Research Journal of Environmental Toxicology

ISSN 1819-3420

Academic Journals Inc.

www.academicjournals.com

Research Journal of Environmental Toxicology 10 (1): 1-15, 2016
ISSN 1819-3420 / DOI: 10.3923/rjet.2016.1.15

Microbial Ecology of Hydrocarbon Degradation in the Soil: A Review

[1]Pooja Pandey, [1]Hardik Pathak and [2]Saurabh Dave
[1]Department of Biotechnology, JECRC University, Jaipur, Rajasthan, India
[2]Department of Chemistry, JECRC University, Jaipur, Rajasthan, India

Corresponding Author: Pooja Pandey, Department of Biotechnology, JECRC University, Jaipur, Rajasthan, India

ABSTRACT

Soil microbial assortment is affected by hydrocarbon perturbation, thus selective enrichment of hydrocarbon degraders occurs. Hydrocarbons interact with the terrestrial ecosystem and soil microorganisms shaping the fate of the contaminants relative to their chemical nature and microbial degradative capabilities, respectively. Microbial methods for monitoring bioremediation of hydrocarbons include chemical, biochemical and microbiological molecular indices that measure rates of microbial activities to show that in the end the target goal of pollutant reduction to a safe and acceptable level. While it is broadly accepted that bacteria and fungi are chief mediators in hydrocarbon degradation, bacteria have been revealed to be more versatile than fungi and therefore may play a greater role during biodegradation of hydrocarbons. Biodegradation via algae, yeast and protozoans are also found important in degrading wide range of these petroleum hydrocarbons. Microbial degradation by *Arthrobacter*, *Mycobacterium*, *Pseudomonas*, *Rhodococcus*, *Aspergillus, Penicillium*, *Chlorella*, *Cyanobacteria* and *Candida* can be considered as a key component in the cleanup strategy for hydrocarbon remediation. This brief review will inspect hydrocarbon degradation by microorganisms under different ecosystems.

Key words: Pollutants, bioremediation, hydrocarbons, microbial degradation

INTRODUCTION

Pollution of the biosphere has increased strongly since the commencement of the industrial revolution. Petroleum industry has played an imperative role in the world economy and society but it has also caused quite a lot of negative environmental impacts around the world. The modern petroleum industry had its beginning in Romania and oil was also recovered from a well, sunk in Pennsylvania by Colonel Drake in 1859 (Alloway and Ayres, 1993). Quantitatively, the organic pollutants are mainly made up of hydrocarbons in their various forms. In broad-spectrum, hydrocarbons are classified into aliphatic (mainly n-alkane), aromatics as well as monoaromatics such as BTEX (benzene, toluene, ethylbenzene and xylenes) and polycyclic aromatics (i.e. PAH i.e., polycyclic aromatic hydrocarbons) and asphaltics (Atlas, 1981). The toxicity of petroleum hydrocarbons depend on the solubility and the bioavailability of the hydrocarbons. In the past, it was implicit that the water soluble fractions of the aromatics and polyaromatics were the most harmful and consequently these compounds were the molecules for considering in toxicological studies. They are assumed to be mutagenic, teratogenic and carcinogenic (Keith and Telliard, 1979). More than ever the polyaromatic hydrocarbons with 4 or 5 rings are known carcinogens (Cerniglia, 1992). The non aromatic substances in the petroleum were not considered very harmful

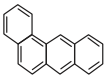
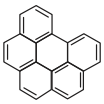
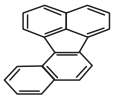
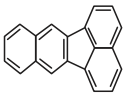
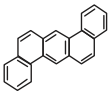
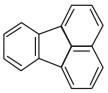
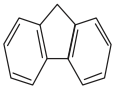
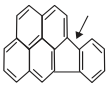
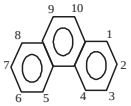
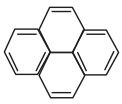
and alkanes and cycloalkanes are now also taken into account (Peterson, 1994). Hydrophobic hydrocarbons are toxic for microorganisms by accumulation in the membrane, which causes the loss of membrane integrity (Sikkema *et al*., 1995).

Biodegradation is an important process in petroleum toxicology because it changes both the nature and concentration of the chemical compounds. It is one of the forms of bioremediation to treat soils, water or sediments contaminated with PAHs. Microorganisms involved for biodegradation should be indigenous to the contaminated area or site (Das and Chandran, 2011). Diverse range of microorganisms has the ability to clean up the hydrocarbon contaminated sites (Atlas, 1978). Microbes convert the chemical compounds into energy, cell mass and biological waste products (Rahman *et al*., 2002). Hydrocarbons degrading microorganisms are extensively distributed in soil habitats. Many researchers are of the opinion that certain bacteria isolates are capable of degrading PAHs of particular note. *Escherichia coli*, *Alcaligenes* sp. and *Thiobacter subterraneus* were efficient isolates for degrading anthracene and phenanthrene. Some microorganisms mainly from the genera *Pseudomonas* and *Mycobacterium* have been found capable of transforming and degrading PAHs under aerobic conditions (Mrozik *et al*., 2003). It is also evident that anthracene could be completely mineralized by *Sphingomonas*, *Nocardia*, *Beijerinckia*, *Paracoccus* and *Rhodococcus* with dihydriol as the initial oxygenated intermediate (Teng *et al*., 2010). Evidence have been accumulating to propose that certain microorganisms namely; *Bacillus subtilis*, *Pseudomonas* a*eruginosa* and *Torulopsis bombicola* could generate bioremediation surfactants such as surfactin, rhamolipid and sophorolipid capable of improving bioremediation by solubilizing PAHs into the aqueous medium which enhance their bioavailability for degradation (Cottin and Merlin, 2007).

In the identical way, hydrocarbons degrading cyanobacteria, molds and yeasts have been reported to be wide spread in many habitats and also implicated in hydrocarbon degradation (Chaillan *et al*., 2004). The present review emphasizes an outline of the current knowledge of microbial PAH catabolism and the mechanism involved in PAHs degradation by different microorganisms.

Polycyclic aromatic hydrocarbons: Polyaromatic hydrocarbons (PAHs) are environmental pollutants in the soil, water and air. They and their derivatives are prevalent products of incomplete combustion of organic materials and from anthropogenic activities (Mrozik *et al*., 2003; Lundstedt, 2003). These compounds are a class of harmful organic chemicals consisting of two or more fused benzene rings in linear, angular and cluster arrangements (Juckpech *et al*., 2012). For example, naphthalene is the simplest with two rings and is the most soluble of the PAHs (Mrozik *et al*., 2003). On the other hand, benzo[a]pyrene, a typical high molecular weight PAHs with five rings, is one of the most recalcitrant and toxic PAHs (Li *et al*., 2010). The chemical properties of individual PAHs are reliant in part upon molecular size (that is their no. of aromatic rings) as well as their molecular topology (that is their pattern of aromatic linkage). An increase in size and angularity of PAH molecular commonly results in an allied increase in their hydrophobicity and electrochemical stability which contributes to its persistence (Loick *et al*., 2009). Polycyclic aromatic hydrocarbon are comparatively neutral to stable with moderately low solubility in water but are extremely lipophilic, where most of them have low vapour pressure. In fact, PAHs are known to exhibit essentially toxic effects and classified as priority pollutants by the US Environmental Protection Agency (USEPA). Due to diverse ranges of the number of carbon atoms, petroleum products have different physicochemical properties which make them differ in their behavior in the environment. Table 1 shows the 17 PAHs priority pollutants according to USEPA.

Table 1: Physical and chemical properties of PAHs arranged on the basis of their toxicities

PAHs	Chemical formula	Chemical structure	Molecular weight (g mol^{-1})	Melting point (°C)	Boiling point (°C)	Density (g cm^{-3})	Solubility in water
Acenaphthalene	$C_{12}H_{10}$		154.21	95	96.2	1.222	0.4 mg/100 mL
Acenaphthylene	$C_{12}H_8$		152.20	92-93	265-275	0.8987	Insoluble
Anthracene	$C_{14}H_{10}$		178.23	218	340	1.25	Insoluble
Benzo(a)anthracene	$C_{18}H_{12}$		228.2879	158	438	1.19	0.010 mg L^{-1}
Benzo(a)pyrene	$C_{20}H_{12}$		252.31	179	495	1.24	0.2-6.2 μg L^{-1}
Benzo(e)pyrene	$C_{20}H_{12}$		252.31	178-179	310-312	1.286	6.3×10^{-3} mg L^{-1}
Benzo(b)fluoranthene	$C_{20}H_{12}$		252.3093	168	-	1.286	0.0012 mg L^{-1}
Benzo(ghi)perylene	$C_{22}H_{12}$		276.3307	278	500	1.378	2.6×10^{-4} mg L^{-1}
Benzo(j)fluoranthene	$C_{20}H_{12}$		252.3093	165	-	1.286	6.76×10^{-3} mg L^{-1}
Benzo(k)fluoranthene	$C_{20}H_{12}$		252.31	217	-	1.286	-
Chrysene	$C_{18}H_{12}$		228.28	254	448	1.274	Insoluble
Dibenz(ah)anthracene	$C_{22}H_{14}$		278.3466	262	-	1.232	5×10^{-4} mg L^{-1}
Fluoranthene	$C_{16}H_{10}$		202.26	110.8	375	1.252	265 μg L^{-1}
Fluorene	$C_{13}H_{10}$		166.223	116-117	295	1.202	1.992 mg L^{-1}
Indeno (1,2,3-cd) pyrene	$C_{22}H_{12}$		276.3	163.6	530		0.062 mg L^{-1}
Phenanthrene	$C_{14}H_{10}$		178.23	101	332	1.18	1.6 mg L^{-1}
Pyrene	$C_{16}H_{10}$		202.25	145-148	404	1.271	0.135 mg L^{-1}

Microorganisms involved in petroleum hydrocarbon degradation

Bacterial degradation: Biodegradation of hydrocarbons can be accomplished by various microorganisms. Several bacteria are able to degrade PAHs as their sole carbon source. The common biochemical pathways for the bacterial degradation of PAHs such as naphthalene, phenanthrene, anthracene and acenaphthene have been well investigated. Biodegradation mechanisms require the presence of molecular oxygen to initiate the enzymatic attack of PAH rings. In the initial step, Dioxygenase (Aromatic) and Monooxygenase (Aliphatic) catalyzed oxidation reactions. Dioxygenase enzyme breaks the benzene ring and formed cis-dihydrodiols as early byproduct. It is a multi component enzyme system which involved many coenzyme and metal ions (as a co factor) (Peng *et al*., 2008). Table 2 summarizes the bacterial sp. involved in biodegradation.

Majority of hydrocarbons utilizing bacteria metabolize either aliphatic or aromatic hydrocarbons. These bacteria are able to adapt too many different hydrocarbons as their energy source. The most simplest and soluble PAH is Naphthalene and microorganisms which are able to utilize Naphthalene are relatively easy to isolate (Mrozik *et al*., 2003). Davies and Evans (1964) were investigated the biochemical sequence and enzymatic reactions leading to the degradation of naphthalene. General naphthalene-degrading bacteria include *Pseudomonas* sp., *Vibrio* sp., *Mycobacterium* sp., *Marinobacter* sp., *Sphingomonas* sp., *Rhodococcus* sp., *Micrococcus* sp (Pawar *et al*., 2013). These metabolize aromatic substrates by first oxygenating the aromatic ring to form a diol (two alcohol groups). This mechanism (Fig. 1) involved multicomponent enzyme system (naphthalene dioxygenase) which attacks on the aromatic ring to form cis-(1R, 2S)-dihydroxy-1,2-dihydronaphthalene (cis-naphthalene dihydrodiol) (Kiyohara *et al*., 1994). The cis-naphthalene dihydrodiol is subsequently dehydrogenated to 1,2-dihydroxynaphthalene by a cis-dihydrodiol dehydrogenase (Goyal and Zylstra, 1997). It ultimately, metabolized to salicylate via 2-hydroxy-2H-chromene-2-carboxylic acid, cis-o-hydroxybenzal pyruvate and 2-hydroxy-benzaldehyde (Kiyohara *et al*., 1994). Furthermore, 1,2-dihydroxynaphthalene is non enzymatically oxidized to 1,2-naphthaquinone (Auger *et al*., 1995). Salicylate is typically decarboxylated to catechol, which is further metabolized by ring fission in meta and ortho pathways. Fuenmayor *et al*. (1998), reported that salicylate is further converted to gentisate by salicylate-5-hydroxylase.

Recently, Amenu (2014), identified that Naphthalene degrader *Pseudomonas* sp. S3 and F3 at optimum pH and temperature 7 and 37ºC respectively. After 7 days of incubation biodegradation efficiency for F3 was 61.11% of naphthalene. Several studies have designated that genes which encode for naphthalene oxidation in *Pseudomonas* are found on plasmids (Mrozik *et al*., 2003). There are three identified plasmids that determine the degradation of naphthalene: NAH7, NPL1 and pND but the plasmid which has been considered most intensively is NAH7 in

Table 2: Bacterial sp. which is involved in Biodegradation (Bamforth and Singleton, 2005)

Compounds	Microorganisms
Alkanes	*Pseudomonas* sp., *Bacillus* sp., *Acinetobacter calcoaceticus* and *Micrococcus* sp., *Candida Antarctica*, *Nocardia erythroplis*, *Ochrobactrum* sp. and *Acinetobacter* sp., *Serratia marcescens*, *Candida tropicalis*, *Alcaligene sodorans*, *Arthrobacter* sp. and *Rhodococcus* sp.
Mono-aromatic hydrocarbons	*Brevibacillus* sp., *Pseudomonas* sp., *Bacillus* sp., *B. stereothermophilus* and *Vibrio* sp., *Corynebacterium* sp., *Ochrobactrum* sp. and *Achromobacter* sp.
Poly-aromatic hydrocarbons	*Alcaligenes odorans*, *Sphingomonas paucimobilis*, *Achromobacter* sp. and *Mycobacterium* sp., *Pseudomonas* sp., *Mycobacterium flavescens*, *Rhodococcus* sp., *Arthrobacter* sp. and *Bacillus* sp., *Burkholderia cepacia*, *Xanthomonas* sp. and *Alcaligenes*
Resins	*Pseudomonas* sp., Members of *Vibrionaceae, Enterobacteriaceea* and *Moraxella* sp.

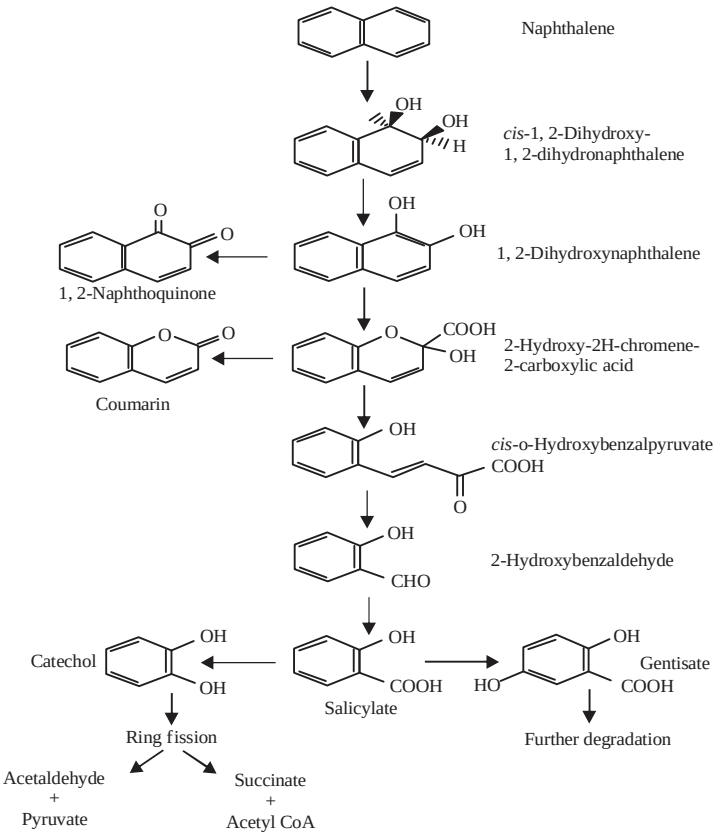


Fig. 1: Multicomponent enzyme system metabolizes naphthalene by *Pseudomonas* sp. (Kiyohara *et al*., 1994)

Pseudomonas putida (Malatova, 2005). Pawar *et al*. (2013), suggested that PAH-degradative gene in majority of PAH degrading bacteria were highly homologous to the naphthalene gene (nah gene) present in NAH7 plasmid of *Pseudomonas putida* strain G. *Pseudomonas* is well known degrader of three and four ring PAHs (Bamforth and Singleton, 2005). For example, phenanthrene was degraded by *Pseudomonas* sp. strain PP2 via a dioxygenase initiated and it converted into the naphthalene degradation pathway (Parales and Haddock, 2004). Phenanthrene has bay and K regions able to form an epoxide, which is suspected to be an ultimate carcinogen (Bamforth and Singleton, 2005). For above reason, it is used as a model substrate to study the catabolic pathway of bay and K-region containing carcinogenic such as benzo[a]pyrene, benzo[a]anthracene and chrysene (Bamforth and Singleton, 2005). In general, bacterial degradation of phenanthrene (Fig. 2) is initiated by 3,4-dioxygenation to yield cis-3,4-dihydroxy-3,4-dihydrophenanthrene, which undergoes enzymatic dehydrogenation to 3,4-dihydroxyphenanthrene (Seo *et al*., 2007). The cleaved product is metabolized into 1-hydroxy-2-naphthoic acid, 1,2-dihydroxynaphthalene and finally into salicylic acid (Mrozik *et al*., 2003). However, salicylic acid can also be converted into catechol (Samanta *et al.,* 1999). They investigate the degradation of phenanthrene by *Brevibacterium* sp. HL4 and *Pseudomonas* sp. DLC-P11and observed 1napthol intermediate during its degradation. *Brevibacterium* sp. HL4 degraded phenanthrene via 1-hydroxy-2-naphthoic acid, 1-naphthol and salicylic acid, whereas *Pseudomonas* sp. DLC-P11 degraded phenanthrene via the formation of

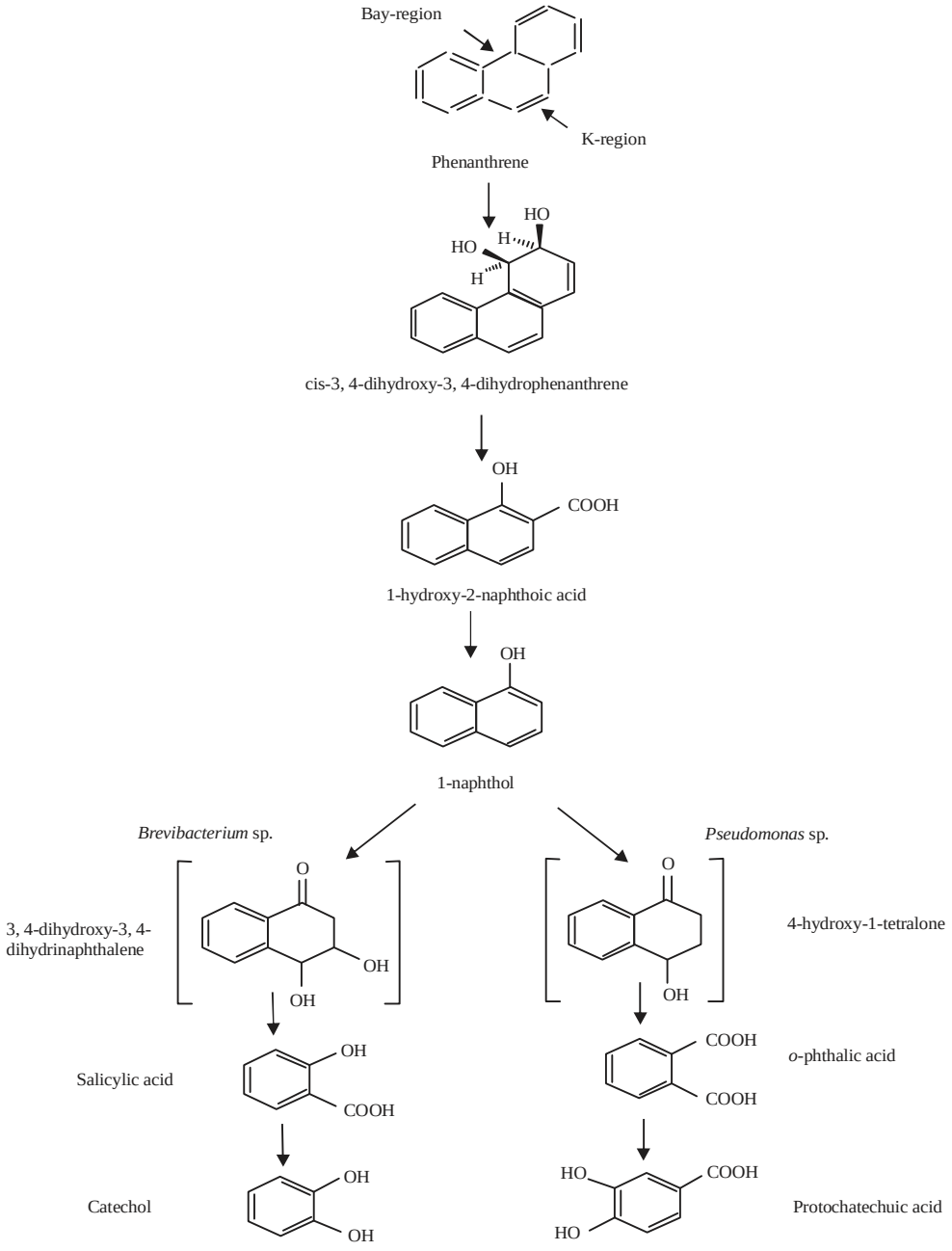


Fig. 2: Degradation of phenanthrene in catechol and protochatechic acid by *Brevibacterium* sp. and *Pseudomonas* sp. (Mrozik *et al*., 2003)

1-hydroxy-2-naphthoic acid, 1-naphthol and o-phthalic acid (Samanta *et al*., 1999). Phenanthrene degradation was also observed by Deveryshetty and Phale (2009), they found phenanthrene degradation by *Pseudomonas* sp. strain PPD via the 'Phthalic acid' route. The key enzyme of mechanism is 1-hydroxy-2-naphthoic acid dioxygenase (HNDO). These results suggest that 1-HNDO of *Pseudomonas* sp. strain PPD has an extradiol-type ring-cleaving dioxygenase system.

Apart from *Pseudomonas* strains, various microorganisms *Mycobacterium*, *Rhodococcus* and *Nocardia* also metabolizes several PAHs. *Mycobacterium* involved both monooxygenation and

dioxygenation with the formation of both cis and trans-1,2-dihydrodiols in the ratio of 25:1 (Mrozik *et al*., 2003). The reaction is catalyzed by cytochrome P450 monooxygenase which forms naphthalene 1,2-oxide. Afterwards it converted to the trans-diol by an epoxide hydrolase enzyme (Mrozik *et al*., 2003). *Mycobacterium* sp. also involved in phenanthrene degradation at different sites of the molecule, apparently via both dioxygenase and monooxygenase attack on the aromatic nucleus. The resulting anthracene cis-1,2-dihydrodiol is dehydrogenated to 1,2-dihydroxyanthracene (Moody *et al*., 2001). According to Heitkamp *et al*. (1988), Majority of *Mycobacterium* sp. are also known for the degradation of pyrene and benzo(a)pyrene. *Mycobacterium* sp. also mineralized pyrene which involves ring oxidation and ring fission. Formation of cis-4,5-pyrenedihydrodiol and trans-4,5-pyrenedihydrodiol and pyrenol are the product of ring oxidation and 4-hydroxyperinaphthenone, 4-phenontheroic acid, phthalic acid and cinnamic acid are product of ring fission. Presence of 4 cis- and trans -4,5-dihydrodiols suggest multiple pathways for the initial oxidative attack of pyrene resulted in the formation of mentioned diols at 4,5 positions (k region).

Vila *et al*. (2001) reported that *Mycobacterium* sp. strain AP1 is capable to form a novel metabolite known 6,6-dihydroxy-2-2-biphenyl dicarboxylic acid, which demonstrates a new metabolic pathway and involves the cleavage of both central rings of pyrene. The schematic pathway proposed for the degradation of pyrene by *Mycobacterium* sp. strain AP1 is shown in Fig. 3.

Fungal degradation: Several fungi have an ability to degrade persistent pollutants (Haritash and Kaushik, 2009). Spellman (2008), reported that it (like bacteria) can metabolize dissolved organic matter as they are chief organisms responsible for the decomposition of carbon in the biosphere. Similarly, Matavulj and Molitoris (2009) concluded that fungi are equipped with extracellular multi enzyme complexes, which involves breakdown of natural polymeric compounds by means of their hyphal systems. Hyphal system is able to colonize and penetrate substrates rapidly and to transport and redistribute nutrients within their mycelium.

Fungal degradation of PAHs, can be carried out by two groups of fungi, non ligninolytic and ligninolytic fungi (Bamforth and Singleton, 2005). *Chrysosporium pannorum*, *Cunninghamella elegans* and *Aspergillus niger* are non-ligninolytic fungi which involved cytochrome P450 monooxygenase enzyme-mediated oxidative pathway for PAH degradation (Sutherland *et al*., 1995). *Pleurotus ostreatus* and *Antrodia vaillantii* are White rot fungi which produces ligninolytic enzymes and involved in oxidation of lignin present in wood and other organic matter (Bamforth and Singleton, 2005). Lignolytic enzymes system consist of Lignin Peroxidases (LP), Manganese dependent peroxidases (MnP) and laccases (Haritash and Kaushik, 2009).

Hadibarata *et al*. (2013) investigated degradation of naphthalene by a white rot fungus *Pleurotus eryngii*. It cleaved C1 and C4 position of naphthalene to give 1,4-Naphthaquinone by dioxygenation mechanism. 1,4-Naphthaquinone convert into benzoic acid and finally converted into Catechol by the combination of decarboxylation and hydroxylation process.

Degradation of phenanthrene involves cytochrome P450 mediated oxidation and later it will mediated by lignin peroxidases enzymes (Bezalel *et al.,* 1997) (Fig. 4).

Leitao (2009) reported metabolism of pyrene by *Penicillium janthinellum* SFU 403, a strain isolated from petroleum-contaminated soils. The first step of degradation involves formation of monophenols, diphenols, dihydrodiols and quinones. It degrade Pyrene (Fig. 5) via hydroxylation to 1-pyrenol hydroxylate to form 1-pyrenol, followed by 1,6 and 1,8-pyrenequinones (Wang and Zhao, 2007).

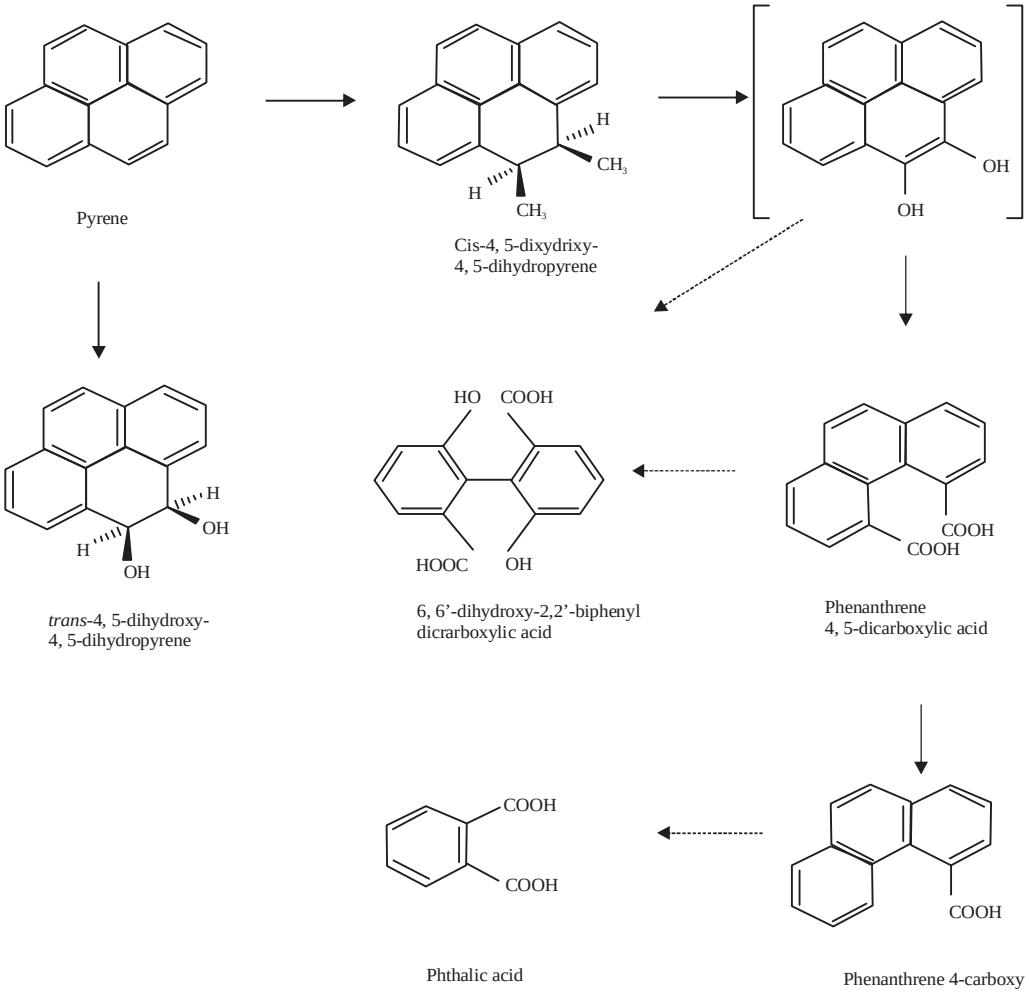


Fig. 3: Schematic pathway proposed for the degradation of pyrene in phthalic acid and phenanthrene 4-carboxy by *Mycobacterium* sp. AP1 (Mrozik *et al*., 2003)

Clemente *et al*. (2001) investigated degradation of PAH by thirteen deuteromycete ligninolytic fungal strains and identified the degree of degradation depends on activity 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 found by the strain 710. Ali *et al*. (2012) identified *Aspergillus terreus* as superior for ligninolytic enzyme production. For maximum production of lignin peroxidase and manganese peroxidases optimum temperatures are 33.6 and 33.1°C and pH are 4.1 and 5.8, respectively. Using optimum condition it was able to degrade 98.5% of naphthalene and 91% of anthracene in soil models.

Algal degradation: Several research has confirmed the involvement of fresh algae (e.g., *Chlorella vulgaris*, *Scenedesmus platydiscus*, *S. quadricauda* and *S. capricornutum*) in degradation of PAHs (Wang and Zhao, 2007). Prokaryotic and eukaryotic photoautotrophic marine algae (i.e.,

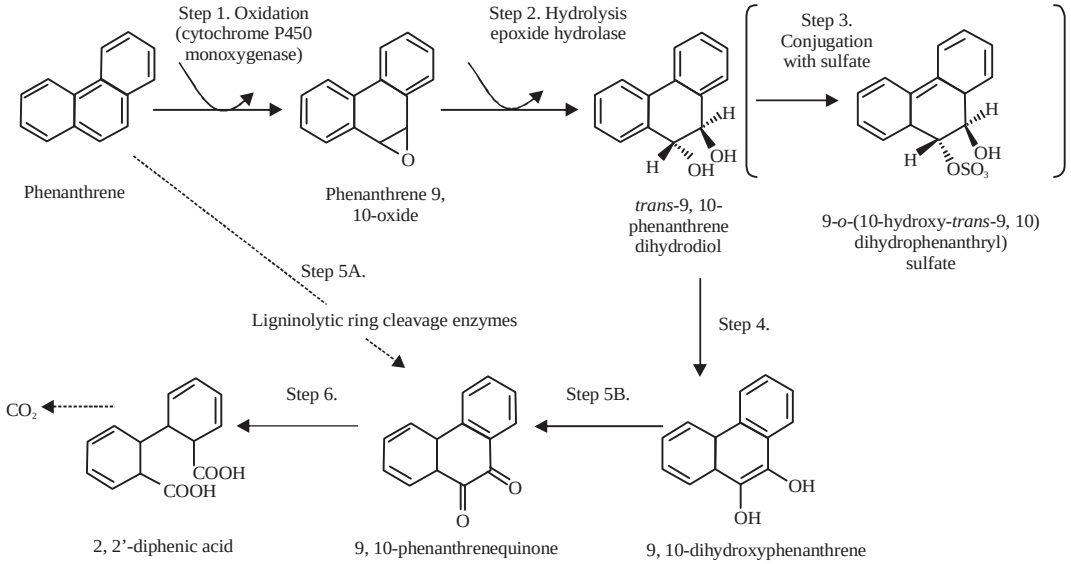


Fig. 4: Proposed pathway for the degradation of phenanthrene into 2,2'-diphenic acid by the ligninolytic fungus *Pleurotus ostreatus* (Bezalel *et al*., 1997)

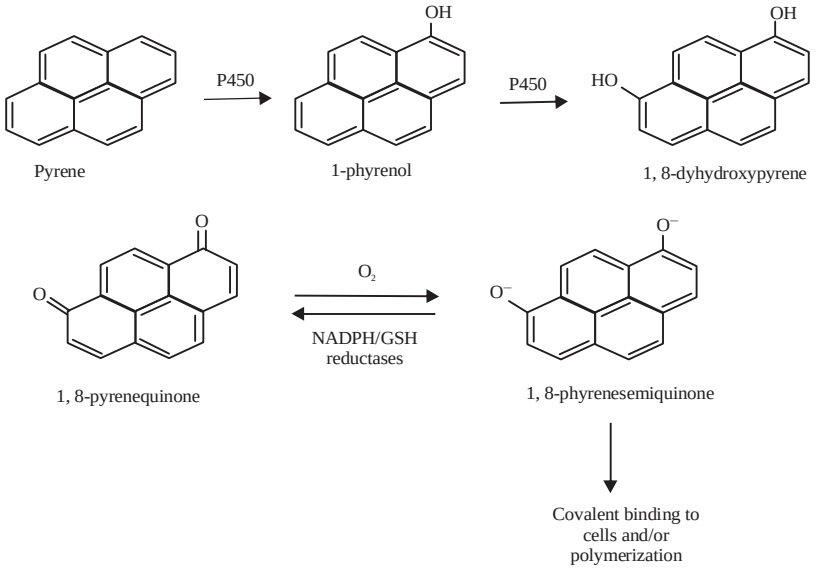


Fig. 5: Metabolism of Pyrene into 1,8-pyrenequinone by *P. janthinellum* SFU403 (Leitao, 2009; Launen *et al*., 1999)

Cyanobacteria, Green algae and Diatoms) are well known to metabolize naphthalene by a series of metabolites (Haritash and Kaushik, 2009). Cerniglia *et al*. (1980), investigated the role of Cyanobacteria (blue-green algae) in naphthalene degradation. It produces four major metabolites, 1-naphthol, 4-hydroxy-4 tetralone, cis naphthalene dihydrodiol and trans-naphthalene dihydrodiol at concentrations which were non toxic (Fig. 6).

The potential of algal-bacterial microcosms of *Pseudomonas migulae* and *Sphingomonas yanoikuyae* were studied for phenanthrene degradation (Haritash and Kaushik, 2009).

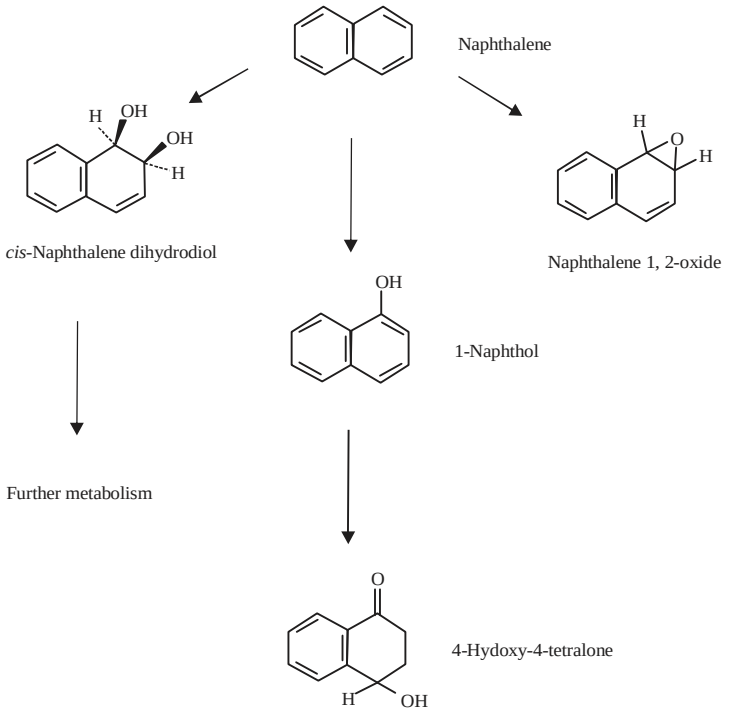


Fig. 6: Metabolism of naphthalene into 4-Hydroxy-4-tetralone by the *Cyanobacterium oscillatoria* sp., strain JCM (Cerniglia *et al*., 1980)

Ueno *et al*. (2008) studied the degradation of fluoranthene, pyrene and a mixture of fluoranthene and pyrene by *Chlorella vulgaris*, *Scenedesmus platydiscus*, *Scenedesmus quadricauda* and *Selenastrum capricornutum*. The PAHs removal in 7 days of treatment was 78 and 48%, respectively by *S. capricornutum* and *C. vulgaris*.

Yeast degradation: Several yeasts may utilize aromatic compounds as growth substrates but more significant is their ability to convert aromatic substances cometabolically. Some species such as the soil yeast *Trichosporon cutaneum* possess specific energy dependent uptake systems for aromatic substrates (e.g., for phenol) (Mortberg and Neujahr, 1985). According to Miranda *et al*. (2007), Yeasts are also able to utilize aliphatic hydrocarbons occurring in crude oil and petroleum products and typical representatives of alkane-utilizing yeasts include *Candida lipolytica*, *C. tropicalis*, *Rhodoturularubra aurantiaca* and *Aureobasidion* (*Trichosporon*) *pullulans*. *Rhodotorula aurantiaca* and *C. ernobii* were found able to degrade diesel oil. Leelaruji *et al*. (2013) reported *Aureobasidium pollulans* and var. *melanogenum* are lipolytic yeast have subsequent ability to degrade naphthalene (24.4%), anthracene (37.3%), pyrene (27.3%) and benzo(a)pyrene (45.95%) via laccase production. Hesham *et al*. (2006) identified yeast strain AEH capable of degrading naphthalene (5.36 mg L^{-1}), phenanthrene (5.04 mg L^{-1}) and chrysene (1.54 mg L^{-1}) within 2, 10, 10 days, respectively. In combinations, yeast strain AEH degrades, naphthalene and phenanthrene (3.79 and, 4.20 mg L^{-1} within 10 days, respectively) and chrysene and benzo(a)pyrene (3.37 and 1.91 mg L^{-1} within 10 days, respectively). In a binary system, all of the other 3 PAHs could be utilized as the carbon source for the cometabolic degradation of benzo(a)pyrene with naphthalene as the best one.

Yeasts are also known for playing a significant role in the removal of toxic heavy metals via biosorbtion method. Wang and Chen (2006) demonstrated that yeasts are capable of accumulating heavy metals such as Cu(II), Ni(II), Co(II), Cd(II) and Mg(II) and are superior metal accumulators compared to certain bacteria. *Pichia anomala* is able to remove Cr(VI) (Bahafid *et al*., 2011) and the biosorption of Cr(VI) is occurs by live and dead cells of three yeasts species: *Cyberlindnera fabianii*, *Wickerhamomyces anomalus* and *C. tropicalis* (Bahafid *et al.,* 2013). Several yeast strains *S. cerevisiae*, *P. guilliermondii*, *Rhodotorula pilimanae*, *Yarrowiali polytica* and *Hansenula polymorpha* have been reported to reduce Cr(VI) to Cr(III) (Ksheminska *et al*., 2006). In addition, the tolerance of *P. guilliermondii* to chromate was found to depend on its capacity for extracellular reduction of Cr(VI) and Cr(III) chelation (Ksheminska *et al.,* 2008). Most studies, have reported the efficiency of immobilized cells of yeasts in metals removal, one example is *Schizosaccharomyces pombe* for copper removal (Subhashini *et al*., 2011).

Protozoa degradation: Protozoa are not a good biodegrader as fungi, bacteria and algae. However, their population has been shown to significantly reduce the number of bacteria available for hydrocarbon removal. It means their presence in a biodegradation system may not always be beneficial (Stapleton and Singh, 2002). Overall, due to limited evidence it does not play an ecologically significant role in the degradation of hydrocarbons in the environment as algae and fungi (Rogerson and Berger, 1981).

The protozoa are the main grazer on the degrading bacteria for organic contaminants, so the interaction between protozoa and degrading bacteria will affect the result of bacteria degradation directly. Mattison *et al*. (2005) constructed a model for the food chain in order to study the influence of grazing bacteria of protozoa flagellate *Heteromita globosa* on the biodegradation of benzene and methylbenzene.

Chen *et al*. (2007) reported protozoa infusorians can accelerate the biodegradation of heterogenous substances in the environment such as PAH. For example, the degradation rate of naphthalene can be improved 4 times than before. There are several possible hypotheses about the mechanism of protozoa accelerating biodegradation of organic contaminants, which mainly include the following six parts: (1) The nutrient mineralization which improves the turnover of nutrients, (2) Bacteria activation which controls the quantity grazes the aged cells or excretes active substance, (3) Selective grazing which reduces the competition to the resource and space and thus is good for the growth of degrading bacteria, (4) Physical disturbance which can increase oxygen content and the surface of degraded matters, (5) Direct degradation which can excrete special enzymes participating in the degradation and (6) Co-metabolism which offers energy and carbon resource for the bacteria during the degradation (Chen *et al*., 2007).

CONCLUSION

The emerging science and technology of bioremediation offers an alternative method to detoxify contaminants. There are various ranges of microorganisms involved in the process of bioremediation. Apart of bacteria, white rot fungi also play a significant role in biodegradation of PAHs. They have a key enzymes lignin peroxides and manganese peroxidase which can convert these PAHs into less harmful substances. Several algae have also capability to degrade wide range of PAHs. Although, protozoa not directly involved in the process of biodegradation but it can influence the rate of biodegradation. The present review is an attempt to explore an answer or advancement and biological questions part event to bioremediation to petroleum hydrocarbons.

ACKNOWLEDGMENT

I wish to express my sincere gratitude to Department of Biotechnology of JECRC University for giving me an opportunity for doing my PhD.

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