ENZYMATIC PROCESSING OF LIGNIN IN THE PRODUCTION OF LIGNOCELLULOSIC COMPOSITES

JOLANTA BATOG and ALOJZY PRZEPIERA

Institute of Natural Fibres & Medicinal Plants
Wojska Polskiego 71b, 60-630 Poznan, Poland

Received June 1, 2011

A lot of attention is paid to the utilization of the chemical activity of lignocellulosic materials for creating a natural bonding force inside them. The oxidizing enzymes, *i.e*. laccase and peroxidase, can be used for bonding lignocellulosic materials by oxidation of phenol compounds – especially lignin.
The study on obtaining board composites using raw materials (flax, hemp), laccase enzyme and its mediators (ABTS, HBT, NHA) was conducted at the Institute of Natural Fibres & Medicinal Plants (INF&MP). The results obtained showed that the board composites bonded by laccase have better strength than in the absence of laccase, and also that the application of laccase mediators improves the enzymatic oxidation of lignin.
The study, aimed at obtaining boards after the activation process by laccase, on using urea-formaldehyde resin, created/evidenced the possibility of a significant reduction in the use of synthetic bonding agents for manufacturing board composites.
Bonding of lignocellulosic composites by oxidizing enzymes has a positive effect on the environment.

Keywords: laccase, lignin, lignocellulosic composites

INTRODUCTION

Due to a desirable combination of their chemical and physical properties, annual plants, including the fibrous ones, *i.e*. flax or hemp, constitute a source of raw materials for manufacturing lignocellulosic composites. The chemical structure of lignocellulosic materials (cellulose, hemicellulose and lignin) suggests possible reactions that should lead to creating durable chemical bonds. Most of the activation methods are based on the exposure of the materials to oxidizing compounds – *e.g.* ozone, hydrogen peroxide and peroxyacetic acid. Enzymatic processing is particularly interesting, as it works similarly with the oxidizing compounds, but in a much milder to the environment manner and under safer conditions. The action of oxidizing enzymes, such as peroxidase or laccase, on lignocellulosic materials activates the phenolic compounds – especially lignin, and can be effectively used for bonding lignocellulosic raw materials. The oxidizing enzyme laccase is classified as a blue copper-protein. At present, the role played by laccase in higher plants has not been fully explained. Generally, it is assumed that laccase takes part in lignin degradation.[1-2] On the other hand, the role of laccase in lignin biosynthesis remains unclear.[3-4]

In the course of an enzyme-initiated recombination of radicals, the monomers of lignin undergo polymerization to a three-dimensional aromatic polymer (Fig. 1).

Additionally, the application of laccase mediators, *i.e*. low-molecular compounds, active in the reactions of laccase with lignin, such as ABTS and HBT, improves the enzymatic oxidation of lignin.[5] Most likely, in the oxidation process, mediators act as electron carriers (Fig. 2).

EXPERIMENTAL

The study aimed at determining the processing parameters of the activation methods for raw materials and pressing parameters for bonding lignocellulosic boards. The conditions of bonding lignocellulosic composites were determined by using flax shives (cellulose – 35.4%, hemicellulose – 25.1%, lignin – 31.0%), laccase obtained from *Aspergillus sp*. and laccase mediators – ABTS [2,2'-azino-bis(3-

ethylbenzothiazoline-6-sulphonate)], HBT (1-hydroxybenzotriazole), and NHA (N-hydroxyacetanilide) (Fig. 3).

In addition to laccase and its mediators, the activation mixtures may contain buffers – acetate or phosphate, whose role is to create the desired pH of the medium and to improve the effects of raw material activation by laccase. The pH values of the acetate buffers used in the experiments were of 4.75 and 5.71, while the pH of the phosphate buffer – 7.21. The evaluation of the activation degree of flax shives and of the bonding trials was carried out by measuring the oxygen consumption during the activation process (with an Oxi 340i oxygen meter), and by determining the bending strength of boards (acc. to PN-EN 310:1994).

The study also aimed at producing boards from flax shives after activation by laccase, on using a lower amount of urea-formaldehyde resin. The additions of bonding agent used in the experiments were of 3, 4, 5, and 6% dry substance of urea-formaldehyde resin.

The bending strength of the obtained boards was determined according to standard PN-EN 310:1994.

Photos of cross-sections of the flax boards were taken with a S-3400N Hitachi Scanning Electron Microscope (SEM).

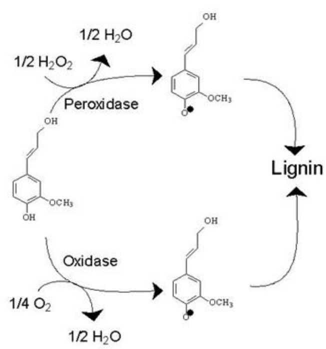


Figure 1: Enzymatic oxidation of phenol compounds

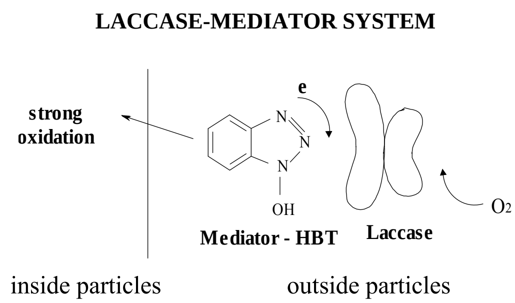
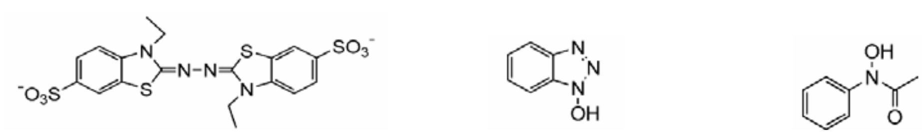


Figure 2: Role of mediator in the reaction of laccase with lignin



ABTS HBT NHA

Figure 3: Laccase mediators

RESULTS AND DISCUSSION

The results of the study showed that the lignocellulosic raw materials are susceptible to laccase, so that they may be possibly bonded into board composites.

Board composites bonded by laccase have better strength than those produced without using laccase, as the application of laccase mediators improves the enzymatic oxidation of lignin.

The best parameters for activating flax shives with laccase and for bonding them to obtain boards are as follows:[6]

- processing: pH 7.21 (phosphate buffer), temperature – 40 °C, time – 30 min, laccase – 12 LAMU/g d.s. (LAMU – the activity unit), NHA – 25 µM/g d.s.,
- pressing: temperature – 180 °C, time – 20 min, pressure – 2.5 MPa.

Figure 4 shows the measurement of oxygen consumption for the evaluation of the raw material oxidation process. The value of oxygen consumption is of 16%, which indicates that flax shives are easy to activate by laccase.[7]

The values of bending strength of the boards are presented in Figure 5, where the control sample was prepared without using laccase.

As a result, boards with lower amounts of synthetic resin were produced, yet with much better bending strength than the composites bonded only by laccase. It was found out that a higher bending strength was obtained when the flax shives were activated with laccase and NHA mediator, and also that the addition of bonding agent was of 6% dry substance of urea-formaldehyde resin. A clear advantage of obtaining boards from flax shives activated by laccase, comparatively with synthetic resins alone, is the reduced emission of toxic substance, *i.e.* formaldehyde.

Photos of cross-sections of flax boards are presented in Figure 6.

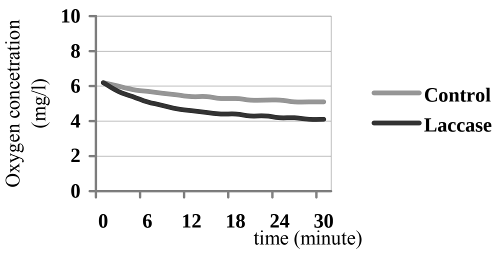


Figure 4: Oxygen consumption during flax shives activation with laccase

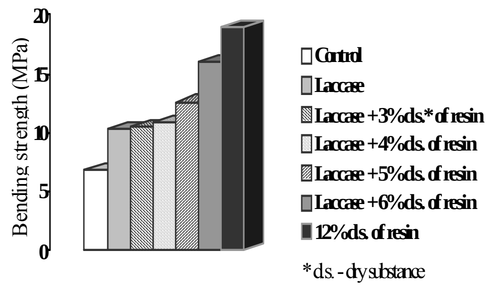
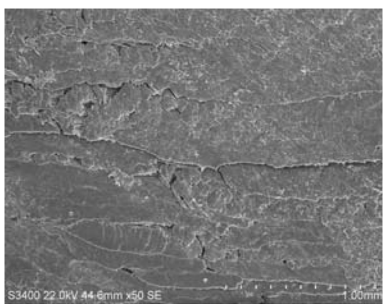
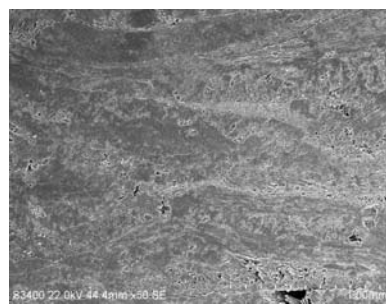


Figure 5: Bending strength of flax boards



Laccase



12% d.s. of resin

Figure 6: Cross-sections of flax boards (SEM)

CONCLUSIONS

1. The best method and conditions for activating flax shives with laccase involve the utilization of NHA as a mediator in a phosphate buffer pH of 7.21.
2. Oxygen consumption shows that flax shives are easy to activate by laccase.
3. A significant reduction in the amounts of synthetic bonding agents used for manufacturing board composites is possible by the application of laccase.
4. Bonding of lignocellulosic composites by laccase has a positive effect on the environment, *i.e.* it contributes to the reduction of formaldehyde emission during production and use.

REFERENCES

[1] S. Kawai, T. Umezawa, M. Shimada and T. Higuchi, *FEBS Lett.*, **236**, 309 (1988).
[2] H. Wariishi, N. Morohoshi and T. Haraguchi, *Mokuzai Gakkaishi*, **33**, 892 (1987).
[3] W. Bao, D. M. O'Malley, R. Whetten and R. R. Sederoff, *Science*, **260**, 672 (1992).
[4] D. M. O'Malley, R. Whetten, W. Bao, C. L. Chen and R. R. Sederoff, *Plant J.*, **4**, 751 (1993).
[5] H. P. Call and I. Mücke, *J. Biotechnol.*, **53**, 163 (1997).
[6] J. Batog, R. Kozlowski and A. Przepiera, *Mol. Cryst. Liq. Cryst.*, **484**, 35 (2008).
[7] J. Batog, R. Kozlowski and A. Przepiera, *Annals of Warsaw University of Life Sciences – SGGW, Forestry and Wood Technology*, **63**, 28 (2008).