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# Does bioavailability limit biodegradation? A comparison of hydrocarbon biodegradation and desorption rates in aged soils

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**Abstract**

In order to determine whether bioavailability limits the biodegradability of petroleum hydrocarbons in aged soils, both the biodegradation and abiotic desorption rates of PAHs and *n*-alkanes were measured at various time points in six different aged soils undergoing slurry bioremediation treatment. Alkane biodegradation rates were always much greater than the respective desorption rates, indicating that these saturated hydrocarbons apparently do not need to be dissolved into the aqueous phase prior to metabolism by soil microorganisms. The biodegradation of PAHs was generally not mass-transfer rate limited during the initial phase, while it often became so at the end of the treatment period when biodegradation rates equaled abiotic desorption rates. However, in all cases where PAH biodegradation was not observed or PAH removal temporarily stalled, bioavailability limitations were not deemed responsible for this recalcitrance since these PAHs desorbed rapidly from the soil into the aqueous phase. Consequently, aged PAHs that are often thought to be recalcitrant due to bioavailability limitations may not be so and therefore may pose a greater risk to environmental receptors than previously thought.

## Introduction

It is commonly believed that bioavailability limitations are responsible for the slow and/or incomplete biodegradation of petroleum hydrocarbons in aged soils and sediments. Several arguments support this claim: First, it has been shown that hydrophobic contaminants with increasing age become sequestered in the soil matrix via partitioning into soil organic matter or diffusion into nanopores, thereby becoming less extractable and bioavailable to microorganisms and other ecological receptors (Alexander 1995, 2000; Chung & Alexander 1998, 1999; Hatzinger & Alexander 1995, 1996; Huesemann 1997; Kelsey & Alexander 1997; Kelsey et al. 1997; Liste & Alexander 2002; Luthy et al. 1997; Macleod & Semple 2000; Madsen 2003; Nam & Alexander 1998; Pignatello & Xing 1996; Schwartz & Scow 2001; Tang & Alexander 1999). Second, mathematical models that couple biodegradation with mass-transfer processes such as desorption from soil organic matter, partitioning from NAPLs into the aqueous phase, or pore diffusion, have often been successfully used to either curve-fit or predict the observed biodegradation behavior of hydrophobic contaminants, leading to the conclusion that mass-transfer rate or bioavailability limitations are responsible for slow biodegradation kinetics (Bosma et al. 1997; Geerdkink et al. 1996; Ghoshal & Luthy 1996, 1998; Ghoshal et al. 1996; Ramaswami & Luthy 1997; Ramaswami et al. 1997; Scow & Alexander 1992; Shor et al. 2003; Wick et al. 2001; Zhang et al. 1998). Third, the observation that contaminant desorption kinetics often resemble in appearance the biphasic biodegradation behavior (i.e., fast initial drop followed by a much slower long-term decrease in contaminant

concentrations) has led to the interpretation that desorption controls biodegradation processes (Williamson et al. 1997).

A few investigators have measured both contaminant desorption and biodegradation kinetics during bioremediation treatment to determine whether bioavailability limitations are responsible for the slow or incomplete biodegradation of aged soils. For example, Berg et al. (1998) and Loehr & Webster (2000) measured the abiotic release rate of PAHs at various times during bioremediation treatment and found that both the rate and extent of PAH release decreased with increasing treatment time. Since hardly any PAHs were released after seven months of treatment, Berg et al. (1998) concluded that slow PAH biodegradation was caused by the equally slow abiotic desorption of these contaminants. Carmichael et al. (1997) observed that phenanthrene and chrysene desorption rates were much greater than biodegradation rates in freshly spiked soils but that desorption rates were slower or equal to mineralization rates in aged soils with high organic matter content, indicating that bioavailability limitations control PAH biodegradation processes in some soils. By contrast, Cornelissen et al. (1998) measured the desorption kinetics of 15 PAHs from sediments before and after bioremediation and found that while some PAHs rapidly desorbed and biodegraded, others did not degrade at all despite the fact that they were readily bioavailable as indicated by large rapidly desorbing fractions (up to 55%) at the end of biotreatment. Similarly, Huesemann et al. (2002) observed that after 90 weeks of slurry treatment, a number of high molecular weight PAHs were recalcitrant to biodegradation although they were released abiotically to a significant degree indicating that microbial factors rather than the lack of bioavailability limited the biodegradation of these PAHs.

The belief that slow or incomplete biodegradation of hydrocarbons in aged soils is primarily caused by bioavailability limitations has significant implications for risk assessment. If indeed the residual PAHs that remain in aged soils and sediments even after long and extensive biotreatment are recalcitrant to biodegradation because they are sequestered and therefore not available for uptake and metabolism by microorganisms, it can easily be argued that a lack of bioavailablity implies also a lack of toxicity and risk to potential environmental receptors. In short, if recalcitrance is due to bioavailability limitations, residual soil contaminants are unlikely to pose serious risks to the environment which, in turn, is likely to translate into significant cost-savings for liable parties because soils with residual contaminants can be left in place without further treatment. Indeed, numerous investigators have demonstrated a decrease not only in contaminant concentration but also bioavailability, leachability, and toxicity with increasing biotreatment time and concluded that 'environmentally acceptable endpoints' were reached at the end of the treatment period although residual contaminants were still present in the soil (Kelsey & Alexander 1997; Linz & Nakles 1997; Loehr & Webster 1997; Loehr et al. 2000a, b; Olivera et al. 1998; Stroo et al. 2000; Tabak et al. 2003; Weissenfels et al. 1992).

While the concept that bioavailability limitation is the principal cause for recalcitrance is appealing, both mechanistically and economically, it must be borne in mind that most studies mentioned above provide only indirect and circumstantial evidence. Only very few studies have focused directly on assessing the importance of mass-transfer limitations by measuring both abiotic release rates and biodegradation rates during bioremediation treatment (Berg et al. 1998; Carmichael et al. 1997; Cornelissen et al. 1998; Huesemann et al. 2002, 2003; Loehr & Webster 2000). It is therefore the objective of this research to determine the role of bioavailability limitations during the slurry bioremediation of PAHs and alkanes in aged soils by directly comparing abiotic desorption and biodegradation rates for respective hydrocarbons at various times during the treatment period. In the context of this study, we regard bioremediation as limited by bioavailability if the observed HC biodegradation rates are similar in magnitude to the respective HC desorption rates. Conversely, bioremediation is considered to be limited by microbial factors if desorption rates are significantly greater than biodegradation rates.

## Materials and methods

### *Aging procedure for soils*

As shown in Table 1, six different soils characterized by a wide range of physical properties (i.e.,

*Table 1.* Characteristics of the six aged, hydrocarbon contaminated soils used in biodegradation experiments

| Soil name | Soil properties | Contaminant type | Aging duration |
|---|---|---|---|
| Belhaven | 20.2% TOC<br>4% Clay | HC mixture of 16 HCs<br>@ ca. 16,000 mg/kg | 51 months<br>(in laboratory) |
| Cullera | 3.1% TOC<br>42% Clay | HC mixture of 16 HCs<br>@ ca. 16,000 mg/kg | 51 months<br>(in laboratory) |
| Sassafras | 1.6% TOC<br>10% Clay | HC mixture of 16 HCs<br>@ ca. 16,000 mg/kg | 51 months<br>(in laboratory) |
| Minas loam | Sandy loam soil | Minas crude oil<br>@ ca. 50,000 mg/kg | 21 months<br>(in laboratory) |
| Bunker C sand | 59% Sand, 31% Silt<br>10% Clay | Bunker C fuel<br>@ ca. 2,500 mg O&G/kg | >5 years<br>(field site) |
| Coal tar sand | NA | Coal tar<br>@ ca. 50,000 mg O&G/kg | >10 years<br>(field site) |

sand, silt, clay, and organic matter content), contaminant composition (i.e., defined hydrocarbon mixtures, crude oil, coal tar, or Bunker C fuel), and aging history (i.e., aged either in the laboratory or under field conditions) were used in this study (Huesemann et al. 2003). All values are noted as weight percent, on dry soil basis. A set of three soils, i.e., Belhaven, Cullera, and Sassafras were air dried, sieved (<2 mm), sterilized using $\gamma$ radiation, moisture adjusted, and spiked with a mixture of 16 model hydrocarbons (dissolved in ethyl-benzene) consisting of eight *n*-alkanes ($C_9$, $C_{10}$, $C_{12}$, $C_{14}$, $C_{16}$, $C_{20}$, $C_{22}$, and $C_{24}$), pristane, 1-octadecene, four PAHs (naphthalene, phenanthrene, pyrene, and benzo(*a*)pyrene), *cis*-decalin, and methyl-cylco-hexane at a final soil concentration of ≈1000 mg/kg each. After evaporation of the solvent, the moisture of all three soils was adjusted to 70% of field capacity. The spiked soils were then transferred to glass jars that were tightly capped and subsequently kept in the dark at room temperature for a 51 month aging period. A sandy loam soil was spiked with a Minas crude oil (32° API gravity) at a final concentration of ≈50,000 mg/kg by mixing 500 g dry soil with 25 g crude oil dissolved in 100 ml methylene chloride and then evaporating the solvent in a rotary evaporator flask to ensure the even coverage of all soil particles. After adjustment of the moisture content to ≈75% of field capacity, the spiked soil was transferred to a glass jar which was flushed with nitrogen, tightly capped, and subsequently stored in the dark at room temperature in the laboratory for 21 months.

It should be noted, as discussed by Madsen (2003), that soil sterilization by $\gamma$ radiation as well as spiking of contaminants dissolved in solvents does not exactly simulate the aging process in field soils and therefore may introduce experimental artifacts that must be considered when drawing conclusions from these biodegradation experiments in terms of the behavior of actual field soils. In order to address this limitation, an aged (>5 years) Bunker C contaminated sandy field soil from a Navy site at Port Hueneme, CA, and an aged (>10 years) coal-tar contaminated sand from a disposal ditch at a coal-gasification plant in Iowa were also used in this study. The gravimetric oil and grease and tar contents of the Bunker C and coal tar contaminated soils were 0.25% and 5%, respectively.

### *Slurry bioreactor operation and sampling*

The aged soils were bioremediated in 2 l slurry reactors for either 182 days (Sassafras), 270 days (Belhaven, Cullera, Bunker C and coal tar contaminated soils), or 354 days (Minas crude oil contaminated soil). To initiate biotreatment, all reactors were inoculated with 50 ml of a mixed bacterial culture that had been enriched in

hydrocarbon degraders adapted to Minas crude oil as a sole carbon source.

The enrichment culture was carried out by adding 20 g of an aged crude oil contaminated soil (i.e., Richland topsoil) which was used in an earlier study (Huesemann et al. 2002) as an inoculum to 100 ml sterile Bushnell Haas mineral medium. The resulting soil slurry was mixed vigorously on a shaker table for 1 h, centrifuged at 500 g for 15 min, and the supernatant was transferred to a 1 l Erlenmeyer flask. 1 ml of Minas crude oil was then added as a sole carbon source, together with 900 ml sterile Bushnell Haas medium, to initiate the growth of hydrocarbon degrading bacteria. The enrichment culture was continuously mixed at 100 rpm and aerated with humidified air at 500 ml/min. Additional crude oil (1 ml), together with sterile nutrient medium containing ammonium nitrate and potassium phosphate to maintain a C/N/P ratio of 100/5/1, was added every 7 days for 6 weeks to continue the enrichment process. The number of hydrocarbon degraders in the final enriched culture which served as an inoculum (50 ml) for all bioreactors was $10^9$ per ml (see method below).

The slurry consisted of ≈20% solids by weight, and the aqueous medium contained ammonium nitrate and potassium phosphate to maintain a C/N/P ratio of 100/1/0.2 (wt) (Huesemann 1994). Calcium chloride (1.11 g/l) was also added as a dispersion agent to avoid the clotting of clayey materials, and the pH was maintained between 6 and 7 throughout the treatment via addition of NaOH. Each soil slurry was placed into a 2 l Erlenmeyer flask (bioreactor) and mixed at 500 rpm with a propeller driven by an electric motor. Humidified air was sparged through a diffuser stone into the slurry to maintain a dissolved oxygen concentration above 2 mg/l. No attempt was made to monitor the bioreactor off-gases for volatilization losses since the subsequent data analysis was restricted primarily to high molecular weight PAHs and alkanes that do not volatilize to any significant extent at the ambient laboratory temperature (Huesemann et al. 1995).

At specified time intervals, representative slurry samples (≈25 ml) were taken from the reactors, transferred to 30 ml glass centrifuge tubes, and centrifuged at 2000 rpm (850 g) for 30 min. The supernatant was carefully removed and stored for future use in the abiotic release rate assay (see below). The wet soil pellet in the bottom of the centrifuge tube was mixed vigorously with a spatula and a 1.5 g subsample was taken for the gravimetric determination of the moisture content (Method 2540B 1989). Another representative 1.5 g of wet soil was removed from the centrifuge tube, transferred to a 250 ml Quarpak glass jar and extracted for subsequent hydrocarbon (HC) analyses as described below. The wet soil that remained in the centrifuge tube was used for the determination of abiotic HC release rates as outlined next.

*Abiotic release rate assay*

The rate of release of PAHs and n-alkanes from soil slurries under abiotic conditions was measured using procedures that were adapted from those reported by Williamson et al. (1998). Approximately 8 g of pre-wetted (=3 g dry wt) Amberlite XAD-2 resin (Supelco, Bellefonte, PA) and 0.3 g of sodium azide (microbial poison) were added to the centrifuge tube which contained the remaining soil pellet. A specified volume of the reserved supernatant was returned to the centrifuge tube to recreate a slurry with the same solids density as the original bioreactor slurry (i.e., ≈20% solids by weight). A teflon-lined cap was tightly screwed onto the centrifuge tube which was then rolled on a modified rock roller at ≈100 rpm. During this intensive mixing process, the XAD resin absorbs 'instantaneously' any hydrophobic contaminants that are released into the aqueous phase from the soil particles by either desorption or dissolution.

Since the presence of XAD resin maximizes the contaminant concentration gradient at the water-soil interface by maintaining hydrocarbon concentrations in the water phase close to zero, the measured abiotic release rates are probably close to maximum achievable rates in a given soil slurry (Loehr & Webster 2000) and therefore possibly greater than 'real' desorption rates in bioreactor slurries where bacteria – because of metabolic insufficiencies – may not be able to keep the concentration of dissolved hydrocarbons in the water phase to a minimum. Thus, the contaminant mass-transfer rates 'sensed' by individual bacterial cells could potentially be smaller than those measured in the bulk slurry using XAD resin. Nevertheless, in the absence of measurements of hydrocarbon mass-transfer rates in the immediate surroundings

of the bacterial cell – which nobody has yet figured out how to do – it is implicitly assumed in the subsequent data analysis that hydrocarbon biodegradation is limited by microbial metabolism if abiotic desorption rates are significantly greater than biodegradation rates.

Based on independently performed measurements of the maximum sorptive capacity of XAD resin, it was assured that the XAD was never close to being saturated with the released hydrocarbons during the performance of the abiotic release assays. The centrifuge tube was removed from the rollers at the same time as the next slurry sample was taken from the bioreactor. This enables the direct comparison of both biodegradation and abiotic release rates during exactly the same time interval. In order to facilitate the separation of XAD resin from the soil particles, 3 g of NaCl was added to the centrifuge tube that was then centrifuged three times at 2000 rpm for 30 min. Between each centrifugation, the XAD resin floating on top of the supernatant was gently mixed to remove any potential soil particles still adhering to the resin without disturbing the soil pellet. After the third centrifugation, the floating XAD resin and most of the supernatant were poured into a 250 ml Quarpak glass jar. Most of the supernatant was transferred back to the centrifuge tube via a pipette and the clean XAD resin was extracted and analyzed for hydrocarbons as described below. The soil pellet and the supernatant remaining in the centrifuge tube were spun at 2000 rpm for 30 min and after carefully decanting the supernatant, the soil pellet was transferred to a 250 ml Quarpak glass jar for extraction and analysis of hydrocarbons as described next.

*Hydrocarbon analyses*

For each slurry bioreactor sampling event, a set of three different samples were generated for subsequent HC analyses: (a) soil from the bioreactor; (b) soil remaining after exposure to XAD resin; and (c) XAD resin. Both soils and XAD resin were extracted by adding 100 g of sodium sulfate (drying agent) and 100 ml of methylene chloride to the respective 250 ml Quarpak glass jars and by rolling them for 12 h at $\approx$100 rpm. For the Belhaven, Cullera, and Sassafras soils and the respective XAD resins, the resulting solvent extracts were analyzed for PAHs and *n*-alkanes *via* GC-MS using standard EPA methods 3540, 3630C, and 8270 as a guideline (EPA 1986). For the Bunker C, coal-tar, and Minas crude oil contaminated soils and the respective XAD resins, the extracts were separated *via* silica column chromatography into an aromatic and saturated hydrocarbon fraction that were subsequently analyzed for PAHs, *n*-alkanes, and biomarker compounds (only for Minas) *via* GC-MS as described in detail by Wang et al. (1994). In order to minimize the effects of sampling errors that occur in heterogeneous soil slurries, all PAH concentrations in the Minas crude oil contaminated soil were normalized to the respective concentrations of the conservative biomarker 17$\alpha$,21$\beta$-hopane (Butler et al. 1991). Using the gravimetric moisture content of the original wet soil pellet, all HC concentrations were calculated as ng per g of dry soil and then normalized to the respective PAH or alkane concentrations in the initial bioreactor slurry. A comparison of the HC masses in the initial soil slurry sample with the respective HC masses in both the XAD resin and the soil slurry after XAD exposure indicated that, with few exceptions, a mass balance of better than 100 $\pm$ 10% was achieved for most HCs.

*Comparison of biodegradation rates and abiotic release rates*

The biodegradation rate for a specific compound between sampling times $t_n$ and $t_{n+1}$ is readily visualized as the slope between the bioreactor soil slurry concentration points $C_n$ and $C_{n+1}$ in a $C$–$t$ graph (see solid ●---● lines in Figures 1–7). Similarly, the abiotic release rate is the slope between the bioreactor soil slurry concentration point $C_n$ and the concentration of the respective hydrocarbon that remains in the soil after exposure to XAD resin during the time interval $t_n$ to $t_{n+1}$ (see dashed ○- - -○ lines in Figures 1–7). While the solid ●---● lines show the change in HC concentrations in the soil slurry bioreactor with treatment time, the dashed ○- - -○ lines show the change in HC concentration in a soil slurry sample taken from the bioreactor for measurement of abiotic desorption rates for a limited time interval using XAD resin. Given that the duration of the abiotic desorption test was exactly the same as the time interval between taking samples from the slurry bioreactor, it is then possible to directly compare biodegradation and desorption rates of

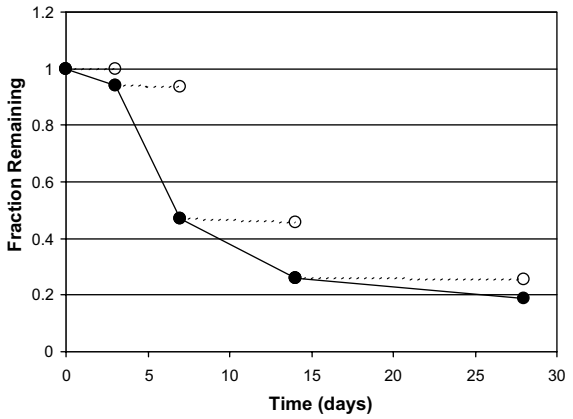


*Figure 1.* Fraction of hexadecane as a function of time in Belhaven soil undergoing slurry biodegradation (●) or abiotic desorption treatment (○).

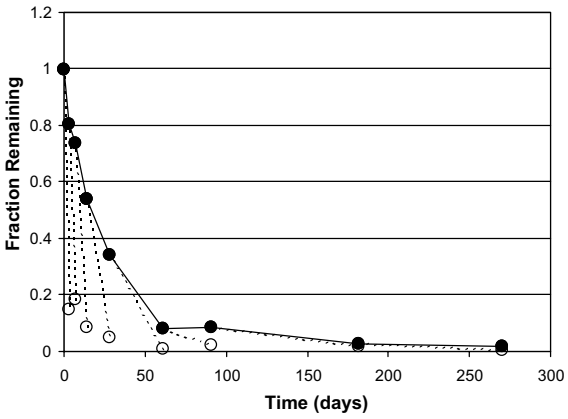


*Figure 3.* Fraction of pyrene as a function of time in Bunker C contaminated soil undergoing slurry biodegradation (●) or abiotic desorption treatment (○).

specific HCs during this time interval by comparing the slopes of the solid ●- - -● and dashed ○- - -○ lines, respectively.

The hydrocarbon mass that remains in the soil after XAD exposure was determined both directly by extraction and analysis (see above) and indirectly as the difference between the hydrocarbon mass in the initial soil and the hydrocarbon mass absorbed to the XAD resin. Because analytical precision was not high enough to quantify the extremely small amounts of abiotically released *n*-alkanes from the difference in the respective concentrations in the initial soil and the XAD exposed soil, the *n*-alkane concentrations in the XAD exposed soil were instead calculated *via* mass-balancing using the precisely measured amounts of *n*-alkanes that were absorbed by the XAD resin. The concentrations of all PAHs in the XAD exposed soils were plotted as the averages of the respective measured concentrations in the XAD exposed soils and calculated concentrations that were obtained via mass balancing (i.e., PAH mass in XAD exposed soil = PAH mass in the initial soil − PAH mass in XAD resin). Finally, the reproducibility of the abiotic release assay was successfully confirmed by performing all procedures and analyses for duplicate bioreactor samples that were taken at time zero, day 14, and at the end of the experiment.

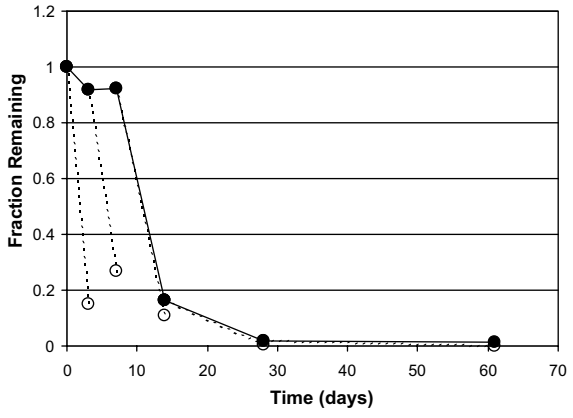


*Figure 2.* Fraction of phenanthrene as a function of time in Belhaven soil undergoing slurry biodegradation (●) or abiotic desorption treatment (○).

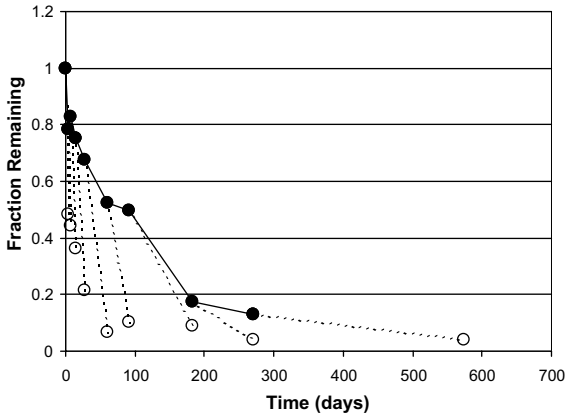


*Figure 4.* Fraction of benzo(*e*)pyrene as a function of time in Bunker C contaminated soil undergoing slurry biodegradation (●) or abiotic desorption treatment (○).

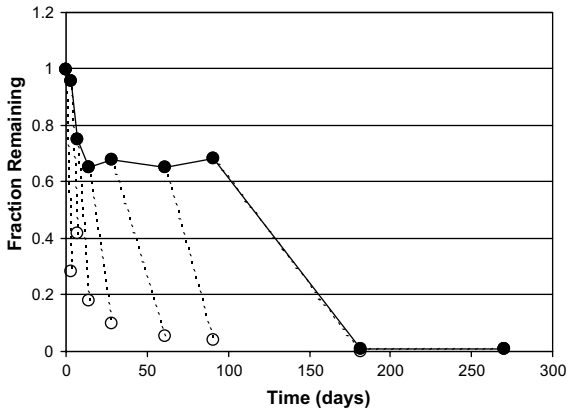


*Figure 5* Fraction of pyrene as a function of time in Cullera soil undergoing slurry biodegradation (●) or abiotic desorption treatment (○).

### *Enumeration of total heterotrophs and hydrocarbon degraders*

At various time intervals a 1 ml slurry sample was removed from the bioreactor and microbial counts for total aerobic heterotrophs and hydrocarbon degraders were determined according to the tube dilution methods described earlier by Huesemann (1994). For the enumeration of hydrocarbon degraders, Minas crude oil was used as the sole carbon source in these bacterial growth assays.

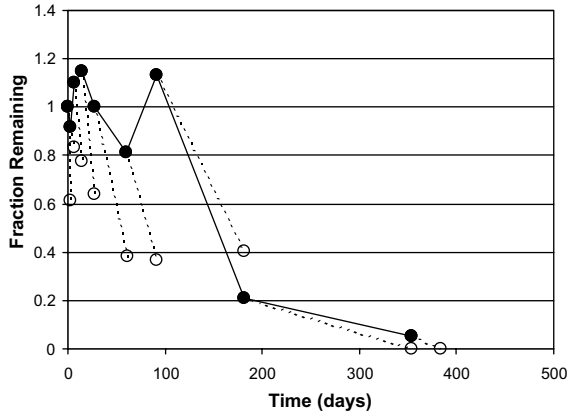


*Figure 6*. Fraction of $C_4$-phenanthrene as a function of time in Minas crude oil contaminated soil undergoing slurry biodegradation (●) or abiotic desorption treatment (○).

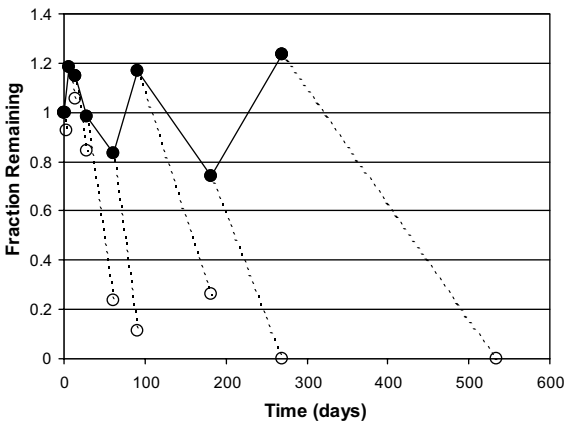


*Figure 7*. Fraction of benzo(*a*)pyrene as a function of time in Cullera soil undergoing slurry biodegradation (●) or abiotic desorption treatment (○).

## Results and discussion

### *Alkanes*

As shown in Figure 1, *n*-hexadecane ($C_{16}$) biodegraded rapidly and less than 10% of it remained in Belhaven soil after 270 days of treatment. However, when bioreactor slurry samples were subjected to abiotic release rate tests, hardly any hexadecane was desorbed or dissolved from the soil particles and taken up by the XAD resin. This is not surprising considering that hexadecane has an extremely low aqueous solubility (i.e., $S = 0.9$ $\mu$g/l (Sutton & Calder 1974)) and a very high octanol–water partition coefficient (i.e., $\log K_{ow} = 8.25$ (Coates et al. 1985)). It is interesting to note that even five and six ring PAHs, which are often considered to be extremely hydrophobic, are much more water soluble and desorb much more readily (see also Figures 2–7) than hexadecane (e.g., the solubility and $\log K_{ow}$ for benzo(*a*)pyrene is 3.8 $\mu$g/l and 6.04, respectively (MacKay et al. 1992)).

Since the biodegradation rates of hexadecane and all other *n*-alkanes (data not shown) were at all times significantly greater than the respective abiotic release rates, it seems that *n*-alkanes do not have to be dissolved in the aqueous phase in order to be biodegraded by soil microorganisms. Apparently, bacteria are able to overcome the mass-transfer limitations by either solubilizing *n*-alkanes with the help of excreted biosurfactants or

by facilitating the direct uptake of these highly insoluble compounds across lipophilic membranes (Alexander 2000; Cameotra et al. 1983; Erickson & Nakahara 1975; Jordan et al. 1999; Miller & Bartha 1989; Velankar et al. 1975).

Several investigators have also reported cases where contaminant biodegradation rates were greater than the respective desorption rates. For example, Thomas et al. (1986) observed that octadecane biodegraded faster than it was desorbed into the aqueous medium. Similarly, it was found that *n*-alkylamines bound to clay (Wszolek & Alexander 1979) and biphenyl sorbed to polyacrylic beads (Calvillo & Alexander 1996) were mineralized rapidly while desorption of these compounds occurred at a much slower rate. Finally, Rijnaarts et al. (1990) observed that initial rates of pesticide biodegradation were consistently higher than initial rates of desorption which led these investigators to conclude that microorganisms were able to enhance intraparticle mass transfer, possibly by entering into small pores and thereby increasing the concentration gradients for desorption.

*Polynuclear aromatic hydrocarbons*

Compared to the extremely slow and insignificant desorption of the highly hydrophobic *n*-alkanes, all PAHs, including high molecular weight compounds such as benzo(*a*)pyrene, benzo(*e*)pyrene, and perylene, desorbed much more rapidly and extensively in all six aged soils (see Figures 2–7). For a given soil, the rate of abiotic desorption in the initial slurry sample ($t = 0$) was inversely related to the magnitude of the respective PAH's octanol–water partition coefficient (data not shown), confirming that the degree of hydrocarbon lipophilicity strongly influences the abiotic release from aged soils.

As shown in Figure 2, phenanthrene biodegraded fast and completely after a seven day lag period in aged Belhaven soil. During this initial lag period, phenanthrene was readily bioavailable as indicated by its fast and extensive abiotic desorption in soil slurry samples that were taken from the bioreactor at various time points (i.e., $t = 0$, 3, and 7 days). Most likely, phenanthrene, although readily bioavailable, was not immediately biodegraded because a lag period of approximately 7 days was required to build up sufficiently high numbers of phenanthrene degrading bacteria. Apparently, as soon as specific hydrocarbon degraders reached high enough numbers, phenanthrene biodegradation commenced and the rate of biodegradation equaled *exactly* the rate of desorption, indicating that during this period phenanthrene biodegradation was mass-transfer rate limited (i.e., phenanthrene was biodegraded as fast as it was desorbed).

The behavior of pyrene during slurry bioremediation of aged Bunker C contaminated soil is somewhat similar to phenanthrene in aged Belhaven soil with the exception that no lag period was observed and that the biodegradation rate was much slower, i.e., it took ≈270 days for pyrene to be completely biodegraded (Figure 3). During the initial phase of the bioremediation treatment (i.e., the first 100 days), abiotic release rates were significantly greater than biodegradation rates indicating that pyrene removal was reaction-rate limited, i.e., controlled by the speed of microbial metabolism. By contrast, during the final phase (i.e., from 100 to 270 days), the rate of biodegradation was almost exactly the same as the rate of desorption, indicating that pyrene degradation was mass-transfer rate limited.

As was mentioned earlier in the Introduction, it is often believed that the recalcitrance of high molecular weight PAHs is due to their limited bioavailability. However, as shown in Figure 4 for Bunker C contaminated soil, the five ring PAH benzo(*e*)pyrene was rapidly and extensively released abiotically throughout the 270 day treatment period indicating that the slow biodegradation of this compound was due to microbial factors. Since the rate of biodegradation at the end of the treatment period (i.e., day 182 to day 270) was almost exactly the same as the rate of desorption in the period following termination of bioremediation treatment (i.e., from day 270 to day 574), it is likely that benzo(*e*)pyrene biodegradation would have become mass-transfer limited if slurry treatment would have continued beyond 270 days.

The biodegradation of pyrene in aged Cullera clay, as well as in Belhaven, Sassafras, and the coal tar contaminated soil (data not shown), was not as straightforward as the one shown earlier in Figure 3 for aged Bunker C contaminated soil. In Cullera soil, pyrene was biodegraded rapidly to about 65% of its initial concentration but, subse-

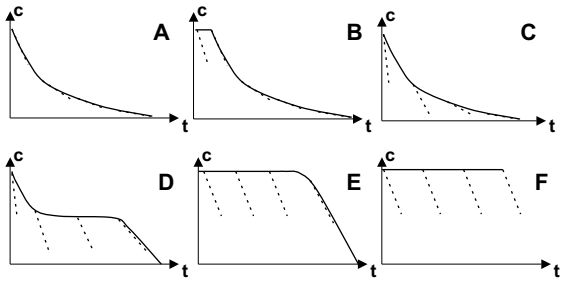


*Figure 8*. Conceptual summary of observed PAH biodegradation and desorption scenarios: (—) Biodegradation, (···) Desorption. See text for detailed description of all scenarios.

quently, biodegradation stalled for almost 100 days (Figure 5). During this period, pyrene was readily bioavailable as indicated by fast a-biotic desorption rates. After 91 days, pyrene degraded rapidly and was completely gone by day 182. It is interesting to note that the abiotic release rate during this time period (i.e., from day 91 to day 182) was exactly the same as the biodegradation rate, indicating that pyrene biodegradation was mass-transfer rate limited. It is not clear why pyrene biodegradation first stalled for 100 days and then continued. It is possible, as discussed later (see also Figure 9), that the initial drop in pyrene concentration was due to cometabolic biodegradation, while the subsequent time period until day 91 was needed to build up significant numbers of bacteria that could use pyrene as a sole source of carbon for growth. As soon as pyrene degraders reached high enough concentrations, they biodegraded pyrene as fast as it was desorbed from the soil particles.

A long period of acclimation was also observed for crude oil component $C_4$-phenanthrene (i.e., phenanthrene containing four alkyl carbons) during slurry bioremediation of aged Minas crude oil contaminated soil (Figure 6). For the first 91 days, no significant $C_4$-phenanthrene biodegradation was observed although this alkyl-PAH rapidly desorbed from the soil particles into the aqueous phase. After day 91, $C_4$-phenanthrene was degraded at almost the same rate as it was desorbed indicating that biodegradation was mass-transfer limited during this period. $C_4$-phenanthrene was almost completely biodegraded at the end of the treatment period (i.e., at day 354).

Despite the fact that all soil slurry biotreatments were carried out for rather long time periods (i.e., at least 270 days), in numerous cases it was found that five and six ring PAHs did not biodegrade at all, although they were readily bioavailable as indicated by their rapid abiotic release rates. For example, the five ring PAH benzo(*a*)pyrene was not biodegraded during the 270 day treatment period in Cullera soil, even though it was readily desorbed and taken up by XAD resin (Figure 7). Thus, it appears that microbial factors rather than bioavailability limitations were responsible for the recalcitrance of higher molecular weight PAHs. This was also the conclusion of Cornelissen et al. (1998) who found that five and six ring PAHs did not biodegrade despite their rapid and extensive desorption from contaminated sediments.

The large number of experimental data precludes the presentation of more than just the typical cases shown in Figures 2–7. However, a rigorous analysis of all data revealed that the biodegradation and desorption behavior of various 3, 4, 5, and 6 ring PAHs in the different aged soils follows only six distinct patterns. As shown in Figure 8, these six behaviors can be categorized as follows: Case A represents situations where biodegradation rates equal desorption rates indicating that microbial PAH removal is mass-transfer limited throughout the treatment. Case B is similar to Case A with the exception that a short initial lag phase is necessary to build up microbial populations of respective PAH degraders. In Case C, desorption rates are much greater than biodegradation rates at the beginning of the treatment indicating that microbial metabolism is limiting

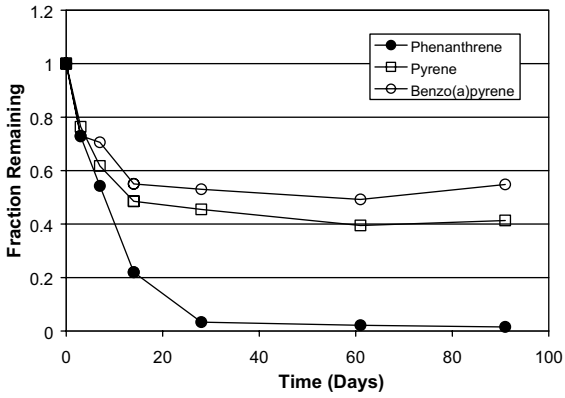


*Figure 9*. Biodegradation of phenanthrene, pyrene, and benzo(*a*)pyrene in coal tar contaminated soil.

the speed of PAH removal. By contrast, PAH biodegradation becomes mass-transfer limited at the end of the treatment as biodegradation rates equal desorption rates. In Case D, PAH biodegradation successfully starts but then stalls, despite significant PAH bioavailability, for a long period to suddenly commence and continue to completion at a speed limited by the desorption rate. Case E is similar to Case D with the exception that no initial drop in PAH concentration is observed. Finally, in Case F, no PAH biodegradation is observed throughout the treatment period despite the fact that the PAH is readily bioavailable as indicated by the rapid and extensive desorption into the aqueous phase.

In general, Cases A and B were observed for 3 ring PAHs, Case C for 3 and 4 ring PAHs, Case D for 4 and 5 ring PAHs, and Cases E and F for 5 and 6 ring PAHs. Specifically, Case F which is most important from a risk perspective, was observed for benzo(*a*)pyrene in Belhaven, Cullera, Sassafras, and Bunker C contaminated soils, benzoperylene for Minas crude oil and Bunker C contaminated soils, and indenopyrene for the Bunker C contaminated soil. In summary, the following general conclusions can be drawn from the experimental data: if PAH biodegradation occurs, the rate of removal may or may not be limited by the desorption rate. In most cases, the PAH biodegradation kinetics is limited by microbial factors during the initial phase while it often becomes mass-transfer limited during the final phase of the bioremediation treatment. More interestingly, when PAH biodegradation does not occur, it was *never* due to bioavailability limitations since PAHs were always rapidly and extensively desorbed into the water phase. This prompts the question: Why are some of the 4, 5, and 6 ring PAHs, despite their ready bioavailability, recalcitrant to microbial degradation?

### *Possible reasons for the recalcitrance of bioavailable PAHs*

Since this research was originally designed to focus on the effects of mass-transfer limitations rather than the potential effects of microbial factors on hydrocarbon bioremediation, it is only possible to speculate why many fully bioavailable PAHs are not at all or only partially biodegraded. One possible reason for the initial partial biodegradation of 4 and 5 ring PAHs as shown in Figure 8 (Case D) is that biodegradation proceeds as long as a cometabolic substrate is present and stops as soon as this substrate has been removed *via* biodegradation.

As shown in Figure 9, during the first two weeks of coal tar contaminated soil slurry treatment, both pyrene and benzo(a)pyrene biodegradation proceeds rapidly as long as phenanthrene is still present in the soil. As soon as phenanthrene has been completely biodegraded, the biodegradation of pyrene and benzo(*a*)pyrene stalls. The same biodegradation behavior was also observed in Belhaven, Sassafras, and Cullera soil where initially pyrene only biodegraded as long as phenanthrene was still present (data not shown). By contrast, when no phenanthrene was present as in the original Minas crude oil, probably because it was lost by evaporation during the spiking procedure, none of the 4 and 5 ring PAHs were removed at all at the beginning of bioremediation treatment.

These data indicate that low molecular weight PAHs such as phenanthrene stimulate the cometabolic biodegradation of high molecular weight PAHs such as pyrene and benzo(*a*)pyrene, a phenomenon that has also been observed by other investigators (Ho et al. 2000; Kanaly & Bartha 1999; Keck et al. 1989; Mannisto et al. 1996; Molina et al. 1999; Stringfellow et al. 1995). Specifically, several studies have shown that the addition of phenanthrene promotes the cometabolic biodegradation of pyrene (Ho et al. 2000; Mannisto et al. 1996; Stringfellow et al. 1995).

Although cometabolism may explain the initial and rapid, but incomplete, biodegradation of 4 and 5 ring PAHs (see Case D in Figure 7), it is not clear why biodegradation suddenly commences on its own following long lag periods of 100 days or more (see Cases D and E). It is possible that this time is required for the selection and buildup of microbial populations (consortia) capable of using the respective high molecular weight PAH*s* as a sole source of carbon for growth (Boonchan et al. 2000; Kanaly et al. 2002). Alternatively, it is also conceivable that some type of unknown inhibitory compounds such as polar biodegradation intermediates restricted the growth and metabolism of certain PAH degrading microorganisms. Since the present study was not specifically designed to elucidate the dynamics of particular PAH degrading

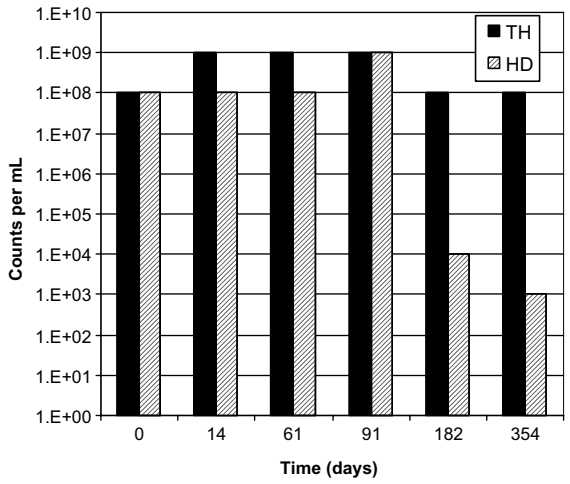


*Figure 10*. Counts of total aerobic heterotrophs (TH) and hydrocarbon degraders (HD) during bioremediation of Minas crude oil contaminated soil.

microorganisms during biotreatment, but rather the role of mass-transfer limitations, it is only possible to speculate that some unknown microbial factors rather than limited PAH bioavailability are responsible for the temporary delay in (Cases D and E) or complete absence of (Case F) PAH biodegradation.

Figure 10 shows the number of both total aerobic heterotrophs (TH) and hydrocarbon degraders (HD) at different times during slurry treatment of Minas crude oil contaminated soil. As was observed in an earlier slurry bioremediation study (Huesemann et al. 2002) and also in all six aged soils used here, both TH and HD counts are similar at time zero and increase slightly during the first phase of treatment (i.e., until day 91). During the second half of the treatment period (i.e., after day 91), TH counts remain constant while the number of HD plummets by six orders of magnitude. Clearly, a profound shift in the composition of microbial populations is taking place as the treatment proceeds. While initially a very large fraction of bacteria consists of HD, the latter make up only $\approx 0.001\%$ of TH at the end of treatment.

Considering that the hydrocarbon degrader assay counts a wide variety of both alkane and PAH degraders, it is not possible to make any definite statements about the population sizes of specific PAH degrading microbes. Nevertheless, it appears unlikely that the observed crash in HD populations can explain the temporary delay in PAH degradation (i.e., Cases D and E, Figure 8) since it occurs during the time period when PAH degradation is (finally) taking place. Apparently, the selection and buildup of specific PAH degrader populations occurs during the time when HD counts are high (i.e., until day 91). It is possible that as soon as these specific PAH degraders remove the respective PAHs, insufficient hydrocarbon substrates remain to support a large population of HD which then collapses at the end of the treatment period.

## Implications for risk assessment

As was pointed out in the Introduction, it is often assumed that slow and incomplete hydrocarbon degradation is caused by mass-transfer limitations. Our experimental data confirm that indeed PAH biodegradation during the final phase of bioremediation treatment is slow because it is limited by the equally slow rates of desorption of the respective hydrocarbons (see Cases A–E in Figure 8). However, our results clearly indicate, for the conditions of this study, that it is incorrect to attribute either the temporary or complete absence of PAH biodegradation to bioavailability limitations. In all cases where PAH biodegradation was either completely absent (Case F, Figure 8) or temporarily stalled (Cases D and E, Figure 8), these hydrocarbons were always bioavailable as indicated by fast abiotic release rates. Because of their ready bioavailability, these recalcitrant PAHs, some of which are known carcinogens, could pose a significant risk to environmental receptors. Consequently, it is inappropriate to automatically assume that the residual PAHs that remain in the soil after extensive bioremediation treatment are recalcitrant because they are not bioavailable and therefore unlikely to pose a risk to the environment. It is particularly tempting to reach this erroneous conclusion in cases that exhibit the typical biphasic behavior that is characteristic of fast and slow release desorption kinetics while in reality biodegradation stops not because of limited bioavailability but because of some unknown microbial factors such as lack of cometabolic substrates or insufficient numbers of hydrocarbon degrading populations (see Case D, Figure 8).

Instead of assuming that incomplete biodegradation is automatically due to bioavailability limitations, it would be more appropriate to measure abiotic contaminant release rates to confirm that residual contaminants are not bioavailable and that therefore a given soil is unlikely to pose a serious risk to the environment. In the absence of such specific testing, it is inappropriate to assume that residual contaminants are recalcitrant because of bioavailability limitations and that they are therefore not posing a significant risk to environmental receptors.

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## References

Alexander M (1995) How toxic are toxic chemicals in soil? Environ. Sci. Technol. 29(11): 2713–2717

Alexander M (2000) Aging, bioavailability and overestimation of risk from environmental pollutants. Environ. Sci. Technol. 34(2): 4259–4265

Berg MS, Loehr RC & Webster MT (1998) Release of petroleum hydrocarbons from bioremediated soils. J. Soil Contam. 7(6): 675–695

Boonchan S, Britz ML & Stanley GA (2000) Degradation and mineralization of high-molecular-weight polycyclic aromatic hydrocarbons by defined fungal-bacterial cocultures. Appl. Environ. Microbiol. 66(3): 1007–1019

Bosma TNP, Middeldorp PJM, Schraa G & Zehnder AJB (1997) Mass transfer limitation of biotransformation: quantifying bioavailability. Environ. Sci. Technol. 31(1): 248–252

Butler EL et al. (1991) Hopane: a new chemical tool for the measurement of oil biodegradation. In: Hinchee RE & Olfenbuttel RF (Eds) On-Site Bioreclamation (pp 515–521). Butterworth Heinemann, Stoneham, MA

Calvillo YM & Alexander M (1996) Mechanism of microbial utilization of biphenyl sorbed to polyacrylic beads. Appl. Microbiol. Biotechnol. 45: 383–390

Cameotra SS, Singh HD, Hazarika AK & Baruah JN (1983) Mode of uptake of insoluble solid substrates by microorganisms. II. Uptake of solid *n*-alkanes by yeast and bacterial species. Biotechnol. Bioeng. 25: 2945–2956

Carmichael LM, Christman RF & Pfaender FK (1997). Desorption and mineralization kinetics of phenanthrene and chrysene in contaminated soils. Environ. Sci. Technol. 31(1): 126–132

Chung N & Alexander M (1998) Differences in sequestration and bioavailability of organic compounds aged in dissimilar soils. Environ. Sci. Technol. 32(7): 855–860

Chung N & Alexander M (1999) Effect of concentration on sequestration and bioavailability of two polycyclic aromatic hydrocarbons. Environ. Sci. Technol. 33(20): 3605–3608

Coates M, Connell DW & Barron DM (1985) Aqueous solubility and octanol to water partition coefficients of aliphatic hydrocarbons. Environ. Sci. Technol. 19(7): 628–632

Cornelissen G, Rjgterink H, Ferdinandy MMA & Van Noort PCM (1998) Rapidly desorbing fractions of PAHs in contaminated sediments as a predictor of the extent of bioremediation. Environ. Sci. Technol. 32(7): 966–970

EPA SW-846 (1986) Test methods for evaluating solid waste, physical/chemical methods. U.S. environmental protection agency, Office of Solid Waste, 3rd ed

Erickson LE & Nakahara T (1975) Growth in cultures with two liquid phases: hydrocarbon uptake and transport. Proc. Biochem. June Issue, 9–13

Geerdkink MJ, Van Loosdrecht MCM & Luyben KCAM (1996) Model for microbial degradation of nonpolar organic contaminants in a soil slurry reactor. Environ. Sci. Technol. 30(3): 779–786

Ghoshal S & Luthy RG (1996), Bioavailability of hydrophobic organic compounds from nonaqueous-phase liquids: the biodegradation of naphthalene from coal tar. Environ. Toxicol. Chem. 15(11): 1894–1900

Ghoshal S & Luthy RG (1998) Biodegradation kinetics of naphthalene in nonaqueous phase liquid–water mixed batch systems: comparison of model predictions and experimental results. Biotechnol. Bioeng. 57(3): 356–366

Ghoshal S, Ramaswami A & Luthy RG (1996) Biodegradation of naphthalene from coal tar and heptamethylnonane in mixed batch systems. Environ. Sci. Technol. 30(4): 1282–1291

Hatzinger PB & Alexander M (1995) Effect of aging of chemicals in soil on their biodegradability and extractability. Environ. Sci. Technol. 29(2): 537–545

Hatzinger PB & Alexander M (1997) Biodegradation of organic compounds sequestered in organic solids or in nanopores within silica particles. Environ. Toxicol. Chem. 16(11): 2215–2221

Ho Y, Jackson M, Yang Y, Mueller JG & Pritchard PH (2000) Characterization of fluoranthrene and pyrene degrading bacteria isolated from PAH contaminated soils and sediments. J. Ind. Microbiol. Biotechnol. 24: 100–112

Huesemann MH (1994) Guidelines for land-treating petroleum hydrocarbon-contaminated soils. J. Soil Contam. 3(3): 299–318

Huesemann MH (1997) Incomplete hydrocarbon biodegradation in contaminated soils: limitations in bioavailability or inherent recalcitrance?. Bioremed. J. 1(1): 27–39.

Huesemann MH, Rhodes IA, Rice-Jackson LM, Sterna LL & Moore KO (1995) Changes in hydrocarbon composition

during slurry bioremediation of diesel-based drill cuttings. In: Proceedings of IGTs Sixth International Symposium on Gas, Oil & Environmental Biotechnology, Colorado Springs, CO, (pp 97–118)

Huesemann MH, Hausmann TS & Fortman TJ (2002) Microbial factors rather than bioavailability limit the rate and extent of PAH biodegradation in aged crude oil contaminated model soils. Bioremed. J. 6(4): 321–336

Huesemann MH, Hausmann TS & Fortman TJ (2003) Assessment of bioavailability limitations during slurry biodegradation of petroleum hydrocarbons in aged soils. Environ. Toxicol. Chem. 22(12): 2853–2860

Jordan RN, Nichols EP and Cunningham AB (1999) The role of (bio)surfactant sorption in promoting the bioavailability of nutrients localized at the solid–water interface. Water Sci. Technol. 39(7): 91–98

Kanaly RA & Bartha R (1999) Cometabolic mineralization of benzo(*a*)pyrene caused by hydrocarbon additions to soil. Environ. Toxicol. Chem. 18(10): 2186–2190

Kanaly RA, Harayama S & Watanabe K (2002) Rhodanobacter sp. Strain BPC1 in a benzo[*a*]pyrene-mineralizing bacterial consortium. Appl. Environ. Microbiol. 68(12): 5826–5833

Keck J, Sims RC, Coover M, Park K & Symons B (1989) Evidence for cooxidation of polycyclic aromatic hydrocarbons in soil. Water Res. 23(12): 1467–1476

Kelsey, JW & Alexander M (1997) Declining bioavailability and inappropriate estimation of risk of persistent compounds. Environ. Toxicol. Chem. 16(3): 582–585

Kelsey JW, Kottler BD & Alexander M (1997) Selective chemical extractants to predict bioavailability of soil-aged organic chemicals. Environ. Sci. Technol. 31(1): 214–217

Linz DG & Nakles DV (1997) Environmentally Acceptable Endpoints in Soil. American Academy of Environmental Engineers, Annapolis, MD

Liste HH & Alexander M (2002) Butanol extraction to predict bioavailability of PAHs in soil. Chemosphere 46(7): 1011–1017

Loehr, RC & Webster MT (1997) Changes in toxicity and mobility resulting from bioremediation processes. Bioremed. J. 1(2): 149–163

Loehr RC & Webster MT (2000) Decreased release of PAHs from soils as a result of field bioremediation. Pract. Period. Hazard. Toxic Radioact. Waste Manage. 4(3): 118–125

Loehr RC, Webster MT & Smith JR (2000a) Fate of treated and weathered hydrocarbons in soil – long-term changes. Pract. Period. Hazard. Toxic Radioact. Waste Manage. 4(2): 53–59

Loehr RC, Webster MT, Johnston DL, Kegel EM & Meyers JD (2000b) Remediation of petroleum refinery waste pond contents: a case study. Pract. Period. Hazard. Toxic Radioact. Waste Manage. 4(4): 140–147

Luthy RG et al. (1997) Sequestration of hydrophobic organic contaminants by geosorbents. Environ. Sci. Technol. 31(12): 3341–3347

Jiminez IY & Bartha R (1996) Solvent-augmented mineralization of pyrene by a Mycobacterium sp.. Appl. Environ. Microbiol. 62(7): 2311–2316

MacKay D, Shiu WY & Ma KC (1992) Illustrated Handbook of Physical–Chemical Properties and Environmental Fate for Organic Chemicals, Vol. II. Lewis Publishers, Chelsea, MI

MacLeod CJA & Semple KT (2000), Influence of contact time on extractability and degradation of pyrene in soils. Environ. Sci. Technol. 34(23): 4952–4957

Madsen EL (2003) Report on bioavailability of chemical wastes with respect to the potential for soil bioremediation. Environmental Protection Agency, National Center for Environmental Research. http: //es.epa.gov/ncer/publications/overview/bioavailability_complete_book.pdf

Mannisto MK, Melin ES, Puhakka JA & Ferguson JF (1996) Biodegradation of PAH mixtures by a marine sediment enrichment. Polycyc. Aromat. Compd. 11: 27–34

Method 2540 B (1989) Total solids dried at 103–105 °C. In: Standard Methods for the Examination of Water and Wastewater, 17th ed. (pp 272–273)

Miller RM & Bartha R (1989) Evidence from liposome encapsulation for transport-limited microbial metabolism of solid alkanes. Appl. Environ. Microbiol. 55(2): 269–274

Molina M, Araujo R & Hodson RE (1999) Cross induction of pyrene and phenanthrene in a Mycobacterium sp. isolated from polycyclic aromatic hydrocarbon contaminated river sediments. Can. J. Microbiol. 45: 520–529

Nam K & Alexander M (1998) Role of nanoporosity and hydrophobicity in sequestration and bioavailability: tests with model solids. Environ. Sci. Technol. 32(1): 71–74

Olivera FL, Loehr RC, Cason-Coplin B, Eby H & Webster MT (1998) Prepared bed land treatment of soils containing diesel and crude oil hydrocarbons. J. Soil Contam. 7(6): 657–674

Pignatello JJ & Xing B (1996) Mechanisms of slow sorption of organic chemicals to natural particles. Environ. Sci. Technol. 30(1): 1–11

Ramaswami A & Luthy RG (1997) Mass transfer and bioavailability of PAH compounds in coal tar NAPL – slurry systems. 1. Model development. Environ. Sci. Technol. 31(8): 2260–2267

Ramaswami A, Ghoshal S & Luthy RG (1997) Mass transfer and bioavailability of PAH compounds in coal tar NAPL – slurry systems. 2. Experimental evaluations. Environ. Sci. Technol. 31(8): 2268–2276

Rijnaarts HHM, Bachmann A, Jumelet JC & Zehnder AJB (1990) Effect of desorption and intraparticle mass transfer on the aerobic biomineralization of α–hexachlorocyclohexane in a contaminated calcareous soil. Environ. Sci. Technol. 24(9): 1349–1354

Schwartz E & Scow KM (2001) Repeated inoculation as a strategy for the remediation of low concentrations of phenanthrene in soil. Biodegradation 12(3): 201–207

Scow KM & Alexander M (1992) Effect of diffusion on the kinetics of biodegradation: experimental results with synthetic aggregates. Soil Sci. Soc. Am. J. 56: 128–134

Shor LM, Liang W, Rockne KJ, Young LY, Taghon GL & Kosson DS (2003) Intra-aggregate mass transport-limited bioavailability of polycyclic aromatic hydrocarbons to *Mycobacterium* Strain PC01. Environ. Sci. Technol. 37(8): 1545–1552

Stringfellow WT, Chen S-H & Aitken MD (1995) Induction of PAH degradation in a phenanthrene-degrading pseudomonad. In: Hinchee RE, Brockman FJ & Vogel CM (Eds) Microbial Processes for Bioremediation (pp 83–89). Battelle Press, Columbus, OH

Stroo HF, Jensen R, Loehr RC, Nakles DV, Fairbrother A & Liban CB (2000) Environmentally acceptable endpoints for PAHs at a manufactured gas plant site. Environ. Sci. Technol. 34(18): 3831–3838

Sutton C & Calder JA (1974) Solubility of higher molecular weight *n*-parafins in distilled water and seawater. Environ. Sci. Technol. 8(7): 654–657

Tabak HH, Lazorchak JM, Lei L, Khodadoust AP, Antia JE, Bagchi R & Suidan MT (2003) Studies on bioremediation of polycyclic aromatic hydrocarbon-contaminated sediments: bioavailability, biodegradability and toxicity issues. Environ. Toxicol. Chem. 22(3): 473–482

Tang J & Alexander M (1999) Mild extractability and bioavailability of polycyclic aromatic hydrocarbons in soil. Environ. Toxicol. Chem. 18(12): 2711–2714

Thomas JM, Yordy JR, Amador JA & Alexander M (1986) Rates of dissolution and biodegradation of water-insoluble organic compounds. Appl. Environ. Microbiol. 52(2): 290–296

Velankar SK, Barnett SM, Houston CW & Thompson AR (1975) Microbial growth on hydrocarbons – some experimental results. Biotechnol. Bioeng. 17: 241–251

Wang Z, Fingas M & Lee K (1994) Fractionation of a light crude oil and identification and quantification of aliphatic, aromatic and biomarker compounds by GC-FID and GC-MS, Part I and Part II. J. Chromatogr. Sci. 32: 361–382

Weissenfels WD, Klewer H-J & Langhoff J (1992) Adsorption of polycyclic aromatic hydrocabons (PAHs) by soil particles: influence on biodegradability and biotoxicity. Appl. Microbiol. Biotechnol. 36: 689–696

Wick LY, Colangelo T & Harms H (2001) Kinetics of mass transfer-limited bacterial growth on solid PAHs. Environ. Sci. Technol. 35(2): 354–361

Williamson DG, Loehr RC & Kimura Y (1997) Measuring release and biodegradation kinetics of aged hydrocarbons from soils. In: Alleman BC, Leeson A (Eds) In Situ and On-Site Bioremediation, Vol. 5 (pp 605–610). Battelle Press, Columbus, OH

Williamson DG, Loehr RC, & Kimura Y (1998) Release of chemicals from contaminated soils. J. Soil Contam. 7(5): 543–558

Wszolek PC & Alexander M (1979) Effect of desorption rate on the biodegradation of *n*-alkylamines bound to clay. J. Agri. Food Chem. 27(2): 410–414

Zhang W, Bower EJ & Ball WP (1998) Bioavailability of hydrophobic organic contaminants: effects and implications of sorption-related mass transfer on bioremediation. Ground Water Monitor. Rev. Winter Issue, 126–138.