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Biodegradation of polycyclic aromatic hydrocarbons

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Abstract

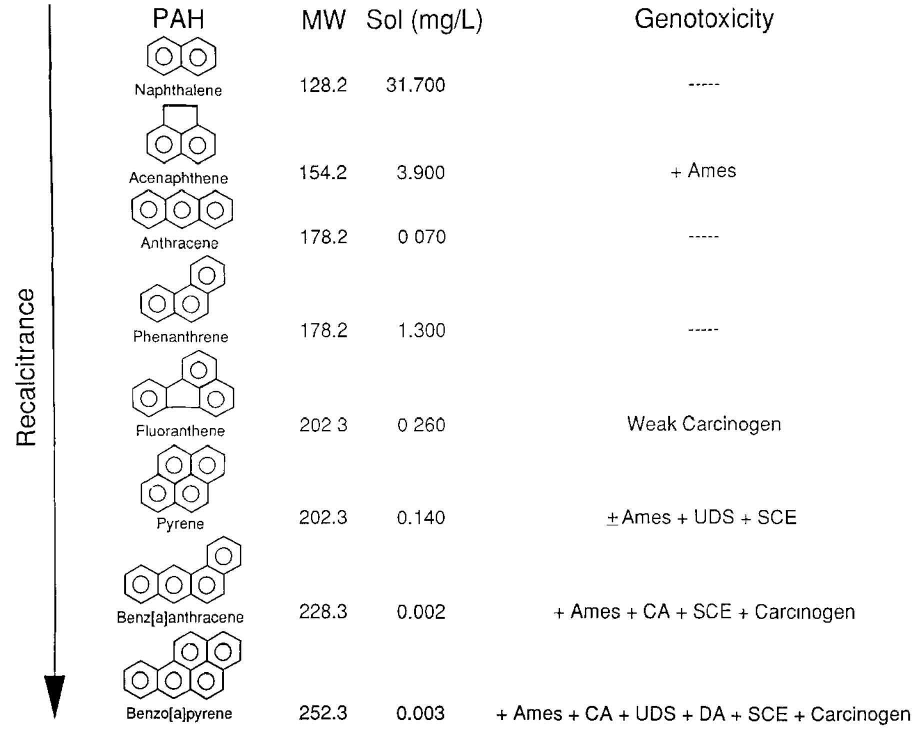
The intent of this review is to provide an outline of the microbial degradation of polycyclic aromatic hydrocarbons. A catabolically diverse microbial community, consisting of bacteria, fungi and algae, metabolizes aromatic compounds. Molecular oxygen is essential for the initial hydroxylation of polycyclic aromatic hydrocarbons by microorganisms. In contrast to bacteria, filamentous fungi use hydroxylation as a prelude to detoxification rather than to catabolism and assimilation. The biochemical principles underlying the degradation of polycyclic aromatic hydrocarbons are examined in some detail. The pathways of polycyclic aromatic hydrocarbon catabolism are discussed. Studies are presented on the relationship between the chemical structure of the polycyclic aromatic hydrocarbon and the rate of polycyclic aromatic hydrocarbon biodegradation in aquatic and terrestrial ecosystems.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) constitute a class of hazardous organic chemicals consisting of three or more fused benzene rings in linear, angular and cluster arrangements (Fig. 1). PAHs mostly occur as a result of fossil fuel combustion, as by-products of industrial processing, and during the cooking of foods (Lijinsky 1991) (Table 1). PAHs enter the environment from a multiplicity of sources which include: direct aerial fallout, chronic leakage of industrial or sewage effluents, accidental discharges during the transport, use and disposal of petroleum products, or from natural sources such as oil seeps and surface water run-off from forest and prairie fire sites (Giger & Blumer 1974; LaFlamme & Hites 1978). More specifically, industrial effluents from coal gasification and liquefaction processes, waste incineration, coke, carbon black, and other petroleum-derived products release high quantities of PAHs into the environment (Table 1). Exposure to PAHs constitutes a significant health risk for people living in industrialized areas of the world (Thakker et al. 1985; Dipple et al. 1990). PAHs are on the United States Environmental Protection Agency's priority pollutant list since some are known carcinogens and mutagens (Keith & Telliard 1979) (Fig. 1).

PAHs are hydrophobic compounds and their persistence within ecosystems is due chiefly to their low water solubility. PAHs rapidly become associated with sediments (Hites et al. 1977, 1980; Gschwend & Hites 1981; Means et al. 1980), where they may become buried and persist until degraded, resuspended, bioaccumulated, or removed by dredging. The lipophilicity, environmental persistence, and genotoxicity increase as the molecular size of PAHs increases up to 4 or 5 fused benzene rings (Fig. 1) and toxicological concern shifts towards chronic toxicity, primarily carcinogenesis (Jacob et al. 1986; Miller & Miller 1981). The possible fates of PAHs in the environment include volatilization, photooxidation, chemical oxidation, bioaccumulation, adsorption to soil particles, leaching and microbial degradation (Fig. 2).

The concentration of PAHs in the environment



PAH	MW	Sol (mg/L)	Genotoxicity
Naphthalene	128.2	31.700	-----
Acenaphthene	154.2	3.900	+ Ames
Anthracene	178.2	0.070	-----
Phenanthrene	178.2	1.300	-----
Fluoranthene	202.3	0.260	Weak Carcinogen
Pyrene	202.3	0.140	±Ames + UDS + SCE
Benz[a]anthracene	228.3	0.002	+ Ames + CA + SCE + Carcinogen
Benzo[a]pyrene	252.3	0.003	+ Ames + CA + UDS + DA + SCE + Carcinogen

Fig. 1. Chemical structures, physical and toxicological characteristics of polycyclic aromatic hydrocarbons. The symbols are: (DA) DNA adducts, (SCE) sister chromatid exchange, (CA) chromosomal aberrations, (Ames) *Salmonella typhimurium* reversion assay, (UDS) unscheduled DNA synthesis, (–) not genotoxic.

varies widely, depending on the level of industrial development and contamination with petroleum products (Jones et al. 1989; Wild et al. 1991a, 1991b). For example, PAH contamination ranges from a low of 5 ng/g of soil in an undeveloped area to 1.79×10^6 ng/g at an oil refinery. Additionally, the concentration of PAHs in marine sediments can exceed 10^5 ng/g in urban estuaries (Johnson & Larsen 1985). A major source of PAH contamination is creosote because PAHs represent approximately 85–90% of creosote constituents. A representative example of a capillary column gas chromatographic analysis of an organic solvent extract of a PAH-contaminated soil from a creosote waste site is presented in Fig. 3.

Recent studies have shown that microbiological degradation of PAHs is the major process that results in the decontamination of sediment and surface soil (Sims et al. 1990). These compounds can be totally degraded (mineralized) or be partially transformed (Fig. 2) by either a community of microorganisms or by a single microorganism (Gib-

Table 1. Major sources and transport mechanisms of PAHs in the environment.

Natural oil seeps
Refinery and oil storage wastes
Accidental spills from oil tankers and other ships
Municipal and urban wastewater discharge runoff
River-borne pollution
Atmospheric fallout of fly ash particulates
Petrochemical industrial effluents
Coal tar and other coal processing wastes
Automobile engine exhausts
Combustion of fossil fuels (gasoline, kerosene, coal, diesel fuel)
Smoked, charcoal broiled, or pan fried foods
Tobacco and cigarette smoke
Forest and prairie fires
Rural and urban sewage sludge
Refuse and waste incineration
Coal gasification and liquefaction processes
Creosote and other wood preservative wastes
Commercial and pleasure boating activities

Reference: IARC (1972–1990) Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol 1–49, International Agency for Research in Cancer, Lyons, France.

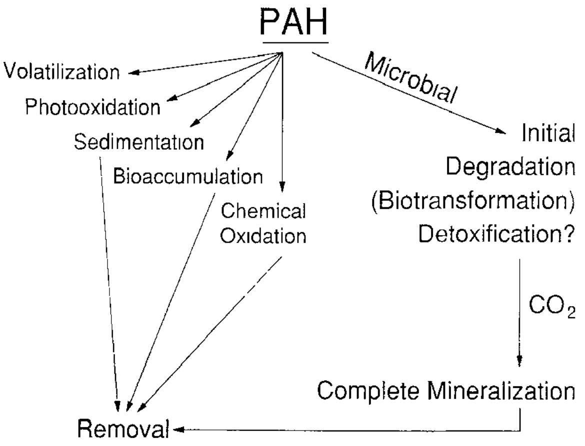


Fig. 2. Schematic representation of the environmental fate of polycyclic aromatic hydrocarbons.

son & Subramanian 1984; Cerniglia 1984; Cerniglia & Heitkamp 1989). Bioremediation of soils contaminated with PAHs is an alternative means of detoxifying hazardous waste, which may replace traditional methods such as incineration (Aronstein et al. 1991; Ellis et al. 1991). This review provides recent information on the general principles of the microbial degradation of PAHs, with special emphasis on the biochemical mechanisms for the degradation.

General principles of polycyclic aromatic hydrocarbon metabolism

The metabolism of PAHs by pure cultures of microorganisms and cometabolic transformations by mixed microbial communities have been studied for almost 80 years. However, in order for bioremediation to be considered a viable technology for the decontamination of PAH-contaminated sites, we need to understand more about the microorganisms, enzymatic processes and the environmental conditions necessary to optimize the degradation of these compounds. The known principles in the metabolism of PAHs are summarized below:

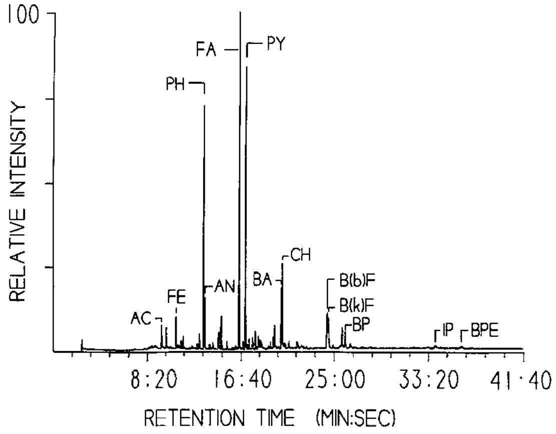


Fig. 3. Capillary gas chromatographic trace of a polycyclic aromatic hydrocarbon contaminated soil sample. PAHs and their symbol abbreviations are: (AC) Acenaphthene, (ACY) Acenaphthylene, (AN) Anthracene, (BPE) Benzo[ghi]perylene, (BP) Benzo[a]pyrene, (B(b)F) Benzo[b]fluoranthene, (B(k)F) Benzo[k]fluoranthene, (BA) Benz[a]anthracene, (CH) Chrysene, (DBA) Dibenz[a,h]anthracene, (FA) Fluoranthene, (FE) Fluorene, (IP) Indeno[1,2,3-cd]pyrene, (NA) Naphthalene, (PH) Phenanthrene, (PY) Pyrene.

1. A wide variety of bacteria, fungi and algae have the ability to metabolize PAHs (Tables 2–4). Generally, the rate of degradation of PAHs is inversely proportional to the number of rings in the PAH molecule. Thus, the lower weight PAHs are biodegraded more rapidly than the higher weight compounds (Cerniglia & Heitkamp 1989).
2. The microbial degradation of PAHs such as naphthalene, phenanthrene, anthracene and acenaphthene has been well documented and the biochemical pathways have been elucidated (Cerniglia 1984; Cerniglia et al. 1992; Gibson & Subramanian 1984; Schocken & Gibson 1984; Pothuluri et al. 1992b). Less is known about the microorganisms capable of utilizing high molecular weight PAHs as sole sources of carbon and energy (Barnsley 1975; Heitkamp & Cerniglia 1988, 1989; Heitkamp et al. 1988; Kelley & Cerniglia 1991; Mueller et al. 1990; Walter et al. 1991; Weissenfels et al. 1990, 1991).
3. Biodegradation mechanisms, both procaryotic and eucaryotic, require the presence of molecular oxygen to initiate enzymatic attack on the PAH rings (Gibson et al. 1968; Chapman 1979; Dagley 1971, 1975; Gibson & Subramanian 1984). Other enzyme systems, such as methane monooxygenases (Colby et al. 1977, 1978; Dalton et al. 1981) and lignin peroxidases (Bumpus 1989; Bumpus et al. 1985; Gold et al. 1989;

Haemmerli et al. 1986; Hammel 1989; Aust 1990) may also be important in the catabolism of PAHs. There is also increasing interest and speculation concerning anaerobic decomposition of PAHs (Vogel & Grbic-Galic 1986; Grbic-Galic & Vogel 1987; Mihelcic & Luthy 1987, 1988).

4. The involvement of plasmids in the degradation of toluene, naphthalene, biphenyl and phenanthrene has been reported (Williams 1981; Zylstra & Gibson 1991; Zylstra et al. 1990). Some catabolic genes, including regulatory genes, have been extensively analyzed (Zylstra et al. 1991). However, little is known about the genetic and regulatory mechanisms involved in the bacterial catabolism of high molecular weight PAHs.
5. Lower molecular weight PAHs, such as naphthalene and phenanthrene, are degraded rapidly in sediments, whereas higher molecular weight PAHs, such as benz[a]anthracene, chrysene or benzo[a]pyrene, are quite resistant to microbial attack. The potential biodegradation rates for PAHs are higher in PAH-contaminated sediments than in pristine sediments (Bauer & Capone 1985, 1988; Heitkamp & Cerniglia

Table 2. Polycyclic aromatic hydrocarbons oxidized by different species of bacteria.

Compound	Organisms	References
Naphthalene	*Acinetobacter calcoaceticus, Alcaligenes denitrificans, Mycobacterium* sp., *Pseudomonas* sp., *Pseudomonas putida, Pseudomonas fluorescens, Pseudomonas paucimobilis, Pseudomonas vesicularis, Pseudomonas cepacia, Pseudomonas testosteroni, Rhodococcus* sp., *Corynebacterium renale, Moraxella* sp., *Streptomyces* sp., *Bacillus cereus*	Ryu et al. 1989; Weissenfels et al. 1990, 1991; Kelley et al. 1991a; Dunn & Gunsalus 1973; Davies & Evans 1964; Foght & Westlake 1988; Jeffrey et al. 1975; Mueller et al. 1990; Kuhm et al. 1991; Walter et al. 1991; Dua & Meera 1981; Tagger et al. 1990; Garcia-Valdes et al. 1988; Trower et al. 1988; Grund et al. 1992; Cerniglia et al. 1984; Barnsley 1983
Acenaphthene	*Beijerinckia* sp., *Pseudomonas putida, Pseudomonas fluorescens, Pseudomonas cepacia, Pseudomonas* sp.	Chapman 1979; Schocken & Gibson 1984; Ellis et al. 1991
Anthracene	*Beijerinckia* sp., *Mycobacterium* sp., *Pseudomonas putida, Pseudomonas paucimobilis, Pseudomonas cepacia, Rhodococcus* sp., *Flavobacterium* sp., *Arthrobacter* sp.	Colla et al. 1959; Akhtar et al. 1975; Jerina et al. 1976; Evans et al. 1965; Ellis et al. 1991; Weissenfels et al. 1991; Foght & Westlake 1988; Walter et al. 1991; Mueller et al. 1990; Savino & Lollini 1977
Phenanthrene	*Aeromonas* sp., *Alcaligenes faecalis, Alcaligenes denitrificans, Arthrobacter polychromogenes, Beijerinckia* sp., *Micrococcus* sp., *Mycobacterium* sp., *Pseudomonas putida, Pseudomonas paucimobilis, Rhodococcus* sp., *Vibrio* sp., *Nocardia* sp., *Flavobacterium* sp., *Streptomyces* sp., *Streptomyces griseus, Acinetobacter* sp.	Kiyohara et al. 1976, 1982, 1990; Weissenfels et al. 1990, 1991; Keuth & Rehm 1991; Jerina et al. 1976; Colla et al. 1959; West et al. 1984; Kiyohara & Nagao 1978; Heitkamp & Cerniglia 1988; Guerin & Jones 1988, 1989; Treccani et al. 1954; Evans et al. 1965; Foght & Westlake 1988; Mueller et al. 1990; Sutherland et al. 1990; Ghosh & Mishra 1983; Savino & Lollini 1977; Trower et al. 1988; Barnsley 1983
Fluoranthene	*Alcaligenes denitrificans, Mycobacterium* sp., *Pseudomonas putida, Pseudomonas paucimobilis, Pseudomonas cepacia, Rhodococcus* sp., *Pseudomonas* sp.	Kelley & Cerniglia 1991; Walter et al. 1991; Weissenfels et al. 1991; Foght & Westlake 1988; Mueller et al. 1989, 1990
Pyrene	*Alcaligenes denitrificans, Mycobacterium* sp., *Rhodococcus* sp.	Heitkamp et al. 1988; Walter et al. 1991; Weissenfels et al. 1991; Grosser et al. 1991
Chrysene	*Rhodococcus* sp.	Walter et al. 1991
Benz[a]anthracene	*Alcaligenes denitrificans, Beijerinckia* sp., *Pseudomonas putida*	Gibson et al. 1975; Mahaffey et al. 1988; Weissenfels et al. 1991
Benzo[a]pyrene	*Beijerinckia* sp., *Mycobacterium* sp.	Gibson et al. 1975; Heitkamp & Cerniglia 1988; Grosser et al. 1991

Table 3. Polycyclic aromatic hydrocarbons oxidized by different species of fungi.

Compound	Organisms	References
Naphthalene	*Absidia glauca, Aspergillus niger, Basidiobolus ranarum, Candida utilis, Choanephora campincta, Circinella* sp., *Claviceps paspali, Cokeromyces poitrassi, Conidiobolus gonimodes, Cunninghamella bainieri, Cunninghamella elegans, Cunninghamella japonica, Emericellopsis* sp., *Epicoccum nigrum, Gilbertella persicaria, Gliocladium* sp., *Helicostylum piriforme, Hyphochytrium catenoides, Linderina pennispora, Mucor hiemalis, Neurospora crassa, Panaeolus cambodginensis, Panaeolus subbalteatus, Penicillium chrysogenum, Pestalotia* sp., *Phlyctochytrium reinboldtae, Phycomyces blakesleeanus, Phytophthora cinnamomi, Psilocybe cubensis, Psilocybe strictipes, Psilocybe stuntzii, Psilocybe subaeruginascens, Rhizophlyctis harderi, Rhizophlyctis rosea, Rhizopus oryzae, Rhizopus stolonifer, Saccharomyces cerevisiae, Saprolegnia parasitica, Smittium culicis, Smittium culisetae, Smittium simulii, Sordaria fimicola, Syncephalastrum racemosum, Thamnidium anomalum, Zygorhynchus moelleri*	Cerniglia & Gibson 1977; Cerniglia et al. 1978; Smith & Rosazza 1974; Cerniglia & Crow 1981; Ferris et al. 1973
Anthracene	*Bjerkandera* sp., *Cunninghamella elegans, Phanerochaete chrysosporium, Ramaria* sp., *Rhizoctonia solani, Trametes versicolor*	Cerniglia 1982; Cerniglia & Yang 1984; Hammel et al. 1991; Sutherland et al. 1992; Field et al. 1992
Acenaphthene	*Cunninghamella elegans*	Pothuluri et al. 1992b
Phenanthrene	*Cunninghamella elegans, Phanerochaete chrysosporium, Trametes versicolor*	Cerniglia & Yang 1984; Cerniglia et al. 1989; Morgan et al. 1991; Sutherland et al. 1991; Bumpus 1989; Hammel et al. 1992
Fluoranthene	*Cunninghamella elegans*	Pothuluri et al. 1990, 1992a
Pyrene	*Cunninghamella elegans, Phanerochaete chrysosporium*	Cerniglia et al. 1986; Hammel et al. 1986
Benz[a]anthracene	*Cunninghamella elegans*	Cerniglia et al. 1980d
Benzo[a]pyrene	*Aspergillus ochraceus, Bjerkandera adusta, Bjerkandera* sp., *Candida maltosa, Candida tropicalis, Chrysosporium pannorum, Cunninghamella elegans, Mortierella verrucosa, Neurospora crassa, Penicillium* sp., *Phanerochaete chrysosporium, Ramaria* sp., *Saccharomyces cerevisiae, Trametes versicolor, Trichoderma viride*	Cerniglia & Gibson 1979, 1980a, 1980b; Ghosh et al. 1983; Cerniglia & Crow 1981; Lin & Kapoor 1979; Bumpus et al. 1985; Wiseman & Woods 1979; Field et al. 1992

Table 4. Polycyclic aromatic hydrocarbons oxidized by different species of cyanobacteria and algae.

Compound	Organisms	References
Naphthalene	*Oscillatoria* sp. (strain JCM), *Oscillatoria* sp. (strain MEV), *Microcoleus chthonoplastes, Nostoc* sp., *Anabaena* sp. (strain CA), *Anabaena* sp. (strain 1F), *Agmenellum quadruplicatum, Coccochloris elabens, Aphanocapsa* sp., *Chlorella sorokiniana, Chlorella autotrophica, Dunaliella tertiolecta, Chlamydomonas angulosa, Ulva fasciata, Cylindrotheca* sp., *Amphora* sp., *Nitzschia* sp., *Synedra* sp., *Navicula* sp., *Porphyridium cruentum*	Cerniglia et al. 1979, 1980a, 1980b, 1982; Narro et al. 1992a
Phenanthrene	*Oscillatoria* sp. strain JCM, *Agmenellum quadruplicatum*	Narro et al. 1992b
Benzo[a]pyrene	*Selenastrum capricornutum*	Warshawsky et al. 1988, 1990

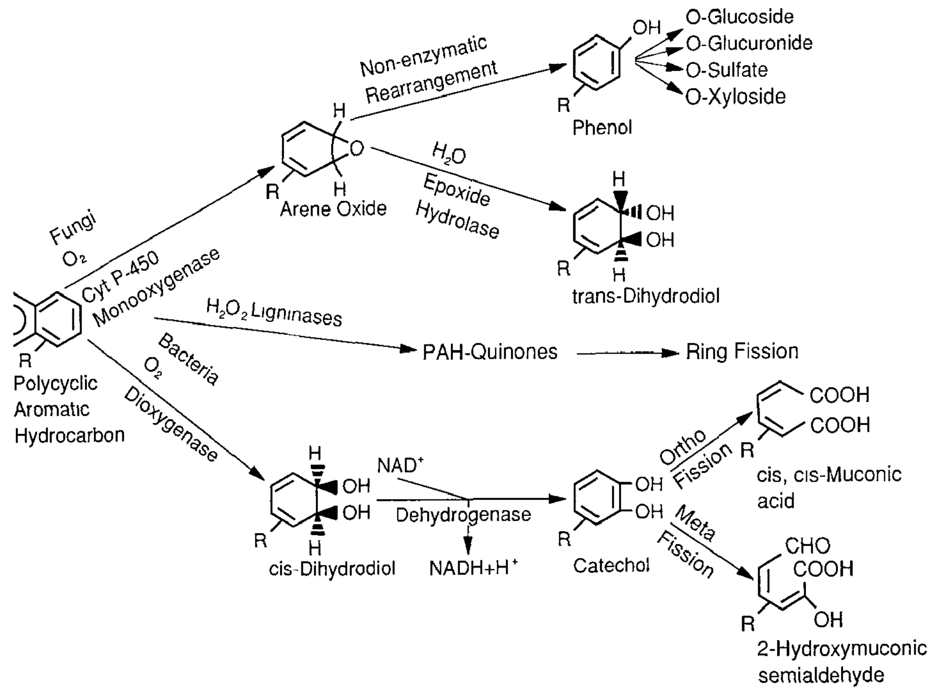


Fig. 4. Pathways for the microbial catabolism of polycyclic aromatic hydrocarbons.

1987; Herbes & Schwall 1978; Foght & Westlake 1988; Keck et al. 1989; Wang et al. 1990).

6. Filamentous fungi hydroxylate PAHs as a prelude to detoxification, whereas most bacteria oxidize PAHs as a prelude to ring fission and carbon assimilation (Cerniglia 1984; Cerniglia et al. 1992; Sutherland 1992). Similar to bacteria, fungal metabolism of PAHs is also highly regio-and stereoselective (McMillan et al. 1987, 1988; Cerniglia 1984; Cerniglia & Gibson 1980a, 1980b; Cerniglia et al. 1983a, 1983b, 1984, 1990).
7. Microbial degradation of PAHs in aquatic and terrestrial ecosystems is influenced strongly by a wide variety of abiotic and biotic factors which include: temperature, pH, soil type, aeration, nutrients, depth, diffusion, microbial adaptations, bioavailability, previous chemical exposure, water availability, sediment toxicity, physico-chemical properties of the PAH, concentration of the PAH and seasonal factors (Manilal & Alexander 1991; Atlas 1991; Wang et al. 1990; Shiaris 1989; Cerniglia & Heitkamp 1989; Wild et al. 1991a, 1991b).

Metabolism of polycyclic aromatic hydrocarbons

Hydroxylation of benzenoid aromatic compounds always involves the incorporation of molecular oxygen (Gibson & Subramanian 1984). Procaryotic microorganisms utilize dioxygenase enzymes to incorporate both atoms of molecular oxygen into the aromatic nucleus to form *cis*-dihydrodiols (Gibson et al. 1990) (Fig. 4). *Cis*-dihydrodiols are stereoselectively dehydrogenated by *cis*-dihydrodiol dehydrogenases (Patel & Gibson 1974), which rearomatize the benzene nucleus to form dihydroxylated intermediates. Subsequent enzymatic fission of the aromatic ring by procaryotic organisms is also catalyzed by highly regio- and stereoselective dioxygenases (Gibson & Subramanian 1984). As a prerequisite for this reaction, the aromatic ring must contain two hydroxyl groups placed either *ortho* or *para* to each other (Evans et al. 1965; Gibson et al. 1968; Barnsley 1976a, 1976b; Chapman 1979). If the hydroxyl moieties are *ortho* to each other, oxygenolytic ring cleavage can occur either between the two hydroxyl groups, by *ortho* or intradiol cleaving dioxygenases, or adjacent to the two hydroxyl groups, by *meta* or extradiol cleaving dioxygenases (Fig. 5).

A considerable amount of research on the microbial degradation of naphthalene, including the elucidation of the metabolic pathways, enzymatic mechanisms and genetic regulation, has been conducted (Davies & Evans 1964; Tattersfield 1927; Tausson 1927; Treccani et al. 1954; Catterall et al. 1971; Dunn & Gunsalus 1973; Barnsley 1976a, 1976b, 1983; Boronin et al. 1980; Cane & Williams 1982; Connors & Barnsley 1982; Ensley & Gibson 1983; Garcia-Valdes et al. 1988; Jeffrey et al. 1975; Kiyohara & Nagao 1978; Patel & Gibson 1974; Schell 1983; Tagger et al. 1990; Utkin et al. 1990). Despite the results on naphthalene degradation, there is little information on the genetic and physiological basis for the microbial degradation of higher-molecular weight PAHs. Thus, naphthalene was chosen as an example to illustrate the general principles outlined above.

Pseudomonas putida PpG7 (Dunn & Gunsalus 1973) and *Pseudomonas putida* strain NCIB 9816 (Davies & Evans 1964; Yen & Serdar 1988) metabolize naphthalene through the pathway shown in Fig. 6. The first step in the pathway involves the formation of *cis*-(1*R*, 2*S*)-dihydroxy-1,2-dihydronaphthalene (naphthalene *cis*-1,2-dihydrodiol) (Jeffrey et al. 1975). This reaction is catalyzed by a multicomponent enzyme system, designated naphthalene dioxygenase (Ensley et al. 1982) in Fig. 6. An iron-sulfur flavoprotein, reductase (Haigler & Gibson 1990a), initially accepts electrons from NADH or NADPH and then transfers them to a 2Fe.2S ferredoxin, designated ferredoxin NAP (Haigler & Gibson 1990b). Ferredoxin NAP in turn reduces the terminal iron-sulfur protein, designated ISP NAP (Ensley & Gibson 1983), which catalyzes the oxidation of naphthalene to naphthalene *cis*-1,2-dihydrodiol (Fig. 6). The genes for the individual components of naphthalene dioxygenase in *Pseudomonas putida* PpG7 and NCIB 9816 are encoded on the NAH7 plasmid (Yen & Gunsalus 1982; Serdar & Gibson 1989) and have been designated nahAa, nahAb, and nahAcAd (Fig. 6). Ensley et al. (1987) have cloned the nahAcAd gene in *E. coli* and shown the expression of naphthalene dioxygenase activity. The nucleotide sequence for the naphthalene dioxygenase genes from *P. putida* strains has recently been reported (Simon et al. 1992).

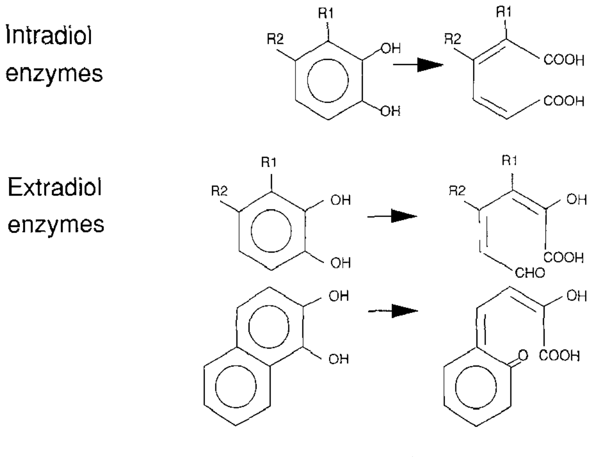


Fig. 5. *Ortho*-(intradiol) and *meta* (extradiol)-ring fission pathways for the catabolism of polycyclic aromatic hydrocarbons.

Figure 4 shows the different routes of initial oxidative attack of PAHs by eucaryotic and procaryotic microorganisms. A diverse group of ligninolytic and non-ligninolytic fungi have the ability to oxidize PAHs (Table 3). The sites of enzymatic attack, mechanisms, chemical intermediates and pathways for the metabolism of several PAHs by eucaryotic microorganisms have been extensively reviewed (Cerniglia 1984; Holland et al. 1986; Cerniglia et al. 1992; Sutherland 1992). The best studied non-ligninolytic fungus, *Cunninghamella elegans*, utilizes cytochrome P-450 monooxygenase enzymes to incorporate one atom of molecular oxygen into the aromatic nucleus and reduce the remaining atom to water (Cerniglia 1984). The resulting arene oxide intermediate can undergo further metabolism by epoxide hydrolase to form a dihydrodiol with the *trans*-configuration or can undergo nonenzymatic rearrangement to form a phenol, which can be conjugated with sulfate, glucuronic acid, or glucose (Fig. 4). *C. elegans* has been shown to oxidize numerous PAHs and their methyl, nitro, and fluoro-substituted derivatives to *trans*-dihydrodiols, phenols, quinones, tetralones and conjugates of these primary metabolites (Table 5). Although small amounts of mutagenic and carcinogenic metabolites are found during the metabolism of PAHs, most fungal transformation

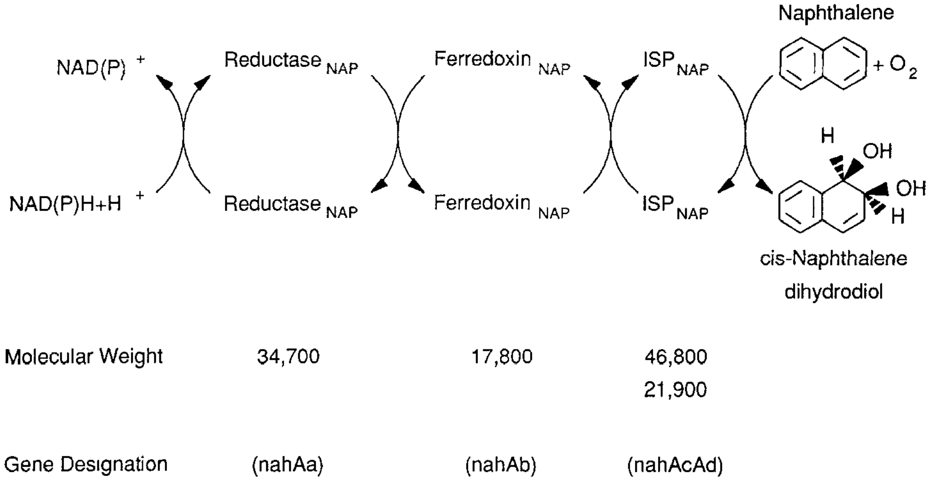


Fig. 6. Oxidation of naphthalene to naphthalene *cis*-1,2-dihydrodiol by naphthalene dioxygenase. (ISP = iron-sulfur protein).

products formed by *C. elegans* are less mutagenic than the parent compound and thus detoxify the PAHs (Cerniglia et al. 1985a, b).

Table 5. Metabolites produced from polycyclic aromatic hydrocarbons by fungi.

Compound	Metabolites	References
Naphthalene	1-Naphthol, 2-naphthol, naphthalene *trans*-1,2-dihydrodiol, 4-hydroxy-1-tetralone, 1,2-naphthoquinone, 1,4-naphthoquinone, sulfate and glucuronide conjugates	Ferris et al. 1973; Smith & Rosazza 1974; Cerniglia & Gibson 1977, 1978; Cerniglia et al. 1978, 1983a, 1985a
Acenaphthene	1-Acenaphthenone, 1,2-acenaphthenedione, *cis*-1,2-dihydroxyacenaphthene, *trans*-1,2-dihydroxyacenaphthene, 1,5-dihydroxyacenaphthene, 1-acenaphthenol, 6-hydroxyacenaphthenone	Pothuluri et al. 1992b
Anthracene	Anthracene *trans*-1,2-dihydrodiol, 1-anthrol, anthraquinone, phthalate, glucuronide, sulfate and xyloside conjugates	Cerniglia 1982; Cerniglia & Yang 1984; Sutherland et al. 1992; Field et al. 1992; Hammel et al. 1991
Phenanthrene	Phenanthrene *trans*-1,2-dihydrodiol, phenanthrene *trans*-3,4-dihydrodiol, phenanthrene *trans*-9,10-dihydrodiol, glucoside conjugate of 1-phenanthrol, 3-,4- and 9-hydroxyphenanthrene, 2,2-diphenic acid	Cerniglia & Yang 1984; Cerniglia et al. 1985a, 1989; Sutherland et al. 1991; Hammel et al. 1992
Fluoranthene	Fluoranthene *trans*-2,3-dihydrodiol, 8 and 9-hydroxyfluoranthene *trans*-2,3-dihydrodiols, glucoside conjugates	Pothuluri et al. 1990
Benz[a]anthracene	Benz[a]anthracene *trans*-3,4-dihydrodiol, benz[a]anthracene *trans*-8,9-dihydrodiol, benz[a]anthracene *trans*-10,11-dihydrodiol, phenolic and tetrahydroxy derivatives of benz[a]anthracene, glucuronide and sulfate conjugates	Cerniglia et al. 1980d, 1985a
Pyrene	1-Hydroxypyrene, 1,6-pyrenequinone, 1,8-pyrenequinone, glucoside conjugates	Hammel et al. 1986; Cerniglia et al. 1986
Benzo[a]pyrene	Benzo[a]pyrene *trans*-4,5-dihydrodiol, benzo[a]pyrene *trans*-7,8-dihydrodiol, benzo[a]pyrene *trans*-9,10-dihydrodiol, benzo[a]pyrene-1,6-quinone, benzo[a]pyrene-3,6-quinone, benzo[a]pyrene-6,12-quinone, 3-hydroxybenzo[a]pyrene, 9-hydroxybenzo[a]pyrene, 7β, 8α, 9α, 10β-tetrahydrobenzo[a]pyrene, 7β, 8α, 9β, 10α-tetrahydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene, benzo[a]pyrene 7,8-dihydrodiol-9,10-epoxide, glucuronide and sulfate conjugates	Haemmerli et al. 1986; Cerniglia & Gibson 1979, 1980a, 1980b; Lin & Kapoor 1979; Wiseman & Woods 1979; Cerniglia et al. 1980c

Recently, Sutherland et al. (1992) reported that another fungus, *Rhizoctonia solani*, metabolizes anthracene to *trans*-1,2-dihydroxy-1,2-dihydroanthracene (anthracene *trans*-1,2-dihydrodiol) and three xyloside conjugates. The formation of xyloside conjugates suggests that *R. solani* may be as useful as *C. elegans* in detoxifying PAHs.

Several research groups have demonstrated that white rot fungi are able to degrade PAHs and have potential for the bioremediation of PAH-polluted soils and sediments (Aust 1990; Bumpus 1989; Gold et al. 1989; Hammel 1989; Haemmerli et al. 1986; Sanglard et al. 1986). The best studied strain, *Phanerochaete chrysosporium*, produces extracellular lignin peroxidases that not only degrade lignin-related compounds but also catalyze the one-electron oxidation of PAHs to quinones (Fig. 4). Degradation of ^{14}C-labeled PAHs to minor amounts of $^{14}CO_2$ and polar metabolites has also been demonstrated (Bumpus et al. 1985). The lignin peroxidases have been shown to oxidize PAHs that have ionization potentials less than about 7.6 eV (Haemmerli et al. 1986).

Hammel et al. (1992) reported that the ligninolytic fungus, *P. chrysosporium*, oxidizes phenanthrene at its C-9 and C-10 positions to give 2,2′-diphenic acid as a ring cleavage product. Their results indicate that *P. chrysosporium* utilizes a different pathway from *C. elegans* and bacteria, since the major site of enzymatic attack by most bacteria is at the C-3 and C-4 positions of phenanthrene. Recently, Sutherland et al. (1991) reported that *P. chrysosporium* under nonligninolytic conditions metabolizes phenanthrene to phenols and *trans*-dihydrodiols, similarly to *C. elegans*. This observation indicates that several enzymatic mechanisms may occur in *P. chrysosporium* for the initial oxidative attack on PAHs (Hammel et al. 1992).

Procaryotic and eucaryotic photoautotrophs (Table 4) may be an exception to the oxidation mechanisms illustrated in Fig. 4, since both *cis*- and *trans*-dihydrodiols have been detected as transformation products formed from PAHs by cyanobacteria and green algae (Cody et al. 1984; Lindquist & Warshawsky 1985; Warshawsky et al. 1988; Narro et al. 1992a, 1992b) (Table 6). Cerniglia et al. (1979, 1980a, 1980b, 1980c) have shown that the marine cyanobacterium *Oscillatoria* sp. strain JCM oxidizes naphthalene under photoautotrophic conditions, predominantly to 1-naphthol. Deuterated naphthalene-labeling experiments indicate that 1-naphthol is formed through a naphthalene 1,2-oxide intermediate rather than by direct hydroxylation (Narro et al. 1992a). Another marine cyanobacterium, *Agmenellum quadruplicatum* PR-6, has also been shown to oxidize phenanthrene via a monooxygenase to form *trans*-9,10-dihydroxy-9,10-dihydrophenanthrene (phenanthrene *trans*-9,10-dihydrodiol) and 1-methoxyphenanthrene as major metabolites. The metabolism of naphthalene and phenanthrene by cyanobacteria is similar to that reported for mammalian and fungal enzymes. The freshwater green alga, *Selenastrum capricornutum*, grown under photoautotrophic conditions, oxidizes benzo[a]pyrene to *cis*-dihydrodiols (Table 6) with the predominant isomer being *cis*-11,12-dihydroxy-11,12-dihydrobenzo[a]pyrene (benzo[a]pyrene *cis*-11,12-dihydrodiol). The formation of *cis*-dihydrodiols suggests a dioxygenase similar to that found in heterotrophic procaryotes rather than the monooxygenase found in cyanobacteria and eucaryotes (Warshawsky et al. 1988, 1990). Further studies are necessary to characterize the enzymes involved in PAH oxidation by algae.

Methanotrophs have been shown to transform a variety of xenobiotics, including alkanes, alkenes and aromatic compounds (Colby & Dalton 1976; Colby et al. 1977, 1978; Dalton et al. 1981). Recently, Brusseau et al. (1990) reported that *Methylococcus trichosporium* OB3b oxidizes naphthalene to 1- and 2-naphthol. The exact mechanism of PAH degradation in methanotrophs has not been determined.

Biodegradation of high molecular weight PAHs

The persistence and bioavailability of PAHs in the environment depend on the physical and chemical characteristics of the PAH as well as the composition and chemical characteristics of the sediment (Wang et al. 1990; Park et al. 1990; Sims et al. 1990). The recalcitrance of PAHs for microbial

Table 6. Metabolites produced from PAHs by different species of cyanobacteria and algae.

Compound	Metabolites	References
Naphthalene	1-Naphthol, 4-hydroxy-1-tetralone, naphthalene *cis*-1,2-dihydrodiol	Cerniglia et al. 1979, 1980a, 1980b; Narro et al. 1992a
Phenanthrene	Phenanthrene *trans*-9,10-dihydrodiol, 1-methoxyphenanthrene	Narro et al. 1992b
Benzo[a]pyrene	Benzo[a]pyrene *cis*-4,5-dihydrodiol, benzo[a]pyrene *cis*-7,8-dihydrodiol, benzo[a]pyrene *cis*-9,10-dihydrodiol, benzo[a]pyrene *cis*-11,12-dihydrodiol	Cody et al. 1984; Lindquist & Warshawsky 1985; Warshawsky et al. 1990

degradation increases directly with the molecular weight and the octanol: water partition coefficient (log Kow) and inversely with aqueous water solubility (Fig. 1) since high molecular weight PAHs are more slowly desorbed and therefore less available for biological uptake (Aronstein et al. 1991). Calculated half-lives of PAHs in sediments vary between laboratory and field studies (Wild et al. 1991a, 1991b). This variation is probably a consequence of differences in the environmental parameters of the site, experimental design and methodology, biological components, and season of sampling. For example, PAH degradation is influenced by environmental factors, including temperature, pH, nutrients, oxygen and water availability, salinity and soil type (Atlas 1991). In general, low molecular-weight PAHs, such as naphthalene (Heitkamp et al. 1987, 1991), have short half-lives, whereas the high molecular weight PAHs, such as benzo[a]pyrene, are recalcitrant and persist indefinitely (Fig. 7). Also, half-lives tend to be shorter in PAH-contaminated sediments than in pristine sediments, which is probably due to the elevated populations of PAH-degrading bacteria in the contaminated sediments (Herbes & Schwall 1978; Shiaris 1989; Guerin & Jones 1989).

Although microbial metabolic pathways for degradation of PAHs containing up to three rings have been proposed (Gibson & Subramanian 1984), until recently little information has been available concerning the ability of microorganisms to metabolize the larger, more recalcitrant, high molecular weight PAHs. Biotransformation of the tetracyclic PAHs fluoranthene and pyrene by eukaryotes has been reported (Table 3) and bacteria that are able to oxidize or co-oxidize fluoranthene and pyrene have been isolated (Table 2). Information on the biochemical pathways for microbial catabolism of higher molecular weight PAHs is scarce.

Research in this laboratory has demonstrated the ability of *Mycobacterium* sp. PYR-1 to substantially mineralize fluoranthene, naphthalene and pyrene in pure culture (Kelley & Cerniglia 1991; Kelley et al. 1991a, 1991b; Heitkamp et al. 1988) and to significantly enhance mineralization of fluoranthene and pyrene in sediments containing indigenous microorganisms (Kelley & Cerniglia 1991; Heitkamp & Cerniglia 1989). Figures 8 and 9 show the proposed pathways for the catabolism of fluoranthene and pyrene by *Mycobacterium* sp. PYR-1. These reaction sequences show that fluoranthene and pyrene biodegradation can range from initial oxygenation and the formation of dead-end products to complete mineralization. From a toxicological standpoint, the question which needs to be addressed is whether high molecular weight PAH biodegradation intermediates in the environ-

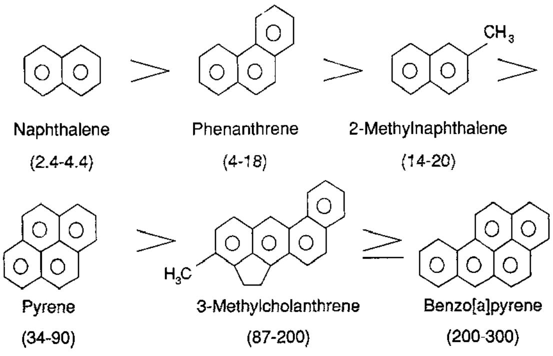


Fig. 7. Half-lives in weeks for the microbial degradation of polycyclic aromatic hydrocarbons. Arrows indicate an increase in recalcitrance with ring structure.

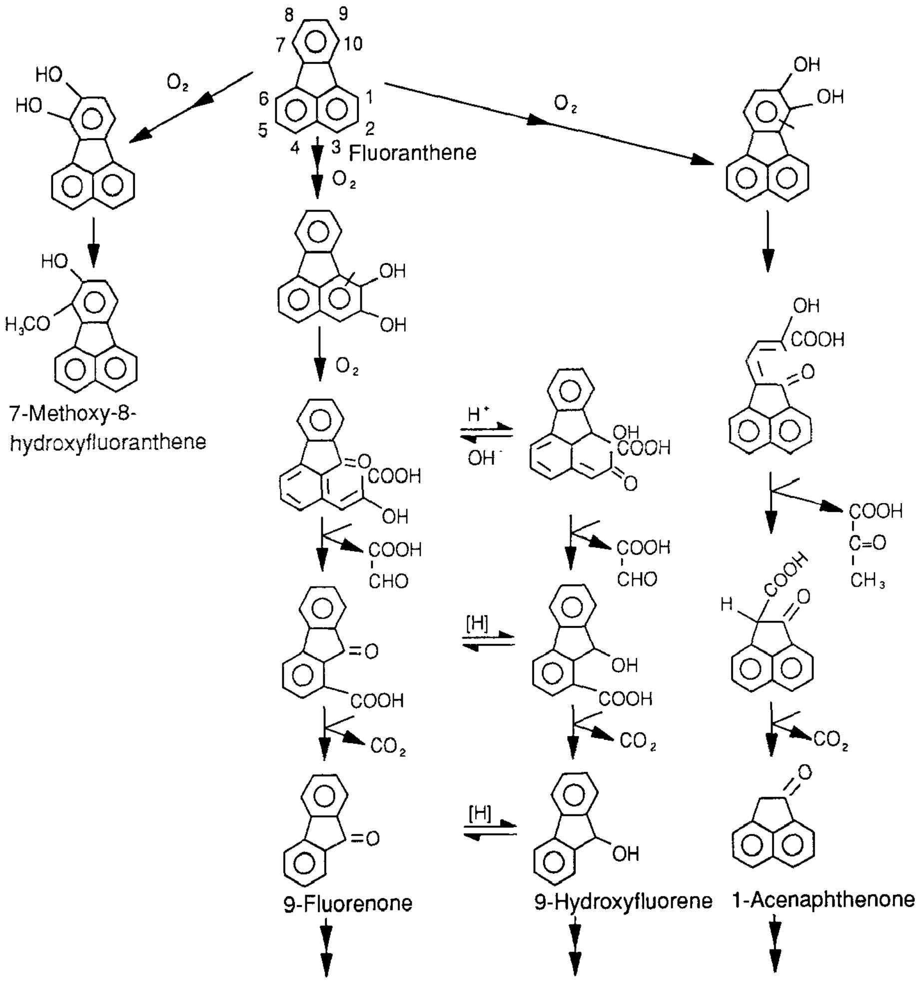


Fig. 8. Pathways proposed for the metabolism of fluoranthene by *Mycobacterium* sp. PYR-1.

ment represent a human health risk and safety problem.

Conclusion

Microbial degradation of PAHs is thought to be the major process involved in effective site bioremediation. The number of PAH compounds now known to be biodegraded has increased; however, the number of isolates of microorganisms that can completely mineralize high molecular weight PAHs, such as benzo[a]pyrene, is limited. Four and five-ring PAHs are very recalcitrant due to their low water solubilities and the resonance energies of their structures.

Microorganisms capable of rapidly mineralizing the more recalcitrant and potentially carcinogenic PAHs need to be isolated and characterized. Although the degradative pathways for PAH catabolism have been widely studied, there have been relatively few investigations on the regulatory mechanisms involved in controlling the microbial degradation of these ubiquitous pollutants.

Most studies regarding the toxicity and biodegradability of PAHs have been conducted on single compounds to minimize the number of variables. PAHs exist in environmental samples as complex mixtures, the concentration of each PAH at polluted sites depends upon the source of the sample, susceptibility to microbial degradation, and abiotic and environmental factors. Generally, the rates of

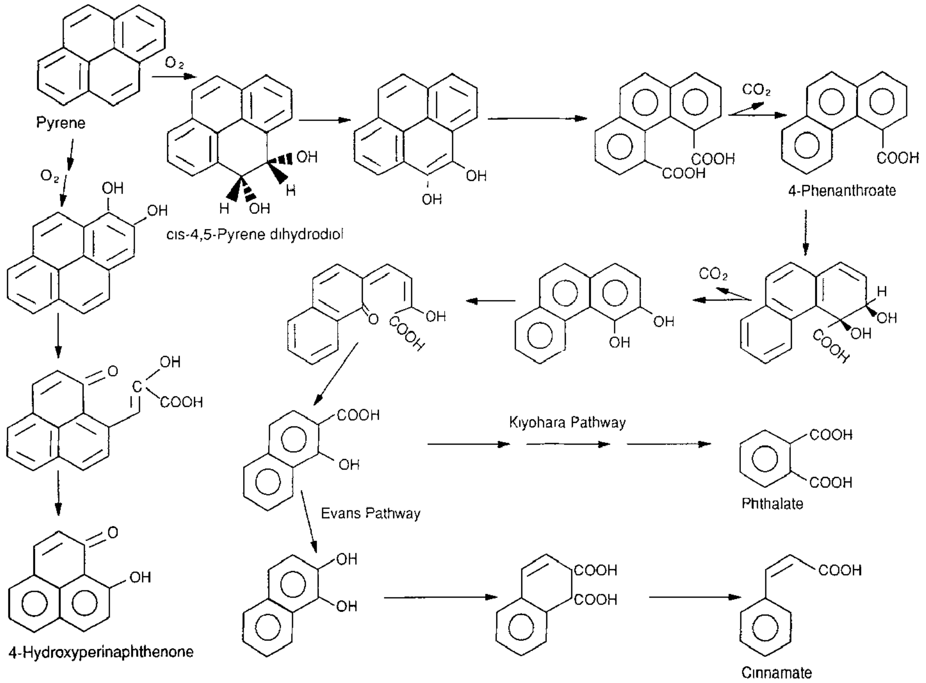


Fig. 9. Pathways proposed for the metabolism of pyrene by *Mycobacterium* sp. PYR-1.

biodegradation of individual hydrocarbons have been found to be related to their chemical structure, degree of aromaticity, concentration and physicochemical properties. Biodegradation of a component in an industrial mixture also depends to a large extent on the qualitative hydrocarbon content of the mixture, preexposure conditions and concentration. Little is known about the biodegradation of complex mixtures of PAHs, especially the effect of one PAH component on the biodegradability of another. Further experiments are required with mixtures of PAHs to determine the full degradative potential of microorganisms and the mechanisms by which they metabolize PAHs.

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