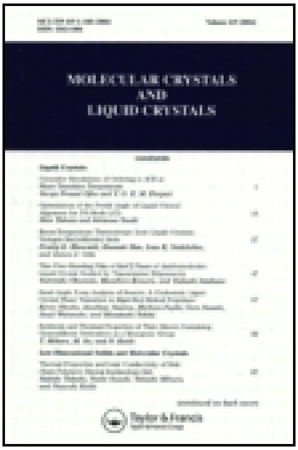

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J. Batog [a] , R. Kozlowski [a] & A. Przepiera [a]
[a] Institute of Natural Fibres, Poznan, Poland

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Lignocellulosic Composites Bonded by Enzymatic Oxidation of Lignin

J. Batog, R. Kozlowski, and A. Przepiera
Institute of Natural Fibres, Poznan, Poland

Recently, research has been carried out on the possibility of using biotic factors for the activation of natural bonding forces present in lignocellulosic materials in order to reduce the use of synthetic adhesives. A new generation of commercially available oxidizing enzymes can be effectively used for bonding lignocellulosic raw materials through oxidation of phenol compounds. Additionally, the application of enzyme mediators improves the enzymatic oxidation of lignin.

Conditions of lignocellulosic composite bonding were determined on materials from annual plants – hemp, flax and oxidizing enzyme – laccase and its mediators – ABTS, HBT, NHA.

Bonding of lignocellulosic composites by oxidizing enzymes has a positive effect on the environment.

Keywords: laccase; laccase mediators; lignocellulosic composites; oxidation of lignin

INTRODUCTION

Recently, a lot of attention has been paid to use the chemical activity of wood to create in it a natural bonding force. Chemical structure of wood (cellulose, hemicellulose and lignin) suggests possible reactions that should lead to creating durable chemical bonds.

Most of activation methods are based on exposure of wood – especially lignin – to oxidizing compounds. However, especially interesting is the enzymatic processing of wood, which works similarly to oxidizing compounds but in much milder and safer manner to the environment.

Address correspondence to R. Kozlowski, Institute of Natural Fibres, ul. Wojska Polskiego 71B, Poznan 60-630, Poland E-mail: whc@inf.poznan.pl

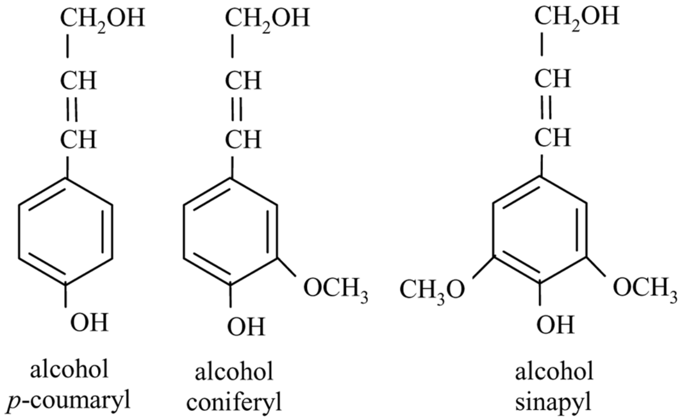

FIGURE 1 Lignin monomers: *p*-coumaryl, coniferyl and sinapyl alcohols.

In commercial scale a new generation of oxidizing enzymes has been introduced – i.e., laccase, peroxidase, which can be used for biosynthesis of aromatic compounds [2,9].

The idea of bonding wood fibres via laccase-catalysed activation of the intercellular lamella was first conceived by Körner [7] and the relevant process was described in a patent application by Kharazipour *et al.* [6].

Lignin is a polymer synthesized from three monomers – p-coumaryl, coniferyl and sinapyl alcohol [1]. They form a chain of nine carbon atoms arranged in a phenol ring with a lateral propane chain. These units have 0 to 2 methoxyl groups attached to the phenol group in ortho position (Fig. 1).

The laccase enzyme can be used for bonding lignocellulosic materials by oxidation of phenol compounds – Figure 2.

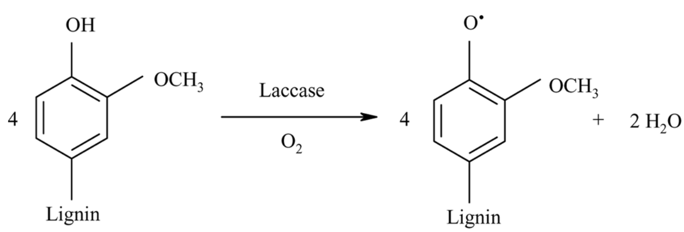

FIGURE 2 Enzymatic oxidation of phenol compounds.

In the course of an enzyme-initiated recombination of radicals, the monomers of lignin undergo polymerization to a three-dimensional, aromatic polymer.

Recently, due to shortage in wood supply, more and more attention has been paid to the use of lignocellulosic wastes from annual fibrous plants (flax, hemp, kenaf, jute) as substitutes for wood products. The advantage of these materials is the shape and slenderness of particles.

Experiments were designed to set up a method of activation and bonding process of raw material by determining processing and pressing parameters. Enhancement of laccase activity was achieved by addition of its mediators and processing medium (by using buffers and an organic solvent) and additional processing by an oxidizing compound. [3–4,8]. Laccase mediators are low-molecular compounds active in reactions of laccase with lignin [5].

EXPERIMENTAL

The following materials were used for the study:

- materials from annual fibrous plants – Table 1
  - hemp hurds – after decortication process
  - flax shives – after dew retting
- oxidizing enzymes – different laccases obtained from *Aspergillus sp.*
  - Laccase 1 and Laccase 2 (activity unit for Laccase 1 – LACU and for Laccase 2 – LAMU)
- laccase mediators – Figure 3
  - ABTS – Diammonium 2,2′-azino-bis(3-ethylbenzo-thiazoline-6-sulphonate)
  - HBT – 1-Hydroxybenzotriazole hydrate
  - NHA – 3′-Hydroxyacetanilide.

TABLE 1 Content of Cellulose, Hemicellulose and Lignin in Raw Materials

Material	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hemp hurds	37.84	29.81	28.97
Flax shives	35.37	25.07	31.00

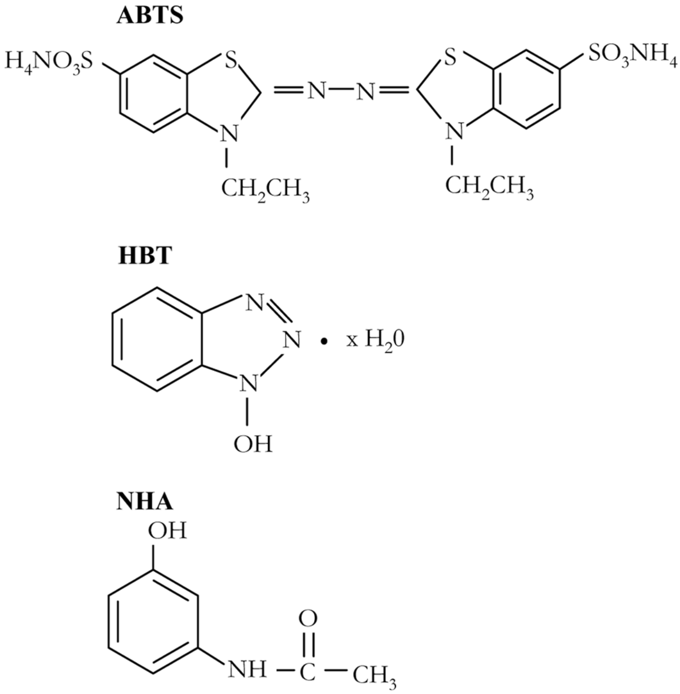

FIGURE 3 Laccase mediators: ABTS, HBT and NHA.

Activation mixtures in addition of laccase and its mediators may contain different substances – buffers and an organic solvent. Their role is to create desired pH of the medium and improve effects of raw material activation by laccase.

The pH of acetate buffers used in the experiments were 4.75 and 5.71 and pH of phosphate buffer –7.21. Dioxane solution was used as an organic solvent.

To improve laccase efficiency on lignin even further, additional processing of raw materials was carried out at the beginning of activation process – treatment with peroxyacetic acid – a compound with strong oxidizing properties.

The bending strength of the obtained boards was determined (according to standard PN-EN 310:1994) by the efficiency of mediators, buffers, dioxane and peroxyacetic acid during the processing of the raw materials with laccase.

Control samples were prepared without using laccase.

Enzymatic oxidation processes of lignocellulosic raw materials were studied by measuring the oxygen consumption (with the use of oxygen meter Oxi 340i with oxygen sensor CellOx 325) during the process of raw material activation.

RESULTS AND DISCUSSION

The enhancement of lignocellulosic raw materials activation by laccase was obtained by addition of mediators – ABTS, HBT and NHA, by creation of the reaction stable processing medium (use of buffers) and, in case of flax shives, also by using dioxane solution (processing medium) and additional treatment with peroxyacetic acid.

The best methods and conditions of lignocellulosic raw materials processing with laccase are as follows:

- hemp hurds are processed using ABTS mediator – for Laccase 1 – in buffered solution, while for Laccase 2 – in water solution,
- flax shives are processed in buffered solution – for Laccase 1 – laccase alone and additionally processed with peroxyacetic acid, while for Laccase 2 – using NHA mediator.

Parameters of activating hemp hurds with laccase and their bonding to obtain board composites are shown in Table 2 and the values of their bending strength are presented in Table 3.

Moreover, parameters of activating flax shives with laccase and their bonding to obtain board composites are shown in Table 4 and the values of their bending strength are presented in Table 5.

Lignocellulosic raw materials processed with laccase show the bending strength of 7–8 MPa for hemp hurds (control samples – 4 MPa) and 10 MPa for flax shives (controls samples – 6–7 MPa).

TABLE 2 Parameters of Hemp Hurds Activation with Laccase and their Bonding

Parameter	Laccase 1	Laccase 2
pH of processing	pH 4.75 acetate buffer	pH 6.5
Temperature of processing (°C)	40	
Time of processing	1 hour	
Dose of laccase*	3 LACU/g d.s.	6 LAMU/g d.s.
Addition of mediator (μM/g d.s.)	ABTS – 40	
Temperature of pressing (°C)	180	
Time of pressing	20 minutes	
Pressure of pressing (MPa)	2.5	

*LACU/LAMU – activity unit of Laccase 1/Laccase 2.

TABLE 3 Bending Strength of Hemp Boards

Board	Bending strength (MPa)
Control – Laccase 1	4.18
Laccase 1	8.02
Control – Laccase 2	4.32
Laccase 2	7.11

The enzyme – laccase shows different effect on the raw materials, Laccase 1 is better on hemp hurds, while Laccase 2 on flax shives.

Measurement of oxygen consumption (Table 6) for evaluation of raw material oxidation process shows that for:

- hemp hurds – oxidation reaction is more effective for Laccase 1 than Laccase 2,
- flax shives – oxidation reaction is more effective for Laccase 2 than Laccase 1,

The results of the study allowed for determination of vulnerability of lignocellulosic raw materials to laccase and showed the possibility of bonding them in board composites.

The results of the study were boards with good bending strength and environmentally friendly.

In the future there is a possibility of significant reduction in use of synthetic bonding agents in manufacturing lignocellulosic composites.

TABLE 4 Parameters of Flax Shives Activation with Laccase and their Bonding

Parameter	Laccase 1	Laccase 2
pH of processing	pH 5.71 acetate buffer	pH 7.21 phosphate buffer
Temperature of processing (°C)	50	40
Processing by peroxyacetic acid	1% d.s. 10 minutes	–
Time of processing	30 minutes	
Dose of laccase*	6 LACU/g d.s.	12 LAMU/g d.s.
Addition of mediator (μM/g d.s.)	–	NHA – 25
Temperature of pressing (°C)	180	
Time of pressing	20 minutes	
Pressure of pressing (MPa)	2.5	

*LACU/LAMU – activity unit of Laccase 1/Laccase 2.

TABLE 5 Bending Strength of Flax Boards

Board	Bending strength (MPa)
Control – Laccase 1	7.48
Laccase 1	10.38
Control – Laccase 2	6.85
Laccase 2	10.34

TABLE 6 Oxygen Consumption (%)

Material	Laccase 1	Laccase 2
Hemp hurds	10	8
Flax shives	10	16

CONCLUSIONS

1. Lignocellulosic raw materials bonded by laccase show better strength than without using laccase. Higher bending strength values were obtained for flax shives than for hemp hurds.
2. Application of laccase mediators improves the enzymatic oxidation of lignin – mediator ABTS in processing of hemp hurds with Laccase 1 and Laccase 2, mediator NHA in processing of flax shives with Laccase 2.
3. Oxygen consumption values indicate that flax shives are easier to activate by enzyme Laccase 2 than hemp hurds.
4. Bonding lignocellulosic composites by laccase is environmentally friendly, because traditional adhesives – urea-formaldehyde resins can be eliminated.

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