Approaches for Evaluating the Production Potential of High Volume Products in Microbial Systems

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Project Goals: Metabolic models can be used to engineer biofuel production strains, where models can find bottlenecks in metabolic pathways, identify important regulatory interactions, and suggest perturbations to force a microorganism to produce or utilize more of a compound of interest. Metabolic modeling will be used to improve conversion of sugars derived from lignocellulosic biomass into specialty biofuels using different microbial platforms, including *Saccharomyces cerevisiae* and *Zymomonas mobilis*. Here we compared different chemical products (including biofuels) by evaluating their chemical production potential based on model-predicted maximum theoretical yields and productivities.

In order to identify the most promising candidates for next-generation biofuels and bioproducts, it is critical to evaluate the production potential of different chemical products in a variety of host organisms. Genome-scale metabolic models offer the ability to efficiently screen a wide variety of chemicals for production in different biological systems; however, traditional constraint-based modeling approaches have focused on calculating the maximum theoretical yields. This value often occurs at an unacceptably low biomass growth rate which results in low productivities. In order to alleviate this problem, a new approach has been developed which instead identifies the maximum theoretical productivity of a chemical production strain in a reactor. The productivity depends upon the type of reactor (*e.g.* chemostat or batch) and the mode of operation (*e.g.* continuous or induced).

This productivity analysis was applied to a large database of High Production Volume (HPV) chemicals totaling more than 3,500 chemicals. Productivity was evaluated in both *Escherichia coli* and *Saccharomyces cerevisiae* with the inclusion of heterologous reactions for non-native metabolites from reaction databases (*e.g.* KEGG and MetaCyc). This analysis shows differences between the maximum possible production rate and the maximum potential productivity. These differences could affect the screening criteria which would otherwise over- or under-estimate the true chemical production potential for different chemical products. Furthermore, the productivity captures differences in reactor types and conditions, allowing for development of strains in the most productive system. Further methods for identifying genetic strategies (*i.e.* growth coupling via gene knockouts) for achieving the maximum potential productivity have also been developed and can be used to design strains which achieve high productivities.

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