



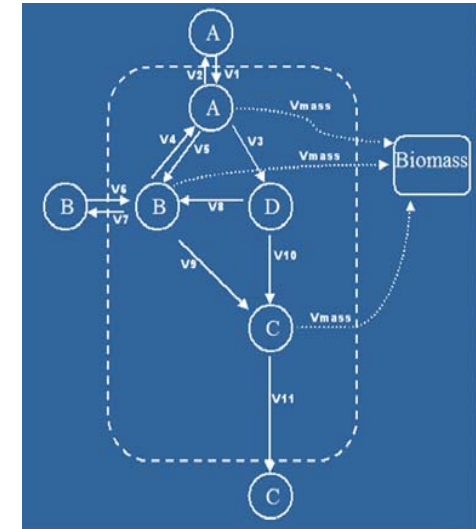
Validation of the Flux Balance Model of *Pichia pastoris*

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ABSTRACT

Validated *in silico* metabolic models have become an in-demand biotech product, as they allow for accurate virtual simulations and the manipulation of an organism's biological processes. One organism whose metabolic model would prove especially useful is the methylotrophic yeast *Pichia pastoris*. *P. pastoris* is widely used as a recombinant protein expression system, and construction of a metabolic model for *P. pastoris* would allow for the up/down regulation of key processes, opening the door for more efficient protein production. Such a model would be valuable in industrial-scale recombinant production and pharmaceutical development. Here at KGI, a Team Masters Project has collaborated with Genomatica to create a *P. pastoris* model in their SimPheny program by utilizing a modeling technique called flux balance analysis. Flux balance models quantify all the reactions in the metabolic pathway through stoichiometry, using mass and energy balances as constraints and thereby avoiding complicated kinetics. The main objective of our project was to validate the TMP model by comparing the predictions generated by SimPheny with experimental data collected through a *P. pastoris* fermentation. Samples were taken throughout the fermentation to examine oxygen consumption, carbon dioxide production, feed consumption, cell density, and biomass composition. This experimental data will be used in the future to assess the accuracy of the SimPheny simulation. *P. pastoris* growth rates from published literature were also compared to SimPheny predictions, and the model was found to be fairly accurate, especially for the experiments and modeling done by Jahic et. a. (2002) and Ren et. al. (2003). Additionally, single gene deletion simulations predicting mutant strain growth on specific carbon sources were run. From this data, essential and nonessential genes were identified, as well as carbon-source specific loci. Such substrate-specific genes can be examined as potential promoter targets for expression systems, such as the AOX1/methanol system currently used. Inconsistencies and inaccuracies in the model were also identified by examining deletion simulation results, and will need to be corrected pending the release of a complete *P. pastoris* annotation.



Pichia pastoris

