Chapter 10
Microbial Degradation of PAHs: Organisms and Environmental Compartments

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10.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) represent a major class of organic compounds (Xue and Warshawsky 2005), that consist of over 100 individual moieties (Rehmann et al. 2008). Because of their toxicity and wide spread occurrence, PAH represent one of the most important groups of environmental pollutants (Eggen and Majcherczyk 1998). They consist of two or more fused benzene rings in linear, angular or cluster arrangements. The persistence of these chemicals in the environment is mainly due to their low solubility in water and stable polycondensed aromatic structure. Hydrophobicity and recalcitrance of PAHs to microbial degradation generally increase as the molecular weight increases. Besides being toxic to animals, some PAHs with four or more benzene rings, such as benzo[*a*]anthracene, chrysene and benzo[*a*]pyrene, have been shown to be carcinogenic (Bezalel et al. 1996).

Historically, human exposures to a variety of these complex mixtures have been associated with an increased incidence of cancer cases. Many PAHs and their heterocyclic analogs have been found to be tumorigenic in humans or experimental animals (Xue and Warshawsky 2005). Early, in 1761, the physician John Hill documented the high incidence of nasal cancer as consequence of excessive use of tobacco snuff (Cerniglia 1984). Pott (1775) reported high rate of scrotal skin cancer in chimney sweeps (compounds contained in the soot). Yamagiwa and Ichikawa (1915) induced tumors on the ears of rabbits by repeated application of coal tar. Due to their toxic, mutagenic and/or carcinogenic properties, sixteen PAHs (Table 10.1) have

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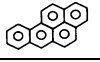
Table 10.1 General characteristics and structures of the PAHs (16 PAHs listed as priority pollutants by USEPA)

PAH (USEPA)	CAS no.	Molecular formula	Structure	Molecular weight (g/mol)	Meelting point (°C)[a]	Vapor pressure (P^s/Pa)[a]	Solubility in water (g/m^3)[a]	log K_{ow} [a]	IARC group [b]
Naphthalene	91-20-3	$C_{10}H_8$		128.171	80.26	10.4	31	3.37	2B
Acenaphtylene	208-96-8	$C_{12}H_8$		152.192	91.8	0.9	16.1	4.00	
Acenaphthene	83-32-9	$C_{12}H_{10}$		154.207	93.4	0.3	3.80	3.92	3
Fluorene	86-73-7	$C_{13}H_{10}$		166.218	114.77	0.09	1.90	4.18	3
Phenanthrene	85-01-8	$C_{14}H_{10}$		178.229	99.24	0.02	1.10	4.57	3
Anthracene	120-12-7	$C_{14}H_{10}$		178.229	215.76	0.001	0.045	4.54	3
Pyrene	129-00-0	$C_{16}H_{10}$		202.250	150.62	0.0006	0.132	5.18	3
Fluoranthene	206-44-0	$C_{16}H_{10}$		202.250	110.19	0.00123	0.26	5.22	3
Chrysene	218-01-9	$C_{18}H_{12}$		228.288	255.5	5.70×10^{-7}	0.002	5.60	2B
Benzo(a)Anthracene	56-55-3	$C_{18}H_{12}$		228.288	160.5	2.80×10^{-5}	0.011	5.91	2B
Benzo(b)Fluoranthene	205-99-2	$C_{20}H_{12}$		252.309	168		0.0015	5.80	2B
Benzo(k)Fluoranthene	207-08-9	$C_{20}H_{12}$		252.309	217	5.20×10^{-8}	0.0008	6.00	2B
Benzo(a)Pyrene	50-32-8	$C_{20}H_{12}$		252.309	181.1	7.00×10^{-7}	0.0038	6.04	1

(continued)

Table 10.1 (continued)

PAH (USEPA)	CAS no.	Molecular formula	Structure	Molecular weight (g/mol)	Meelting point (°C)[a]	Vapor pressure (P^s/Pa)[a]	Solubility in water (g/m^3)[a]	log K_{ow} [a]	IARC group [b]
Benzo(g,h,i)Perylene	191-24-2	$C_{22}H_{12}$		276.330	272.5		0.00026	6.40	3
Indeno(1,2,3-cd)Pyrene	193-39-5	$C_{22}H_{12}$		276.330	162				2B
Dibenzo(a,h)Anthracene	50-70-53	$C_{22}H_{14}$		278.346	269.5	3.70×10^{-10}	0.0006	6.75	2A

Source: [a] Mackay et al. (2006), [b] IARC (2010)

been listed as priority pollutants by US Environmental Protection Agency (EPA), which should be regularly monitored in terrestrial and aquatic ecosystems (Chen et al. 2008), their concentration does not exceed the threshold in soil and water.

The main sources of PAHs in the environment include: incomplete combustion at higher temperatures of recent and fossil organic matter (pyrolytic source), slow maturation of organic matter under geochemical gradient conditions (petrogenic source) and short-term diagenetic degradation of biogenic precursors (diagenesis) (Neff 1979; McElroy et al. 1989). As a result of anthropogenic activities as accidental oil leaks and spills, contamination of air, soil, freshwater (surface water and groundwater), and marine environments has been often reported (Bumpus 1989). They are unique contaminants in the environment because they are generated continuously by the inadvertently incomplete combustion of organic matter, for instance in forest fires, home heating, traffic, and waste incineration (Johnsen et al. 2005).

The majority of PAHs released into the environment are subjected to processes like volatilization, chemical oxidation, bioaccumulation and adsorption on soil particles. The main pathway for their removal is currently considered to be microbial transformation and degradation (Ambrosoli et al. 2005). PAHs were in the past considered to be recalcitrant to biodegradation in the absence of oxygen as electron acceptor, but they have recently been proven to be biodegradable under a variety of anaerobic conditions. There are many studies on anaerobic microbial degradation of aliphatic and monoaromatic hydrocarbons. However, only a few studies are available on the anaerobic biodegradation of polyaromatic hydrocarbons (Fuchedzhieva et al. 2008).

The extent and rate of biodegradation depend on many factors including pH, temperature oxygen, microbial population, previous microbial acclimation, accessibility of nutrients, chemical structure, cellular transport properties, and chemical partitioning in growth the medium (Haritash and Kaushik 2009).

In the last decades, biological degradation of PAHs has aroused significant interest since these contaminants have been included among priority pollutants in the United States (Code of Federal Regulation 1982). It has been suggested that the persistence of PAHs in the environment might be due to their low susceptibility to nucleophilic attack owing to the presence of dense clouds of pi-electrons on both sides of the ring structures. In addition, some physical properties of PAHs, such as low aqueous solubility and high solid/water distribution ratios, hamper microbial degradation of these compounds, thereby resulting in their accumulation in soils and sediments (Covino et al. 2010a). PAHs also are usually associated with contaminants, such us BTEX, aliphatic hydrocarbons and heavy metals, which make their biodegradation more difficult (Reineke 2001).

As biodegradation is one of the main routes of PAHs removal from the environment, in this chapter a global point of view of the microbial degradation of PAHs in different environmental compartments has been provided.

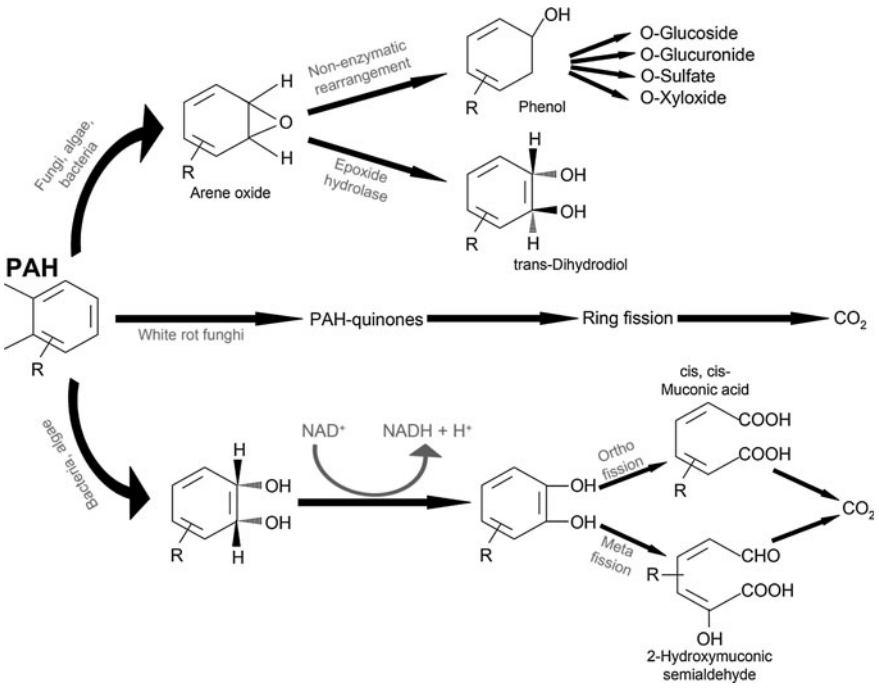


Fig. 10.1 General pathways for PAHs degradation (Redraw from Bamforth and Singleton 2005)

10.2 PAHs Degrading Organisms

The PAH-degrading microorganisms could be algae, bacteria and fungi. It involves breakdown of organic compounds through biotransformation into less complex metabolites, and through mineralization into inorganic minerals, H_2O, CO_2 (aerobic) or CH_4 (anaerobic) (Haritash and Kaushik 2009)

Schematic metabolic pathways to degrade PAHs of these microorganisms are shown in the Fig. 10.1. In aerobic pathways, the main strategy of microorganisms to degrade aromatic pollutants is to use a range of peripheral enzymes, which convert the substances to a key intermediate. Anaerobic degradation of aromatic compounds is carried out by phototrophic bacteria, by fermenting, manganese-reducing, iron-reducing, nitrate-reducing, and sulphate-reducing bacteria, and by methanogenic consortia (Bosma et al. 2001). Metabolism of organic compounds by respiration leads to a much more efficient use of potential chemical energy than anaerobic conversion (Reineke 2001). Although both aerobic and anaerobic biodegradation contribute significantly to remove aromatic pollutants from the environment, the aerobic mechanism is preferred because the process is faster and

Table 10.2 A list of PAH utilizing bacteria, fungi, cyanobacteria and microalgae

Some PAHs utilizing bacteria	Some PAHs utilizing fungi		Some PAHs utilizing cyanobacteria and microalgae
Acinetobacter calcoaceticus	*Absidia glauca*	*Mortierella ramanniana*	*Agmenellum quadruplicatum*
Acinetobacter sp.	*Acheremonium* sp.	*Mortierella verrucosa*	*Agmenellum* sp.
Aeromonas sp.	*Aspergillus niger*	*Mucor hiernalis*	*Anabaena* sp. (strain CA)
Agrobacterium sp.	*Aspergillus ochraceus*	*Mucor racemosus*	*Anabaena* sp. (strain 1F)
Alcaligenes denitrificans	*Aspergillus* sp.	*Neurospora crassa*	*Amphora* sp.
Alcaligenes faecalis	*Basidiobolus ranarum*	*Penicillum chrysogenum*	*Aphanocapsa* sp.
Arthrobacter polychromogenes	*Candida maltosa*	*Penicillum* sp.	*Chlorella autothrophica*
Arthrobacter sp.	*Candida tropicalis*	*Pestalotia* sp.	*Chlorella sorokiniana*
Bacillus cereus	*Candida utilis*	*Phanaerochaete chrysosporium*	*Chorella vulgaris*
Bacillus sp.	*Choanephora campinter*	*Phanaerochaete sordid*	*Chlamydomonas angulosa*
Beijerinckia sp.	*Chrysosphorum pannorum*	*Phlebia radiate*	*Coccochloris elabrens*
Burkhoderia sp.	*Circinella* sp.	*Phlyctochytrium reinboldtae*	*Cylindrotheca* sp.
Corynebacterium renale	*Claviceps paspali*	*Phytophthora cinnamomi*	*Dunaliella tertiolecta*
Flavobacterium sp.	*Cokeromices poitrassi*	*Rhizoctonia solani*	*Microcoleus chthonoplastes*
Haemophilus sp.	*Conidiobolus gonimodes*	*Rhizophlyctis harderi*	*Navicula* sp.
Klebuiella sp.	*Cunninghamella bainieri*	*Rhizopus oryzae*	*Nitzschia* sp.
Micrococcus sp.	*Cunninghamella blakesleeana*	*Rhizopus stolonifer*	*Nostoc* sp.
Moraxella sp.	*Cunninghamella echinulata*	*Saccharomices cerevisiae*	*Oscillatoria sp.* (strain JMC)
Mycobacterium flavescens	*Cunninghamella elegans*	*Saprolegnia parasitica*	*Oscillatoria sp.* (strain MEV)
Mycobacterium sp.	*Cunninghamella japonica*	*Smittium culicis*	*Phrototheca zopfii*
Nocardia sp.	*Emencellopsis* sp.	*Smittium culisetae*	*Porphyridium cruentum*
Paenobacillus sp.	*Epicoccum nigrum*	*Smittium simuli*	*Scenedesmus platydiscus*
Pseudomonas cepacia	*Fusarium oxyporum*	*Sordana fimicola*	*Selenastrum capricornutum*
Pseudomonas fluorescens	*Gilbertella persicaria*	*Syncephalastrum racemosum*	*Skeletonema costatum*
Pseudomonas paucimobilis	*Gliocadium* sp.	*Thamnidium anomalum*	*Synedra* sp.

(continued)

Table 10.2 (continued)

Some PAHs utilizing bacteria	Some PAHs utilizing fungi		Some PAHs utilizing cyanobacteria and microalgae
Pseudomonas putrid	*Helicostylum piriforme*	*Thichocladium canadense*	
Pseudomonas testeroni	*Hyphochytrium catenoides*	*Trichoderma viride*	
Pseudomonas vesicularis	*Irpex lateus*	*Verticillum* sp.	
Pseudomonas sp.	*Linderina pennispora*	*Zygorhynchus moelleri*	
Rhodococcus sp.			
Rhodotorula glutinis			
Staphylococcus auriculans			
Sphingomonas paucimobilis			
Sphingomonas sp.			
Streptomyces griseus			
Streptomyces sp.			
Vibrio sp.			

Source Modified after Cerniglia (1993)

substantive and the initial introduction of oxygen into the aromatic hydrocarbons via hydration in the anaerobic processes is thermodynamically highly unfavorable. As a result, aerobic catabolism of aromatic pollutants is more prevalent in the biosphere (Cao et al. 2009).

10.2.1 Aerobic

10.2.1.1 Bacteria

Bacteria are able to utilize PAHs as the sole source of carbon and energy (Reineke 2001). They exist as gram-positive and gram-negative bacteria with the ability to metabolize some PAHs (Cerniglia 1993).

According to Johnsen et al. (2005), bacteria growing in suspended, shaken cultures with crystalline PAHs in concentrations exceeding the aqueous solubility as the sole source of energy and carbon exhibit characteristic growth curves. These curves can be divided into three phases: exponential phase (maximum rate limited by the physiology of the bacteria, bioavailability number >1), pseudo-linear growing (maximum rate limited by the concentration of PAHs, bioavailability

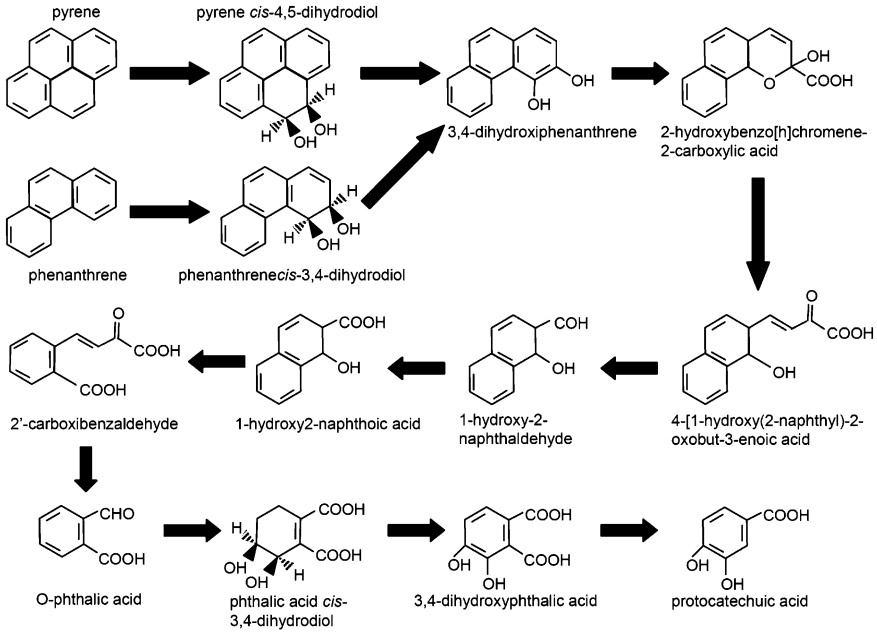


Fig. 10.2 Phenanthrene biodegradation pathway in *Mycobacterium* species (Redrawn from Pagnout et al. 2007)

number <1) and pseudo-stationary. However, real heterogeneous media do not have the same characteristics as these idealized conditions have.

Most of PAH-degrading bacteria oxidize PAHs using dioxygenases. A few bacteria, such as *Mycobacterium* sp. are also capable of oxidizing the PAHs aromatic rings via cytochrome P_{450} monooxygenase enzyme to form *trans*-dihydrodiols rather than *cis*-dihydrodiols (Cao et al. 2009) (Fig. 10.1). Principal well-know PAHs utilizing bacteria are shown in Table 10.2. In the Fig. 10.2, is shown a bacterial biodegradation pathway (Pagnout et al. 2007).

Some authors found that the co-metabolism is an important feature of the degradation of PAH. In some cases, there exists an inhibition on the biodegradation when two or more PAHs are presents while in other cases, an enhancement in the biodegradation is observed. Bouchez et al. (1995) conducted a study where six bacterial strains were isolated. Each of these strains was capable of using PAHs as sole carbon and energy source, at least one of them: naphthalene, phenanthrene, anthracene, fluorene, fluoranthene and pyrene. When more than one PAH were present in the test, an inhibition of the degradation occurs. They related this to PAH specific inhibitory capacity effect and solubility. Dean-Ross et al. (2002) found that *Mycobacterium flavescens* biodegraded fluoranthene in the presence of pyrene, although metabolization of pyrene was slower in the presence of

fluoranthene than in its absence. On the other hand, *Rhodococcus* sp. metabolized fluoranthene in the presence of anthracene, although the presence of fluoranthene slowed the rate of anthracene biodegradation. Walter et al. (1991) found that *Rhodococcus* sp. UW1 could use phenanthrene, anthracene, fluoranthene and chrysene as sole sources of carbon and energy, whereas naphthalene and fluorene were only co-metabolized.

In the field of interaction between photosynthetic organisms and bacteria, Anokhina et al. (2004) studied the consumption of phenanthrene in soil by model plant–microbial associations including natural and transconjugant plasmid-bearing rhizospheric strains of *Pseudomonas fluorescens* and *P. aureofaciens* degrading polycyclic aromatic hydrocarbons. They found that the inoculation of barley seeds with both natural and transconjugant plasmid-bearing *Pseudomonas* strains were able to degrade PAH protecting plants from the phytotoxic action of phenanthrene and favored its degradation in soil. Yutthammo et al. (2010) investigated PAHs biodegradation by Phyllosphere bacteria of ornamental plants (mainly consisting of bacteria, such as *Acinetobacter*, *Pseudomonas*, *Pseudoxanthomonas*, *Mycobacterium,* and uncultured bacteria). They studied ten evergreen ornamental plants: *Ixora* sp. (ixora), *M. paniculata* (orange jasmine), *W. religiosa* (water jasmine), *Bougainvillea* sp. (bougainvillea), *Jasminum sambac* (L.) Ait. (jasmine), *Codiaeum variegatum* (Croton), *Ficus* sp. (ficus), *Streblus asper* Lour. (toothbrush tree), *Pseuderanthemum graciliflorum* (Nees) Ridl. (blue crossandra), and *Hibiscus rosa-sinensis* L. (hibiscus). The results indicated that phyllosphere bacteria on unsterilized leaves, were able to enhance the activity of leaves for phenanthrene removal. Daane et al. (2001) carried out a study that indicated that the rhizosphere of salt marsh plants contained a diverse population of PAH-degrading bacteria, and the use of plant-associated microorganisms has the potential for bioremediation of contaminated sediments. One year later, Daane et al. (2002) described *Paenibacillus naphthalenovorans* sp. nov., a naphthalene-degrading bacterium from the rhizosphere of salt marsh plants. Other synergistic studies (Borde et al. 2003) have also demonstrated a relationship in algal-bacterial microcosms for the treatment of phenanthrene.

There are some studies focusing on the effect of concentration. (Mahanty et al. 2010) observed that *Mycobacterium frederiksbergense* degraded anthracene, naphthalene and pyrene. Experiments were conducted according to a 2^3 factorial design at the low (1 mg l^{-1}) and high (50 mg l^{-1}) levels of the PAHs in combination, in an artificial media (Bushnell Hass (BH)). The results showed that PAH removals varied 54–81% when each PAH was at low concentrations in the mixture and 67–89% at their higher concentration combinations.

Taking into consideration bioremediation studies, Das and Mukherjee (2007) found that *B.subtilis* DM-04 and *P. aeruginosa* M and NM strains could be useful in bioremediation of sites highly contaminated with crude petroleum-oil hydrocarbons. The thermophilic nature of these bacteria could add further advantage for their use in bioremediation of petroleum contaminated soils in tropical countries. Lin et al. (2010) found that a novel *Bacillus fusiformis* (BFN) isolated from the sludge in petroleum-contaminated wastewater can be used to effectively

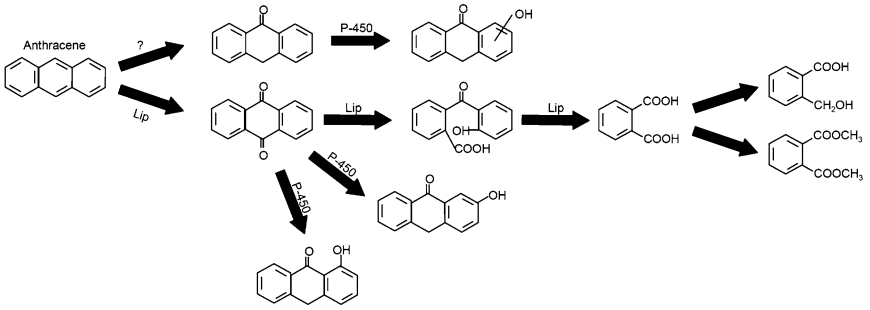


Fig. 10.3 Anthracene degradation by *I. lacteus* (Redrawn from Catjthaml et al. 2002)

biodegrade naphthalene. It emerged that the degradation of naphthalene rose to 99.1% within 4 days under optimum conditions (temperature 30°C, pH 7.0, inoculum concentration of 0.2% and C/N ratio of 1 at initial naphthalene concentration of 50 mg/L). According to Navarro et al. (2008), *Sphingomonas* sp. is able to degrade anthracene, phenanthrene, pyrene and benzo[a]pyrene, in aqueous deoxyribonucleic acid solution. In their study, aqueous DNA solution was applied for the remediation of a PAH-contaminated soil by sequential soil washing and lixiviates biodegradation.

The toxicity of PAHs metabolites during bacterial degradation has been little studied. It has been reported that the metabolites of some PAHs are potentially more bioavailable and could be more toxic than the precursors. Oxy-PAHs accumulated were more toxic and more persistent than the parent compounds, highlighting the importance of complete mineralization of PAHs without accumulation of the catabolic intermediates (Cao et al. 2009).

10.2.1.2 Fungi

A diverse group of lignolytic and non-lignolytic fungi are able to oxidize PAH (Table 10.2). Fungi do not utilize PAHs as a sole source of carbon and energy, but transform PAHs co-metabolically (Cerniglia 1993).

The microbial degradation by lignolytic fungi has been intensively studied during past few years due to their ability to degrade lignin (an amorphous and complex biopolymer with an aromatic structure similar to the aromatic molecular structure of some environmental pollutants) by producing extracellular enzymes with very low substrate specificity. This makes them suitable for degradation of aromatic compounds, such as PAHs (Valentín et al. 2006; Haritash and Kaushik 2009). Fungal degradation pathway has been reflected in Fig. 10.3 (Catjthaml et al. 2002).

Non-Lignolytic Fungi

According to Reineke (2001), a variety of non-lignolytic fungi have been found to transform polycyclic aromatic hydrocarbons to metabolites that are similar to those produced by mammalian enzymes. Only a few fungi appear to have the ability to catabolize PAHs to CO_2. Non-lignolytic fungi metabolize PAHs by citochrome P450 monoxygenase and epoxide hydrolase-catalysed reactions to form *trans*-dihydrodiols. Other metabolites formed include phenols, quinines and conjugates (Cerniglia and Sutherland 2001).

Non-lignolytic fungi, such as *Cunninghamella elegans* and *Penicillium janthinellum* can metabolize a variety of PAHs to polar metabolites (Potin et al. 2004). Also *Cladosporium sphaerospermum* was able to degrade PAHs in non-sterile soils. *Cunningamella elegans* degraded naphthalene, acenaphthene, anthracene, phenanthrene, benzo[a]pyrene, benzo[a]anthracene, fluoranthene and pyrene (Reineke 2001). Some of these fungi that metabolize polycyclic aromatic hydrocarbons are summarized in Table 10.2, and are included into different classes: Zygomycetes, Ascomycetes, Blastomycetes, Hyphomycetes, and Coelomycetes.

Lignolytic Fungi

Ligninolytic fungi possess an extracellular degradation system which is capable of breaking down lignin (Kirk and Farrell 1987). White-rot fungi can degrade a wide range of organopollutants and the degradative activity is because of the lignin-degradating systems of these fungi (Haritash and Kaushik 2009). Many authors believe that white-rot fungi metabolize PAHs through the extracellular ligninolytic enzymes, including lignin peroxidases, Mn-peroxidase, versatile peroxidase, and laccase. The precise role of these enzymes in PAH degradation has not yet been determined; however, it has been shown that only laccase-producing fungi can mineralize PAHs to CO_2 and H_2O (Pozdnyakova et al. 2010).

Covino et al. (2010b) have conducted a study to assess PAH-degradation capability of *L. tigrinus* strain CBS 577.79 in liquid cultures, to clarify the possible involvement of laccase and MnP in the degradation process and to identify the fungal PAH degradation products. They used two media: malt extract glucose (MEG) and N- limited medium (NKLM). They obtained that Laccase activity was predominant on MEG while Mn-peroxidase (MnP) was preferentially produced in LNKM. The identification of degradation products showed the presence of several PAH derivatives, presumably derived from the action of lignin-modifying enzymes. In 2000, Márquez-Rocha (2000) found that the white rot fungus, *Pleurotus ostreatus*, metabolized four soil adsorbed polycyclic aromatic hydrocarbons: pyrene, anthracene and phenanthrene and were mineralized after 21 d. However, benz[a]pyrene was also oxidized, but not mineralized. They also found that biodegradation was increased when surfactant Tween 40 was added.

A factor that is necessary to take into consideration is the salinity. Valentín et al. (2006), have been studied the application of lignolytic fungi to detoxification

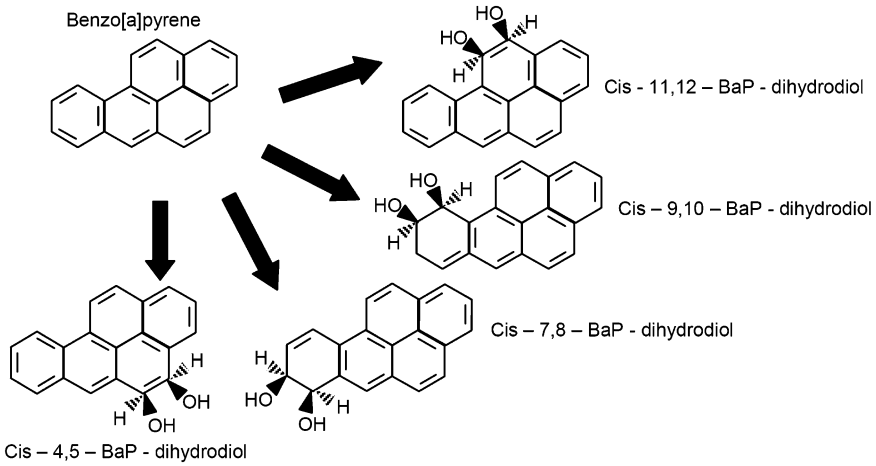


Fig. 10.4 Algal transformation products of benzo[a]pyrene (Redrawn from Juhasz and Naidu 2000)

of marine sites contaminated with PAHs, evaluating the effects of the high salinity associated with coastal areas on fungal growth, production of enzymes and ligninolytic activity. The study was carried out with four PAHs: phenanthrene, fluoranthene, pyrene and chrysene. They found an extensive inhibitory effect on PAH degradation ranging from strong for some fungi to not appreciable for others.

In biosorption context, Chen et al. (2010) carried out an experiment to elucidate biosorption of PAHs to fungal biomass and the relative contributions of biosorption and biodegradation to the total removal of PAHs by white-rot fungi. They studied five PAHs: naphthalene, acenaphthene, fluorene, phenanthrene and pyrene. In this study, the conclusion was that biosorption of PAHs by white-rot fungi is very important process influencing the fate of PAHs in the environment. The partitioning of PAHs into fungal biomass is the primary mechanism of biosorption, and sorption capabilities (Koc, carbon-normalizated partition coefficients) are linearly related to Kow (octanol–water partition coefficients).

Other factor to be taken into consideration is the culture media. Pozdnyakova et al. (2010) have carried out some experiments to study the influence of cultivation conditions on pyrene degradation by the fungus *Pleurotus ostreatus* D1. They found that in Kirk's medium, about 65.6 ± 0.9% of the initial pyrene was metabolized after three weeks, and that in basidiomycetes rich medium, *P. ostreatus* D1 metabolized up to 89.8 ± 2.3% of pyrene within three weeks. In the first case, pyrene-4,5-dihydrodiol was accumulated where as it did not exist in the latter case. Experiments were also conducted to determine the effect of fungal inoculum, biomass and glucose concentrations on fluoranthene, pyrene and chrysene degradation by *Bjerkandera* sp. BOS55 (Valentín et al. 2007). This work showed the capability of the white-rot fungus to degrade PAHs in soil slurry phase

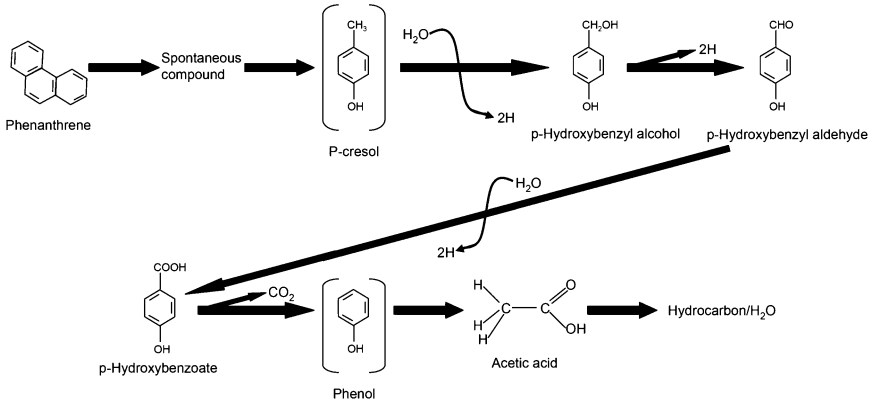


Fig. 10.5 Proposed pathways for anaerobic biotransformation of phenanthrene by sulfate-reducing bacteria (Redrawn from Tesai et al. 2009)

bioreactors. They found that the use of free mycelia seems to be beneficial for the degradative action of the fungus. Thus, degradation of the PAHs was enhanced between 13 and 29% by using mycelium as inoculum compared to fermentation with pellets inoculation. They also found that a small change in the initial glucose concentration in the range 20–3 g/L did not exert a significant effect on PAH degradation while the initial biomass exerted a larger effect on PAH degradation compared to the other two studied parameters. In the study of very concentrated media, Bumpus (1989) demonstrated that *P. chrysosporium* is able to degrade PAHs present in anthracene oil (heavy oil which distils over from coal tar). Analysis by capillary gas chromatography and high-performance liquid chromatography showed that at least 22 PAHs, including all of the most abundant PAH components present in anthracene oil, underwent 70–100% disappearance during 27 days of incubation under nutrient nitrogen–limited media. An experiment was conducted by Kotterman et al. (1994) to optimize the biodegradation of anthracene by *Bjerkandera* sp. with respect to O_2, N, and C. They concluded that the supply of O_2 was the most important factor in the biodegradation of anthracene.

Bioremediation of polluted liquid and solid matrices with lignolytic fungi has been widely studied for last several years. There are some works on comparison of different organisms. A study to assess the PAH-biodegradation potential of *L. tigrinus* CBS 577.79 on real solid matrices derived from a wood treatment plant in view of its possible use in mycoremediation applications was carried out by Covino et al. (2010a). They compared the results with *Irpex lacteus.* They found that *L. tigrinus* was able to colonize and detoxify solid PAH-contaminated matrices under non-sterile conditions. Its growth and degradation performances were invariably higher than those of *I. lacteus.*

Like in other situations, the factor of the aged in the matrix is important. Eggen and Majcherczyk (1998) have studied the degradation and metabolisms of

benzo[a]pyrene in soil inoculated with *P. ostreatus*. Two strains of *P. ostreatus* were used in a mineralization study with [^{14}C]benzo[a]pyrene. They observed that in aged soil contaminated with creosote, *P. ostreatus* removed benzo[a]pyrene most extensively in the very first month. Elimination of spiked ^{14}C-benzo[a]pyrene was higher than benzo[a]pyrene originally present, and 40% removal of the first compound was observed. Although mineralization was low (1%), but there was a significant inoculation effect (with white rot fungi) on mineralization as compared to control.

10.2.1.3 Microalgae and Cyanobacteria

It has been known that Cyanobacteria and eukaryotic algae have the capacity to metabolize PAHs. As Cyanobacteria are the largest and most widely distributed group of photosynthetic prokaryotes on earth (Reuter and Müller 1993), it has been decided to include this group in a specific section, together with microalgae. Figure 10.4 shows an algal biodegradation pathway (Juhasz and Naidu 2000).

Some researchers studied the difference in species of cyanobacteria and micro algae in relation to PAHs degradation. Cerniglia et al. (1980) studied the ability to metabolize naphthalene of nine cyanobacteria, four green microalgae, one red microalgae, and two diatoms. All these organisms oxidized naphthalene under photoautotrophic conditions. Hong et al. (2008) found that the tolerance of *Skeletonema costatum* to phenanthrene and fluoranthene was greater than that of *Nitzschia sp.*, and that the toxic effect of fluoranthene on *S. costatum* and *Nitzschia sp.* was higher than that of phenanthrene. They also found that the accumulation and degradation abilities of *Nitzschia* sp. were higher than those of *S. costatum*. Lei et al. (2007) studied the efficiency of four microalgal species (*Chlorella vulgaris*, *Scenedesmus platydiscus*, *Scenedesmus quadricauda*, and *Selenastrum capricornutum)* to remove fluoranthene, pyrene, and a mixture of fluoranthene and pyrene. They observed that PAH removal was algal species specific and also PAH-dependent. The most effective specie in removing and transforming PAHs was *S. Capricornutum* and the least effective was *C. vulgaris.*

In the field of metabolic pathways, Warshawsky et al. (1995) carried out a study with different culture lamps and found that under gold light, metabolites of Benzo[a]pyrene (BaP) produced by *Selenastrum capricornutum* were the dihydrodiols of which the 11,12-dihydrodiol was the major metabolite. Under white light, at low doses of BaP, the major metabolite was 9,10-dihydrodiol. With increasing dose, the ratio of dihydrodiols to quinones decreased to less than two. Similarly, with increasing light energy output, from gold to white to UV-A in the PAH absorbing region, BaP quinone production was increased. According to Narro et al. (1992a), the marine cyanobacterium *Oscillatoria* sp. strain JCM oxidized naphthalene predominantly to 1-naphthol. Other study of Narro et al. (1992b) showed that under photoautotrophic growth conditions, the marine cyanobacterium *Agmenellum quadruplicatum* PR-6 metabolized phenanthrene to form trans-9,

10-dihydroxy-9,10-dihydrophenanthrene (phenanthrene trans-9,10-dihydrodiol) and 1-methoxyphenanthrene as the major ethyl acetate-extractable metabolites.

Others works were carried out to study the interaction with metals. Ke et al. (2010) investigated the effects of some metals on biosorption and biodegradation of five mixed polycyclic aromatic hydrocarbons, by *Selenastrum capricornutum*. They found that for low molecular weight PAHs, both metal dosage and exposure time had a significant, positive effect on their removal, while for high molecular weight PAHs, the presence of metals did not affect the removal efficiency. Some of the cyanobacteria and microalgae that metabolize polycyclic aromatic hydrocarbons are summarized in Table 10.2.

10.2.2 Anaerobic and Anoxic Conditions

During past two decades, anaerobic biodegradation of aromatic pollutants has been a subject of extensive research (Cao et al. 2009). According to them, some bacteria which are involved in PAHs anaerobic degradation are *Acidovorax* sp., *Bordetella* sp., *Pseudomonas* sp., *Sphingomonas* sp., *Variovorax* sp., *P. stutzeri* and *Vivrio pelagiusrelated*.

Anaerobic degradation is a slower process than aerobic limited by several other factors and present in natural environments and so it cannot be overlooked. For petroleum hydrocarbons, even though aerobic processes are generally used, anaerobic biodegradation is significant under certain circumstances (e.g., O_2-depleted aquifers, oil spilled marshes) (Zhang and Bennett 2005). However, biochemical mechanism of anaerobic biodegradation of PAHs has not yet been elucidated (Haritash and Kaushik 2009). An anaerobic degradation pathway has been shown in Fig. 10.5 (Tesai et al. 2009).

Rockne et al. (2000) showed that highly-enriched, denitrifying cultures could mineralize bicyclic and polycyclic aromatic hydrocarbons. Mineralization was nitrate dependent and was sustainable over several feedings and the cultures produced N_2O, a denitrification product, when was supplied with PAHs as the sole carbon and energy source. Ambrosi et al. (2005) conducted an experiment by adding fluorene, phenanthrene and pyrene to soil samples in order to investigate the anaerobic degradation potential of PAHs under denitrifying conditions. The study was made with the soil alone or enriched with nitrate, as electron acceptor, PAHs and, in some samples, glucose or acetate. The principal variable was the specific PAHs tested, because the molecular conformation prevails over other parameters in controlling the degradation. The next variable in importance was the addition of other carbon sources as different of the PAHs. They concluded that anaerobic biodegradation of PAHs is possible both through fermentative and respiratory metabolism, provided that low molecular weight co-metabolites and suitable electron acceptors (nitrate) are present.

There are also a few studies on co-metabolism. A study carried out by Chang et al. (2008) in mangrove sediments, with phenantrene and pyrene, demonstrates that the anaerobic degradation of PAH was enhanced by the addition of acetate, lactate, pyruvate, sodium chloride, cellulose, or zero-valent iron. However, it was inhibited by the addition of humic acid, di-(2-ethylhexyl) phthalate (DEHP), nonylphenol, or heavy metals. Marine sediment biodegradation was also studied by Kim et al. (2008) who chose lactate as a supplementary carbonaceous substrate to stimulate degradation of PAHs. Results showed that lactate amendment enhanced biodegradation rates of PAHs in the sediments by 4–8 times, and caused a significant shift in archaebacterial community in terms of structure and diversity with time. Li et al. (2009) studied mangrove sediments of Hong-Kong, concretely the vertical distribution and anaerobic biodegradation of polycyclic aromatic hydrocarbons in these sediments. They found close relationships between bacterial population sizes and the concentration of anaerobic electron acceptors. The dominant electron acceptor was sulfate followed by Fe(III). Mn(IV) was at very low concentrations and nitrates were nearly exhausted at sediment depths greater than 6 cm.

The anaerobic treatment of sewage sludge is of great importance as reported by several authors. In the context of anaerobic sludge treatment, it is important to take into consideration the control of sludge quality (particularly its concentration in trace organic pollutants), so as to limit potential risks owing to sludge land application. The anaerobic removal of 13 Polycyclic Aromatic Hydrocarbons (PAHs) was measured in five continuous anaerobic digestors with different feed sludge (Barret et al. 2010), to elucidate if bioavailability and/or co-metabolism limit their biodegradation during the anaerobic digestion of contaminated sludge. In their work, they demonstrated that aqueous PAHs (sum of free and sorbed-to-DCM PAHs) were bioavailable. It was also demonstrated that bioavailability is not the only influencing factor. Indeed, PAHs biodegradation resulted from a combination of bioavailability and co-metabolism. Bernal-Martínez et al. (2005) combined anaerobic digestion and ozonation to remove PAH from urban sludge. The digested sludge with known PAH concentration, was ozonized in a fed batch reactor. The ozonation of anaerobically digested sludge improved the PAH removal rate (61%). An additional enhancement (up to 81%) of the PAH removal rate was obtained by the addition of hydrogen peroxide during ozonation. Similar performances were achieved by the use of surfactants which enhanced PAH bioavailability.

10.3 Environmental Compartments

The spread of chemicals is caused by high production and release rates combined with their relative mobility in air, water, soil and biota, and their stability against biotic and abiotic transformation (Bosma et al. 2001). Highly heterogenic spatial distribution of PAHs in the different compartments depends on several characteristics, i.e. sources location, abundance of emission, the

atmospheric/marine circulation regimen, etc. Therefore, it is of special concern for the study of biodegradation of PAHs in the environmental compartments on which they can be located.

10.3.1 Soils

According to Johnsen et al. (2005), in soil, PAHs are heterogeneously distributed and may be adsorbed on organic particles in small pores which makes them inaccessible to microorganisms, or otherwise occluded by the multitude of solid soil constituents. The spatial distribution of soil microorganisms is also heterogeneous and often autocorrelated at scales from millimetres to tens of metres, and similar scales of heterogeneity apply to geophysical and geochemical parameters (Bengtsson et al. 2010).

The levels of PAHs, like in all compartments, are very heterogeneous from non-polluted areas to high polluted. For example, Nadal et al. (2004) obtained the sum of the 16 PAHs levels between 1002 and 112 ng/g (dry weight) for samples collected near chemical industries and unpolluted sites, respectively, in Tarragona (Spain). Nam et al. (2008) determined PAHs levels from background locations in the UK and Norway. They found that in the UK, the concentrations ranged between 42 and 11200 μg kg^{-1} dry weight, and in Norway, ranged between 8.6 and 1050 μg kg^{-1} dry weight. In urban soils of Beijing (China), Tang et al. (2005) found concentrations ranging from less than 366–27825 ng g^{-1}. In Poland, in arable soils, Maliszewska-Kordybach et al. (2008) obtained that the content of Σ16PAHs ranged from 80–7264 μg kg^{-1}.

Soil is the most extensively studied compartment for PAHs biodegradation. There are different factors that influence degradation in soils, like the presence of aged PAHs, the inoculums, the species presents in soil and the characteristics of the soil.

Related to the aging process, Huesenmann et al. (2004) reported that aged PAHs, that are often thought to be recalcitrant due to bioavailability limitations, may not be so and therefore, may pose a greater risk to environmental receptors than previously thought. Bogan and Sullivan (2003) studied the degradation of phenanthrene and pyrene by *Mycobacterium austroafricanum* strain GTI-23. They studied six different soils and observed that for both, phenanthrene and pyrene, an increase in contact time led to higher sequestration and lower biodegradation, and that TOC content was the most important parameter governing these processes.

In the field of inoculums studies, Arias et al. (2008) worked on the production and fate of PAHs metabolites in soil, as metabolites are potentially more toxic than the parental products. They developed a static soil microcosm system and an analytical methodology for detection of PAHs in soils and their oxidation products. They used this system with a soil contaminated with phenanthrene (as a model PAH) and 1-hydroxy- 2-naphthoic acid, diphenic acid, and phthalic acid (as putative metabolites). They studied this with and without inoculation of strain

Mycobacterium sp. AP1. In inoculated microcosms, 35% of the added phenanthrene was depleted, 19% being recovered as CO_2 and 3% as diphenic acid. The latter, together with other two unidentified metabolites, was accumulated in the soil. Somtrakoon et al. (2008) carried out a study to enhance biodegradation of anthracene in acidic soil by inoculating *Burkholderia* sp. VUN10013 and found that the indigenous microorganisms in the pristine acidic soils have limited ability to degrade anthracene, and bacterial inoculation significantly enhanced anthracene degradation in such acidic soils. Yuan et al. (2002) studied the inoculation of non-local microorganisms in the soil to investigate the differences in biodegradation. They observed that the consortium used was capable of biodegrading five PAHs and found that the remaining PAH amount after 40-day incubation period ranged from 92.6–96.9% in the autoclaved control bottles; from 90.1–94.6% in non-autoclaved bottles and without consortium; from undetectable to 13.5% in non-autoclaved bottles and with PAH-adapted consortium.

Taking into consideration different degrading species and their previous exposure or not to the pollutants, some studies have been carried out. Johnsen and Karlson (2005) estimated the PAH degradation capacity of 13 soils ranging from pristine locations to heavily polluted industrial sites to investigate, if the PAHs degradation capacity depends or not on previous PAH exposure (concretely phenanthrene and pyrene). They found that densities of phenanthrene degraders reflected previous PAH exposure, whereas pyrene degraders were detected only in the most polluted soils. They found that the time to 10% mineralization of added ^{14}C phenanthrene and ^{14}C pyrene was inversely correlated to the initial PAH pollution.

According to the characteristics of the soil, Hwang and Cutright (2004) studied a relationship between soil characteristics and subsequent desorption and biodegradation of pyrene. They found that fraction of expandable clay minerals was correlated to the achievable desorption and biodegradation. It could be possible to that the microbial movement and adhesion to clays turned out to a great extent of the soil-phase biodegradation. The maximum achievable desorptions were 30.2, 10.4, and 1.0 mg/kg for soils which were 1.7, 2.2, and 4.4 wt% of expandable clays (smectite and vermiculite), respectively. The total (soil + water) biodegradation reached to 65, 78.3, and 81.8% of the initial concentration (100 mg/kg) for the three soils, respectively. Nam and Alexander (1998) studied the role of sequestration and bioavailability (of phenanthrene) to the nanoporosity and the hydrophobicity. They found results according to their previous hypothesis, which was that sequestration and reduced bioavailability occur when hydrophobic compounds enter into nanopores having hydrophobic surfaces. Nam and Kim (2002) studied the role of loosely bound humic substances in the bioavailability of aged phenanthrene. They observed that in the humic-mineral fraction occurred major sequestration and, for that reason, humic and fulvic acids may act like a barrier to the biodegradation.

There are several studies of the role of surfactants in enhancing biodegradability of PAHs in soils. Many PAHs sorb strongly onto soil, which limits their availability for microbial degradation. Surfactants have been shown to enhance

desorption and solubilisation of PAHs (Wilson and Jones 1993). Sobish et al. (2000) found that with surfactant combinations T10 and T15 (mixtures of a non-ionic hydrophilic and a non-ionic hydrophobic component) the biodegradation was enhanced. With the addition of T10:1(1%) to a different types of soils, they found in the first one, having an overall low bioavailability of PAH, a reduction of 16 PAHs by 36%, while in other soils they were reduced by 44% and by 90%. Chang et al. (2008) evaluated the effects of chemical interactions on naphthalene biodegradation in the surfactant-soil/water systems. They found that addition nonionic surfactants (TX-100 and Brij35) used as the concentration of monomer or micelle, can influence naphthalene biodegradation. Kim et al. (2001) investigated the effect of non-ionic surfactants on the solubility and biodegradation of PAHs in the soil slurry phase. They found that the PAH solubility was linearly proportional to the surfactant concentration above the critical micelle concentration.

10.3.2 Sediments

Photochemical reactions are not possible in the subsurface, where microbial-mediated transformations constitute the dominant removal mechanism of persistent organic compounds (Bosma et al. 2001). The microbial biodegradation of PAHs in the sediments (like in soils) has been widely studied, due to the presence of different groups of microorganisms that are able to degrade these compounds and due to different PAH levels.

10.3.2.1 Marine Sediments

Because of their low water solubility and hydrophobicity, PAHs in the marine environment rapidly become associated with organic and inorganic suspended particles (Chiou et al. 1998) and they are subsequently deposited into sediments. As PAH solubility decreases with increasing molecular weight, bioaccumulation of PAHs from sediments by marine organisms is generally greater for the lower molecular weight and more water soluble PAHs (Djomo et al. 1996; Porte and Albaigués 1994).

PAH levels in marine sediments are highly heterogeneous depending on geographical location. For example, PAH concentrations as high as 48000 ng/g were found in Venice lagoon (LaRocca et al. 1996), levels between 5700 and 8500 ng/g were found in the Mediterranean littoral of France-Spain (Baumard et al. 1999) and levels of approximately 800 ng/g were found in Todos Santos Bay, Mexico (Macías-Zamora et al. 2002). Shao et al. (2010) carried out an investigation of the deep-sea sub-surface environment, concretely in sub-surface sediments on the Mid-Atlantic Ridge. They found that the total concentration of PAHs was 445 ng/g dry wt sediment, in the sediment 2.7 m beneath the bottom surface at a water depth of 3962 m on the Mid-Atlantic Ridge. The concentrations of phenanthrene and

fluorene were relatively high. In addition, PAH-degrading bacteria were found within the sediments.

In marine sediments, some of the most important factors in PAHs biodegradations are size of inoculum, salinity characteristic of the sediment and pre-exposure of the organisms. There has been growing concern over the mounting concentration of PAHs in the marine environment. One of the most studied areas is the mangrove, important estuarine wetland closely tied to human activities and subject to PAHs contamination (Haritash and Kaushik 2009). Chen et al. (2008) studied the degradation of phenathrene by *Sphingomonas* sp., a bacterial strain isolated from mangrove sediment. They found that in this case, salinity was the most significant factor in biodegradation, followed by inoculum size. They noted that the phenanthrene biodegradation could be best described by the first order rate model, with a kinetic constant of 0.1185 h^{-1}, under the optimal condition (30°C, 15 ‰ salinity, a carbon/nitrogen ratio of 100 : 1 and an inoculum size of 10^6 MPN g^{-1} sediment). They found that the biodegradation was lower with more or less salinity than optimal, and with smaller inoculum size than optimal. Chen et al. (2010) studied the degradation of phenanthrene in mangrove sediments, but with different inocula (*Sphingomonas* sp., a mixture of *Sphingomonas* sp. and *Mycobacterium* sp., and without inoculum), different salinities, different sediment types, and presence of other PAHs. They observed that optimal phenanthrene biodegradation could take place in clay loam sediment slurry at low salinity (5–15‰) with the inoculation of both *Sphingomonas* sp. and *Mycobacterium* sp., and that the presence of other PAHs had little impact in the degradation process.

Tadros and Hughes (1997) investigated the degradation of PAHs by indigenous mixed and pure cultures isolated from coastal sediments. They found two bacterial species degraders of PAHs in their sediments: *Pseudomonas* sp. and *Ochrobactrum* sp. They also found a relationship between bacteria concentration and the degradation of the compounds. The degradations of naphthalene and fluorene were 98.7% and 90.4%, respectively by indigenous mixed microorganisms, both used as sole grown substrate (after 8 days of incubation). Hinga (2003) studied subtidal marine sediments sampled after a fuel oil spill. It was found that the degradation rate of low molecular weight (LMW) polycyclic aromatic hydrocarbons (PAH) was correlated to the sediment total organic carbon (TOC).

10.3.2.2 Freshwater Sediments

Among organic pollutants, polycyclic aromatic hydrocarbons (PAHs) are common in freshwater ecosystems and particularly in river sediments where they accumulate (Quantin et al. 2005).

Jackson and Pardue (1999) investigated the biodegradation of Louisiana "sweet" crude oil in Lousiana's freshwater marshes and found degradation rates of 6.8% per day for the measured polycyclic aromatic hydrocarbon fractions (i.e. naphthalene, methylated naphthalenes, phenanthrene, and methylated phenanthrenes). Yuan et al. (2001) carried out a study in the laboratory to determine

the potential biodegradation of phenanthrene in the river sediment (from Keelung River). They studied seven different sediments, and found half-lives ranged from 0.61 to 5.78 days when the concentration of phenanthrene was 5 μg/g. Moreover, they studied the addition of different substances and found that acetate, pyruvate and yeast extract did not have a significant impact on the biodegradation, but ammonium, sulphate and phosphate had an influence. Quantin et al. (2005) studied the biodegradation of river sediments under oxic and anoxic conditions, and also investigated whether input of fresh organic material (cellulose) could enhance biodegradation. They observed aerobic degradation for the PAHs, but they did not find anaerobic degradation. They observed that total dissipation of 12 PAHs reached a maximum of 78% in the NOM biotic treatment (natural organic matter as the only source of carbon) under aerobiosis after 180 days. Also they found that cellulose addition stimulated both aerobic and anaerobic respiration, but had no effect on PAH dissipation (disappearance).

10.3.3 Water

In water environments, the solubility of PAHs is low, and decreases with increasing molecular weight (solubilities are shown in Table 10.1). In sea water, the solubility of each PAH is less than in fresh water due to salting out effect. PAHs biodegradation studies conducted in the aquatic environment have been carried out mainly with cyanobacteria and algae.

Due to their high hydrophobicity, PAHs tend to interact with non-aqueous phases and natural organic matter also, as a consequence, they are poorly bioavailable for microbial degradation (Buchholz et al. 2007). The biodegradation in water environment has not been studied as widely as in other compartments (like sediments or soils). In this compartment, the biodegradation has been less studied in seawater than in freshwater.

10.3.3.1 Seawater

Like in others compartments, PAHs levels in surface seawater are highly heterogeneous. Witt (2002) found a concentrations of PAHs up to 16,600 pg/L in Central Baltic Sea. In Bahia Blanca estuary (Argentina), levels were found between undetected to more than 4 μg/L (Arias et al. 2009).

Hong et al. (2008) studied the degradation of phenanthrene and fluoranthene by *Skeletonema costatum* and *Nitzschia* sp. (from a mangrove aquatic ecosystem). They found that the microalgal species showed comparable or higher efficiency in the removal of the mixture of these two PAHs than each one singly, suggesting that the presence of one PAH stimulated the degradation of the other. They further noted that in the case of *Nitzschia* sp. the degradation of phenanthrene in single condition was 3.73% and in mixed condition 4.75% and the degradation of

fluoranthene in single condition was 3.33%and in mixed conditions was 3.59%. In the case of *S. costatum*, they observed no significant difference for fluoranthene degradation between single and mixed conditions, but the degradation of phenanthrene in mixed conditions was significantly higher than in single condition (38 and 16%, respectively). Cerniglia et al. (1980) compared the ability of nine cyanobacteria, four green microalgae and two diatoms, some isolated from seawater and some from freshwater to metabolize naphthalene and found that all these organisms oxidized naphthalene under photoautotrophic conditions (between 0.5 and 2.4% in 24 h). Narro et al. (1992b) studied the degradation of phenanthrene by the marine cyanobacterium *Agmenellum quadruplicatum.* They concluded that the marine cyanobacterium *A. quadruplicatum* PR-6 metabolized phenanthrene to form phenanthrene trans-9,10-dihydrodiol and 1-methoxyphenanthrene as the major metabolites. Poeton et al. (1999) studied the biodegradation of PAH by marine bacteria in water, and the effect of solid phase. They found linear relationship between aqueous PAH and biomass concentration. They also found that the coefficients for this model were different with and without sediments, and that the biodegradation was higher in presence of sediments (with sediment present, degradation rates for phenanthrene and fluoranthene were 2.1 to 3.5 and 2.1 to 5.3 times faster, respectively).

10.3.3.2 Freshwater

Levels of different PAHs in freshwater are very heterogeneous. For example, Wang et al. (2009) found in the Three Gorges Reservoir (Yangtze River) levels of total PAHs between 13.8 and 97.2 ng L^{-1}. Fernandes et al. (1997) found PAH level in the Seine River and its estuary, ranging from 4 to 36 ng L^{-1}. Countway et al. (2003) carried out a study in the York River and its estuary, and obtained levels of PAHs between 2.09 and 122.85 ng L^{-1} (with the salinity in these points around 13). Smith et al. (1991) investigated levels of PAHs in waters from three rivers which traverse the largest cities in south-eastern Australia in range of <0.3 to 525 ng L^{-1}.

Ke et al. (2010) studied biodegradation of fluorene, phenanthrene, fluoranthrene, pyrene and benzo[a]pyrene by green freshwater alga *Selenastrum capricornutum*, and the influence of the exposure to metals on the biodegradation process. For the PAHs studied, they only found influence of metal dosage and exposure time on the removal of fluorene and phenanthrene (up to 99 and 87% from the medium in seven days). Warshawsky et al. (1988) also studied *Selenastrum capricornutum* with respect to biodegradation of benzo[a]pyrene. They found that *S. capricornutum* produced cisvicinal dihydrodiols from molecular oxygen via a dioxygenase enzyme pathway. It was the first example for characterization of a dioxygenase pathway in a freshwater alga of a five ring carcinogenic PAH. Lei et al. (2007) investigated the degradation of fluoranthene and pyrene by four freshwater algae: *Chlorella vulgaris, Scenedesmus platydiscus, Scenedesmus quadricauda*, and *Selenastrum capricornutum*. Percentage remaining in medium

after seven days of both PAHs with the different algae was: *Chlorella vulgaris* 23.8%, *Scenedesmus platydiscus* 11.8%, *Scenedesmus quadricauda* 3.4%, and *Selenastrum capricornutum* 2.0%. Xia et al. (2006) studied biodegradation of chrysene, benzo(a)pyrene and benzo(g,h,i)perylene in the Yellow river. As this river is the most large turbid in the world, they studied the effect of suspended sediment on the biodegradation. They found that the biodegradation rates of PAHs increased with the sediment content in the water. For chrysene (with initial concentration of 3.80 μg/L), biodegradation rate constants were 0.053, 0.084 and 0.111 d^{-1}, when the sediment contents were 0, 4 and 10 g/L, respectively).

10.4 Conclusions

The PAH degrading microorganisms from different taxonomic groups (mainly bacteria, microalgae and fungi), produce different metabolites on degradation and carry out degradation in different environmental compartments. In general, PAHs with low molecular weight are more degraded than PAHs with high molecular weight. The compartment most studied is soil, followed by sediment. The water is less studied than soil and sediment, and seawater less than freshwater. In soil and sediment, the main variables are size of inoculum, degrading species, and characteristics of soil/sediment. In the sediment also, salinity is important. In water, some of the factors that influence the biodegradation are degrading species, sediments content in the water and presence of other compounds or other PAHs. In the future, it would be interesting to carry out an extensive study of different compartments (making emphasis on the effect of different variables), especially those compartments which are yet less studied.

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