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THE USE OF VARIOUS YEAST STRAINS FOR REMOVAL OF PINE WOOD EXTRACTIVE CONSTITUENTS

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ABSTRACT

The yeast strains *Trichosporon pullulans, Cryptococcus albidus*, *Sporobolomyces salmonicolor*, and *Debaryomyces occidentalis* var. *occidentalis* isolated from plant material were cultured in a medium containing pine sawdust. Their ability to degrade extractives from Pinus sylvestris was examined by HPLC analysis and FTIR spectroscopy of acetone extracts obtained by the extraction of pine wood before and after yeast treatment. According to the obtained results, it was clearly demonstrated that the used yeast microorganisms remove about 60 % of wood extractives, mainly fatty and resin acids, steryl esters and triglycerides. The novel biotechnological method for pretreatment of wood by using yeasts resulting in reduction of lipophilic extractives could increase pulp quality and decrease effluent toxicity as well as minimize troubles caused by resins in pulp production.

KEY WORDS: pinewood, extractives, yeast microorganisms, FTIR, HPLC

INTRODUCTION

The content of extractives in most wood is very low, usually under 5%, in comparison with that of the main component cellulose, lignin, and hemicellulose. The extractives are lower molecular compounds, distributed in the lumen or specific tissues such as resin canal, and do not combine with the components constructing cell walls (Hon and Shiraishi 2001). The main types of extractives are resins and fats (fatty acid, ester type and resin acid, free COOH) and polyphenols (glycoside reductive type aglycone oxidative type).

Extractives and especially resin and fatty acids can cause pitch problems in paper making and increase toxicity of the waste water in pulp mills. Traditionally, pich deposits in pulping processes have been reduced by debarking and seasoning logs and wood chips and by adding pitch control agents (Allen et al. 1991). As an alternative to the natural biodegradation of wood extractives, the biological control of pitch problems by treatment with different microorganisms have been suggested in recent years. A biotechnological approach is based on the use of wood-

inhabiting fungi to degrade wood lipophilic compounds (Farrell et al. 1993). Some studies reported several fungal strains (i.e. *Trametes versicolor*) which were very efficient to remove softwood extractives and also detoxified the effluents from mechanical pulping of softwood (Wang et al. 1995, Bertaud et al. 2002).

One of the most obvious application of white rot fungi is in biobleaching and biopulping in the pulp and paper industry to replace environmentally unfriendly chemicals. In our previous research some yeast organisms as *Sporobolomyces roseus* were used for wood pretreatment in order to modify the lignin wood component (Košíková and Sláviková 2004). In this work four yeast strains were examined from the viewpoint of their ability to remove extractives from *Pinus sylvestris* before pulping.

MATERIALS AND METHODS

Cultivation and yeast microorganisms

The yeast strains: *Trichosporon pullulans* 30-1-10, *Debaryomyces occidentalis* var. *occidentalis* 47-1-15, *Sporobolomyces salmonicolor* 19-4-21 and *Cryptococcus albidus* 17-4-38, and were maintained on malt agar slants at 5° C in the Culture Collection of Yeasts, Institute of Chemistry, Slovak Academy of Sciences, Bratislava. They were cultured in a medium containing 6.7 g Yeast Nitrogen Base (Difco), and pine sawdust (2 g) per liter of solution in distilled water. The pH was adjusted to 6.5. The medium was sterilized by autoclaving at 121 °C for 15 min. The strains were cultivated in flasks (500 ml) containing 250 ml of medium. The inoculation was performed with 4 ml of cells suspension (10^6 cells per ml). Incubation was proceeded on rotary shakers at 28 °C for 28 days. All experiments were done in triplicate.

The growth of the yeasts was determined by the nitrogen content of dry solid product separated from the cultivation medium by centrifugation. Protein was calculated from the nitrogen content (% N x 6.25) assayed on an elemental analyser (Model 240 Perkin-Elmer).

Extraction

The pine wood samples before and after treatment with yeast strains were extracted in Soxhlet with acetone for 6 hour.

FTIR spectroscopy

The IR measurements were performed with FTIR spectrometer NICOLET Magna 750 operating at 4 cm resolution using KBr pellets.

HPLC analysis

The extracted compounds were analyzed by HPLC using gradient elution in combination with a 35°C thermostated LiChrospher 100 RP-18 column with DAD detection. Analysis of the crude extract after filtration starting with 3 minutes solvent A (MeCN : H_2O : HOAc = 90:10:0.1) followed by a linear gradient in 15 minutes to solvent B (MeCN : MeOH : CH_2Cl_2 = 60:8:40).

RESULTS AND DISCUSSION

A new biotechnological approach of this work is based on the use of yeast strains to degrade wood lipophilic compounds. The effect of different yeast microorganisms (Table 1) on removal lipophilic extractives from pine wood sawdust was examined. Biological treatment was performed by inoculating of pine sawdust in cultural medium for 28 days without glucose because it was revealed in our previous paper (Košíková and Sláviková 2004) that the effect of yeasts on modification of lignin was more significant in the absence of carbon source. The yeast biomass was obtained from the cultivation medium by centrifugation together with the sawdust due to low size of wood particles (under 0.35 mm). Therefore, the amount of yeast biomass was determined based on the nitrogen content of dry solid product separated from the cultivation medium. All experiments were repeated three times. The differences in the determined values did not exceed 5 %. The treatment of sawdust without yeasts had no influence on the content of extractives during cultivation process. Tab. 1 summarized the data concerning the biomass production as well as the amount of removed extractives by various yeast microorganisms. The observed growth of biomass was relatively low. It ranged from 0.72 to 2.56 g/L.

Tab. 1: Evaluation of lignin effect on the growth of biomass and removal of extractives from pine wood

Species	Biomass g/L	Residual extractives %
Trichosporon pullulans	1.15	40.81
Debaryomyces occidentalis var. *occidentalis*	2.56	40.54
Sporobolomyces salmonicolor	0.72	39.19
Cryptococcus albidus	0.96	37.03

The values of the determined extractives from the yeast-treated wood were corrected for the yeast of biomass (Tab. 1). The obtained results clearly demonstrated that a yeast treatment of pine sawdust results in the significant reduction of lipophilic wood extractives. All used yeast strains removed approximately 60 % of extractives from pinewood although the differences in the yield of biomass were observed. This could be explain by the different ability of the individual yeast strains to remove the extractives.

The corresponding acetone extracts of the yeast-treated wood samples were characterized by FTIR spectroscopy. The spectra of acetone extracts from native pine wood and those obtained by extraction of wood samples after cultivation with various yeast strains are illustrated in Fig. 1. The assignment of the individual absorption bands in the spectra was performed by comparison with the spectra of models (Fig. 2) for different types of extractives: palmitic acid

(resin acid), cholestyroleat (steryl esters) and stigmasterol (sterols). Absorption bands at 3400 cm^{-1} correspond to δ OH aliphatic carboxyl acids; at 2927 and 2854 cm^{-1} asymmetric and symmetric C-H stretching, respectively; 1743 cm^{-1} C=O stretching vibration of esters and 1700 cm^{-1} C=O stretching vibration of acids; 1460 and 1382 cm^{-1} $-CH_2$ and $-CH_3$ deformation; 1163 the C-O frequency from ester groups; 1159 and 960 cm^{-1} C-O stretching of ester groups trans and cis; 840 and 700 cm^{-1} alkene cis isomers; 824 cm^{-1} C-H bending vibration. The comparison of the spectra of acetone extracts from yeast treated wood with those shown in Fig. 2, corresponding to extractive model compounds allowed to characterize the structural changes of extractives during biological treatment.

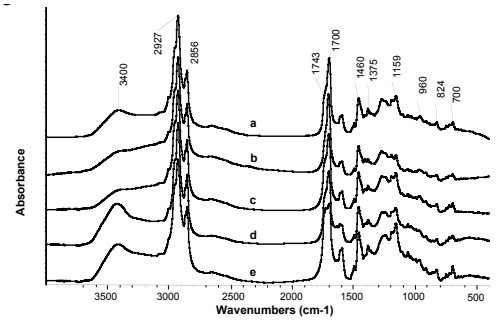

Fig. 1: FTIR spectra of the extractives from pine wood
a – acetone extract of native wood
b – acetone extract of wood treated with T. pullulans
c – acetone extract of wood treated with D. occidentalis var. occidentalis
d – acetone extract of wood treated with Cr. albidus
e – acetone extract of wood treated with S. salmonicolor

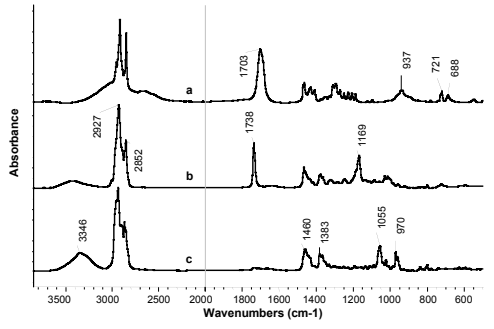

Fig. 2: FTIR spectra of extractive model compounds
a – palmitic acid
b – cholestyroleat
c – stigmasterols

The differences between the spectrum of acetone extract from native wood and those of yeast treated wood samples show that tested microorganisms contribute to significant degradation of extractives as it results from decrease of the absorption bands in the region 1650-1800 cm^{-1}. The obtained results indicate the high efficiency of used strains from the viewpoint of fatty and resin acids degradation as well as steryl esters and triglycerides removal. The decrease in sterols was not so evident.

In addition, the acetone extracts of native and yeast-treated pine wood were analyzed and quantified by HPLC. The values summarized in Tab. 2 show that the used strains decrease significantly the content of extractives in pinewood. This finding is in agreement with the results obtained by FTIR analysis.

Tab. 2: HPLC analysis of an acetone extract of native pine wood and after a four week treatment with yeast strains (mg/g wood)

Wood sample	Fatty and resin acids	Sterols	Steryl esters	Triglycerides	Total extractives
Native wood	24.99	0.43	2.17	0.46	28.05
Wood treated with *Trichosporon pullulans*	6.56	0.40	0.34	-	7.30
Wood treated with *Debaryomyces occidentalis* var. *occidentalis*	5.42	0.43	0.16	-	6.01

CONCLUSIONS

Four different yeast strains were used for the elimination of apolar extractives in *Pinus sylvestris*. According to the obtained results, it was clearly demonstrated that the used microorganisms were able to remove about 60 % of wood extractives. FTIR and HPLC analyses of acetone extractives isolated from yeast-treated wood samples confirm the partial degradation of resin and fatty acids as well as steryl esters during biological treatment. This novel biological method based on the application of the selected yeast strains was shown to be very effective for the removal of lipophilic extractives from wood.

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