**Optimization of Isobutanol Production by *Zymomonas mobilis***

Indro Ghosh[1]* (inghosh@wisc.edu), and **Robert Landick**[1]

[1]Great Lakes Bioenergy Research Center, University of Wisconsin-Madison

https://www.glbrc.org/research/conversion

**Project Goals:**

**We are optimizing the production of the 'Next-Gen' biofuel isobutanol by *Zymomonas mobilis*, a bacterial species capable of highly efficient fermentation. By expressing a novel set of genes, we have demonstrated production of isobutanol by *Z. mobilis*. To fully harness the rapid and efficient Glucose metabolism of *Z. mobilis*, we are eliminating metabolic bottlenecks in the conversion of Glucose to Isobutanol. Specifically, we will (1) exploit an engineered link between isobutanol production and growth in *Escherichia coli* to estimate best ratio of enzyme expression, (2) measure levels of intermediate metabolites in *E. coli* and *Z. mobilis* to identify bottlenecks, (3) adjust expression levels of pathway genes, and (4) disrupt competing metabolic pathways to maximize intracellular carbon diversion towards isobutanol. Applying this strategy of identifying bottlenecks before remedying them in an iterative fashion will greatly improve titers of isobutanol produced by *Z. mobilis* and help develop an industrially relevant producer strain.**

Efficient production of isobutanol by microbes on an industrial scale is of great interest. Isobutanol has been produced previously in *E. coli* and *Saccharomyces cerevisiae* using five genes; an overproduced valine precursor: α-ketoisovalerate (using *alsS, ilvC,* and *ilvD*) is converted to isobutanol (using *kivd,* and *adhA*). Predictions based on measured enzyme kinetics indicate that efficient isobutanol bioproduction will occur only when bottlenecks within the pathway are eliminated, and when synthesis of enzymes imposes minimal burden to cells. Optimizing enzyme expression levels will accomplish both of these requirements and allow efficient microbial production of isobutanol.

We have demonstrated production of isobutanol by *Z. mobilis* by expressing enzymes (*kivd, adhA*) that convert naturally produced α-ketoisovalerate into isobutanol. To increase titers of produced isobutanol, we are expressing additional genes (*alsS, ilvC, ilvD*) to convert pyruvate to isobutanol in *Z. mobilis*, something that is complicated by the frequent rejection of foreign genes by the innate immune systems of *Z. mobilis*. We are also testing multiple strategies to inactivate *Z. mobilis* Pdc, in order to redirect carbon flux away from the naturally produced ethanol, towards isobutanol. These approaches will hypothetically enable high titer isobutanol production from *Z. mobilis*.

Expression of an optimal ratio of enzymes in *Z. mobilis* will be aided by metabolic intermediate level measurements and additional studies in *E. coli*. A preliminary OptSSeq[1] experiment has

indicated that expression of *ilvC* is essential for efficient pathway function in *E. coli*, in line with expectations based on enzyme kinetics. Upon expressing an isobutanol producing cassette in *Z. mobilis*, we will measure levels of enzymes produced, and also levels of intermediate metabolites. Significant metabolite accumulations will be indicative of bottlenecks, and will guide the design of subsequent cassette iterations to remedy identified bottlenecks. An optimized strain developed using these strategies will be an industrially relevant isobutanol producer.

## Publications


1. Ghosh IN, Landick R (2016) OptSSeq: High-Throughput Sequencing Readout of Growth Enrichment Defines Optimal Gene Expression Elements for Homoethanologenesis. ACS synthetic biology 5:1519-1534



*This work was funded by the DOE Great Lakes Bioenergy Research Center.*
*(DOE BER Office of Science DE-FC02-07ER64494)*