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Fungal enzymatic degradation of industrial effluents – A review

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Abstract

Environmental and economic affable technology that is mechanized by the enzymes of microbes is bioremediation. Microorganisms like bacteria, fungi and plant life produce large number of digestive enzymes that has been researched and reported regarding their involvement in degrading the persistent pollutants which are lethal to human life. Fungal enzymes possess advantages over additional resources owing to their applicability and sustenance, even in deviant and unfavorable environmental circumstances. Research in exploring these enzymes helped a lot in contributing towards sustainable development of bioprocess technology to reduce the noxiousness of the pollutants. This review is an attempt offering descriptive information on the digestive enzymes from fungi, their application in degrading wide variety of contaminants. Advanced methods like using immobilized fungal cells or enzymes in beads or carriers have received good success and thus future holds promise for development of genes required in cloning for the decolorization and decomposition of dyes as well as bioengineered fungi that can utilize pollutants or various industrial effluents as substrates.

Key words – Bioremediation – Enzymes – Enzyme activity – Extracellular enzymes – fungal degradation – Laccases – Lipases and Peroxidases

Introduction

Toxic pollutant elimination from industrial effluents is practiced over several decades, nevertheless physico-chemical processes like oxidation and reduction evaporation, chemical precipitation, filtration, reverse osmosis, electrochemical treatment and ion-exchange are restricted owing to their cost effectiveness and do not guarantee satisfactory treatment of the effluent**.** Furthermore, these processes are generally strenuous and pricey, because of the intricacy and volume of effluents released during production process (Crini & Lichtfouse 2019).

These reasons have necessitated requirement for development of novel and advanced technologies which accentuate the destruction of pollutants otherwise not effective through conventional method of disposal. Previous two decades have witnessed notable rise in search of eco-friendly and cost-effective alternatives for conventional methods of handling wastes. Among the identified technologies which were promising were those which have meticulously mimicked the time-tested natural systems that were successful in restoring environments to their original status upon objectionable distresses. Indeed, the process of self-restoration ability by nature, which is infinite, was in fact responsible for birth to the concept.

These technologies involve utilizing microorganisms like bacteria, yeast, algae and fungi and

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algae for treating industrial effluents, which obtained attention and reputation in the last few decades owing to their enhanced performance and less cost, steady effect and easy retrieval of treasured metals (Rehman & Shakoori 2001, Wang et al. 2001). Termed as bioremediation this technology is regarded to end up being the most appropriate approach for treating industrial effluents as it provides long lasting remedy for clean-up and also removal of poisonous pollutants via metabolic reactions mediated by organisms (Pearce & Ollerman 1998).

Deemed to be possessing beneficial enzymes, microorganisms, have great variation between genera in ability of production of specific enzymes, which is dependent on specific medium and pH. During the recent years, utilizing microorganisms for sources of significant industrial enzymes has gained stirred attention for assessment of their enzymatic action. (Saranraj & Stella 2013, Akpan et al. 1999, Pandey et al. 2000, Abu et al. 2005). Microbial enzymes have remarkable potential for degradation of diversed nature of organic compounds (Alexander 1981). Owing to the following advantages enzymes from microbes are preferred over plants to animal sources:

1. Economic in production
2. More manageable and anticipated enzyme contents
3. Dependable supplies of raw material with stable composition which can be easily arranged

The biology of fungi, their financial worth and pathogenic features are not new to the civilization. Fungi are recognized for their greater abilities of generating vide varieties of proteins, acids and other catabolic products, over all their entire enzymes have made them more soughted out more than microbial enzymes their capability to adapt in serious environmental constrains (Kües 2015, Kadri 2017, Quintella et al. 2019). Fungi have been utilized for varied applications ranging from food fermentation to production of pharmaceuticals. The biodegradation of undesirable components by fungi involve converting them into tolerable, useful or harmless products (Tripathi et al. 2007).

Fungi are regarded as nature's most active agents for decomposition of waste as they secrete a variety of extracellular enzymes, forming an indispensable element in soil-food-web (Rhodes 2012). Among other decompositions of lignocellulose is regarded to be the most crucial process in the carbon cycle of earth (Rhodes 2013). Indeed, these are the solitary organisms which can decompose wood on earth, especially 'white-rot-fungi' are unique, as they require lignin for their growth which acts as a carbon source (Kirk et al. 1976). Table 1 presents the materials that are degraded by fungi.

Table 1 Materials degraded by Fungi (Kirk et al. 1976)

Wood	Plastics	Library Materials
Wooden airfacts	Wool	Wall Paintings
Stored Paper	Wrapping Papers	Electro insulating materials
Textiles	Glass Surfaces	Coal
Leather	Concrete	Ground waste rubber materials

In order to achieve fruitful mycoremediation (remediation using fungi), selection of correct fungal species for targeting specific pollutant is necessary (Matsubara et al. 2006, Rhodes 2014). Table 2 presents a few important industrial digestive enzymes from fungi (Martin & Christopher 1990)

Table 2 presents a few important industrial digestive enzymes from fungi (Martin & Christopher 1990).

Enzyme a	**EC number b**	**Source**	**Intra/extra -cellular c**	**Scale of production d**	**Industrial use**
Fungal enzymes					
α-Amylase	3.2.1.1	*Aspergillus*	E	++	Baking

Table 2 Continued.

Enzyme [a]	EC number [b]	Source	Intra/extra -cellular [c]	Scale of production [d]	Industrial use
Aminoacylase	3.5.1.14	*Aspergillus*	I	-	Pharmaceutical
Glucoamylase[k]	3.2.1.3	*Aspergillus*	E	+++	Starch
Catalase	1.11.1.6	*Aspergillus*	I	-	Food
Cellulase	3.2.1.4	*Trichoderma*	E	-	Waste
Dextranase	3.2.1.11	*Penicillium*	E	-	Food
Glucose oxidase	1.1.3.4	*Aspergillus*	I	-	Food
Lactase	3.2.1.23	*Aspergillus*	E	-	Dairy
Lipase [e]	3.1.1.3	*Rhizopus*	E	-	Food
Rennet[m]	3.4.23.6	*Mucor miehei*	E	++	Cheese
Pectinase[n]	3.2.1.15	*Aspergillus*	E	++	Drinks
Pectin lyase	4.2.2.10	*Aspergillus*	E	-	Drinks
Protease[m]	3.4.23.6	*Aspergillus*	E	+	Baking
Raffinase[o]	3.2.1.22	*Mortierella*	I	-	Food

[a] the names in common usage are given. As most industrial enzymes consist of mixtures of enzymes, these names may vary from the recommended names of their principal component. Where appropriate, the recommended names of this principal component are given below
[b] The EC number of the principal component
[c] I - intracellular enzyme; E - extracellular enzyme
[d] +++ > 100-ton year^{-1}; ++ > 10-ton year^{-1}; + > 1-ton year^{-1}; - < 1-ton year^{-1}
[e] triacylglycerol lipase
[f] chymosin
[g] Endo-1,3(4)-β-glucanase
[h] xylose isomerase
[i] subtilisin
[j] α-dextrin endo-1,6-α-glucosidase
[k] glucan 1,4-α-glucosidase
[l] β-galactosidase
[m] microbial aspartic proteinase
[n] polygalacturonase
[o] α-galactosidase
[p] β-fructofuranosidase

Industrial application of Fungi Enzymes

α-Amylase

First enzyme to be produced industrially in 1894, amylase (Crueger & Crueger 1984) is the enzyme that breaks down starch into sugar. Owing to their intended technological implication and monetary benefits, amylase have received a pronounced attention. α – amylase is used for production of glucose commercially (Hema et al. 2006). Successful applications of microbial amylases include starch saccharification, baking, textile, paper and pulp, brewing and detergent industries along with advanced applications for medical, clinical and analytical chemistry (Saranraj & Stella 2013).

Aminoacylase

Utilized for the creation of L-amino acids aminoacylases are involved in industrial settings from late 1950s (Sato & Tosa 2010, Birnbaum et al. 1952). Fungi belonging to the genus *Hypomyces*, *Fusarium*, *Auricularia*, *Pythium*, and *Menisporopsis* are capable of producing D-aminoacylase. Microbes release specific enzymes like D or L aminoacylase (Little child 2015) and these compounds help in producing amino acids (Drauz 1997), contains nitrogen acts as an pharmaceutically active compounds.

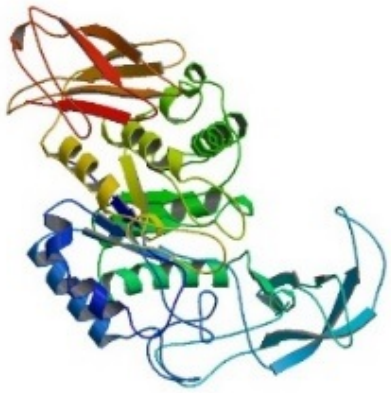

Fig. 1 – Structure of Alpha-Amylase Precursor (Reproduced with permission from Hwang et al. 1997)

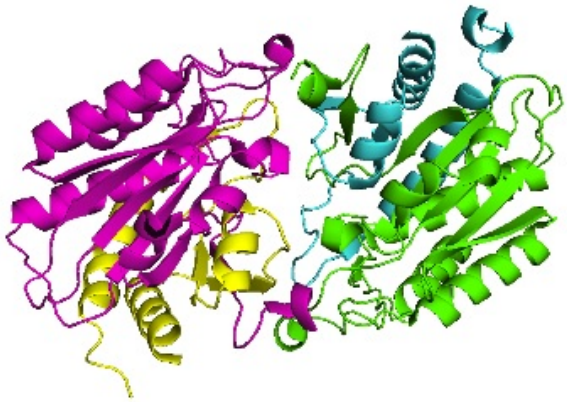

Fig. 2 – Quaternary structure of an Aminoacylase (Reproduced with permission from en.wikipedia.org)

Glucoamylase

One of the oldest and widely used biocatalyst in food industry is Glucoamylase (GA). Glucose is obtained from non-reducing ends of starch by hydrolysing α-1, 4 glycosidic bonds and it even has a ability to hydrolyse α-1, 6 linkages at a inferior level (Mertens & Skory 2006). Saccharification of partially processed starch/dextrin to glucose is a single of the major program of glucoamylase, which is a necessary substrate for abundant fermentation procedures and range of beverage and meals industries. For industrial purposes glucoseacylase has been traditionally produced from filamentous fungi as they secrete huge quantities of the enzyme extracellularly (Kumar & Satyanarayana 2009). Among other fungi involved in production of the enzyme important for industrial application involve *Rhizopus oryzae*, *Aspergillus niger* and *Aspergillus awamori* (Coutinho & Reilly 1997).

Catalase

Catalase is an ubiquitous enzyme can degrade hydrogen peroxide into oxygen and water generally present in aerobic organisms and acts against deleterious properties of reactive oxygen species like Hydroxyl radicals, Superoxide anions etc. (Susmitha et al. 2013). Two types of large-size subunit catalases (L1 and L2) are present in filamentous ascomycetes. L2-type is extracellular in natures which are usually induced by various stressors; on the other hand, L1-type accumulates in asexual spores and is not inducible (Hansberg et al. 2012). Major applications of catalases include bleaching of cotton fabrics (Alexandra et al. 2002), food industry (Hengge 1999); contact lens hygiene (Cook & Worsley 1996).

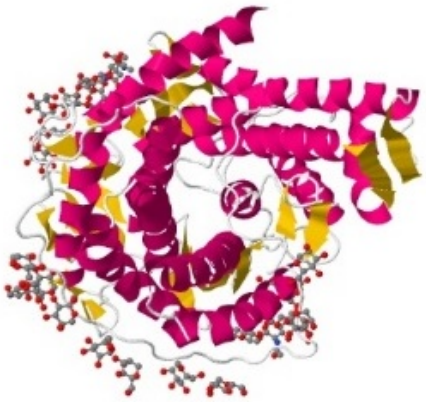

Fig. 3 – Refined crystal structures of Glucoamylase from Aspergillus Awamori Var. X100 (Reproduced with permission from Aleshin et al. 1994)

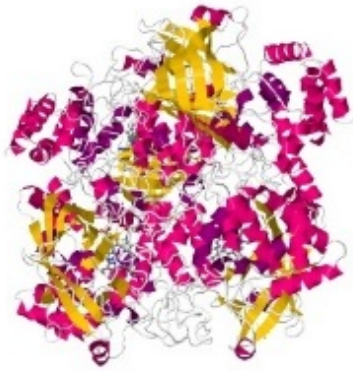

Fig. 4 – Catalase A from *Saccharomyces Cerevisiae* (Reproduced with permission from Maté et al. 1999)

Cellulase

Structurally simpler in comparison to cellulase bacterial systems, cellulosomes fungal cellulases (Bayer et al. 1994, 1998) are those that characteristically have two different provinces: a catalytic domain (CD) and a cellulose binding module (CBM), hinged by short poly-linker region to catalytic domain at N-terminal (Carvalho et al. 2003, Bayer et al. 2004). Owing to enzyme complexity system and enormous industrial prospective, cellulases became prospective for exploration by speculative and industrialized groups. Microbial cellulases reported impending application in numerous industries including textile, pulp and paper, laundry, food, brewing, agriculture and biofuel production (Kuhad et al. 2011).

Dextranase

Endo-dextranase enzyme from glucosyl transferases family, dextranase is produced from certain strain of microorganism. Dextranase can hydrolyze dextran to low-molecular-weight polysaccharides, it favourably cleaves the 1, 6-A- glucosidic linkages in dextran and its degradation products. These have major important applications in medicine (Yu-Qi et al. 2017) and sugarcane processing (Gibriel et al. 2014).

Glucose oxidase

Gluconic acid is obtained by oxidation of β-d-glucose and it is catalysed by Glucose oxidase

(GOX). through employing molecular oxygen for accepting electron along with producing hydrogen peroxide simultaneously. It is considered as safe and its production from *A. niger* has been basis for several industrial applications. GOX from microbes has been receiving great attention owing to its extensive uses in pharmaceutical, chemical, beverage, food, biotechnology, clinical chemistry and many other industries (Bankar et al. 2009). One of its novel applications comes from its electrochemical activity which makes it a significant constituent in glucose sensors and impending in fuel cell applications that have increased its demand in recent years (Wong et al. 2008).

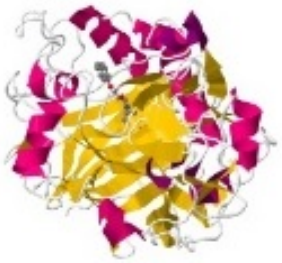

Fig. 5 – Catalytic modules of Cel7D from Phanerochaete chrysosporium as a chiral selector: Structural studies of its complex with the b-blocker (R)-propranolol (Reproduced with permission from Muñoz et al. 2003)

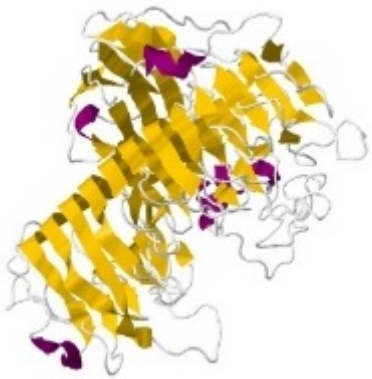

Fig. 6 – Dex49A from Penicillium minioluteum (Reproduced with permission from Larsson et al. 2003)

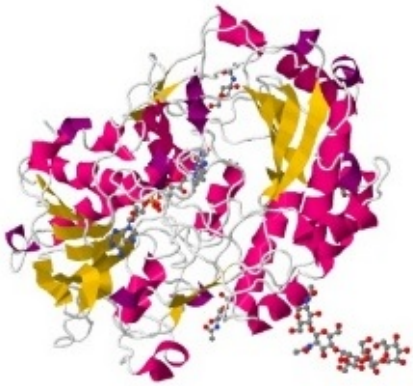

Fig. 7 – Glucose Oxidase from Penicillium Amagasakiense (Reproduced with permission from Wohlfahrt et al. 1999)

Lactase

Lactase is an enzyme which brings about break down of milk sugar lactose to glucose and galactose (Borglum & Sternberg 1972). This have been broadly studied during the recent years (Mehaia & Cheryan 1987), owing to the technique of enzyme immobilization that has paved new and motivating possibilities for use of this sugar. Lactase applications involve in bread production (Pomeranz & Miller 1963), thickness decreasing of frozen milk products (Stimpson 1954), enlightening animal fodders (Stimpson 1957) and ice cream containing milk solids will be enhanced (Sampey & Neubeck 1955). Having safe use history this is being subjected to numerous safety tests lactase obtaining from *A. niger*, *A. oryzae* and *Kluyveromyces lactis* are deliberated to be harmless.

Fig. 8 – Crystal structure of MGS-M5, a lactate dehydrogenase enzyme from a Medee basin deep-sea metagenome library (Reproduced with permission from Alcaide et al. 2015)

Lipases

Lipase belongs to the class of hydrolyses. Fatty acids and glycerol are obtained by catalysing the hydrolysis of triglycerides over an oil-water suspension, which also catalyse hydrolysis and Trans esterification of other esters including the production of esters and also exhibiting enation selective properties. Lipase has a capability to perform precise chemical transformations, stability and Broad spectrum substrate specificity (Dutra et al. 2008, Griebeler et al. 2011; owing to these properties it has enhanced popularity in the applications of detergent, food, cosmetic, organic synthesis, and pharmaceutical industries (Park et al. 2005, Gupta et al. 2007, Grbavčić et al. 2007, Franken et al. 2009). Advanced applications include their activity as biocatalysts in lipid technology that have been used insitu and exsitu complex manufacturing applications (Joseph et al. 2008). Among other microorganisms fungoid types are specifically cultured in solidstate fermentation (SSF) for production of lipases (Abada 2008, Dutra et al. 2008).

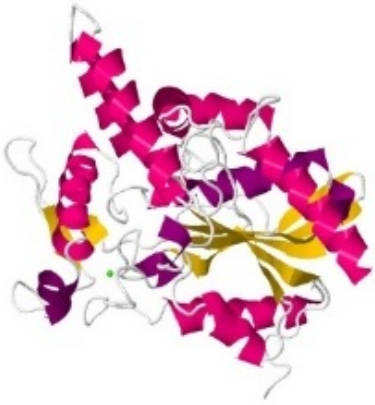

Fig. 9 – Structure of Lipase (Reproduced with permission from Kim et al. 1997)

Rennet

Microbial rennets obtained from various microorganisms, which are commercially promoted in the trade names like as Hanilase, Fromase, Rennilase, Marzyme, etc. are being promoted since the 1970s, these which have demonstrated acceptable for manufacture of different kinds of cheese. Apart from various strains of species of *Mucor* that are frequently used for manufacture of microbial rennets others include *Endothia parasitica*, *Aspergillus oryza*e, *Irpex lactis*, *Rhizomucor pusillus and R. miehei*. Presently microbial rennet is used in nearly 1/3rd of the cheese manufactured worldwide.

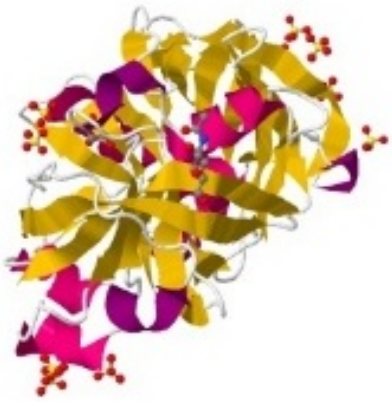

Fig. 10 – Camel chymosin at 1.6A resolution (Reproduced with permission from Langholm Jensen et al. 2013)

Pectinase

Pectinase present in the cell walls of plants. Pectinase is a polysaccharide substrate, which splits the pectin into of polygalacturonic acid into monogalacturonic acid by linking of glycosidic linkages. Peels of the fruits which are made of pectin layer can be easily assimilated by microbial pectinases in order to extract juices from the fruits. Hence, pectinase enzymes are generally used in processes that involve break down of plant materials, like speeding up the extraction of fruit juice. Currently nearly all the pectinolytic enzymes utilized for industrial purposes are produced by fungi comprising of *Rhizopus stolonifer*, *Fusarium oxysporum*, *Aspergillus* sp., *Alternaria mali*, *Penicillium italicum*, *Neurospora crassa* and many others. Orange peels are used as substrate for the growth of *Aspergillus niger* (Beulah et al. 2015, Abe et al. 1988, Aguilar & Huitron 1987).

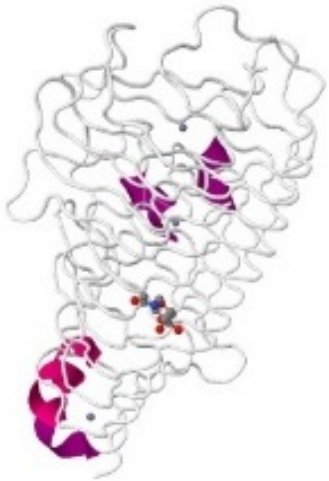

Fig. 11 – Endo-Polygalacturonase II from Aspergillus niger (Reproduced with permission from van Santen et al. 1999)

Pectin lyase

Middle lamella of higher plants and cell walls, contain pectin lyase enzyme which acts upon peptic materials and immensely leading for technological innovations and also helps in the productivity and reduce economic costs. About 25% of the world's enzyme production is solely from this enzyme (Alkorta et al. 1998, Kashyap et al. 2001). This enzyme is proved to be very important in applications for industries like food, paper and textile (Yadav et al. 2009). Pectic enzymes that are utilized in food industry, for commercial purpose have a mixture of oxidoreductase, hemicellulose, proteases, cellulases and glycosidases (Krop & Pilnik 1974, Mc Feeters 1985).

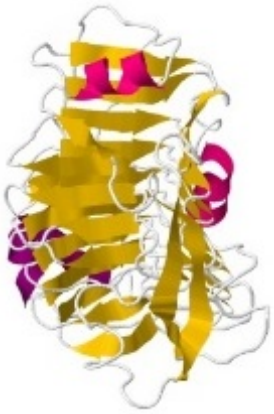

Fig. 12 – Pectin Lyase A (Reproduced with permission from Mayans et al. 1997)

Protease

These are assembly of enzymes, whose catalytic purpose is to hydrolyze peptide bonds of proteins and break down them into polypeptides or free amino acids (Deng et al. 2010). They are associated in a many physiological reactions ranging from basic digestion of food proteins to highly controlled cascades (Hooper 2002, Oseni 2011).

Industrial application of these enzymes includes photographic industries, food, leather, tanning, pharmaceutical, detergents industries and waste treatment (Jellouli et al. 2009, Bhaskar et al. 2007, Gupta et al. 2002). These are divided into 3 groups, acid, neutral and alkaline proteases based on their acid-base behavior. Acid proteases are those that perform finest at pH range of 2.0-5.0, these are typically produced by fungi. Favoured source of enzyme is microorganisms as these are rapid in growth, entail partial space for cultivation and also has ease for heritable manipulation in order achieve novel enzymes with changed properties desirable for numerous applications (Kocher & Mishra 2009).

Fig. 13 – Extracellular metalloproteinases from Aspergillus (Reproduced with permission from Fernández et al. 2013)

Raffinase

An enzyme that hydrolyzes raffinose, yielding fructose in the reaction is raffinase. Nearly 40% of totally obtainable enzymes such as raffinase, amylases, dextranase, lactase, pectinase and cellulose are from filamentous fungi especially Aspergillus spp. (Archer & Peberdy 1997). Industrial applications include food (Singh & Kayastha 2013).

Fig. 14 – Crystal structure of alpha-galactosidase I from Mortierella vinacea (Reproduced with permission from Fujimoto et al. 2009)

Application of Fungal Enzymes for treating industrial effluents

Capable of producing rare enzymes proficient in performing under chemically difficult conditions fungi are efficient in exploiting minimal living conditions at large (Viswanath et al. 2008, Shraddha et al. 2011). Possessing characteristics of high selectivity and efficiency along with environmentally benevolent reactions, enzymes have achieved significant attention for removal of environmental and industrial pollutants. Among such enzymes that were studied, fungal extracellular enzymes of two major classes i.e., peroxidases (lignin and manganese) and laccases have been extremely exploited for studies of pollutant removal including toxic phenolic compounds and recalcitrant xenobiotics. Innumerable studies have been reported during the recent years focussing on improvement of fabrication of these enzymes, screening of novel fungal strains, amendments in evolution conditions, utilizing inducers and search for economically viable growth substrates like food and agricultural wastes (Viswanath et al. 2014). Bioremediation of industrial effluents via enzymatic approach involves conversion of toxic and complex chemical substances into safe compounds through removal of few functional groups either in-vivo or in-vitro processes (Venkatesagowda et al. 2012, Messias et al. 2009, Balaji et al. 2014). The following discussion provides an insight into fungal enzymes utilized for treatment of industrial effluents. Table 3 provides an overview of various pollutants in industrial effluents degraded by respective fungal enzymes.

Table 3 Fungal enzymes employed for degradation of various pollutants in industrial effluents

Fungus	Enzyme	Industrial Pollutant	References
Caldariomyces fumago	Chloro peroxidase	Phenols	Wannstedt et al. 1990
Coprinus cinereus	Peroxidease	Phenols	Budde et al. 2001
C.cinereus	Peroxidase and H_2O_2	Phenols	Kauffmann et al. 1999
Pleurotus eryngii	Versatile peroxidase	Phenols and Hydroquinones	Gómez-Toribio et al. 2001
P.eryngii	Versatile peroxidase	2,4-DCP	Rodriguez et al. 2004
Bjerkanderaadusta	Versatile peroxidase	PCP	Davila-Vazquez et al. 2005
P.eryngii	Versatile peroxidase	Benzyl, Cinnamyl,napthyl, andaliphatic, unsaturated alcohols	Guillen et al. 1992

Table 3 Continued.

Fungus	Enzyme	Industrial Pollutant	References
Penicillium simplicissimum	Aryl-alcoholoxidase	Vanilly alcohol	De Jong et al. 1992
Penicillium simplicissimum	Methyl transferase	Phenols, benzoic acids, -OH substituents, etc.	Jeffers et al. 1997
Aspergillus nidulans	N-Acetyl-6-hydroxy tryptophan oxidase	Cersol	Birse & Clutterbuck 1990
Chrysonilia sitophila	LiP type III	Bleach Plant	Dezotti et al. 1995
Chrysonilia sitophila	Free LiP, LiP III	Bleach Plant	Ferrer et al. 1991
Phanerochaete chrysosporium	MnP	Bleach Plant	Michel et al. 1991
Coriolus versicolor	Laccase	Bleach Plant	Davis & Burns 1990
Laccase			
C.versicolor	Laccase	2,4,6-TCP,0.5-5mM	Ullah et al. 2000
T.versicolor	Guaiacol	2,4,5-TCP, 2mM	Roper et al. 1995
Pleurotus ostreatus	Laccase	2,6- Dimethoxyphenol, 1mM	Hublik & Schinner 2000
panus tigrinus	MnP	2,4,6-TCP,0.5-5mM	Leontievsky et al. 2000
T.versicolor	Laccase	Anthracene, 15mg/l	Johannes et al. 1996
Pycnosporus cinnabrinus	Laccase	Benzo [α] pyrene 1,6-3,6 and 6,12-quinones	Rama et al. 1998
Coriolopsis gallica	Laccase	Anthracene.20µM	Pickard et al. 1999
Trametes versicolor	Laccase	Delignification	Paice et al. 1996
Pycnosporus cinnabrinus	Laccase	Delignification	Balakshin et al. 2001
Trametes villosa	Laccase	Delignification	Chakara & Ragauskas 2001
Coriolus versicolor	Laccase	Delignification	Balakshin et al. 2001
Peroxidase			
P.chrysosporium	LiP	BenBenzo [α] pyrene	Haemmerli et al. 1986
Phanerochaete chrysosporium	MnP	Fluorene,20µM	Bogan et al. 1996
Nematoloma frowardii	MnP	Anthracene, Pyrene, Fluoranthene	Ute & Fritsche 1997
Trametes versicolor	MnP	Phenethrene	Collins & Dobson 1996
Phanerochaete chrysosporium	Peroxidase	Methylated Lignin	Tien & Kirk 1983
Nematoloma frowardii	Peroxidase	Milled Straw	Hofrichter et al. 1999
Nematoloma frowardii	Peroxidase	DHP	Steffen et al. 2000
S.coronilla	Peroxidase	Non-Phenolic lignin	Kapich et al. 2005
P. chrysosporium	Crude LiP	Azure B, Tropaeolino, Orange2	Cripps et al. 1990
Bzerkanderaadusta	MnP	Reactive Blue15	Heinfling-Weidtmann et al. 2001
Bjerkanderaadusta sp.	MnP	Orange2	Lopez et al. 2002
P. chrysosporium	MnP	Crystal Violet polyR:478	Moldes et al. 2003
White:Rot Fungus	LiP	AcidBlue 25, Indigo Carmine, Reactive Blue	Young & Yu 1997

Lipases

Triacylglycerol acylhydrolases catalysing hydrolysis of triacylglycerol to fatty acids and glycerol are called Lipases (Thakur 2012). Apart from hydrolysis of triglycerides, lipases catalyze diversity of chemical reactions including esterification, acidolysis, trans-esterification and aminolysis. Infact lipase is often used for catalysing hydrolysis of varied substrates which are not natural to obtain selective enantio- and regio substrates (Wang et al. 2015, Facchini et al. 2016).

Fungal species having capability to secrete lipases that deteriorate fats and oils are found in soils polluted with discharge from the products of oil and dairy (Niyonzima & More 2014).

Lipases have been explored extensively for treating waste water (Dauber & Boehnke 1993). Deterioration of polyvinyl alcohol films and bioremediation of polluted soils was possible by lipase from *Aspergillus niger* and *Aspergillus terreus* respectively (Jecu et al. 2010, Mahmoud et al. 2015). Lipase from other species that have shown effective bioremediation are *Aspergillus ibericus* and *Aspergillus uvarum* (Salgado et al. 2016). Essentially lipolytic enzyme from *Aspergillus niger* that have been isolated from soil polluted by oil have reported to degrade polyaromatic hydrocarbons (Mauti et al. 2016, Margesin et al. 1999, Riffaldi et al. 2006, Prasad & Manjunath 2011).

Mechanism of Lipase Activity – Reactions of lipolytic activity occur at the interface of lipid-water, resulting in formation of balance among monomeric, emulsified, and micellar states by lipolytic substrates (Sharma et al. 2011). Triglyceride being the chief constituent of fat or natural oil hydrolyzes successively to monoacylglycerol, fatty acids, glycerol and diacylglycerol among which glycerol and fatty acids are extensively utilized as raw materials. Ex: monoacylglycerol utilized in pharmaceutical cosmetic and food industries as emulsifying agent. A report on hydrolysis of triolein produced from *Candida rugose* lipase in oil-water biphasic system has proved to be efficient. Lipase adsorbs first on oil-water interface of the bulk water phase and then breaks triolein ester bonds resulting in production of monoolein, diolein and glycerol consecutively. Oleic acid is released at each successive reaction stage in the process of catalysis. Since glycerol released is in hydrophilic nature it gets dissolved in aqueous phase (Hermansyah et al. 2007).

Laccases

Belonging to minor group of enzymes, laccases are named – the blue copper proteins or the blue copper oxidases (Xu 1996, Thurston 1994) among others. These are oxidoreductases that oxidize diphenols along with other associated substances using molecular oxygen as electron acceptor (Viswanath et al. 2008, Thurston 1994). Their utilization as biocatalysts for organic synthesis has been ignored previously, perhaps as they were unavailable commercially (Riva 2006, Baldrian 2006, Joo et al. 2008).

Laccases are predominantly abundant in numerous white-rot fungi engaged in metabolism of lignin (Bourbonnais et al. 1995). Fungal laccases degrade lignin and remove potentially toxic phenols produce during the degradation of lignin (Thurston 1994). Additionally, fungal laccases are assumed engage in the release of dihydroxynaphthalene melanins, which are darkly pigmented polymers produced by organisms opposed to environmental stress (Henson et al. 1999) and morphogenesis of fungi by catalysing development of extracellular pigments (Zhao & Kwan 1999). With reference to their use in biotechnology, fungal laccases gained extensive applications, extending from effluent decolouration and pulp bleaching detoxification, organic synthesis, elimination of phenolics in wines, biosensors, obstructing functions of dye transfer in washing powders and detergents and synthesis of composite medical compounds, numerous of which have been patented (Yaver et al. 2001). Enhancement in biotechnological utilization of laccase was possible with induction of laccase-mediators that are proficient to oxidise non-phenolic compounds, which are or else scarcely or not oxidised by only enzyme (Couto & Herrera, 2007, Mendonça et al. 2008).

Mechanism of Laccase Activity – Laccased catalysed reactions continue by monoelectronic oxidation of suitable substrate molecule into equivalent reactive radical (Riva 2006). Process of redox reaction is possible with support of group of copper ions which form catalytic central of the enzyme (Wong et al. 2008). Of specific interest are laccases with respect to impending applications, owing to their competences of oxidizing extensive range of environmentally dangerous substrates.

Hazardous compounds recognised to have carcinogenic effect owing to their persistence in nature are pentachlorophenols (PCP), polycyclic aromatic hydrocarbons (PAH), 1,1,1-trichloro-2,2-bis (4-chlorophenyl)ethane (DDT), polychlorinated biphenyls (PCB), ethylbenzene, benzene, xylene, toluene and trinitrotoluene (TNT). These substances are transformed by fungi through

bioremediation (Riva 2006). Laccase from ectomycorrhizal fungus *Xerocomus chrysenteron* responds to DDT pressure in various ways, signifying huge prospective for biodegradation or mineralization of DDT (Chao et al. 2008). Biodegradation of 2, 4-dichlorophenol using surface response methodology by laccase from *Pleurotus* sp. is one among latest applications established by Bhattacharya & Banerjee (2008). BPA oxidation using laccase weakens binding of environmental estrogen to ER-alpha resulting in losing all its ER-alpha-dependent impact on cells of cancer propagation Bolli et al. (2008).

Aqueous solutions contaminated by BPA were bioremediated using laccase from *Trametes versicolor* which is immobilized on hydrophobic membranes in non-isothermal bioreactors (Ricupito et al. 2009).

Xenobiotic compounds that are oxidized by laccases include polycyclic aromatic hydrocarbons (Tekere et al. 2007, Pozdnyakova et al. 2004, Koschorreck et al. 2008), pesticides (Gorbatova et al. 2006, Torres et al. 2003, Ford et al. 2007), and chlorinated phenolics (Colao et al. 2006, Bollag et al. 2003). Furthermore, polycyclic aromatic hydrocarbons, arising from oil deposits were also seen to be degraded by laccases (Hu et al. 2007, Pointing 2001, Galli et al. 2008).

The underlying phenomenon of removal of xenobiotics encompasses pollutant enzymatic oxidation to free quinones or radicals, which experience polymerization and fractional precipitation (Dec & Bollag 2000). Laccase from white-rot fungus, *Trametes hirsuta*, was utilized to oxidize alkenes (Niku-Paavola & Viikari 2000). The oxidation is result of a double-stage process where in enzyme catalyses primary substrate oxidation first, when a mediator is inserted to reaction, it oxidizes secondary substrate, alkene, to the equivalent ketone or aldehyde. Furthermore, laccase is also successful in immobilizing pollutants of soil by combining to humic substances of soil by process which is homologous to synthesis of humic acid in soils (Bollag & Myers 1992). Other xenobiotics which can be immobilized in similar way include phenolic compounds and anilines such as 3,4-dichloroaniline, 2,4,6-trinitrotoluene or chlorinated phenols (Ahn et al. 2002). The immobilization results in lowering biological accessibility of the xenobiotics and hence reducing their toxicity.

Lacaases also find importance in decolorization of dyes. Owing to their chemical structure, dyes are resistant to waning upon exposure to light, water and other chemicals, hence maximum of them are problematic to decolourize owing to their synthetic origin (Poots et al. 1976). Apart from decolorization these are also utilized for bleaching textiles and also for synthesizing dyes (Setti et al. 1999). Lacasses from *Pycnoporus sanguineus* have partially decolorized two azo dyes along with wide-ranging decolorization of 2 triphenylmethane dyes (bromophenol blue and malachite green) (Pointing & Vrijmoed 2000).While, laccase from *Trametes hirsute* are able to degrade dyes like triarylmethane, indigoid, azo and anthraquinonic dyes that are utilized in dyeing textiles along with 23 industrial dyes (Couto SR, Herrera et al. 1999).

Laccases are indespensible in treating bleach kraft pulp effluetns or detoxification of agricultural derivatives comprising coffee pulp or olive mill wastes (D'Annibale et al. 2000). Isolated from the fungus *Flavodon flavus*, laccase has shown decolourization of wastewater from kraft paper mill bleach plant (Raghukumar 2000). Similarly purified laccase from white-rot basidiomycete, *T. villosa* degraded bisphenol A, an endocrine-disrupting chemical (Setti et al. 1999). Nonylphenols resulting from partial biodegradation of nonylphenol polyethoxylates (NPEOs) are released into the environment, chiefly because of their incomplete elimination in effluent treatment units (Ying et al. 2002). These are resistant to biodegradation over their parental compounds resulting of being piled up in rivers around world leading to bioacculumation and biomagnification (Heemken et al. 2001, Junghanns et al. 2005). Aquatic hyphomycete *Clavariopsis aquatic* that secretes laccases has demonstrated to degrade xenoestrogen nonylphenol.

Peroxidases

Understood to be omnipresent enzymes, peroxidases catalyze lignin oxidation along with other phenolic substances at the cost of hydrogen peroxide (H_2O_2) in the existence of a mediator. These can be either haem or nonhaem proteins (Kim et al. 2002, Xu 1996). The most studied

peroxidases owing to their great prospect to degrade toxic substances are lignin peroxidase (LiP), manganese-dependant peroxidase (MnP), and versatile peroxidase (VP). Use of enzymes certainly have potential advantages which includes allowing degradation process under trivial biological conditions, applicability at both high and low contaminant concentrations over an extensive range of temperature, pH and salinity; suitability to unamenability to loading effects; biorefractory compounds; no acclimatization of biomass and decrease in sludge volume over all ease of regulating process (Nicell et al. 1993).

Fungal heme protein lignin peroxidase (LiP) produces radical cations by oxidizing dimethylated aromatic compounds. These are produced by most white-rot fungi, like *Phanerochaete flavido-alba* (Ben Hamman et al. 1999), *Bjerkandera* sp. strain BOS55 (ten Have et al. 1998), *Trametes trogii* (Vares & Hatakka 1997), *Phlebia ochraceofulva* (Vares et al. 1993), and *Phlebia tremellosa* (Vares et al. 1992). In fact numerous forms of isozymes were identified in *P. chrysosporium* cultures and also in great number of other white-rot fungi (e.g., *Trametes versicolor*, *Bjerkandera adusta*, *Phlebia radiata*). The activity and quantity of LiP isozymes produced by *P. chrysosporium* vary from 2 to 15, which are dependent on the strain, medium composition, method of cultivation and age of the culture.

Also, a glycosylated heme-containing extracellular peroxidase Manganese Peroxide has similar catalytic cycle as that of LiP and horseradish peroxidase (HRP), but uses absolute Mn (II) as a substrate which is widespread in lignocellulose and soil. Secreted in numerous forms in microenvironments by white-rot fungi and some soil litter–decomposing fungi manganese peroxidase is produced by around 56 fungi in liquid and/or solid-state fermentation as compiled by Hofrichter (2002). Distinct group of Basidiomycetes, like families Coriolaceae, Meruliaceae, Polyporaceae, and the soil litter families Strophariaceae and Tricholomataceae secrete MnP. Nearly 11 isozymes of MnP are reported to be formed by *Ceriporiopsis subvermispora* (Urzua et al. 1995, Lobos et al. 1994). Five isozymes in *P. chrysosporium* MP-1 have been detected (Kirk & Cullen 1998). For both LiP and MnP nitrogen-deficient conditions favour their production.

Both LiP and MnP has been extensively explored for their capabilities of treating various industrial effluents containing complex and varied composition of organic and toxic substances.

Mechanism of Peroxidases Activity – Steadiness of recombinant and innate peroxidases by *Phanerochaete chrysosporium* were investigated and found that one-electron oxidation by the enzymes will result in production of cation radicals of the contaminants. An impulsive chemical reaction like C-C cleavage of hydroxylation of cation radical's results in the formation of additional hydrophilic products. Metabolism of these products and cometabolism in the existence of appropriate carbon source will lead to the formation of CO_2 by these fungi. The mechanism of action of these enzymes result from complex process of oxidation, reduction, methylation, and hydroxylation (Nie et al. 1999).

Both LiP and MnP extracellular peroxidases are capable of cleaving several PAHs in vitro. These vary from each other in the variety of reducing substrates. Great yield in PAH quinones was improved when incubated with extracellular enzymes of white-rot fungi containing great levels of MnP (Field et al. 1992). It has been recognised that one-electron oxidations of several PAHs by Mn (III) suggests the role of MnP of *P. chrysosporium* in the breakdown of PAHs in vivo. Lignin peroxidase (LiP) and manganese peroxidase (MnP) produced by *Phanerochaete chrysosporium* contribute to decolourization of Olive Mill Wastes (Sayadi & Ellouz 1995). Aromatic rings are cleaved by these enzymes leading to prospective colour removal from dyes. Dyes like Azure B, Tropaeolin O, and Orange II were partially decolorized within 20 minutes by crude LiP in nitrogen-limited cultures of *P. chrysosporium* (Cripps et al. 1990). It was also reported that dye decolorization enhanced with higher LiP concentrations (Wu et al. 1996). Addition of Veratryl alchol (VA) enahces the degradation and decolorization rates of dyes as reported by Ollikka et al. 1993 where in addition of VA improved the decolorization of Reactive Red from 22 to 96% within a duration of 3 minutes. Different sources of LiP have also shown similar efficiency as reported by Christian et al. 2005 where LiP from *Trametes versicolor* decolorized Remazol Brilliant Blue R

(RBBR) in the presence or absence of VA (Ferreira et al. 2000). LiP from *P. chrysosporium* oxidizes sulfonated azo dyes, generating sulfophenyl hydroperoxides (Chivukula et al. 1995).

Another major area of application of LiP and MnP is in degradation of lignin, which has attracted their use in degradation of paper and pulp mill effluents.White-rot fungi are naturally principal lignin degraders owing to presence of precise genes for the enzymes (LiP and MnP) essential for depolymerisation of lignin, other enzymes including laccase and hydrogen peroxide–generating enzymes and reactive oxygen species (ROSs).

Production of these enzymes can be in diverse combinations, signifying multiple strategies for biodegradation of lignin. In course of lignin degradation oxidation of phenolic compounds to phenoxy radicals happens through lignin-degrading enzymes, whereas oxidation of nonphenolic compounds through cation radicals. Depending on the trend of enzyme production, Hatakka (1994) categorised white-rot fungi into 3 major classes:

(1) The lignin–manganese peroxidase group (LiP-MnP) (e.g., *Phlebia radiata* and *Phanerochaete chrysosporium*),
(2) The manganese peroxidase–laccase group (e.g., *Rigidoporus lignosus* and *Dichomitus squalens*), and
(3) The lignin peroxidase–laccase group (e.g., *Junghuhnia separabilima* and *Phlebia ochraceofulva*).

Diversity of oxidases like glucose-1-oxidase, glucose-2-oxidase or pyranose-2-oxidase, glyoxal oxidase, methanol oxidase, veratryl alcohol oxidase are produced by white-rot fungi in addition to LiPs, MnPs, and laccases, which are sources of H_2O_2 used by peroxidases in lignin degradation. It is reported that Glucose-1-oxidase and glucose-2-oxidase play an significant role in the ligninolytic system of *P. chrysosporium* by producing H_2O_2. Cellobiose dehydrogenase (CDH), widely produced by both white-rot and brown-rot Basidiomycetes in the family of Coniophoraceae, and some soft-rot fungi and molds have been listed by Henriksson et al. (2000). This enzyme is an extracellular flavin- and heme-containing which can reduce O_2 and produce H_2O_2.

Other agents aiding in lignin degradation are identified as reactive oxygen species (ROSs), which are required owing to quite big lignocellulolytic enzymes which are difficult in penetrating lignified wood cell walls, hence employing these low-molecular-weight agents towards decay initiation. Being diffusible and small enough in penetrating and aiding in the function of depolymerisation of wood cell walls as hypothesized during the mid of the twentieth century. Though maximum work is reported on hydroxyl radicals (· OH), other ROSs, like peroxyl radicals (ROO ·) and hydroperoxyl radicals (· OOH) may also be engaged in fungal attack. Yet supplementary biological ROS agents contain singlet oxygen, hypochlorous acid, and peroxynitrite.

Miscellaneous Enzymes

Produced both by brown - and white-rot fungi oxalate acts as organic acid chelator. Produced at same time as MnP through liquid cultures of *Phanerochaete chrysosporium*, LiP and MnP can decompose oxalate in the occurrence of VA or Mn (II), respectively. Electron transfer is the meachnism involved in reaction among enzymatically formed VA+• and oxalate, decarboxylating oxalate and reducing VA+• back to VA. Coexistence of oxalate with lignin-degrading enzyme systems indicates inhibition of lignin degradation as small amount of oxalate is observed to decrease lignin content significantly in bio bleaching experiments (Vares et al. 1992).

Conclusion

Utilization of fungi for industrial wastewater treatment is an older process which is not a new technology. Though fungi are efficient in treating many wastewaters, nevertheless it is to be understood that still there are numerous sorts of wastewaters which are not degraded by fungi attributed either to slow growth of fungi or trouble in using them for bioreactors. While as new manufacturing products are used, newer varieties of effluents will be generated which may be amenable to degradation by fungi. During contemporary years, action mechanism of white-rot

fungi associated to organic pollutants was understood to offer great potential for utilization of treating industrial effluents like paper and pulp, textiles, dyes and PCB degradation.

Advanced methods like using immobilized fungal cells or enzymes in beads or carriers have received good success, resulting in reduction of treatment cost. Past decades have witnessed considerable work focussing on screening of fungi and their genes for degradation of various wastes but failed in isolating needs that need specific objectives. Hence, research in this area has opened new prospects for a variety of molecular biology studies related to biodegradation. Future holds promise for development of genes required in cloning for the decolorization and decomposition of dyes as well as bioengineered fungi that can utilize pollutants or various industrial effluents as substrates. Protein engineering is also being considered to enhance catalytic properties of enzymes that will lead to a new generation of tailor-made enzymes to meet specific demands.

Conflict of Interest:

All the authors declare no conflict of interest.

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