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A singular value decomposition approach for the derivation of the Michaelis-Menten equation

Un enfoque de descomposición en valores singulares para la derivación de la ecuación de Michaelis-Menten

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Abstract
The Michaelis-Menten (MM) equation is traditionally derived by taking a quasi-steady state assumption (QSSA) for the intermediate complex or a chemical equilibrium assumption for the transformation of substrate into the intermediate complex. The validity of these assumptions has been subjected to intense research in recent decades, where the use of tools from singularly perturbed systems has played a central role. The present work aims to explore an approach to derive the MM equation from a singular value decomposition (SVD) analysis of the MM kinetics. The idea is to consider singular values as scaling factors to convert the MM equations into a singularly perturbed system. The results showed that the MM equation can be obtained from the boundary-layer system for a sufficient separation of the time scale represented by singular values. Such a boundary-layer system can be interpreted as resulting from a linear combination of the traditional complex QSSA and substrate equilibrium assumption (SEA). The SVD methodology combined with results from singularly perturbed systems can be used for extended MM kinetics. To this end, the case of the autocatalytic MM kinetics was considered as a worked example. Numerical examples were used to illustrate the theoretical findings.
Keywords: Michaelis-Menten; multiscale; singular values.

Resumen
La ecuación de Michaelis-Menten (MM) se deriva tradicionalmente tomando una suposición de estado casi estable (QSSA) para el complejo intermedio o una suposición de equilibrio químico para la reacción de substrato en complejo. La validez de estos supuestos ha sido objeto de intensas investigaciones en las últimas décadas, donde el uso de herramientas de sistemas singularmente perturbados ha jugado un papel central. El presente trabajo tiene como objetivo explorar un enfoque para derivar la ecuación MM a partir de un análisis de descomposición de valores singulares (SVD) de la cinética MM. La idea es considerar valores singulares como factores de escala para convertir las ecuaciones MM en un sistema singularmente perturbado. Los resultados mostraron que la ecuación MM se puede obtener del sistema de capa límite para una separación suficiente de la escala de tiempo representada por valores singulares. Dicho sistema de capa límite puede interpretarse como resultado de una combinación lineal del complejo tradicional QSSA y la suposición de equilibrio del sustrato. La metodología SVD combinada con los resultados de sistemas singularmente perturbados se puede utilizar para la cinética MM extendida. Para ello, se consideró como ejemplo trabajado el caso de la cinética MM autocatalítica. Se utilizaron ejemplos numéricos para ilustrar los hallazgos teóricos.
Palabras clave: Michaelis-Menten, multiescala, valores singulares.

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1 Introduction

The Michaelis-Menten (MM) reaction scheme is the most widely used in biochemistry. It describes the conversion of a substrate (S) into a product (P) by the catalyzing action of an enzyme (E) via an intermediate binding complex (C). The scheme of the Michaelis-Menten (MM) kinetics can be represented as follows:

$$\mathrm{E} + \mathrm{S} \xrightarrow{k_1} \mathrm{C} \tag{1a}$$

$$\mathrm{C} \xrightarrow{k_{-1}} \mathrm{E} + \mathrm{S} \tag{1b}$$

$$\mathrm{C} \xrightarrow{k_c} \mathrm{E} + \mathrm{P} \tag{1c}$$

The first step (1.a) represents the formation of the complex intermediate from the binding of the enzyme to the substrate, the second step is the reversibility of the first step, and the third step is the decomposition of the complex to form products and reestablish the enzyme. The parameters k_1 and k_{-1} represent the rate constants of the forward and backward complex formation, and the parameter k_c represents the rate constant of the product formation from the complex intermediate. By departing from the law of mass action, the dynamical behavior of the species involved in the MM scheme is governed by the following differential equations:

$$\frac{dS}{dt} = -k_1 ES + k_{-1} C, \quad S(0) = S_0 \tag{2a}$$

$$\frac{dE}{dt} = -k_1 ES + (k_{-1} + k_c) C, \quad E(0) = E_0 \tag{2b}$$

$$\frac{dC}{dt} = k_1 ES - (k_{-1} + k_c) C, \quad C(0) = 0 \tag{2c}$$

$$\frac{dP}{dt} = k_c C, \quad P(0) = 0 \tag{2d}$$

Here, for simplicity the same letter was used to denote the species and the respective concentration. The above differential equations are not independent since the following conservation laws are satisfied:

$$E + C = E_0 \tag{3a}$$

$$S + C + P = S_0 \tag{3b}$$

The analysis of the system (2) focuses on the first three differential equations since the rate of product formation can be obtained from the availability of the complex dynamics. One can use the invariant given by Eq. (3.a) to reduce the system (2.a)-(2.c) to the following two expressions:

$$\frac{dS}{dt} = -k_1 E_0 S + k_1 CS + k_{-1} C, \quad S(0) = S_0 \tag{4a}$$

$$\frac{dC}{dt} = k_1 E_0 S - k_1 CS - (k_{-1} + k_c) C, \quad C(0) = 0 \tag{4b}$$

In practice, the concentration of the complex intermediate C is rarely accessible for measurements. Since the quantification of the rate of product generation (Eq. (2.d)) is commonly the task, one would like to dispose of a mathematical expression depending only on the measurable substrate concentration S. Michaelis and Menten (1913) assumed that the substrate is in instantaneous chemical equilibrium with the complex, which implies the following relationship:

$$k_1 E_0 S \approx k_{-1} C \tag{5}$$

This assumption, called as substrate equilibrium assumption (SEA), can be used in Eq. (2.d) to obtain an approximation for the rate of product generation:

$$\frac{dP}{dt} \approx \frac{k_c E_0 S}{K_S + S} \tag{6}$$

where

$$K_S = \frac{k_{-1}}{k_1} \tag{7}$$

is the substrate dissociation equilibrium constant. Subsequently, Briggs and Haldane (1925) considered a different way by assuming a quasi-steady-state assumption (QSSA) on the rate of complex generation, which states that $dC/dt \approx 0$ for large times. In terms of Eq. (4.b), one has

$$C \approx \frac{E_0 S}{K_M + S} \tag{8}$$

where

$$K_M = \frac{k_{-1} + k_c}{k_1} \tag{9}$$

is the so-called Michaelis constant linked to the substrate-enzyme affinity. Therefore, the rate of product generation can be approximated by the well-known MM equation:

$$\frac{dP}{dt} = \frac{k_c E_0 S}{K_M + S} \tag{10}$$

It is noted that the QSSA can be reduced to the SEA when $k_c \ll k_{-1}$, such that $K_M \approx K_S$.

The approximate models given by eqs. (6) and (10) are simple in structure and for a century they have found wide acceptance in the physiology and biochemistry fields, among many others. While the application of the MM equation was increasing, theorists hesitated on how to find a justification for the assumptions (mainly the Briggs and Haldane's QSSA) leading to the MM equation. Heineken *et al.* (1967) proposed a formal approach based on singular perturbations by considering the ratio E_0/S_0 as a small parameter. The underlying idea is that there are two-time scales involved in the MM dynamics, such that the Tikhonov's theorem is valid for obtaining an approximate solution that

is valid for large times. Reich and Sel'Kov (1974) and Schauer and Heinrich (1979) advanced on the singular perturbation analysis of the MM kinetics by considering more involved expressions for the singularity parameter (e.g., E_0/K_M). Segel (1988) estimated the characteristic time scales for the fast and slow dynamics and proposed a sufficient condition

$$\frac{E_0}{K_M + S_0} \ll 1 \tag{11}$$

to guarantee the fulfillment of the QSSA. The methodology of Segel (1988) is based on the computation of fast and slow time scales, and so it is quite flexible for the analysis of more general forms of the MM kinetics, including the kinetics of suicide substrates (Burke *et al*., 1990), zymogen activation (Eilertsen *et al*., 2018a), coupled enzyme kinetics (Eilertsen *et al*., 2018b) and open Michaelis-Menten reaction mechanism (Eilertsen *et al*., 2022). Borghans *et al*. (1986) showed that the validity of the QSSA can be extended by using a coordinate change where the total substrate concentration $S + C$ is considered instead of the sole substrate concentration S, and reported the following condition

$$\frac{KE_0}{(K_M + E_0 + S_0)^2} \ll 1 \tag{12}$$

Here, $K = k_c/k_1$. This approach has attracted some interest and is called as the total QSSA (tQSSA in short) (Tzafriri and Edelman, 2007). Despite important theoretical advances, the validity of the QSSA has been a matter of discussion. Schnell (2014) provided a critical discussion on the validity of the QSSA and postulated that a reactant stationary assumption is the required condition for the validity of the Michaelis-Menten equation to estimate kinetic parameters. Patsatzis and Goussis (2019) argued that the MM equation is not sufficient to describe the MM kinetics and proposed an algorithmic approach to compute a MM equation that is valid under multiscale conditions. They used the computational singular perturbation (CSP) algorithm to derive a new validity condition for the MM equation:

$$\frac{E_0 - C}{K_R + S + K} \ll 1 \tag{13}$$

In this way, the MM equation validity depends on the (S, C)-trajectory. For instance, for small times one may have small values of $E_0 - C$ and the above inequality is satisfied. However, for large times the substrate concentration is so depleted that the condition (13) is not fulfilled. Recently, Rao and Heynderickx (2021) provided a critical discussion of the validity of the MM equation for complex enzyme kinetic mechanisms.

The QSSA is a well-accepted approach for computing reduced-order models of reacting kinetics. In the case of the MM mechanism, the QSSA has offered a simple route to compute reduced-order models that can be used in practice. However, the notion that the QSSA should be applied to the intermediate complex is still unclear. The present study aims to provide a generalization of the QSSA based on singular value decomposition (SVD) and singular perturbation analysis (Hoppensteadt, 1974) of the MM kinetics. The task is to show that the MM kinetics can be approximated by a MM equation under sufficient separation of scaling factors obtained from SVD.

2 SVD analysis of the MM kinetics

The system (4) has a particular structure that can be exploited to analyze the multiscale dynamics of the substrate and complex concentrations. To this end, it is convenient to normalize the substrate and complex concentrations. Following Heineken *et al*. (1967), the substrate and complex concentrations are normalized by the initial conditions S_0 and E_0, respectively. The dimensionless variables are given by

$$x \equiv \frac{S}{S_0} \tag{14a}$$

$$y \equiv \frac{C}{E_0} \tag{14b}$$

Then, the system (4) can be expressed as follows:

$$\frac{dx}{dt} = -k_1 E_0 x + k_1 E_0 xy + k_{-1}\left(\frac{E_0}{S_0}\right)y, \quad x(0) = 1 \tag{15a}$$

$$\frac{dy}{dt} = k_1 S_0 x - k_1 S_0 xy - (k_{-1} + k_c)y, \quad y(0) = 0 \tag{15b}$$

In matrix form, one has

$$\begin{pmatrix} \frac{dx}{dt} \\ \\ \frac{dy}{dt} \end{pmatrix} = \begin{pmatrix} -k_1 E_0 & k_1 E_0 & k_{-1}\left(\frac{E_0}{S_0}\right) \\ k_1 S_0 & -k_1 S_0 & -(k_{-1} + k_c) \end{pmatrix} \begin{pmatrix} x \\ xy \\ y \end{pmatrix} \tag{16}$$

Importantly, all the elements of the matrix

$$M \equiv \begin{pmatrix} -k_1 E_0 & k_1 E_0 & k_{-1}\left(\frac{E_0}{S_0}\right) \\ k_1 S_0 & -k_1 S_0 & -(k_{-1} + k_c) \end{pmatrix} \tag{17}$$

have the same units; namely, t^{-1}, such that all operations (e.g., sums of elements) involving the elements of the matrix M will be consistent with physical units. The matrix M can be considered as an operator that maps the vector of rate functions

$$F(x,y) = \begin{pmatrix} x \\ xy \\ y \end{pmatrix} \tag{18}$$

in the vector of reaction rates

$$r(x,y) \equiv \begin{pmatrix} \frac{dx}{dt} \\ \\ \frac{dy}{dt} \end{pmatrix} \tag{19}$$

That is, $M : F(x,y) \rightarrow r(x,y)$. In this way, the matrix M transforms the information carried out by the rate functions $F(x,y)$ in the reaction rates $r(x,y)$. In the following, we will consider SVD of the matrix M to assess the strength and directions of the information transformation of the reaction kinetics. The SVD of the matrix M implies that the transformation $r(x,y) = MF(x,y)$ can be rewritten as

$$r(x,y) = U\Sigma V^T F(x,y) \tag{20}$$

where U and V are respectively 2×2 and 3×3 orthonormal matrices corresponding to the left and the right eigenvectors of the matrix M and $\Sigma = \text{diag}(\sigma_1, \sigma_2)$ is a 2×3 with $\sigma_1 \geq \sigma_2$ being the singular values. Orthonormality implies that $U^{-1} = U^T$, such that Eq. (20) can be written as

$$U^T r(x,y) = \Sigma V^T F(x,y) \tag{21}$$

In terms of the differential equations (15), one has the following expressions:

$$u_{11}\frac{dx}{dt} + u_{21}\frac{dy}{dt} = \sigma_1(v_{11}x + v_{21}xy + v_{31}y) \tag{22a}$$

$$u_{12}\frac{dx}{dt} + u_{22}\frac{dy}{dt} = \sigma_2(v_{12}x + v_{22}xy + v_{32}y) \tag{22b}$$

Where u_{ij} $i,j = 1,2$ are the elements of the matrix U and v_{ij};$i = 1,2,3$;$j = 1,2$ are the elements of the matrix V. In this representation, $u_{11}x + u_{21}y$ and $u_{12}x + u_{22}y$ can be seen as pseudo-species resulting from the linear combination of the real species, while the parameters $\sigma_1 v_{11}$, $\sigma_1 v_{21}$, $\sigma_1 v_{31}$, $\sigma_2 v_{12}$, $\sigma_2 v_{22}$ and $\sigma_2 v_{32}$ can be interpreted as pseudo-rate constants. Since the elements of the matrix M have units of 1/time and the matrices U and V are orthonormal (i.e., the norm of the columns is normalized to one), the singular values have units of 1/time. In this way, the inverse of the singular values σ_1 and σ_2 can be seen as characteristic time scales obtained from SVD of the matrix M. Thus, the singular values σ_1 and σ_2 factoring the right-hand sides of Eq. (22) can be seen as characteristic scaling factors that weight the impact of the reaction functions $F(x,y)$ in the reaction rates $r(x,y)$. Consider the following parameter:

$$\varepsilon = \frac{\sigma_2}{\sigma_1} \tag{23}$$

where the singular values are positive and $\sigma_1 \geq \sigma_2$, then one has that $0 < \varepsilon \leq 1$. In this way, the system (22) can be expressed as follows:

$$\varepsilon\left(u_{11}\frac{dx}{dt} + u_{21}\frac{dy}{dt}\right) = \sigma_2(v_{11}x + v_{21}xy + v_{31}y) \tag{24a}$$

$$u_{12}\frac{dx}{dt} + u_{22}\frac{dy}{dt} = \sigma_2(v_{12}x + v_{22}xy + v_{32}y) \tag{24b}$$

Suppose that $\varepsilon \ll 1$. Then, the differential system given by Eq. (24) can be analyzed as a singularly perturbed system with ε acting as the perturbation parameter (Hoppensteadt, 1974). The fast (i.e., reduced) system is obtained by taking the fast time variable

$$t_{fast} = \frac{t}{\varepsilon} \tag{25}$$

to give

$$u_{11}\frac{dx}{dt_{fast}} + u_{21}\frac{dy}{dt_{fast}} = \sigma_1(v_{11}x + v_{21}xy + v_{31}y) \tag{26a}$$

$$u_{12}\frac{dx}{dt_{fast}} + u_{22}\frac{dy}{dt_{fast}} = \varepsilon\sigma_2(v_{12}x + v_{22}xy + v_{32}y) \tag{26b}$$

and allowing $\varepsilon \rightarrow 0$ to obtain

$$u_{11}\frac{dx}{dt_{fast}} + u_{21}\frac{dy}{dt_{fast}} = \sigma_1(v_{11}x + v_{21}xy + v_{31}y) \tag{27a}$$

$$u_{12}\frac{dx}{dt_{fast}} + u_{22}\frac{dy}{dt_{fast}} = 0 \tag{27b}$$

Fraser (1988) has pointed out that model reduction of the MM kinetics is synonymous with approximating the model dynamics on an invariant manifold. In this sense, Eq. (27.b) can be integrated to give an expression for the fast fiber (Zagarakis *et al*., 2004):

$$\varphi_{fast}(x,y) = u_{12}(x-1) + u_{22}y \tag{28}$$

That is, for short times the trajectories of the MM kinetics are constrained to evolve on the fast fiber $\varphi_{fast}(x,y)$. It is noted that the fast fiber represents a straight line with slope u_{12}/u_{22} in the plane (x,y). On the other hand, the slow (i.e., boundary-layer) system is obtained from Eq. (24) by allowing $\varepsilon \rightarrow 0$; namely,

$$\sigma_2(v_{11}x + v_{21}xy + v_{31}y) = 0 \tag{29a}$$

$$u_{12}\frac{dx}{dt} + u_{22}\frac{dy}{dt} = \sigma_2(v_{12}x + v_{22}xy + v_{32}y) \tag{29b}$$

Then, the slow manifold is given by

$$\varphi_{slow}(x,y) = v_{11}x + v_{21}xy + v_{31}y \tag{30}$$

Interestingly, the fast fiber is given by a linear combination of the reaction rates $r(x,y)$, whilst the slow fibers (Eq. (27.b)) by a linear combination of the rate functions $F(x,y)$. A system trajectory in the phase plane $(x(t), y(t))$ converges to the slow manifold (30) for large times. In this way, Eq. (30) corresponds to a family of σ-isoclines (Calder and Siegel, 2008), which have the characteristic of being tangent to the trajectories at the equilibrium point.

Eq. (30) leads to the following relationship that is satisfied for large times:

$$y = -\frac{\left(\frac{v_{11}}{v_{21}}\right)x}{\left(\frac{v_{31}}{v_{21}}\right)+x} \tag{31}$$

Since $dp/dt = k_c C$, $S = S_0 x$ and $C = E_0 y$, one has that the expression for the slow manifold (30) can be written as a relationship between substrate and complex concentrations:

$$C = -\frac{E_0\left(\frac{v_{11}}{v_{21}}\right)S}{\left(\frac{v_{31}}{v_{21}}\right)S_0+S} \tag{32}$$

which implies that the rate of product generation for large times can be approximated by

$$\frac{dP}{dt} = -\frac{k_c E_0\left(\frac{v_{11}}{v_{21}}\right)S}{\left(\frac{v_{31}}{v_{21}}\right)S_0+S} \tag{33}$$

This expression has the structure of a MM equation where

$$V_{\max} = -k_c E_0\left(\frac{v_{11}}{v_{21}}\right) \tag{34a}$$

$$K_{aff} \equiv \frac{v_{31}}{v_{21}}S_0 \tag{34b}$$

play the role of the maximum velocity and the substrate affinity constant. In this way, by necessity, one has the ratio $v_{11}/v_{21} < 0$ to have $V_{\max} > 0$. Otherwise, the MM-like equation $dP/dt = -k_c E_0 (v_{11}/v_{21}) S/[(v_{31}/v_{21})S_0 + S]$ would predict that the product is being removed from the reacting system, which is a contradiction. Extensive numerical computations show that $v_{11} = -v_{21}$, such that $v_{11} = -v_{21}$ and $V_{\max}$ reduces to the classical expression $V_{\max} = k_c E_0$. However, we were unable to give rigorous proof for the equality $v_{11} = -v_{21}$. One concludes that the behavior of the rate of product generation for long times can be reduced to a MM equation as long as a sufficient separation (i.e., sufficiently small values of the perturbation parameter ε) of the scaling factors σ_1 and σ_2 is exhibited.

The reduction of the MM kinetics model to the MM equation was obtained from the boundary-layer system (29), resulting in the slow manifold given by Eq. (30) or equivalently Eq. (31). The slow manifold approximation could be heuristically obtained by considering that the quantity $u_{11}x + u_{21}y$ does not change on the time scale over which the product is formed. Such assumption would mean that

$$v_{12}x + v_{22}xy + v_{32}y \approx 0 \tag{35}$$

An interpretation of the above approximation can be given as follows. In the original analysis, Michaelis and Menten (1913) assumed that the substrate is in instantaneous chemical equilibrium with the complex. In terms of the expression (15.a), the SEA assumption implies that $-k_1 E_0 x + k_1 E_0 xy + k_{-1}\left(\frac{E_0}{S_0}\right)y \approx 0$. On the other hand, Briggs and Haldane (1925) took an alternative route by assuming that the concentration of the intermediate complex does not change on the timescale of product formation. In terms of Eq. (15.b), The Briggs and Haldane's QSSA implies that $k_1 S_0 x - k_1 S_0 xy - (k_{-1} + k_c)y \approx 0$. The condition (34) derived from the SVD analysis can be seen as a general form of the SEA and the complex QSSA. In this regard, one can consider the boundary-layer system (29) as a generalized QSSA for the MM kinetics. That is, rather than applying the QSSA for the substrate or the complex species, Eq. (33) indicates that the generalized QSSA applies for a linear, non-necessarily convex combination of the individual QSSA.

It should be noted that the classical QSSA derived by Briggs and Haldane (1925) depends only on the initial enzyme concentration E_0 (see Eq. (8)). In contrast, the QSSA assumption derived from the SVD analysis depends also on the initial substrate concentration S_0 (see Eq. (33)). In this way, Eq. (29) defines a bundle of slow manifolds that depend on the initial substrate concentration. That is, for a given set of numerical values of the kinetics constants, the slow manifold is also dependent on the initial condition S_0. In this way, a trajectory starting at S_0 would converge to a fiber of the slow manifold bundle.

2.1 *Validity sufficient conditions*

A key question is to establish sufficient conditions on the kinetics constant to have a large separation of the scaling factors. The singular values of the matrix M (see Eq. (15)) are computed from the eigenvalues of the matrix MM^T given by

$$MM^T = \begin{pmatrix} 2k_1^2E_0^2 + k_{-1}^2\left(\frac{E_0}{S_0}\right)^2 & -2k_1^2S_0E_0 - k_{-1}(k_{-1}+k_c)\left(\frac{E_0}{S_0}\right) \\ -2k_1^2S_0E_0 - k_{-1}(k_{-1}+k_c)\left(\frac{E_0}{S_0}\right)^2 & 2k_1^2S_0^2 + (k_{-1}+k_c)^2 \end{pmatrix} \tag{36}$$

The singular values of M are the squared root of the eigenvalues of the above matrix. The trace and determinant of MM^T are given by

$$tr = 2k_1^2(E_0^2 + S_0^2) + k_{-1}^2\left(\frac{E_0}{S_0}\right)^2 + (k_{-1}+k_c)^2 \tag{37a}$$

$$det = 2k_1^2E_0^2(k_{-1}+k_c)^2 + 2k_1^2k_{-1}^2E_0^2 - 4k_1^2k_{-1}(k_{-1}+k_c)E_0^2 - k_{-1}^2(k_{-1}+k_c)^2\left(\frac{E_0}{S_0}\right)^2 \tag{37b}$$

Introduce the following dimensionless parameters:

$$\alpha = \frac{k_1 E_0}{k_c} \tag{38a}$$

$$\beta = 1 + \frac{S_0^2}{E_0^2} \tag{38b}$$

$$\gamma = \frac{k_{-1}}{k_c} \tag{38c}$$

Then, eqs. (37.a) and (37.b) become

$$tr = k_c^2 \varphi_1(\alpha,\beta,\gamma) \tag{39a}$$

$$det = k_c^4 \varphi_2(\alpha,\beta,\gamma) \tag{39b}$$

where

$$\varphi_1(\alpha,\beta,\gamma) = 2\alpha^2\beta + \left(\frac{\gamma}{\beta-1}\right)^2 + (\gamma+1)^2 \tag{40a}$$

$$\varphi_2(\alpha,\beta,\gamma) = 2\alpha^2(\gamma+1)^2 + 2\alpha^2\gamma^2 - 4\alpha^2\gamma(\gamma+1) - \left(\frac{\gamma^2}{\beta-1}\right)(\gamma+1)^2 \tag{40b}$$

The eigenvalues of the matrix MM^T are given as the roots of the polynomial $\lambda^2 - tr\lambda + det = 0$, such that the singularity parameter is given by

$$\varepsilon^2 = \frac{\varphi_1(\alpha,\beta,\gamma) - \sqrt{\varphi_1(\alpha,\beta,\gamma)^2 - 4\varphi_2(\alpha,\beta,\gamma)}}{\varphi_1(\alpha,\beta,\gamma) + \sqrt{\varphi_1(\alpha,\beta,\gamma)^2 - 4\varphi_2(\alpha,\beta,\gamma)}} \tag{41}$$

Then, a general condition for the validity of the MM-like equation (33) is

$$\frac{\varphi_1(\alpha,\beta,\gamma) - \sqrt{\varphi_1(\alpha,\beta,\gamma)^2 - 4\varphi_2(\alpha,\beta,\gamma)}}{\varphi_1(\alpha,\beta,\gamma) + \sqrt{\varphi_1(\alpha,\beta,\gamma)^2 - 4\varphi_2(\alpha,\beta,\gamma)}} \ll 1 \tag{42}$$

The above inequality is quite complex and involves the three dimensionless parameters α, β and γ. Two limiting cases can be obtained as follows:

a) Assume that $\gamma \ll 1$ and $\alpha \gg 1$. The first assumption implies that the complex formation reaction is weakly reversible. Then, $\varphi_1(\alpha,\beta,\gamma) = O(2\alpha^2\beta)$ and $\varphi_2(\alpha,\beta,\gamma) = O(2\alpha^2)$, such that

$$\varepsilon^2 \approx \frac{\alpha\beta - \sqrt{\alpha^2\beta^2 - 2}}{\alpha\beta + \sqrt{\alpha^2\beta^2 - 2}} \tag{43}$$

One has that $\varepsilon \ll 1$ for $\alpha \gg 1$. That is, $\alpha \gg 1$ is a sufficient condition for the validity of the MM-like equation (31). From Eq. (38.a), such a condition can be expressed as

$$\frac{E_0}{K_{VSC}} \gg 1 \tag{44}$$

where K_{VSC} is the Van Slyke-Culen constant. In the context of the reversible QSSA (rQSSA), the above inequality was derived by Segel and Slemrod (1989) by other analysis routes based on singular perturbation analysis.

b) Assume that $\gamma \ll 1$ and $\beta \gg 1$. As in the above item, $\varphi_1(\alpha,\beta,\gamma) = O(2\alpha^2\beta)$ and $\varphi_2(\alpha,\beta,\gamma) = O(2\alpha^2)$, such that the magnitude order of the singularity parameter is given by Eq. (41). Similarly, $\varepsilon^2 \ll 1$ for $\beta \gg 1$, which in terms of Eq. (38.b), $1 + \frac{S_0^2}{E_0^2} \gg 1$, equivalently,

$$\frac{S_0}{E_0} \gg 1 \tag{45}$$

That is, the MM-like equation (31) is valid when the initial substrate concentration is sufficiently high relative to the initial enzyme concentration. This validity condition was derived by Laidler (1955) in the framework of the standard QSSA (sQSSA).

The above analysis showed that the SVD recasts some validity conditions already reported in the literature. However, such conditions are only particular cases of the general validity condition given by Eq. (42).

2.2 *Numerical illustrations*

Let us illustrate the above findings with some numerical computations. Figure 1 illustrates the behavior of the SVD perturbation parameter $\varepsilon = \sigma_2/\sigma_1$ with respect to the dimensionless parameters α and γ (see Eq. (38)). The results were obtained by fixing $S_0 = 1.0$ and $k_c = 1.0$, and for four different values of the dimensionless parameter: (a) $\beta = 1.25$, (b) $\beta = 1.5$, (c) $\beta = 2.0$, and (d) $\beta = 3.0$. Values of ε that are of the order of 0.01 were obtained for $\alpha > 10$ and $\gamma > 10$, suggesting that the MM equation is valid for large values of the parameters α and γ. Besides, the region of validity would decrease when the parameter β is increased (i.e., when the ratio E_0/S_0 was increased). That is, the high concentration of enzyme relative to the substrate concentration limits the validity of the MM equation. For instance, it has been reported that the concentration of the enzyme trisophosphate isomerase is about 2.5-fold higher than the concentration of its substrate dihydroxyacetone-phosphate in rabbit muscle (Albe *et al.*, 1990). Tzafriri and Edelman (2007) proposed the tQSSA framework to study the validity of the MM equation under enzyme excess concentration relative to the Michaelis constant and derived stringent sufficient conditions. Yun and Han (2020) used extensive numerical simulations to claim that the MM equation is valid only when $E_0 < 0.01K_M$, and that for higher enzyme concentrations the approximate MM kinetics follows a non-MM equation. Figure 1 suggests that the MM equation is still valid as long as the parameters α and γ are sufficiently large.

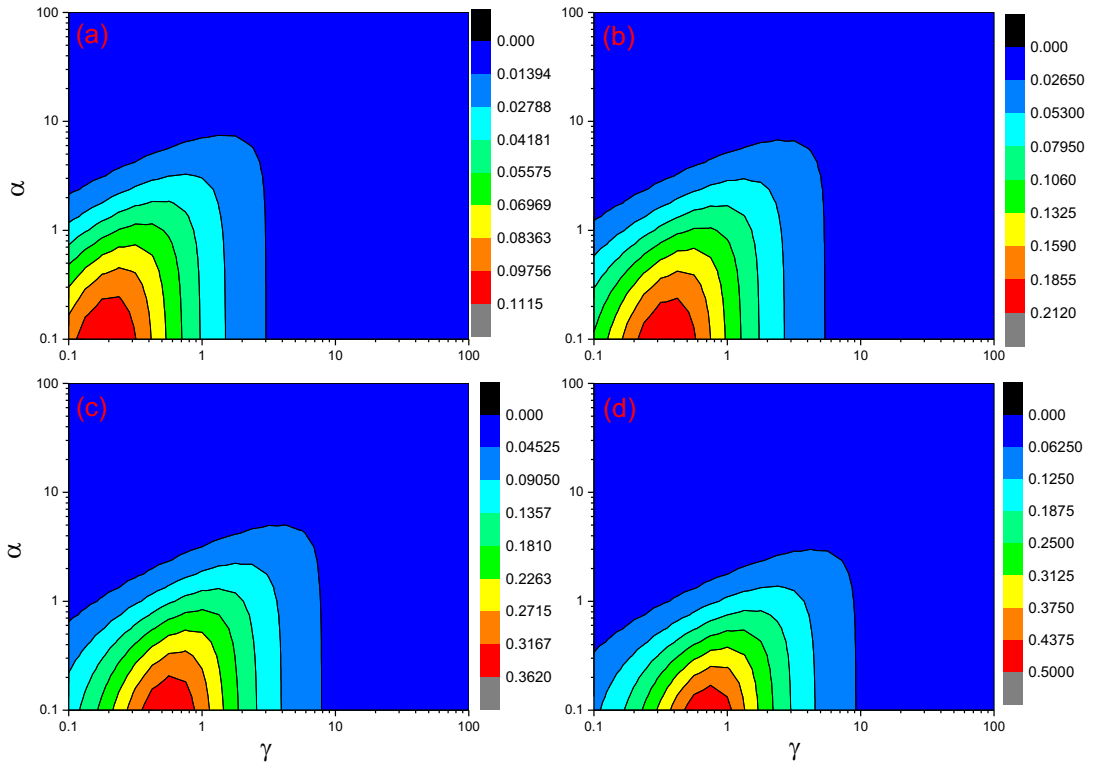

Figure 1. Behavior of the SVD perturbation parameter $\varepsilon = \sigma_2/\sigma_1$ with respect to the dimensionless parameters α and γ. The results were obtained by fixing $S_0 = 1.0$ and $k_c = 1.0$. (a) $\beta = 1.25$, (b) $\beta = 1.5$, (c) $\beta = 2.0$, and (d) $\beta = 3.0$.

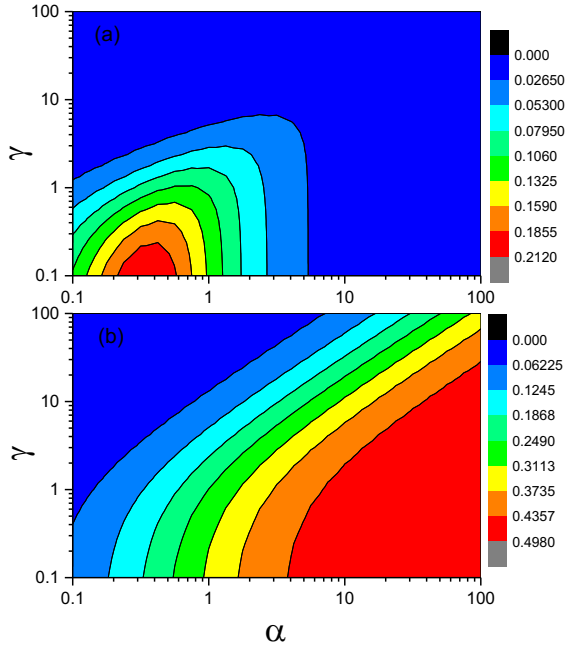

Figure 2. Comparison between (a) the small parameter $\varepsilon = \sigma_2/\sigma_1$ obtained with SVD analysis and (b) the Seagel's ratio $E_0/(K_M+S_0)$. The results were obtained for $S_0 = 1.0$, $k_c = 1.0$ and $\beta = 1.5$. The MM equation is valid for small values (cold regions) of the SVD and Seagel's parameters.

Figure 2 compares the small parameter $\varepsilon = \sigma_2/\sigma_1$ obtained with SVD analysis with the Segel's ratio $E_0/(K_M+S_0)$ (see Eq. (11)). The results were obtained for $S_0 = 1.0$, $k_c = 1.0$ and $\beta = 1.5$. The MM equation is valid for small values (cold regions) of the SVD and Segel's parameters. Figure 2 shows that, at least for such parameter values, the SVD condition predicts a wider range for the validity of the MM equation. For instance, the Segel's condition would predict that the MM equation is not valid for large values of the parameter α, whereas the SVD condition predicts the validity of the MM equation. Overall, the results in Figure 1 illustrate that the MM equation approximation can be used for a more general set of rate constants and initial conditions than those predicted by the standard Segel's condition (11).

One question is how the Michaelis constant (9) compares with the affinity constant predicted by the SVD analysis (see Eq. (34.b)). Figure 3 presents the comparison between Eq. (9) and Eq. (34.b) for different combinations of the kinetics and initial condition parameters. Except for the variation of the ratio E_0/S_0 where the Michaelis constant does not change with E_0/S_0, Eq. (9) and Eq. (34.b) yield quite similar values, suggesting that the MM equation may be applicable for a wider range of kinetics parameters than those predicted by the Segel's condition given by Eq. (11).

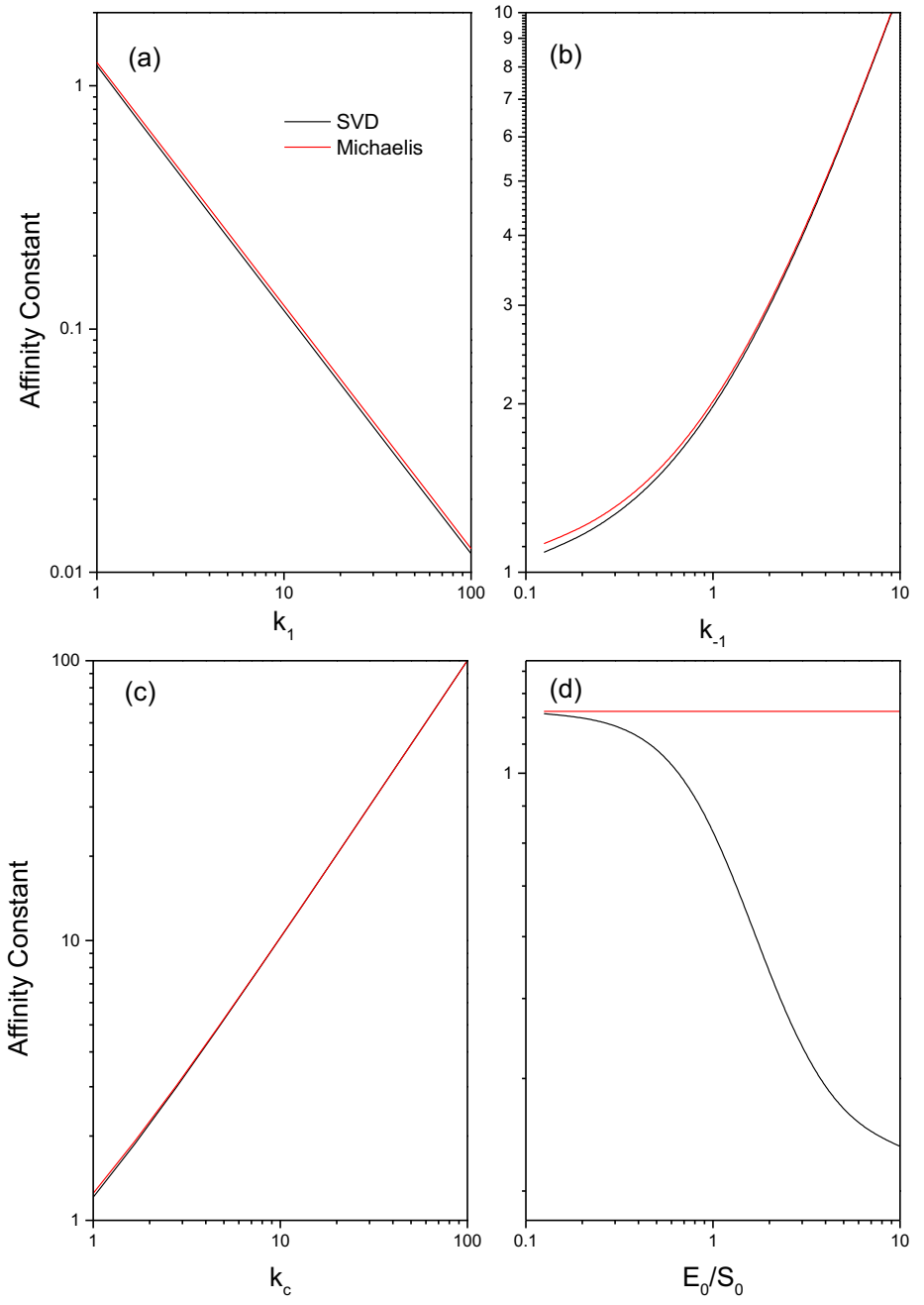

Figure 3. Behavior of the affinity constant with respect to the different kinetics parameters for the prediction made with the SVD analysis and the Michaelis constant. (a) $k_{-1} = 0.25$, $k_c = 1.0$, $S_0 = 1.0$ and $E_0 = 0.25$. (b) $k_1 = 1.0$, $k_c = 1.0$, $S_0 = 1.0$ and $E_0 = 0.25$. (c) $k_1 = 1.0$, $k_{-1} = 0.25$, $S_0 = 1.0$ and $E_0 = 0.25$. (d) $k_1 = 1.0$, $k_{-1} = 0.25$, $k_c = 1.0$ and $S_0 = 1.0$.

The SVD analysis showed that the substrate-complex phase-plane trajectory follows the slow manifold $\varphi_{fast}(x,y)$ (Eq. (28)) for small times, and the fast manifold $\varphi_{slow}(x,y)$ (Eq. (30)) for large times. To illustrate the above feature, consider the parameter values $k_1 = 1.0$, $k_{-1} = 0.25$, $k_c = 1.0$, $E_0 = 0.25$ and $S_0 = 1.0$. In this case, the SVD perturbation parameter is $\varepsilon = 0.0966$, the affinity constant is $K_{aff} = 1.2106$ (Eq. (34.b)) and the Michaelis constant is $K_M = 1.25$. Figure 4.a presents the behavior of the exact trajectory computed with the differential equations (4). For small times, the trajectory is tangential to the slow manifold, while for large time (approaching the equilibrium point) the trajectory approaches the slow manifold. The slow manifold was computed as $C = -E_0(v_{11}/v_{21})S/[(v_{31}/v_{21})S_0 + S]$ (Eq. (33)) and for comparison, the slow manifold obtained with the classical QSSA assumption was computed as

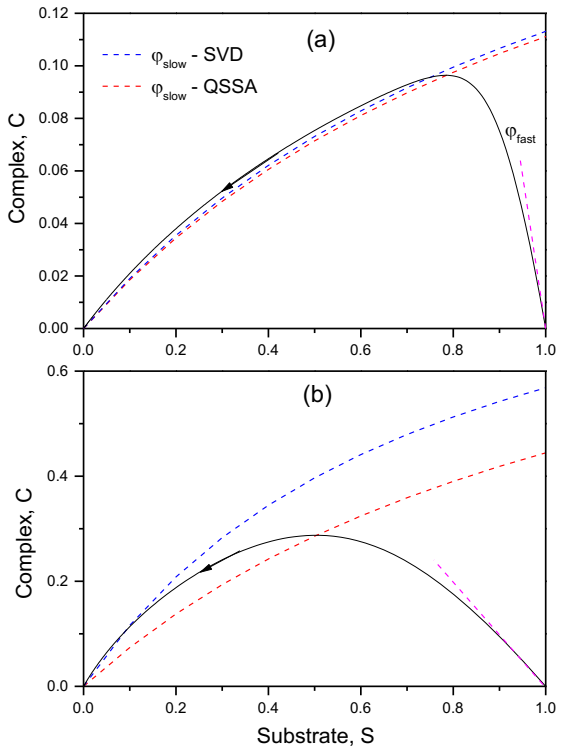

Figure 4. (a) Behavior of the substrate-complex trajectories respect to the SVD estimated slow invariant (dotted lines) manifold given by Eq. (26). (a) $k_1 = 1.0$, $k_{-1} = 0.25$, $k_c = 1.0$, $S_0 = 1.0$ and $E_0 = 0.25$. (b) $k_1 = 1.0$, $k_{-1} = 1.0$, $k_c = 1.0$, $S_0 = 1.0$ and $E_0 = 1.0$.

$C = -E_0 S/(K_M + S)$. The difference between the SVD analysis and the QSSA predictions is very small, particularly for large times. It can be noted that the system trajectory is tangent to the slow manifold for large times (i.e., when the trajectory approaches the equilibrium point), which illustrates the fact that the slow manifold function corresponds to a family of σ-isoclines (Calder and Siegel, 2008) depending on the initial substrate condition S_0 via the computations of the SVD of the matrix M (see Eq. (17)). For mild times, the trajectory evolves in a band close to the fast manifold, such that the MM equation can be considered as an acceptable approximation for the MM kinetics. Now, let us consider the same parameters, but with $E_0 = 1.0$. In this case, the initial substrate and enzyme concentrations are of the same order, a situation that has been considered as not following the MM equation approximation (Yun and Han, 2020). The corresponding SVD perturbation parameter is $\varepsilon = 0.2672$, which cannot be considered as sufficiently small to have a sufficient separation of time scales. The affinity constant is $K_{aff} = 0.829$ and the Michaelis constant is $k_M = 1.25$, which exhibits a marked difference, which is reflected in the graph of the corresponding slow manifold (Figure 4.b). The system trajectory is tangential to the fast manifold for small times and to the slow manifold predicted by the SVD analysis for large times. However, the trajectory is not tangential to the slow manifold predicted by the QSSA, which suggests that the classical MM equation is not an acceptable approximation for the MM kinetics for large values of the initial enzyme-to-substrate concentration ratio. Finally, the numerical results depicted in Figure 4 are in line with the SVD analysis, which concluded that the system trajectory approaches asymptotically (i.e., for very long time) the slow manifold. However, for mild times the slow manifold is not an exact approximation for the system trajectory, but rather only an approximation reflected in the MM equation.

3 Autocatalytic MM kinetics

The analysis described in the above section showed that, under suitable conditions, there exists a sufficient separation of the scaling factors, which can be exploited to reduce the dimensionality of the differential equations describing the MM kinetics. In turn, for a large time the generation of product can be approximated by a MM equation. An interesting question is whether the SVD analysis can be extended to more general forms of the MM kinetics scheme.

Consider the following autocatalytic MM kinetics:

$$\mathrm{E} + \mathrm{S} \xrightarrow{k_1} \mathrm{C} \tag{46a}$$

$$\mathrm{C} \xrightarrow{k_{-1}} \mathrm{E} + \mathrm{S} \tag{46b}$$

$$\mathrm{C} \xrightarrow{k_c} 2\,\mathrm{E} + \mathrm{P} \tag{46c}$$

The difference with the classical MM kinetics is in the third step where the enzyme is both regenerated and produced by the decomposition of the intermediate complex to give products. Kinases that activate themselves are examples of reactions that meet the kinetics scheme (45) (Bishop and Quian, 2010). The application of the law of mass action to the kinetics scheme (46) gives the following set of differential equations:

$$\frac{dS}{dt} = -k_1 ES + k_{-1} C, \quad S(0) = S_0 \tag{47a}$$

$$\frac{dE}{dt} = -k_1 ES + (k_{-1} + 2k_c) C, \quad E(0) = E_0 \tag{47b}$$

$$\frac{dC}{dt} = k_1 ES - (k_{-1} + k_c) C, \quad C(0) = 0 \tag{47c}$$

$$\frac{dP}{dt} = k_c C, \quad P(0) = 0 \tag{47d}$$

This system admits the following conservation law:

$$S + E + 2C = S_0 + E_0 \tag{48}$$

such that the dimensionless equations for the substrate

and the complex can be written as follows:

$$\frac{dx}{dt} = -k_1(S_0 + E_0)x + k_1 S_0 x^2 + 2k_1 E_0 xy + k_{-1}\left(\frac{E_0}{S_0}\right)y, \quad x(0) = 1 \tag{49a}$$

$$\frac{dy}{dt} = k_1\frac{(S_0 + E_0)S_0}{E_0}x - k_1\left(\frac{S_0^2}{E_0}\right)x^2 - 2k_1 S_0 xy - (k_{-1} + k_c)y, \quad y(0) = 0 \tag{49b}$$

In this case, the vector of rate functions $F(x,y)$ is

$$F(x,y) = \begin{pmatrix} x \\ x^2 \\ xy \\ y \end{pmatrix} \tag{50}$$

and the matrix M is given by

$$M = \begin{pmatrix} -k_1(S_0 + E_0) & k_1 S_0 & 2k_1 E_0 & k_{-1}\left(\frac{E_0}{S_0}\right) \\ k_1\frac{(S_0+E_0)S_0}{E_0} & -k_1\left(\frac{S_0^2}{E_0}\right) & -2k_1 S_0 & -(k_{-1}+k_c) \end{pmatrix} \tag{51}$$

Following the SVD decomposition of the matrix M, and assuming sufficient separation of the singular values, one obtains the following expression for the slow invariant manifold:

$$\varphi_{slow}(S,C) = v_{11}x + v_{21}x^2 + v_{31}xy + v_{41}y \tag{52}$$

Then, the dimensionless complex concentration in the slow invariant manifold can be expressed as

$$y = -\frac{x(v_{11} + v_{21}x)}{v_{41} + v_{31}x} \tag{53}$$

Since $dP/dt = k_c C$, and after using the normalization relationships given by Eq. (13), one obtains the following expression:

$$\frac{dP}{dt} = -\frac{k_c E_0 S}{\frac{v_{41}}{v_{31}}S_0 + S}\frac{(v_{11}S_0 + v_{21}S)}{v_{31}S_0} \tag{54}$$

This equation has the structure of the MM equation corrected by the factor $(v_{11}S_0 + v_{21}S)/(v_{31}S_0)$. In this way, the approximate rate of product generation can be described by a second-order MM equation where the factor $(v_{11}S_0 + v_{21}S)/(v_{31}S_0)$ arises because of the autocatalytic kinetics. In contrast to the MM equation, the approximate autocatalytic equation (54) approaches a first-order kinetics rate rather than a zero-order kinetics rate when the substrate concentration is very high. Figure 5 illustrates the behavior of the substrate-complex trajectories for two substrate initial conditions with $E_0 = 0.25$, $k_1 = 1$, $k_{-1} = 0.02$ and $k_{cat} = 5$, such that the decomposition of the intermediate complex to form products is the faster reaction step. For long times, the trajectories converged to the slow invariant manifold defined by Eq. (52).

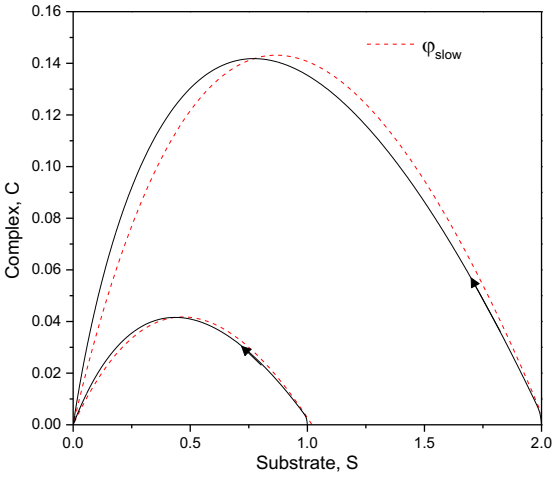

Figure 5. Phase-portrait of the substrate-complex trajectories for the autocatalytic MM kinetics for $k_1 = 1.0$, $k_{-1} = 0.25$, $k_c = 5.0$, $E_0 = 0.02$.

Conclusions

The SVD offered a suitable framework for the multiscale analysis of the MM kinetics. It was shown that the dynamics of the MM kinetics can be reduced to a MM equation for a sufficient separation of the singular values of the rate constant matrix. Using results from singularly perturbed systems, the QSSA was applied to a linear, non-convex combination of the substrate and intermediate complex rates, rather than to a single species. Hence, one obtains a generalization of the classical QSSA commonly used for the MM kinetics. The methodology exhibited certain flexibility for application to a general MM kinetics, such as the autocatalytic case where the resulting effective equation for the substrate generation is a second order equation. Overall, the analysis presented in this work should be seen as a first step towards the development of general QSSA for the reduction of complex kinetics. The SVD approach used in the present work is rather general, although non-necessarily affordable for analytical and algebraical handling. For instance, the analysis can be used for the similar case of the Monod equation (Meraz *et al.*, 2022).

Although our study provided valuable insights on the validity of the MM equation, it has the main limitation that the validity conditions described in Subsection 2.1 depend only on the kinetics parameters and the initial conditions. Such criteria apply for conditions close to the equilibrium points. If the task is to obtain a MM equation valid along a trajectory, our sufficient conditions are not valid and other analysis

strategies should be pursued (Patsatzis and Goussis, 2019, 2023).

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