Interannual Climate Variability Affects the Microbial Response to and Fermentability of Lignocellulosic Biofuel Crops

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Project Goals: To design and implement a "Feedstock-to-Fuel" analytical pipeline that can be used to investigate how variables such as agronomic conditions, feedstock variability, pretreatment chemistry, and microorganism growth and productivity interact with each other during biofuel production.

Fundamental studies on lignocellulosic biofuel production are often limited in their scope, either focusing on individual stages of the process and/or a single feedstock, or spanning only a few stages and variables. In these evaluations, the impacts of variables within an earlier stage (for example, agronomic practices) on the results obtained in later stages (such as fermentation yields and efficiency) are often inferred based on assumptions that are widely accepted, but have little experimental evidence to support their validity. Indeed, results from these studies are typically shortsighted and have limited use in guiding the improvement of upstream operations. There is a need for fundamental studies that also have a systems-wide scope, however, these studies are very challenging to implement. By taking advantage of the breadth of expertise and resources within the Great Lakes Bioenergy Research Center (GLBRC) we were able to design and implement a "Feedstock-to-Fuel" analytical pipeline that allows us to perform the types of analyses needed to investigate how upstream variables such as agronomic conditions, feedstock variability, and pretreatment chemistry interact with each other and influence the properties of the fermentation media (enzymatic hydrolysate) and ultimately the genetic response, growth and productivity of the fermentation microorganisms.

In the first of these studies we investigated the impact of interannual climate variability on downstream biofuel production processes. Corn stover and switchgrass were collected at the UW Ag Research Station in Arlington, WI during three years with significantly different precipitation profiles (2010, 2012, and 2013). These were chosen to represent a wet, dry, and an average year, respectively, when compared to the 30-year climate norms for Arlington, WI. During fermentation of AFEX-treated biomass hydrolysates, *Zymomonas mobilis* 2032 showed no major difference in response, with the exception of slightly lower cell growth in the switchgrass hydrolysates. *Saccharomyces cerevisiae* Y128 likewise showed reduced growth in the switchgrass hydrolysates, but was completely unable to grow in hydrolysate generated from 2012 switchgrass (SG). Statistical analysis of the chemical composition of the hydrolysates (common organic acids, phenolic-derived inhibitors, and minerals) did not implicate any compounds for the poor performance. Chemical genomic analysis of the yeast response to these hydrolysates pointed to inhibition of ergosterol biosynthesis as a reason for the growth inhibition. Further investigation revealed the presence of high quantities of pyrazines and imidazoles in both the acetone extract and hydrolysate of the pretreated 2012 switchgrass. Both classes of compounds are derived from reactions of soluble sugars with ammonia and are known inhibitors of ergosterol biosynthesis. Of these compounds, 2-methylpyrazine was particularly inhibitory to yeast growth. Addition of these compounds to a synthetic hydrolysate at the same concentrations as in the actual hydrolysate also inhibited growth of *S. cerevisiae* Y128. During the drought year, soluble sugars accumulated in the switchgrass, possibly because of incomplete utilization of the sugars due to early senescence or as an osmotic stress response, and were ultimately degraded during pretreatment to chemicals that were severely inhibitory to yeast growth. These results show a complex interplay between the weather impacts on biomass crop composition, pretreatment chemistry, and the response of the microorganisms during fermentation.

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