

## Comparative Studies of Diverse Feedstocks and Identification of High Levels of Hemicellulose and Degradation Inhibitors That Impact Microbial Biofuel Synthesis

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**Project Goals: A variety of biomass feedstocks could be used for producing bio-based chemicals and biofuels. However, chemical compositions of various plant biomass, and even same feedstocks grown under different conditions, can vary widely. 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. Compositional and functional analyses determined that high concentrations of pyrazines and imidazoles generated from excess soluble sugars in drought switchgrass during pretreatment partially contributed to this microbial inhibition. We then expanded our studies to five different feedstocks and would like to know how the levels of lignocellulose-derived inhibitors varied and how their impact on microbial response and fermentation performance, especially for xylose utilization. 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 will allow us to identify feedstock-specific differences.**

To investigate how much the known compounds and lignocellulose-derived inhibitors vary in different feedstocks and how these variations affect microbial response and biofuel synthesis, hydrolysates produced from five different feedstocks, including corn stover, switchgrass, miscanthus, sorghum, and mixed prairie, were compared by chemical composition analysis, chemical genomics, and microbial fermentation. To avoid the variability of different growth conditions, all feedstocks were harvested in the same year (Year 2014) from the same location, and then were pretreated with the same Ammonia Fiber Expansion method (AFEX). Hydrolysates were produced and then used to study microbial responses using chemical genomics and microbial fermentation.

Two ethanologens, *Saccharomyces cerevisiae* and *Zymomonas mobilis*, showed poor xylose utilization in corn stover hydrolysate compared to other feedstock hydrolysates. Chemical genomics also revealed a larger microbial stress response to corn stover hydrolysate. Comparison of the hydrolysate composition revealed the highest levels of several aromatic compounds in corn stover hydrolysate, including coumaric acid, coumaroyl amide, ferulic acid, and feruloyl amide. Supplementation of these inhibitors into a non-inhibitory hydrolysate inhibits

xylose utilization. These results indicate that elevated concentrations of hydroxycinnamic acid derived compounds inhibit xylose utilization in corn stover hydrolysate.

Chemical analysis of untreated feedstocks revealed that high concentrations of hydroxycinnamic acid-derived inhibitors originate from untreated corn stover and AFEX pretreatment. Furthermore, the levels of these inhibitors in the untreated biomasses and their hydrolysate varied both between feedstocks and between harvest years for the same feedstock. Year 2014 corn stover has the highest xylan/glucan content ratio, perhaps indicating joint regulation of the deposition of these compounds within the cell wall. Our results indicate that high hemicellulose content in year 2014 corn stover result in high levels of these inhibitors found in its hydrolysate, causing the poor microbial fermentation performance, especially xylose utilization. Although a higher hemicellulose content in feedstocks would be expected to contribute to greater ethanol yields, it may be linked to higher levels of inhibitors in hydrolysates and reduced microbial fermentation performance and xylose utilization.

## References

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