

## **Biorefinery of Lignocellulosic Carbohydrates: Production of Lipid and Ethanol through Engineered Microbial Conversion**

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**Project Goals:** CABBI intends to develop efficient ways to grow bioenergy crops, innovate green bioprocessing technologies to transform biomass into valuable platform chemicals including lipids, sugars and alcohols, and market the resulting biofuels and other bioproducts.

Lignocellulose is regarded as renewable biomass for the production of biofuels and biochemicals. Glucose and xylose are the major sugars which can be recovered from cellulose and hemicellulose by hydrolysis. These cellulosic sugars can be used to produce biofuels and value-added bioproducts, such as lipids and ethanol. Bioenergy sorghum has been seen as a potential energy crop due to its high biomass productivity, drought tolerance, and wide environmental adaptability (Rooney et al., 2007, Cheng et al., 2019). In this study, lipids and ethanol were produced from bioenergy sorghum syrups using engineered yeasts. Bioenergy sorghum was hydrothermally pretreated at 50% solids loading in a continuous reactor system. Pretreated biomass was mechanically refined using a burr mill to further improve biomass accessibility for hydrolysis. Fed-batch enzymatic hydrolysis was conducted with 50% w/v solids loading to achieve 230 g/L sugar concentration. Different strains of *Rhodosporidium toruloides* were used to ferment sugars into lipids, and the highest lipid yield of 9.2 g/L was observed. The lipid yield was improved to 17.8 g/L by shifting the C/N ratio from 80 to 1700 to trigger the lipid production in the second-stage fermentation. For ethanol production, the engineered *Saccharomyces cerevisiae* SR8 δ-ADH6 was utilized to co-ferment glucose and xylose. Additionally, the effects of nutrient media (YP, YNB/Urea, and Urea), cellulosic sugar concentration, and sulfite addition were investigated to optimize the ethanol yield from sorghum syrups. The sugar concentration significantly affected the ethanol yield. The optimal ethanol yield at 73.3% was obtained from the fermentation of YNB/Urea broth consisting of 34 g glucose/L and 17 g xylose/L.

### **References:**

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- 2, Cheng, M.-H., Dien, B.S., Lee, D.K., Singh, V. (2019) Sugar production from bioenergy sorghum by using pilot scale continuous hydrothermal pretreatment combined with disk refining. *Bioresource Technology* 289: 121663.

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