Synthetic Biochemistry: Making Biofuels and Commodity Chemicals the Cell-Free Way

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The goal of the project is to develop cell-free production of isobutanol and terpene biofuels via commercially viable enzyme systems that can produce continuously for at least 1 week at volumetric productivities >1.5 g/L/hr, in > 90% yield and reach titers that lead to product phase separation. Significant enzyme engineering has been done on a large scale (*e.g.* on at least 10-20 individual enzymes) and the current isobutanol system is being scaled up for high-volume production.

Considerable effort is currently directed to engineer micro-organisms to produce useful chemicals. The greatest potential environmental benefit of metabolic engineering will be the production of high volume commodity chemicals, such as biofuels. Yet the high yields and concentrations required for the economic viability of low-value chemicals are particularly hard to achieve in microbes due to the myriad competing biochemical pathways and product toxicity. We are developing an alternative approach, which we call synthetic biochemistry. Synthetic biochemistry throws away the cells and builds biochemical pathways in reaction vessels using complex mixtures of isolated enzymes and extracted cofactors. As the only pathway in the vessel is the desired transformation, yields can approach 100%. The challenge for synthetic biochemistry is to replace the complex regulatory systems that exist in cells in a simplified form. We have designed and built highly robust systems that can operate continuously for long periods of time. We are working to improve longevity further and to scale synthetic biochemistry reactors. So far we have generated a highly stable enzyme system that provides continuous production of isobutanol, with titers of ~65 g/L and productivity of 1.6 g/L/hr.

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