Closing the Carbon Cycle: Design, Optimization and Scaling-Up Production of Carbon-Negative Platform Chemicals

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Project Goals: Non-model organisms have unique traits and offer significant advantages and benefits for biomanufacturing. One example is gas fermenting acetogens capable of converting low cost waste feedstocks to fuels and chemicals, deployed today at commercial scale for conversion of steel mill emissions to ethanol. Yet, engineering these non-model organisms is challenging due to lower transformation and recombination efficiencies, longer cycle times and a more limited set of genetic tools compared to model organisms *E. coli* or yeast.

Cell-free systems can guide and accelerate non-model organism strain development. We are establishing a new interdisciplinary venture, the clostridia Foundry for Biosystems Design (cBioFAB) that combines advancements in cell-free and *Clostridium* engineering metabolic engineering to develop industrial-robust production strains for conversion of lignocellulosic biomass to next-generation biofuels and bioproducts such as acetone, butanol, 3-hydroxybutyrate (3-HB), 1,3-butanediol (1,3-BDO) or monoethylene glycol (MEG).

Acetone and isopropanol are important industrial bulk and platform chemicals, exclusively produced from fossil resources today. We have developed a sustainable and commercially relevant route from abundant, low-cost waste feedstocks—such as industrial waste gases or biomass syngas—by engineering autotrophic acetogen, *Clostridium autoethanogenum*. To achieve this, we constructed and screened a combinatorial biosynthetic pathway library using genes derived from a historical industrial strain collection and enzyme engineering. To optimize flux, we performed strain engineering using omics analysis, kinetic modelling, and cell-free prototyping to identify competing interactions between heterologous enzymes and native metabolism. We developed and scaled up a continuous fermentation process in an industrial pilot plant, consistently demonstrating high selectivities (~90%) and productivities (~3 g/L/h) for extended periods (>3 weeks). Life cycle analysis confirmed significant (>165%) greenhouse gas

savings. We show that acetogens, despite living on the edge of life, can be efficient cell factories for chemicals production.

References

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