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Does De-aromatization of Lignin Increase its Bioavailability?

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Abstract

Efficient conversion of lignin to useful molecular building blocks has been elusive. One promising approach is partial de-polymerization of lignin and subsequent bioconversion of the mono, di, and poly-aromatic breakdown products into value-added compounds.[1,2] Work to date using this approach has examined growth of individual organisms and also microbial communities on partially depolymerized lignin streams. Various processes have been explored to depolymerize lignin, such as acid- or base-catalysis, and reductive and oxidative processes.[3] Much of the prior work has focused on processes that leave the aromatic rings largely intact, with the goal of utilizing aromatic catabolic pathways in soil microbes to funnel the carbon into central metabolism. However, conversion is generally low. With *P. putida*, a conversion host that has been extensively explored for lignin bioconversion, conversion is limited largely to monomeric aromatic species,[4-6] yet most depolymerization processes result in only a small fraction of monomeric products.[7] In this work, we consider whether de-aromatization of lignin might lead to higher biological conversions of the carbon. In particular, we wish to determine whether de-aromatization leads to more facile conversion of oligomeric or polymeric fragments by microbial communities. We extensively de-aromatized lignin from corn stover using an oxidative process involving chelator-mediated Fenton (CMF) chemistry[8] and also treated the same lignin with a base-catalyzed depolymerization (BCD) process. While both processes result in water-soluble material, CMF is extensively de-aromatized whereas BCD retains much aromaticity. We measured the conversion of the two water-soluble lignin streams by two microbial communities.

Objective

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

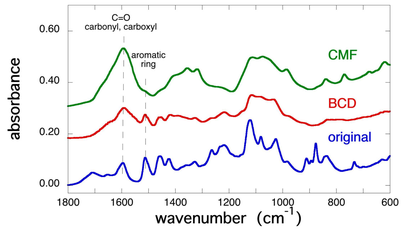
Materials and Methods

Lignin. Lignin was isolated from corn stover using an alkaline process followed by enzymatic hydrolysis of sugars at the Advanced Biofuels Process Demonstration Unit at Lawrence Berkeley National Lab. A portion of the lignin was de-aromatized using successive chelator-mediated Fenton (CMF) reactions (4 mM $FeCl_3$, 4 mM 1,2 dihydroxybenzene (DHB) 0.5% H_2O_2, at initial pH of 6 at RT). Following each reaction the pH was increased to 6 with NaOH and another aliquot of 0.5% H_2O_2 was added. De-aromatized lignin was isolated by centrifugation after adjusting the pH to 7 followed by freeze-drying of the supernatant. Another portion of the alkaline corn stover lignin was treated by base catalyzed depolymerization (BCD) using 2% NaOH at 120 C for 1 hr followed by neutralization with HCl and centrifugation to remove the insoluble portion.

Culture conditions. One community was isolated from compost and the second community had accumulated on the lignin stored in non-sterile laboratory ambient conditions. Both communities had been adapted to growing on CMF lignin in the presence of minimal M9 media with 0.4% glucose prior to this work and had been stored as glycerol stocks. Overnight cultures were grown in sterile LB media at 30 ºC and then pelleted, resuspended in M9, and used to innoculate the test cultures consisting of 50 ml of M9 + 0.4% glucose at 30 ºC. CMF and BCD lignins at 50 mg/ml were filtered through 0.2 um filters and added to the cultures to give a nominal concentration of 5 mg/ml. 0.4% glucose was added at each time point for which samples were collected.

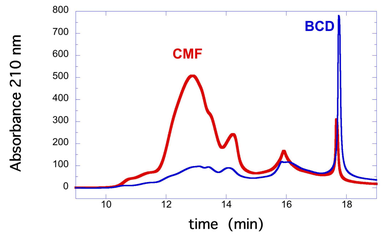
Characterization of CMF and BCD lignins by FTIR, 31P NMR and aqueous SEC

FTIR indicates nearly complete de-aromatization for CMF and partial de-aromatization for BCD

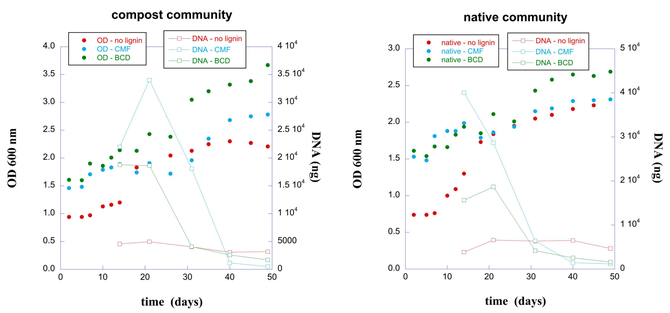


31P NMR indicates higher COOH content for CMF than for BCD. CMF is more hydrophilic.

Aqueous SEC indicates lower Mw for BCD than for CMF

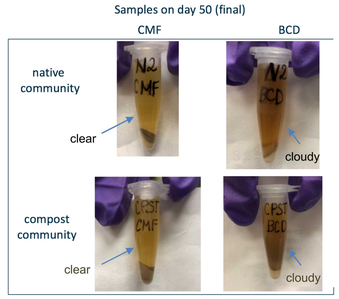


OD and DNA versus culture time

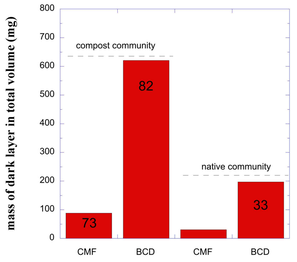


DNA content was measured using PicoGreen after extraction and purification using DNeasy PowerSoil kit (Qiagen) for 200 µl samples taken at various time points. DNA content peaked between 10 and 20 days. For both microbial communities, DNA content was much higher for cultures with the lignin-derived material than for cultures without BCD or CMF lignin. The peak DNA content was much higher for CMF than for BCD lignin.

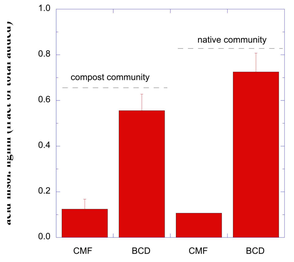
Much higher fraction of BCD lignin was rendered insoluble than for CMF



For BCD a gel-like layer remains that does not pellet upon centrifugation



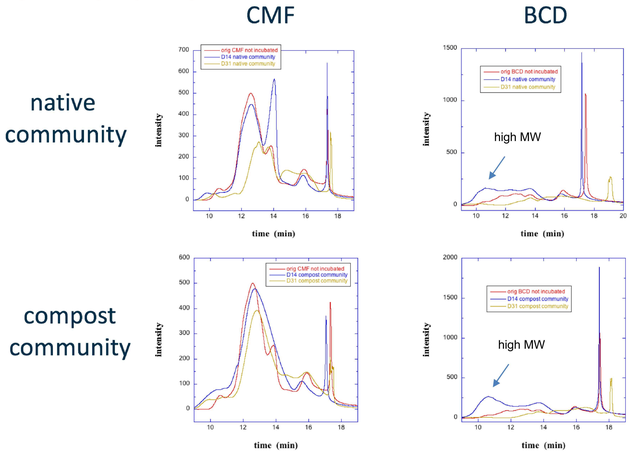
numbers indicate % of the mass solubilized by acid hydrolysis



after acid hydrolysis

Aqueous SEC of soluble CMF and BCD

The plots to the right show aqueous size exclusion chromatograms for the 0.2 mm filtered supernatant of each culture at day 14 and day 31 compared to the original. These results show that for BCD a high molecular weight component develops at intermediate times that disappears at later times when it becomes retained by the filter.



Conclusions

- much higher fraction of lignin was rendered insoluble for BCD
- higher peak DNA content for CMF
- SEC of soluble lignin shows increased high MW for BCD lignin

These results indicate that bioavailability is greater for CMF lignin than for BCD lignin

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Acknowledgments

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