539d Sensitivity and Bifurcation Analysis of the Metabolism of *Escherichia Coli* at Optimal Enzyme Levels

Francisco G. Vital-Lopez, Costas D. Maranas, and Antonios Armaou

Cells are comprised of thousands of components with specific functions such as genes, enzymes and metabolites. The intricate interactions between these components make cells very complex systems. Due to this complexity, the design of microorganisms with specific capabilities is a difficult endeavor that requires systematic and efficient procedures. Even though a framework that takes in account all the features of the cell is not available yet, numerous researchers in this field have focused their efforts on uncovering the mechanisms that govern the cellular processes such as gene expression and metabolism. The study of metabolism is especially important in the optimization of bioprocesses. The main approaches in the analysis of the metabolism are based on the stoichiometry or in the kinetics of the enzymatic reactions that take place in the cell. The first methodologies characterize the space, constrained by the stoichiometry, of possible flux distributions [1, 2]. On the other hand, kinetic models can provide more accurate outcomes. In spite of kinetic parameters being difficult to obtain, large-scale models are now available and it is expected their number, complexity and accuracy will increase in the coming years [3]. Kinetic models allow us to determine the enzyme levels needed to accomplish optimal production of a given metabolite [4, 5]. Moreover, kinetic models make possible the analysis of stability of the predicted states, an issue that is important in view of the fact that biological systems may exhibit not only monotonic stable states but also bistable switching threshold phenomena, oscillations and chaotic behavior [6].

Since in practice it is extremely difficult to modulate the levels of all the enzyme of a cell, Nikolaev et al. developed a large-scale optimization framework to select the most promising subsets of enzymes and determine their optimal levels [4]. They demonstrated its application on a kinetic model of central carbon metabolism of *Escherichia coli* for the production of serine [5]. In this work we analyze the sensitivity and robustness of the states obtained at the optimal enzyme levels as determined by Nikolaev et al. We construct bifurcation diagrams considering the level of one and two of the selected enzymes as parameters. Accordingly, we determine the suitable parameter values for which the system operates in a region of high productivity but far enough of an undesired critical point. We also investigate the feasibility that the states can be driven to operate in a limit cycle and compare the productivity in each case.

[1] Schilling, C. H., Schuster, S., Palsson, B. O., and Heinrich, R., (1999). Metabolic pathway analysis: basic concepts and scientific applications in the post-genomic era. *Biotechnol. Prog.*, 15, 296-303.

[2] Pharkya, P., Burgard, A. P., and Maranas, C. D. (2003). Exploring the overproduction of amino acids using the bilevel optimization framework OptKnock. *Biotechnology and Bioengineering*. 84, 887-899.

[3] Chassagnole, C., Noisommit-Rizzi, N., Schmid, J. W., Mauch, K., and Reuss, M. (2002). Dynamic modeling of the central carbon metabolism of *Escherichia coli. Biotechnol. Bioeng.*, 79(1), 53-73.

[4] Nikolaev, E. V., P. Pharkya, C. D. Maranas and A. Armaou. Optimal selection of enzyme levels using large-scale kinetic models, *Proceedings of 16th I.F.A.C. World Congress*, to appear, Prague, Czech Republic, 2005.

[5] Visser, D., Schmid, J. W., Mauch, K., Reuss, M., and Heijnen, J. J. (2004). Optimal re-design of primary metabolism in *Escherichia coli* using linlog kinetics. *Metabolic Engineering*, 6, 378-390.

[6] Lloyd, D., Aon, M. A., and Cortassa, S. (2001). Why homeodynamics, not homeostasis? *The Scientific World*, 1, 133-145.