MICROBIAL METABOLISM OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) : A Review

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ABSTRACT:Polycyclic aromatic hydrocarbons (PAHs) are the class of hydrocarbons containing two or more fused aromatic hydrocarbons. They can persist in the environment due to their low water solubility. Polycyclic aromatic hydrocarbons are relatively abundant in the environment and toxic to mammals and aquatic organisms as they can be carcinogenic or mutagenic. PAHs have accumulated in the environment mainly as a result of anthropogenic activities such as the combustion of fossil fuels. Interest has surrounded the occurrence and distribution of PAHs due to their potentially harmful effects to human health. Some microorganisms were found to be capable of transforming and degrading PAHs and these abilities may be useful in removing them from the environment. This paper discusses the main micro-organisms involved in these transformations, the major aerobic and anaerobic breakdown pathways and the factors affecting microbial metabolism of polycyclic aromatic hydrocarbons.

KEYWORDS: aerobic, anaerobic, aromatic, bacterial, fungal, degradation, Polycyclic Aromatic Hydrocarbons (PAH), , metabolism, micro-organisms,.

1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a class of organic compounds that consist of two or more fused benzene rings and/or pentacyclic molecules that are arranged in various structural configurations. They are highly recalcitrant molecules that can persist in the environment due to their hydrophobicity and low water solubility [1]. Some representative PAHs are shown in Fig 1.

PAHs are ubiquitous in the natural environment, and originate from two main sources: these are natural (biogenic and geochemical) and anthropogenic [2].

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It is the latter source of PAHs that is the major cause of environmental pollution and hence the focus of many bioremediation programmes.

PAHs naturally occur in fossil fuels such as coal and petroleum, but are also formed during the incomplete combustion of organic materials such as coal, diesel, wood and vegetation [3],[4].

This results in airborne PAH contamination, which is the main route for PAH transport over long distances [5].

Point sources of PAHs can originate from petroleum and diesel spills and from industrial processes such as coal liquefaction and gasification during coke production [6]. For example, creosotes and coal tar, which are by-products of coking, contain significant quantities of PAHs (eg creosote contain up to 85% PAHs). More minor sources of PAHs include tobacco smoke and burnt food.

Natural processes can also provide a source of PAHs, such as volcanic

eruptions and forest fires. In addition, PAHs can have a geochemical origin as they are formed during pyrolysis, which involves the exposure of sediments to high temperatures during sediment diagenesis [7].

PAHs are widely distributed in soils and sediments, groundwater and the atmosphere. They have been detected in marine sediments and intertidal sediments, gas works site soils, sewage sludge contaminated soils, aquifers and groundwater and in atmospheric deposits such as vehicle exhaust fumes [4].

Point sources of PAH contamination are the most significant environmental concern. Though the areas contaminated are relatively small in size, the contaminant concentration at these sites is often high and associated with co-contaminants such as benzene, toluene, ethylene and xylene (BTEX) compounds, heavy metals and aliphatic hydrocarbons, which can hinder remediation efforts. Soils can be contaminated with between 1 μg kg−1 and 300 g kg−1 PAHs [8] depending on the source of contamination (eg old coal gasification sites have the higher levels stated). Atmospheric levels of PAHs resulting from the incomplete combustion of materials such as coal and wood have been found to be between 60 μgm^{-3} and $3mgm^{-3}$ air.

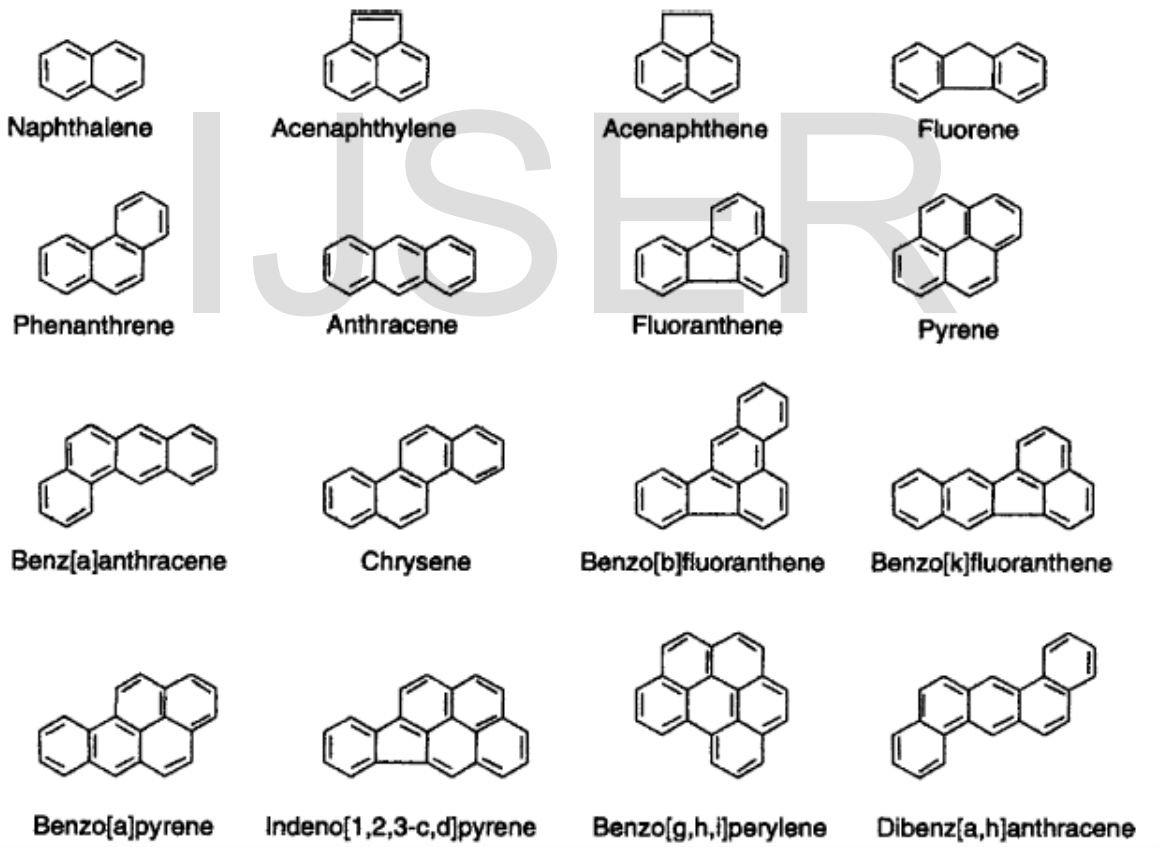

Fig. 1: chemical structures of the 16 polycyclic aromatic hydrocarbons[9].

2.CHEMICAL AND PHYSICAL PROPERTIES OF PAHS

Polycyclic aromatic hydrocarbons (PAHs) are a group of compounds containing carbon and hydrogen, composed of two or more fused aromatic rings in linear, angular, and cluster arrangements. Many PAHs contain a "bay-region" and a "K-region". The bay- and K-region epoxides, which can be formed metabolically, are highly reactive both chemically and

biologically. Phenanthrene is the simplest aromatic hydrocarbon which contains these regions. The bay-region of phenanthrene is a sterically hindered area between carbon atoms 4 and 5 and the K-region is the 9, 10 double bond, which is the most oleinic aromatic double bond with high electron density (Fig. 1). According to the Schmidt-Pullman electronic theory, K-region epoxides should be more carcinogenic than the parent hydrocarbon.

Low-molecular weight (LMW) PAHs (two or three rings) are relatively volatile, soluble and more degradable than are the higher molecular weight compounds. Highmolecular weight (HMW) PAHs (four or more rings) sorb strongly to soils and sediments and are more resistant to microbial degradation. Because of solid state, high molecular weight and hydrophobicity, expressed as its log P value between 3 and 5, PAHs are very toxic to whole cells [10]. Some properties of selected PAHs are presented in Table 1.

Table 1 : Chemical and physical properties of some selected PAHs

Compound	No. C atoms	Molecular weight	Melting point (°C)	Boiling point (°C)	Solubility in water (mg/l)
Naphthalene	10	128.2	80.2	218.0	30.6
Acenaphthalene	12	154.2	96.0	278.0	3.9
Phenanthrene	14	178.2	100.0	339.0	1.2
Anthracene	14	178.2	217.0	340.0	0.7
Pyrene	16	202.26	150.4	393.0	0.145
Fluoranthene	16	202.26	108.8	383.0	0.262
Chrysene	18	228.29	253.8	431.0	0.003

3. PERSISTENCE OF PAHs IN THE ENVIRONMENT

The persistence of PAHs in the environment is dependent on a variety of factors, such as the chemical structure of the PAH, the concentration and dispersion of the PAH and the bioavailability of the contaminant. In addition, environmental factors such as soil type and structure, pH and temperature and the presence of adequate levels of oxygen, nutrients and water for the activity of the pollutant-degrading microbial community will control the time that PAHs persist in the environment.

In general, the higher the molecular weight of the PAH molecule, the higher the hydrophobicity and toxicity, and the longer the environmental persistence of the molecule.1 In addition the 'age' of the contaminant in the soil/sediment matrix plays a significant role in the biodegradability of PAHs in soil [11].

A study using phenanthrene as a model PAH showed that phenanthrene mineralisation and therefore biodegradability was significantly reduced with time of ageing. The association of PAHs with co-pollutants such as hydrocarbons and heavy metals is another factor that can prolong their residence time in the environment.

Aliphatic hydrocarbons and BTEX compounds are readily biodegradable by

the *in situ* microbial community relative to the more complex chemical structures of the PAHs. This results in the depletion of available oxygen in the surrounding environment and the onset of anaerobicity. Though recent work has shown that there is a real potential for the biodegradation of PAHs in the absence of molecular oxygen, details regarding the efficiency and scale of PAH degradation in anaerobic environments is still limited, with rates of anaerobic organic matter oxidation up to an order of magnitude less than those under aerobic conditions [12]. In addition, it is possible that the presence of heavy metals in soil could inhibit microbial growth and hence limit the metabolism of contaminants under anaerobic conditions.

4. Toxicity of PAHs

It has long been known that PAHs can have serious deleterious effects to human health, with the physician John Hill first recognising the link between the use of snuff and nasal cancer in 1761. Following
The relative toxicity of PAHs can be measured using LD50 values (the lethal dose in 50% of cases). These are expressed as milligrams of toxic material per kilogram of the subject's body weight that will cause death in 50% of cases. It is important to specify the route by which the toxic material was administered to the test animal (such as oral or intraperitoneal), and the animal upon which the toxic material was tested (ie rat, mouse).
PAHs are also suspected carcinogens but are not thought to be genotoxic unless they are 'activated' by mammalian enzymes to reactive epoxides and quinones. This occurs via a cytochrome P450 monooxygenase enzyme-mediated reaction that oxidises the aromatic ring to form epoxide and diol–epoxide reactive intermediates. Harvey, 1996 reported that these intermediates may undergo one of at least four different mechanisms of oxidation and/or hydrolysis before the intermediates combine with and/or attack DNA to form covalent adducts with DNA. this discovery, research into the toxic effects that PAHs have upon mammalian health has continued, with many PAHs displaying acute carcinogenic, mutagenic and teratogenic properties. Benzo[*a*]pyrene is recognised as a priority pollutant by the US Environmental Protection Agency as this compound is known to be one of the most potently carcinogenic of all known PAHs [13]. When ingested, PAHs are rapidly absorbed into the gastrointestinal tract due to their high lipid solubility.A major route of PAH uptake is via dermal absorption as highlighted by a study of 12 coke-oven workers [14]. An estimated 75% of the total absorbed amount of PAHs (specifically pyrene) entered the body through the skin, highlighting this as a major exposure route of PAHs. The rapid absorption of PAHs by humans results in a high potential for biomagnification in the food chain. In general, the greater the number of benzene rings, the greater the toxicity of the PAH [1].
DNA adducts can lead to mutations of the DNA, resulting in tumours.

5. MICROBIAL METABOLISM OF PAHS

There are three fundamentally different mechanisms in the aerobic metabolism of PAHs by microorganisms (Fig 2) and specific details of bacterial and fungal (ligninolytic and non-ligninolytic) PAH metabolism are discussed below. The basis of these mechanisms is the oxidation of the aromatic ring, followed by the systematic breakdown of the compound to PAH metabolites and/or carbon dioxide.
Anaerobic metabolism of PAHs is thought to occur via the hydrogenation of the aromatic ring.
PAH-degrading microorganisms are ubiquitously distributed in the natural environment, such as in soils (bacteria and non-ligninolytic fungi) and woody materials (ligninolytic fungi). Many PAH contaminated soils and sediments host active populations of PAH-degrading bacteria. For example, phenanthrene-degrading bacteria were isolated from

PAH-contaminated mangrove sediments in Hong Kong [15]. These isolates were able to degrade phenanthrene under a range of salinities both in pure and mixed cultures. Anaerobic environments, eg municipal sewage sludges [16] and marine sediments,[17] can also host a diverse array of PAH-degrading bacteria.
Unlike non-ligninolytic fungi, the ligninolytic fungi, such as *Phanerochaete chrysosporium*, are commonly associated with woody materials and are not commonly found in soils. However, these fungi can be enriched in a soil by the addition of straw, wood chips and other ligninrich substrates. A thorough listing of microorganisms capable of PAH degradation is provided by [18].

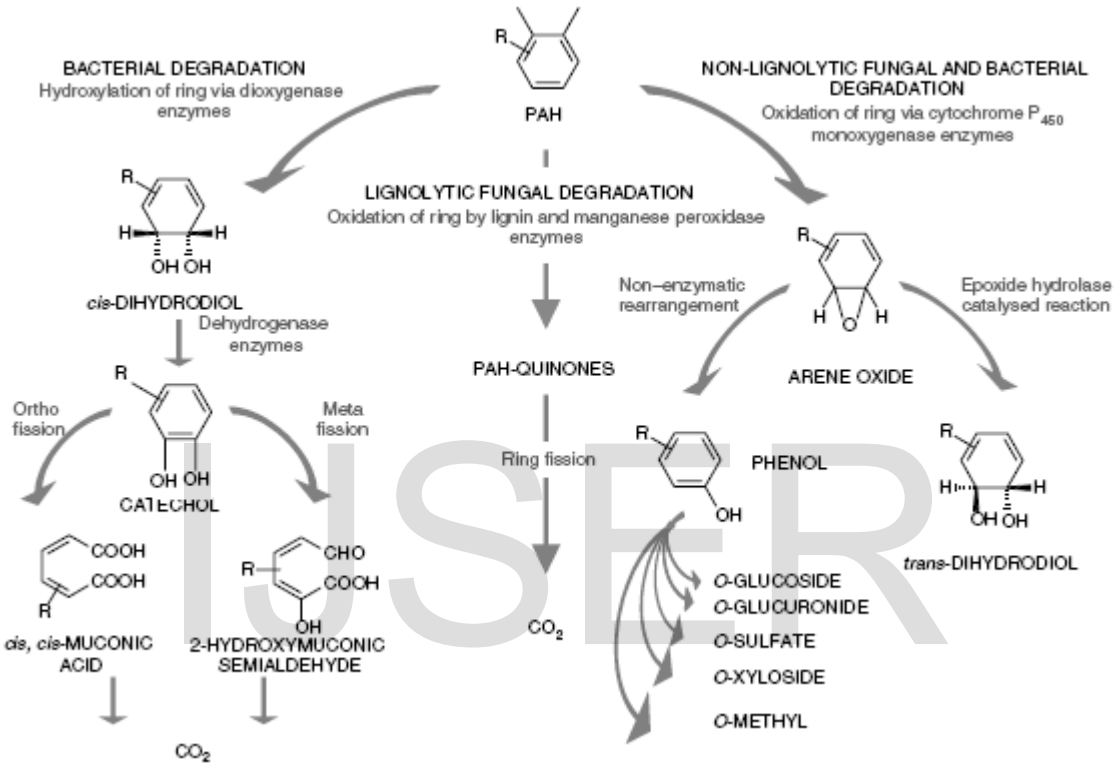

Fig 2: the three main pathways for poly aromatic hydrocarbon degradation by fungi and bacteria [9].

5.1 Bacterial metabolism of PAHs

The principal mechanism for the aerobic bacterial metabolism of PAHs is the initial oxidation of the benzene ring by the action of dioxygenase enzymes to form *cis*-dihydrodiols. These dihydrodiols are dehydrogenated to form dihydroxylated intermediates, which can then be further metabolised via catechols to carbon dioxide and water. The metabolic pathways and enzymatic reactions involved in the microbial degradation of naphthalene have been studied in detail with an example pathway of naphthalene transformation given in Fig 3.
There is a large diversity of bacteria that are able to oxidise naphthalene using dioxygenase enzymes, including organisms from the genus *Pseudomonas* and *Rhodococcus* .
A few bacteria are also capable of oxidising PAHs by the action of the cytochrome P-450 monoxygenase enzyme to form *trans*-dihydrodiols such as *Mycobacterium* sp [19]. [20] reported the bioremediation of PAH-contaminated oil-based drill-cuttings using spent white-rot fungi, (*Pleurotus ostreatus)* under laboratory conditions. [12] reported the ability of marine methanotrophs in degrading PAHs via the action of the methane monoxygenase gene. It is thought however that these are minor mechanisms

compared with the activity of the dioxygenase enzymes.

The toxicity of naphthalene metabolites generated during bacterial degradation has been little studied. The metabolites of naphthalene, such as naphthalene dihydrodiols, have a higher water solubility than naphthalene and are therefore potentially more bioavailable, and could pose a greater toxicity than the These metabolites were considerably more toxic than the naphthalene precursor. In comparison to the naphthalene metabolites produced by humans and some fungi, naphthalene intermediates generated during the cytochrome P_{450}-mediated oxidation by *Mycobacterium* sp generate *trans*-dihydrodiols that could reasonably be expected to show minimal toxicity to humans.

5.2 Fungal metabolism of PAHs

There are two main types of fungal metabolism of PAHs; these are mediated by the non-ligninolytic and ligninolytic fungi (also known as the white-rot fungi). The majority of fungi are non-ligninolytic, as they do not grow on wood, and therefore have no need for the lignin peroxidase enzymes that are produced by the ligninolytic fungi. However, many ligninolytic fungi such as *Phanerochaete chrysosporium* and *Pleurotus ostreatus* can produce both non-ligninolytic and ligninolytic type enzymes, but it is unclear to what degree each enzyme contributes to the breakdown of the PAH molecule.

5.2.1 Non-ligninolytic fungi

The first step in the metabolism of PAHs by non ligninolytic fungi is to oxidise the aromatic ring in a cytochrome P450 monoxygenase enzyme catalysed reaction to produce an arene oxide.16 This route is similar to the mammalian metabolism of PAHs. In comparison to the oxidation of the aromatic ring by dioxygenase enzymes to form *cis*dihydrodiols, the monoxygenase enzyme incorporates only one oxygen atom onto the ring to form an arene oxide. This is subsequently hydrated via an epoxide-hydrolase catalysed reaction to form a *trans*dihydrodiol [22].

In addition, phenol derivatives may be produced from arene oxides by the non-enzymatic rearrangement of the compound, which can act as substrates for subsequent sulfation or methylation, or conjugation with glucose, xylose, or glucuronic acid. Although most non-ligninolytic fungi are not capable of the complete mineralisation of PAHs, these PAH-conjugates are generally less toxic and more soluble than their respective parent compounds. For example,[23] and [1] demonstrated this with the degradation of fluoranthene by the non-ligninolytic fungal species *Cunninghamella elegans.* The metabolites 3-fluoranthene-*β*-glucopyranoside, 3-(8-hydroxy-fluoranthene)-*β*-glucopyranoside, fluoranthene *trans*-2,3-dihydrodiol and 8-hydroxy-fluoranthene- *trans*-2,3-dihydrodiol showed no mutagenic effects to a rat liver homogenate fraction, and 9-hydroxy-fluoranthene-*trans*-2,3-dihydrodiol was considerably less toxic than fluoranthene.

Chrysosporium pannorum, cunninghamella elegans and *Aspergillus niger* are examples of non-ligninolytic fungi that use a P_{450} monoxygenase enzyme-mediated oxidative pathway for PAH degradation. An example pathway of the cytochrome P_{450}-mediated oxidation of phenanthrene is detailed in steps 1 to 4 of Fig 4. The latter steps (5 and 6) are thought to be mediated by lignin peroxidase enzymes [24].

5.2.2 Ligninolytic fungi

White-rot fungi are a group of fungi that produce ligninolytic enzymes involved in the oxidation of lignin present in wood and other organic matter. There are two types of ligninolytic enzymes; these are peroxidases and laccases[25]. These enzymes are secreted extracellularly, and oxidise organic matter via a non-specific radical based reaction [26]. There are two main types of peroxidase enzyme

This generates a selection of PAH-quinones and acids rather than dihydrodiols (see steps 5 and 6, Fig 4). There is significant interest surrounding the use of ligninolytic fungi to degrade PAHs, as they have low substrate specificity and are therefore able to degrade even the most recalcitrant of compounds. Also, the enzymes involved are extracellular, and are theoretically able to diffuse into the soil/sediment matrix and potentially oxidise PAHs with low bioavailability. Degradation studies of the ligninolytic fungi have shown that PAHs may be degraded by a combination of ligninolytic enzymes, cytochrome P450 monooxygenases, and epoxide hydrolases that can result in the complete mineralisation of the compound. Degradation studies of high molecular weight PAHs such as pyrene and benzo[*a*]pyrene by ligninolytic fungi (ie *Phanerochaete chrysosporium* and *Pleurotus ostreatus*) have suggested that a combination of ligninolytic and non-ligninolytic enzymes may be the key to the complete mineralisation of these recalcitrant compounds [24].

Substantial research has focused upon the potential of this group of fungi to remediate PAH-contaminated materials. Bioremediation trials that used ligninolytic fungi to remediate PAH-contaminated soils and sediments have shown mixed results. [27] used four white-rot fungi species to degrade a coal-tar-contaminated soil. Although the soil in this study was supplemented with straw (as a substrate for the fungi), the indigenous soil depending on their reducing substrate type, lignin peroxidase (LP) and manganese peroxidase (MnP), both of which are capable of oxidising PAHs [25]. Laccases, which are phenol oxidase enzymes, are also capable of oxidising PAHs.

Under ligninolytic conditions, white-rot fungi can oxidise PAHs by generating free radicals (i.e hydroxyl free radicals) by the donation of one electron,16 which oxidises the PAH ring. microorganisms were more successful in PAH degradation than the introduced fungal species. In another study by [28] that monitored the potential of white-rot and brown-rot fungi to degrade a PAH-contaminated soil, *Pleurotus ostreatus* and *Antrodia vaillantii* were used to inoculate an artificially-contaminated soil to degrade a range of PAHs. The *P ostreatus* fungal inoculum significantly increased the degradation of PAHs relative to the unamended soils, but resulted in the accumulation of potentially toxic PAH metabolites. As this white-rot fungus also inhibited the *in situ* microbial populations within the soil, this may have prevented the complete mineralisation of the PAHs, resulting in the accumulation of PAH metabolites. They suggested that a fungal–bacterial consortium would be beneficial to the decontamination of this soil. As the brown-rot fungus *A vaillantii* showed equal if not better PAH degradation than *P ostreatus*, and did not generate dead-end PAH metabolites, it was suggested that this fungus could be exploited as a valuable inoculum in bioremediation trials. In order to increase the efficiency of white-rot fungi in the remediation of PAH-contaminated soil, [29] designed a two-stage pilot scale reactor that initially extracted PAHs from a contaminated soil and subsequently treated the extracted PAHs in a fungal bioreactor. This fungal bioreactor utilised *P chrysosporium*, and was successful in degrading high molecular weight PAHs such as benzo[*a*]pyrene.

[30] also reported the ability of *P. Chrysosporium* to degrade PAH-contaminted soils. They reported a biodegradation efficiency of up to 100% for phenanthrene and less than 100% for other three and four-ringed polyaromatic hydrocarbons.

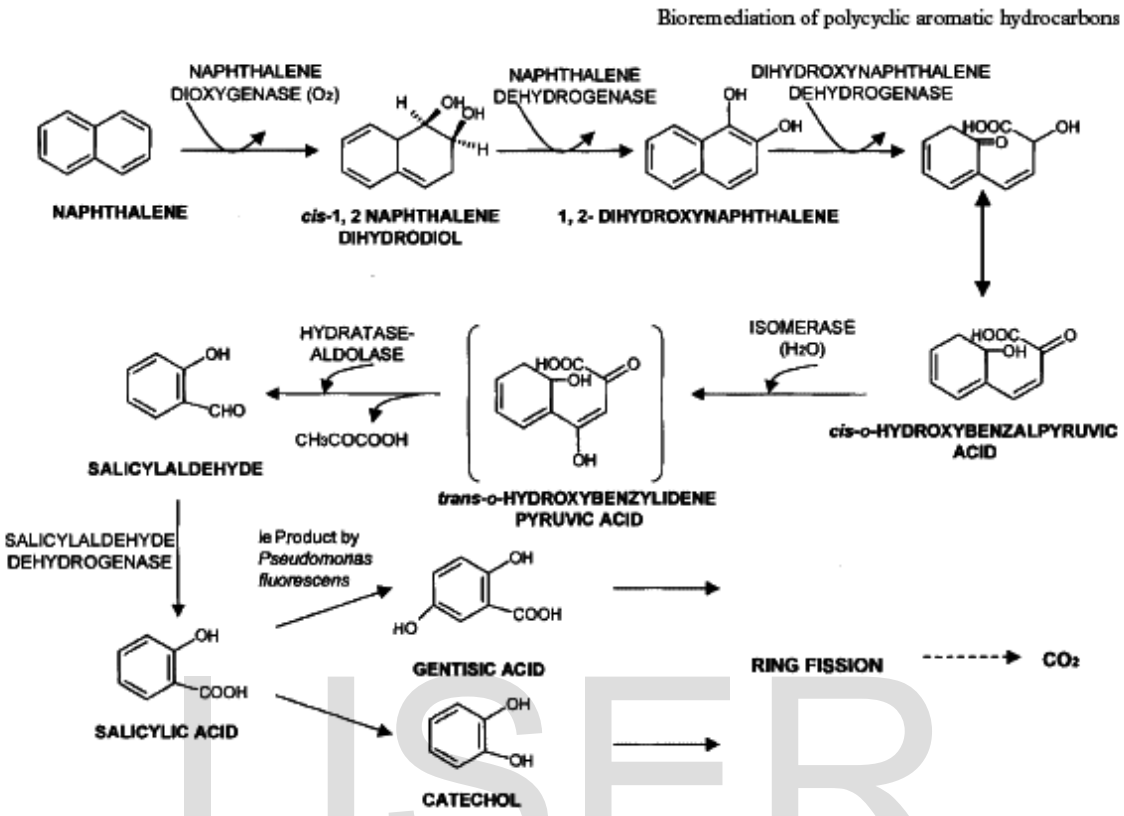

Fig 3: the main pathways in the aerobic degradation of naphthalene by bacteria [9].

6. Anaerobic metabolism of PAHs

PAHs are a common contaminant of anaerobic environments such as aquifers and marine sediments. Even aerobic environments such as contaminated soils, sediments and groundwater can develop anaerobic zones. This is due to the organic contaminant stimulating the *in situ* microbial community, resulting in the depletion of molecular oxygen during aerobic respiration. This oxygen is not replenished at the same rate as its depletion, which results in the formation of anaerobic zones proximal to the contaminant source [31].

It was not until recently that the potential of microorganisms to degrade PAHs in the absence of molecular oxygen has been recognised. Previous studies have tended to focus upon the thermodynamically more favourable aerobic processes of bioremediation of recalcitrant organic compounds such as PAHs, whereby molecular oxygen is incorporated into the aromatic ring prior to the dehydrogenation and subsequent PAH ring cleavage.

In the absence of molecular oxygen, alternative electron acceptors such as nitrate, ferrous iron and sulphate are necessary to oxidise these aromatic compounds, with recent research clearly demonstrating that PAH degradation will occur under both denitrifying [32] and sulfate-reducing anaerobic conditions [33]. The mechanisms of anaerobic PAH degradation are still tentative, though [33] [34] have proposed a mechanism for the anaerobic degradation of naphthalenes.

The first step is the carboxylation of the aromatic ring to 2-naphthoic acid, which may activate the aromatic ring prior to hydrolysis. Stepwise reduction of 2-naphthoic acid via a series of hydrogenation reactions results in decaclin-2-carboxylic acid which is subsequently converted to decahydro-2-

naphthoic acid. There may be other mechanisms for anaerobic naphthalene degradation, however these have not yet been elucidated. For example, it is proposed that the initial step in anaerobic naphthalene degradation under sulfate-reducing conditions occurs via a hydroxylation reaction to form a naphthol intermediate.

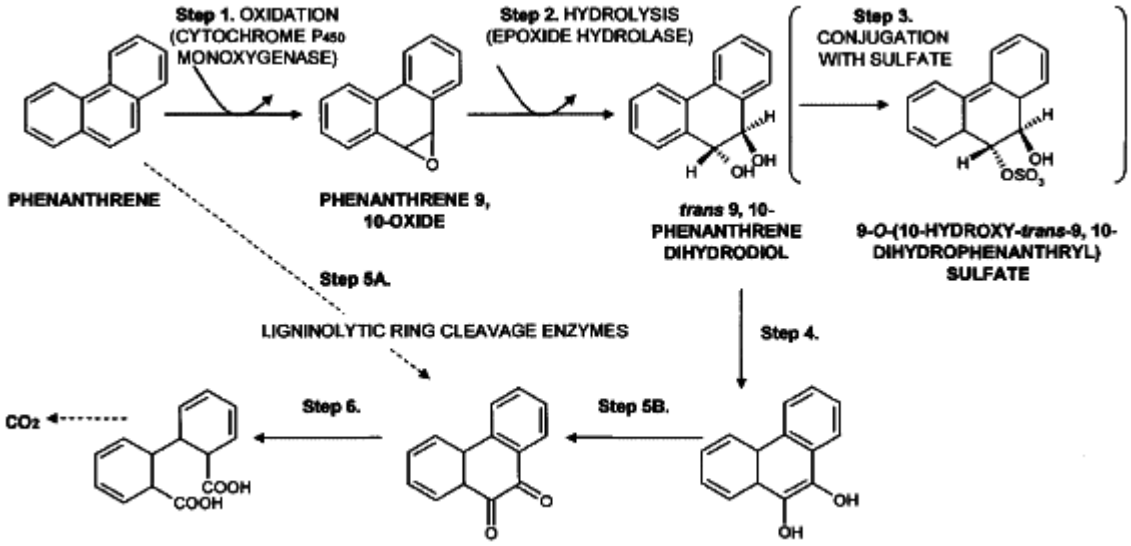

Fig 4: proposed pathway for the degradation of phenanthrene by the ligninolytic fungus *Pleurotus ostreatus* [24].

Conclusion

The fate of polycyclic aromatic hydrocarbons in nature is of great environmental concern due to their toxic, mutagenic and carcinogenic properties. Thus there is interest in understanding the physicochemical processes and microbial degradation reactions that affect the mobility and fate of these compounds in the environment. The pathways of microbial metabolism of PAHs have been established and it is known that many environments contain microbes capable of reducing PAH concentrations. The biodegradation of PAHs can then be considered on one hand to be part of the normal carbon cycle, and on the other hand as the removal of man-made pollutants from the environment.

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