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Depolymerization of Lignin for Biological Conversion Through Sulfonation and a Chelator-Mediated Fenton Reaction

Michael S. Kent,[1,2] Daniella V. Martinez [2], Alberto Rodriguez,[1,2] Miranda Juarros,[2] Estevan J. Martinez,[2] Todd M. Alam,[2] Blake A. Simmons,[1,3] Kenneth L. Sale, [1,2] Steven W. Singer[1,3]

[1]Deconstruction Division, Joint BioEnergy Institute, Emeryville, CA, [2]Sandia Nat. Labs, Livermore CA and Abq, NM, [3]Lawrence Berkeley Nat. Lab. Berkeley CA

Abstract

Generating value from lignin through depolymerization and biological conversion to valuable fuels, chemicals, or intermediates has great promise but is limited by several factors including lack of cost-effective depolymerization methods, toxicity within the breakdown products, and low bioconversion of the breakdown products.[1] High yield depolymerization of natural lignins requires cleaving carbon-carbon bonds.[2] We report that a chelator-mediated Fenton (CMF) reaction efficiently cleaves C-C bonds at or near room temperature in sulfonated polymers and that repolymerization can be minimized through control of the reaction conditions. This method was used to depolymerize lignosulfonate from Mw = 28,000 g/mol to Mw = 800 g/mol. The breakdown products were characterized by FTIR, NMR, and GC-MS and evaluated for bioavailability. The breakdown products are rich in acid, aldehyde, ether, and alcohol functionality but largely devoid of aromaticity. A panel of monocultures were tested for growth on the breakdown products. Growth at a low level was observed for several monocultures on the depolymerized LS in absence of glucose. Much stronger growth was observed in the presence of 0.2% glucose. These results suggest that this method may be promising for biological conversion of lignin into higher value chemicals or intermediates.

Objective

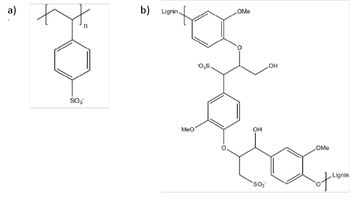
Increase bioavailability of lignin for biological conversion into useful fuels, chemicals, or intermediates.

Materials and Methods

Polystyrene sulfonate (PSS) was obtained from Polymer Standards Service (63,900, PDI < 1.2). Lignosulfonate DP-4397 (LS) was a gift from Borregaard.

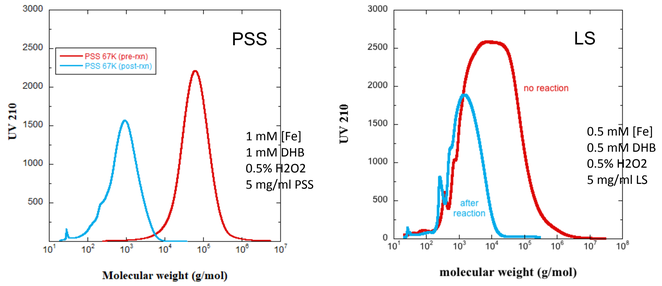
Culture conditions. Tryptic soy broth (Sigma-Aldrich) was prepared as 30 g/L in water and autoclaved at 121 ºC for 20 minutes. A 10x yeast nitrogen base medium without amino acids (SIGMA-Aldrich) was prepared according to the manufacturer's specifications and supplemented with Complete Supplement Mixture (MP Biomedicals, USA) at a final concentration of 80 mg/L. The pH of the resulting medium (YNB+CSM) was adjusted to a value of 6 with 10 N NaOH and the solution was filter sterilized (0.45 µm cellulose-acetate membrane). Depolymerized LS at a concentration of 5 mg/mL was also filtered (0.45 µm cellulose-acetate membrane), mixed at a 9:1 v/v ratio with the 10x YNB+CSM stock solution, and used for microbial cultivations.

To start the cultivations, all organisms were inoculated from agar plates and grown for 24 hours in tryptic soy broth at 30 ºC. The cells were then centrifuged, resuspended in water, and transferred at an initial OD of approximately 0.1 to 1 to 48-well plates containing cultivation medium (500 µL final volume per reaction). The plates were covered with an AeraSeal sealing film (Excel Scientific, USA) and a plastic lid to prevent evaporation and incubated at 30 ºC with shaking at 300 rpm for 60 h.

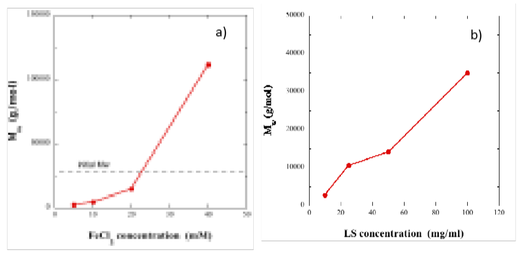


Chemical structures of a) polystyrene sulfonate and b) lignosulfonate

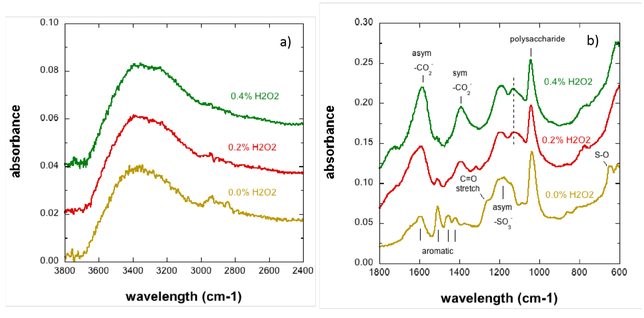
CMF reaction cleaves C-C bonds in sulfonated polymers at or near room T[3-5]



Aqueous SEC for PSS and LS before and after CMF reaction at room T. Depolymerization of PSS demonstrates that CMF reaction cleaves C-C bonds. For LS, Mw decreases from 28,000 g/mol to 800 g/mol.

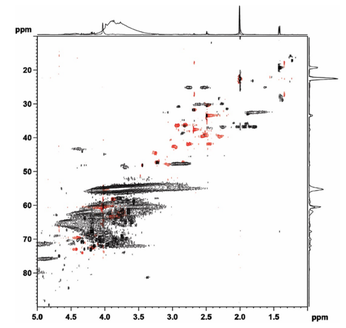


Post-reaction M_w for LS as a function of a) $[FeCl_3]$ (with $[H_2O_2]$ = 1%, [LS] = 5 mg/ml, [DHB] = 4 mM), and b) [LS] (with $[FeCl_3]$ = 10 mM, [H2O2] = 1%, [DHB] = 4 mM). Both series were at room T. Depolymerization is best achieved at low [Fe] and low [LS].

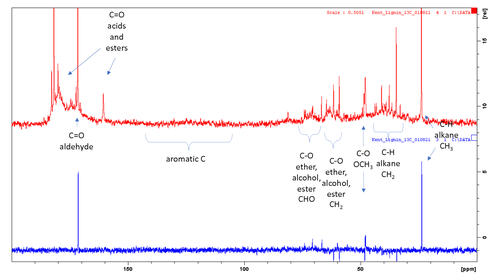


FTIR spectra for LS as a function of [LS] (a, b) with $[H_2O_2]$ = 0.5%, and as a function of $[H_2O_2]$ (c, d) with 5 mg/ml LS. Reactions were performed with $[FeCl_3]$ = [DHB] = 0.5 mM at 40 ºC. CMF reaction opens the aromatic rings of LS and generates COOH groups.

NMR of LS breakdown products



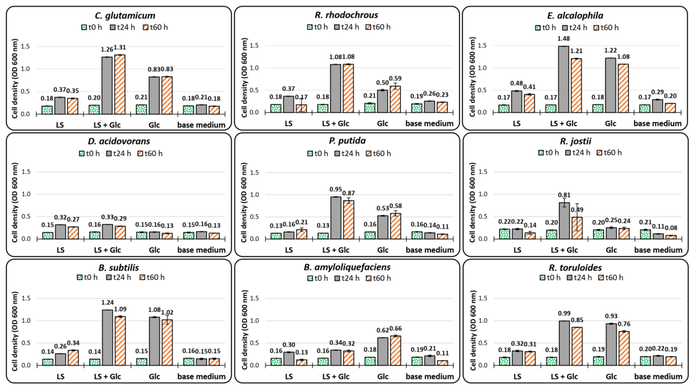
^1H-^{13}C HSQC NMR spectra for LS (black) and products for CMF reaction of LS with $[FeCl_3]$ = 1 mM, [DHB] = 10 mM, $[H_2O_2]$ = 1% at 40 ºC (red). CMF opens aromatic rings in LS.



Quantitative ^{13}C NMR (top) and INEPT NMR spectra (bottom) for products of CMF reaction of LS with $[FeCl_3]$ = 1 mM, [DHB] = 10 mM, $[H_2O_2]$ = 1% at 40 ºC. Nearly half the carbon atoms are carbonyls, and very little aromaticity is present.

Growth of organisms on LS breakdown products

The plots to the right show growth of monocultures in the LS breakdown stream or base medium in the presence or absence of glucose. The results show that several organisms can grow on the LS material, alone or in combination with glucose.



Conclusions

- CMF reaction cleaves C-C bonds in sulfonated polymers at room T
- Repolymerization can be minimized at low [Fe] and low [LS}
- CMF reaction also opens the aromatic rings of LS and generates acids and aldehydes
- Some organisms are able to grow on the LS breakdown products in the presence of glucose.
- This method is promising for biological conversion of lignin into higher value chemicals or intermediates.

References

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Acknowledgments

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