Systems biology towards a continuous platform for biofuels production

Adam J. E. Freedman[1], Jason T. Boock[2], Geoffrey Tompsett[3], **Kristala L. J. Prather[2], Michael T. Timko[3]** and **Janelle R. Thompson*[1] (jthompson@mit.edu)**

[1]Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge MA; [2]Chemical Engineering, Massachusetts Institute of Technology, Cambridge MA; [3]Chemical Engineering, Worcester Polytechnic Institute, Worcester, MA

Project Goals: Environmental strain isolation provides an opportunity to discover organisms with unique growth characteristics and physiological traits to overcome bioprocess challenges including end product toxicity, culture contamination and low temperature growth. We hypothesize that an environmental isolate of *B. megaterium* SR7, which we recovered from a deep subsurface supercritical carbon dioxide ($scCO_2$) well, will provide bioprocess advantages when cultured in a dual-phase reactor of growth media and $scCO_2$. We are working towards the following goals (1) Develop *B. megaterium* into a bioproduction host for biofuels (2) Engineer de novo pathways for biosynthesis of longer chain fuels in *B. megaterium* and (3) Develop and model a two-phase stripping chemostat for continuous biosynthesis and in situ extraction of biofuels.

Abstract: To develop strain SR7 for use in dual-phase bioreactors we have carried out genome sequencing and functional annotation, physiological growth characterization, and established a toolset for genetic modification. After sequencing *B. megaterium* SR7's 5.45 Mbp genome, natural metabolite profiles affirmed the strain's use of glycolysis and the TCA cycle for anaerobic energy generation, as expected by functional genomic annotations. Process improvements, including optimized minimal media formulation and altered mixing regimes, established consistent growth at 1 atm CO_2 as a higher throughput model system for $scCO_2$ conditions. Based on findings from 1 atm CO_2 cultures, we amended $scCO_2$ media obtaining improved growth under $scCO_2$.

We developed a genetic system for strain SR7 using a protoplast-based transformation protocol employing a *B. megaterium* compatible plasmid that is maintained in SR7 for at least 100 hours in both aerobic and anaerobic cultures. We also identified xylose- and IPTG-inducible promoters capable of 10- to 250-fold inducible heterologous protein expression under aerobic and 1 atm CO_2 conditions; the specific IPTG promoter has not been demonstrated in *B. megaterium* previously. We engineered SR7 to produce isobutanol by introducing a two-enzyme (2-ketoisovalerate decarboxylase (KivD) and alcohol dehydrogenase (Adh)) pathway and feeding 2-ketoisovalerate. The two-step conversion occurs at approximately 70-80% substrate conversion in aerobically and 1 atm CO_2 grown cells. However, the intermediate aldehyde was found to accumulate at short culture times. Due to the high partition coefficient for the aldehyde to the $scCO_2$ phase, five alternative alcohol dehydrogenases were tested. A variant was found that lowered the build-up of isobutyraldehyde and resulted in conversion of 2-ketoisovalerate to isobutanol above 85%.

In addition to systems work on SR7, we designed and constructed a bench-scale, continuous flow, pressurized fermentation system centered on a 300-mL high-pressure

chemostat that is equipped with specialized features for monitoring cell growth by optical density and measuring pH. Recent abiotic experimental modeling of butanol extraction by $scCO_2$ has enabled us to obtain mass transfer coefficients that will inform our chemostat design in order to minimize build-up of toxic products. We have begun process level energy balance calculations, aiming to identify conditions that optimize the energy return of fuel products relative to input energy costs.

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