We previously demonstrated that copper-catalyzed alkaline hydrogen peroxide (Cu-AHP) pretreatment of woody biomass substantially improves delignification and enzymatic digestibility relative to uncatalyzed AHP. To enhance the Cu-AHP process, we introduced an alkali pre-extraction step prior to the Cu-AHP pretreatment of hybrid poplar, and this two-stage pretreatment improved glucose yields relative to standard Cu-AHP from 63% to 86%. Secondly, to utilize the \( \text{H}_2\text{O}_2 \) more efficiently, fed-batch addition of \( \text{H}_2\text{O}_2 \) was performed over the course of 10 h. This also resulted in an increase in the glucose yields (75%) compared to the standard Cu-AHP pretreatment (63%). Importantly, combining these strategies resulted in glucose yields of 96% of theoretical maximum. Experiments were also performed to evaluate the impact of different concentrations of enzyme, \( \text{H}_2\text{O}_2 \), and catalyst loading on the modified fed-batch, two-stage Cu-AHP pretreatment process. Our results demonstrated that we could substantially lower the chemical inputs while still maintaining high product yields.

In another approach to improve sugar yields and reduce the chemical inputs of the two-stage Cu-AHP process, we tested the Cu-AHP pretreatment technique with hybrid poplar engineered to contain readily cleavable ester bonds in the backbone of lignin (Zip-Lignin™). This modification of the cell wall was specifically designed by other members of the Great Lakes Bioenergy Research Center (GLBRC) to facilitate the deconstruction of hardwoods via alkaline pretreatment methods. Preliminary results reveal higher glucose yields from the engineered hybrid poplar relative to the wild-type line. Alternatively, the enzyme loading on Cu-AHP pretreated Zip-Lignin poplar could be decreased while maintaining glucose yields at ~80%. Together, these results highlight the potential for Zip-Lignins to reduce the recalcitrance of woody biomass and improve alkaline pretreatments.