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Undamped oscillations in bacterial glycolysis models

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Abstract−The exotic dynamical behaviors exhibited in chemical reaction systems, such as multiple steady states, undamped oscillations, chaos, and so on, often result from unstable steady states. A bacterial glycolysis model is studied, which involves the generation of adenosine triphosphate (ATP) in a flow system and consists of eight species and ten reactions. A minimum subnetwork of the bacterial glycolysis model is determined to exhibit an unstable steady state with a positive real eigenvalue, which gives rise to undamped oscillations for a small perturbation. A set of rate constants and the corresponding unstable steady state are computed by using a positive real eigenvalue condition. The phenomena of oscillations and bifurcation are discussed. These results are extended to the bacterial glycolysis model and several parent networks.

Key words: Chemical Reaction Network, Oscillation, Positive Real Eigenvalue, Glycolysis, Bifurcation

INTRODUCTION

Some exotic dynamic phenomena in chemical reactors, such as unstable steady states, undamped oscillations and multiple steady states, have been shown to occur under both non-isothermal and isothermal conditions [Chang et al., 1989; Kim and Rhee, 1989; Chuang et al., 2004]. There are a growing number of experimental systems in which abnormal behavior is exhibited in isothermal reactors [Geiseler and Bar-Eli, 1981; Orbán and Epstein, 1985; Hung et al., 1995; Dutt and Müller, 1996]. This is to say that instabilities derive not from thermal effects but rather from the intricacy of chemistry itself. The identification of such reaction mechanisms is important, since it not only helps experimentalists to determine reaction mechanisms but also helps engineers to design more efficient reaction processes.

Some results of the chemical reaction network theory have been based upon classification of reaction networks by means of a non-negative integer index called the deficiency. The work of Horn [1972], Horn and Jackson [1972], and Feinberg [1972] led to the deficiency zero theorem: for any deficiency zero network, no matter what the positive rate constants are, there is at most one positive steady state, that steady state is stable and there does not exist any cyclic solution. However, the stable property of deficiency zero networks is not shared in a general way by networks of higher deficiency. The deficiency one theorem [Feinberg, 1987] and the deficiency one algorithm [Feinberg, 1988] provide means to distinguish between those (deficiency one) mechanisms which can exhibit multiple steady states and those which cannot. Besides these deficiency-oriented theories, the subnetwork analysis [Li, 1998; Chuang et al., 2004] and the SCL graph method [Schlosser and Feinberg, 1994] can also be applied to determine the possibility of multiple steady states. The stoichiometric network analysis developed by Clarke [1980] is a useful tool to study the stability of complex reaction networks. Based on stoichiometric network analysis, conditions for the occurrence of a Hopf bifurcation in reaction networks are proposed in papers [Eiswirth et al., 1991, 1996].

Biological cell systems, which usually consist of many species and reactions, can also give rise to those complex reaction behaviors [Hatzimanikatis and Bailey, 1997; Li, 1998; Chuang et al., 2004; Ho et al., 2005]. A rudimentary description of glycolysis, a process by which a carbon source is digested by a cell, is formulated based upon pathways pertinent to bacteria by Hatzimanikatis and Bailey [1997]. Under certain assumptions, the steady states of the glycolytic system are simplified and described by three coupled non-linear equations. They showed that the system admitted multiple steady states for some kinetic parameters. In this work, the complex glycolytic system with eight species and ten reactions is determined to admit unstable steady states with positive real eigenvalues, which approach undamped oscillations for a small perturbation. A positive real eigenvalue condition in the appendix is applied, which gives a necessary and sufficient condition for the determination of an unstable steady state having a positive real eigenvalue in general isothermal reaction networks. The undamped oscillations in a subnetwork and several extended networks are also determined and discussed.

THEORY

The example of bacterial glycolysis model, simplified by Hatzimanikatis and Bailey [1997], arises from participation of ATP and adenosine diphosphate (ADP) and from the involvement of phosphoenolpyruvate (PEP) in the glycolysis pathway. The overall reaction is Glucose+2ADP→2Pyruvate+2ATP, which contains the following elementary steps:

$$\begin{aligned} &S+E \rightarrow G+P \\ &S+T \rightarrow G+D \\ &G+T \rightarrow F+D \\ &F+2D \rightleftarrows 2E+2T \\ &E+D \rightarrow P+T \\ &M+T \rightleftarrows 2D \\ &D \rightleftarrows T \end{aligned} \tag{1}$$

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(The symbols S and P correspond to extracellular glucose and to pyruvate; G corresponds to the pool of glucose 6-phosphate and fructose 6-phosphate; F corresponds to fructose 1,6-diphosphate; E corresponds to phosphoenolpyruvate; M corresponds to adenosine mono-phosphate (AMP), D to ADP, and T to ATP, respectively.)

The assumptions used in this work are: 1) the reactants, glucose and AMP, and the product, pyruvate, continue to diffuse or flow in and out of a cell, while the other species are all constrained inside a cell; 2) enzyme activities depend only on the concentrations of their substrates and the cell is not growing; 3) the reaction system is well mixed and mass action kinetics is followed. Thus, the glycolysis in a reaction region inside of a cell including material transportation terms is represented by a reaction network (2) and its corresponding dynamical ordinary differential equations are listed in Eq. (3).

$$\begin{aligned}
&A_1 \rightleftarrows 0 \rightleftarrows A_8\\
&A_4 \rightarrow 0\\
&A_1+A_2 \rightarrow A_3+A_4\\
&A_1+A_5 \rightarrow A_3+A_7\\
&A_3+A_5 \rightarrow A_6+A_7\\
&A_6+2A_7 \rightleftarrows 2A_2+2A_5\\
&A_2+A_7 \rightarrow A_4+A_5\\
&A_8+A_5 \rightleftarrows 2A_7\\
&A_7 \rightleftarrows A_5
\end{aligned} \tag{2}$$

(A_1: S, A_2: E, A_3: G, A_4: P, A_5: T, A_6: F, A_7: D, A_8: M, and 0: zero complex)

$$\begin{aligned}
\frac{dc_1}{dt} &= k_{0\to A_1} - k_{A_1\to 0}c_1 - k_{A_1+A_2\to A_3+A_4}c_1c_2 - k_{A_1+A_5\to A_3+A_7}c_1c_5\\
\frac{dc_2}{dt} &= -k_{A_1+A_2\to A_3+A_4}c_1c_2 + 2k_{A_6+2A_7\to 2A_2+2A_5}c_6c_7^2 - 2k_{2A_2+2A_7\to A_6+2A_7}c_2^2c_5^2\\
&\quad - k_{A_2+A_7\to A_4+A_5}c_2c_7\\
\frac{dc_3}{dt} &= k_{A_1+A_2\to A_3+A_4}c_1c_2 - k_{A_3+A_5\to A_6+A_7}c_3c_5 + k_{A_1+A_5\to A_3+A_7}c_1c_5\\
\frac{dc_4}{dt} &= -k_{A_4\to 0}c_4 + k_{A_1+A_2\to A_3+A_4}c_1c_2 + k_{A_2+A_7\to A_4+A_5}c_2c_7\\
\frac{dc_5}{dt} &= -k_{A_3+A_5\to A_6+A_7}c_3c_5 + 2k_{A_6+2A_7\to 2A_2+2A_5}c_6c_7^2 - 2k_{2A_2+2A_7\to A_6+2A_7}c_2^2c_5^2\\
&\quad + k_{A_2+A_7\to A_4+A_5}c_2c_7 - k_{A_8+A_5\to 2A_7}c_8c_5 + k_{2A_7\to A_8+A_5}c_7^2\\
&\quad + k_{A_7\to A_5}c_7 - k_{A_5\to A_7}c_5 - k_{A_1+A_5\to A_3+A_7}c_1c_5\\
\frac{dc_6}{dt} &= k_{A_3+A_5\to A_6+A_7}c_3c_5 - k_{A_6+2A_7\to 2A_2+2A_5}c_6c_7^2 + k_{2A_2+2A_7\to A_6+2A_7}c_2^2c_5^2\\
\frac{dc_7}{dt} &= k_{A_3+A_5\to A_6+A_7}c_3c_5 - 2k_{A_6+2A_7\to 2A_2+2A_5}c_6c_7^2 + 2k_{2A_2+2A_7\to A_6+2A_7}c_2^2c_5^2\\
&\quad - k_{A_2+A_7\to A_4+A_5}c_2c_7 + 2k_{A_8+A_5\to 2A_7}c_8c_5 - 2k_{2A_7\to A_8+A_5}c_7^2\\
&\quad - k_{A_7\to A_5}c_7 + k_{A_5\to A_7}c_5 + k_{A_1+A_5\to A_3+A_7}c_1c_5\\
\frac{dc_8}{dt} &= k_{0\to A_8} - k_{A_8\to 0}c_8 - k_{A_8+A_5\to 2A_7}c_8c_5 + k_{2A_7\to A_8+A_5}c_7^2
\end{aligned} \tag{3}$$

where c_i, i=1, 2, …, 8 denote the concentrations of species A_1, A_2, …, A_8 within the reaction region of a cell and $k_{i\to j}$ is a rate constant for reaction i→j in network (2).

The last seven lines in Eq. (2) are the elementary steps in mechanism Eq. (1). The first two lines in Eq. (2) display the inflow of reactants and the outflow of remaining reactants and the products. In reaction network terms [Glansdorff and Prigogine, 1971], to account for the inflow of A_1 and A_8 in the feed stream, the pseudo-reactions 0→A_1 and 0→A_8 are added to true chemistry Eq. (1). (The physical meaning of "0" (zero complex) represents the surroundings.) Compared with the dynamical equations in Eq. (3), the rate constants $k_{0\to A_1}$ and $k_{0\to A_8}$ are assigned, respectively, to be equal to c_1^f/θ and c_8^f/θ (The symbol c_i^f denotes the feed concentration of species i(=1, 8) and θ denotes the residence time.). Also to account for the outflow of A_1, A_8 and A_4 in the effluent stream, pseudo-reactions $A_1\to 0$, $A_8\to 0$ and $A_4\to 0$ are added to true chemistry Eq. (1). The flow rates $k_{A_1\to 0}$, $k_{A_8\to 0}$ and $k_{A_4\to 0}$ are all assigned to be equal to the reciprocal of residence time $1/\theta$. Thus, in reaction network terms, we consider the reactions given in Eq. (1) operating in an open system to be modeled by reaction network (2), instead of Eq. (1).

The complex reaction network (2) has deficiency two. According to the deficiency-oriented theories, a network must have a deficiency of at least one to give rise to multiple steady states or an unstable steady state. Thus, we are interested to know whether there exists a deficiency one "minimum" subnetwork of the deficiency two network (2) consisting of the same eight species as the network (2), which admits multiple steady states or an unstable state. All such deficiency one subnetworks were analyzed by the deficiency one algorithm and none admits steady state multiplicity. A subnetwork is determined to exhibit an unstable steady state with a positive real eigenvalue by using a positive real eigenvalue condition. It is obtained by deleting the reaction $A_1+A_5\to A_3+A_7$ from network (2) and displayed below.

$$\begin{aligned}
&A_1 \rightleftarrows 0 \rightleftarrows A_8\\
&A_4 \rightarrow 0\\
&A_1+A_2 \rightarrow A_3+A_4\\
&A_3+A_5 \rightarrow A_6+A_7\\
&A_6+2A_7 \rightleftarrows 2A_2+2A_5\\
&A_2+A_7 \rightarrow A_4+A_5\\
&A_8+A_5 \rightleftarrows 2A_7\\
&A_7 \rightleftarrows A_5
\end{aligned} \tag{4}$$

The network (4) is used to introduce some terminology used in the positive real eigenvalue condition (formal definitions can be found in Feinberg [1987, 1988]). We will use the symbol N to denote the number of species in a network under consideration. Thus, for network (4) N=8. By $\Re^N$ we shall mean the usual vector space of N-tuples of real numbers. The standard basis for $\Re^N$ will be denoted $\{\mathbf{A}_1, \mathbf{A}_2, ..., \mathbf{A}_N\}$.

The complexes of a network are the objects that appear before and after reaction arrows. Thus the set of complexes for network (4) is {0, A_1, A_8, A_4, A_1+A_2, A_3+A_4, A_3+A_5, A_6+A_7, A_6+2A_7, $2A_2+2A_5$, A_2+A_7, A_4+A_5, A_8+A_5, $2A_7$, A_7, A_5}. Given a network with N species, we shall associate with each complex a vector in $\Re^N$. Consider network (4), with complex 0 (zero) we associate the complex vector $\mathbf{0}$ in $\Re^8$; with complex A_6+2A_7 we associate the complex vector $\mathbf{A}_6+2\mathbf{A}_7$; and so on.

We shall write $y_i\to y_j$ (or the abbreviation i→j) to indicate the reaction whereby the complex y_i reacts to complex y_j. We denote the set of reactions in a network by the symbol R. Thus the set of reactions in network (4) is R={0→A_1, $A_1\to 0$, 0→A_8, $A_8\to 0$, $A_4\to 0$, $A_1+A_2\to A_3+A_4$, $A_3+A_5\to A_6+A_7$, $A_6+2A_7\to 2A_2+2A_5$, $2A_2+2A_5\to A_6+2A_7$, $A_2+A_7\to A_4+A_5$, $A_8+A_5\to 2A_7$, $2A_7\to A_8+A_5$, $A_7\to A_5$, $A_5\to A_7$}. We reserve the symbol r for the number of distinct reactions in a network. For network (4), r=14. A reaction $y_i\to y_j$ is said to be reversible if its reverse reaction $y_j\to y_i$ is also in the net-

work; otherwise, the reaction $y_i \rightarrow y_j$ is irreversible. We shall call reversible reactions $y_i \rightleftarrows y_j$ a reversible reaction pair. The symbol p indicates the number of distinct reversible reaction pairs in a network. The number of irreversible reactions in a network is r−2p. For network (4), there are five (p=5) reversible reaction pairs and four (r−2p=14−10) irreversible reactions.

With each reaction of the network we associate a reaction vector in $\Re^N$ obtained by subtracting the "reactant" complex vector $\mathbf{y}_i$ from the "product" complex vector $\mathbf{y}_j$, i.e., $\mathbf{y}_j-\mathbf{y}_i$. Consider network (4) for reaction $0 \rightarrow A_1$; the corresponding reaction vector in $\Re^8$ is $\mathbf{A}_1-\mathbf{0}$ (=$\mathbf{A}_1$). The set of reaction vectors for network (4) is $\{\pm\mathbf{A}_1, \pm\mathbf{A}_8, -\mathbf{A}_4, \mathbf{A}_3+\mathbf{A}_4-\mathbf{A}_1-\mathbf{A}_2, \mathbf{A}_6+\mathbf{A}_7-\mathbf{A}_3-\mathbf{A}_5, \pm(2\mathbf{A}_2+2\mathbf{A}_5-\mathbf{A}_6-2\mathbf{A}_7), \mathbf{A}_4+\mathbf{A}_5-\mathbf{A}_2-\mathbf{A}_7, \pm(2\mathbf{A}_7-\mathbf{A}_8-\mathbf{A}_5), \pm(\mathbf{A}_5-\mathbf{A}_7)\}$.

We shall say that a reaction network has rank *s* if there exists a linearly independent set of *s* reaction vectors for the network and there exists no linearly independent set of s+1 reaction vectors. The set of eight reaction vectors $\{-\mathbf{A}_1, -\mathbf{A}_8, \mathbf{A}_3+\mathbf{A}_4-\mathbf{A}_1-\mathbf{A}_2, \mathbf{A}_6+\mathbf{A}_7-\mathbf{A}_3-\mathbf{A}_5, 2\mathbf{A}_2+2\mathbf{A}_5-\mathbf{A}_6-2\mathbf{A}_7, \mathbf{A}_4+\mathbf{A}_5-\mathbf{A}_2-\mathbf{A}_7, 2\mathbf{A}_7-\mathbf{A}_8-\mathbf{A}_5, \mathbf{A}_5-\mathbf{A}_7\}$ for network (4) is linearly independent, but any set of nine reaction vectors for network (4) is linearly dependent. Thus, the rank of network (4) is eight, and for it we write s=8.

The stoichiometric subspace for a network is the span of its reaction vectors. We shall reserve the symbol S_t to designate the stoichiometric subspace for a network. It is clear that a stoichiometric subspace is a linear subspace of $\Re^N$. The dimension of a stoichiometric subspace is equal to the rank s of its network. The stoichiometric subspace for network (4) is the span generated by the reaction vectors $\{-\mathbf{A}_1, -\mathbf{A}_8, \mathbf{A}_3+\mathbf{A}_4-\mathbf{A}_1-\mathbf{A}_2, \mathbf{A}_6+\mathbf{A}_7-\mathbf{A}_3-\mathbf{A}_5, 2\mathbf{A}_2+2\mathbf{A}_5-\mathbf{A}_6-2\mathbf{A}_7, \mathbf{A}_4+\mathbf{A}_5-\mathbf{A}_2-\mathbf{A}_7, 2\mathbf{A}_7-\mathbf{A}_8-\mathbf{A}_5, \mathbf{A}_5-\mathbf{A}_7\}$ which is $\Re^8$.

We shall say that a vector $\phi \in \Re^N$ is sign compatible with (or the abbreviation s.c.w.) a vector $\sigma \in \Re^N$ if sign σ_L=sign ϕ_L, L=1, 2, ..., N. This means that σ_L is positive if ϕ_L is positive, σ_L is negative if ϕ_L is negative, and σ_L is zero if ϕ_L is zero.

Suppose a network under consideration has r distinct reactions with p reversible reaction pairs and r−2p irreversible reactions. A spanning subnetwork of the network under consideration is a network consisting of all the r−2p irreversible reactions and one and only one reaction of each p reversible reaction pairs. There are r−p (=(r−2p)+p) reactions in a spanning subnetwork. We shall reserve the symbol F to denote the set of reactions in a spanning subnetwork. The network shown in (5) is a spanning subnetwork of network (4), since it consists of the four irreversible reactions and one reaction of the reversible reaction pair in network (4). For it we write $F=\{A_1 \rightarrow 0,\ A_8 \rightarrow 0,\ A_4 \rightarrow 0,\ A_1+A_2 \rightarrow A_3+A_4,\ A_3+A_5 \rightarrow A_6+A_7,\ A_6+2A_7 \rightarrow 2A_2+2A_5,\ A_2+A_7 \rightarrow A_4+A_5,\ A_8+A_5 \rightarrow 2A_7,\ A_7 \rightarrow A_5\}$.

$$\begin{aligned} &A_1 \rightarrow 0 \leftarrow A_8\\ &A_4 \rightarrow 0\\ &A_1+A_2 \rightarrow A_3+A_4\\ &A_3+A_5 \rightarrow A_6+A_7\\ &A_6+2A_7 \rightarrow 2A_2+2A_5\\ &A_2+A_7 \rightarrow A_4+A_5\\ &A_8+A_5 \rightarrow 2A_7\\ &A_7 \rightarrow A_5 \end{aligned} \tag{5}$$

A network having one or more than one reversible reaction pairs (p≥1) will contain more than one spanning subnetwork. In what follows it will be understood that we have chosen to work with a fixed (but arbitrary) spanning subnetwork in each case under study. For a chosen spanning subnetwork, we shall construct a set of corresponding spanning-subnetwork vectors $\{\mathbf{d}^{(1)}, \mathbf{d}^{(2)}, \ldots, \mathbf{d}^{(r-p-s)}\}$ in a vector space $\Re^R$. Let $\{\boldsymbol{\omega}_{i\rightarrow j}: i \rightarrow j \in R\}$ be the standard basis for $\Re^R$. These spanning-subnetwork vectors are the r−p−s linearly independent (nonzero) solutions to the vector equation

$$\sum_{i\rightarrow j \in F} d^{(L)}_{i\rightarrow j}(\mathbf{y}_j-\mathbf{y}_i)=\mathbf{0}, \qquad L=1, 2, \ldots, r-p-s \tag{6}$$

(A solution is a family of numbers $\{d^{(L)}_{i\rightarrow j}: i \rightarrow j \in F\}$.). Then, $\mathbf{d}^{(L)}$, L=1, 2, …, r−p−s are vectors defined in the following way:

$$\mathbf{d}^{(L)}=\sum_{i\rightarrow j \in F} d^{(L)}_{i\rightarrow j}\boldsymbol{\omega}_{i\rightarrow j} \tag{7}$$

Consider network (4). For an arbitrary spanning subnetwork, there is one (r−p−s=14−5−8=1) spanning-subnetwork vector $\mathbf{d}^{(1)}$ in $\Re^R$. Let network (5) be the chosen spanning subnetwork. By Eq. (6), we have

$$\begin{aligned} &d^{(1)}_{A_1\rightarrow 0}(\mathbf{0}-\mathbf{A}_1)+d^{(1)}_{A_8\rightarrow 0}(\mathbf{0}-\mathbf{A}_8)+d^{(1)}_{A_4\rightarrow 0}(\mathbf{0}-\mathbf{A}_4)\\ &\quad+d^{(1)}_{A_1+A_2\rightarrow A_3+A_4}(\mathbf{A}_3+\mathbf{A}_4-\mathbf{A}_1-\mathbf{A}_2)\\ &\quad+d^{(1)}_{A_3+A_5\rightarrow A_6+A_7}(\mathbf{A}_6+\mathbf{A}_7-\mathbf{A}_3-\mathbf{A}_5)\\ &\quad+d^{(1)}_{A_6+2A_7\rightarrow 2A_2+2A_5}(2\mathbf{A}_2+2\mathbf{A}_5-\mathbf{A}_6-2\mathbf{A}_7)\\ &\quad+d^{(1)}_{A_2+A_7\rightarrow A_4+A_5}(\mathbf{A}_4+\mathbf{A}_5-\mathbf{A}_2-\mathbf{A}_7)\\ &\quad+d^{(1)}_{A_8+A_5\rightarrow 2A_7}(2\mathbf{A}_7-\mathbf{A}_8-\mathbf{A}_5)+d^{(1)}_{A_7\rightarrow A_5}(\mathbf{A}_5-\mathbf{A}_7)=\mathbf{0} \end{aligned}$$

A set of nonzero linearly independent solutions to the above equation is:

$$\begin{aligned} &d^{(1)}_{A_1\rightarrow 0}=-1,\ d^{(1)}_{A_8\rightarrow 0}=0,\ d^{(1)}_{A_4\rightarrow 0}=2,\ d^{(1)}_{A_1+A_2\rightarrow A_3+A_4}=1,\ d^{(1)}_{A_3+A_5\rightarrow A_6+A_7}=1,\\ &d^{(1)}_{A_6+2A_7\rightarrow 2A_2+2A_5}=1,\ d^{(1)}_{A_2+A_7\rightarrow A_4+A_5}=1,\ d^{(1)}_{A_8+A_5\rightarrow 2A_7}=0,\ d^{(1)}_{A_7\rightarrow A_5}=-2 \end{aligned} \tag{8}$$

Let $\mathbf{c}=[c_1, c_2, \ldots, c_N]$ be a composition vector in $\overline{\mathbf{P}}^N$ (non-negative orthant of $\Re^N$) for species A_L, L=1, 2, ..., N. In general, the set of isothermal mass action differential equations describing the behavior of a reaction network can be written:

$$\frac{d\mathbf{c}}{dt}=\mathbf{f}(\mathbf{c})=(f_1(\mathbf{c}), \ldots, f_N(\mathbf{c}))=\sum_{i\rightarrow j \in R} k_{i\rightarrow j}\left(\prod_{L=1}^{N} c_L^{y_{iL}}\right)(\mathbf{y}_j-\mathbf{y}_i) \tag{9}$$

where $\mathbf{y}_i$ and $\mathbf{y}_j$ denote, respectively, the reactant and product complex, y_{iL} denoting the stoichiometric coefficient of species A_L in reactant complex y_i, and $k_{i\rightarrow j}$ denoting the rate constant for reaction i→j. The mass action differential equations for network (4) are

$$\begin{aligned} \frac{dc_1}{dt}&=k_{0\rightarrow A_1}-k_{A_1\rightarrow 0}c_1-k_{A_1+A_2\rightarrow A_3+A_4}c_1c_2\\ \frac{dc_2}{dt}&=-k_{A_1+A_2\rightarrow A_3+A_4}c_1c_2+2k_{A_6+2A_7\rightarrow 2A_2+2A_5}c_6c_7^2-2k_{2A_2+2A_5\rightarrow A_6+2A_7}c_2^2c_5^2\\ &\quad-k_{A_2+A_7\rightarrow A_4+A_5}c_2c_7\\ \frac{dc_3}{dt}&=k_{A_1+A_2\rightarrow A_3+A_4}c_1c_2-k_{A_3+A_5\rightarrow A_6+A_7}c_3c_5\\ \frac{dc_4}{dt}&=-k_{A_4\rightarrow 0}c_4+k_{A_1+A_2\rightarrow A_3+A_4}c_1c_2+k_{A_2+A_7\rightarrow A_4+A_5}c_2c_7\\ \frac{dc_5}{dt}&=-k_{A_3+A_5\rightarrow A_6+A_7}c_3c_5+2k_{A_6+2A_7\rightarrow 2A_2+2A_5}c_6c_7^2-2k_{2A_2+2A_5\rightarrow A_6+2A_7}c_2^2c_5^2\\ &\quad+k_{A_2+A_7\rightarrow A_4+A_5}c_2c_7-k_{A_8+A_5\rightarrow 2A_7}c_8c_5+k_{2A_7\rightarrow A_8+A_5}c_7^2\\ &\quad+k_{A_7\rightarrow A_5}c_7-k_{A_5\rightarrow A_7}c_5\\ \frac{dc_6}{dt}&=k_{A_3+A_5\rightarrow A_6+A_7}c_3c_5-k_{A_6+2A_7\rightarrow 2A_2+2A_5}c_6c_7^2+k_{2A_2+2A_5\rightarrow A_6+2A_7}c_2^2c_5^2 \end{aligned} \tag{10}$$

$$\frac{dc_7}{dt} = k_{A_1+A_7\to A_6+A_7}c_3c_5 - k_{A_6+2A_7\to 2A_2+2A_5}c_6c_7^2 + k_{2A_2+2A_5\to A_6+2A_7}c_2^2c_5^2 - k_{A_2+A_7\to A_4+A_5}c_2c_7 + 2k_{A_8+A_5\to 2A_7}c_8c_5 - 2k_{2A_7\to A_8+A_5}c_7^2 - k_{A_7\to A_5}c_7 + k_{A_5\to A_7}c_5$$

$$\frac{dc_8}{dt} = k_{0\to A_8} - k_{A_8\to 0}c_8 - k_{A_8+A_5\to 2A_7}c_8c_5 + k_{2A_7\to A_8+A_5}c_7^2$$

By a steady state of a reaction system we shall mean a composition $\mathbf{c}^*$ satisfying $\mathbf{0}=\mathbf{f}(\mathbf{c}^*)$. By a positive steady state we shall mean a steady state at which all species concentrations are positive. Only positive steady states are considered in this article.

For each composition $\mathbf{c}\in\overline{P}^N$ the right side of differential Eq. (10) is a linear combination of all reaction vectors in its network. This is to say that $d\mathbf{c}/dt$ always lies in the stoichiometric subspace S_t of the network under consideration. Suppose $\gamma=\mathbf{c}-\mathbf{c}^*$ is a vector of composition change around a positive steady state $\mathbf{c}^*$. Then it leads to $\gamma\in S_t$. Any vector $\gamma\in S_t$ of a given network can be represented by reaction vectors in the reaction set F for an arbitrary chosen spanning subnetwork:

$$\gamma=[\gamma_1,\ \gamma_2,\ \ldots,\ \gamma_N]=\sum_{i\to j\in F}\gamma_{i\to j}(\mathbf{y}_j-\mathbf{y}_i) \tag{11}$$

RESULTS AND DISCUSSION

A positive real eigenvalue condition provides a necessary and sufficient condition for the determination of an unstable steady state having a positive real eigenvalue with its eigenvector lying in the stoichiometric subspace for the reaction network under study. For a given reaction network, we choose an arbitrary spanning subnetwork and construct its spanning-subnetwork vectors. Following the condition, a system of equations and inequalities is constructed. We then solve the system. If a set of qualified solutions exists, there is a set of positive rate constants such that the corresponding isothermal mass action differential equations for the given network admit an unstable steady state with a positive real eigenvalue. Otherwise, no matter what positive rate constants the system might have, the differential equations cannot exhibit any positive-real-eigenvalue positive steady state.

Consider network (4). By the spanning-subnetwork vector in Eq. (5) and the positive real eigenvalue condition, a set of inequalities and equations is constructed in Eq. (12):

For reversible reaction $A_1\rightleftarrows 0$ with $A_1\to 0\in F$,

$[\xi_1\mu_1+\alpha_1](-1)-\gamma_{A_1\to 0}$ and $[\xi_1\cdot 0+\alpha_1](-1)-\gamma_{A_1\to 0}$ are s.c.w. $-\mu_1$ (12a)

For reversible reaction $A_8\rightleftarrows 0$ with $A_8\to 0\in F$,

$[\xi_1\mu_8+\alpha_1]\cdot 0-\gamma_{A_8\to 0}$ and $[\xi_1\cdot 0+\alpha_1]\cdot 0-\gamma_{A_8\to 0}$ are s.c.w. $-\mu_8$ (12b)

For reversible reaction $A_6+2A_7\rightleftarrows 2A_2+2A_5$ with $A_6+2A_7\to 2A_2+2A_5\in F$,

$[\xi_1(\mu_6+2\mu_7)+\alpha_1]-\gamma_{A_6+2A_7\to 2A_2+2A_5}$ and $[\xi_1(2\mu_2+2\mu_5)+\alpha_1]-\gamma_{A_6+2A_7\to 2A_2+2A_5}$ are s.c.w. $2\mu_2+2\mu_5-\mu_6-2\mu_7$ (12c)

For reversible reaction $A_8+A_5\rightleftarrows 2A_7$ with $A_8+A_5\to 2A_7\in F$,

$[\xi_1(\mu_8+\mu_5)+\alpha_1]\cdot 0-\gamma_{A_8+A_5\to 2A_7}$ and $[\xi_1(2\mu_7)+\alpha_1]\cdot 0-\gamma_{A_8+A_5\to 2A_7}$ are s.c.w. $2\mu_7-\mu_8-\mu_5$ (12d)

For reversible reaction $A_7\rightleftarrows A_5$ with $A_7\to A_5\in F$,

$[\xi_1\mu_7+\alpha_1](-2)-\gamma_{A_7\to A_5}$ and $[\xi_1\mu_5+\alpha_1](-2)-\gamma_{A_7\to A_5}$ are s.c.w. $\mu_5-\mu_7$ (12e)

For irreversible reaction $A_4\to 0$,

$\xi_1>0$ (12f)

$[\xi_1(\mu_4)+\alpha_1]-\gamma_{A_4\to 0}=0$ (12g)

For irreversible reaction $A_1+A_2\to A_3+A_4$,

$\xi_1>0$ (12h)

$[\xi_1(\mu_1+\mu_2)+\alpha_1]-\gamma_{A_1+A_2\to A_3+A_4}=0$ (12i)

For irreversible reaction $A_3+A_5\to A_6+A_7$,

$\xi_1>0$ (12j)

$[\xi_1(\mu_3+\mu_5)+\alpha_1]-\gamma_{A_3+A_5\to A_6+A_7}=0$ (12k)

For irreversible reaction $A_2+A_7\to A_4+A_5$,

$\xi_1>0$ (12l)

$[\xi_1(\mu_2+\mu_7)+\alpha_1]-\gamma_{A_2+A_7\to A_4+A_5}=0$ (12m)

From Eq. (11), the eigenvector $\gamma\in S_t$ is represented by

$$\begin{aligned}\gamma=&[\mathbf{A}_1](-\gamma_{A_1\to 0}-\gamma_{A_1+A_2\to A_3+A_4})\\&+[\mathbf{A}_2](-\gamma_{A_1+A_2\to A_3+A_4}+2\gamma_{A_6+2A_7\to 2A_2+2A_5}-\gamma_{A_2+A_7\to A_4+A_5})\\&+[\mathbf{A}_3](\gamma_{A_1+A_2\to A_3+A_4}-\gamma_{A_3+A_5\to A_6+A_7})\\&+[\mathbf{A}_4](-\gamma_{A_4\to 0}+\gamma_{A_1+A_2\to A_3+A_4}+\gamma_{A_2+A_7\to A_4+A_5})\\&+[\mathbf{A}_5](-\gamma_{A_3+A_5\to A_6+A_7}+2\gamma_{A_6+2A_7\to 2A_2+2A_5}+\gamma_{A_2+A_7\to A_4+A_5}-\gamma_{A_8+A_5\to 2A_7}+\gamma_{A_7\to A_5})\\&+[\mathbf{A}_6](\gamma_{A_3+A_5\to A_6+A_7}-\gamma_{A_6+2A_7\to 2A_2+2A_5})\\&+[\mathbf{A}_7](\gamma_{A_3+A_5\to A_6+A_7}-2\gamma_{A_6+2A_7\to 2A_2+2A_5}-\gamma_{A_2+A_7\to A_4+A_5}+2\gamma_{A_8+A_5\to 2A_7}-\gamma_{A_7\to A_5})\\&+[\mathbf{A}_8](-\gamma_{A_8\to 0}-\gamma_{A_8+A_5\to 2A_7})\end{aligned} \tag{13a}$$

It is required that μ is sign compatible with γ. This implies that

$$\begin{aligned}&\mu_1 \text{ is s.c.w. } -\gamma_{A_1\to 0}-\gamma_{A_1+A_2\to A_3+A_4}\\&\mu_2 \text{ is s.c.w. } -\gamma_{A_1+A_2\to A_3+A_4}+2\gamma_{A_6+2A_7\to 2A_2+2A_5}-\gamma_{A_2+A_7\to A_4+A_5}\\&\mu_3 \text{ is s.c.w. } \gamma_{A_1+A_2\to A_3+A_4}-\gamma_{A_3+A_5\to A_6+A_7}\\&\mu_4 \text{ is s.c.w. } -\gamma_{A_4\to 0}+\gamma_{A_1+A_2\to A_3+A_4}+\gamma_{A_2+A_7\to A_4+A_5}\\&\mu_5 \text{ is s.c.w. } -\gamma_{A_3+A_5\to A_6+A_7}+2\gamma_{A_6+2A_7\to 2A_2+2A_5}+\gamma_{A_2+A_7\to A_4+A_5}-\gamma_{A_8+A_5\to 2A_7}+\gamma_{A_7\to A_5}\\&\mu_6 \text{ is s.c.w. } \gamma_{A_3+A_5\to A_6+A_7}-\gamma_{A_6+2A_7\to 2A_2+2A_5}\\&\mu_7 \text{ is s.c.w. } \gamma_{A_3+A_5\to A_6+A_7}-2\gamma_{A_6+2A_7\to 2A_2+2A_5}-\gamma_{A_2+A_7\to A_4+A_5}+2\gamma_{A_8+A_5\to 2A_7}-\gamma_{A_7\to A_5}\\&\mu_8 \text{ is s.c.w. } -\gamma_{A_8\to 0}-\gamma_{A_8+A_5\to 2A_7}\end{aligned} \tag{13b}$$

The inequalities and Eq.s (12) and (13b) are solved to determine an unstable steady state with a real positive eigenvalue. According to Eqs. (12) and (13b), we assume the sign patterns of $-\mu_1$, $-\mu_8$, $2\mu_2+2\mu_5-\mu_6-2\mu_7$, $2\mu_7-\mu_8-\mu_5$, $\mu_5-\mu_7$, μ_1, …, μ_8. Eqs. (12) and (13b) with the assumed sign patterns form a linear system of inequalities and equations. Indeed, it is always linear for any reaction network with r−p−s=1. Mathematically, a linear system of inequalities and equations can be solved by the Simplex method, even if consisting of many unknowns. A computer program was developed to solve this example, for which the number of sign patterns considered is $3^{13}-1$. Among them only four different sets of solutions are found. A set of solutions displayed in Eq. (14) is used to do the following analysis without any particular reason. According to the formula in the Appendix, Eq. (14) is used to construct a set of rate constants in Eq. (15), an unstable steady state $\mathbf{c}^*$ in Eq. (16) having a positive real eigenvalue (λ=1) with its eigenvector $\gamma\in S_t$. Note the equality of the flow rate $k_{A_1\to 0}$, $k_{A_8\to 0}$ and $k_{A_4\to 0}$ in Eq. (15), which is consistent with a CFSTR picture.

$$\begin{aligned}&\mu_1=-12.99999,\ \mu_2=14.99999,\ \mu_3=-1,\ \mu_4=10.99999,\\&\mu_5=13.99999,\ \mu_6=44.99998,\ \mu_7=5.999995,\ \mu_8=-1,\\&\xi_1=1,\ \alpha_1=-14.49999,\\&\gamma_{A_1\to 0}=12.58695,\ \gamma_{A_8\to 0}=-1.913043,\ \gamma_{A_4\to 0}=-6.999995,\\&\gamma_{A_1+A_2\to A_3+A_4}=-12.49999,\ \gamma_{A_3+A_5\to A_6+A_7}=-1.5,\\&\gamma_{A_6+2A_7\to 2A_2+2A_5}=-2.499999,\ \gamma_{A_2+A_7\to A_4+A_5}=6.499996,\ \gamma_{A_8+A_5\to 2A_7}=2,\ \gamma_{A_7\to A_5}=0\end{aligned} \tag{14}$$

$$A_1 \underset{1.1471573}{\overset{21.999958}{\rightleftarrows}} 0 \underset{21.999958}{\overset{1.9130433}{\rightleftarrows}} A_8$$

$$A_4 \xrightarrow{21.999958} 0$$

$$A_1 + A_2 \xrightarrow{2242.5017} A_3 + A_4$$
$$A_3 + A_5 \xrightarrow{1.2727301} A_6 + A_7 \quad (15)$$
$$A_6 + 2A_7 \underset{1984537.3}{\overset{74520.314}{\rightleftharpoons}} 2A_2 + 2A_5$$
$$A_2 + A_7 \xrightarrow{90.000045} A_4 + A_5$$
$$A_8 + A_5 \underset{71.999592}{\overset{321.99983}{\rightleftharpoons}} 2A_7$$
$$A_7 \underset{29.750039}{\overset{0.74999834}{\rightleftharpoons}} A_5$$

c_1^*=0.00668898, c_2^*=0.06666644, c_3^*=10.999999, c_4^*=0.09090926,
c_5^*=0.07142848, c_6^*=0.02222221, c_7^*=0.16666714, c_8^*=0.08695668. (16)

The reaction network (15) has deficiency one. The deficiency one algorithm [Feinberg, 1972] is used to show that it cannot admit multiple steady states no matter what rate constants it might have. It is interesting to know where this unstable steady state $\mathbf{c}^*$ in Eq. (16) goes for a small perturbation without approaching any other steady state. Fig. 1 shows the concentration (c_1, c_3 and c_7)-time (t) plot with the rate constants in Eq. (15). A sustained oscillation is obtained and it has a period about 2,200 sec. For network (15), five different initial conditions (I.C. 1, 2, …, 5 or points K, L, …, O) are numerically simulated and the phase portrait (c_3 vs. c_7) is shown in Fig. 2. The I.C. 1 is the unstable steady state $\mathbf{c}^*$ in Eq. (16). All the five initial conditions approach an anticlockwise stable limiting cycle (R→S→T→R→…), which is marked by the bold curve in Fig. 2.

Network (15) is a deficiency one subnetwork of network (2). Can the sustained oscillations of subnetwork (15) be extended to its parent network (2)? In the study of steady state multiplicity in complex reaction networks, a parent network often (but not always) also admits multiple steady states if one of its deficiency one subnetworks consisting of the same number of species exhibits steady state multiplicity. To extend the multiplicity from a subnetwork to a parent network, sometimes the parameters in the system of inequalities and equations remain the same without even a change; sometimes

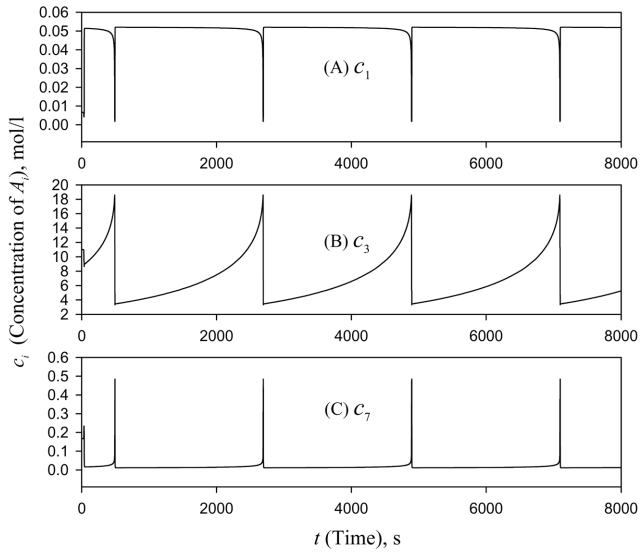

Fig. 1. The concentrations (c_1, c_3 and c_7) - time (t) plot with the rate constants in reaction network (15).

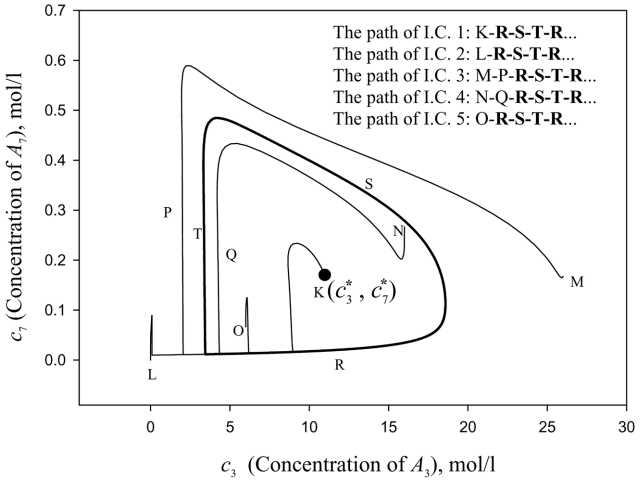

Fig. 2. The phase portrait for the reaction network (15).

only a little change is required. For the cases of parameters without a change, the subnetwork analysis [Li, 1998] provides some sufficient conditions for the capacity of multiple steady states in a network of deficiency greater than one if one of its subnetworks admits steady state multiplicity. One example [Chuang et al., 2004] is shown for the case that a little bit of change for the parameters is required. With a similar concept, we think the parameters in Eq. (14) for the subnetwork (15) can be used to study sustained oscillations for its parent network (2).

Consider the network (2) of deficiency two, r−p−s=15−5−8=2; we have two spanning-subnetwork vectors $\mathbf{d}^{(1)}$, $\mathbf{d}^{(2)}$ and the system of inequalities and equations constructed by the positive real eigenvalue condition becomes nonlinear, for which the Simplex method cannot be applied. Based on the vector μ and γ in Eq. (14), we adjust some of these parameters to satisfy the positive real eigenvalue condition for the network (2). The modified vectors μ and γ are shown in Eq. (17), which are used to construct a set of rate constants in Eq. (18), an unstable steady state $\mathbf{c}^*$ in Eq. (19) having a positive real eigenvalue (λ=1) with its eigenvector $\gamma \in S_r$. Comparing Eq. (14) with Eq. (17), the vector μ remains unchanged and only the values of $\gamma_{A_1 \to 0}$, $\gamma_{A_8 \to 0}$, $\gamma_{A_1+A_2 \to A_3+A_4}$ and $\gamma_{A_2+A_7 \to A_4+A_5}$ are tuned slightly. It implies that the solution Eq. (14) for the linear system of inequalities and equations derived from the positive real eigenvalue condition of the deficiency one subnetwork (15) can be extended to be a set of solution for that of the deficiency two parent network (18) with only a slight modification.

μ_1=−12.99999, μ_2=14.99999, μ_3=−1, μ_4=10.99999,
μ_5=13.99999, μ_6=44.99998, μ_7=5.999995, μ_8=−1,
ξ_1=1, α_1=−14.49999, ξ_2=0.02, α_2=0, (17)
$\gamma_{A_1 \to 0}$=12.54208, $\gamma_{A_8 \to 0}$=−1.928978, $\gamma_{A_4 \to 0}$=−6.999995,
$\gamma_{A_1+A_2 \to A_3+A_4}$=−12.47999, $\gamma_{A_3+A_5 \to A_6+A_7}$=−1.5, $\gamma_{A_6+2A_7 \to 2A_2+2A_5}$=−2.499999,
$\gamma_{A_2+A_7 \to A_4+A_5}$=6.289996, $\gamma_{A_8+A_5 \to 2A_7}$=2, $\gamma_{A_7 \to A_5}$=0, $\gamma_{A_1+A_5 \to A_3+A_7}$=0.01.

$$A_1 \underset{1.1506088}{\overset{27.160431}{\rightleftharpoons}} 0 \underset{27.160431}{\overset{1.9289784}{\rightleftharpoons}} A_8$$
$$A_4 \xrightarrow{27.160431} 0$$
$$A_1 + A_2 \xrightarrow{2250.4377} A_3 + A_4$$
$$A_3 + A_5 \xrightarrow{1.6361681} A_6 + A_7 \quad (18)$$

$$A_6 + 2A_7 \underset{2303435.2}{\overset{50067.446}{\rightleftarrows}} 2A_2 + 2A_5$$

$$A_2 + A_7 \xrightarrow{62.611960} A_4 + A_5$$

$$A_8 + A_5 \underset{48.373866}{\overset{505.44446}{\rightleftarrows}} 2A_7$$

$$A_7 \underset{38.141090}{\overset{0.61475304}{\rightleftarrows}} A_5$$

$$A_1 + A_5 \xrightarrow{32.368340} A_3 + A_7$$

c_1^*=0.00554516, c_2^*=0.07933311, c_3^*=10.969999, c_4^*=0.07363654,
c_5^*=0.05571419, c_6^*=0.02222221, c_7^*=0.20333381, c_8^*=0.07102164. (19)

Fig. 3 shows the concentration (c_8)-time (t) plot with the variation of rate constant $k_{A_2+A_7\to A_4+A_5}$. As $k_{A_2+A_7\to A_4+A_5}$ equals to 7.5; the system goes to a stable steady state though a damped oscillation. It gives rise to a stable limiting cycle as $k_{A_2+A_7\to A_4+A_5}$ equals to 7.6 and 10 with an increase of the oscillating cycle time and the amplitude of c_8 as increasing the rate constant $k_{A_2+A_7\to A_4+A_5}$. Fig. 4 displays the bifurcation plot of $k_{A_2+A_7\to A_4+A_5}$ vs. c_8. As $k_{A_2+A_7\to A_4+A_5}\leq 7.5$, the system admits

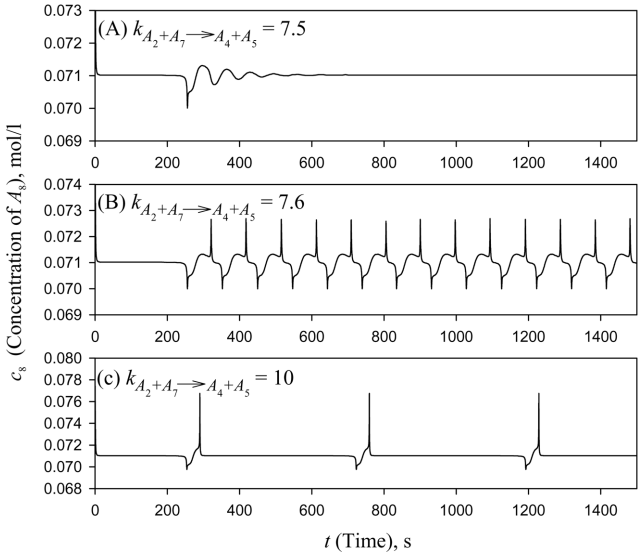

Fig. 3. The concentration (c_8) - time plot for the reaction network (18) with a different rate constant $k_{A_2+A_7\to A_4+A_5}$.

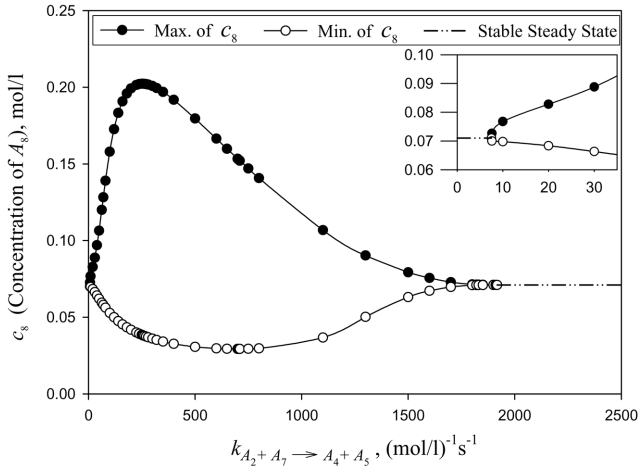

Fig. 4. The bifurcation plot ($k_{A_2+A_7\to A_4+A_5}$ vs. c_8) for the reaction network (18).

a stable steady state and the stable state concentration c_8 is equal to 0.07102. It is depicted in the enlarged plot at the upper-right corner of Fig. 4. As $7.6\leq k_{A_2+A_7\to A_4+A_5}\leq 255$, the amplitude of c_8 in the limiting cycle (indicated by the max. and min. value in Fig. 4) increases with an increase of the rate constant $k_{A_2+A_7\to A_4+A_5}$. As $255<k_{A_2+A_7\to A_4+A_5}\leq 1917$, the amplitude of c_8 in the limiting cycle decreases with an increase of the rate constant $k_{A_2+A_7\to A_4+A_5}$. As $k_{A_2+A_7\to A_4+A_5}$ is greater than about 1918, the oscillations damp and the stable state concentration c_8 has the same value as $k_{A_2+A_7\to A_4+A_5}\leq 7.5$.

Consider a family member of network (2). The addition of a pair of reversible reactions $A_1+2A_5 \rightleftarrows A_6+2A_7$ to network (2) leads to a deficiency three network. Thus, r−p−s=17−6−8=3 and three spanning-subnetwork vectors can be computed. According to the positive real eigenvalue condition, a nonlinear system of inequalities and equations can be constructed. Similarly, a set of solutions to the nonlinear system can be easily obtained by modifying the vector γ in Eq. (17) slightly. The parameters in Eq. (20) are a set of solutions, which is only a little different from those in Eq. (17) for the values of $\gamma_{A_3+A_5\to A_6+A_7}$. Eq. (20) is used to construct a set of rate constants in Eq. (21a) and an unstable steady state $\mathbf{c}^*$ in Eq. (21b) having a positive real eigenvalue (λ=1) with its eigenvector $\gamma\in S_t$.

μ_1=− 12.99999, μ_2=14.99999, μ_3=− 1, μ_4=10.99999,
μ_5=13.99999, μ_6=44.99998, μ_7=5.999995, μ_8=− 1,
ξ_1=1, α_1=− 14.49999, ξ_2=0.02, α_2=0, ξ_3=0.009, α_2=− 0.009 (20)
$\gamma_{A_1\to 0}$=12.54208, $\gamma_{A_8\to 0}$=− 1.928978, $\gamma_{A_4\to 0}$=− 6.999995,
$\gamma_{A_1+A_2\to A_3+A_4}$=− 12.47999, $\gamma_{A_3+A_5\to A_6+A_7}$=− 1.60791,
$\gamma_{A_6+2A_7\to 2A_2+2A_5}$=−2.499999, $\gamma_{A_2+A_7\to A_4+A_5}$=6.289996,
$\gamma_{A_8+A_5\to 2A_7}$=2, $\gamma_{A_7\to A_5}$=0, $\gamma_{A_1+A_5\to A_3+A_7}$=0.01, $\gamma_{A_1+2A_5\to A_6+2A_7}$=0.

$$A_1 \underset{1.1506088}{\overset{27.160431}{\rightleftarrows}} 0 \underset{27.160431}{\overset{1.9289784}{\rightleftarrows}} A_8$$

$$A_4 \xrightarrow{27.160431} 0$$

$$A_1 + A_2 \xrightarrow{2250.4377} A_3 + A_4$$

$$A_3 + A_5 \xrightarrow{1.4385347} A_6 + A_7 \qquad (21a)$$

$$A_1 + 2A_5 \underset{4.4049983}{\overset{538.00568}{\rightleftarrows}} A_6 + 2A_7 \underset{1777571.4}{\overset{67543.954}{\rightleftarrows}} 2A_2 + 2A_5$$

$$A_2 + A_7 \xrightarrow{68.687408} A_4 + A_5$$

$$A_8 + A_5 \underset{58.217085}{\overset{444.04363}{\rightleftarrows}} 2A_7$$

$$A_7 \underset{33.505695}{\overset{0.67440459}{\rightleftarrows}} A_5$$

$$A_1 + A_5 \xrightarrow{2.8434523} A_3 + A_7$$

c_1^*=0.00554516, c_2^*=0.07933311, c_3^*=10.86208, c_4^*=0.07363654,
c_5^*=0.06342206, c_6^*=0.01982421, c_7^*=0.18534880, c_8^*=0.07102164.(21b)

Fig. 5 shows a two-parameter (Flow Rate, $k_{A_7\to A_5}$) plane for different values of the rate constant $k_{A_1+2A_5\to A_6+2A_7}$ (=538.00568 and 14000) for network (21a). The area inside the cusp (marked with OSC) stands for an undamped oscillations region, and outside of the cusp (marked with SS) denotes a stable steady state region. Fig. 5 shows that, to maintain the existence of the sustained oscillations under a fixed rate constant $k_{A_1+2A_5\to A_6+2A_7}$, the smaller the flow rate is, the smaller the rate constant $k_{A_7\to A_5}$ is required and the narrower the range of it exists. To maintain the existence of the sustained oscillations under a higher rate constant $k_{A_1+2A_5\to A_6+2A_7}$, it is required to increase the flow

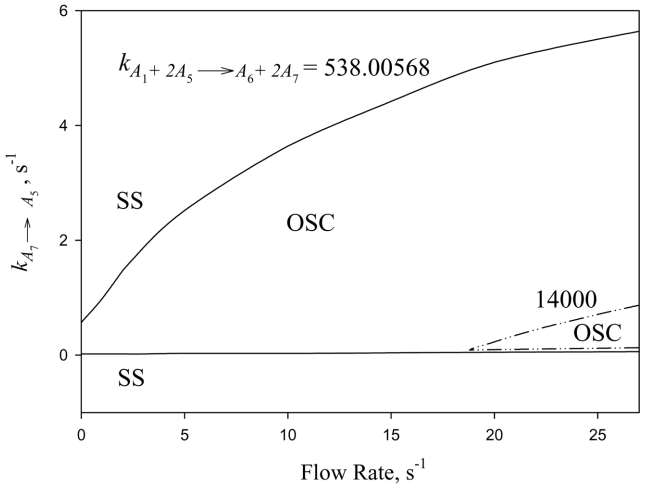

Fig. 5. The locus of the oscillation bifurcation for network (21) in the two-parameter (Flow Rate, $k_{A_7 \rightarrow A_5}$) plane for different values of the rate constant $k_{A_1+2A_5 \rightarrow A_6+2A_7}$ (=538.00568 and 14000).

rate and to decrease the rate constant $k_{A_7 \rightarrow A_5}$. It also shows that the higher the rate constant $k_{A_1+2A_5 \rightarrow A_6+2A_7}$ is, the narrower the range of the sustained oscillations.

The parameters in Eq. (14) satisfy the positive real eigenvalue condition derived from the deficiency one subnetwork (4). Parameters in Eqs. (17) and (20) are the modification of Eq. (14). They can be extended to be the solutions to the positive real eigenvalue condition for the parent networks in Eqs. (2) and (21), respectively. The subnetwork analysis extends directly the capacity of multiple steady states of a subnetwork to its parent networks without changing any of the parameters. According to the analysis, the extended reactions can be represented by a linear combination of the reversible reactions $y_i \rightleftarrows y_j$ in the subnetwork with $\mathbf{y}_i \cdot \boldsymbol{\mu} \neq \mathbf{y}_j \cdot \boldsymbol{\mu}$ (The symbol "·" indicates the standard dot product.). Similarly, one can prove that the above condition also works for the case of the positive real eigenvalue problems (Although the vector $\boldsymbol{\mu}$ has a different definition in each problem, the system of inequalities and equations for both problems forms a similar structure.). Thus, applying the same parameters in Eqs. (17), (14) and (20) without modifying any one of them, the capacity of admitting an unstable steady state with a positive real eigenvalue for reaction networks in Eqs. (2), (4) and (21) can be extended directly to their parent networks. Take network (4) for an example. The parameters in Eq. (14) can be used to show that its family members displayed in network (22) also have the capacity to exhibit unstable steady states with positive real eigenvalues. The first eight lines of network (22) are just the deficiency one subnetwork (4). The parameters a, b, …, e in the last line of network (22) are any real numbers. A negative parameter means the reverse of the reaction arrow. The last line of reaction network (22) describes any of the reactions which can be represented by a linear combination of the five reactions on the right-hand side of the equation. These five reactions are reversible in the subnetwork (4) and have the property of $\mathbf{y}_i \cdot \boldsymbol{\mu} \neq \mathbf{y}_j \cdot \boldsymbol{\mu}$ (For the vector $\boldsymbol{\mu}$ in Eq. (14), it leads to $\mu_1 \neq 0$, $\mu_8 \neq 0$, $\mu_6+2\mu_7 \neq 2\mu_2+2\mu_5$, $\mu_8+\mu_5 \neq 2\mu_7$ and $\mu_7 \neq \mu_5$.). The extended reactions derived by the last line of the network (22) might consist of many irreversible reactions and reversible reaction pairs. Thus, the network (22) might have high deficiency. In a similar way, the capacity of admitting an unstable steady state with a positive real eigenvalue for the networks in Eqs. (2) and (21) can also be extended to their family networks by the parameters in Eqs. (17) and (20), respectively.

$$
\begin{aligned}
&A_1 \rightleftarrows 0 \rightleftarrows A_8\\
&A_4 \rightarrow 0\\
&A_1+A_2 \rightarrow A_3+A_4\\
&A_3+A_5 \rightarrow A_6+A_7\\
&A_6+2A_7 \rightleftarrows 2A_2+2A_5\\
&A_2+A_7 \rightarrow A_4+A_5\\
&A_8+A_5 \rightleftarrows 2A_7\\
&A_7 \rightleftarrows A_5\\
&y_i \rightarrow y_j = a(A_1 \rightarrow 0)+b(A_8 \rightarrow 0)+c(A_6+2A_7 \rightarrow 2A_2+2A_5)\\
&\qquad +d(A_8+A_5 \rightarrow 2A_7)+e(A_7 \rightarrow A_5)
\end{aligned}
\tag{22}
$$

CONCLUSIONS

A complex bacterial glycolysis model is studied, which consists of eight nonlinear coupled equations. A minimum subnetwork of the bacterial glycolysis model is determined to exhibit an unstable steady state with a positive real eigenvalue, which gives rise to undamped oscillations for a small perturbation. The phenomena of oscillations and bifurcation are discussed. These results are extended to the bacterial glycolysis model and several parent networks.

The positive real eigenvalue condition is applied in this work, which provides a necessary and sufficient condition for the determination of an unstable steady state having a positive real eigenvalue for general reaction networks. The advantage of this analysis is without knowing the values of rate constants in advance, which are usually difficult to determine precisely. For a deficiency one network, the positive real eigenvalue condition constructs a linear system of inequalities and equations, which can be solved by the Simplex method. The capacity of admitting an unstable steady state with a positive real eigenvalue for a subnetwork might be extended to its parents.

The results of this work might help us to study the complex reaction networks in other biological systems. From a metabolic engineering point of view, it can be also applied to investigate glycolysis in other organisms, such as mammalian cells and yeast, even if they have different stoichiometry. At the course of the breakdown of glucose to obtain energy in cells, this work mainly focuses on glycolysis. An oxidative catabolism system, which couples a glycolysis model, a citric acid cycle and a respiratory chain, is currently under investigation. It involves not only the ATP generation from glucose but also the conversion of pyruvate to carbon dioxide and water. The results of this work serve as a guide to this kind of complex reaction network.

APPENDIX

1. Positive Real Eigenvalue Condition

Consider an N-species reaction network with reaction set R and stoichiometric subspace S,. Suppose the network has rank s and r reactions with p reversible reaction pairs. Let the reaction set for an arbitrary spanning subnetwork be F and let $\{\mathbf{d}^{(1)}, \mathbf{d}^{(2)}, \ldots, \mathbf{d}^{(r-p-s)}\}$ be

a set of corresponding spanning-subnetwork vectors. Then the corresponding isothermal mass action differential equations for the given network have the capacity to admit an unstable steady state having a positive real eigenvalue with its eigenvector in the stoichiometric subspace S_t, if and only if there exist a nonzero vector $\gamma \in S_t$, a vector $\mu \in \Re^N$ which is sign compatible with γ, and also numbers $\xi_1, \xi_2, \ldots, \xi_{r-p-s}, \alpha_1, \alpha_2, \ldots, \alpha_{r-p-s}$ satisfying the following two conditions:

(i) For all reversible reaction $y_i \rightleftarrows y_j \in R$ with $y_i \rightarrow y_j \in F$,

$\sum_{L=1}^{r-p-s} [\xi_L(\mathbf{y}_i \cdot \mu) + \alpha_L] d_{i\rightarrow j}^{(L)} - \gamma_{i\rightarrow j}$ and $\sum_{L=1}^{r-p-s} [\xi_L(\mathbf{y}_i \cdot \mu) + \alpha_L] d_{i\rightarrow j}^{(L)} - \gamma_{i\rightarrow j}$ are sign compatible with $(\mathbf{y}_j - \mathbf{y}_i)\cdot\mu$.

(ii) For all irreversible reactions $y_i \rightarrow y_j \in R$,

$\sum_{L=1}^{r-p-s} \xi_L d_{i\rightarrow j}^{(L)} > 0$ and $\sum_{L=1}^{r-p-s} [\xi_L(\mathbf{y}_i \cdot \mu) + \alpha_L] d_{i\rightarrow j}^{(L)} - \gamma_{i\rightarrow j} = 0$.

If the answer is yes, the vectors γ, μ and the numbers ξ_i, α_i, i=1, …, r−p−s are used to construct a set of positive rate constants $\{k_{i\rightarrow j}, i\rightarrow j \in R\}$ and an unstable steady state $\mathbf{c}^*$ having a positive real eigenvalue λ (>0) with its corresponding eigenvector $\gamma \in S_t$. They are:

$$\mathbf{c}^* = [c_1^*, \ldots, c_N^*] = \frac{\gamma}{\mu} = \left[\frac{\gamma_1}{\mu_1}, \ldots, \frac{\gamma_N}{\mu_N}\right], \tag{A.1}$$

c_L^*=any positive real number if $\gamma_L = \mu_L = 0$.

$$k_{i\rightarrow j} = \frac{\lambda \kappa_{i\rightarrow j}}{\prod_{L=1}^{N} (c_L^*)^{y_{iL}}} \qquad \forall y_i \rightarrow y_j \in R, \tag{A.2}$$

where λ=any positive real number. The variable $\kappa_{i\rightarrow j}$ is evaluated by:

For all irreversible reactions $i \rightarrow j \in R$,

$$\kappa_{i\rightarrow j} = \sum_{L=1}^{r-p-s} \xi_L d_{i\rightarrow j}^{(L)} \tag{A.3}$$

For all reversible reactions $i \rightleftarrows j \in R$ with $i \rightarrow j \in F$ and $\mathbf{y}_i \cdot \mu \neq \mathbf{y}_j \cdot \mu$,

$$\kappa_{j\rightarrow i} = \frac{\sum_{L=1}^{r-p-s} [\xi_L(\mathbf{y}_i \cdot \mu) + \alpha_L] d_{i\rightarrow j}^{(L)} - \gamma_{i\rightarrow j}}{(\mathbf{y}_j - \mathbf{y}_i)\cdot\mu} \tag{A.4}$$

$$\kappa_{i\rightarrow j} = \frac{\sum_{L=1}^{r-p-s} [\xi_L(\mathbf{y}_j \cdot \mu) + \alpha_L] d_{i\rightarrow j}^{(L)} - \gamma_{i\rightarrow j}}{(\mathbf{y}_j - \mathbf{y}_i)\cdot\mu} \tag{A.5}$$

For all reversible reactions $i \rightleftarrows j \in R$ with $i \rightarrow j \in F$ and $\mathbf{y}_i \cdot \mu \neq \mathbf{y}_j \cdot \mu$,

$$\kappa_{i\rightarrow j} > 0, \ \kappa_{j\rightarrow i} > 0 \tag{A.6}$$

$$\kappa_{i\rightarrow j} - \kappa_{j\rightarrow i} = \sum_{L=1}^{r-p-s} \xi_L d_{i\rightarrow j}^{(L)} \tag{A.7}$$

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NOMENCLATURE

$A_1, A_2, \ldots$: chemical species

$c_1, c_2, \ldots$: molar concentrations for species $A_1, A_2, \ldots$ [mol/*l*]

$\mathbf{c}^*$: composition vectors at unstable steady states [mol/*l*]

$\mathbf{d}^{(L)}$: a set of spanning-subnetwork vectors in $\Re^R$

F : reaction set in a spanning subnetwork

$k_{i\rightarrow j}$: rate constant for reaction $i \rightarrow j$ $[(\text{mol/}l)^{1\text{-reaction order}} \cdot s^{-1}]$

$k_{i\rightarrow 0}$: flow rate $[s^{-1}]$

L : (=r−p−s), number of linearly independent solutions to Eq. (6)

N : number of species in a reaction network

$\overline{P}^N$: non-negative orthant of $\Re^N$

p : number of distinct reversible reaction pairs in a network

R : reaction set

$\Re^R$: vector space for r-tuples ($i \rightarrow j$) of real numbers with reaction $i \rightarrow j$ in the reaction set R

r : number of distinct reactions in a network

S_t : stoichiometric subspace for a network

s : number of ranks in a network

t : time [s]

$\mathbf{y}_i, \mathbf{y}_j$: complex vectors

Greek Letters

α_i : variables used in the positive real eigenvalue condition

γ : the nonzero real eigenvector

$\kappa_{i\rightarrow j}$: variables defined in Eqs. (A.3)-(A.7)

λ : a positive real eigenvalue

μ : variables defined in Eq. (A.1)

$\omega_{i\rightarrow j}$: the standard basis for vector space $\Re^R$

ξ_i : variables used in the positive real eigenvalue condition

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