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ORIGINAL PAPER

# Globally attractive oscillations in open monosubstrate allosteric enzyme reactions

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**Abstract** Here we study the dynamical properties of glycolytic and other similar biochemical oscillation-generating processes by means of the analysis of a model proposed by Golbdeter and Lefever (Bioph J 13:1302–1315, 1972) in a reduced form proposed by Keener and Sneyd (Mathematical physiology, chap 1, Springer Verlag, Berlin, 2009). After showing that the orbits of the system are bounded, we give some conditions for the existence of oscillations and for the global arrest of them. Then, after deriving an equivalent Lienard-Newton's equation we assess uniqueness and the global stability of the arising limit cycle. Finally, we shortly investigate the possibility of breaking of the spatial symmetry. Some biological remarks end the work.

**Keywords** Limit cycles · Glycolysis · Global stability · Lienard's equation · Turing's bifurcations

## 1 Introduction

The discovery of damped [5] and undamped [28] oscillations in glycolytic process was a turning point in modern biochemistry. Indeed, apart the paramount relevance of glycolysis for the life [1], thanks to this discovery it became clear maybe for the first time that one must not only "understand the sequence of molecular transformation among reaction pathways, but" one "must understand the regulation of *rates* of transformation" [31].

Moreover, those experimental discoveries were the backbone of the applications of nonlinear theories in chemistry and biology, through the introduction—framed in its

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theory of non-equilibrium thermodynamics—of the concepts of breaking of temporal (and spatial) simmetries by I. Prigogine and its school [26,27].

As a consequence, a large body of literature investigated and is investigating these oscillations, both experimentally [8,16,25,32] and theoretically [11,17], so that that glycolytic oscillations may be considered "the" prototypical biochemical oscillations [12].

Since sixties [29,12,18], it was hypothesized (and there is nowadays a large consensus, supported by many experimental evidences, to this hypothesis) that the key step for the onset of such oscillations involves the allosteric enzyme phosphofructokinase (PFK1), and in particular the positive feedback exerted on PFK1 by its product: the ADP.

In 1968 E.E. Selkov proposed a pioneering mathematical model of this feedback on PFK [29]. Although quite approximated, and although developed in a stage where the knowledge of this biochemical process was relatively small, Selkov's model is able to well reproduce glycolytic oscillations in yeast. Moreover, it triggered a number of further studies [11,30].

However, as we mentioned above, as far as the experimental knowledge of the glycolysis progressed, new and more detailed kinetic models were proposed for the onset of the oscillations in glycolysis. Among them, of remarkable relevance is the Golbeter-Lefever model [9], which, in a more general setting, describes the activation of a generic allosteric enzyme by its product in the framework of the Monod-Whyman-Changeaux theory [21].

The Godbeter-Lefever model is able to catch finer details of the onset and of behaviour of the glycolytic oscillations. For example, experiments [14] shows that a key factor in triggering the oscillations is the supply rate of the substrate: if this rate is under a small threshold or if it is over a higher threshold the system tends to steady state, and there is onset of periodicity only for intermediate values. Mathematically, this means that assuming as bifurcation parameter the supply rate there are two Hopf bifurcations points. Whereas the Goldbeter-Lefever model correctly reproduces this behavior, the Selkov's model shows a unique bifurcation, the higher, and the range of the supply rate inducing a bounded oscillation is quite narrow.

The aim of this work is to investigate both the global stability of the steady state of the Goldbeter-Lefever model and to assess the uniqueness of its sustained oscillations.

Indeed, a topic that is of relevance in the study of biochemical oscillations is the assesment of their robustness respect to large deviations of the involved chemical compounds, which was first stressed by Prigogine (see [26,27] and references therein). This is a biologically interesting subject, since complex biochemical reactions may lead to the presence of multiple oscillatory domains [26,10,2] or also a continuum of possible oscillations, as in the pioneering Lotka model for undamped chemical oscillations [20,26]. Summarizing, the result of a perturbation may induce totally different oscillatory behaviours, as well as the extinction of the oscillations.

Recently, we faced these problem in glycolysis by showing, through a mathematical analysis, that the Selkov model predicts a unique limit cycle for the ATP and ADP [4]. Here we extend our study by means of the more realistic and more general Goldbeter-Lefever model, although this implies a more complex, and to some degree less analytical, mathematical analysis.

Symmetrically, a related problem is finding the conditions for the global arrest of oscillations, that we develop here in some depth.

At the best of our knowledge the only other analysis on uniqueness of limit cycles in glycolisis is a brief example (treated from a pure mathematical point of view) in [15] and regarding a quite simpler model [6].

## 2 The Goldbeter–Lefever's model

In [9] it has been proposed an ODE-based model of glycolisis and other open mono-substrate allosteric enzyme reactions (see also [27] ). In the GoldbeterLefever model the enzyme is a dimer having two possible states: active ($R$) and inactive ($T$). The substrate $x$ can bind to both $R$ and $T$, the product $y$ only to $T$. There is a constant influx of the substrate $x$ and a first order reaction removal of the product $y$ (or, equivalently, a first order degradation rate of the product). According to experimental data, there is fast equilibration of the enzyme w.r.t. the metabolites, thus reducing the model to a bidimensional ODE system [9]. In [18], it was shown that under the following hypotheses:

**Ha** The substrate binds uniquely to the active form of the enzyme;
**Hb** If the substrate binds to $R$ the formation of the product is preferred to the dissociation;
**Hc** The inactivation of the enzyme ($R \to T$) is preferred to its activation ($T \to R$),

the Goldbeter-Lefever model can be with good approximation reduced to the following [18]:

$$\begin{aligned} \frac{dx}{d\tau} &= \nu - x(1+y)^2 \\ \frac{dy}{d\tau} &= x(1+y)^2 - \eta y \end{aligned} \tag{1}$$

where : $\widehat{x}(\tau)$ and $y(\tau)$ are adimensional concentration of, respectively, the substrate (ATP for glycolysis) and the product (ADP for glycolysis) at time $\tau$; $\nu$ is the supply rate of the substrate into the system and $\eta$ is the removal rate of the product. The function $V(x, y) = x(y+1)^2$ in model (1) is the conversion rate from the substrate to the product. Note that $V(x, y)$ is unbounded, which does not allow to apply the general theorems proposed in [7].

## 3 Model, orbits and attractors: first properties

Some nice works have appeared where some very general families of biochemical models are studied. Those papers, for example [7,24,3] are interesting since they catch common features of apparently very different specific, but have the limit of giving general results. Studies as the present work are, instead, model-specific, which is a limit since one may not catch important basic biochemical facts, but they allow to infer results that are more detailed.

For example, system (1) belongs to the very general family of models studied in [24], where some very general result for such systems is given, with reference to boundedness and existence and local stability of limit cycles. However, here we are interested in giving some global results that are model specific and analytical, thus we shall proceed by transforming our system in another that is more amenable to analytical treatment. Indeed, by means of the following transformation of the time variable:

$$dt = (1 + y^2(\tau))^2 d\tau,$$

we obtain a new system:

$$\begin{aligned} x'(t) &= \frac{\nu}{(1+y)^2} - x \\ y'(t) &= x - \eta \frac{y}{(1+y)^2}, \end{aligned} \tag{2}$$

which is topologically equivalent to (1), and which we shall use for our mathematical analysis.

It is straightforward to verify that the solutions of (2) are positive. Moreover, since the orbits of the original system (1) are bounded [24], it follows that they are bounded. Moreover:

**Proposition 3.1** *The orbits of* (2) *are bounded and the set:*

$$\Omega = \left\{ (x, y) | 0 \le x \le \nu, y \ge 0, \frac{\nu}{\eta} \le x + y \le \nu + \frac{\nu}{\eta}, y \ge x - \frac{\nu + \eta}{2} \right\}$$

*is positively invariant and attractive.*

*Proof* Preliminarily, we note that the differential inequality $x' \le \nu - x$ implies that:

$$\limsup_{t \to +\infty} x(t) \le \nu$$

yielding that the set $A = [0, \nu] \times \mathbb{R}_+$ is positively invariant and attractive. Moreover, from $(x+y)'(t) = (\nu - \eta y)(1+y)^{-2}$ it follows that for $y > \nu\eta^{-1}$ it is $(x+y)'(t) < 0$. Thus, let us consider a generic point $P = (\widehat{x}, \widehat{y}) \in A$ with $\widehat{y} > \nu\eta^{-1}$ and , and let us consider the line $x + y = \widehat{x} + \widehat{y}$ on which $P$ lies. It is easy matter to show that the family of sets: $\Omega_w = \{(x, y) \in A | x + y \le w = \widehat{x} + \widehat{y}\}$ is positively invariant for $w \ge \nu(1+\eta^{-1})$. Since this property also holds for all the points belonging to the orbit stemming from the generic point $P$ and such that $y(t) > \nu\eta^{-1}$, it follows the global attractiveness of $\Omega_{\nu(1+\eta^{-1})}$.

Finally, observe now that: (i) the inequality $(x+y)'(t) = (\nu - \eta(x+y))(1+y)^{-2}$ implies:

$$\liminf_{t \to +\infty} (x(t) + y(t)) \ge \frac{\nu}{\eta};$$

(ii) the inequality $y'(t) - x'(t) \geq 2(x - y) - \nu - \eta$ implies:

$$\liminf_{t \to +\infty} (y(t) - x(t)) \geq -\frac{\nu + \eta}{2}.$$

From these two lim inf and from the global attractiveness of $\Omega_{\nu(1+\eta^{-1})}$ it follows our claim on $\Omega$. □

Thus, in the following we shall only study (2) in $\Omega$.

By setting $x' = 0$ we obtain the x-nullcline:

$$x = a(y) := \frac{\nu}{(1 + y)^2},$$

and by setting $y' = 0$ we obtain the y-nullcline:

$$x = b(y) := \frac{\eta y}{(1 + y)^2} = \frac{\eta}{\nu} y a(y),$$

from which it immediately follows that there is a unique equillibrium $EQ = (x_e, y_e)$ where:

$$y_e = \frac{\nu}{\eta}, x_e = a(y_e) = \frac{\eta^2 \nu}{(\eta + \nu)^2}.$$

We recall here that such an chemical equilibrium " is regarded as a *reference* state describing the total absence of self-organization" [23]. Thus it is important to assess how the values of the two kinetic parameters $\eta$ and $\nu$, which are both linked to irreversible phenomena, may determine the onset of such organization. This is illustrated in the following proposition:

**Proposition 3.2** *If*

$$\eta < 27$$

*then EQ is locally asymptotically stable (LAS). If* $\eta > 27$ *there exist three real functions:*

$$\varphi(\eta) = Arg\left(-\frac{3\sqrt{3}}{\sqrt{\eta}} + i\frac{\sqrt{27 - \eta}}{\sqrt{\eta}}\right) \in \left(\frac{\pi}{2}, \pi\right) \tag{3}$$

$$q_1(\eta) = -1 + 2\sqrt{\eta} cos\left(\frac{2\pi - \varphi(\eta)}{3}\right) \in \mathbb{R}_+ \tag{4}$$

$$q_2(\eta) = -1 + 2\sqrt{\eta} cos\left(\frac{\varphi(\eta)}{3}\right) \in \mathbb{R}_+ \tag{5}$$

*such that:* (I) *if* $\nu \in J = (\nu_1(\eta), \nu_2(\eta)) = (\eta q_1(\eta), \eta q_2(\eta))$ *then EQ is unstable and there is at least a LAS limit cycle;* (II) *if* $\nu \in (0, \nu_1(\eta)) \cup (\nu_2(\eta), +\infty)$ *then EQ is LAS.*

*Proof* Linearising at EQ one gets the following characteristic polynomial: $\lambda^2 + C(\eta, \nu)\lambda + C_0(\eta, \nu)$, where:

$$\begin{aligned} C(\eta, \nu) &= b'(y_e) + 1 = \eta^4 + (1-\nu)\eta^3 + 3\nu\eta^2 + 3\nu^2\eta + \nu^3 \\ C_0(\eta, \nu) &= b'(y_e) - a'(y_e) = \frac{\eta}{\nu} a(y_e) > 0. \end{aligned} \tag{6}$$

Thus it follows that the sign of $C(\eta, \nu)$ rules the stability of EQ: if $C(\eta, \nu) >$ the EQ is LAS, if $C(\eta, \nu) <$ then EQ is unstable and, thanks to the boundedness of the orbits [13] there is at least a LAS limit cycle.

Now, by setting $q = \eta^{-2}\nu^2$ we have that $C(\eta, \nu) = \eta^3 K(q)$ where:

$$K(q) = q^3 + 3q^2 + (3-\eta)q + (1+\eta). \tag{7}$$

It follows that the study of stability of EQ coincides with the study of the sign of $K(q)$ for $q > 0$. Since $K(0) > 0$, we have to find the minimum of $K(q)$. The minimum is located at $q_{min} = -1 + \sqrt{\eta/3}$ so that:

$$K(q_{min}) = \frac{2}{3}\eta\left(3 - \sqrt{\frac{\eta}{3}}\right).$$

Thus, if $\eta < 27$ then $K(q) > 0$ for all $q > 0$ and EQ is LAS. If $\eta > 27$, the cubic curve $K(q)$ has two positive real roots $q_1(\eta) \in (0, q_{min})$ and $q_2(\eta) > q_{min}$, and if $q \notin [q_1, q_2]$ (i.e. $\nu \notin (\eta q_1, \eta q_2)$) then again EQ is LAS, whereas if $q \in (q_1, q_2)$ (i.e. $\nu \notin (\eta q_1, \eta q_2)$) then EQ is unstable. Finally, the Cardano's formula for third degree algebrical equations allows to esplicitely compute $q_1$ and $q_2$. □

Moreover, thanks to proposition 3.1 and to the above result, it easily follows that:

**Corollary 3.3** *In case of unstability of EQ, for all initial condition in $\mathbb{R}^2_+$ the orbits will be attracted by a LAS limit cycle, i.e. the oscillating regime is global.*

*Remark* We stress here that with respect to the informative but informal study of the model given in classical reference book [18], the results of proposition 3.2 and of corollary 3.3 provide further both formal and quantitative information of some biochemical interest. From the formal point of view, we have rigourously shown that there is at least a LAS limit cycle and that the cycling property of the system is general for all positive initial conditions (or, equivalently, for whatever large meaningful perturbations). This is an interesting result also from the biochemical point of view, since it shows that the oscillations are robust, although there might be multiple attracting oscillations. The uniqueness of the attractor will be investigated in the next section.

From a quantitative point of view, we have provided the following informations: (i) the value of a threshold $\eta_0 = 27$, under which the local stability of he equilibrium is independent of the substrate supply rate; (ii) the exact analytical expression of the curves $\nu_1(\eta)$ and of $\nu_2(\eta)$ that form the border of the parametric region corresponding to an oscillating regime.

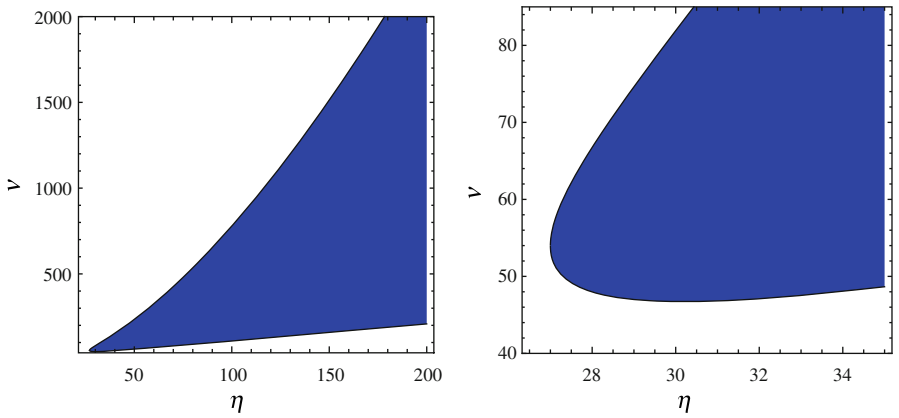


**Fig. 1** *Upper panel*: region of local asymptotic stability (*white*) and of unstability, i.e. where there are oscillations, (*dark*) for $(\eta, \nu) \in (0, 200] \times (0, 2000]$. *Lower panel*: zoom in the window $(\eta, \nu) \in (26.5, 35] \times (40, 85]$

In Fig. 1 we plotted the parametric regions of local stability (white) and of unstability-oscillations (gray), which are separated by the Hopf curve:

$$\gamma = \{(\nu, \eta) \in R_+^2 | C(\nu, \eta) = 0\}.$$

Indeed, as far as Hopf bifurcations are concerned, it holds that:

**Proposition 3.4** *Assuming $\nu$ as bifurcation parameter, at $C(\eta, \nu) = 0$ there is a Hopf bifurcation provided that $(\eta, \nu) \neq (27, 54)$. Assuming $\eta$ as bifurcation parameter, at $C(\eta, \nu) = 0$ there is a Hopf bifurcation provided that $(\eta, \nu) \neq (\eta_*, \nu_*) \approx (30.176, 46.730)$.*

*Proof* In case that the supply rate $\nu$ is the bifurcation parameter, the Hopf non-zero speed condition [13] reads:

$$Re\left(\frac{d\lambda}{d\nu}\right)\Big|_{(\eta,\nu)\in\gamma} = -2\frac{C_0(\eta, \nu)}{C^2(\eta, \nu) + 4C_0^2(\eta, \nu)}\frac{\partial C}{\partial \nu}\Big|_{(\eta,\nu)\in\gamma} \neq 0.$$

From $C(\eta, \nu) = \eta^3 K(q; \eta)$ we have:

$$\frac{\partial C}{\partial \nu}\Big|_{(\eta,\nu)\in\gamma} = \eta^2 K'\left(q_j(\eta)\right) \neq 0,\ j = 1, 2$$

provided that $q_j(\eta) \neq q_{min}$, since there $K'(q_{min}) = 0$. Thus, there is a Hopf bifurcation at $C(\eta, \nu)\Big|_{(\eta,\nu)\in\gamma}$ provided that $(\eta, \nu) \neq (27, 54)$, where $\nu_1(27) = \nu_2(27) = 54$.

In case that $\eta$ is the bifurcation parameter, we have that it must hold:

$$Re\left(\frac{d\lambda}{d\nu}\right)\Big|_{(\eta,\nu)\in\gamma} = -2\frac{C_0(\eta, \nu)}{C^2(\eta, \nu) + 4C_0^2(\eta, \nu)}\frac{\partial C}{\partial \eta}\Big|_{(\eta,\nu)\in\gamma} \neq 0.$$

We preliminarily observe that at the point (27, 54) there is a Hopf bifurcation since there:

$$\left.\frac{\partial C}{\partial \eta}\right|_{(\eta,\nu)=(27,54)\in\gamma} = -19683 < 0.$$

In the other points lying in $\gamma$, by applying the theorem of implicit functions it yields that:

$$\left.\frac{\partial C}{\partial \eta}\right|_{(\eta,\nu)\in\gamma} = \left.\frac{\partial C}{\partial \nu}\right|_{(\eta,\nu)\in\gamma} \frac{d\nu_j(\eta)}{d\eta},$$

where $\nu_j(\eta) = \eta q_j(\eta)$. Thus, there is Hopf bifurcation w.r.t. the parameter $\eta$ in all $\gamma$, except for the extremal point $(\eta_*, \nu_*)$ of the curve $\nu_1(\eta)$. This point is easily calculated by numerically solving the system $C(\eta, \nu) = 0$ and $\partial_\eta C(\eta, \nu) = 0$ □

*Remark* As far as the local stability of the limit cycles arising at the two Hopf bifurcations is concerned, it is possible to verify, after long symbolic and numerical calculations, that they are supercritical.

As far as the global stability of EQ is concerned, here we note that:

**Proposition 3.5** *If*

$$\eta \leq 27 \tag{8}$$

*then EQ is GAS.*

*Proof* By applying the Dulac-Bendixon theorem we have that:

$$Div(x', y') = -\frac{y^3 + 3y^2 + y(3-\eta) + 1 + \eta}{(1+y)^3} = -\frac{K(y)}{(1+y)^3}.$$

Thus proceeding as the the previous propositions we have that if (8) holds than EQ is GAS in $\Omega$. □

*Remark* Proposition 8 has the following biochemical interpretation: if the removal rate of the product is under the threshold $\eta_0 = 27$, no form of temporal self-organization is possible. However large the supply rate of the substrate may be, all perturbation will vanish and the equilibrium will be re-established.

## 4 A Newton-Lienard equation equivalent to the model

The first equation of (2) yields $x = y' + b(y)$, and substituting in the first equation of the system, one obtains:

$$y'' = -f(y)y' - g(y) \tag{9}$$

or equivalently:

$$\begin{aligned} \frac{dy}{dt} &= v \\ \frac{dv}{dt} &= -f(y)v - g(y). \end{aligned} \tag{10}$$

System (10) is formally a Newton's system ruling, in the phase space $(y, v)$, the dynamics of a point of mass 1, position $y(t)$ and velocity $v(t) = y'(t)$ subject to a force $\phi(y) = -g(y)$ where

$$g(y) = b(y) - a(y) = \frac{\eta}{\nu} a(y) \left( y - \frac{\nu}{\eta} \right) \tag{11}$$

and to a position-dependent damping $\delta(y, v) = -f(y)v$, where the damping coefficient is:

$$f(y) = 1 + b'(y) = 1 + \eta \frac{y-1}{(1+y)^3} = \frac{K(y;\eta)}{(1+y)^3}, \tag{12}$$

and for $\eta > 27$ it is is signum-varying, and its integral:

$$F(y) = \int_{y_e}^{y} f(w)dw = \left( y - \frac{\nu}{\eta} \right) + b(y) - b\left( \frac{\nu}{\eta} \right) \tag{13}$$

is ubounded. In Newton's system (10), the force is anti-symmetric w.r.t. the equilibrium position $y_e$ (i.e. $(y - y_e)\phi(y) < 0$ for $y - y_e \neq 0$) and, as a consequence, the potential energy associated to the force $\phi(y)$ is positive for $y \neq y_e$:

$$G(y) = \int_{y_e}^{y} g(w)dw = \frac{\eta + \nu}{y+1} + \eta \log(y+1) - \eta \left( \log\left( \frac{\eta + \nu}{\eta} \right) + 1 \right) \tag{14}$$

and it is 'U-shaped'.

*Remark* As far as this "potential energy" $G(y)$ is concerned, we remark here that it is a purely mathematical definition. However, it might be of some interest to ascertain if it might have a physical meaning, which is outside the aims of this work.

Recently, for Newton-Lienard equations, Tzy-Wai Hwang and Hsin-Jung Tsai proposed in [15] an interesting sufficient criterion to exclude periodic solutions. In our case the criterion reads:

**Proposition 4.1** *Assume that there exist* $K_1, K_2 \in \mathbb{R}$ *such that:*

$$f(y) + g(y)\left(K_1 + K_2 F(y)\right) > 0 \tag{15}$$

*then there are no closed orbits in* $\Omega$.

The above proposition allows us to claim that:

**Proposition 4.2** *Let it be* $\eta > 27$. *If* $\nu \in (0, \eta q_1(\eta)) \cup (\eta q_2(\eta), +\infty)$ *then EQ is GAS in* $\Omega$.

*Proof* By setting condition (15) with $(K_1, K_2) = (-H, 0)$ and after some elementary algebra the constraint (15) becomes:

$$\Psi(y) = (1+y)^2 + \eta\frac{1-y}{1+y} > H\eta\left(y - \frac{\nu}{\eta}\right) \tag{16}$$

idest:

$$\frac{K(y)}{y+1} > H\eta\left(y - \frac{\nu}{\eta}\right).$$

For $\eta > 27$ the function $\Psi(y)$, as it is easy to verify, is convex and it has a unique negative minimum at a point $y_{min}$, and it is $\Psi(y) \leq 0$ for $y \in [q_1(\eta), q_2(\eta)]$. Thus, thanks to the properties of convex functions,both for $(\nu/\eta) < q_1(\eta)$ and for $(\nu/\eta) > q_2(\eta)$ if we choose:

$$H = \Psi'\left(\frac{\nu}{\eta}\right),$$

we have that the constraint (16) holds for all $y > 0$. □

*Remark* The above proposition and proposition 3.5 allow to infer that if, for given $(\eta, \nu)$, the equilibrium state is locally stable, then it also is globally stable. Moreover, note that the case $\eta < 27$ corresponds to the case of a damping function $f(y)$ that is positive for all $y > 0$.

## 5 Uniqueness of limit cycle

Before starting the study of the uniqueness of the limit cycles that arise in case of unstability of the equilibrium point, it is useful to remark that:

*Remark* Since the orbits of system (2) are bounded by the positively invariant and attractive set $\Omega$, and since in that set there is a unique equilibrium point, it follows that if there is a unique limit cycle and it is LAS then the cycle is also globally attractive.

A number of theorems exist that assess the uniqueness of limit cycle solution for (10), but the most celebrated, although giving a very strict condition, is the Zhang's theorem [33] that in a more applicable form due to Yang Kuang and H.I. Freedman [19] reads:

**Proposition 5.1** **[19]** *Considering a Newton's canonical system with* $f(y)$ *and* $g(y)$ *continuous and differentiable in* $(a_1, a_2)$, *where:* $-\infty \leq a_1 < a_2 \leq +\infty$. *Thus, if it*

*exists a* $y_e \in (a_1, a_2)$ *such that* $g(y_e) = 0$ *and* $(y - y_e)g(y) > 0$, *and, moreover, the function*

$$\varrho(y) = \frac{f(y)}{g(y)}$$

*is non-decreasing in* $(a_1, y_e) \cup (y_e, a_2)$ *then there is at most limit cycle in the region* $a_1 < y < a_2$ *and it is locally stable, if it exists.*

In our case, straightforward calculations yield that:

$$\varrho(y) = \frac{K(y)}{\eta(1+y)\left(y - \frac{\nu}{\eta}\right)},\ \varrho'(y) = \frac{A(y)}{(y+1)^2(y\eta - \nu)^2} \tag{17}$$

where:

$$A(y) = y^4\eta + y^3(2\eta - 2\nu) + y^2\left(\eta^2 - 6\nu\right) + y\left(-2\eta^2 - 2\eta - 6\nu\right) \\ -\eta^2 + (2\eta - 2)\nu - \eta.$$

Let us denote the four roots of the fourth-degree algebrical equation $A(y) = 0$ as $Y_1(\eta, \nu), Y_2(\eta, \nu), Y_3(\eta, \nu), Y_4(\eta, \nu)$, which are analytically calculable. Noting that

$$A(0) = 2(\eta - 1)\nu - \eta(1 + \eta),$$

one straightforwardly may show that:

**Proposition 5.2** *Let EQ be unstable. Thus, if*

$$\nu > \frac{\eta(\eta + 1)}{2(\eta - 1)}, \tag{18}$$

*and if for* $j = 1, 2, 3, 4$*:*

$$Y_j(\eta, \nu) \notin A = \left\{ y \in R \;\middle|\; 0 \le y \le \nu\left(1 + \frac{1}{\eta}\right) \right\} \tag{19}$$

*then there is a unique and limit cycle that is globally attractive.*

Observe that:

(I) The constraint (18–19), although when written *in extenso* is of very difficult readability, is not a restriction and it is the natural condition guaranteeing that the function $\varrho(y)$ is increasing;
(II) In the parameter space $(\eta, \nu)$ the region where (18–19) is fulfilled might be a subset of the oscillation region.

However, as far as the point (II) is concerned, after symbolically implementing the Sturm's algorithm, we numerically assessed that in the set $S = (\eta, \nu) \in (0, 200] \times (0, 2000]$ (the same in the left part of Fig. 1) the uniqueness region fills all the oscillation region. Similarly, we also tested a large number of points in the oscillatign region externally to $S$, and in all cases constraint (18–19) was fulfilled.

## 6 Onset of spatial self-organization

Here we shall briefly study the onset of spatial self-organization through Turing bifurcations, particularly in the case where the spatially homogenous system is not temporally self-organized. Assuming that the substrate and product diffuse in one-dimension in $[0, L]$, and denoting with the symbol $r$ the spatial variable, the Eq. 1 become:

$$\begin{aligned} \partial_\tau x(\tau, r) &= \nu - x(1+y)^2 + D_x \partial_r^2 x \\ \partial_\tau y(\tau, r) &= \alpha x(1+y)^2 - \eta y + D_y \partial_r^2 y \end{aligned} \tag{20}$$

with no-flux border conditions: $\partial_r(x, y)(\tau, 0) = \partial_r(x, y)(\tau, L) = 0$. Linearizing at equilibrium $(x, y) = (x_e, y_e) + (X, Y)$, $|(X, Y)| << 1$, and expanding $(X, Y)$ in spatial Fourier series, the time-varying coefficients of the series $(X_M(\tau), Y_M(\tau))$ $M \in \mathbb{N}$ are ruled by the following systems:

$$\begin{aligned} X'_M(\tau) &= -\left(d_x M^2 + \left(\frac{\eta+\nu}{\eta}\right)^2\right) X_M - \frac{2\nu\eta}{\nu+\eta} Y_M \\ Y'_M(\tau) &= +\left(\frac{\eta+\nu}{\eta}\right)^2 X_M - \left(d_y M^2 + \left(\eta - \frac{2\nu\eta}{\nu+\eta}\right)\right) Y_M \end{aligned} \tag{21}$$

where $(d_x, d_y) = (\pi/L)^2 (D_x, D_y)$ and $M \in \mathbb{N}$. Thus, there is onset of spatial self-organization provided that it exists at least a $M \in \mathbb{N}$ such that the solutions of $\lambda_M^2 + h_1(N)\lambda_M + h_0(M) = 0$ have positive real part, where:

$$\begin{aligned} h_1(M) &= (d_x + d_y)M^2 + \frac{C(\eta, \nu)}{\eta^2(\nu+\eta)} \\ h_0(M) &= M^4 d_x d_y + M^2 \frac{\left(\eta^3 d_x(\eta - \nu) + d_y(\eta+\nu)^3\right)}{\eta^2(\eta+\nu)} + \frac{(\eta+\nu)^2}{\eta}. \end{aligned}$$

Coherently with the general theory of Turing bifurcations [22], it follows that if there is breaking of the temporal symmetry ($C(\eta, \nu) < 0$) then one can find modes $M$ corresponding to the breaking of the spatial symmetry. However, in the case where $C(\eta, \nu) > 0$, defining $\theta = d_y/d_x$ [9], we may rewrite $h_0(M)$ as follows:

$$h_0(M) = M^4 d_x d_y + M^2 d_x \frac{\left(C(\eta, \nu) + (\theta - 1)(\eta+\nu)^3\right)}{\eta^2(\eta+\nu)} + \frac{(\eta+\nu)^2}{\eta},$$

implying that for $\theta \geq 1$ (as for glycolysis [9]) there is no Turing bifurcation: the diffusion alone is not able to induce spatial structures. In the case $\theta \in (0, 1)$ there may be instabilities provided that it exists at least a $M \in \mathbb{N}$ such that:

$$d_y < -\frac{\eta\left(M^2\eta^2 d_x(\eta-\nu)+(\eta+\nu)^3\right)}{M^2(\eta+\nu)\left(M^2\eta^2 d_x+(\eta+\nu)^2\right)}.$$

## 7 Concluding remarks

In this work we proposed an analytical study of the Golbeter-Lefever model [9,18] in the reduced form proposed in [18] by Keener and Sneyd for describing open mono-substrate allosteric enzyme reactions [9,18] in the case that an allosteric enzyme is activated by its product, as for the phosphofructokinase in the glycolytic process.

By transforming the original model in another that is topologically equivalent, we showed that the orbits of the system are bounded.

Then, we studied the phenomenon of the arrest and onset of oscillations, i.e. the establishment vs breaking of temporal simmetry. We obtained that:

- **R1** if the removal rate of the product ($\eta$) is lower than a well-identified threshold then the arrest of the oscillations is global;
- **R2** If that rate exceeds the threshold, then the parameters space is split in two subregion and for each value of $\eta$ two threshold arise for the supply rate of the substrate ($\nu$)
- **R3** If $\nu$ is external to the interval determined by these two rates, then the arrest is again globally attractive;
- **R4** If the supply rate is between the two thresholds, then the system has at least one stable limit cycle, i.e. there is onset of temporal self-organization.

The case **R4**, however, cannot exclude the presence of two locally stable cycles (birhytmicity), or even more than two, i.e. there might be multiple choices for the self-organization. From a kinetic point of view, this case physically means that the periodic behavior of the process and, in particular, its period may depend not only on the kinetic features (such as the substrate supply rate and the product removal rate), but also on the initial (or, equivalently, the *post-perturbation*) concentrations of substrate and product.

Therefore, we investigated the uniqueness of the cycle and we obtained an analytical condition for guaranteeing this property. By applying this criterion, our computations suggest that, in parameters space, in the region guaranteeing the existence of the periodic solution it is also guaranteed its uniqueness. This is an interesting biochemical result since it means that the system when cycling, after large perturbations, will not only recover the original period, but also the concentrations will follow the original dynamical law. Thus, this implies that the Goldbeter-Lefever model predicts that the dynamical features of the self-sustained oscillations of glycolysis (and other chemical oscillations described by the model in study) only depend on the systems parameters, since their behaviour is independent of the initial conditions.

Similar considerations apply to the case of constant equilibrium, which, under the appropriate conditions above described for the parameters, cannot qualitatively change.

Then, we briefly investigated the onset of spatial self-organization in absence of temporal self-organization. The results we obtained are coherent with those illustrated for the complete model in [9]. This suggests that the reduced model of [18] retains important properties of the full modelization.

Finally, it is appropriate to recall that all the physical inferences we obtained in this study are valid under the conditions of validity of the model (1), namely the hypotheses **Ha**, **Hb** and **Hc** illustrated in Sect. 2.

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