Absolute Quantitation of Intracellular Metabolite Concentrations in *C. thermocellum* and *T. saccharolyticum*

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Project Goals: The BioEnergy Science Center (BESC) is focused on the fundamental understanding and elimination of biomass recalcitrance. BESC's approach to improve accessibility to the sugars within biomass involves (1) designing plant cell walls for rapid deconstruction and (2) developing multi-talented microbes or converting plant biomass into biofuels in a single step [consolidated bioprocessing (CBP)]. BESC research in biomass deconstruction and conversion targets CBP by studying thermophilic anaerobes to understand novel strategies and enzyme complexes for biomass deconstruction and manipulating these microorganisms for improved conversion, yields, and biofuel titer. BESC researchers provide enabling technologies in biomass characterization, 'omics, modeling and data management in order to (1) understand chemical and structural changes within biomass and (2) to provide insights into biomass formation and conversion mechanisms.

To provide quantitative metabolomics data that supports the development of mathematical metabolic models in *C. thermocellum* and *T. saccharolyticum*, we have used LC-MS/MS and an isotope-ratio based approach to measure intracellular metabolite concentrations of central carbon metabolites (Glycolysis, TCA cycle, amino acids, nucleotides, cofactors, etc.) in *C. thermocellum* and several engineered *T. saccharolyticum* strains. The strains were grown on minimal media on cellubiose. We have now quantitated a set of ~100 metabolites. Our results show large differences in the metabolome of *C. thermocellum* vs. *T. saccharolyticum* and point to metabolic bottlenecks and inefficiencies that could be corrected via metabolic engineering. These measurements and results will facilitate the development of quantitative and dynamic models of *T. saccharolyticum* and *C. thermocellum* metabolism and ethanol fermentation pathways.

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