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Effects of Fungal and Enzymatic Treatments on Isolated Lignins and on Pulp Bleachability

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Summary

Modifications of wood powder lignin (WPL) and black liquor lignin (BLL) were studied with growing cultures and cell-free lignin peroxidases and laccase of *Phlebia radiata*. WPL was easily degraded by growing cultures of *Phlebia radiata*. The modifications achieved by using these enzymes were, however, less marked. Only laccase caused a slight change in the molecular mass distribution of WPL. BLL was very resistant even to fungal attack, although some changes in the molecular mass distribution of the dissolved fraction were detected. The capability of the lignin-modifying enzymes of *Phlebia radiata* to improve bleachability of kraft and peroxyformic acid pulps was also tested. Bleachability of kraft pine pulps was improved only when laccase was used after hemicellulase treatment. Hemicellulases apparently increase both lignin extractability and the accessibility of lignin to lignin-modifying enzymes. In the peroxyformic acid pulps the more oxidized lignin was further oxidized by lignin peroxidases, as indicated by a decrease in brightness.

Introduction

Microbial degradation of lignin is mainly accomplished by basidiomycetes. In nature, the biodegradation of lignin is the rate-limiting step of the overall carbon cycle. The microbial degradation is catalyzed by enzymes, which can be produced under suitable conditions in bioreactors. Since the discovery of the first lignin peroxidase enzyme in the culture broth of *Phanerochaete chrysosporium* (Tien and Kirk 1983; Glenn *et al.* 1983), the application of lignin-modifying enzymes especially in the pulp and paper industry has attracted great interest. Low production levels have limited the use of lignin-modifying enzymes on polymeric lignin substrates. Production methods for lignin-modifying enzymes have recently been improved (Linko 1988; Kantelinen *et al.* 1989; Polvinen *et al.* 1991) but for possible large-scale applications, cloning of these enzymes to more efficient production hosts is inevitable. Hitherto laccase is the only lignin-modifying enzyme which has been expressed in active form when cloned to another eukaryote (Saloheimo and Niku-Paavola 1991).

Characterization of the reactions catalyzed by lignin-modifying enzymes has usually been carried out with low molecular mass lignin model compounds. Only a few reports have been published of studies concerning polymeric, native or synthetic and industrial lignins. Both depolymerization and polymerization have been reported. Tien and Kirk (1983) observed depolymerization when treating methylated lignin with lignin peroxidase. By contrast, straw lignin preparations and milled wood lignin were polymerized during lignin peroxidase treatment (Haemmerli *et al.* 1986). Veratryl alcohol was found to enhance the polymerization. Modifications in the absorption spectra of kraft lignin after the use of laccase and lignin peroxidase were reported by Niku-Paavola (1987). Kern *et al.* (1989) concluded that no clear indication of either polymerization or depolymerization of lignin in wood or DHP-lignin by lignin peroxidase could be detected when analyzed by ^{13}C NMR spectra of pyrolysis-gas chromatography-mass spectrometry. The physical state of spruce milled wood lignin as well as the presence of veratryl alcohol was shown to have a major effect on the reactions of lignin peroxidase (Kurek *et al.* 1990). Colloidal lignin was polymerized during lignin peroxidase treatment. When veratryl alcohol was added, some depolymerization was also detected. Synthetic DHP lignin was shown to be polymerized by horseradish peroxidase, lignin peroxidase and laccase (Kondo *et al.* 1990). Moreover, when the lignin was converted to its glycoside it remained unchanged or was slightly depolymerized in the enzymatic treatments. Labelled monomeric coniferyl alcohol was polymerized quantitatively by lignin peroxidase (Sarkanen *et al.* 1991). Further exposure of the dehydropolymerizate to lignin peroxidase resulted in an increase in the degree of the polymerization. Hammel and Moen (1991), however, were able to de-

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