

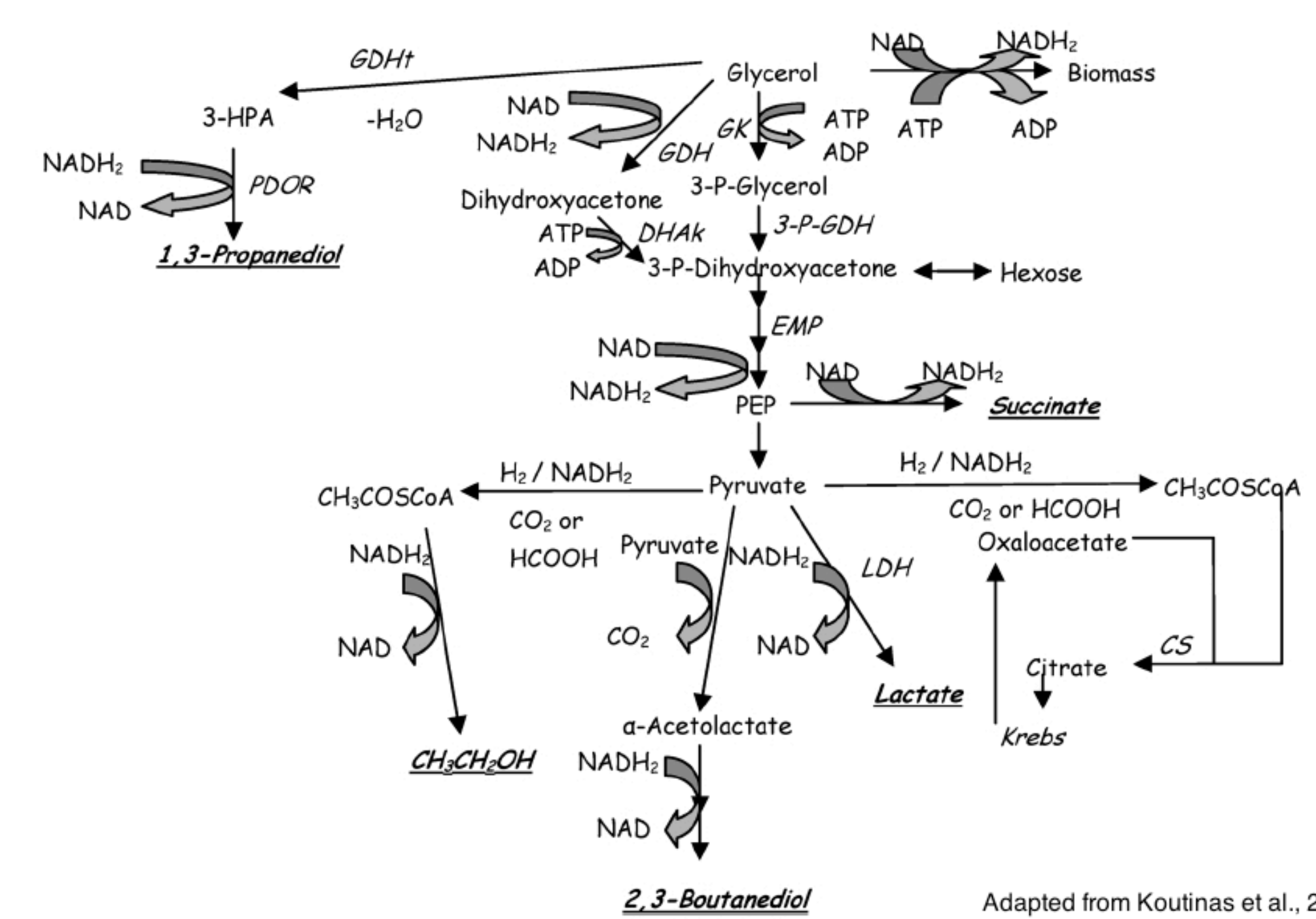
Representing batch fermentations combining Flux Balance Analysis and dynamic modeling

Olavarria, K. and Gomez, J.G.C.

Instituto de Ciências Biomédicas, Universidade de São Paulo, Brasil

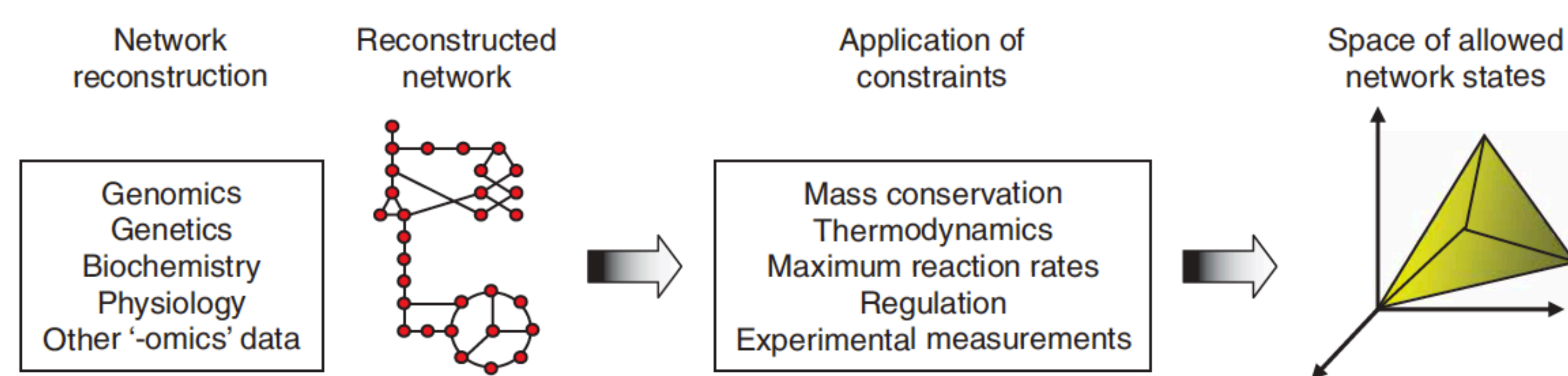
Summary: Flux Balance Analysis (FBA) is a popular tool to understand, analyze and represent some important aspects of the metabolism such as the mass conservation, cofactor balances and yields. Because FBA requires the existence of metabolic steady state and is “blind” to the changes in the metabolite concentrations, its application to analyze batch fermentations needs further considerations. In this panel we aim to show the combination of FBA with dynamical analysis to represent the temporal changes in the concentration of the carbon sources and some relevant products during a batch fermentation. Specifically, we considered the case where changes in the pH of the culture triggered the generation of some product. We illustrate the application of the developed tool to the mixed fermentation by *Klebsiella*.

Fermentation pathways in *Klebsiella*



Adapted from Koutinas et al., 2014

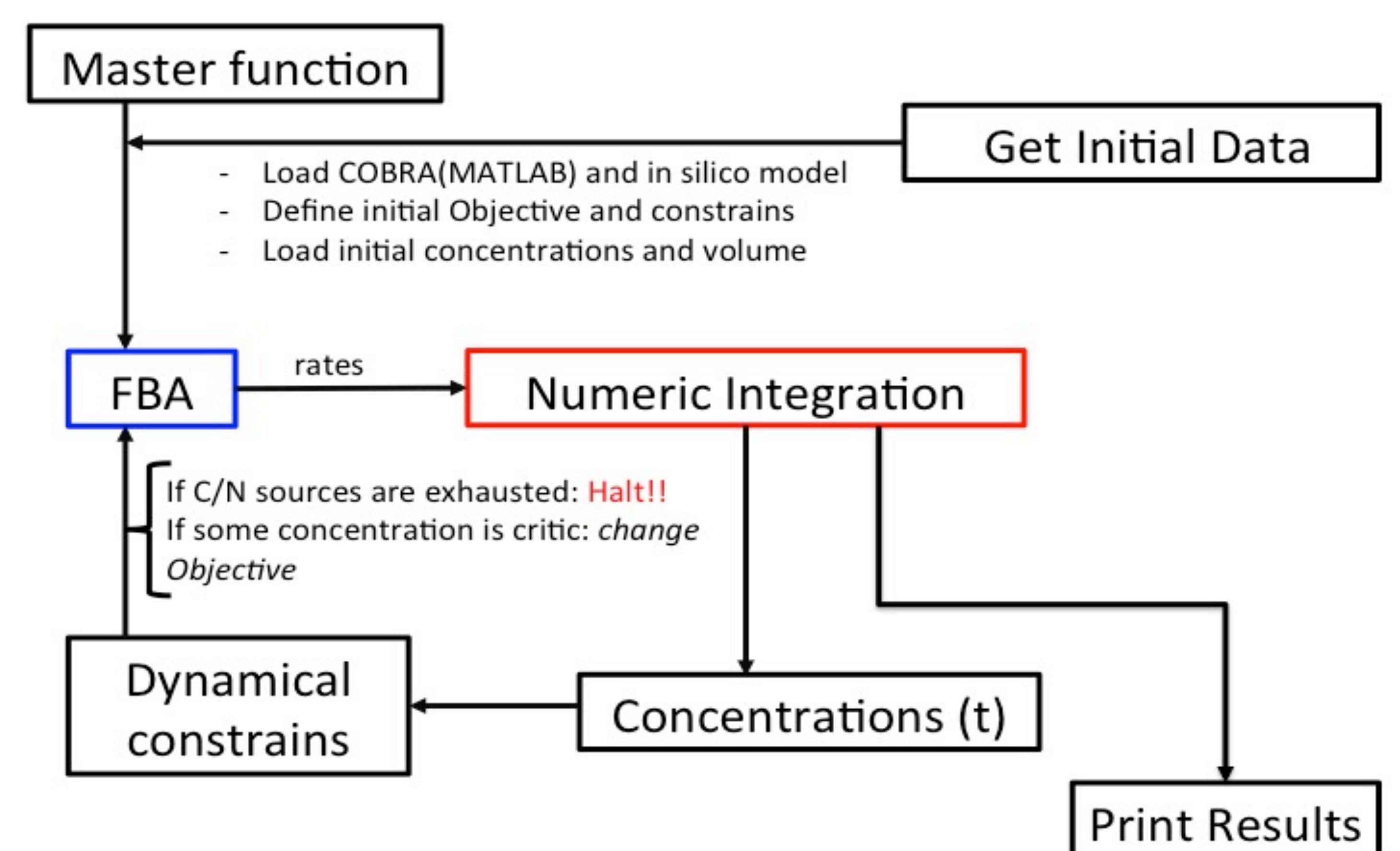
Flux Balance Analysis



Adapted from Schelleberger et al., 2011

FBA was performed with the COBRA toolbox (Schelleberger et al., 2011). The Ecolicore *in silico* model was modified to incorporate the fermentation pathways of *Klebsiella*, the Entner-Doudoroff pathway and the glycerol assimilation pathway. The Ecolicore originally comprises 95 reactions and 72 metabolites and it is freely available from the web site of the Systems Biology Research Group from the University of California San Diego. The numeric integration protocol was modified from Sánchez et al., 2014.

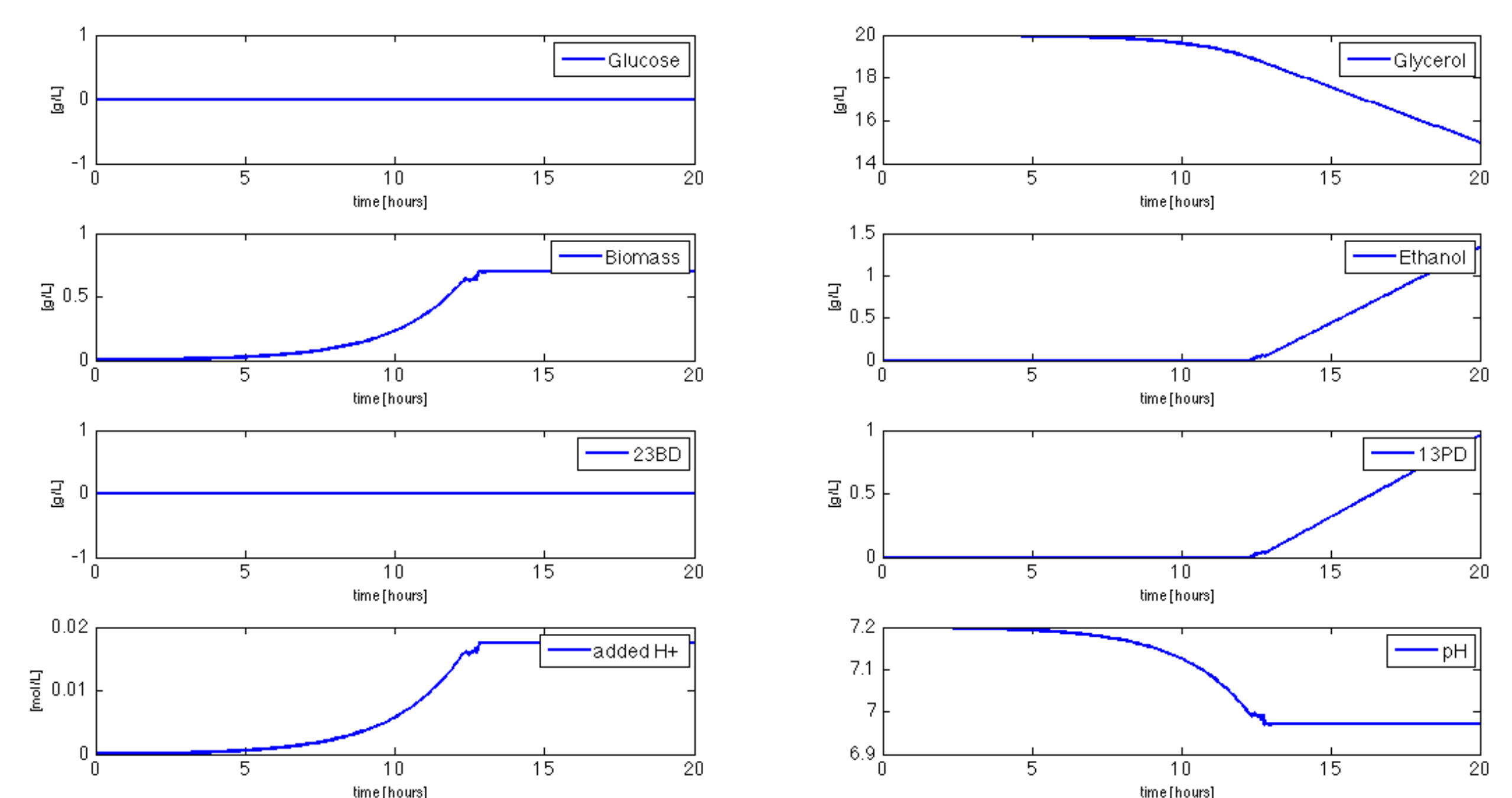
Algorithm



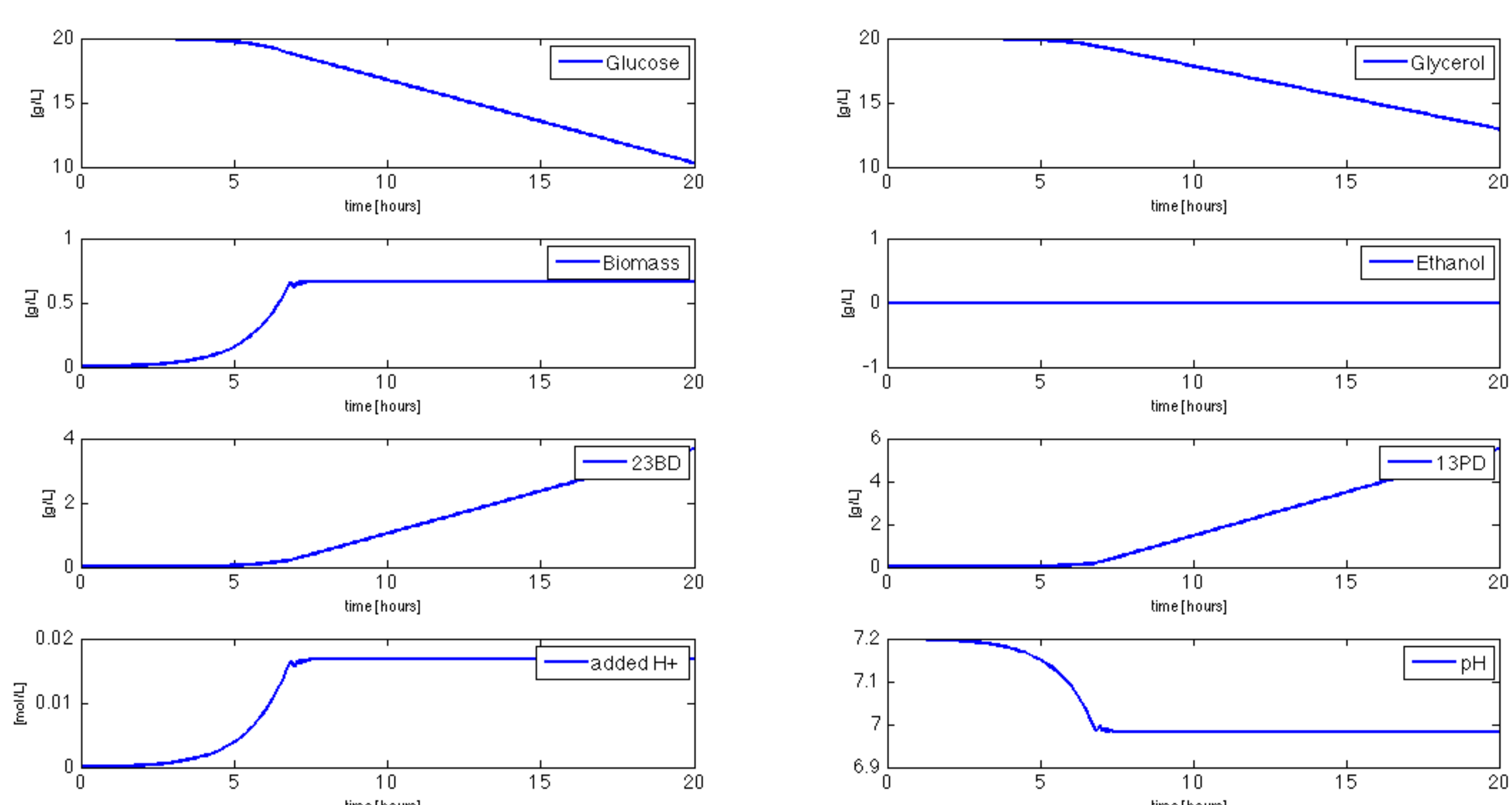
It had been reported an increase in the generation of dienes by *Klebsiella* when the pH of the culture media is decreased (Celińska and Grajek, 2009). It is believed that the generation of dienes allows the reoxidation of NADH without produce further organic acids that low the pH.

We calculated the amount of H⁺ generated during the cell growth. Considering a determinate culture volume, buffer concentration and pKa, we calculated the temporal changes in the pH. Once the culture arrive to a critic pH of 7, the simulation procedure change the **Objective** from mazimization of biomass formation to minimization of H⁺ generation.

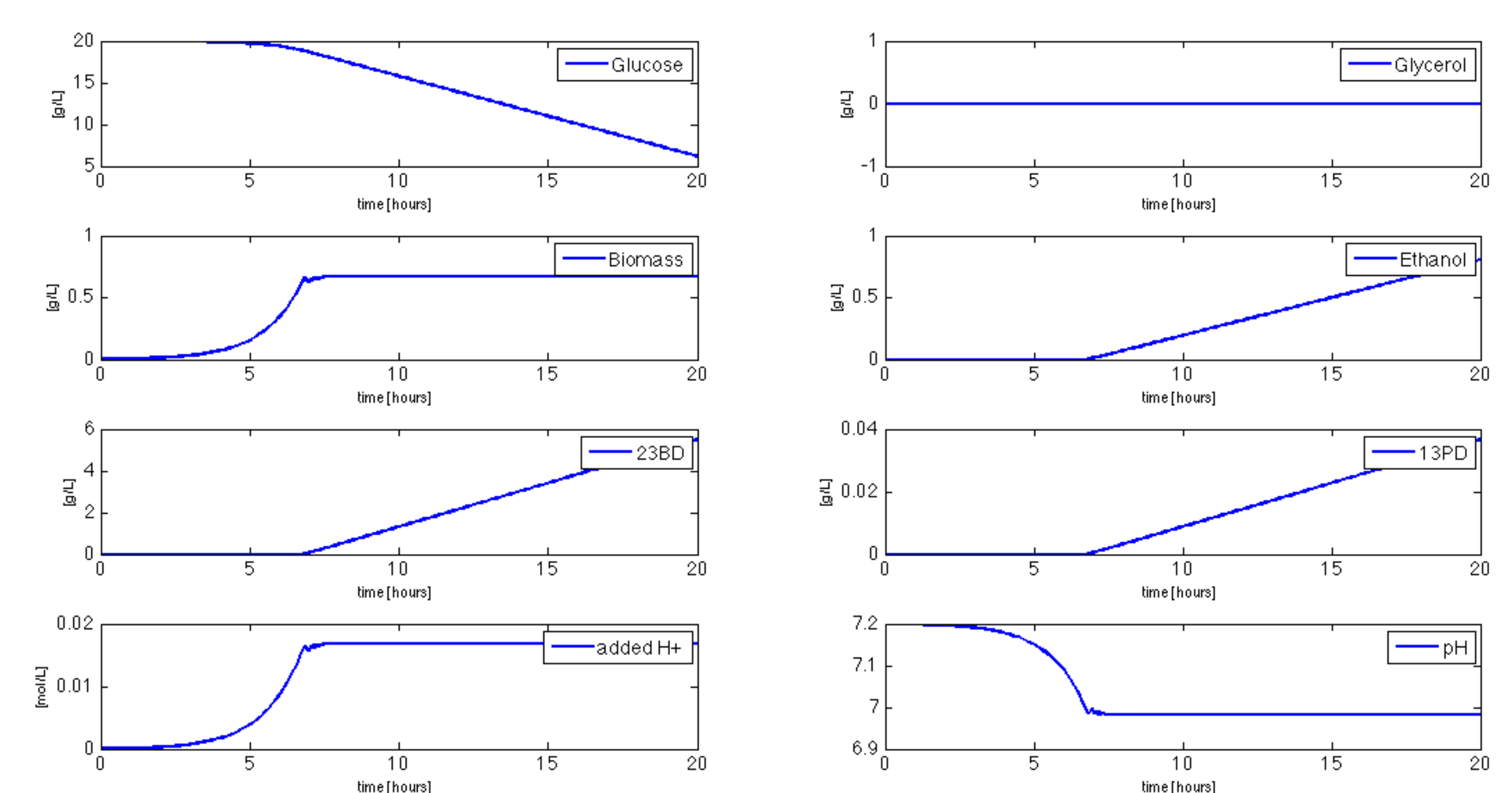
Glycerol



Co-feeding Glucose+Glycerol



Glucose



References:

- Celińska E. and W. Grajek. 2009. *Biotechnology Advances* 27, 715–725
- Koutinas, A. A. et al. 2014. *Chem Soc Rev* 43, 2587–2627.
- Orth et al. 2010. *Nat. Biotechnology* Vol. 28 No. 3.
- Sánchez et al. 2014. *Metab Eng* 25, 159–173.
- Schellenberger J. et al. 2011. *Nat. Prot.* Vol. 6, No. 9: 1290–1307.

Conclusion and Perspective:

We developed a computational tool able to perform FBA considering a dynamical change in the Objective function according with changes in the concentration of extracellular metabolites. It is required further refinement of this tool and test it against experimental data.

Funding: FAPESP 13/50357-2 and 13/24087-8