**479e Dynamic Flux Balance Analysis of Yeast Primary Metabolism in Fed-Batch Culture**

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Flux balance models of intracellular metabolism have received considerable attention over the past decade because they allow cellular physiology to be analyzed in the absence of enzyme kinetics. The metabolic reaction network and the stoichiometry of the individual reactions are reconstructed from a combination of genomic, biochemical and/or physiological data. Both small-scale pathway models and genome-scale cellular models have been developed for the yeast Saccharomyces cerevisiae. A common problem is that the set of linear algebraic equations are underdetermined because the number of unmeasured fluxes exceeds the number of stoichiometric equations. Flux balance analysis (FBA) is performed by posing a linear programming problem where a cellular objective such as growth rate maximization is assumed. Yeast models ranging from small-scale pathway descriptions to genome-scale reconstructions of whole cell metabolism have been validated with FBA.

Classical FBA requires that uptake rate measurements of the growth limiting substrates serve as inputs to the metabolic network. However most fermentations are carried out in a batch or fed-batch mode where only extracellular metabolite concentrations are measured. The dynamic flux balance analysis (DFBA) method was developed to incorporate extracellular metabolite dynamics and substrate uptake kinetics within the FBA framework. A flux balance description of intracellular metabolism is combined with dynamic mass balances on extracellular substrates and products to predict cellular behavior when the extracellular environment changes with time. DFBA has been used to simulate the dynamics of batch and fed-batch fermentations. A powerful feature of DFBA is that regulatory mechanisms such as saturation of the glucose transporter, growth inhibition by ethanol and the Crabtree effect can be incorporated via the substrate uptake rate kinetics. In this sense DFBA can be considered as a structured extension of unstructured kinetic modeling where the growth rate and product yields are computed from a mechanistic description of intracellular metabolism.

The objective of this paper is to develop a dynamic flux balance model of yeast primary metabolism and to utilize this model to evaluate the tradeoffs between cell growth and ethanol formation in fed-batch culture. Our computational studies are performed with a published flux balance model of S. cerevisiae primary metabolism. As originally formulated the uncompartmented flux model included 98 metabolites and 99 reactions involved in the following metabolic pathways and processes: membrane transport, glycolysis, pentose phosphate cycle, citric acid cycle, glyoxylate shunt, oxidative phosphorylation, fermentation, amino acid synthesis and polymerization, nucleotide synthesis, RNA synthesis, fatty acid synthesis, polysaccharide synthesis and biomass formation. By omitting reactions thought to be unimportant for fed-batch growth on glucose minimal media, the flux model was reduced to 85 reaction fluxes. We formulate stoichiometric equations for 68 metabolites assumed to remain at metabolic steady state. The underdetermined flux balance model is solved using linear programming where the cellular objective is maximization of growth rate.

A dynamic flux balance model is formulated by augmenting the stoichiometric equations with dynamic mass balance equations for extracellular glucose, ethanol, and liquid and gas phase oxygen. Simple Michaelis-Menten kinetics are used to model the oxygen uptake rate, while the glucose uptake rate is assumed to be inhibited by high extracellular glucose and ethanol concentrations. Oxygen solubility is modeled using Henry's law. A sequential solution strategy is used to dynamically simulate cellular growth and ethanol production for various glucose and oxygen feeding policies. For example air is fed at a constant flow rate during the aerobic portion of batch to increase the biomass concentration, and then the air flow is eliminated at a predetermined time to achieve anaerobic growth conditions that increase carbon flux to ethanol. We use the Matlab integration code ode15s and the Matlab compatible linear program code MOSEK to implement the sequential solution strategy. Our initial results demonstrate that

the final ethanol concentration is very sensitive to the air switching time. Additional simulation results will be described in the presentation.