Analysis of a Stiff Limit Cycle: Glycolysis in Saccharomyces cerevisiae

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***Abstract*— A 22-D glycolysis model is analyzed with CSP, when it exhibits an oscillatory (limit cycle) behavior. Due to the action of a number of fast dissipative timescales and of significant decouplings, it is shown that the limit cycle lies in a 3-D subdomain.**

I. Introduction

The construction of complex mathematical models in biology and genetics demands the development of particular algorithmic tools for the acquisition of the desired physical understanding. As a result, a number of methodologies have recently been developed in order to construct simplified models that are of low dimension but retain all the significant features of the full model. These methodologies have been employed successfully for the analysis of a large number of problems in the field of biochemistry, e.g. [1]-[5].

Simplification of large and complex nonlinear mathematical models is mainly based on the presence of very fast dissipative time scales, which quickly become exhausted, allowing slower scales to characterize the evolution of the physical process. These fast time scales do not affect the progress of the system directly, but they simply constrain its evolution in a low dimensional space. This situation is usually defined as stiffness and the low dimensional space, where the system evolves according to the slow time scales, is defined as a slow manifold.

Here, the CSP algorithm [6], [7] will be employed for the analysis of a model describing the glycolysis cycle of intact yeast cells as a *homogeneous* two-phase (intracellular/extracellular) system [8]; the kinetics of which involves 24 reactions among 22 metabolites, as shown in Table I. Being one of the most significant topics in biochemistry, glycolysis has been the subject of extensive study.

For the 22-dimensional glycolysis model and the oscillatory regime examined here, CSP analysis shows that the long term evolution takes place along a 3-dimensional limit cycle. This feature is the result of (i) the existence of two conservation laws, (ii) the development of ten dissipative fast time scales, which force the trajectory to move on a 10-dimensional slow manifold and (iii) the effective decoupling on this manifold of three dimensions from the remaining seven; the latter being practically decoupled from all other dimensions of the problem as well.

TABLE I
Reactions in the detailed model [8]

1	$\leftrightarrow Glc_x$
2	$Glc_x \leftrightarrow Glc$
3	$Glc + ATP \rightarrow G6P + ADP$
4	$G6P \leftrightarrow F6P$
5	$F6P + ATP \rightarrow FBP + ADP$
6	$FBP \leftrightarrow GAP + DHAP$
7	$DHAP \leftrightarrow GAP$
8	$GAP + NAD^+ \leftrightarrow BPG + NADH$
9	$BPG + ADP \leftrightarrow PEP + ATP$
10	$PEP + ADP \rightarrow Pyr + ATP$
11	$Pyr \rightarrow ACA$
12	$ACA + NADH \rightarrow EtOH + NAD^+$
13	$EtOH \leftrightarrow EtOH_x$
14	$EtOH_x \rightarrow$
15	$DHAP + NADH \rightarrow Glyc + NAD^+$
16	$Glyc \leftrightarrow Glyc_x$
17	$Glyc_x \rightarrow$
18	$ACA \leftrightarrow ACA_x$
19	$ACA_x \rightarrow$
20	$ACA_x + CN_x^- \rightarrow$
21	$\leftrightarrow CN_x^-$
22	$G6P + ATP \rightarrow ADP$
23	$ATP \rightarrow ADP$
24	$ATP + AMP \leftrightarrow 2\ ADP$

II. The Limit Cycle

The governing equations are of the form of the N-dim. system:

$$\frac{d\mathbf{y}}{dt} = \mathbf{Q}^{-1}\left(\mathbf{S}_1 R^1 + ... + \mathbf{S}_N R^N\right) = \mathbf{g}(\mathbf{y}) \qquad (1)$$

where the elements of the N-dim. column vector $\mathbf{y}$ are the concentrations of the metabolites (mM), t is time (min), the N-dim. column state vector $\mathbf{S}_k$ and the scalar R^k denote the stoichiometric vector and rate, respectively, of the k-th reaction (see [8] for the expressions for the reaction rates). The $N \times N$ matrix $\mathbf{Q}$ is diagonal, its entries equaling either unity for the intracellular metabolites or the ratio of the extracellular volume to the total volume of intracellular cytosol, y_{vol}, for the extracellular ones.

The oscillatory behavior of the glycolysis model is displayed in Fig. 1, where the evolution of the concentration of nicotinamide adenine dinucleotide (NADH) for the period $0 < t < 100$ min is displayed; the behavior of the other metabolites being similar. This oscillatory motion develops as various transient components die-out, is characterized by a frequency $\omega_{ch} = 2\pi/T \approx 10$ min^{-1} and evolves around a limit cycle. As is depicted in Fig. 2, for the interval $450 < t < 500$ min in which all fast

initial transients are exhausted, fully oscillatory motion is established along a limit cycle at sufficiently long times; the structure of the cycle suggesting that it occupies a low-dimenisional subspace.

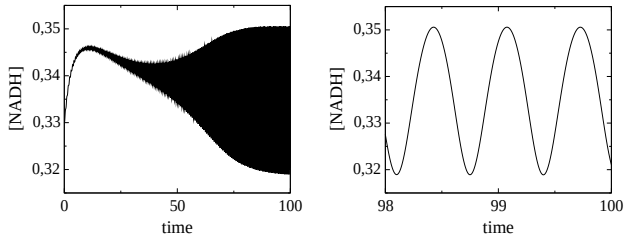

Fig. 1. The evolution of the NADH concentration (mM) with time (min) during the period $0 < t < 100$ min. On the right, magnification when fully oscillatory motion is established.

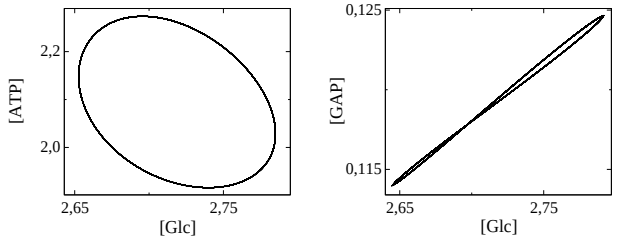

Fig. 2. The trajectory on the $[Glc]$ - $[ATP]$ and the $[Glc]$ - $[GAP]$ planes, during the period $450 < t < 500$ min.

III. CSP Results

Using CSP, various simplified models can be constructed when the solution evolves along the limit cycle, providing different levels of accuracy. Shown in Fig. 3 is the accuracy provided when six or ten fast modes are considered exhausted (M=6 or 10). Since $\tau_{11} \approx \omega_{ch}$, M=10 provides the maximum simplification possible.

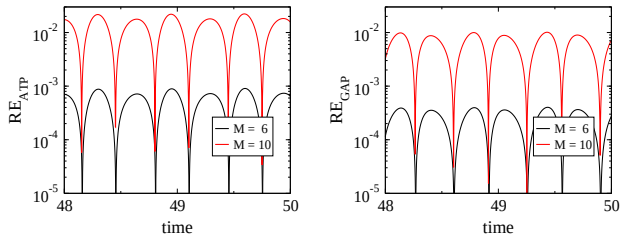

Fig. 3. M=6, 10. The relative error of $[ATP]$ and $[GAP]$ when comparing the solutions of the full and simplified models.

The better accuracy provided by the M=6 simplified model, relative to the one provided by the M=10 one, is due to the fact that a larger time scale gap exists in this case; i.e., τ_6/τ_7 is smaller than τ_{10}/τ_{11}.

Considering the M=10 case, CSP data indicate that the ten fast time scales affect the most the ten variables:

$$\mathbf{y}^r = ([BPG],[GAP],[AMP],[PEP],[F6P],[NADH],[DHAP],[ACA],[Glc],[EtOH])^T$$

where [X] denotes the concentration of X in mM, the rates of change of which relate to that of the remaining twelve variables with the relation:

$$\frac{d\mathbf{y}^r}{dt} = \mathbf{G}_s^r \frac{d\mathbf{y}^s}{dt} \tag{2}$$

where $\mathbf{G}_s^r$ is a $N \times M$ matrix [9] and

$$\mathbf{y}^s = ([ATP],[G6P],[ADP],[FBP],[NAD^+],[Glyc],[Pyr],[Glc_x],[EtOH_x],[Glyc_x],[ACA_x],[CN_x^+])^T$$

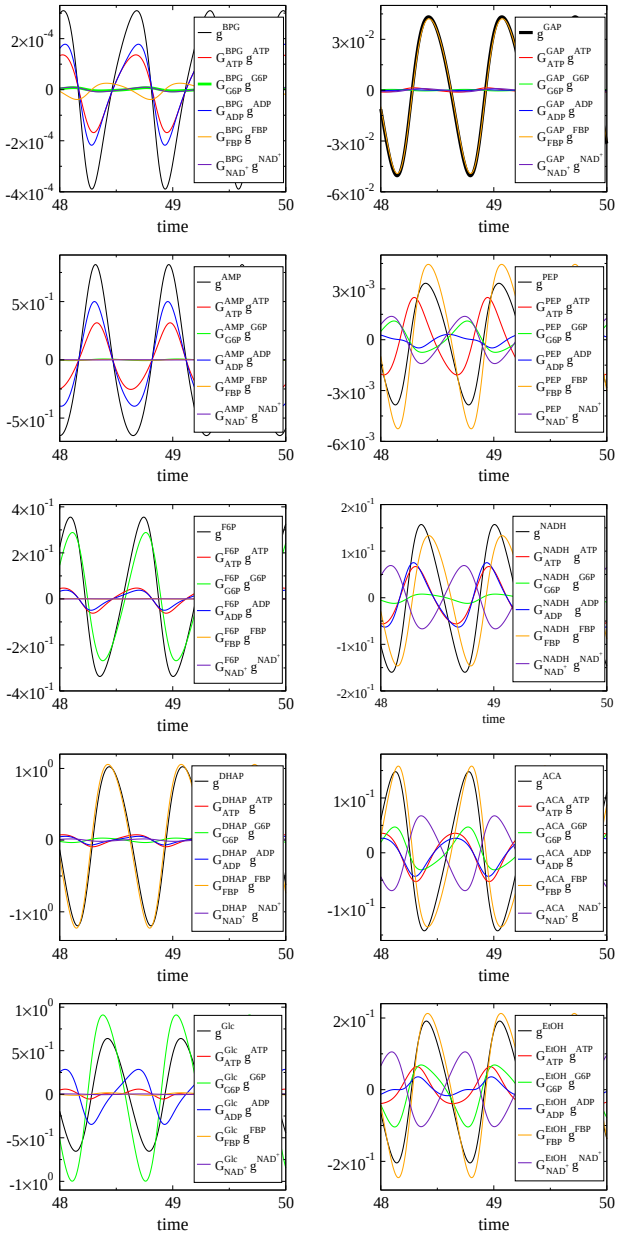

Fig. 4. M=10. The evolution in time (min) of the M components in the LHS of Eq. (2) and the most important additive terms of the corresponding components in the RHS; i.e. g^i (i=1,M) and of the largest $G_k^i g^k$ (k-1,N-M).

Shown in Fig. 4 are the ten components in the LHS of Eq. (2) along with the most important additive terms of the corresponding components in the RHS. Inspection of the displayed data reveals that the rate of change of the

variables in $\mathbf{y}^r$ depends on the rate of change of only five elements of $\mathbf{y}^s$, namely:

$$\mathbf{y}^{s1} = ([ATP], [G6P], [ADP], [FBP], [NAD^+])^T$$

being independent on the rate of change of the rest:

$$\mathbf{y}^{s2} = ([Glyc], [Pyr], [Glc_x], [EtOH_x], [Glyc_x], [ACA_x], [CN_x^+])^T$$

Further analysis indicates that the rate of change of $\mathbf{y}^{s2}$ decouples not only from $\mathbf{y}^r$ but from $\mathbf{y}^{s1}$ too. The validity of this statement is demonstrated by the results displayed in Fig. 5, where the solution of the original model is compared with that of a perturbed model; the latter consisting of the original model in which the magnitude of the rate of change of the variables in $\mathbf{y}^{s2}$ is increased by 20% for all times after $t = 25$ min. Both solutions were computed on the basis of initial conditions lying on the limit cycle.

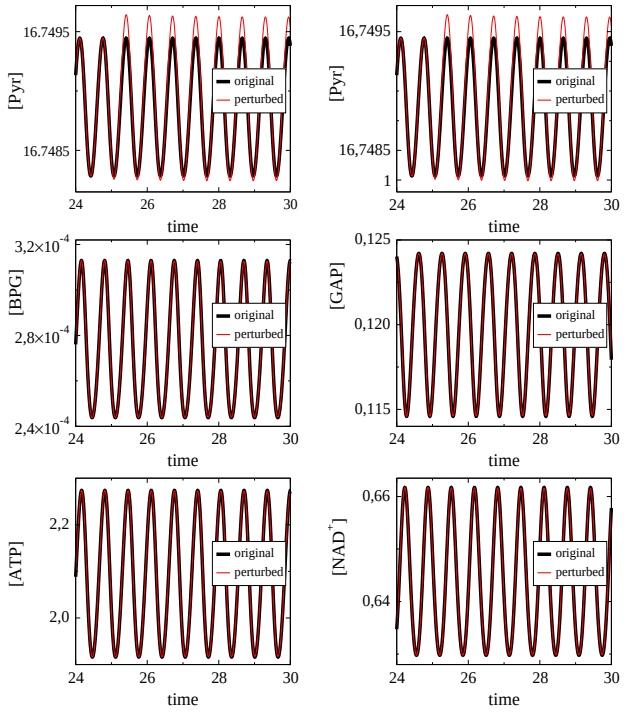

Fig. 5. The effects on the concentration of the metabolites in $\mathbf{y}^{s2}$ ([$Glyc$], [Pyr]), $\mathbf{y}^r$ ([BPG], [GAP]) and $\mathbf{y}^{s1}$ ([ATP], [NAD^+]) of a 20% perturbation in the magnitude of the rate of change of the seven components in $\mathbf{y}^{s2}$ imposed from $t = 25$.

In other words, in the perturbed model the governing equations for $\mathbf{y}^r$ and $\mathbf{y}^{s1}$ are similar to the ones in the original model, while the governing equation for $\mathbf{y}^{s2}$ is initially, up to $t = 25$ min, similar to that of the original model, say:

$$\frac{\mathbf{y}^{s2}}{dt} = \mathbf{g}^{s2}(\mathbf{y}^r, \mathbf{y}^{s1}, \mathbf{y}^{s2}) = \mathbf{g}^{s2}(\mathbf{y}) \quad (3)$$

being replaced for all subsequent times, $t \geq 25$ min, by the equation:

$$\frac{\mathbf{y}^{s2}}{dt} = 1.20\ \mathbf{g}^{s2}(\mathbf{y}^r, \mathbf{y}^{s1}, \mathbf{y}^{s2}) = 1.20\ \mathbf{g}^{s2}(\mathbf{y}) \quad (4)$$

The results displayed in Fig. 5 show that the perturbation imposed from $t = 25$ is immediately felt by the components in $\mathbf{y}^{s2}$, such as $[Glyc]$ and $[Pyr]$. Regarding the components in $\mathbf{y}^r$, such as $[BPG]$ and [GAP], Fig. 5 verifies that the imposed perturbation has no effect on them. Moreover, Fig. 5 indicates that the imposed perturbation has no effect in the components of $\mathbf{y}^{s1}$, such as $[ATP]$ and $[NAD^+]$.

These results indicate that a 5-dimensional simplified model can be constructed for the accurate simulation of the glycolysis process along the limit cycle.. This size can be further reduced by taking into account the two conservation laws:

$$[NAD^+] + [NADH] = \text{const.}$$
$$[ATP] + [ADP] + [AMP] = \text{const.}$$

that involve variables in $\mathbf{y}^r$ and $\mathbf{y}^{s1}$, so that a 3-dimensional simplified model can be constructed, involving the rate of change of [ATP], [G6P] and [ADP] only.

IV. Conclusions

A demonstration on the usefulness of the $N \times M$ matrix $\mathbf{G}_s^r$ was presented, in identifying the couplings operating along the limit cycle. This matrix, measuring the sensitivity of the variables in $\mathbf{y}^r$ with respect to those in $\mathbf{y}^s$ [9], identifies the couplings enforced by the fast time scales as the solution relaxes and then moves on the slow manifold.

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