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A study for multiple steady states of biochemical reactions under substrate and product inhibition

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Abstract

This paper combines Sturm's method with the tangent analysis method to solve a biochemical reaction involving multiplicity. This method can easily derive the necessary conditions for multiplicity. In addition, we find a starting bifurcation point for multiplicity which cannot be obtained by the tangent method alone. Moreover, a start-up strategy is suggested to obtain a high conversion and unique steady state in four selected kinetic models of biochemical reactions, with inhibition.

Keywords: Biochemical reaction; Multiplicity; Start-up; Inhibition

1. Introduction

Many biochemical reactions involving free or immobilized cells and enzymes as catalysts follow complex kinetic schemes and do not obey simple Michaelis–Menten kinetics. Substrate-inhibited and product-inhibited reactions are common examples. The various kinetic models that can be used for many biochemical systems have been summarized previously [1]. Many of these kinetic models indicated the possibility of the existence of multiplicity [2]. Past works have been confined mainly to the commonly used substrate-inhibited kinetic models [3,4]. Bruns et al. [5] and Sadana et al. [6] have analyzed more complex kinetic schemes. Ramachandran et al. [7] have analyzed the conditions for the occurrence of multiplicity. Their derivation methods are the same as the tangent method [8–11]. Although the elementary catastrophe theory [12] and the singularity theory [13] are extensively used to solve the steady-state multiplicity in reaction engineering, the tangent analysis method is still a powerful technique. This method has been referred to not only in biochemical reaction systems but also in chemical reaction engineering. Lin [8,10] discussed the exact multiplicity criteria for autocatalytic reactions in perfectly mixed Continuously Stirred Tank Reactor (CSTR); however, the criteria can only be applied to the situation when CSTR is perfectly mixed. In recent years, the effects of macromixing and micromixing of two unpremixed feeds on the necessary and sufficient conditions for multiplicity in a CSTR have been studied [14,15].

The existence of steady-state multiplicity may have several practical implications. For example, multiplicity may lead to washout and its existence places constraints on the start-up and control policies needed to maintain the system at a desired state. The start-up conditions will influence the transient yield or selectivity of the desired product. When multiplicity exists, different initial conditions may approach different steady states. Thus, it is important to predict the conditions for multiplicity to occur. Liou and Chien [16] have recently used the discriminator roots of the characteristic equation to solve the start-up problem of an autocatalytical reaction. However, their method cannot be used when the order of conversion in the characteristic equation is above 2. The same problem can also be found in the tangent analysis method developed [9,11].

Takoudis et al. [17] used Sturm's method [18] to study multiple steady states in reaction-controlled surface-catalyzed reactions. So far, combining the tangent method with Sturm's method to study steady-state multiplicity has never been reported. On the other hand, the method of the discriminator root of the characteristic equation [19,20] has not been used in combination with the tangent method to solve steady-state multiplicity in chemical engineering.

In this paper, we combine Sturm's method with the tangent method to solve multiple steady states of complex biochemical reaction with substrate and product inhibition in a CSTR. The simulated results show that our work can find not only the necessary conditions for multiplicity but also

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can a starting bifurcation point for multiple steady-states of four schemes of substrate-inhibited kinetic reactions. A start-up strategy to obtain a high conversion and unique steady state is also suggested.

2. Deriving the multiplicity region in terms of substrate concentration

The behavior of a completely back-mixed reactor can be represented in dimensionless form as

$$\alpha(1-a) = f(a) \tag{1}$$

where a represents the dimensionless substrate concentration and has values from 0 to 1, $f(a)$ represents the dimensionless rate, and α a dimensionless parameter which accounts for the residence time in the reactor.

To illustrate the existence of multiplicity, consider Fig. 1 as a graphical solution to Eq. (1). It consists of a rate curve $f(a)$ against (a). The points of intersection of this curve with a straight line of $y=\alpha(1-a)$ represent the steady-state solutions to Eq. (1). This straight line has a slope of $(-\alpha)$ and passes through the point [1,0].

If $f(a)$ is a monotonically increasing function of the substrate concentration, then only one such intersection is possible, implying a unique steady state. This is the situation for the common Michaelis–Menten kinetics without substrate inhibition. It is evident that, if the value of α is either $<\alpha_-$ or $>\alpha^+$, then only one steady state exists. Here, α^+ and α_- are the negative slopes of the two lines which are tangential to the rate curve from the point [1,0]. Hence, the necessary condition is that two tangents must be drawn to the rate curve from the point [1,0]. A sufficient condition for multiplicity is that the value of $(-\alpha)$ should lie in between the slopes of these two tangents. These conditions are now represented mathematically.

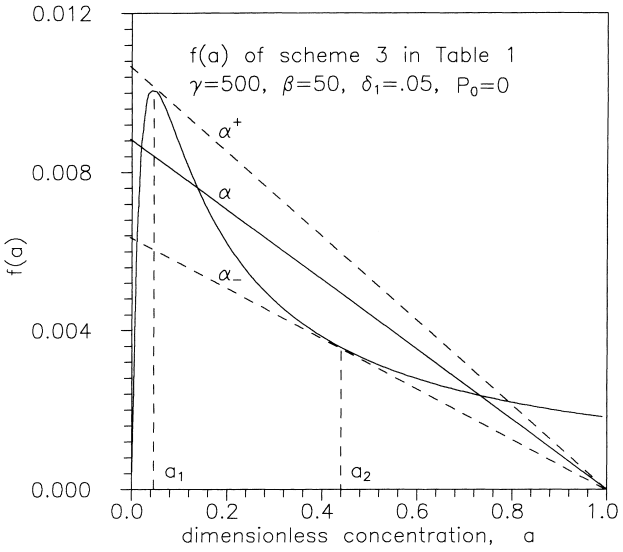

Fig. 1. Schematic representation of the tangent analysis method.

Suppose the tangents touch the rate curve at points a_1 and a_2. The slope of the straight line (dashed lines in Fig. 1) must be equal to the slope of the rate curve at that point. This leads to the following equations:

$$f'(a_1) = \frac{f(a_1)}{a_1 - 1} \tag{2}$$

and

$$f'(a_2) = \frac{f(a_2)}{a_2 - 1} \tag{3}$$

The necessary condition is therefore that a_1 and a_2 should be real, i.e. the equation

$$F(a) = f'(a) - \frac{f(a)}{a-1} = 0 \tag{4}$$

should have two real roots in the interval $0<a<1$. Eq. (4) is called the characteristic equation.

Slopes of the two dashed lines $-\alpha^+$ and $-\alpha_-$ can be described as

$$\alpha^+ = \frac{f(a_1)}{1-a_1} \tag{5}$$

$$\alpha_- = \frac{f(a_2)}{1-a_2} \tag{6}$$

Hence, a sufficient condition for multiplicity is that

$$\alpha_- < \alpha < \alpha^+ \tag{7}$$

From the above derivation of the tangent analysis method, the necessary condition for multiplicity is that Eq. (4) should have two real roots, a_1 and a_2, in the interval [0,1] and a sufficient condition for multiplicity is that $\alpha_- < \alpha < \alpha^+$. If the system parameter violates one of the above necessary and sufficient conditions, the system is unique.

3. Deriving the necessary conditions for multiplicity

In general, when the order of dimensionless concentration a in Eq. (4) is larger than 2, the necessary conditions for multiplicity (the existence condition of a_1 and a_2 in [0,1]) cannot be easily obtained. Lin [9,11] used the plot of the characteristic equation and employed an implicit criterion to derive the exact uniqueness and multiplicity criteria of an nth order reaction and a binary reaction in a non-adiabatic CSTR. However, these systems are of the three or fourth order of a in the characteristic equation. Ramachandran et al. [7] also derived the third or fourth order of the dimensionless concentration a in a characteristic equation for the complex biochemical reactions. In their work, the conditions for the existence of a_1 and a_2 have not been discussed and were only used to plot the simulated results for multiplicity. Sadana et al. [6] used an empirical equation to express the necessary conditions for the multiplicity of a biochemical reaction.

Here, we combine Sturm's method with the tangent analysis method to study the condition for the existence of a_1 and a_2.

Sturm's method is outlined as follows [17,18]:

Given a real algebraic equation

$$F(X) = a_0X^n + a_1X^{n-1} + \cdots + a_{n-1}X + a_n = 0 \quad (a_0 \neq 0) \tag{8}$$

without multiple roots, let $N(x)$ be the number of the sign changes (disregarding vanishing terms) in the sequence of functions $F_0=F(X)$, $F_1(X)=\mathrm{d}F(X)/\mathrm{d}x$, $F_i(X)=-$remainder $(F_{i-2}(X)/F_{i-1}(X))$ for $i>1$; $F_n(X)\neq 0$ is a constant. Then, the number of real roots of Eq. (8) located between two real numbers a and $b>a$ which are themselves not roots of Eq. (8) is equal to $N(a)-N(b)$.

If $F(X)$ have multiple roots, $F(X)$ and $\mathrm{d}F(X)/\mathrm{d}x$ have a common divisor; in this case, $F_n(X)$ is not a constant and $N(a)-N(b)$ is the number of real roots between a and b, where each multiple root is counted only once.

Here, we use Sturm's method to find the necessary conditions for multiplicity (i.e. the existence of a_1 and a_2 in the range of [0,1]) of the third and fourth order of a in the characteristic equation $F(a)=0$ (Eq. (4)).

Case A: The order of a in the characteristic equation is 3. Let

$$F_0(a) = F(a) = Ba^3 + Ca^3 + Da + E = 0 \tag{9}$$

$$F_1(a) = 3Ba^2 + 2Ca + D = 0 \tag{10}$$

$$F_2(a) = k_1a + k_2 \tag{11}$$

$$F_3(a) = k_3 \tag{12}$$

where B ($B>0$), C and D are coefficients and

$$k_1 = \frac{2C^2}{9B} - \frac{2D}{3}$$

$$k_2 = \frac{CD}{9B} - 4$$

$$k_3 = \frac{(2C - 3Bk_2)/k_1}{k_1 - D}$$

We focus on the number of real roots located between 0 and 1 equal to the number of sign change of $N(0)-N(1)$ of Eq. (9). On the other hand, the necessary conditions for multiplicity are two real roots, a_1 and a_2, in [0,1] of Eq. (9) from Section 2. Furthermore, the properties of Eq. (9) are cases (i) $F(0)>0$, $F(1)>0$ or (ii) $F(0)<0$, $F(1)<0$ and $F(\infty)=\infty$, $F(-\infty)=-\infty$, $F(-\infty)=-\infty$ when two real roots exist in the range [0,1]. Note that, when $N(0)-N(1)=0$ exists, a_1 and a_2 do not exist in [0,1], and when $N(0)-N(1)=1$ and $a_1=a_2$ in [0,1] exists, a start bifurcation point for multiplicity occurs. It implies that, when case (i) $F(0)>0$, $F(1)>0$ occurs, the value of $N(0)-N(1)$ jumps from 0 to 2, or when case (ii) $F(0)<0$, $F(1)<0$ occurs, and the value of $N(0)-N(1)$ jumps from 1 to 3, and $F(\infty)=\infty$, $F(-\infty)=-\infty$ are the necessary conditions for multiplicity when the order of a in the characteristic equation is 3.

Case B: The order of a in the characteristic equation is 4. Let

$$F_0(a) = F(a) = Aa^4 + Ba^3 + Ca^3 + Da + E = 0 \tag{13}$$

$$F_1(a) = 4Aa^3 + 3Ba^2 + 2Ca + D = 0 \tag{14}$$

$$F_2(a) = k_4a^2 + k_5a + k_6 \tag{15}$$

$$F_3(a) = k_7a + k_8 \tag{16}$$

$$F_4(a) = k_9 \tag{17}$$

where A ($A>0$), B, C, D and E are coefficients, and

$$k_4 = \frac{3B^2}{16A} - \frac{C}{2}$$

$$k_5 = \frac{2BC}{16A} - \frac{3D}{4}$$

$$k_6 = \frac{BD}{16A} - E$$

$$k_7 = \frac{4Ak_6}{k_4} + \frac{((3B - 4Ak_5)/k_4)k_5}{k_4 - 2C} \tag{18}$$

$$k_8 = \frac{k_6((3B - 4Ak_5)/k_4)}{k_4 - D}$$

$$k_9 = \frac{(k_5 - k_8k_4)/k_7}{k_7}k_8 - k_6$$

The properties of Eq. (13) are case (i) $F(0)>0$, $F(1)>0$ or case (ii) $F(0)<0$, $F(1)<0$ and $F(\infty)=\infty$, $F(-\infty)=\infty$ when two real roots exist in the range [0,1]. It implies that, when case (i) $F(0)>0$, $F(1)>0$ occurs, and the value of $N(0)-N(1)$ jumps from 0 to 2 or when case (ii) $F(0)<0$, $F(1)<0$ occurs, and the value of $N(0)-N(1)$ jumps from 1 to 3, and $F(\infty)=\infty$, $F(-\infty)=\infty$ are the necessary conditions for multiplicity when the order of a in the characteristic equation is 4.

4. A start-up strategy for a high conversion and unique steady state

The system parameters can be classified into three types: (a) the reaction parameters as the reaction rate constants; (b) the start-up parameter as an initial dimensionless substrate concentration and (c) the operating parameter as the residence time. Thus, if a reaction engineer has chosen the biochemical reaction type and the reaction parameters, the rest is to determine the start-up and operating parameters for obtaining a high conversion and unique steady state. A start-up strategy to obtain a high conversion and unique steady state is suggested as follows:

Step 1. The biochemical reaction type and the reaction parameters (k, E, K_i, K_i', K_m, K_p and k') are determined by a reaction engineer.

Step 2. The uniqueness can be obtained by choosing a start-up parameter (a design initial dimensionless substrate

concentration S_0^{design}) to violate the necessary conditions ($N(0)-N(1)=2$ for Sturm's method) for multiplicity when the order of a is 3 or 4.

Step 3. Once uniqueness is guaranteed from Step 2, a high conversion is obtained when a small value of operating parameter α ($\alpha=K_mF/kEV$) is chosen by adjusting V or F.

5. Application to various kinetic schemes

Several kinetic schemes in Table 1 are analyzed with regard to establishing the necessary and sufficient conditions for multiplicity. For each kinetic scheme, the dimensionless parameters necessary to characterize the system are defined, the corresponding dimensionless rate form is given and the expression for $F(a)$ is presented in Table 1.

Scheme 1

It represents the most common type of substrate kinetics. Typical examples include hydrolysis of sucrose by invertase [20], hydrolysis of benzyl penicillin by amidase [21] and phenol degradation [22].

Substituting the data in Table 2 of Hill and Robinson [22], $kE=0.295\,h^{-1}$, $K_i=0.0026\,l\,mg^{-1}$, $K_m=41.2\,mg\,l^{-1}$ and $S_0=1000$, 1178 or $1400\,mg\,l^{-1}$, respectively, into the $F(a)$ of Scheme 1 in Table 1, we obtain

$$S_0 = 1000,\quad [F_0(0), F_1(0), F_2(0), F_3(0)] = [+, 0, -, -],\quad N(0) = 1$$
$$[F_0(1), F_1(1), F_2(1), F_3(1)] = [+, +, +, -],\quad N(1) = 1$$

$$S_0 = 1178,\quad [F_0(0), F_1(0), F_2(0), F_3(0)] = [+, 0, -, 0],\quad N(0) = 1$$
$$[F_0(1), F_1(1), F_2(1), F_3(1)] = [+, +, +, 0],\quad N(1) = 0$$

$$S_0 = 1400,\quad [F_0(0), F_1(0), F_2(0), F_3(0)] = [+, 0, -, +],\quad N(0) = 2$$
$$[F_0(1), F_1(1), F_2(1), F_3(1)] = [+, +, +, +],\quad N(1) = 0$$

From the above results, the system is unique when $S_0 \le 1178\,mg\,l^{-1}$. Fig. 2 shows multiplicity occurring when $S_0 > S_0^{\text{design}} = 1178\,mg\,l^{-1}$. Note that the value of $N(0)-N(1)$ jumping from 0 to 2 is the necessary condition for multiplicity.

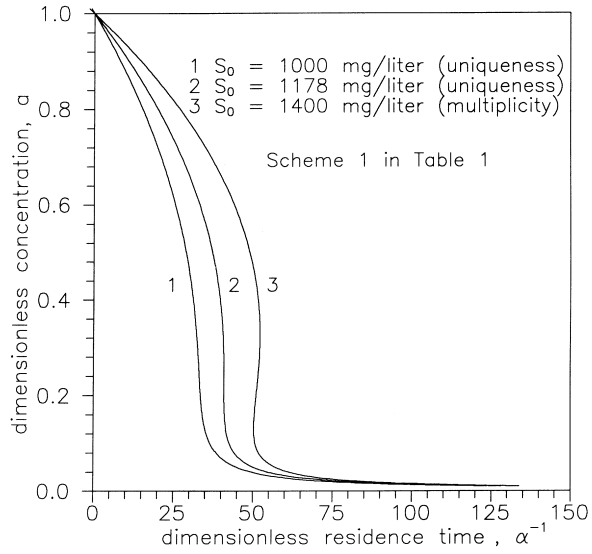

Fig. 2. Schematic representation of the example of Scheme 1 in Table 1.

Scheme 2

It represents an empirical relationship to account for substrate inhibition. Using the data in Table VIII of Edwards [1], $kE=0.5265\,h^{-1}$, $K_m=0.1138\%$ (w/v), $K_i'=3.501\%$ (w/v) and S_0 (% w/v)=5, 5.65 or 7, respectively, into $F(a)$ of Scheme 2 in Table 1, we obtain

$$S_0 = 5,\quad [F_0(0), F_1(0), F_2(0), F_3(0)] = [+, -, -, -],\quad N(0) = 1$$
$$[F_0(1), F_1(1), F_2(1), F_3(1)] = [+, +, +, -],\quad N(1) = 1$$

$$S_0 = 5.65,\quad [F_0(0), F_1(0), F_2(0), F_3(0)] = [+, -, -, 0],\quad N(0) = 1$$
$$[F_0(1), F_1(1), F_2(1), F_3(1)] = [+, +, +, 0],\quad N(1) = 0$$

$$S_0 = 7,\quad [F_0(0), F_1(0), F_2(0), F_3(0)] = [+, +, -, +],\quad N(0) = 2$$
$$[F_0(1), F_1(1), F_2(1), F_3(1)] = [+, +, +, +],\quad N(1) = 0$$

Table 1
Rate forms and the corresponding equation of $f(a)$ and $F(a)$ for various kinetic schemes

Scheme No.	Kinetic scheme	Dimensionless parameters	$f(a)$	$F(a)$
1	$kES/(K_m+S+K_iS^2)$ [20–22]	$\alpha=K_mF/kEV$, $\beta=S_0/K_m$, $\gamma=K_iS_0^2/K_m$	$a/(1+\beta a+\gamma a^2)$	$a^3+((\beta-\gamma)/2\gamma)a^2+1/2\gamma$
2	$kES\exp(-s/K_i')/(K_m+S)$ [1]	α, β and $\psi=S_0/K_i'$	$a\exp(-\psi a)/(a+\beta a+\gamma a^2)$	$a^3+((1/\psi)+(1/\beta)-1)a^2-(a/\beta)+(1/\psi\beta)$
3	$kES/(K_m+S+K_iS^2)\times 1/(1+K_pP)$ [7]	α, β, γ and $\delta_1=K_pS_0$; $J=1+\delta_1(1+p_0)$	$a/(1+\beta a+\gamma a^2)\times 1/(J-\delta_1 a)$	$3\gamma\delta_1a^4+2(\beta\delta_1-\gamma\delta_1-\gamma J)a^3+(J\gamma-\beta J-\beta\delta_1+\delta_1)a^2-J$
4	$(kES+k'ES^2)/(K_m+S+K_iS^2)$ [1]	α, β, γ as above and $\delta=k'S_0/k$	$(a+\delta a^2)/(1+\beta a+\gamma a^2)$	$\delta a^4+2a^3+((\beta\delta-\gamma+\beta-\delta)/\gamma)a^2+(2/\gamma)\delta a+1/\gamma$

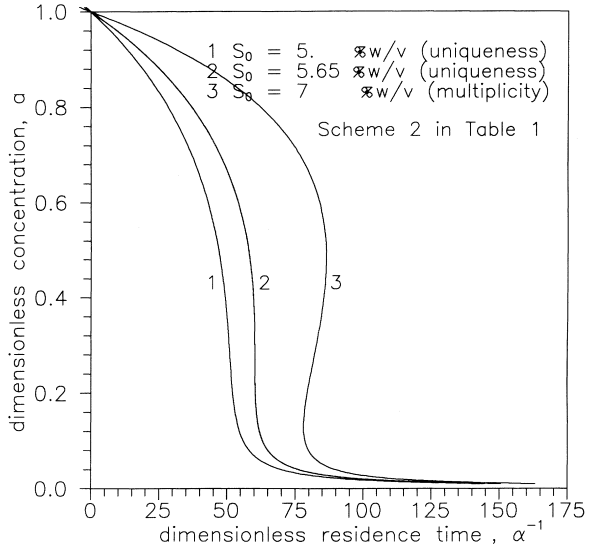

Fig. 3. Schematic representation of the example of Scheme 2 in Table 1.

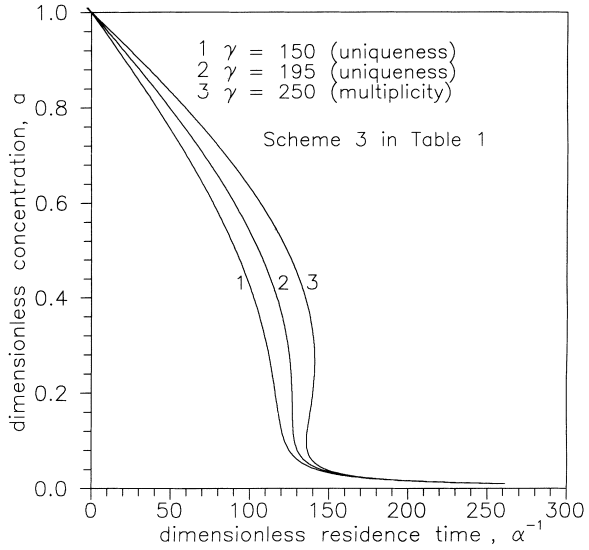

Fig. 4. Schematic representation of the example of Scheme 3 in Table 1.

From the above results, the system is unique when $S_0 \leq 5.65\%$ (w/v). Fig. 3 shows multiplicity occurring when $S_0 > S_0^{\text{design}} = 5.65\%$ (w/v).

Scheme 3

It represents a situation where both substrate and product of the reaction p inhibit the rate. The dimensionless product concentration p can be expressed in terms of the dimensionless substrate concentration a. Using an overall material balance,

$$p = p_0 + 1 - a \tag{19}$$

where p_0 is the dimensionless inlet product concentration. The dimensionless rate form $f(a)$ presented in Table 1 can be obtained from Eq. (19).

Same as Scheme 1 in Table 1, the value of $N(0)-N(1)$ jumping from 0 to 2 is the necessary condition for multiplicity. Substituting the simulated data $\beta=10$, $\delta_1=0.6$ and $p_0=0$, $J=1+\delta_1(1+p_0)=1.6$ and $\gamma=150$, 195 or 250, respectively, into $F(a)$ of Scheme 3 in Table 1, we obtain

$$\gamma = 150, \quad [F_0(0), F_1(0), F_2(0), F_3(0), F_4(0)] = [-, 0, +, -, -], \quad N(0) = 2$$
$$[F_0(1), F_1(1), F_2(1), F_3(1), F_4(1)] = [-, -, +, +, -], \quad N(1) = 2$$

$$\gamma = 195, \quad [F_0(0), F_1(0), F_2(0), F_3(0), F_4(0)] = [-, 0, +, -, 0], \quad N(0) = 2$$
$$[g_0(1), g_1(1), g_2(1), g_3(1), g_4(1)] = [-, -, +, +, 0], \quad N(1) = 1$$

$$\gamma = 250, \quad [F_0(0), F_1(0), F_2(0), F_3(0), F_4(0)] = [-, 0, +, -, +], \quad N(0) = 3$$
$$[F_0(1), F_1(1), F_2(1), F_3(1), F_4(1)] = [-, -, +, +, +], \quad N(1) = 1$$

From the above results, the system is unique when $\gamma \leq 195$. Fig. 4 shows multiplicity occurring when $\gamma > \gamma^{\text{design}} = 195$.

Scheme 4

It arises from certain complex mechanisms, such as enzymes existing in two different forms, two substrate systems, enzyme with multiple sub-sites, and some examples for these are given [23,24].

Substituting the data in Table VI of Edwards [1], $kE=0.2395\,\text{h}^{-1}$, $K_i=0.3209\,\text{mM}^{-1}$, $K_\text{m}=0.2879\,\text{mM}$, $k'=0.022\,\text{mM}^{-1}\,\text{h}^{-1}$ and $S_0=10$, 17 or 20 mM, respectively, into $F(a)$ of Scheme 4 in Table 1, we obtain

$$S_0 = 10, \quad [F_0(0), F_1(0), F_2(0), F_3(0), F_4(0)] = [+, +, +, -, -], \quad N(0) = 1$$
$$[F_0(1), F_1(1), F_2(1), F_3(1), F_4(1)] = [+, +, +, +, -], \quad N(1) = 1$$

$$S_0 = 17, \quad [F_0(0), F_1(0), F_2(0), F_3(0), F_4(0)] = [+, +, +, -, 0], \quad N(0) = 1$$
$$[F_0(1), F_1(1), F_2(1), F_3(1), F_4(1)] = [+, +, +, +, 0], \quad N(1) = 0$$

$$S_0 = 20, \quad [F_0(0), F_1(0), F_2(0), F_3(0), F_4(0)] = [+, +, -, -, +], \quad N(0) = 2$$
$$[F_0(1), F_1(1), F_2(1), F_3(1), F_4(1)] = [+, +, +, +, +], \quad N(1) = 0$$

Fig. 5 shows multiplicity occurring when $S_0 > S_0^{\text{design}} = 17\,\text{mM}$. Therefore, we can choose an S_0 to let $S_0 > S_0^{\text{design}} = 17\,\text{mM}$; then, the system is unique.

On the other hand, an analytical criterion for this is difficult to derive due to the quadratic nature of $F(a)$ of Scheme 4 in Table 1. The results of this calculation for various ranges of the parameters p, r and δ to ensure the existence multiplicity are summarized in Table 1 of Sadana et al. [6]. It is

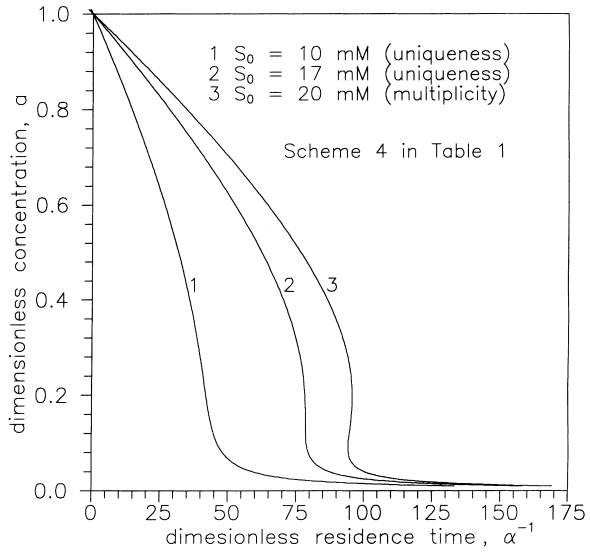

Fig. 5. Schematic representation of the example of Scheme 4 in Table 1.

seen that, for a given value of p, r and δ, there exists a minimum value of γ below which multiplicity is absent. The value of γ can also be expressed by an empirical equation

$$\gamma = 28.86 + 2.16\beta - 4.01 \times 10^{-3}\beta^2 + \delta(25.78 + 1.78\beta - 2.73 \times 10^{-3}\beta^2) \quad (20)$$

Eq. (20) is purely empirical and merely represents the data in Table 1 of Sadana et al. [6]; the only advantage of it being that it can be used readily to examine whether multiplicity exists. On the other hand, our work can obtain the exact criteria for multiplicity. Fig. 6 shows that Eq. (20) is too conservative for a reactor design to obtain a unique steady state except γ=1. It is demonstrated that our method is superior to the method of Sadana et al. [6].

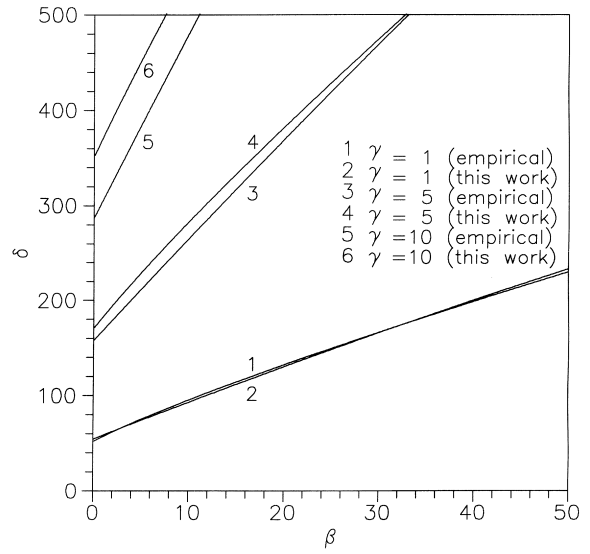

Fig. 6. Schematic representation of a minimum value of γ above which multiplicity occurs for Scheme 4 in Table 1 as per our work and Eq. (20).

6. Conclusion

Combining Sturm's method with the tangent analysis method can easily find the necessary conditions for multiplicity which cannot be obtained by using the tangent analysis method alone when the order of dimensionless initial concentration is above 2. Moreover, we can find a start bifurcation point for steady-state multiplicity. In addition, our method can suggest a start-up strategy to obtain a high conversion and unique steady state. Our results can be helpful for design, start-up and control of biochemical systems with substrate and product inhibition.

7. Nomenclature

a	dimensionless concentration (S/S_0)
a_1, a_2	dimensionless concentration corresponding to the points at which the tangent from [1,0] touches the rate curve
E	an enzyme concentration (mol l^{-1})
$f(a)$	dimensionless rate form
F	flow rate of the reactant (h^{-1})
$F(a)$	function defined by Eq. (4)
J	parameter defined in Table 1
k	rate constants for the enzyme reaction in Table 1 (h^{-1})
k'	rate constants for the enzyme reaction in Table 1 (h^{-1} mM^{-1} for Scheme 4)
K_i	substrate inhibition constants in Table 1 (mg^{-1} for Scheme 1, (mol/l)$^{-1}$ for Scheme 3, mM^{-1} for Scheme 4)
K_i'	substrate inhibition constant in Table 1 (mol l^{-1} for Scheme 2)
K_m	substrate inhibition constant in Table 1 (mg for Scheme 1, % w/v for Scheme 2, mM for Scheme 4)
K_p	product inhibition constant in Table 1 (l mol^{-1} for Scheme 3)
$N(a)$	the number of sign change of $F(a)$ of Sturm's method
p, p_0	dimensionless concentration of product in the reactor and incoming feed, respectively, (p/S_0, p_0/S_0) for Scheme 3 in Table 1
P, P_0	concentration of product in the reactor and incoming feed, respectively, in Table 1 (mol l^{-1} for Scheme 3)
S, S_0	substrate concentration in the reactor and incoming feed, respectively, in Table 1 (mg for Scheme 1, % w/v for Scheme 2, mol l^{-1} for Scheme 3, mM for Scheme 4)
S_0^{design}	substrate concentration to avoid multiplicity (mg for Scheme 1, % w/v for Scheme 2, mol l^{-1} for Scheme 3, mM for Scheme 4)
V	volume of the reactor (l)

Greek symbols

α	dimensionless reciprocal of the residence time defined as $K_m F/kEV$
α^+, α_-	upper and lower bounds of α within which multiplicity exists
β, γ, δ, δ_1	parameters defined in Table 1

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