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The Effects of Conservation Laws in Metabolic Networks

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***Abstract*—Metabolism is characterized by intricate networks of interactions between biochemical fluxes, metabolic compounds, and regulatory interactions. To investigate and eventually understand the dynamic behaviors arising from such networks of interactions and their associated parameter values is often not possible by intuitive reasoning alone. In this paper, we study a class of metabolic networks, where the conservation laws can be used to identify the parameter range for normal living. The general results are verified by a simplified model of the Calvin cycle in photosynthesis.**

***Index Terms*—Metabolic networks; conservation law; cellular dynamics; Calvin cycle; parameter variations.**

I. Introduction

Recent years have witnessed dramatic changes in the metabolic networks. The cellular organisms can be modeled using an entire metabolic map representing all metabolic reactions that take place in the cell to understand its behavior in certain environmental conditions. The prediction of the cellular behaviors gives valuable opportunities for agricultural, industrial, and medical purposes.

There are a number of approaches to model metabolic networks. One of the most used modeling techniques is the Flux Balance Analysis (FBA), by which models can be built with respect to stoichiometry of the reactions that take place in the cellular organisms and predictions can be made using linear programming whose objective is the maximization of certain products or the minimization of consumption of certain metabolites. Recently, the constraint-based (including the flux-balance constraints and energy-balance constraints) optimization approaches have been proposed to discuss various metabolic networks. The internal metabolism of the substrate is modeled as a linear programming problem and variations in the environment are modeled with a set of ordinary differential equations. When taking the conservation laws into consideration, the model can be illustrated as a set of differential algebraic equations. The algebraic equations can cause singularity-induced, saddle-node, and Hopf bifurcation [1], [2].

Photosynthesis is a complex system that includes lots of biophysical and biochemical reactions, such as absorption of light energy, conversion of light energy to chemical energy, and some other biochemical reactions involved in the photosynthetic carbon metabolism [3], [4]. It is generally difficult to model the complete photosynthetic process, and the carbon in crop yield is mainly from the CO_2 fixation during the photosynthetic carbon metabolic process, so the photosynthetic carbon metabolism becomes a focal point of wide attention. From systematic viewpoint, the photosynthetic carbon metabolism can be viewed as a bio-molecular network which has many important dynamical characteristics, such as the oscillation driven by variation of external condition, the effect of each enzyme, and steady states. Various models of photosynthesis process have been proposed and their dynamical behaviors have also be investigated [5], [6], [7], [8], [9]. In [10] Zhu proposed a simple model of the Calvin cycle which has two key characteristics of the Calvin cycle: the autocatalytic cycle and the utilization of photosynthate. For a group of fixed parameters, Zhu found that the model has multiple steady states from numerical computation, but only one is physiologically feasible.

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In order to get higher photosynthesis rates which means generating higher yield potential, we want to have a higher CO_2 assimilation or fixation rate. Hence, one can study how to drive or improve the system so that it can run into the steady state while obtaining the relatively highest CO_2 assimilation rate. Meanwhile, there are many conservation laws in photosynthesis, we should take them into consideration. The rest of the paper is organized as follows. We describe the generally theoretical result in general metabolic networks with conservation laws in section 2. Section 3 is devoted to explanation of the effect of parameter variations on the reaction rates and steady states. In section 4, a simplified model of Calvin cycle in photosynthesis will be discussed to illustrate the theoretical framework. Conclusions and future works are presented in section 5.

II. The rate equations of metabolic networks

An overarching attribute of metabolic networks is their inherent robustness and ability to cope with ever-changing environmental conditions. Despite this flexibility, network stoichiometry and connectivity do establish limits/barriers to the coordination and accessibility of reactions. Many paper, e.g., [11], [12], [13], [14], [15], [16], [17], discussed metabolic networks using the flux balance analysis methods or conservation laws. We know that a metabolic network can be modeled by a lot of differential algebraic equations and these equation models provide a largely complete skeleton of the metabolic reactions present in an organism. There are many methods to deal with differential algebraic equations, so that we can use these methods to get some instructive advice to the control principle of the real metabolic networks. Examination of the structural and topological properties of metabolic networks is important at both the conceptual level to reveal the organizational principles of metabolic interactions within cellular networks and at the practical level for more effectively focusing engineering interventions and ensuring the consistency of the underlying reconstructions.

Many metabolic networks can be modeled as the following reaction rate equations

$$\frac{ds_i}{dt} = \sum_{j=1}^{M} a_{ij} v_j, \quad i = 1, \cdots, N \tag{1}$$

with conservation laws

$$\sum_{k} s_k = const. \tag{2}$$

for some k, where s_i $(i = 1, \cdots, N)$ represent the notation of the substrates or metabolites, a_{ij} $(i = 1, \cdots, N, j = 1, \cdots, M)$ are the reaction coefficients, and v_j $(j = 1, \cdots, M)$ is the reaction rate of the j-th reaction. In metabolic networks, there are generally many conservation laws, e.g., the total amount of each enzyme, the sum of [ATP] and [ADP] and so on. About differential algebraic equations (DAEs), there are many works discussing about their bifurcations, such as in [1], [2], new methods were presented for calculating the multi-parameter singularity-induced, saddle-node and Hopf bifurcation boundary associated with the parameter-dependent DAEs. There are also many works discussing about the equilibria of the DAEs system. Most of them get the equilibria through solving the algebraic equations or just through numerical simulation. In general, when saying the system reaches its equilibria, we often mean that the right sides of the equations reach to zeros, i.e.,

$$\sum_{j=1}^{M} a_{ij} v_j = 0, \quad i = 1, \cdots, N. \tag{3}$$

Using (2) and (3), the relationship between the reaction rates v_j $(j = 1, \cdots, M)$ can be obtained. If the relationship of the reaction rates of under the equilibria is known, then we can adjust the rates to make the system reach the equilibria we want by adjusting the parameter values. In this paper, we consider the parameter changes that cause robustness to perturbations, i.e., a perturbation to some specific parameters can destabilize the metabolic system and thus induce its collapse. If the perturbation causes abrupt changes in some metabolites so that the other metabolites will close to zero in (2), the metabolic system can collapse due to a rapid increase or decrease in some metabolites, thus causing apoptosis. The collapse of the system may be caused by some state variables close to the conserved quantity and thus reduce the attraction region of the stable equilibria. Small perturbation will induce collapse of the whole system. Therefore, the conservation laws can be used to identify the parameter range so that the metabolic system can operate normally.

III. The effect of parameter variations to reaction rates and stability

In biology, many processes often involve many parameters, and the importance of these parameters in determining system behaviors must be assessed so as to gain deep insight into designing principles of living organisms. However, many parameters driving the biological system behaviors vary extensively, therefore it is generally difficult to analyze the effects of individual parameters in a systematic way. We know that how the stability of the steady states is affected by the parameter variations. The increase or decrease of the steady state due to the change of a specific parameter can be obtained. The metabolic networks can also be modeled with the following ordinary differential equations:

$$\dot{x_i} = f_i(x, p), \quad i = 1, \cdots, n, \tag{4}$$

where $x = (x_1, x_2, \cdots, x_n)^T$ is the variable vector and $p = (p_1, p_2, \cdots, p_m)^T$ is parameter vector. The sign of $\frac{\partial f_i}{\partial p_j}$ can be used to illustrate the effect of p_j variation to x_i, that means when the sign is positive increasing the value of p_j can make the value of x_i increase faster. On the contrary, if the sign is negative that means increase the value of p_j will decrease the value of x_i. If p_j is not the direct parameter in the differential equation of x_i, we can get the sign of $\frac{\partial f_i}{\partial p_j}$ through the product

of the sign of the path from some x_j to x_i and the effect p_j variation on its own output x_j.

We get the steady state of the system by setting the right side of the equations to zero and solve the algebraic equations. Sometimes it is hard to obtain the accurate solution of algebraic equations and the problem can be solved through numerical simulation to get the numerical solutions. But in most cases we do not need to know the specific value of steady states, and what we want to know is their change with parameter variation. From the above analysis we can know that how the stability of the steady states is affected by the parameter variations and which stable steady state will persist. The increase or decrease of the steady state due to the change of a specific parameter can also be obtained. In [19], [20], [21], [22], [23], all have also mentioned about this.

IV. The simplified model of Calvin cycle in photosynthesis

Photosynthesis is inherently a complex system. It includes biophysical and biochemical reactions associated with light energy absorption, conversion of light energy into chemical energy in the form of ATP (adenosine triphosphate) and NADPH (Nicotinamide adenine dinucleotide phosphate), and complex biochemical reactions involved in the photosynthetic carbon metabolism. There are many processes involved in photosyntehic carbon metabolism, including the Calvin cycle, photorespiratory metabolism (PCOP), starch synthesis, and Suc synthesis [3], [9]. In this paper, we consider only the Calvin cycle and the rate of each reaction depends nonlinearly on the concentrations of its substrates and products [10]. The model is described by ordinary differential equations as follows:

$$\frac{dRuBP}{dt} = v_{13} - v_1, \quad (5)$$

$$\frac{dPGA}{dt} = 2v_1 - v_2 - v_5, \quad (6)$$

$$\frac{dDPGA}{dt} = v_2 - v_3, \quad (7)$$

$$\frac{dGAP}{dt} = v_3 - v_4 - v_6, \quad (8)$$

$$\frac{dRu5P}{dt} = 0.6v_4 - v_{13}. \quad (9)$$

It has been shown that there is only one physiologically feasible equilibrium under the same external conditions [10]. Such a simplified model of the photosynthetic Calvin cycle keeps the key characteristics of photosynthetic carbon metabolism: the autocatalytic cycle, the utilization of photosynthate, and the generation of sinks etc.

The model of the Calvin cycle with the phosphate feedback mechanism is considered. In this model, ATP is consumed in two steps, i.e. from PGA to DPGA and from Ru5P to RuBP. In these two steps, ADP is released and one phosphate is tied up in the sugar compound. Reformation of ATP need both ADP and Pi as substrates. The phosphate is replenished only when the photosynthetic product, the sugars are utilized by sinks and phosphate is released. In this way, the phosphate exerted a feedback control mechanism. It is imaginable that if the sink capacity is too low, i.e. when v_5 is too low, the phosphate can not be effectively released, the ATP will be diminished and the rate of the CO_2 absorption will inevitably decrease. However, v_5 can not be too high, since the substrate levels of the Calvin cycle will decrease when the rate of v_5 is too high. The reaction rates $v_i s$ are composed of the substrates and parameters and we can explain such a phenomenon by analyzing the effects of the parameters. In order to guarantee stability of the equilibria, the parameters are restricted into some ranges and accordingly the reaction rates are limited too. In other words, the reaction rates can neither be too high nor too low by adjusting the parameter values based on the conservation law.

In this paper, we still consider this model and made some minor changes to consider how to identify parameter ranges based on the conservation law. In other words, different from the constant ATP in [10], ATP is assumed to be a state variable and its dynamics is assumed to obey the Michaelis-Menten kinetics and can be written as follows

$$\frac{dATP}{dt} = v_{16} - v_2 - v_{13}, \quad (10)$$

where the photorespiratory process is not included [9].

Due to the importance of ATP and ADP in photosynthesis, both of them are assumed to be state variables. During the photosynthesis, the total concentration of the adenylate nucleotides, i.e., the sum of ATP and ADP is assumed to remain constant [9]

$$ATP + ADP = const. \quad (11)$$

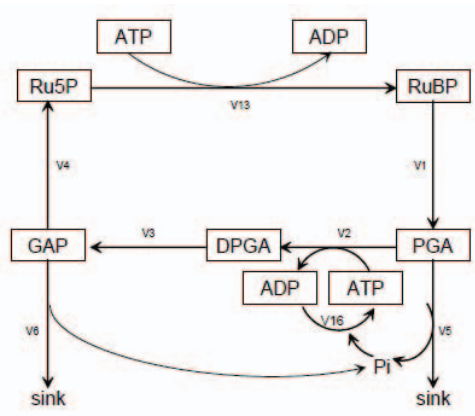

Fig. 1. The simplified model of Calvin cycle in photosynthesis. The full name of the abbreviation of the variable are: RuBP: Ribulose 1,5-biphosphate; PGA: 3-Phosphoglycerate; DPGA: 1,3-bisphosphoglycerate; GAP: Glyceraldehyde 3-phosphate Ru5P Ribulose 5-phosphate; ATP: adenosine triphosphate; ADP:adenonisine disphosphate; and P_i: phosphoric acid.

The model of Calvin cycle is thus composed of differential equations (5)-(10) and algebraic equation (11)(here we set $ATP + ADP = 1$). The graphic representation of the Calvin cycle is shown in Fig. 1. The reactions take the form

1. $RuBP + CO_2 \rightarrow 2PGA$,
2. $PGA + ATP \rightarrow ADP + DPGA$,
3. $DPGA + NADPH \rightarrow GAP + P_i + NADP$,

4. $GAP \rightarrow 0.6Ru5P$,
5. $PGA \rightarrow Sink$,
6. $GAP \rightarrow Sink$,
13. $Ru5P + ATP \rightarrow RuBP + ADP$,
16. $ADP + P_i \rightarrow ATP$.

In the above ordinary differential equations, the reaction rates v_i are expressed as follows

$$v_1 = \frac{V_{1max} \times RuBP}{K_{m1} + RuBP}, \quad (12)$$

$$v_2 = \frac{V_{2max} \times PGA \times ATP}{(K_{m21} + PGA)(K_{m22} + ATP)}, \quad (13)$$

$$v_3 = \frac{V_{3max} \times DPGA}{K_{m3} + DPGA}, \quad (14)$$

$$v_4 = \frac{V_{4max} \times GAP}{K_{m4} + GAP}, \quad (15)$$

$$v_5 = \frac{V_{5max} \times PGA \times ATP}{(K_{m51} + PGA)(K_{m52} + ATP)}, \quad (16)$$

$$v_6 = \frac{V_{6max} \times GAP}{K_{m6} + GAP}, \quad (17)$$

$$v_{13} = \frac{V_{13max} \times Ru5P \times ATP}{(K_{m131} + Ru5P)(K_{m132} + ATP)}, \quad (18)$$

$$v_{16} = \frac{V_{16max} \times ADP \times P_i}{(K_{m161} + ADP)(K_{m162} + P_i)}. \quad (19)$$

Under normal conditions, the system (5)-(11) gets its steady state with the given parameters' value. The reaction rates depend nonlinearly on the substrates and parameters. In order to gain the relationship between the reaction rates v_i, by setting the right-hand side of the ordinary differential equations (5)-(10) to be zero, we we can obtain the result that all the other seven reaction rates in the simplified model can be expressed by v_5 as follows

$$v_1 = \frac{3}{8}v_5, \quad v_2 = -\frac{1}{4}v_5, \quad v_3 = -\frac{1}{4}v_5, \quad v_4 = \frac{5}{8}v_5,$$
$$v_6 = -\frac{7}{8}v_5, \quad v_{13} = \frac{3}{8}v_5, \quad v_{16} = \frac{1}{8}v_5.$$

From the relationship we can deduce that when v_5 is in the reasonable range, increasing its value will increase v_1, which means the rate of CO_2 absorption will be increased. We choose parameter V_{16} to make the numerical simulation of this model, we can see that when V_{16} is too low, the value of variable PGA will be increasing and thus induce collapse. On the other hand, when V_{16} is too high, ATP will increase fast and also induce collapse, as shown in Figs.2 and 3. The value of parameters are $V1max = 3.78$, $V2max = 12.75$, $V3max = 5.04$, $V4max = 3.05$, $V5max = 0.1$, $V6max = 1.49$, $V13max = 8$, $Km1 = 1$, $Km21 = 0.240$, $Km22 = 0.390$, $Km3 = 0.5$, $Km4 = 0.84$, $Km51 = 0.75$, $Km52 = 0.75$, $Km6 = 5$, $Km131 = 0.15$, $Km132 = 0.059$, $Km161 = 0.0142$, and $Km162 = 0.3$. In Fig.2, at the low limit value, the value of PGA increase fast and cause ADP is close to the conserved quantity and in Fig.3 at the high limit value ATP is close to the conserved quantity. Due to the conservation law, the continual change in some specific parameters will cause concentrations

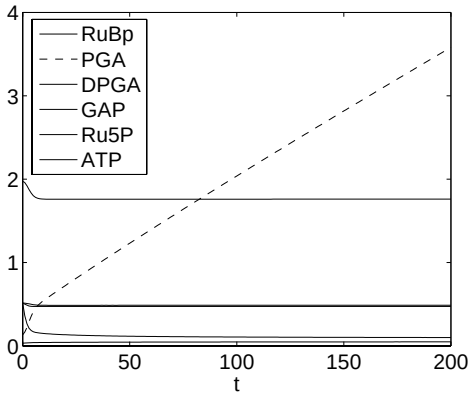

Fig. 2. The system collapses due to fast increase in PGA at $V16max = 6.0$. In this situation, the value of ADP will soon close to the conserved quantity and cause the collapse of the whole system.

of some components close zero or the conserved quantity. Then the attraction region will shrink very small perturbation will cause the collapse of the whole system, as shown in Figs.2 and 3. When taking V_{5max} as a parameter and changing it to $V_{5max} = 10$, the state variables RuBP, PGA, DPGA, GAP, and Ru5P all soon close to zero except ATP=1, i.e., the conserved quantity, as shown in Fig.4.

From the above analysis, we can conclude that once some threshold is exceeded, the whole system can not operate normally and even collapse can occur. This result is in accordance with the real phenomenon. Therefore, the conservation law can be use to determine the parameter range so that the whole system can operate normally. The effects of other parameter variations can be analyzed similarly. For example, the parameter V_{5max} also must be constrained in a reasonable range, it cannot be too large otherwise the PGA will soon be converted to ATP, and all other substrates reach zero besides ATP, which is close to the conserved quantity.

V. Conclusion

The changes in the concentration of metabolites in the environment are represented by ordinary differential equations and intracellular cell metabolism is represented by a set of algebraic equations. In this paper, we have discussed the relationship between the reaction rates in metabolic networks and the effect of parameter variations to reaction rates and stability. The relationship of the reaction rates in the simplified Calvin cycle model and the effect of parameter variations to the whole system has also been got, and due to the conservation of photosynthesis structure the value of parameters must be stay in reasonable range to keep the process operate normally. In the following work, what we want to do is how to quantify the attraction region of the stable equilibria, then we can describe quantitatively the robustness of the system by the attraction region. There are more and more work to do with the constraints-based metabolic model, what we want to do in the future is the systematic analysis of conservation relations

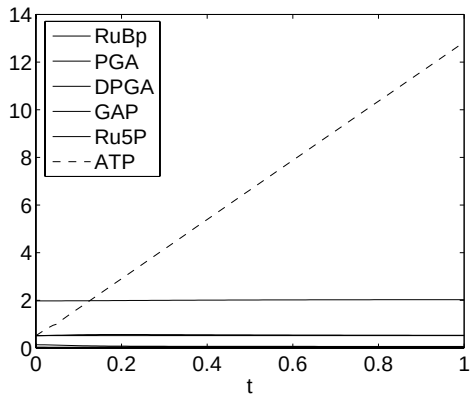

Fig. 3. The system collapses due to fast increase in ATP at $V_{16max} = 26$. Due to the conservation laws ATP+ADP=1, when the value of V_{16max} beyond the upper range, the ATP will soon close to the conserved quantity 1 and the whole system collapse.

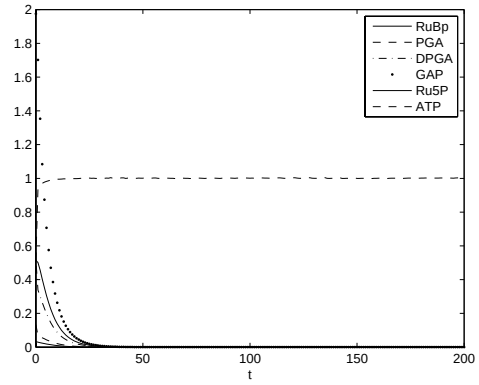

Fig. 4. The system collapses due to state variables soon close to zero at $V_{5max} = 10$.

of metabolic networks. Moreover, photosynthesis is a very complex network, it will be further studied in our future work.

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