Comparative Studies of Diverse Lignocellulosic Feedstocks for Microbial Biofuel Synthesis and the Impact on the Biorefinery

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Project Goals: Feedstocks can vary widely in terms of their biomass yields and quality characteristics (chemical composition, moisture content, etc.), which can affect microbial biomass-to-biofuel conversion efficiency. Previously, we found that elevated levels of lignocellulose-derived inhibitors in hydrolysates produced from pretreated switchgrass harvested from a drought year greatly inhibited the growth of *Saccharomyces cerevisiae*. We then expanded our studies to five different feedstocks, including two annuals (corn stover and energy sorghum) and three perennials (switchgrass, miscanthus, and mixed prairie), and evaluated the variability in microbial response and fermentation performance related to levels of lignocellulose-derived inhibitors. We found that the process ethanol yield varied among these five feedstocks during the microbial fermentation process using both yeast and bacterium (*Zymomonas mobilis*). However, both biomass quality and biomass yield affected the field-scale ethanol yields, the amount of ethanol produced per acre. Biomass quality was the main driver of the ethanol yields for high yielding crops, such as miscanthus. Biomass yield was the main driver for the ethanol yields for low-productivity crops, such as mixed prairie. Our results suggest that a lignocellulosic refinery may use a variety of feedstocks with a range of quality without a major negative impact on ethanol yields.

A variety of biomass feedstocks could be used for producing bio-based chemicals and biofuels. However, refineries that convert biomass into fuels often rely on just one feedstock. It would greatly benefit refinery operation if more than one feedstock can be used. To investigate how the diversity of potential biofuel cropping systems and feedstock supply might affect process and field-scale ethanol yields, we processed and experimentally quantified ethanol production from five different herbaceous feedstocks: two annuals (corn stover and energy sorghum) and three perennials (switchgrass, miscanthus, and mixed prairie). We previously found that *Saccharomyces cerevisiae* were significantly impaired for anaerobic fermentation of hydrolysates produced from pretreated switchgrass harvested from a drought year compared to non-drought years, as well as from pretreated corn stover from the same drought year. To avoid interannual variability, we have used lignocellulosic hydrolysates derived from five different plant feedstocks harvested from the same location and timeframe, as well as pretreated in an identical manner. This allowed us to identify feedstock-specific differences. We studied how the

levels of lignocellulose-derived inhibitors varied in these different feedstock hydrolysates and how their impact on microbial response and fermentation performance using both a yeast (*Saccharomyces cerevisiae* Y128) or a bacterium (*Zymomonas mobilis* 8b). Overall, the process ethanol yield showed some variability across years and feedstocks. A low process ethanol yield for corn stover was determined to result from inhibition of xylose utilization by unusually elevated levels of hydroxycinnamates (*p*-coumaric and ferulic acids) in the untreated biomass and their amide derivatives in the resulting hydrolysates. However, the field-scale ethanol yield from each feedstock was dependent on both biomass quality and cropping system productivity. Biomass yield had a greater influence on the ethanol yield for low-productivity crops, while biomass quality was the main driver for ethanol yields from high-yielding crops. Most feedstocks fall within a similar range of process ethanol yield, particularly for the more resistant strain *Z. mobilis* 8b. This supports the claim that the refinery can successfully diversify its feedstock supply, enabling many social and environmental benefits that can accrue due to landscape diversification.

References

1. Ong, R. G., A. Higbee, S. Bottoms, Q. Dickinson, D. Xie, S. A. Smith, J. Serate, E. Pohlmann, A. D. Jones, J. J. Coon, T. K. Sato, G. R. Sanford, D. Eilert, L. G. Oates, J. S. Piotrowski, D. M. Bates, D. Cavalier, and Y. Zhang. 2016. Reaction products of drought stressed bioenergy crops inhibit microbial biofuel production. Biotechnology for Biofuels. 9:237.
2. Y. Zhang, L. G. Oates, J. Serate, D. Xie, E. Pohlmann, Y. Bukhman, S. D. Karlen, M. K. Young, A. Higbee, D. Eilert, G. R. Sanford, J. S. Piotrowski, D. Cavalier, J. Ralph, J. J. Coon, T. K. Sato, and R. G. Ong. 2018. Diverse lignocellulosic feedstocks can achieve high field-scale ethanol yields while providing flexibility for the biorefinery and landscape-level environmental benefits. GCB Bioenergy 10:825-840.

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