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Review

Biodegradation aspects of Polycyclic Aromatic Hydrocarbons (PAHs): A review

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ABSTRACT

PAHs are aromatic hydrocarbons with two or more fused benzene rings with natural as well as anthropogenic sources. They are widely distributed environmental contaminants that have detrimental biological effects, toxicity, mutagenecity and carcinogenicity. Due to their ubiquitous occurrence, recalcitrance, bioaccumulation potential and carcinogenic activity, the PAHs have gathered significant environmental concern. Although PAH may undergo adsorption, volatilization, photolysis, and chemical degradation, microbial degradation is the major degradation process. PAH degradation depends on the environmental conditions, number and type of the microorganisms, nature and chemical structure of the chemical compound being degraded. They are biodegraded/biotransformed into less complex metabolites, and through mineralization into inorganic minerals, H_2O, CO_2 (aerobic) or CH_4 (anaerobic) and rate of biodegradation depends on pH, temperature, oxygen, microbial population, degree of acclimation, accessibility of nutrients, chemical structure of the compound, cellular transport properties, and chemical partitioning in growth medium. A number of bacterial species are known to degrade PAHs and most of them are isolated from contaminated soil or sediments. *Pseudomonas aeruginosa*, *Pseudomons fluoresens*, *Mycobacterium* spp., *Haemophilus* spp., *Rhodococcus* spp., *Paenibacillus* spp. are some of the commonly studied PAH-degrading bacteria. Lignolytic fungi too have the property of PAH degradation. *Phanerochaete chrysosporium*, *Bjerkandera adusta*, and *Pleurotus ostreatus* are the common PAH-degrading fungi. Enzymes involved in the degradation of PAHs are oxygenase, dehydrogenase and lignolytic enzymes. Fungal lignolytic enzymes are lignin peroxidase, laccase, and manganese peroxidase. They are extracellular and catalyze radical formation by oxidation to destabilize bonds in a molecule. The biodegradation of PAHs has been observed under both aerobic and anaerobic conditions and the rate can be enhanced by physical/chemical pretreatment of contaminated soil. Addition of biosurfactant-producing bacteria and light oils can increase the bioavailability of PAHs and metabolic potential of the bacterial community. The supplementation of contaminated soils with compost materials can also enhance biodegradation without long-term accumulation of extractable polar and more available intermediates. Wetlands, too, have found an application in PAH removal from wastewater. The intensive biological activities in such an ecosystem lead to a high rate of autotrophic and heterotrophic processes. Aquatic weeds *Typha* spp. and *Scirpus lacustris* have been used in horizontal–vertical macrophyte based wetlands to treat PAHs. An integrated approach of physical, chemical, and biological degradation may be adopted to get synergistically enhanced removal rates and to treat/remediate the contaminated sites in an ecologically favorable process.

Contents

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1. Introduction

PAHs are aromatic hydrocarbons with two or more fused benzene rings. They are formed during the thermal decomposition of organic molecules and their subsequent recombination. Incomplete combustion at high temperature (500–800 °C) or subjection of organic material at low temperature (100–300 °C) for long periods result in PAH production. They occur as colorless, white/pale yellow solids with low solubilities in water, high melting and boiling points and low vapour pressure (Table 1). With an increase in molecular weight, their solubility in water decreases; melting and boiling point increase and vapour pressure decreases [1,2]. The chemical structures of some commonly studied PAHs are given in Fig. 1. The common sources of PAHs in environment include natural as well as anthropogenic. Natural sources are forest and rangeland fires, oil seeps, volcanic eruptions and exudates from trees. Anthropogenic sources of PAH include burning of fossil fuel, coal tar, wood, garbage, refuse, used lubricating oil and oil filters [3], municipal solid waste incineration and petroleum spills and discharge. They are ubiquitously present contaminants which are toxic, mutagenic and carcinogenic [4]. PAHs were, perhaps, the first recognized environmental carcinogens. They do not degrade easily under natural conditions. Persistence increases with increase in the molecular weight. They have gathered significant concern because of their presence in all components of environment, resistance towards biodegradation, potential to bio-accumulate and carcinogenic activity. Though they are the chief pollutants of air [5], soil acts as the ultimate depository of these chemicals. Their fate in environment includes volatilization, photo-oxidation, chemical oxidation, adsorption on soil particles, leaching and microbial degradation [6].

The hazards associated with the PAHs can be overcome by the use of conventional methods which involve removal, alteration, or isolation of the pollutant. Such techniques involve excavation of contaminated soil and its incineration or containment. These technologies are expensive, and in many cases transfer the pollutant from one phase to another. On the other hand, bioremediation is the tool to transform the compounds to less hazardous/non-hazardous forms with less input of chemicals, energy, and time [7,8]. Although PAH may undergo adsorption, volatilization, photolysis, and chemical degradation, microbial degradation is the major degradation process [9,10]. Microbes are known for their catabolic activity in bioremediation, but changes in microbial communities are still unpredictable and the microbial community is still termed as a 'black box' [11]. The PAH-degrading microorganism could be algae, bacteria, and fungi. It involves the 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). The bioremediation of a pollutant and its rate depends on the environmental conditions, number and type of the microorganisms, nature and chemical structure of the chemical compound being degraded. Thus, to devise a bioremediation system, a number of factors are to be counted for. Both bacteria and fungi have been extensively studied for their ability to degrade xenobitics including PAHs. The extent and rate of biodegradation depends on many factors including pH, temperature, oxygen, microbial population, degree of acclimation, accessibility of nutrients, chemical structure of the compound, cellular transport properties, and chemical partitioning in growth medium [12].

2. Microbial degradation

2.1. Bacteria

Bacteria are the class of microorganisms actively involved in the degradation of organic pollutants from contaminated sites. A number of bacterial species are known to degrade PAHs. Most of them, representing biodegradation efficiency, are isolated from contaminated soil or sediments. Long-term petrochemical waste discharge harbours bacteria capable of degrading PAH to a considerable extent. Among the PAH in petrochemical waste, Benzo(a)pyrene is considered as the most carcinogenic and toxic. Studies have shown that bacteria can degrade BaP when grown on an alternative carbon source in liquid culture experiments. Ye et al. [13] observed a 5% decrease in BaP concentration after 168 h during incubations with *Sphingomonas paucimobilis* strain EPA 505. They also observed that resting cells of *S. paucimobilis* strain EPA 505 grown on nutrient agar supplemented with glucose, result in significant evolution of $^{14}CO_2$ (28%) indicating hydroxylation and ring cleavage of the 7,8,9,10-benzo ring. Aitken et al. [14] isolated 11 strains from a variety of contaminated sites (oil, motor oil, wood treatment, and refinery) with the ability to degrade BaP. The organisms were identified as at least three species of *Pseudomonas*, as well as *Agrobacterium*, *Bacillus*, *Burkholderia* and *Sphingomonas* species. BaP has been reported to be degraded by other bacteria including *Rhodococcus* sp., *Mycobacterium*, and mixed culture of *Pseudomonas* and *Flavobacterium* species [15–17]. Heitkamp et al. [18] described a bacterial isolate which was able to mineralize pyrene. Romero et al. [19] isolated *Pseudomonas aeruginosa* from a stream heavily polluted by a petroleum refinery. The species was found to be actively growing over high dosages of phenanthrene with complete removal of the pollutant in a period of 30 days. Rehmann et al. [20] isolated a *Mycobacterium* spp., strain KR2 from a PAH contaminated soil of a gaswork plant, which was able to utilize pyrene as sole source of carbon and energy. The isolate metabolized up to 60% of the pyrene added (0.5 mg ml^{-1}) within 8 days at 20 °C. Cis-4,5-pyrene dihydrodiol, 4-5-phenanthrene dicarboxylic acid, 1-hydroxy-2-naphthoic acid, 2-carboxybenzaldehyde, phthalic acid, and protocatechuic acid were identified as degradation products and a degradation pathway for pyrene was also suggested (Fig. 2). Yuan et al. [21] isolated six gram negative strains of bacteria from a petrochemical waste disposing site having the capacity of degrading acenaphthene, fluorene, phenanthrene, anthracene, and pyrene by 70–100% in a period of 40 days of initial treatment. Two of the six strains isolated were *Pseudomons fluoresens* and *Haemophilus* spp., the rod-shaped bacteria. Dean-Ross et al. [22] isolated two bacterial strains (*Mycobacterium flavescens* and *Rhodococcus* spp.) from sediments of River Grand Calumet from two

Table 1
Physical–chemical properties of Polycyclic Aromatic Hydrocarbons (PAHs).

S. No.	Name	M.F.	CAS registry No.[a]	B.Pt. (°C)[a]	M.Pt. (°C)[a]	V.P. (Pa at 25 °C)	Aqueous solubility (mg/l)[b]	IARC[c] group
1	Benzo[k]fluoranthene	$C_{20}H_{12}$	207-08-9	480	215.7	5.2×10^{-8}	–	2B
2	Anthracene	$C_{14}H_{10}$	120-12-7	342	216.4	1×10^{-3}	0.015	3
3	Benzo[b]fluoranthene	$C_{20}H_{12}$	205-99-2	481	168.3	6.7×10^{-5}	–	2B
4	Benzo(e)pyrene	$C_{20}H_{12}$	192-97-2	493	178.7	4×10^{-7}	–	3
5	Fluoranthene	$C_{16}H_{10}$	206-44-0	375	108.8	1.2×10^{-3}	0.25	3
6	Naphthalene	$C_{10}H_8$	91-20-3	218	80.2	11	30	n.e.
7	Phenanthrene	$C_{14}H_{10}$	85-01-8	340	100.5	2×10^{-2}	1–2	3
8	Benzo[ghi]perylene	$C_{22}H_{12}$	191-24-2	500	277	6×10^{-8}	–	3
9	Pyrene	$C_{16}H_{10}$	129-00-0	150.4	393	6.0×10^{-4}	0.12–0.18	3

[a] [156].
[b] [157].
[c] [4].

different locations. Both the bacteria were found to be capable of PAH degradation with the initial reaction rates of 0.044 mg l^{-1} for the K_s for pyrene mineralization by *M. flavescens* and 0.470 μg l^{-1} for the K_s for anthracene mineralization by *Rhodococcus species*. The study also proposed the degradation pathway of fluoranthene. In both strains, metabolism of fluoranthene occurred on the fused ring of fluoranthene molecule, producing 9-fluorenone-1-carboxylic acid.

There has been growing concern over the mounting concentration of PAHs in marine environment. Mangrove sediments, important estuarine wetlands are closely tied to human activities and are subjected to PAH contamination. Bacteria isolated from the mangrove sediments are known to degrade phenanthrene from 42% to 78% with different degradation potential depending upon the different sediments [23]. Romero et al. [19] studied phenanthrene degradation by microorganisms isolated from a contaminated

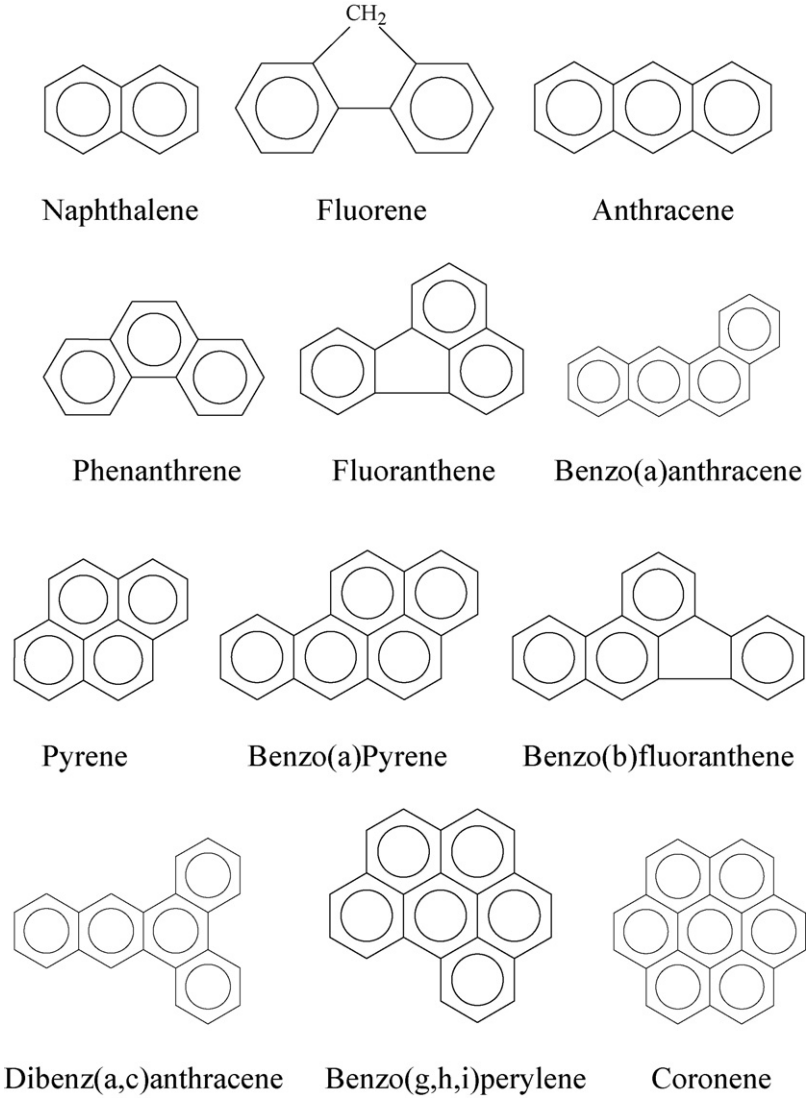

Fig. 1. Chemical structures of some commonly studied PAHs.

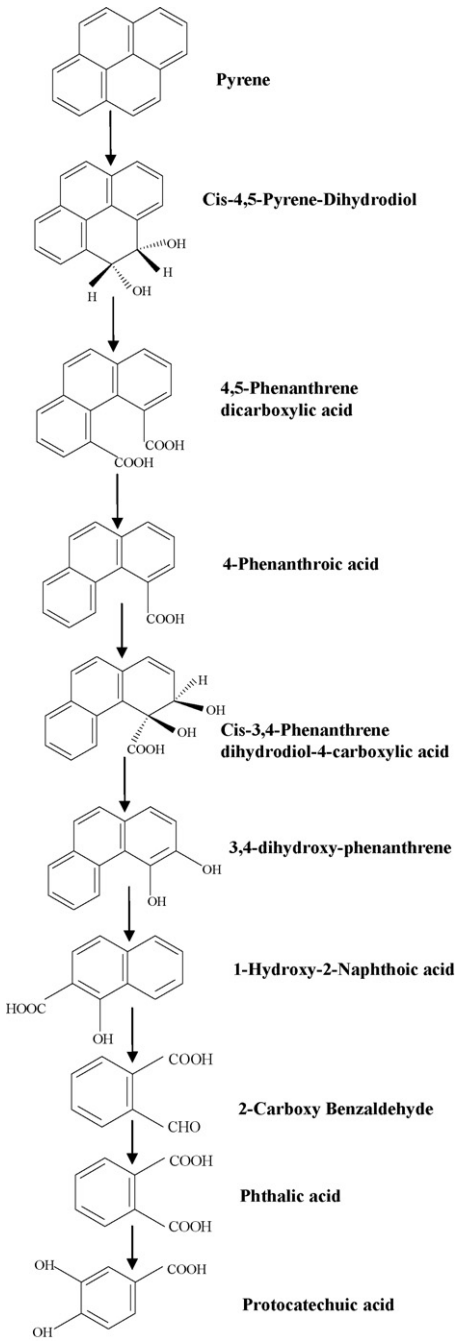

Fig. 2. Proposed degradation of Pyrene by *Mycobacterium* sp. Strain KR2 [20].

stream. *Rhodotorula glutinis* and *Pseudomonas aeruginosa* were the prevailing microorganisms utilizing phenanthrene. Cells of these microorganisms were inoculated in liquid mineral basal medium and almost complete phenanthrene transformation was observed in the experiment during the 1-month incubation period, for both microorganisms. It was seen that *R. glutinis* was as active as *P. aeruginosa* at growing on phenanthrene; the aromatic hydrocarbon degradation correlated directly to microbial density and biomass increase [19]. A study carried out for decontamination of soil polluted with PAHs by means of mixed microbial culture of the bacteria from the genera *Acenitobacter* and *Klebsiella* showed that within 6 months a significant reduction of 98% of the total PAH content took place. The concentration of the PAHs with three and four rings decreased to 0.5% of the original content in the soil. The concentration of most five to seven ring PAHs with carcinogenic properties decreased to 3% of the original value. Daane et al. [24] isolated and characterized PAH-degrading bacteria associated with the rhizosphere of salt marsh plants. They categorized the isolated bacteria into three main bacterial groups—gram-negative *pseudomonas*; gram-positive, non-spore forming *nocardioforms*; and the gram-positive, spore-forming group, *Paenibacillus*. They observed that phenanthrene-enriched isolates are able to utilize a greater number of PAHs than are the naphthalene-enriched isolates. Later, they established that bacteria belonging to genus *Paenibacillus*, isolated from the petroleum-contaminated sediment and salt marsh rhizosphere can use naphthalene or phenanthrene as sole carbon source and can degrade the PAHs [25]. Verrhiest et al. [26] studied interaction between a PAH mixture and microbial communities in natural freshwater sediment. They observed that benzo(k)fluoranthene concentration in the sediment was stable for over 28 days, whereas phenanthrene and fluoranthene remained from 3% to 6%. The study showed that (a) PAH induce perturbations in the microbial communities in terms of density and metabolism, (b) indigenous bacteria might be use for toxicity assessment, and (c) native microorganisms of sediments seem to have a high capacity of PAH degradation, depending on the physicochemical properties and the availability of substances present.

2.2. Fungi

Several fungi are known to have the property of degradation of persistent pollutants. Cutright studied the kinetics involved in PAH degradation by the fungi *Cunninghamella echinulata var. elegans* and suggested that for a first order reaction-system the rate of change in contaminant concentration is proportional to the concentration of contaminant in soil and the time prediction tool in degradation is dependent on the microorganisms, the contaminant type and its concentration [27]. The microbial degradation by lignolytic fungi has been intensively studied during the past few years [28] and due to the irregular structure of lignin, lignolytic fungi produce extracellular enzymes with very low substrate specificity, making them suitable for degradation of different compounds. The lignolytic system consists of three main enzyme groups with lignin peroxidase, manganese dependent peroxidase, phenoloxidases (lacases, tyrosinases), and H_2O_2-producing enzymes. Experiments with purified enzymes proved that lignolytic enzymes are able to degrade PAHs [29]. It has been observed that lignolytic enzymes perform a one electron radical oxidation, producing cation radicals from contaminants followed by appearance of quinines [30]. A study by Clemente et al. [31] investigated degradation of PAH by thirteen deuteromycete ligninolytic fungal strains and found that the degree of dedradation varies with a variation of lignolytic enzymes. Maximum degradation of naphthalene (69%) was observed by the strain 984 having Mn-peroxidase activity, followed by strain 870 (17%) showing lignin peroxidase and laccase activities. Phenanthrene degradation of 12% was observed with strain 870 with Mn-peroxidase and laccase activities. A good level of degradation of anthracene (65%) was observed by the strain 710. Recently, soil fungi have been studied regarding their ability to degrade Polycyclic Aromatic Hydrocarbons (PAHs) and produce ligninolytic enzymes under microaerobic and very-low-oxygen conditions [32]. Low-molecular-weight PAHs (2–3 rings) were found to be degraded most extensively by *Aspergillus* sp., *Trichocladium canadense*, and *Fusarium oxysporum*. For high-molecular-weight PAHs (4–7 rings), maximum degradation has been observed by *T. canadense*, *Aspergillus* sp., *Verticillium* sp., and *Achremonium* sp. Such studies have found that fungi have a great capability to degrade a broad range of PAHs under low-oxygen conditions.

The monooxygenase system of cytochrom P-450 generating epoxides may also be involved in degradation. The epoxides can be

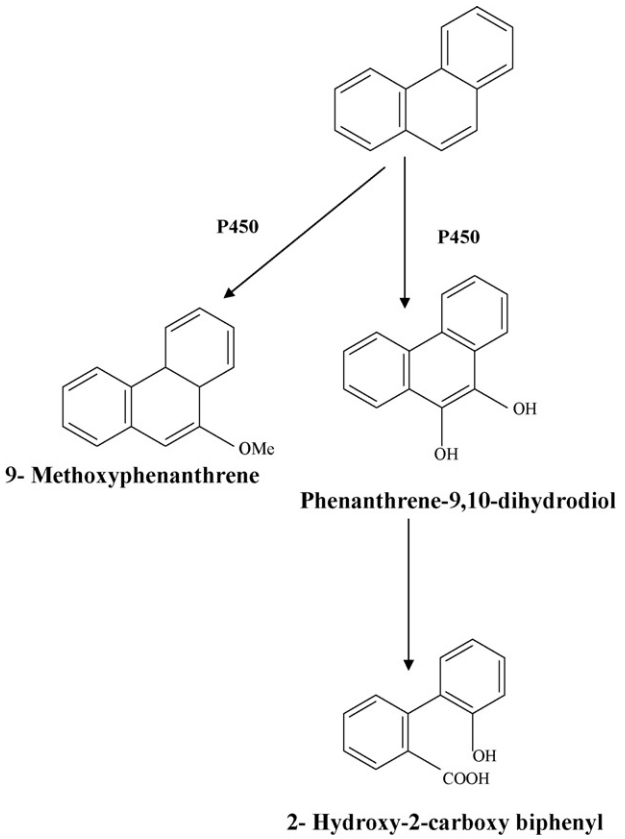

Fig. 3. Fungal degradation pathway of Phenanthrene using *Irpex lacteus* [33].

rearranged into hydroxyl derivatives or they may be hydrolyzed to vicinal dihydrodiols. Cajthaml et al. [33] studied fungal degradation products of PAHs by lignolytic fungus *Irpex lacteus* and found that the major degradation products of anthracene and phenanthrene were anthraquinone and phenanthrene-9,10-dihydrodiol, respectively (Fig. 3). The study also proposed the pathway for degradation of anthracene and phenanthrene [33].

White-rot fungi (WRF) can degrade a wide range of organopollutants and the degradative activity is because of the lignin-degrading systems of these fungi. Boyle et al. [34] found that white-rot fungi growing in soil did not degrade significant amounts of PAHs. However, in liquid culture they degraded many PAHs. The effect of nutrient nitrogen was also assessed because nitrogen sources are frequently added during bioremediation, but nitrogen can inhibit the lignin-degrading system of WRF [35]. Addition of soil, sawdust, or ground alfalfa inhibited formation of the polar metabolites, but had little effect on mineralization. Degradation of Benzo(a)pyrene by WRF in liquid medium was faster by fungi that had been exposed to PAHs, suggesting that the degaradative system was inducible. Degradation in soil was increased by some surfactants, these increasing the B(a)P concentration in the soil moisture. The responsible factors for increased degradation could be increased accessibility of B(a)P to degaradative system or induction of WRF degaradative system [34]. Among hundreds of WRF displaying lignolytic activity, *Phanerochaete chrysosporium*, *Bjerkandera adusta*, and *Pleurotus ostreatus* have been extensively studied. Intermediate compounds as quinones, hydroxyl- and dihydroxy-PAH have been isolated in degradation experiments, but it is not clear whether they accumulate as dead-end products. Accumulation of PAH-quinones was reported in liquid cultures of *P. chrysosporium* and *B. adusta* [36,37] and in soil by *P. ostreatus* [38]. Eggen and Majcherczyk concluded that in aged soil contaminated with creosote, *P. ostreatus* removed Benzo(a)pyrene most extensively in first month [39]. The most abundant fungi in polluted environments are yeasts [40,41] and they can oxidize PAH with alternative carbon sources. The rate of degradation of phenanthrene by *Rhodotorula glutinis*, yeast isolated from contaminated stream was found to be almost equal to the degradation by bacteria *Pseudomonas aeruginosa* [19].

2.3. Algae

Degradation of PAHs requires a consortium of microorganisms and algae is no exception. Prokaryotic and eukaryotic photoautotrophic marine algae (i.e. cyanobacteria, green algae, and diatoms) are known to metabolize naphthalene to a series of metabolites [42–44], though there are indications that cis-hydroxylation of naphthalene by cyanobacteria, *Oscillatoria* and *Agmenellum* spp. involve pathways similar to fungus [42–46]. BaP is known to be transformed to diols, and quinones by marine algae in a period of 5–6 days. Warshawsky et al. found that *Selenastrum capricornutum*, a freshwater green alga metabolizes BaP to cis-dihydrodiols using a dioxygenase enzyme system as found in heterotrophic prokaryotes. It was observed that *S. capricornutum* produced 11,12-dihydrodiol under gold light and 9,10-dihydrodiol under white light. With increasing light energy from gold to white to UV-A in PAH-absorbing region, BaP quinone production increased. The study also concluded that only green algae almost completely metabolized BaP to dihydrodiols, whereas yellow algae and blue green algae failed in metabolizing the PAH. Higher doses of PAHs prove phytotoxic to algae [47,48]. Certain algae have been reported to enhance the removal fluoranthene and pyrene when present with bacteria. Borde et al. [49] first reported case of photosynthesis-enhanced biodegradation of toxic aromatic pollutants by algal–bacterial microcosms in a one-stage treatment. The potential of algal–bacterial microcosms for the biodegradation of salicylate, phenol and phenanthrene was studied. *Pseudomonas migulae* and *Sphingomonas yanoikuyae* were studied for phenanthrene degradation. The green alga *Chlorella sorokiniana* was cultivated in the presence of the pollutants at different concentrations, showing increasing inhibitory effects in the order salicylate < phenol < phenanthrene. A substantial removal (>85%) was recorded only in the systems inoculated with both algae and bacteria and incubated under continuous lighting. Such studies have demonstrated synergistic relationships in the algal–bacterial microcosms. Recently different microalgal species have been reported to degrade fluoranthene and pyrene [50]. The study of fluoranthene, pyrene, and a mixture of fluoranthene and pyrene by *Chlorella vulgaris*, *Scenedesmus platydiscus*, *Scenedesmus quadricauda*, and *Selenastrum capricornutum* has shown that removal is algal species-specific and toxicant-dependent. PAHs removal in 7 days of treatment was 78% and 48%, respectively by *S. capricornutum* and *C. vulgaris*. The removal efficiency of fluoranthene and pyrene in a mixture higher than the respective single compound, suggesting that the presence of one PAH stimulated the removal of the other PAH. A heterotrophic green microalgal strain *Prototheca zopfii* immobilized in polyurethane foam has also been reported to help accumulation of mixture of PAHs in the matix [51], whereas the free living cells of the alga can reduce PAHs and n-alkanes [52]. Hong et al. [53] studied the accumulation and biodegradation of phenanthrene and fluoranthene by the algae enriched from a mangrove aquatic ecosystem. The isolated microalgal species *S. costatum* and *Nitzschia* sp. were capable of accumulating and degrading the two typical PAHs simultaneously. The accumulation and degradation abilities of *Nitzschia* sp. were higher than those of *S. costatum*. Degradation of fluoranthene by the two algal species was slower, indicating its recalcitrance. The microalgal species also showed comparable or higher efficiency in the removal of the mixture than phenanthrene or fluoranthene singly, suggesting that the presence of one PAH stimulated the degradation of the other. The studies and

results obtained show that alga is suitable for PAH bioremediation and it acts co-metabolically with bacteria.

3. Enzymes in degradation

Enzymes involved in the degradation of PAHs are oxygenase, dehydrogenase and lignolytic enzymes. Fungal lignolytic enzymes are lignin peroxidase, laccase, and manganese peroxidase. They are extracellular and catalyze radical formation by oxidation to destabilize bonds in a molecule [54,55]. Laccase and Mn-dependent peroxidase are present abundantly in spent mushroom compost (SMC), whereas the production of ligninase is reported to be low and addition of SMC enhances the rate of PAH-degradation. Bogan and Lamar found that the disappearance of PAHs showed a strong correlation with the ionization potentials (IPs) [56]. The IP values, referring to the energy required to remove an electron and to form a cation radical are 8.12 for naphthalene, 8.03 for phenanthrene, 7.21 for benzo(a)pyrene, and 7.31 for benzo(g,h,i)perylene [57]. A one-electron oxidation of PAHs can take place by peroxidases (IP $\leq$ 7.35 eV), laccase (IP $\leq$ 7.45 eV), Mn-dependent peroxidase (IP $\leq$ 8.19 eV), and ligninase (IP $\leq$ 7.55 eV) [57,58]. These enzymes are active at different temperatures. Most of the enzymes have optimum activity at mesophilic temperatures and it decreases with very high and low temperatures. Some of the enzymes are reported to be active even at extremes of temperatures. Only laccase activity is detected at 5 °C. The optimum temperature is 45 °C for laccase, but its activity drops to 30% at 5 °C while 31% activity is found at 75 °C. On the other hand, the activity of Mn-dependent peroxidase is high even at 75 °C. Farnet et al. [59] reported that the activity of laccase of fungus *Marasmius quercophilus* is optimal at 80 °C. Enzymes also show substrate specificity but lignolytic enzymes are non-specific acting on phenolic and non-phenolic organic compounds via the generation of cation radicals after one electron oxidation [60,61]. *Pleurotus laccase* produces hydroxyl radicals [62] while Mn-dependent peroxidase of fungus *Nematoloma forwardii* degrades a broad spectrum of PAHs and aliphatic substances directly to carbon-di-oxide and polar fission products [29]. A generalized degradation pathway of PAHs has been proposed in different studies. A phthalic derivative is produced as one of the ring fission products of PAHs by white rot fungi and bacteria [58,63]. The derivatization of phthalate results into carbon-di-oxide or highly polar metabolites and the lignolytic enzymes and ozonation/photocatalytic oxidation act by free radical attack on the organopollutants [64]. Thus, the intermediates of these three methods are ring opening phthalic derivatives and aliphatics such as pentadecane, hexadecane, and nonadecane [61,64].

A higher dose of PAHs in the substrate may also affect the activity/rate of microbial degradation. Verrhiest et al. [26] while studying the interaction between a PAH mixture and microbial communities in natural freshwater sediment established that PAH dose has no effect on the microbial community in sediments up to a range of 30 mg PAH/kg. The PAHs had an effect at higher concentration owing to partial inhibition of the leucine–aminopeptidase activity. The b-glucosidase activity was stimulated by the organic pollutants at the same concentration. Schutzendubel et al. [65] found that during only 3 days of incubation, *Bjerkandera adusta* removed 56% and 38% of Fluorene and anthracene, while *Pleurotus ostreatus* degraded 43% and 60% of these compounds; other PAH were degraded to a lower extent. Except for anthracene in cultures of *P. ostreatus*, all PAH were removed uniformly during the cultivation time but Fluorene and anthracene were degraded faster than other PAH. The fungi produced valuable activity of manganese-dependent peroxidase but laccase was secreted only by *P. ostreatus* and was strongly induced by the addition of milled wood. The production of the oxidative enzymes did not correlate directly to the metabolism of PAH. Both fungi showed a very low activity of LiP during the whole incubation period; the enzyme was not induced by milled wood. *P. ostreatus* in the BSM (basic medium) medium showed activity of MnP only at the end of cultivation. The addition of wood inhibited production of the enzyme in younger cultures and increased the activity after 27 days. Laccase activity was detected only in cultures of *P. ostreatus*. The first maximum of activity was reached after 22 days in BSM cultures and BSMW (basic medium with milled wood) cultures, respectively. In cultures of both fungi, only a very low and no significant cresolase activity of the tyrosinase could be detected. In the case of *P. ostreatus*, the highest level of anthracene elimination was observed in 12-day-old cultures (62%). A second maximum in removal of anthracene was detected after 39 days (18%). Fluorene was degraded to a high degree in a 7-day-old culture (42%) and was practically uniformly removed over the whole cultivation time. Other PAHs were degraded at an almost constant rate during the 48 days of cultivation ($1 \pm 12\%$). Cultures supplemented with milled wood showed much lower degradation values: only anthracene (max 23% after 17 days), fluorene ($19 \pm 30\%$) and partially phenanthrene ($0 \pm 8\%$) were degraded.

A limitation of essential co-substrates for the monitored enzymes could be a factor confounding a clear correlation between the degradation of PAH and the secretion of oxidative enzymes. Similar to the role of H_2O_2 and manganese availability, co-substrates for the mediated oxidation by laccase could be essential in observing a clear correlation of PAH degradation and enzyme activity [66,67]. In the growth phase of fungi and in the absence of necessary extracellular enzymes and/or co-substrates, the degradation of PAH could take place preferentially by aromatic compound uptake. High oxidative potential generated by extracellular enzymes in later stages can enhance PAH degradation or displace the previous mechanism.

4. Oxygen: determining the path

The biodegradation of PAHs has been observed under both aerobic and anaerobic conditions. The microbial communities in contaminated sediments and soils exist under anaerobic conditions and biotransformation of pollutants is observed under such conditions. The anaerobic biodegradation of PAHs is a slow process, and its biochemical mechanism has not yet been elucidated [68,69]. These pathways initiate the biodegradation of PAHs by introducing both atoms of molecular oxygen into the aromatic nucleus, the reaction being catalyzed by a multicomponent dioxygenase which consists of a reductase, a ferredoxin and an iron–sulfur protein [70]. Studies have shown that two- and three-ring PAHs can be degraded anaerobically [69,71,72], but there is lack of evidences if it is true for PAHs with more than three rings. PAHs are known to dissipate under nitrate- and sulfate-reducing conditions. A study by Ambrosoli et al. [73] reported anaerobic PAH degradation in soil by a mixed bacterial consortium under denitrifying conditions and concluded that anaerobic biodegradation of fluorene, phenanthrene and pyrene, seems to be possible both through fermentative and respiratory metabolism, provided that low molecular weight co-metabolites and suitable electron acceptors (nitrate) are present.

Recently, novel anaerobic biotransformation pathways of fluorene and phenanthrene by sulfate-reducing bacteria (SRB) have been proposed (Fig. 4) [74]. The SRB was enriched from anaerobic swine wastewater sludge and it could biotransform 88% of fluorene and 65% of phenanthrene in a 21 days period of incubation. It was observed that sulfate reduction was coupled with biotransformation of fluorene and phenanthrene. Fluorene and phenanthrene were biotransformed through a sequence of hydration and hydrolysis reactions followed by decarboxylation with the formation of *p*-cresol (only in the phenanthrene system) and

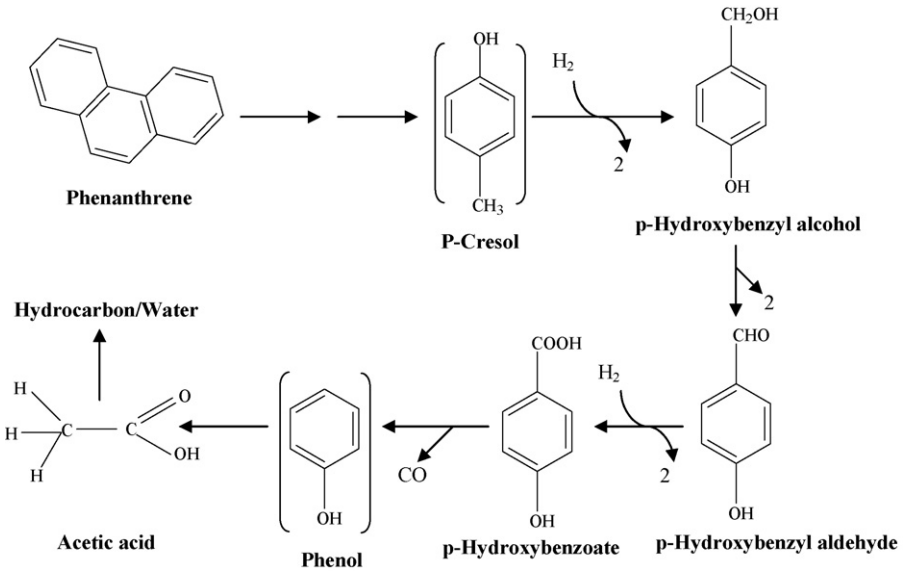

Fig. 4. Proposed anaerobic biotransformation pathway of phenanthrene by sulfate-reducing bacteria [74].

phenol. PAH degradation has also been observed in sediments derived from freshwater aquifers coupled to Fe (III) reduction [75]. There have been several studies on anaerobic degradation of naphthalene as compared to other PAHs. The rate and extent of PAH degradation is correlated with the number of benzene rings and the presence/absence of side chains [76]. Though the anaerobic degradation is comparatively a slower process and is limited by several other factors, it has the advantage of being adopted in natural environments and cannot be overlooked. The sulfate-reducing bacteria were found to be involved in degradation of phenanthrene under anaerobic conditions [77]. Later, it was added that sulfate reducing bacteria constitute a major microbial component in two-to four-ring PAH degradation, but the methanogen and vanomycin microbial populations are also involved [78]. The addition of microbial inhibitors like bromoethanesulfonic acid (BESA) (a selective methanogen inhibitor), molybdate (a selective sulfate-reducing bacteria inhibitor), or vanomycin (a selective eubacteria inhibitor) delayed PAH degradation. However, it was inhibited by the addition of humic acid, di-(2-ethylhexyl) phthalate (DEHP), nonylphenol, or heavy metals. Another study reported that the anaerobic degradation of PAH was enhanced by the addition of acetate, lactate, pyruvate, sodium chloride, cellulose, or zero-valent iron [79]. The biodegradation process of hydrocarbons, under anaerobic and reducing conditions, can be hypothesized into three major steps. Initially high concentration of aromatic hydrocarbons is partly degraded under nitrate and sulfate-reducing conditions to form low molecular weight organic acids as metabolic intermediates. Organic acids act as ligands complexing insoluble Fe (III) oxides in the aquifer and mobilizing Fe (III). Finally, the mobilized Fe (III) is available for iron reducing bacteria and intensifies the degradation of aromatic hydrocarbons [80].

Microbial community in the contaminated sediments appears to be adapted for PAH oxidation in situ as ^{14}C-labelled PAH was oxidized to $^{14}CO_2$ without a lag. In contrast, there is little, if any anaerobic, PAH oxidation in sediments without prior hydrocarbon contamination. Sediments not polluted with high levels of hydrocarbons can readily be adapted for rapid anaerobic PAH oxidation once they are exposed to PAH. The emergence of the capacity for anaerobic PAH oxidation is accompanied by an increase in anaerobic PAH degraders. This suggests that the appropriate PAH-degrading microorganisms are present, albeit in low numbers, even in sediments that are only exposed to low PAH inputs. If PAH concentrations increase, these organisms can respond with an increase in their activity. Thus, it seems likely that most harbor sediments will develop the capacity for anaerobic PAH degradation when high levels of PAHs are introduced into the sediments as the result of petroleum contamination. Therefore, if the introduction of the PAHs can be subsequently reduced, the sediments may be capable of significant self-purification, even under anaerobic conditions [81].

Pure cultures of several anaerobic nitrate-reducing [82,83] and sulfate-reducing [84] bacteria, capable of degrading PAHs, have been identified. Most of the bacteria are from the genus *Pseudomonas*. The *Pseudomonas* genus comprises many aerobic naphthalene-degrading bacteria and accounts for 86.9% of the hydrocarbon-degrading microorganisms found in gasoline contaminated aquifers [85]. Phylogenetic analysis of the PAH-degrading sulfur-reducing bacteria revealed that the organism belonged to the *Desulfobacterium* genus closely related (96.9% sequence identity) to the strain mXyS 1 which has the ability to grow by anaerobic m-xylene degradation [86].

From all the studies carried out to date with different aromatic compounds, such as phenols, cresols, anilines, benzoates, toluene, benzene, xylenes, nitroaromatic and chlorinated compounds, and many others, it can be concluded that anaerobic bacteria follow a strategy that is similar to that of aerobic bacteria [87]. First, the diverse aromatic compounds are transformed into a few central intermediates. Subsequently, the aromatic ring is activated and cleaved, and the resulting non-cyclic compounds are converted into central metabolites. Under anaerobic conditions, the major intermediates are benzoate (or benzoyl-CoA) and, to a lesser extent, resorcinol and phloroglucinol [87,88]. Reactions involved in the channelling processes that lead to the central intermediates include carboxylations, decarboxylations, hydroxylations, reductions, reductive dehydroxylations, deaminations, dechtorinations, aryl ether cleavages, and lyase reactions. The aromatic central intermediates are reductively attacked, as proposed by Evans in 1977 [89], and cleaved by hydrolysis. The resulting non-cyclic products are transformed by p-oxidation to central metabolites.

5. Effect of substrate, pretreatment and amendments

The organic pollutants which are in prolonged contact of the soil are bound to the soil particles and show reduced bioavailability

towards biodegradation. The phenomenon is known as sequestration. The phenomenon of contaminant sequestration has recently been a topic of discussion due to the anticipated impact of this process. Since the particles are inaccessible to the solution phase and are partially immobilized, they pose less risk/threat to the environment and human health and their remediation carries unnecessary economic burden with minimal health and safety benefits [90]. Several mechanisms have been proposed to describe sequestration of contaminants into/onto organic matter. Different studies [91,92] explain that the interaction of the contaminant molecule and soil particle begins with the partitioning of the molecule into/onto humic acid or fulvic acid polymer layers present at the surface of soil particle. Later, the molecule diffuses into the three-dimensional micropores of the particles which are present in the humin core of the particle and partially inaccessible to the solution phase by the overlying layer of polymeric humic acid and fulvic acid [93]. The outer layer of the core is lipophilic and, therefore, binds the organic pollutant strongly rendering it recalcitrant [94]. Later it was concluded that organic matter content is not the only factor responsible for sequestration. Cation exchange capacity (CEC), micropore volume, soil texture, and surface area, too, play a role in it [95]. Studies have also been reported in contradiction to the earlier model of sequestration. White et al. found that extraction of soluble organic matter (humic acid and fulvic acid), leaving humin, increased the extent of PAH sequestration [96]. A study by Bogan and Sullivan, demonstrated that addition of fulvic acid or a material rich in fulvic acid can result in higher rate of contaminant degradation [90]. It concluded that the sequestration of contaminant molecule is primarily because of the humin fraction. The members of genus *Mycobacterium* are widely used in bioremediation of aged contaminated sites. It has been established that mycobacteria have exceptionally lipophilic surfaces which makes them suitable organisms for the uptake of bound pollutants from the soil particles. They are also known to have good catabolic efficiency towards PAHs up to five benzene rings [97–99].

The biological degradation/extraction of a pollutant can be enhanced by physical/chemical pretreatment of contaminated soil. The slow rate of degradation in soil is primarily due to the slow rate of desorption of contaminants from soil particles and not due to the slow rate of degradation by the microorganisms. The reasons for slow desorption are slow diffusion of contaminants through the pore liquid and through the soil organic matter. In order to increase the rate of diffusion, the soil is subjected to thermal or chemical treatment prior to the microbial remediation. An increase in temperature can decrease the soil–water partition coefficient and as a result, dissolution of contaminants in water is observed. The partition-coefficient of PAHs decreases by 20–30% for every 10° rise in temperature between 5 and 45 °C [100,101]. Apart from it, the mass transfer within the soil increases with increase in temperature. The mass transfer depends on the effective diffusion coefficient, which is proportional to the diffusion coefficient and inversely proportional to the partition coefficient [102,103]. The diffusion coefficient of water increases by 4–5 times with an increase in temperature from 20 to 120 °C and results in about 150 times increase in the effective diffusion coefficient. Bonten et al. [104] studied the effects of short-term heating on subsequent biodegradation of PAHs. Heating at 120 °C for 1 h increased the degree of degradation after 21 days of an aged PAH contamination from 9.5% to 27%. Lower temperatures resulted in smaller increases. Chemical pretreatment for organic soil contaminants is addition of an organic solvent which increases the rate of mass transfer of hydrophobic compounds in soil. Such an increase in the rate of mass transfer may lead to redistribution of contaminants from sites exhibiting slow desorption rate to those exhibiting a fast one. The most prominent effect of soaking with an organic solvent is a change in partition coefficient. The soil–solvent partition coefficient decreases exponentially with an increase in acetone concentration [105,106]. A 4:1 acetone–water mixture can desorb more than 95% of all the PAHs present within an hour [107]. Bonten et al. [104] studied that soaking of the contaminated sludge in 4:1 (v/v) acetone–water mixture increased the degree of degradation from 9.5% to 20.4% as a result of dissolution of PAHs. Another chemical technique is the oxidation with ozone or hydrogen peroxide in combination with UV-radiation. Usual chemical methods involve a heavy input of chemicals and formation of harmful residues, whereas UV-ozone treatment produces no significant toxic products and it can destroy more than 90% of PAHs [108].

Introduction of a group of natural microbial strains or a genetically engineered variant to treat contaminated soil is termed 'bioaugmentation' and it can enhance the rate of degradation. Addition of biosurfactant-producing microbes can enhance the bioavailability of PAHs. Addition of biosurfactant-producing bacteria (i.e. *Pseudomonas aeruginosa*) and addition of light oils can increase the bioavailability of PAHs and metabolic potential of the bacterial community. Addition of oils includes the inorganic nutrients and bacterial strains capable of degrading PAHs co-metabolically (i.e. *Sphingomonas paucimobilis*) [109]. Surfactant compounds produced by *Pseudomonas aeruginosa* can increase the concentration of PAHs in the aqueous phase of the system. Increases in aqueous concentrations are generally in direct proportion to the amount of surfactant present. Inclusion of *Pseudomonas aeruginosa* surfactant in the land farm operation can increase the accessibility of PAHs to soil bacteria [110].

Addition of a carbon source as a nutrient in contaminated soil is known to enhance the rate of pollutant degradation by stimulating the growth of microorganisms responsible, termed as 'biostimulation'. It has been suggested that the addition of carbon in the form of pyruvate stimulates the microbial growth and enhances the rate of PAH degradation [111]. It did not show diauxic growth and accelerated the adaptation of *P. putida* G7 to naphthalene and enhanced the rate of in situ bioremediation. Mushroom compost and spent mushroom compost (SMC) are also applied in treating organopollutant contaminated sites [112,113]. Addition of SMC results in enhanced PAH-degrading efficiency (82%) as compared to the removal by sorption on immobilized SMC (46%). It is observed that the addition of SMC to the contaminated medium reduced the toxicity, added enzymes, microorganisms, and nutrients for the microorganisms involved in degradation of PAHs [114]. In a study by Guerin [115], polycyclic aromatic hydrocarbons contaminated soil from a creosoting plant was remediated using an ex situ land treatment process. The process involved soil mixing, aeration, and fertilizer addition. The indigenous PAH utilizing microorganisms were shown to increase during the treatment process, indicating that biostimulation was effective. The most extensive degradation was apparent with the 2- and 3-ring PAH, with decreases of 97% and 82%, respectively. The higher molecular weight 3- and 4-ring PAHs were degraded at slower rates, with reductions of 45% and 51%, respectively. Six-ring PAHs were degraded the least with average reductions of 35%.

Laboratory experiments have shown that the rate of biodegradation is more in liquid medium under constant steering than in soil/medium with soil added in it. The difference is due to the tendency of PAH particles to adsorb on soil particles and this renders reduced availability to microorganisms [21]. It has been observed that soils amended with municipal and petroleum sludge have higher rate of PAH degradation than the rate in soil alone. Since, the sludge is rich in number of microorganisms and level of different nutrients, they favour vigorous growth of microbes and enhanced biodegradation rates. The decrease in nitrate, sulfate, and phosphate content in the medium accounted for the consumption as nutrients during biodegradation.

Biodegradation of phenanthrene is also affected by the salinity of degradation medium. Bacteria isolated from mangrove sediments can degrade phenanthrene [23] and it was observed that bacterial growth is directly proportional to the phenanthrene concentration. But, the rate of degradation of PAH was low in the medium with high salinity and it was more in medium with less salinity. The inhibition due to salinity was more when bacteria were present individually and the effect was reduced when mixed culture of all the bacteria was used. It was also reported that the addition of glucose, as a carbon source, reduced the inhibition by salinity.

6. Inhibition and co-metabolism

Most of the studies have concentrated on specific biodegradation rates with single substrate system under aerobic conditions. Since PAHs are present in mixtures, the effect of substrate interaction in biodegradation is important in understanding the fate of PAHs. Some studies have presented the evidence that substrate interaction affect the biodegradation of PAHs by pure and mixed cultures. Sometimes, high molecular weight PAHs after low molecular weight PAHs have been utilized/degraded [109], while high concentration of naphthalene may have inhibited degradation of other PAHs due to toxicity [116]. Stringfellow and Aitken found competitive inhibition of phenanthrene degradation by naphthalene, methylnaphthalene, and fluorene in binary mixtures using two pure cultures [117]. They concluded that the occurrence of competitive inhibition observed with two different pseudomonas species might be common among PAH-degrading organisms. The presence of phenanthrene is reported to inhibit degradation of pyrene [118]. In studies with pure denitrifying isolates, the presence of naphthalene enhanced both phenanthrene and pyrene degradation, whereas phenanthrene apparently inhibited pyrene degradation, though the observations were not confirmed with metabolite analysis. Bacterial degradation of anthracene by *Rhodococcus* spp. and pyrene degradation by *M. flavescens* has also been reported to be inhibited by the presence of fluoranthene in the medium [22]. Sometimes specific strains of microorganisms may also cause inhibition. The effect of surfactant-like compounds, produced by the microorganisms, when growing on aromatic hydrocarbons, solubilizes the PAH and leads to an increase in concentration in the medium. It could also, at times, cause inhibition of the degradation process [119,120]. Some of the studies have reported no effect of substrate on degradation. Tsai et al. [74] reported that fluorene and phenanthrene can be degraded by sulfate-reducing bacteria without any inhibition. The degradation rates of fluorene and phenanthrene in the single compound systems were 0.136 and 0.09 d^{-1}, respectively. When both fluorene and phenanthrene were spiked into the system, the k_1 of fluorene and phenanthrene were reduced to 0.098 and 0.072 d^{-1}, respectively. The rate of fluorene and phenanthrene degradation was higher in the single compound system compared to the mixed one. The reason for the inhibition of microbial activity was attributed to high concentration of total PAHs. However, other studies have reported even the stimulation of degradation of PAHs when present in mixtures. The biodegradation of PAHs in varying mixture combinations by pure culture of *Pseudomonas putida* strain KBM-1 under aerobic conditions showed that the presence of naphthalene (2-ringed PAH) stimulated phenanthrene (3-ringed PAH) degradation 5-fold and pyrene (4-ringed PAH) degradation 2-fold. Findings which report co-metabolism have also been made. Yuan et al. [21] reported that the degradation efficiency of microorganisms is more vigorous when acenaphthene, fluorene, phenanthrene, anthracene, and pyrene are present simultaneously compared to the rate of degradation when the PAHs are present individually because the presence of all five compounds provides more carbon source, or cross acclimation may enhance the rate of biodegradation.

7. Kinetics

The properties of soil determine the activity of its microflora which is responsible for the degradation of polycyclic aromatic hydrocarbons (PAHs). Moreover, soil properties influence the strength of the interactions between the PAHs and individual soil components. The introduction of sewage sludge into the soil changes these properties which, in turn, changes environmental conditions [121]. The range and rate of changes in the content of individual PAHs determined on the basis of the half-lives exhibited that they depend on the properties of sewage sludge and sewage sludge dose. Apart from direct microbiological degradation, losses resulting from leaching and volatilization, part of PAHs can be converted to bound residues [122], and some part may have been sequestered into inaccessible microsites in the soil–sewage sludge matrix through aging [95]. Though a lot of work has been done on bioremediation of contaminated soils and the dissipation of PAHs from them, the study of kinetics involved during the process is still in infancy. Cutright [27] undertook a study to determine the specific degradation rates for the bioremediation of PAH-contaminated soils. The kinetics associated with the fungi *Cunninghamella echinulata* in conjunction with different nutrient supplements was investigated. It was observed that for a first order kinetics system, the rate of change in contaminant was proportional to its concentration in soil. The prediction of time for bioremediation of contaminated soil is dependent on the microorganisms, contaminant type and its concentration. Further, the development of more accurate kinetic model requires the monitoring of biomass, respiration studies, and study of interactions of different organisms between them. Though bioremediation has high rate of success, but the kinetics is still not fully understood and the kinetics become more complicated when fungi are used for bioremediation. As explained in earlier section, the different enzymes involved in fungal degradation have optimal activity at different temperatures and some of them are active even at very high or low temperatures. Thus, monitoring the kinetics for different fungal strains is difficult but majority of them have good degradation capacities in a mesophilic range. The degradation rate can be enhanced by pretreatment at a high temperature which results in volatilization and decrease in the soil–water partition coefficient, as a result which dissolution of contaminants increases enhancing the rate of degradation. As far as molecular weight of PAH is concerned, a limited number of bacteria have been identified that can grow in pure cultures on PAHs with five or more aromatic rings (high molecular weight (HMW) PAHs) because the high retention of these compounds by the solid soil phase results in very low mass-transfer rates of HMW-PAHs to the bacterial cells to match the basic metabolic requirements cells. Thus, the degradation rate of HMW-PAHs is slow [123]. An important factor for the PAH-degradation activity of bacteria is the pH of soil. The shift of the pH from 5.2 to 7.0 can significantly enhance the rate of PAH degradation by strain BA 2 [124]. Neutralization of soil is generally favorable for the degradation of mineral oil components by bacteria [125]. However, a pH of 5.2 should not lead to total inhibition of activity. Maximum PAH oxidation rates and optimum specific bacterial growth are obtained near pH 7.0 and 30 °C [126].

8. PAHs removal by composting, wetlands and phytoremediation

There are several microorganisms which can degrade a variety of contaminants and even the supplements. It has been

observed that the addition of straw, compost, manure, etc. helps to enhance degradation by improving soil texture, oxygen transfer, and providing energy to the microbial population. Wischmann and Steinhart [127] reported that in unamended soil, only aromatics with up to three fused benzene rings were degraded, whereas soil supplementation with compost helped to enhance elimination of all compounds monitored. Substantial residues after 15 weeks were only found for benzo(a)anthracene, chrysene, and benzo(a)pyrene, with 11%, 19%, and 54% respectively. Accumulation of ketonic and quinonic degradation products such as 9-fluorenone, anthracene-9,10-dione, 2-methylanthracene-9,10-dione, and benz(a)anthracene-7,12-dione was observed in unamended soil material. In mixtures with compost, short-term concentration maxima of such products correlated well with enhanced contaminant elimination. They concluded that supplementation of contaminated soils with compost materials can enhance biodegradation without long-term accumulation of extractable polar and more available intermediates. Wong et al. [128] studied the amendment of pig manure with soil spiked with PAHs. The increase in pig manure amendment can enhance the amount of soluble organic carbon, nitrogen and phosphorus which in turn increases the number of thermophilic, mesophilic and PAH-degrading bacteria in early stage of composting. Amendment of pig manure is beneficial to PAH removal during composting and removal efficiency increased up to 90%. Pig manure application at a dose of 25% is reported to have maximum removal of phenanthrene and anthracene. The 3-ring PAHs can be reduced during composting and maximum degradation takes place in first 3 weeks with a removal rate up to 95%, whereas 4-ring PAHs are degraded at a much slower rate with a reduction of 90% after 5 weeks of composting. The slow degradation rate might be due to high molecular weight and organic carbon partition coefficient [129]. Furthermore, significant degradation of pyrene occurs after most of the 3-ring PAHs have been removed [130].

Phytoremediation is defined as the use of green plants to remove pollutants from the environment to render them harmless. Plants can take the pollutants up and accumulate them in roots of foliage. It is an *in situ*, solar energy regulated technique, which minimises environmental disturbance and reduces costs [131]. Moreover, it particularly suits to the treatment of large areas of surface contamination, when other methods may not be as effective. Apart from it, large number of microorganisms is associated with rhizosphere which results in microbial degradation and co-metabolism [131]. Several species of grass such as *Agropyron smithii*, *Bouteloua gracilis*, *Cyanodon dactylon*, *Elymus Canadensis*, *Festuca arundinacea*, *Festuca rubra*, *Melilotus officinalis*, etc. are also known to degrade PAHs [132,133]. Results of an investigation indicated that grasses and legumes enhance the removal of PAHs from contaminated soils by. The plants included the legume alfalfa and three grasses: tall fescue, sudangrass, and switchgrass. Pyrene and anthracene were used as PAH contaminants. Planted soils had significantly lower concentrations of the PAHs than the unplanted soils, with 30–40% more degradation in the planted soils [134]. A mix of eight prairie grasses was studied in sandy loam soils to determine the degradation of four PAHs (benzo[*a*]pyrene, benzo[*a*]anthracene, dibenzo[*a,h*]anthracene, and chrysene) stimulated by plant growth. The grasses included big bluestem, little bluestem, Indiangrass, switchgrass, Canada wild-rye, side oats grama, blue grama, and western wheatgrass. PAH disappearance was consistently greater in planted units compared to unplanted controls, indicating that phytoremediation enhanced the removal of these compounds from contaminated soil. The biodegradation was greatest for benzo[*a*]anthracene followed by chrysene, benzo[*a*]pyrene, and finally dibenzo[*a,h*]anthracene. This ranking correlated with the water solubility of the PAH compounds; i.e., the more water-soluble the compound the greater its disappearance from the soil [135]. Soil planted with ryegrass was observed to lose a greater amount of a mixture of hydrocarbons than soil that was unplanted. The hydrocarbon mixture included *n*-alkanes (C10, C14–C18, C22, C24), as well as pristane, hexadecane, phenanthrene, anthracene, fluoranthene, and pyrene. After 22 weeks, the initial extractable hydrocarbon concentration of 4330 mg total hydrocarbon per kg soil decreased to less than 120 mg per kg soil (97% reduction) in planted soils, but to only 790 mg per kg soil (82% reduction) in unplanted soil. Larger microbial numbers and activity in the planted versus unplanted soil led the authors to conclude that plant roots enhanced biodegradation of the hydrocarbons by stimulating the soil microbes [136]. Another indirect role that plants play in the degradation of petroleum hydrocarbons is the release of enzymes from roots. These enzymes are capable of transforming organic contaminants by catalyzing chemical reactions in soil. Ref. [137] identified plant enzymes as the causative agents in the transformation of contaminants mixed with sediment and soil. The identified enzyme systems included dehalogenase, nitroreductase, peroxidase, and laccase. These findings suggest that plant enzymes may have significant spatial effects extending beyond the plant itself and temporal effects continuing after the plant has died [138]. The phytoremediation potential of two cold-hardy plants, Arctared red fescue and annual ryegrass, planted together in soil contaminated with either crude oil or diesel has also been examined [139]. Results indicated that contaminated soils planted with the two species had significantly lower concentrations of total petroleum hydrocarbon (TPH) compared to unplanted controls. The initial crude oil concentration for planted treatments and unplanted controls was approximately 6200 mg TPH per kg soil, while the initial diesel concentration was approximately 8350 mg TPH per kg. After 640 days, crude oil contaminated soil planted with both species had 1400 mg TPH per kg soil (77% reduction), while the unplanted control contained 2500 mg TPH per kg soil (60% reduction). Likewise, diesel-contaminated soil planted with both species had 700 mg TPH per kg soil (92% reduction) after 640 days compared to 2200 mg TPH per kg soil (74% reduction) for the unplanted control. Rasmussen and Olsen [140] studied the efficiency of orchard grass (*Dactylis glomerata*) towards PAH-removal. The study reported that a soil/sand mixture vegetated with orchard grass exhibited high treatment efficiency with an input from the microbial catabolic degradation by plant exudates.

Wetlands, too, have found an application in PAH removal from wastewater. Specific macrophytes, microflora and microfauna are the characteristic feature of wetlands. The intensive biological activities in such an ecosystem lead to a high rate of autotrophic and heterotrophic processes. Aquatic weeds *Typha* spp. and *Scirpus lacustris* have been used in horizontal–vertical macrophyte based wetlands to treat phenanthrene [141]. The removal of phenanthrene is found to be greater than 99.9%. During the degradation of phenanthrene, 1-hydroxy-2-napthoic acid (HNA) has been identified as an initial conversion product. HNA is a naphthalene derivative and originates from phenanthrene. The occurrence of HNA as an intermediate metabolite in phenanthrene degradation indicates the presence of bacterial microflora. Pilot-scale wetlands have been used for the treatment of PAH-contaminated water [142], particularly fluoranthene, and the possible role of fungi in these ecosystems has been investigated. Giraud et al. isolated 40 fungal species from a contaminated wetland and a control wetland [142]. They reported that fluoranthene was degraded efficiently by 33 species while only 2 species were able to remove anthracene over 70%. The most frequently isolated species were *Absidia cylindrospora*, *Mucor hiemalis*, *Aspergillus fumigatus*, *Cladosporium cladosporoides*, *Fusarium solani*, and *Trichoderma viride*. No PAHs were detected when analyzing the effluent water of the constructed wetland. An analysis of microbial population showed an increase in fungal population in the contaminated system when

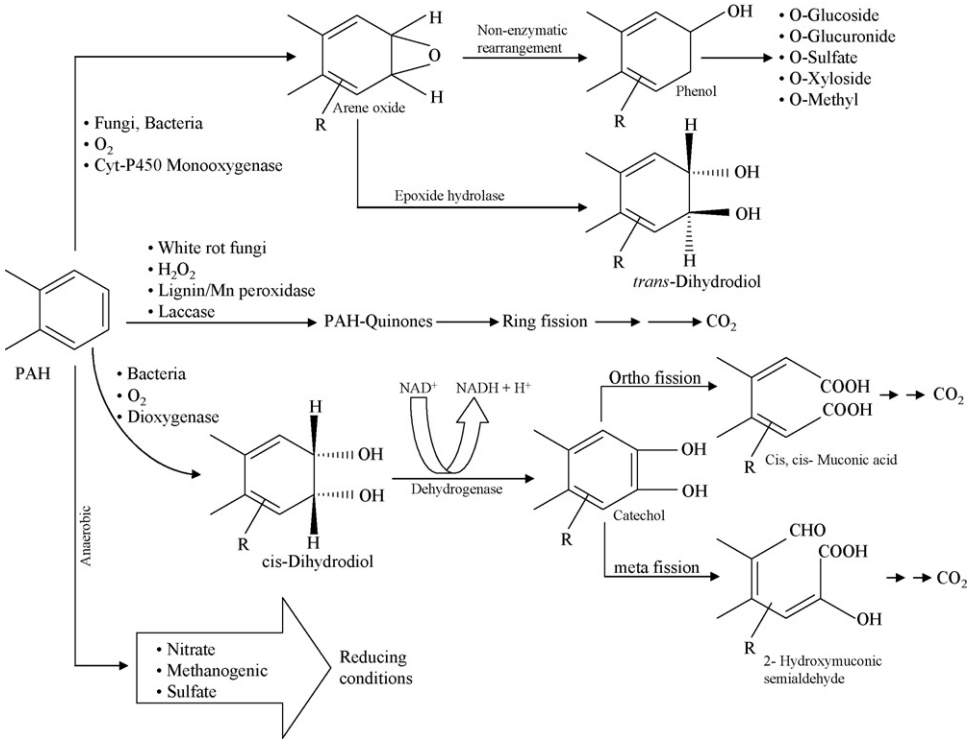

Fig. 5. Proposed pathway for microbial catabolism of polycyclic aromatic hydrocarbons [158].

compared to a control pilot wetland. Most of the fungi isolated from the wetland degraded PAH efficiently under lab conditions too. Some of the fungal species were reported to have inducible degradative capacities, since their ability to degrade the PAH increased with the increase in degree of contamination. The studies till date suggest that phytoremediation is an emergent and novel technique for contaminant removal and can be adopted in combination with microorganisms to maximize the rate of removal. Finally, phytoremediation may be applied with relative ease using existing agricultural practices at contaminated sites [143].

9. Microbial genetic adaptations

The biodegradation of PAH has been extensively studied and many microbial strains have been isolated for their ability to metabolise PAH with varying degradation rates. Pre-exposure of a microbial community to hydrocarbons, either from anthropogenic sources or from natural sources is important in determining the rate of PAH degradation. This phenomenon, which results in an increase in the hydrocarbon-oxidizing potential of the community on pre-exposure to high doses of contaminant, is known as adaptation. Several studies have reported adaptation and increase in degradation rate on PAH. Caparello and LaRock [144] studied mineralization of hexadecane and degradation of n-alkane mixtures by bacteria in several surface water and sand samples and concluded that areas with greater hydrocarbon burdens had higher hydrocarbon-oxidizing activity. Sayler et al. [145] showed that exposure of freshwater sediments to a synthetic oil accelerated the rate of polyaromatic hydrocarbon (PAH) mineralization. Induction and depression of enzymes, genetic changes, and selective enrichment have been defined as three mechanisms for adaptation of microbial communities to chemical contaminants [125]. The primary genetic mechanism for the adaptation of the microbial community is the amplification of genes which are involved in the metabolism of the chemical contaminant by selective enrichment and gene transfer [146]. Monitoring of adaptation to hydrocarbons has been made possible by the development of DNA probes specific for the genes encoding hydrocarbon-catabolic pathways. Colony hybridization technique has shown a correlation between the enhanced rates of PAH mineralization in oil-contaminated sediments and an increase in the number of colonies containing DNA sequences which hybridized to TOL (toluate oxidation) and NAH (naphthalene oxidation) plasmid probes [147]. The catabolic pathway in Pseudomonas putida G7 states that the first enzyme is a dioxygenase, which converts the aromatic hydrocarbon to the cis-dihydrodiol. The dioxygenases responsible for the aerobic oxidation of lower molecular weight aromatic hydrocarbons have many similarities, suggesting that they have a common evolutionary origin [148,149]. Genes encoding the dioxygenases of such compounds have been cloned and sequenced, such as those belonging to the TOL and NAH families [150,151]. On the other hand, there is little information about bacterial genes encoding proteins for the degradation of higher molecular weight PAH, including phenanthrene, anthracene, pyrene and fluoranthene. The highly homologous naphthalene degradation genes of the respective nah, *pah*, *ndo* and *dox* operons in some soil pseudomonads are also involved in transformation of phenanthrene and anthracene [151–153]. The cloned PAH catabolizing genes from non-pseudomonads is reported in *Comamonas testosteroni* strains that have the ability to degrade naphthalene, phenanthrene, and anthracene [154].

Polymerase Chain Reaction (PCR) and DNA hybridization have also proved useful in detection of polycyclic aromatic hydrocarbon degradation genes in different soil bacteria [155]. Different strains of Pseudomonas, Mycobacterium, Gordona, Sphingomonas, Rhodococcus and Xanthomonas which degrade polycyclic aromatic hydrocarbons (PAH) have been characterized for genes encoding degradation enzymes for PAH. Genomic DNA from these strains was hybridized with a fragment of *ndoB*, coding for the large iron sulfur protein of the naphthalene dioxygenase from *Pseudomonas putida*. A group of seven naphthalene-degrading Pseudomonas strains showed strong hybridization with the ndoB probe, and five

Gordona, Mycobacterium, Rhodococcus and Pseudomonas strains able to degrade higher molecular weight PAH showed weaker hybridization signals [155]. This suggests a molecular relationship between genes coding for PAH catabolism in various PAH-degrading bacterial taxa, which could be used to evaluate the PAH-degradation potential of mixed populations. The expansion of the DNA probe method to the detection of genes encoding the catabolism of other classes of hydrocarbons, detection of specific RNAs, and recombinant DNA technology can help develop bacterial and fungal strains with improved capability for hydrocarbon metabolism and suitability as seed organisms for the metabolism of PAHs.

10. Conclusion

Bioremediation is the tool to transform the compounds to less hazardous/non-hazardous forms with less input of chemicals, energy, and time. It is an approach to degrade/remove pollutants in an eco-friendly manner. PAH-contaminated sites can be remediated using the microorganisms—algae, bacteria, and fungi individually or in combination [158] (Fig. 5). The bioremediation of a pollutant and the rate at which it is achieved depends on the environmental conditions, number and type of the microorganisms, nature and chemical structure of the chemical compound being degraded. Thus, to devise a bioremediation system, several factors are responsible which need to be addressed and explored. There are a number of bacterial species isolated from different environments and capable of degrading PAHs. Acclimatization of these species can serve as a key for enhanced degradation. The induction of degradation capacity by exposing the microbes to higher levels of pollutants may, at times, result in genetic adaptability/changes responsible for higher rate of removal. The major group of fungi responsible for PAH-degradation is of white rot fungi. They have a battery of enzymes lignin peroxidase and manganese peroxidase which converts PAH to less harmful and simpler forms. Many algal species have got the property to biotransform the pollutants to less hazardous ones. Apart from it, some plants can also degrade the PAHs. A universal, consistent, and efficient system can be devised by simultaneous application of all the microorganisms to get synergistically enhanced rates. Pretreatment of the medium or addition of supplements should be promoted to increase the availability and to regulate the degradation kinetics. Monitoring and regulation of the environmental factors in specific areas can help the plants/microorganisms sustain even in adverse environments. An integrated approach of physical, chemical, and biological degradation should be adopted to treat/remediate the contaminated sites in an ecologically favorable process. The emission/disposal of PAH containing waste should be reduced/avoided and its reuse and recovery should be promoted. Control at the source can significantly reduce harmful levels in environment and the strategies involved in mitigation and remediation.

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