Alkaline Pretreatment Effectively Reduces Recalcitrance of Zip-Lignin Poplar

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https://www.glbrc.org/research/deconstruction

Project Goals: To compete with traditional petroleum refineries, cellulosic bio-refineries must achieve high carbohydrate-to-fuel yields and facilitate lignin valorization to commodity products. The Great Lakes Bioenergy Research Center (GLBRC) adopted a two-fold strategy for improving enzymatic deconstruction of hardwoods via alkaline pretreatment. First, the GLBRC has refined the alkaline pretreatment processes employed to reduce biomass recalcitrance. These efforts have resulted in the development of two highly effective alkaline pretreatments: Extractive Ammonia (EA) and copper bipyridine-catalyzed alkaline hydrogen peroxide (Cu-AHP) pretreatment. Second, the GLBRC has genetically engineered poplar to contain readily cleavable ester bonds in the backbone of lignin (Zip-Lignin™). This modification of the cell wall was designed specifically to facilitate deconstruction of hardwoods via alkaline pretreatment and subsequent enzymatic hydrolysis.

EA pretreatment takes advantage of the properties of liquid ammonia for modifying the crystalline structure of cellulose from native cellulose I_β (CI) to cellulose III_I (CIII) as well as selectively extracting part of the lignin, leaving most of the carbohydrates intact in a single process stream. Previous studies demonstrated that the CIII allomorph is responsible for increasing enzymatic hydrolysis rates by 2-5 fold[1, 2] relative to the native CI. Furthermore, lignin is well known to inhibit both enzymes and microorganisms required to convert lignocellulosic biomass into biofuels. The combined effect of lignin extraction and CIII formation is responsible for a significant improvement in biomass conversion at low enzyme loading and high solid loading enzymatic hydrolysis compared to ammonia fiber expansion (AFEX™) pretreatment. In this work, EA pretreatment was performed using low and high severities on two Zip poplar lines. Enzymatic hydrolysis of the EA pretreated Zip poplar lines at 1% glucan loading, using 15 mg protein per g glucan enzyme loading, revealed glucan and xylan conversions up to ~80%. Relative to the wild-type line, the incorporation of Zip-Lignin into poplar improved glucan conversions by approximately 8% in this preliminary study.

Alkaline hydrogen peroxide (AHP) has been shown to be an effective pretreatment for both herbaceous feedstocks and woody biomass. The utility of this approach, however, has been limited by the prohibitively high oxidant loadings (e.g., 250-2000 mg H_2O_2 per g biomass). We recently discovered that adding small amounts of copper 2,2'-bipyridine complexes [Cu(bpy)] as

catalysts during AHP pretreatment (Cu-AHP) resulted in substantially improved delignification and enhanced sugar yields following enzymatic hydrolysis at modest oxidant loadings i.e., ~100 mg H_2O_2 per g biomass.[3] We subsequently demonstrated that copper co-localized with disrupted regions of the cell wall following pretreatment of poplar, providing indirect evidence for copper catalyzing the oxidation and removal of lignin.[4] With an aim to increase the effectiveness of the Cu-AHP process on woody biomass and further reduce chemical inputs, we utilized Zip-Lignin poplar as a substrate. Our preliminary results revealed 12% higher glucose yields (78% to 90%) relative to the wild-type line. Alternatively, the enzyme loading on Cu-AHP pretreated Zip-Lignin poplar could be decreased by 2/3 while still maintaining glucose yields at 78%. Together, these results highlight the significant potential for Zip-Lignins to reduce the recalcitrance of woody biomass.

References

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