism that do not proceed through salicylate as an intermediate (Sutherland *et al.*, 1995). Little is known about the metabolic pathways for the metabolism of other PAH, or about the inducibility of their degradation.

The ability to use salicylate or other small, water-soluble molecules to induce PAH degradation represents a potentially powerful method to enhance the degradation of the HMW PAH that do not serve as growth substrates for bacteria. In addition to its role as an inducer, salicylate can also be used as a growth substrate so that populations of PAH-degrading bacteria might be increased as well. The addition of salicylate to soil has been proposed and tested as a means of increasing the population of naphthalene degraders and correspondingly enhancing naphthalene degradation (Ogunseitan *et al.*, 1991; Ogunseitan and Olson, 1993). Salicylate addition has also been studied as a means of sustaining the activity and population density of a bacterium introduced to soil (Colbert *et al.*, 1993a; Colbert *et al.*, 1993b). Both salicylate and phthalate (an intermediate in a pathway for phenanthrene degradation that differs from the one shown in Figure 2.2) were shown to sustain PAH-degrading activity by freshwater organisms in short-term experiments (Tittle *et al.*, 1995). Salicylate was added to pilot-scale slurry-phase reactors used to treat contaminated soil from a wood-treatment site (Brown *et al.*, 1995), but there was no control reactor in which salicylate was not added with which to make a comparison. Overall, the effects of salicylate and other potential inducing substrates on HMW PAH degradation have not been evaluated extensively.

3. EXPERIMENTAL MATERIALS AND METHODS

Experimental Design

The intention of this work was to investigate the biological treatment of contaminated soil from an MGP site. Slurry-phase bioremediation was chosen as the laboratory model of biological treatment for several reasons. First, slurry-phase treatment is both a full-scale technology (U.S. EPA and U.S. Air Force, 1993; U.S. EPA, 1996a) and is readily scaled down for evaluation at the bench-scale, so that results obtained in the laboratory would be pertinent to larger-scale systems. Second, slurry-phase bioremediation provides a high degree of mixing, which is believed to improve the transfer of PAH and other hydrophobic chemicals from the soil to the aqueous phase and hence increase rates of biodegradation. As a result, slurry-phase reactors can be used to evaluate the inherent biological treatability of a soil in a relatively short period of time (Vernieri *et al.*, 1992; U.S. EPA, 1993; Ghoshal and Luthy, 1996). Finally, slurry systems facilitate the examination of amendments that might stimulate biodegradation processes. With respect to the influence of proposed amendments on PAH degradation, the results obtained in slurry reactors should qualitatively be applicable to other forms of biological treatment.

We began the project by identifying a source of contaminated soil and preparing the soil for use in the experimental work. Based on results from preliminary soil analysis we decided to focus on seven of the PAH in the slurry-phase reactors: phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benz[*a*]anthracene and benzo[*a*]pyrene. Phenanthrene and anthracene were chosen as representative low molecular weight PAH (each of these is a three-ring compound) that should be readily biodegradable. Fluoranthene and pyrene were chosen because they are among the group of compounds we classify as high molecular weight (they are both four-ring compounds) and were present at the highest concentrations among the 16 PAH analyzed. Chrysene, benz[*a*]anthracene and benzo[*a*]pyrene were chosen because all three are carcinogens, and we have experience with bacterial degradation of these compounds in other ongoing work; in addition, all three as well as pyrene were available in radiolabeled form so that we could study the ability of the microbial communities in the slurry phase reactors to mineralize these compounds.

Experimental work was conducted in two phases. In Phase I, PAH removal was evaluated as a function of solids residence time (SRT) in the reactor. Two reactors were operated side-by-side at SRTs of 18 and 35 d. Based on findings from Phase I, the 18 d SRT was selected for further study in Phase II. In Phase II we implemented several changes based on experience gained in Phase I, including modification of the bench-scale reactor operation, adoption of a more efficient soil extraction technique, and refinement of the analytical method used to quantify PAH. In Phase II we also evaluated the effects of amendments (nonionic surfactant and supplemental carbon sources) on degradation of PAH in treated soil removed from the reactor. We focused on treated soil because conventional slurry-phase treatment should remove the most readily degradable fraction of the PAH; amendments would be required to remove only the most recalcitrant fraction of the PAH.

A single soil sample was used throughout the project. The soil was homogenized as a single batch but was prepared for use in two separate batches, which were used over periods that roughly corresponded to Phases I and II as described above. Although the use of a homogenized

soil sample was not completely representative of field conditions, screening and blending are often done in field operations. The use of a homogenized soil source was important to ensure that changes in reactor performance or that differences between experimental treatments were due to experimental conditions other than variability in soil composition.

Soil Source and Preparation

The soil was obtained from an MGP site in Greenville, SC, owned by Duke Power Company that began to undergo excavation in the summer of 1995. Duke Power personnel had already collected several large samples of contaminated soil during site assessment work and shipped a five gallon sample to us on August 10, 1995.

The soil sample contained standing water, which was decanted prior to further handling. The drained soil was air dried and homogenized in a covered outdoor location by spreading it out on a metal pan (1.3 m x 1.3 m), blending thoroughly and sieving every day for five days. Blending was achieved by first piling the soil into a cone and splitting the pile into quarters. Two quarters from opposite sides of the cone were combined and mixed by hand with a trowel, the remaining two quarters were similarly combined and blended, and the two halves were combined and blended again (Head, 1992). Some of the pile was then sieved through a No. 3 sieve (7 mm, or 0.26 in) and the remainder was returned to the shipping container for long-term storage. Single particles not passing the sieve were rejected, while clay clumps and aggregates were broken up manually until they passed through the sieve. Only a small fraction of the sample was rejected.

After air drying and homogenizing, the moisture content of the soil was determined by drying triplicate 25 g (wet wt.) samples in an oven at 105°C for 24 hr. The soil was then distributed into glass bottles in 100 g (wet wt.) aliquots, each of which was used for batch feeding the reactors on a selected time schedule. Bottles were stored cold (approximately 6°C) until needed for feeding.

The remaining soil in the container was stored in the cold until March, 1996, when it was sieved and distributed in the same manner described above.

Reactor Design and Operation

Phase I. Two bench-scale slurry-phase bioreactors were constructed. Each reactor consisted of a jacketed, cylindrical glass vessel with a 3 L working volume; a variable-speed mixer with integral thermocouple providing digital readout of mixing speed, torque and temperature (Lightnin LabMaster; Fisher Scientific, Pittsburgh, PA); and an air diffuser supplied with breathing quality compressed air. The air flow rate was controlled through a two-stage regulator and gas flow meter, and the air was humidified before entering the reactor by passing it through a gas washing bottle filled with water. The two reactors were mounted on a wooden box frame in a fume hood, and the temperature in each reactor was controlled at 22°C by circulating water from a constant temperature bath through the reactor jacket.

Each slurry-phase reactor was started by placing 441 g of soil (dry wt.) into 2.5 L of potassium phosphate buffer (25 mM, pH 7) containing 10 mM ammonium chloride, then beginning aeration; the final solids concentration in the reactors was 15% by weight. The reactors were operated in

batch mode for several weeks, after which semi-continuous operation was initiated. The desired solids residence time in each reactor was achieved by removing one-fifth of the reactor contents and feeding the same volume of untreated soil and buffer solution either once per week (35 d SRT) or twice per week (18 d SRT). After several weeks of semi-continuous operation, about half the contents of each reactor were removed and placed into the other reactor. The reactor contents were blended in this manner to ensure that each reactor contained approximately the same biomass during the startup period.

The air flow rate was set at 10 - 20 mL/min, which was estimated to provide much more oxygen than would be required to completely oxidize all of the PAH in the soil. Mixing was maintained at 100 rpm to minimize horizontal movement of the mixer shaft. This relatively slow mixing speed led to some stratification of heavy and light particles in the reactors, but the layer of heavier particles on the bottom of each reactor was subject to modest agitation. The mixing speed was increased to 350 rpm prior to sampling to resuspend the heavier particles.

The duration of Phase I was from November, 1995, through March, 1996.

Phase II. Only the reactor with the 18 d SRT was operated during Phase II of the study. As a result of several occasions in which a jacketed reactor broke, the contents of the original reactor were transferred to an unjacketed glass vessel with a thicker wall than the jacketed reactors. The mixer was also replaced with one less susceptible to horizontal movement of the shaft, which permitted the use of higher mixing speeds. Routine mixing was at 375 rpm and for sampling was increased to 425 rpm. Although temperature in the reactor was not controlled, it was measured during sampling events and normally was in the range of
20 - 25°C. All other operating conditions were the same as in Phase I.

Allowing time for the bioreactor to adjust to modified operating conditions, routine sampling in Phase II began in July, 1996, and proceeded through November, 1996, at which time the soil sample was depleted. All of the experiments involving the addition of amendments to the treated soil were done with soil obtained from the reactor during Phase II.

Feed soil used in Phase II was from the second batch of the original soil sample. During Phase II we also began to use new methods of soil extraction and PAH analysis, so that the data on feed soil and reactor performance obtained from Phase II are not directly comparable to the data from Phase I; analytical issues are discussed in more detail below. During Phase II all treated soil samples were analyzed in triplicate.

Sampling and Sample Preparation

A vacuum sampling device was constructed from glass tubing and a stoppered graduated cylinder (1000 mL), as shown in Figure 3.1. When ready to sample, the wide-bore glass tube was placed into the reactor and the vacuum was applied until 535 mL of slurry was collected in the cylinder.

The soil slurry was poured into four 250 mL stainless steel centrifuge bottles. The bottles were centrifuged at 5,000 rpm for 20 min in a Sorval (DuPont, Wilmington, DE) refrigerated centrifuge at 10°C. The water layer was decanted and tested for pH, and the soil pellet was transferred to a

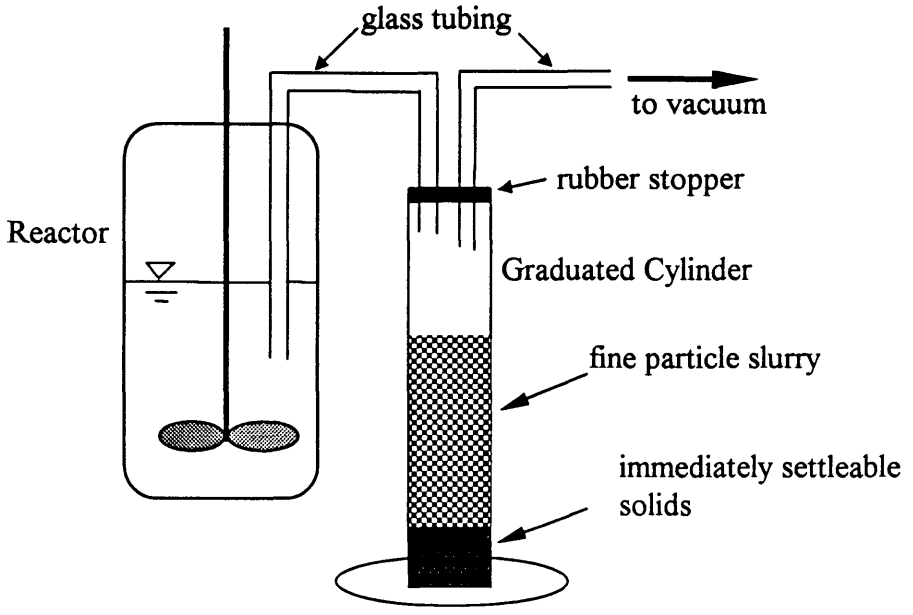


Figure 3.1. Reactor sampling device. The working volume of the reactor was 3000 mL and the volume of the graduated cylinder was 1000 mL.

beaker and weighed. A 30 g subsample was oven-dried at 105°C for 24 hr to determine the moisture content.

Soil Extraction

In Phase I the soil was extracted for analysis using the soxhlet method (method 3540 in EPA/SW846; U.S. EPA, 1986). A shake-flask method was used to extract the soil during Phase II. The two extraction techniques were compared during the transition between the two phases of the project, when the first batch of soil from the original soil sample was still being used.

Soxhlet extraction. A 20 g subsample of moist soil (centrifuged sample for the treated soil) was placed in a glass beaker and mixed vigorously with 20 g of anhydrous sodium sulfate (prepared in accordance with method 3540 of EPA/SW846). From this mixture 20 g was placed in a glass extraction thimble lined with glass wool (pre-rinsed with acetone and methylene chloride). A 1 mL volume of an internal standard solution (deuterated anthracene, 100 mg/L in methylene chloride) was added directly to the extraction thimble for analysis of recovery.

The extraction thimble was placed in a soxhlet apparatus with approximately 250 mL of methylene chloride in the round-bottom flask and extracted for 18 - 24 hr. The apparatus then was cooled and the extract was passed through a drying column (10 cm x 1.5 cm dia.) containing anhydrous sodium sulfate. The volume of the extract was then raised to exactly 250 mL. A 1 mL volume of the extract was diluted into acetonitrile and filtered through a 1.6 μm pore-size glass-fiber filter. The glassware and filter were rinsed three times with acetonitrile yielding a total

acetonitrile volume of 20 mL, or a 1:20 dilution of the original methylene chloride extract. An aliquot of the acetonitrile solution was further diluted 1:10 for a total dilution of 1:200. Both acetonitrile solutions were analyzed by high pressure liquid chromatography (HPLC).

Recovery of the internal standard averaged 94 ± 4.9% (n=14), indicating that little loss of PAH analytes occurred during the sample handling procedures.

Shake-flask extraction. The following method was adapted from a method provided by G. Sayles of the EPA National Risk Management Research Laboratory in Cincinnati, OH. The method was developed as a means of improving the throughput of soil samples for analysis and substantially reducing the volume of solvent used compared to the soxhlet method (personal communication: G.D. Sayles,).

The moist soil sample was mixed with anhydrous sodium sulfate, the quantity of which depended on moisture content. The ratio of sodium sulfate to moist soil was approximately 2.5:1 for the treated soils, which typically had a moisture content of 50%, and 0.5:1 for the feed soils, which typically had a moisture content of 8%. In general triplicate subsamples of the soil/sodium sulfate mixture from each sample were extracted and analyzed.

For each subsample the soil and sodium sulfate mixture was weighed into a 60 mL serum bottle. The amounts added to each bottle (10 g for treated soils and 5 g for feed soils) corresponded to approximately 1.5 g of dry solids from the original soil sample. Eight 6 mm dia. borosilicate glass beads (rinsed with methylene chloride), 1 mL of internal standard solution (100 mg/L of deuterated anthracene in methylene chloride), 10 mL of acetone, and 10 mL of methylene chloride were added to the serum bottle. The glass beads improved extraction through abrasion and disaggregation of the soil. Acetone and methylene chloride were chosen as the extraction solvents based on their availability, compatibility, and effectiveness in extracting PAHs from this soil.

The serum bottle was sealed with a Teflon septum and aluminum crimp seal, placed on a reciprocating shaker table at a 45° angle and shaken for 24 hours at 35°C. The bottle was then removed from the shaker and spun in a centrifuge (Fisher Marathon 21K/R) at 4000 rpm for 10 min at 10°C. The extract was removed from the bottle by syringe and diluted from 1:50 to 1:300 into acetonitrile for HPLC analysis.

A preliminary test with different size aliquots of dry solids was conducted to evaluate the effect of soil quantity on PAH extraction and analysis. Dry solid amounts between 0.5 g and 15 g gave statistically indistinguishable results. Another preliminary experiment was conducted in which six successive extractions of the same treated soil sample were performed. For all of the PAH compounds of interest, at least 97% of the total amount extracted was obtained in the first extraction. Concentrations in the sixth extract were either non-detectable or less than 0.05% of the total.

Comparison of soil extraction methods. PAH concentrations determined after Soxhlet or shaker extraction of aliquots from the first batch of soil are compared in Table 3.1. It is clear that, for these samples, the shaker method provided superior extraction compared to the soxhlet method. Consequently, the shaker method was adopted for sample analysis during Phase II of the project.

Table 3.1. Concentrations of PAH in untreated soil determined with two different extraction techniques.

	Concentration (mg/kg)	
Compound	**Soxhlet [a]**	**Shaker [b]**
phenanthrene	26 ± 8.9	50 ± 9.2
anthracene	5.0 ± 1.9	6.2 ± 1.7
fluoranthene	43 ± 7.4	75 ± 5.1
pyrene	76 ± 20	190 ± 13
benz[*a*]anthracene	56 ± 15	130 ± 10
chrysene	66 ± 13	140 ± 8.0
benzo[*a*]pyrene	34 ± 6.0	72 ± 6.9

[a] Mean and standard deviation of eight samples.

[b] Mean and standard deviation of three sub-samples from each of three bottles containing untreated soil (nine extractions total).

Liquid-phase Extractions

Supernatant from centrifuged samples of treated soil was extracted and analyzed to confirm that significant amounts of PAH were not present in the aqueous phase or in suspension. A 100 mL volume of supernatant was mixed with 50 mL methylene chloride in a separatory funnel and shaken vigorously for 2 min. After 10 min of settling the residual emulsion was broken mechanically with a glass stirring rod. The organic layer was removed and filtered through glass wool followed by an anhydrous sodium sulfate drying column. The aqueous layer was extracted again with a second 50 mL volume of methylene chloride and filtered through glass wool and the drying column. If present, the residual solvent-water emulsion was removed from the separatory funnel and passed through the drying column. The solvent phases were then combined for analysis.

Analysis of Extracts and Other Liquid Samples

All PAH analyses were conducted by HPLC using a Waters (Milford, MA) 600E system controller, model 717 autosampler and model 470 scanning fluorescence detector. Separation was achieved with a Supelcosil LC-PAH column (25 cm x 3.2 mm dia, 5 μm particle size; Supelco, Bellefonte, PA) and a C_{18} pre-column which was preceded by a 0.5 μm pore-size stainless steel frit. An Alltech (Deerfield, IL) 330 column heater was used to maintain a constant

column temperature of 30°C. The mobile phase was 60:40 acetonitrile: water from 0 - 5 min, a linear gradient to 100% acetonitrile from 5 - 15 min, and 100% acetonitrile from 15 - 35 min.

PAH concentrations were quantified by calibration against 4 - 5 dilutions of an external standard PAH mixture prepared gravimetrically in acetonitrile. The standard curve for deuterated anthracene was prepared from serial dilutions of a certified standard solution (Fisher Scientific, Pittsburgh, PA), in some cases in the same mixture as the other seven PAH.

The HPLC fluorescence detector could be programmed to vary the excitation and emission wavelengths during an individual analytical run, so that these wavelengths could be programmed to optimize the selectivity and sensitivity for each PAH of interest. The most difficult compounds to quantify were chrysene and benz[*a*]anthracene. These compounds eluted at nearly the same time and appeared to co-elute with other fluorescing species (presumably other PAH), so that during Phase I the two peaks overlapped with a broad, unresolved peak. Chrysene and benz[*a*]anthracene were quantified during Phase I by counting the area under each peak extended to the baseline, which probably overestimated the actual concentration. During Phase II the fluorescence program was modified slightly to increase the selectivity for these two compounds, although some sensitivity was lost as a result. The fluorescence programs used in Phases I and II are shown in Table 3.2.

Table 3.2. Fluorescence program for HPLC detector.

		Wavelength (nm)	
Compound(s)	**Time (min)**	**Excitation**	**Emission**
phenanthrene	0	259	370
anthracene	11.0	252	405
fluoranthene	12.5	284	460
pyrene	13.9	336	398
benz[*a*]anthracene and chrysene	15.2	265	380/390 [a]
benzo[*a*]pyrene	18.5	378	406

[a] Emission wavelength used in Phases I and II, respectively.

Quantification limits for the PAH analytes of interest are summarized in Table 3.3, in terms of both the concentration in the injected sample and the concentration in the original soil sample. All quantification limits were well below the concentrations of the analytes in the untreated soil.

Analysis of a Reference Soil

A reference PAH-contaminated soil was purchased from Resource Technology Corp. (Laramie, WY; lot number RQ103) and analyzed according to the procedures described above for extraction and analysis during Phase II. The soil was sent with a certificate of analysis for the 16 priority

Table 3.3. Quantification limits for HPLC analysis of PAH.

Compound	Quantification Limit	
	Liquid, μg/L	Soil, mg/kg [a]
phenanthrene	1.0	0.67
anthracene	0.5	0.33
fluoranthene	1.0	0.67
pyrene	1.0	0.67
benz[*a*]anthracene	0.5	0.33
chrysene	1.0	0.67
benzo[*a*]pyrene	0.5	0.33

[a] Assumes 20 mL extraction solvent per 1.5 g dry soil, 1:50 dilution of extract in acetonitrile. A lower limit could be achieved with less dilution of the extract. If greater dilution were used in a given experiment, the actual detection limit is reported with the data.

pollutant PAH, based on interlaboratory analyses under a certification program contract with EPA. Results from our analysis of the seven PAH of interest are compared to the certified analysis in Table 3.4. The concentrations overlapped within one standard deviation for each of the seven analytes quantified.

PAH Mineralization

During Phase I, the ability of microorganisms in the soil slurry to mineralize five different PAH was tested using ^{14}C-labeled compounds. Each compound was tested in triplicate in sterile 40 mL glass vials, each containing 15 mL of tap water buffer (TWB; Stringfellow and Aitken, 1994). The target PAH was added to each vial at a concentration corresponding to approximately 80% of its solubility in water (Mackay *et al.*, 1992). The amount of radiolabeled compound added to each of the vials corresponded to approximately 20,000 disintegrations/min (dpm) except for vials containing benzo[*a*]pyrene, which contained 10,000 dpm. The remaining amount of the PAH (if any) was added in unlabeled form. The molar quantities of labeled and unlabeled compound added were as follows: 176 picomoles (pmol) for ^{14}C-chrysene; 200 pmol for ^{14}C-benzo[*a*]pyrene; 280 pmol ^{14}C-pyrene + 11.71 nmol pyrene, 166 pmol ^{14}C-benz[*a*]anthracene + 633 pmol benz[*a*]anthracene, 1.1 nmol ^{14}C-phenanthrene + 106 nmol phenanthrene.

Vials containing the individual PAH were inoculated with 5 mL of soil slurry from the reactor operated with the 18-d SRT. Triplicate killed controls were prepared by adding 0.5 mL of 20% H_3PO_4 to the vials containing the various PAH prior to addition of the soil slurry. Triplicate uninoculated controls were prepared by adding 20 mL of TWB and no soil slurry. Each vial was sealed with a cap containing a teflon septum from which was suspended a plastic bucket containing a fluted strip of Whatman No. 1 filter paper soaked in 200 μL of 2N KOH, which

Table 3.4. Analysis of reference PAH-contaminated soil.

Compound	Concentration, mg/kg	
	Measured [a]	Certified Value [b]
phenanthrene	2,130 ± 101	1,920 ± 493
anthracene	486 ± 28	431 ± 103
fluoranthene	1,460 ± 70	1,430 ± 401
pyrene	1,300 ± 64	1,080 ± 341
benz[*a*]anthracene	221 ± 15	264 ± 58
chrysene	251 ± 15	316 ± 71
benzo[*a*]pyrene	97.9 ± 7.3	96.5 ± 28.6

[a] Mean and standard deviation of triplicate samples (mean reported to three significant figures).

[b] Mean and standard deviation from interlaboratory analyses (number of laboratories not known), as provided by Resource Technology Corp. Mean reported to three significant figures. For the interlaboratory analyses the soil was extracted by the soxhlet method and analyzed by gas chromatography/mass spectrometry (GC/MS).

served as a carbon dioxide trap. The vials were then incubated in the dark for 48 hr at 25°C on a rotary shaker.

After shaking, 0.5 mL of 20% H_3PO_4 was injected through the septa into the vials containing the live samples and the uninoculated controls. All of the vials were then placed back on the rotary shaker for another 24 hr. After this period, the fluted filter paper strips were removed from each vial and placed in scintillation vials containing 9 mL of ScintiSafe® (Fisher) scintillation cocktail. A 5 mL aliquot of the solution from each incubation vessel was also removed and added to a scintillation vial containing 9 mL of cocktail. A 15 mL volume of acetonitrile was then added to each of the incubation vessels, then 5 mL aliquots were removed and added to scintillation vials containing 9 mL of cocktail. All the scintillation vials were then counted on a Packard Instruments (Downers Grove, IL) Tri-Carb 1900TR scintillation counter.

A second experiment was conducted during Phase II in which the mineralization of benzo[*a*]pyrene was evaluated in the presence and absence of a supplemental carbon source (succinate or salicylate). Soil slurry containing approximately 0.2 g (dry wt.) of soil solids was removed from the reactor and added to 50 mL of M9 mineral salts buffer (Sambrook *et al.*, 1989). Replicate vials containing 10 mL of M9 buffer and 200 pmol ^{14}C-benzo[*a*]pyrene (approximately 10,000 dpm) with no additional carbon source, 250 μM salicylate or 430 μM succinate were each inoculated with 1 mL of the soil suspension, then covered with aluminum foil, teflon septum and screw cap. Each vial also contained an internal tube which held a piece of filter paper soaked in KOH; this method of trapping ^{14}CO2 was found to be superior to the method

described above, which resulted in significant amounts of PAH sorption to the plastic buckets in the controls. The vials were incubated for 96 hr on a rotary shaker at 25°C, then 250 μL of 20% H_3PO_4 was injected through the septum and the vials shaken for another 24 hr. After shaking, the filter paper was removed from each vial, added to scintillation cocktail and counted as described above. Killed controls were also prepared as described above.

Surfactant Sorption and PAH Solubilization

Before conducting experiments to study the effect of a nonionic surfactant (Brij 30) on biodegradation of PAH, we evaluated surfactant sorption to the soil and the solubilization of PAH in both the untreated and treated soils.

Kinetics. To evaluate the rate of surfactant sorption and PAH solubilization, three 500-mL erlenmeyer flasks were prepared for each soil tested; two of the flasks contained surfactant and the third was a control without surfactant. Each test flask contained 30 g (dry wt.) soil solids, 15 g/L of Brij 30, phosphate buffer stock (pH 7), and sodium azide at a concentration of 1% (wt:wt) in a volume of 200 mL. The control flask contained the same components except surfactant. The solids and buffer concentrations were chosen to replicate conditions in the slurry reactor, and azide was added to preclude biodegradation of the surfactant during the incubation period. The flasks were placed on a rotary shaker at 200 rpm at room temperature and sampled at several intervals over a 15 d period. Flasks were sampled by allowing the solids to settle for 5 min, then 3 mL of supernatant was removed and placed into a syringe fitted with a 0.8 μm pore-size polycarbonate filter. The first 2 mL of filtrate was wasted and the final 1 mL was injected into an amber glass vial. Aliquots of 0.1 mL were then diluted into varying volumes of reagent water for surface tension analysis and another 0.1 mL aliquot was diluted into acetonitrile for analysis of PAH concentration. Surfactant concentrations were quantified by surface tension measurement using a du Nouy ring tensiometer (Fisher) in comparison to a standard curve prepared with a range of Brij 30 concentrations below the critical micelle concentration (approximately 8 mg/L).

The rate of surfactant sorption was quantified by assuming first-order mass transfer:

$$\frac{dS}{dt} = k_s \cdot (S - S_e) \; ; \quad S(t=0) = S_0 \tag{3.1}$$

where S is surfactant concentration in the liquid phase; k_s is the observed first-order mass transfer rate coefficient for surfactant sorption; and S_e is the surfactant concentration in the liquid phase at equilibrium. The solution to eq. 3.1 is:

$$S(t) = S_e + (S_0 - S_e) \cdot e^{-k_s \cdot t} \tag{3.2}$$

Eq. 2 was fit to the data on surfactant concentration vs. time with both k_s and S_e as unknowns, using ProStat (Poly Software International, Salt Lake City, UT).

Two models were used to analyze the PAH solubilization data. In the first model a varying surfactant concentration was assumed, since the surfactant was sorbing to the soil over the same period in which PAH solubilization was occurring. The second model was a simple first-order

mass transfer model in which the variation in surfactant concentration was ignored. In both cases the form of the equation describing PAH concentration as a function of time was:

$$\frac{dP}{dt} = k_p \cdot (P_e - P)\ ; \quad P(t=0) = 0 \tag{3.3}$$

where P is the concentration of the PAH of interest in the liquid phase; k_p is the observed first-order mass transfer coefficient for PAH solubilization; and P_e is the PAH concentration in the liquid phase at equilibrium. For the model in which variations in surfactant concentration were ignored, P_e was considered to be unknown but constant, so that the solution to eq. 3.3 was:

$$P(t) = P_e \cdot \left(1 - e^{-k_p \cdot t}\right) \tag{3.4}$$

The parameters P_e and k_p for each of the PAH of interest were estimated by fitting eq. 3.4 to the PAH concentration data using ProStat.

For the model in which the variation of surfactant concentration was taken into account, the value of P_e in eq. 3.3 is described by the relationship between surfactant concentration and the saturation concentration of a given PAH compound in the liquid phase (Grimberg *et al.*, 1995):

$$P_e = P_{e,a} + (S - CMC) \cdot q_m \tag{3.5}$$

where $P_{e,a}$ is the saturation concentration of the PAH in the aqueous phase in the absence of surfactant, CMC is the critical micelle concentration of the surfactant, and q_m is the effective solubilization capacity of the surfactant for the given PAH compound (mass PAH solubilized per unit mass of surfactant). Under the experimental conditions used, however, $S >> CMC$ and $P_e >> P_{e,a}$, so that eq. 3.5 was simplified to:

$$P_e = S \cdot q_m \tag{3.6}$$

The time-dependent surfactant concentration was determined with eq. 3.2. With a variable P_e, however, there was no analytical solution to eq. 3.3. In this case the parameters k_p and q_m were estimated by fitting eqs. 3.3 and 3.6 to the PAH and surfactant concentration data using the least-squares optimization procedure in ModelMaker (Cherwell Scientific Publishing, Palo Alto, CA).

Equilibria. Liquid-phase concentrations of surfactant and PAH at equilibrium were measured as a function of surfactant dose for both the untreated and treated soils. Based on the results from the kinetics experiment described above, equilibrium was assumed to be reached within 48 hr. For each soil, duplicate serum bottles were prepared for each of seven different surfactant doses up to 15 g/L and one pair was prepared without surfactant. Each bottle contained 3 g (dry wt.) of soil solids, surfactant, phosphate buffer stock (pH 7), and sodium azide (1%, wt:wt) in a volume of 20 mL. The soil and buffer concentrations were chosen to replicate conditions in the slurry reactor. Each bottle was crimp-sealed with a teflon septum and placed on a rotary shaker (200 rpm) at room temperature. After shaking for 48 hr the bottles were centrifuged at 4000 rpm for 30 min, then 3 mL of supernatant was removed, syringe-filtered, and diluted for surface tension

and PAH analysis as described above. In two cases involving very high dilution of the sample from the treated soil, the surface tension data were inconsistent with the PAH solubilization data and therefore were rejected.

Effects of Amendments on PAH Biodegradation

The effects of adding surfactant, supplemental carbon sources (salicylate and/or phthalate) or both on PAH degradation in treated soil removed from the slurry-phase reactor was tested in triplicate for each condition. Each of a series of 500 mL erlenmeyer flasks contained 10 g (dry wt.) of centrifuged soil solids removed from the slurry reactor in a final volume of 67 mL. In those flasks receiving one or more amendments, the following concentrations were used: surfactant (Brij 30) at 8.0 g/L; sodium salicylate at 40 mg/L (250 μM); and potassium hydrogen phthalate at 51 mg/L (250 μM). Killed controls with and without surfactant were prepared by adding sodium azide to a final concentration of 1% (wt:wt). Eight glass beads were added to each flask to improve mixing, the flasks were covered with laboratory film to permit oxygen transfer while minimizing water losses to evaporation, then the flasks were placed on a rotary shaker (200 rpm) at 20°C. For most of the experimental conditions, samples of the liquid phase were collected every 24 hr and analyzed for surfactant concentration via surface tension measurement. On the fifth day the contents of each flask were transferred to serum bottles for centrifugation, the aqueous phase was decanted, and the solid phase was extracted and analyzed as described above. The aqueous phase was syringe-filtered and analyzed for PAH and surfactant as described above.

Chemicals

PAH were purchased from various suppliers and were the highest grade available. (9-^{14}C)Phenanthrene (8.3 mCi/mmol), (4,5,9,10-^{14}C)pyrene (32.3 mCi/mmol), and (7-^{14}C)benzo[*a*]pyrene (26.6 mCi/mmol) were purchased from Sigma (Milwaukee, WI). (5,6,11,12-^{14}C)Chrysene (47.6 mCi/mmol) and (5,6-^{14}C)benz[*a*]anthracene (54.6 mCi/mmol) were obtained from Chemsyn Science Laboratories (Lenexa, KS). All radiolabeled chemicals had purities > 98%. Solvents were all HPLC or spectrophotometric grade and were purchased from various suppliers. Brij 30 was purchased from Sigma. All other chemicals were ACS reagent grade or equivalent.

4. PAH REMOVAL DURING SLURRY-PHASE TREATMENT

Phase I Reactor Performance

Routine operation of the reactors began in November, 1995. The reactor operated at the 35 d SRT was shut down in March, 1996, and sampling for the reactor operated at the 18 d SRT was stopped in mid-May, 1996. Mean concentrations of the seven PAH of interest in the treated soil from both reactors are compared in Table 4.1 to the concentrations in the untreated soil.

Table 4.1. Concentrations of selected PAH in treated soil in comparison to feed soil.

	Concentration (mg/kg)		
Compound [a]	**Feed Soil** [b]	**18 d SRT Reactor** [c]	**35 d SRT Reactor** [d]
phenanthrene	26 ± 8.9	7.5 ± 5.5 [e]	13 ± 31
anthracene	5.0 ± 1.9	1.7 ± 1.3 [e]	3.7 ± 6
fluoranthene	43 ± 7.4	15 ± 11 [e]	19 ± 17 [e]
pyrene	76 ± 20	40 ± 37 [e]	42 ± 35 [e]
benz[*a*]anthracene	56 ± 15	38 ± 32 [f]	41 ± 30 [f]
chrysene	66 ± 13	37 ± 29 [e]	39 ± 31 [e]
benzo[*a*]pyrene	34 ± 6.0	20 ± 14 [e]	17 ± 12 [e]

[a] All samples extracted by soxhlet method (see Section 3).

[b] Mean and standard deviation of 8 samples.

[c] Mean and standard deviation of 18 samples from November, 1995, to May, 1996.

[d] Mean and standard deviation of 12 samples from November, 1995, to March, 1996.

[e] Significantly different than the concentration in the feed soil, t-test, $p < 0.05$.

[f] Significantly different than the concentration in the feed soil, t-test, $0.05 < p < 0.10$.

Significant PAH removal was observed in both reactors, but the removals were less than 50% for pyrene, benz[*a*]anthracene, chrysene, and benzo[*a*]pyrene. There were no statistically significant differences between reactors for any of the compounds (t-test, $p < 0.05$), so that there did not appear to be an advantage to operating at the higher SRT.

PAH in solution or associated with colloids in the liquid phase were measured in three centrifuged samples obtained from the 18 d SRT reactor (Table 4.2). Except for benzo[*a*]pyrene, each compound was well below the aqueous solubility of the pure compound. The actual solubility of each PAH would be lower than the pure-compound solubility in accordance with its mole fraction in the NAPL phase (Yeom *et al.*, 1995), which was unknown. However, the low measured concentrations in the aqueous phase suggest that degradation of these compounds was probably limited by rates of mass transfer.

Table 4.2. Concentrations of selected PAH in the liquid phase from centrifuged samples of treated soil obtained from the 18 d SRT reactor.

Compound	Concentration (μg/L)	
	Liquid phase [a]	Aqueous Solubility [b]
phenanthrene	$< 4.2 \pm 2.6$ [c]	1,100
anthracene	0.5 ± 0.2	70
fluoranthene	$< 1.1 \pm 0.5$ [c]	260
pyrene	$< 3.3 \pm 0.1$ [c]	130
benz[*a*]anthracene	< 1 [d]	11
chrysene	< 1.1 [e]	4
benzo[*a*]pyrene	$< 3.5 \pm 0.7$ [c]	3.8

[a] Mean and standard deviation of three samples collected on different dates in May, 1996. For sets with one or more samples below the detection limit, the detection limit itself was used in calculating the mean.

[b] Solubility of the pure compound; see Table 2.1.

[c] One sample was below the detection limit of 1 μg/L.

[d] All three samples were below the detection limit of 1 μg/L.

[e] Two samples were below the detection limit of 1 μg/L.

From inspection of the data in Table 4.1, it is clear that there was considerably more variability in the treated soil than in the untreated soil, even though the treatment process provided mixing as well as some equalization over the solids residence time. Variability in the treated soil was attributed to a noticeable phase separation that occurred in the reactors, in which soil particles (particularly sand) separated from black, globular particles several mm in diameter. Similar observations have been made by others treating PAH-contaminated soil in slurry-phase reactors (Simpkin and Giesbrecht, 1994; Blackburn *et al.*, 1995; Brown *et al.*, 1995).

The phase separation observed in the treated soil was evaluated by wet-sieving soil slurry with #16 mesh (1.2 mm) and #48 mesh (0.3 mm) sieves. The particles retained by the 1.2 mm sieve (Figure 4.1) were black, globular, and had a tar-like consistency. The particles retained by the 0.3 mm sieve (Figure 4.2) consisted of both sand and small, uniform black particles, which were much more rigid than the larger black globules. Particles passing the 0.3 mm sieve (not shown) were fine clay and were not conducive to settling. In six slurry samples, $53 \pm 8\%$ of the dry mass was associated with the fine particles and $47 \pm 8\%$ of the dry mass was associated with particles larger than 0.3 mm. One of the sieved slurry samples was subjected to PAH analysis, and 98% of the PAH (the seven PAH analyzed) were found in the particles larger than 0.3 mm. A similar analysis of one feed (untreated) soil sample indicated that 59%

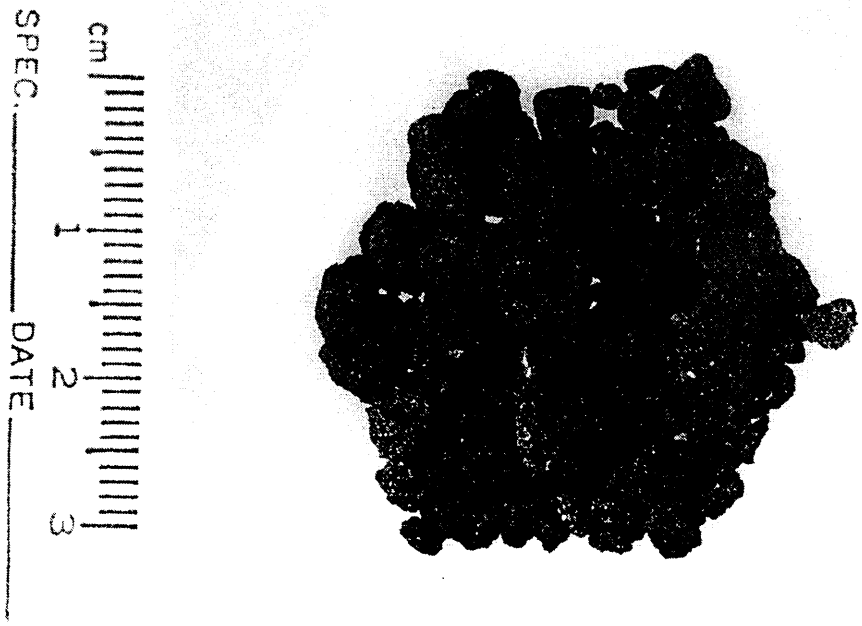


Figure 4.1. Slurry particles retained by 1.2 mm sieve.

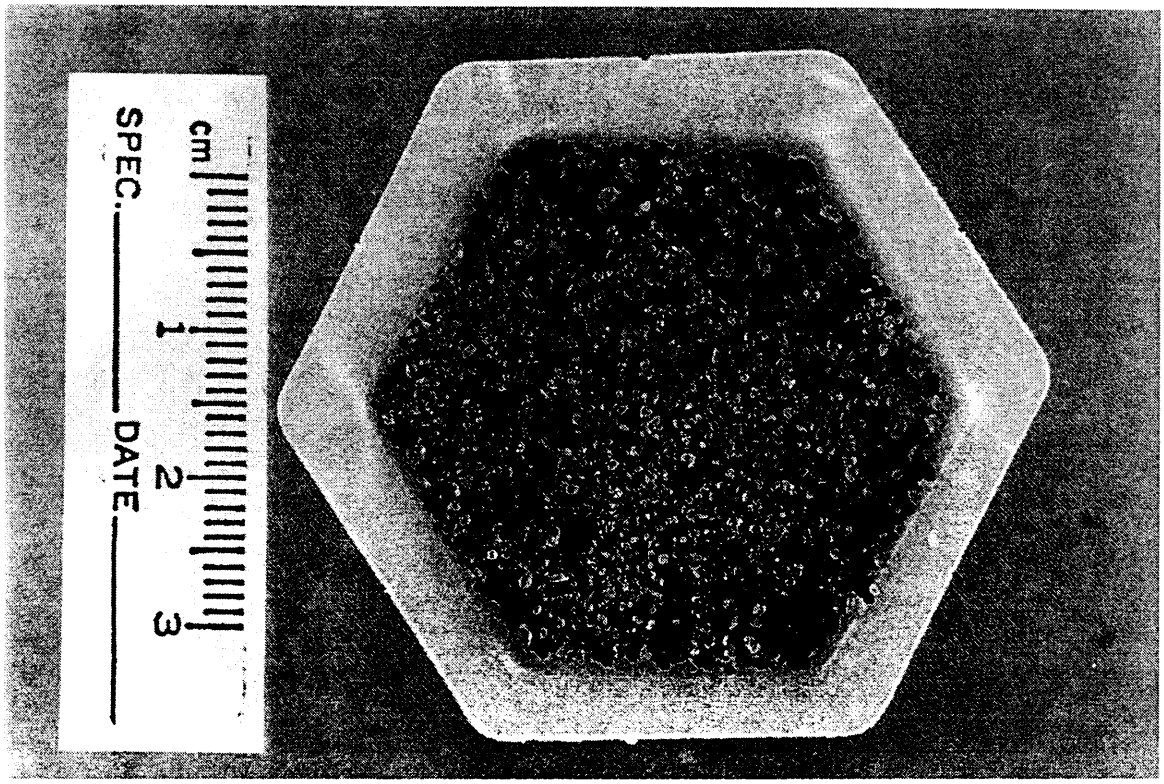


Figure 4.2. Slurry particles retained by 0.3 mm sieve.

of the total dry mass and 71% of the PAH were associated with particles larger than 0.3 mm. Thus, it appears that the mixing regime used during Phase I led to significant aggregation of tar or other NAPL in the slurry-phase reactors. The association of the majority of PAH with larger particles is contrary to experience with soil washing, in which the majority of hydrophobic contaminants is associated with fine particles (Berg *et al.*, 1994; Castaldi, 1994; Jerger *et al.*, 1994).

The removal of PAH indicated by the data shown in Table 4.1 was assumed to occur by biodegradation in the reactors. The only other potentially significant mechanism of removal would have been volatilization, which was not believed to be significant for the seven compounds followed. Others have found volatilization to be insignificant during slurry-phase treatment of PAH-contaminated soils (Otte *et al.*, 1994; Brown *et al.*, 1995). Also, in subsequent work using a closed vessel operated at much higher mixing speeds than used in Phase I, we have measured vapor-phase concentrations and found that volatile losses are insignificant for the seven compounds of interest (Roy, 1997).

Mineralization of PAH

To verify that there were microorganisms in the reactors capable of biodegrading PAH, we evaluated mineralization of five selected compounds by a slurry inoculum obtained from the 18 d SRT reactor after routine operation began (Table 4.3). All of the compounds except benzo[*a*]pyrene were mineralized in this experiment, indicating that the slurry contained active PAH-degrading biomass. The lack of mineralization of benzo[*a*]pyrene as well as the presence of liquid-phase concentrations in the reactor at approximately the solubility limit (Table 4.2) suggests that degradation of benzo[*a*]pyrene may have been limited by biological activity rather than mass transfer. Lack of mineralization of benzo[*a*]pyrene has been observed in other studies on the PAH-degrading activity of soil and sediment microbial communities (Herbes and Schwall, 1978; Goodin and Webber, 1995; Grosser *et al.*, 1995; Carmichael and Pfaender, 1997; Tiehm *et al.*, 1997) or bacteria isolated from contaminated soils (Weissenfels *et al.*, 1991; Walter *et al.*, 1991; Kästner *et al.*, 1994; Dean-Ross and Cerniglia, 1996). In one case, benzo[*a*]pyrene mineralization by a microbial community in a PAH-contaminated soil occurred only after 28 d of incubation (Grosser *et al.*, 1991).

Overall, the findings from Phase I were that (1) the reactors contained active PAH-degrading biomass; (2) increased solids residence time (beyond 18 d) in the reactor did not significantly increase PAH removal; (3) mixing in the reactors was inadequate and led to aggregation of tar or some other separate phase containing the majority of the PAH; (4) removal of benzo[*a*]pyrene probably was limited by biological activity; and (5) removal of the other six PAH that were followed probably was limited by mass transfer.

Phase II Reactor Performance

Slurry from the 18 d SRT reactor operated during Phase I was placed into a new reactor which was mixed at a substantially higher rate than during Phase I. A different batch of feed soil was used during Phase II as well. Phase separation of the reactor contents was not observed during Phase II, most likely as a result of the higher mixing speed.

Table 4.3. Mineralization of selected PAH by soil slurry. [a]

Compound	Recovery of Added ^{14}C as $^{14}CO_2$, % [b]		
	Live	Killed Control [c]	Uninoculated
phenanthrene	40 ± 1.1	0.3 ± 0.1	0.3 ± 0.1
pyrene	52 ± 0.9	0.3 ± 0.2	0.6 ± 0.1
chrysene	20 ± 1.9	0.3 ± 0.1	1.0 ± 0.8
benz[*a*]anthracene	8.5 ± 0.3	0.4 ± 0.2	1.0 ± 0.1
benzo[*a*]pyrene	0.4 ± 0.0	0.5 ± 0.2	0.5 ± 0.3

[a] Five mL of soil slurry from the 18 d SRT reactor was added to 15 mL buffer containing the compound of interest at approximately 80% of its aqueous solubility. Live samples and uninoculated controls were acidified with phosphoric acid after 48 hr of incubation.

[b] Mean and standard deviation of triplicate samples.

[c] Inoculated with soil but acidified with phosphoric acid at the beginning of incubation.

Routine sampling of the reactor began in July, 1996, and continued through the beginning of November, 1996. Approximately one month after routine sampling began we noticed a substantial decrease in the concentrations of fluoranthene, pyrene, benz[*a*]anthracene, and chrysene in the treated soil, and the lower concentrations were sustained through the remainder of Phase II operation. The data for pyrene are illustrated in Figure 4.3(a).

The apparently sudden decrease in concentration for several of the PAH was believed to be due to a change in the biological activity in the reactor, for several reasons: (1) there were no changes in the operation of the reactor during Phase II; (2) all of the samples from Phase II were analyzed as a single batch in random order and the recovery of internal standard was consistent for all of the samples; and (3) concentrations of benzo[*a*]pyrene did not change during the same period of operation (Figure 4.3(b)). Concentrations of phenanthrene also did not change and concentrations of anthracene decreased slightly during Phase II operation (data not shown). It is possible that the change in treated soil concentrations for some of the PAH may have been due to decreases in the feed soil concentrations of these PAH, but we do not have feed soil analyses that span the entire period of Phase II operation. All of the feed soil analyses were from the latter part of Phase II.

The concentrations of all seven PAH of interest during the latter part of Phase II operation (August 4 - November 8, 1996) are summarized in Table 4.4. Relatively good removal was obtained for all of the compounds except anthracene, which was present at a low initial concentration, and benzo[*a*]pyrene, for which there was no net removal over three months. The poor removal of benzo[*a*]pyrene during Phase II is consistent with the absence of

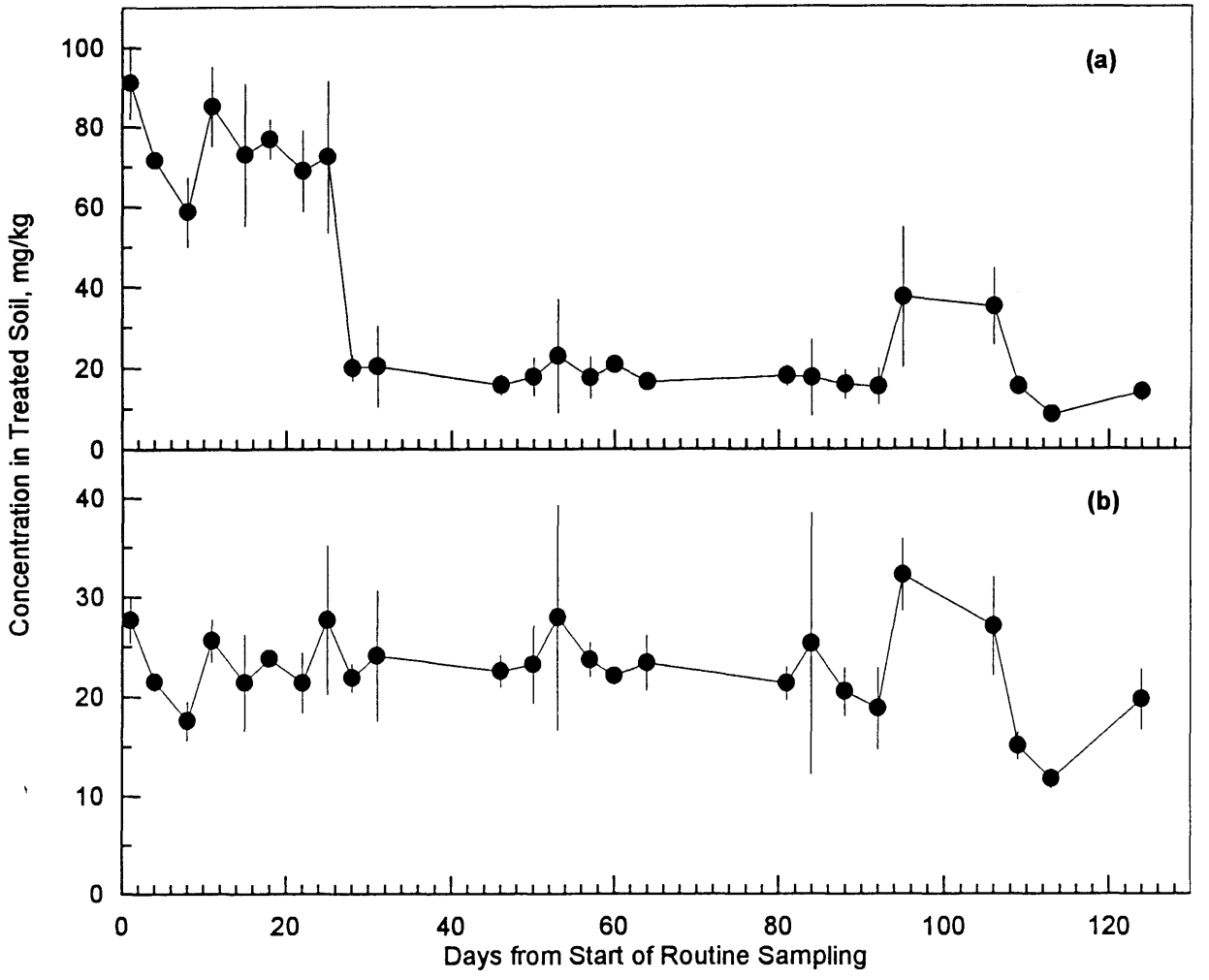


Figure 4.3. Concentrations of **(a)**pyrene and **(b)** benzo[*a*]pyrene during Phase II operation. Data are means and standard deviations of triplicates.

mineralization of benzo[*a*]pyrene in the experiment conducted during Phase I (Table 4.3) and the near-saturating concentration of benzo[*a*]pyrene in the liquid phase during Phase I (Table 4.2). These combined results suggest that the reactor contained little or no biomass capable of degrading benzo[*a*]pyrene at significant rates, that benzo[*a*]pyrene degradation was inhibited by the presence of competing substrates or by other conditions in the reactor, or that benzo[*a*]pyrene degradation is inherently far slower than that of other PAH.

Comparison of the reactor performance during Phase II (Table 4.4) with that from Phase I (Table 4.1) indicates that, in general, removal of most of the PAH was better during Phase II. A notable exception is benzo[*a*]pyrene, for which there essentially was no net removal during Phase II. The improved performance during Phase II may have been due to the increased mixing speed used relative to that used in Phase I, or may have been due to a long-term acclimation of the microbial community which manifested as sudden increases in removal for several PAH during the latter part of Phase II.

Table 4.4. Performance of the slurry-phase bioreactor during the last three months of Phase II.

Compound	Feed Soil, mg/kg [a]	Treated Soil, mg/kg [b]	% Reduction
phenanthrene	16 ± 9.0	2.5 ± 2.5	84
anthracene	3.8 ± 3.1	2.2 ± 1.0	42
fluoranthene	38 ± 6.6	< 5.0 ± 4.8 [c]	> 87
pyrene	89 ± 13	19 ± 5.6	79
benz[*a*]anthracene	16 ± 1.6	6.3 ± 1.5	61
chrysene	7.5 ± 1.3	< 1.6 ± 1.4 [d]	> 79
benzo[*a*]pyrene	22 ± 1.7	22 ± 3.9	0

[a] Mean and standard deviation of six samples.

[b] Mean and standard deviation of 17 samples. For sets with one or more samples below the detection limit, the detection limit itself was used in calculating the mean.

[c] Six samples were below the detection limit of 1.0 mg/kg.

[d] Fourteen samples were below the detection limit of 1.0 mg/kg.

Remediation standards applied to the treatment of PAH-contaminated soil are generally site-specific. However, the lack of benzo[*a*]pyrene degradation during Phase II suggests that the removal of benzo[*a*]pyrene and, perhaps, other high molecular weight PAH that were not analyzed might govern the technical feasibility of bioremediation at a given site. Thus, strategies to enhance PAH degradation during slurry-phase or other forms of biological treatment may need to be developed.

5. EFFECTS OF AMENDMENTS ON PAH REMOVAL FROM TREATED SOIL

During Phase II of the study we began to evaluate two strategies for enhancing PAH degradation: the addition of a nonionic surfactant (Brij 30) and the addition of supplemental carbon sources. Both strategies were envisioned to provide further PAH removal after an initial period of slurry-phase treatment, based on the assumption that enhancements would be required only for the more recalcitrant fraction of the PAH contamination.

Surfactant addition might be appropriate if PAH biodegradation were limited primarily by mass transfer into the aqueous phase. The envisioned process would involve treating the soil removed from the slurry-phase reactor in a subsequent batch or semi-continuous process. The treated soil entering the second reactor would already be enriched with a microbial community capable of degrading PAH, but not previously exposed to the surfactant. As discussed in Section 2, this situation is likely to minimize biodegradation of the surfactant during the critical period in which the surfactant influences the mass transfer of PAH. If the second reactor were not operated as a strictly batch process, then it would eventually develop a significant community of surfactant degraders. In this case, the surfactant might have to be added in an intermediate reactor (unaerated) preceding the second-stage bioreactor. The only surfactant considered in this study was Brij 30, a hydrophobic, nonionic surfactant. Brij 30 was shown recently to enhance the rate of crystalline phenanthrene dissolution to a much greater extent than other nonionic surfactants (Grimberg *et al.*, 1995). Experiments with the surfactant focused first on quantifying its sorption to the soil, its influence on PAH solubilization rates, and the equilibrium of PAH solubilization in the absence of biodegradation.

If HMW PAH degradation is limited primarily by a loss of growth substrates and the consequent starvation of the PAH-degrading microbial community, then the addition of appropriate carbon sources alone might increase the degradation of the HMW compounds. Such carbon sources could be added at any time (or at repeated intervals) during the operating period of a semi-continuous or batch slurry-phase process, or could be added in a second-stage bioreactor. If HMW PAH degradation were limited both by starvation of the microbial community and by mass transfer, then carbon-source supplementation would have to be combined with a means of improving mass transfer, such as surfactant addition. In this case, the operating schemes described above should be used to minimize surfactant biodegradation.

Solubilization of PAH by a Nonionic Surfactant

Surfactant sorption. As discussed in Section 2, when surfactants are added to soil some of the amount added will sorb to the soil. Thus, to quantify the effects of a surfactant on PAH solubilization it is first necessary to quantify the rate and extent of surfactant sorption.

The rate of surfactant sorption to both the untreated soil and the treated soil at a fixed initial surfactant concentration is illustrated in Figure 5.1. In both cases the surfactant concentration declined steadily over the first 24 hr and remained stable through 72 hr for the untreated soil and through 96 hr for the treated soil. Based on the results of these experiments, it was assumed that the surfactant reached equilibrium with the soil within 48 hr. For the applied surfactant dosage

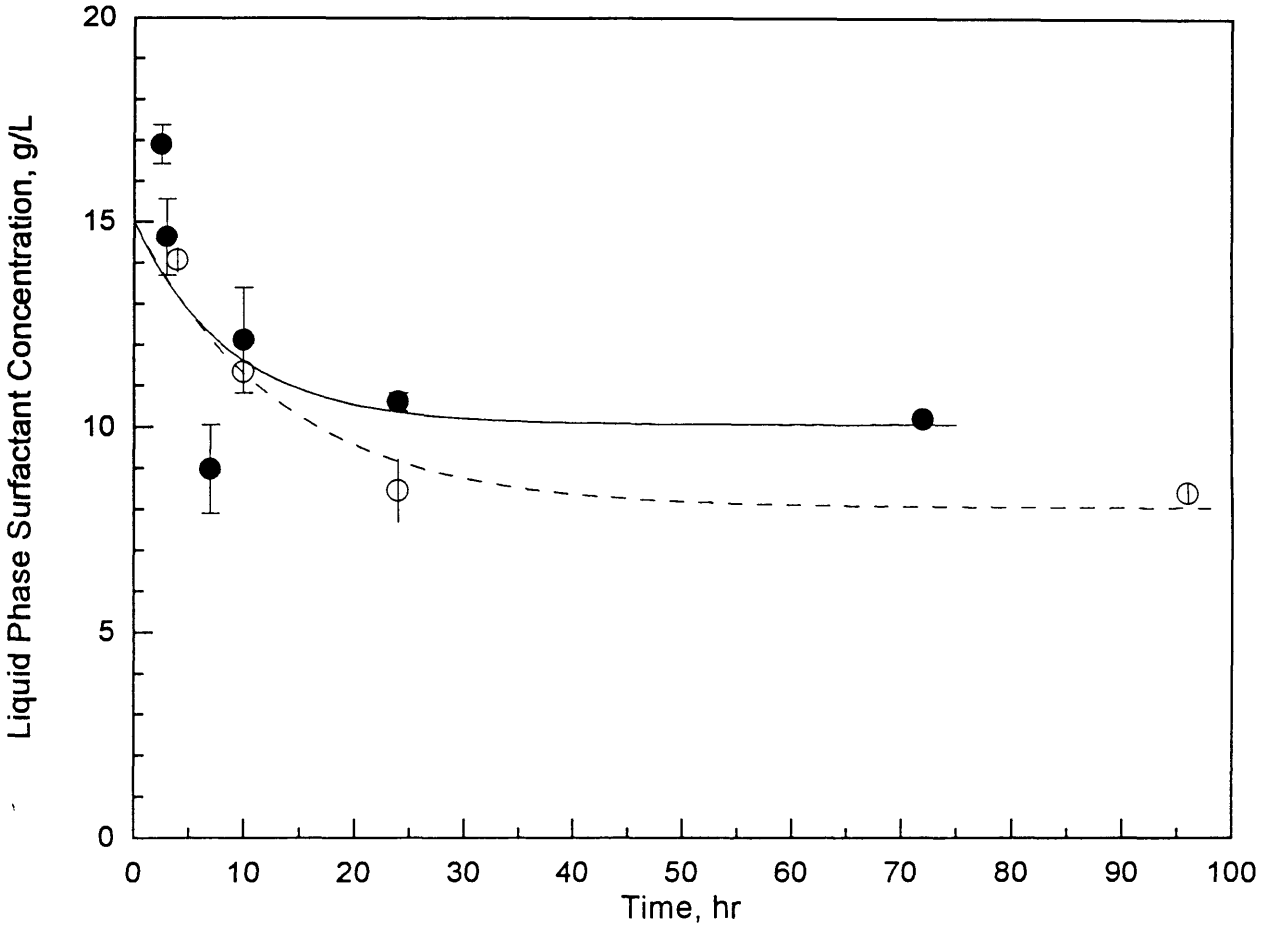


Figure 5.1. Liquid phase surfactant concentration as a function of time in batch experiments with untreated (•) and treated (o) soils. Data points and vertical bars represent means and standard deviations, respectively, of duplicate measurements. The solid and dashed lines represent best fits to eq. 3.2 for the untreated and treated soils, respectively. Flasks contained 30 g (dry wt.) soil solids, phosphate buffer (pH 7), and sodium azide at a concentration of 1% (wt:wt) in a volume of 200 mL. The initial dose of Brij 30 was 15 g/L.

used in this experiment (15 g/L or 100 mg/g dry soil), first-order sorption rates were estimated to be 0.12 hr^{-1} for the treated soil ($r^2 = 0.58$) and 0.076 hr^{-1} for the treated soil ($r^2 = 0.96$).

The extent of surfactant sorption after 48 hr was evaluated as a function of surfactant dose for both the untreated and treated soils. Results are shown in Figure 5.2. The untreated soil appears to have reached a maximum sorption capacity at a surfactant dose of 25 mg/g dry soil, beyond which the concentration in the liquid phase increased linearly with increasing dose. The slope of the linear increase in liquid-phase surfactant concentration vs. surfactant dose was near unity, indicating that all of the surfactant added above the maximum amount sorbed to the soil was available in the liquid phase. Maximum sorption capacities of soil for nonionic surfactants have been observed by others (Liu *et al.*, 1992; Edwards *et al.*, 1994b).

More surfactant sorbed to the treated soil (approximately 47 g/g dry soil) than to the untreated soil, although there were fewer data for the treated soil. In addition to the data shown in Figure 5.2, two higher surfactant doses were tested with the treated soil but the liquid phase surfactant concentration measurements were rejected as inaccurate based on an analysis of the liquid phase PAH concentration data (shown below) and because they deviated substantially (higher) from a 1:1 correspondence with surfactant dose. The higher sorption of surfactant in the treated soil than in the untreated soil in the equilibrium experiment is consistent with the sorption rate data after 24 hr shown in Figure 5.1. Increased surfactant sorption after slurry-phase treatment is probably due to increased surface area of the soil solids, which does not appear to be offset by potential decreases in NAPL content resulting from biodegradation.

PAH solubilization kinetics. Liquid-phase concentrations of PAH were measured as a function of time in the same experiment in which surfactant concentration was followed with time (Figure 5.1). The PAH data are summarized in Figure 5.3. Liquid-phase concentrations were followed over 72 hr for the untreated soil and over 96 hr for the treated soil, but only the data over the first 24 hr are shown. For the untreated soil there were no significant differences between concentrations at 24 hr and at 72 hr. For the treated soil, the concentrations at 96 hr were slightly higher than at 24 hr but the greatest changes in concentration occurred over the first 24 hr. These observations differ from those of Yeom *et al.* (1995), who noted that a rapid period of PAH solubilization from a tar-contaminated soil was followed by a slower period of solubilization for as long as 380 hr at high surfactant concentrations. However, these earlier observations were made with "soil" that contained 75% organic matter (Yeom *et al.*, 1995).

In both the untreated and treated soils, PAH concentrations approached saturation within 10 hr and were above 80% of saturation within three to four hours. Although these experiments were conducted in shake flasks, the conditions simulate those in a mechanically mixed reactor. Thus, it appears that PAH solubilization is rapid, even in treated soil in which a substantial fraction of the original mass of PAH was removed by biodegradation. The time scale over which solubilization occurred in these experiments is expected to be far shorter than the scale over which PAH biodegradation would occur. It is also expected to be shorter than the scale over which surfactant degradation would occur in the absence of a microbial community adapted to surfactant degradation.

Little previous work has been done on the effects of surfactants on solubilization of hydrophobic chemicals in contaminated soils, so that there is little information on how surfactants might influence mass transfer rates under field conditions. In the only previous study in which an attempt was made to quantify the influence of surfactants on rates of PAH solubilization in contaminated soil (Yeom *et al.*, 1996), the authors assumed that the surfactant acted primarily by influencing the diffusion of PAH through the NAPL matrix. However, we believe that surfactants are more likely to influence PAH transfer into the aqueous phase by enhancing solubilization of the NAPL phase, and therefore chose to evaluate the experimental data by assuming that PAH solubilization can be described by first-order kinetics (Grimberg *et al.*, 1995):

$$\frac{dP}{dt} = k_p \cdot (P_e - P)\,; \quad P(t=0) = 0 \tag{3.3}$$

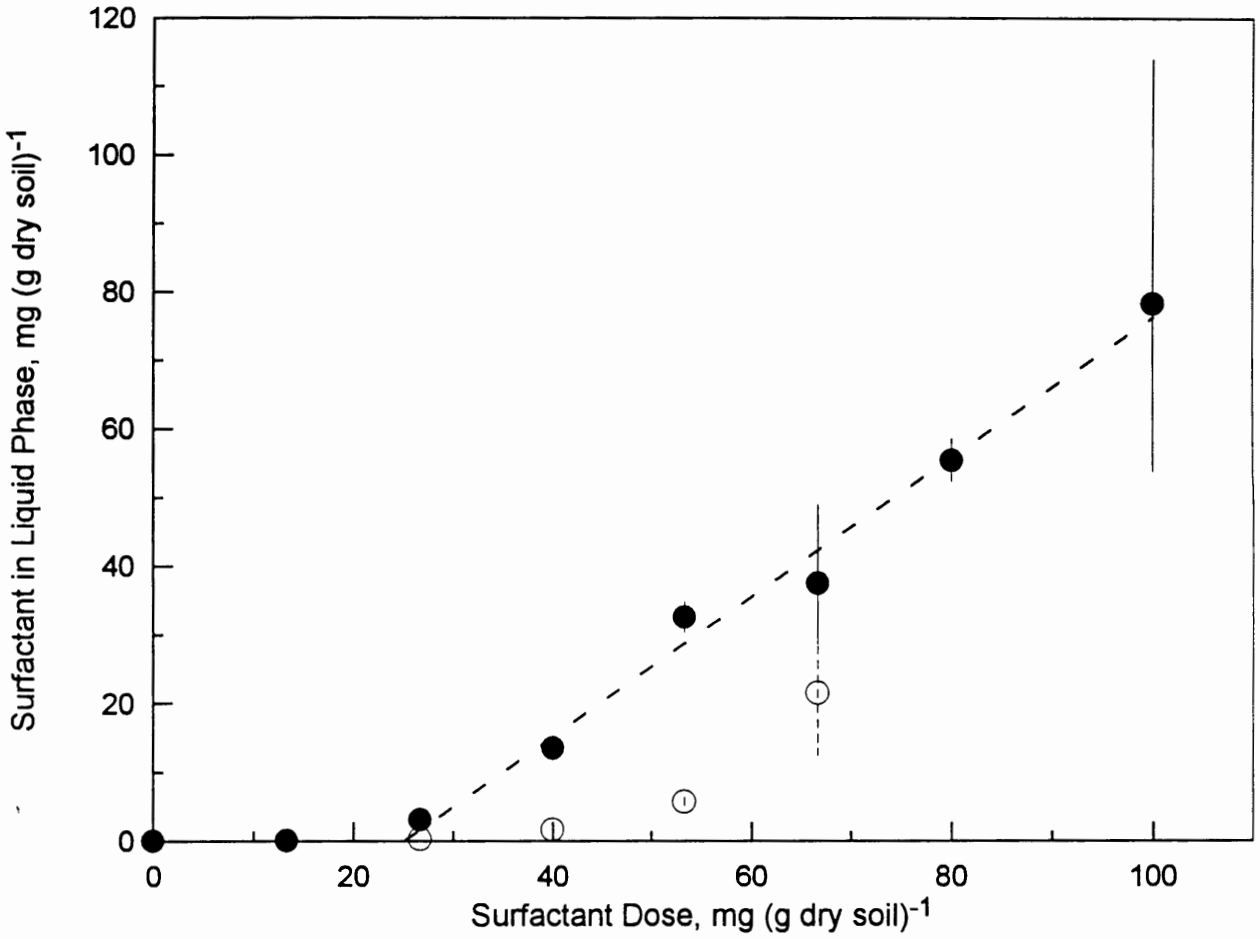


Figure 5.2. Equilibrium liquid-phase surfactant concentration as a function of initial surfactant dose in batch experiments with untreated (•) and treated (o) soils. Data points and vertical bars represent means and standard deviations, respectively, of duplicate measurements. Flasks contained 3.0 g (dry wt.) soil solids, phosphate buffer (pH 7), and sodium azide at a concentration of 1% (wt:wt) in a volume of 20 mL. Flasks were incubated for 48 hr before measuring surface tension in the liquid phase. The dashed line is the line of best fit for the untreated soil at surfactant doses higher than 20 mg/g, and has a slope of 1.02.

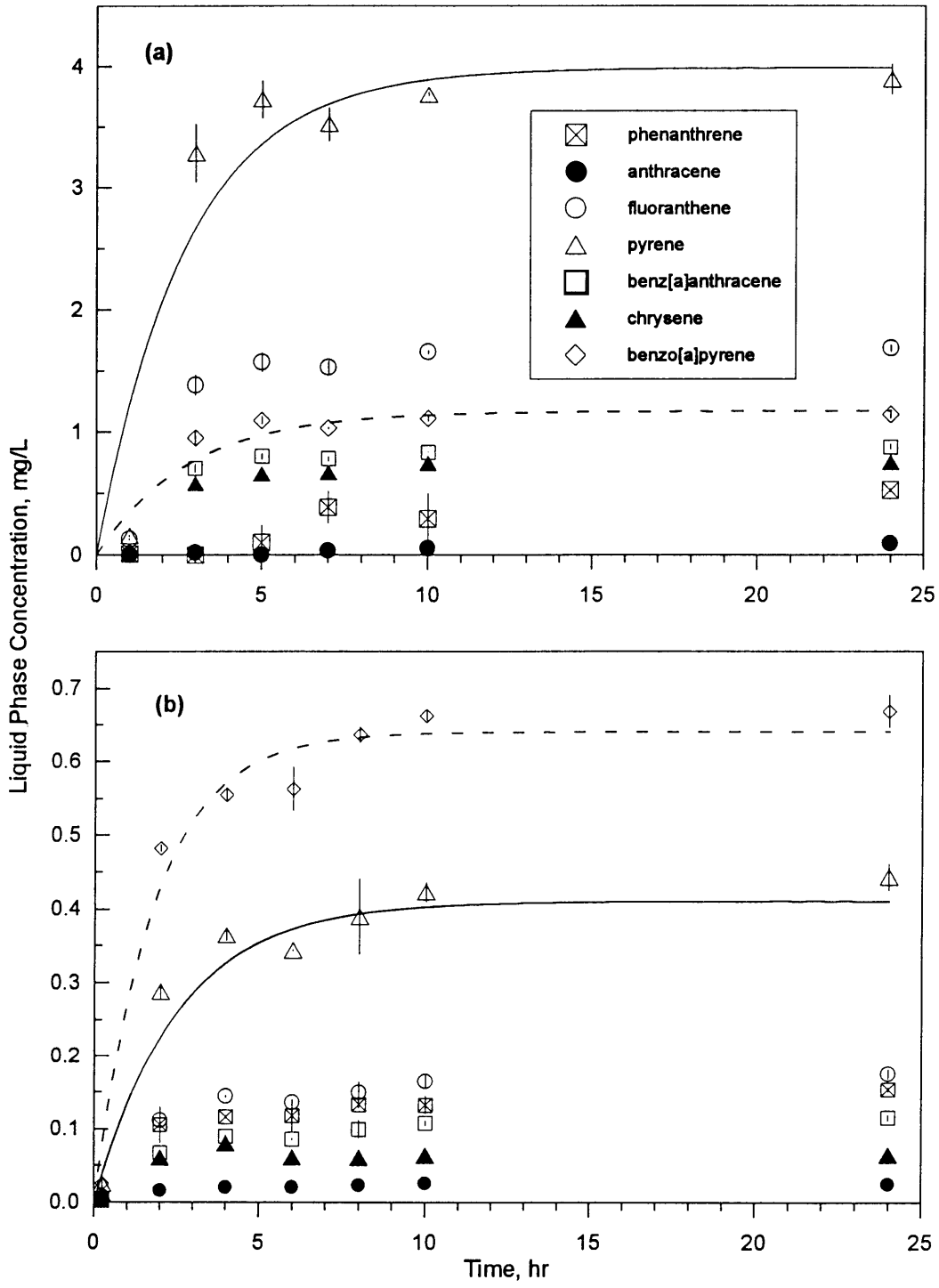


Figure 5.3. Solubilization of PAH in **(a)**untreated and **(b)** treated soils in batch experiments upon the addition of 15 g/L (100 mg/g dry solids) Brij 30. Data are means and standard deviations of duplicates and correspond to the data shown in Figure 5.1. Solid and dashed lines are fits of a first-order mass transfer model (eq. 3.4) to the data for pyrene and benzo[*a*]pyrene, respectively.

where P is the concentration of the PAH of interest in the liquid phase; k_p is the observed first-order mass transfer coefficient for PAH solubilization; and P_e is the PAH concentration in the liquid phase at equilibrium. In the simplest form of this model, P_e is assumed to be constant (i.e., the liquid phase saturation concentration is assumed to be constant), in which case the solution to eq. 3.3 is:

$$P(t) = P_e \cdot \left(1 - e^{-k_p \cdot t}\right) \tag{3.4}$$

Fits of equation 3.4 to the data for pyrene and benzo[*a*]pyrene are shown in Figure 5.4.

Since a significant fraction of the surfactant sorbed to the soil over the same period during which PAH solubilization occurred (Figure 5.1), we also evaluated a model which considered simultaneous surfactant sorption and solubilization of PAH by the surfactant remaining in the liquid phase. We fit this model to the experimental data and compared it to the fit obtained by assuming a constant surfactant concentration in the liquid phase. The fits for both models are summarized in Tables 5.1 and 5.2 for each of the PAH analyzed. The simpler model, which ignores the variation in surfactant concentration, resulted in a better fit than the more complex model. However, the fits are based on relatively few data points, particularly during the initial period of the experiments in which concentrations of both the surfactant and the PAH were changing most rapidly. In general the fits were better for the treated soil than for the untreated soil, probably because there were more data obtained during early time points with the treated soil.

For a given soil type (untreated or treated), the five compounds solubilized to the greatest extent resulted in very similar observed mass transfer coefficients (k_p, model 1 or model 2). This result suggests that, for each soil, the PAH were solubilized from a relatively homogeneous source (e.g., a tar phase). Huesemann (1997) has suggested that mass transfer of hydrocarbons out of soil into an aqueous phase is dominated by a compound's hydrophobicity (K_{ow}), so that mass transfer rates would decrease with increasing hydrophobicity; this effect was not observed for the treated soil in particular (Table 5.2), in which the seven PAH of interest have K_{ow} values that vary by 1.5 orders of magnitude (Table 2.1). The similarity of mass transfer coefficients within a given soil type is consistent with findings that the aqueous-side mass transfer dominates the kinetics of PAH dissolution from NAPLs (Kraatz *et al.*, 1997). For a given PAH, results from the different soils cannot be compared directly because the effective liquid-phase concentrations of the surfactant were different.

Equilibria of PAH solubilization. The solubilization of individual PAH from both the untreated and treated soils is shown in Figure 5.4. In a system containing only excess hydrophobic chemical (e.g., a NAPL) and water, there is usually a linear relationship between solubilized hydrophobic chemical and surfactant concentration above the CMC (Rosen, 1989). For both soil types in this study, however, it appears that the relationship between surfactant dosage and PAH concentration in the liquid phase was not linear.

Table 5.1. Best fit parameter values for kinetics of PAH solubilization from untreated soil.

	Model 1 [a]			Model 2 [b]		
Compound	k_p (hr^{-1})	P_e (mg/L)	r^2	k_p (hr^{-1})	q_m (mg/g)	r^2
phenanthrene	0.10	0.56	0.74	0.0026	0.10	0.82
anthracene	0.05	0.14	0.94	ND	ND	ND
fluoranthene	0.37	1.72	0.86	0.21	0.16	0.86
pyrene	0.37	3.99	0.83	0.22	0.38	0.83
benz[*a*]anthracene	0.58	0.84	0.74	0.25	0.081	ND
chrysene	0.48	0.73	0.75	0.22	0.071	0.11
benzo[*a*]pyrene	0.36	1.17	0.83	0.21	0.11	0.83

[a] First order model assuming constant surfactant concentration in the liquid phase; k_p and P_e are best fits to eq. 3.4.

[b] First order model accounting for variability in the liquid-phase surfactant concentration; k_p and q_m (effective solubilization capacity of the surfactant for the PAH) are best fits to eq. 3.6. ND, not determined (model did not converge on a unique pair of parameter values for anthracene data and an r^2 value could not be calculated for the fit to benz[*a*]anthracene data).

Table 5.2. Best fit parameter values for kinetics of PAH solubilization from treated soil. [a]

	Model 1			Model 2		
Compound	k_p (hr^{-1})	P_e (mg/L)	r^2	k_p (hr^{-1})	q_m (mg/g)	r^2
phenanthrene	0.56	0.14	0.89	0.23	0.013	0.83
anthracene	0.45	0.02	0.95	0.22	0.023	0.93
fluoranthene	0.50	0.16	0.95	0.23	0.016	0.87
pyrene	0.51	0.41	0.94	0.23	0.040	0.87
benz[*a*]anthracene	0.40	0.11	0.95	0.19	0.011	0.90
chrysene	1.13	0.07	0.85	0.74	0.00055	0.86
benzo[*a*]pyrene	0.56	0.64	0.96	0.27	0.060	0.89

[a] Notes as in Table 5.1.

In a system containing soil, water and surfactant, and in the absence of an excess hydrophobic chemical phase, hydrophobic chemicals will eventually reach an equilibrium between the aqueous, micellar, and soil phases (Edwards *et al.*, 1994a; 1994b). The relationship between surfactant concentration and hydrophobic solute concentration in the liquid phase is not linear in such systems (Edwards *et al.*, 1994a; 1994b). When the aqueous solubility of a hydrophobic chemical is very low relative to its solubilization in surfactant micelles, and when the range of surfactant concentrations is well above the CMC, the equilibrium liquid-phase concentration of the hydrophobic chemical (C_e) can be approximated by:

$$C_e = \left(q_{s,0} \cdot w_s\right) \cdot \frac{S_e}{K_{sm} + S_e} \tag{5.1}$$

where $q_{s,0}$ is the initial concentration of the hydrophobic chemical in the soil, w_s is the weight of soil per unit liquid volume, K_{sm} is a partition coefficient describing the partitioning of the chemical between the surfactant micelles and soil, and S_e is the surfactant concentration in the liquid phase at equilibrium.

Yeom *et al.* (1995) used eq. 5.1 to quantify the relationship between PAH and surfactant concentrations in the liquid phase in a soil heavily contaminated with coal tar. However, it is not clear that equilibrium relationships in soils that contain a NAPL phase are the same as in the absence of the NAPL. As discussed in Section 2, recent evidence suggests that PAH have a much higher affinity for the NAPL (creosote) in a contaminated soil than for the soil organic matter (Rutherford *et al.*, 1997). It is also likely that most, if not all, of the constituents in a NAPL can be solubilized, so that the bulk NAPL phase will decrease in mass with increasing surfactant concentration. A precise description of the equilibrium condition would require knowledge of the partitioning of a given PAH between soil organic matter, the NAPL, and the micelles (still ignoring the contribution of aqueous solubility). A confounding factor is that the composition of the NAPL could change if individual constituents are solubilized to different extents in the surfactant micelles. For complex NAPLs such as tars, neither the complete composition nor the solubilization capacities of individual constituents in the surfactant of interest would be known. Thus, it is virtually impossible to predict PAH distributions between the liquid and soil phases precisely.

The effect of a surfactant on solubilization of PAH from a complex NAPL might be reasonably estimated if it is assumed that the bulk composition of the NAPL at equilibrium is the same in the micellar phase as in the soil. Furthermore, if it is assumed that the bulk NAPL phase itself attains an equilibrium between the soil (e.g., soil organic matter) and the micelles, the solubilized concentration of an individual constituent of the NAPL would still be described by eq. 5.1. Under such conditions the value of K_{sm}, the partition coefficient for the individual PAH between the soil and micellar phases, would be independent of the compound (i.e., the same for all compounds). The solubilization curves for liquid phase concentrations normalized by the initial soil concentration would also be similar for all compounds.

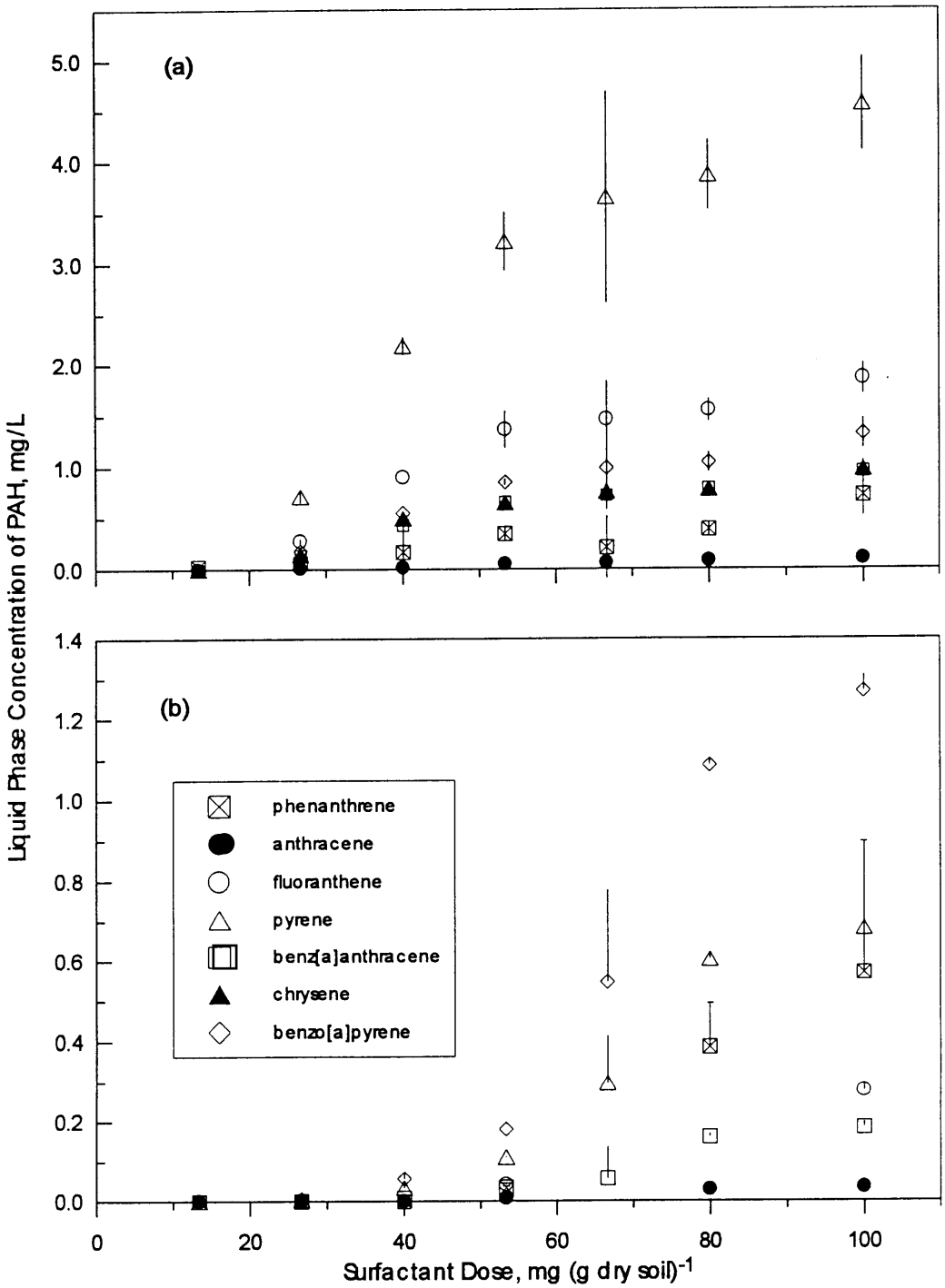


Figure 5.4. Liquid-phase PAH concentrations at equilibrium as a function of initial surfactant dose in **(a)**untreated and **(b)** treated soils. Experimental conditions were as described in Figure 5.2. Error bars (standard deviations) in panel (b) are shown in the positive direction only. All data for chrysene in the treated soil were below the detection limit. Note: surfactant dose x 0.15 = applied liquid concentration in g/L.

The solubilization data from Figure 5.4 are shown in normalized form in Figure 5.5. For the untreated soil, there is substantial overlap in the normalized concentrations (including the error) of all of the PAH except chrysene. There are fewer data for the treated soil, but with the error values the concentrations of all of the PAH also seem to overlap. The similarities in the normalized solubilization curves for most of the PAH are expressed quantitatively in the estimated K_{sm} values, which are summarized in Table 5.3. In comparing the estimated K_{sm} values in Table 5.3, it should be kept in mind that the estimates were based on the mean PAH concentrations in the soil, and did not account for the variability in these concentrations. While there are greater differences in the estimated K_{sm} values for the treated soil than for the untreated soil, there is no trend with K_{ow} of the PAH (the listed order of the compounds is roughly in order of increasing K_{ow}).

Table 5.3. Estimated K_{sm} values for individual PAH in untreated and treated soils.

	K_{sm} (g/L) [a]	
Compound	**Untreated Soil**	**Treated Soil**
phenanthrene	30 ± 7.8	0.9 ± 3.5
anthracene	49 ± 6.9	60 ± 13
fluoranthene	19 ± 3.7	12 ± 1.6
pyrene	18 ± 3.3	22 ± 3.8
benz[*a*]anthracene	15 ± 2.4	28 ± 5.1
chrysene	2.9 ± 0.5	ND [b]
benzo[*a*]pyrene	16 ± 2.5	12 ± 2.0

[a] Best fit value and 95% confidence interval from non-linear regression of eq. 5.1 to the liquid-phase PAH concentration data. Only the mean value of $q_{s,0}$ was used in the fitted equation, so that the error estimate for K_{sm} does not take into account the error in $q_{s,0}$.

[b] ND, not determined.

It is not clear why the equilibrium behavior of chrysene was so different than that of the other PAH in the solubilization experiment with untreated soil. The difference suggests either that chrysene had a substantially stronger affinity for the surfactant micelles than did the other compounds, or that it was not part of a homogeneous NAPL phase, as was assumed for all of the compounds. There are not enough data to distinguish between these, or other, possibilities.

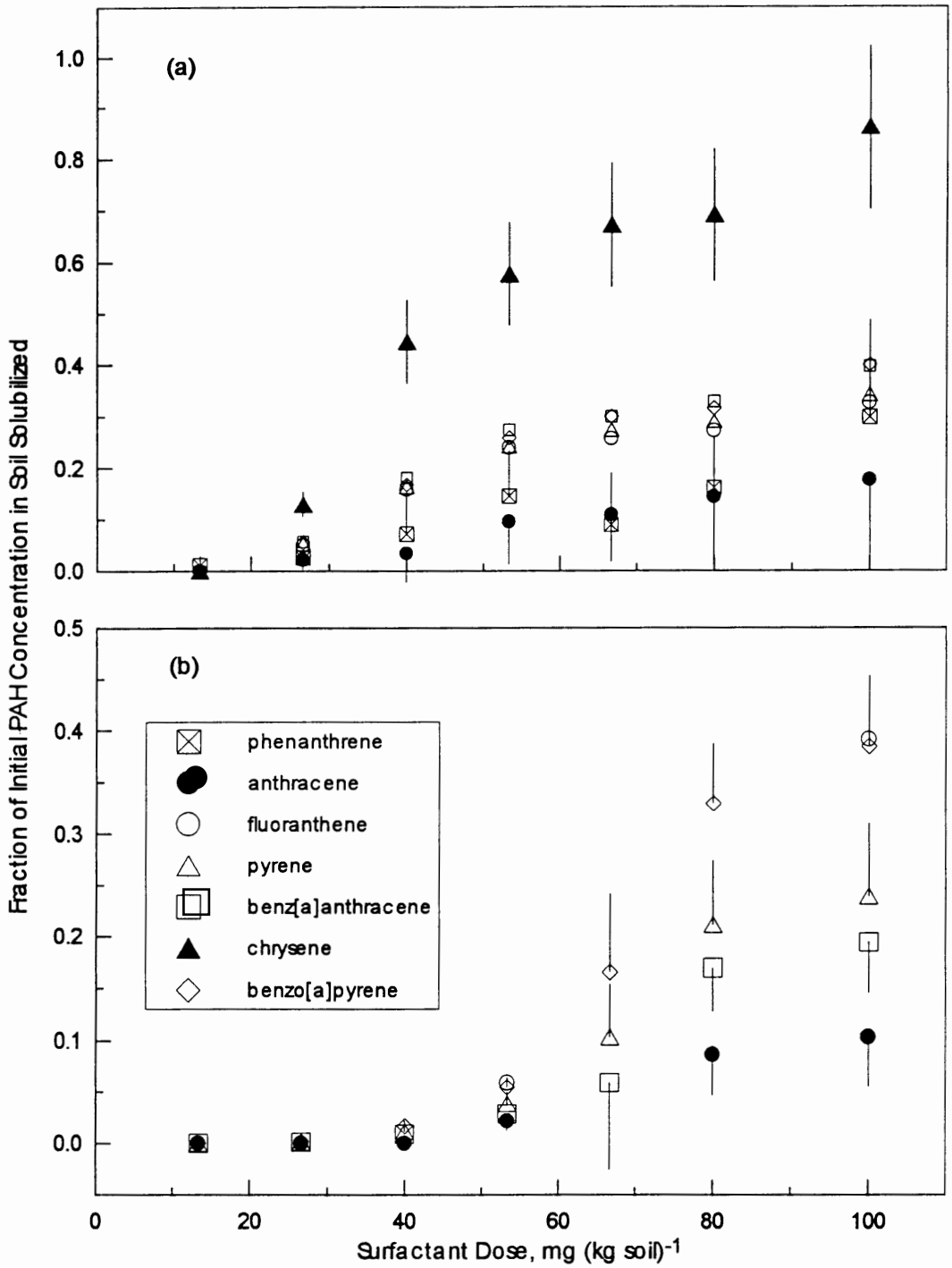


Figure 5.5. Data from Figure 5.4 normalized by the initial PAH concentration in the soil for **(a)** untreated soil and **(b)** treated soil. Error bars (shown in one direction only except for chrysene in panel [a]) represent combined errors in both the liquid phase concentrations and the initial soil concentration. Error bars in panel (a)are shown only for anthracene, phenanthrene and chrysene. Data for phenanthrene are omitted from panel (b) because of their wide scatter. Error bars for the two data points for fluoranthene are also omitted from panel (b) for clarity.

Effects of a Nonionic Surfactant and Carbon-source Supplements on PAH Biodegradation

An experiment was conducted to evaluate the effects of Brij 30 and supplemental carbon sources (salicylate and phthalate) on the biodegradation of PAH in treated soil removed from the slurry-phase reactor. The soil was mixed continuously for five days in shake flasks at a concentration that simulated the concentration in the slurry reactor (15% wt:wt). A five-day period was chosen because we believe that any positive effect of amendments should be manifested over a short period of time if their addition is to be cost-effective. Considering the extent to which PAH biodegradation occurred during the 18 day SRT of the reactor in the absence of amendments (Section 4), it seemed that any positive effects of the amendments would be observable within five days of batch treatment. The results of this experiment are summarized in Table 5.4.

Table 5.4. Effects of amendments on biodegradation of PAH in treated soil in a batch system. [a]

	Concentration After Five Days (mg/kg soil) [b]			
Amendment(s) [c]	**Anthracene**	**Pyrene**	**Benz[*a*]-anthracene**	**Benzo[*a*]-pyrene**
azide	3.00 ± 0.87	23.9 ± 7.9	8.27 ± 2.79	28.4 ± 9.7
none	1.75 ± 0.65 [d]	14.0 ± 5.8 [d]	5.53 ± 1.93	23.5 ± 6.3
salicylate	2.02 ± 0.75	15.9 ± 4.4 [d]	6.29 ± 1.38	26.1 ± 5.5
phthalate	1.99 ± 0.19 [d]	15.3 ± 2.7 [d]	6.61 ± 0.72	25.5 ± 2.7
salicylate, phthalate	1.75 ± 0.17 [e]	15.8 ± 1.1 [d]	6.11 ± 0.14	24.5 ± 2.1
azide, surfactant	1.91 ± 0.21 [d]	16.5 ± 1.2 [d]	5.05 ± 0.23 [d]	19.5 ± 1.4 [d]
surfactant	2.14 ± 0.34	18.1 ± 2.7	5.52 ± 0.76	19.8 ± 2.2
surfactant, salicylate, phthalate	2.53 ± 0.69	17.0 ± 0.5	5.25 ± 0.27	19.6 ± 0.9

[a] Soil slurry was removed from the reactor, centrifuged, then distributed to replicate flasks (10 g dry weight each) containing phosphate buffer, inorganic nutrients and other amendments as indicated in a final volume of 67 mL. The flasks were shaken continuously on a rotary shaker for five days.

[b] Sum of the concentrations in the soil and in the liquid phase (expressed as an equivalent soil concentration). Liquid-phase concentrations were below the detection limit for all samples that did not contain surfactant. Data are means and standard deviations of triplicates.

[c] Flasks amended with sodium azide (1% wt:wt) served as killed controls. Salicylate and phthalate were added at 250 μM. The surfactant (Brij 30) was added at 54 mg/g soil. All amendments were added as a single dose at the start of the incubation period.

[d] Significantly lower than killed control amended with azide only (*t*-test, $p < 0.10$).

[e] Significantly lower than killed control amended with azide only (*t*-test, $p < 0.05$).

Results are shown in Table 5.4 only for those PAH which had residual concentrations in the soil above the detection limit (2 mg/kg for this experiment) in all three replicate flasks. Most of the

flasks had soil concentrations below the detection limit for phenanthrene, fluoranthene and chrysene. In the flasks to which no amendments were added, slight removal of anthracene and pyrene seemed to occurr over the five-day batch incubation period in comparison to the abiotic control, but the difference between these samples was significant only at the 10% level. Such a modest decrease in PAH concentration with increased incubation time might not have been noticeable during the operation of the slurry reactor in Phase I, in which no significant difference in PAH concentration was observed between solids residence times of 18 and 35 d. For benz[*a*]anthracene and benzo[*a*]pyrene the differences between the samples and the killed control were not statistically significant (*t*-test, $p > 0.1$); this finding is consistent with data from the slurry-phase reactor, in which long-term removal of benzo[*a*]pyrene did not occur (Section 4). The addition of salicylate or phthalate did not enhance removal of the PAH over the five-day experimental period. Dissolved oxygen concentrations in all flasks remained at 70 - 80% of saturation, and the pH remained at approximately 7, for the duration of the experiment.

There was no removal of the PAH in the surfactant-amended samples relative to the abiotic control, either with or without the addition of salicylate and phthalate. The abiotic controls with surfactant added had significantly lower residual PAH concentrations than did the controls without surfactant. We suspect that PAH losses in the surfactant-amended controls occurred as a result of increased rates of volatilization, since liquid phase concentrations of the PAH were far higher than in the controls without surfactant.

In the flasks containing surfactant, there were substantial differences between the flasks to which sodium azide was added and those receiving only the surfactant. First, the apparent liquid phase concentrations of surfactant were very different, as were the liquid-phase concentrations of PAH (Figure 5.6; benzo[*a*]pyrene is shown as a representative example of the PAH data). In addition, the liquid phases in the flasks that were not amended with azide were dark black, whereas the liquid phases in the azide-amended flasks were slightly yellow but translucent. The dark color of the liquid phases in the flasks that did not receive azide probably was due to emulsification of the tar and other nonaqueous phases in the soil. To check this possibility, the liquid phases were pooled, passed through filters of various pore sizes, and analyzed by HPLC (Table 5.5). While the PAH concentration was unaffected by filtration through filters with 0.7 μm or 0.45 μm pore sizes, it appears that approximately half of the PAH were retained on filters with 0.2 μm pores. Much of the material retained on the filters at each pore size were solid particles. Thus, it is not clear that the PAH were truly "solubilized" in this experiment except in the abiotic controls.

The difference between azide-amended flasks and those receiving only surfactant can also be seen by comparing the measured liquid-phase PAH concentrations to those predicted from equilibrium considerations, using eq. 5.1 and the estimated K_{sm} values from Table 5.3; note that these K_{sm} values were determined in flasks that also contained sodium azide to inhibit biological activity. As expected, the predicted liquid-phase PAH concentrations in flasks amended with azide were close to the measured values, whereas the predicted concentrations in flasks not containing azide were much lower than the measured values after the first day (Figure 5.7).

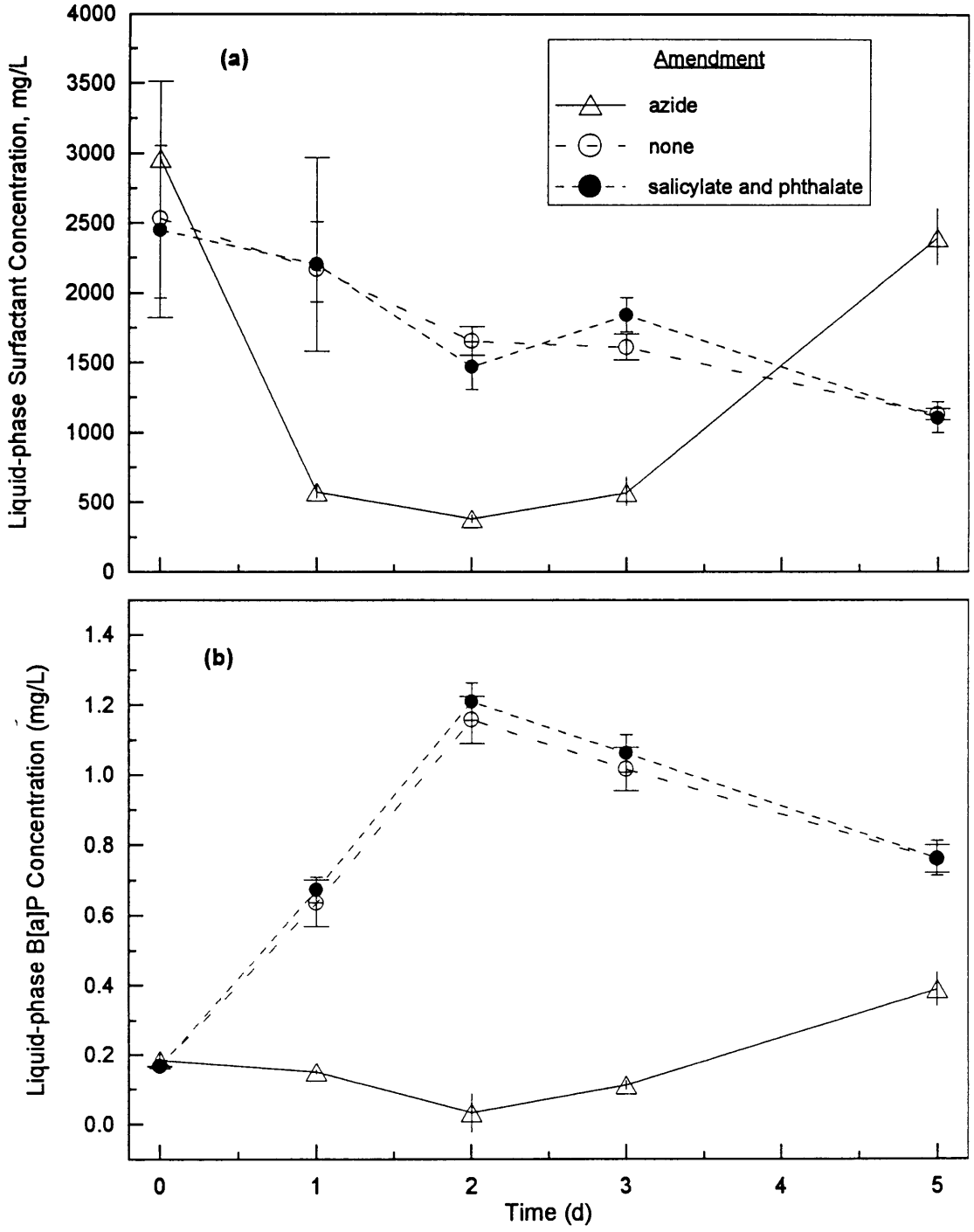


Figure 5.6. Concentrations of **(a)**Brij 30 and **(b)** benzo[*a*]pyrene as a function of time in liquid phases of flasks amended with surfactant. Other amendments are as shown in the legend; experimental conditions are described in notes to Table 5.4. Data represent means and standard deviations of triplicates.

Table 5.5. Effects of filtration on concentrations of PAH in combined liquid phases from flasks not amended with sodium azide.

	Liquid-phase Concentration, mg/L [a]				
	Filter Type				
Compound	**None**	**0.7 μm [b]**	**0.4 μm [c]**	**0.4 μm [d]**	**0.2 μm [b]**
phenanthrene	0.180	0.178	0.169	0.163	0.083
anthracene	0.057	0.060	0.054	0.052	0.026
fluoranthene	0.400	0.407	0.395	0.382	0.192
pyrene	1.21	1.23	1.19	1.15	0.58
benz[*a*]anthracene	0.264	0.260	0.252	0.036	0.118
chrysene	0.309	0.272	0.272	0.273	0.000
benzo[*a*]pyrene	1.14	1.12	1.10	1.07	0.59

[a] Analysis of a single sample (pooled liquid phases from all flasks not amended with azide). Filtrations were sequential (e.g., the liquid filtered through the 0.45 μm filter was the filtrate from the 0.7 μm filter). Experimental conditions are described in notes to Table 5.4.

[b] polycarbonate filter

[c] cellulose filter

[d] glass fiber filter

The reasons for the difference between the characteristics of the liquid phases in the flasks containing sodium azide and those without azide are not known. One possible explanation is that the substantial increase in ionic strength caused by the addition of sodium azide affected the chemistry of the soil/water/surfactant system. The addition of the same concentration of sodium azide to surfactant solutions in the absence of soil had no effect on the surface tension of the solutions (data not shown). However, it is possible that ionic interactions with the soil minerals and/or the soil organic matter could have significantly influenced the interaction of the solid phase with either the NAPL or the surfactant. Another explanation is that biological activity in the flasks not amended with azide caused the observed emulsification. It would be possible to distinguish between these explanations by adding another electrolyte, such as sodium chloride, to an otherwise "live" system, or by using another method to inhibit biological activity that did not simultaneously alter the ionic strength of the medium. These experiments have not yet been done.

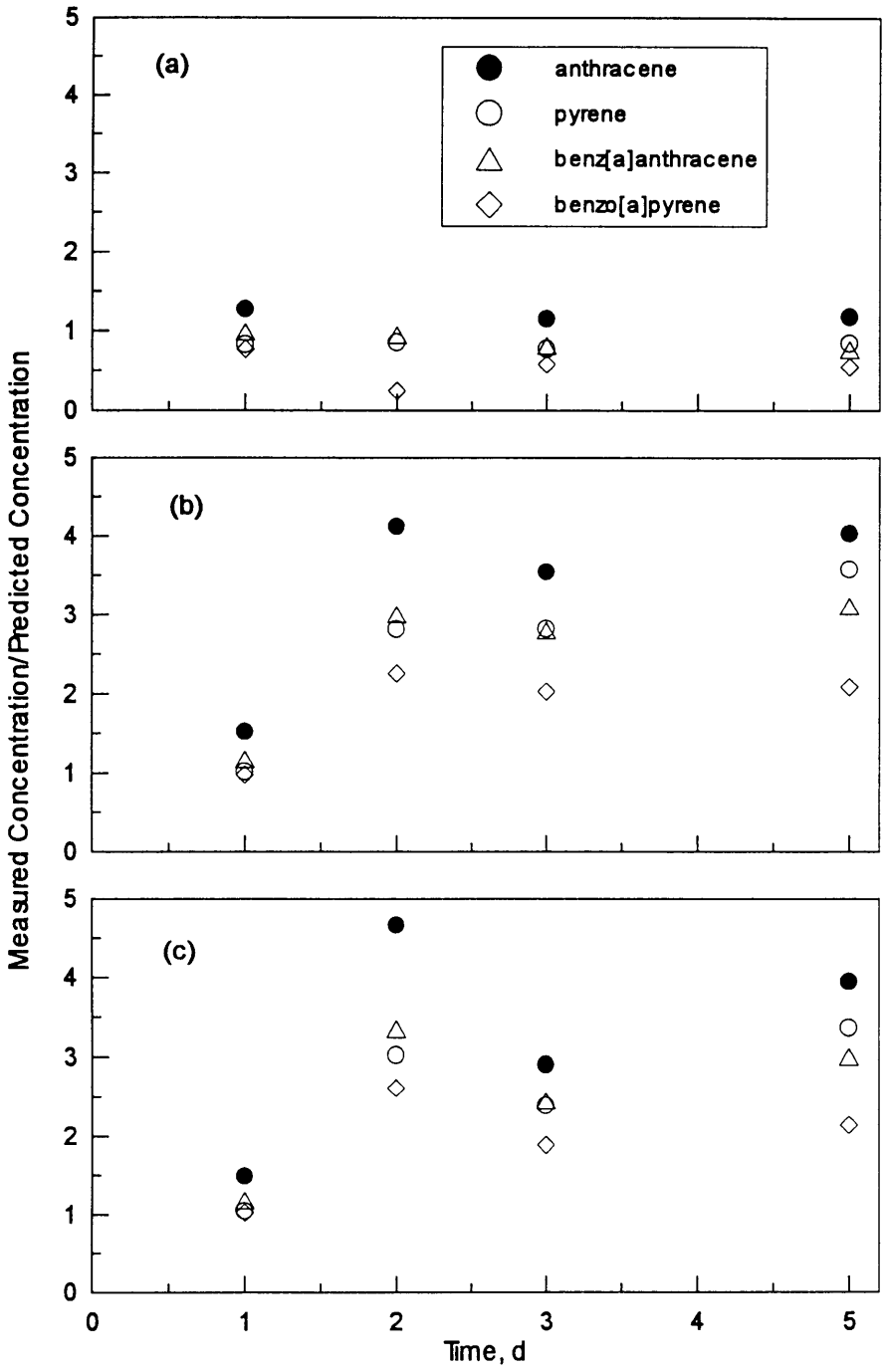


Figure 5.7. The ratio of measured to predicted liquid-phase PAH concentrations in flasks containing surfactant plus **(a)** sodium azide, **(b)** no other amendments, or **(c)** salicylate and phthalate. Predicted values were determined with eq. 5.1 using measured values of micelle concentration (S_e; Figure 5.5), PAH concentrations in the soil (values for the azide-amended controls without surfactant; Table 5.4) and estimated values of K_{sm} (Table 5.3). Experimental conditions are described in notes to Table 5.4. Data before day 1 are omitted because the systems would not have been in equilibrium.

Effects of Salicylate on Benzo[*a*]pyrene Mineralization

Benzo[*a*]pyrene was the only PAH of several tested that was not mineralized in an experiment conducted during Phase I (Section 4). We therefore re-evaluated the potential for mineralization of benzo[*a*]pyrene by the slurry removed from the reactor during Phase II, specifically to determine if mineralization could be stimulated by the addition of salicylate. Results are shown in Table 5.6. There was little recovery of $^{14}CO_2$ under any of the experimental conditions, but the two sets of vials containing salicylate did result in significantly higher amounts of radiolabel recovered in the CO_2 traps. There is no reason to believe that the vials containing salicylate systematically resulted in higher amounts of volatilization than did the other vials, so we believe that slight but measurable amounts of $^{14}CO_2$ were produced in these vials. However, the amount of ^{14}C recovered in the CO_2 traps in all the vials was less than the potential impurities in the ^{14}C-benzo[*a*]pyrene (stated by the manufacturer to be < 2%). Therefore, no conclusions can be drawn from this experiment regarding the potential presence of benzo[*a*]pyrene-degrading microorganisms in the soil slurry.

Table 5.6. Effects of supplemental carbon sources on benzo[*a*]pyrene mineralization [a]

Condition	^{14}C Recovered in CO_2 Traps, %
killed control [b]	0.36 ± 0.15
no amendment	0.34 ± 0.11
salicylate [c]	1.10 ± 0.25
succinate [d]	0.39 ± 0.03
salicylate [c] + succinate [d]	1.18 ± 0.42

[a] Vials contained diluted slurry from the reactor (approximately 4 mg dry solids) in 10 mL of M9 buffer (with or without a supplemental carbon source) containing radiolabeled benzo[*a*]pyrene at a concentration of approximately 80% of its solubility in water. Vials were incubated for 96 hr before quenching with acid and counting the radiolabel in the CO_2 traps. Substantial increases in biomass were observed in vials containing either or both of the supplemental carbon sources (salicylate or succinate) but were not quantified.

[b] Acidified to pH 2 with H_3PO_4.

[c] Salicylate added at 250 μM.

[d] Succinate added at 430 μM.

Conclusions on the Effects of Amendments on PAH Degradation

The surfactant Brij 30 was able to solubilize a substantial fraction of the PAH initially present in the soil, both for the untreated soil and the treated soil. However, relatively large quantities of the surfactant sorbed to the soil (25 g surfactant/kg soil solids for the untreated soil and approximately 48 g surfactant/kg soil solids for the treated soil) before appreciable concentrations

were observed in the liquid phase. A less hydrophobic surfactant would sorb less to the soil but also would solubilize less hydrocarbon per unit surfactant in the liquid phase. Thus, selection of a surfactant of the appropriate hydrophobicity depends on the specific application.

For an application such as soil flushing or soil washing, in which the surfactant would not be reused, the optimum surfactant would be that which minimizes total surfactant requirements (cost) in both the solid and liquid phases while removing the desired quantity of contaminant. For the application envisioned in conjunction with biodegradation of the contaminant (i.e., the surfactant effectively is reused until the desired reduction in contaminant concentration is achieved), the effect of the surfactant on rates of contaminant mass transfer into the liquid phase must be considered as well. We observed that Brij 30 resulted in rapid solubilization of the PAH of interest, reaching approximately 80% of equilibrium in the liquid phase within three to four hours. This rate of increase in liquid phase concentrations is far higher than would be required to stimulate biodegradation, for which a residence time or reaction period of several days would be adequate. Thus, it probably would be acceptable to use a surfactant resulting in slower rates of mass transfer for a given liquid-phase concentration if that surfactant sorbed to the soil much less significantly than did Brij 30.

Rates of mass transfer from a nonaqueous phase to an aqueous phase often depend on both an effective mass transfer coefficient and the equilibrium concentration of the solute in the aqueous phase. The overall influence of a surfactant on mass transfer rates therefore can involve effects on either or both of these parameters. For example, the solubilization capacity of nonionic surfactants for phenanthrene (i.e., the equilibrium concentration of solubilized phenanthrene per unit surfactant concentration above the CMC) depends strongly on the hydrophobicity of the surfactant, but the mass transfer coefficient is essentially independent of surfactant hydrophobicity (Grimberg *et al.*, 1995). Thus, rates of PAH mass transfer in the contaminated soil used in this study probably would have been within the same order of magnitude for surfactants of varying hydrophobicity. Accordingly, a surfactant substantially less hydrophobic than Brij 30 probably would have resulted in acceptable rates of PAH solubilization with far lower losses due to sorption to the soil.

The experiments conducted with surfactants alone were performed under conditions intended to preclude confounding effects of biological activity, particularly biodegradation of the PAH, the surfactant, or both. Sodium azide at a concentration of 1% (wt:wt) was used for this purpose, but in a subsequent experiment there were significant differences in liquid-phase characteristics between systems amended with azide and those that were not. In the presence of azide, both the apparent surfactant concentrations and the PAH concentrations in the liquid phase were lower than in systems to which azide was not added. In the absence of azide it appeared that emulsification was the primary mechanism by which the PAH were transferred into the liquid phase. Contaminants solubilized in micelles, which are extremely small and fluid, are expected to exchange rapidly with the aqueous phase (Grimberg *et al.*, 1995). In contrast, emulsified or particulate organics suspended in the liquid phase may still require dissolution or desorption across an interface, so that the transfer of PAH into the aqueous phase may be substantially slower than when they are solubilized in micelles. It is important to understand the distinctions between these mechanisms of mass transfer, as they may have very different impacts on PAH bioavailability. Many experiments involving the addition of surfactants to soil systems are

performed with either sodium azide or mercuric chloride as inhibitors of biological activity, and our results suggest that changes in ionic strength associated with the use of these agents may influence the results of the experiments.

A single experiment was conducted to test the effects of surfactants and supplemental carbon sources on PAH degradation in treated soil removed from the slurry-phase reactor. Neither the surfactant, the carbon sources, nor the combined additives stimulated PAH biodegradation over a five-day period. After five days there appeared to be saturating concentrations of the PAH in the liquid phases of the flasks containing surfactant; because of the emulsified and particulate nature of the contaminants in the liquid phase, however, it is possible that dissolution into the aqueous phase was still rate-limiting in these flasks. We do not know if the surfactant itself had a negative effect on the biomass in the system during this experiment.

There were various indications that benzo[*a*]pyrene was the least degradable of the PAH investigated. A final experiment to evaluate whether the slurry biomass was capable of mineralizing benzo[*a*]pyrene indicated that mineralization might be possible but, if so, at rates much slower than for the other PAH tested. Our research on pure cultures of PAH-degrading bacteria isolated from contaminated soils does not indicate that benzo[*a*]pyrene mineralization is less frequent than the mineralization of other high molecular weight compounds, but instead may be slower in those organisms capable of mineralizing the high molecular weight compounds (unpublished data).

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