

## **Investigating the Effects of Environmental Stress on Feedstock Quality and Biofuel Production by Field-to-Fuel Optimization Research Pipeline**

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### **Project Goals**

Our previous work suggests that feedstock composition can change in response to drought stress, resulting in the production of inhibitory compounds that impede conversion to biofuels by yeast. We established the Field-to-Fuel Optimization research group to better understand the impacts of drought and other environmental stresses on feedstock yield and composition to their downstream effects on microbial biofuel production.

### **Abstract**

In order to establish an economically productive and sustainable bioenergy industry, biorefineries will need to produce biofuels and bioproducts from renewable plant feedstocks consistently from year to year. However, the quality and quantity of biomass used in such pipeline can be profoundly impacted by numerous environmental factors. Previously, we determined that yeast fermentation of lignocellulosic hydrolysates from switchgrass grown during a drought year (2012) in Wisconsin was severely impaired compared to switchgrass from a non-drought year (2010) (Ong *et al.*, 2016). Based on this, we hypothesized that drought and other environmental stresses could alter feedstock composition, which may subsequently impact downstream biomass deconstruction and conversion into biofuels. To directly test this hypothesis, we formed a multi-disciplinary, integrative research pipeline whose goal is to provide crucial knowledge that can enable lignocellulosic biofuel industries to identify biomass growth conditions that negatively impact microbial conversion into biofuels, and determine agronomic practices and microbial engineering strategies that mitigate this inhibition. Together, we designed and planted experimental field sites at five different locations in Michigan and Wisconsin with a variety of bioenergy feedstocks under different treatment conditions (drought, nitrogen depletion, fungicide treatment, etc.) for three growing seasons (2018-2020). These sites included subplots of switchgrass fitted with rainout shelters that siphon off natural rainwater, allowing us to mimic drought conditions and directly compare with control switchgrass samples.

As a first test of our research pipeline, we processed and analyzed 2018 switchgrass samples that were either grown under a rainout shelter (“rainout” treatment) or outside the shelter (“ambient” treatment). We developed and utilized customized pretreatment and enzymatic hydrolysis methods to process small amounts of harvested rainout and ambient switchgrass from a total of 19 separate plots across locations. Next, we performed small-scale anaerobic fermentation experiments and found that yeast fermentation and biofuel production was impaired in rainout switchgrass hydrolysates compared to paired ambient hydrolysates from multiple sites in WI and MI. However, inhibition of biofuel production was not consistently found across all plots at some sites. Additional investigation determined that the dry biomass yields of rainout switchgrass were not consistently lower than their paired ambient controls, suggesting variable effectiveness of individual rainout shelters. By modifying the rainout shelters, we were able to achieve significantly reduced biomass yields for rainout switchgrass across all locations in 2020, indicating significant drought-stress. Future studies are planned to evaluate the rainout and ambient samples from 2020 and compare to the results obtained from 2018. Finally, as part of this project, we developed web applications to track the large number of biomass samples and share their associated data from planting through harvest, deconstruction, and fermentation between groups. In the future, we will apply this Field-to-Fuel Optimization research pipeline to determine whether other environmental conditions that impact biomass and biofuel yields, biochemically identify inhibitory plant molecules induced by the conditions, and uncover genetic engineering strategies that enable biofuel-producing microbes to overcome the inhibition.

## References

Ong, R.G., *et al.* (2016). Inhibition of microbial biofuel production in drought-stressed switchgrass hydrolysate. *Biotechnol Biofuels*. 2016 Nov 8;9:237. doi: 10.1186/s13068-016-0657-0

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