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ФЕРМЕНТАТИВНАЯ МОДИФИКАЦИЯ ЛИГНОЦЕЛЛЮЛОЗНЫХ ВЕЩЕСТВ ДЛЯ ПРОИЗВОДСТВА ВОЛОКОННЫХ ПЛИТ

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Цель исследования – разработка ферментных систем для модификации древесно-волоконных материалов, а также материалов на основе однолетних растений для производства бесклеевых волоконных плит. Проект направлен, во-первых, на разработку ферментативного процесса модификации и, во-вторых, на разработку целлюлазно-гемицеллюлозных комплексов на основе барды после производства спирта в качестве субстрата и индуктора. Полученные результаты показывают, что возможно заменить синтетические смолы на систему гидролитических ферментов, которая активирует и поперечно сшивает волокна, в частности при производстве волоконных плит средней плотности.

Introduction

Fibre boards such as MDF (Medium Density Fibreboard), HDF (High Density Fibreboard) 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 approx. 45 %, most MDF was used in laminate flooring. The second largest customer is the furniture industry at 25 %, followed by timber construction with approx. 11 % of MDF consumption (MDF-Magazin 2006).

For the production of these materials, fibres are used which are manufactured by thermo-mechanical pulping (TMP) 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 unfavourable 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 these, there are political efforts for more economic independence from petrochemicals.

State of the art concerning the enzymatic modification of wood PULPS

Use of Phenoloxidases

In the past, the biotechnological activation of the fibre 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 fibre surface by the creation of phenoxy radicals and subsequent polymerisation and their use for fibre 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 wet content (>50%) and enzyme dosages due to their small redox potential.

Use of Hydrolases (Cellulases/Hemicellulases)

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

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

Mechanisms of the enzymatic modification of lignocellulosic fibres with hydrolytic enzymes

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

There are still unanswered questions on the following points:

Which enzymes of the complex are necessary?

Which enzyme is the "bottle neck", 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?

Experiments

Investigations into the optimisation of the applied enzyme complex

The following investigations were made to get knowledge concerning the effect on the enzymatic modification.

Comparison of different enzyme complexes both with and without effect on the modification of lignocellulosic fibres

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

Investigation into the adsorption of selected enzyme components into the lignocellulosic fibre material.

The enzyme concentrates investigated contain an abundance of different enzyme components which have not been examined in detail. Up to now, 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 excreted enzyme complexes are different in composition. We investigated one enzyme (No. 1 in table 1) with reduced effect and three enzyme samples (No. 2–4 in table 1) with good effect on "the binding ability". The cellulolytic and hemicellulolytic activities of the enzymes investigated are indicated in

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. Beside endoglucanases and cellobiohydrolases, β-glucosidases that convert the cellobiose to glucose are the last member of the cellulose degradation complex [6].

In all enzyme preparation we were able to find β-glucosidases with differing degrees of activity. It was apparent that in the extract which is unsuitable for the fibre modification there is somewhat more activity than in Cellupract AL 100. Apart from cellulolytic activities, these enzyme preparations contain various activities for the degradation of hemicellulose. However, since hemicellulose is not as homogeneously developed as the cellulose, its decomposition **requires** more **heterogeneous** enzymes such as xylanases, xylosidases, acetyl xylan esterases, glucuronosidases, 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-beta-1.4 xylanase catalyzes the hydrolysis of the xylan backbone, while the в xylosidase promotes the further degradation to xylose [7]. In all four concentrates very high activities of the endoxylanases could be detected. In 3 out of 4 samples xylosidase activities are visible. In the same 3 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 alpha-L-arabinosidase activity was visible in the preparation SIAB 03.

The result of these investigations is that the differences between the analyzed activities of these four enzyme

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

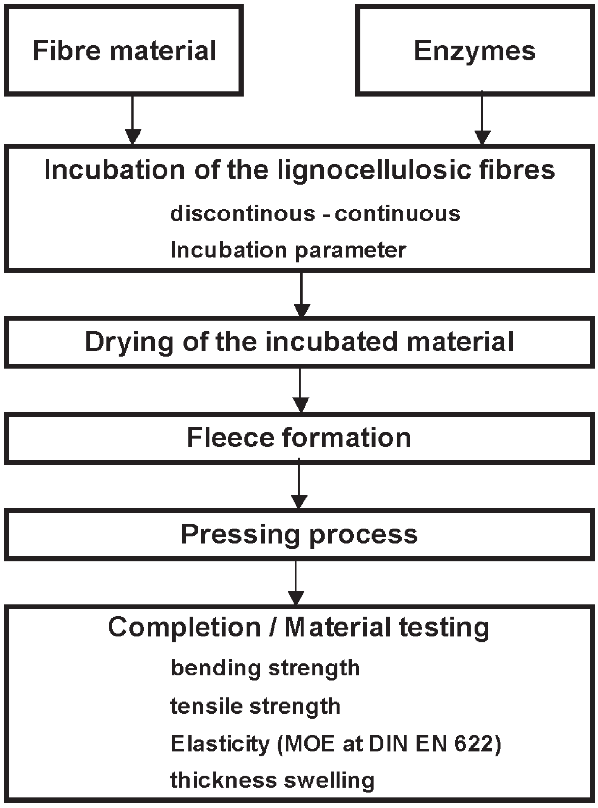

Fig. 1. Scheme of the procedures for the production of MDF by enzymatic modification

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 (see Fig. 2).

Investigations into the enzymatic modification of lignocellulosic fibres 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. Fibre pulp was produced by thermo-mechanical refining. The fibre material was dried up to a wet content of 4 %.

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

Bending strength
Internal bond (IB)
Tensile strength
MOE (Modulus of elasticity)
24 h thickness swelling

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

In an up-scaling pilot-scale trial, incubated hemp and wood fibres 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 fibre incubation proved that wheat bran enzymes, as well as those fermented with lactose or stillage subtrate, are suitable for fibre 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.

With comparison of the individual activities, one especially significant point was an almost linear correlation between CMC activity and the bending strength of the MDF produced. Xylanase and filter paper activities did not show any significant influence. Correlation between CMC activity and bending strength of MDF is shown at Fig. 2.

Altogether, it can be stated that an enzymatic modification of lignocellulose fibres from wheat and barley straw, 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 % to 10 % (related to dry fibre mass). Strength properties of MDF produced with incubated fibres of different sources are shown at Fig. 3.

However, the mechanical properties depend on the kind of fibre material. In particular, the use of fibre material from hemp shives leads to a better strength of glue-free MDF compared with pine wood fibres. These results were verified during up-scaling trials in a plant for the production of door skins. Here, the enzyme complexes developed by the SIAB were proved suitable for the production of standard products.

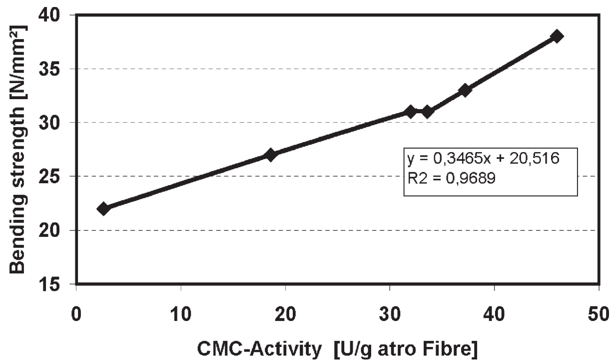

Fig. 2. Correlation between CMC activity and bending strength of MDF

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 up-scaling 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 agent should be tested. The integration of the new procedure into the traditional fibre board production process should be possible without large capital expenditure.

Investigations into the production of enzyme complexes up to pilot scale for the enzymatic modification of lignocellulosic materials

The investigations aimed at the production of cellulase complexes different in composition and with a wide spectrum of hemicellulases. As substrate and inducer for the enzyme production media on basis of thin stillage as well as native stillage from the 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 which exhibits a switched-off carbon-catabolite repression. Native undiluted stillage resulted in highest enzyme activities. The comparison between thin stillage and native stillage shows, that the last one is suitable for the enzyme production and therefore promising as substrate. The enzymes produced on native stillage with addition of waste cellulose were used for the enzymatic modification of hemp fibres in an industrial plant (Fig. 5).

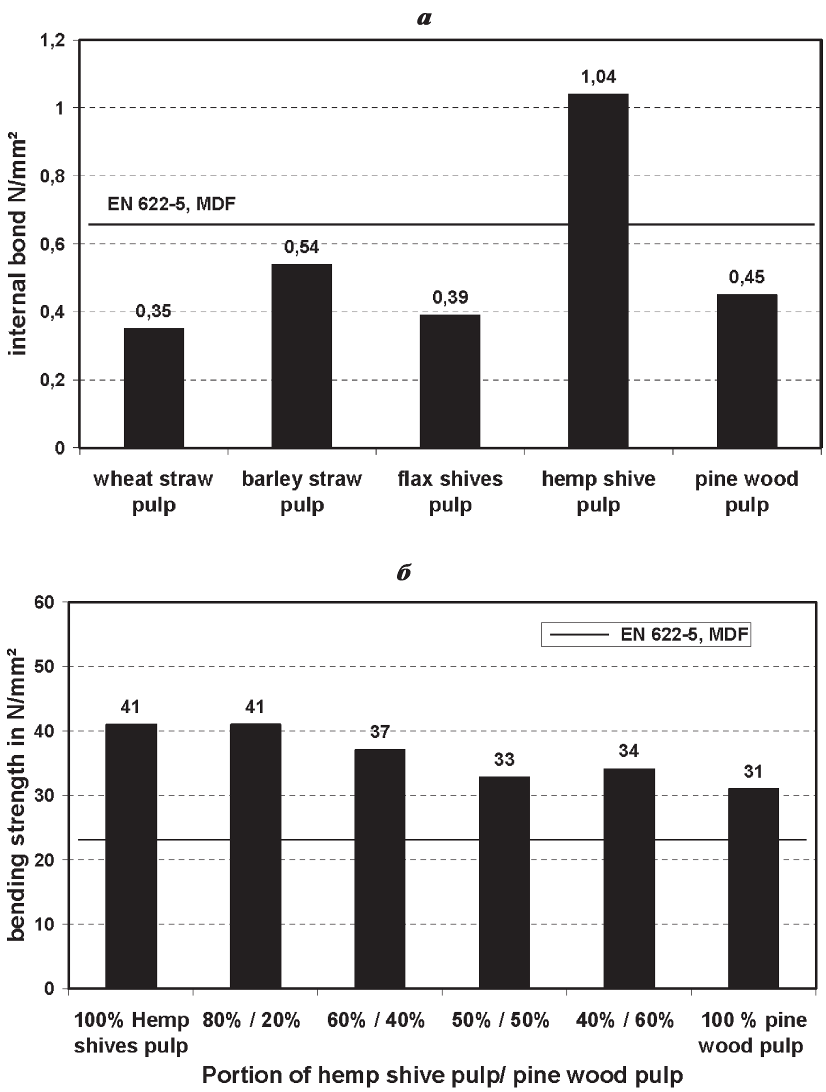

Fig. 3. Strength properties of MDF produced with incubated fibres of different sources

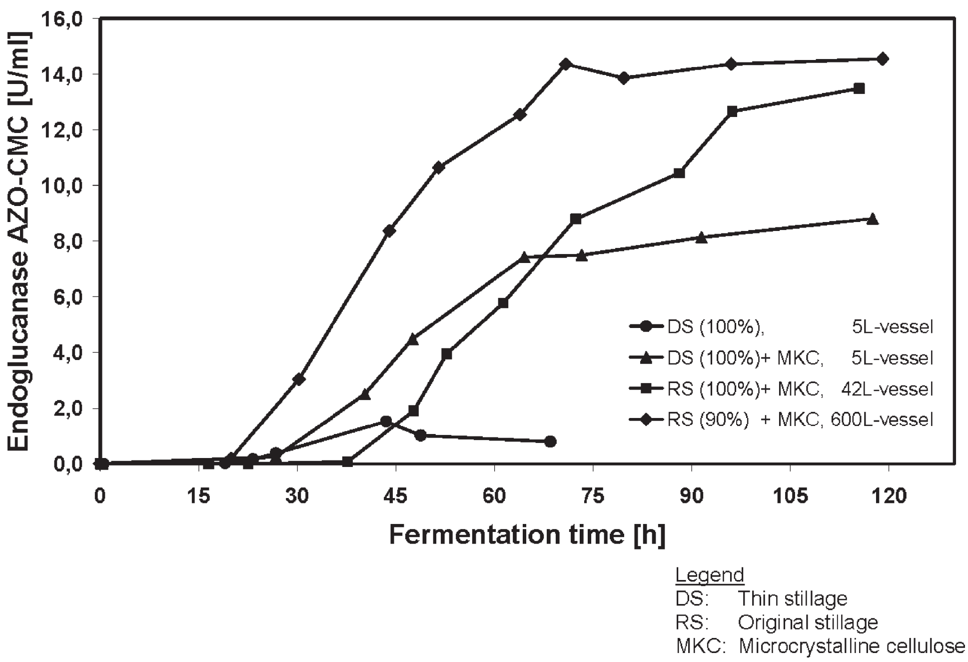

Fig. 4. Formation of endoglucanase on different stillage-medium

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

Conclusions

Stillage is a favoured fermentation substrate for the production of xylanase /cellulase enzyme complexes. The enzymatic modification of wood fibre material as well as lignocellulosic fibre materials from annual plants using hydrolytic enzymes leads to improved material properties. The mechanical properties of the products depend on the kind of fibre materials. By comparing the single enzyme activities, in particular, it was possible to prove an almost linear correlation of the endoglucanase activity with the bending strength of MDF. The results were verified in an up-scaling 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 fibres from different annual and perennial plants requires different enzyme complexes which should be adapted in cellulase/ hemicellulase composition to the substrate. The commercial realisation 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 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 the distillation as fermentation substrate without additional sterilization.

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ENZYMATIC MODIFICATION OF LIGNOCELLULOSIC SUBSTANCES FOR THE PRODUCTION OF FIBRE BOARDS

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The object of the research is the development of enzyme systems for the enzymatic modification of wood fibre materials as well as fibre materials from annual plants for the production of glue-free fibre boards. The project is aimed first at the process development for the enzymatic modification and secondly 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 bio-catalytic cross-linking of the fibres with hydrolytic enzyme systems, in particular for the production of medium-density fibre boards.