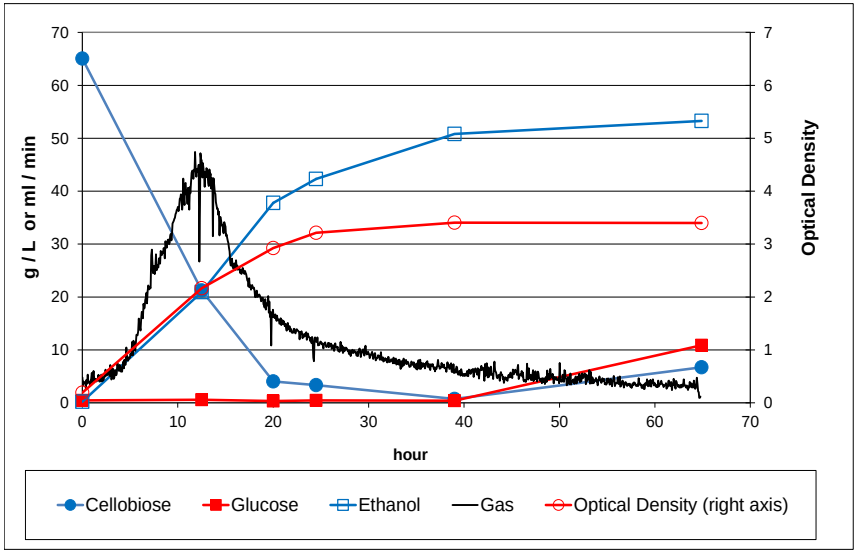
Improved thermophilic anaerobe achieves high biofuel titers from wood and cellulose

U.S. DEPARTMENT OF ENERGY | Office of Science

Background

- The thermophilic, anaerobic bacterium *T. saccharolyticum* digests hemicellulose and utilizes the major sugars present in biomass.
- It was previously engineered to produce ethanol at yields equivalent to yeast, largely by typical knockouts for undesired fermentation products (e.g., lactate, acetate).
- While saccharolytic anaerobes have been studied as potential biomass-fermenting organisms, development for commercial ethanol production have not been reported.



Approach

- The final strain M2886 was generated by removing genes for exopolysaccharide synthesis, the regulator *perR*, and reintroduction of phosphotransacetylase and acetate kinase into the methyglyoxal synthase gene. It was also subjected to multiple rounds of adaptation and selection, resulting in mutations later identified by resequencing.

Outcome

- We achieved the highest ethanol titers to date from *Thermoanaerobacterium saccharolyticum* during a four-year project to develop it for industrial production of ethanol from pretreated hardwood. The highest ethanol titer achieved was 70 g/L in batch culture with a mixture of cellobiose and maltodextrin.
- In a “mock-hydrolysate” SSF with Sigmacell-20, glucose, xylose, and acetic acid, an ethanol titer of 61 g/L was achieved, at 92% of theoretical yield. Fungal cellulases were rapidly inactivated under these conditions and had to be supplemented with cellulosomes from *C. thermocellum*. Ethanol titers of 31 g/L were reached in a 100 liter SSF of pretreated hardwood and 26 g/L in a fermentation of a hardwood hemicellulose extract.

Significance

- This study demonstrates that thermophilic anaerobes are capable of producing ethanol at high yield and at titers greater than 60 g/L from purified substrates, but challenges were encountered with existing fungal cellulases and inhibitors from pretreatment. The high titers and yields we observed support the feasibility of using engineered thermophiles for industrial ethanol production if challenges associated with pretreatment inhibitors can be avoided.

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Herring *et al.*, “Strain and bioprocess improvement of a thermophilic anaerobe for the production of ethanol from wood.” *Biotech Biofuels* (2016), accepted.