EFFECT OF ENZYMATIC TREATMENT ON TOXICITY OF LIGNOCELLULOSIC COMPOSITES

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Abstract
The study on obtaining board composites using flax shives, laccase and its mediators was conducted at the Institute. The degree of raw material activation and bonding trials were evaluated by measuring the oxygen consumption during the activation process and the bending strength of the boards. The board composites have higher strength than in the absence of laccase and the application of laccase mediators improves the enzymatic oxidation of lignin. Recently, the study aimed at producing boards from flax shives after the activation process by laccase and with the use of lower amount of urea-formaldehyde resin. The bending strength and the formaldehyde content in the obtained boards were determined. A clear advantage of obtaining board composites from flax shives activated by laccase, compared with synthetic resins only, is the reduction in emission of formaldehyde.

1 Introduction
Currently wood industry is facing ever growing global competition, stringent regulations concerning environment protection and new market demands. The demand for wood derived boards has grown steadily over the last years due to the needs of its end users i.e. housing, furniture and floor industries. Synthetic resins used during their production, primarily urea formaldehyde (UF), melamine urea formaldehyde (MUF) and phenolic formaldehyde (PF) resins constitute about 10% of the board mass and come from non-renewable sources i.e. crude oil and gas. The use of these resins is linked with several negative effects – the change in availability and costs related to varying costs of the resources, formaldehyde emissions (carcinogenic for people) during production and use of the boards and limited possibility of reuse or combustion for obtaining energy [1].

Therefore, attention has been drawn to reducing the share of this type of bonding agents and search has been undertaken for new solutions in existing processing of wood both for cellulose and for wood derived products. The use of natural chemical reactivity of wood has become focus of research. Main wood components are cellulose, hemicellulose and lignin. The structure and chemical composition of wood indicates a few possible reactions, which should result in forming durable chemical bonds. Researchers started studying methods of activating wood for obtaining active functional groups. Most activating methods for wood involve subjecting it to action of oxidizing compounds such as ozone, hydrogen peroxide,
peroxyacetic acid, and nitric acid. The highest susceptibility to activating agents is proposed for lignin. This is linked to the fact that both in solid and shredded wood strongly lignified components of wood tissue (i.e. middle lamella and primary cell membrane) are the most easily accessible for external factors. Lignin is a polymer synthesized from three monomers – p-coumaryl, coniferyl and sinapyl alcohol (Figure 1).

![Figure 1. Lignin monomers - p-coumaryl, coniferyl and sinapyl alcohol.](image)

In lignin molecules, apart from hydroxyl groups, functional groups occur or may be formed – carbonyl and carboxyl groups, which under specific conditions are able to react creating chemical bonds of energy much higher than this of hydrogen bonds.

The most promising example of activation methods is enzymatic treatment of wood, which has high potential of creating specific reactions, enables to develop processes less harmful for the environment, and reducing resources consumption and finally reducing costs [2]. The action of enzymes on wood activates them in a similar way to the oxidizing compounds, but it is much milder and safer for the environment. Recently, a new generation of oxidizing enzymes has been introduced in commercial scale i.e. laccase and peroxidase.

Laccases are oxidoreductases that oxidize various aromatic compounds - phenols, diphenols, polyphenols, aminophenols, polyamides, using oxygen as electron acceptor and producing water as by-product [3]. The interest in these long-known enzymes (first described in the 19th century) has gradually grown because of their exceptional usefulness in numerous biotechnological applications. Laccases can show both lignolytic and polymerizing ability [4-5]. The substrate range for laccase can be widened with laccase mediator systems (LMS) and include both phenolic and non-phenolic compounds. In these systems the mediator is first oxidized by laccase and then it diffuses into the cell wall oxidizing the lignin inaccessible to laccase (Figure 2) [6]. Oxidation of non-phenolic lignin units by laccase, depending on the mediator, can occur after electron transfer, hydrogen atom of the radical, or as an effect of a ionic mechanism [7].

![Figure 2. Proposed role of mediator in reaction of laccase with lignin.](image)
High accessibility of laccases at moderate price and wide oxidizing abilities make them useful for a large number of applications [8-9]. Most published research and laccase applications in wood industry concern production of pulp and paper [10]. New studies deal with modification of lignocellulosic raw materials, which process can go in two directions – laccase enhanced biografting of phenols and other compounds and in situ crosslinking of lignin molecules by laccase [10-11].

Another laccase feature can be useful in modification of wood fibres, namely, its ability to form reactive radicals in lignin (Figure 3) [12-13].

This direction was assumed in a laboratory and pilot scale trials based on lignin treatment with enzymes in the production of fibreboards and MDF [14-18]. Basically, such a process makes use of lignin ability of forming phenoxy radicals, which couple when fibres are pressed into boards. Additional factors that can contribute to improvement of bonding can be molecular entanglement on the surface, condensation of hemicellulose degradation products and hydrogen bonds.

Moreover, the current trend towards using renewable resources causes that annual fibrous plants become used more and more often for the production of composite materials – i.e. known for centuries flax, hemp, jute and kenaf. These raw materials are characterized with desirable physical and mechanical properties – the shape and slenderness of molecules, mechanical strength and also chemical composition, which is similar to this of wood. Additionally, they are abundant, cheap and renewable [19].

2 Materials and testing methods

Having this in mind, the Institute has studied at a laboratory scale the method of enzymatic bonding of board composites from fibrous annual plants – flax shives (cellulose 35.4%, hemicellulose 25.1%, lignin 31%), with the use of laccase from Aspergillus sp. and its mediators - ABTS, HBT, NHA (Figure 4).

Activation mixtures, in addition to laccase and its mediators, may contain phosphate buffer of pH 7.21. Its role is to create the desired pH of the medium and improve the effects of raw material activation by laccase. Control samples were prepared without using laccase.

The study aimed at determining processing parameters of activation methods for raw materials and pressing parameters for bonding boards.
Enzymatic oxidation process of flax shives was studied by measuring the oxygen consumption during the process of raw material activation (we used oxygen meter Oxi 340i). The resistance to bending of the obtained boards was the determinant of mediator efficiency in processing of raw materials with laccase.

Recently, there is a possibility of significant reduction in use of synthetic bonding agents in manufacturing board composites. The study aimed at obtaining boards from flax shives after the activation process by laccase and with the use of lower amount of urea-formaldehyde resin. The degrees of bonding used in the experiments were 3, 4, 5, 6% dry substance of urea-formaldehyde resin. The bending strength of the obtained boards was determined (acc. to PN-EN 310:1994). The toxicity of the boards was based on determination of formaldehyde content (acc. to PN-EN 120:1994).

3 Results
It was found that lignocellullosic materials show susceptibility to the laccase action. The increase of bending strength was observed for the board samples with laccase treatment and the use of mediators – HBT and NHA enhanced the lignin oxidation process. The values of bending strength of boards are presented in Figure 5.

The best method and conditions of bonding flax shives with laccase are observed for using NHA mediator [20]. The parameters for activating and bonding to obtain boards are as follows:
- processing – pH 7.21 (phosphate buffer), temp. 40°C, time 30 min., laccase 12 LAMU/g d.s., NHA – 25 μM/g d.s.,
- pressing – temp. 180°C, time 20 min., pressure 2.5 MPa.
Figure 6 shows the results from the measurement of oxygen consumption for evaluation of raw material oxidation process. The value of oxygen consumption is 16% what indicates that flax shives are easy to activate by laccase [21].
The study showed that there is a possibility of significant reduction in use of urea-formaldehyde resin in manufacturing board composites by application of laccase. The values of bending strength of boards from flax shives after the activation process by laccase and with the use of lower amount of urea-formaldehyde resin are presented in Figure 7 [22].

It was found that the higher bending strength was obtained when flax shives were activated with the laccase and NHA mediator and the degree of bonding was 6% dry substance of urea-formaldehyde resin. Figure 8 shows the formaldehyde content in obtained boards.

A clear advantage of obtaining the boards from flax shives activated by laccase, in comparison with only synthetic resins, is the reduction in emission of formaldehyde.

4 Conclusions
The processing of flax shives with laccase results in significant increase of the bending strength, which is even higher after using laccase mediators: HBT and especially NHA. As a result of the study a possibility of significant reduction in use of synthetic bonding agent in manufacturing board composites has been created. Bonding of lignocellulosic composites by laccase has a positive effect on the environment by reduction of formaldehyde emission in production and use.
References


