Improving pretreatment inhibitor tolerance in *Clostridium thermocellum*

U.S. DEPARTMENT OF ENERGY | Office of Science

Background

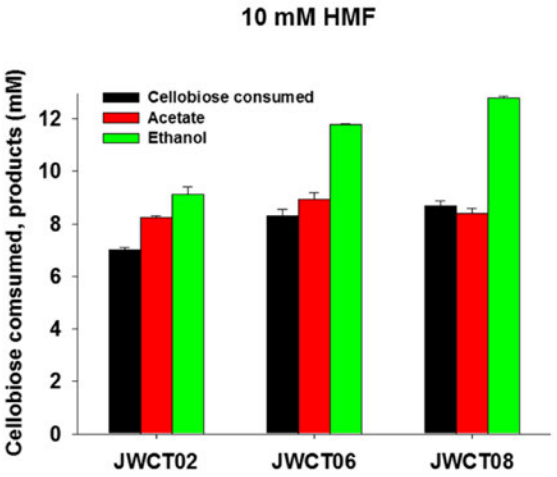
- Lignocellulosic biomass is generally pretreated with chemicals and heat to improve subsequent solubilization; however, microbial inhibitors including furfurals are generated from this process.
- Engineering increased tolerance to inhibitors in a leading BESC consolidated bioprocessing microbe, *C. thermocellum*, will improve robustness during conversion of pretreated biomass.

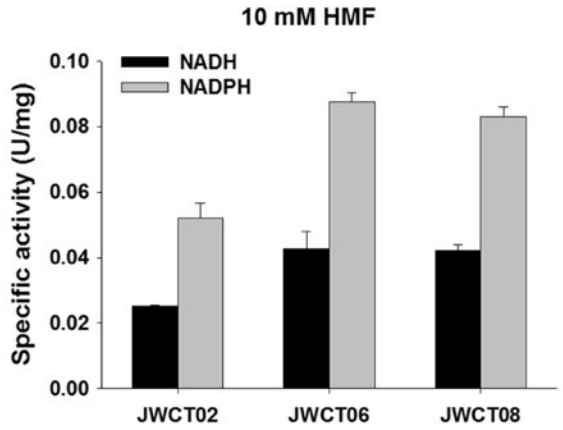
Approach

- Previous BESC research identified an enzyme (BdhA) from a thermophilic microbe that reduces furfural and 5-hydroxymethyl furfural (HMF) toxicity.
- Novel shuttle vectors were constructed to express the *BdhA* gene (*Teth39_1597*) from two different strong promoters in *C. thermocellum*.
- The recombinant strains were tested for tolerance to furan aldehydes.

Outcome

- BdhA was expressed in the background strain (JWCT02) using either the S-layer or enolase recombinant promoters in strains JWCT06 and JWCT08, respectfully.
- Activity assays confirmed that overexpression of BdhA in *C. thermocellum* resulted in higher hydroxymethylfurfural reduction rates, especially with added NADPH.
- Improved substrate utilization and higher ethanol titers were observed in the *BdhA* expression strain in the presence of 10 mM HMF.





Significance

- Bioconversion performance in the presence of common pretreatment inhibitors was improved through targeted strain engineering thus reducing barriers to achieving a robust consolidated bioprocessing strategy with *Clostridium thermocellum*.

Kim S-K, Groom J, Chung D, Elkins J, Westpheling J., "Expression of a heat-stable NADPH-dependent alcohol dehydrogenase from *Thermoanaerobacter pseudethanolicus* 39E in *Clostridium thermocellum* 1313 results in increased hydroxymethylfurfural resistance," *Biotechnol. Biofuels*. 10:66, 2017. DOI 10.1186/s13068-017-0750-z

BESC BioEnergy Science Center