Biodegradation of Polycyclic Aromatic Hydrocarbons (PAHs) by fungal enzymes: A review.

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are a large group of chemicals. Their sources can be either natural or anthropogenic. They represent an important concern due to their widespread distribution in the environment, their resistance to biodegradation, their potential to bioaccumulate and their harmful effects. In fact, natural resources polluted with PAHs usually lead to mutagenic and carcinogenic impacts in fresh-water, marine-water and terrestrial species. Several pilot treatments have been implemented to prevent further economic consequences and deterioration of soil and water quality. As a promising option, fungal enzymes are regarded as a powerful choice for potential degradation of PAHs. Their rate of degradation depends on many factors, such as environmental conditions, fungal strain, nature of the fungal enzyme and nature and chemical structure of the PAH among others. *Phanerochaete chrysosporium*, *Pleurotus ostreatus* and *Bjerkandera adusta* are most commonly used for the degradation of such compounds due to their production of ligninolytic enzymes as lignin peroxidase, manganese peroxidase and laccase. The rate of biodegradation depends on many culture conditions, such as temperature, oxygen, accessibility of nutrients and agitated or shallow culture. Moreover, the addition of biosurfactants can strongly modify the enzyme activity. The removal of PAHs is dependent on the ionization potential. The study of the kinetics is not completely comprehended, and it becomes more challenging when fungi

are applied for bioremediation. Degradation studies in soil are much more complicated than liquid cultures because of the heterogeneity of soil, thus, many factors should be considered when studying soil bioremediation, such as desorption and bioavailability of PAHs. Different degradation pathways can be suggested. The peroxidases are heme-containing enzymes having common catalytic cycles. One molecule of hydrogen peroxide oxidizes the resting enzyme withdrawing two electrons. Subsequently, the peroxidase is reduced back in two steps of one electron oxidation. Laccases are copper-containing oxidases. They reduce molecular oxygen to water and oxidize phenolic compounds.

Keywords: Polycyclic aromatic hydrocarbons (PAHs), Biodegradation, Fungi, Enzymes

Abbreviations

P: *Phanerochaete*

C: *Coriolus*

T: *Trametes*

P: *Pleurotus*

I: *Irpex*

LiP: Lignin peroxidase

MnP: Mn-peroxidase

VP: Versatile peroxidase

LAC: Laccase

ABTS: 2,2-azinobis(3-ethylbenzthiazoline-6-sulfonic acid)

VA: 3,4-dimethoxybenzyl alcohol, veratryl alcohol

PAHs: Polycyclic aromatic hydrocarbons

ANT: Anthracene

PHE: Phenanthrene

FLU: Fluorene

PYR: Pyrene

FLA: Fluoranthene

CHR: Chrysene

B[a]P: Benzo[a]pyrene

B[a]A: Benzo[a]anthracene

IP: Ionization potential

WRF: White-rot fungi

1 Introduction

Polycyclic aromatic hydrocarbons (PAHs), or polyarenes, are a large group of chemicals with two or more fused aromatic rings in linear, angular or clustered arrangements. PAHs with less than six aromatic rings are often denominated as small PAHs and those containing more than six aromatic rings are often called large PAHs (Haritash and Kaushik, 2009). They occur as colorless, white/pale yellow solids with low solubility in water, high melting and boiling points and lower vapour pressure as seen in Table 1. With an increase in molecular weight, their solubility in water decreases; melting and boiling point increases and vapour pressure decreases (Patnaik, 2007).

The widespread occurrence of PAHs is due to their generation from the incomplete combustion or pyrolysis of numerous organic materials, such as coal, oil, petroleum gas, and wood. PAHs exhibit the most structural variety in nature compared to any other class of non-halogenated molecules in the eco- and biosphere. Moreover, with continued oil production and transport, the quantities of these hydrocarbons in water and sediment will keep increasing (Arun et al., 2008). Fate of PAHs in the environment includes volatilization, photo-oxidation, chemical oxidation, adsorption on soil particles and leaching (Haritash and Kaushik, 2009). They are difficult to degrade in natural matrices and their persistence increases with their molecular weight. Therefore, these compounds represent an important concern due to their widespread presence in the environment, their resistance towards biodegradation, their potential to bio-accumulate and their mutagenic and carcinogenic effects that occurs by breathing air containing PAHs in the workplace, or by coming in contact with air, water, or soil near hazardous waste sites, or by drinking contaminated water or milk etc. (Lei et al., 2007; Albanese et al., 2014; Wang et al., 2014a; Zhao et al., 2014).

Potential treatments have been implemented to prevent further economic consequences and deterioration of soil and water quality. Among such treatments, bioremediation initiatives promise to deliver long lasting and low cost solutions for PAHs degradation. Biodegradation of hydrocarbons was carried out either by bacteria (Cybulski et al., 2003; Arulazhagan and Vasudevan, 2011; Mao et al., 2012; Hamamura et al., 2013; Sun et al., 2014; Cébron et al., 2015; Darmawan et al., 2015; Ferreira et al., 2015; Okai et al., 2015; Singh et al., 2015), fungi (K. G. Wunch et al., 1999; Li et al., 2005; Chan et al., 2006; Elisabet Aranda, 2009; Hadibarata et al., 2009; Hadibarata and Kristanti, 2014; Bonugli-Santos et al., 2015; Cébron et al., 2015; Jové et al., 2015; Marco-Urrea et al., 2015; Mineki et al., 2015; Simister et al.,

2015; Young et al., 2015) or algae (Chan et al., 2006; Diaz et al., 2014; Luo et al., 2014). As a result of such a large experience, the fungi emerge as a powerful choice for degradation of polyaromatic hydrocarbons. They have advantages over bacteria due to their capability to grow on a large spectrum of substrates and at the same time, they produce extracellular hydrolytic enzymes, which can penetrate the polluted soil and remove the hydrocarbons (Balaji and Ebenezer, 2008; Messias et al., 2009; Venkatesagowda et al., 2012).

The rate of bioremediation of a pollutant depends on the environmental conditions, type of microorganism, as well as the nature and chemical structure of the compound to be removed. Therefore, to develop a bioremediation process, a number of factors are to be taken into account. The level and rate of biodegradation of PAHs by fungal enzymes relies upon growth factors, such as, oxygen, accessibility of nutrients, and enzyme optimum conditions like pH, temperature, chemical structure of the compound, cellular transport properties, and chemical partitioning in growth medium (Singh and Ward, 2004).

Enzymatic biodegradation of polycyclic aromatic hydrocarbons by fungal strains have not been significantly reviewed, to the best of our knowledge. The main objective of the present review is to understand the enzymatic biodegradation of PAHs using fungal strains. In this sense, rate and pathways of biodegradation of PAHs are strongly related to the environmental conditions for the enzymatic activity and also for the fungal growth., It is also dependent on the system where the degradation takes place either ex-situ or in-situ, and on the nature and chemical structure of the pollutant. Also, the mechanisms of enzymes degrading PAHs should be highlighted. Therefore, to build a complete scenario of enzymatic fungal bioremediation many factors need to be considered at the time.

2 Fungal enzymes

Davis et al., (1993) demonstrated that all fungi have innate efficiency to degrade PAHs. Ligninolytic fungi have been extensively studied for the past few years (Haritash and Kaushik, 2009) because they produce extracellular enzymes with extremely reduced substrate specificity. This evolved due to the irregular structure of lignin but resulted in the ability to also degrade and mineralize various organopollutants (Hatakka, 1994; Vyas et al., 1994a; Hammel, 1995). Latest research showed that extracellular peroxidases of these fungi are responsible for the initial oxidation of PAHs (Acevedo et al., 2011; Betts, 2012; Li et al., 2014; Zhang et al., 2015). Fungal lignin peroxidases oxidize a number of PAHs directly, while fungal manganese peroxidases co-oxidize them indirectly through enzyme-mediated

lignin peroxidation. (Vyas et al., 1994a) have tested several white rot fungi and suggested that all of them oxidize anthracene to anthraquinone. The ligninolytic system contains three principal enzyme groups, i.e. lignin peroxidase (LiP), Mn-dependent peroxidase (MnP), phenol oxidase (laccase, tyrosinase), and H_2O_2 producing enzymes (Novotný et al., 2004a). Ligninolysis is oxidative, it is induced by high oxygen ranges in the culture medium, and is part of the organism secondary metabolism; it is expressed under nutrient limiting conditions, generally nitrogen (Haemmerli et al., 1986; Hammel et al., 1986; Sanglard et al., 1986; Novotný et al., 2004a) and their physiology has been broadly studied. Thus, there is an elaborated comprehension of the ligninolytic mechanisms of basidiomycetes (Hatakka, 1994; Thurston, 1994). Novotný et al., (2004b) studied the degradation amounts and enzymatic activities of MnP, LiP and laccase in different species of ligninolytic fungi cultivated in liquid medium and soil and their impact on some xenobiotics including PAHs. They showed that degradation of anthracene and pyrene in spiked soil by *Phanerochaete chrysosporium*, *Trametes versicolor* and *Pleurotus ostreatus* depends on the MnP and laccase levels secreted into the soil. Thus, fungal degradation of PAHs is not as fast or effective as bacteria, but they are very non-specific and have the capability to hydroxylate a large variety of xenobiotics. Furthermore, many fungi are naturally living in soil litter and could grow into the soil and propagate through the solid matrix to remove the PAHs. These criteria make the ecological role of ligninolytic fungi in bioremediation (Lee et al., 2014; Winquist et al., 2014; Kristanti and Hadibarata, 2015; Lee et al., 2015). In addition to MnP, LiP and laccase other fungal enzymes, such as Cytochrome P450 monooxygenase, epoxide hydrolases, lipases, proteases and dioxygenases have been extensively studied for their ability to degrade PAHs (Bezalel et al., 1997; Balaji et al., 2014).

3 Different species of fungus enzyme-degrading PAHs

Since the effectiveness of bioremediation depends on the selection of species with matching properties, a vast range of fungi have been investigated for their capability to metabolize PAHs up to six rings and the metabolite secretion is strain-dependent. In fact, as reported by Pothuluri et al., (1992), *Cunninghamella elegans* degraded acenaphtene to its corresponding metabolites: 1-acenaphthenone, 1,2-acenaphthenedione, *cis*-1,2-dihydroxyacenaphthene, *trans*-1,2-dihydroxyacenaphthene, 1,5-dihydroxyacenaphthene, 6-hydroxyacenaphthenone, also anthracene is degraded to anthracene *trans*-1,2-dihydrodiol 1-anthrol, 9,10-anthraquinone, phthalate, glucuronide, sulfate and xyloside conjugates of hydroxylated intermediates using the strains: *Bjerkandera* sp, *Cunninghamella elegans*, *Naematoloma*

frowardii, Phanerochaete chrysosporium, Phanerochaete laevis, Pleurotus ostreatus, Pleurotus sajor-caju, Ramaria sp, *Rhizoctonia solani, Trametes versicolor* (Cerniglia, 1982; Cerniglia and Yang, 1984; Bezalel et al., 1996; Johannes and Majcherczyk, 2000) (Table 2).

Recently, Jové et al., (2016) have conducted a comparative study on degradation efficiency of anthracene by three ligninolytic white-rot fungi (*P.chrysosporium*, *Irpex lacteus* and *P.ostreatus*) and three non-ligninolytic fungi, and have shown that *P. chrysosporium* exhibited higher degradation efficiency of 40% compared to 38% with *Irpex lacteus* and less than 30% of anthracene removal with *P. ostreatus*. Balaji et al., (2014) studied the capability of different fungal strains to secrete extracellular enzymes, such as lipase, laccase, peroxidase and protease, PAHs contaminated soil solution was used as the unique carbon source. The best lipase production was observed in *Penicillium chrysogenum* (112 U ml^{-1}), followed by *Lasiodiplodia theobromae* VBE1 (100 U ml^{-1}). However, *Colletotrichum gleosporioides* was unable to produce lipase enzyme during PAHs degradation, because of the toxic impact of PAHs in contaminated soil. The best laccase production was observed in *P. chrysogenum (*79 U ml^{-1}) and *Aspergillus fumigatus* (73 U ml^{-1}), while moderate peroxidase activity (52 U ml^{-1}) was noticed in *Mucor racemose* and *Rhizopus stolonifer*. Similar results were reported by Venkatesagowda et al., (2012) and Thiyagarajan et al., (2008) with a highest lipase production of 108 U ml^{-1} observed by *L. theobromae* and peroxidase production of 516 U ml^{-1} observed by *Coprinus* sp. The studies of Balaji and Ebenezer, (2008) and Banu and Muthumary, (2005) revealed highest lipase production by *C. gleosporioidies* in solid-state fermentation. Lee et al., (2014) investigated the efficiency of 150 taxonomically and physiologically diverse white rot fungi in a variety of biotechnological procedures, such as dye decolorization which corresponds to the beginning of lignin metabolism and is considered as a prediction of its capability to remove recalcitrant organopollutants, such as PAHs (Antonella Anastasi, 2009; Barrasa et al., 2009), gallic acid reaction which can be carried out to rank the fungi by their capability to degrade the PAHs, ligninolytic enzymes, and tolerance to four different PAHs: phenanthrene, anthracene, fluoranthene, and pyrene. All the fungi in this study produced three ligninolytic enzymes, LiP, MnP, and laccase. Nevertheless, since the ligninolytic enzyme activities of the fungi were analyzed in a nitrogen-limited condition, higher enzyme activity did not correlate with higher efficiency in the dye decolorization and gallic acid tests. Moreover, marine-derived fungi such as *Aureobasidium pullulans, Mucor sp.*, *Aspergillus* sp. AS 58, *Pichia guilliermondii* M-30, *Aspergillus niger* etc. can be considered as a source of enzymes of environmental interest. Bonugli-Santos et al., (2015) has reported that these strains produce hydrolytic and/or oxidative enzymes, such as alginate, lyase, amylase,

cellulase, chitinase, glucosidase, inulinase, keratinase, ligninase, lipase, nuclease, phytase, protease, and xylanase. These enzymes have an optimal temperature from 35 to 70 °C, and an optimal pH from 3.0 to 11.0. For marine-derived fungal strains, salinity has to be taken into account in screening and production.

Almost all the fungi produce LiP, MnP and laccase but at different rates under the same culture conditions. As a result, the efficiency of enzymatic bioremediation is strongly dependent on the type of the fungal strain. Among all these studied fungi displaying lignolytic activity, the white rot fungi *Phanerochaete chrysosporium*, *Pleurotus ostreatus* and *Bjerkandera adusta* have been intensively studied and have shown higher potential to metabolize PAHs (Haritash and Kaushik, 2009).

3.1 *Phanerochaete chrysosporium*

The potential of *Phanerochaete chrysosporium* fungi for use in PAHs bioremediation was first reported by Bumpus et al., (1985) who stated that this white-rot basidiomycete partly degraded benzo[a]pyrene to carbon dioxide. Accordingly, several studies have been carried out to degrade PAHs by *P. chrysosporium* under ligninolytic, nutrient-sufficient, or other induced culture conditions by the ligninolytic extracellular enzymes or intracellular catabolism (Syed and Yadav, 2012; Gu et al., 2015) Besides, *P. chrysosporium* was reported to be a potent candidate for PAHs degradation due to its special physiological characteristics and active adsorption sites. In fact, *P.chrysosporium* can adsorb PAHs compound in its mycelial pellets owing to its abundant conjugated structures (C=C and aromatic components), numerous chemical groups (–OH, –COO–, O–C=O, –NH2, CO–NH) and high carbon content (Gu et al., 2015). In fact, the capability to degrade PAH was attributed to the generation of an extracellular lignin degrading enzyme (ligninase) throughout secondary metabolism (Sanglard et al., 1986). Studies on xenobiotic degradation by *P. chrysosporium* implied a non-specific battery of enzymes produced by this fungus that degrades the lignin polymer as the main agent in pollutant metabolism. The key enzymatic constituents of the ligninolytic system of *P. chrysosporium* are thought to be lignin peroxidase (LiP) and manganese peroxidase (MnP) (Bogan and Lamar, 1995a). LiPs are ideal candidates to be the catalysts of preliminary PAH oxidation in *P. chrysosporium*, and for anthracene, which is a LiP substrate (Hammel et al., 1992a). Nevertheless, many of the organopollutants degraded by *P. chrysosporium* are not LiP substrates. As an example, phenanthrene was initially found not to be a LiP substrate (Hammel et al., 1986), but later was claimed to undergo degradation in nutrient limited *P. chrysosporium* cultures (Bumpus, 1989a). *P. chrysosporium* strains are also able to degrade a

large variety of PAHs even under nitrogen limiting conditions (Andreoni et al., 2004; Bumpus et al., 1985). Bumpus, (1989) demonstrated that *P. chrysosporium* is able to cleave 70 to 100% of at least 22 PAHs by substrate disappearance. Most of them profuse in anthracene oil within a period of 27 days of incubation with nitrogen-limited cultures of the fungus. Actually, the radiolabeled carbon of [^{14}C]phenanthrene, which was the prevalent compound of this mixture, was oxidized to $^{14}CO_2$. Moreover, HPLC experiments and mass balance analysis revealed the conversion of [^{14}C]phenanthrene to more polar and water-soluble metabolites. These results were corroborated by other researchers who have shown that besides [^{14}C]phenanthrene degradation, this fungus is able to oxidize [^{14}C]2-methylnaphthalene, [^{14}C]biphenyl, and [^{14}C]benzo[a]pyrene to $^{14}CO_2$ (Sanglard et al., 1986). Lee et al., (2010) demonstrated the potential of two strains of *Phanerochaete sordida* (KUC8369, KUC8370) among seventy-nine screened white rot strains to degrade considerably higher amount of phenanthrene and fluoranthene than the strains of *P. chrysosporium* and have also proven that the strain KUC8369 was the best degrader of fluoranthene despite the fact that it produced lower MnP than *P. chrysosporium.* Phenanthrene metabolism in ligninolytic *P. chrysosporium* was different from the pathway of most bacteria. The PAH was cleaved between positions 3 and 4, and also differed from the process in non-ligninolytic fungi and other eukaryotes, unable to cause PAH ring fission. In fact, the ligninolytic fungus, *P. chrysosporium*, oxidizes phenanthrene at its C-9 and C10 position to give 2,2'- diphenic acid as a ring cleavage product. On the other hand, the major site of enzymatic attack by most bacteria is at the C-3 and C-4 position of phenanthere. Also, *P. chrysosporium*, under non-ligninolytic conditions, metabolizes phenanthrene to phenols and trans-dihydrodiols. This observation proves that several enzymatic mechanisms may occur in *P.chrysosporium* for the initial oxidative attack on PAHs (Cerniglia and Yang, 1984).

The potential of *P. chrysosporium* in PAHs degradation is regarded to their extracellular enzymes. Therefore LiP and MnP. LiPs are ideal candidates to be the catalysts of preliminary PAH oxidation in *P. chrysosporium*. Nevertheless, it is suggested that other LiP-independent mechanisms need to exist for the initial oxidation of PAHs which are not lignin peroxidase substrates. Also, different pathways for PAHs degradation by *P. chrysosporium* can be considered.

3.2 *Pleurotus ostreatus*

P. ostreatus, considered as white-rot fungi has been well documented for its capacity to degrade PAHs (Bezalel et al., 1996, 1997; Tortella et al., 2015). Bezalel et al., (1996) reported that the white rot fungi, *P. ostreatus* cleaves a wide variety of PAHs, including phenanthrene, with small correlation between PAH degradation and extracellular laccase, manganese peroxidase, or manganese-independent peroxidase activities. The same authors demonstrated in a subsequent study that *P. ostreatus* is able to metabolize phenanthrene to phenanthrene trans-9,10-dihydrodiol and 2,2'-diphenic acid as well as mineralizing it to CO_2. The formation of phenanthrene trans-9R,10R-dihydrodiol, in which only one atom of oxygen originated from molecular oxygen, this indicates that *P. ostreatus* initially oxidizes phenanthrene stereoselectively, via a cytochrome P-450 monooxygenase and an epoxide hydrolase rather than a dioxygenase intervenes to form the dihydrodiol (Bezalel et al., 1997). Schützendübel et al., (1999) studied the degradation of polycyclic aromatic hydrocarbons (PAHs) with *Pleurotus ostreatus* in liquid cultures for the duration of 7 weeks. It removed 43% and 60% of fluorene and anthracene after only 3 days of incubation. Phenanthrene, fluoranthene and pyrene were degraded uniformly during the 7 weeks but to a lower level than fluorene and anthracene, and their degradation rate reached a maximum of 15%. The removal of anthracene at a different rate than fluorene implies the synchronized existence of a minimum of two different degradation pathways. In this study, an addition of milled wood to the culture increased the secretion of MnP and laccase, but no increase in the degradation of PAHs was recorded. Possibly, for MnP, this can be due to the lack of H_2O_2 during the secretion time which would inhibit the oxidation process (Field et al., 1992). The same reason could explain the clear correlation between the degradation of PAHs and laccase activity since there is a limitation of co-substrates for the oxidation mediated by this enzyme.

3.3 *Bjerkandera adusta*

Other than *Pleurotus ostreatus*, Schützendübel et al., (1999) investigated the degradation of phenanthrene, fluoranthene, pyrene, fluorene and anthracene with *Bjerkandera adusta* in the same culture conditions. This fungus degraded 56% and 38% of fluorene and anthracene, while other PAHs were removed uniformly but to a lower rate. LiP and MnP activity was not detected during this oxidation and the ending quinones resulting from this oxidation were not observed as final products (Field et al., 1992). These results suggest a new pathway than the

typical extracellular ones, as described for the removal of phenanthrene by *Phanerochaete chrysosporium* (Sutherland et al., 1991) and *Pleurotus ostreatus* (Lea Bezalel et al., 1996a), and the higher oxidative potential produced in latter phase by these enzymes can increase PAHs degradation.

Another study by Wang et al., (2002) investigated the usefulness of a chemically modified manganese peroxidase with cyanuric chloride-activated methoxypolyethylene glycol, produced by *Bjerkandera adusta*. The modified and native enzymes demonstrated identical catalytic properties in the oxidation of Mn(II) and other substrates including veratryl alcohol, guaiacol, 2,6-dimethoxylphenol, and 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonate). However, the modified enzyme exhibited higher level of resistance to denaturation by hydrogen peroxide and stability to organic solvents such as N,N-dimethylformamide, acetonitrile, methanol, ethanol and tetrahydrofuran. Likewise, the modified enzyme demonstrated better stability to higher temperatures and lower pH than the native enzyme. The same author showed in later study that the oxidation rate of PAHs, such as anthracene, pyrene, benzo[a]pyrene and PAHs with ionization potentials of 7.43 eV or lower, decreased in the presence of manganous ions in the purified manganese-lignin peroxidase (MnLiP) hybrid isoenzyme from *Bjerkandera adusta.* Therefore, chemical modification of manganese peroxidase from *B. adusta* improved its effectiveness.

4 Culture conditions

Natural resources polluted with PAHs usually raise their mutagenic and carcinogenic impact in fresh-water, marine-water and terrestrial species. This leads to unfavorable conditions for growth of even indigenous microorganisms. To overcome this limitation and obtain the highest enzyme production, culture conditions and inducers should be optimized (Balaji et al., 2014). Generally, microorganisms require suitable growth conditions (e.g. carbon source, nutrients, temperature, pH, redox potential and oxygen content which, strongly affect their growth (Adams et al., 2015). White rot fungi, in turn, appear to act because of their ability to penetrate contaminated soils with their hyphae and the production of extracellular oxidases (Wang et al., 2009).

Hadibarata and Kristanti, (2014) reached higher rates of degradation of n-eicosane by adding glucose as a carbon source for *Trichoderma* S019 strain. In this regard, the incubation time was found to be critical parameter influencing the degradation rate, for instance, Zafra et al., (2015b) have found that *Trichoderma asperellum* could degrade 74 % of phenanthrene, 63 %

of pyrene, and 81 % of benzo[a]pyrene after 14 days of incubation at concentration of 1000 mg kg^{-1}. Moreover, *Peniophora incarnata* KUC8836 was able to degrade up to 95.3 % of phenanthrene and 97.9 % of pyrene after 2 weeks of incubation (Lee et al., 2014) and showed higher degradation rate of creosote at higher concentration of 229.49 mg kg^{-1} (Lee et al., 2015). Furthermore, the concentration of the contaminant represents a key point which influenced the degradation of PHAs. Zafra et al., (2015a) have shown that the concentration of contaminant had a selective pressure on hydrocarbon-degrading organisms, and higher PAHs is a growth-limiting for microorganisms which developed a response against PAHs regarding cell membrane structure, mycelia pigmentation, and sporulation alterations. Likewise, the growth of fungi is inhibited in highly contaminated soils and overall remediation by fungi is extremely slow, needing many days or even more than a month as reported by Drevinskas et al., (2016). In this sense Balaji et al., (2014) also tested different carbon sources for lipase production by *P. chrysogenum* and *L. theobromae* and cellulose in *M. racemosus* and sucrose induced the highest activity in these species. Similarly, nitrogen sources have to be taken into account, yeast extract was found to be the best inducer of maximum lipase production in the mentioned strains. Dharmsthiti and Kuhasuntisuk, (1998) also demonstrated that yeast extract, as a supplemental source increased lipase production by *Pseudomonas aeruginosa* LP602 in lipid-rich wastewater treatment. Moreover, Mineki et al., (2015) investigated the degradation of PAHs with *Trichoderma/Hypocrea* genus which used pyrene as sole source of carbon, and found that the growth of the strain and pyrene-degrading activity was enhanced to 27% and 24-25% compared with the control after incubation for 7 and 14 days, respectively, by adding 0.02% yeast extract, 0.1% sucrose, or 0.1% lactose. Garapati and Mishra, (2012) also reported the relevance of nutrients in biodegradation of hydrocarbon by a fungal strain Ligninolytic enzymes can be regulated by aromatic compounds, such as different dyes and PAHs, that way fungi can use these aromatic compounds as unique source of carbon (Yang et al., 2011). In fact, it is unwise to test ligninolytic enzyme activity when screening species with highest PAH removal in the absence of substrate as the use of xenobiotic as substrates can induce the enzyme activity (Lee et al., 2014). This technique is attainable for fungal species that produce ligninolytic enzymes with higher efficiency, such as *Bjerkandera adusta* KUC9107 and *Skeletocutis perennis* KUC8514 for LiP production; *Phanerochaete velutina* KUC8366 and *Phanerochaete sp.* KUC9015 for MnP production; and *Cerrena consors* KUC8416 and 8421 for laccase production (Moreira et al., 2006). Hofrichter et al., (1998), found that a wide spectrum of aromatic compounds was in part mineralized by the manganese peroxidase (MnP) system of the white rot fungus

Nematoloma frowardi and that mineralization was enhanced by peptide glutathione GSH (a natural peptide produced by eukaryotic cells which protects cells against reactive oxygen species and free radicals) and depended on the ratio of MnP activity to concentration of GSH. This suggests that carbon and nitrogen are essential for enzyme activity and consequently affects PAHs degradation. Furthermore, PAHs themselves can be used as a substrate and at the same time, as a nutrient source for enzymes inducing their activity reducing costs of culturing.

Simultaneously, the effect of different surfactants can affect PAHs removal. Balaji et al., (2014) showed that lipase production was maximized with Triton X-100 boosting activities to 68 U ml^{-1} in *P. chrysogenum*, 72 U ml^{-1} in *M. racemosus* and 62 U ml^{-1} in *L. theobromae* VBE1. Also, Gopinath et al., (2013) highlighted the relevance of surfactant in lipase production and its numerous applications. The highest lipase activity by *Metarhizium anisopliae*, occurs when Tween 80 and SDS were applied (Ali et al., 2009). Likewise, Chen et al., (2006) investigated the effect of surfactants on PAHs degradation by white rot fungi in soil water system, and found that Triton X-100 and SDS restrained the removal of PAHs. Moreover, biosurfactants enhance the removal of PAHs (Arun et al., 2008). Thus, using surfactants is generally effective for the biodegradation process, but previous testing is needed to prevent the inhibition in some cases.

Different temperatures were detected for optimal enzymes activity. Most of the enzymes have highest activity at mesophilic temperatures and it declines with very high and reduced temperatures. Several enzymes are claimed to be active even at extreme temperatures. At a temperature of 5 °C, only the laccase activity is detected. The optimum temperature for laccase activity is 45 °C, but it declines to 30% at 5 °C, and 31% at 75 °C. However, the activity of Mn-dependent peroxidase was higher even at 75 °C (Haritash and Kaushik, 2009). Farnet et al., (2000) have shown that the activity of fungus, *Marasmius quercophilus* laccase was the highest at 80 °C.

The extracellular enzyme release and polycyclic aromatic hydrocarbons (PAHs) removal in agitated and shallow stationary liquid cultures of *Phanerochaete chrysosporium* requires the addition of two inducers of lignin peroxidase (LiP) and manganese peroxidase (MnP), veratryl alcohol and Tween-80, respectively (Ding et al., 2008). However, if shallow stationary cultures are used, they also produce enzyme since it increased the contacting area between cells and oxygen without shear stress, while agitated cultures increased biodegradation rate by aiding interphase mass transfer of PAHs into aqueous phase. Simultaneously, they are recognized as inhibitors to the production of ligninolytic enzymes due to shear stress on

mycelia. The use of a LiP stimulator, veratryl alcohol, did not increase PAH degradation but considerably improved LiP activity. In contrast, Tween-80 enhanced MnP secretion and PAH degradation in shallow stationary cultures. On the other side, high PAH degradation was noticed in agitated cultures in the absence of apparent LiP and MnP activities. Same results were proved by Schützendübel et al., (1999), who mentioned that degradation of fluorene, anthracene, phenanthrene, fluoranthene and pyrene are not associated with the production of extracellular enzymes by *Pleurotus ostreatus* and *Bjerkandera adusta*. Similar results were described by Lea Bezalel et al., (1996a) et Verdin et al., (2004) who found degradation of PAH in fungal cultures in the absence of LiP and MnP activities. Mohammadi et al., (2009) have also tested the effect of the incubation mode on anthracene biodegradation and it was revealed that the culture agitation clearly increased the biodegradation capacity of bagasse immobilized fungal cells despite the repressive effect of culture agitation on the ligninase activity.

All these outcomes indicated that extracellular peroxidase activities are not directly associated with the PAH degradation, and the increased solubility may be essential in the enhancement of PAH degradation rather than enzyme activity and hence suggested the possibility of producing other oxidative and hydrolytic enzymes that were not analyzed but could probably have degraded PAHs. Another suggestion is the PAHs degrading role of intracellular enzymes, such as tyrosinases and dioxygenases secreted into the culture filtrate by different fungi (Milstein et al., 1983).

Enzyme immobilization allows an alternative procedure that enables an increase in the steady state of enzymes and significant environmental tolerance. Immobilized enzyme, which can be established by several methods, such as adsorption, entrapment, and covalent bonding based on chemical/physical mechanisms, has enahnced activity and stability. The immobilized enzyme can withstand a wider range of temperatures and pH, as well as significant substrate concentration changes; this makes the complex much more resistant to severe environments (Dodor et al., 2004). This results in a longer lifetime and higher productivity per active unit of enzyme. Immobilization enables the enzyme to be recycled, and such an approach is much better suited for hydrophobic PAHs-contaminated soil bioremediation (Chang et al., 2015).

5 Ionization potential

Bogan and Lamar, (1995a) defined the behavior of intact fungus and the MnP-based lipid peroxidation system with respect to a larger variety of creosote PAHs. The disappearance of three- to six-ring creosote PAH components from intact fungal cultures and throughout lipid

peroxidation in vitro was reported. In each of these cases, the approach is shown to be mainly dependent on IP, indicating that the contribution of one or more than one electron oxidants is included over the entire range of PAHs tested. One-electron oxidation of PAHs occurs by peroxidases (IP≤7.35 eV), Mn dependent peroxidase (IP≤8.19 eV), ligninase (IP≤7.55 eV) and laccase (IP≤7.45 eV) (Cavalieri et al., 1983). The IP values, referring to the energy needed to eliminate 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 (Table 1) (Cavalieri et al., 1983). PAHs like benz[a]anthracene, pyrene, and anthracene, that have ionization potential <7.35 eV, are LiP substrates, whereas PAHs, such as phenanthrene and benzo[e]pyrene that have ionization potentials >7.35 eV, cannot be LiP substrates. The products of the enzymatic oxidation are PAH quinones. For example, benzo[a]pyrene is oxidized to its 1,6-, 3,6-, and 6,12-quinones, pyrene to its 1,6- and 1,8-quinones, and anthracene to 9,10-anthraquinone (Hammel, 1995). Vyas et al., (1994a) has suggested that *P. ostreatus* and *T. versicolor* produce enzyme(s) other than lignin peroxidase capable of oxidizing compounds with higher ionization potential, such as anthracene.

6 Kinetics

The characteristics of the soil determine the diversity and activity of its microflora which is responsible for the degradation of polycyclic aromatic hydrocarbons and any other compound. In addition, soil characteristics influence the strength of interactions between the PAHs and individual soil compounds. Cutright, (1995) indicated that *Cunninghamella echinulata var. elegans* efficiently degrades PAHs in the presence of these nutrients while any other indigenous microorganisms are not. Moreover, for a first-order reaction system, the rate of change in contaminant concentration is proportional to the contaminant concentration in the soil and time prediction tool in degradation depends on the microorganism, the contaminant type and its concentration.

The prediction of time for bioremediation of polluted soil is based mostly on the microorganisms, pollutant type and its concentration. Furthermore, the improvement of more appropriate kinetic model needs the monitoring of biomass, respiration studies, and investigation of interactions of different organisms. Although bioremediation has a larger rate of success than synthetic methods, still the kinetics is not completely understood, and the kinetics becomes more challenging when fungi are applied for bioremediation (Haritash and Kaushik, 2009). As described previously, the different enzymes involved in fungal

degradation have maximum activity at different temperatures and some of them are active even at extreme temperatures. Therefore, monitoring the kinetics for various fungal strains is complicated, but most of them have good degradation capacities in a mesophilic range. The degradation rate can be improved by pretreatment at a high temperature which results in volatilization and decrease in the soil–water partition coefficient, as a result dissolution of pollutants increases the degradation rate.

7 Soil and liquid cultures

Most research studies on the extracellular enzyme activity produced by fungi have primarily been focusing on experiments in liquid culture (Ruiz-Dueñas et al., 1999; Kwang Ho Lee, 2004; Eibes et al., 2006a; Rodrigues et al., 2008; Mäkelä et al., 2009; Dashtban et al., 2010). Nevertheless, some interest has been given to the changes of the enzymes produced by fungal strains during PAHs degradation in different soil types (Wang et al., 2009). When fungi get into non-sterile soil, they must compete with indigenous soil microbes for nutrients and the mycelia of the fungi may be affected. As a consequence, the production of enzymes may be influenced by more complicated elements (McErlean et al., 2006; Wang et al., 2014a).

Boyle et al., (1998) showed that white rot fungi growing in soil presented low amounts of degraded polyaromatic hydrocarbons (PAHs), even though they did degrade some other organopollutants. Nevertheless, in liquid culture, they degraded several PAHs. The latter interpretation was supported by Novotný et al., (2004b) who have demonstrated that the importance of higher fungal enzyme levels for effective degradation of recalcitrant compounds was better revealed in liquid media in comparison to the same strains growing in soil. Liquid culture reports have documented the degradation and/or mineralization of an extensive range of PAHs with phenanthrene, fluorene, benzofluorene, anthracene, fluoranthene, pyrene, benz[a]anthracene, and benzo[a]pyrene, among them (Bogan and Lamar, 1995a).

PAHs existing in soil are largely differentiated because of the higher heterogeneity of the soil structure (Li et al., 2007). Furthermore, the forms of the sorbed contaminants in soils are an essential element that affects the degradation (Yang et al., 2009). A significant spectrum of PAHs is biodegradable in aqueous culture but they are not biodegradable in soil. This fraction is classified as persistent residue with highly reduced bioavailability (Cornelissen et al., 2005).

PAHs molecules can be divided into three categories considering the desorption and bioavailability: easily desorbing and available fraction; the hardly desorbing and available fraction; and the irreversible and completely unavailable fraction (Li et al., 2007). Therefore, at the beginning of degradation, PAHs are quickly desorbed, and the desorption could possibly not present a limitation for biodegradation. The sorbed forms or the bioavailability of a contaminant are controlled by the characteristics of the contaminant and the soil, along with the contact time between the contaminant and the soil (Wang et al., 2014b).

Wang et al., (2009) observed a degradation of phenanthrene, pyrene and benzo[a]pyrene in soils by *Phanerochaete chrysosporium.* The highest activity of LiP and MnP reached 1.92 U g^{-1}. Their high molecular size with higher ring number, reduced aqueous solubility and large octanol/water partition coefficient (K_{ow}) of these compounds, makes them firmly combined and entrapped in soil micropores or soil organic matter (SOM) matrix. This results in the limitation on their biodegradability by enzymes. Huesemann et al., (2003) confirmed that the low rates of PAHs biodegradation were due to their low bioavailability in soil and low mass transfer rate of hydrophobic organic contaminants to the aqueous phase rather than deficiency in microorganisms degrading them. Furthermore Wang et al., (2009) also demonstrated that the degradation of pyrene by *P. chrysosporium* decreased with increasing SOM content, confirming that the SOM content can negatively affect the bioavailability of PAHs (Gill and Arora, 2003). While the maximum of LiP and MnP activities increased.

Also sorption of contaminants makes them less bioavailable with elevated contact time (aging) in the soil (Antizar-Ladislao et al., 2006; Li et al., 2008). The decrease in bioavailability induced by aging fluctuates with the contaminants and soils and the mechanisms are still to be investigated in deep (Northcott and Jones, 2001; Nam and Kim, 2002; Watanabe et al., 2005). Some studies suggested that the contaminants are slowly transported from easily desorbing and bioavailable sites to hardly desorbing and less bioavailable sites where they accumulate throughout aging, and even to irreversible and non-bioavailable sites, which leads to reduced decontamination rates (Northcott and Jones, 2001; Sun et al., 2008).

Furthermore, sterilization of the soil is typically employed when investigating biodegradation in laboratory experiments to ensure that the degradation capability is high. This situation is not applied in bioremediation field. Few studies have described the effects of soil sterilization on the bioavailability of sorbed contaminants and changes in SOM (Northcott and Jones, 2001; Nam et al., 2003; Kelsey et al., 2010). Wang et al., (2014) observed that sterilization

increased the degradation of pyrene because of the removal of competition from indigenous microbes.

8 Degradation Pathways

An understanding of the process for PAHs degradation in fungal strains would be a crucial step in the clarification of the enzymatic mechanisms. Ligninolytic enzymes undergo a one electron radical oxidation, producing aryl cation radicals from contaminants followed by generation of quinones (Vyas et al., 1994b; Cerniglia, 1997a). As an example, the intact culture of *P. chrysosporium* degraded anthracene to anthraquinone (Hammel et al., 1991). Anthraquinone was further degraded to phthalic acid and carbon dioxide. Purified forms of lignin peroxidase and manganese peroxidase likewise were able to oxidize anthracene, pyrene, fluorene and benzo[a]pyrene to the corresponding quinones (Haemmerli et al., 1986; Hammel et al., 1986, 1991; Hammel, 1992; Bogan and Lamar, 1996) (Figure 1).

Also, the crude and the purified ligninase of *Phanerochaete chrysosporiu*m oxidize the benzo(a)pyrene into three soluble organic compounds, which are benzo(a)pyrene 1,6 -, 3,6-, and 6,12-quinones (Haemmerli et al., 1986). These facts support the suggestion that lignin-degrading enzymes were peroxidases, mediating oxidation of aromatic compounds through aryl cation radicals. The ligninase which was unstable in the presence of hydrogen peroxide could be stabilized by inclusion of veratryl alcohol to the reaction mixture. The oxidation of benzo(a)pyrene was stimulated by this alcohol. Likewise, Hammel et al., (1986) studied the oxidation of polycyclic aromatic hydrocarbons and dibenzo[p]-dioxins and found that the lignin peroxidase (ligninase) of *P. chrysosporium* catalyze the oxidation of a wide range of lignin-related compounds. Tests with pyrene as the substrate revealed that pyrene-1,6-dione and pyrene-1,8-dione are the main oxidation products (84% of total as determined by high performance liquid chromatography), and gas chromatography/mass spectrometry analysis of ligninase-catalyzed pyrene oxidations executed in the presence of H_2O revealed that quinone oxygens occur from water. Whole cultures of *P. chrysosporium* oxidized pyrene to these quinones in transient step. Experiments with dibenzo[p]dioxin and 2-chlorodibenzo[p]dioxin revealed that they are also substrates for ligninase. The immediate product of dibenzo[p]dioxin oxidation was the dibenzo[p]dioxin cation radical, which was noticed in enzymatic reactions by its electron spin resonance and apparent absorption spectra. The cation radical mechanism of ligninase thus was applied besides lignin, to other environmentally important aromatics. Hammel, (1992) also studied the oxidation pathway of phenanthrene and

phenanthrene-9,10-quinone (PQ) by the ligninolytic fungus, *Phanerochaete chrysosporium* at their C-9 and C-10 positions to result in a ring-fission product, 2,2'-diphenic acid (DPA), which was identified in chromatographic and isotope dilution experiments. DPA formation from phenanthrene was relatively higher in reduced nitrogen (ligninolytic) cultures than in high-nitrogen (non-ligninolytic) cultures and was not present in uninoculated cultures. The oxidation of PQ to DPA included both fungal and abiotic process, and was not affected by the amount of nitrogen added, and cleaved rapidly than phenanthrene to DPA. Phenanthrene-trans-9,10-dihydrodiol, which was earlier shown to be the major phenanthrene metabolite in non-ligninolytic *P. chrysosporium* cultures, was not formed in the ligninolytic cultures. Therefore, phenanthrene degradation by ligninolytic *P. chrysosporium* proceeded in sequence from phenanthrene to PQ and then to DPA, involving both ligninolytic and non-ligninolytic enzymes, and is not initiated by a common microsomal cytochrome P-450. The extracellular lignin peroxidases of *P. chrysosporium* were not able to oxidize phenanthrene *in vitro* and consequently were also less likely to catalyze the first step of phenanthrene degradation *in vivo*. Both phenanthrene and PQ were mineralized to identical range by the fungus, which supported the intermediacy of PQ in phenanthrene degradation, but both compounds were mineralized considerably less than the structurally associated lignin peroxidase substrate pyrene. Hammel et al., (1991) reported that *Phanerochaete* was generally different from the bacteria, which proceeded through AC cis-1,2-dihydrodiol instead of AQ and has been suggested to yield salicylate rather than phthalate as a monocyclic cleavage product. The formation of quinone to prepare the aromatic ring for cleavage is an uncommon biodegradation approach and was showed to be of general significance in *P. chrysosporium*. LiPs have also been involved in the degradation of polychlorinated phenols by this organism. The monooxygenase system of cytochrome P-450 producing epoxides can also be included in degradation of PAHs. The epoxides can be rearranged into hydroxyl derivatives or can be hydrolyzed to vicinal dihydrodiols. Ligninolytic fungus, *Irpex lacteus* degraded anthracene and phenanthrene and the main degradation products were anthraquinone and phenanthrene-9,10-dihydrodiol, respectively as shown in Figure 2. The study also suggested the degradation pathway of anthracene and phenanthrene (Cajthaml et al., 2002). Thus, several systems are involved in the degradation of PAHs with fungal enzymes including intracellular cytochrome P450 and extracellular lignin peroxidase, manganese peroxidase and laccase.

The biodegradation of PAHs was studied under aerobic and anaerobic conditions. Fungal strains were grown on PAHs under static aerobic conditions for 6 and 10 days. The highest

587 degradation of naphthalene (69%) was performed by a strain that had MnP activity, followed
588 by strain that showed lignin peroxidase and laccase activities. Likewise, it was found that
589 highest degradation of phenanthrene (12%) was observed with the strain that contained MnP
590 and laccase activities (Clemente et al., 2001). Soil fungi *Aspergillus* sp., *Trichocladium*
591 *canadense*, and *Fusarium oxysporum* degrade polycyclic aromatic hydrocarbons low-
592 molecular-weight PAHs (2-3 rings) and produce ligninolytic enzymes also under
593 microaerobic and highly reduced oxygen conditions, but ligninolytic enzyme activities can
594 vary among fungi and PAHs. Under microaerobic conditions, the 3 species demonstrated at
595 least one of the assayed ligninolytic activities (LiP, MnP, laccase). In contrast, under very-
596 low-oxygen conditions, ligninolytic enzyme activity was frequently not observed (Silva et al.,
597 2009).

598 Marco-Urrea et al., (2015), described the biodegradation pathways of PAHs using non-
599 ligninolytic fungi. These non-ligninolytic strains showed a particular type of resistance to
600 different amounts of PAHs. The most common mechanism of PAHs transformation is the
601 intracellular accumulation followed by the degradation, akin to benzo[a]pyrene degradation
602 using intracellular enzymes of the strain *Fusarium solani* (Fayeulle et al., 2014). Likewise,
603 extracellular enzymes, such as laccase, can also be produced by some of these non-
604 ligninolytic fungi. but they are not as effective as intracellular enzymes degrading PAHs. The
605 phase I of degradation pathway includes the formation of oxidized metabolites, such as
606 hydroxyl-, dihydroxy-, dihydrodiol- and quinone-derivatives followed by the phase II which
607 includes the conjugation with sulfate-, methyl-, glucose-, xylose- or glucuronic acid groups.
608 These metabolites are less harmful than the original PAHs (Cerniglia and Sutherland, 2010).

609 **9 Mechanism of degradation with enzymes**

610 **9.1 Characteristics of ligninolytic enzymes**

611 **9.1.1 Characteristics of peroxidases**

612 A couple of extracellular oxidative enzymes are responsible for lignin degradation:
613 peroxidases and laccases (phenol oxidases). Both peroxidases were initially found in *P.*
614 *chrysosporium* (Tien and Kirk, 1983). A number of other fungi also possess these enzymes,
615 while others have either one or the other (Mester and Tien, 2000). In the majority of species,
616 peroxidases are generally recognized to be families of isozymes occurring as extracellular
617 glycosylated proteins which may enhance their stability (Thurston, 1994). The ratio between

the isozymes varies with the culture age and the culture conditions (Leisola et al., 1987; Bogan and Lamar, 1995b). They need hydrogen peroxide to oxidize lignin and lignin-related compounds. Their molecular weights and isoelectric points range from 35 to 47 kDa and 2.8 to 5.4 kDa, respectively (Leisola et al., 1987; Bogan and Lamar, 1995b; Johansson et al., 2002). The peroxidases are single heme-containing enzymes (protoporphyrin IX) so that the absorption spectrum of the native enzyme has a very particular absorbance maximum at 406-409 nm (ten Have and Teunissen, 2001). The peroxidases are divided into two different types depending on their different substrate spectra: manganese peroxidase (MnP), for which Mn(II) is best reducing substrate and the lignin peroxidase (LiP). LiP oxidizes non-phenolic and phenolic aromatic compounds (ten Have and Teunissen, 2001).

9.1.2 Characteristics of laccase

Laccase belongs to the copper oxidase family that is able to catalyze the oxidation of phenols, polyphenols and anilines, which are largely dispersed in higher plants, fungi and bacteria (Tavares et al., 2006). The enzyme is typically larger than peroxidases, having a molecular weight around and above 60 kD and have acidic isoelectric points (ten Have and Teunissen, 2001). As with other extracellular enzymes, laccases are glycosylated. As an alternative to H_2O_2, laccases use dioxygen as an oxidant, reducing it by four electrons to water. These types of enzymes have four copper per enzyme, that represents three different types, and consequently, every type has a different role in the oxidation of laccase substrates (Messerschmidt and Huber, 1990). The type 1 copper is suggested to be included in the reaction with the substrates. It has an absorption maximum at the wavelength of 610 nm which gives the enzyme the typical blue color. The type 2 copper and the two type 3 coppers cluster in a triangular form which is involved in the binding and in the reduction of O_2 as well as the storage of electrons coming from the reducing substrates. The type 2 copper does not present visible absorbance, while the type 3 coppers have an absorption maximum at 330 nm (Mester and Tien, 2000; ten Have and Teunissen, 2001). It was demonstrated that, in the presence of suitable mediators, laccase is capable to oxidize a considerably larger range of compounds, such as PAHs (Peng et al., 2015).

9.1.3 Mediators of laccase Laccase has been well studied for its capacity to oxidize PAHs, xenobiotic and phenolic lignin model compounds (Majcherczyk et al., 1998; Peng et al., 2015). Earlier, its application was limited because of the low oxidation potential, thus, in the presence of an appropriate mediator; laccases show higher oxidation capability resulting in

numerous biotechnological applications involving oxidation of non-phenolic lignin compounds and detoxification of various environmental pollutants (Upadhyay et al., 2016; Khambhaty et al., 2015). Recently, laccase has found applications in other sectors, such as in the design of biosensors and nanotechnology (Li et al., 2014; Upadhyay et al., 2016). Besides, they are used in the decolourization and detoxification of industrial effluents and the treatment of wastewater (Viswanath et al., 2014; Chandra and Chowdhary, 2015). These mediators include 1-hydrobenzotriazole (1-HBT) (Majcherczyk et al., 1998a), 2,2`-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (Bourbonnais et al., 1997), and violuric acid (Xu et al., 2000), but also natural mediators have been explored, such as phenol, aniline, 4-hydroxybenzoic acid, 4-hydroxybenzyl alcohol (Johannes and Majcherczyk, 2000), 3-hydroxyanthranilate (Eggert et al., 1996). These natural mediators were as effective as the synthetic coumpounds (Johannes and Majcherczyk, 2000). Other natural compounds including cysteine, methionine, and reduced glutathione, containing sulfhydryl groups, were also efficient as mediator compounds (Johannes and Majcherczyk, 2000).

Many reports have studied the oxidation of PAH by purified fungal laccases (Table 3). LACs of *T. versicolor*, *C. hirsutus, P. ostreatus*, and *Coriolopsis gallica* were the most studied.

For example, *T. versicolor* LAC, in combination with HBT, was capable to oxidize two PAHs, acenaphthene and acenaphthylene; ABTS did not clearly affect the oxidation rate. LAC without mediator oxidized about 35% of the acenaphthene and only 3% of acenaphthylene. The principal products obtained after incubation were 1,2-acenaphthenedione and 1,8-naphthalic acid anhydride (Johannes et al., 1998). The purified LAC of *T. versicolor* did not transform PHE. The supplementation of a redox mediator, ABTS or HBT, to the reaction mixture improved the degradation of PHE by LAC about 40% and 30%, respectively (Han et al., 2004). LAC produced by *C. hirsutus* catalyzed The oxidation of five PAHs: FLA, PYR, ANT, B[a]P, and PHE in the presence of the redox mediators ABTS and HBT. B[α]P was the most effectively oxidized substrate In the system mediated by ABTS, , while ANT was the most effectively oxidized substrate in the one mediated by HBT. There was no clear correlation between the IP and the oxidation of the substrates. The rate of oxidation by LAC of *C. hirsutus* varied from 10.9 to 97.2% depending on the PAHs examined. The oxidation of FLA and PYR by *C. hirsutus* LAC was effective and ranged from 37.9 to 92.7%. PYR which is one of the least oxidizable PAHs was still oxidized until 40% in the presence of all the mediators.

LAC from *T. versicolor* catalyzed the *in vitro* oxidation of ANT and B[a]P which have IPs $\leq$7.45 eV. The presence of ABTS improves the oxidation of ANT while it is crucial for the

684 oxidation of B[a]P. Anthraquinone was recognized as the major end product of ANT
685 oxidation (Sutherland et al., 1991). Consequently, the PAH-oxidizing abilities of LAC differ,
686 depending on the fungal species from which it was produced (Cho et al., 2002).
687 ANT was entirely degraded by the LAC of *Ganoderma lucidum* fungus in the absence of a
688 redox mediator. At the same time and in the presence of the mediator, this same LAC
689 degraded B[a]P, FLU, acenapthene, acenaphthylene, and B[a]A at a rate ranging from 85.3%
690 to 100% (Pozdnyakova, 2012; Pozdnyakova et al., 2006).
691 The degradation of B[a]P by purified LAC of *Pycnoporus cinnabarinus* was investigated. The
692 reaction required the presence of the exogenous ABTS as a mediator. Almost all of the
693 substrate (95%) was transformed within 24 hours. The enzyme principally oxidized the
694 substrate to benzo[a]pyrene-1,6-, 3,6- and 6,12-quinones (Rama et al., 1998).
695 The effect of different mediators on LAC action was investigated by Pickard et al., (1999).
696 Different PAHs were oxidized by *Coriolopsis gallica* LAC such as B[a]P, 9-
697 methylanthracene, 2-methylanthracene, ANT, biphenylene, acenaphthene, and PHE. 9-
698 methylanthracene was the most rapidly oxidized substrate. There was no apparent correlation
699 between the ionization potential of the substrate and the first-order rate constant for substrate
700 degradation *in vitro* by adding ABTS. The effects of mediating substrates were studied
701 furthermore by applying ANT as a substrate. A synergistic effect of HBT and ABTS was
702 detected. In fact, HBT supported approximately one-half ANT oxidation rate that ABTS
703 supported, whereas HBT with ABTS enhanced the oxidation rate nine-fold, compared with
704 the oxidation rate supported by only ABTS (Pickard et al., 1999).
705 The white-rot fungi generate yellow form of LAC during solid-state fermentation of a
706 substrate containing natural lignin. The active center of this enzyme is transformed by the
707 products of lignin degradation. Consequently, LAC becomes capable to catalyze the oxidation
708 of nonphenolic compounds in the absence of mediators (Pozdnyakova et al., 2006a). The rate
709 of degradation using the yellow LAC produced by *P. ostreatus* was also detected. The
710 naphthalene derivatives α- and β-naphthols, α-nitroso-β naphthol, α-hydroxy-β-naphthoic
711 acid, and α- naphthylamine were all appropriate LAC substrates despite yellow LAC did not
712 catalyze the degradation of the two-ring PAH naphthalene. Yellow LAC oxidized all the
713 PAHs of three to five rings such as ANT, PYR, FLU, FLA, PHE, and perylene, with rates of
714 degradation ranging from 40% to 100%. The efficiencies were greater than that observed for a
715 blue LAC from the same fungus without and with ABTS and HBT mediators.. The same
716 product of ANT oxidation and several unknown products of FLU oxidation were noticed in
717 solutions of various solvents (Pozdnyakova, 2012; Pozdnyakova et al., 2006).

9.2 Catalytic cycle of peroxidases

LiP and MnP have a common catalytic cycle, as also observed for other peroxidases (ten Have and Teunissen, 2001). One molecule of H_2O_2 oxidizes the native enzyme by withdrawing two electrons, creating compound I. The latter could be reduced back to two single-electron oxidation steps to the native form through an intermediate compound II. In the case of LiP, reduction of compound II is the rate-limiting step in the catalytic cycle. For this reason, this compound is regarded to be less effective than LiP compound I. As the reduction of compound II is relatively slow, it is available for longer time for a reaction with H_2O_2 resulting in inactive enzyme, identified as compound III which is characterized to be a complex between LiP and superoxide (Cai and Tien, 1992). Other fungal enzymes could provide the needed hydrogen peroxide for peroxidase activity. As part of their ligninolytic system, white-rot fungi produce H_2O_2-generating oxidases (Kirk and Farrell, 1987), such as glucose oxidases, glyoxal oxidase, and aryl alcohol oxidase. White-rot fungi that lack H_2O_2-generating oxidases depend on the oxidation of physiological organic acids, such as oxalate and glyoxylate which indirectly results in H_2O_2 (ten Have and Teunissen, 2001). Also, the reduction of quinones to their equivalent hydroquinones and the subsequent autoxidation or enzymatically catalyzed oxidation may generate H_2O_2 due to the involvement and reduction of O_2 (Muñoz et al., 1997).

9.2.1 MnP

MnP is distinct from the other peroxidases due to the framework of its binding site. MnP oxidizes Mn^{2+} to Mn^{3+}, which cannot be substituted by other metals at physiological concentrations (Glenn et al., 1986). At the time of the discovery of MnP, it was revealed that a number of aliphatic organic acids including lactate and oxalate induced Mn^{2+} oxidation rate (Glenn et al., 1986; Matsubara et al., 1996). These organic acids, e.g., oxalate and to a lower degree malonate and glyoxylate were demonstrated to be produced as *de novo* metabolites by white-rot fungi (Dutton and Evans, 1996). These acids are able to chelate Mn^{3+} resulting in a comparatively stable complex. The complexed Mn^{3+} can then oxidize phenolic lignin model compounds and many phenols via phenoxy radical configuration (Jensen et al., 1994). Beside phenolic structures, the MnP system has also been observed to oxidize nonphenolic lignin model compounds (Hofrichter et al., 1998).

PAH degradation studies revealed that MnP from *I. lacteus* was capable to effectively degrade three- and four-ring PAHs, including phenanthrene, anthracene and fluoranthene. MnP

produced by *Anthracophyllum discolor*, degraded pyrene (>86%), and anthracene (>65%) alone or in mixture, and also degraded fluoranthene and phenanthrene but less effectively (<15.2% and <8.6%, respectively) (Acevedo et al., 2011). MnP-catalyzed oxidation of PAHs resulted in respective quinones. Anthrone, which is an expected intermediate was formed during the degradation of anthracene by MnP, and it was followed by the production of 9,10-anthraquinone. Anthraquinone has earlier been revealed as the typical oxidation product in *in vitro* reactions of peroxidases. More oxidation resulted in the production of phthalic acid, as it was shown in ligninolytic cultures of *P. chrysosporium* (Hammel et al., 1991). The characteristic ring-cleavage product 2-(2_-hydroxybenzoyl)-benzoic acid shows that MnP is capable to cleave even the aromatic ring of a PAH molecule. One single report was found suggesting that MnP does not oxidize anthracene in the presence of Mn^{2+} (Vazquez-Duhalt et al., 1994).

Since the high hydrophobicity of PAHs significantly inhibits their degradation in liquid media, MnP degraded anthracene, dibenzothiophene, and pyrene in the presence of acetone (36% v/v), which is a miscible organic solvent. Anthracene was degraded to phthalic acid and had the highest degradation rate, followed by dibenzothiophene and then pyrene (Eibes et al., 2006b).

Degradation of PAHs by crude MnP produced by *Nematoloma frowardii* was experimented on separate PAHs: PHE, ANT, PYR, FLA, and B[a]A and then on a mixture of different PAHs: PHE, ANT, PYR, FLA, CHR, B[a]A, B[a]P, and benzo[b]fluoranthene. The oxidation of PAHs was enhanced in the presence of glutathione which is a mediator substance capable to generate reactive thiyl radicals. Products of glutathione-mediated MnP mineralization were: 14C-PYR (7.3%), 14C-ANT (4.7%), 14C-B[a]P (4.0%), 14C-B[a]A (2.9%), and 14C-PHE (2.5%) (Sack et al., 1997c). The induction effect of reduced glutathione (GSH) was also investigated by Thomas Günther, (1998) and showed an increase of the oxidative strength of MnP. As a consequence anthracene was fully reduced and 60% of pyrene was degraded after only 24h.

Therefore, alternative redox mediators, increasing the oxidative effect of MnP have been investigated. MnP was capable to oxidize FLU which has a high IP value (8.2 eV) and creosote which is a complex PAHs mixture in the presence of Tween-80. Also, Tween-80 enable MnP produced by *Stropharia coronilla* to oxidize a large amount of B[a]P into polar fragments (Steffen et al., 2003).

9.2.2 LiP

LiP is able to oxidize several phenolic and non-phenolic substrates with calculated ionization potential, a measure for the ease to abstract an electron from the highest filled molecular orbital, up to 9.0 eV (ten Have and Teunissen, 2001). LiP has been revealed to entirely oxidize methylated lignin and lignin model compounds as well as several polyaromatic hydrocarbons (Hammel et al., 1992a). Among the oxidation reactions catalyzed by LiP are the cleavage of Cα-Cβ and aryl Cα bond, aromatic ring opening, and demethylation (Kaal et al., 1995). One secondary metabolite, veratryl alcohol (VA), has been the focus of many studies. VA is a rich substrate for LiP and increases the oxidation of otherwise weak or terminal LiP substrates (Ollikka et al., 1993). Three main roles of VA have been recommended so far. As defined earlier, VA could behave as a mediator in electron-transfer reactions. Secondly, VA is a good substrate for compound II; for that reason, VA is important for completing the catalytic cycle of LiP through the oxidation of terminal substrates.127 Thirdly, VA prevents the H_2O_2-dependent inactivation of LiP by reducing compound II back to native LiP. In addition, if the inactive LiP compound III is established, the intermediate VA^+ is able to reduce LiP compound III back to its native form (ten Have and Teunissen, 2001).

Purified LiP from *P. chrysosporium* had been shown to attack B[a]P using one-electron abstractions, causing unstable B[a]P radicals which undergo further spontaneous reactions to hydroxylated metabolites and many B[a]P quinones (Haemmerli et al., 1986). benzo[a]pyrene-1,6-, 3,6-, and 6,12-quinones were detected as the products of B[a]P oxidation by *P. chrysosporium* LiP. At the same time with the appearance of oxidation products, LiP was inactivated. Similarly to all peroxidases, LiP is inhibited by the presence of hydrogen peroxide (Valderrama et al., 2002); the addition of VA to the reaction mixture could stabilize the enzyme. The oxidation rate is ameliorated more than 14 times in the presence of VA, and the most of the enzyme activity was retained during the B[a]P oxidation (Haemmerli et al., 1986).

Most of reports on the oxidation of PAHs with LiP concentrated on LiP from *P. chrysosporium* as shown in Table 3. Anthraquinone is the major product of anthracene oxidation by LiP produced by *P. chrysosporium* (Field et al., 1996). Hammel et al., (1986a) demonstrated that LiP produced by *P. chrysosporium* catalyzes the degradation of certain PAHs with IP<7.55 eV. As a consequence, H_2O_2-oxidized states of LiP are more oxidizing than the analogous states of standard peroxidases.

Studies on pyrene as a substrate showed that pyrene-1,6-dione and pyrene-1,8-dione are the principle oxidation products. Gas chromatography/mass spectrometry analysis of LiP-

816 catalyzed pyrene oxidation done in the presence of H_2O_2 revealed that the quinone oxygens
817 come from water. The one-electron oxidative mechanism of LiP is relevant to lignin and
818 lignin-related substructures as well as certain polycyclic aromatic and heteroaromatic
819 contaminants. The oxidation of pyrene by entire cultures of *P. chrysosporium* also generated
820 these quinones. As a result, it can be concluded that LiP catalyzes the first step in the
821 degradation of these compounds by entire cultures of *P. chrysosporium* (Hammel et al.,
822 1986).
823 Vazquez-Duhalt et al., (1994) utilized LiP from *P. chrysosporium* to investigate the oxidation
824 of anthracene, 1-, 2-, and 9- methylanthracenes, acenaphthene, fluoranthene, pyrene,
825 carbazole, and dibenzothiophene. Among the studied compounds, LiP was able to oxidize
826 compounds with IP<8 eV. The greatest specific activity of PAHs oxidation was shown when
827 pHs are between 3.5 and 4.0. The reaction products involve hydroxyl and keto groups. The
828 product of anthracene oxidation was 9,10-anthraquinone. The products of LiP oxidation of 1-
829 and 2-methylanthracene were 1- and 2-methylanthraquinone, respectively.
830 9,10-anthraquinone, 9-methyleneanthranone, and 9-methanol-9,10- dihydroanthracene were
831 the products detected by from the oxidation of 9-methylanthracene (Vazquez-Duhalt et al.,
832 1994). Anthraquinone resulting from carbon-carbon bond cleavage of 9-methylanthracene,
833 was also observed. The mass spectra of the two products resulting from acenaphthene
834 correspond to 1-acenaphthenone and 1-acenaphthenol. The comparison of the GC-mass
835 spectrometry analysis of dibenzothiophene oxidation by LiP with a sample of authentic
836 dibenzothiophene sulfoxide resulted in sulfoxide. The UV spectrum of the product of pyrene
837 oxidation most closely fitted that of 1,8- pyrenedione. In spite fluoranthene and carbazole
838 were oxidized, their products were not established (Vazquez-Duhalt et al., 1994).
839 Torres et al., (1997) studied LiP, cytochrome *c*, and hemoglobin for oxidation of PAHs in the
840 presence of hydrogen peroxide and demonstrated that LiP oxidized anthracene, 2-
841 methylanthracene, 9- hexylanthracene, pyrene, acenaphthene, and benzo[a]pyrene; the
842 unreacted compounds included chrysene, phenanthrene, naphthalene, triphenylene, biphenyl,
843 and dibenzofuran. The oxidation of the aromatic compounds by LiP matched with their IPs;
844 only those compounds that had IPs<8 eV were transformed. The reaction products from the
845 three hemoproteins (LiP, cytochrome *c*, and hemoglobin) were principally quinones, which
846 suggest that the three biocatalysts have the same oxidation mechanism. The resulting product
847 from anthracene was anthraquinone, and the resulting product from 2-methylanthracene was
848 2-methylanthraquinone. The ending products for pyrene and benzo[a]pyrene oxidation were
849 pyrenedione and benzo[a]pyrenedione, respectively. The mass spectra results of the products

from acenaphthene degradation catalyzed by LiP correlated well with 1-acenaphthenone and 1-acenaphthenol (Torres et al., 1997).

Expriments on the catalytic properties of ligninolytic enzymes demonstrates that degradation by LiP is restricted to certain range of compounds according to their IP values. Furthermore, the catalytic activities of MnP and LAC are extended to the following factors (a) the presence of some natural and synthetic mediators such as ABTS for LAC and gluthatione for MnP and LAC; (b) the modification of the active center of LAC during fermentation of a fungi on lignin-containing natural substrates; (c) the combination of PAH oxidation with lipid peroxidation (MnP and LAC). Therefore, MnP and LAC can be considered as the most effective in PAHs oxidation since their role extends to the initial oxidation and production of quinones (Pozdnyakova, 2012).

$$Ferric\ enzyme + H_2O_2 \overset{k_1}{\rightarrow} Compound\ I + H_2O \quad (1)$$

$$Compound\ I + RH \overset{k_2}{\rightarrow} Compound\ II + R^{\circ} \quad (2)$$

$$Compound\ II + RH \overset{k_3}{\rightarrow} Ferric\ enzyme + R^{\circ} + H_2O \quad (3)$$

$$Compound\ II + RH \overset{K_J}{\leftrightarrow} Compound\ II - - - RH \rightarrow k_3$$

$$Ferric\ enzyme + R^{\circ} + H_2O \quad (4)$$

$$Compound\ II + H_2O_2 \rightarrow Compound\ III \quad (5)$$

*RH represents the reducing substrate and R° represents the reducing substrate after one electron oxidation

9.2.3 Catalytic cycle of laccase

Laccases are known to catalyze the oxidation of a significant variety of phenolic compounds and aromatic amines (Peng et al., 2015). When certain substrates can potentially provide two electrons such as ABTS, laccases carry out one-electron oxidation. As a result, radicals are produced which undergo subsequent non-enzymatic reactions as seen in Equation 6.

$$4RH + O_2 \rightarrow 4R + 2H_2O \quad (6)$$

Hundreds of studies have been done on the characteristics of fungal laccases. And most of the research has been investigating tree laccases or other copper-containing oxidases (Tollin et al., 1993).

Even though, the redox potential of laccases (0.5-0.8 V) does not favor the oxidation of non-phenolic compounds, numerous studies have demonstrated that laccases are capable of oxidizing compounds which have redox potentials higher than that of the enzyme. In these studies, ABTS, 1- hydroxybenzotriazole (HOBT) or 3-hydroxyanthrani- late were applied as a cooxidant/mediator, and non-phenolic lignin, veratryl alcohol, and PAH were oxidized (Collins and Dobson, 1996; Eggert et al., 1996; Bourbonnais et al., 1997; Majcherczyk et al., 1998a). The enzyme kinetic background of these reactions is still not identified.

10 Conclusions

Enzymatic bioremediation is the tool to convert PAHs to less harmful/non-harmful forms with less chemicals, energy, and time. It is a solution to degrade/remove contaminants in an eco-friendly way. From the early to the current research, vast range of fungi have proved their efficiency in the bioremediation of PAH-contaminated wastes through enzymes, such as MnP, LiP, laccase and other fungal enzymes, such as Cytochrome P450 monooxygenase, epoxide hydrolases, lipases, protease and dioxygenases.

The enzymatic bioremediation of a pollutant and the rate at which it is reached relies upon the environmental conditions, number and type of the microorganisms, characteristics of the chemical compound to degrade. Hence, to improve the degradation rate and develop a bioremediation system, various factors are accountable which need to be dealt with and are to be investigated, such as pretreatment at high temperature.

Powerful and cost-effective bioremediation should involve either entire mineralization of the PAHs or at minimum biotransformation to less harmful compounds. Generally, fungal rates of degradation of PAHs are slow and inefficient compared to bacteria; however, since numerous fungi have the ability to hydroxylate a wide variety of PAHs, their ecological role could be significant since these polar intermediates can be mineralized by soil bacteria or detoxified to simpler non-hazardous compounds. Additionally, fungi have an advantage over bacteria since the fungal mycelium could grow into the soil and spread itself through the solid matrix to degrade the PAHs. To improve and empower biodegradative potential of fungi, substantial

research on the enzymes included in PAH degradation pathways and on the molecular genetics and biochemistry of catabolic pathways is required.

Acknowledgements

The authors are sincerely thankful to the Natural Sciences and Engineering Research Council of Canada (Discovery Grant 355254, CRD Grant and Strategic Grant 447075) for financial support. The views or opinions expressed in this article are those of the authors.

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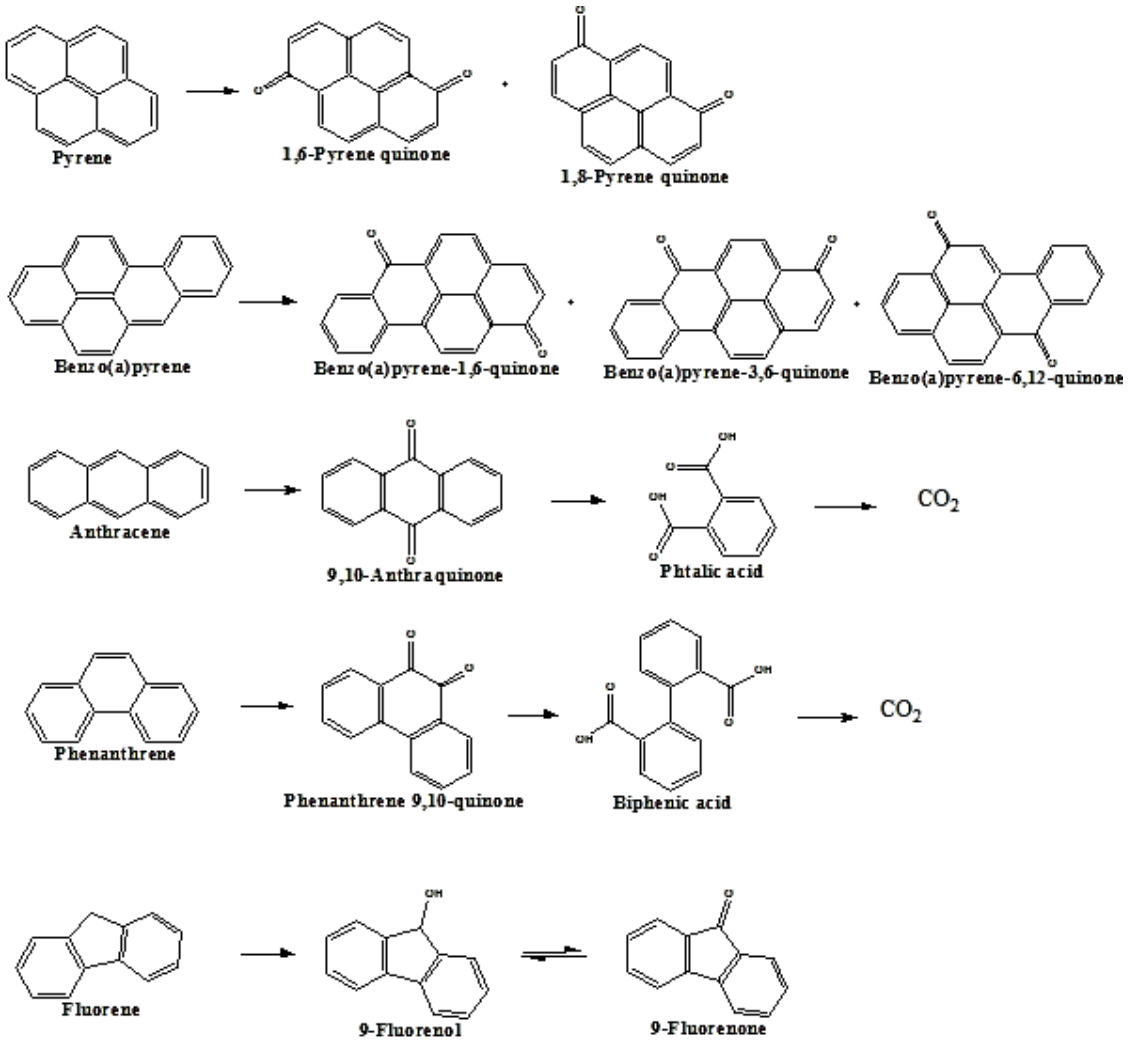


Fig.1. Oxidation of polycyclic aromatic hydrocarbons by ligninolytic fungi

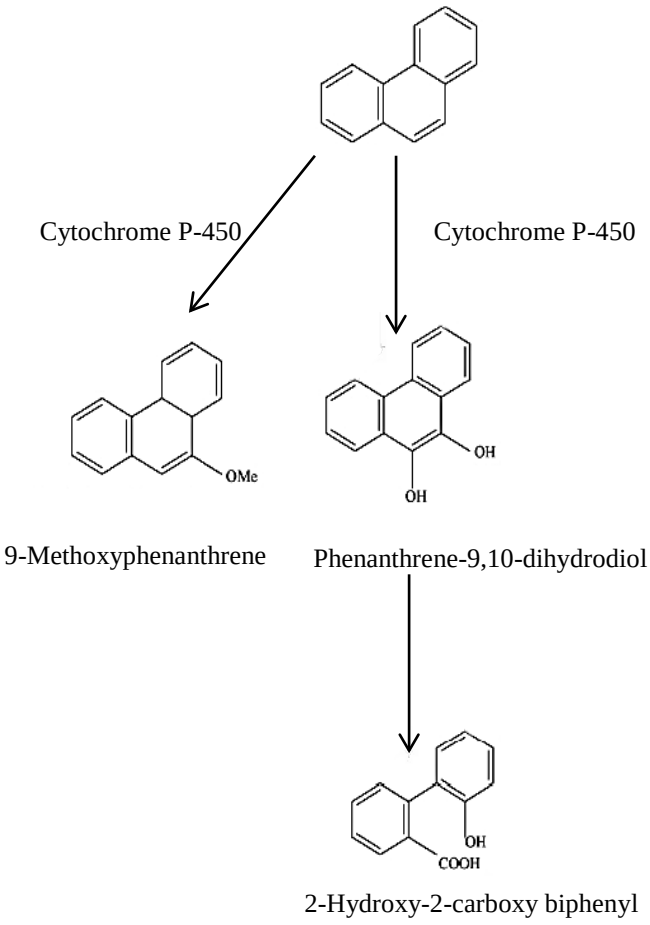


Fig.2. Degradation pathway of phenanthrene using the fungus, *Irpex lacteus* (Modified from Cajthaml et al., 2002)

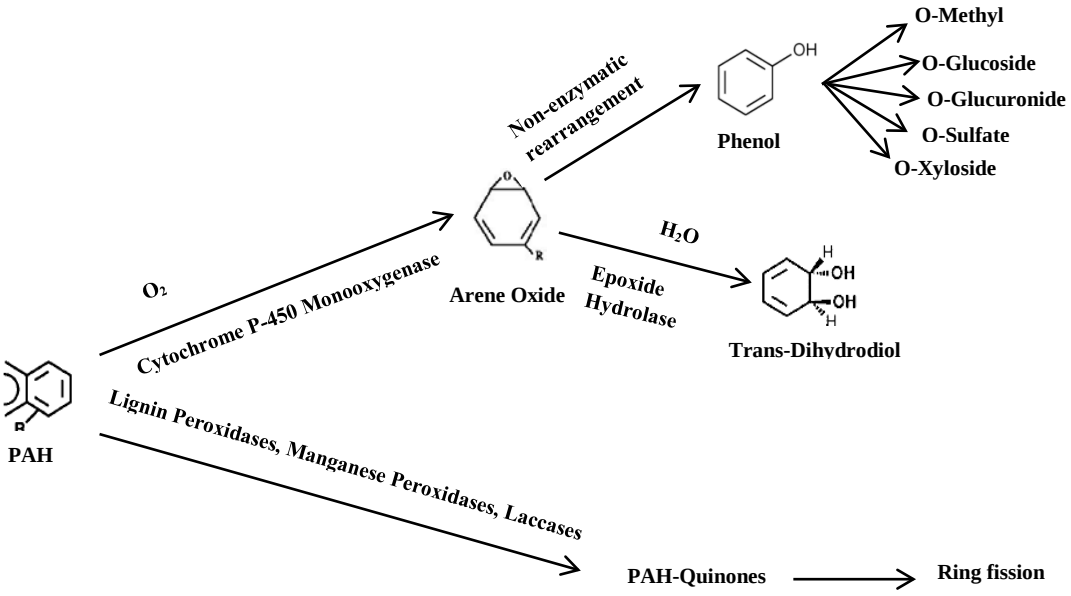
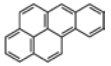
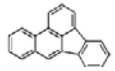
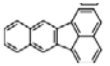


Fig.3. Different pathways for the fungal metabolism of polycyclic aromatic hydrocarbons

Table 1: Physical-chemical characteristics of different polycyclic aromatic hydrocarbons

Compound	formula	Mol. wt. (g mol^{-1})	CAS registry No.	Vapour Pressure (Pa at 25°C)	Boiling Point (°C)	Melting Point (°C)	Aqueous solubility (mg/l)	Ionization Potential(eV)[a]	Structure
Naphthalene	C10H8	128	91-20-3	11.9	218	80.2	30	-	
Anthracene	C14H10	178	120-12-7	3.4×10^{-3}	340	216.4	0.015	7.43	
Phenanthrene	C14H10	178	85-01-8	9.07×10^{-2}	339-340	100.5	1-2	8.03	
Fluoranthene	C16H10	202	206-44-0	1.08×10^{-3}	375-393	108.8	0.25	7.90	
Pyrene	C16H10	202	129-00-0	5.67×10^{-4}	360-404	393	0.12-0.18	7.53	
benz[a]anthracene	C18H12	228	56-55-3	14.7 x 10^{-3}	438	162	0.0057	<7.35	
benz[a]pyrene		252	50-32-8	0.37 x 10^{-6}	495	179	0.0038	<=7.45	
Benzo[b]fluoranthene	C20H12	252	205-99-2	1.07×10^{-5}	168	168.3	-	7.70	
Benzo[k]fluoranthene	C20H12	252	207-08-9	1.28×10^{-8}	217	215.7	-	7.48	
Benzo(ghi)perylene	C22H12	276	191-24-2	1.33×10^{-8}	525	277	-	7.31	

[a]IPs for all the PAHs except benzo[b]fluoranthene and benzo[k]fluoranthene are from (Pysh and Yang, 1963). The IPs were determined by the polarographic oxidation method. IPs for benzo[b]fluoranthene and benzo[k]fluoranthene are from the modified neglect of diatomic overlap calculations of (Simonsick and Hites, 1986).

Table 2: Polycyclic aromatic hydrocarbons oxidized by different species of fungi and their corresponding metabolites

Compounds	Microorganisms	References	Metabolites	References
Acenaphtene	*Cunninghamella elegans*	(J V Pothuluri et al., 1992)	1-Acenaphthenone, 1,2-Acenaphthenedione, *cis*-1,2-Dihydroxyacenaphthene, *trans*-1,2-Dihydroxyacenaphthene, 1,5-Dihydroxyacenaphthene, 6-Hydroxyacenaphthenone	(J V Pothuluri et al., 1992)
Anthracene	*Bjerkandera* sp, *Cunninghamella elegans, Naematoloma frowardii, Phanerochaete chrysosporium, Phanerochaete laevis, Pleurotus ostreatus, Pleurotus sajor-caju, Ramaria* sp, *Rhizoctonia solani, Trametes versicolor*	(L. Bezalel et al., 1996; Bogan and Lamar, 1995; Cerniglia and Yang, 1984; Hammel et al., 1992a; Johannes and Majcherczyk, 2000; Kotterman et al., 1998; Sack and Günther, 1993)	Anthracene *trans*-1,2-Dihydrodiol 1-Anthrol, 9,10-Anthraquinone, Phthalate, Glucuronide, Sulfate and Xyloside conjugates of hydroxylated intermediates	(Lea Bezalel et al., 1996a; Cerniglia, 1982; Cerniglia and Yang, 1984; Collins and Dobson, 1996; Field et al., 1992; Hammel et al., 1991; Johannes et al., 1996; Sutherland et al., 1992)
Phenanthrene	*Aspergillus niger, Cunninghamella elegans, Naematoloma frowardii, Phanerochaete chrysosporium, Phanerochaete laevis, Pleurotus ostreatus, Syncephalastrum racemosum, Trametes versicolor*	(L. Bezalel et al., 1996; Bogan and Lamar, 1996; Bumpus, 1989; Cerniglia, 1997; Hammel et al., 1992a; Kotterman et al., 1998; Sack and Günther, 1993)	Phenanthrene *trans*-1,2-dihydrodiol Phenanthrene *trans*-3,4-dihydrodiol Phenanthrene *trans*-9,10-dihydrodiol Glucoside conjugate of 1-phenanthrol 1-,2-,3-,4-, and 9-phenanthrol 1-methoxyphenanthrene, Phenanthrene-9,10-quinone 2,2□-Diphenic acid	(Lea Bezalel et al., 1996b; Casillas et al., 1996; Cerniglia et al., 1989; Cerniglia and Yang, 1984; Hammel et al., 1992b; Sack et al., 1997a, 1997b; Sutherland et al., 1991)

Fluorene	*Cunninghamella elegans, Laetiporus sulphureus, Phanerochaete chrysosporium, Pleurotus ostreatus, Trametes versicolor*	(L. Bezalel et al., 1996; B W Bogan et al., 1996; Bogan and Lamar, 1996; Sack and Günther, 1993)	9-Fluorenone 9-Fluorenol 2-Hydroxy-9-fluorenone	(Lea Bezalel et al., 1996a; Bill W. Bogan et al., 1996; Pothuluri et al., 1993)
Fluoranthene	*Cunninghamella elegans, Naematoloma frowardii, Laetiporus sulphureus, Penicillium* sp, *Pleurotus ostreatus*	(Sack and Günther, 1993)	Fluoranthene *trans*-2,3-dihydrodiol, 8 and 9-Hydroxyfluoranthene *trans*-2,3-dihydrodiols, Glucoside conjugates of hydroxylated intermediates	(JAIRAJ V. Pothuluri et al., 1992; Pothuluri et al., 1990)
Pyrene	*Aspergillus niger, Agrocybe aegerita, Candida parapsilopsis, Crinipellis maxima, Crinipellis perniciosa, Crinipellis stipitaria, Crinipellis zonata, Cunninghamella elegans, Fusarium oxysporum, Kuehneromyces mutablis, Marasmiellus ramealis,* *Marasmius rotula, Mucor* sp, *Naematoloma frowardii, Penicillium janczewskii,* *Penicillium janthinellum, Phanerochaete chrysosporium, Pleurotus ostreatus,* *Syncephalastrum racemosum,* *Trichoderma harzianum*	(L. Bezalel et al., 1996; Hammel et al., 1986; Manilla-Pérez et al., 2011)	1,6-Pyrenequinone 1,8-Pyrenequinone Glucoside conjugates 1-Pyrenol 1,6-dihydroxypyrene 1,8-dihydroxypyrene 1-Pyrene sulfate 1-Hydroxy-8-pyrenyl sulfate 6-Hydroxy-1-pyrenyl sulfate Pyrene *trans*-4,5-Dihydrodiol	(Lea Bezalel et al., 1996a; Cerniglia et al., 1986; Hammel et al., 1986; Lange et al., 1996; Launen et al., 1995; Sack et al., 1997a)
Benzo[a]anthracene	*Candida krusei, Cunninghamella elegans, Phanerochaete chrysosporium Phanerochaete laevis,*	(Bill W. Bogan et al., 1996;	Benz[*a*]anthracene *trans*-3,4-dihydrodiol, Benz[*a*]anthracene *trans*-8,9-dihydrodiol,	(Cerniglia et al., 1994; C. E. Cerniglia et al., 1980)

	Pleurotus ostreatus, Rhodotorula minuta, Syncephalastrum racemosum, Trametes versicolor	Cerniglia, 1984)	Benz[*a*]anthracene *trans*-10,11-dihydrodiol, Phenolic and tetrahydroxy derivativesof benz[*a*]anthracene, Glucuronide and Sulfate conjugates of hydroxylated intermediates	
Benzo[a]pyrene	*Aspergillus ochraceus, Bjerkandera adusta, Bjerkandera* sp, *Candida maltosa,* *Candida maltosa, Candida tropicalis, Chrysosporium pannorum, Cunninghamella elegans, Mortierella verrucosa, Naematoloma frowardii, Neurospora crassa, Penicillium janczewskii, Penicillium janthinellum, Phanerochaete chrysosporium, Phanerochaete laevis, Pleurotus ostreatus, Ramaria* sp, *Saccharomyces cerevisiae,* *Syncephalastrum racemosum, Trametes versicolor, Trichoderma* sp, *Trichoderma* *viride*	(L. Bezalel et al., 1996; Bogan and Lamar, 1996; Bumpus et al., 1985; Haemmerli et al., 1986; Sack and Günther, 1993)	Benzo[*a*]pyrene *trans*-4,5-dihydrodiol Benzo[*a*]pyrene *trans*-7,8-dihydrodiol Benzo[*a*]pyrene *trans*-9,10-dihydrodiol Benzo[*a*]pyrene-1,6-quinone Benzo[*a*]pyrene-3,6-quinone Benzo[*a*]pyrene-6,12-quinone 3-Hydroxybenzo[*a*]pyrene 9-Hydroxybenzo[*a*]pyrene 7b,8a,9a,10b-tetrahydrobenzo[*a*]pyrene, 7b,8a,9a,10b-tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene, Benzo[*a*]pyrene 7,8-dihydrodiol-9,10-epoxide, Glucuronide and Sulfate conjugates of hydroxylated intermediates	(Carl E. Cerniglia et al., 1980; Cerniglia and Gibson, 1980a, 1980b, 1979; Haemmerli et al., 1986; Launen et al., 1995)
Chrysene	*Cunninghamella elegans, Penicillum janthinellum, Syncephalastrum racemosum*	(Kiehlmann et al., 1996; Pothuluri et al., 1995)	2-Chrysenyl sulfate 2-Hydroxy-8-chrysenylsulfate Chrysene *trans*-1,2-dihydrodiol	(Kiehlmann et al., 1996; Pothuluri et al., 1995)

Benzo[e]pyrene	*Cunninghamella elegans*	(Pothuluri et al., 1996)	3-Benzo[*e*]pyrenyl sulfate	(Pothuluri et al., 1996)
			10-Hydroxy-3-benzo[*e*]pyrenyl sulfate	
			Benzo[*e*]pyrene-3-0-b-glucopyranoside	

Table 3: Polycyclic aromatic hydrocarbons oxidation by different enzymes

Enzymes	Microorganisms	PAHs	Products	References
LiP	*P. chrysosporium*	B[a]P	B[a]P-1,6-quinone B[a]P-3,6-quinone B[a]P-6,12-quinone	(Haemmerli et al., 1986; Torres et al., 1997)
		ANT	9,10-anthraquinone	(Field et al., 1996; Torres et al., 1997; Vazquez-Duhalt et al., 1994)
		PYR	PYR-1,6-dione; PYR-1,8-dione	(Hammel et al., 1986; Torres et al., 1997; Vazquez-Duhalt et al., 1994)
		FLA	ND	(Vazquez-Duhalt et al., 1994)
		1-methylanthracene	1-methylanthraquinone	(Vazquez-Duhalt et al., 1994)
		2-methylanthracene	2-methylanthraquinone	(Torres et al., 1997; Vazquez-Duhalt et al., 1994)
		9-methylanthracene	9-anthraquinone; 9-methyleneanthranone; 9-methanol-9,10-dihydroanthracene	(Vazquez-Duhalt et al., 1994)
		Acenaphthene	1-acenaphthenone; 1-acenaphthenol	(Torres et al., 1997; Vazquez-Duhalt et al., 1994)
		Dibenzothiophene	dibenzothiophene sulfoxide	(Vazquez-Duhalt et al., 1994)
MnP	*Anthracophyllum discolor*	PYR; ANT; FLA; PHE	ND	(Acevedo et al., 2010)
		PHE; ANT; FLA; PYR	9,10-anthraquinone	(Baborová et al., 2006)
	I. lacteus	ANT	anthrone; 9,10-anthraquinone; 2-(2_-hydroxybenzoyl)-benzoic acid; phthalic acid	(Eibes et al., 2006; Field et al., 1996; Hammel et al., 1991; Moen and Hammel, 1994)
	P. chrysosporium	FLU	9-fluorenone	(Bill W. Bogan et al., 1996)

		PHE	PHE-9,10-quinone; 2,2_-diphenic acid	(Moen and Hammel, 1994)
		dibenzothiophene	4-methoxybenzoic acid	(Eibes et al., 2006)
	Nematoloma frowardii (*Phlebia* sp.)	PHE; ANT; PYR; FLA; CHR; B[a]A; B[a]P; benzo[b]fluoranthene	CO2 from PHE; ANT; PYR; B[a]A; B[a]P	(Sack et al., 1997c; Thomas Günther, 1998)
	Stropharia coronilla	ANT; B[a]P	9,10-anthraquinone; CO2; B[a]P-1,6-quinone	(Steffen et al., 2003, 2002)
LAC	*C. hirsutus*	ANT; PHE; PYR; FLA; B[a]P B[a]P; ANT; PHE; FLU;	ND	(Cho et al., 2002)
	Coriolopsis gallica	9-methylanthracene; 2-methylanthracene; Acenaphthene; carbazole; N-ethylcarbazole; Dibenzothiophene	9-fluorenone; dibenzothiophene sulfone	(Bressler et al., 2000; Pickard et al., 1999)
	Ganoderma lucidum	ANT; FLU; B[a]A; B[a]P; Acenaphthene; Acenaphthylene	ND	(Hunsa Punnapayak, 2009)
	P. ostreatus	ANT; PHE; FLU; PYR; FLA; perylene	9,10-anthraquinone; 9-fluorenone	(Pozdnyakova et al., 2006)
	Pycnoporus cinnabarinus	B[a]P	B[a]P-1,6-quinone; B[a]P-3,6-quinone; B[a]P-6,12-quinone	(Rama et al., 1998)
	T. versicolor	Acenaphthene; PHE; ANT; Acenaphthylene, B[a]P; ANT; FLA; PYR; B[a]A; CHR; perylene;	1,2-acenaphthenedione 1,8-naphthalic acid anhydride; 9,10-anthraquinone; PHE-9,10-quinone, 2,2_-diphenic	(Binková and Šrám, 2004; Böhmer et al., 1998; Cañas et al., 2007; Collins et al., 1996; Johannes et al., 1998; Johannes and Majcherczyk, 2000; Majcherczyk et al., 1998)

benzo[b]fluoranthene;	acid; B[a]P-1,6-quinone;
	B[a]P-3,6-quinone;
benzo[k]fluoranthene; FLU	B[a]P-6,12-quinone