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Effects of periodic input on the quasi-steady state assumptions for enzyme-catalysed reactions

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Abstract. In this paper we investigate the validity of a quasi-steady state assumption in approximating Michaelis-Menten type kinetics for enzyme-catalysed biochemical reactions that are subject to periodic substrate input.

1. Introduction

One of the fundamental features of enzymatic reactions is enzyme saturation. At saturation of an enzyme, the reaction rate catalysed by the enzyme reaches an apparent maximum, and a further increase of substrate concentration does not appear to enhance the reaction rate. This feature is captured by Michaelis-Menten formalism [5] which laid the foundation for classical enzyme kinetics. The derivation of this formalism is based on certain assumptions, principally, the quasi-steady state assumption (QSSA) (see e.g. [12], [13]). The validity of these assumptions has been the subject of much recent attention. For example, it was initially proposed that a necessary condition for the standard or classical (sQSSA) assumption is that the initial substrate concentration greatly exceeds that of the enzyme. This situation often arises in laboratory experiments, but is less common for reactions *in vivo*. Consequently, a new, weaker necessary condition on the initial concentrations has been derived (see [13]). Moreover, when the initial enzyme concentration greatly exceeds that of the substrate concentration, a reverse quasi-steady state assumption (rQSSA) can be made ([8], [13] and below). By a change of variables, a “total” QSSA can sometimes be used (see [1]).

Although much effort has been made to elucidate the conditions for applying the sQSSA or the rQSSA to derive Michaelis-Menten formalism, almost all previous work has concentrated on the reactions with no substrate input. Therefore, strictly speaking, the conclusions drawn are only valid for isolated enzymatic reactions. In a living system, large numbers of enzymatic reactions are networked in

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a complex manner. Pathways can be unidirectional, reversible, branched, or cyclic [16]. A particular enzymatic reaction is embedded in such a pathway, taking product molecules from the previous reaction step and supplying substrate to the next step. Moreover, a living system may take up substrates from external sources and releases products to them. Therefore, all enzymatic reactions in a living system are subject to substrate input and product removal. In order to understand the function of biochemical reactions *in vivo* using enzyme kinetics, it is therefore essential to determine the validity of this approach under such conditions.

In [14, 15], as a reasonable and biologically relevant first step, we investigated the effects of a constant substrate input on the derivation and validity of classical quasi-steady state assumptions. Necessary conditions for the validity of these assumptions were derived and were shown to be dependent on the input I . In fact the sQSSA gets “better”, in the sense that the necessary condition for validity is relaxed by increasing I , and thus the sQSSA is valid for a wider range of parameters and initial data (indeed, for arbitrary initial data, provided I is chosen sufficiently large). On the other hand, it was shown that if the rQSSA is valid for the system without input, then calculations of the error in product production rate suggest that the introduction of an input of a certain magnitude could render the rQSSA invalid. It was noted that, even in the case where the introduction of (a possible sufficiently large) input maintains or widens the applicability of a QSSA, the time scales associated with the transient and QSS periods, the form of the quasi-steady state solution itself, and the associated reaction rate equation are all dependent on I . Therefore the formalism established in the absence of input *does not*, in general, provide accurate information regarding systems with input.

In this paper we extend our investigation to a time-dependent input. In general, all enzymatic reactions in a living system are subject to fluctuating input. These fluctuations may stem from varying environmental conditions, or from the interactions of the reactions. For example, plant photosynthesis is subject to light intensity fluctuations and the processes of acquiring carbon resources are time-dependent. Moreover, since a particular reaction is always embedded in a pathway, its input is the output of preceding reactions. As such, any reaction is forced by other reactions, for example, oscillations generated in glycolysis can force the pentose phosphate pathway. A full understanding of enzymatic reactions *in vivo* must therefore consider the fluctuating nature of substrate input. In this work, we will investigate one such class of fluctuation, namely, periodic inputs.

In order to isolate the effects of oscillations in input, we shall consider throughout periodic inputs that have a fixed positive average value over a time period. We therefore consider the following reaction scheme:

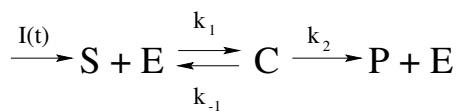


Fig. 1. The enzyme-substrate reaction with substrate input

subject to a substrate input, $I(t)$, of the form

$$I(t) = I_0(1 + \epsilon \sin(\omega t)), \quad t \geq 0. \quad (1.1)$$

Here E , S , C , and P denote, respectively, the free enzyme, free substrate, enzyme-substrate complex and the product concentrations. Also, I_0 is a positive constant and ϵ and ω are non-negative parameters. The principal period of the input is $\frac{2\pi}{\omega}$ and the average of $I(t)$ over a time period is I_0 . As $I(t)$ has to be nonnegative at all times, we restrict ϵ to satisfy $0 \leq \epsilon \leq 1$. If $I_0 = 0$, the reaction shown in Figure 1 reduces to the classic (isolated) enzyme-substrate reaction proposed in [5] by Michaelis and Menten. When $\epsilon = 0$, $I(t) \equiv I_0$ and the reaction reduces to that studied in [14]. We are interested in the effects of the size and frequency of input oscillations on the validity of a QSSA to be defined below.

We will first consider the dynamics of the full system and then compare these with the dynamics of a reduced system formed by using a QSSA. First deriving the model system in Section 2, we consider small and large perturbations in Section 3, discussing both transient and asymptotically periodic behaviour. In Section 4 we discuss the errors involved in using the QSSA, considering their dependence on both amplitude and frequency of input. In Section 5, we conclude with a brief discussion.

2. Enzyme-Substrate Reactions with Periodic Input

The time evolution of the concentrations of the reactants in Figure 1 can be described by the following system of ordinary differential equations:

$$\frac{dE}{dt} = -k_1ES + k_{-1}C + k_2C, \quad (2.1a)$$

$$\frac{dS}{dt} = I(t) - k_1ES + k_{-1}C, \quad (2.1b)$$

$$\frac{dC}{dt} = k_1ES - k_{-1}C - k_2C, \quad (2.1c)$$

together with the decoupled equation,

$$\frac{dP}{dt} = k_2C. \quad (2.1d)$$

The case $I(t) \equiv 0$, has been intensively studied (see [1], [8], [9], [11], [12], [13] and the references therein), where questions regarding the nature and validity of the standard and reverse quasi-steady state assumptions were posed and answered. The initial data usually considered in this case are:

$$E(0) = E_0, \quad S(0) = S_0, \quad C(0) = 0 \quad \text{and} \quad P(0) = 0. \quad (2.2)$$

In [14], it is assumed that at first the system is in its asymptotic (positive, stable, steady) state $E = E_0$, $S = \hat{S}$, $C = \hat{C}$ and for completeness it is taken that $P = 0$. (With a constant, positive input, the product is being continually produced at a rate k_2C , we can assume, however, that this is being removed from the reaction at an equal rate and therefore the net product concentration “in the reaction at steady

state", is zero.) It is then assumed that a perturbation to this system is made by introducing a further quantity of substrate, denoted by S_0 , and dynamics of the relaxation of the system back to the steady state are investigated. The appropriate initial data for this model system are therefore

$$E(0) = E_0, \quad S(0) = S_0 + \hat{S}, \quad C(0) = \hat{C} \quad \text{and} \quad P(0) = 0, \quad (2.3)$$

with $\hat{S} = K_M I_0 / k_2 E_0$ and $\hat{C} = I_0 / k_2$, where $K_M = (k_2 + k_{-1}) / k_1$. On using this and noting that $E(t) + C(t) = \text{const.} = E_0 + \hat{C}$, in this case the system (2.1a) - (2.1c) reduces to

$$\frac{dS}{dt} = I_0 - k_1(E_0 + \hat{C} - C)S + k_{-1}C, \quad (2.4a)$$

$$\frac{dC}{dt} = k_1(E_0 + \hat{C} - C)S - k_1 K_M C. \quad (2.4b)$$

Note that in the case $I_0 = 0$, system (2.4) and data (2.3) reduce to the classical system with data (2.2). Making the classical or standard quasi-steady state assumption (sQSSA), $dC/dt = 0$, reduces (2.4) to the differential-algebraic system,

$$\frac{dS}{dt} = I_0 - \frac{k_2(E_0 + \hat{C})S}{S + K_M}, \quad C = \frac{(E_0 + \hat{C})S}{S + K_M}, \quad S(0) = S_0 + \hat{S}. \quad (2.5)$$

A necessary condition for the validity of the sQSSA is shown in [14] to be

$$E_0 \ll S_0 + \hat{S} + K_M. \quad (2.6)$$

If this condition is satisfied, it was shown there that system (2.4) undergoes a rapid transient in which the substrate concentration is almost unaltered and then the dynamics of the system closely follow those of the reduced system (2.5).

Let us now consider the full system with input given by (1.1). Following similar arguments to those outlined above, it is straight forward to show that if we consider the initial data given in (2.3), then (2.1) can be rewritten as

$$\frac{dS}{dt} = I(t) - k_1(E_0 + \hat{C} - C)S + k_{-1}C, \quad (2.7a)$$

$$\frac{dC}{dt} = k_1(E_0 + \hat{C} - C)S - k_1 K_M C. \quad (2.7b)$$

The initial data (2.3) combined with system (2.7), models the situation where the reaction system (2.4) is resting in its positive steady state i.e. $(S, C) = (\hat{S}, \hat{C})$. System (2.4) is then perturbed by the introduction of a periodic perturbation to the substrate input and an initial input of amplitude S_0 . The subsequent dynamics are thus captured by (2.7) with initial data (2.3).

Now on making the sQSSA with respect to system (2.7), we obtain the differential-algebraic system

$$\frac{dS}{dt} = I(t) - \frac{k_2(E_0 + \hat{C})S}{S + K_M}, \quad C = \frac{(E_0 + \hat{C})S}{S + K_M}, \quad S(0) = S_0 + \hat{S}. \quad (2.8)$$

One would expect that the solutions of (2.5) and (2.8) would be similar, at least for ϵ sufficiently small. Therefore, given that the dynamics of (2.5) have been well studied in [14] (indeed, the solution can be expressed in a closed form), in the following we analyse the applicability of (2.5) (rather than the more complex system (2.8)) as a valid approximation to (2.7).

3. System Dynamics

3.1. Time Scales

We begin by investigating the effects of small perturbations on the time scales for system (2.7), using similar arguments as those detailed in [14]. We first suppose that the necessary condition, $E_0 \ll S_0 + \hat{S} + K_M$, for the applicability of the sQSSA (2.5) to the $\epsilon = 0$ ($I = I_0$) case holds in all that follows. For ϵ sufficiently small, we therefore anticipate that (2.7) will display a rapid transient phase followed by some form of quasi steady behaviour. During the transient phase, system (2.7) must have $S \approx S(0) = \hat{S} + S_0$. In this case, (2.7b) can be solved directly to yield $C(t) = C_1 + C_2 \exp(-ht)$ for constants C_1 and C_2 , and for which $h = k_1(S_0 + \hat{S} + K_M)$. The time scale for the fast transient is therefore defined to be $t_f := 1/h$ (essentially the relaxation time). Notice that this time scale is unaltered by the introduction of the perturbation.

Next, we assume a QSS period exists in which $dC/dt \approx 0$ and moreover that (2.5) provides a reasonable approximation to the dynamics of (2.7) during this phase. The time scale associated with this period can be calculated thus (see also [13] and [14])

$$t_s^\epsilon := \frac{|S_{\max} - S_{\min}|}{\left| \frac{dS}{dt} \right|_{\max}},$$

where the maximum in the denominator is calculated via (2.5). It is straightforward to show that this maximum occurs when $S = S_0 + \hat{S}$. Moreover, since the right hand side of (2.7a) is bounded above and below respectively by the functions $I_0(1 \pm \epsilon) - k_1(E_0 + \hat{C} - C)S + k_{-1}C$, then

$$|S_{\max} - S_{\min}| \leq |S_0 + \hat{S} - \hat{S} + O(\epsilon)| = S_0 + O(\epsilon).$$

Therefore

$$t_s^\epsilon \approx \frac{S_0 + \hat{S} + K_M + O(\epsilon)}{k_2 E_0} = t_s + O(\epsilon),$$

where t_s is the corresponding time scale for the $I = I_0$ case (see [14]).

The first necessary condition for the validity of the QSSA (2.5) is that $t_f \ll t_s^\epsilon$, i.e.,

$$k_2 E_0 \ll k_1 (S_0 + \hat{S} + K_M)^2 + O(\epsilon). \tag{3.1}$$

The second necessary condition requires the change in substrate during the transient be small (recall this was how t_f was calculated). To investigate this, it is

computationally easier to follow [14] and make the change of variables $U = S - \hat{S}$, $V = C - \hat{C}$. Thus, system (2.7) reduces to

$$\frac{dU}{dt} = \epsilon I_0 \sin(\omega t) - k_1(E_0 - V)U + (k_1\hat{S} + k_{-1})V, \quad (3.2a)$$

$$\frac{dV}{dt} = k_1(E_0 - V)U - k_1(\hat{S} + K_M)V, \quad (3.2b)$$

and the appropriate initial data are

$$U(0) = S_0, \quad V(0) = 0. \quad (3.2c)$$

Following similar arguments to those detailed in [14], it can be shown that the relative change in substrate can be estimated by

$$\left| \frac{\Delta U}{S_0} \right| \approx t_f \frac{1}{S_0} \left| \frac{dU}{dt} \right|_{max},$$

where $|dU/dt|_{max}$ is calculated using (3.2a) with $V = C - \hat{C} = 0$ (noticing that $|dU/dt|$ is decreasing in the first instance). Hence,

$$\left| \frac{\Delta U}{S_0} \right| \approx \frac{k_1 E_0 S_0 + O(\epsilon)}{k_1 S_0 (S_0 + \hat{S} + K_M)}.$$

Therefore, as we require $|\Delta U/S_0| \ll 1$, this implies

$$E_0 + O(\epsilon) \ll S_0 + \hat{S} + K_M. \quad (3.3)$$

For ϵ sufficiently small, this second condition is stronger than the first and is therefore the validity condition we require. Hence, in this case the necessary condition for the application of (2.5) as a valid approximation to the dynamics of system (2.7) remains $E_0 \ll S_0 + \hat{S} + K_M$. In what follows we chose parameter values so that this condition holds.

3.2. Numerical Integration

It is straightforward to numerically integrate system (2.7) and we used the MATLAB integration routine “ode45”.

For ϵ sufficiently small, the dynamics of (2.7) can be separated into three stages, see Figure 2. Initially, the system undergoes a fast transient as predicted, during which the substrate concentration S remains approximately unaltered but there is a rapid increase in complex concentration. In the second stage, again as predicted above, the dynamics of the full system follow very closely those of the approximate system (2.5). The time scales for the transient and QSS periods discussed above are shown on the figure and it can be seen that both remain good estimates in the case $0 < \epsilon \ll 1$.

Finally, in a third phase, the dynamics appear to tend to a periodic cycle with period $\frac{2\pi}{\omega}$, i.e. the same period as the input oscillation, around the fixed point (\hat{S}, \hat{C}) of system (2.5), see Figure 3. Notice that on increasing the frequency ω , the limit cycle reduces in size (see also later). The asterisk represents the fixed point (\hat{S}, \hat{C}) .

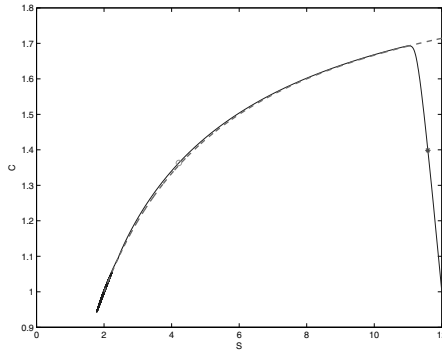


Fig. 2. The complex concentration C versus the substrate concentration S (continuous curve) given by (2.7) and the QSS approximation (dotted curve) given by (2.5). The asterisk and circle denote the fast and slow time scales t_f and t_s , respectively. Here $E_0 = 1$, $S_0 = 10$, $I_0 = 1$, $k_1 = k_{-1} = k_2 = 1$ and $\epsilon = 0.05$, $\omega = 0.1\pi$.

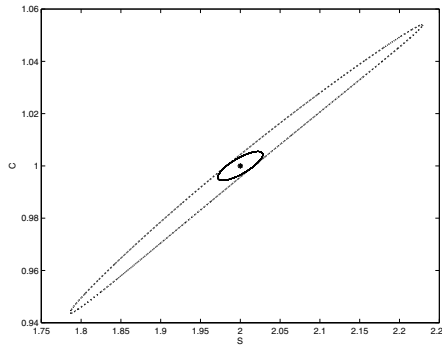


Fig. 3. The periodic solution of system (2.7) for $\omega = 0.1\pi$ (dotted line) and $\omega = \pi$ (continuous line). The steady state (\hat{S}, \hat{C}) of (2.5) is shown by the asterisk. Here $\epsilon = 0.05$ and all other parameter values are as in Fig. 2.

For ϵ not small, there is no explicit method of verifying the applicability of the QSSA equations (2.5). However, by numerically integrating system (2.7), we found that the system behaviour is in many respects very similar to that for small ϵ . The dynamics are again divided into three phases. As can be seen in Fig. 4, a rapid transient is followed by a period in which the the solution remains close to the C -nullcline (the sQSS nullcline) and then finally settles to seemingly periodic behaviour again with period $2\pi/\omega$ (see Fig. 5).

3.3. Existence and Uniqueness of Periodic Solutions

It is straight forward to show that with $\epsilon = 0$, the system does not display cyclic behaviour and therefore the periodic behaviour captured by the numerical simulations discussed above must arise as a result of the periodic forcing of the system input. We shall now prove that a positive periodic solution for the system (2.7)

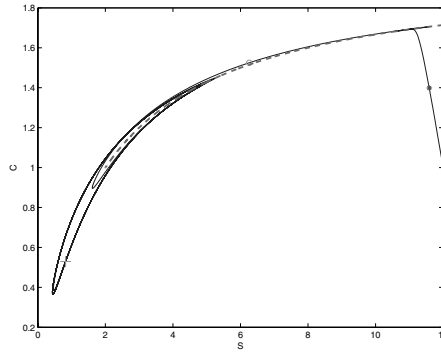


Fig. 4. The complex concentration C versus the substrate concentration S (continuous curve) given by (2.7) and the QSS approximation (dotted curve) given by (2.5). The asterisk, circle and cross denote the fast time scale t_f , the slow time scale t_s and t_3 respectively (see text). Here $\epsilon = 1$ and all other parameter values are as in Fig. 2.

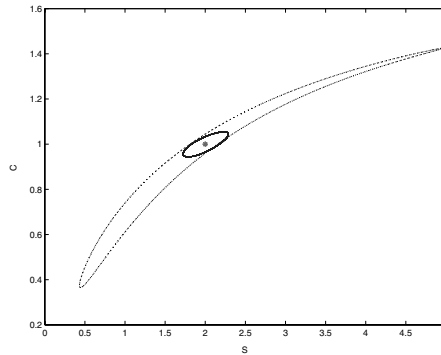


Fig. 5. The periodic solution of system (2.7) for $\omega = 0.1\pi$ (dotted line) and $\omega = \pi$ (continuous line). The steady state (\hat{S}, \hat{C}) of (2.5) is shown by the asterisk. Here $\epsilon = 1$ and all other parameter values are as in Fig. 2.

does exist for all values of $\epsilon \leq 1$ with period equal to that of the input oscillation. Moreover, for ϵ sufficiently small, we show that the periodic solution is unique.

First, notice that the quadrant $\{(S, C) : S \geq 0, C \geq 0\}$ is invariant for the flow generated by (2.7) for any value of $\epsilon, 0 \leq \epsilon \leq 1$. Also, it is straight forward to show that any periodic solution of (2.7) must lie in the interior of this quadrant.

Second, clearly, any periodic solution $(U(t, \epsilon), V(t, \epsilon))$ of (3.2) defines a periodic solution $(U(t, \epsilon) + \hat{S}, V(t, \epsilon) + \hat{C})$ of (2.7).

If $\epsilon = 0$, then (3.2) has a unique steady state, $(U, V) = (0, 0)$, which, after a little algebra, can be shown to be a locally stable node. Let $X = (U, V)$ and rewrite (3.2) in the form

$$\frac{dX}{dt} = F(t, X, \epsilon), \tag{3.4}$$

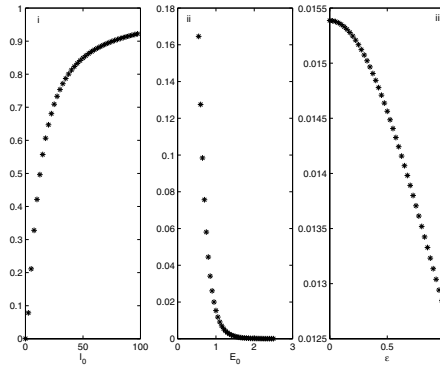


Fig. 6. The largest modulus eigenvalue of the matrix $M(T)$ (see text) plotted for ranges of values of (i) I_0 , (ii) E_0 and (iii) ϵ . All other parameters (and those not being varied) are as in Fig. 2.

where F is the column vector with elements given by the right hand side of (3.2). Clearly, F is a smooth function of (t, X, ϵ) and F has period $\frac{2\pi}{\omega}$ in t i.e.

$$F\left(t + \frac{2\pi}{\omega}, X, \epsilon\right) = F(t, X, \epsilon), \quad \forall t \geq 0.$$

We have the following result.

Theorem 1 *For any value of ϵ , $0 \leq \epsilon \leq 1$, system (2.7) has at least one positive $2\pi/\omega$ -periodic solution. Moreover, for ϵ sufficiently small, the $2\pi/\omega$ - periodic solution is unique and varies continuously with ϵ .*

Proof. We first consider the case where ϵ is sufficiently small. By considering the flow generated by F , it is straightforward to show that when $\epsilon = 0$, the only solution of (3.4) that is periodic, is the trivial solution, $X = 0$. Let $\eta \in \mathbb{R}^2$ and let $\psi(t, \eta, \epsilon)$ be the solution of (3.4) that satisfies the initial condition

$$\psi(0, \eta, \epsilon) = \eta.$$

The existence of such a solution is assured by the smoothness of F . Given the periodicity of F , for the solution ψ to be $\frac{2\pi}{\omega}$ -periodic, it is necessary and sufficient that

$$\psi\left(\frac{2\pi}{\omega}, \eta, \epsilon\right) - \eta = 0. \tag{3.5}$$

By the above arguments, $(\eta, \epsilon) = (0, 0)$ satisfies this equation. The Jacobian matrix of (3.5) with respect η is

$$\psi_\eta\left(\frac{2\pi}{\omega}, \epsilon, \eta\right) - I_{2 \times 2},$$

where $I_{2 \times 2}$ is the 2×2 identity matrix.

The determinant of this matrix cannot be calculated directly. However, the function $\psi_\eta(t, 0, 0)$ solves

$$\psi'_\eta(t, 0, 0) = F_X(t, \psi(t, 0, 0), 0)\psi_\eta(t, 0, 0), \quad \psi_\eta(0, 0, 0) = I_{2 \times 2}.$$

This problem is equivalent to

$$\frac{d\mathcal{U}}{dt} = -k_1(E_0 - \hat{C})\mathcal{U} + (k_1\hat{S} + k_{-1})\mathcal{V}, \quad (3.6a)$$

$$\frac{d\mathcal{V}}{dt} = k_1(E_0 - \hat{C})\mathcal{U} - k_1(\hat{S} + K_M)\mathcal{V}. \quad (3.6b)$$

$$\mathcal{U}(t + \frac{2\pi}{\omega}) = \mathcal{U}(t), \quad \mathcal{V}(t + \frac{2\pi}{\omega}) = \mathcal{V}(t), \quad (3.6c)$$

which has only the trivial solution $(\mathcal{U}, \mathcal{V}) = (0, 0)$. Thus, the Jacobian matrix $\psi_\eta(\frac{2\pi}{\omega}, 0, 0) - I_{2 \times 2}$ is nonsingular. Hence, by The Implicit Function Theorem, equation (3.5) is uniquely solvable in a neighbourhood of $\epsilon = 0$ and the result follows.

When ϵ cannot be assumed small, we refer to the original system (2.7) directly. Let

$$D = \{(S, C) \mid 0 \leq S \leq S_1, 0 \leq C \leq C_1\};$$

$$S_1 = K_m \left[\frac{E_0 + \hat{C}}{\delta} - 1 \right]; \quad C_1 = E_0 + \hat{C} - \delta,$$

where δ is chosen sufficiently small such that $S_1 > 2I_0K_m/(k_2E_0)$. Then, it can be shown that D is an invariant region for the flow generated by (2.7) in the sense that given any $(S_0, C_0) \in D$, the solution $(S(t, S_0), C(t, C_0)) \in \text{int}D$ for all $t > 0$. Hence defining the Poincaré map $P : \mathbb{R}^2 \rightarrow \mathbb{R}^2$ by

$$P((S(0, S_0), C(0, C_0))) = (S(2\pi/\omega, S_0), C(2\pi/\omega, C_0)),$$

we see that $(S_0, C_0) \in D$ implies $P((S_0, C_0)) \in D$. Moreover, clearly P is a continuous mapping. The result follows by a standard application of the Brouwer Fixed Point Theorem to the map P (see e.g. [17]). \square

3.4. Stability

The numerical simulations indicate that the periodic solution shown to exist above is (at least linearly asymptotically) stable. In order to obtain more information regarding linear stability we adopt the standard approach of Floquet theory (see e.g. [2]). Again, it is easier to refer to the transformed system (3.2) for this purpose.

Let $\mathbf{U}^* = (U^*, V^*)$ be a $\frac{2\pi}{\omega}$ periodic solution of (3.2). Then linearizing (3.2) about this solution, we obtain the system

$$\frac{d\mathbf{x}(t)}{dt} = A(t)\mathbf{x}(t), \quad (3.7)$$

where $\mathbf{x}(t) = [x(t) \ y(t)]^T$ and $A(t)$ denotes the periodic matrix

$$A(t) = \begin{bmatrix} -k_1(E_0 - V^*) & k_1(\hat{S} + U^*) + k_{-1} \\ k_1(E_0 - V^*) & -k_1(\hat{S} + U^* + K_M) \end{bmatrix}.$$

Letting

$$B(t) = \begin{bmatrix} X_1(t) & X_2(t) \\ Y_1(t) & Y_2(t) \end{bmatrix},$$

be the fundamental matrix of (3.7) that satisfies $B(0) = I_{2 \times 2}$, the general solution of (3.7) can be written

$$\mathbf{x}(t) = B(t)\mathbf{x}(0).$$

Hence,

$$\mathbf{x}(nT) = C^n \mathbf{x}(0), \quad \text{where } T = \frac{2\pi}{\omega} \quad \text{and } C = B(T).$$

Therefore, the eigenvalues of C (the Floquet multipliers) determine the linear stability of the periodic solution. These are linearly stable if both eigenvalues satisfy $|\lambda| < 1$, and unstable if at least one of them has absolute value larger than 1. As is common, the eigenvalues of this matrix cannot be determined explicitly. However, we were able to compute a numerical approximation to them as outlined below.

Firstly, the system (3.2) is solved numerically, (we again used the MATLAB routine “ode45”) to pick up a point on the periodic solution, say (U_p, V_p) . Then the system (3.2) and two copies of system (3.7) are solved simultaneously with the initial data

$$(U(0), V(0), x_1(0), y_1(0), x_2(0), y_2(0)) = (U_p, V_p, 1, 0, 0, 1). \quad (3.8)$$

The numerical values of U and V thus calculated will always be on the periodic solution (to within the accuracy of the numerical method) and hence at each time t , the matrix

$$M(t) = \begin{bmatrix} x_1(t) & x_2(t) \\ y_1(t) & y_2(t) \end{bmatrix}, \quad (3.9)$$

will be an approximation to $B(t)$, and the eigenvalues of C approximated by those of $M(T)$ (which can again be computed using MATLAB). In Fig. 6 the largest $|\lambda|$ are plotted for a range of values of I_0 , E_0 and ϵ , respectively. Observe that in each case $|\lambda|$ remains strictly less than 1 with, in fact, the maximum modulus decreasing with increase E_0 or ϵ , indicating an increase in the “strength of attraction” of the limit cycle. This result was repeated for a range of the other parameter values, strongly suggesting that the periodic solution is linearly stable.

We note the dependence of the eigenvalues on I_0 and ϵ is at first view somewhat surprising. Recall that both these parameters give a measure of the size of the forcing amplitude (although the former represents the mean input over a period and the latter measures the maximum perturbation from this mean) and so one’s first

guess would be that the cycle would become more or less stable in a manner that was determined by either of these interchangeably. However, it appears that the periodic solution becomes a stronger attractor (the largest eigenvalue decreases) as the amplitude is increased via increasing ϵ with the reverse happening if the amplitude is increased via increasing I_0 . However, we note that the initial data and therefore the system dynamics are dependent on I_0 whereas only the forcing amplitude about the mean depends on ϵ . It seems therefore that these two parameters play quite different roles in the dynamics. Finally, that the periodic solution becomes a stronger attractor with increasing ϵ is perhaps to be expected, as the forcing term becomes more dominant in the dynamics.

4. Error Estimates for the QSSA

4.1. Amplitude Dependence

The numerical simulations discussed above show that the solution trajectory remains “close” to the QSS nullcline. We now provide a quantitative measure of this closeness by investigating how errors in product formation vary with the parameter ϵ .

Denote by C_{ss} and \dot{P}_{ss} , respectively, the complex concentration calculated using the approximation (2.5) and the product production rate derived therefrom using (2.1d). Then

$$\dot{P}_{ss}(t) = k_2 C_{ss}(t) = \frac{k_2(E_0 + \hat{C})S(t)}{S(t) + K_M}.$$

We shall consider a measure of error based on the following scaled absolute error in the production rate (see [13] and [14]):

$$\mathcal{E} := \frac{\dot{P} - \dot{P}_{ss}}{\dot{P}_{max}},$$

where \dot{P}_{max} denotes the maximum value of the product production rate which, by (2.1d), it is equal to $k_2(E_0 + \hat{C}) = k_2E_0 + I_0$. Thus,

$$\mathcal{E}(t) = \frac{k_2(C(t) - C_{ss}(t))}{k_2E_0 + I_0}.$$

Numerical computations of the full system suggest this error will be oscillatory and this is easily confirmed by plotting the function $\mathcal{E}(t)$ (data not shown). This error function gives at any instant (during the second phase) the closeness of fit to the QSS nullcline. A “total error” over a period (of the substrate input) can therefore be defined by

$$\mathcal{E}_{QSS}^{tot} := \frac{\omega}{2\pi} \int_T^{T+\frac{2\pi}{\omega}} |\mathcal{E}(t)| dt. \quad (4.1)$$

Here T is a representative time for which the dynamics are in the second phase and we use $T = t_s$, with t_s defined above (for the parameter values used here $t_s = 14$; numerical computations show this to be reasonable). Figure 7 shows a calculation

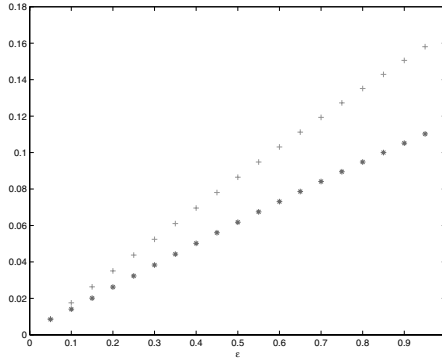


Fig. 7. The total errors \mathcal{E}_{QSS}^{tot} (asterisks) and \mathcal{E}_3^{tot} (crosses) in product formation plotted versus ϵ . Other parameter values as in Fig. 2.

of this error as a function of ϵ . Notice that the error is an increasing, almost linear function of ϵ . For small ϵ , as predicted above, the errors are small and hence the QSSA (2.5) can be viewed as a good approximation. Even at the extreme value of $\epsilon = 1$ (recall this represents input values oscillating between 0 and $2I_0$), the total relative error is less than 12% and for $\epsilon \leq 0.5$ this figure drops to less than 7%.

The biochemical reaction represented in Fig. 1 is most likely to be found *in vivo* in its asymptotic state. This is because *in vivo* reactions have in general evolved over long periods time. Consequently, transient processes have died out with a stable steady state being reached; steady-state behaviour is often observed from *in vivo* measurements. In the case of a constant input, this is the steady state (\hat{S}, \hat{C}) and for periodic input, it is the periodic solution discussed above. It is of interest therefore to measure the “difference” in system output in these two cases. We therefore extend the “quasi-steady state” forward in time to include the fixed point (\hat{S}, \hat{C}) of system (2.5). Thus we can define the “total phase three” error as

$$\mathcal{E}_3^{tot} = \frac{\omega}{2\pi} \int_{t_3}^{t_3 + \frac{2\pi}{\omega}} \left| \frac{k_2(C(t) - \hat{C})}{k_2 E_0 + I_0} \right| dt, \tag{4.2}$$

where t_3 is chosen sufficiently large so that the solution is in the third phase (t_3 was chosen to be 100 here). This error is also plotted in Fig 7 as a function of ϵ . It is also an increasing and almost linear function of ϵ and again shows that this error remains small but slightly larger than the phase 2 error for a significant range of ϵ (see below).

The errors detailed above are a stern test of the applicability of the sQSSA. From the viewpoint of biological experiments, an interesting alternative would be to estimate a true average error. For example, it is common that reactions rate estimates are obtained experimentally by measuring the total amount of product formed in

a given time from which an average product production rate is derived. Hence we define the average errors to be

$$\langle \mathcal{E} \rangle_{QSS} := \frac{\omega}{2\pi} \int_{t_s}^{t_s + \frac{2\pi}{\omega}} \mathcal{E}(t) dt \quad \text{and} \quad \langle \mathcal{E} \rangle_3 := \frac{\omega}{2\pi} \int_{t_3}^{t_3 + \frac{2\pi}{\omega}} \frac{k_2(C(t) - \hat{C})}{k_2 E_0 + I_0} dt.$$

The results are shown in Fig 8. As would be expected, these errors are smaller than those detailed above. However, what we could not have deduced by the more crude error calculations, is the following. These average errors are significantly smaller. The 2nd phase average error is again linearly increasing in ϵ , but at its largest (corresponding to oscillations ranging between 0 and $2I_0$) is less than 7%. The error in the 3rd phase is almost negligible ($O(10^{-9})$). (Figure 8 shows the 3rd phase error scaled by a factor of 10^6 .) The 3rd phase error slightly decreases with ϵ . Notice also the average errors remain positive for all values of ϵ . We conclude therefore that the solution of the full system (2.7) essentially “behaves on average” like the reduced system (2.5). However, over a period, (2.5) slightly under estimates the product production rate (for this set of parameter values at least). The phase 3 errors are particularly small indicating that “fixed” point (\hat{S}, \hat{C}) provides a good, average approximation from which to calculate system output. The fact that this error reduces with increasing ϵ is a result of the periodic oscillations becoming more symmetric in their orbit around the point (\hat{S}, \hat{C}) .

4.2. Frequency Dependence

In all the above error calculations, the frequency of input oscillation was held fixed. An interesting result was found when we considered the dependence of the average errors $\langle \mathcal{E} \rangle$ upon ω . We set $\epsilon = 1$, its extreme value and varied ω between 0.05π and π . It was found that the second phase error $\langle \mathcal{E} \rangle_{QSS}$ decreases rapidly with increasing ω , see Fig. 9. The reason for such behaviour is that the system cannot respond fast enough to higher frequency oscillations. Hence for a fixed forcing amplitude the output oscillations decrease in amplitude with increasing frequency.

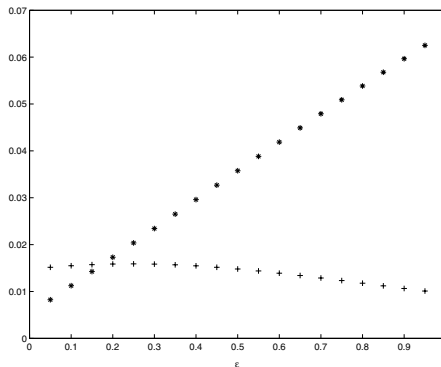


Fig. 8. The average errors $\langle \mathcal{E} \rangle_{QSS}$ (asterisks) and $\langle \mathcal{E} \rangle_3 \times 10^6$ (crosses) in product formation rate plotted versus ϵ . Other parameter values as in Fig. 2.

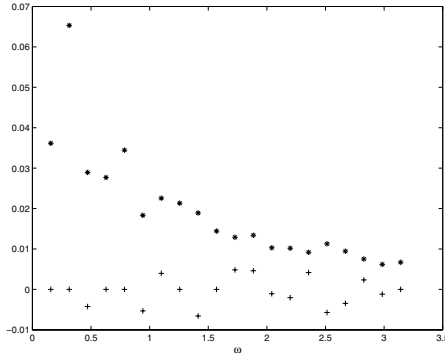


Fig. 9. The average errors $\langle \mathcal{E} \rangle_{QSS}$ (asterisks) and $\langle \mathcal{E} \rangle_3$ (crosses) as a function of ω . $\epsilon = 1$, other parameter values are as in Fig. 2.

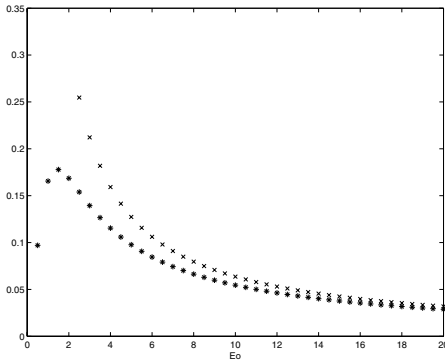


Fig. 10. The total errors \mathcal{E}_3^{tot} (asterisks) and the approximation (4.3) (crosses) as functions of E_0 . $\epsilon = 1$, other parameter values are as in Fig. 2.

The phase 3 error $\langle \mathcal{E} \rangle_3$ was again significantly smaller than the phase 2 error for all values of ω . Further investigations revealed that the apparent oscillations in this error are almost certainly an artifact of the averaging procedure, namely the fixed start time t_3 .

4.3. Errors when the QSSA is “Invalid”

It was shown above that a periodic solution exists for all parameter values. Numerical calculations indicate that these are stable. It is of interest therefore to investigate whether the error analysis discussed above is applicable in the third (periodic) phase to a wider set of parameter values than those determined by the necessary conditions for the applicability of the sQSSA, namely (2.6). As an illustrative example, we discuss the behaviour as E_0 is varied. Similar behaviour can be observed by varying other system parameters. Figure 10 shows the total error (4.2) as a function of E_0 .

With all other parameters fixed, condition (2.6) is violated for high values of E_0 and hence in this case the QSSA cannot strictly be applied. However, from fig. 10, it is clear that the errors reduce with increasing E_0 . This can be explained by as follows.

As E_0 is increased the system dynamics display an initial transient which involves a rapid decrease of substrate to a level very close to \hat{S} . Quasi steady behaviour is then seen with little further change in substrate concentration. This is exactly the reverse quasi-steady state behaviour predicted for high enzyme concentrations as illustrated in for example Fig. 8 of [14] and discussed in detail in [8] for the no-input case. During the third phase, the change in substrate level remains small. Formally then, for E_0 sufficiently large, we see that on letting $S = \hat{S} + O(1/E_0^2)$, substituting into (2.7a) and equating terms of $O(1)$, we get

$$0 = I(t) - k_1 E_0 \hat{S} + k_{-1} C.$$

From this it follows that

$$C = \hat{C} - \frac{I_0}{k_{-1}} \epsilon \sin \omega t + O(1/E_0^2),$$

i.e. to first order, C performs oscillations of period $2\pi/\omega$ centred around \hat{C} . Substituting this expression for C into (4.2) yields

$$\mathcal{E}_3^{tot} = \frac{2I_0\epsilon}{\pi k_{-1}} \frac{1}{E_0} + O\left(\frac{1}{E_0^2}\right), \quad (4.3)$$

and the dependence on E_0 is clear.

From fig. 10, it is seen that the total phase three error also decreases for decreasing E_0 , sufficiently small. For E_0 sufficiently small, the sQSS phase of the dynamics dominates. Therefore from (2.7b), given $S = \hat{S} + O(1)$, it is straight forward to show that $C = \hat{C} + O(E_0^2)$ and hence $\mathcal{E}_3^{tot} = O(E_0^2)$.

5. Discussion

Biochemical rhythms are ubiquitous characteristics of living organisms. The periods of such rhythms range from seconds to days (see e.g. [3]). In this paper, we have studied a classic enzymatic reaction subjected to periodic input. Since this input may be either from other enzymatic reactions or external environments, the frequency of input may vary greatly depending on the biochemical context. In biochemical networks, the amplitude of input to an enzymatic reaction is generally controlled by the maximum enzyme activities of preceding enzyme reactions. Therefore, the amplitude of such inputs is usually limited to a certain range. The form of input as given in (1.1) captures these two distinguishing features.

In this paper we have attempted to understand the possible effects of periodic perturbations to substrate input on the full dynamics of the classic enzyme-catalysed reaction system and the quasi-steady state assumptions derived therefrom. Given that necessary conditions for the validity of the sQSSA for systems with constant substrate input have now been established [14], our aim here was to see

how good this approximation remains when the substrate input is subject to periodic fluctuations. Such behaviour may represent more fully the situation *in vivo* where it is likely that either imposed periodicity is prevalent in biochemical networks, or simply that a constant input is subject to some “noise”. We conclude that the sQSSA derived in [14] remains a very good approximation to the full dynamics for a wide range of input amplitudes and frequencies. Moreover, when the system is in its third, periodic phase, the extended QSSA, $(S, C) = (\hat{S}, \hat{C})$ introduced here, provides an accurate means by which to calculate product production rates *even when the conditions for the applicability of the sQSSA are significantly violated*.

The sQSSA is most accurate when the input performs high frequency, small amplitude oscillations around a positive mean value. This accuracy remains high even in the case of large perturbations, provided the frequency of oscillation is sufficiently high. Hence, greatest care must be taken in applying this approximation, where it is known or expected that the input oscillations will be of significant amplitude and, more importantly, of lower frequency.

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