ARTICLE IN PRESS

JOURNAL OF ENVIRONMENTAL SCIENCES XX (2016) XXX–XXX

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jes

www.jesc.ac.cn

Review

Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: A review

Tayssir Kadri[1], *Tarek Rouissi*[1], *Satinder Kaur Brar*[1,*], *Maximiliano Cledon*[1], *Saurabhjyoti Sarma*[1], *Mausam Verma*[2]

1. *INRS-ETE, Université du Québec, 490 Rue de la Couronne, Québec, QC G1K 9A9, Canada*
2. CO_2 *Solutions Inc., 2300, rue Jean-Perrin, Québec, QC G2C 1T9, Canada*

ARTICLE INFO

Article history:
Received 29 March 2016
Revised 22 August 2016
Accepted 23 August 2016
Available online xxxx

Keywords:
Polycyclic aromatic hydrocarbons (PAHs)
Biodegradation
Fungi
Enzymes

ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are a large group of chemicals. 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. Several pilot treatments have been implemented to prevent economic consequences and deterioration of soil and water quality. As a promising option, fungal enzymes are regarded as a powerful choice for degradation of PAHs. *Phanerochaete chrysosporium*, *Pleurotus ostreatus* and *Bjerkandera adusta* are most commonly used for the degradation of such compounds due to their production of ligninolytic enzymes such 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.
© 2016 The Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. Published by Elsevier B.V.

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.

* *Corresponding author*. E-mail: satinder.brar@ete.inrs.ca (Satinder Kaur Brar).

http://dx.doi.org/10.1016/j.jes.2016.08.023
1001-0742/© 2016 The Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. Published by Elsevier B.V.

Please cite this article as: Kadri, T., et al., Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: A review, J. Environ. Sci. (2016), http://dx.doi.org/10.1016/j.jes.2016.08.023

UNCORRECTED PROOF

ARTICLE IN PRESS

Contents

Introduction . . . 0
1. Fungal enzymes . . . 0
2. Different species of fungus enzyme-degrading PAHs . . . 0
2.1. *P. chrysosporium* . . . 0
2.2. *P. ostreatus* . . . 0
2.3. *B. adusta* . . . 0
3. Culture conditions . . . 0
4. Ionization potential . . . 0
5. Kinetics . . . 0
6. Soil and liquid cultures . . . 0
7. Degradation pathways . . . 0
8. Mechanism of degradation with enzymes . . . 0
8.1. Characteristics of ligninolytic enzymes . . . 0
8.1.1. Characteristics of peroxidases . . . 0
8.1.2. Characteristics of laccase . . . 0
8.1.3. Mediators of laccase . . . 0
8.2. Catalytic cycle of peroxidases . . . 0
8.2.1. MnP . . . 0
8.2.2. LiP . . . 0
8.2.3. Catalytic cycle of laccase . . . 0
9. Conclusions . . . 0
Uncited reference . . . 0
Acknowledgments . . . 0
References . . . 0

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 vapor pressure as seen in Table 1. With an increase in molecular weight, their solubility in water decreases; melting and boiling point increases and vapor 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 occur 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 (W. 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 rely upon

Please cite this article as: Kadri, T., et al., Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: A review, J. Environ. Sci. (2016), http://dx.doi.org/10.1016/j.jes.2016.08.023

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 has 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, the 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.

1. 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., 1994; 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 have 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 *P. chrysosporium*, *Trametes versicolor* and *P. 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., 2015a, 2015b). 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).

Table 1 – Physical–chemical characteristics of different polycyclic aromatic hydrocarbons.

Compound	Formula	Mol. wt. (g/mol)	CAS registry No.	Vapor pressure (Pa at 25°C)	Boiling point (°C)	Melting point (°C)	Aqueous solubility (mg/l)	Ionization potential (eV) [a]	Structure
Naphthalene	$C_{10}H_8$	128	91-20-3	11.9	218	80.2	30	–	
Anthracene	$C_{14}H_{10}$	178	120-12-7	3.4×10^{-3}	340	216.4	0.015	7.43	
Phenanthrene	$C_{14}H_{10}$	178	85-01-8	9.07×10^{-2}	339–340	100.5	1–2	8.03	
Fluoranthene	$C_{16}H_{10}$	202	206-44-0	1.08×10^{-3}	375–393	108.8	0.25	7.90	
Pyrene	$C_{16}H_{10}$	202	129-00-0	5.67×10^{-4}	360–404	393	0.12–0.18	7.53	
Benz[*a*]anthracene	$C_{18}H_{12}$	228	56-55-3	14.7×10^{-3}	438	162	0.0057	<7.35	
Benz[*a*]pyrene		252	50-32-8	0.37×10^{-6}	495	179	0.0038	≤7.45	
Benzo[b]fluoranthene	$C_{20}H_{12}$	252	205-99-2	1.07×10^{-5}	168	168.3	–	7.70	
Benzo[k]fluoranthene	$C_{20}H_{12}$	252	207-08-9	1.28×10^{-8}	217	215.7	–	7.48	
Benzo(ghi)perylene	$C_{22}H_{12}$	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).

Please cite this article as: Kadri, T., et al., Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: A review, J. Environ. Sci. (2016), http://dx.doi.org/10.1016/j.jes.2016.08.023

ARTICLE IN PRESS

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

Compounds	Microorganisms	References	Metabolites	References
Acenaphtene	*Cunninghamella elegans*	(Pothuluri et al., 1992a, 1992b)	1-Acenaphthenone, 1,2-Acenaphthenedione, *cis*-1,2-Dihydroxyacenaphthene, *trans*-1,2-Dihydroxyacenaphthene, 1,5-Dihydroxyacenaphthene, 6-Hydroxyacenaphthenone	(Pothuluri et al., 1992a, 1992b)
Anthracene	*Bjerkandera* sp., *Cunninghamella elegans, Naematoloma frowardii, Phanerochaete chrysosporium, Phanerochaete laevis, Pleurotus ostreatus, Pleurotus sajor-caju, Ramaria* sp., *Rhizoctonia solani, Trametes versicolor*	(Bezalel et al., 1996a, 1996b, 1996c; Bogan and Lamar, 1995; Cerniglia and Yang, 1984; Hammel et al., 1992; 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	(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*	(Bezalel et al., 1996a, 1996b, 1996c; Bogan and Lamar, 1996; Bumpus, 1989; Cerniglia, 1997a, 1997b; Hammel et al., 1992; 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	(Bezalel et al., 1996b; Casillas et al., 1996; Cerniglia et al., 1989; Cerniglia and Yang, 1984; Hammel et al., 1992; Sack et al., 1997a, 1997b; Sutherland et al., 1991)
Fluorene	*Cunninghamella elegans, Laetiporus sulphureus, Phanerochaete chrysosporium, Pleurotus ostreatus, Trametes versicolor*	(Bezalel et al., 1996a, 1996b, 1996c; Bogan et al., 1996a, 1996b; Bogan and Lamar, 1996; Sack and Günther, 1993)	9-Fluorenone 9-Fluorenol 2-Hydroxy-9-fluorenone	(Bezalel et al., 1996a; Bogan et al., 1996a, 1996b; 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	(Pothuluri et al., 1992a, 1992b; Pothuluri et al., 1990)
Pyrene	*Aspergillus niger, Agrocybe aegerita, Candida parapsilopsis, Crinipellis maxima, Crinipellis perniciosa, Crinipellis stipitaria, Crinipellis zonata, Cunninghamella elegans, Fusarium oxysporum,*	(Bezalel et al., 1996a, 1996b, 1996c; Hammel et al., 1986; Manilla-Pérez et al., 2011)	1,6-Pyrenequinone 1,8-Pyrenequinone Glucoside conjugates 1-Pyrenol 1,6-dihydroxypyrene	(Bezalel et al., 1996a; Cerniglia et al., 1986; Hammel et al., 1986; Lange et al., 1996; Launen et al., 1995; Sack et al., 1997a)

Please cite this article as: Kadri, T., et al., Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: A review, J. Environ. Sci. (2016), http://dx.doi.org/10.1016/j.jes.2016.08.023

ARTICLE IN PRESS

		Kuehneromyces mutablis, Marasmiellus ramealis, Marasmius rotula, Mucor sp., *Naematoloma frowardii, Penicillium janczewskii, Penicillium janthinellum, Phanerochaete chrysosporium, Pleurotus ostreatus, Syncephalastrum racemosum, Trichoderma harzianum*		1,8-dihydroxypyrene 1-Pyrene sulfate 1-Hydroxy-8-pyrenyl sulfate 6-Hydroxy-1-pyrenyl sulfate Pyrene *trans*-4,5-Dihydrodiol	
1	Benzo[a]anthracene	*Candida krusei, Cunninghamella elegans, Phanerochaete chrysosporium Phanerochaete laevis, Pleurotus ostreatus, Rhodotorula minuta, Syncephalastrum racemosum, Trametes versicolor*	(Bogan et al., 1996a, 1996b; Cerniglia, 1984)	Benz[*a*]anthracene *trans*-3,4-dihydrodiol, Benz[*a*]anthracene *trans*-8,9-dihydrodiol, Benz[*a*]anthracene *trans*-10,11-dihydrodiol, Phenolic and tetrahydroxy derivativesof benz[*a*]anthracene, Glucuronide and Sulfate conjugates of hydroxylated intermediates	(Cerniglia et al., 1994; Cerniglia et al., 1980a, 1980b)
2	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*	(Bezalel et al., 1996a, 1996b, 1996c; 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	(Cerniglia et al., 1980a, 1980b; Cerniglia and Gibson, 1979, 1980a, 1980b; Haemmerli et al., 1986; Launen et al., 1995)
3	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)
4	Benzo[e]pyrene	*Cunninghamella elegans*	(Pothuluri et al., 1996)	3-Benzo[*e*]pyrenyl sulfate 10-Hydroxy-3-benzo[*e*]pyrenyl sulfate Benzo[*e*]pyrene-3-0-b-glucopyranoside	(Pothuluri et al., 1996)

Q6

PROOF

Please cite this article as: Kadri, T., et al., Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: A review, J. Environ. Sci. (2016), http://dx.doi.org/10.1016/j.jes.2016.08.023

ARTICLE IN PRESS

2. 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. (1992a, 1992b), *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., *C. elegans*, *Naematoloma frowardii*, *P. chrysosporium*, *Phanerochaete laevis*, *P. ostreatus*, *Pleurotus sajor-caju*, *Ramaria* sp., *Rhizoctonia solani*, *T. versicolor* (Cerniglia, 1982; Cerniglia and Yang, 1984; Bezalel et al., 1996a, 1996b, 1996c; Johannes and Majcherczyk, 2000) (Table 2).

Q18 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 *I. 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), followed by *Lasiodiplodia theobromae* VBE1 (100 U/mL). 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) and *Aspergillus fumigatus* (73 U/mL), while moderate peroxidase activity (52 U/mL) 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 observed by *L. theobromae* and peroxidase production of 516 U/mL 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 Q19 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) have 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 *P. chrysosporium*, *P. ostreatus* and *Bjerkandera adusta* have been intensively studied and have shown higher potential to metabolize PAHs (Haritash and Kaushik, 2009).

2.1. *P. chrysosporium*

The potential of *P. 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, 1995). 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., 1992). 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, 1989). *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) Q20 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.

ARTICLE IN PRESS

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 phenanthrene. 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.

2.2. *P. ostreatus*

P. ostreatus, considered as white-rot fungi has been well documented for its capacity to degrade PAHs (Bezalel et al., 1996a, 1996b, 1996c, 1997; Tortella et al., 2015). Bezalel et al. (1996a, 1996b, 1996c) 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 *P. 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.

2.3. *B. adusta*

Other than *P. ostreatus*, Schützendübel et al. (1999) investigated the degradation of phenanthrene, fluoranthene, pyrene, fluorene and anthracene with *B. 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 *P. chrysosporium* (Sutherland et al., 1991) and *P. ostreatus* (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 *B. 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 *B. adusta*. Therefore, chemical modification of manganese peroxidase from *B. adusta* improved its effectiveness.

3. 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

ARTICLE IN PRESS

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. 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 (Lee et al., 2015a, 2015b). 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 *B. 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 *N. frowardii* 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 in *P. chrysogenum*, 72 U/mL in *M. racemosus* and 62 U/mL 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). Q22 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). Q23 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 *P. 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

Please cite this article as: Kadri, T., et al., Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: A review, J. Environ. Sci. (2016), http://dx.doi.org/10.1016/j.jes.2016.08.023

ARTICLE IN PRESS

557 mentioned that degradation of fluorene, anthracene, phenan-
558 threne, fluoranthene and pyrene are not associated with the
559 production of extracellular enzymes by *P. ostreatus* and *B. adusta*.
560 Similar results were described by Bezalel et al. (1996a) and Verdin
561 et al. (2004) who found degradation of PAH in fungal cultures in
562 the absence of LiP and MnP activities. Mohammadi et al. (2009)
563 have also tested the effect of the incubation mode on anthracene
564 biodegradation and it was revealed that the culture agitation
565 clearly increased the biodegradation capacity of bagasse
566 immobilized fungal cells despite the repressive effect of culture
567 agitation on the ligninase activity.
568 All these outcomes indicated that extracellular peroxidase
569 activities are not directly associated with the PAH degrada-
570 tion, and the increased solubility may be essential in the
571 enhancement of PAH degradation rather than enzyme activity
572 and hence suggested the possibility of producing other
573 oxidative and hydrolytic enzymes that were not analyzed
574 but could probably have degraded PAHs. Another suggestion
575 is the PAHs degrading role of intracellular enzymes, such as
576 tyrosinases and dioxygenases secreted into the culture filtrate
577 by different fungi (Milstein et al., 1983).
578 Enzyme immobilization allows an alternative procedure
579 that enables an increase in the steady state of enzymes and
580 significant environmental tolerance. Immobilized enzyme,
581 which can be established by several methods, such as adsorp-
582 tion, entrapment, and covalent bonding based on chemical/
583 physical mechanisms, has enhanced activity and stability. The
584 immobilized enzyme can withstand a wider range of tempera-
585 tures and pH, as well as significant substrate concentration
586 changes; this makes the complex much more resistant to severe
587 environments (Dodor et al., 2004). This results in a longer lifetime
588 and higher productivity per active unit of enzyme. Immobiliza-
589 tion enables the enzyme to be recycled, and such an approach is
590 much better suited for hydrophobic PAHs-contaminated soil
591 bioremediation (Chang et al., 2015).

593 4. Ionization potential

594 Bogan and Lamar (1995) defined the behavior of intact fungus
595 and the MnP-based lipid peroxidation system with respect to a
596 larger variety of creosote PAHs. The disappearance of three- to
597 six-ring creosote PAH components from intact fungal cultures
598 and throughout lipid peroxidation *in vitro* was reported. In each
599 of these cases, the approach is shown to be mainly dependent
600 on IP, indicating that the contribution of one or more than one
601 electron oxidants is included over the entire range of PAHs
602 tested. One-electron oxidation of PAHs occurs by peroxidases
603 (IP $\leq$ 7.35 eV), Mn dependent peroxidase (IP $\leq$ 8.19 eV), ligninase
604 (IP $\leq$ 7.55 eV) and laccase (IP $\leq$ 7.45 eV) (Cavalieri et al., 1983).
605 The IP values, referring to the energy needed to eliminate an
606 electron and to form a cation radical are 8.12 for naphthalene,
607 8.03 for phenanthrene, 7.21 for benzo(a)pyrene, and 7.31 for
608 benzo(g,h,i)perylene (Table 1) (Cavalieri et al., 1983). PAHs like
609 benz[a]anthracene, pyrene, and anthracene, that have ioniza-
610 tion potential <7.35 eV, are LiP substrates, whereas PAHs, such
611 as phenanthrene and benzo[e]pyrene that have ionization
612 potentials >7.35 eV, cannot be LiP substrates. The products of
613 the enzymatic oxidation are PAH quinones. For example,
614 benzo[a]pyrene is oxidized to its 1,6-, 3,6-, and 6,12-quinones,
615 pyrene to its 1,6- and 1,8-quinones, and anthracene to
616 9,10-anthraquinone (Hammel, 1995). Vyas et al. (1994) has
617 suggested that *P. ostreatus* and *T. versicolor* produce enzyme(s)
618 other than lignin peroxidase capable of oxidizing compounds
619 with higher ionization potential, such as anthracene.

5. Kinetics 620

622 The characteristics of the soil determine the diversity and
623 activity of its microflora which is responsible for the degra-
624 dation of polycyclic aromatic hydrocarbons and any other
625 compound. In addition, soil characteristics influence the
626 strength of interactions between the PAHs and individual
627 soil compounds. Cutright (1995) indicated that *Cunninghamella*
628 *echinulata var. elegans* efficiently degrades PAHs in the
629 presence of these nutrients while any other indigenous micro-
630 organisms are not. Moreover, for a first-order reaction system,
631 the rate of change in contaminant concentration is proportional
632 to the contaminant concentration in the soil and time prediction
633 tool in degradation depends on the microorganism, the contam-
634 inant type and its concentration.
635 The prediction of time for bioremediation of polluted soil is
636 based mostly on the microorganisms, pollutant type and its
637 concentration. Furthermore, the improvement of more appropri-
638 ate kinetic model needs the monitoring of biomass, respiration
639 studies, and investigation of interactions of different organisms.
640 Although bioremediation has a larger rate of success than
641 synthetic methods, still the kinetics is not completely under-
642 stood, and the kinetics becomes more challenging when fungi are
643 applied for bioremediation (Haritash and Kaushik, 2009). As
644 described previously, the different enzymes involved in fungal
645 degradation have maximum activity at different temperatures
646 and some of them are active even at extreme temperatures.
647 Therefore, monitoring the kinetics for various fungal strains is
648 complicated, but most of them have good degradation capacities
649 in a mesophilic range. The degradation rate can be improved by
650 pretreatment at a high temperature which results in volatiliza-
651 tion and decrease in the soil–water partition coefficient, as a
652 result dissolution of pollutants increases the degradation rate.

6. Soil and liquid cultures 654

655 Most research studies on the extracellular enzyme activity
656 produced by fungi have primarily been focusing on experi-
657 ments in liquid culture (Ruiz-Dueñas et al., 1999; Kwang Ho
658 Lee, 2004; Eibes et al., 2006a; Rodrigues et al., 2008; Mäkelä
659 et al., 2009; Dashtban et al., 2010). Nevertheless, some interest
660 has been given to the changes of the enzymes produced by
661 fungal strains during PAHs degradation in different soil types
662 (Wang et al., 2009). When fungi get into non-sterile soil, they
663 must compete with indigenous soil microbes for nutrients and
664 the mycelia of the fungi may be affected. As a consequence, the
665 production of enzymes may be influenced by more complicated
elements (McErlean et al., 2006; Wang et al., 2014a). Q24
667 Boyle et al. (1998) showed that white rot fungi growing
668 in soil presented low amounts of degraded polyaromatic
669 hydrocarbons (PAHs), even though they did degrade some
670 other organopollutants. Nevertheless, in liquid culture, they

Please cite this article as: Kadri, T., et al., Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: A review, J. Environ. Sci. (2016), http://dx.doi.org/10.1016/j.jes.2016.08.023

UNCORRECTED PROOF

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, 1995).

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).

PAH 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 *P. chrysosporium*. The highest activity of LiP and MnP reached 1.92 U/g. 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 PAH 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

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

Please cite this article as: Kadri, T., et al., Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: A review, J. Environ. Sci. (2016), http://dx.doi.org/10.1016/j.jes.2016.08.023

ARTICLE IN PRESS

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. (2014a, 2014b) observed that sterilization increased the degradation of pyrene because of the removal of competition from indigenous microbes.

7. 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., 1994; 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; Hammel et al., 1991; Hammel, 1992; Bogan and Lamar, 1996) (Fig. 1).

Also, the crude and the purified ligninase of *P. chrysosporium* 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, *P. 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, *I. lacteus* degraded anthracene and phenanthrene and the main degradation products were anthraquinone and phenanthrene-9,10-dihydrodiol, respectively as shown in Fig. 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 (Fig. 3).

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 degradation of naphthalene (69%) was performed by a strain that had MnP activity, followed by strain that showed lignin peroxidase and laccase activities. Likewise, it was found that highest degradation of phenanthrene (12%) was observed with the strain that contained MnP and laccase activities (Clemente et al., 2001). Soil fungi *Aspergillus* sp., *Trichocladium canadense*, and *Fusarium oxysporum* degrade polycyclic aromatic hydrocarbons low-molecular-weight PAHs (2–3 rings) and produce ligninolytic enzymes also under microaerobic and

UNCORRECTED PROOF

Please cite this article as: Kadri, T., et al., Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: A review, J. Environ. Sci. (2016), http://dx.doi.org/10.1016/j.jes.2016.08.023

ARTICLE IN PRESS

Cytochrome P-450

Cytochrome P-450

OMe

OH

OH

9-Methoxyphenanthrene

Phenanthrene-9,10-dihydrodiol

OH

COOH

2-Hydroxy-2-carboxy biphenyl

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

highly reduced oxygen conditions, but ligninolytic enzyme activities can vary among fungi and PAHs. Under microaerobic conditions, the 3 species demonstrated at least one of the assayed ligninolytic activities (LiP, MnP, laccase). In contrast, under very-low-oxygen conditions, ligninolytic enzyme activity was frequently not observed (Silva et al., 2009).

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

8. Mechanism of degradation with enzymes

8.1. *Characteristics of ligninolytic enzymes*

8.1.1. *Characteristics of peroxidases*

A couple of extracellular oxidative enzymes are responsible for lignin degradation: peroxidases and laccases (phenol oxidases). Both peroxidases were initially found in *P. chrysosporium* (Tien and Kirk, 1983). A number of other fungi also possess these enzymes, while others have either one or the other (Mester and Tien, 2000). In the majority of species, peroxidases are generally recognized to be families of isozymes occurring as extracellular 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, 1995). They need hydrogen peroxide to

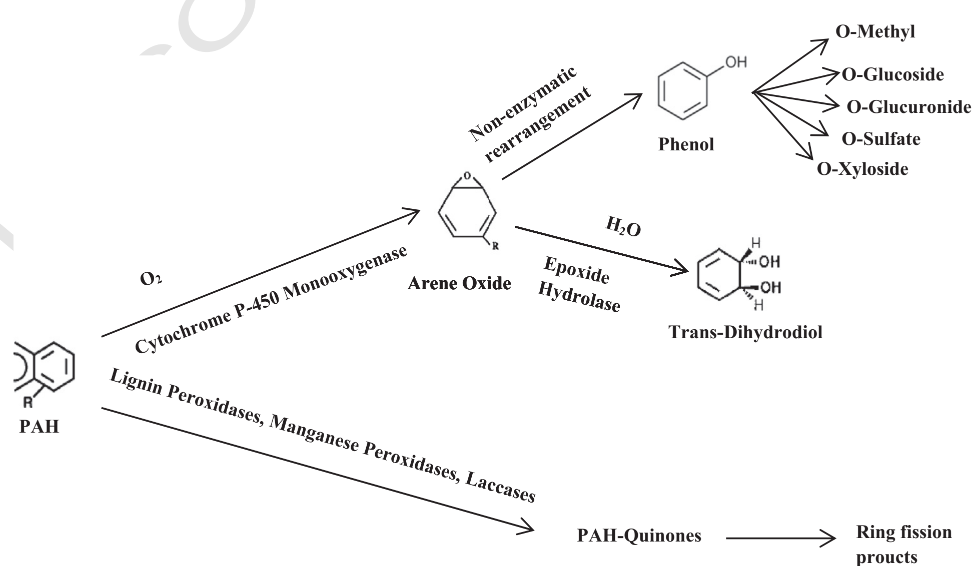

Fig. 3 – Different pathways for the fungal metabolism of polycyclic aromatic hydrocarbons.

Please cite this article as: Kadri, T., et al., Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: A review, J. Environ. Sci. (2016), http://dx.doi.org/10.1016/j.jes.2016.08.023

ARTICLE IN PRESS

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, 1995; 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).

8.1.2. 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).

8.1.3. 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 compounds (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 ≤7.45 eV. The presence of ABTS improves the oxidation of ANT while it is crucial for the oxidation of B[a]P. Anthraquinone was recognized as the major end product of ANT oxidation (Sutherland et al., 1991). Consequently, the PAH-oxidizing abilities of LAC differ, depending on the fungal species from which it was produced (Cho et al., 2002).

ANT was entirely degraded by the LAC of *Ganoderma lucidum* fungus in the absence of a redox mediator. At the same time and in the presence of the mediator, this same LAC degraded B[a]P, FLU, acenapthene, acenaphthylene, and B[a]A at a rate ranging from 85.3% to 100% (Pozdnyakova, 2012; Pozdnyakova et al., 2006a, 2006b).

The degradation of B[a]P by purified LAC of *Pycnoporus cinnabarinus* was investigated. The reaction required the presence of the exogenous ABTS as a mediator. Almost all of the substrate (95%) was transformed within 24 hr. The enzyme principally oxidized the substrate to benzo[a]pyrene-1,6-, 3,6- and 6,12-quinones (Rama et al., 1998).

The effect of different mediators on LAC action was investigated by Pickard et al. (1999). Different PAHs were oxidized by *C. gallica* LAC such as B[a]P, 9-methylanthracene, 2-methylanthracene, ANT, biphenylene, acenaphthene, and PHE. 9-methylanthracene was the most rapidly oxidized substrate. There was no apparent correlation between the ionization potential of the substrate and the first-order rate

Please cite this article as: Kadri, T., et al., Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: A review, J. Environ. Sci. (2016), http://dx.doi.org/10.1016/j.jes.2016.08.023

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)
	I. lacteus	PHE; ANT; FLA; PYR	9,10-Anthraquinone	(Baborová et al., 2006)
		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	(Bogan et al., 1996a, 1996b)
		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	CO_2 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; CO_2; B[a]P-1,6-quinone	(Steffen et al., 2002, 2003)
LAC	*C. hirsutus*	ANT; PHE; PYR; FLA; B[a]P	ND	(Cho et al., 2002)
	Coriolopsis gallica	B[a]P; ANT; PHE; FLU; 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., 2006a, 2006b)
	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; benzo[b]fluoranthene; benzo[k]fluoranthene; FLU	1,2-Acenaphthenedione 1,8-Naphthalic acid anhydride; 9,10-Anthraquinone; PHE-9,10-quinone, 2,2_-Diphenic acid; B[a]P-1,6-quinone; B[a]P-3,6-quinone; B[a]P-6,12-quinone	(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)

Please cite this article as: Kadri, T., et al., Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: A review, J. Environ. Sci. (2016), http://dx.doi.org/10.1016/j.jes.2016.08.023

ARTICLE IN PRESS

constant for substrate degradation *in vitro* by adding ABTS. The effects of mediating substrates were studied furthermore by applying ANT as a substrate. A synergistic effect of HBT and ABTS was detected. In fact, HBT supported approximately one-half ANT oxidation rate that ABTS supported, whereas HBT with ABTS enhanced the oxidation rate nine-fold, compared with the oxidation rate supported by only ABTS (Pickard et al., 1999).

The white-rot fungi generate yellow form of LAC during solid-state fermentation of a substrate containing natural lignin. The active center of this enzyme is transformed by the products of lignin degradation. Consequently, LAC becomes capable to catalyze the oxidation of nonphenolic compounds in the absence of mediators (Pozdnyakova et al., 2006a). The rate of degradation using the yellow LAC produced by *P. ostreatus* was also detected. The naphthalene derivatives α- and β-naphthols, α-nitroso-β naphthol, α-hydroxy-β-naphthoic acid, and α- naphthylamine were all appropriate LAC substrates despite yellow LAC did not catalyze the degradation of the two-ring PAH naphthalene. Yellow LAC oxidized all the PAHs of three to five rings such as ANT, PYR, FLU, FLA, PHE, and perylene, with rates of degradation ranging from 40% to 100%. The efficiencies were greater than that observed for a blue LAC from the same fungus without and with ABTS and HBT mediators. The same product of ANT oxidation and several unknown products of FLU oxidation were noticed in solutions of various solvents (Pozdnyakova, 2012; Pozdnyakova et al., 2006a, 2006b).

8.2. 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).

8.2.1. 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 *N. 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 24 hr.

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).

ARTICLE IN PRESS

8.2.2. 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., 1992). Among the oxidation reactions catalyzed by LiP are the cleavage of $C\alpha$–$C\beta$ and aryl $C\alpha$ 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. 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. Similar 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. (1986) 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-catalyzed pyrene oxidation done in the presence of H_2O_2 revealed that the quinone oxygens come from water. The one-electron oxidative mechanism of LiP is relevant to lignin and lignin-related substructures as well as certain polycyclic aromatic and heteroaromatic contaminants. The oxidation of pyrene by entire cultures of *P. chrysosporium* also generated these quinones. As a result, it can be concluded that LiP catalyzes the first step in the degradation of these compounds by entire cultures of *P. chrysosporium* (Hammel et al., 1986).

Vazquez-Duhalt et al. (1994) utilized LiP from *P. chrysosporium* to investigate the oxidation of anthracene, 1-, 2-, and 9- methylanthracenes, acenaphthene, fluoranthene, pyrene, carbazole, and dibenzothiophene. Among the studied compounds, LiP was able to oxidize compounds with IP < 8 eV. The greatest specific activity of PAHs oxidation was shown when pHs are between 3.5 and 4.0. The reaction products involve hydroxyl and keto groups. The product of anthracene oxidation was 9,10-anthraquinone. The products of LiP oxidation of 1- and 2-methylanthracene were 1- and 2-methylanthraquinone, respectively.

The 9,10-anthraquinone, 9-methyleneanthranone, and 9-methanol-9,10-dihydroanthracene were the products detected by from the oxidation of 9-methylanthracene (Vazquez-Duhalt et al., 1994). Anthraquinone resulting from carbon–carbon bond cleavage of 9-methylanthracene, was also observed. The mass spectra of the two products resulting from acenaphthene correspond to 1-acenaphthenone and 1-acenaphthenol. The comparison of the GC–mass spectrometry analysis of dibenzothiophene oxidation by LiP with a sample of authentic dibenzothiophene sulfoxide resulted in sulfoxide. The UV spectrum of the product of pyrene oxidation most closely fitted that of 1,8-pyrenedione. In spite fluoranthene and carbazole were oxidized, their products were not established (Vazquez-Duhalt et al., 1994).

Torres et al. (1997) studied LiP, cytochrome *c*, and hemoglobin for oxidation of PAHs in the presence of hydrogen peroxide and demonstrated that LiP oxidized anthracene, 2-methylanthracene, 9-hexylanthracene, pyrene, acenaphthene, and benzo[a]pyrene; the unreacted compounds included chrysene, phenanthrene, naphthalene, triphenylene, biphenyl, and dibenzofuran. The oxidation of the aromatic compounds by LiP matched with their IPs; only those compounds that had IPs < 8 eV were transformed. The reaction products from the three hemoproteins (LiP, cytochrome *c*, and hemoglobin) were principally quinones, which suggest that the three biocatalysts have the same oxidation mechanism. The resulting product from anthracene was anthraquinone, and the resulting product from 2-methylanthracene was 2-methylanthraquinone. The ending products for pyrene and benzo[a]pyrene oxidation were 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).

Experiments on the catalytic properties of ligninolytic enzymes demonstrate 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 glutathione 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 PAH oxidation since their role extends to the initial oxidation and production of quinones (Pozdnyakova, 2012).

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

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

Please cite this article as: Kadri, T., et al., Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: A review, J. Environ. Sci. (2016), http://dx.doi.org/10.1016/j.jes.2016.08.023

UNCORRECTED PROOF

ARTICLE IN PRESS

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

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

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

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

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

8.2.3. *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 Eq. (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.

9. 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.

Uncited reference

Q29

Clar and Schoental, 1964

Acknowledgments

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.

REFERENCES

Adams, G.O., Fufeyin, P.T., Okoro, S.E., Ehinomen, I., 2015. Bioremediation, biostimulation and bioaugmention: a review. Int. J. Environ. Biorem. Biodegrad. 3, 28–39.

Baborová, P., Möder, M., Baldrian, P., Cajthamlová, K., Cajthaml, T., 2006. Purification of a new manganese peroxidase of the white-rot fungus *Irpex lacteus*, and degradation of polycyclic aromatic hydrocarbons by the enzyme. Res. Microbiol. 157, 248–253. http://dx.doi.org/10.1016/j.resmic.2005.09.001.

Balaji, V., Ebenezer, P., 2008. Optimization of extracellular lipase production in *Colletotrichum gloeosporioides* by solid state fermentation. Indian J. Sci. Technol. 1, 1–8.

Balaji, V., Arulazhagan, P., Ebenezer, P., 2014. Enzymatic bioremediation of polyaromatic hydrocarbons by fungal consortia enriched from petroleum contaminated soil and oil seeds. J. Environ. Biol. Environ. Biol. India 35, 521–529.

Banu, N., Muthumary, J.P., 2005. Mycobiota of sunflower seeds and samples collected from vegetable oil refinery located in Tamilnadu, India. Mycol. Prog. 4, 195–204.

Barrasa, J.M., Martínez, A.T., Martínez, M.J., 2009. Isolation and selection of novel basidiomycetes for decolorization of recalcitrant dyes. Folia Microbiol. (Praha) 54, 59–66.

Betts, W.B., 2012. Biodegradation: Natural and Synthetic Materials. Springer Science & Business Media.

Bezalel, L., Hadar, Y., Cerniglia, C.E., 1996a. Mineralization of polycyclic aromatic hydrocarbons by the white rot fungus *Pleurotus ostreatus*. Appl. Environ. Microbiol. 62, 292–295.

Bezalel, L., Hadar, Y., Fu, P.P., Freeman, J.P., Cerniglia, C.E., 1996b. Initial oxidation products in the metabolism of pyrene, anthracene, fluorene, and dibenzothiophene by the white rot fungus *Pleurotus ostreatus*. Appl. Environ. Microbiol. 62, 2554–2559.

Bezalel, L., Hadar, Y., Fu, P.P., Freeman, J.P., Cerniglia, C.E., 1996c. Metabolism of phenanthrene by the white rot fungus *Pleurotus ostreatus*. Appl. Environ. Microbiol. 62, 2547–2553.

Bezalel, L., Hadar, Y., Cerniglia, C.E., 1997. Enzymatic mechanisms involved in Phenanthrene degradation by the white rot fungus *Pleurotus ostreatus*. Appl. Environ. Microbiol. 63, 2495–2501.

UNCORRECTED PROOF

ARTICLE IN PRESS

Binková, B., Šrám, R.J., 2004. The genotoxic effect of carcinogenic PAHs, their artificial and environmental mixtures (EOM) on human diploid lung fibroblasts. Mutat. Res. Mol. Mech. Mutagen. 547, 109–121.

Bogan, B.W., Lamar, R.T., 1995. One-electron oxidation in the degradation of creosote polycyclic aromatic hydrocarbons by *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. 61, 2631–2635.

Bogan, B.W., Lamar, R.T., 1996. Polycyclic aromatic hydrocarbon-degrading capabilities of *Phanerochaete laevis* HHB-1625 and its extracellular ligninolytic enzymes. Appl. Environ. Microbiol. 62, 1597–1603.

Bogan, B.W., Schoenike, B., Lamar, R.T., Cullen, D., 1996a. Manganese peroxidase mRNA and enzyme activity levels during bioremediation of polycyclic aromatic hydrocarbon-contaminated soil with *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. 62, 2381–2386.

Bogan, B.W., Lamar, R.T., Hammel, K.E., 1996b. Fluorene oxidation in vivo by *Phanerochaete chrysosporium* and in vitro during manganese peroxidase-dependent lipid peroxidation. Appl. Environ. Microbiol. 62, 1788–1792.

Böhmer, S., Messner, K., Srebotnik, E., 1998. Oxidation of phenanthrene by a fungal laccase in the presence of 1-hydroxybenzotriazole and unsaturated lipids. Biochem. Biophys. Res. Commun. 244, 233–238. http://dx.doi.org/10.1006/bbrc.1998.8228.

Bonugli-Santos, R.C., dos Santos Vasconcelos, M.R., Passarini, M.R., Vieira, G.A., Lopes, V.C., Mainardi, P.H., et al., 2015. Marine-derived fungi: diversity of enzymes and biotechnological applications. Front. Microbiol. 6.

Bourbonnais, R., Paice, M.G., Freiermuth, B., Bodie, E., Borneman, S., 1997. Reactivities of various mediators and laccases with Kraft pulp and lignin model compounds. Appl. Environ. Microbiol. 63, 4627–4632.

Boyle, D., Wiesner, C., Richardson, A., 1998. Factors affecting the degradation of polyaromatic hydrocarbons in soil by white-rot fungi. Soil Biol. Biochem. 30, 873–882.

Bressler, D.C., Fedorak, P.M., Pickard, M.A., 2000. Oxidation of carbazole, N-ethylcarbazole, fluorene, and dibenzothiophene by the laccase of *Coriolopsis gallica*. Biotechnol. Lett. 22, 1119–1125.

Bumpus, J.A., 1989. Biodegradation of polycyclic hydrocarbons by *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. 55, 154–158.

Bumpus, J.A., Tien, M., Wright, D., Aust, S.D., 1985. Oxidation of persistent environmental pollutants by a white rot fungus. Science 228, 1434–1436.

Cai, D., Tien, M., 1992. Kinetic studies on the formation and decomposition of compounds II and III. Reactions of lignin peroxidase with H_2O_2. J. Biol. Chem. 267, 11149–11155.

Cajthaml, T., Möder, M., Kacer, P., Sasek, V., Popp, P., 2002. Study of fungal degradation products of polycyclic aromatic hydrocarbons using gas chromatography with ion trap mass spectrometry detection. J. Chromatogr. A 974, 213–222.

Cañas, A.I., Alcalde, M., Plou, F., Martínez, M.J., Martínez, Á.T., Camarero, S., 2007. Transformation of polycyclic aromatic hydrocarbons by laccase is strongly enhanced by phenolic compounds present in soil. Environ. Sci. Technol. 41, 2964–2971. http://dx.doi.org/10.1021/es062328j.

Casillas, R.P., Crow Jr., S.A., Heinze, T.M., Deck, J., Cerniglia, C.E., 1996. Initial oxidative and subsequent conjugative metabolites produced during the metabolism of phenanthrene by fungi. J. Ind. Microbiol. 16, 205–215.

Cavalieri, E.L., Rogan, E.G., Roth, R.W., Saugier, R.K., Hakam, A., 1983. The relationship between ionization potential and horseradish peroxidase/hydrogen peroxide-catalyzed binding of aromatic hydrocarbons to DNA. Chem. Biol. Interact. 47, 87–109.

Cébron, A., Beguiristain, T., Bongoua-Devisme, J., Denonfoux, J., Faure, P., Lorgeoux, C., et al., 2015. Impact of clay mineral, wood sawdust or root organic matter on the bacterial and fungal community structures in two aged PAH-contaminated soils. Environ. Sci. Pollut. Res. 1–15.

Cerniglia, C.E., 1982. Initial reactions in the oxidation of anthracene by *Cunninghamella elegans*. J. Gen. Microbiol. 128, 2055–2061.

Cerniglia, C.E., 1984. Microbial metabolism of polycyclic aromatic hydrocarbons. In: Laskin, A.I. (Ed.), Advances in Applied Microbiology. Academic Press, pp. 31–71.

Cerniglia, C.E., 1997a. Fungal metabolism of polycyclic aromatic hydrocarbons: past, present and future applications in bioremediation. J. Ind. Microbiol. Biotechnol. 19, 324–333. http://dx.doi.org/10.1038/sj.jim.2900459.

Cerniglia, C.E., 1997b. Fungal metabolism of polycyclic aromatic hydrocarbons: past, present and future applications in bioremediation. J. Ind. Microbiol. Biotechnol. 19, 324–333.

Cerniglia, C.E., Gibson, D.T., 1979. Oxidation of benzo [a] pyrene by the filamentous fungus *Cunninghamella elegans*. J. Biol. Chem. 254, 12174–12180.

Cerniglia, C.E., Gibson, D.T., 1980a. Fungal oxidation of benzo (a) pyrene and (±)-trans-7, 8-dihydroxy-7, 8-dihydrobenzo (a) pyrene. J. Biol. Chem. 255.

Cerniglia, C.E., Gibson, D.T., 1980b. Fungal oxidation of (±)-9, 10-dihydroxy-9, 10-dihydrobenzo [a] pyrene: formation of diastereomeric benzo [a] pyrene 9, 10-diol 7, 8-epoxides. Proc. Natl. Acad. Sci. 77, 4554–4558.

Cerniglia, C.E., Sutherland, J.B., 2010. Degradation of polycyclic aromatic hydrocarbons by fungi. Handbook of Hydrocarbon and Lipid Microbiology. Springer, pp. 2079–2110.

Cerniglia, C.E., Yang, S.K., 1984. Stereoselective metabolism of anthracene and phenanthrene by the fungus *Cunninghamella elegans*. Appl. Environ. Microbiol. 47, 119–124.

Cerniglia, C.E., Mahaffey, W., Gibson, D.T., 1980a. Fungal oxidation of benzo [a] pyrene: formation of (−)-trans-7, 8-dihydroxy-7, 8-dihydrobenzo [a] pyrene by *Cunninghamella elegans*. Biochem. Biophys. Res. Commun. 94, 226–232.

Cerniglia, C.E., Dodge, R.H., Gibson, D.T., 1980b. Studies on the fungal oxidation of polycyclic aromatic hydrocarbons. Bot. Mar. 23, 121–124.

Cerniglia, C.E., Kelly, D.W., Freeman, J.P., Miller, D.W., 1986. Microbial metabolism of pyrene. Chem. Biol. Interact. 57, 203–216.

Cerniglia, C.E., Campbell, W.L., Freeman, J.P., Evans, F.E., 1989. Identification of a novel metabolite in phenanthrene metabolism by the fungus *Cunninghamella elegans*. Appl. Environ. Microbiol. 55, 2275–2279.

Cerniglia, C.E., Gibson, D.T., Dodge, R.H., 1994. Metabolism of benz [a] anthracene by the filamentous fungus *Cunninghamella elegans*. Appl. Environ. Microbiol. 60, 3931–3938.

Chan, S.M.N., Luan, T., Wong, M.H., Tam, N.F.Y., 2006. Removal and biodegradation of polycyclic aromatic hydrocarbons by *Selenastrum capricornutum*. 25, 1772–1779.

Chandra, R., Chowdhary, P., 2015. Properties of bacterial laccases and their application in bioremediation of industrial wastes. Environ. Sci. Process. Impacts 17, 326–342.

Chang, Y.-T., Lee, J.-F., Liu, K.-H., Liao, Y.-F., Yang, V., 2015. Immobilization of fungal laccase onto a nonionic surfactant-modified clay material: application to PAH degradation. Environ. Sci. Pollut. Res. 1–12.

Chen, J., Wang, X.J., Hu, J.D., Tao, S., 2006. Effect of surfactants on biodegradation of PAHs by white-rot fungi. Huan Jing Ke Xue 27, 154–159.

Cho, S.-J., Park, S.J., Lim, J.-S., Rhee, Y.H., Shin, K.-S., 2002. Oxidation of polycyclic aromatic hydrocarbons by laccase of *Coriolus hirsutus*. Biotechnol. Lett. 24, 1337–1340.

Clar, E., Schoental, R., 1964. Polycyclic Hydrocarbons. Springer.

Clemente, A.R., Anazawa, T.A., Durrant, L.R., 2001. Biodegradation of polycyclic aromatic hydrocarbons by soil fungi. Braz. J. Microbiol. 32, 255–261.

Collins, P.J., Dobson, A.D., 1996. Oxidation of fluorene and phenanthrene by Mn (II) dependent peroxidase activity in

ARTICLE IN PRESS

whole cultures of *Trametes* (Coriolus) *versicolor*. Biotechnol. Lett. 18, 801–804.
Collins, P.J., Kotterman, M., Field, J.A., Dobson, A., 1996. Oxidation of Anthracene and benzo[a]pyrene by laccases from *Trametes versicolor*. Appl. Environ. Microbiol. 62, 4563–4567.
Cornelissen, G., Gustafsson, Ö., Bucheli, T.D., Jonker, M.T., Koelmans, A.A., van Noort, P.C., 2005. Extensive sorption of organic compounds to black carbon, coal, and kerogen in sediments and soils: mechanisms and consequences for distribution, bioaccumulation, and biodegradation. Environ. Sci. Technol. 39, 6881–6895.
Cutright, T.J., 1995. Polycyclic aromatic hydrocarbon biodegradation and kinetics using *Cunninghamella echinulata var. elegans*. Int. Biodeterior. Biodegrad. 35, 397–408. http://dx.doi.org/10.1016/0964-8305(95)00046-1.
Cybulski, Z., Dziurla, E., Kaczorek, E., Olszanowski, A., 2003. The influence of emulsifiers on hydrocarbon biodegradation by Pseudomonadacea and Bacillacea strains. Spill Sci. Technol. Bull. 8, 503–507.
Darmawan, R., Nakata, H., Ohta, H., Niidome, T., Takikawa, K., et al., 2015. Isolation and evaluation of PAH degrading bacteria. J. Bioremed. Biodegr. 6, 2.
Dashtban, M., Schraft, H., Syed, T.A., Qin, W., 2010. Fungal biodegradation and enzymatic modification of lignin. Int. J. Biochem. Mol. Biol. 1, 36–50.
Davis, M.W., Glaser, J.A., Evans, J.W., Lamar, R.T., 1993. Field evaluation of the lignin-degrading fungus *Phanerochaete sordida* to treat creosote-contaminated soil. Environ. Sci. Technol. 27, 2572–2576. http://dx.doi.org/10.1021/es00048a040.
Dharmsthiti, S., Kuhasuntisuk, B., 1998. Lipase from *Pseudomonas aeruginosa* LP602: biochemical properties and application for wastewater treatment. J. Ind. Microbiol. Biotechnol. 21, 75–80.
Diaz, M., Mora, V., Pedrozo, F., Nichela, D., Baffico, G., 2014. Evaluation of native acidophilic algae species as potential indicators of polycyclic aromatic hydrocarbon (PAH) soil contamination. J. Appl. Phycol. 27, 321–325.
Ding, J., Cong, J., Zhou, J., Gao, S., 2008. Polycyclic aromatic hydrocarbon biodegradation and extracellular enzyme secretion in agitated and stationary cultures of *Phanerochaete chrysosporium*. J. Environ. Sci. 20, 88–93.
Dodor, D.E., Hwang, H.-M., Ekunwe, S.I., 2004. Oxidation of anthracene and benzo [a] pyrene by immobilized laccase from *Trametes versicolor*. Enzym. Microb. Technol. 35, 210–217.
Drevinskas, T., Mickienė, R., Maruška, A., Stankevičius, M., Tiso, N., Mikašauskaitė, J., et al., 2016. Downscaling the in vitro test of fungal bioremediation of polycyclic aromatic hydrocarbons: methodological approach. Anal. Bioanal. Chem. 408, 1043–1053.
Dutton, M.V., Evans, C.S., 1996. Oxalate production by fungi: its role in pathogenicity and ecology in the soil environment. Can. J. Microbiol. 42, 881–895.
Eggert, C., Temp, U., Dean, J.F.D., Eriksson, K.-E.L., 1996. A fungal metabolite mediates degradation of non-phenolic lignin structures and synthetic lignin by laccase. FEBS Lett. 391, 144–148.
Q33 Eibes, G., Cajthaml, T., Moreira, M.T., Feijoo, G., Lema, J.M., 2006. Enzymatic degradation of anthracene, dibenzothiophene and pyrene by manganese peroxidase in media containing acetone. Chemosphere 64, 408–414.
Elisabet Aranda, R.U., 2009. Conversion of polycyclic aromatic hydrocarbons, methyl naphthalenes and dibenzofuran by two fungal peroxygenases. Biodegradation 21, 267–281.
Farnet, A.M., Criquet, S., Tagger, S., Gil, G., Petit, J.L., 2000. Purification, partial characterization, and reactivity with aromatic compounds of two laccases from *Marasmius quercophilus* strain 17. Can. J. Microbiol. 46, 189–194.
Fayeulle, A., Veignie, E., Slomianny, C., Dewailly, E., Munch, J.-C., Rafin, C., 2014. Energy-dependent uptake of benzo [a] pyrene and its cytoskeleton-dependent intracellular transport by the telluric fungus *Fusarium solani*. Environ. Sci. Pollut. Res. 21, 3515–3523.
Ferreira, L., Rosales, E., Sanromán, M.A., Pazos, M., 2015. Preliminary testing and design of permeable bioreactive barrier for phenanthrene degradation by *Pseudomonas stutzeri* CECT 930 immobilized in hydrogel matrices. J. Chem. Technol. Biotechnol. 90, 500–506.
Field, J.A., De Jong, E., Costa, G.F., De Bont, J.A., 1992. Biodegradation of polycyclic aromatic hydrocarbons by new isolates of white rot fungi. Appl. Environ. Microbiol. 58, 2219–2226.
Field, J.A., Vledder, R.H., van Zelst, J.G., Rulkens, W.H., 1996. The tolerance of lignin peroxidase and manganese-dependent peroxidase to miscible solvents and the in vitro oxidation of anthracene in solvent: water mixtures. Enzym. Microb. Technol. 18, 300–308.
Garapati, V.K., Mishra, S., 2012. Hydrocarbon degradation using fungal isolate: nutrients optimized by combined grey relational analysis. Int. J. Eng. Res. Appl. 2, 390–399.
Gill, P., Arora, D., 2003. Effect of culture conditions on manganese peroxidase production and activity by some white rot fungi. J. Ind. Microbiol. Biotechnol. 30, 28–33.
Glenn, J.K., Akileswaran, L., Gold, M.H., 1986. Mn(II) oxidation is the principal function of the extracellular Mn-peroxidase from *Phanerochaete chrysosporium*. Arch. Biochem. Biophys. 251, 688–696.
Gopinath, S.C., Anbu, P., Lakshmipriya, T., Hilda, A., 2013. Strategies to characterize fungal lipases for applications in medicine and dairy industry. Biomed. Res. Int. 2013.
Gu, H., Luo, X., Wang, H., Wu, L., Wu, J., Xu, J., 2015. The characteristics of phenanthrene biosorption by chemically modified biomass of *Phanerochaete chrysosporium*. Environ. Sci. Pollut. Res. 22, 11850–11861.
Hadibarata, T., Kristanti, R.A., 2014. Potential of a white-rot fungus *Pleurotus eryngii* F032 for degradation and transformation of fluorene. Fungal Biol. 118, 222–227.
Hadibarata, T., Tachibana, S., Itoh, K., 2009. Biodegradation of chrysene, an aromatic hydrocarbon by *Polyporus* sp. S133 in liquid medium. J. Hazard. Mater. 164, 911–917.
Haemmerli, S.D., Leisola, M.S., Sanglard, D., Fiechter, A., 1986. Oxidation of benzo (a) pyrene by extracellular ligninases of *Phanerochaete chrysosporium*. Veratryl alcohol and stability of ligninase. J. Biol. Chem. 261, 6900–6903.
Hamamura, N., Ward, D.M., Inskeep, W.P., 2013. Effects of petroleum mixture types on soil bacterial population dynamics associated with the biodegradation of hydrocarbons in soil environments. FEMS Microbiol. Ecol. 85, 168–178.
Hammel, K.E., 1992. Oxidation of aromatic pollutants by Lijfnin-degrading fungi and their extracellular peroxidases. Met. Ions Biol. Syst. 28 Degrad. Environ. Pollut. Microorg. Their Met. 28, 41.
Hammel, K.E., 1995. Mechanisms for polycyclic aromatic hydrocarbon degradation by ligninolytic fungi. Environ. Health Perspect. 103, 41–43.
Hammel, K.E., Kalyanaraman, B., Kirk, T.K., 1986. Oxidation of polycyclic aromatic hydrocarbons and dibenzo[p]-dioxins by *Phanerochaete chrysosporium* ligninase. J. Biol. Chem. 261, 16948–16952.
Hammel, K.E., Green, B., Gai, W.Z., 1991. Ring fission of anthracene by a eukaryote. Proc. Natl. Acad. Sci. U. S. A. 88, 10605–10608.
Hammel, K.E., Gai, W.Z., Green, B., Moen, M.A., 1992. Oxidative degradation of phenanthrene by the ligninolytic fungus *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. 58, 1832–1838. Q34
Han, M.-J., Choi, H.-T., Song, H.-G., 2004. Degradation of phenanthrene by *Trametes versicolor* and its laccase. J. Microbiol. Seoul Korea 42, 94–98.
Haritash, A.K., Kaushik, C.P., 2009. Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review. J. Hazard. Mater. 169, 1–15. Q35

UNCORRECTED PROOF

ARTICLE IN PRESS

1623 Hatakka, A., 1994. Lignin-modifying enzymes from selected white-rot fungi: production and role from in lignin degradation. FEMS Microbiol. Rev. 13, 125–135.

1626 Hofrichter, M., Scheibner, K., Schneegaß, I., Fritsche, W., 1998. Enzymatic combustion of aromatic and aliphatic compounds by manganese peroxidase from *Nematoloma frowardii*. Appl. Environ. Microbiol. 64, 399–404.

1630 Huesemann, M.H., Hausmann, T.S., Fortman, T.J., 2003. Assessment of bioavailability limitations during slurry biodegradation of petroleum hydrocarbons in aged soils. Environ. Toxicol. Chem. 22, 2853–2860.

1634 Hunsa Punnapayak, S.P., 2009. Polycyclic aromatic hydrocarbons (PAHs) degradation by laccase from a tropical white rot fungus *Ganoderma lucidum*. Afr. J. Biotechnol. 8, 5897–5900. http://dx.doi.org/10.4314/ajb.v8i21.66070.

1638 Jensen, K.A., Evans, K.M.C., Kirk, T.K., Hammel, K.E., 1994. Biosynthetic pathway for veratryl alcohol in the ligninolytic fungus *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. 60, 709–714.

1642 Johannes, C., Majcherczyk, A., 2000. Natural mediators in the oxidation of polycyclic aromatic hydrocarbons by laccase mediator systems. Appl. Environ. Microbiol. 66, 524–528.

1645 Johannes, C., Majcherczyk, A., Hüttermann, A., 1996. Degradation of anthracene by laccase of *Trametes versicolor* in the presence of different mediator compounds. Appl. Microbiol. Biotechnol. 46, 313–317.

1649 Johannes, C., Majcherczyk, A., Hüttermann, A., 1998. Oxidation of acenaphthene and acenaphthylene by laccase of *Trametes versicolor* in a laccase-mediator system. J. Biotechnol. 61, 151–156.

1652 Johansson, T., Nyman, P.O., Cullen, D., 2002. Differential regulation of mnp2, a new manganese peroxidase-encoding Gene from the ligninolytic fungus *Trametes versicolor* PRL 572. Appl. Environ. Microbiol. 68, 2077–2080.

1656 Jové, P., Olivella, M.À., Camarero, S., Caixach, J., Planas, C., Cano, L., De Las Heras, F.X., 2015. Fungal biodegradation of anthracene-polluted cork: a comparative study. J. Environ. Sci. Health A 1–8.

1660 Kaal, E.E.J., Field, J.A., Joyce, T.W., 1995. Increasing ligninolytic enzyme activities in several white-rot Basidiomycetes by nitrogen-sufficient media. Bioresour. Technol. 53, 133–139.

1663 Kelsey, J.W., Slizovskiy, I.B., Peters, R.D., Melnick, A.M., 2010. Sterilization affects soil organic matter chemistry and bioaccumulation of spiked p, p′-DDE and anthracene by earthworms. Environ. Pollut. 158, 2251–2257.

Q36 W., K.G., A., W., B, J.W., 1999. Mineralization of benzo[a]pyrene by Marasmiellus troyanus, a mushroom isolated from a toxic waste site. Microbiol. Res. 154, 75–79.

1670 Khambhaty, Y., Ananth, S., Sreeram, K.J., Rao, J.R., Nair, B.U., 2015. Dual utility of a novel, copper enhanced laccase from Trichoderma aureoviridae. Int. J. Biol. Macromol. 81, 69–75.

1673 Kiehlmann, E., Pinto, L., Moore, M., 1996. The biotransformation of chrysene to trans-1,2-dihydroxy-1,2-dihydrochrysene by filamentous fungi. Can. J. Microbiol. 42, 604–608. http://dx.doi.org/10.1139/m96-081.

1677 Kirk, T.K., Farrell, R.L., 1987. Enzymatic "combustion": the microbial degradation of lignin. Annu. Rev. Microbiol. 41, 465–501.

1679 Kotterman, M.J., Vis, E.H., Field, J.A., 1998. Successive mineralization and detoxification of benzo[a]pyrene by the white rot fungus *Bjerkandera* sp. strain BOS55 and indigenous microflora. Appl. Environ. Microbiol. 64, 2853–2858.

1683 Kristanti, R.A., Hadibarata, T., 2015. Biodegradation and identification of transformation products of fluorene by Ascomycete fungi. Water Air Soil Pollut. 226, 1–6.

1686 Kwang Ho Lee, S.G.W., 2004. Micromorphological characteristics of decayed wood and laccase produced by the brown-rot fungus *Coniophora puteana*. J. Wood Sci. 50, 281–284.

1689 Lange, B., Kremer, S., Anke, H., Sterner, O., 1996. Metabolism of pyrene by basidiomycetous fungi of the genera *Crinipellis*, *Marasmius*, and *Marasmiellus*. Can. J. Microbiol. 42, 1179–1183.

1692 Launen, L., Pinto, L., Wiebe, C., Kiehlmann, E., Moore, M., 1995. The oxidation of pyrene and benzo [a] pyrene by nonbasidiomycete soil fungi. Can. J. Microbiol. 41, 477–488.

1695 Lee, H., Choi, Y.-S., Kim, M.-J., Huh, N.-Y., Kim, G.-H., Lim, Y.W., et al., 2010. Degrading ability of oligocyclic aromates by *Phanerochaete sordida* selected via screening of white rot fungi. Folia Microbiol. (Praha) 55, 447–453.

1699 Lee, H., Jang, Y., Choi, Y.-S., Kim, M.-J., Lee, J., Lee, H., et al., 2014. Biotechnological procedures to select white rot fungi for the degradation of PAHs. J. Microbiol. Methods 97, 56–62.

1702 Lee, H., Jang, Y., Lee, Y.M., Lee, H., Kim, G.-H., Kim, J.-J., 2015a. Enhanced removal of PAHs by *Peniophora incarnata* and ascertainment of its novel ligninolytic enzyme genes. J. Environ. Manag. 164, 10–18.

1706 Lee, H., Yun, S.Y., Jang, S., Kim, G.-H., Kim, J.-J., 2015b. Bioremediation of polycyclic aromatic hydrocarbons in creosote-contaminated soil by *Peniophora incarnata* KUC8836. Bioremediat. J. 19, 1–8.

1710 Lei, A.-P., Hu, Z.-L., Wong, Y.-S., Tam, N.F.-Y., 2007. Removal of fluoranthene and pyrene by different microalgal species. Bioresour. Technol. 98, 273–280.

1713 Leisola, M.S., Kozulic, B., Meussdoerffer, F., Fiechter, A., 1987. Homology among multiple extracellular peroxidases from *Phanerochaete chrysosporium*. J. Biol. Chem. 262, 419–424.

1716 Li, P., Li, H., Stagnitti, F., Wang, X., Zhang, H., Gong, Z., et al., 2005. Biodegradation of pyrene and Phenanthrene in soil using immobilized fungi *Fusarium* sp. Bull. Environ. Contam. Toxicol. 75, 443–450.

1720 Li, J., Sun, H., Zhang, Y., 2007. Desorption of pyrene from freshly-amended and aged soils and its relationship to bioaccumulation in earthworms. Soil Sediment Contam. 16, 79–87.

1724 Li, X., Li, P., Lin, X., Zhang, C., Li, Q., Gong, Z., 2008. Biodegradation of aged polycyclic aromatic hydrocarbons (PAHs) by microbial consortia in soil and slurry phases. J. Hazard. Mater. 150, 21–26.

1727 Li, X., Wang, Y., Wu, S., Qiu, L., Gu, L., Li, J., Zhang, B., Zhong, W., 2014. Peculiarities of metabolism of anthracene and pyrene by laccase-producing fungus *Pycnoporus sanguineus* H1. Biotechnol. Appl. Biochem. 61, 549–554.

1731 Luo, L., Wang, P., Lin, L., Luan, T., Ke, L., Tam, N.F.Y., 2014. Removal and transformation of high molecular weight polycyclic aromatic hydrocarbons in water by live and dead microalgae. Process Biochem. 49, 1723–1732.

Q37 Majcherczyk, A., Johannes, C., Hüttermann, A., 1998. Oxidation of polycyclic aromatic hydrocarbons (PAH) by laccase of *Trametes versicolor*. Enzym. Microb. Technol. 22, 335–341.

1738 Mäkelä, M.R., Hildén, K., Hatakka, A., Lundell, T.K., 2009. Oxalate decarboxylase of the white-rot fungus *Dichomitus squalens* demonstrates a novel enzyme primary structure and non-induced expression on wood and in liquid cultures. Microbiol. Read. Engl. 155, 2726–2738.

1743 Manilla-Pérez, E., Lange, A.B., Luftmann, H., Robenek, H., Steinbüchel, A., 2011. Neutral lipid production in *Alcanivorax borkumensis* SK2 and other marine hydrocarbonoclastic bacteria. Eur. J. Lipid Sci. Technol. 113, 8–17.

1747 Mao, J., Luo, Y., Teng, Y., Li, Z., 2012. Bioremediation of polycyclic aromatic hydrocarbon-contaminated soil by a bacterial consortium and associated microbial community changes. Int. Biodeterior. Biodegrad. 70, 141–147.

1751 Marco-Urrea, E., García-Romera, I., Aranda, E., 2015. Potential of non-ligninolytic fungi in bioremediation of chlorinated and polycyclic aromatic hydrocarbons. New Biotechnol.

1754 Matsubara, M., Suzuki, J., Deguchi, T., Miura, M., Kitaoka, Y., 1996. Characterization of manganese peroxidases from the hyperlignolytic fungus IZU-154. Appl. Environ. Microbiol. 62, 4066–4072.

1758 Messerschmidt, A., Huber, R., 1990. The blue oxidases, ascorbate oxidase, laccase and ceruloplasmin modelling and structural relationships. Eur. J. Biochem. 187, 341–352.

UNCORRECTED PROOF

ARTICLE IN PRESS

1761 Messias, J.M., da Costa, B.Z., de Lima, V.M.G., Dekker, R.F.H., Rezende, M.I., Krieger, N., et al., 2009. Screening Botryosphaeria species for lipases: production of lipase by *Botryosphaeria ribis* EC-01 grown on soybean oil and other carbon sources. Enzym. Microb. Technol. 45, 426–431.

1766 Mester, T., Tien, M., 2000. Oxidation mechanism of ligninolytic enzymes involved in the degradation of environmental pollutants. Int. Biodeterior. Biodegrad. 46, 51–59.

1769 Milstein, O., Vered, Y., Shragina, L., Gressel, J., Flowers, H.M., Hüttermann, A., 1983. Metabolism of lignin related aromatic compounds by *Aspergillus japonicus*. Arch. Microbiol. 135, 147–154.

1773 Mineki, S., Suzuki, K., Iwata, K., Nakajima, D., Goto, S., 2015. Degradation of polyaromatic hydrocarbons by fungi isolated from soil in Japan. Polycycl. Aromat. Compd. 35, 120–128.

1776 Moen, M.A., Hammel, K.E., 1994. Lipid peroxidation by the manganese peroxidase of *Phanerochaete chrysosporium* is the basis for phenanthrene oxidation by the intact fungus. Appl. Environ. Microbiol. 60, 1956–1961.

1780 Mohammadi, A., Enayatzadeh, M., Nasernejad, B., 2009. Enzymatic degradation of anthracene by the white rot fungus *Phanerochaete chrysosporium* immobilized on sugarcane bagasse. J. Hazard. Mater. 161, 534–537.

1784 Moreira, P.R., Bouillenne, F., Almeida-Vara, E., Malcata, F.X., Frere, J.M., Duarte, J.C., 2006. Purification, kinetics and spectral characterisation of a new versatile peroxidase from a *Bjerkandera* sp. isolate. Enzym. Microb. Technol. 38, 28–33.

1788 Muñoz, C., Guillén, F., Martínez, A.T., Martínez, M.J., 1997. Laccase isoenzymes of Pleurotus eryngii: characterization, catalytic properties, and participation in activation of molecular oxygen and Mn2+ oxidation. Appl. Environ. Microbiol. 63, 2166–2174.

1792 Nam, K., Kim, J.Y., 2002. Role of loosely bound humic substances and humin in the bioavailability of phenanthrene aged in soil. Environ. Pollut. 118, 427–433.

1795 Nam, K., Kim, J.Y., Oh, D.I., 2003. Effect of soil aggregation on the biodegradation of phenanthrene aged in soil. Environ. Pollut. 121, 147–151.

1798 Northcott, G.L., Jones, K.C., 2001. Partitioning, extractability, and formation of nonextractable PAH residues in soil. 1. Compound differences in aging and sequestration. Environ. Sci. Technol. 35, 1103–1110.

1802 Novotný, Č., Svobodová, K., Erbanová, P., Cajthaml, T., Kasinath, A., Lang, E., et al., 2004a. Ligninolytic fungi in bioremediation: extracellular enzyme production and degradation rate. Soil Biol. Biochem. 36, 1545–1551.

1806 Novotný, Č., Svobodová, K., Erbanová, P., Cajthaml, T., Kasinath, A., Lang, E., Šašek, V., 2004b. Ligninolytic fungi in bioremediation: extracellular enzyme production and degradation rate. Soil Biol. Biochem., enzymes in the environment: activity. Ecol. Appl. 36, 1545–1551. http://dx.doi.org/10.1016/j.soilbio.2004.07.019.

1811 Okai, M., Kihara, I., Yokoyama, Y., Ishida, M., Urano, N., 2015. Isolation and characterization of benzo [a] pyrene-degrading bacteria from the Tokyo Bay area and Tama River in Japan. FEMS Microbiol. Lett. 362, fnv143.

1815 Ollikka, P., Alhonmäki, K., Leppänen, V.M., Glumoff, T., Raijola, T., Suominen, I., 1993. Decolorization of azo, triphenyl methane, heterocyclic, and polymeric dyes by lignin peroxidase isoenzymes from *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. 59, 4010–4016.

1820 Patnaik, P., 2007. A Comprehensive Guide to the Hazardous Properties of Chemical Substances. John Wiley & Sons.

1822 Peng, X., Yuan, X.-Z., Liu, H., Zeng, G.-M., Chen, X.-H., 2015. Degradation of polycyclic aromatic hydrocarbons (PAHs) by laccase in rhamnolipid reversed micellar system. Appl. Biochem. Biotechnol.

1826 Pickard, M.A., Roman, R., Tinoco, R., Vazquez-Duhalt, R., 1999. Polycyclic aromatic hydrocarbon metabolism by white rot fungi and oxidation by *Coriolopsis gallica* UAMH 8260 laccase. Appl. Environ. Microbiol. 65, 3805–3809. 1829

1830 Pothuluri, J.V., Freeman, J.P., Evans, F.E., Cerniglia, C.E., 1990. Fungal transformation of fluoranthene. Appl. Environ. Microbiol. 56, 2974–2983.

1833 Pothuluri, J.V., Freeman, J.P., Evans, F.E., Cerniglia, C.E., 1992a. Fungal metabolism of acenaphthene by *Cunninghamella elegans*. Appl. Environ. Microbiol. 58, 3654–3659.

1836 Pothuluri, J.V., Heflich, R.H., Fu, P.P., Cerniglia, C.E., 1992b. Fungal metabolism and detoxification of fluoranthene. Appl. Environ. Microbiol. 58, 937–941.

1839 Pothuluri, J.V., Freeman, J.P., Evans, F.E., Cerniglia, C.E., 1993. Biotransformation of fluorene by the fungus *Cunninghamella elegans*. Appl. Environ. Microbiol. 59, 1977–1980.

1842 Pothuluri, J.V., Selby, A., Evans, F.E., Freeman, J.P., Cerniglia, C.E., 1995. Transformation of chrysene and other polycyclic aromatic hydrocarbon mixtures by the fungus Cunninghamella Elegans. Can. J. Bot. 73, 1025–1033.

1846 Pothuluri, J.V., Evans, F.E., Heinze, T.M., Cerniglia, C.E., 1996. Formation of sulfate and glucoside conjugates of benzo[e]pyrene by *Cunninghamella elegans*. Appl. Microbiol. Biotechnol. 45, 677–683.

Q38 Pozdnyakova, N.N., 2012. Involvement of the ligninolytic system of white-rot and litter-decomposing fungi in the degradation of polycyclic aromatic hydrocarbons, involvement of the ligninolytic system of white-rot and litter-decomposing fungi in the degradation of polycyclic aromatic hydrocarbons. Biotechnol. Res. Int. 2012 (2012), e243217.

1856 Pozdnyakova, N.N., Rodakiewicz-Nowak, J., Turkovskaya, O.V., Haber, J., 2006a. Oxidative degradation of polyaromatic hydrocarbons catalyzed by blue laccase from *Pleurotus ostreatus* D1 in the presence of synthetic mediators. Enzym. Microb. Technol. 39, 1242–1249.

1861 Pozdnyakova, N.N., Rodakiewicz-Nowak, J., Turkovskaya, O.V., Haber, J., 2006b. Oxidative degradation of polyaromatic hydrocarbons and their derivatives catalyzed directly by the yellow laccase from *Pleurotus ostreatus* D1. J. Mol. Catal. B Enzym. 41, 8–15.

1866 Pysh, E.S., Yang, N.C., 1963. Polarographic oxidation potentials of aromatic compounds. J. Am. Chem. Soc. 85, 2124–2130.

1868 Rama, R., Mougin, C., Boyer, F.-D., Kollmann, A., Malosse, C., Sigoillot, J.-C., 1998. Biotransformation of bezo[a]pyrene in bench scale reactor using laccase of *Pycnoporus cinnabarinus*. Biotechnol. Lett. 20, 1101–1104.

1872 Rodrigues, M.A.M., Pinto, P., Bezerra, R.M.F., Dias, A.A., Guedes, C.V.M., Cardoso, V.M.G., et al., 2008. Effect of enzyme extracts isolated from white-rot fungi on chemical composition and in vitro digestibility of wheat straw. Anim. Feed Sci. Technol. 141, 326–338.

1877 Ruiz-Dueñas, F.J., Guillén, F., Camarero, S., Pérez-Boada, M., Martínez, M.J., Martínez, Á.T., 1999. Regulation of peroxidase transcript levels in liquid cultures of the ligninolytic fungus *Pleurotus eryngii*. Appl. Environ. Microbiol. 65, 4458–4463.

1881 Sack, U., Günther, T., 1993. Metabolism of PAH by fungi and correlation with extracellular enzymatic activities. J. Basic Microbiol. 33, 269–277.

1884 Sack, U., Heinze, T.M., Deck, J., Cerniglia, C.E., Cazau, M.C., Fritsche, W., 1997a. Novel metabolites in phenanthrene and pyrene transformation by *Aspergillus niger*. Appl. Environ. Microbiol. 63, 2906–2909.

1888 Sack, U., Heinze, T.M., Deck, J., Cerniglia, C.E., Martens, R., Zadrazil, F., et al., 1997b. Comparison of phenanthrene and pyrene degradation by different wood-decaying fungi. Appl. Environ. Microbiol. 63, 3919–3925.

1892 Sack, U., Hofrichter, M., Fritsche, W., 1997c. Degradation of polycyclic aromatic hydrocarbons by manganese peroxidase of *Nematoloma frowardii*. FEMS Microbiol. Lett. 152, 227–234.

1895 Sanglard, D., Leisola, M.S., Fiechter, A., 1986. Role of extracellular ligninases in biodegradation of benzo (a) pyrene by *Phanerochaete chrysosporium*. Enzym. Microb. Technol. 8, 209–212. 1898

UNCORRECTED PROOF

ARTICLE IN PRESS

Schützendübel, A., Majcherczyk, A., Johannes, C., Hüttermann, A., 1999. Degradation of fluorene, anthracene, phenanthrene, fluoranthene, and pyrene lacks connection to the production of extracellular enzymes by *Pleurotus ostreatus* and *Bjerkandera adusta*. Int. Biodeterior. Biodegrad. 43, 93–100.
Silva, I.S., Grossman, M., Durrant, L.R., 2009. Degradation of polycyclic aromatic hydrocarbons (2–7 rings) under microaerobic and very-low-oxygen conditions by soil fungi. Int. Biodeterior. Biodegrad. 63, 224–229.
Simister, R.L., Poutasse, C.M., Thurston, A.M., Reeve, J.L., Baker, M.C., White, H.K., 2015. Degradation of oil by fungi isolated from Gulf of Mexico beaches. Mar. Pollut. Bull.
Simonsick, W.J., Hites, R.A., 1986. Characterization of high molecular weight polycyclic aromatic hydrocarbons by charge exchange chemical ionization mass spectrometry. Anal. Chem. 58, 2114–2121.
Singh, A., Ward, O.P., 2004. Biodegradation and Bioremediation. Springer Science & Business Media.
Singh, P., Parmar, D., Pandya, A., 2015. Parametric optimization of media for the crude oil degrading bacteria isolated from crude oil contaminated site. Int. J. Curr. Microbiol. App. Sci. 4, 322–328.
Steffen, K.T., Hatakka, A., Hofrichter, M., 2002. Removal and mineralization of polycyclic aromatic hydrocarbons by litter-decomposing basidiomycetous fungi. Appl. Microbiol. Biotechnol. 60, 212–217.
Steffen, K.T., Hatakka, A., Hofrichter, M., 2003. Degradation of benzo[a]pyrene by the litter-decomposing Basidiomycete *Stropharia coronilla*: role of manganese peroxidase. Appl. Environ. Microbiol. 69, 3957–3964.
Sun, H., Wang, C., Huo, C., Zhou, Z., 2008. Semipermeable membrane device-assisted desorption of pyrene from soils and its relationship to bioavailability. Environ. Toxicol. Chem. SETAC 27, 103–111.
Sun, K., Liu, J., Gao, Y., Jin, L., Gu, Y., Wang, W., 2014. Isolation, plant colonization potential, and phenanthrene degradation performance of the endophytic bacterium *Pseudomonas* sp. Ph6-gfp. Sci. Rep. 4.
Sutherland, J.B., Selby, A.L., Freeman, J.P., Evans, F.E., Cerniglia, C.E., 1991. Metabolism of phenanthrene by *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. 57, 3310–3316.
Sutherland, J.B., Selby, A.L., Freeman, J.P., Fu, P.P., Miller, D.W., Cerniglia, C.E., 1992. Identification of xyloside conjugates formed from anthracene by *Rhizoctonia solani*. Mycol. Res. 96, 509–517.
Syed, K., Yadav, J.S., 2012. P450 monooxygenases (P450ome) of the model white rot fungus *Phanerochaete chrysosporium*. Crit. Rev. Microbiol. 38, 339–363.
Tavares, A.P.M., Coelho, M.A.Z., Agapito, M.S.M., Coutinho, J.A.P., Xavier, A., 2006. Optimization and modeling of laccase production by *Trametes versicolor* in a bioreactor using statistical experimental design. Appl. Biochem. Biotechnol. 134, 233–248.
Ten Have, R., Teunissen, P.J.M., 2001. Oxidative mechanisms involved in lignin degradation by white-rot fungi. Chem. Rev. 101, 3397–3414.
Thiyagarajan, A., Saravanakumar, K., Kaviyarasan, V., 2008. Optimization of extracellular peroxidase production from *Coprinus* sp. Indian J. Sci. Technol. 1, 1–5.
Q39 Thomas Günther, U.S., 1998. Oxidation of PAH and PAH-derivatives by fungal and plant oxidoreductases. J. Basic Microbiol. 38, 113–122.
Thurston, C.F., 1994. The structure and function of fungal laccases. Microbiology 140, 19–26. http://dx.doi.org/10.1099/13500872-140-1-19.
Tien, M., Kirk, T.K., 1983. Lignin-degrading enzyme from the hymenomycete *Phanerochaete chrysosporium* Burds. Science (Washington) 221, 661–662.
Tollin, G., Meyer, T.E., Cusanovich, M.A., Curir, P., Marchesini, A., 1993. Oxidative turnover increases the rate constant and extent of intramolecular electron transfer in the multicopper enzymes, ascorbate oxidase and laccase. Biochim. Biophys. Acta 1183, 309–314.
Torres, E., Tinoco, R., Vazquez-Duhalt, R., 1997. Biocatalytic Oxidation of Polycyclic Aromatic Hydrocarbons in Media Containing Organic Solvents. Water Sci. Technol., Environmental BiotechnologySelected Proceedings of the International Conference on Environmental Biotechnology Vol. 36 pp. 37–44.
Tortella, G., Durán, N., Rubilar, O., Parada, M., Diez, M.C., 2015. Are white-rot fungi a real biotechnological option for the improvement of environmental health? Crit. Rev. Biotechnol. 35, 165–172.
Upadhyay, P., Shrivastava, R., Agrawal, P.K., 2016. Bioprospecting and biotechnological applications of fungal laccase. 3 Biotech 6, 1–12.
Valderrama, B., Ayala, M., Vazquez-Duhalt, R., 2002. Suicide inactivation of peroxidases and the challenge of engineering more robust enzymes. Chem. Biol. 9, 555–565.
Vazquez-Duhalt, R., Westlake, D.W., Fedorak, P.M., 1994. Lignin peroxidase oxidation of aromatic compounds in systems containing organic solvents. Appl. Environ. Microbiol. 60, 459–466.
Venkatesagowda, B., Ponugupaty, E., Barbosa, A.M., Dekker, R.F.H., 2012. Diversity of plant oil seed-associated fungi isolated from seven oil-bearing seeds and their potential for the production of lipolytic enzymes. World J. Microbiol. Biotechnol. 28, 71–80.
Verdin, A., Sahraoui, A.L.-H., Durand, R., 2004. Degradation of benzo[a]pyrene by mitosporic fungi and extracellular oxidative enzymes. Int. Biodeterior. Biodegrad. 53, 65–70.
Viswanath, B., Rajesh, B., Janardhan, A., Kumar, A.P., Narasimha, G., 2014. Fungal laccases and their applications in bioremediation. Enzyme Res. 2014.
Vyas, B.R.M., Bakowski, S., Šašek, V., Matucha, M., 1994. Degradation of anthracene by selected white rot fungi. FEMS Microbiol. Ecol. 14, 65–70. Q40
Wang, Y., Vazquez-Duhalt, R., Pickard, M.A., 2002. Purification, characterization, and chemical modification of manganese peroxidase from *Bjerkandera adusta* UAMH 8258. Curr. Microbiol. 45, 77–87.
Wang, C., Sun, H., Li, J., Li, Y., Zhang, Q., 2009. Enzyme activities during degradation of polycyclic aromatic hydrocarbons by white rot fungus *Phanerochaete chrysosporium* in soils. Chemosphere 77, 733–738.
Wang, C., Sun, H., Liu, H., Wang, B., 2014a. Biodegradation of pyrene by *Phanerochaete chrysosporium* and enzyme activities in soils: effect of SOM, sterilization and aging. J. Environ. Sci. (China) 26, 1135–1144.
Wang, C., Sun, H., Liu, H., Wang, B., 2014b. Biodegradation of pyrene by *Phanerochaete chrysosporium* and enzyme activities in soils: effect of SOM, sterilization and aging. J. Environ. Sci. 26, 1135–1144.
Watanabe, N., Schwartz, E., Scow, K.M., Young, T.M., 2005. Relating desorption and biodegradation of phenanthrene to SOM structure characterized by quantitative pyrolysis GC–MS. Environ. Sci. Technol. 39, 6170–6181.
Winquist, E., Björklöf, K., Schultz, E., Räsänen, M., Salonen, K., Anasonye, F., Cajthaml, T., Steffen, K.T., Jørgensen, K.S., Tuomela, M., 2014. Bioremediation of PAH-contaminated soil with fungi — from laboratory to field scale. Int. Biodeterior. Biodegrad. 86, 238–247 Part C.
Xu, F., Kulys, J.J., Duke, K., Li, K., Krikstopaitis, K., Deussen, H.J., Abbate, E., Galinyte, V., Schneider, P., 2000. Redox chemistry in laccase-catalyzed oxidation of N-hydroxy compounds. Appl. Environ. Microbiol. 66, 2052–2056.
Yang, Y., Hunter, W., Tao, S., Gan, J., 2009. Microbial availability of different forms of phenanthrene in soils. Environ. Sci. Technol. 43, 1852–1857.
Yang, Y., Ma, F., Yu, H., Fan, F., Wan, X., Zhang, X., Jiang, M., 2011. Characterization of a laccase gene from the white-rot fungi

UNCORRECTED PROOF

ARTICLE IN PRESS

Trametes sp. 5930 isolated from Shennongjia nature Reserve in China and studying on the capability of decolorization of different synthetic dyes. Biochem. Eng. J. 57, 13–22.

Young, D., Rice, J., Martin, R., Lindquist, E., Lipzen, A., Grigoriev, I., Hibbett, D., 2015. Degradation of bunker C fuel oil by white-rot fungi in sawdust cultures suggests potential applications in bioremediation. PLoS One 10, e0130381.

Zafra, G., Absalón, A.E., Cortés-Espinosa, D.V., 2015a. Morphological changes and growth of filamentous fungi in the presence of high concentrations of PAHs. Braz. J. Microbiol. 46, 937–941.

Zafra, G., Moreno-Montaño, A., Absalón, Á.E., Cortés-Espinosa, D.V., 2015b. Degradation of polycyclic aromatic hydrocarbons in soil by a tolerant strain of *Trichoderma asperellum*. Environ. Sci. Pollut. Res. 22, 1034–1042.

Zhang, S., Ning, Y., Zhang, X., Zhao, Y., Yang, X., Wu, K., et al., 2015. Contrasting characteristics of anthracene and pyrene degradation by wood rot fungus *Pycnoporus sanguineus* H1. Int. Biodeterior. Biodegrad. 105, 228–232.

Zhao, Z., Zhang, L., Cai, Y., Chen, Y., 2014. Distribution of polycyclic aromatic hydrocarbon (PAH) residues in several tissues of edible fishes from the largest freshwater lake in China, Poyang Lake, and associated human health risk assessment. Ecotoxicol. Environ. Saf. 104, 323–331.

UNCORRECTED PROOF

Please cite this article as: Kadri, T., et al., Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: A review, J. Environ. Sci. (2016), http://dx.doi.org/10.1016/j.jes.2016.08.023