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Examination of fungal oxidases potential for lignin valorisation

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PURPOSE OF THE ABSTRACT

Lignocellulosic (LC) biomass represents one of low-cost, abundant and renewable materials with a high potential for the conversion into numerous chemicals or precursors for polymer synthesis. The major components of the LC biomass are cellulose, hemicellulose and lignin. Lignin is an amorphous three-dimensional polymer of phenylpropanoid units which are interconnected by seven types of bonds. Due to its aromatic, highly functionalized structure and abundancy, lignin is considered to be a very promising renewable and sustainable feedstock for the production of the chemicals, especially bio-aromatics. The most problematic step in the valorisation chain of lignin is its efficient depolymerisation. The efficient depolymerisation would provide an access to the new platform of lignin-based aromatic monomers or oligomers, which would be used to replace fossil-based feedstock for the numerous industrial applications.1

The enzymatic lignin depolymerisation represents one of the many possibilities to produce monoaromatics or lignin oligomers. Laccase (free or immobilized) is one of the commonly used enzyme to achieve oxidative lignin depolymerisation. An alternative to this costly enzymatic treatment is to use lignin as a substrate for fungi exploiting enzyme oxidative activity in situ. This approach requires a more complex downstream processing, however represents one of the promising strategies to overcome the challenge of lignin depolymerisation using environmentally friendly process.2

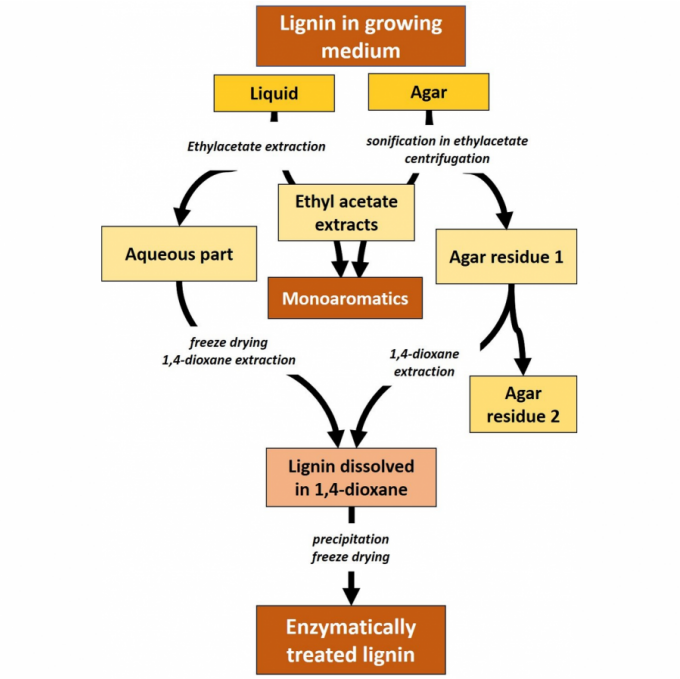
In this study, fungal strains belonging to Cunninghamella elegans NRCIB Ce1, Fomitopsis pinicola NRCIB Fp1, Trametes versicolor NRCIB Tv1 and Absidia sp. NRCIB Asp1 were used from the collection of the Plant Pathology Laboratory. All strains were grown in liquid 2 % malt extract or in malt extract with 1 % agar supplemented with 1 % lignin (ethanol organosolv) at 25 °C for 2 weeks. A stirring speed of 200 rpm was used to grow the microorganisms in the liquid medium. If necessary, samples were freeze-dried at -60 °C before further dowstream processing, separating reaction products. Two specific sample preparation protocols were developed as shown in Figure 1. The extraction-precipitation combination enabled separation of monoaromatic compounds and the remaining lignin assigned as ?enzymatically treated lignin'. Treated lignin samples were characterized using size-exlusion chromatography (SEC), to obtain molecular weight distributions (MWDs), while the quantitative 31P NMR analysis provided information about the functional OH group distribution. The ethylacetate extracts containing monoaromatics were accordingly analysed using GC-MS.

SEC data analysis of the samples cultivated in liquid media disclosed a negligible variation within the MWDs of the enzymatically treated lignin samples, however showing the tendency to form larger/condensed lignin structures. Syringaldehyde detected with GC-MS analysis was produced as the main monoaromatic product, however in moderate concentrations. The addition of agar to the cultivation media reasonably affected lignin

depolymerisation, which reflected as formation of even more condensed lignin structures compared to the ones formed in liquid media. A higher variaty of monoaromatics (vanilin, syringaldehyde, coniferyl aldehyde, propylguaiacol, sinapyl aldehyde, methyl syringol, 3,5-dimethoxy-4-hydroxyphenylacetic acid) detected in agar samples and the larger lignin fragments formed point towards the presence of a simultaneous condensation reactions within the reactive intermediates. Overall, fungal oxidases, especially, the ones originating from Fomitopsis pinicola and Trametes versicolor strain demonstrated the uppermost efficiency depolymerising lignin macromolecule.

There is an obvious potential of organosolv lignin depolymerisation using fungal oxidases and at the same time an illustrative example of both natural sources affecting each other and producing desired aromatic monomers.

FIGURES



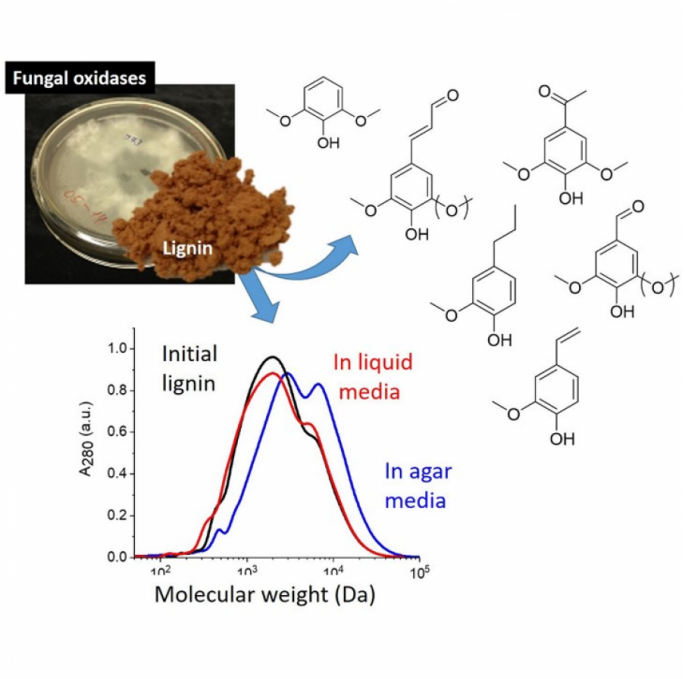


FIGURE 1
Sample preparation procedure.
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FIGURE 2
Fungal oxidase-assisted lignin depolymerisation
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KEYWORDS
lignin depolymerisation, | fungal oxidase | size-exclusion chromatography | 31P NMR

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