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Enzymatic Modification of Lignocellulosic Substances for the Production of Fiberboards[1]

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Abstract—The object of the research is the development of enzyme systems for the enzymatic modification of wood fiber materials as well as fiber materials from annual plants for the production of glue-free fiberboards. The project is aimed first at the process development for the enzymatic modification and second at the development of cellulase/hemicellulase complexes on the basis of stillage as a substrate and inducer for the enzymatic modification. The results demonstrate that it is possible to substitute synthetic resins by means of activation and biocatalytic cross-linking of fibers with hydrolytic enzyme systems, in particular, for the production of medium-density fiberboards.

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INTRODUCTION

Fiberboards such as MDF (medium-density fiberboard), HDF (high-density fiberboard), and insulation materials are variously used in the furniture and packaging industry, in the construction industry, and in automotive manufacture. The production of MDF in Europe in 2005 rose around 13.7% to 13.5 million mi (EPF 2006). With a share of approximately 45%, most MDF was used in laminate flooring. The second largest customer is the furniture industry at 25%, followed by timber construction with approximately 11% of MDF consumption (MDF-Magazin 2006).

For the production of these materials, fibers are used that are manufactured by thermomechanical pulping of wood chips. In Europe, softwood is dominantly used as a raw material. In the wood pulp and paper industry, there are fears of increasing costs for raw materials. It could be interesting to explore new sources of raw materials, in particular, the use of agricultural residues, such as wheat straw and shives of flax and hemp. In addition, rising prices of synthetic bonding agents, as well as official restrictions on material emissions of volatile substances (known as VOCs), in particular, of formaldehyde, are leading to unfavorable market conditions [1].

This discussion is a chance for the development of emission-reduced products for the market. Demand already exists from large furniture manufacturers and the wood-based materials industry. Apart from this, there are political efforts for more economic independence from petrochemicals.

[1] The article is published in the original.

STATE OF THE ART CONCERNING THE ENZYMATIC MODIFICATION OF WOOD PULPS

Use of Phenoloxidases

In the past, the biotechnological activation of fiber materials with phenol-oxidizing enzymes (laccase, lignin peroxidase, manganese peroxidase) was examined in particular. The procedures dealt with the enzymatic activation of the lignin on the fiber surface by the creation of phenoxy radicals and their subsequent polymerization and use for fiber cross-linking [2].

So far, however, both the enzyme costs and the manufacturing procedures are still unsuitable for highly productive processes. The procedure has not yet attained any use in general practice. A substantial disadvantage for the use of these enzyme systems is their long incubation times (several hours) as well as the use of high moisture content (>50%) and enzyme dosages due to their small redox potential.

Use of Hydrolases (Cellulases/Hemicellulases)

On the basis of positive results with an increase in the tensile strength of test sheets made of thermomechanical pulp, tests were carried out on the use of hydrolytic enzymes for fiber incubation and the improvement of MDF properties [3, 4].

The incubation of softwood pulp with such enzymes led to the reactivation of the fiber surface with partial hydrolysis of the carbohydrates. With a lower dosage and an incubation time of 20 min, fiberboards with standard strength properties were produced.

Activities of the enzyme concentrates

Enzyme concentrate	Xylanase, IU/ml	Cellulase (FPA), IU/ml	Endoglucanase (Azo-CMC), U/ml
Xylanase from *T. reesei*	2.081	n.d.	58.8
Dyadic xylanase XL conc.	10.545	18.5	371
CelluPract AL 100	1.332	14.2	292
SIAB 03	6.725	93.8	289

Mechanisms of the Enzymatic Modification of Lignocellulosic Fibers with Hydrolytic Enzymes

The mechanism that leads to the increase in fiber binding strength during the enzymatic modification is still unknown. The hydrolases that are successful in enzymatic modification are in all cases enzyme complexes composed of various cellulase and hemicellulase components. Pure single-enzyme components were not found to be successful in any enzymatic modification.

Several unanswered questions remain:

Which enzymes of the complex are necessary?

Which enzyme is the "bottleneck" related to the modification?

What is the optimum composition of the enzyme complex?

How to assay the complex in a manner adapted to the application?

EXPERIMENTAL

Investigations into the Optimization of the Applied Enzyme Complex

The following investigations were carried out to learn more about the effect on the enzymatic modification:

Comparison of different enzyme complexes both with and without an effect on the modification of lignocellulosic fibers.

Investigations concerning the composition and the activity of enzyme components in those enzyme complexes.

Investigations into the adsorption of selected enzyme components into the lignocellulosic fiber material.

The enzyme concentrates investigated contain an abundance of different enzyme components that have not been examined in detail. At present, about 22 extracellular cellulase and hemicellulase components have been described in *Trichoderma reesei* [5]. Depending on what media and inducers are used for the cultivation of these fungi, the secreted enzyme complexes are different in composition. We investigated one enzyme (table, no. 1) with a reduced effect and three enzyme samples (table, nos. 2–4) with a good effect on the "binding ability." The cellulolytic and hemicellulolytic activities of the enzymes investigated are indicated in the table.

The analysis was performed via native PAGE at alkaline pH with 100 μg of protein per lane, followed by detection with 4-methylumbelliferyl-coupled substrates for the respective enzyme activities. In addition to endoglucanases and cellobiohydrolases, β-glucosidases that convert cellobiose to glucose are the last member of the cellulose degradation complex [6].

In all enzyme preparations, we were able to find β-glucosidases with differing degrees of activity. It was apparent that in the extract that is unsuitable for fiber modification there is somewhat more activity than in CelluPract AL 100. Apart from cellulolytic activities, these enzyme preparations have various activities for the degradation of hemicellulose. However, since hemi-

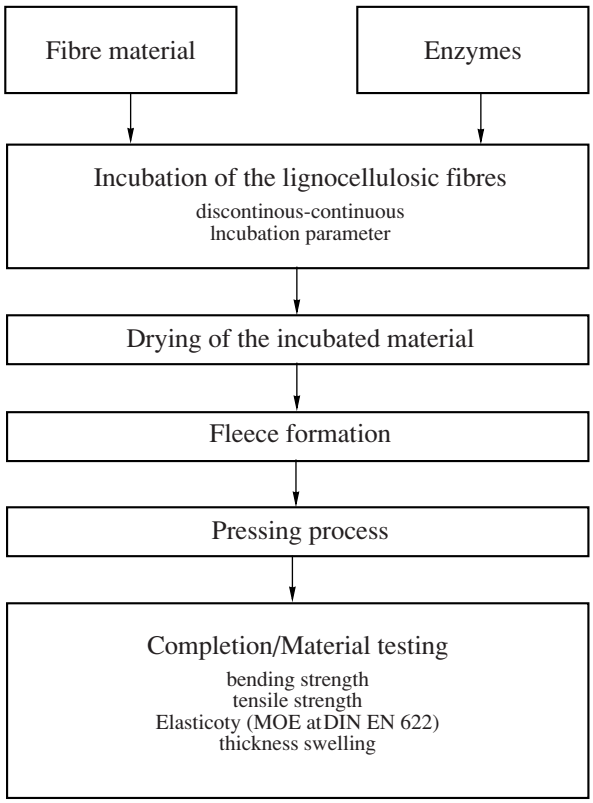

Fig. 1. Scheme of the procedures for the production of MDF by enzymatic modification.

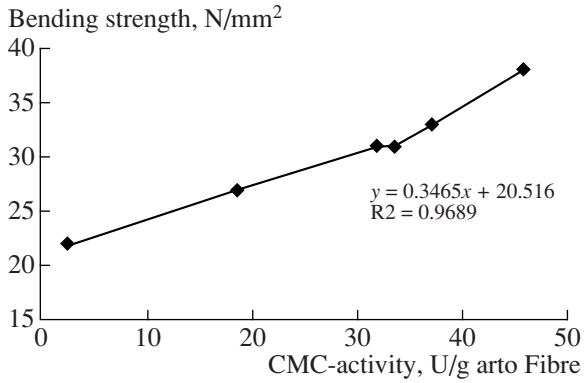

Fig. 2. Correlation between CMC activity and MDF bending strength.

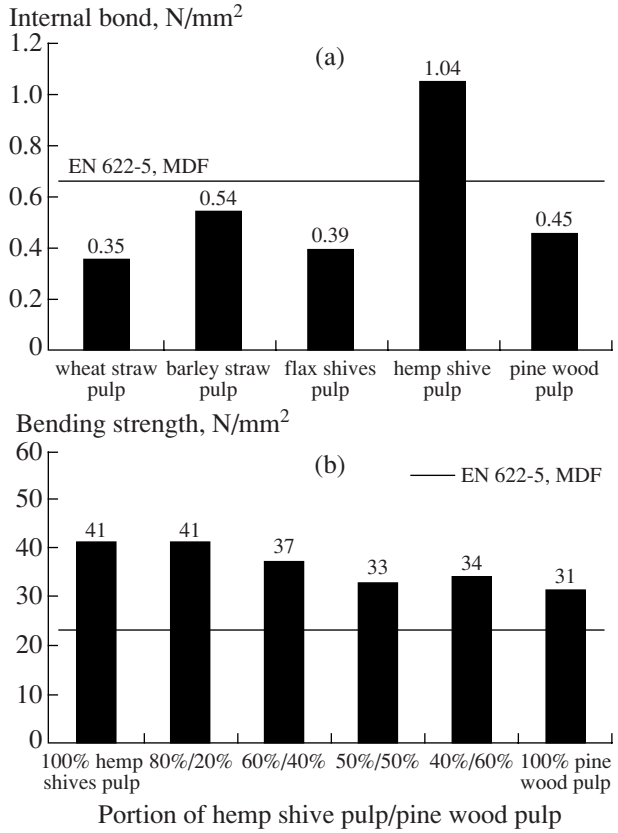

Fig. 3. Strength properties of MDF produced with incubated fibers of different sources.

cellulose is not as homogeneously developed as cellulose, its decomposition requires more heterogeneous enzymes such as xylanases, xylosidases, acetyl xylan esterases, glucuronidases, mannanases, arabinosidases, and others. During these investigations, only some of these activities could be tested because of the unavailability of specific substrates for a couple of enzymes. Endo-β-1.4 xylanase catalyzes the hydrolysis of the xylan backbone, while xylosidase promotes further degradation to xylose [7]. In all four concentrates, very high activities of endoxylanases could be detected. In three out of four samples, xylosidase activities are visible. In the same three samples, we could find α-galactosidase. The concentrate with the missing activities had quite a good effect on the bending strength of MDF boards. No mannosidase activity was provable in all four preparations and only a trace of α-*L*-arabinosidase activity was visible in the SIAB 03 preparation.

The result of these investigations is that the differences between the analyzed activities of these four enzyme concentrates could not explain the differences between their "activation" suitability. At present, only the endoglucanase activity measured by degradation of Azo-CMC substrate possibly correlates with the binding strength of MDF boards (Fig. 2).

Investigations into the Enzymatic Modification of Lignocellulosic Fibers from Annual and Perennial Plants for the Production of Building Materials

Different lignocellulosic plants, such as wheat and barley straw, flax and hemp shives, and pine wood chips, were used as raw material for the research. Fiber pulp was produced by thermomechanical refining. The fiber material was dried up to a moisture content of 4%.

A buffered enzyme solution was sprayed on the fibers. After an incubation period of 20 min, the fibers were formed into a mat and hot pressed to form MDF. The following material properties were examined:

Bending strength

Internal bond (IB)

Tensile strength

Modulus of elasticity (MOE)

Thickness swelling (24 h)

The scheme of the procedures for the production of MDF by enzymatic modification is shown in Fig. 1.

In an upscaling pilot-scale trial, incubated hemp and wood fibers were used for the production of door skins.

RESULTS AND DISCUSSION

The results achieved significant progress in the optimization of enzyme production and material production. The results of the fiber incubation proved that wheat bran enzymes, as well as those fermented with lactose or stillage substrate, are suitable for fiber incubation. Concerning the generated enzyme components, no disadvantages arise from the use of stillage, meaning that stillage provides an inexpensive substance. With the addition of induction additives, e.g., waste cellulose, the enzyme formation can be increased and the composition of the enzyme complexes can be purposefully varied.

In comparison of the individual activities, one especially significant point was an almost linear correlation

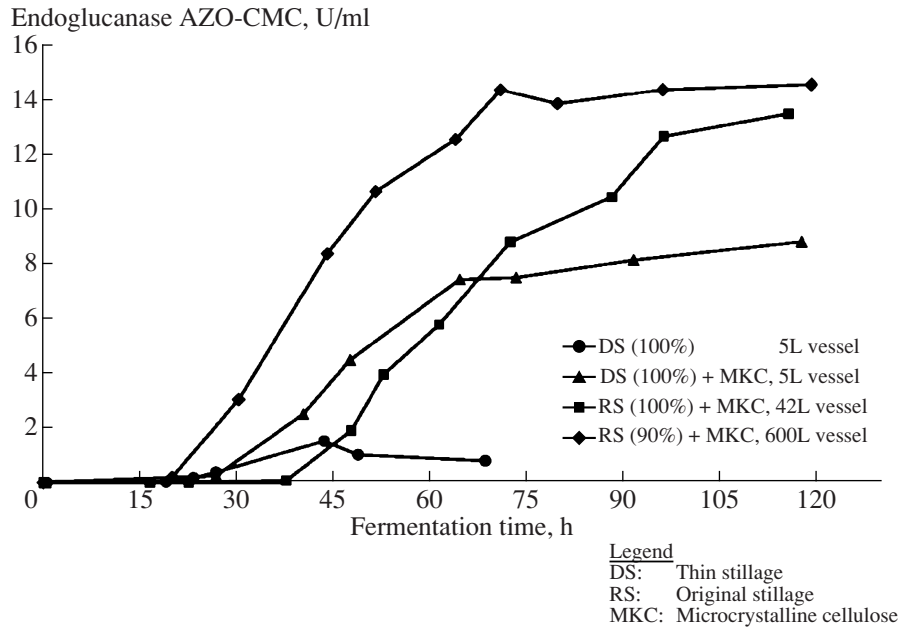

Fig. 4. Formation of endoglucanase on different stillage media.

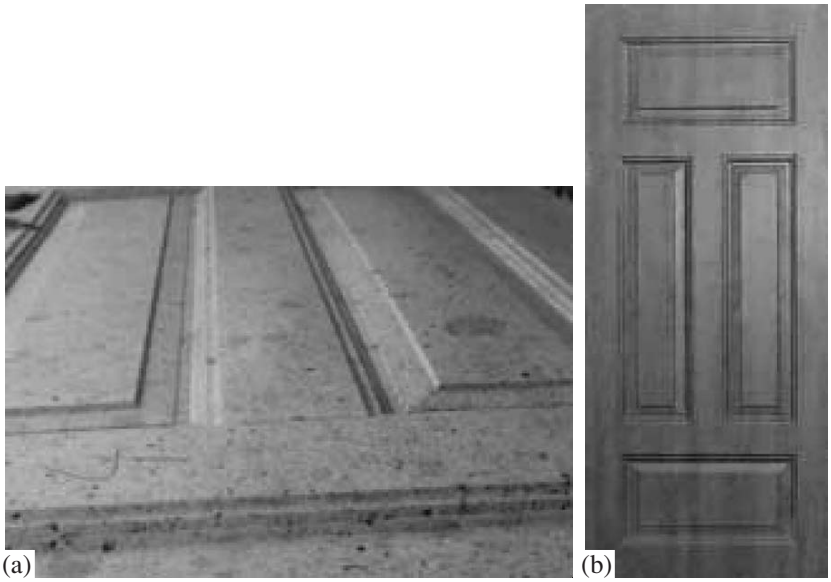

Fig. 5. Door skin and doors made of incubated hemp shive pulp.

between CMC activity and the bending strength of the MDF produced. Xylanase and filter paper activities did not show any significant influence. The correlation between CMC activity and MDF bending strength is shown in Fig. 2.

Altogether, it can be stated that enzymatic modification of lignocellulosic fibers from wheat and barley straw or flax or hemp shives with hydrolytic enzymes leads to improved material properties. In particular, it is shown that material quality standards can be obtained with an enzyme dosage of only 7–10% (related to dry fiber mass). The strength properties of MDF produced with incubated fibers of different sources are shown in Fig. 3.

However, the mechanical properties depend on the kind of fiber material. In particular, the use of fiber material from hemp shives leads to a better strength of glue-free MDF compared with pine wood fibers. These results were verified during upscaling trials in a plant for the production of door skins. Here, the enzyme

complexes developed by SIAB were proved suitable for the production of standard products.

For special applications in MDF production, such as door skins, the use of resins as binders exhibits disadvantages because of adhesion and impurities on the press plates. In such cases, the enzymatic modification already has a beneficial effect. However, the upscaling investigations are still at an early stage. The process parameters need further optimization. The efficiency of the enzymes has to be optimized and the use of hydrophobic agents should be tested. The integration of the new procedure into the traditional fiberboard production process should be possible without large capital expenditures.

Investigations into the Production of Enzyme Complexes up to Pilot Scale for the Enzymatic Modification of Lignocellulosic Materials

The investigations were aimed at the production of cellulase complexes different in composition and with a wide spectrum of hemicellulases. As substrates and inducers for the enzyme production, media based on thin stillage as well as native stillage from a distillery were used. To enhance the induction of cellulase and xylanase components, different waste celluloses were added to the medium. The formation of endoglucanase dependent on different stillage media is demonstrated in Fig. 4.

The used strain was a *T. reesei* mutant strain that exhibits switched-off carbon catabolite repression. Native undiluted stillage resulted in the highest enzyme activities. The comparison between thin stillage and native stillage shows that the latter is suitable for enzyme production and therefore promising as a substrate. The enzymes produced on native stillage with the addition of waste cellulose were used for the enzymatic modification of hemp fibers in an industrial plant (Fig. 5).

CONCLUSIONS

Stillage is a favored fermentation substrate for the production of xylanase /cellulase enzyme complexes. The enzymatic modification of wood fiber material, as well as lignocellulosic fiber materials from annual plants, using hydrolytic enzymes leads to improved material properties. The mechanical properties of the products depend on the kind of fiber materials. By comparing the activities of single enzymes, in particular, it was possible to prove an almost linear correlation of endoglucanase activity with MDF bending strength. The results were verified in an upscaling attempt for the production of door skins.

The suitability of the enzyme complexes for the production of standard door skins could be proved. For further optimization, the enzymatic modification of lignocellulosic fibers from different annual and perennial plants requires different enzyme complexes, which should be adapted in cellulase/hemicellulase composition to the substrate. The commercial realization of the enzymatic modification processes is substantially determined by the enzyme costs. The procedure is economical for special applications, but not yet for bulk products. The following methods look promising to reduce the enzyme costs:

Stillage (natural) as a cheap substrate/inducer for enzyme production.

Use of fermentation broth without expensive downstream processing.

Reduction of the enzyme dosage by substrate-adapted enzyme complexes.

Use of hot stillage coming from distillation as a fermentation substrate without additional sterilization.

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