1. INTRODUCTION

Rapid industrial expansion is one of the main reasons for the increase in pollution in densely populated and less developed regions of the world. In recent years, the increase in demand and availability of labours in less developed countries encouraged different chemical industries at large and small-scale level. One such example of the small-scale industry is the textile and dyeing industries. India has a long custom of producing a variety of textiles and is the second largest sector in the country after agriculture. From total Indian exports, around 15 % are contributed substantially by the Indian textile industries (Chaudhary 2011).

The Indian textile industry is labour intensive and is one of the largest employment providers which employ around 92 million skilled and unskilled workers (Karhad 2014). Furthermore, globalization of Indian textile industry also promoted “total industrial production” factor of this sector. The Indian textile industry contributes around 9 % of GDP and 35 % of foreign exchange earnings by exporting textiles and its related products (Kumar and Bharathi 2011).

The textile industry in most of the countries are undergoing drastic changes as globalization of textiles led to international competition in environmental friendly manufacturing and use of textile products. Commercial dyes are available with 20 to

80 % concentration of active ingredients in liquid dispersions, granules, pastes, and powders (Idris *et al.* 2013). All these dyes are recalcitrant in nature and are the major source of water pollution in developing countries.

During recent years awareness of environmental problems has increased considerably and, has become a major issue in the international textile trade as thousands of different dyes and auxillary chemicals are used in order to produce quality products (Jacob and Azariah 2000). About 0.7 million tons of an annual consumption of synthetic dyes have been reported, from which two-third of the total dyestuff market alone accounts for textile industry (Deveci *et al.* 2004; Gregorio *et al.* 2010). Moreover, the growth of the worldwide textile industry has seen a proportionate increase in the use of synthetic dyes, and this led to rise in pollution due to the effluent contaminated with dyestuff released into rivers and natural ponds (Pandey *et al*. 2007).

1.1 Dyes

A dye is a coloured substance that has an affinity to the substrate to which it is being applied. Such substances with considerable colouring capacity are widely employed in the textile, pharmaceutical, food, cosmetics, plastics, photography and paper industries. Since prehistoric period, people have been using colourants to dye furs, textiles and other objects. These natural substances were mainly obtained from plants, vegetables or animals. In the textile industry, natural dyes have been mostly replaced by synthetic dyes as they possess a varied range of colours, intensity, ease of manufacture, fastness and resistance to fading by physical, chemical and microbial agents (Wesenberg *et al*. 2003). Moreover, the growth of the worldwide textile industry in the years since then had seen a commensurate increase in the use of such synthetic dyes.

1.1.1 Classification of dyes

Dyes may be classified by their usage or application method or by their chemical structure.

a. Classification of dyes based on their use or application method

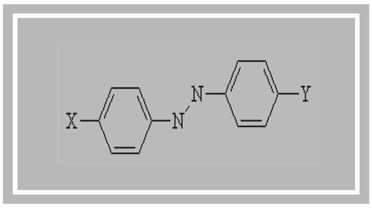
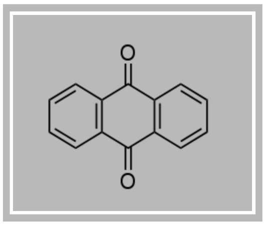
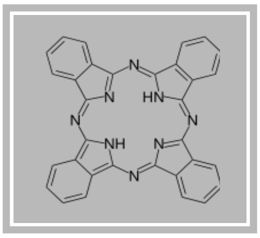
Classification of the dyes by their use or application is the principal system adopted by the Colour Index (Hunger 2003). The dye classes presented are considered most interesting for dyeing different textile fibres and shows structural interrelationships of dyes that are assigned to different classes by the Colour Index (Table 1).

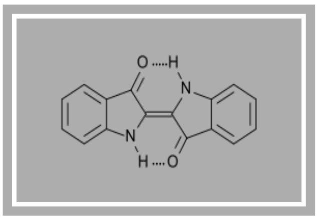
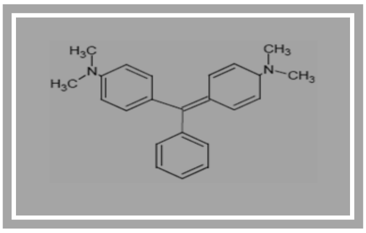
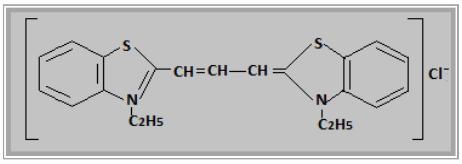
Class	Chemical type	Principal Textile Substrate	Pollutants associated with Dyes
a. Water Soluble Dyes			
Acid	Low molecular dyes with a mono-azo, di-azo or anthraquinone structure	Wool, Nylon, Silk	Colour, organic acids, unfixed dyes
Basic	Azo, Azine, Xanthene, Cyanine, Anthraquinone, Hemicyanine, Diphenylmethane	Polyester, modified Nylon	Colour
Direct	Azo, Phthalocyanine, Stilbene, Oxazine	Cotton, Rayon, Nylon, Leather	Colour, salt, unfixed dye, cationic fixing agents, surfactant, defoamer, leveling and retarding agents, finish, diluents etc.
Reactive	Azo, Phthalocyanine, Anthraquinone, Formazan, Oxazine	Cotton, Silk, Nylon, Wool	Colour, salt, alkali, unfixed dye, surfactants, defoamer, diluents
b. Water Insoluble Dyes			
Disperse	Azo, Styryl, Nitro, Benzodifuranone, Anthraquinone	Polyester	Colour, organic acids, carriers, leveling agents, phosphates, defoamers, lubricants, dispersants, delustrants, diluents
Sulphur	Indeterminate structure	Cotton, Rayon	Colour, alkali, oxidizing agent, reducing agent, unfixed dye
Vat	Anthraquinone (including polycyclic quinine), Indigoids	Cotton, Rayon, Wool	Colour, alkali, oxidizing agents, reducing agents

Table 1: Classification of dyes based on their use or application (Hunger 2003).

b. Classification of dyes based on their chemical structure

Dyes are composed of a group of atoms known as chromophores, which are responsible for the dye colour. These chromophore-containing centers are based on diverse functional groups, such as azo, anthraquinone, phthalocyanine and others as shown in (Table 2).

Chromophore	Chemistry	Properties
Azo	Distinguishes by presence of one or more azo group **(-N=N-)**, bound between two nitrogen atom that are usually bound by an aromatic group. 	Now days, Azo dyes are widely used because of their good performance and cost effectiveness.
Anthraquinone	Term anthraquinone, refers to a specific isomer 9,10 anthraquinone (IUPAC: 9,10-dioxoanthracene) wherein the keto groups are located on the central ring. 	These dyes are known for their good fastness and light fastness. The major drawback is that they are expensive (Hunger 2003) and are used to some extent to provide red and blue shade as other dyes cannot provide such combination properties that are offered by anthraquinone dyes.
Phthalocyanine	This group of dyes is constituted by a ring structure of aromatic or benzopyrrole (indole) rings with a metal ion in the center. 	The colours are extremely stable and strong, making them cost effective.

Indigoid	Indole structture is built up by a ring closure benzene derivatives C-N or C-C bond formation. 	Indigo is still widely used to colour denim, mostly due to its gradual fading characteristic which gives paler blue shades.
Di- and Triarylcarbenium	Belong to the class of polymethine dyes and can be considered as branched polymethines. The branches are created by two aryl rings in which polymethine is incorporated and bonded by another R group to central methine carbon atom. 	Dyes are bright and strong but are generally deficient in light fastness.
Polymethine	Methine dyes and polyene dyes that are characterized by a chain of methine groups that forms a system of conjugated double bonds. 	Commercial use of these dyes is restricted to sensitizing dyes for silver halide photography and as infrared absorbers for optical data storage and other bio-imaging application.
Cationic dyes	Cationic dyes carries a localized or delocalized positive charge in their molecule. The salt forming counter ion is the colourless anion of low molecular mass inorganic or organic acid. Many of these dyes can be converted into water-insoluble dye bases by addition of alkali, for this reason they were formerly known as basic dyes.	Initially they were used for dyeing paper, leather, silk etc. Their high brilliance and colour strength is partly compensated for their poor light-fastness on these materials. Therefore, they gained much greater importance for dyeing acid- modified polyester and polyamide fibres.

Sulphur Compounds	These dyes are manufactured by heating aromatic or heterocyclic compounds such as amines, phenols or nitro compunds with sulphur or alkali metal polysulphides.	Sulphur dyes constitute the class of dyes for producing cost-effective tertiary shades especially black on cellulosic fibres.
Metal complexes	Metal complexes are often formed with azo dyes; most used metals in metal complex dyes are chromium and copper, iron, cobalt and nickel. The most important groups involved in complex forming around the metal ion are hydroxyl (-OH), carboxyl (-CO_2) and amino groups (-NH-).	Dyeing of wool in dark shades with high fastness.
Fluorescent dyes and other chromophores e.g. stilbene dyes, formazan dyes, quinophthalone dyes, nitro- & nitroso and triphenodioxazine dyes	This dyes produce a pure colour dictated by their absorption and emission spectra and have high molar extinction and quantum yield. These requirements are met by very few dyes; therefore, are not widely used.	The disadvantage is their poor light-fastness

Table 2: Classification of dyes based on their chemical structure (Hunger 2003).

1.2 Environmental impact of synthetic dyes

Today, synthetic dyes have conquered industries like textile, paper, pharmaceutics, cosmetics, food, paints, printing and others by their expansive applications (Marmion 1991). In the textile manufacturing industry, every year up to 50 % of dyes are lost in effluents due to the inefficiency of the dyeing process during the dyeing and finishing operations (Vaidya and Datye 1982; Yang *et al*. 2009).

Considering the account of production by volume and number, worldwide production of 60-70 % organic dyes are constituted by azo dyes, thus making it as the largest group of colourants (Carliell *et al*. 1998; Bafana *et al*. 2011; Chequer *et al*. 2013). These are the most common dyes spilled into the environment as they are lucrative and easily synthesized. It is noteworthy that the majority of the success story of azo dyes over the natural dyes and other textile dyestuff is because of their great structural diversity, high fastness properties in terms of light and wetness, stability and availability of varied range of colours (Chang *et al*. 2004; Saratale *et al*. 2011).

Azo dyes possess toxic end products such as benzidines, 1, 4 phenylenediamine, sulphonated and unsulphonated compounds and aryl amine derivatives. These products not only have a pessimistic effect on the wastewater but are also obstinate to biodegradation and have been proven as potent carcinogens and mutagens (Chung and Stevens 1993; Carliell *et al.* 1995). Available literature also indicates that the aquatic flora and fauna are also severely affected by the acute toxic effects such as reduction in sunlight penetration; which results in a consequent decrease in the photosynthetic activity, dissolved oxygen concentration and water quality caused by the indecent expulsion of textile dye effluents (Vandevivere *et al*. 1998; Saratale *et al*. 2011). This raises a vital stipulation for the development of multistep management concepts which not only assures irreversible decolourization but also mineralization of azo dyes.

1.3 Scope for treatment of different dye removal techniques

It has become increasingly important to understand the environmental fate of these pollutants. An upsurge of a new substantial industry for environmental restoration is led today by the appreciation as environmental pollution is a worldwide threat to public health (Aust and Benson 1993). However, in the recent years a number of treatment methods have been projected for treatment of coloured textile dyes and effluents (Fig. 1).

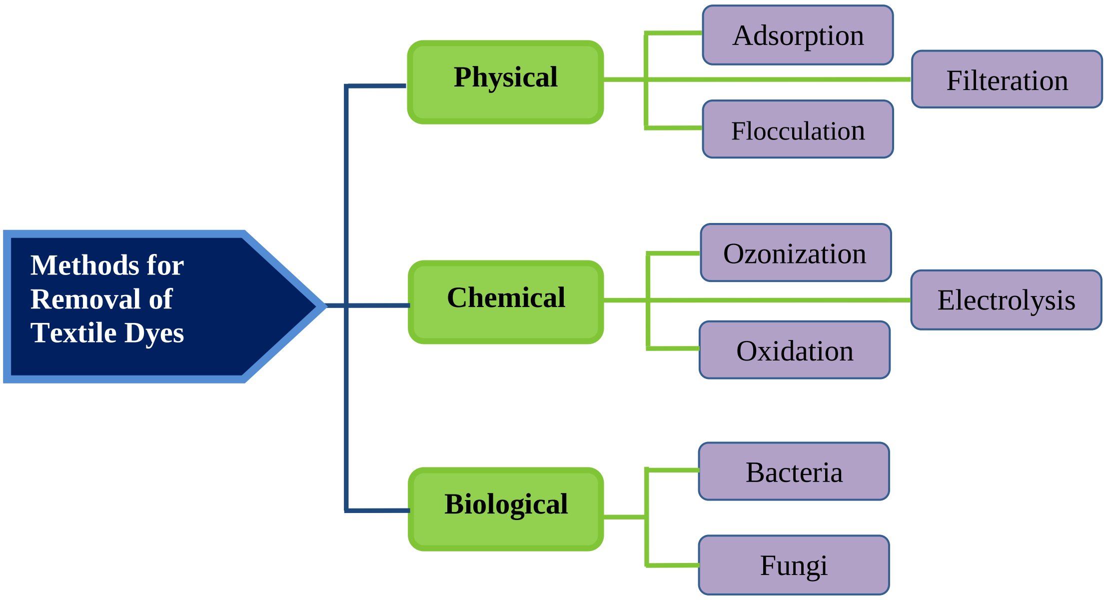


Figure: 1 Treatment methods for the removal of textile dyes.

1.3.1 Physical methods

Physical methods based on adsorption renders criteria for selection of adsorbents based on the characteristics like the possibility of adsorbent regeneration and high affinity for various dyes (Karcher *et al.* 2002). Field scale applications of adsorption technology are limited due to its excessive maintenance cost and the eco-friendly disposal of spent adsorbents (Verma *et al*. 2012).

In flocculation methods, effective removal of sulphur and disperse dyes has been observed, whereas acid, reactive, direct and vat dyes shows low flocculation capacity (Marmagne and Coste 1996; Vandevivere *et al*. 1998). Although, filteration method can be used for filtering and recycling of mercerising, bleaching and pigment rich wastewaters; these method accounts for the use of highly expensive membrane filters and requires different pre-treatments depending upon the type of influent wastewaters (Robinson *et al*. 2001; Akbari *et al*. 2006; Verma *et al*. 2012). These make the process more expensive and thereby limit the application.

1.3.2 Chemical methods

Chemical methods such as ozonization have high reactivity with azo dyes, but its practical application is limited due to its low capacity to remove C.O.D, high ozone cost and incompetence towards dispersed dyes and other water insoluble dyes (Alaton *et al*. 2002; Anjaneyulu *et al*. 2005). In advanced oxidation processes, many oxidizing agents like ozone and hydrogen peroxide are used in combination with TiO_2, ZnO_2, Mn, Fe and other catalysts to enable the destruction of dye molecule, and making them prone to degradation. However, advanced oxidation processes like Electrochemical oxidation and Fenton's oxidation have been reported to have a very high rate of colour removal, but generates iron oxide sludge which limits the process (Saxe *et al.* 2006; Archna *et al*. 2012).

In brief, physicochemical techniques inherits drawback such as high investment cost, production of large amount of sludge causing land pollution, restoration, secondary pollutants, interactions with other wastewater constituents thus, being unable to completely remove the recalcitrant azo dyes (Forgacs *et al.* 2004; Zhang *et al*. 2004; Mohan *et al*. 2007; Tastan *et al*. 2010). Stringent regulations by government for the discharge of coloured effluents from textile and dyestuff manufacturers has today raised a serious concern for the environmental regulatory agencies in several countries because no single conventional technology can completely remove all classes of dyes.

1.3.3 Biological methods

Today, decolourization of azo dyes is a challenge for textile as well as wastewater treatment industries because of strict rules and regulation. Perusal of literature suggests that biological methods provide a great potential advantage over the conventional treatment methods. By this method, decolourization of dyes may take place in two ways: i) either by adsorption on the microbial biomass (biosorption) or ii) by bioremediation of dyes by the cells (Zhou and Zimmermann 1993). Therefore, it is necessary to clarify the concepts of biosorption and bioremediation. In the process of biosorption, the solutes gets bind to the biomass by processes which do not involve metabolic energy or transport (Tobin *et al*. 1994). In contrast, in the process of bioremediation, the dye molecule is broken down into its derivatives by microbes like bacteria, algae, yeast and fungi which are either naturally occurring or introduced deliberately.

In recent years, bioremediation is a key research area in the environmental sciences and being cost-effective and it is receiving much attention for treatment of textile dye (Sugumar and Thangam 2012). Bioremediation includes biodegradation and biotransformation. Biodegradation addresses a biologically mediated breakdown of chemical compounds. This process ends achieving a complete mineralization, as the original dye structure is destroyed and the microbial cells tears the pollutant into water, carbon dioxide and/or any other inorganic end products (Reshma *et al.* 2011). Therefore, formulations can be made and launched in large quantities into polluted sites once these microorganisms such as bacteria and fungi having ability to degrade toxic waste are isolated and cloned.

Biodegradation by bacteria

A number of researchers have investigated the ability of bacteria to metabolize azo dyes (Cao *et al.* 1993; McMullan *et al.* 2001; Claus *et al.* 2002; Bhaskar *et al.* 2003). Different groups of bacteria have the capability to decolourize azo dyes under conventional anaerobic, facultative anaerobic and aerobic conditions (Saratale *et al.* 2011). Under anaerobic conditions, decolourization also involves the azo bond cleavage i.e. a transfer of four-electrons (reducing equivalents), which proceeds through two stages at the azo linkage, which is referred as dye reduction. Due to this reduction, the azo dyes are not only degraded but also gets converted into aromatic compounds (Blümel *et al.* 2002), which may be toxic, mutagenic, and possibly carcinogenic (Pinheiro *et al.* 2004). Therefore, it is necessary that these carcinogenic aromatic amines have to be degraded aerobically to achieve complete degradation of azo byes. On the other hand, they are not readily metabolized (Claus *et al.* 2002) and the substituent compounds of azo dyes contain sulpho and nitro groups, which are recalcitrant to aerobic bacterial degradation. Furthermore azo reductases being very specific in nature, cleaves only azo bonds of selective dyes (Zimmermann *et al*. 1982; Zimmermann *et al*. 1984). Biodegradable components of industrial wastewater can be removed by aerobic treatment, but according to Joshi *et al.* (2004) it is futile in degrading xenobiotic compounds such as dyes.

Biodegradation by fungi

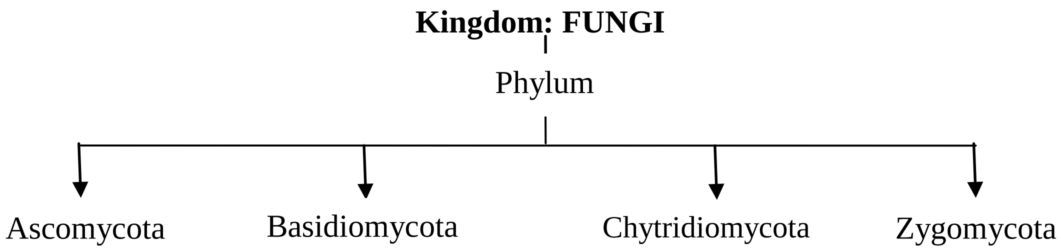
Fungi have been acknowledged as the most effective microorganisms in biodegradation due to their innate ability to produce low specific extracellular enzymes that are capable of hydrolyzing recalcitrant organic compounds (Couto 2009; Kaushik and Malik 2009; Idris *et al*. 2013). Fungi play a vital role in maintaining the environmental equilibrium, and their ubiquitous nature not only enables them to colonize all matrices *viz*. air, soil and water, but also adapt their metabolism to varying carbon and nitrogen sources (Saratale *et al.* 2011; Anastasi *et al*. 2013). The key factor addressing a flourishing bioremediation by fungi relies upon its availability, act of adsorption and degradation, and their ability to amalgamate with environmental condition that promotes a clean-up process with low impact on native biota (Jurado *et al*. 2011).

1.4 Physiology of fungi and its classification

A fungus is a eukaryotic, multicellular organism consisting of a dense hyphal network called mycelium, which ramifies through the substrate. The fungal hyphae are long, tubular, filamentous structure with a growing tip. Their cell wall is made up of complex polysaccharides called fungal chitins. The heterogeneity in lifestyle of fungi is reflected by their complex reproduction, either sexually or asexsually depending on the environmental conditions. According to Ainsworth *et al.* (1990), the kingdom fungi are divided into two divisions: a) Myxomycota (plasmodial forms- cytoplasm without cell wall) and b) Eumycota (mycelial forms).

Myxomycota consist of 4-classes: Acrasiomycetes, Hydromyxomycetes, Myxomycetes and Plasmodiophoromycetes; while Eumycota is divided into five subdivision: Mastigomycotina, Zoomycotina, Ascomycotina, Basidiomycotina and Deteromycotina.

However, Hawksworth *et al.* (1995) revised the Ainsworth's classification and divided it into four phylum. According to him the sub-division Deuteromycotina represents asexual stages of Ascomycota and some Basidiomycota, and was not classified as a monophyletic group.



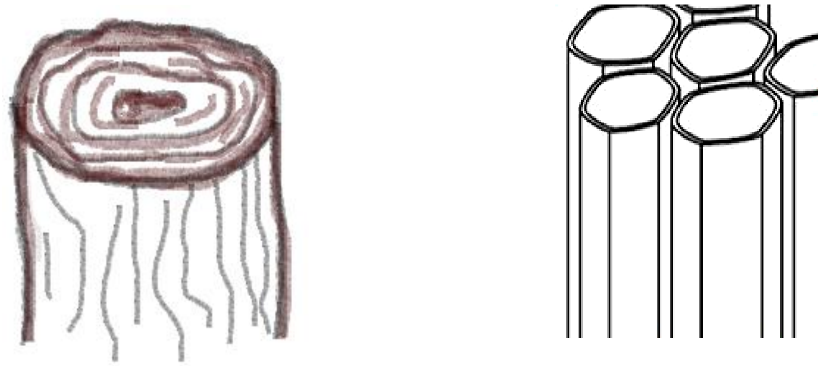
An estimated fungal biodiversity of the world is about 1.5 million species (Manoharachary *et al*. 2005). The ability of fungi to establish both mutualistic and pathosistic relationships with plants and other organisms and habitat diversity accounts a significant evidence of an extravagant evolutionary success of this heterogenous group (Anastasi *et al.* 2013).

Among the different modes of obtaining organic compounds for the growth, saprophytic and symbiotic nature of fungi having the capacity to decompose organic waste plays a major role in maintaining the carbon cycle (Sanchez 2009). This saprophytic nature is mainly attributed by wood degrading fungi which utilize plant biomass/ wood logs as their food source; thus decrease its strength and stability and impose a huge timber loss (Bento *et al.* 2014).

1.5 Wood degrading fungi

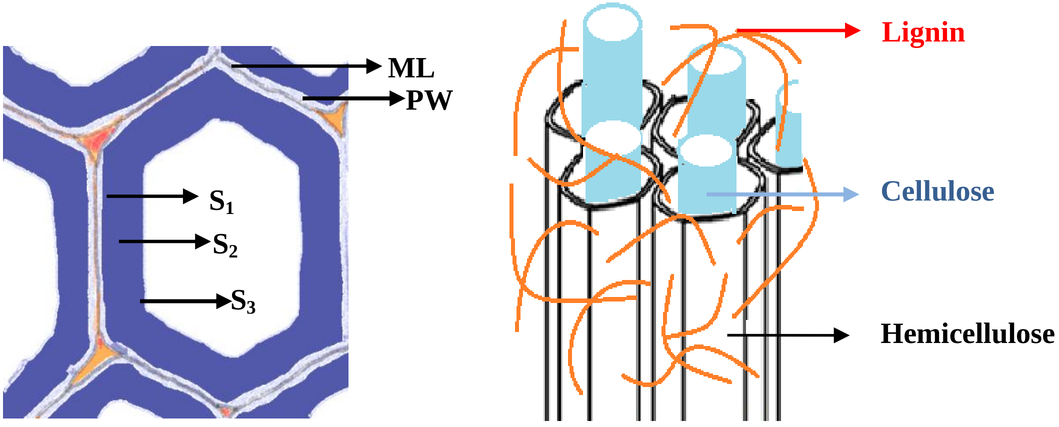
Saprophytism is the most essential lifestyle for the microbes. The procurement of nutrients during the course of evolution and development becomes a greater challenge for survival of fungi. Thus, to cope up with these challenges, fungi developed certain physiological and cellular mechanisms; to suffice their energy need by invading dead wood/plant biomass and decompose it.

Wood is featured as the natural, most profuse and renewable source of energy. Its cell wall is mainly composed of a mixture of lignocellulosics. These lignocellulosics consist of cellulose (ca. 40 %), hemicellulose (ca. 20± 30 %), and lignin (ca. 20±30 %) (Sjostrom 1993; Tuomela *et al*. 2000). Cellulose, the major wood component is a linear polymer with high molecular weight, while hemicelluloses are more complex bearing side chains of monosaccharaides and acetyl groups compared to cellulose. Both cellulose and hemicellulose determines the tensile strength of wood. Lignin is a complex polymer of phenylpropanoid units that arbitrates the adhesion between the fibres (Mai *et al*. 2004). The arrangement of cell wall component of wood is shown in (Fig. 2).



Wood

Plant cell



Plant cell wall

Cell wall components

Figure 2: Wood and its cell wall components (modified from, Schwarze 2007).

Wood degrading fungi with their unique non-specific enzyme system renders accessibility of carbohydrates stored in cell walls by effectively removing the lignin barrier and enabling gain of energy rich cellulose (Dashtban *et al.* 2010). Mostly, basidiomycetes also referred as white-rot fungi are involved in completely mineralization of the lignin. This non-specific enzyme system of wood degrading fungi with their ability to complete degradation of lignin have tremendously contributed to the biotechnological researches to their potential in bioremediation of organic pollutants, xenobiotic compounds, ethanol production and in bio-pulping of wood in the paper industry (Anastasi *et al.* 2013).

Various factors such as environmental conditions, nutrient availability, specification of fungal species and wood characteristics may be liable for wood degradation (Blanchette 1991; Perestelo *et al*. 1999; Luna *et al*. 2012). During the process of wood degradation, each fungus shows characteristic pattern of decay depending on its taxonomic group; due to the chemical and physical changes occurring in the wood cell walls. On the basis of the chemistry and morphology of decay patterns wood degrading fungi are classified into: Brown rot, Soft rot and White rot fungi (Worrall *et al*. 1997; Anagnost 1998; Schwarze 2007; Koyani *et al*. 2010).

1.5.1 Brown rot

Brown rot fungi cause rapid depolymerisation of hemicelluloses, cellulose and degradation of cell wall carbohydrates, leaving behind a lignin-rich brown coloured wood (Hammel *et al*. 2002). In the early stage of decay, the fibrous texture is lost due to removal of cellulose, while in the advanced stages of decay, wood is often cubically cracked and appears brown in colour and it is degraded via both non-enzymatic and enzymatic systems (Goodell 2003). These fungi employ cellulolytic enzymes for degradation of hemicelluloses and cellulose while lignin remains as such because of absence lignin degrading enzymes (Kobakhidze *et al.* 2012). With regard to attack on wood species, these fungi are most prevalent on conifers such as pines and firs. Most of the brown rot species are restricted to the group of basidiomycetes (Pointing *et al.* 2003).

Initiation of decay occurs by incursion of fungal hyphae through the cell lumen nearly associated with the S_3 layer. According to Lee *et al.* (2004), S_3 layer appears to resist degradation by brown rot fungi, while the first signs of rapid degradation by brown rot decay are visible in the outer regions of the cell wall, i.e. in the middle lamella, S_1, and the outer parts of S_2 layer (Irbe *et al*. 2006 ; Fackler *et al*. 2010). Considering the structural and chemical properties, majority of wood destroying brown rot fungi retain lignin while hemicellulose in the wood substrate appears to induce the synthesis of cellulose degrading enzymes (Kirk and Highley 1973; Nilsson 1974; Highley 1977; Blanchette 1983) or possibly a non-protein degrading system (Koenigs 1974). Compared to the crystalline cellulose, hemicelluloses are amorphous and surround the cellulose fibrils (Zabel and Morell 1992). According to Ringman *et al.* (2013), the fungus induces the production of hydroxyl radicals within the polymer matrix. These hydroxyl radicals react with hemicelluloses and lignin. Due to higher accessibility, hemicelluloses are degraded first and expose the cellulose (Curling *et al.* 2002; Irbe *et al.* 2006; Fackler *et al.* 2010), later these hydroxyl radicals start to depolymerize the cellulose, beginning with its paracrystalline moieties. Therefore, they are able to evade lignin mineralization and straightforwardly degrade the polysaccharides (Koenig *et al.* 2010; Martinez *et al.* 2011; Ringman *et al.* 2013) leading to an extensive removal of S_1 and S_2 layers (Liese 1970; Rayner and Boddy 1988; Eriksson *et al.*1990).

1.5.2 Soft rot

The term soft rot was originally proposed by Savory in (1954), for the decay caused by cellulose-degrading micro-fungi. Although, the major food sources are cellulose and hemicelluloses, soft rot fungi can degrade all three wood components *viz* lignin, cellulose and hemicellulose. Ascomycetes, Deuteromycetes and some bacteria features soft rot decay. Available literature indicates that some white and brown-rot basidiomycetes can cause facultative soft-rot decay (Schwarze 2007; Koyani *et al.* 2010; Pramod *et al.* 2015). Wood turns brown crumbly in dry environments while in wet environments it becomes very soft when attacked by soft rot fungi. According to Daniel and Nilsson (1998), soft rot susceptibility is inversely correlated with the wood lignin content; thus soft rot is mainly observed in hardwood having around 18-20 % of lignin content having guaiacyl units in hardwoods as compared to 25-28 % of lignin having guaiacyl and syringyl units in softwood, which is slightly higher.

The term soft rot is typically associated with formation of chains of distinct cavities with conical ends within the fibre wall of wood cell and also the formation of erosion troughs of cell wall erosion (Skyba 2008). However, similar cavities formation was reported by some white rot fungi (Duncan 1960; Daniel *et al.* 1992; Schwarze *et al.* 1995; Schwarze 2007; Koyani *et al.* 2010; Pramod *et al.* 2015) thus making this concept bit complicated. Therefore, to distinguish the soft rot decay, the mode of their attack is categorized into two types: Type 1 and Type 2 (Corbett 1965; Hale and Eaton 1985a, b).

In Type 1 attack, the cavity formation occurs in S_2 layer when the penetrating hyphae is aligned along the cellulose microfibrils in the fibre wall, either by forming a T- branch or simple bending of hyphae i.e. L-bending. Further repetition of this process leads to the formation of interconnected chain of cavities. Type 2 attack is referred as erosion attack (Corbett 1965). The hyphae growing in the cell lumen secretes enzymes, which accomplishes the cell wall erosion/erosion troughs in entire secondary wall.

In case of softwood, the S_2 layer is eventually degraded while S_1 layer and middle lamellae remain unaffected while lignin is degraded to some extent (Daniel and Nilsson 1989; Nilsson *et al*. 1989). This makes the decay pattern distinct from white rot wherein the middle lamella is extensively degraded. Formation of erosion trough is initiated within the lamina towards the middle lamellae i.e. in the immediate environs of hyphae growing on the luminal surface (Koyani *et al.* 2010). The extent of soft rot decay varying from small erosion trough to a complete removal of secondary cell wall layer not only cause cellulose degradation, but also loss in the wood strength in advance stage of decay.

1.5.3 White rot

White rot decay of wood is caused by basidiomycetes and some ascomycetes (Sutherland and Crawford 1981; Schwarze *et al*. 2000a). During the course of decay by white rot fungi, wood assumes bleached appearance due to removal of lignin and wood appears like laminated spongy structure (Anastasi *et al*. 2009). White rot fungi are able to fragment and further metabolize all the major structural polymers of wood and other lignocellulosic i.e. lignin, cellulose, and hemicellulose; causing accidental collapse and damages (Kirk and Cullen 1998). The ability to oxidize phenolic compounds extracellularly differentiates the white rot fungi from brown rot species (Evans and Hedger 2001). Bavendamm test has been used for this purpose, to screen the white rot fungi by polyphenol activity (Bavendam 1928). Depending on the pattern of lignin removal white rot fungi is categorised as selective delignification and simultaneous rot (Blanchette 1991). In selective delignification, in early stages of degradation hemicelluloses and lignin are preferentially attacked, while in simultaneous rot cellulose, hemicellulose and lignin are removed uniformly (Koyani *et al.* 2010).

The distinctive features of white rot fungi to completely depolmerize and mineralize lignin using the delignifying extracellular ligninolytic enzymes, taxonomically characterize it as a group of higher heterogenous fungi (Eriksson *et al*. 1990; Hatakka 2001). This developed strategy of white rot fungi to degrade the most recalcitrant compounds (i.e. lignin) is remarkable and renders the promising ability of bioremediation to white rot fungi to degrade or transform a large variety of aromatic pollutants (Martinez *et al.* 2004; Bianco and Perrotta 2015).

1.6 Lignin

1.6.1 Composition and Distribution of lignin within cell wall of vascular plants

Lignin is a major, an integral constituent and natural polymer of wood cell wall of vascular plants. It acts as cementing material for cellulose and hemicellulose which are densely packed within the protective matrix of lignin; therefore, endowing rigidity and stiffness to the vascular elements (Shrivastava *et al.* 2014). These lignified elements are necessary for the transport of water, minerals, and photosynthetic products.

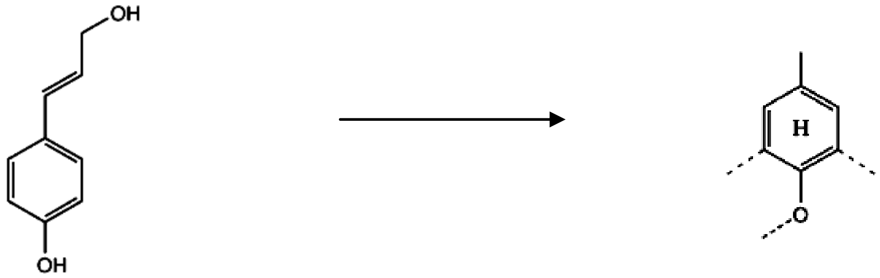
In hardwoods, lignin accounts for 25 % of cell wall composition (Schwarze 2007), whereas lignin content of gymnosperm wood is higher as compared to angiosperms. At the time of maturation of wood cells, lignin gets infused in all cell wall layers together with middle lamella. It is estimated that about 80 % of lignin is in-filled in the rounded cell corners of compound middle lamella (Fig. 2), and remaining 20 % is equally distributed in the primary and secondary wall layers (Schwarze 2007).

1.6.2 Lignin Structure

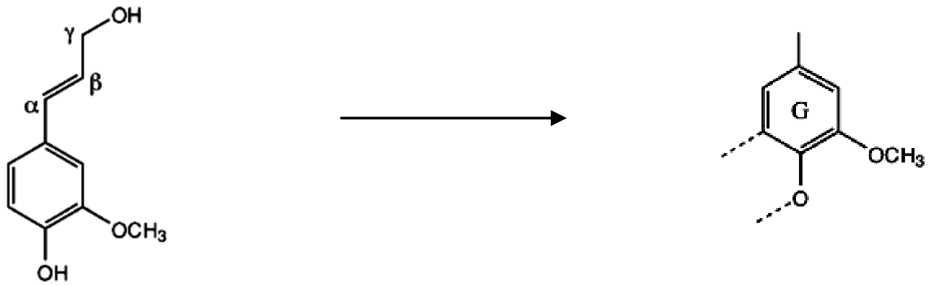
Lignin is highly insoluble complex branched polymers derived from oxidative-coupling of monolignols that constitutes the three-dimentional network. These monolignols are the three phenylpropanoid precursors: i) *p*-coumaryl alcohol, ii) *p*-coniferyl alcohol and iii) *p*-sinapylalcohol.

These precursors are joined by C-C and ether linkages. According to Wen *et al*. (2013), further via combination of free radical coupling as shown in they assemble into an intricate racemic macromolecule and gives rise to the corresponding phenylpropanoid units: *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl units (S) (Fig. 3).

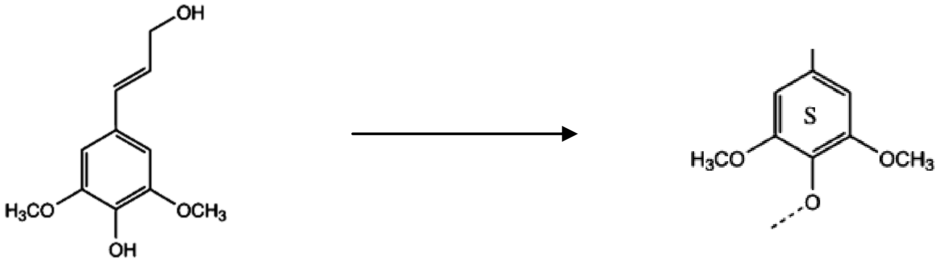
Phenylpropanoid precursors **Corresponding phenylpropanoid units**



p-coumaryl alcohol *p*-hydroxyphenyl units



p-coniferyl alcohol guaiacyl units



p-sinapyl alcohol syringyl units

Figure 3: Corresponding phenylpropanoid H, G and S units.

Polymerization of these phenylpropanoid units leads to formation of complex lignin structure (Fig. 4).

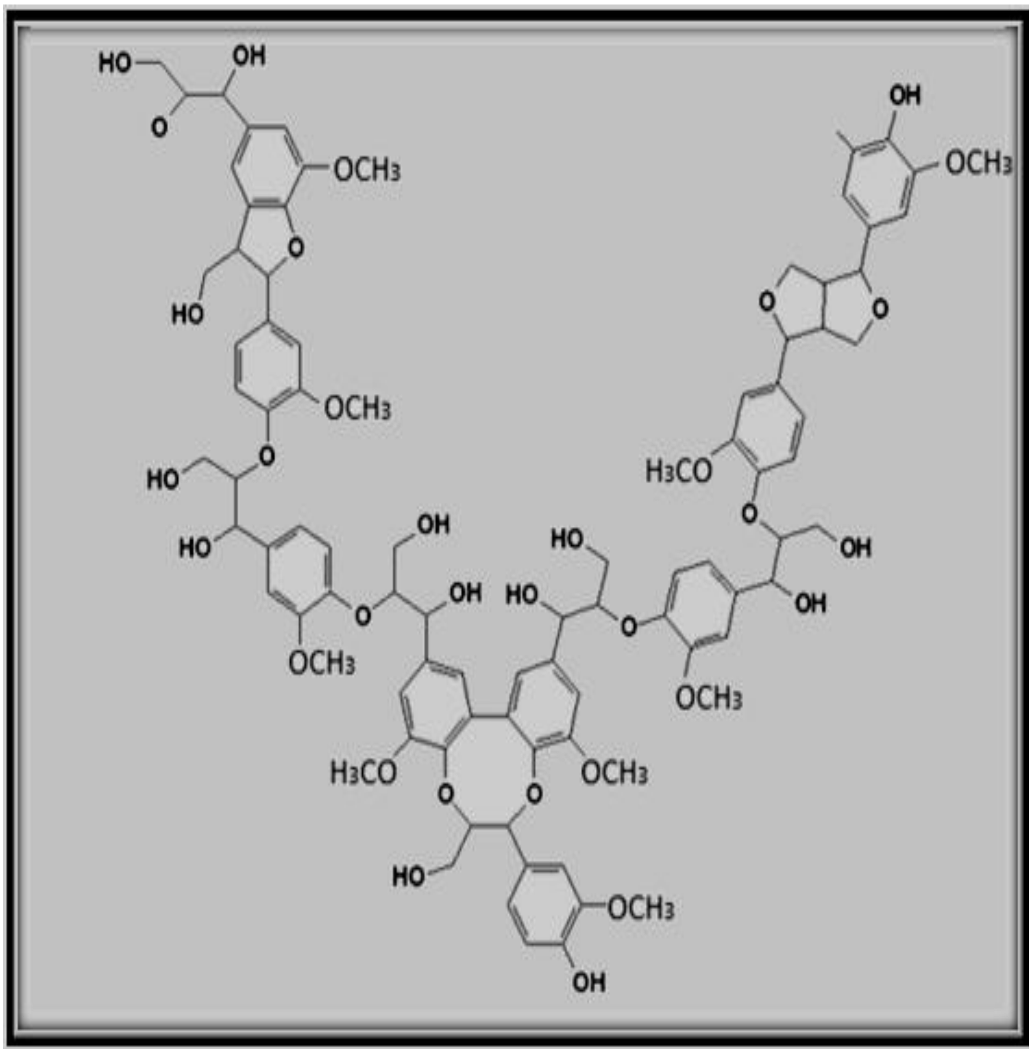
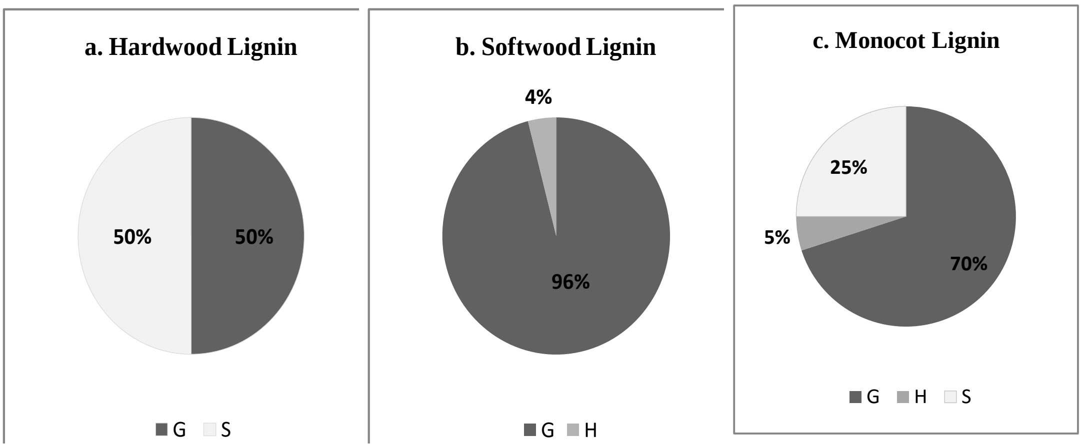


Figure 4: Structure of lignin formed by the polymerization of phenylpropanoid units (modified from, Quiroz-Castañeda and Folch-Mallol 2011).

Structurally, lignin content differs in monolignols compositions in different wood types such as Softwoods (Gymnosperms and Conifers), Hardwoods (Angiosperms and deciduous trees etc.) and in Monocots (grasses) (Sjostrom 1993; Pekka 2000).

The hardwood lignin is composed of G and S units in equal ratios G:S = 50:50 (Fig. 5a). In contrast, softwood lignin is composed of G units, and small amounts of H units G:H = 96:4 (Fig. 5b) while monocot lignin include three units with H-units still comparatively minor, G:S:H = 70:25:5 (Fig. 5c) making its structure apparently more intricate (Wong 2009; Paliwal *et al*. 2012).


a. Hardwood Lignin

b. Softwood Lignin

c. Monocot Lignin

Figure 5: Pie chart representation of percent (G:H:S) lignin units composition in different wood types: a. Hardwood, b. Softwood and c. Monocots.

Due to its compositional and structural complexity until now, the characteristics of lignin have not yet been completely elucidated (Kang *et al*. 2012).

1.7 Lignin degradation

Lignin is a heterogeneous class of compounds (Fengel and Wegener 1989) and is the second (after cellulose) most abundantly annually produced natural polymer on terrestrial earth and the most abundant aromatic material accounting for about 40 % of the solar energy stored in plants (Leonowicz *et al*. 1999). The removal of lignin matrix, from cellulose and hemicelluloses enables the microorganisms for subsequent use of these carbohydrates. This degradation of lignin proves to be a key step for closing the carbon cycle. Increasing research interest on the degradation of lignin is also accounts the importance of wood and other lignocellulosics as a renewable resource for the production of paper products, feeds, chemicals and fuels (Ruqayyah *et al*. 2011).

The highly intricate structure of lignin that lacks the regular hydrolysable bonds is poorly degraded by most of the microorganisms (Aro *et al*. 2005; Betcheva *et al*. 2007). On the other hand, to overcome the complications arising in lignin degradation, microbes need to cope up with following confronts of the lignin structure: i). Lignin being a hefty polymer, it desires the enzymatic system to be essentially extracellular to degrade it. ii). Lignin structure is constructed with interconnected and stable carbon-carbon and ether bonds, so the action mechanism of the enzymatic degradation needs to oxidative and not hydrolytic. and iii). Since lignin consists of an assortment of stereo-irregular units, it requires enzymes with less specificity than hydrolytic enzymes required for cellulose/hemicellulose degradation (Sethi *et al*. 2013; Isroi *et al*. 2011; de Souza 2013). According to ten Have and Teunissen (2001), the only known organisms that can completely break down lignin to carbon dioxide and water are white-rot fungi (Leatham 1986; Kirk and Farrell 1987).

1.8 Action mechanism of lignin degradation by white rot fungi

White rot fungi include most members of Basidiomycotina, in addition to a few Ascomycotina (Eriksson *et al*. 1990). White-rot fungi belonging to the subdivision Basidiomycotina attack either hardwood or softwood while Ascomycotina probably degrade only hardwood (Kirk and Farrell 1987). Compared to other organisms, white-rot fungi degrade lignin extensively with a very fast pace, which apparently facilitates enzyme access to carbohydrates (de Koker *et al*. 2000; Mohamed *et al*. 2013). The hyphae penetrate the cell wall by means of ligninolytic enzymes, which are released from the hyphal tip (Schwarze *et al*. 2004). White rot postulates a unique ability to degrade the most intractable polymer (lignin) along with other components of the cell wall by means of either a. Selective delignification and b. Simultaneous decay (Blanchette 1984; Maloy and Murray 2001, Schwarze 2007; Koyani *et al*. 2010; Sanghvi *et al*. 2013; Koyani *et al*. 2013).

a. Selective delignification

During selective delignification, in the decay process lignin is degraded earlier than cellulose or hemicelluloses which occurs at a distance from fungal hyphae (Blanchette *et al*. 1997), resulting in defibration by dissolution of the middle lamella so that cells tends to separate in the early stage of decay, and remarks attack on hemicelluloses and lignin (Worrall *et al.* 1997; Srivilai *et al.* 2013), with limited attack on cellulose (Guerra *et al*. 2003).

Ultrastructural studies showed that middle lamella and cell corners are the last areas to be degraded as lignin was first removed from the secondary wall near the lumen and later throughout the secondary wall toward the middle lamella (Agosin *et al.* 1990). Low molecular weight compounds which are smaller than enzymes are responsible for overall lignocellulose biodegradation in case of selective delignification (Blanchette *et al.* 1997; Goodell *et al.* 1997; Messner *et al.* 2003;

Arantes *et al.* 2011). This is because lignocellulolytic enzymes cause only extracellular degradation of macromolecular constituents in the intact wood cell as they are too large (Arantes and Milagres 2009). Selective degradation is more prone in wood species which have a low lignin content and whose lignin have the largest amount of ß-aryl ether bonds and a high syringyl/guaiacyl ratio (Agosin *et al.* 1990). Later, Schwarze (2007) revealed that ß-aryl ether-linked syringyl units are more specifically degraded by selectively delignifying fungi than the guaiacyl ones.

b. Simultaneous Decay

In simultaneous decay, decomposition of lignin takes place close to the hyphae and results in erosion of the cell wall from the lumen surface. Delignification is prominent due to the simultaneous removal of all three components cellulose, hemicelluloses and lignin (Blanchette and Reid 1986; Anagnost 1998, Luna *et al.* 2012). In general cell wall thinning occurs from the lumen outwards middle lamella due to the coalescence of the erosion troughs induced by numerous hyphae (Liese 1970; Schwarze *et al.* 1995). This exceptional ability of the white rot fungi to completely depolymerize and mineralize the lignin is attributed to the concomitant actions of their nonspecific enzyme system, including extracellular oxidoreductases, low-molecular-mass metabolites, and dehydrogenases, plus activated oxygen species (Jurado *et al.* 2011). These non-specific extracellular enzyme systems consist of ligninolytic enzymes.

1.9 Ligninolytic enzymes of white rot fungi

During the process of lignin removal white rot fungi produce the ligninolytic/lignin modifying enzymes. These enzymes include hemeperoxidases and phenol oxidases, which are responsible for generating highly reactive free radicals that undergo a complex series of spontaneous cleavage reactions which is a key step in lignin degradation (Reddy and Mathew 2001). Heme peroxidases include **Manganese peroxidases** (MnP, E.C.1.11.1.13) and **Lignin peroxidase** (LiP, E.C.1.11.1.14). MnP act on phenolic units through the oxidation of Mn^{2+} to Mn^{3+} as well as on non-phenolic units in presence of second mediators. In contrast, LiP is able to oxidize directly non-phenolic lignin units, whereas phenol oxidases include **Laccase** (EC 1.10.3.2) that catalyze the oxidation of polyphenols and methoxy-substituted phenols as well as aromatic amines (Jurado *et al.* 2011). The limited levels of carbon and nitrogen often provokes the secretion and synthesis of these ligninolytic enzymes. Production of peroxidases is generally repressed by agitation but optimal at high oxygen tension, while agitation, organic solvents and aromatic compounds improve the production of laccase (Galhaup *et al*. 2002a). The following (Fig. 6) shows the schematic representation of lignin degradation by the ligninolytic enzymes of white rot fungi.

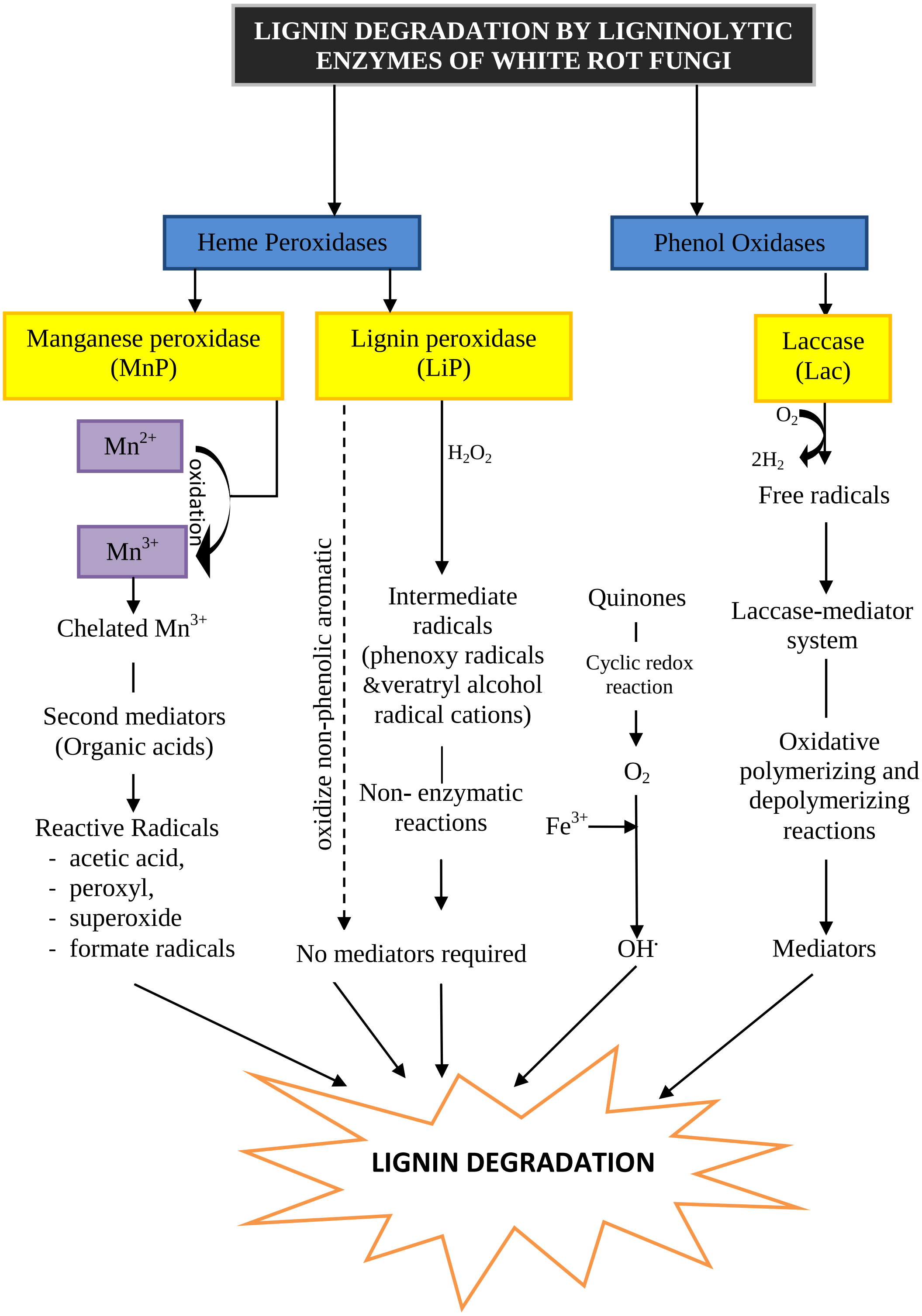


Figure 6: Schematic representation of overall processes involved in lignin degradation by of white rot fungi (modified from, Dashtban *et al.* 2010).

This suggests that there is more than one ecologically successful strategy for the degradation of lignin. Different ligninolytic fungi produce dissimilar combinations of these enzymes, but not all of these three major enzymes are needed to degrade lignin (Hatakka 1994, 2001).

a. Heme Peroxidases

The peroxidases MnP and LiP were discovered later after the discovery of Laccase (before beginning of 1980s). These peroxidases require H_2O_2 for their catalytic activity to promote the oxidation of lignin into free radicals, which subsequently undergo spontaneous reactions with oxygen or water, which leads to depolymerisation. H_2O_2 is generated by glyoxal oxidase (GLOX, E.C.1.2.3.5), aryl-alcohol oxidases (AAO, E.C.1.1.3.7), and cellobiose oxidase or by non-enzymatic systems *via* the Fenton reaction (Pekka 2000). H_2O_2 is the precursor of the hydroxyl radical, the strongest oxidizing agent produced by fungi and also participates in its own in lignocellulose degradation *via* the iron-catalyzed Haber–Weiss reaction (Jurado *et al.* 2011).

Manganese Peroxidase (MnP)

Manganese peroxidase (Mn(II): hydrogen-peroxide oxidoreductase, EC 1.11.1.13) are extracellular glycoproteins with an iron protoporphyrin IX (heme) prosthetic group. This heme protein has a highly specific Mn^{2+} binding site acting as a mediator for MnP and belongs to the class II peroxidase group in basidiomycetes fungi (Jarvinen *et al*. 2012). MnP possess molecular weights ranging between 32 and 62.5 kDa and optimum from pH of 4-7 (Urek and Pazarlioglu 2004; Baborova *et al.* 2006; Asgher *et al.* 2008) is reliant on H_2O_2 that necessitates Mn^{2+} to oxidize mono-aromatic phenols and aromatic dyes (Kuwahara *et al*. 1984; Bonugli-Santos *et al*. 2010).

Mechanism of MnP action

Manganese peroxidase catalyzes the H_2O_2-dependent oxidation of lignin (Wariishi *et al*. 1991) and its derivatives (Lackner *et al*. 1991; Gold and Alic 1993). Mn^{2+} is used as reducing substrate by Mn-dependent peroxidases Mn^{3+}. MnP catalyzes the peroxide dependent oxidation of Mn^{2+} by MnP-compound I and MnP-compound II to Mn^{3+}. During this process of oxidation, Mn^{2+} is chelated by bidentate organic acid chelators such as glycolate or oxalate (Kishi *et al.* 1994), which stabilize ion Mn^{3+} and endorse its discharge from the enzyme (Glenn and Gold 1985; Glenn *et al.* 1986;

Wariishi *et al.* 1988; Kishi *et al.* 1994; Kirk and Cullen 1998) as represented by the following reaction (Fig. 7):

- Initiation of catalytic cycle addition of H_2O_2 to the native enzyme

$$\mathbf{MnP + H_2O_2 \longrightarrow MnP\text{-}compound\ I + H_2O}$$

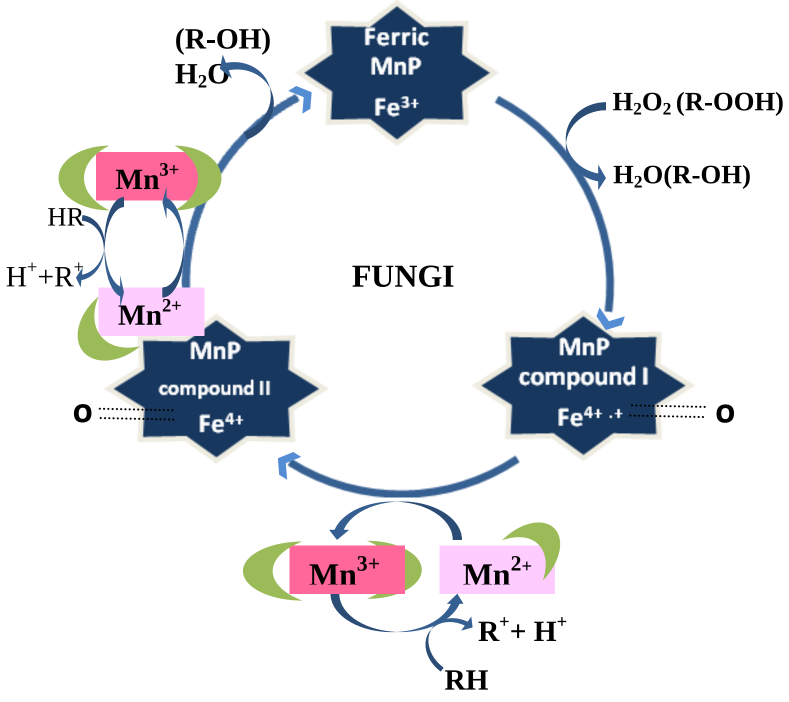
- Addition of Mn(II) rapidly reduces MnP-compound I to MnP-compound II.

$$\mathbf{MnP\text{-}compound\ I + Mn^{2+} \longrightarrow MnP\text{-}compound\ II + Mn^{3+}}$$

- Mn(II) is an obligatory redox coupler for the enzyme and completes the cycle by reducing MnP-compound II to generate the native enzyme, thus Mn^{2+} binds with oxalate chelators and oxidizes to form chelated Mn^{3+} which diffuses from the enzyme surface and in turn oxidizes the phenolic substrate.

$$\mathbf{MnP\text{-}compound\ II + Mn^{2+} \longrightarrow MnP + H_2O + chelated\ Mn^{3+}}$$

This step closes the cycle and the input of H_2O_2 results in the formation of H_2O and two $Mn3^+$ chelated (Wariishi *et al.* 1992).



☾-Chelators, ✷ -Heme, - **RH**- Aliphatic or aromatic hydrocarbon and RH to R^++ H^+- spontaneous reaction

Figure 7: Schematic diagram representing mechanism of MnP action (modified from, Wariishi *et al.* 1988; Wariishi *et al.* 1992; Kuan *et al.* 1993; Kishi *et al.* 1994; Kirk and Cullen 1998).

On the other hand, manganese peroxidase postulates properties of both oxidase and peroxidase, and therefore catalyze phenolic lignin model compounds by chelated Mn^{3+} and also non-phenolic lignin by formation of reactive radicals (Steffen *et al*. 2003; Shin *et al*. 2005; Singh *et al.* 2011a; Xu *et al.* 2012).

Phenolic substrates catalyzed by MnP

Chelated Mn^{3+} mediates the oxidation of organic substrates. It also oxidizes lignin model compounds in the absence of the enzyme (Forrester *et al.* 1988). These organic substrates include phenolic substrate, dimmers, carboxylic acids, amines, dyes and phenolic lignins (Hofrichter 2002).

Non-Phenolic substrates catalyzed by MnP

Under certain physiological conditions Mn^{3+} chelator becomes a weak oxidant limited to the oxidation of phenolic lignin structures and fails to oxidize non-phenolic lignin components (Reddy *et al*. 2003a; Wong 2009; Paliwal *et al.* 2012). Therefore, during oxidation of non-phenolic substrates by Mn^{3+}, second mediators (like thiols) - reactive radicals are formed. Further, substrate oxidation is initiated via abstraction of hydrogen rather than electron with oxidation of thiol to thiyl by enzyme generated Mn^{3+} (Wariishi *et al.* 1988; Paliwal *et al.* 2012).

The enzyme generated Mn(III) also couples with peroxidation of lipids, lipid peroxy radical intermediates are capable of oxidizing non-phenolic lignin model compounds occurring by peroxidation of unsaturated lipids by MnP in presence of Mn^{2+} (Breen and Singleton 1999). This mechanism involves the addition of O_2 to form peroxy radicals and non-enzymatic degradation (Wong 2009).

$$\mathbf{Mn^{2+} + 2H+ + H_2O_2 \rightarrow 2\ Mn^{3+} + 2H_2O}$$

In fungi lacking H_2O_2-generating oxidases, the lignin-degrading efficiency of the fungi is increased because these radicals can be used by MnP as a source of peroxides (Dashtban *et al*. 2010). Thus, this oxidase activity enables MnP to depolymerize their natural substrate, i.e. lignin as well as recalcitrant xenobiotics (Scheibner *et al*. 1997; Van Aken *et al*. 1999) and textile dyes (Heinfling *et al*. 1998). Furthermore, H_2O_2 could also be utilized by lignin peroxidase, which is H_2O_2 dependent for catalytic activity.

Lignin Peroxidase (LiP)

Tien and Kirk (1983), discovered lignin peroxidase (LiP) which is characterized by its low pH optima 2-5, high redox potential and molecular mass varying from 37 to 50 kDa for different white rot fungal species (Hirai *et al*. 2005; Asgher *et al*. 2006). LiPs consequently oxidize not only the usual peroxidase substrates such as phenols and anilines, but also a variety of non-phenolic lignin structures and other aromatic ethers that resemble the basic structural unit of lignin. Therefore, they are considered powerful oxidants than typical peroxidases (Kersten 1990). The key reaction of lignin degradation by LiP is that it uses H_2O_2 (Mai *et al*. 2004) along with veratryl alcohol for the activity and is capable of oxidizing and cleaving lignin and lignin model compounds (ten Have and Teunissen 2001; Paliwal *et al* .2012).

Mechanism of LiP action

LiP uses a two-step mechanism to interact with its substrate as shown in the following:

- The ferric form of the enzyme, usually referred to as native enzyme Fe (III), is oxidized by $2e^-$ by H_2O_2 cleaved at the O–O bond to a form LiP-compound I[Fe(IV)=O.+] intermediate that exists as a ferry iron porphyrin radical cation, [Fe(IV)=O.+].

Native enzyme Fe(III)+ H_2O_2= LiP-compound I [Fe(IV)=O.+] + H_2O.

- LiP-compound I can be reduced by one electron by chemicals having a suitable reduction potential aromatic substrates such as veratryl alcohol (VA) is oxidized by LiP-compound by $1e^-$ resulting into LiP-Compound II [Fe(IV)=O], and an aromatic cation radical ($VA^{\cdot+}$).

LiP-compound I [Fe(IV)=$O^{\cdot}$+] + VA= LiP-Compound II [Fe(IV)=O] + $VA^{\cdot}$+

LiP-Compound II [Fe(IV)=O]+ VA= Native enzyme Fe(III)+ $VA^{\cdot}$+

Further, LiP-compound II, which is more reduced in turn oxidize substrates by $1e^-$ and returns the enzyme to its native stage thus completing the catalytic cycle. In some cases this is also possible by a direct $2e^-$ reduction.

On the other hand, LiP-II reacts with H_2O_2 resulting into inactive Lip-compound III (Ferric supero complex) in the presence of excess H_2O_2 and in the absence of a reducing substrate; furthur LiP-compound III by oxidation with a VA radical cation to veratryl aldehyde through the displacement of superoxide from the active site is converted to native enzyme (Renganathan and Gold 1986; Khindaria 1995; Wong 2009).

LiP-compound I and LiP-compound II oxidizes VA to veratryl alcohol radical cation intermediate $VA^{\cdot}+$ (Bietti *et al*. 1998; Khindaria 1995). According to Paliwal *et al.* (2012), C4 ether breakdown causes conversion of phenolic compounds (guaiacol, vanillyl alcohol, catechol, syringic acid, acetosyringone etc.) to phenoxy radicals with the reduction of LiP-compound I and LiP-compound II (Fig 8). These intermediate radicals in presence of oxygen:

i) Undergo non-enzymatic reactions such as radical coupling and polymerization, side-chain cleavage, demethylation, intramolecular addition and rearrangement and degrade lignin polymer and/or,

ii) Subjected to $C\alpha$–C_β breakdown, yielding p-quinones. Subsequently, this quinones undergo cyclic redox reaction and activates oxygen by quinone reductase enzyme resulting in the reduction of ferric ion by superoxide cation radical. Reoxidation of reduced ferric iron by reduction of H_2O_2 produces highly reactive oxidants hydroxyl free radical (OH·) which initiates the attack on lignin.

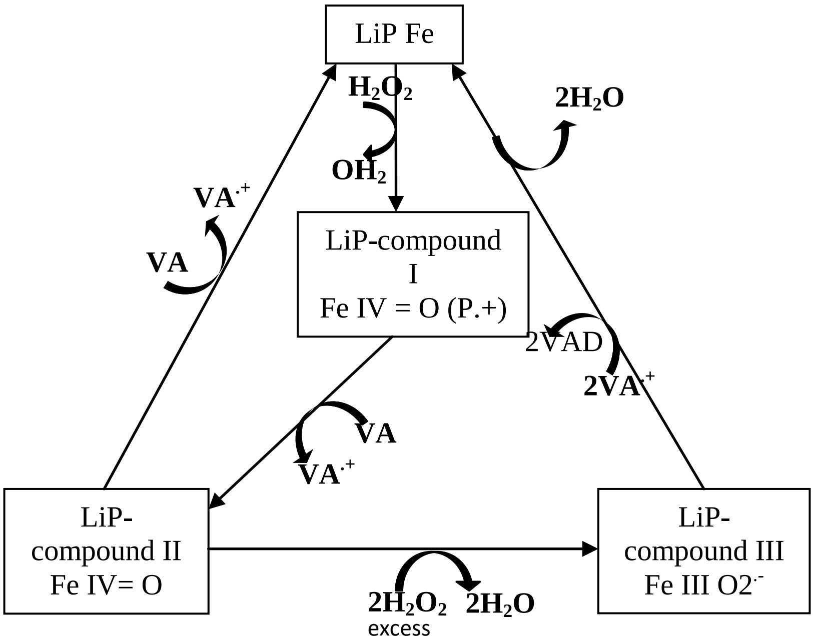


Figure 8: **Schematic diagram representing mechanism of LiP action (modified from, Paliwal *et al*. 2012).**

LiP directly undergoes oxidation of non-phenolic aromatic substrates unlike MnP which requires the presence of mediators due to its high redox potential and degrade lignin polymer (Dashtban *et al*. 2010).

b. Phenol Oxidases

Laccase (Lac)

Laccase (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) belonging to the copper oxidase family. It is mainly characterised by presence of copper atoms in the catalytic centre usually called multi-copper oxidase. Fungi belonging to the classes of Ascomycetes, Deuteromycetes and Basidiomycetes mainly account the presence of laccase (Gnanasalomi and Gnanadoss 2013). Laccases can degrade lignin in the absence of lignin peroxidase and manganese peroxidase (Mayer and Staples 2002). Compared to the peroxidases, laccases are larger and are involved in the detoxification of phenol compounds produced during lignin degradation and acts synergistically with peroxidases and other enzymes in the breakdown of lignin (Leatham and Stahmann 1981; Eggert *et al*. 1997) and have molecular weight around ±60 kD (Call and Muecke 1997). Laccases contain four coppers per enzyme, representing three different types: Type 1, Type 2 and Type 3, each having a distinct role in the oxidation of laccase substrates.

Type 1(T1) gives a typical blue colour to the enzyme and is involved in the reactions with the substrates. It contains one Cu atom per molecule, located in domain 3 with the copper lying in a shallow depression on the surface of the enzyme. Type 2(T2) like type 1 has one Cu atom. Along with Type 3 coppers (having two Cu atoms, T3), it clusters in triangular form at the interface between domains 1 and 3 with each domain providing ligand residues for the coordination of the copper atoms.

The enzyme catalyzes four 1e− oxidations of a reducing substrate at the primary electron acceptor site, T1. The electrons of reducing substrates from the T1 site are transferred to T2/T3 trinuclear centre resulting in the reduction of dioxygen to water (Gianfreda 1999). Oxidation of lignin by fungal laccase has been studied intensively since the early 1970s (Bourbonnais *et al*. 1995). Like peroxidase, laccase oxidizes both phenolic with broad substrate specificity that catalyzes the oxidation of phenols, anilines, and aromatic thiols (Thurston 1994) as well as non-phenolic compounds. Like MnP, laccase also requires presence of redox mediator for oxidation of non-phenolic compounds as they cannot be oxidized by laccase directly if the redox potentials are greater than 1.3 V (Bourbonnais and Paice 1990).

Mechanism of Lac action

Lac catalysis is initiated at the T1 site of primary electron acceptor due to reduction of the reducing substrate by the copper. This results in conversion of enzyme from fully oxidized/resting to fully reduced state by a successive 4e− oxidation, due to the transfer of the electrons extorted from the reducing substrate to the trinuclear site T2/T3 (Fig. 8).

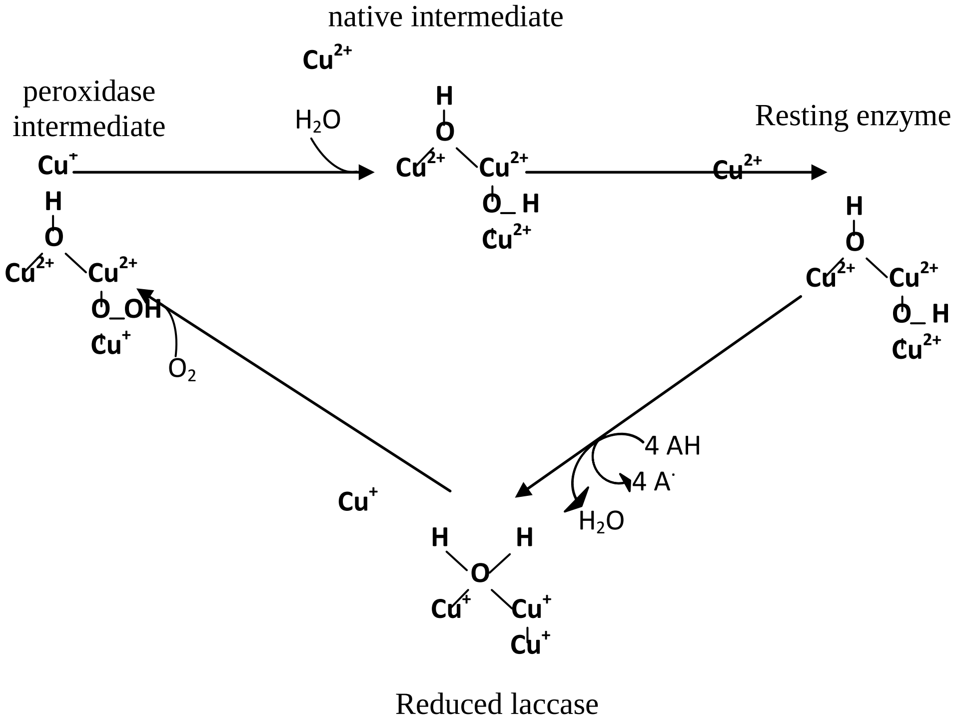


Figure 9: Schematic diagram representing mechanism of Lac action (modified from, Wong 2009).

By the formation of bound oxygen the dioxygen reductions ocurs in two steps:

- The first step results in the formation of H_2O_2 intermediate (free radicals) linked between oxidized T3 and the reduced T2 copper sites, due to 2e− transferred from T3 with the binding of dioxygen molecule to the trinuclear site T2/T3. Further, with the transfer of final electron from the T2 copper, H_2O_2 intermediate decays to an oxygen radical followed by a 2e− reductive cleavage of the O–O bond with the release of a water molecule (Palmer *et al*. 2001; Lee *et al*. 2002).

- Later on at the final step, O_2^- is released as a second water molecule and all four copper centres are oxidized.

In case of oxidation of phenolic compounds such as vanillyl glycol, 4, 6-di(t-butyl) guaiacol and syringaldehyde to form phenoxy radicals, laccase uses molecular oxygen of phenolic compounds as the electron acceptor to remove protons. By the formation of phenoxy radicals, the degradation of phenolic β-1 lignin substructure model takes place resulting Cα oxidation, Cα–Cβ cleavage, alkylaryl cleavage, and aromatic ring cleavage (Lundquist and Kristersson 1985; Faure *et al.* 1996; Kawai *et al.* 1999a; Wong 2009).

As discussed earlier in this chapter, laccase directly cannot oxidize non-phenolic lignin compounds, due to low redox potential. However, with the presence of low molecular weight redox mediators, laccase can oxidize the non-phenolic compounds (Li *et al.* 1999). According to Woolridge (2014) these mediators are ABTS (Bourbonnais and Paice 1990; Muheim *et al*. 1990), 1-hydrobenzotriazole (1-HBT) (Li *et al*. 1998; Kawai *et al*. 1999b; Srebotnik and Hammel 2000), NHA (Woolridge 2014) and violuric (Li *et al*. 1999; Xu *et al*. 2000). The combination of laccase plus a low molecular weight redox mediator is known as the laccase mediator system (LMS). Thus, low substrate specificity together of laccase with presence of O_2 specificity makes it suitable and attractive for industrial application (Mayer and Staples 2002; Baldrian 2006).

1.10 Application of ligninolytic enzymes of white rot fungi in different industrial sectors

In the recent time, a drastic increase in the demand for ligninolytic enzymes have been scaled up due to the biotechnological significance of these enzymes, which have replaced the conventional chemical processes of several industries (Maciel *et al.* 2010). These groups of enzymes produced by white rot fungi are highly versatile in nature and they find applications in a wide variety of industries viz. Nanotechnology, Pharmaceutical, Medical, Cosmetics, Paper and Pulp, Food, Bioremediation of Textile Dyes, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), Xenobiotics, etc. as shown in (Fig. 10).

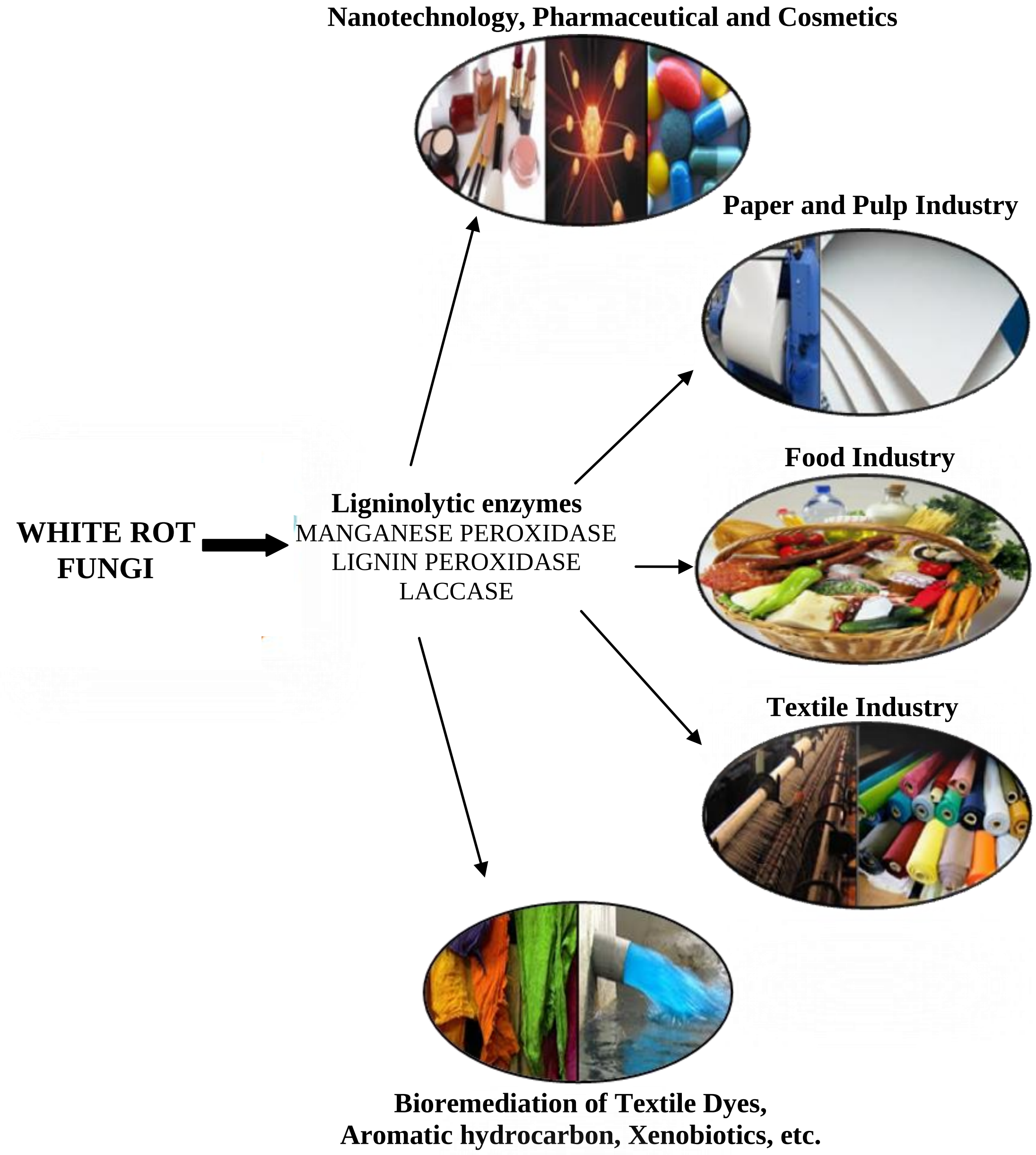


Figure 10: Application of ligninolytic enzymes in different industrial sectors.

1.11 White rot fungi as a potential tool in biodegradation approach

The application of white-rot fungi and their enzymes in biotechnology is not only restricted to the transformation or disruption of the lignin barrier from plant material, but also used for the transformation of other molecules. White rot basidiomycetes involved in lignin degradation can participate in the oxidation of environmental pollutants that are structurally related to lignin and are undesirable due to their estrogenic, mutagenic, and/or carcinogenic properties on the biota (Jurado *et al.* 2011).

These pollutants include aromatic and aliphatic xenobiotic compounds, together with environmental pollutants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), textile dyes, etc. The main mechanism of biodegradation employed by white rot fungi however, is the lignin degradation system of enzymes (Sharma *et al.* 2013). Ligninolytic enzymes work extracellularly allowing the access for many of the non-polar, non-soluble toxic compounds and bind non-specifically (Christian *et al.* 2005). Thus, peroxidases and laccases by means of non-specific free radical formation (Pointing 2001; Law *et al*. 2003), cleaves the C-C and C-O bonds of lignin and xenobiotics.

Perusal of literature indicates that white rot basidiomycetes play pivotal role in timber damage by degrading highly complex natural molecule like lignin. Environmental pollution is a worldwide threat to public health. Biological degradation, has become an increasingly popular treatment of agricultural and industrial waste for both economic and ecological reasons, (Milala *et al.* 2009; Soliman *et al.* 2013). If applied in environmentally sustainable green and white biotechnology processes, significant progress can be achieved in lignocellulose degradation by white-rot basidiomycetes fungi and their enzymes. Therefore, main objectives of the present study are as follows:

OBJECTIVES OF THE STUDY

- Isolation, purification and characterization of rot fungi.
- Optimization of growth media.
- Screening of pure cultures for various enzymes.
- Production of enzymes by Solid State Fermentation.
- Isolation, purification (complete/partial) and characterization of different enzymes.
- To study enzyme kinetics (effect of temperature, pH, etc.).
- Molecular characterization of enzymes through electrophoresis.
- Optimization of its degrading and decolourizing ability.
- *In vitro* testing of isolated fungi for wood decay.