Thermodynamic analysis of *C. thermocellum* glycolysis using deuterated water (^2H_2O) during high substrate loading fermentations

Melanie M Callaghan*[1,2] (callaghan2@wisc.edu), Tyler B Jacobson[1,2], David M Stevenson[1,2], Daniel G Olson[1,3], Lee R lynd[1,3], Daniel Amador-Noguez[1,2], and **Gerald A. Tuskan**[2]

[1]University of Wisconsin-Madison, Madison, WI; [2]Center for Bioenergy Innovation, Oak Ridge National Laboratory, TN; [3]Dartmouth College, Hanover, NH.

cbi.ornl.gov

The Center for Bioenergy Innovation (CBI) vision is *to accelerate domestication of bioenergy-relevant, non-model plants and microbes to enable high-impact innovations at multiple points in the bioenergy supply chain.* CBI addresses strategic barriers to the current bioeconomy in the areas of 1) high-yielding, robust feedstocks, 2) lower capital and processing costs via consolidated bioprocessing (CBP) to specialty biofuels, and 3) methods to create valuable byproducts from the lignin. CBI will identify and utilize key plant genes for growth, composition, and sustainability phenotypes as a means of achieving lower feedstock costs, focusing on poplar and switchgrass. We will convert these feedstocks to specialty biofuels (C4 alcohols, C6 esters and hydrocarbons) using CBP at high rates, titers and yield in combination with cotreatment, pretreatment or catalytic upgrading. CBI will maximize product value by *in planta* modifications and biological funneling of lignin to value-added chemicals.

Clostridium thermocellum is a highly efficient cellulolytic anaerobe bacterium for use in CBP of biomass which can be metabolically engineered to produce C_2 and C_4 alcohols. We continue to work at improving titer, yield and rate for these conversions. Thermodynamics constitutes a key determinant of flux and enzyme efficiency in metabolic networks. A biochemical reaction with a strong thermodynamic driving force will achieve a higher net flux given a fixed amount of enzyme than one closer to equilibrium. Within a pathway, steps closer to equilibrium will be the least enzyme efficient. Thermodynamic analysis can therefore provide unique insights in synthetic pathway design by identifying bottlenecks, pinpointing the enzymes for which changes in activity will have the largest effect on flux, and predicting the most efficient route for product synthesis. Previously, we used ^{2}H-glucose and ^{13}C-glucose as isotope tracers to investigate the *in vivo* reversibility and thermodynamics of the central metabolic networks of *C. thermocellum*, *T. saccharolyticum,* and anaerobically grown *Escherichia coli*. We found that the glycolytic pathway in *C. thermocellum* operates remarkably close to thermodynamic equilibrium, with an overall drop in Gibbs free energy 5-fold lower than that of *T. saccharolyticum* or anaerobically grown *Escherichia coli* [1, 2]. We now hypothesize that the limited thermodynamic driving force of glycolysis in *C. thermocellum* limits ethanol titers in high substrate loading fermentations. To test this hypothesis, we are developing the use of deuterated water (^2H_2O) as a cost-efficient tracer to measure how the thermodynamics of *C. thermocellum*'s glycolytic and fermentative pathways change dynamically during high substrate loading fermentations. Here, we present the initial results of this novel isotope-tracer approach. This work will aid in the construction of accurate metabolic models that incorporate thermodynamic constraints, identify potential bottlenecks, and guide fast rational engineering of microbial networks.

References/Publications

1. Jacobson TB, Korosh TK, Stevenson DM, Foster C, Maranas C, Olson DG, Lynd LR, Amador-Noguez D. 2020. *In vivo* thermodynamic analysis of glycolysis in *Clostridium thermocellum* and *Thermoanaerobacterium saccharolyticum* using ^{13}C and ^{2}H tracers. mSystems 5:e00736-19. doi: 10.1128/mSystems.00736-19
2. Cui J, Stevenson D, Korosh T, Amador-Noguez D, Olson DG, Lynd LR (2020). Developing a cell-free extract reaction (CFER) system in *Clostridium thermocellum* to identify metabolic limitations to ethanol production. Front. Energy Res. 8:72. doi: 10.3389/fenrg.2020.00072

The Center for Bioenergy Innovation is a U.S. Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science.