

## **Consolidated Bioprocessing of Cellulose to Isobutanol in *Clostridium thermocellum***

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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.

CBP has the potential to reduce biofuel or biochemical production costs by processing cellulose hydrolysis and fermentation simultaneously without the addition of pre-manufactured cellulases. In particular, *Clostridium thermocellum* is a promising thermophilic CBP host because of its high cellulose decomposition rate. Here we report the engineering of *C. thermocellum* to produce isobutanol. Metabolic engineering for isobutanol production in *C. thermocellum* is hampered by enzyme toxicity during cloning, time-consuming pathway engineering procedures, and slow turnaround in production tests. In this work, we first cloned essential isobutanol pathway genes under different promoters to create various plasmid constructs in *Escherichia coli*. Then, these constructs were transformed and tested in *C. thermocellum*. Among these engineered strains, the best isobutanol producer was selected and the production conditions were optimized. We confirmed the expression of the overexpressed genes by their mRNA quantities. We also determined that both the native ketoisovalerate oxidoreductase (KOR) and the heterologous ketoisovalerate decarboxylase (KIVD) expressed were responsible for isobutanol production. We further found that the plasmid was integrated into the chromosome by single crossover. The resulting strain was stable without antibiotic selection pressure. This strain produced 5.4 g/L of isobutanol from cellulose in minimal medium at 50°C within 75 h, corresponding to 41% of theoretical yield.

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