The Effect of Lignin Modifying Enzymes on the Molecular Weight Distribution of Kraft Lignin

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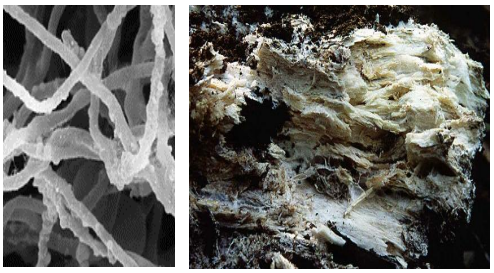
OBJECTIVES

- To determine the effect of several enzymes and cofactors on the molecular weight distribution of treated Kraft lignin.
- To use High-Performance Size Exclusion Chromatography (HPSEC) coupled to Multi-Angle Laser Light Scattering (MALLS) detection for molecular weight analysis of lignin.

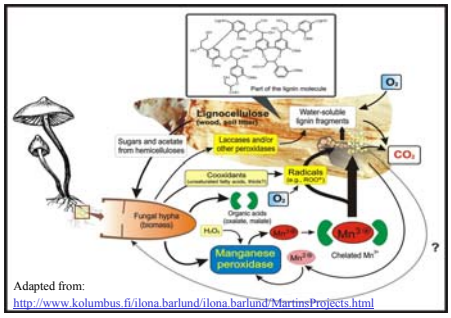
INTRODUCTION

Lignin, second only to cellulose as a source of fixed carbon in the biosphere, is generally considered to be highly resistant to rapid biological degradation. White rot fungi, the primary lignin decomposers, produce various extracellular redox enzymes and electron transfer mediators, which are thought to facilitate degradation of polymeric lignin. We have used *Trametes cingulata* (ATCC) and *Phanerochaete chrysosporium* (Sc26) in this set of experiments.



Phanerochaete chrysosporium

Although enzymes such as lignin peroxidase, manganese-dependent peroxidase, and laccase are extracellular, confirmation of direct enzyme-lignin interaction is unclear. Enzyme-mediated electron transfer from lignin molecules to oxidized cofactors such as Mn^{+3} and H_2O_2 is thought to be enhanced by the ability of these low molecular weight compounds to penetrate the wood ultrastructure and carry out oxidation of lignin *in situ*, where steric hindrances make direct enzyme interaction unlikely.



Adapted from:
http://www.kolumbus.fi/ilona.barlund/ilona.barlund/MartinsProjects.html

EXPERIMENTAL SET-UP

The entire set of experiments was done with duplicates samples.

Different broth and cofactors

Broth samples were derived from fungal growth (*T. cingulata* or *P. chrysosporium*) on lignin, concentrated, and diafiltered using an Amicon PM10 membrane. Lignin samples (NREL and UM) were combined with NREL or UM broth and various cofactors such as hydrogen peroxide, dithiothreitol (DTT), Fe, Cu or Mn, and then analyzed on days 1, day 4 and day 12 to check for molecular weight reduction. Each lignin was combined with either of the two broths and then supplemented with any one of the cofactors.

The total reaction volume was 1.5 ml, which comprised of 100 μL of broth, 15 μL of the cofactor and 1.385 ml of 5.0 g/L lignin sample. 5.0 g/L lignin alone was used as control. Samples with only broths and without any cofactors were also prepared as secondary controls when examining the effect of cofactors.

ABBREVIATIONS

UML – University of Minnesota lignin

UMB – University of Minnesota broth

NL– National Renewable Energy Laboratory lignin

Sc26 - induced *Phanerochaete chrysosporium* (Sc26)

Sc26wo – *Phanerochaete chrysosporium* (Sc26) not induced

Induced growth studies

- 1.0 g/L lignin in acetate buffer (pH 5.2) or Tris buffer (pH 8.0) was used to examine the effect of pH. *T. cingulata* (ATCC) and *P. chrysosporium* (Sc26) were used for inoculation of the experimental set that was not induced. *Trametes cingulata* (ATCC) and *Phanerochaete chrysosporium* (Sc26) cultures grown in the previous set of experiments with lignin were filtered and used for the induced set. Samples were withdrawn periodically and filtered through 0.45 μm nylon Acrodisc filters before analysis. 1.0 g/L lignin in the respective broths without fungal inoculation was used as control.
- The total experimental volume was 15 ml with two different concentrations (1x and 5x) of enzyme used for both sets. An additional set of experiments with hydrogen peroxide added to the sample was also prepared due to the enhancement of molecular weight reduction seen from previous experiments.

Analysis

- High-Performance Liquid Chromatography –
  - Asahipak GS-320. (Shodex)
  - 100 μL sample loop, 0.1 M NaOH (pH 12) @ 0.5 ml/min.
  - Series 1050 Multi-wavelength Detector (Hewlett Packard).
  - Dawn EOS Multi-Angle Laser Light Scattering detector (Wyatt Technology, Santa Barbara, California).
  - Optilab DSP Interferometric Refractometer (Wyatt Technology).

RESULTS

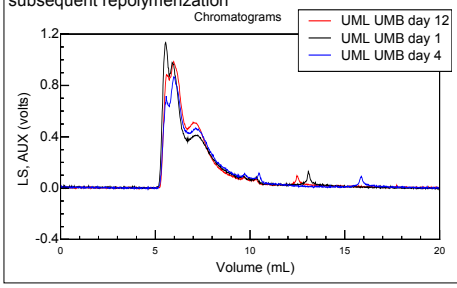
Plots

Dashed curves – Interferometric Refractometer overlays

Continuous curves – Light Scattering detector overlays

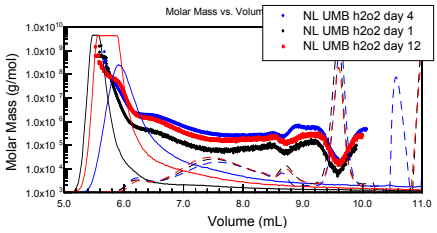
Effect of broth

Depolymerization was seen only when NREL lignin was coupled with NREL broth and when UM lignin was coupled with UM broth. Depolymerization was however followed by subsequent repolymerization



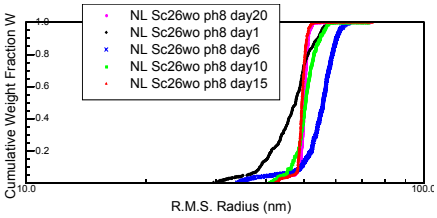
Effect of cofactors

Hydrogen peroxide was the only cofactor which showed NREL lignin depolymerization for NREL as well as UM broth



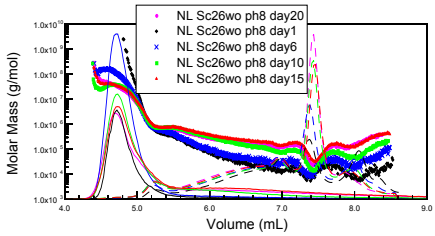
Change in root mean square radius

The distribution in the radius of gyration became narrower with time, indicating that molecular conformation changed to a more uniform molecular shape.



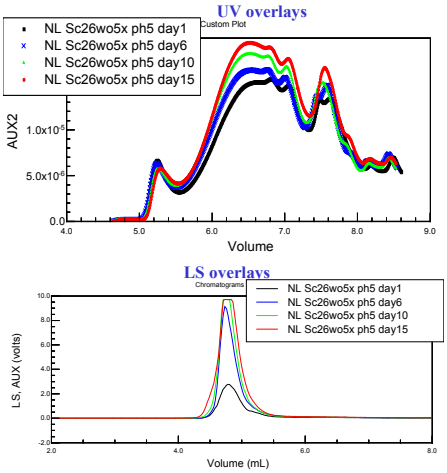
Growth studies

For both induced cultures and cultures that were not induced it was seen that there was initial lignin polymerization followed by subsequent depolymerization.



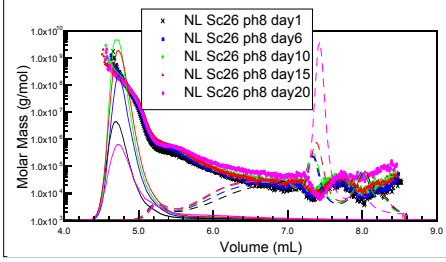
Effect of pH

Even though the UV overlays as shown below hint at a molecular weight reduction it can be seen from the light scattering overlays that the aggregate peaks increase. The lowering in peaks is due to aggregation at pH 5. It is important to reduce this instability due to aggregation by maintaining a higher pH.



Induced growth

Cultures from the previous set of experiments were filtered and used for these set of experiments. There wasn't a significant difference between the two cultures.



CONCLUSIONS

- The induced cultures showed more lignin depolymerization for the specific lignin samples in which they were initially grown.
- H_2O_2 enhanced the depolymerization for both types of lignin studied.
- The distribution in the radius of gyration became narrower with time, indicating that molecular conformation changed to a more uniform molecular shape.
- Aggregation of lignin at pH below 8.0 reduces the reliability of the molecular weight reduction data obtained.

ACKNOWLEDGEMENTS

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