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Application of *Phanerochaete chrysosporium 1038* – Enzyme Complex and Laccase in Biobleaching of Flax Fibers

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Summary: Bleaching processes in textile industry require to keep fibers tenacity, partially to preserve the pectin and reducing the lignin content, that gives color to row flax fibers. The use of lignocellulose-degrading enzymes from basidiomycete *Phanerochaete chrysosporium 1038* strain and pure Laccase from Biocatalyst in flax fibers treatment was studied. The whiteness of enzymatically-processed fibers was significantly improved and the residual quantity of nondegraded lignin was less than obtained with chemical processing. The structural changes in the flax fibers during enzyme treatment were determined with IR spectroscopy, which confirmed the lignin degradation.

Keywords: Laccase, *Phanerochaete chrysosporium 1038*, Flax Fibres.

1. INTRODUCTION

The major chemical ingredients of row flax fibres are cellulose, pectin and lignin. It is well known that pectin keeps together flax cells that form the row fibre. The "adhesive" between these cells contains lignin that gives the specific braun-gray colour of flax. In order to bleach the flax and to keep the fibre tenacity high enough it is necessary to remove the lignin and partially to preserve the pectin. The problem of the classical treatment with alkalis and oxidizers is that these chemicals can hydrolyse not only the pectin and the lignin but the cellulose itself and to decrease drastically the material strength. Research on lignin biodegradation has become of great interest because lignin is the most abundant renewable material next to cellulose. Lignin is a highly branched and heterogeneous three-dimensional structure made up of phenylpropanoid units, which are interlinked through a variety of different bonds. Due to this natural polymer is poorly biodegraded [3].

Therefore studies of lignin biodegradation have been carried out mostly using white-rot fungi, which produce extracellular lignin-modifying enzymes such as laccases and peroxidases (lignin peroxidases and manganese paroxidases) [8, 9, 11]. One application of white-rot fungi and their oxidative enzymes is in biobleaching and biopulping in the pulp and paper industry, where they can replace environmentally harmful chemicals as well as saving mechanical pulping energy cost biotransformation and bioremedation.

Laccases are blue copper oxidases that catalyse the one-electron oxidation of phenolic and other electron-rich substrates. Reaction catalyse by laccase are the cleavage of alkyl-phenyl, Cα - Cβ bonds and phenolyc lignin dimers; demethoxylation and depolymerization [2].

The relationship between lignin molecule degradation and decoloriztion of linen will be studied. In vivo delignification experiments will run in parallel to physiological studies in order to investigate the role of physiological factors which affect the lignilytic activities of enzymes [7, 12].

The objective of this work is to study the possibility of flax fibers bleaching by pure laccase and enzyme complex from *Phanerochaete chrysosporium 1038* combined with peroxide treatment aimed to obtaining fibers with high whiteness and relatively well preserved tenacity.

2. MATERIALS AND METHODS

Strain and Media

The strain *Phanerochaete chrysosporium* has been deposited in the National Bank of Industrial Microorganisms and Cell Cultures, Bulgaria as a strain N1038, was used in the experiment. The cultivation of *Ph. chrysosporium 1038* was carried out in a medium as described in [6]. The sterile glucose solution (20 $g.dm^{-3}$) was sterilized separately and was added to the growth medium. All chemicals were supplied by Merck (Germany).

Cultivation conditions

The fungus strain *Phanerochaete chrysosporium 1038* was cultivated (as a batch culture) on a rotary shaker at 140 rpm for 120 h at 30°C.

To enhance production of ligninolytic enzymes in the extracellular fluid, veratryl alcohol (2.5 mM) [10] were added respectively 48 hours after the start point of cultivation. At the end of cultivation the extracellular culture liquid (CL) was separated by centrifugation at 4000 rpm and concentrated (30 times) by ultrafiltration using a YM10 (YM 10, Amicon Corp.,) membrane. Further, the concentrated solution was dialysed against 5 mM sodium tartaric buffer (pH = 4.5) for 24 h at 4°C [14].

Enzymatic treatment
Unbleached flax fibers from "Rylski len-AD" Bulgaria were placed in a vessel, containing CL in bath ratio 1:20. The samples were treated at different temperatures for 1-48 h.

Separately a samples, treated with CL from *Ph. chrysosporium 1038*, flax fibers were bleached in the range of 30-60°C for 1-5 h with 0.1g/l laccase (EC 1.10.3.2 *Trametes sp*. Laccase L603P from Biocatalyst; 0.125 g protein per g solid) in 100 mmol Na-acetate/acetic acid buffer pH = 5.0. All samples were bleached with 1% H_2O_2 (o.w.f.) at 80°C for 15 min. in bath ratio 1:10 without shaking. Some samples were treated with H_2O_2 at 80°C for 1 h (like references).

Analytical measurements
The bleaching effect of the treatments was measured by Datacolor equipment and CIELab parameters lightness (L) evaluate the effectiveness of enzyme treatment. Untreated flax fibers were used as a reference sample.

Activity of Laccase is defined as μmol of substrate (0.098 mmol guaiacol) oxidized by 0.002 g laccase l^{-1} in 0.05 M Na-acetate/acetic acid buffer pH 5 per min. Laccase activity was assayed spectrophotometrically [5] by measuring the increase of the absorbance at 465 nm ($\varepsilon_{465} = 26.6 \times 10^4$ $mol^{-1}cm^{-1}$).

The enzyme activity of ligninolytic enzymes in CL was determined as described in [4] and the total activity was 195 U/mg.
FTIR spectra were recorded on a Perkin-Elmer Spectrum 1000 FTIR spectrophotometer, resolution 2 cm^{-1}. All spectra were normalised between absorbances of 0 and 1 in the region of 1710-1600 cm^{-1}.

Each spectrum was analysed by second derivatization and curve fitting. The spectra were transferred to a personal computer and subjected to a line shape analysis by the Grams-software [1].

χ – Kappa Number assay

The content of lignin in samples studied was characterized by χ – Kappa Number assay. The flax fibers (1g each) were placed in a vessel containing 500 cm^3 distilled water at 25°C with careful stirring to which 100 cm^3 0.1M $KmnO_4$ and 100 cm^3 4N H_2SO_4 were added. The stirring duration was 10 min. Then 20 cm^3 0.1N KI were added to the solution and was titrated with 0.2N $Na_2S_2O_3$. The Kappa number was measured by SCAN method (SCAN C 1:00) [13].

3. RESULTS AND DISCUSSION

The experiment involved a treatment of flax fibers with CL, obtained from *Ph. chrysosporium 1038* and pure laccase at different temperatures. After that, all samples were bleached with H_2O_2. Colorimetric results, indicating efficiency in increasing the whiteness of fibers, are shown on Fig. 1. The maximum value of lightness is by temperature 40°C for pure laccase (Fig. 1a). The lightness of flax fibers, treated with CL showed maximum value by temperature 37°C, which is optimal for the investigated fungus strain (Fig. 1b). For the samples treated with a combination of enzyme and sodium peroxide bleaching, the same dependence could be observed.

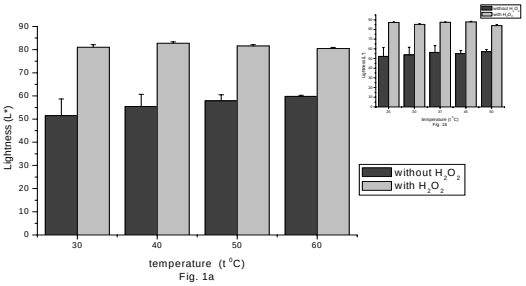

Fig. 1. The degree of lightness of treated fibers with Laccase (Fig. 1a) and *Ph. chrysosporium* (Fig. 1b).

On the Fig. 2 is detected the effect of temperature on the laccase activity from pure enzyme. It can be seen the maximal enzyme activity was found at 40°C.

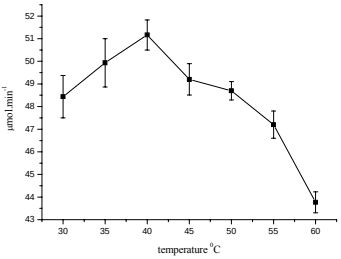

Fig. 2. Temperature dependence of laccase activity

Another important parameter for the study process is Kappa-number which evaluate the quality of residual bound lignin in the samples (Fig. 3a and Fig. 3b). The lowest quantity of residual lignin was found after 5 h of treatment. However the highest degree of lightness in all treatments was found after 24 hours of reaction. The reason for this difference can be the process of dessorption of degraded lignin fragments which still possess phenolic nature.

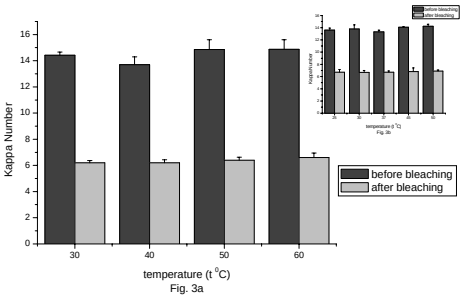

Fig. 3. Change of lignin content in flax fibers through Kappa Number with Laccase (Fig. 3a) and *Ph. chrysosporium 1038* (Fig. 3b). Reference – untreated – Kappa = 15.23; target sample - H_2O_2 bleached (80°C/1h; 1.5g/l); Kappa = 5.5

The FTIR of flax fibers treated with laccase showed the following key peaks (Fig. 4). The peak at 1158 cm^{-1} shows decrease of phenolic groups. The signals for aromatic rings also decrease (peak 1603 cm^{-1}). The differences were observed by peak 1714 cm^{-1} - aliphatic carbonyl bending lowered, but some increase of a new aromatic carbonyl groups was found at peak 1670 cm^{-1}. The structural changes in the flax fibers during enzyme treatment, determined with FTIR spectroscopy, confirmed the lignin degradation.

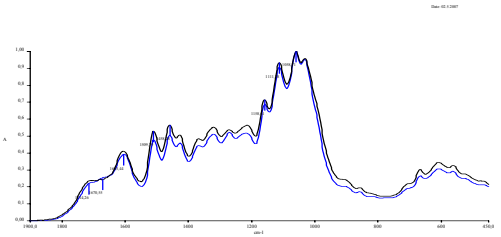

Fig. 4. FTIR spectrum of flax fibers treated with laccase

4. CONCLUSIONS

The results clearly indicated that the application of enzyme complex obtained after cultivation of *Phanerochaete chrysosporium 1038* for bleaching of flax fibers, can be enough efficient to compete with classic hydrogen peroxide bleaching method or more efficient and modern but expensive method with pure laccase treatment. The structural changes in the flax fibers during enzyme treatment with laccase, determined with FTIR spectroscopy, confirmed the lignin degradation.

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