

## Quantitative Network Analysis in *Clostridium thermocellum* using $^{13}\text{C}$ and $^2\text{H}$ -tracers

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**Project Goals: 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 will address 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 and C6 esters) using CBP at high rates, titers and yield in combination with cotreatment or pretreatment. CBI will maximize product value by *in planta* modifications and biological funneling of lignin to value-added chemicals.**

Recent advances in genetic engineering have enabled production of a small suite of specialty biofuels at a commercial scale using industrially relevant organisms such as *Saccharomyces cerevisiae* and *Escherichia coli*. However, industrial biotechnology has been hampered by low titers, yields, and productivities (1). Product toxicity has long been cited to be one of the main limitations to achieving high product titers and has often been linked to physiochemical effects on the cell membrane (2–4). However, thermodynamic constraints can also limit the metabolic potential of a given host to produce a particular compound (5) by affecting the rate of a biochemical reaction or pathway through the flux-force relationship (6).

Thermophilic, cellulolytic anaerobic bacteria such as *Clostridium thermocellum* have a pyrophosphate dependent metabolism in close proximity to thermodynamic equilibrium (7), which is thought to help with ATP yield over traditional glycolysis (8). This energetic efficiency may be causing the current ethanol titer limit of  $\approx 30$  g/L produced by metabolically engineered strains of *C. thermocellum*, which is in stark contrast to the 70 g/L titer achieved by metabolically engineered strains of the hemicellulose-fermenting *Thermoanaerobacterium saccharolyticum* (9).

In order to test the relationship of the thermodynamic driving force to product titers, we used metabolic flux analysis in conjunction with flux ratio analysis to compare the Gibbs free energy values and fluxes of wild-type *C. thermocellum* and *T. saccharolyticum* to metabolically

engineered strains in the presence of isotopically labelled ethanol. In the present study we identify GAPDH, TPI, and ADH as close to thermodynamic equilibrium in wild-type *C. thermocellum*, compare the effects of alternative pyruvate oxidation pathways towards increasing the thermodynamic driving force and altering fluxes of fermentation pathways, and suggest further engineering strategies to overcome metabolic bottlenecks.

## References

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