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Utilization of bioremediation processes for the treatment of PAH-contaminated sediments

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The widespread contamination of aquatic sediments by polycyclic aromatic hydrocarbons (PAHs) has created a need for cost-effective remediation processes. Many common PAHs are biodegradable, leading to studies investigating the potential of sediment bioremediation. This article reviews several factors that currently complicate the implementation of sediment bioremediation processes: the effect of complex mixtures of contaminants on the rate and extent of degradation observed, the bioavailability of PAHs in sorbed- and nonaqueous-phase, and methods being evaluated to enhance degradation/availability (surfactant-enhanced solubility, nutrient addition, and bioaugmentation).

Keywords: review; biodegradation; polycyclic aromatic hydrocarbons; sediments

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are among the most common organic contaminants of aquatic sediments [21,39–41,55]. Direct human exposure to PAHs in bottom sediment is minimal; however, due to the tendency of PAHs to accumulate in the food chain [50,56,66], their release during dredging operations, episodes of high hydraulic scouring, or leaching from confined disposal facilities, poses a threat to aquatic ecosystems [38]. Bioremediation is one method that may reduce the risk of sediment-associated PAHs. Among the sediment bioremediation processes that have been investigated are the use of slurry reactors [22,24], landfarming (after dredging), and beach-surface bioremediation of oil spills [62,73]. In any of these applications, bioremediation is complicated by two factors. First is the need to biodegrade a complex mixture of contaminants with varying ring number and molecular weight. For example, sediment contamination from manufactured gas plant site soils typically include 2-, 3-, 4-, and 5-ringed PAHs (along with non-aromatic hydrocarbons) [23]. Second, contaminants must be made available to the degrading consortia. PAHs are hydrophobic and sorb strongly to the organic matter in sediments [37,43,68], and oil-phase contamination represents an even larger sink for PAH-partitioning in many cases [19,20]. The bulk of the contaminant mass thus resides in a phase separate from the degrading organisms, and the rate of mass transfer can control process efficacy [9,10].

In recent years, there have been considerable research efforts to characterize the impact of these complicating factors in bioremediation processes and to modify systems to overcome potential barriers in application. In many cases, these studies have built upon earlier and more fundamental work investigating the metabolism of PAHs by microorganisms. The purpose of this paper is to review recent advances in our understanding of the biodegradation of PAH mixtures and PAH availability as they relate to sediment bioremediation.

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Biodegradation of PAH mixtures

It is well established that many individual polycyclic aromatic hydrocarbons are degraded by bacteria [16,35,36,52,76,77]. Recently, there has been increased interest in developing an understanding of microbial degradation processes when contaminants are present in complex mixtures. A mixture of contaminants in a bioremediation system may result in inhibition, cometabolism, augmentation, or no effect at all. Laboratory studies using defined mixtures of PAHs have begun to address the problems raised by the presence of more than one contaminant [6,8,67,70,71,77,80]. In these studies, combinations of individual effects have been observed. For example, both cometabolism and inhibition have been observed in the degradation of a simple mixture of phenanthrene and fluorene by a *Pseudomonas* sp [8]. In addition to systematic laboratory studies, information on biodegradation of mixtures can be inferred from studies using contaminated sediments from field sites [24,48]. In this section, information on laboratory studies that specifically evaluated mixtures' effects is presented. Because most studies utilizing sediments from contaminated sites are complicated by factors in addition to multiple contaminants (eg bioavailability, experimental protocols, and environmental factors), results obtained in these experiments are presented in a separate section.

Inhibition is the reduction in the rate and/or extent of degradation of one compound by the presence of another, and it is the most common effect noted in the degradation of PAH mixtures. For example, Tiehm and Fritzche [70] observed the inhibition of pyrene degradation by fluorene in a pure culture of a *Mycobacterium* sp grown on pyruvate. Inhibition has been noted in cases when only one [8] or

both [67,71] PAHs are a carbon and energy source. Inhibition suggests that multiple PAHs are degraded using common enzymatic pathways, leading to competition for the active sites of enzymes [8,67]. Bouchez *et al* [8] suggested that changes in enzyme induction could cause inhibition, as one compound represses the synthesis of enzymes needed to degrade the other. Also, the aqueous solubility of individual compounds may influence the inhibition patterns observed. Several studies [8,67,70] have reported that more soluble PAHs inhibit the degradation of the less soluble ones, although no such correlation was observed in other reports [6,70,80]. The use of mixed rather than pure cultures to degrade mixtures has the potential to mitigate observed inhibition effects. Mixed cultures can display complementary degradative action and have a greater tolerance for toxic products, where intermediate products can act as substrates for other bacterial activity [8,71].

Cometabolism, as defined by Dalton and Stirling, is 'the transformation of a non-growth substrate in the obligate presence of a growth substrate or another transformable compound' [15]. Certain PAHs are degraded by cometabolism. For example, recent studies of PAH mixtures have demonstrated the cometabolic degradation of fluorene [8,67,70,77]. Cometabolism may be an important fate process when larger, and often more recalcitrant, PAHs are present in mixtures of more readily degraded, smaller PAHs. This was noted by Bouchez *et al* when a pure culture of a *Pseudomonas* sp was unable to use fluoranthene as a sole carbon source, but was able to cometabolize it in the presence of phenanthrene [8]. Work by Ye *et al* [80], focusing exclusively on the degradation of four- and five-ringed PAHs by *Sphingomonas paucimobilis* isolated on fluoranthene, found that some high molecular weight PAHs can be degraded through primary metabolism; however, the study did not address the potential inhibitory effect of smaller PAHs on PAH metabolism by *S. paucimobilis*.

Augmentation describes cases in which degradation of one compound is enhanced by the presence of another. Bouchez *et al* [8] found that fluorene augmented the degradation of phenanthrene by a pure culture of a *Rhodococcus* sp. Tiehm and Fritzche [70] observed an increase in the degradation of pyrene in the presence of phenanthrene by a pure culture of a *Mycobacterium* sp amended with surfactants. Without the increased solubility imparted by surfactants, augmentation did not occur, but the increase in pyrene degradation was not cometabolism (pyrene was degraded when present individually). This observation was attributed to increased growth of the bacteria on phenanthrene, and the increased biomass resulted in augmentation of pyrene degradation.

Mixtures of PAHs do not always produce any of these observable effects. For example, the degradation of phenanthrene, when present in high concentration, was not affected by the presence of fluorene, fluoranthene or pyrene [70]. When there was limited PAH in aqueous solution, inhibition was observed. In a study by Ye *et al* [80], the presence of PAHs, including benzo[*a*]anthracene, benzo[*b*]fluoranthene, chrysene, dibenzo[*a,h*]anthracene and fluoranthene, did not affect the degradation of benzo[*a*]pyrene by *Sphingomonas paucimobilis*. Only benzo[*b*]fluoranthene caused noticeable reduction in the degradation of benzo[*a*]pyrene. Ye *et al* suggested two explanations for their observations: the compounds fed did not compete for the active sites of the enzyme responsible for benzo[*a*]pyrene degradation, or there are different enzymes responsible for degradation of the different PAHs.

Controlled laboratory experiments on the effects of mixtures in the presence of sediments are less common than those in aqueous solution only. Generally these studies show little or no effect of mixtures on degradation [6,36]. Heitkamp and Cerniglia [36] observed that mineralization of 2-methylnaphthalene and phenanthrene in sediment-containing microcosms was not affected by the presence of pyrene or benzo[*a*]pyrene. Bauer and Capone [6] noted that there was no effect on the degradation of each compound in pairs of naphthalene, anthracene, and phenanthrene in sediment slurry reactors. Mixed cultures were used, as was the case in the Heitkamp and Cerniglia study. This was very different to the situation observed by Bouchez *et al* [8], where naphthalene was strongly inhibitory to the degradation of other PAHs, even in mixed cultures. The interpretation of these studies is complicated by the question of bioavailability, which is further discussed in the next section. To understand the degradation of mixtures in the presence of sediments requires an understanding of the partitioning of individual compounds. Using the studies conducted by Heitkamp and Cerniglia [36] as an example, pyrene and benzo[*a*]pyrene are considerably more sorptive than 2-methylnaphthalene and phenanthrene. Thus the aqueous phase concentrations of pyrene and benzo[*a*]pyrene will be much lower than either 2-methylnaphthalene and phenanthrene, decreasing the potential for competitive inhibition.

PAH bioavailability

Biodegradation rates of many organic pollutants, including PAHs, are often described by concentration-dependent rate expressions such as Monod kinetics or first-order models [25,27,46,63,65]. These evaluations require that the substrate is 'available' to the organism, which implies that the contaminant resides in aqueous solution. In contaminated sediments, PAHs are found in the sorbed phase, and in cases of severe contamination, PAHs can exist in a non-aqueous phase liquid (NAPL). Assuming that biodegradation rates are proportional to the concentration of the dissolved contaminants, sorption to sediment organic matter or partitioning into an oil phase may control biodegradation by maintaining low aqueous phase contaminant concentrations [20]. The partitioning of PAHs to sediments strongly correlates with the organic carbon content of the sediment and the contaminant's relative hydrophobicity, typically described by its octanol/water partition coefficient (K_{ow}) [42]. Physical parameters influence this equilibrium partitioning of PAHs and the organic fraction, in particular temperature [59]. PAHs exhibit a wide range of octanol/water partition coefficients. For example, naphthalene's log K_{ow} (3.37) [31] is almost 5400 times less than that of benzo[*g,h,i*]perylene (log K_{ow} = 7.10) [79]. Thus the aqueous phase concentration of individual contaminants in sediment/water systems, and the mass of an individual contaminant available for bioremediation, vary greatly. For this

reason there are considerable concerns on the ability to bioremediate sediments containing primarily low solubility and strongly hydrophobic 4-ring and larger PAHs.

In addition to partitioning, biodegradation can be impacted by the flux rate of contaminants from a non-bioavailable to the dissolved phase. When the non-bioavailable phase is represented by PAH adsorbed to a sediment particle, the flux of contaminants to the aqueous phase can be influenced by several parameters: the solid phase/bulk water phase concentration gradient, the thickness of the liquid diffuse layer, the porosity of the particle, and the particle diameter. In bioremediation, the desorption gradient is established through the metabolism of aqueous phase PAHs by bacteria in the bulk solution, the diffuse layer, or attached to the sediment surface [32]. Diffusion of PAHs into the sediment particles complicates the desorption process. Sediments contain small pores that are inaccessible to microorganisms, but are large enough for contaminants to diffuse into, and subsequently adsorb. To remediate the contaminated soil completely, diffusion of PAHs sorbed in these pores must occur, and the rate of this intraparticle diffusion can control the rate and extent of remediation achieved [26]. Harms and Zehnder [32] investigated the ability of attached bacteria to create a desorption gradient following the adsorption of 3-chlorodibenzofuran to Teflon particles. The rate of desorption in systems containing active microbes was compared to that of systems in which desorption was established though flushing with contaminant-free medium. Interestingly, these studies demonstrated that the presence of attached bacteria accelerated desorption rates, primarily due to the increased concentration gradient that results from a decrease in desorption path length (ie the formation of a steeper concentration gradient).

While sediment-associated PAHs are at least partially desorbed and available to the microorganisms in treatment systems, there is considerable interest in the extent to which bioremediation (or other processes that rely on complete transfer of contaminants from a sorbed to aqueous phase) is capable of decreasing contaminant mass initially in the sorbed state. The widely reported physiochemical phenomena of slow or 'irreversible' adsorption recently reviewed by Pignatello and Xing [60] may control process efficacy and not microbiological considerations. This does not dismiss concerns regarding the ability of microbes to degrade specific contaminants in a complex mixture, but if the flux of contaminants from the sediment to the aqueous phase is sufficiently low, the maintenance of an actively growing bioreactor may become impossible. This is of primary concern in establishing the endpoints of treatment possible with 'aged' materials. Diffusion into and out of sediment micropore structures requires extended time periods. Thus, aged materials are more extensively permeated, and reversing the process can be slow. This was demonstrated by Hatzinger and Alexander [33], who added phenanthrene and 4-nitrophenol to sterile sediments and inoculated them with a *Pseudomonas* strain after varying periods of contaminant/sediment contact. Mineralization of contaminants was inversely related to the time of contact allowed.

The presence of a non-aqueous phase liquid (NAPL) also represents a partitioning-sink for PAHs in sediments, and bioavailability of PAHs in NAPLs, such as coal tar, is an area of current research. Because NAPLs represent a complex mixture of biodegradable organics, some studies have employed surrogate non-biodegradable NAPLs (eg heptamethylnonane and dibutyl phthalate) as carriers of target PAHs to simplify the evaluation of bioavailability. In general, these studies demonstrated that the flux of PAHs from a non-aqueous phase is sufficient to support growth, and under certain laboratory conditions the mass transfer of contaminants from the non-aqueous phase is more rapid than biodegradation rates [18]. The specific findings of these studies, including the influence of NAPL composition on biodegradation rates/extent and the influence of NAPL surface area on availability, are discussed in the following paragraphs.

Ortega-Calvo *et al* [57] investigated the rate of ^{14}C-phenanthrene solubilization and mineralization in systems where heptamethylnonane and dibutyl phthalate were used, individually and in mixtures, as NAPLs. The rate of phenanthrene partitioning was influenced significantly ($P = 0.05$) by the composition of the NAPL. The relative rate of phenanthrene partitioning was reported to be most rapid with heptamethylnonane, followed by a 1:1 volumetric mixture of heptamethylnonane and dibutyl phthalate, and slowest in dibutyl phthalate alone. Rates of mineralization were always less than partitioning rates; however, mineralization rates were significantly greater in the heptamethylnonane-only systems as compared to systems containing 50% or 100% dibutyl phthalate. Rates of partitioning and mineralization were influenced by factors including the volume of NAPL added and mixing in experimental systems. As the volume of NAPL was increased, rates of partitioning decreased, presumably due to a decrease in the surface area:volume ratio. As expected, mixing systems increased partitioning rates. In mixed systems, the extent of phenanthrene mineralization observed over a 92-h experiment ranged from 5.1% with 5 ml of dibutyl phthalate-only NAPL to 20.3% in a 0.5-ml heptamethylnonane system.

Ghoshal *et al* [27] conducted studies on the extent of ^{14}C-naphthalene mineralization in mixed systems containing either heptamethylnonane or coal tar as the NAPL. When coal tar was used as the NAPL, it was introduced either as a 'globule' or absorbed in uniform microporous silica beads (mean diameter = 250 μm, average pore diameter = 140 Å, pore volume 1.2 ml g^{-1}). When heptamethylnonane was used, the NAPL was contained in a 34-mm i.d. glass tube fused to the bottom of the flask that had slots at the base to allow phenanthrene transfer to the bulk medium. In all cases, the rates of dissolution were measured directly and rates of biodegradation determined by curve-fitting of mineralization data. Results of these experiments demonstrated that the form of the coal tar NAPL (eg globule or in beads) influenced the mass transfer rates and the rate-controlling phenomena (dissolution or biodegradation). In systems with coal tar contained in porous beads, biodegradation was slower than mass transfer. When coal tar was present as a globule, mass transfer was slower than biodegradation. In heptamethylnonane systems the rates of biodegradation were consistently less than the rates of mass transfer. The extent of biodegradation was assessed when mineralization was no longer observed (approximately 70

days for heptamethylnonane systems and approximately 150 days for coal tar NAPL systems; extent not provided for coal tar–silica bead systems). In coal tar globules, 6–7% remained in the NAPL phase with 70–73% converted to CO_2. Naphthalene was more available in the heptamethylnonane systems as only 0.3–0.7% remained in the non-aqueous phase with 53–70% converted to CO_2.

There is limited information on microbial factors that may influence bioavailability in either the adsorbed or NAPL phase. Guerin and Boyd [28] conducted studies to evaluate the bioavailability of naphthalene in soil systems inoculated with two bacterial species: *Pseudomonas putida* ATCC 17484, and a soil isolate designated NP-Alk. The NP-Alk inocula degraded naphthalene in soil slurries, but at a rate slower than predicted from aqueous phase-dependent kinetic relationships determined in soil-free systems. Conversely, the *Pseudomonas* sp consistently degraded naphthalene in soil systems at a rate equal to or greater than predicted from soil-free systems. No mechanisms for the difference in bioavailability were proposed, but this study demonstrates that bioavailability may be species-specific. In NAPL systems, the ability of an organism to adhere to, and perhaps solubilize, the NAPL may be critical [18,51]. Additionally, the ability to produce biosurfactants may also aid in NAPL-phase biodegradation through enhanced solubility or emulsification of the hydrocarbon and increasing surface area.

Field and pilot studies

The studies cited above were all controlled laboratory experiments. Results are available from field or pilot studies, but interpretation of potential mixtures and/or bioavailability effects is more complicated due to the number/phase of contaminants present, differences in experimental systems, differences in reporting results, and environmental factors (temperature etc). Studies assessing the biodegradation of PAH under various conditions (landfarming, unsaturated soil/sediment with no mixing; slurry systems where sediments are saturated with water and mixed continuously; and sediment microcosms where sediments are saturated and static) are summarized in Table 1. Microbial activity is reported as either percent degradation (measured by compound disappearance), percent mineralization, or the normalized rate/half-life of degradation.

Due to the variety of PAH compounds, experimental conditions, materials, and measures of activity commonly used, it is difficult to derive specific interactions from mixtures of compounds, or the true extent of degradation possible. In general, high degrees of biodegradation (extent or rate) for 2- and 3-ring PAHs are observed. Significant biodegradation of 4- to 6-ring compounds is sometimes observed, but it is generally less than that of the 2- and 3-ring PAHs present [2]. Complete degradation of high molecular weight PAHs is rarely observed. Their lower bioavailability and/or depletion of inducing substrates may thus control the efficacy of sediment bioremediation processes.

A comprehensive report of pilot-scale slurry reactor operations using a creosote-contaminated soil was prepared by Lewis [48]. In this study, five well-mixed slurry reactors were operated for 12 weeks treating contaminated soils. Systems were augmented with nutrients, *Pseudomonas fluorescens*, *Pseudomonas stutzeri*, and an *Alcaligenes* sp. Samples were taken for PAH analysis, and off-gas from reactors was monitored with vapor traps to account for volatilization losses. At the end of the study, total PAH concentrations had dropped an average of 93.4% (±3.2%). The degradation of 2- and 3-ringed compounds was considerably faster than that of the 4- to 6-ringed compounds. After 2 weeks, 95.9% (±1.9) of the 2- and 3-ring compounds had been removed, as compared to only 81.6% (±3.9) of the 4- to 6-ring compounds. Volatile emissions were observed in the first 5 days of operation (primarily composed of benzene and related monoaromatics), and then levels dropped to below detection limits. These results demonstrate the potential of bioremediation under optimal mass transfer conditions. Few laboratory studies duplicate these successes, however, perhaps because of poor mixing, variability in inocula, water content, or other unknown factors. With little fundamental information on microbes that degrade 4- to 6-ring PAHs, it is difficult to determine the factors critical to their activity.

A full-scale treatment system at the French Limited Superfund site in Harris County, Texas [22,24], was used to remediate lagoon sediments containing a mixture of organic contaminants including PCBs, PAHs, benzene, toluene, ethylbenzene and xylenes (BTEX), chlorinated solvents, and pesticides. Nutrients and oxygen were supplied to enhance rates of microbial degradation. Design considerations include the use of pressurized oxygen eductors to minimize volatilization losses. The solids were maintained in suspension with centrifugal pumps and mixers fixed on floating platforms and mounted to boom arms. The units were moved throughout the lagoon to facilitate mixing and mass transfer. The project was successfully completed with high removal efficiencies of the organics mixture at considerable cost savings as compared to more traditional technologies ($55 million for bioremediation versus $120 million for incineration).

Enhancement of the rate and extent of PAH biodegradation

Surfactant-enhanced bioremediation

Since PAH desorption is a critical factor in the rate and extent of sediment bioremediation, the potential of nonionic surfactants to facilitate this process has been investigated (nonionic surfactants are less toxic than anionic or cationic surfactants). Surfactants may enhance the bioavailability of sorbed PAHs by decreasing the capillary forces in the sediment matrix [11] or by increasing the apparent aqueous solubility of contaminants at concentrations above their critical micelle concentration (CMC) [17]. By increasing PAH solubility, surfactants increase the fraction of soluble compound and presumably the amount available for microbial uptake [29,49].

Many studies have attempted to delineate the effect of surfactants in enhancing the aqueous solubility of PAHs sorbed to a soil matrix [1,17,49]. The apparent enhanced solubilization is commonly attributed to the increased concentration gradient at the soil–water interface, created by the large partitioning of hydrophobic organic compounds

Table 1 Comparison of PAH degradation in soils and sediments in various treatment systems

Description	Soil/sediment	Compound (number of rings)	Degradation*	Time	Reference
Sediment microcosms, not shaken Indigenous microorganisms	Sediment from oil-contaminated stream	2 – Naphthalene	1.0[a]	N/A	Herbes and Schwall [37]
		3 – Anthracene	8.5×10^{-3}		
		4 – Benzo[*a*]anthracene	1.2×10^{-5}		
		5 – Benzo[*a*]pyrene	2.0×10^{-6}		
Uncontaminated sediment spiked with PAH	Sediment from uncontaminated river bed	2 – Naphthalene	8.0×10^{-6}		
		3 – Anthracene	3.0×10^{-6}		
		4 – Benzo[*a*]anthracene	4.0×10^{-8}		
		5 – Benzo[*a*]pyrene	3.0×10^{-7}		
Simulated landfarming Indigenous microorganisms Sludge (w/PAHs) added to soil	Oily sludge from a petrochemical waste treatment facility Sandy loamy soil from a landfarm	3 – Fluorene	98.5	1280 days	Bossert *et al* [7]
		Phenanthrene	99.8		
		Anthracene	93.1		
		4 – Fluoranthene	95.3		
		Pyrene	14.4		
		Benz[*a*]anthracene	98.5		
		Chrysene	96.9		
		5 – Benzo[*b*]fluoranthene	20.6		
		Benzo[*j*]fluoranthene	20.6		
		Benzo[*k*]fluoranthene	70.1		
		Benzo[*a*]pyrene	44.4		
Sediment microcosms with intermittent shaking Indigenous microorganisms	Redfish Bay, TX Sediments with higher natural PAH content	2 – Naphthalene	68.5[b]	8 weeks	Heitkamp and Cerniglia [34]
		3 – Phenanthrene	43.0		
		4 – Pyrene	15.1		
		5 – Benzo[*a*]pyrene	3.1		
Spiked with ^{14}C-labeled PAH	Lake Chicot, AK Sediments chronically exposed to pesticides	2 – Naphthalene	56.5	8 weeks	
		3 – Phenanthrene	48.1		
		4 – Pyrene	5.3		
		5 – Benzo[*a*]pyrene	0.5		
	DeGray Reservoir, AK Pristine sediments	2 – Naphthalene	54.5	8 weeks	
		3 – Phenanthrene	22.3		
		4 – Pyrene	<0.2		
		5 – Benzo[*a*]pyrene	<0.2		
Simulated landfarming Indigenous microorganisms Creosote waste mixed with sandy soil	Kidman fine sandy soil	3 – Fluorene	3.81[c]	N/A	Keck *et al* [44]
		Phenanthrene	1.65		
		Anthracene	2.04		
		4 – Fluoranthene	1.00		
		Pyrene	1.65		
		Chrysene	2.96		
		5 – Benzo[*b*]fluoranthene	ND		
		Benzo[*k*]fluoranthene	8.89		
		Benzo[*a*]pyrene	ND		
Simulated slurry systems Enrichment culture Soil contaminated with PAH	Soil from wood impregnation and coking plants	2 – Naphthalene	85	28 days	Wiessenfels *et al* [78]
		3 – Fluorene	95		
		Phenanthrene	97		
		Anthracene	68		
		4 – Fluoranthene	95		
		Pyrene	94		
		Chrysene	78		
		5 – Benzo[*a*]anthracene	87		
		Benzo[*a*]pyrene	74		
Simulated landfarming Indigenous microorganisms Soil spiked with PAH dissolved in dichloromethane	Kidman sandy loam soil	2 – Naphthalene	1.0[c]	N/A	Park *et al* [58]
		3 – Anthracene	63.8		
		Phenanthrene	7.6		
		4 – Fluoranthene	179.5		
		Pyrene	123.8		
		Chrysene	176.7		
		5 – Benzo[*a*]pyrene	147.1		

Table 1 Continued

Description	Soil/sediment	Compound (number of rings)	Degradation*	Time	Reference
Sediment/slurry system Indigenous microorganisms Continuous shaking Compound disappearance monitored Creosote-contaminated soils	Sandy subsurface and subsoil	2 – Naphthalene	100	30 days	Mueller *et al* [53]
		3 – Acenaphthylene	100		
		Acenaphthene	99.8		
		Fluorene	99.9		
		Phenanthrene	99.6		
		Anthracene	99.5		
		4 – Fluoranthene	99.3		
		Pyrene	77.1		
		Chrysene	94.7		
		5 – Benzo[*a*]pyrene	70.7		
		Benzo[*a*]anthracene	85.7		
Simulated landfarming Commercial and indigenous inoculum Sediments contaminated with PAHs	Contaminated sediments from Old Inger (CERCLA) site, LA	*Commercial inoculum*		61 days	Catallo and Portier [12]
		3 – Acenaphthene	1.00[c]		
		Anthracene	2.26		
		Phenanthrene	4.36		
		4 – Pyrene	1.78		
		5 – Benzo[*b*]fluoranthene	2.84		
		Indigenous microorganisms			
		3 – Acenaphthene	1.00		
		Anthracene	2.38		
		Phenanthrene	3.50		
		4 – Pyrene	2.24		
		5 – Benzo[*b*]fluoranthene	3.14		
Simulated landfarming Indigenous microorganisms Soil spiked with PAH dissolved in dichloromethane	Land soil, 3–10 cm from surface 74% sand, 21% silt, 5% clay	3 – Fluorene	1.32[a]	150 days	Leduc *et al* [47]
		Acenaphthylene	1.39		
		Acenaphthene	1.03		
		Anthracene	1.00		
Simulated landfarming Indigenous microorganisms Soil contaminated with PAHs	Tar contaminated, manufacturing gas plant site soil	2 – Napthalene	20	3 months	Erickson *et al* [23]
		3 – Acenaphthylene	53		
		Acenaphthene			
		Anthracene			
		Fluorene			
		Phenanthrene			
		4 – Benzo[*a*]anthracene	44		
		Chrysene			
		Fluoranthene			
		Pyrene			
		5 – Benzo[*b*]fluoranthene	45		
		Benzo[*k*]fluoranthene			
		Benzo[*a*]pyrene			

*Percent degraded unless otherwise stated. Superscript refers to all values within the subsection in which it appears.
[a]Relative rate of degradation.
[b]Percent mineralized.
[c]Relative half lives.

into the micellar pseudophase. Yeom *et al* [81] however, proposed two mechanisms to explain the surfactant-mediated release of sorbed PAHs: the increase of the concentration gradient at the soil–water interface, and the increase of the diffusivity of PAHs due to swelling of the soil organic matrix. From the experimental and model results it was concluded that the penetration of surfactant molecules, followed by swelling of the soil–tar matrix, was responsible for the increase in diffusivity and was the primary factor for the enhanced PAH release from the soil.

There are mixed reports regarding the ability of surfactants to enhance biodegradation. Some investigators have observed inhibition of PAH biodegradation in the presence of nonionic surfactants [4,45,69]; others have observed increased biodegradation with the addition of nonionic surfactants at concentrations below their CMC [3,4] or in the presence of surfactant micelles [11,29,64]. Tsomides *et al* [72] demonstrated that these confounding results may stem from experimental conditions. In these experiments, the effect of enhanced phenanthrene solubility on biodegradation rate and extent was studied in microcosms containing Houston Ship Channel sediments and Triton X-100 at levels above its CMC. Initially, the surfactant inhibited degradation as compared to surfactant-free controls. After 5 days, rapid mineralization in the surfactant systems was observed, and at that time a statistically significant ($P = 0.05$) increase in mineralization was observed in surfactant-amended systems as compared to controls. At the end of the experiment (22 days) there was no

statistical difference between surfactant systems or surfactant-free controls.

Since surfactant micelles represent a separate, suspended, pseudo-phase, the potential exists for the formation of a nonavailable PAH fraction during surfactant-enhanced solubilization of contaminants. Guha *et al* [30] studied the biodegradation kinetics of phenanthrene partitioned into micellar-phase nonionic surfactants. An effective bioavailable fraction of the micellar-phase phenanthrene was defined using four different surfactants, evaluated under various experimental conditions, and modeled mathematically. The results indicated that a fraction of the micellar-phase phenanthrene was directly bioavailable (implying the direct mass transfer from the micelle to the bacterial cell) and that this fraction decreases with increase in micelle concentration. It was also hypothesized that the fraction available is a function of the bacterial culture (hydrophobicity of the cell surface) and the type of surfactant (length of the hydrophilic chain).

The effect of surfactants on sediment bioremediation systems is thus unclear. Certainly surfactants may inhibit degradation, and therefore their addition may not be advisable without requisite laboratory testing. A further consideration in this area is the extensive partitioning of surfactants to sediments and its impact on cost. Enhanced PAH solubility occurs after the critical micelle concentration is reached. The sorption of surfactant to sediments must be overcome before micelle formation occurs, affecting the volume of surfactant required and the economics of the process.

Nutrient addition

Studies investigating the impact of nutrient addition on PAH bioremediation have been performed largely in connection with oil spill clean-up. Laboratory [13,54,74] and field studies [61,62,73,75] of the biodegradation of oil have found that nutrient addition increases the rate of biodegradation over that of unamended controls. However, in open systems such as a contaminated beach, there may be sufficient nutrients provided by tidal action or high background levels of nitrogen and phosphorus [75]. The effect of nutrient enhancement on biodegradation of beach material contaminated during the *Exxon Valdez* oil spill was investigated using three types of fertilizer: commercially available slow release formulations, an oleophilic fertilizer, and water-soluble fertilizer applied as a solution [61]. Two to three weeks after application of the oleophilic fertilizer, Inipol EAP 22, the oiled cobblestones on the beach were visibly cleaner than in control areas [61]. Oleophilic fertilizers are designed to concentrate nutrients at the oil/water interface, making them available to microorganisms at the oil surface [5]. However, the contribution of Inipol's oleophilic nature to bioremediation was not clear from the Prince William Sound field study [62]. Churchill *et al* [13] found that Inipol EAP 22 possessed surface-active characteristics in their experiments with hexadecane, octadecane, toluene, and 2-methylnaphthalene and pure bacterial cultures. Their results suggest that the surfactant properties of Inipol increased the bioavailability of hydrocarbons and that Inipol's success in the *Exxon Valdez* clean-up effort may have been significantly influenced by its surface-active properties. In closed systems (eg slurry reactors) nutrients must be added to meet the demands of maintaining an active population. For example, Lewis [48] added 177 mg L^{-1} nitrogen as ammonia and 27 mg L^{-1} phosphorus as orthophosphate in order to obtain the optimal ratio of total organic carbon to nutrients.

Bioaugmentation

To increase the biodegradation of contaminants, the addition of PAH-degrading bacteria has been suggested. Certain bacterial properties advantageous to PAH remediation have been identified by Mueller *et al* [54] and include the production of biosurfactants and the tendency of organisms to attach to surfaces. Augmentation of laboratory systems with PAH-degrading cultures is commonplace, but rarely can these results be translated to field studies. In work on oil spill remediation, the results of laboratory [54,74] and field studies [73,75] indicate that bioaugmentation does not significantly increase bioremediation rates over those produced by nutrient addition alone. In these studies, nutrients, not hydrocarbons were the limiting factor. Adding more microorganisms without additional nutrients did not therefore improve the rate of degradation [75]. The removal of biomass by tidal and wave action is suggested as a reason for the limited usefulness of bioaugmentation as well [54,75]. In experiments in slurry systems where washout would not occur, and isolates were added to the system, there was no attempt to determine the survivability of the augmented microorganisms [48]. Without this information, it is impossible to confirm the influence of bioaugmentation in slurry systems.

Discussion

Bioremediation of PAH-contaminated sediments is a challenging application of bioengineering with currently undetermined efficacy. Numerous studies have demonstrated the ability of microorganisms to degrade many of the PAHs of interest, but complicating factors encountered in field application have not been equally well addressed. This is particularly true for higher molecular weight PAHs containing four or more rings. Studies addressing the effects of mixtures, bioavailability, and enhancement of biodegradation most often focus either on the readily biodegradable 2- and 3-ring compounds or use complex media such as crude oil or coal tar, making interpretation of findings difficult and perhaps case-specific. Moreover, the use of pure cultures or uncharacterized inocula make the extrapolation of results to field systems tenuous.

To assess accurately the potential success of bioremediation at sites containing PAH-contaminated sediments, it is critical that we understand the factors that control biodegradation of higher molecular weight compounds in a complex medium. In particular, the effects of PAH mixtures and availability on the rate and extent of degradation needs to be clarified. Information from field studies shows that significant losses of 4- and 5-ring PAHs may occur in various bioremediation applications. Little is known about the mechanisms by which this occurs, how to predict the extent to which it occurs, or the ability to enhance degradation. Interestingly, there is little information on the microbial ecology of mixed culture PAH bioremediation systems.

Temporal changes in individual PAH concentrations and their net distribution may serve as a selective pressure for population shifts in the PAH-degrading population. Quantitative ecological studies in field systems would provide critical insight into this currently uncharacterized area, and clarify potential mixtures' effects, identify species-specific factors that impact bioavailability of high molecular weight PAH biodegradation (ie biosurfactant production or other physiological factors), and provide the basis for process enhancement through bioaugmentation.

The need to understand these systems better should not detract from the potential that bioremediation has demonstrated. Laboratory studies have shown that under ideal conditions, bioremediation is capable of rapidly decreasing the mass of many common PAHs in sediment/water and NAPL/water systems. In field studies the disappearance of many high molecular weight PAHs has been observed, although to varying extents. Ongoing research in slow release desorption processes will greatly assist in the assessment of attainable bioremediation endpoints. Fundamental advances in the elucidation of PAH metabolism will continue to expand the knowledge base for bioremediation processes. For example, the general assumption that aerobic conditions are required for PAH metabolism is now in question. Recent studies have demonstrated PAH mineralization under sulfate-reducing conditions [14]. The rates observed in this study were slow in comparison to aerobic processes, and certainly the metabolic process is less well understood. However, under conditions of slow mass transport, rapid rates of contaminant degradation are unnecessary, and with greater understanding of anaerobic metabolism it may be possible to improve the rates observed. Continued progress in these fundamental areas of physiochemical processes and microbiology, combined with the requisite studies in model laboratory and field systems will resolve many current uncertainties regarding the potential and efficacy of bioremediating PAH-contaminated sediments.

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