Chapter 7
Bacterial Degradation of High Molecular Weight Polynuclear Aromatic Hydrocarbons

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7.1 Introduction

7.1.1 Sources of PAHs in the Environment

Polycyclic aromatic hydrocarbons (PAHs) are a group of organic compounds consisting of two and more than two fused benzene rings. PAHs are naturally present in the fossil fuel. However, the increased level of PAHs in the environment over the last few decades is due to the huge increment in production and use of petroleum and petroleum products. Point sources of PAHs originate from accidental discharges during production, transportation and disposal of petroleum and its products and industrial processes such as, liquefaction and gasification of coal and waste incineration. Creosote and coal tar, which are by-products of coking, are rich source of PAHs containing 85–90% of it (Cerniglia 1992).

At contaminated sites, PAHs are often present along with other contaminants, such as, BTEX (benzene, toluene, ethyl-benzene and xylene) compounds, aliphatic hydrocarbons and heavy metals. Depending on the source of contamination, the level of PAHs in soil has been found to range from 1 to 300 μg/kg. Air-borne PAHs originating from incomplete combustion of organic materials can give rise to high PAH levels in the atmosphere (60 $\mu g/m^3$–3 mg/m^3 of air) (Bamforth and Singleton 2005). PAHs as well as numerous PAH derivatives, i.e., alkylated PAHs, nitro-PAHs, oxygenated PAHs, quinones, hydroxy and hydroxynitro compounds, are formed during incomplete combustion of various fuels such as, coal, gasoline, diesel and biofuels (Mukherji et al. 2002). Natural processes, such as volcanic

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eruption and forest fires are other sources of PAHs in the environment. PAHs are also formed through geochemical processes, such as, exposure of sediments to high temperature during sediment diagenesis.

7.1.2 Physical and Chemical Properties of PAHs

PAHs are a group of compounds composed of two or more fused aromatic rings in linear, angular, and cluster arrangements as shown in Fig. 7.1. Based on structural diversity, PAHs can be broadly categorized into alternant and non-alternant classes. Alternant PAHs contain only fused benzenoid rings (e.g., anthracene, phenanthrene and pyrene), whereas non-alternant PAHs contain four or five carbon aromatic rings in addition to benzene ring (e.g., fluorene and fluoranthene) (Harvey 1991). PAHs can also be classified as low molecular weight (LMW) and high molecular weight (HMW) PAHs depending upon the number of aromatic rings. PAHs containing three or less than three aromatic rings are known as LMW PAHs (e.g., naphthalene and phenanthrene) and PAHs having greater than three aromatic rings are known as HMW PAHs (e.g., pyrene, fluoranthene, chrysene). Most of the PAHs have melting points above room temperature and boiling points above 100°C and are characterized by relatively low aqueous solubility.

Greater thermodynamic stability of PAHs arises from the delocalization of π-electron density. Thermodynamic stability and low aqueous solubility make them recalcitrant in the environment. The impact of PAH structure on its chemical behavior can generally be categorized as follows: PAHs with a linear structure are more unstable compared to their angular counterparts. Increased size and angularity of the PAH structure increase hydrophobicity and electrochemical stability which, in turn, affect their chemical and photochemical reactivity, as well as their ionization potential, vapor pressure, solubility and adsorption characteristics. The environmental fate of a PAH molecule depends on the number of aromatic rings and also on the pattern of ring linkage (Peters et al. 1999). The persistence of PAHs increases with an increase in the number of benzene rings and this is inversely correlated with the environmental biodegradation rates of PAHs.

Some PAHs contain a "bay-region" and a "K-region" (Mrozik et al. 2003). The bay- and K-region epoxides are reported to be highly reactive. Both these regions are present in phenanthrene as illustrated in Fig. 7.2. The bay-region of phenanthrene is between carbon atoms 4 and 5 whereas the K-region is between the carbon atoms 9, 10. The bay-region is a sterically hindered area. According to the Schmidt-Pullman electronic theory, K-region epoxides are likely to be more carcinogenic than the parent hydrocarbon.

LMW PAHs are characterized by higher volatility, higher solubility and greater ease of degradation than the higher molecular weight PAHs. HMW PAHs strongly sorb on to soil and sediments due to their high hydrophobicity and are therefore less available to indigenous microorganisms for degradation. The association of a contaminant with organic matter in soil (expressed as the organic matter

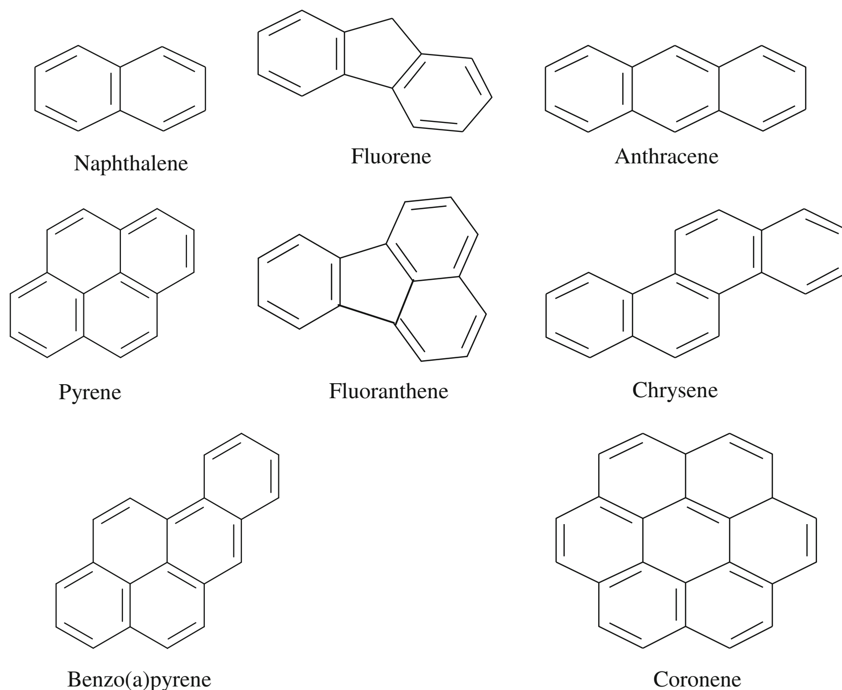

Fig. 7.1 Chemical structure of some polynuclear aromatic hydrocarbons

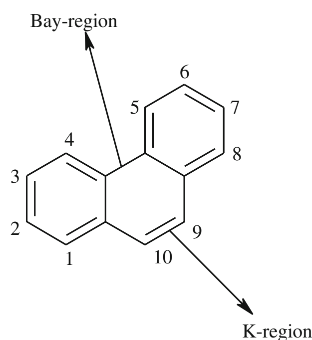

Fig. 7.2 Structure of phenanthrene showing bay-region and K-region

normalized partition coefficient K_{oc}) is a function of hydrophobicity of the compound which is expressed in terms of the octanol–water partitioning coefficient K_{ow}. Some properties of selected PAHs are presented in Table 7.1.

7.1.3 Toxicity of PAHs

Removal of PAHs from the environment is a major concern since they can exert acute as well as chronic toxicity (Juhasz et al. 2000). The highly hydrophobic and ipophilic

Table 7.1 Physical and chemical properties of selected PAHs

PAHs	Phenanthrene	Fluorene	Fluoranthene	Pyrene
Molecular formula	$C_{14}H_{10}$	$C_{13}H_{10}$	$C_{16}H_{10}$	$C_{16}H_{10}$
Molecular weight	178.22	166.21	202.26	202.26
Boiling point (°C)	340	295	375	404
Melting point (°C)	101	116.5	105–110	151.2
Density (g/cc)	1.179	1.202	1.252	1.271
No. of aromatic rings	3	3	4	4
Aq. solubility* (mg/L)	1.18	1.89	0.24	0.13

*Aqueous solubility is reported at 25°C
Source Yaws (1999) and Material Safety Data Sheets

nature of PAHs is responsible for their accumulation in the fatty tissue. Thus, PAHs are biomagnified through the food chain. Lower molecular weight PAHs impose greater acute toxicity because of their higher aqueous solubility compared to the higher molecular weight PAHs. PAHs can enter the human body through dermal exposure and food intake. It has been found that PAHs, especially, pyrene can get absorbed through dermal administration (75%). Acute toxicity is often reported based on lethal dose required to kill 50% of the test animals ($LD_{50,}$ mg/Kg-Body weight) in animal assays. LD_{50} value of naphthalene based on mice bioassay with oral administration is found to lie in the range of 533–710 mg/Kg while that for phenanthrene is 750 mg/Kg. The LD_{50} value for anthracene, fluoranthene, pyrene and benzo(a)pyrene (BaP) are $>$ 450, 100, 514 and 232 mg/Kg, respectively, based on mice bioassays with intraperitonial exposure (Bamforth and Singleton 2005). Exposure through inhalation may occur near combustion sources since PAHs are formed during incomplete combustion of carbonaceous fuels. Bacterial assays, human cell mutagenicity assays, animal assays and epidemiological studies have demonstrated the mutagenicity and carcinogenicity of PAHs associated with combustion and ambient aerosols. Although unsubstituted PAHs are not directly genotoxic to the mammalian system, a cytochrome P450 monooxygenase enzyme mediated reaction activates them to reactive epoxides that are genotoxic (Mukherji et al. 2002). However, various substituted PAHs are directly genotoxic. USEPA has listed 17 PAHs as priority pollutants for their toxic, mutagenic and carcinogenic properties. BaP is recognized as a potent human carcinogen while several other PAHs, such as, chrysene, are also classified as possible human carcinogens (IARC 1983, 1998).

7.2 Bioremediation of PAHs

Over the past two decades, numerous researchers have demonstrated the ability of microorganisms, such as, bacteria, fungi and algae to degrade PAHs (Cerniglia 1992). Such PAH-degrading microorganisms are commonly found in soil and in the aquatic environment. The first step in bacterial degradation of PAHs involves an initial oxidative attack in the presence of dioxygenase enzyme to form

cis dihydrodiol. The introduction of two hydroxy groups facilitates further degradation. Cleavage of the aromatic ring also occurs in the presence of molecular oxygen. Microbial degradation can potentially transform toxic PAHs, into benign compounds at a relatively low cost. Hence, biodegradation has gained attention for the clean-up of PAH contaminated sites. Bioremediation was adopted at numerous PAH contaminated Superfund sites in United States. Biodegradation of PAHs composed of three rings is well established and the degradation of HMW PAHs (composed of more than three rings) by bacteria has also been reported. Several bacterial genera have been identified for their ability to degrade PAHs, including *Pseudomonas*, *Alcaligenes, Mycobacterium*, *Rhodococcus*, *Sphingomonas* and *Cycloclasticus*. However, the microbial degradation of PAHs containing four or more aromatic rings is energetically less favorable compared to degradation of LMW PAHs (Mueller et al. 1997). BaP has been the subject of comprehensive studies on biodegradation due to its carcinogenicity (Kanaly and Harayama 2000).

7.2.1 Degradation of Four Ring PAHs

Of the four ring PAHs, degradation of fluoranthene, pyrene, chrysene, and benzo(*a*)anthracene has been investigated to varying degree. Fluoranthene, a non-alternant PAH containing a five carbon aromatic ring, is observed to be metabolized by a variety of bacteria, and pathways describing its biodegradation have been proposed.

7.2.1.1 Degradation of Fluoranthene by Bacteria

In 1990, two independent groups of researcher reported the isolation of a single organism capable of utilizing fluoranthene as a sole source of carbon and energy. Weissenfels et al. (1990) demonstrated the isolation of the soil microorganism *Alcaligenes denitrificans* strain WW1, which biodegraded fluoranthene at a rate of 0.3 mg/ml per day for an initial concentration of 1 mg/ml. It could also utilize pyrene and benzo(*a*)anthracene by cometabolism. A dioxygenase pathway was involved as confirmed by identification of some metabolites. Three metabolites of fluoranthene biodegradation by strain WW1, 7-hydroxyacenaphthylene, 7-acenaphthenone, and 3-hydroxymethyl-4,5-benzo-coumarine, were identified by UV, mass spectroscopy and NMR spectroscopic methods (Weissenfels et al. 1991).

The bacterium *Sphingomonas paucimobilis* EPA505 reported by Mueller et al. (1990) was isolated from a seven member bacterial community. This culture was capable of utilizing fluoranthene as a sole source of carbon and energy. Use of a non-ionic surfactant Tween 80 (200 mg/L) increased the aqueous solubility of fluoranthene from 1 to 28 μM and decreased the generation time of the strain from 21 to 11 h. A resting cell suspension of EPA505 grown on fluoranthene was also capable of utilizing other four ring and five ring PAHs, such as, benzo(*b*)fluorene, benzo(*a*)anthracene, chrysene and pyrene.

In batch cultures with 1.66 mg/L fluoranthene, Kelley and Cerniglia (1991) reported that *Mycobacterium* sp. strain PYR-1 was capable of degrading greater than 78% fluoranthene within 5 days. When added to soil and water microcosms, *Mycobacterium* strain caused seven-fold increase in mineralization of fluoranthene over the indigenous microorganisms. Although mineralization of fluoranthene to CO_2 was rapid, some metabolites were identified: 8-hydroxy-7-methoxyfluoranthene, 9-hydroxyfluorene, 9-fluorenone, 9-fluorenone-1-carboxylic acid, 1-acenaphthenone, 9-hydroxy-1-fluorene carboxylic acid, phthalic acid, 2-carboxybenzaldehyde, benzoic acid, phenyl acetic acid, and adipic acid.

7.2.1.2 Degradation of Pyrene by Bacteria

The first report of pyrene degradation as sole source of carbon and energy was demonstrated for a *Rhodococcus* sp. strain UW1 (Walter et al. 1991). Within 2 weeks, 72% (initial concentration 500 mg/L) was mineralized to CO_2. A metabolite of molecular formula $C_{16}H_{10}O_4$ was identified. It was formed as a result of recyclization of the direct *meta*- fission product of pyrene. This strain could also utilize chrysene as sole source of carbon and energy.

Various *Mycobacterium* species have been reported for their pyrene degrading ability and the pathways have also been hypothesized based on identification of metabolites. Heitkamp and Cerniglia (1988) isolated a *Mycobacterium* sp. from sediments near a hydrocarbon source. This culture could mineralize pyrene when grown in mineral salts medium supplemented with organic nutrients. Pyrene induced culture mineralized over 60% of radiolabeled pyrene within 96 h. Enzymes responsible for pyrene catabolism seemed to be inducible, since a long lag phase in pyrene mineralization was observed in cultures grown in the absence of pyrene. In contrast, no pyrene mineralization was observed in non-induced culture. Seven metabolites of pyrene metabolism were detected by high-performance liquid chromatography. This included three ring oxidation products, *cis*-4,5-pyrene dihydrodiol, *trans*-4,5-pyrene dihydrodiol, and pyrenol. Four ring fission products were also observed, i.e., 4-hydroxyperinaphthenone, 4-phenanthroic acid, phthalic acid, and cinnamic acid. 4-phenanthroic acid was the major metabolite. Pyrenol was possibly formed by non-enzymatic dehydration of pyrene dihydrodiols or by oxidative metabolism of pyrene by the *Mycobacterium*. Multiple pathways for initial oxidative attack on pyrene was suggested since both *cis*- and *trans*-4,5-dihydrodiols were identified as metabolites. Further studies confirmed that both dioxygenase and monooxygenase enzymes were secreted by this microorganism. Sediment microcosms from where this strain was isolated showed enhanced mineralization of various PAHs, including pyrene and BaP when inoculated with the *Mycobacterium* strain (Heitkamp and Cerniglia 1989). Although this strain could utilize pyrene as primary substrate, pyrene degradation was inhibited by increasing the organic nutrients in the microcosm. Nutrient depletion was possibly caused by the utilization of organic nutrients by the rapidly growing indigenous microorganisms.

Later, other *Mycobacterium* species, i.e., *Mycobacterium* sp. strain RJGII-145 (Grosser et al. 1991), *Mycobacterium flavescens* (Dean-Ross and Cerniglia 1996) and *Mycobacterium* sp. strain KR2 (Rehmann et al. 1998) have been reported to utilize pyrene as a sole source of carbon and energy. *Mycobacterium* sp. strain RJGII-135 was isolated by Grosser et al. (1991) from soil near a coal gasification plant. It utilized pyrene as sole source of carbon and energy. When the pyrene induced culture was reintroduced in the soil containing pyrene, enhanced mineralization of pyrene was observed. Pyrene mineralization reached 55% within 2 days, compared to 1% for the indigenous population. Metabolites of pyrene degradation were also identified through further studies (Schneider et al. 1996). Three stable intermediates, 4-phenanthrene carboxylic acid, 4,5-pyrene dihydrodiol, 4,5-phenanthrene dicarboxylic acid were formed within 4–8 h after the start of the experiment.

Mycobacterium sp. strain BB1 was isolated from a former coal gasification site (Boldrin et al. 1993). Fluoranthene and pyrene were used as sole source of carbon and energy by this culture. It exhibited a maximum growth rate of 1.2 mg/L/h for large crystals of pyrene and 5.6 mg/L/h for small crystals during exponential growth in a 1.6 L fermenter with an initial concentration of 0.5 g/L of pyrene. This strain was used to examine the effects of various culture conditions, such as degradation of pyrene at low dissolved oxygen concentrations (Fritzsche 1994), utilization of PAHs in mixtures (Tiehm and Fritzsche 1995) and degradation of pyrene in presence of non-ionic surfactants. Jimenez and Bartha (1996) used a *Mycobacterium* sp. in solvent-augmented mineralization of pyrene. The cells, which physically adhered to solvent droplets, showed 8.5 times faster rate of pyrene degradation compared to cells in suspension. Another *Mycobacterium* sp. strain CH1 isolated from PAH contaminated freshwater sediments could mineralize pyrene and fluoranthene as the sole carbon and energy source. This culture was also capable of growth on a wide range of branched alkanes and n-alkanes (Churchill et al. 1999). *Gordona* sp. strain BP9 and *Mycobacterium* sp. strain VF1 were isolated from hydrocarbon-contaminated soil and each was capable of utilizing fluoranthene and pyrene as sole carbon and energy sources (Kastner et al. 1994). Reintroduction of BP9 into soil after growth on pyrene (200 mg/L) as sole source of carbon and energy exhibited six-fold increase in pyrene metabolism compared to native un-inoculated soil microorganisms (Kastner et al. 1998). The rate and extent of pyrene degradation by various pure cultures are listed in Table 7.2.

7.2.2 Limitations in Degradation of PAHs in Soil

Many microorganisms are metabolically capable of mineralizing or partially transforming PAHs in the environment. Some fungi can produce extracellular enzymes, but for most of the bacteria, the pollutants must cross the cell membrane to get direct access to the enzymes or at least attach to the membrane bound enzymes. With the exception of some bacterial species that can attach directly to the interface between the organic and aqueous phase, in general for degradation to

Table 7.2 Rate and extent of pyrene degradation by pure cultures

Bacterial strains	μ_{max} (1/h)	K_s	Y	Maximum biodegradation rate (mg/ml d)	% Degradation (Initial conc., mg/L)	Time (day)	Reference
Rhodococcus sp. UW1	ND	ND	ND	0.08	72% (500 mg/L)	14	Walter et al. (1991)
M. flavescens ATCC 700033	ND	ND	ND	0.806	38.8% (50 mg/L)	14	Dean-Ross and Cerniglia (1996)
Mycobacterium sp. KR2	ND	ND	ND	0.037	60% (500 mg/L)	8	Rehmann et al. (1998)
Pseudomonas sp. strain LP1	0.018	ND	ND	0.159	68% (100 mg/L)	30	Obayori et al. (2008)
P. aeruginosa LP5	0.024	ND	ND	0.118	67% (100 mg/L)	30	Obayori et al. (2008)
P. aeruginosa LP5	0.017	ND	ND	0.096	47% (100 mg/L)	30	Obayori et al. (2008)

take place, the contaminants must be solubilized in the aqueous phase. The two major mass transfer limitations are caused by: (a) compound availability and (b) crossing of the membrane.

7.2.2.1 Compound Availability

Bioavailability of a compound depends on its physico-chemical properties. Aqueous solubility plays the most important role in determining the potential for bioremediation. Solubility of PAHs is decreased with increase in the number of rings. Very low aqueous solubility and high hydrophobicity (high K_{ow}) are the two governing factors responsible for the recalcitrance of four and greater than four ring PAHs. Higher ring PAHs often exist in a separate phase, i.e., as components of non-aqueous phase liquids (NAPLs) or remain strongly sorbed on to soil. Mukherji and Weber (1998, 2001) demonstrated that mass transfer of naphthalene from a NAPL affected its biodegradation rate. However, some cultures are reported to overcome bioavailability limitations by virtue of enhanced cell surface hydrophobicity (CSH) as discussed later.

7.2.2.2 Crossing of Membrane

Another challenge is for PAHs to cross the cell membrane of the microorganism for gaining access to the enzymes present inside the cell. Crossing the membrane is required even for membrane bound enzymes since they are located on the inner side of the cell membrane. Both sides of the membrane are considered fairly polar due to

the presence of polar head groups on the phospholipids that comprise the membrane while the center region is non-polar in nature due to the fatty acid moieties of the phospholipids. In addition to the polarity obstacles, transport through the cell membrane is also affected by the membrane associated proteins and peptidoglycan. In gram-negative bacteria, an additional outer membrane needs to be crossed. For a contaminant to overcome all the cell barriers and access the enzymes inside the cell, it must either be selectively taken into the cell, or it must have the right combination of polarity, size, and functional groups to get across the cell membrane. Bressler and Gray (2003) surveyed the literature and determined the maximum biodegradation rate of various PAHs under aerobic condition. The maximum biodegradation rate was correlated with log K_{ow}. PAHs with log K_{ow} values above three demonstrated reduced biodegradation. This limitation is mainly due to the poor aqueous solubility and adsorption properties of these compounds, however, the repulsion of these highly non-polar compounds by the polar regions of the cell membrane cannot be ignored. Compounds with log K_{ow} values below one also demonstrated reduced rates of biodegradation possibly due to reduced ability of these extremely polar compounds to diffuse across the non-polar regions of the cell membrane.

Bressler and Gray (2003) derived an expression for maximum flux through the membrane (F_{max}) as a function of the octanol water partition coefficient (K_{ow}), molecular weight (MW) and aqueous solubility (C_{aq}) of a compound (Eq. 7.1) and computed the values for some known environmental contaminants. They assumed the membrane crossing step as the rate limiting step in biodegradation assuming that the intracellular concentration of a compound is maintained as zero by the active enzyme and demonstrated a linear relationship between the maximum reported biodegradation rate and the maximum membrane flux for various environmental contaminants and fitted a regression line using the data set.

$$F_{max} = 0.003\left(K_{ow}C_{aq}/\,MW^{0.5}\right) \tag{7.1}$$

They concluded that compounds with biodegradation rate falling significantly above the regression line (such as, toluene and phenol) are not transported by diffusion through the lipid bilayer. In contrast, those compounds having biodegradation rate falling significantly below the regression line are limited by the rate of enzymatic conversion rather than by membrane permeation. Biodegradation of fluorene, chrysene and several other PAHs falling on the regression line are limited by the membrane permeation. In contrast, the maximum biodegradation rate of the four ring PAHs, fluoranthene and pyrene was well below the predicted line. Thus, biodegradation of pyrene and fluoranthene is limited by the synthesis of key enzymes.

7.2.3 Surfactant Enhanced Biodegradation of HMW PAHs

Contradictory and inconclusive results have been observed regarding the effects of surfactants on the biodegradation of PAHs at surfactant concentration below and above the critical micelle concentration (CMC) (Margesin

and Schinner 1999). Some studies showed an enhanced effect in which not only LMW PAHs were degraded successfully, but even compounds containing four and more than four rings were also degraded effectively. In contrast, in other studies, degradation was found to be inhibited. While some researchers believe that PAHs solubilized within surfactant micelles are completely bioavailable, yet others believe that they are only partially bioavailable or not at all bioavailable (Guha and Jaffe 1996; Luning Prak and Pritchard 2002). Volkering et al. (1995) reported a strong inhibition in biodegradation of polynuclear aromatic hydrocarbons (PAHs) in the presence of surfactants above their CMC. Surfactants may also directly interact with the bacterial cell surfaces, the interaction being dependent both on the type of surfactant and the type of microorganisms. Efroymson and Alexander (1991) reported that Triton X-100 prevented the adherence of cells to an organic-aqueous interface, however, this had a beneficial effect on naphthalene biodegradation by *Arthrobacter* sp. Since naphthalene has relatively high aqueous solubility, it was available both from the aqueous phase as well as from the organic-water interface. Hindered adherence may have adversely affected the uptake of HMW PAHs with very low aqueous solubility. However, Triton X-100 is also reported to promote the attachment of *Burkholderia* cultures to the NAPL-water interface (Mohanty 2010). Some surfactants are toxic to bacterial cultures while enhanced solubilization of toxic components from a multicomponent NAPL phase such as petroleum caused by the surfactants, may adversely affect bacterial cultures.

Thibault et al. (1996) used two pyrene degrading soil *Pseudomonas* species in soil reinoculation experiments to test the effects of four surfactants, namely, Simple Green, Biosolve, Witconol SN70 and Sodium dodecyl sulphate (SDS). Witconol SN70 caused maximum enhancement in solubilization and mineralization of pyrene under unsaturated conditions. However, when pyrene degraders were inoculated into soil slurries, degradation of pyrene was highest in the absence of surfactants. It was concluded that increased solubilization of pyrene in the soil slurry, caused toxicity to the microorganisms.

Boonchan et al. (1998) evaluated various surfactants for their impact in improving the biodegradation of four, five and seven ring PAHs by *Strenotrophomonas maltophilia* VUN 10,010, isolated from manufactured gas plant site soil. All the cationic and anionic surfactants tested were found to be highly toxic for the bacterial strains while the Tween series was utilized as growth substrate. Five non-ionic surfactants (Brij 35, Igepal CA-630, Triton X-100, Tergitol NP-10, and Tyloxapol) increased the apparent solubility of fluoranthene, pyrene and B(a)P at least 250 fold at 10 g/L surfactant concentration. They were less toxic and were not used as growth substrates. Pyrene and also the five and seven ring PAHs were utilized by the culture as sole source of carbon and energy. Not only the solubility, but also the rate of pyrene degradation by strain VUN 10,010 was enhanced by the addition of the four non-ionic surfactants (5–10 g/L). However, an inhibition in degradation rate was observed with Igepal CA-630 (5 g/L). The specific growth rate of VUN 10,010 on pyrene was increased by 67% in the presence of 10 g/L

Brij 35 or Tergitol NP-10. The addition of Brij 35 and Tergitol NP-10 to media containing a single HMW PAH (four and five benzene rings) as the sole carbon source increased the maximum specific PAH degradation rate and decreased the lag period normally seen for PAH degradation. The addition of Tergitol NP-10 to VUN 10,010 cultures which contained a PAH mixture (three to seven benzene rings) substantially improved the overall degradation rate of each PAH and increased the specific growth rate of VUN 10,010 by 30%. Their results suggested that besides the improvement in dissolution rate, surfactants may also facilitate the transport of PAHs through the cell membrane.

Doong and Lei (2003) observed a correlation between the polyoxyethylene chain length and the solubilization efficiency of surfactants. Four non-ionic surfactants and one anionic surfactant were found to enhance the bioavailability of naphthalene, phenanthrene and pyrene with efficiencies ranging from 21.1 to 60.6, 33.3 to 62.8 and 26.8 to 70.9%, respectively. The trend in degradation efficiency was as follows: Brij 30 > Triton X-100 > Tween 80 > Brij 35. The HLB values of Brij 30, Triton X-100, Tween 80 and Brij 35 were 9.7, 13.5, 15.0 and 16.9, respectively. With increase in HLB value, the degradation potential of the surfactant was decreased. Triton X-100 and Brij 30 were utilized as sole source of carbon and energy by *Pseudomonas putida*. Although the dissolution of PAHs was increased, Brij 35 and Tween 80 inhibited the growth of *P. putida*. Increase in surfactant concentration also decreased the mineralization rate of pyrene possibly due to mass transfer limitation from the micelle. Mass transfer processes are proportional to concentration gradient of PAHs inside the micelle so that when the PAHs are diluted in a larger micellar mass, transfer to the cells may decrease. Another possibility may be catabolic repression due to preferential utilization of the surfactant.

Willumsen and Arvin (1999) studied the impact of Triton X-100 on fluoranthene degradation by *S. paucimobilis* EPA505. Although the surfactant enhanced mineralization of fluoranthene, it was much lower compared to the increase in solubilization. A model which described the degradation kinetics of Triton X-100 solubilized fluoranthene by *S. paucimobilis* EPA505 cells was also developed after accounting for micellar solubilization, metabolite accumulation and its bioavailability considerations. In the presence of Triton X-100 and calcium, the mineralization rate of fluoranthene by *S. paucimobilis* EPA505 was almost doubled (Willumsen and Karlson 1998). In contrast, absence of calcium inhibited fluoranthene mineralization possibly due to the adverse effect of Triton X-100 on the cytoplasmic membrane.

In a study involving the Tween series surfactants (Tween 20, Tween 40, Tween 60 and Tween 80), Kim and Weber (2003) reported enhanced solubilization of phenanthrene at different doses of surfactant above their CMC. However, the micelle solubilized phenanthrene was not bioavailable to *S. paucimobilis* EPA 505. Preferential microbial uptake of the hydrophobic part of the chemical surfactant caused destabilization of the micelles due to an imbalance in the amphiphilic molecule. Thus, phenanthrene was released from the micellar core such that its biodegradation was increased.

7.3 Bacterial Mechanisms for Overcoming Bioavailability Limitation

It is generally believed that most bacteria can access and degrade pollutants only when they are dissolved in water. However, some PAH-degrading bacteria are reported to overcome the bioavailability limitations for enhancing uptake through (1) direct interfacial uptake and (2) uptake facilitated by secretion of biosurfactants.

7.3.1 Direct Interfacial Uptake

In direct interfacial uptake, bacteria attach itself to the surface of the NAPL droplets which are larger in size compared to the bacterial cell. Substrate uptake takes place at the point of contact through diffusion. CSH plays an important role in direct uptake. Microorganisms capable of direct uptake can modify their cell surface outer membrane to enhance CSH. In this case, substrate uptake does not depend on the dissolution rate. Some microorganisms have surface structures such as, fimbriae and capsules which help them to attach to the NAPL-water interface such that they can directly uptake substrate from a separate phase.

CSH is an important parameter affecting the adhesion behavior of bacterial cells to surfaces and interfaces. CSH is commonly measured by determining the contact angle (Busscher et al. 1984) of a uniform layer of cells. Some other assays for hydrophobicity include hydrophobic interaction chromatography (Dillon et al. 1986), salt aggregation test (Lindahl et al. 1981), polystyrene microsphere attachment (Rosenberg 1981) and microbial attachment to microsphere (Zita and Hermansson 1997). The bacterial adhesion to hydrocarbons (BATH) assay (Rosenberg et al. 1980) is also used for determining the potential of a culture for direct interfacial uptake.

BATH assay is a simple and quantitative method for determining the degree of adherence of bacterial cells to a specific liquid hydrocarbon, i.e., n-hexadecane when an aqueous suspension of bacterial cells is mixed with the hydrocarbon. Adherence is directly related to change in absorbance of the aqueous suspension with respect to the control. However, BATH assay results are affected by solution phase interaction between the NAPL and bacterial culture, hence it is not a true measure of CSH. Bacterial cell surface charge is reported to vary with pH, growth medium, culture age and ionic strength. At a certain pH, the maximum adherence occurs when either the bacterial culture or the NAPL droplet is uncharged. Busscher et al. (1995) reported the zeta potential of various solvents such as, n-hexadecane, toluene and xylene ranges from -63 to -18 mV at pH 7. Chakraborty et al. (2010) demonstrated the effect of NAPLs of varying composition serving as growth substrate on adherence measured in BATH assay and surface charge of three bacterial strains *Burkholderia cepacia* (ES1) and

Burkholderia multivorans (NG1 and HN1). All the cultures used depicted growth on the NAPLs composed of n-hexadecane, naphthalene, phenanthrene and pyrene in varying proportion. Change in NAPL used as growth substrate affected both the zeta potential and CSH of the cultures (based on contact angle measurements), which in turn affected their adherence to the NAPL-water interface determined using the BATH assay. Zeta potential was found to be close to zero at pH 2 for all the cultures. For each culture a strong inverse correlation was observed between zeta potential and adherence to n-hexadecane. Thus, BATH assay results are the outcome of both hydrophobic and electrostatic interaction between the bacterial culture and NAPL phase.

Some bacterial cultures are capable of inducing high CSH when only hydrocarbon/oil is provided as sole substrate. This enhancement in hydrophobicity is reported to facilitate the direct interfacial uptake of hydrocarbons from NAPLs and the uptake of solid hydrocarbons. Most PAHs other than naphthalene and substituted naphthalenes have low aqueous solubility of the order of 1 mg/L or less so that direct interfacial uptake may provide a distinct advantage to the microorganism utilizing it as a substrate. Induction of CSH has been demonstrated by various researchers by growing cultures both in the presence of a readily degradable soluble substrate and in the presence of hydrocarbons/oil. Subsequently, the CSH is determined through contact angle measurements or BATH assay. *Pseudomonas* sp. strain PP2 grown on phenanthrene depicted greater adherence to aliphatic (hexane and heptane) and aromatic hydrocarbons (benzene and xylene) compared to the dextrose grown cells (Prabhu and Phale 2003). These changes were attributed to growth associated with production of an extracellular biosurfactant. *Mycobacterium* sp. LB501T is reported to form a biofilm on solid anthracene provided as sole substrate (Wick et al. 2002). Such a biofilm formation was not observed when glucose was provided as a co-substrate along with anthracene. This was attributed to surface modifications that caused enhancement in hydrophobicity of anthracene grown cells. In adhesion experiments, anthracene grown cells demonstrated 1.5–8.0 fold greater adherence to Teflon and up to 70 times greater adherence to anthracene surfaces compared to glucose grown cells (Wick et al. 2002). This activity was linked to specific changes in the phospholipid fatty acid (PLFA) and glycolipid fatty acid (GLFA) patterns (Wick et al. 2003). In another study, mineralization of fluoranthene by a *Mycobacterium* strain was facilitated by direct attachment to fluoranthene provided as sole substrate (Willumsen and Karlson 2001). High CSH and adherence to emulsified solvent droplets containing dissolved pyrene facilitated its mass transfer to the degrading bacteria, during solvent-augmented biodegradation by a *Mycobacterium* sp. (Jimenez and Bartha 1996).

Similar enhancement in hydrophobicity and attachment to NAPLs has been reported for uptake of aliphatic hydrocarbons from NAPLs, such as n-hexadecane, diesel and petroleum (Amin et al. 1996; Mohanty and Mukherji 2008). Thus, cultures can actively regulate their CSH as a strategy for optimizing the uptake of substrates characterized by low aqueous solubility. In addition to extracellular biosurfactants, the extracellular polymeric substances (EPS) associated with

bacterial cells may play a role in bioavailability enhancement and enhanced attachment to NAPLs. The role of EPS in affecting attachment to NAPLs was implicated for an alkane degrading *Rhodococcus* strain Q15 (Whyte et al. 1999). For *Rhodococcus* sp. strain 094, a good correlation was observed between CSH, alkane oxidation and EPS activity (Berdholt et al. 2002). EPS release in the stationary phase was correlated with decrease in hydrophobicity and emulsification of the culture broth. Membrane bound vesicles with some emulsifying activity were responsible for CSH in *Acinetobacter venetianus* RAG-1 and *Acinetobacter* sp. strain H01-N (Leahy et al. 2003). Mohanty and Mukherji (2008) demonstrated the effect of growth substrates by conducting the BATH assay using two oil degrading bacterial cultures *B. cepacia* ES1 and *Exiguobacterium aurantiacum* AS1 with two different NAPL types, i.e., diesel and n-hexadecane. For both the cultures, adherence to a NAPL phase was highest when the corresponding NAPL was used for culture growth. For dextrose grown cultures of *B. cepacia* and *E. aurantiacum*, negligible adherence to NAPL phase was observed irrespective of the NAPL phase used in the assay.

7.3.2 Production of Biosurfactants and PAH Uptake

Production of biosurfactant is another mechanism adopted by certain bacterial species to degrade complex petroleum hydrocarbons. Like chemical surfactants, biosurfactants are amphiphilic molecules having both hydrophilic and hydrophobic regions which help them in attachment to different surfaces. Biosurfactants are categorized into groups based on their MW (Ron and Rosenberg 2002). LMW surfactants are typically glycolipids where the carbohydrate group is attached to long chain alkyl acids or lipoproteins. Rhamnolipids, trehalolipids and sophorolipids are a few known biosurfactants that come under this category. Several species of *Pseudomonas* are reported to produce rhamnolipids. External addition of rhamnolipids was reported to have varying impacts on CSH of bacterial cultures degrading phenanthrene, i.e., *Bacillus subtilis* BUM and *P. aeruginosa* P-CG3, which, in turn, was found to alter the relative contribution of the two cultures in phenanthrene biodegradation (Zhao et al. 2011). External addition of a sophorolipid mixture produced by *Candida bombicola* has been shown to enhance phenanthrene biodegradation by *Pseudomonas yanoikuyae* through enhanced solubilization (Schippers et al. 2000).

HMW biosurfactants are composed of polysaccharides, proteins, lipopolysaccharides, lipoproteins or a mixture of these polymers. They are less effective in reducing interfacial tension compared to LMW biosurfactants. However, they can create a hydrophilic coat around the oil droplets which inhibits coalescence. The most studied are surfactants produced by different species of the genera *Acienetobacter*. Emulsan produced by *Acinetobacter* RAG-1 and alasan produced by *Acinetobacter radioresistens* are a complex mixture of heteropolysaccharides and proteins. One of the protein moieties of 45 kDa in alasan has

been studied extensively and reported to be the most important structural component determining the activity of the complex. It contributed to the formation of stable oil–water emulsion (Navon-Venezia et al. 1998; Toren et al. 2001). Only 500 μg/ml of alasan increased the aqueous solubility of phenanthrene, fluoranthene and pyrene by 6–25 times. The presence of alasan more than doubled the mineralization rate of the PAHs by *S. paucimobilis* EPA 505 (Barkay et al. 1999).

Biosurfactants are usually produced during the stationary growth phase and this is induced by molecular signals involved in quorum sensing (Ron and Rosenberg 2002). Emulsification of oil by biosurfactants increases bioavailability by increasing the surface area. Biosurfactants are also reported to cause adhesion/detachment of bacteria by regulating their CSH. Biosurfactants may be cell bound or may be released into the medium. Cell bound biosurfactants may be bound to the hydrophobic region in the outer layer of the cell surface thereby exposing the hydrophilic part to the outside and thus the bacteria will be able to interact with hydrophilic surfaces but not hydrophobic surfaces (Nue 1996). The reverse orientation is also possible, so that the surfactant is bound through the hydrophilic part to the cell surface thereby exposing the hydrophobic part to the environment. This would allow the bacteria to interact with hydrophobic surfaces only. CSH of *Pseudomonas aeruginosa* was increased by the presence of cell bound biosurfactant rhamnolipids whereas, for *Acinetobacter* strain, reduced CSH has been reported due to presence of cell bound bioemulsifier (Nue 1996).

Deziel et al. (1996) first reported the production of rhamnolipid biosurfactant by *P. aeruginosa* 19 SJ for facilitating growth on the PAHs, naphthalene and phenanthrene. Maximum extracellular biosurfactant production was observed at the onset of the stationary phase when high cell density limited the availability of the substrate. Production of the biosurfactant enhanced solubilization of the substrate. Prabhu and Phale (2003) also indicated the role of extracellular biosurfactants in phenanthrene biodegradation. Bordoloi and Konwar (2009) reported biosurfactant production by various strains of *P. aeruginosa* isolated from petroleum contaminated soil in Assam, India. All the strains were capable of degrading the PAHs, fluorene, phenanthrene and pyrene as sole substrate. The biosurfactants produced by the various strains lowered the surface tension up to 30–32 mN/m and was characterized by CMC in the range 100–110 mg/L. Differences were observed in the nature of the various biosurfactants. The lipopeptide biosurfactant produced by strains MTCC 7815 and 7812 depicted the best pyrene solubilizing activity. Strain MTCC 8163 produced a biosurfactant that was proteino-starchy in nature, while strains MTCC 8165 and MTCC 7814 produced structurally complex biosurfactants comprising of protein, carbohydrates and lipids. Enhanced solubilization by the biosurfactants was linked to sustained growth on the PAHs. *B. subtilis* DM04 and a muccoidal (M) and non-muccoidal (NM) strain of *P. aeruginosa* isolated from petroleum contaminated soil in north-eastern India were reported to degrade pyrene as sole carbon and energy source with the help of biosurfactants that enhanced solubilization (Das and Mukherjee 2007).

7.4 Degradation of HMW PAHs and Substrate Interaction Effects

In the environment, PAHs exist as complex mixtures along with a multitude of other compounds, such as, in creosote and coal tar. In this scenario, one compound may affect the rate and extent of degradation of another compound, e.g., through increasing the biomass growth, competitive inhibition and co metabolism. These effects are referred as substrate interaction effects. As observed by various researchers, the net result could be an increase in the rate of biodegradation as a result of enhanced biomass growth (Beckles et al. 1998), a decrease in biodegradation rate due to competitive inhibition or hindrance in enzyme induction (Guha et al. 1999; Molina et al. 1999), or no noticeable effect because the opposing effects cancel out (Beckles et al. 1998; Guha et al. 1999). Degradation of a difficult to degrade substance may be enhanced due to enhancement in the biomass population caused by an easily degradable substrate. The interactions and effects encountered in a multi-substrate system are a function of the microbial community, the type of culture (mixed versus pure) and the physiological state of the community. In general, the biodegradation rates of the more degradable and abundant compounds are reduced due to competitive inhibition, while enhanced biodegradation occurs for the recalcitrant PAHs due to simultaneous increase in biomass growth on multiple substrates. Studies by Guha et al. (1999), using naphthalene, phenanthrene and pyrene as sole substrate, and in binary and ternary mixtures revealed that in ternary mixture, biodegradation of naphthalene was inhibited while the biodegradation rates of phenanthrene and pyrene were enhanced. Knightes and Peters (2006) studied the interactions in binary mixtures and in a complex mixture using nine PAHs, i.e., naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, 2-ethylnaphthalene, phenanthrene, anthracene, pyrene, fluorene and fluoranthene. They reported both positive and negative interactions between the substrates in these multi-substrate systems.

Competitive inhibition, i.e., retardation in rates of degradation of a substrate may be caused as a result of competition for the same enzyme by other substrates. For example, *cis*-naphthalene dihydrodiol dehydrogenase has a broad substrate specificity and oxidizes *cis*-dihydrodiols of many PAHs beside naphthalene so that a competitive inhibition is observed in naphthalene biodegradation. Such negative effects due to competitive inhibition may be observed where at least one step in the multistep transformation of a PAH occurs through a common enzyme. Biodegradation pathways for a wide range of PAHs are similar, and a dioxygenase enzyme is involved in the initial step of aerobic biodegradation. Thus, competitive inhibition is quite prevalent. The degradation of phenanthrene by *Pseudomonas stutzeri* P-16 and *P. saccharophila* P-15 isolated from a creosote contaminated soil was inhibited by naphthalene, 1-methylnaphthalene, 2-methylnaphthalene and fluorene. These cultures could also utilize naphthalene, 1-methylnaphthalene and 2-methylnaphthalene as sole substrate but were unable to utilize fluorene (Stringfellow and Aitken 1995). Bouchez et al. (1995)

observed that a *Pseudomonas* sp. strain S Ant Mu5 degraded anthracene to a greater extent when it is supplied alone compared to when it is supplied in a mixture with fluorene.

Positive effects and enhanced degradation of substrate may occur as a result of proliferation of biomass on multiple substrates. Since degradation rate is a function of the biomass concentration, the biodegradation rate for each compound in a mixture may be increased if common bacterial cultures are responsible for their utilization. The presence of a suitable PAH substrate or pre-exposure to suitable PAH can facilitate the degradation of other PAHs through enzyme induction. Bauer and Capone (1988) observed enhanced degradation of PAHs as a result of pre-exposure to other aromatic hydrocarbons. Naphthalene and phenanthrene enhanced the degradation of various other PAHs through cross acclimation (Bauer and Capone 1988). Exposure to certain compounds may block enzyme induction. Such malefic association has been reported for a pyrene degrading *Rhodococcus* strain S Pyr Na 1 in the presence of either phenanthrene or fluoranthene (Bouchez et al. 1995). In another study, phenanthrene degradation by several strains was found to decrease due to lack of pre-exposure to specific PAHs (Molina et al. 1999). McLellan et al. (2002) reported that the addition of phenanthrene as a second substrate could increase benzo[a]pyrene metabolism by *Mycobacterium* sp. strain RGJII-135 up to six-fold. Pyrene and phenanthrene were potent inducers of benzo[a]pyrene degradation activity.

Co-metabolism of a non-growth substrate can occur in the presence of a growth substrate, however, the products of co-metabolic transformation may hinder degradation of the growth substrate. Partial co-metabolic transformation of HMW PAHs can yield more water soluble intermediates which may be degraded by other bacterial strains. PAH-degrading bacteria, *Burkholderia* sp. VUN10013 was found to utilize and degrade low MW PAHs, such as phenanthrene and anthracene, but not the HMW PAHs, pyrene, fluoranthene, chrysene and benzo[a]pyrene (Somtrakoon et al. 2008). However, when present in a mixture with phenanthrene or anthracene, the HMW PAHs, pyrene and fluoranthene could be co metabolically degraded by this strain. Phenanthrene was better at enhancing the transformation of the non-growth substrates compared to anthracene. Presence of the HMW PAHs adversely affected the degradation of anthracene, but not that of phenanthrene. Bouchez et al. (1995) investigated the degradation of PAHs in binary mixtures using six bacterial strains, including two *Rhodococcus* spp. capable of growth on pyrene and fluoranthene. All individual strains were capable of transforming the PAHs co-metabolically and both inhibition and synergistic interactions were observed. The ability of *Pseudomonas* sp. strain S Phe Na 1 to degrade phenanthrene was partially inhibited by the transformation products formed by fluorene co metabolism. Moreover, the presence of fluoranthene as a second PAH slightly decreased (92%) the degradation of phenanthrene by *Pseudomonas* sp. strain S Phe Na 1. Inhibition was most commonly observed when the added PAH was more water soluble than the PAH added originally. It was also observed that mineralization yields were higher and biomass yields were lower for HMW PAH-degrading bacteria than for LMW PAH-degrading bacteria.

Luning Prak and Pritchard (2002) reported the degradation of mixtures of PAHs, pyrene, fluoranthene and phenanthrene by *S. paucimobilis* EPA 505 in the presence of the surfactant, Tween 80. This strain could utilize both phenanthrene and fluoranthene as growth substrates, but could not utilize pyrene. In mixtures, phenanthrene was preferentially degraded over fluoranthene, and preference for pyrene was the least. Competing substrates hindered degradation, however, after degradation of the competing substrate, the other PAHs were degraded at rate higher than or comparable to the single-substrate system.

7.5 Degradation of PAHs in Presence of Other Hydrocarbons

The effect of the presence of aliphatics on the rate and extent of degradation of PAHs has not been studied extensively. Ghosh et al. (2010) demonstrated negligible degradation of pyrene present in a NAPL along with other constituents, i.e., n-alkanes and LMW PAHs although the *Sphingomonas* sp. used was grown on pyrene provided as sole source of carbon and energy. Zytner et al. (2006) used a synthetic diesel containing the aliphatics: dodecane, tetradecane, heptadecane, pristane, octadecane and eicosane together contributing to 90%. The remaining 10% was composed of naphthalene and phenanthrene. Biodegradation rate was studied in 1-L bioreactors containing soil spiked with the synthetic diesel/individual compounds. Degradation by heterotrophic microorganisms indigenous to the Elora silt loam soil was studied and the fraction of contaminants remaining was analyzed over time by GC-FID over a period of 30 days. The degradation trend observed for the synthetic diesel fuel was: C12 > naphthalene > C17 > C19 > C20 > phenanthrene. The order changed when the compounds were degraded individually: C17, C19, C20, C12 > phenanthrene > naphthalene. The amount degraded was also changed in the mixture compared to individual compound. For example, phenanthrene degradation decreased to 9% in the synthetic diesel mixture compared to 26% when present individually. This difference may be due to the competitive inhibition.

7.6 Conclusions

Bacterial degradation of HMW PAHs with more than four rings has been conclusively demonstrated in-spite of their structural complexity and low aqueous solubility. Pyrene, fluoranthene and other HMW PAHs can serve as sole source of carbon and energy for naturally occurring microorganisms. The degradation pathways have been inferred for various organisms through identification of metabolites. The degradation rates are often found to be low due to either bioavailability limitations or membrane flux limitations. In engineered systems, the bioavailability limitations may be overcome by the addition of surfactants, however, chemical surfactants are also reported to cause adverse effects on

biodegradation. Bacteria degrading HMW PAHs are often found to induce high CSH that promotes adherence to solid PAHs, NAPLs or sorbed PAHs. Alternatively, some bacteria gain a selective advantage by secretion of biosurfactants. Biosurfactants are reported to increase uptake through enhancement in solubilization, emulsification and also by inducing changes in CSH. The presence of HMW PAHs along with other easily degradable PAHs and other hydrocarbons (i.e., n-alkanes) may adversely affect their degradation due to competitive inhibition. However, beneficial effects such as, cometabolic degradation of HMW PAHs are also reported. Bioavailability limitations together with adverse substrate interaction effects may be largely responsible for the persistence of HMW PAHs in the environment.

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References

Amin M, El-Dien A, Hosny MS, El-Mohammady Y (1996) Involvement of bacterial cell surface hydrophobicity in the biodegradation of petroleum hydrocarbons. J Microbiol 31:1–12

Bamforth SM, Singleton I (2005) Bioremediation of polycyclic aromatic hydrocarbons: current knowledge and future directions. J Chem Technol Biotechnol 80:723–736

Barkay T, Navon-Venezia S, Ron EZ, Rosenberg E (1999) Enhancement of solubilization and biodegradation of polyaromatic hydrocarbons by the bioemulsifier alasan. Appl Environ Microbiol 65(6):2697–2702

Bauer JE, Capone DG (1988) Effects of co-occurring aromatic hydrocarbons on degradation of individual aromatic hydrocarbons in marine sediment slurries. Appl Environ Microbiol 54:1649–1655

Beckles DM, Ward CH, Hughes JB (1998) Effects of mixtures of PAHs and sediments on fluoranthene biodegradation patterns. Environ Toxicol Chem 17:1246–1251

Berdholt H, Bruheim P, Potocky M, Eimhjellen K (2002) Hydrophobicity development, alkane oxidation, and crude-oil emulsification in a *Rhodococcus* species. Can J Microbiol 48:295–304

Boldrin B, Tiehm A, Fritzche C (1993) Degradation of phenanthrene, fluorene, fluoranthene, and pyrene by a *Mycobacterium* sp. Appl Environ Microbiol 59:1927–1930

Boonchan S, Britz MS, Stanley GL (1998) Surfactant-enhanced biodegradation of high molecular weight polycyclic aromatic hydrocarbons by *Stenotrophomonas maltophilia*. Biotechnol Bioeng 59(4):482–494

Bordoloi NK, Konwar BK (2009) Bacterial biosurfactant in enhancing solubility and metabolism of petroleum hydrocarbons. J Hazard Mater 170(1):495–505

Bouchez MD, Blanchet VP, Vandecasteele (1995) Degradation of polycyclic aromatic hydrocarbons by pure strains and by defined strain associations: inhibition phenomena and cometabolism. Appl Microbiol Biotechnol 43:156–164

Bressler DC, Gray MR (2003) Transport and reaction processes in bioremediation of organic contaminants. Int J Chem React Eng 1:1–16

Busscher HJ, Weerkamp AH, van der Mei HC, van Pilt AWJ, de Jong HP, Arends J (1984) Measurement of the surface free energy of bacterial cell surfaces and its relevance for adhesion. Appl Environ Microbiol 48:980–993

Busscher HJ, van der Belt-Gritter B, van der Mei HC (1995) Implications of microbial adhesion to hydrocarbons for evaluating cell surface hydrophobicity 1. Zeta potential of hydrocarbon droplets. Colloids Surf B 5:111–116

Cerniglia CE (1992) Biodegradation of PAHs. Biodegradation 3:351–368

Chakraborty S, Mukherji S, Mukherji S (2010) Surface hydrophobicity of petroleum hydrocarbon degrading *Burkholderia* strains and their interactions with NAPLs and surfaces. Colloids Surf B 78:101–108

Churchill SA, Harper JP, Churchill PF (1999) Isolation and characterization of a *Mycobacterium* species capable of degrading three and four-ring aromatic and aliphatic hydrocarbons. Appl Environ Microbiol 65:549–552

Das K, Mukherjee AK (2007) Differential utilization of pyrene as the sole source of carbon by *Bacillus subtilis* and *Pseudomonas aeruginosa* strains: role of biosurfactants in enhancing bioavailability. J Appl Microbiol 102(1):195–203

Dean-Ross D, Cerniglia CE (1996) Degradation of pyrene by *Mycobacterium flavescens*. Appl Microbiol Biotechnol 46:307–312

Deziel E, Paquette G, Villemur R, Lepine F, Bisaillon J (1996) Biosurfactant production by a soil *Pseudomonas* strain growing on polycyclic aromatic hydrocarbons. Appl Environ Microbiol 62(6):1908–1912

Dillon JK, Fuerst JA, Hayward AC, Davis GHG (1986) A comparison of five methods for assaying bacterial hydrophobicity. J Microbiol Methods 6:13–19

Doong R, Lei W (2003) Solubilization and mineralization of polycyclic aromatic hydrocarbons by *Pseudomonas putida* in the presence of surfactant. J Hazard Mater B96:15–27

Efroymson RA, Alexander M (1991) Biodegradation by an *Arthrobacter* species of hydrocarbons partitioned into an organic solvent. Appl Environ Microbiol 57(5):1441–1447

Fritzsche C (1994) Degradation of pyrene at low defined oxygen concentrations by a *Mycobacterium* sp. Appl Environ Microbiol 60:1687–1689

Ghosh I, Mohanty S, Mukherji S (2010) Comparative study on degradation of a model NAPL by *Sphingomonas* and *Burkholderia* sp. and impact of triton-X 100. In: Proceedings of 9th international conference on hydroscience and engineering (ICHE 2010), IIT, Madras, 2–5 Aug 2010

Grosser RJ, Warshawsky D, Vestal JR (1991) Indigenous and enhanced mineralization of pyrene, benzo[*a*]pyrene, and carbazole in soils. Appl Environ Microbiol 57:3462–3469

Guha S, Jaffe PR (1996) Bioavailability of hydrophobic compounds partitioned into the micellar phase of nonionic surfactants. Environ Sci Technol 30:1382–1391

Guha S, Peters CA, Jaffe PR (1999) Multisubstrate biodegradation kinetics of naphthalene, phenanthrene, and pyrene mixtures. Biotechnol Bioeng 65:491–499

Harvey RG (1991) Polycyclic aromatic hydrocarbons: chemistry and carcinogenicity. Cambridge University Press, Cambridge

Heitkamp MA, Cerniglia CE (1988) Mineralization of polycyclic aromatic hydrocarbons by a bacterium isolated from sediment below an oil field. Appl Environ Microbiol 54: 1612–1614

Heitkamp MA, Cerniglia CE (1989) Polycyclic aromatic hydrocarbon degradation by a *Mycobacterium* sp. in microcosms containing sediment and water from a pristine ecosystem. Appl Environ Microbiol 55:1968–1973

IARC Monograph (1983, 1998) Evaluation of the carcinogenic risk of chemicals to humans: Polynuclear aromatic compounds, Part I, Vol 32. http://monographs.iarc.fr/ENG/Monographs/allmonos49.php

Jimenez I, Bartha R (1996) Solvent-augmented mineralization of pyrene by a *Mycobacterium* sp. Appl Environ Microbiol 62:2311–2316

Juhasz AL, Britz ML, Stanley GA (2000) Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of the microbial degradation of benzo[a]pyrene. Int Biodeter Biodegrad 45:57–88

Kanaly RA, Harayama S (2000) Biodegradation of high molecular-weight PAHs by bacteria. J Bacteriol 182:2059–2067

Kastner M, Breuer-Jammali M, Mahro B (1994) Enumeration and characterization of the soil microflora from hydrocarbon-contaminated soil sites able to mineralize polycyclic aromatic hydrocarbons (PAH). Appl Microbiol Biotechnol 41:267–273

Kastner M, Breuer-Jammali M, Mahro B (1998) Impact of inoculation protocols, salinity, and pH on the degradation of polycyclic aromatic hydrocarbons (PAHs) and survival of PAH-degrading bacteria introduced into soil. Appl Microbiol Biotechnol 64:359–362

Kelley I, Cerniglia CE (1991) The metabolism of fluoranthene by a species of *Mycobacterium*. J Ind Microbiol 7:19–26

Kim HS, Weber WJ Jr (2003) Preferential surfactant utilization by a PAH-degrading strain: effects on micellar solubilization phenomena. Environ Sci Technol 37:3574–3580

Knightes CD, Peters CA (2006) Multisubstrate biodegradation kinetics for binary and complex mixtures of polycyclic aromatic hydrocarbons. Environ Toxicol Chem 25(7):1746–1756

Leahy JG, Khalid ZM, Quintero EJ, Jones-Meehan JM, Heidelberg JF, Batchelor PJ, Colwell RR (2003) The concentrations of hexadecane and inorganic nutrients modulate the production of extracellular membrane-bound vesicles, soluble protein, and bioemulsifier by *Acinetobacter venetianus* RAG-1 and *Acinetobacter* sp. strain HO1-N. Can J Microbiol 49:569–575

Lindahl M, Faris A, Wadstrom T, Hjerten S (1981) A new test based on 'salting out' to measure relative surface hydrophobicity of bacterial cells. Biochim Biophys Acta 677:471–476

Luning Prak DJ, Pritchard PH (2002) Degradation of polycyclic aromatic hydrocarbons dissolved in tween 80 surfactant solutions by *Sphingomonas paucimobilis* EPA 505. Can J Microbiol 48:151–158

Margesin R, Schinner F (1999) Biodegradation of diesel oil by cold-adapted microorganisms in presence of sodium dodecyl sulphate. Chemosphere 38(15):3463–3472

McLellan SL, Warshawsky D, Shann JR (2002) The effect of polycyclic aromatic hydrocarbons on the degradation of benzo[*a*]pyrene by *Mycobacterium* sp. strain RJGII-135. Environ Toxicol Chem 21:253–259

Mohanty S (2010) Surfactant-aided biodegradation of petroleum hydrocarbons from non-aqueous phase liquids by *Burkholderia* sps. Ph.D. Thesis, IIT Bombay, Mumbai, India

Mohanty G, Mukherji S (2008) Enhancement of NAPL bioavailability by induction of cell-surface hydrophobicity in *Exiguobacterium aurantiacum* and *Burkholderia cepacia*. Ind J Biotechnol 7(3):295–306

Molina MR, Araujo RE, Hodson (1999) Cross-induction of pyrene and phenanthrene in a *Mycobacterium* sp. isolated from PAH contaminated river sediments. Can J Microbiol 45:520–529

Mrozik A, Seget ZP, Labuzek S (2003) Bacterial degradation and bioremediation of polycyclic aromatic hydrocarbons. Pol J Environ Stud 12(1):15–25

Mueller JG, Chapman PJ, Blattmann BO, Pritchard PH (1990) Isolation and characterization of a fluoranthene-utilizing strain of *Pseudomonas paucimobilis*. Appl Environ Microbiol 56: 1079–1086

Mueller JG, Devereux R, Santavy DL, Lantz SE, Willis SG, Pritchard PH (1997) Phylogenetic and physiological comparisons of PAH degrading bacteria from geographically diverse soils. Antonie van Leeuwenhoek 71:329–343

Mukherji S, Weber WJ Jr (1998) Mass transfer effects on microbial uptake of naphthalene from complex NAPLs. Biotechnol Bioeng 60(6):750–760

Mukherji S, Weber WJ Jr (2001) Mass transfer effects on microbial uptake of naphthalene from complex NAPLs. Biotechnol Bioeng 75(1):130

Mukherji S, Swain AK, Venkataraman C (2002) Comparative mutagenicity assessment of aerosols in emissions from biofuel combustion. Atmos Environ 36:5627–5635

Navon-Venezia S, Banin E, Ron EZ, Rosenberg E (1998) The bioemulsifier alasan: role of protein in maintaining structure and activity. Appl Microbiol Biotechnol 49:382–384

Nue T (1996) Significance of bacterial surface-active compounds in interaction of bacteria with interfaces. Microbiol Rev 60(1):151–166

Obayori OS, Ilori MO, Adebusoye SA, Oyetibo GO, Amund OO (2008) Pyrene-degradation potentials of *Pseudomonas* species isolated from polluted tropical soils. World J Microbiol Biotechnol 24:2639–2646

Peters CA, Knightes CD, Brown DG (1999) Long-term composition dynamics of PAH-containing NAPLs and implications for risk assessment. Environ Sci Technol 33(24): 4499–4507

Prabhu Y, Phale PS (2003) Biodegradation of phenanthrene by *Pseudomonas* sp. strain PP2: novel metabolic pathway, role of biosurfactant and cell surface hydrophobicity in hydrocarbon assimilation. Appl Microbiol Biotechnol 61(4):342–351

Rehmann K, Noll HP, Steinberg CE, Kettrup AA (1998) Pyrene degradation by *Mycobacterium* sp. strain KR2. Chemosphere 36:2977–2992

Ron EZ, Rosenberg E (2002) Biosurfactants and oil bioremediation. Curr Opin Biotechnol 13:249–252

Rosenberg M (1981) Bacterial adherence to polystyrene: a replica method of screening for bacterial hydrophobicity. Appl Environ Microbiol 42:375–377

Rosenberg M, Gutnic D, Rosenberg E (1980) Adherence of bacteria to hydrocarbons: a simple method for measuring cell surface hydrophobicity. FEMS Microbiol Lett 9:29–33

Schippers C, Geßner K, Müller T, Scheper T (2000) Microbial degradation of phenanthrene by addition of a sophorolipid mixture. J Biotechnol 83(3):189–198

Schneider J, Grosser R, Jayasimhulu K, Xue W, Warshawsky D (1996) Degradation of pyrene, benz[a]anthracene, and benzo[a]pyrene by *Mycobacterium* sp. strain RJGII-135, isolated from a former coal gasification site. Appl Environ Microbiol 62:13–19

Somtrakoon K, Suanjit S, Pokethitiyook P, Kruatrachue M, Lee H, Upatham S (2008) Phenanthrene stimulates the degradation of Pyrene and fluoranthene by Burkholderia sp. VUN 10013. World J Microbiol Biotechnol 24:523–531

Stringfellow WT, Aitken MD (1995) Competitive metabolism of naphthalene, methylnaphthalenes, and fluorene by phenanthrene-degrading *Pseudomonads*. Appl Environ Microbiol 61:357–362

Thibault SL, Anderson M, Frankenberger WT Jr (1996) Influence of surfactants on pyrene desorption and degradation in soils. Appl Environ Microbiol 62:283–287

Tiehm A, Fritzsche C (1995) Utilization of solubilized and crystalline mixtures of polycyclic aromatic hydrocarbons by a *Mycobacterium* sp. Appl Microbial Biotechnol 42:964–968

Toren A, Navon-Venezia S, Ron EZ, Rosenberg E (2001) Emulsifying activities of purified Alasan proteins from *Acinetobacter radioresistens* KA53. Appl Environ Microbiol 67: 1102–1106

Volkering F, Breure AM, Van Andel JG, Reulkens WH (1995) Influence of non-ionic surfactants on bioavailability and biodegradation of polycyclic aromatic hydrocarbons. Appl Environ Microbiol 61(5):1699–1705

Walter U, Beyer M, Klein J, Rehm HJ (1991) Degradation of pyrene by *Rhodococcus* sp. UW1. Appl Microbiol Biotechnol 34:671–676

Weissenfels WD, Beyer M, Klein J (1990) Degradation of phenanthrene, fluorene and fluoranthene by pure bacterial culture. Appl Microbiol Biotechnol 32:479–484

Weissenfels WD, Beyer M, Klein J (1991) Microbial metabolism of fluoranthene: isolation and identification of ring fission products. Appl Microbiol Biotechnol 34:528–535

Whyte LG, Slagman SJ, Pietrantonio F, Bourbonniere L, Koval SF, Lawrence JR, Inniss WE, Greer CW (1999) Physiological adaptations involved in alkane assimilation at a low temperature by *Rhodococcus* sp. strain Q15. Appl Environ Microbiol 65(7):2961–2968

Wick LY, De Munain AR, Springael D, Harms H (2002) Responses of *Mycobacterium* sp. LB501T to the low bioavailability of solid anthracene. Appl Microbiol Biotechnol 58(3): 378–385

Wick LY, Pasche N, Bernasconi SM, Pelz O, Harms H (2003) Characterization of multiple-substrate utilization by anthracene-degrading *Mycobacterium frederiksbergense* Lb501t. Appl Environ Microbiol 59(10):6133–6142

Willumsen PA, Arvin E (1999) Kinetics of degradation of surfactant solubilized fluoranthene by a *Sphingomonas paucimobilis*. Environ Sci Technol 33:2571–2578

Willumsen PA, Karlson U (1998) Effect of calcium on the surfactant tolerance of a fluoranthene degrading bacterium. Biodegradation 9:369–379

Willumsen PA, Karlson U (2001) Bacterial cell surface hydrophobicity affecting degradation of PAHs in NAPLs. In: Proceedings of 6th international *in situ* and on site bioremediation symposium, San Diego, CA, 4–7 June 2001, Battelle Press, Columbus, Ohio, pp 141–147

Yaws CL (1999) Chemical properties handbook. McGraw Hill, New York
Zhao Z, Selvam A, Wong JWC (2011) Effects of rhamnolipids on cell surface hydrophobicity of PAHs degrading bacteria and the biodegradation of phenanthrene. Bioresour Technol 102(5):3999–4007
Zita A, Hermansson M (1997) Effects of bacterial cell surface structures and hydrophobicity on attachment to activated sludge flocs. Appl Environ Microbiol 63:1168–1170
Zytner RG, Slab AC, Stiver WH (2006) Bioremediation of diesel fuel contaminated soil: comparison of individual compounds to complex mixtures. Soil Seed Cont 15:277–297