

Laccase, an Emerging Tool to Fabricate Green Composites: A Review

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In the last two decades, laccases have received much attention from researchers because of their specific ability to oxidize lignin. This function of laccase is very useful for applications in several biotechnological processes, including delignification in the pulp and paper industry and the detoxification of industrial effluents from the textile and petrochemical industries. This review focuses on laccase-mediated fiberboard synthesis. Growing concerns regarding the emission of formaldehyde from wood composites has prompted industrialists to consider the fabrication of green composites. Laccase-mediated fiber treatments oxidize the lignin component without affecting the cellulose structure. As a result, free radicals are generated on the fiber surface, and these can act as potential reactive sites for further cross-linking reactions in board manufacturing. Binderless fiberboards prepared using such methods can be considered as green composites because the manufacturing process involves no additional resin.

Keywords: Laccase structure; Radicals, Crystallinity index; Self-bonding

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INTRODUCTION

Wood is the best example of a natural composite. The polysaccharides, cellulose and hemicellulose, of wood are embedded in a matrix of aromatic lignin (Moniruzzaman and Ono 2013). Likewise, commercial wood composites are made from wood-based materials bonded together with a synthetic adhesive using heat and pressure (Li *et al.* 2007). Therefore, in wood composites, the synthetic adhesive is the only foreign component that is not present in the natural wood. These synthetic adhesives are mostly formaldehyde-based, produced from non-renewable resources such as petroleum and natural gas, and they emit formaldehyde throughout their usable life (Li *et al.* 2009; Moubarik *et al.* 2009). The International Agency for Research on Cancer has classified formaldehyde gas as carcinogenic to humans (IARC 2004). Thus, to fabricate a completely natural wood composite, it should be free of synthetic adhesives. Many alternative methods are being studied, such as using natural adhesives, using lignin- or tannin-based adhesives, or modifying wood fiber to facilitate self-bonding. A number of research attempts have been conducted to reduce or replace the formaldehyde content in adhesives preparation, but none of them have been commercially applicable (Khan *et al.* 2004; Khan and Ashraf

2006). The most successful attempt to utilize lignin as an adhesive for industrial applications was an investigation into substituting the phenol of phenol-formaldehyde (PF) resins with 50 wt% organosolv lignin obtained from groundnut shell lignin (GNSL) (Khan and Ashraf 2006; Nasir *et al.* 2013b).

Fiber modification is a new approach to initiate self-bonding between fibers in fiberboard. Several chemical, mechanical, and biological methods have been suggested to modify the physical, mechanical, or thermoplastic behaviors of cellulosic fibers (Gradwell 2004; Li *et al.* 2007; Nasir *et al.* 2014b). Such methods include alkaline treatment, steam treatment, microwave treatment, enzyme treatment, or high-temperature treatment to modify the physico-chemical behavior of cellulosic fibers. Recently, there has been increasing interest in enzyme-assisted fiber modification. Several enzymes have been identified that specifically act on cellulose, hemicellulose, or lignin without affecting the other components (Chandra and Ragauskas 2002). Enzymatic treatments are often milder, causing less damage to the original structure, and more environmentally friendly compared to chemical treatments (Kunamneni *et al.* 2008a). Laccase is a well-established oxidoreductase enzyme that acts specifically on lignin in cellulose fiber hydrolysis. Research by Felby *et al.* (1997, 2002, 2004) and Kharazipour *et al.* (1997) revealed that wood fibers could be enzymatically activated *in vitro* by laccase enzymes; this treated fiber could be used to produce wood composites with enhanced self-bonding between fibers.

Laccase is a widely distributed enzyme in plants and fungi (Milstein *et al.* 1989). The majority of fungi that produce laccase belong to the class of white rot fungi involved in lignin degradation (Ohkuma *et al.* 2001; Kharazipour *et al.* 2008; Yu *et al.* 2009). Laccase action involves the oxidation of various phenolic polymers present in the lignin structure, with a concomitant reduction of oxygen to water (Witayakran and Ragauskas 2009). Recently, laccase has been utilized in the pulp and paper industry to improve the wet strength of fibers (Felby *et al.* 1997; Lund and Felby 2001; Mattinen *et al.* 2011). Laccase treatments usually involve the application of laccase enzymes to activate the lignin in fibers (a one-component system) or the addition of another component with laccase to act as a potential cross-linking agent (a two-component system) (Gochev and Krastanov 2007). Because laccase enzymes are too large to penetrate the cell wall (50 to 100 kDa), treatments are restricted to surface modification only (Kunamneni *et al.* 2007). Therefore, during enzyme hydrolysis, the free phenolic groups on the fiber surfaces act as potential reactive sites for laccase enzymes to create phenoxy radicals.

Regardless of the mechanisms by which laccase acts upon lignocellulose materials, its application can be very wide such as pulp bleaching, textile-dye bleaching, food improvement, bioremediation of soils and polymer synthesis (Kudanga *et al.* 2011; Widsten and Kandelbauer 2008). All the possible applications of laccase are under intensive investigation in order to replace the hazardous chemical treatments to environmental friendly enzymatic treatment. A successful example of laccase application is in pulp and paper industries, where it not only acts as a bio-bleaching agent but it also enhances the fiber-to-fiber bonding (Giardina *et al.* 2010). Based on the research progress of laccase in pulp and paper industries, it is believed that similar approach fiber to fiber bonding can be achieved in wood composite industries also. This review paper focuses on feasibility to prepare a binderless board, by understanding the laccase structure, lignin-laccase reaction mechanism, and fiber improvement.

BINDERLESS COMPOSITE BOARDS

The main problem with these thermosetting adhesives is the emission of volatile formaldehyde vapor, which is carcinogenic in nature (Que *et al.* 2007; González-García *et al.* 2011). Many developed countries have focused their research on developing wood composites free from formaldehyde-based adhesives. Table 1 summarizes the research and development into fiberboard prepared without formaldehyde-based adhesives.

Table 1. Important Developments in Fiberboard Preparation by Self-Bonding of Fiber

Method	Advantage	Limitation	Reference
Ferric sulfate treatment before pressing	No adhesive	Not resistant to water	Linzell (1945)
Hydrochloric acid + ferrous sulfate sprayed on the wood fiber	High mechanical strength	Corrosive nature	Stofko and Zavarin (1977)
Co-polymerization of lignin with low-molecular mass compounds	Laccase enzyme	Lower intensity of polymerisation	Milstein <i>et al.</i> (1994)
Laccase treatment on natural fiber to activate the lignin content of fiber	Completely eco-friendly	Hydrophilic	Felby <i>et al.</i> (1997)
Laccase mediator (hydroxyl benzotriazole) used to improve self-bonding of fiber	Improved wet strength in paper	Hydrophilic	Wong <i>et al.</i> (2000)
Lignin plasticizing by heating above glass transition temperature (T _g)	Completely eco-friendly	High temperature required	Thielemans <i>et al.</i> (2002)
Native lignin plasticizing by steam explosion	Eco-friendly	Hydrophilic	Salvadó <i>et al.</i> (2003)
Fiberboard prepared from laccase activated lignin	Eco-friendly	Lower strength	Felby <i>et al.</i> (2004)
Binderless fiberboard produced from laccase-treated rubber wood fiber	Eco-friendly	Lower strength / hydrophilic	Nasir <i>et al.</i> (2013a)
Enzyme-hydrolysed lignin (EHL) used in MDF by laccase treatment	Improved mechanical strength	Hydrophilic	Nasir <i>et al.</i> (2014b)
Soy-EHL treated by laccase added to fiberboard	Improved mechanical strength	Lower water resistance	Nasir <i>et al.</i> (2014a)

Thielemans *et al.* (2002) prepared a binderless board by heating and pressing cellulosic fibers at high temperature. Lignin, an amorphous component, starts plasticizing at a high temperature (above 200 °C) and behaves like a thermoplastic resin (Lora and Glasser 2002). Felby *et al.* (1997) and Kharazipour *et al.* (1997) suggested an enzyme-assisted composite fabrication without using any adhesive. Laccase, an oxidoreductase

enzyme, was used to generate free radicals, which were expected to help in either the physical or chemical bonding of fibers by modifying the fiber as well as the lignin structure (Kharazipour *et al.* 1997; Yu *et al.* 2009). Hüttermann *et al.* (2001) prepared a binderless particle board using laccase treatment that exhibited improved tensile strengths but lower water resistance. Much research has been done to develop a completely natural fiberboard by treating fiber with laccase, but none of the methods have been commercialized yet (Milstein *et al.* 1994; Lund and Felby 2001; Felby *et al.* 2004; Nasir *et al.* 2013a; Nasir *et al.* 2014a; Nasir *et al.* 2014b). Felby *et al.* (2002) prepared a laccase-treated binderless board in a pilot-scale production. They found that the mechanical strength was good and comparable to the conventional urea formaldehyde-based resin boards but the dimensional stability was very poor. When the wax was applied in treated fiber to improve the dimensional stability, it inhibited the bonding effect of the enzyme (Felby *et al.* 2002). Thus, binderless boards cannot be regarded as commercially viable until they have been shown to achieve good dimensional stability along with mechanical strength.

LACCASE

Historical Development

Laccase was first discovered by Yoshida (1883) in latex produced from the Japanese lacquer tree (*Rhus vernicifera*) that hardened in the presence of air (Yoshida 1883; Giardina *et al.* 2010). It is widely distributed among various classes of angiosperm, gymnosperm, fungi, and bacteria. While laccase is involved in the synthesis and biopolymerization of lignin in higher plants (Raiskila 2008), it plays a major role in the biodegradation of lignin in wood-rotting fungi (Kunamneni *et al.* 2008b). Although laccase has low redox potential, it can oxidize the phenolic compounds of lignin. This oxidation property of laccase can be improved further by addition of natural or chemical mediators, promoting the oxidation of other the recalcitrant aromatic compounds (Moldes *et al.* 2010; Garcia-Ubasart *et al.* 2011). Because of their wide reaction capability and broad substrate specificity, laccase enzymes possess great biotechnological potential (Kunamneni *et al.* 2008b; Garcia-Ubasart *et al.* 2012). The promising applications of laccase include textile-dye bleaching (Mendonça Maciel *et al.* 2010), pulp bleaching (Valls *et al.* 2010), food improvement (Gochev and Krastanov 2007; Mendonça Maciel *et al.* 2010), bioremediation of soils and water (Murugesan 2003; Bustos-Ramírez *et al.* 2013), polymer synthesis (Wang *et al.* 2009), and the development of biosensors and biofuel cells (Kim *et al.* 2014; Fokina *et al.* 2015).

Laccase is a well-studied oxidoreductase enzyme that acts specifically on lignin and its constituent compounds (such as phenols, polyphenols, anilines, aryldiamines, methoxy-substituted phenols, hydroxyindols, and benzenethiols) (Kunamneni *et al.* 2007; Van de Pas *et al.* 2011). It is a compound containing multiple copper atoms that catalyzes the single-electron oxidation of phenolic compounds with a simultaneous reduction of oxygen to water (Zhou *et al.* 2009; Tian *et al.* 2012). Laccase treatment of lignocellulosic fibers causes many changes to the physical and chemical properties of the fibers (Garcia-Ubasart *et al.* 2012). The rate of laccase-catalyzed oxidation varies with the physical and chemical properties of the substrate.

Molecular Structure

Laccases (*p*-diphenol: dioxygen oxidoreductase, EC 1.10.3.2) are extracellular, monomeric glycoproteins, multinuclear enzymes with carbohydrate contents of 8% to 50% (Gochev and Krastanov 2007). The active site of laccase is comparable to that of ceruloplasmin, ascorbate oxidase, and bilirubin oxidase (Kunamneni *et al.* 2007). The molecular mass of laccase ranges from 50 to 100 kDa, depending on the source and the origin of the enzyme (Widsten and Kandelbauer 2008; Giardina *et al.* 2010). Like other enzymes, laccase has a tertiary structure, as revealed under X-ray crystallography (Piontek *et al.* 2002; Gochev and Krastanov 2007). The function of laccase is dependent on Cu atoms that are arranged in four sets (Enguita *et al.* 2003). These four Cu atoms, having different electron paramagnetic resonance (EPR) signals, exhibit three redox sites, namely Cu I, II, and III, which play a crucial role in the reaction mechanism (Claus 2004). Cu I is an oxidized form that is EPR detectible and emits a blue color at 600 nm (Bertrand *et al.* 2002; Enguita *et al.* 2003). Cu II and Cu III are closely related in structure, but the EPR signal of Cu III is not detectible (Enguita *et al.* 2003). However, laccase shows a very low redox potential (RP) ranges from 0.4 V to 0.8 V, the fungal laccases show the highest redox potential among all the sources of laccase (Gochev and Krastanov 2007)

Reaction Mechanism

Laccases is a low redox potential enzyme; hence it can only oxidize the phenolic compounds (lignin moieties) having lower redox potential than laccase. The addition of mediator can enhance the substrate range, allowing the oxidation of non-aromatic compounds having redox potentials higher than those of the laccases (Kunamneni *et al.* 2008a). Laccase utilizes oxygen as the electron acceptor and removes protons from the phenolic hydroxyl group (Kunamneni *et al.* 2008a). Thus, free radicals are formed on phenolic compounds that can spontaneously rearrange and lead to fission at the C-C or C-O bonds of the alkyl side chains or cause the cleavage of aromatic rings (Dashtban *et al.* 2010). The laccase catalysis mechanism involves three major reaction steps. Initially, Cu I is reduced by a reducing substrate and oxidizes itself (Claus 2004; Kunamneni *et al.* 2008a). The electron generated at Cu I is transferred internally to Cu II, then to Cu III; these three atoms are arranged in a triangular structure relative to each other (Bertrand *et al.* 2002; Claus 2004). In this process, oxygen is reduced to water at the tri-nuclear cluster of copper atoms. The O₂ molecule binds to the tri-nuclear cluster of Cu atoms for asymmetric activation, and it is assumed that this O₂ bond pocket restricts the entry of any other oxidizing agent except O₂ (Dashtban *et al.* 2010; Giardina *et al.* 2010). Thus, a laccase enzyme can reduce one molecule of oxygen to two molecules of water through the single-electron oxidation of various aromatic compounds, such as phenols, polyphenols, anilines, aryl diamines, methoxy-substituted phenols, hydroxyindols, and benzenethiols (Widsten 2002; Zhou *et al.* 2009; Zakzeski *et al.* 2010).

A laccase enzyme can extend its substrate range from phenolic to non-phenolic organic substrates if the laccase is supplemented with a mediator (Gochev and Krastanov 2007; Lee *et al.* 2012). A mediator is generally a small-size compound, able to generate stable radicals during reaction (Cañas and Camarero 2010; Euring *et al.* 2011). These radicals react with various chemical compounds, including non-phenolics, that laccase alone cannot oxidize (Giardina *et al.* 2010). Some of the common mediators are 2,2'-azino-

bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), N-hydroxyphthalimide (NHPI), and 1-hydroxybenzotriazole (HOBt) (Gochev and Krastanov 2007).

LIGNIN

Lignin is a three-dimensional network of three types of phenyl-propanoid monomers with irregular repeating units. The most accepted theory of lignin's biosynthesis is the radical coupling of three basic monolignols: *p*-coumaryl, coniferyl, and sinapyl alcohols (Gang *et al.* 1999; Raikila 2008). The process of lignin polymerization is known as lignification, during which phenolic monomers produce radicals that couple together with other monomer radicals to form oligomers and, ultimately, a phenyl-propanoid polymer (Brunow 2005; Milstein *et al.* 1994). The lignin polymer exhibits various bonding such as β -O-4, β -5, 5-5, 5-3, or α -4 bonds. Among these possibilities for lignin polymerization, β -O-4 and 5-5 are the most common and are responsible for two-thirds of the total bonding (Sernek 2002; Brunow 2005).

Lignin Polymerization

Liu *et al.* (1994) studied lignin polymerization using chemicals and observed that phenolic monomers were polymerized by the catalytic action of phenol oxidases or peroxidases. A similar reaction was observed when lignin was oxidized by laccase enzymes (Boerjan *et al.* 2003). The enzymatic oxidation of lignin with the oxido-reductases laccase and peroxidase has been shown to increase the bonding strength of fibers in MDF (Felby *et al.* 2002; Nasir *et al.* 2013a). The idea of utilizing laccases as oxidizing enzymes for lignin bonding applications was based on the reactivity of phenoxy radicals in the plant cell wall (Barcelo 1997; Wang *et al.* 2013). In native plants, *in-situ* oxido-reductase catalysis activity initiates the polymerization through the cross-linking of phenoxy radicals, and the same technique can be applied in the *in-vitro* bonding of lignocellulosic materials (Cesarino *et al.* 2012; Koch and Schmitt 2013). The laccase activity during delignification can be improved by adding a redox mediator, which increases the range of substrates from phenolic to non-phenolic compounds (Gutiérrez *et al.* 2012; Rosado *et al.* 2012).

Lignin polymerization begins with the oxidation of the phenyl-propane hydroxyl groups (Kuzina *et al.* 2011). The lignin precursors undergo dimerization through enzymatic dehydrogenation, which is initiated by electron transfer and yields resonance-stabilized phenoxy radicals (Chen *et al.* 2006; Vanholme *et al.* 2012; Zhang *et al.* 2012). Figure 1 displays the phenoxy radical formed from laccase oxidation and the different forms of resonance.

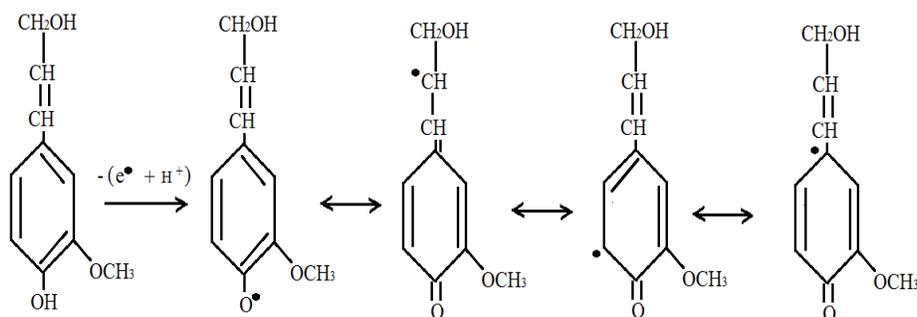


Fig. 1. Generation of resonating phenoxyl radicals by enzymatic dehydrogenation of coniferyl alcohol (adapted from Freudenberg and Neish (1968))

In the resonance structures, the radical changes positions to stabilize the oxidized phenolic compound, but it forms various bonds with other radicals in any of the positions of the unpaired electron (Vanholme *et al.* 2012). Such monolignols, having free radicals, can undergo radical coupling reactions and produce a number of dimers, called dilignols (van Parijs *et al.* 2010; Vanholme *et al.* 2012). The β -O-4 and β -5 bonding types result in a linear polymer structure. However, a branched polymer may form when nucleophilic compounds, such as alcohols, phenolic hydroxyl groups, or water, attack the benzylic carbon of the quinone methide intermediate (Cesarino *et al.* 2012; Rowell 2012).

In the single-electron transfer reaction, lignin molecules are converted into small precursors of lignin called lignols (Boerjan *et al.* 2003). These lignols react further with additional lignol radicals in a typical chain reaction to form bilignols (Boerjan *et al.* 2003). The bilignols then undergo further endwise polymerization instead of combining with one another (Boerjan *et al.* 2003; Mattinen *et al.* 2008). This radical reaction led to a very complex lignin structure, which formed an infinitely random three-dimensional network in the middle lamella of woody plants.

LACCASE APPLICATION IN FIBERBOARD FABRICATION

Currently, laccase is considered one of the most inexpensive and widely available enzymes used in commercial applications (Brijwani *et al.* 2010; Cristóvão *et al.* 2011). Some of these applications are already in practice, such as pulp processing (Virk *et al.* 2012), detoxification of environmental pollutants (Harms *et al.* 2011), preventing wine browning (Osma *et al.* 2010), oxidation of dyes and their precursors (Kumar *et al.* 2011), and producing lignin from cellulosic material (Wang *et al.* 2014). Laccase has been used commercially as a potential delignification agent in pulp production since the last decade (Camarero *et al.* 2007; Rico *et al.* 2014; Wang *et al.* 2014). Relatively recent studies have started to apply laccase in wood composites to prepare completely natural boards (Huttermann *et al.* 2001; Park *et al.* 2001; Felby *et al.* 2002, 2004; Widsten *et al.* 2004; Frihart and Service 2005). Table 2 presents the chronological development of laccase application in fiberboard by various methods.

Lignin polymerization is another approach, under intensive investigation for generating self-bonding between fibers (Mai *et al.* 2004; Savolainen *et al.* 2010). In the

laccase-assisted lignin oxidation, various free radicals of phenols and polyphenols are formed. These free radicals are highly reactive and can participate in polymerization, depolymerization, copolymerization, and grafting (Saastamoinen *et al.* 2012). Since the lignin structure is very similar to that of phenol–formaldehyde (PF) resins, similar polymerization can be achieved (Khan and Ashraf 2006; Laurichesse and Avérous 2014). To transform lignin into an insoluble adhesive, it must be additionally cross-linked; a lower number of free positions in the aromatic nuclei and a lower rate of reactivity limit the utility of lignin as an adhesive (Khan and Ashraf 2006). Furthermore, the methoxy or methoxy-equivalent groups present on the aromatic ring of lignin are considerably less reactive toward hydroxybenzyl alcohol groups than the hydroxyl groups found in phenol (Pizzi 2003; Khan and Ashraf 2006; Schorr *et al.* 2014). Because of these reasons, lignin cannot be utilized as effectively as a potential adhesive as synthetic PF resins. Thus, potential cross-linking agents, such as polyisocyanates (Dunky 2003), epoxides (Zakzeski *et al.* 2010), polyols (Pizzi 2003), polyethyleneimine (Huang and Li 2007), maleic anhydride (Gu and Li 2010), proteins (Frihart 2010; Hamarneh *et al.* 2010), amines (Dunky 2003), or melamine (Amaral-Labat *et al.* 2012), are required to achieve the desired results. Due to the aforementioned concerns for safety and the environment, the use of formaldehyde as a possible agent of lignin polymerization was not included here. So far, all of these procedures, for different reasons, have not led to the development of any major practical applications. Laccase treatment to activate the lignin structure was a new technique developed in the early 1990s (Felby *et al.* 1997; Huttermann *et al.* 2001; Wu *et al.* 2011).

Table 2. Chronological Development of Laccase Applications to Fiberboard Fabrication

Reference	Novelty	Application
Yoshida (1883)	Discovered laccase	Discovered for the first time in latex from the Japanese lacquer tree (<i>Rhus vernicifera</i>)
Yamaguchi <i>et al.</i> (1992)	phenol dehydrogenative polymerization	Achieved bonding among woody-fibers by polymerisation
Bao <i>et al.</i> (1993)	Biosynthesis in plants	Reported laccase in plants, and its role in lignin biosynthesis
Milstein <i>et al.</i> (1994)	Lignin polymerization	Laccase-catalyzed lignin polymerization by radical coupling, alkyl–aryl cleavage, or Ca oxidation
Kharazipour <i>et al.</i> (1997)	Fiber surface activation	Activated wood fibers for the production of wood composites
Bourbonnais <i>et al.</i> (1997)	Laccase mediator	Laccase activity enhanced by a mediator, and it behaved like a nonspecific enzyme
Xu <i>et al.</i> (1998)	Used in pulp	Studied the reaction mechanism and various parameters of the enzyme reaction on pulp
Ikeda <i>et al.</i> (1998)	Radical polymerization	Laccase-induced radical polymerization of the phenoxyl radicals of lignin with or without a mediator
Gianfreda <i>et al.</i> (1999)	Calculated molecular mass	The average molecular mass of laccase was found 60 to 70 kDa. Hence, it is an intracellular cellular treatment and do not affect the cellulose.
Setti <i>et al.</i> (1999) and Lund and Ragauskas (2001)	Oxidative coupling of lignocellulosic molecules	Lund and coworkers reported grafting of 4-hydroxyphenylacetic acid (PAA) and guaiacol sulfonate to kraft lignin

Nyanhongo <i>et al.</i> (2002)	Nonspecific nature of laccase	Laccase can act not only on phenols but also on polyphenols, anilines, aryl diamines, methoxy substituted phenols, hydroxyindols, benzenethiols, <i>etc.</i>
Felby <i>et al.</i> (2004) and Felby <i>et al.</i> (2002)	Binderless board	Manufactured binderless board from fiber treated by laccase
Suurnäkki <i>et al.</i> (2010)	Fiber surface modification	A small amount of enzyme is sufficient for larger-scale activation of pulp fiber
Nasir <i>et al.</i> (2013a)	Fiber treatment parameters and fabrication parameters	Prepared binderless board from laccase treated fiber at different manufacturing parameter.
Schubert <i>et al.</i> (2015)	Synthetic adhesive in combination with Laccase	Optimized the Laccase modified fibers and synthetic binder and reduced the adhesive amount up to 40 %.

Laccase is applied to wood composites with two goals: physical modification of fiber and chemical modification of fiber. The physical modifications may include changes in the crystallinity or the morphology of the fiber surface. Such changes may improve the mechanical strength and facilitate self-bonding of fibers by mechanical interlocking (Winandy and Rowell 2005). The chemical modifications include the activation of the lignin molecules of lignocellulose fibers to induce lignin polymerization reactions (Tamminen *et al.* 2010; Moilanen *et al.* 2011).

Crystallinity Index Improvement

Cellulose is a long, linear chain of D-glucose connected with β -1,4-glycosidic bonds. The hydroxyl groups present in its basic structural unit link laterally by well-organized hydrogen bonding networks, giving rise to a crystalline structure (Janga *et al.* 2012). Though the first crystalline structure of cellulose was proposed by Carl von Nägeli in 1858 (Wilkie 1961), its structure is not yet fully understood because of its complexity (Quintana *et al.* 2015). The crystallinity Index (CrI) of cellulose is one of the most important parameters to study in determining the physical and mechanical behavior of cellulose fibers (Nasir *et al.* 2013a). There are various techniques to calculate the CrI of cellulosic fibers, but their values differ significantly depending on the method used (Bansal *et al.* 2010). Cellulose exists in four different crystalline forms (polymorphs): I, II, III, and IV. Cellulose I is native cellulose as it exists in its natural state, and the rest are all the result of some chemical modification (Ishikawa *et al.* 1997).

The crystallinity of cellulose plays an important role in the accessibility and longevity of cellulosic fiber (Awadel-Karim *et al.* 1999; Schenzel *et al.* 2005). Li and Pickering (2008) and Nasir *et al.* (2013a) studied the effect of laccase on cellulosic fiber and observed an up to 22% and 10% increase in the crystallinity index, respectively. Such an effect is consistent with removal of non-crystalline matter. Figure 2 shows the change in the CrI of rubber wood fiber treated with laccase in different time interval (Nasir *et al.* 2014b).

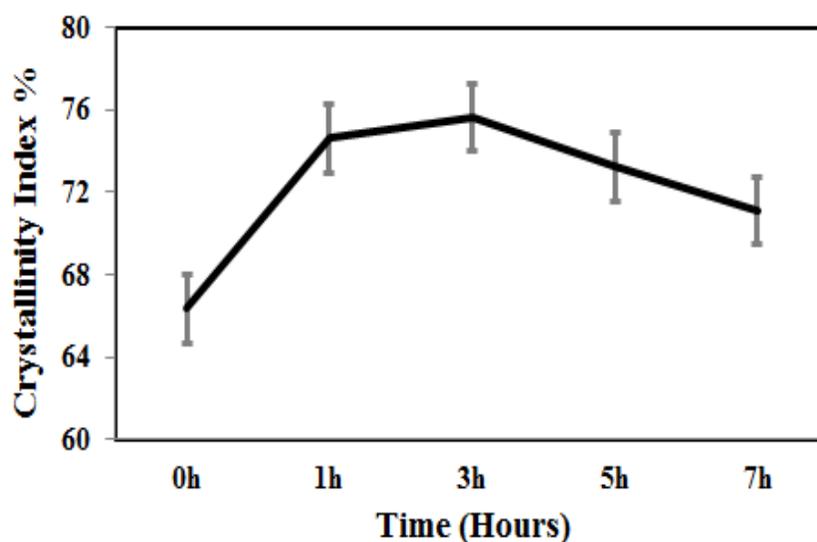


Fig. 2. Crystallinity index (Crl, %) of laccase-treated fiber at different time intervals (adapted from Nasir *et al.* (2014b))

With an increase in the crystalline to amorphous ratio, the rigidity of cellulose fibers increases but the flexibility decreases (Ishikawa *et al.* 1997). Thermogravimetric analysis (TGA) shows an interesting pattern of improved thermal stability when the change in crystallinity is considered (Li and Pickering 2008; Zeng *et al.* 2011). Laccase mediator-based hydrolysis removes the amorphous phenolic and non-phenolic components from the surface but does not affect the microfibril core, which remains crystalline (Quintana *et al.* 2015). Thus, the selective removal of amorphous components from the fiber increases the crystallinity of individual fibers.

Surface Modification

Enzymes can be used in cross-linking/self-bonding of wood fibers to prepare fiberboard without any external adhesive (Felby *et al.* 2002; Widsten and Kandelbauer 2008; Nasir *et al.* 2013a). Nasir *et al.* (2013a) treated rubber wood fiber with laccase and observed a smooth deposition of lignin onto the surface (Fig. 3). An enzymatically modified fiber can improve the inter-bonding strength of fiber in many ways, such as surface smoothness/roughness or adsorption/desorption behavior, that can lead to mechanical interlocking between fibers (Symington *et al.* 2009). Mechanical interlocking is a type of physical force in which two components of distinct interfaces are held together. This mechanism is similar to dovetail joints, where the surface of one component is embedded into another. In a laccase hydrolysis process, along with the breakdown of lignin the precipitation and adsorption of lignin also occurs simultaneously (Maximova 2004; Pribowo *et al.* 2012). A lignin-adsorbed surface can change the sorption characteristics, dimensional stability, and intermolecular adhesion of the fiber (Maximova 2004; Yu *et al.* 2009).

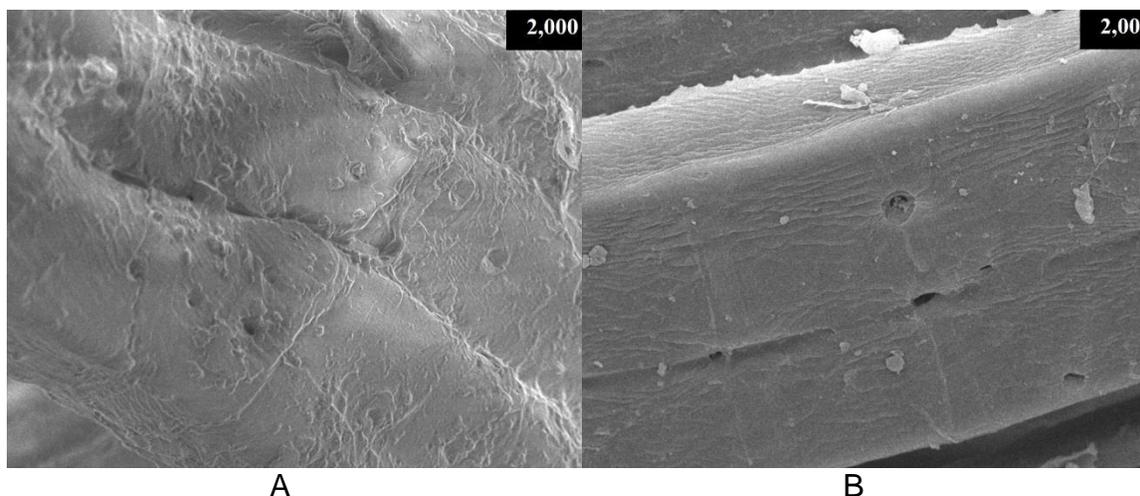


Fig. 3. SEM picture of (A) untreated and (B) treated fiber at 2000 × magnification (adapted from Nasir *et al.* (2013a))

Chemical Modification

Chemical modification involves the direct chemical reaction of components at the interface, either a free radical reaction (Zhou *et al.* 2009), ionic reaction (Shill *et al.* 2012), hydrogen bonding, or carbonyl bonding (Hill and Cetin 2000). Figure 4 shows the possible self-bonding reactions that may occur at the time of hot pressing. Laccase is a well-established approach to generate phenoxy radicals from lignin by the oxido-reduction process (Hüttermann *et al.* 2001; Mai *et al.* 2004). These free radicals undergo polymerization reactions and form a network of polymers by coupling (similar to thermoset adhesives) (Kunamneni *et al.* 2008a; Spulber *et al.* 2014). In recent studies, it has been well established that laccase enzymes obtained from fungi are best suited for the activation of native lignin and accelerating the oxido-reductase coupling of lignin (Ceylan *et al.* 2008; Liu *et al.* 2009; Witayakran and Ragauskas 2009; Bledzki *et al.* 2010; Singh and Singh 2014).

Another approach to improve the self-bonding of the fiber is to bring the copolymer matrix (lignin and hemicellulose) to the surface of the fiber so that it can take part in the auto-adhesion of the fiber when the fiber is pressed at high temperatures. Laccase is a specific enzyme that acts on lignin, but laccase supplemented with a mediator can act on a wide range of substrates (phenolic compounds) (Fillat and Roncero 2010; Lee *et al.* 2012). It can oxidize the variety of organic compounds present in plant cell walls, such as lignin, ortho- and para-diphenols, aminophenols, polyphenols, aryl diamines, polyamines, and some inorganic ions (Mattinen *et al.* 2011). These copolymers of the cell wall exhibit an amphiphilic nature and serve as both adsorbing surfaces and adsorbable amphiphiles (Tian *et al.* 2012). It is evident that a lignocellulosic composite can be formed successfully if the wood surface is coated with a thermoplastic cell matrix such as lignin or hemicellulose (Gradwell 2004; Kumar *et al.* 2009; Tian *et al.* 2012).

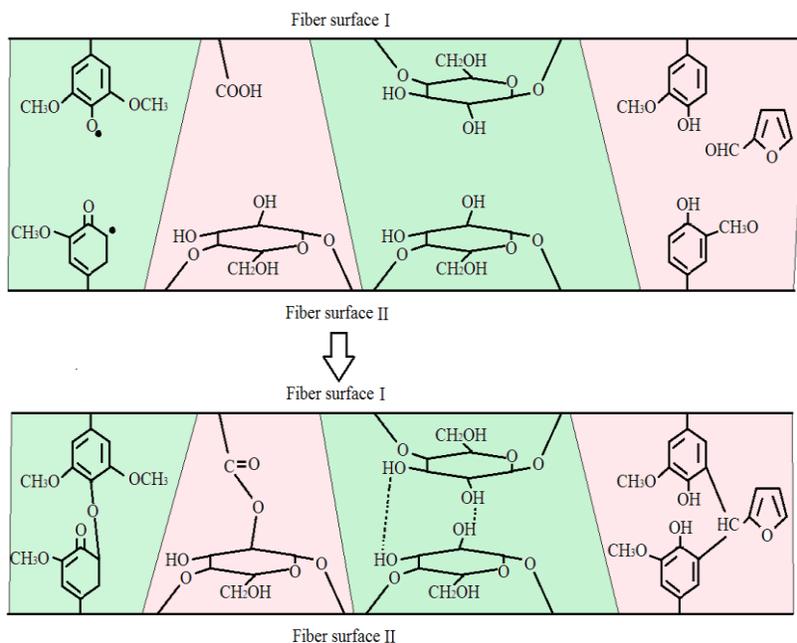


Fig. 4. The possible auto-adhesion reactions between two modified fibers (adapted from Widsten (2002))

Although a treated fiber exhibits improved physical and mechanical properties compared to an untreated fiber, its water resistance properties decrease (Li and Pickering 2008). The untreated fibers are bundled together and the surfaces are covered with non-cellulosic compounds, such as lignin, wax, and pectin, which restrict the water absorption. The removal of such compounds during laccase treatments separates the fiber bundles and exposes hydroxyl groups on the fiber surface. Much research has been proposed on producing wood composites from laccase-modified natural fiber, but fiber modification alone cannot achieve the minimum required strength (Lund and Felby 2001; Felby *et al.* 2002). Thus, an enzyme-treated fiberboard can work excellently in combination with adhesive, or it can reduce the quantity of adhesive used.

CONCLUSIONS

Formaldehyde emission is a serious concern, and positive progress in laccase-based wood composites will lead to the manufacture of an eco-friendly, biodegradable composite. Laccase displays a versatile mode of action and has a tremendous scope for future work. It not only plays a role in the delignification of cellulosic fiber but has the capability to remove other phenolic as well as nonphenolic extractives. It is a potential tool to modify the physio-chemical properties of natural fiber by altering surface morphology, surface deposition, pulp grafting, and delignification. The optimum treated fiber exhibits enhanced crystallinity, improved thermal resistance, and higher mechanical strength that would be transferred to the product into which it is made. A binderless board can be formed either by liberating reactive radicals from lignin or by functionalizing lignocellulosic fibers, but further work is needed to improve the mechanical strength and water resistance. An

integrated approach of fiber modification and lignin polymerization should be studied. Detailed study will be required to determine the radical reaction mechanism of laccase and the stability of oxidized lignin molecules through laccase reaction engineering.

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