

6. Consolidated Bioprocessing of Cellulose to Isobutanol in *Clostridium thermocellum*

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Project Goals: BESC biomass formation and modification research involves working directly with two potential bioenergy crops (switchgrass and Populus) to develop varieties that are easier to break down into fermentable sugars. We are using both testing and generation of large numbers of natural and modified plant samples as well as developing genomics tools for detailed studies into poorly understood cell wall biosynthesis pathways. BESC research in biomass deconstruction and conversion targets consolidated bioprocessing (CBP) by studying model organisms and thermophilic anaerobes to understand novel strategies and enzyme complexes for biomass deconstruction.

Biofuels from cellulose could effectively lower cost compared to those from corn or sugar sources. However, biomass recalcitrance currently limits the use of lignocelluloses. CBP is a potential solution in which biomass hydrolysis and fermentation occur simultaneously. *Clostridium thermocellum* is an attractive thermophilic CBP host because of its high cellulose utilization rate. In addition, isobutanol is an emerging biofuel with comparable energy density to gasoline and compatibility with existing infrastructures. Here, we seek to improve isobutanol titer using an engineered *C. thermocellum*. However, *C. thermocellum* cellulosic isobutanol production includes several challenges: (1) lack of suitable overexpression system and (2) limited enzyme stability at elevated temperatures. We first developed a small scale fermentation condition to quickly test isobutanol production. Then we applied a medium-throughput workflow to design, assemble, transform different plasmids and directly screen *C. thermocellum* recombinants for isobutanol production. Our best-engineered strain, CT24, produced 2 g/L isobutanol from cellulose at 50°C.

In addition, we developed a high-throughput enzyme assay to screen for improved thermostability of keto-isovalerate decarboxylase (KIVD). The evolved enzyme, LLM2, remains active after 20 minute incubation at 56°C. With LLM2 overexpressed, the engineered strain CT230 achieves a 0.6 g/L isobutanol titer at 55°C.

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