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Research review paper

Adhesion improvement of lignocellulosic products by enzymatic pre-treatment

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ABSTRACT

Enzymatic bonding methods, based on laccase or peroxidase enzymes, for lignocellulosic products such as medium-density fiberboard and particleboard are discussed with reference to the increasing costs of presently used petroleum-based adhesives and the health concerns associated with formaldehyde emissions from current composite products. One approach is to improve the self-bonding properties of the particles by oxidation of their surface lignin before they are fabricated into boards. Another method involves using enzymatically pre-treated lignins as adhesives for boards and laminates. The application of this technology to achieve wet strength characteristics in paper is also reviewed.

Contents

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1. Introduction

Wood, agricultural fiber residues (agrifibers), and other woody plants are composite materials whose main components are polysaccharides (cellulose and hemicelluloses) and lignin (Sjöström, 1993; Ververis et al., 2004; Reddy and Yang, 2005). These lignocellulosics constitute an abundant, renewable source of raw material for a wide array of products such as paper and paperboards (Roberts, 1996; Ververis et al., 2004), composite boards such as medium-density fiberboard (MDF) and particleboard, and other adhesively bonded composites such as plywood widely used in the construction and furniture industries (Maloney, 1996; Sellers, 2001; Reddy and Yang, 2005). Regarding lignocellulosic composites, large amounts of increasingly more expensive petroleum-derived adhesives are needed for their manufacture. In addition, formaldehyde emissions during production and end-use of MDF, particleboard, and other adhesively bonded products glued with formaldehyde-containing adhesives such as urea-formaldehyde are a concern for the manufacturers and consumers (Maloney, 1996; Sellers, 2001). The stringent environmental and human health safety regulations and mounting raw material costs have prompted research into reducing the amount of harmful and/or expensive adhesive components and replacing synthetic adhesives with more environmentally-friendly and safer alternatives. In terms of paper products, which may be prepared without adhesives but often require costly resin additives for enhancing wet strength (Roberts, 1996), savings in raw material and chemical costs can be achieved if the basis weight of the products can be reduced without compromising product quality. Alternatively, the durability of the products at a given basis weight could be increased by suitable chemical modification of the fibers.

In the quest for novel adhesion solutions and strength additives for lignocellulosic composite and paper products, a common theme is the attempt to increase the bonding between the fibers or other constituent particles by their chemical modification. An overwhelming majority of the studies in this area are based on oxidative modification of lignin in the particles themselves and/or using added cross-linking material such as technical lignins derived from the paper industry. The activation of lignin for bonding can be carried out by oxidation with phenol-oxidizing enzymes (laccase and peroxidases) derived from white-rot fungi or other sources or by using the fungi as such. The objective of the present review paper is to summarize and evaluate the state-of-the-art of the field of lignocellulosic composite and paper products prepared using enzymatic activation of lignin for adhesion improvement. Although modification of lignin for improving adhesion of wood composites with thermosetting adhesives has also been investigated (Fackler et al., 2008), applications involving synthetic adhesives are beyond the scope of this review.

2. Enzymatic modification of lignin

2.1. Chemical structure of lignin

Lignin is an amorphous, phenolic macromolecule found in the cell walls of vascular plants (Sjöström, 1993). It is made up of *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S)-type phenylpropane units bearing 1–3 free or etherified hydroxyl groups, respectively (Fig. 1). The phenylpropane units are connected by different types of carbon–carbon (e.g. 5–5') and ether (e.g. β-O-4) linkages. Softwood and hardwood lignins are mainly of the G and GS types, respectively, while grasses and other non-wood lignocellulosic plants may contain a significant proportion of H-type lignin.

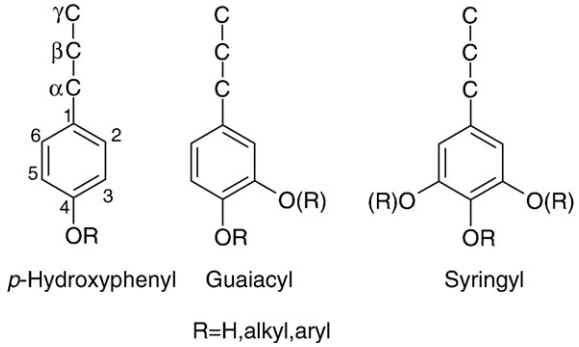

Fig. 1. Lignin phenylpropane units.

2.2. Modification of lignin by laccase and peroxidases

Laccase and peroxidases are phenol-oxidizing enzymes produced by fungi (particularly white-rot) and certain plants, bacteria, and animals. White-rot fungi are basidiomycetes playing an important role in the biodegradation of wood and other lignocellulosic materials (Li, 2003; Regalado et al., 2004). The enzymes expressed by white-rot fungi are able to degrade all the main components of lignocellulosics, i.e., cellulose, hemicelluloses, and lignin (Enoki et al., 1988). From the point of view of adhesive applications based on enzymatic treatments of lignocellulosic materials, which are all more or less based on the enzymatic oxidation and subsequent cross-linking of lignin and/or other plant-derived phenolics such as tannins, it is of interest to examine how the chemical structure of lignin changes upon exposure to laccase (Leonowicz et al., 2001; Li, 2003) and peroxidase (Li, 2003; Regalado et al., 2004). These two types of phenol-oxidizing enzymes catalyze the one-electron oxidation of phenolic groups to phenoxy radicals. They differ in that while laccase catalyze the oxidation of phenolic substrates by dioxygen (O_2), peroxidases such as lignin peroxidase (LiP) and manganese peroxidase (MnP) require hydrogen peroxide as substrate (Fig. 2). The peroxidases most commonly used for modification of lignocellulosic materials are LiP, MnP, and horseradish peroxidase (HRP).

Unlike many other peroxidases, LiP is able to catalyze the oxidation of not only phenolic but also non-phenolic aromatic substrates (Li, 2003), giving rise to aryl cation radical intermediates (Kawai et al., 1987; Higuchi, 1989). Non-enzymatic transformations of lignin radicals may result in depolymerization of the lignin macromolecules, formation of phenolic hydroxyl groups via cleavage of carbon–carbon and ether bonds, and other reactions (Kirk and Chang, 1975; Enoki et al., 1988; Nagieb et al., 1988; Higuchi, 1989; Hatakka et al., 1996). The lignin radicals may also combine, resulting in repolymerization (Hatakka et al., 1996). The investigations of Hatakka et al. (1996) with technical lignins showed the net effects of laccase or MnP treatment in terms of molecular weight and functional group content to be strongly dependent on the reaction parameters such as enzyme dose.

The substrate range of phenol-oxidizing enzymes such as laccase and MnP can be extended to non-phenolic (etherified) lignin units by using redox mediators such as 2,2'-azinobis-(3-ethylbenzenthiazoline-6-sulfonic acid) (ABTS) and 1-hydroxybenzotriazole (HBT). These small molecules are first oxidized by laccase and can then diffuse to cell walls of lignocellulosic materials, where they oxidize lignin phenolic hydroxyl groups and are often themselves regenerated in the process (Bourbonnais and Paice, 1990; Call and Mücke, 1997; Xu et al., 2002; Li, 2003). The mediators thus help overcome the accessibility problem faced by bulky phenol-oxidizing enzymes, which can usually act only on phenolic hydroxyl groups located at the surface of lignocellulosic materials and are unable to penetrate into the cell walls.

The adhesive effect obtained when lignocellulosic materials are treated with phenol-oxidizing enzymes and then pressed into composite or paper products is usually attributed, at least partly, to cross-linking of lignin-based radicals (Fig. 3; see Sections 3–6).

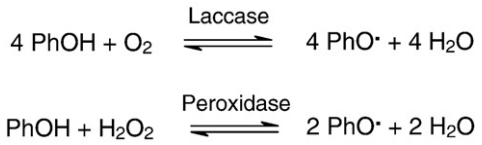

Fig. 2. Laccase- and peroxidase-catalyzed oxidation of phenols.

2.3. Modification of lignin by brown-rot fungi

Brown-rot fungi are a type of basidiomycetous microorganisms able to effectively depolymerize and remove cellulose and hemicelluloses from lignocellulosic materials, as well as oxidizing lignin at a slower rate (Kirk, 1975; Enoki et al., 1988; Goodell, 2003). Some brown-rot fungi are able to produce laccase (Lee et al., 2004). As a consequence, brown-rotted wood comprises mainly modified lignin. These properties make it worthwhile to look at brown-rot fungi as a potential means for producing reactive lignin from wood and other lignocellulosic raw materials for adhesive and other applications. The fungi cause extensive demethylation of methoxyl groups of (particularly phenolic) lignin units; aromatic hydroxylation takes place as well. As a result, brown-rotted lignin (BRL) is high in phenolic hydroxyl groups. The carboxyl content of BRL is also significant, due in part to oxidation of initially formed catechol groups and in part to side-chain oxidation (Kirk, 1975; Jin et al., 1990). Kirk (1975) found the phenolic and carboxyl group contents of BRL to be more than twice as high as those of the corresponding milled wood lignin (MWL), while the BRL contained only two thirds of the methoxyl groups found in the MWL.

In terms of the suitability of BRL treated with a phenol-oxidizing enzyme as an adhesive for lignocellulosic composite materials, some of its characteristics should promote radical formation in the lignin and should thus have a positive impact on adhesion. The increased phenolic hydroxyl content is evidently one of them. The presence of carboxyl groups increases the water solubility of lignin and may thus be beneficial during enzymatic treatment; however, the carboxyl groups may also reduce the dimensional stability of the composite product. Aromatic ring cleavage reduces the number of aromatic radicals (potential cross-linking sites) that can be formed and may thus detract from the adhesive properties of BRL.

3. Manufacture of binderless fiberboard from enzymatically pre-treated lignocellulosic fibers

3.1. Activation of fibers by enzymatic pre-treatment before pressing

In the thermomechanical pulping for the manufacture of fiberboard, the raw material such as wood chips or saw dust is pre-heated at elevated temperature and pressure (~170 °C; ~0.8 MPa) to facilitate fiber separation during the refining stage. Under these conditions, the wood lignin is plasticized and fiber separation occurs along the highly lignified middle lamella region (Back and Salmén, 1982). As a consequence, the surface of the separated fibers is rich in lignin (Widsten et al., 2001, 2002a). In addition, the high-temperature defibration results in lignin depolymerization via cleavage of ether bonds connecting the lignin phenylpropane units (Widsten et al., 2001, 2002a). The depolymerized, water-extractable lignin fraction has a high content of phenolic hydroxyl groups (Widsten et al., 2001, 2002a) and is crucial for the reactivity of lignin toward phenol-oxidizing enzymes such as laccase in terms of oxidation of phenolic hydroxyl groups to phenoxy radicals (Widsten et al., 2002b, 2003a). Low-molecular weight phenols in the extract have been suggested to function as an oxidation mediator enabling radical formation in fiber domains not directly accessible to bulky laccase molecules

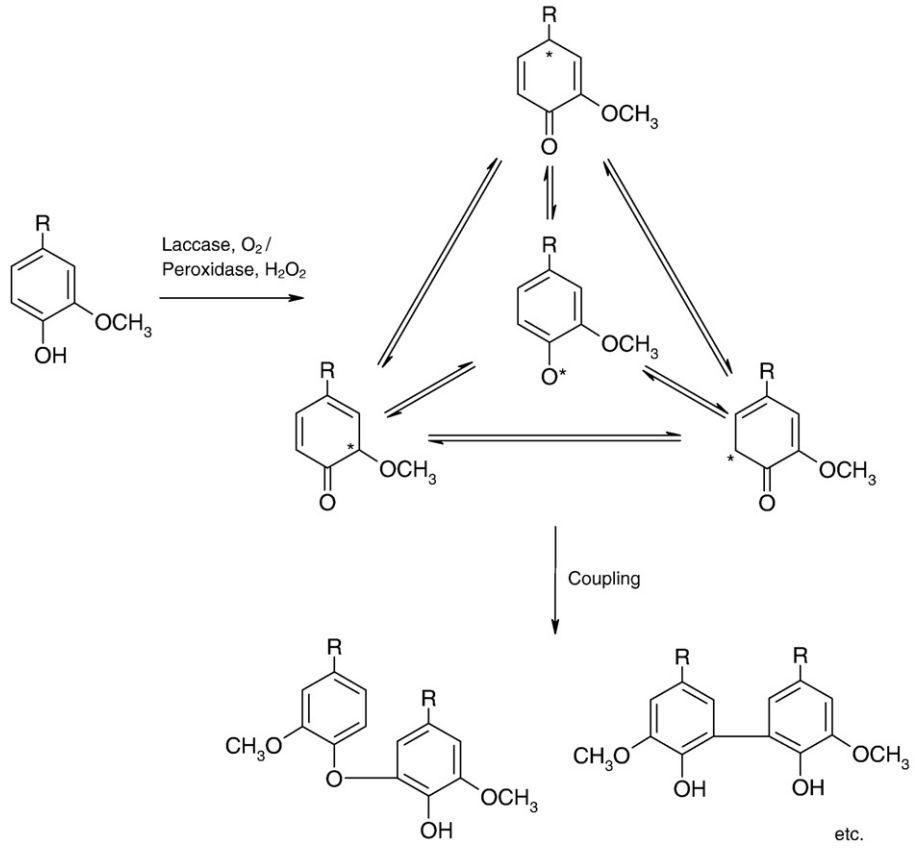

Fig. 3. Coupling reactions of phenoxy radicals on lignocellulosic substrates treated with phenol-oxidizing enzymes.

(Hassingboe et al., 1998). A large increase in radical formation was obtained when laccase was applied together with the synthetic mediator ABTS (Hassingboe et al., 1998). Felby et al. (1997) found the properties of boards prepared from water-extracted and laccase-treated beech fibers to be inferior to those of boards made from unextracted and laccase-treated fibers. Widsten et al. (2003b, 2004) found the properties of hardwood and and softwood MDF to improve with an increase in radical formation in the fibers on laccase treatment. The formation of covalent interfiber bonds by radical coupling during pressing has been suggested to be a major contributor to the improved adhesion effect obtained (Felby et al. 1997; Widsten et al. 2003b; Felby et al., 2004). Increased surface compatibility and entanglement of surface molecules due to deposition of lignin extractives on the surface of fibers incubated with laccase have also been suggested to improve adhesion (Felby et al., 1997; Felby et al., 2004).

3.2. Effect of pre-heating temperature and wood type on fiberboard properties

The amount of depolymerized lignin present in the fibers increases with an increase in wood pre-heating temperature; it also varies from species to species and at a given defibration temperature, hardwood lignin is depolymerized to a greater extent than softwood lignin (Widsten et al., 2001, 2002a). This is probably due to the greater thermolability of the β-aryl ether linkages of syringyl units present in hardwood as compared to the mainly guaiacyl-type softwood lignin (Domburg et al., 1974). The amount of water-extractable aromatic material and the number of phenolic hydroxyl groups in birch fibers increased from 1.5 to 4.2% and 0.2 to 0.43 mmol/g, respectively, when the pre-heating temperature increased from 171 to 196 °C (Widsten et al., 2001). In the case of spruce, the change of pre-heating temperature from 171 to 202 °C resulted in respective increases from 0.8 to 1.5% and from 0.20 to 0.31 mmol/g (Widsten et al., 2002a). Widsten et al. (2002b, 2003a) found the formation of phenoxy radicals and the internal bond (IB) strength of fiberboards (Widsten et al., 2003b, 2004) to increase with increasing pre-heating temperature on laccase treatment of birch and spruce fibers. An increase in pre-heating temperature thus clearly renders wood fibers more reactive toward laccase and improves the board properties.

3.3. Properties of fiberboards prepared from enzymatically pre-treated fibers

The preparation of fiberboards from enzymatically/fungally treated fibers has been investigated on a laboratory- and pilot-scale. In the laboratory-scale experiments by Felby et al. (1997) and Kharazipour et al. (1997, 1998), fibers were either incubated with laccase (Felby et al., 1997; Kharazipour et al., 1997) or peroxidase (Kharazipour et al., 1998) at low consistency in a water medium at a suitable pH or laccase solution was sprayed onto the fibers (Kharazipour et al., 1997). The activated fibers were formed into mats and their water content adjusted to the desired level for wet- or dry-process boards, after which the mats were pressed into 3–6 mm thick fiberboards with densities in the MDF (0.7–0.8 g/cm^3) or high-density fiberboard (HDF, hardboard) (>0.8 g/cm^3) range. Unbehaun et al. (2000) manufactured dry-process MDF from rape seed and pine fibers treated with laccase or enzyme culture media but provide no experimental details. The methods employed in the pilot-scale studies differ in regard of the treatment method (mixing/spraying, addition point). In the study by Widsten et al. (2003b, 2004), laccase was applied by spraying it onto the fibers in the refiner blowline as the fibers were in a fluffed state; the fibers were then air-formed into mats which were hot-pressed into 12-mm MDF. In another pilot-scale study by Felby et al. (2002), laccase was applied during the atmospheric refining stage, after which the fibers were incubated in a rotary bin, dried, air-formed and cold-pressed into mats, and finally hot-pressed into 8-mm HDF. The best results in terms of IB strength obtained in the above-mentioned studies are shown in Table 1.

Laboratory-scale fiberboard production from enzymatically treated fibers has also been undertaken by other research groups using either laccase or enzyme culture media for incubation. Kühne and Dittler (1999) sprayed unspecified lignocellulosic fibers with a commercial laccase or a mixture of brown-rot and white-rot fermentation media, malt solution, and effluent from fiberboard production. The boards prepared with the fungus–malt–effluent mixture had significantly higher mechanical strength and dimensional stability in percentage terms (only relative values were reported) than control boards made from enzymatically treated fibers and controls. Compared to them, the boards made from laccase-treated fibers displayed ~50% lower IB strength and equal dimensional stability. Körner et al. (2001) fermented wood (mainly spruce) chips, treated with a fungicide, with the brown-rot fungus *Coniophora puteana* before defibration. The fibers were dried to a moisture content of 11% and fabricated into dry-process MDF with target density of 0.700 g/cm^3. The boards prepared from chips fermented for 9 h showed ~180% better modulus of rupture (MOR), ~280% better modulus of elasticity (MOE), and 60% lower thickness swell (TS) than those made from non-fermented chips (only relative values were given). In addition, the refining energy was reduced by 40% on 13-h fermentation. Cao et al. (2007) prepared wet-process MDF from pine (*Pinus kesiya*) fibers incubated with laccase. The optimal incubation conditions in terms of IB strength were pH 3.0, 50 °C, 2 h, and a laccase dose of 20 U/g fiber. Similar results were obtained with eucalypt fibers by Wei et al. (2007). The optimal incubation conditions in this case were pH 4.5, 40–45 °C, 1–2 h, and a laccase dose of 20 U/g fiber.

Although the large variability of raw material type and incubation and production conditions complicates the comparison of the work carried out by different research groups, while other studies provide little or no experimental data or only relative values for board properties, some general conclusions can be made. A comparison of the properties of enzymatically glued boards with the specifications of the European standard EN 622-5 for the different thickness ranges of 1.8–12-mm dry-process general purpose MDF for use in dry conditions (e.g., IB 0.60–0.65; thickness swell 15–45%) shows that none of the boards with densities in the MDF range comply with all the requirements for mechanical strength and dimensional stability. While sufficient IB strength can be achieved, the thickness swell and/or bending strength criteria are not met. In terms of higher density boards (0.87–1.04 g/cm^3), boards of high mechanical strength and sufficient dimensional stability meeting European norms for HDF (EN 622-2) have been made on laboratory- and pilot-scale. For patents in the area, see Kharazipour et al. (1993) and Qvintus-Leino et al. (2003).

3.4. Evaluation of the different fiberboard production methods

The method employed in the laboratory-scale studies in which the fibers are first produced in a normal manner and then incubated for an extended period before being fabricated into boards would require major changes to the current industrial manufacturing practices and therefore does not seem industrially viable. Regarding the methods used on a pilot-scale, the application of enzyme in the atmospheric refining stage during the fiber production (Felby et al. 2002) has the advantage that the fibers are not subjected to high temperatures, which may result in premature radical cross-linking and deactivate the enzyme. Nevertheless, the separate incubation stage required reduces the attractiveness of this approach. An advantage of the method of spraying the enzyme onto fibers in the refiner blowline (i.e., at the UF resin application point) (Widsten et al., 2003b, 2004) is that it requires no changes to existing manufacturing facilities and equipment. A drawback of this method is that the conditions prevailing in the blowline (high-temperature and low fiber moisture content) may

Table 1
Fiberboards prepared from enzymatically activated wood fibers

Enzyme	Fiber	Pre-heating		Incubation conditions					Pressing conditions					Board properties					Reference
		P, MPa	*t*, min	Fiber/water	*T*, °C	*t*, h	pH	Enzyme dose, U/g[a]	Mat water content, %	*t*, min	*T*, °C	*P*, MPa	*ρ*, g/cm^3	Thickness, mm	IB, MPa	MOR, MPa	MOE, GPa	24 h TS, %	
Laccase SP 504[b]	Mixed[c]	0.8–1.0	n/a	3.6% w/v (buffer)	35	12	5.0	26,900	De-watered fibers	5	190	1.0	0.78	5.4	0.95			23	Kharazipour et al. 1997
Laccase SP 504[b]	Mixed[c]	0.8–1.0	n/a	34/66 (sprayed)	RT	12			20–25	5	190	1.0	0.77	6.0	0.59			22	Kharazipour et al. 1997
Laccase SP 504[b]	Beech			5%, w/v (water)	20	1	4.5	3.5	33	5	180	n/a	1.04	3	1.55			57	Felby et al. 1997
Laccase SP 504[b]	Beech			5%, w/v (water)	20	1	4.5	3.5	12	5	200	n/a	0.90	3	1.57	44.6	3.36	19	Felby et al. 1997
Laccase[d]	Beech	0.85	4	45/55 (water)[e]	50	0.5	7.0	24	11–13	3.4	200	n/a	0.87	8	0.93	46.0	3.95	46	Felby et al. 2002
Laccase[e]	Aspen	1.2	4	Dry fibers[f]	n/a	n/a		12	7	5	170	n/a	0.75	12	1.02	16.1	2.17	33	
Laccase[e]	Birch	1.2	4	Dry fibers[f]	n/a	n/a		6	6	4.7	190	n/a	0.71	12	0.86	19.8	2.73	39	
Laccase[b]	Spruce	1.6	4	Dry fibers[f]				0.6	7	4.1	170	n/a	0.91	12	1.26	23.5	3.7	39	
Trichoderma reseei medium	Pine								Dry fibers				0.8		0.16	~19		~75	Unbehaun et al. 2000
Laccase	Rape straw								Dry fibers				0.8		0.18	~13		50	Unbehaun et al. 2000
Trametes versicolor medium	Rape straw								Dry fibers				0.8		0.35	20		50	Unbehaun et al. 2000
Peroxidase SP 502[g]	Mixed[c]	0.8–1.0	n/a	6.6%, w/v (buffer)	RT	4	7.0	4200	De-watered fibers	5	190	1.0	0.8	5	0.63	41.7	4.02	28	Kharazipour et al. 1998

Unless otherwise indicated, the boards were prepared on a laboratory scale.
[a] Expressed per g of fiber in units for laccase and peroxidase units (PODU) for peroxidase.
[b] From *Trametes* (*Coriolus*) *versicolor*.
[c] 80% spruce and pine, 20% beech.
[d] From *Myceliophtora thermophila*.
[e] From *Trametes hirsuta*; Pilot-scale production: enzyme added during atmospheric refining at 60 °C; water sprayed in blowline; incubation in rotary bin.
[f] Pilot-scale production: enzyme added by spraying in the refiner.
[g] From *Basidiomycete*; H_2O_2 concentration 10 mM in peroxidase solution (300 PODU/ml).

reduce radical formation and stability in the fibers and deactivate the enzyme. However, the experimental results clearly show that laccase is able to function and/or survive at these harsh conditions to an extent large enough to significantly improve board properties. The higher defibration temperature required to produce fiber reactive enough toward laccase when this method is used is a limiting factor for production plants unable to raise the pre-heating temperature to a suitable level. Regarding energy consumption, the cost of the elevated pre-heating temperature should be more than offset by diminished energy demand during refining. The cost of enzyme should not be prohibitive as the pilot-scale studies show that even laccase doses as low as 0.6 U/g fiber are feasible.

Based on the above, the potentially most practical production method for plants able to elevate the pre-heater temperature to above-standard levels would seem to be the process involving the application of enzyme in the blowline so that a separate incubation stage can be avoided. However, to bring all board properties up to standards, optimization of production parameters may not be enough but a suitable (low-cost) additive able to improve the dimensional stability without impairing mechanical properties may be required. Future studies could address this topic.

4. Manufacture of particleboard using enzymatically activated lignin as an adhesive

4.1. Technical lignins

Technical lignins such as kraft lignin (KL), lignosulfonate (LS), soda lignin (SL), and organosolv lignin (OSL) are isolated from the spent pulping liquors of kraft, sulfite, soda and organosolv pulping processes, respectively. These lignins as well as spent sulfite liquor (SSL) have been pre-treated with phenol-oxidizing enzymes for use as particleboard adhesives, or could be considered for this purpose. The adhesive effect is largely attributed to an oxidative activation (radical formation) of the lignin in the adhesive and on the surface of the wood particles followed by cross-linking via radical coupling during pressing into boards (Haars et al., 1989; Hüttermann et al., 1989). The reactivity of technical lignins toward phenol-oxidizing enzymes is expected to depend on their 1) molecular weight distribution, 2) water-solubility, 3) content of phenolic hydroxyl groups, and 4) number of available phenoxy radical cross-linking sites (phenolic hydroxyls + unsubstituted carbons *ortho* to a phenolic hydroxyl) (Hüttermann et al., 1989). Some properties of technical lignins are given in Table 2. SSL consists of LS (~55%), sugars (~25–30%), and other inorganic and organic substances (Sjöström, 1993). The content of phenolic hydroxyl groups may also increase during treatment with phenol-oxidizing enzymes (Section 2.2).

4.2. Properties of particleboard prepared with enzymatically pre-treated lignin

Despite the higher phenolic hydroxyl content of KL vs. LS (~0.6 vs. ~0.4/C_9 unit), the published work has focused mainly on the use of LS and SSL from the sulfite pulping process. A possible reason for the absence of work with KL is its water-insolubility which reduces its reactivity toward laccase (Hüttermann et al., 1989). This applies also to other essentially water-insoluble lignins (Table 2) such as soda lignin, OSL (not widely available commercially), and MWL (prepared for

Table 2
Selected properties of technical lignins

Lignin type and source	Lignin content, %	Water solubility	Phenolic hydroxyl groups/C9 unit	Radical cross-linking sites/C9 unit[a]	Poly-dispersity (Mw/Mn)	Reference
Pine kraft lignin (Indulin AT, Westvaco Co.)	93	2% (25 °C)	0.57–0.62	0.91	4.32	Cook and Sellers 1989
Red oak organosolv lignin	92	5% (25 °C)	0.60–0.65	0.63	2.46	Cook and Sellers 1989
Hardwood soda/anthraquinone lignin		Low	0.77	0.85		Pizzi et al., 1989
Lignosulfonate		Very high	0.3–0.4			Fengel and Wegener 1984; Pizzi 1994

[a] No. of free phenolic hydroxyl groups+no. of unsubstituted carbons *ortho* to a free phenolic hydroxyl group. The latter is obtained by multiplying the average number of free phenolic hydroxyls by the no. average no. aromatic hydrogens/C9 unit (Cook and Sellers 1989) (assuming that C2 and C6 are unsubstituted and C2 is substituted by the phenylpropane side-chain).

laboratory-scale investigations only). To overcome the solubility problem, laccase treatments have been carried out using immobilized laccase in organic solvent/water mixtures. However, the cost of the organic solvent and the reduced activity and stability of laccase in many organic solvents, and the need to immobilize the laccase (Hüttermann et al., 1989) reduce the attractiveness of this approach. LS, on the other hand, is water-soluble because of its sulfonic acid groups.

Unlike fiberboard, the published enzymatic applications aiming at resin substitution in particleboard are all two-component systems; they are based on the use of technical lignin or other added adhesive. The use of enzymatically activated LS/SSL for adhesion of particleboard was first proposed in the patent of Nimz et al. (1972), which focuses on the use of inorganic oxidants but also mentions the potential use of enzymes, preferably peroxidases, for lignin oxidation. No examples of particleboard prepared with enzymatically treated LS/SSL were provided.

Haars et al. (1989) incubated spray-dried SSL with a culture filtrate of *Trametes versicolor* (Table 3). The transverse tensile strength of particleboards prepared using the activated SSL by pressing at ambient temperature was equal to or higher than that of boards prepared with PF or UF resin by pressing at 190 °C; boards made with non-incubated SSL had 60% lower transverse tensile strength. Despite their high mechanical strength, the boards made with enzymatically treated SSL showed poor dimensional stability because of the hydrophilic sulfonic acid groups in the LS component. An attempt was made to improve the water resistance by incorporation of water-insoluble OSL into the formulation. The SSL–OSL boards, however, had very low mechanical strength.

Hüttermann et al. (1989) manufactured particleboards using enzymatically treated (culture filtrate of *Trametes versicolor*) LS or MWL as the adhesive (Table 3). The transverse tensile strength of the boards exceeded the European standard as well as that of the controls prepared with UF resin, and was somewhat higher with the water-soluble LS than with the water-insoluble MWL. The dimensional stability, on the other hand, was better with MWL. In both cases, the dimensional stability of the boards was higher than that of the control UF-glued boards.

5. Adhesion improvement of paper products by enzymatic pre-treatment of lignin-containing pulps

5.1. Treatments without added lignin (one-component systems)

The prospect of enhancing the mechanical strength of paper products by enzymatic treatment of lignin-containing pulp fibers has sparked research on the topic. Improvement of interfiber bonding without added lignin or other cross-linking agents by activation of lignin present in unbleached pulp by treatment with a phenol-oxidizing enzyme such as laccase is one of the methods investigated. The number of radicals formed in kraft pulps upon laccase treatment is directly proportional to their kappa number (Lund et al., 2003). The lignin located at the fiber surface is particularly prone to oxidation while the lignin in the inner fiber domains is oxidized at a slower rate (Lund et al., 2003).

Despite the radical formation, Lund and Felby (2001) found laccase treatment to have virtually no effect on the dry and wet tensile strength of kraft paper made from unbleached high-yield kraft pulp unless the treatment was performed in the presence of an oxidation mediator. In the latter case, the wet tensile strength of kraft paper was improved with some mediators such as ABTS while others such as HBT proved ineffective. Further gains in wet tensile strength were obtained when heat treatment was combined with the laccase/mediator treatment. The authors attributed the strength improvements to polymerization of lignin in the handsheets and to cross-linking of phenoxy radicals in adjacent fibers. In a study by Wong et al. (1999), a low dose of laccase/HBT improved the strength of paper made from high-yield kraft pulp.

In terms of mechanical pulp, Wong et al. (2000) studied the effect of laccase, cellulose and proteinase treatment on the mechanical

Table 3
Particleboards prepared with enzymatically activated technical lignins

Enzyme	Lignin type	Binder amount, %	Enzyme dose, U/ml[a]	Pressing conditions			Board properties			Reference
				t, min	*T*, °C	*ρ*, g/cm^3	Thickness, mm	Transverse tensile strength, MPa[c]	24-h TS, %	
Trametes versicolor phenol-oxidizing enzymes	MWL[a]	15[b]		5	RT	0.775	19	0.42	19.9	Hüttermann et al., 1989
Trametes versicolor phenol-oxidizing enzymes	LS	15[b]		5	RT	0.774	19	0.47	24.0	Hüttermann et al., 1989
Trametes versicolor culture filtrate	Ca^{2+}-SSL		320	5	24		19	0.64	Below std.	Haars et al. 1989
Trametes versicolor culture filtrate	Mg^{2+}-SSL		320	5	24		19	0.60	Below std.	Haars et al. 1989

[a] Milled wood lignin (MWL) is not obtained from any commercial pulping process but is prepared in small amounts for laboratory-scale experiments.
[b] Two parts lignin, one part enzyme solution.
[c] European standard EN 312-4: 0.35 MPa.

properties of handsheets made from radiata pine pressurized refiner mechanical pulp. While all three enzymes improved tensile stiffness, only the laccase consistently improved tensile and burst indices at a given handsheet density. The presence of an oxidation mediator (HBT) did not improve the mechanical properties but decreased the brightness substantially. Felby et al. (1998) prepared handsheets from laccase-treated unbleached radiata pine thermomechanical pulp (TMP). The dry tensile index of the paper was lower than that of controls made from untreated pulp, and the handsheets disintegrated during cold water soaking so that wet tensile index was unable to be determined.

A patent by Hansen et al. (1995) deals with production of paperboard and corrugated medium from unbleached chemical or semichemical pulp, or recycled fibers. The application of laccase mediator systems to the manufacture of paper products such as corrugated paperboard or containers from lignin-containing pulps has been patented by Lund and Felby (2000).

5.2. Treatments with added lignin (two-component systems)

As with composite boards, the use of technical lignin and other phenolic cross-linkers of wood or non-wood origin has been investigated in relation to paper products.

Elegir et al. (2007) ultrafiltered black liquor from kraft pulping of softwood to obtain a low-molecular weight lignin (UFL), which was incubated with *Trametes pubescens* laccase and used for adhesion improvement of linerboard (kraft liner). The laccase was shown to cross-link the added lignin and the residual lignin in the kraft fibers. As a result, the wet tensile strength of the linerboard was more than doubled while other board properties (dry tensile strength, SCT index, TEA index, Scott bond) were also improved to some extent. The application of laccase together with a mediator (ABTS) allowed even higher wet tensile strength to be obtained but also resulted in a decline in the other board properties.

Yamaguchi et al. (1994) deposited a dehydrogenative polymer (DHP) consisting of enzymatically polymerized vanillic acid on laccase-treated TMP. The tensile strength and water resistance of TMP paper increased, which was ascribed to coupling reactions between fiber lignin and DHP, and to an increase in fiber contact area. Lund and Felby (2001) found the addition of lignin-rich extractives to laccase-treated high-yield kraft pulp to improve the wet tensile strength of kraft paper. In another investigation, Felby et al. (1998) found a paper made from unbleached radiata pine TMP treated with laccase and phenol-sugar adduct (ferulated arabinoxylan) to have superior dry and wet tensile index and higher density than controls. The improved adhesion was ascribed to both chemical and physical factors.

6. Manufacture of wood laminates using brown-rotted lignin as an adhesive

BRL has a significantly higher content of phenolic hydroxyl groups than native wood lignin, which makes it an interesting candidate for adhesive applications based on the reactions of this functional group. Jin et al. (1991) rotted Douglas fir and used it as an adhesive for wood laminates. BRL was used as such or applied together with horseradish peroxidase or a mixture of horseradish peroxidase and laccase. The results (Table 4) show that cedar and sweetgum laminates bonded with BRL and enzymes had considerably higher dry shear strength than the corresponding laminates glued with BRL only, while no such improvements were obtained with pine laminates. In most cases, the incorporation of laccase into the adhesive formulation had a slightly beneficial effect on laminate strength as compared to laminates bonded with BRL and peroxidase only. In all cases, hot-pressing after gluing for 5 min at 149 °C gave markedly better results than cold pressing for the same time. The hot-pressing also improved the water resistance of the laminates; cold-pressed laminates disintegrated rapidly in cold water, whereas those that were hot-pressed survived for 12 h. The high carboxyl content of BRL was considered to be the most likely reason for the low–moderate water resistance. Another problem with the BRL adhesives was the fact that they did not perform nearly as well as the synthetic adhesives.

7. Conclusions and outlook

Before composite and paper products can be produced industrially from enzymatically or fungally treated lignocellulosic particles, there are technological and economical problems to overcome. Prerequisites for industrial implementation include keeping the production cost of enzymes/fungi low and avoiding incurring high investment costs from changes to existing production plants to accommodate the new production methods.

Regarding the manufacture of binderless MDF, the main challenge is to find a method allowing sufficient dimensional stability to be obtained without increasing the board density to HDF level. A small amount of a low-cost additive (with cross-linking properties) may be needed to achieve this, although the boards are then not truly binderless. Other than that, there are no great obstacles for the industrial implementation of binderless MDF production methods as the enzymatic treatment can be performed without changes to manufacturing plants (see Section 3.4 for a discussion).

As for particleboard, the published two-component production methods based on phenol-oxidizing enzymes and technical lignins seem impractical for both economical and technical reasons: the cost of the technical lignin adhesive may be significant, and the fact that the incubation of particles has to be carried out as a separate step makes the methods incompatible with existing manufacturing facilities. There is also the dilemma that the water-soluble technical lignins reduce the dimensional stability of the product, while water-insoluble lignins may not be reactive enough to provide sufficient cross-linking.

In terms of paper products made without additional binder material, rather conflicting results have been obtained by different research groups. It does seem, however, that significant wet strength improvement can be obtained when fibers are treated with a laccase/mediator system. Naturally, the cost of the mediator has to be weighed against that of existing wet strength agents. Future investigations could focus on finding low-cost and effective natural mediators. The two-component systems containing lignin or other cross-linkable material seem technically more promising than the one-component systems but their cost may be prohibitive in relation to existing industrial methods. Another issue to consider is how to implement the methods without major changes to the papermaking facilities.

The method of manufacturing laminated wood from brown-rotted lignin is unsuitable for industrial application as the product properties are clearly inferior to those obtained with synthetic adhesives. The

Table 4
Dry shear strength (MPa) of three wood species glued with BRL and conventional resins (parallel 2-ply laminated wood)

	Western red cedar		Sweetgum		Southern pine	
Resin	Hot press	Cold press	Hot press	Cold press	Hot press	Cold press
Brown-rotted lignin (BRL)	1.65	1.12	2.32	0.29	1.26	D
BRL+HRP+H_2O_2	2.57	1.64	3.30	1.65	1.10	D
BRL+HRP/L+H_2O_2	2.63	1.80	3.00	1.92	1.32	D
Phenol-formaldehyde	–	–	–	–	2.79	–
Phenol-resorcinol-formaldehyde	–	–	–	–	–	3.41

Adapted from Jin et al. (1991).
HRP = horseradish peroxidase; L = laccase; D = delaminated during sample cutting.

fungal treatment may also be too time-consuming and expensive to be feasible.

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