ORIGINAL PAPER

Quasi-Steady-State Laws in reversible model of enzyme kinetics

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Abstract In enzyme kinetics, the Quasi-Steady-State Assumption (QSSA) has been proposed for over 80 years, which plays a very important role in simplifying systems of equations derived from chemical reactions with enzymes. Five years ago, we proved that the QSSA is always true in the simplest model with the second elementary reaction irreversible, and called them as Quasi-Steady-State Laws. Thus, all conclusions based on QSSA have a solid foundation in irreversible case. However, the chemical reactions are not always so simple in many life processes. The second elementary reaction should be reversible in general, and the irreversible case is actually only an approximation. So it is more important and interesting to study the reversible case, and it has already attracted enzymologists for a long time. The basic assumption, i.e. QSSA in this general case has appeared in 1930. We proved this lasting over 80 years assumption in this paper.

Keywords Michaelis–Menten equation · Rate constants of enzyme kinetics · Quasi-Steady-State Assumption

1 Introduction

In 1892, Adrian Brown studied the chemical reaction of hydrolysis of sucrose by yeast β–fructofuranosidase. He found that when the concentration of sucrose is much greater than that of enzymes, the reaction rate is irrelevant to the concentration of sucrose [1]. In 1902, he proposed that this phenomenon can be explained if the reaction

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consisted of many small elementary steps and immediate products had been produced [2]. Meanwhile, Victor Henri [3] proposed two reaction mechanisms which contains only one substrate and one product with an immediate product. One of them became the basic model of enzyme kinetics. The reaction is composed of two elementary steps. In the first step, the substrate S and enzyme E form a complex C with rate k_1 and reversible rate k_{-1}. In the second step which is assumed to be irreversible, the complex decomposes into product P and enzyme E with rate k_2.
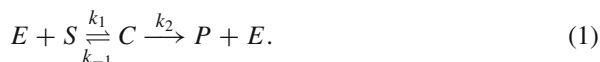
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \xrightarrow{k_2} P + E. \tag{1}$$

Based on the law of mass action, the whole reaction process is determined by the following nonlinear differential equations [4]:

$$\frac{dS}{dt}(t) = -k_1 S(t) E(t) + k_{-1} C(t) \tag{2}$$

$$\frac{dE}{dt}(t) = -k_1 S(t) E(t) + (k_{-1} + k_2) C(t) \tag{3}$$

$$\frac{dC}{dt}(t) = k_1 S(t) E(t) - (k_{-1} + k_2) C(t) \tag{4}$$

$$\frac{dP}{dt}(t) = k_2 C(t) \tag{5}$$

with the initial condition

$$(S(0), E(0), C(0), P(0)) = (S_0, E_0, 0, 0). \tag{6}$$

Because this nonlinear system can not be integrated explicitly, Michaelis and Menten [5] proposed equilibrium assumption in 1913 to simplify this system. However, Briggs and Haldane [6] pointed out that the Michaelis assumption is not always justified. It based on a usually unrealistic condition $k_{-1} \gg k_2$. They proposed the famous assumption Quasi-Steady-State Assumption(QSSA) under a more realistic condition $S_0 \gg E_0$.

By QSSA, they got the famous Michaelis–Menten equation.

$$v_0 = \frac{V_{\max} S}{K_M + S} \tag{7}$$

The Michaelis–Menten equation is considered to provide a good relationship among these rate constants. At the single-molecule level, the enzyme molecule moves according to thermal fluctuation and reacts stochastically with substrate molecules [7,8]. By the statistical analysis of the stochastic behave, Michaelis–Menten equation also holds [9,10]. Recently, Bajzer and Strehler [11] provided a new formula which is more accurate than Michaelis–Menten equation under QSSA.

Since the work of Briggs and Haldane, QSSA has become a fundamental assumption in enzyme kinetics. It has been proven very useful in finding approximate explicit

analytical solutions [12,13] and parameter estimations [14–17]. QSSA was used to reduce the complexity of biochemical systems, such as metabolic processes and genetic regulation processes [18].

Although QSSA is consistent with all the experiments known, we can not ensure that this assumption is also true in undone experiments or numerical computations. In fact, QSSA has been wrongly abused in great extend [19–21]. Laidler [22] first discussed the validity of QSSA, and he thought that the condition $S_0 \gg E_0$ was very important. Segel thought $E_0 \ll S_0 + K_M$ can guarantee the validity of QSSA [4,23]. Recently, Hanson and Schnell [24] found that during the initial transient period, the concentration of substrate is almost unchanged has little relationship with QSSA. Goussis analyzed the relationship of the quasi steady state and partial equilibrium approximations in more general condition [25]. In 2008, we proved mathematically that QSSA is always true when $S_0 \gg E_0$ in the above model [26], which is the first try of applying dynamical systems into the analysis of enzyme catalysis. Then, we call it Quasi-Steady-State Law.

In the above basic model, the second elementary step is irreversible. The chemical reactions are not always so simple in many life processes. The second elementary reaction should be reversible. Even some reaction catalyzed by enzymes which had been considered irreversible, was found to be reversible under certain conditions [27]. But the reversible case was paid less attention for the complexity of the mathematical analyzing. Here, we call it the Reversible Model.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} P + E, \tag{8}$$

where E, S, C, P represent enzyme, substrate, enzyme-substrate complex and product, respectively. And k_1, k_{-1}, k_2, k_{-2} represent the rate constants of corresponding reaction steps.

Based on the law of mass action, the whole reaction process is determined by the following nonlinear differential equations

$$\frac{dS}{dt}(t) = -k_1 S(t) E(t) + k_{-1} C(t) \tag{9}$$

$$\frac{dE}{dt}(t) = -k_1 S(t) E(t) + (k_{-1} + k_2) C(t) - k_{-2} P(t) E(t) \tag{10}$$

$$\frac{dC}{dt}(t) = k_1 S(t) E(t) - (k_{-1} + k_2) C(t) + k_{-2} P(t) E(t) \tag{11}$$

$$\frac{dP}{dt}(t) = k_2 C(t) - k_{-2} P(t) E(t) \tag{12}$$

with the initial condition

$$(S(0), E(0), C(0), P(0)) = (S_0, E_0, 0, 0). \tag{13}$$

where $E(t), S(t), C(t)$ and $P(t)$ denote the concentrations of enzyme, substrate, enzyme-substrate complex and product at time t during the process, respectively.

And the two conservation laws are

$$E(t) + C(t) = E_0, \tag{14}$$
$$S(t) + C(t) + P(t) = S_0. \tag{15}$$

This Reversible Model is more complicated than Henri's irreversible basic model (1). A straightforward method to simplify Reversible Model is to use the assumptions used in the basic model. Thus, a question raised that whether the assumptions used in the basic model are also valid in Reversible Model. Haldane [28] first proposed the QSSA in Reversible Model just like that in the basic model. In 1958, Miller and Alberty [29] derived the exact analytical solutions to this system only for the case $k_1 = k_{-2}$, and they found that the Quasi-Steady-State approximation was a good approximation if $S_0 \gg E_0$. After that, many numerical experiments showed that Quasi-Steady-State approximation was also true in the reversible case [30]. Moreover, many concepts and assumptions derived from Henri's basic model were testified in the reversible case directly, such as total Quasi-Steady-State Assumption (tQSSA) [31,32]. However, the question we asked in [26] is still unanswered for the Reversible Model:

"*Question*: Is QSSA always true for any group of reaction rate constants or if it is only true for the reaction rate constants satisfying some conditions?"

Here, in this paper, we do the same thing as in [26] for the Reversible Model. That is we use qualitative theory of dynamical systems to give a rigorous mathematical proof of QSSA in Reversible Model. But it is surely more complicated, and more interesting. As QSSA is also valid in this case, we call it Quasi-Steady-State Law in Reversible Model (QSSL in RM) from now on.

2 Quasi-Steady State Laws in reversible model

In this section, we repeat the Quasi-Steady-State Assumption as stated in the famous text book again [33]: Under the physiologically common condition that substrate is in great excess over enzyme ($S_0 \gg E_0$), the enzyme-substrate complex C remains approximately constant until the substrate is nearly exhausted with an exception of the transient initial stage of the reaction.

In [26], we stated and proved the two Quasi-Steady-State Laws in the model whose second elementary step is irreversible, i.e. $k_{-2} = 0$.

Quasi-Steady State Law 1: Given any small positive number $\varepsilon > 0$, there is a proper positive number U such that $C(t)$ will go upwards from 0 at $t = 0$ to $E_0 - \varepsilon$ in a period less than ε, then it will stay in the interval between E_0 and $E_0 - \varepsilon$ until $S(t)/S_0 < \varepsilon$, if $S_0 > U$.

Quasi-Steady State Law 2: Given any small positive number $\varepsilon > 0$, there is a proper positive number U such that $|\frac{dC}{dt}(t)|$ will be less than ε after a fast initial period less than ε and keep this state until $S(t)/S_0 < \varepsilon$, if $S_0 > U$.

We have explained in great detail why two versions of Quasi-Steady State Law are required in [26]. For convenience, we restate:

"$C \approx constant$ in a period and $\frac{dC}{dt} \approx 0$ in the same period are not equivalent in general. $C \approx constant$ can not ensure $\frac{dC}{dt} \approx 0$, because $\frac{dC}{dt}$ may oscillate frequently.

Conversely, $\frac{dC}{dt} \approx 0$ can not ensure $C \approx constant$ either, because C may change significantly as time goes by."

When $k_1 > k_{-2} > 0$, i. e. the second step of the model is reversible and the direction of the reaction is mainly from S to P, the above statement in [33] is also true. We also summarize two versions of Quasi-Steady State Laws.

Quasi-Steady State Law 1 in Reversible Model (QSSL1 in RM): Given any small positive number $\varepsilon > 0$, there is a proper positive number U such that $C(t)$ will go upwards from 0 at $t = 0$ to $E_0 - \varepsilon$ in a period less than ε, then it will stay in the interval between E_0 and $E_0 - \varepsilon$ forever.

Quasi-Steady State Law 2 in Reversible Model (QSSL2 in RM): Given any small positive number $\varepsilon > 0$, there is a proper positive number U such that $|\frac{dC}{dt}(t)|$ will be less than ε forever with the exception of a fast initial period less than ε, if $S_0 > U$.

Note that, in this model "C remains approximately constant" forever, not just "until the substrate is nearly exhausted".

3 Proof of QSSL1 in RM

The system (9)–(12) with initial condition (13), is equivalent to the system

$$\frac{dS}{dt} = -k_1 SE + k_{-1}(E_0 - E) \tag{16}$$

$$\frac{dE}{dt} = -k_1 SE + (k_{-1} + k_2)(E_0 - E) - k_{-2}E(S_0 - E_0 - S + E) \tag{17}$$

with initial condition $(S(0), E(0)) = (S_0, E_0)$.

To make the proof more readable, we use $P(S, E)$ and $Q(S, E)$ to denote the righthand polynomials of this system respectively, that is:

$$P(S, E) = -k_1 SE + k_{-1}(E_0 - E) \tag{18}$$

$$Q(S, E) = -k_1 SE + (k_{-1} + k_2)(E_0 - E) - k_{-2}E(S_0 - E_0 - S + E). \tag{19}$$

The equilibrium point (S_s, E_s) of this system satisfies

$$P(S_s, E_s) = 0 \tag{20}$$

$$Q(S_s, E_s) = 0 \tag{21}$$

$k_1 \times (21) + (k_{-2} - k_1) \times (20)$ lead to

$$-k_1 k_{-2} E_s^2 - (k_1 k_{-2}(S_0 - E_0) + k_1 k_2 + k_{-1} k_{-2})E_s + (k_{-1} k_{-2} + k_1 k_2)E_0 = 0. \tag{22}$$

Thus,

$$E_s = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}, \tag{23}$$

where $b = -(k_1k_{-2}(S_0 - E_0) + k_1k_2 + k_{-1}k_{-2})$, $a = -k_1k_{-2}$ and $c = (k_{-1}k_{-2} + k_1k_2)E_0$. It can be seen that

$$E_s = \frac{(-b \pm \sqrt{b^2 - 4ac})(-b \mp \sqrt{b^2 - 4ac})}{2a(-b \mp \sqrt{b^2 - 4ac})} = \frac{2c}{-b \mp \sqrt{b^2 - 4ac}}. \tag{24}$$

We only need to consider the positive case, i.e.

$$E_s = \frac{2c}{-b + \sqrt{b^2 - 4ac}}. \tag{25}$$

According to (20),

$$S_s = \frac{k_{-1}(E_0 - E_s)}{k_1 E_s}. \tag{26}$$

Here, it is obvious that

Lemma 1

$$\lim_{S_0 \to +\infty} E_s = 0. \tag{27}$$

Consider the curve

$$Q(S, E) = 0. \tag{28}$$

This is a quadratic curve with respect to variables S and E. It can be written as

$$-k_{-2}E^2 + (k_{-2} - k_1)SE + (k_{-2}E_0 - k_{-2}S_0 - (k_{-1} + k_2))E \\ + (k_{-1} + k_2)E_0 = 0, \tag{29}$$

which is a hyperbola, and its center $(\hat{S}, \hat{E})$ satisfies

$$\frac{k_{-2} - k_1}{2}\hat{E} = 0 \tag{30}$$

$$\frac{k_{-2} - k_1}{2}\hat{S} + (-k_{-2})\hat{E} + \frac{-k_{-2}(S_0 - E_0) - (k_{-1} + k_2)}{2} = 0 \tag{31}$$

Thus,

$$(\hat{S}, \hat{E}) = \left(\frac{k_{-2}(S_0 - E_0) + (k_{-1} + k_2)}{k_{-2} - k_1}, 0\right).$$

Moreover, the directions (l, m) of asymptotes satisfy:
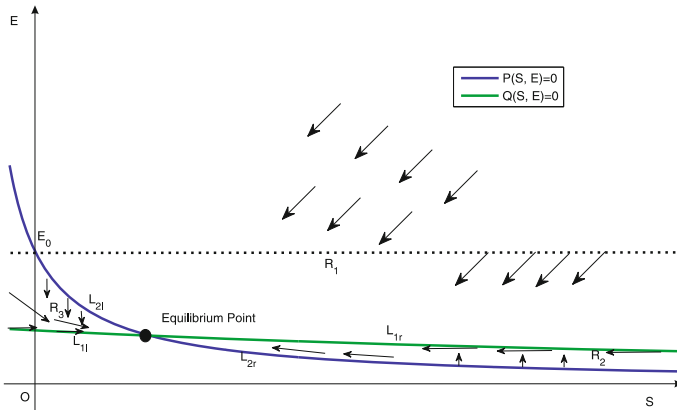
$$(k_{-2} - k_1)lm - k_{-2}m^2 = 0. \tag{32}$$

Fig. 1 The first quadrant of the phase plane: $Q(S, E) = 0$ and $P(S, E) = 0$ are branches of two hyperbolas, respectively, which intersect each other at the equilibrium point. The *arrows* show the phase flow of this dynamical system

They are $l : m = 1 : 0$ and $l : m = \frac{k_{-2}}{k_{-2} - k_1}$.

The curve $P(S, E) = 0$ can be analyzed by the same method. It is a hyperbola with its center $(-k_{-1}/k_1, 0)$. The directions of its asymptotes are $l : m = 1 : 0$ and $l : m = 0 : 1$.

Now the shape of the phase flow of the system (16)–(17) can be figured.

Let L_{1r}, L_{1l}, L_{2r}, L_{2l}, R_1, R_2 and R_3 be the point sets in the first quadrant of the $S - E$ plane described as following (cf. Fig. 1)

$$L_{1r} = \{(S, E) : Q(S, E) = 0, S > S_s\},$$
$$L_{1l} = \{(S, E) : Q(S, E) = 0, 0 < S < S_s\},$$
$$L_{2r} = \{(S, E) : P(S, E) = 0, S > S_s\},$$
$$L_{2l} = \{(S, E) : P(S, E) = 0, 0 < S < S_s\},$$
$$R_1 = \{(S, E) : E > \tilde{E}, (S, \tilde{E}) \in L_1, \text{ and } E > \overline{E}, (S, \overline{E}) \in L_2, S \geq 0\},$$
$$R_2 = \{(S, E) : \tilde{E} > E > \overline{E}, (S, \tilde{E}) \in L_1, (S, \overline{E}) \in L_2, S > S_s\},$$
$$R_3 = \{(S, E) : \tilde{E} > E > \overline{E}, (S, \tilde{E}) \in L_2, (S, \overline{E}) \in L_1, 0 \geq S < S_s\}.$$

Notice that L_{1r}, L_{1l}, L_{2r} and L_{2l} lie on the hyperbolas $Q(S, E) = 0$ or $P(S, E) = 0$ in the first quadrant. It must be that(cf. Fig. 1):

(1) In the region R_1,

$$\frac{dS}{dt}(t) = P(S(t), E(t)) < 0 \tag{33}$$

$$\frac{dE}{dt}(t) = Q(S(t), E(t)) < 0 \tag{34}$$

(2) In the region R_2,

$$\frac{dS}{dt}(t) = P(S(t),\ E(t)) < 0 \tag{35}$$

$$\frac{dE}{dt}(t) = Q(S(t),\ E(t)) > 0 \tag{36}$$

(3) In the region R_3,

$$\frac{dS}{dt}(t) = P(S(t),\ E(t)) > 0 \tag{37}$$

$$\frac{dE}{dt}(t) = Q(S(t),\ E(t)) < 0 \tag{38}$$

(4) On the curve L_{1r},

$$\frac{dS}{dt}(t) = P(S(t),\ E(t)) < 0 \tag{39}$$

$$\frac{dE}{dt}(t) = Q(S(t),\ E(t)) = 0 \tag{40}$$

(5) On the curve L_{1l},

$$\frac{dS}{dt}(t) = P(S(t),\ E(t)) > 0 \tag{41}$$

$$\frac{dE}{dt}(t) = Q(S(t),\ E(t)) = 0 \tag{42}$$

(6) On the curve L_{2r},

$$\frac{dS}{dt}(t) = P(S(t),\ E(t)) = 0 \tag{43}$$

$$\frac{dE}{dt}(t) = Q(S(t),\ E(t)) > 0 \tag{44}$$

(7) On the curve L_{2l},

$$\frac{dS}{dt}(t) = P(S(t),\ E(t)) = 0 \tag{45}$$

$$\frac{dE}{dt}(t) = Q(S(t),\ E(t)) < 0 \tag{46}$$

Observing the phase plane, we can easily obtain the following lemma.

Lemma 2 *Solutions of the system (9)–(12) with initial condition (6) will finally approach the equilibrium point $(S_s,\ E_s)$ as time goes to infinity. There are three possible kinds of trajectories to approach $(S_s,\ E_s)$ (cf. Fig. 2):*
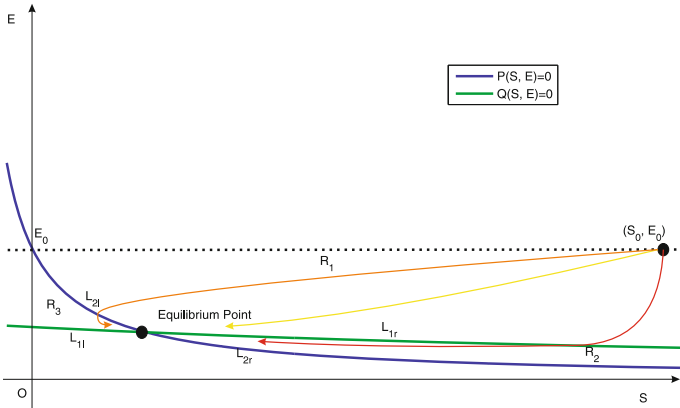
Fig. 2 Three possible kind of trajectories to approach the equilibrium point

1. The solution $(S(t), E(t))$ stays in R_1 forever and finally approaches (S_s, E_s);
2. The solution $(S(t), E(t))$ runs across curve L_{1r} horizontally from right to left, then it stays in region R_2 and approaches (S_s, E_s);
3. The solution $(S(t), E(t))$ runs across curve L_{2l} vertically from top to bottom, then it stays in region R_3 and approaches (S_s, E_s).

Consider the curve $Q(S, E) = R$, where $R \le 0$. It can be written as

$$
\begin{aligned}
-k_{-2}E^2 &+ (k_{-2} - k_1)SE + (k_{-2}E_0 - k_{-2}S_0 - (k_{-1} + k_2))E \\
&+ (k_{-1} + k_2)E_0 - R = 0,
\end{aligned}
\tag{47}
$$

which is also a hyperbola with $(\hat{S}, \hat{E})$ as its center. What we concern is the intersection point $(0, E_R)$ between this curve and the E−axis. Let $S = 0$ in (47), and denote $(k_{-2}E_0 - k_{-2}S_0 - (k_{-1} + k_2))$ as b_1, $((k_{-1} + k_2)E_0 - R)$ as c_1.

$$
E_R = \frac{-b_1 \pm \sqrt{b_1^2 + 4k_{-2}c_1}}{-2k_{-2}}.
\tag{48}
$$

We only need to consider the positive one, which is

$$
E_R = \frac{-b_1 - \sqrt{b_1^2 + 4k_{-2}c_1}}{-2k_{-2}} = \frac{b_1 + \sqrt{b_1^2 + 4k_{-2}c_1}}{2k_{-2}} = \frac{2c_1}{\sqrt{b_1^2 + 4k_{-2}c_1} - b_1}.
\tag{49}
$$

Note that

Lemma 3 Curve $Q(S, E) = R$ $(R < 0)$ is above curve $Q(S, E) = 0$ in the first quadrant (cf. Fig. 3). For fixed $R \le 0$,
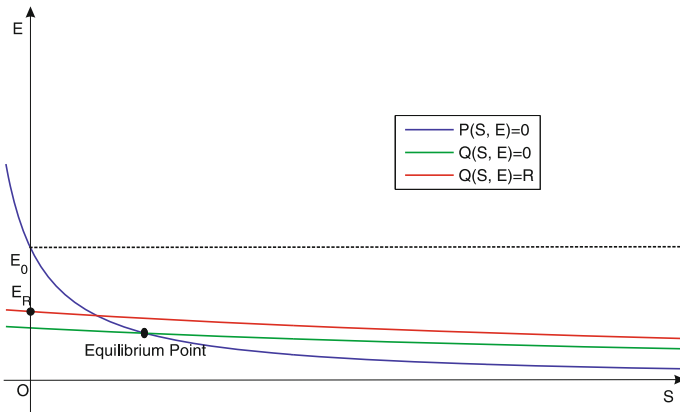
$$
\lim_{S_0 \to +\infty} E_R = 0.
\tag{50}
$$

Fig. 3 Curve $Q(S, E) = R$ $(R < 0)$ (*red*) is above *curve* $Q(S, E) = 0$ (*green*) in the first quadrant (Color figure online)

QSSL1 in RM: Given any small positive number $\varepsilon > 0$, there is a proper positive number U such that $C(t)$ will go upwards from 0 at $t = 0$ to $E_0 - \varepsilon$ in a period less than ε, then it will stay in the interval between E_0 and $E_0 - \varepsilon$ forever.

Proof The reaction starts with $S(0) = S_0$ and $E(0) = E_0$. At this moment, $dE/dt(0) = -k_1 S_0 E_0$. Given small positive number $\varepsilon > 0$, choosing $S_0 > 1/(k_1\varepsilon)$, we can insure the initial point (S_0, E_0) and the equilibrium point (S_S, E_S) is separated by curve $Q(S, E) = -E_0/\varepsilon$. Note that $Q(S, E) = -E_0/\varepsilon$ lies above the curve $Q(S, E) = 0$ in the first quadrant according to Lemma 3. By this restriction, the solution $(S(t), E(t))$ must cross the hyperbola $Q(S, E) = -E_0/\varepsilon$ before approaching the equilibrium point. Denote by t_ε the time to make the solution $(S(t), E(t))$ intersecting with the curve $Q(S, E) = -E_0/\varepsilon$, that is $Q(S(t_\varepsilon), E(t_\varepsilon)) = -E_0/\varepsilon$, then $Q(S(t), E(t)) < -E_0/\varepsilon$ for $0 \leq t < t_\varepsilon$. Hence,

$$t_\varepsilon \leq \frac{E_0}{\frac{E_0}{\varepsilon}} = \varepsilon. \tag{51}$$

By Lemma 3, given any positive number ε and $-E_0/\varepsilon < 0$, there exists a positive U_0, such that, if $S_0 > U_0$, $E_R < \varepsilon$. That leads to the first quadrant part of curve $Q(S, E) = -E_0/\varepsilon$ lies under the horizontal curve $E = \varepsilon$. Thus, in a period less than ε, the solution $(S(t), E(t))$ arrives at the curve $Q(S, E) = -E_0/\varepsilon$ which lies under the horizontal curve $E = \varepsilon$, i. e. $E(t) \leq \varepsilon$. According to Lemma 2, there are only three possible ways to approach the equilibrium point. In Trajectory 1 and Trajectory 3, $E(t)$ is decreasing forever. In Trajectory 2, $E(t)$ will decrease until the solution runs across L_{1r}, and then it will increase to E_S as time goes to infinity. In this case, $E_S < \varepsilon$. Thus, no matter which cases, $E(t) < \varepsilon$, when $t > \varepsilon$.

Here, a choice of

$$U = \max\{U_0, 1/(k_1\varepsilon)\}, \tag{52}$$

completes the proof. □

4 Proof of QSSL2 in RM

In order to prove QSSL 2 in RM, we need to consider the second order differential equation concerning E derived from the system (16)–(17). Let

$$V = \frac{dE}{dt}.$$

Then,

$$
\begin{aligned}
\frac{dV}{dt} &= \frac{d^2 E}{dt^2} \\
&= -2k_{-2}EV + (k_{-2} - k_1)VS + (k_{-2} - k_1)E\frac{dS}{dt} \\
&\quad + (k_{-2}E_0 - k_{-2}S_0 - (k_{-1} + k_2))V,
\end{aligned}
$$

and $V(0) = -k_1 S(0)E(0) + (k_{-1} + k_2)(E_0 - E(0)) - k_{-2}E(0)(S_0 - E_0 - S(0) + E(0)) = -k_1 S_0 E_0$. For

$$
\begin{aligned}
V = \frac{dE}{dt} &= -k_{-2}E^2 + (k_{-2} - k_1)ES + (k_{-2}E_0 - k_{-2}S_0 - (k_{-1} + k_2))E \\
&\quad + (k_{-1} + k_2)E_0, \\
S = (V + k_{-2}E^2 &- (k_{-2}E_0 - k_{-2}S_0 - (k_{-1} + k_2))E \\
&- (k_{-1} + k_2)E_0)/E(k_{-2} - k_1).
\end{aligned}
$$

Thus,

$$
\begin{aligned}
\frac{dV}{dt} &= -2k_{-2}EV \\
&\quad + V(V + k_{-2}E^2 - (k_{-2}E_0 - k_{-2}S_0 - (k_{-1} + k_2))E - (k_{-1} + k_2)E_0)/E \\
&\quad + E(-k_1(V + k_{-2}E^2 - (k_{-2}E_0 - k_{-2}S_0 - k_2)E - k_2 E_0) \\
&\quad + k_{-2}k_{-1}(E_0 - E)) + (k_{-2}E_0 - k_{-2}S_0 - (k_{-1} + k_2))V.
\end{aligned}
$$

The right hand is denoted as $A(E, V)$ which can be treated as a quadratic function of V:

$$
\begin{aligned}
\frac{dV}{dt} = A(E, V) &= \frac{V^2}{E} - \frac{((k_{-2} + k_1)E^2 + (k_{-1} + k_2)E_0)V}{E} \\
&\quad + E(-k_1 k_{-2}E^2 - (k_1 k_{-2}(S_0 - E_0) + k_1 k_2 + k_{-1}k_{-2})E \\
&\quad + (k_{-1}k_{-2} + k_1 k_2)E_0)
\end{aligned}
$$

We get the system

$$\frac{dE}{dt} = V \tag{53}$$

$$\frac{dV}{dt} = A(E, V) \tag{54}$$

with initial condition $(E(0), V(0)) = (E_0, -k_1 S_0 E_0)$.

First, we consider the vector fields on the plane $E - V$. Since $0 < E(t) \leq E_0$ for any t, it is enough to consider the vector fields of this system in the region $0 < E(t) \leq E_0$.

Let the right hands of system (53)–(54) be zero. Then

$$V = 0$$
$$E = \frac{2c}{-b + \sqrt{b^2 - 4ac}} \tag{55}$$

where $b = -(k_1 k_{-2}(S_0 - E_0) + k_1 k_2 + k_{-1} k_{-2})$, $a = -k_1 k_{-2}$ and $c = (k_{-1} k_{-2} + k_1 k_2) E_0$ (cf. Eq. (23)). The negative solution is omitted. The right hand of (55) is just E_s. This is not a coincide, for the system considered in this section is derived from system (16)–(17). To sum up, $(E_s, 0)$ is the singular point of system (53)–(54).

Assume $S_0 > E_0$. For each fixed E, treat $A(E, V) = 0$ as a quadratic equation of V. The constant part of this equation is:

$$E(-k_1 k_{-2} E^2 - (k_1 k_{-2}(S_0 - E_0) + k_1 k_2 + k_{-1} k_{-2})E + (k_{-1} k_{-2} + k_1 k_2)E_0).$$

Denote $a_2 = 1/E$, $b_2 = -((k_{-2} + k_1)E^2 + (k_{-1} + k_2)E_0)/E$ and c_2 as the constant part. Consider the discriminant
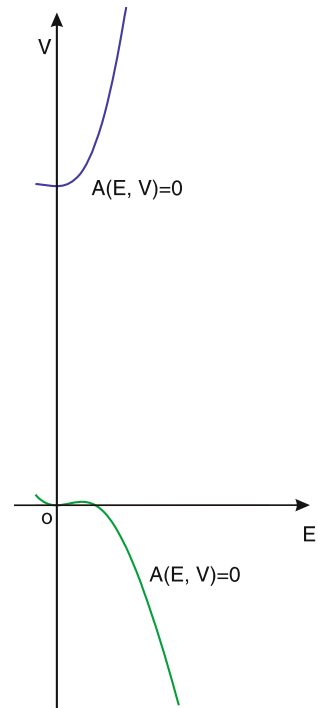
$$\Delta = b_2^2 - 4a_2 c_2.$$

If $E > E_S$, $c_2 < 0$, then $\Delta > 0$ and there is a negative and a positive solution of V. If $0 < E < E_s$,

$$c_2 = E(-k_1 k_{-2} E^2 - (k_1 k_{-2}(S_0 - E_0) + k_1 k_2 + k_{-1} k_{-2})E + (k_{-1} k_{-2} + k_1 k_2)E_0)$$
$$\leq E((k_{-1} k_{-2} + k_1 k_2)E_0) \tag{56}$$

We can get

$$\begin{aligned}
\Delta &= b_2^2 - 4a_2 c_2 \\
&> (((k_{-2} + k_1)E^2 + (k_{-1} + k_2)E_0)^2 - 4(k_{-1}k_{-2} + k_1 k_2)E_0 E^2)/E^2 \\
&> (((k_{-2} + k_1)E^2 + (k_{-1} + k_2)E_0)^2 - 4(k_{-2} + k_1)(k_{-1} + k_2)E_0 E^2)/E^2 \\
&= ((k_{-2} + k_1)E^2 - (k_{-1} + k_2)E_0)^2/E^2 \\
&> 0.
\end{aligned}$$

Fig. 4 The shape of
$A(E, V) = 0$

That is to say, there are two positive solutions of V. For $E = E_0$ and $V = V_0(= -k_1 S_0 E_0)$,

$$
\begin{aligned}
A(E_0, V_0) &= k_1^2 S_0^2 E_0 + k_1 S_0((k_{-2} + k_1)E_0^2 + (k_{-1} + k_2)E_0) \\
&\quad + E_0(-k_1 k_{-2} E_0^2 - (k_1 k_{-2}(S_0 - E_0) + k_1 k_2 + k_{-1} k_{-2})E_0 \\
&\quad + (k_{-1} k_{-2} + k_1 k_2)E_0) \\
&= k_1^2 S_0^2 E_0 + k_1^2 E_0^2 S_0 + k_1(k_{-1} + k_2)E_0 S_0 \\
&> 0
\end{aligned}
$$

Thus, (E_0, V_0) is below the curve $A(E, V) = 0$.

Now, we can sketch the shape of $A(E, V)$ as Fig. 4 and the phase flow of this system as Fig. 5.

From the phase flow, we obtain

Lemma 4 *Solutions of the system* (53)–(54) *with initial condition* $(E(0), V(0)) = (E_0, -k_1 S_0 E_0)$ *will finally approach the equilibrium point* $(E_s, 0)$ *as time goes to infinity. There are two possible kinds of trajectories to approach* $(E_s, 0)$ *(cf. Fig. 6):*

1. *The solution* $(E(t), V(t))$ *will approach* $(E_s, 0)$ *in the way that* $E(t)$ *decreases monotonically to* E_s *and* $V(t)$ *increase monotonically to 0;*
2. *At first,* $E(t)$ *decreases monotonically and* $V(t)$ *increase monotonically to 0. After that, the solution* $(E(t), V(t))$ *runs across the segment* $(0, E_s)$ *on* $E-$*axile*
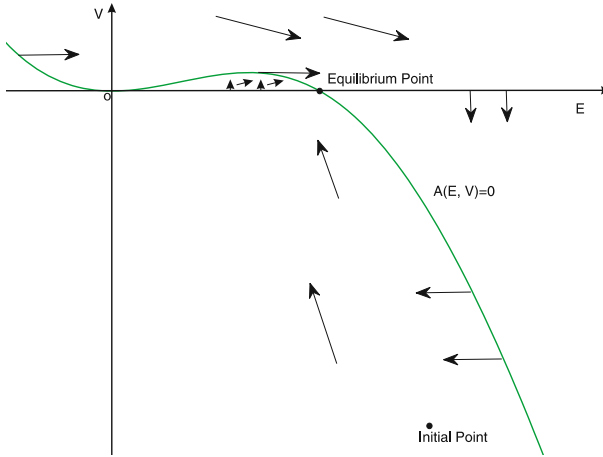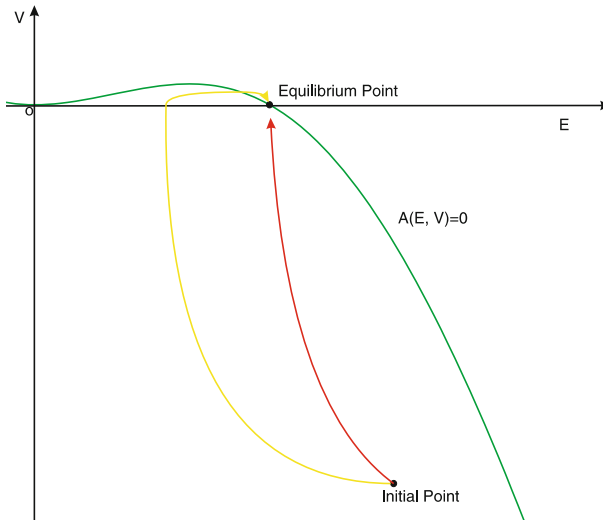
Fig. 5 Phase flow of system (53)–(54)

Fig. 6 Two possible kind of trajectories to approach the equilibrium point

vertically from bottom to top. Then, $E(t)$ and $V(t)$ increase. $(E(t), V(t))$ will run across $A(E, V) = 0$ horizontally from left to right. Finally, $E(t)$ increases to E_s and $S(t)$ decrease to 0.

QSSL2 in RM: Given any small positive number $\varepsilon > 0$, there is a proper positive number U such that $|\frac{dC}{dt}(t)|$ will be less than ε forever with the exception of a fast initial period less than ε, if $S_0 > U$.

Proof Under the condition $0 < E < E_s$, for each E, $A(E, V) = 0$ has two positive solutions of V. Denote them as V_1 and V_2 where $V_1 \leq V_2$. For (56)

$$V_1 V_2 = \frac{c_2}{a_2} \le E^2((k_{-1}k_{-2} + k_1 k_2)E_0) \le E_s^2((k_{-1}k_{-2} + k_1 k_2)E_0).$$

Given the positive number $1 > \varepsilon > 0$, according to Lemma 1, there exists a positive U_1, such that $E_s < \varepsilon/\sqrt{(k_{-1}k_{-2} + k_1 k_2)E_0}$ when $S_0 > U_1$. Under this condition,

$$V_1 V_2 < \varepsilon^2. \tag{57}$$

Then, $V_1 < \varepsilon$, i.e. in Fig. 6 when $S_0 > U_1$, the height of "green hill" on the interval $0 < E < E_s$ is less than ε.

By QSSL1 in RM, there exists a positive U_2, such that when $S_0 > U_2$, $E(t)$ will go downwards from E_0 at $t = 0$ to $\varepsilon^2/2$ in a period t_1 less than $\varepsilon^2/2$, then it will stay in the interval between 0 and $\varepsilon^2/2$ forever.

Lemma 4 states two possible kinds of trajectories. No matter which case, V increases to 0 after the reaction begins. When $S_0 > U_2$, after a period t_1 less than $\varepsilon^2/2$, $E(t)$ go downwards from E_0 at $t = 0$ to $\varepsilon^2/2$. Thus, from t_1, $V(t) = dE/dt(t) < -\varepsilon$ can not last for

$$\frac{\varepsilon^2/2}{\varepsilon} = \frac{\varepsilon}{2}. \tag{58}$$

That is to say, for $t > t_1 + \varepsilon/2$, $V(t) > -\varepsilon$.

If the solution of system (53)–(54) adapts the first kind of trajectories, $V(t)$ will be greater than $-\varepsilon$ and less than 0 for $t > t_1 + \varepsilon/2$, i.e. $|V(t)| < \varepsilon$ for $t > t_1 + \varepsilon/2$. Notice that $t_1 + \varepsilon/2 < \varepsilon$.

For the second kind of trajectories, $V(t)$ can not be greater than "the height of the green hill" (c.f. Fig. 6), which is less than ε if $S_0 > U_1$. Thus, choosing $U = \max\{U_1, U_2\}$ completes the proof. □

5 Numerical examples

To make our laws and proofs easier to understand, we give some numerical examples in this section.

In the following numerical example, we fix the rate constants of (8) as $k_1 = 2, k_2 = 2, k_{-1}$ and $k_{-2} = 1$. We choose $E_0 = 1$ as the initial concentration of enzymes.

5.1 Examples for QSSL1 in RM

According to the proof of QSSL1 in RM, U is chosen by (52). If we choose $\varepsilon = 0.5, 1/(k_1\varepsilon) = 1$. R will be $-E_0/\varepsilon = -2$.

$$E_R = \frac{2c_1}{\sqrt{b_1^2 + 4k_{-2}c_1} - b_1} = \frac{10}{\sqrt{(2 + S_0)^2 + 20} + 2 + S_0}. \tag{59}$$
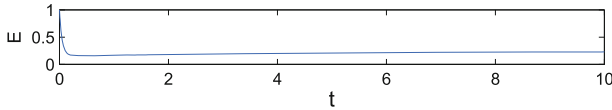
Fig. 7 Concentration of enzyme of the first 10 units of time. Parameters: $k_1 = 2, k_2 = 2, k_{-1}, k_{-2} = 1, E_0 = 1, \varepsilon = 0.5$ and $S_0 = 9$
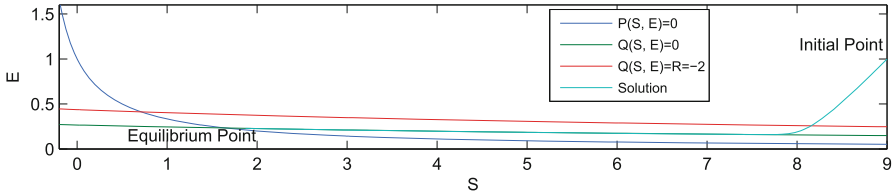

Fig. 8 The relationship of concentrations of enzyme and substrate during the whole reaction. Parameters: $k_1 = 2, k_2 = 2, k_{-1}, k_{-2} = 1, E_0 = 1, \varepsilon = 0.5$ and $S_0 = 9$
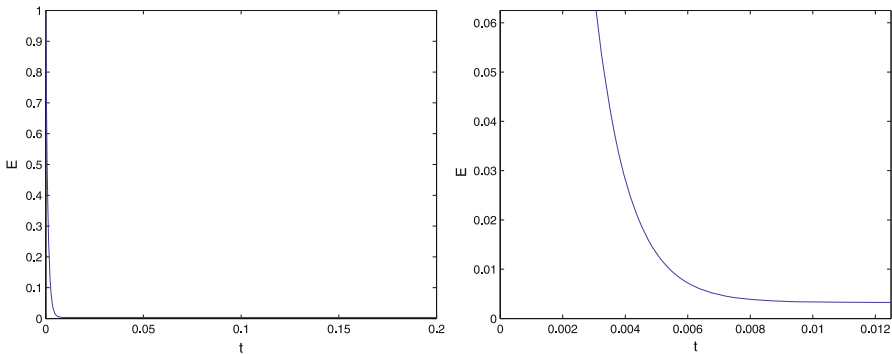

Fig. 9 *Left panel* Concentration of enzyme of the first 0.2 units of time. *Right panel* The enlargement of the *left bottom* part of *left panel*. Parameters: $k_1 = 2, k_2 = 2, k_{-1}, k_{-2} = 1, E_0 = 1, \varepsilon = 0.05$ and $S_0 = 460$

It is easy to prove that if $S_0 > 8$, $E_R < 0.5 = \varepsilon$. So we choose $S_0 = 9$ as the initial condition. $E(t)$ goes from 1 to 0.46 in time 0.05 (cf. Fig. 7). And then, it stays in the interval $[0, 0.5]$ forever (cf. Fig. 8).

If we choose a much smaller $\varepsilon = 0.05$, $1/(k_1\varepsilon) = 10$. R will be $-E_0/\varepsilon = -20$.

$$E_R = \frac{2c_1}{\sqrt{b_1^2 + 4k_{-2}c_1} - b_1} = \frac{46}{\sqrt{(2 + S_0)^2 + 92} + 2 + S_0}. \tag{60}$$

It can be proved that if $S_0 > 458$, $E_R < 0.05 = \varepsilon$. So we choose $S_0 = 460$ as the initial condition. $E(t)$ goes from 1 to 0.045 in time 0.004 (cf. Fig. 9). And then, it stays in the interval $[0, 0.05]$ forever (cf. Fig. 10).

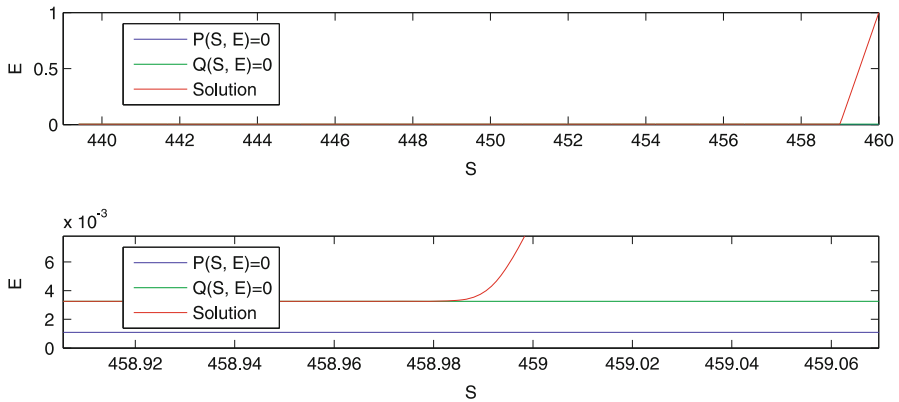Thus, these numerical examples are completely consistently with QSSL1 in RM.

Fig. 10 *Top panel* The relationship of concentrations of enzyme and substrate during the initial 10 units of time. *Bottom panel* The enlargement of the top panel while the solution runs across $Q(S, E) = 0$. Parameters: $k_1 = 2, k_2 = 2, k_{-1}, k_{-2} = 1, E_0 = 1, \varepsilon = 0.05$ and $S_0 = 460$

5.2 Examples for QSSL2 in RM

As the above subsection, we choose $\varepsilon = 0.5$ and $\varepsilon = 0.05$ respectively.

In the proof of QSSL2 in RM, the bound is chosen as the larger one between U_1 and U_2. U_1 is the number that if $S_0 > U_1$, then $E_s < \varepsilon/\sqrt{(k_{-1}k_{-2} + k_1k_2)E_0}$. And U_2 is chosen as the bound in QSSL1 in RM for $\varepsilon^2/2$.

For the case $\varepsilon = 0.5$, U_1 can be fixed as 10 and U_2 can be 38. Thus, we choose $S_0 = 39$ as an example. $|dC/dt|$ runs downwards from 78 to 0.43 in a period less than 0.07 and $|dC/dt| < 0.5$ forever.

For the case $\varepsilon = 0.05$, U_1 can be 111 and U_2 can be 18,398. Thus, we choose $S_0 = 18,400$ as an example. $|dc/dt|$ runs downwards from 36,800 to 0.04 in a period less than 0.0004 and $|dC/dt| < 0.05$ forever.

These examples are totally consistently with QSSL2 in RM.

6 Conclusion

The fundamental assumption QSSA has been proved in [26] and named Quasi-Steady-State Laws for the simplest one substrate one product model. In this paper, we extend the application of QSSA in a more general model, the reversible one substrate one product model. In real life processes, chemical reactions are usually reversible. As in [26], we reexpressed QSSA in two versions:

1. Under the condition that concentration of substrates is in great excess over concentration of enzymes, after the initial transient period, the enzyme-substrate complex remains approximately constant;
2. Under the condition that concentration of substrates is in great excess over concentration of enzymes, after the initial transient period, the rate of enzyme-substrate complex is approximate zero.

We proved that these two versions are both right by the qualitative theory of dynamical systems. Then, we name them as Quasi-Steady-State Law 1 and Quasi-Steady-State Law 2, respectively.

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