Dynamic Models of Biological Systems. A Hotbed for Nonlinear Analysis.

By

Doraiswami Ramkrishna
School of Chemical Engineering
Purdue University
West Lafayette, IN 47907

Chemical engineers have exploited nonlinear analysis of dynamical systems in diverse ways during the last century in the fields of chemical reaction engineering, fluid mechanics and transport processes. The objective of this presentation is to call attention to numerous topics in biological applications that represent opportunity areas for nonlinear analysis. The essential requirement of course is the availability of good dynamic models of such complex systems. While conservation principles and good constitutive supplements have proved sufficient for modeling of physical systems, biological systems add substantially to the challenge through features unique to them.

The unique features of biological systems are related to their capacity, acquired through exposure to eons of evolutionary pressures, to respond to their environment in ways that promote their survival. The consequence is an enormous increase in the diversity of responses of the system that modeling must necessarily represent within a dynamic framework. This requirement is enforced by the uncertainty surrounding our assessment of the organism's evolutionary experience that cannot be resolved without the institution of an interactive program of experiment and theory growing with contributions from each other. From this perspective, the opportunity in this area is not to be viewed simply as a Mecca for modelers for solely intellectual contentment. Rather, it will promote understanding of complex systems through the development of new experimental protocols that will provide a deeper setting for uncovering intriguing behavior. Some examples will be discussed.

The modeling framework involves viewing metabolism as a combination of so-called elementary modes[1] along which external substrates are taken up for metabolic activity relating to the production of various intracellular metabolites, biomass and fermentation products. The elementary modes represent multiple options for the organism. While steady state methodologies postulate a fixed distribution of external substrate among various elementary modes, the cybernetic approach of Ramkrishna and coworkers postulates a distribution *controlled* so that global objectives such as maximization of growth rate or uptake rate of substrate is accomplished. In particular, the hybrid model (Kim et al., 2008[2]; Song and Ramkrishna[3]) postulates cybernetic control of substrate uptakes through elementary modes so that a global objective such as the maximization of

[1] S. Schuster, D. Fell, T. Dandekar, *Nature Biotech*.,**18**, 326-332, 2000
[2] J. Kim, J. Varner, D. Ramkrishna, *Biotechnol. Prog*., in press.
[3] Song, Hyun-Seob and Doraiswami Ramkrishna, "Reduction of a Set of Elementary Modes by Yield Vector Analysis," *Biotechnol & Bioeng.,* in press.

substrate is realized, while internal metabolism in each mode occurs at steady state with respect to the uptake rate through that mode. The resulting dynamic models are equipped to handle large metabolic networks including the role of regulatory processes.

As an example, anaerobic growth of *E coli* is considered in a mixture of glucose and pyruvate. The organism can consume the two substrates either sequentially or simultaneously depending on the concentrations of the two substrates and the organism's preculturing. The metabolic network used for the modeling is shown in Figure 1.

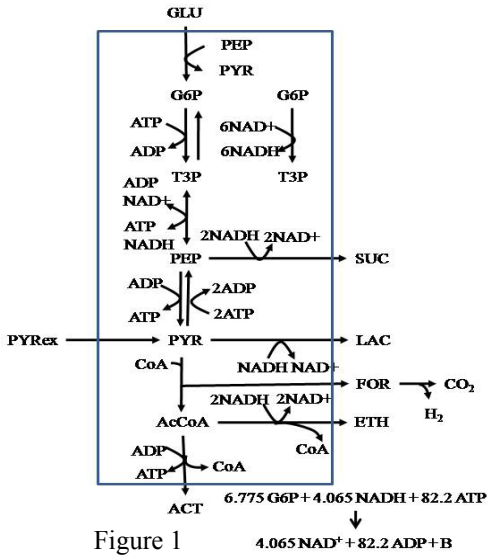

Figure 1

The batch experiments of anaerobic *E.coli* (strain GJT001), obtained from Ka-yiu San's group of Rice University, were implemented to identify the kinetic parameters in the hybrid model.

The reduced metabolic network of anaerobic *E.coli* is shown in "Fig. 1". All biochemical reactions in the reduced network is originated from Young's reduced network.[4] However, some modifications were made based on the information in the website of B.Ø.Palsson's group[5], since the pyruvate uptake and other reactions must be considered additionally.

Applying pseudo steady state assumption to internal species in "Fig. 1"., total 19 elementary flux modes were identified using standard software.[6]

Among a total of 19 elementary modes, 15 were eliminated to finally yield the four elementary flux modes displayed in "Table 1" - accomplished by fitting the cybernetic model parameters to the experimental data on all fermentation products, substrates, and biomass concentrations in batch cultures. To simplify the parameter fitting process, five modes were pre-eliminated as they did not appear to be competitive.

Table 1. Major elementary flux modes of anaerobic *E. coli*

I	Elementary flux mode ($\mathbf{z}_i$)
1	35.76 GLU = 57.96 FOR + 31.01 ACT + 26.95 ETH
2	150.76 PYR = 133.42 FOR + 133.42 ACT
3	13.55 GLU + 66.62 PYR = 80.17 FOR + 75.43 ACT + 4.74 ETH
4	8.81 GLU + 85.59 PYR = 89.65 FOR + 89.65 ACT

The four modes shown in Table 1 were treated as four competing major reactions of metabolism. Each reaction rate and enzyme synthesis were assumed to satisfy Michaelis-Menten kinetics, and those reactions were regulated by cybernetic variables to maximize the total carbon uptake rate. The model was simulated for elucidating the steady state picture as a function of dilution rate and glucose fraction (γ) in the feed. A narrow window of dilution rates was found displaying steady state multiplicity with hysteresis.

[4] J. Young, K. Henne, J. Morgan, A. Konopka, D. Ramkrishna, *Biotech. & Bioeng.*, **100**, 542-559, 2008.
[5] http://gcrg.ucsd.edu/downloads/index.html
[6] http://pinguin.biologie.unijena.de/bioinformatik/networks/metatool/new_metatool/new_metatool.html

This result was verified experimentally as shown in Figure 2. A total of 6 different shift-ups and shift-downs of dilution rates were made to check the predicted hysteresis with 3 steady states.

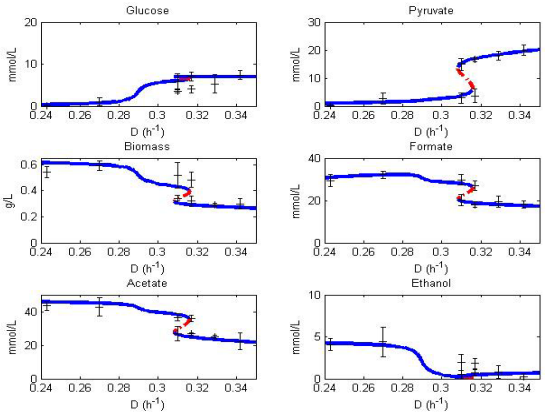

Fig. 2. Steady-state plots of continuous *E. coli* cultures at different dilution rates. (Solid line : Stable steady state plot, Dashed line : Unstable steady state plot, Error bars : 90% confidence interval of experimental data)

For higher concentration fractions γ of pyruvate, the steady state multipli-city was even higher as shown. Thus in Figure 3 are displayed as many as 5 steady states. Experimental verification is currently in progress.

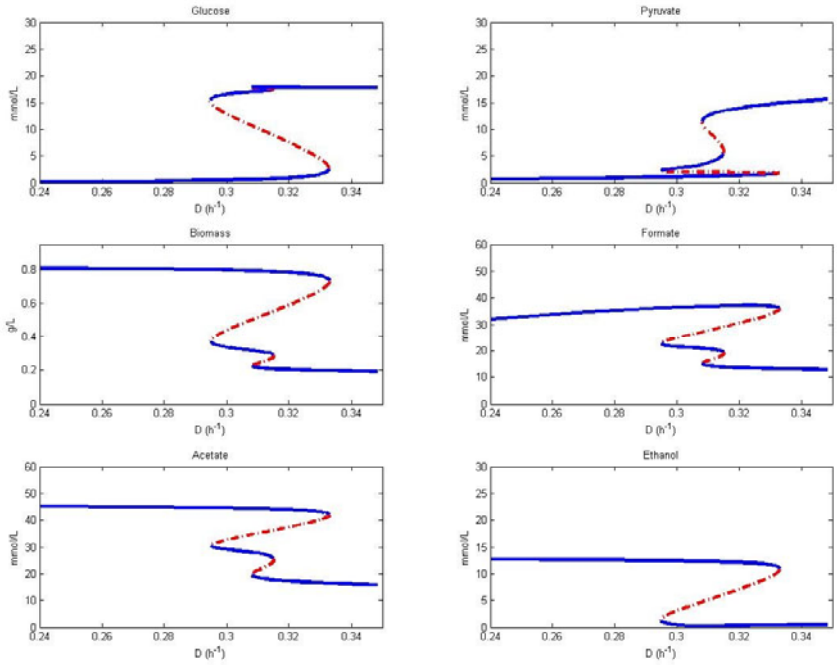

Figure 3. Steady state multiplicity in continuous reactors fed with a mixture of glucose and pyruvate.

The model also predicts oscillatory behavior (see Figure 4below) at low dilution rates with glucose-only feed. (Such dilution rates are indeed encountered in water treatment). With mixed feeds such Hopf bifurcation behavior becomes even more possible. These results require verification.

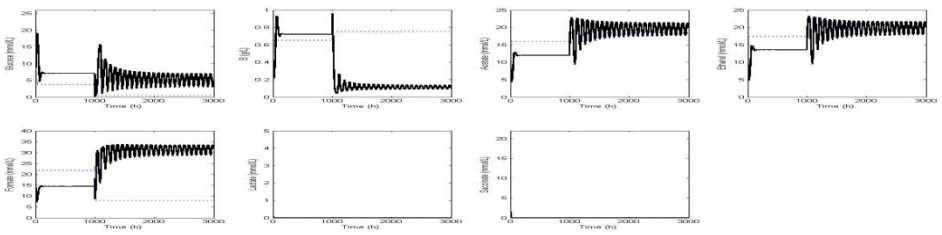

Figure 4. Oscillatory Behavior predicted by the dynamic cybernetic model

Experimental support for the above multiplicity and oscillatory features should not be viewed as a mere test of the implications of some specific nonlinearity. It is a consequence of manipulation of elementary modes by the organism in response to environmental changes. Biological systems have an inherence propensity for such nonlinear behavior because of metabolic regulation.