

Dynamic behavior of double-substrate interactive model

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Abstract

Although modeling microbial growth with one limiting substrate such as Monod model is a common practice, microbes very often consume multiple substances from the environment for their growth. Therefore, the extension of current model to a multi-substrate analysis is needed for practical applications. The dynamic behavior of a double-substrate interactive growth model with Andrew's substrate inhibition model is theoretically discussed in this article. The yield factors considered are either a constant or a linear function of limiting substrate. The simulation indicated that there could be at least three non-trivial steady-state solutions with similar dynamic behavior for all the three cases, i.e. substrate without inhibition, one or both substrate with inhibition. The steady state of highest productivity is always a stable one. The steady state of the lowest productivity could change from a stable mode to an unstable mode while increasing dilution rate. And the limit cycle (sustained oscillation) could appear during the transition. The other steady state is always unstable. For the cases of one or both limiting substrates inhibition, the fourth steady state could appear, and it is always unstable.

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1. Introduction

Cells grow by taking up multiple substances from the environment. These nutrients include different carbon sources and a wide variety of components such as nitrogen, amino acids, vitamins and so on. Therefore, the possibility of multi-substrate limitations is quite frequently encountered in practice. In fact, the situation of multiple limiting substrates is noted in some experiments (Court and Pirt, 1981; Machado and Grady, 1989; Panikov, 1979).

Continuous culture is an important tool to produce the desired products under optimal operating conditions and also to determine the response of microorganisms to the environment. Double-substrate limiting growth is the simplest condition of multiple limiting substrates and needs to be examined first. Those cases have been discussed for the microorganisms of interest, including variation of cellular composition (Boer *et al.*, 2003), accumulation of storage compound (Durner *et al.*,

2001), changing of cell metabolism (Weusthuis *et al.*, 1994) and excretion of metabolic intermediates (Jung *et al.*, 2001). These detail results were reviewed by Zinn *et al.* (2004). From the viewpoint of maximum production, accurate control of the growth nutrients in the culture broth is very important and needs suitable kinetic model, especially in the case of multiple limiting substrates. Therefore, the characteristics of the proposed model need to be analyzed more clearly.

Some mathematical models are developed to describe the growth rate of a biological population with two or more limiting substrates. Bader (1978) classified the mathematical models of double-substrate limiting growth making into two categories. One is the non-interactive model which the growth rate of the biological population is controlled by one limiting substrate at one time. The other, interactive model, describes the growth rate as a function of both two limiting substrates at the same time. Chen and Christensen (1985) developed a unified model which is based on the probability. Cybernetic hypothesis, developed by Ramkrishna and coworkers (Dhurjati *et al.*, 1985; Kompala *et al.*, 1984, 1986), described the response of microorganisms tend to maximize yield among possible substrates, especially, in continuous culture. The situation that nutrients were not necessarily substitutable was noted by Baltzis and Fredrickson (1988) both experimentally and theoretically, which later

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Nomenclature

a, b	constant in the formulation of yield factor Y_2
A, B	functions defined in Eq. (7)
C_{s1}, C_{s2}	two limiting substrate concentrations
C_x	cell concentration
C_{xo}	scaling factor
D	dimensionless dilution rate
D_o	dilution rate
K_{i1}, K_{i2}	model constants defined in Eq. (1)
K_{s1}, K_{s2}	model constants defined in Eq. (1)
K_1, K_2	dimensionless model constant defined in Eq. (3)
P_{m1}, P_{m2}, P_z	functions defined in Eqs. (6a) and (6b)
S_1, S_2	dimensionless two limiting substrate concentrations
t	dimensionless time
t_o	time
X	dimensionless cell concentration
y_1, y_2	yield factors for two limiting substrates
Y_1, Y_2	dimensionless yield factors for two limiting substrates

Greek symbols

λ	eigenvalue
μ	dimensionless specific cell growth rate
μ_s	specific cell growth rate
μ_{sm}	maximum specific cell growth rate

Subscript

F	the value in inlet stream
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resulted in an extension of cybernetic model by Pavlou and Fredrickson (1989). Haas (1994) also proposed a formulation which was based on non-interactive growth process occurring among perfectly substitutable nutrients.

In this article, the dynamic behavior of a double-substrate limited interactive growth model with Andrew's substrate inhibition model (Andrews, 1968) for each substrate proposed by Liu et al. (1993) is discussed, i.e.

$$\mu_s = \frac{\mu_{sm} C_{s1} C_{s2}}{((K_{s1} + C_{s1} + C_{s1}^2)/K_{i1})((K_{s2} + C_{s2} + C_{s2}^2)/K_{i2})} \quad (1)$$

where μ_s is the specific growth rate, μ_{sm} the maximum specific growth rate, C_{s1} and C_{s2} the two limiting substrate concentrations, K_{s1} and K_{s2} the model constants, and K_{i1} and K_{i2} are substrate inhibition constants. When K_{i1} and K_{i2} approach to infinity, inhibition effect is neglected and this model is reduced to McGee et al.'s model (1972) which is simply a product of two Monod models for each substrate. Microbial growth, such as *Lactobacillus plantarum* (Lee et al., 1976), *Saccharomyces cerevisiae* (Borzani et al., 1977), *Saccharomyces carlsbergensis* (Finn and Wilson, 1954) and *Klebsiella aerogenes* (Harrison and Pirt, 1967), in continuous cultures occasionally exhibits oscillatory phenomena. The simple model failed to explain the observed oscillatory behavior in the chemostat. The model with constant

yield term had been evidenced that it could not have any periodic solutions (Crooke et al., 1980). In 1982, the studies of Crooke and Tanner (1982) and Agrawal et al. (1982) first reported that if the yield coefficient increases linearly with substrate concentration, the stable steady state may undergo the Hopf bifurcation and a limit cycle may appear. The single specie fermentation process had been investigated and continue until now (Zhu and Huang, 2006). In this study, we discussed a double-substrate limiting growth model and the yield factor either a constant or a linear function of limiting substrate was used.

2. Theoretical formulation

2.1. Mathematical model

For a continuous bioreactor, system variables are cell concentration (C_x) and two limiting substrate concentrations (C_{s1}, C_{s2}). The governing equations with control variables, dilution rate (D_o) and two feed substrate concentrations (C_{s1F}, C_{s2F}), can be described in the dimensionless forms as:

$$\frac{dX}{dt} = \mu X - DX \quad (2a)$$

$$\frac{dS_1}{dt} = -\frac{\mu X}{Y_1} + D(S_{1F} - S_1) \quad (2b)$$

$$\frac{dS_2}{dt} = -\frac{\mu X}{Y_2} + D(S_{2F} - S_2) \quad (2c)$$

where $X = (C_x/C_{xo}), S_1 = (C_{s1}/K_{s1}), S_2 = (C_{s2}/K_{s2}), D = (D_o/\mu_{sm}), \mu = (\mu_s/\mu_{sm}), t = (t_o/\mu_{sm}), Y_1 = (K_{s1}y_1/C_{xo}), Y_2 = (K_{s2}y_2/C_{xo}), S_{1F} = (C_{s1F}/K_{s1}), S_{2F} = (C_{s2F}/K_{s2})$.

The parameters Y_1 and Y_2 are the dimensionless yield factors (cell/substrate) for the two limiting substrates, respectively. C_{xo} is a scaling factor and t_o is time. The corresponding dimensionless specific growth rate in Eq. (1) becomes:

$$\mu = \frac{S_1 S_2}{(1 + S_1 + K_1 S_1^2)(1 + S_2 + K_2 S_2^2)} \quad (3)$$

where $K_1 = (K_{s1}/K_{i1})$ and $K_2 = (K_{s2}/K_{i2})$.

The yield factor considered in this study was either a constant (Y_1) or a linear function of limiting substrate ($Y_2 = a + bS_2$) (Essajee and Tanner, 1979).

2.2. Steady state

Besides the washout steady state $(X, S_1, S_2) = (0, S_{1F}, S_{2F})$, the non-trivial solution can be expressed as:

$$\mu = D \quad (4a)$$

$$X = Y_1(S_{1F} - S_1) \quad (4b)$$

$$X = Y_2(S_{2F} - S_2) \quad (4c)$$

By substituting the growth Eq. (3) into Eq. (4a), one may have:

$$\frac{1}{D} = \left(\frac{1}{S_1} + 1 + K_1 S_1 \right) \left(\frac{1}{S_2} + 1 + K_2 S_2 \right) \quad (5a)$$

And the following equation can be obtained from Eqs. (4b) and (4c) by eliminating X .

$$S_1 = S_{1F} - \frac{Y_2(S_{2F} - S_2)}{Y_1} = S_{1F} - \frac{(a + bS_2)(S_{2F} - S_2)}{Y_1} \quad (5b)$$

Eq. (5a) is a hyperbola in the $S_1 - S_2$ phase plane when $K_1 = K_2 = 0$ as discussed by Liu *et al.* (1993) and Eq. (5b) is a parabolic curve with a right open end and the vertex is at P_m (P_{m1}, P_{m2}), where

$$P_{m1} = -\frac{(a + bS_{2F})^2 - 4bS_{1F}Y_1}{4bY_1} \quad \text{and} \quad P_{m2} = -\frac{a - bS_{2F}}{2b} \quad (6a)$$

The curve described by Eq. (5b) intersects with the line $S_2 = 0$ at $(P_z, 0)$, where

$$P_z = S_{1F} - \frac{aS_{2F}}{Y_1} \quad (6b)$$

in the $S_1 - S_2$ phase plane, the steady-state solutions are the intersection points of these two curves, i.e. Eqs. (5a) and (5b).

2.3. Local stability and bifurcation theory

The criterion of a local stability is that the real parts for all eigenvalues of the Jacobian matrix at the respective steady state are negative (Verhulst, 1990). The Jacobian matrix can be expressed as:

$$\mathbf{J} = \begin{pmatrix} \mu - D & -\frac{\partial\mu}{\partial S_1}X & -\frac{\partial\mu}{\partial S_2}X \\ -\frac{\mu}{Y_1} & -\frac{\partial\mu X}{\partial S_1 Y_1} - D & -\frac{\partial\mu X}{\partial S_2 Y_1} \\ -\frac{\mu}{Y_1} & -\frac{\partial\mu X}{\partial S_1 Y_2} & -\frac{\partial(\mu/Y_2)}{\partial S_2}X - D \end{pmatrix}$$

The local stability of the continuous bioreactor with double limiting substrates can be analysis as follows:

(I) For non-trivial solution:

The eigenvalues are the roots of the characteristic equation of Eqs. (2a)–(2c), i.e. $(\mathbf{J} - \lambda\mathbf{I}) = 0$.

$$(\lambda + D)(\lambda^2 + A\lambda + B) = 0 \quad (7)$$

in which

$$A = D + \left(\left(\frac{\partial\mu}{\partial S_2} \right) \left(\frac{1}{Y_2} \right) + \left(\frac{\partial\mu}{\partial S_1} \right) \left(\frac{1}{Y_1} \right) \right) X - \left(\frac{\mu X}{Y_2^2} \right) \left(\frac{\partial Y_2}{\partial S_2} \right) \quad (7a)$$

$$B = DX \left(\left(\frac{\partial\mu}{\partial S_2} \right) \left(\frac{1}{Y_2} \right) + \left(\frac{\partial\mu}{\partial S_1} \right) \left(\frac{1}{Y_1} \right) \right) - \frac{\mu X^2}{Y_1 Y_2^2} \left(\frac{\partial\mu}{\partial S_1} \right) \left(\frac{\partial Y_2}{\partial S_2} \right) \quad (7b)$$

When Y_2 is a constant, the three roots of the characteristic equation are:

$$-D, \quad -D, \quad - \left(\left(\frac{\partial\mu}{\partial S_2} \right) \left(\frac{1}{Y_2} \right) + \left(\frac{\partial\mu}{\partial S_1} \right) \left(\frac{1}{Y_1} \right) \right) X \quad (8)$$

In this case, there is no Hopf bifurcation possible (Leah, 1988).

When Y_2 is a linear function of limiting substrate, the local stability of non-washout steady state can be determined by the sign of

$$\left(\frac{\partial\mu}{\partial S_2} \right) \left(\frac{1}{Y_2} \right) + \left(\frac{\partial\mu}{\partial S_1} \right) \left(\frac{1}{Y_1} \right).$$

Since

$$\frac{\partial\mu}{\partial S_1} = \frac{1 - K_1 S_1^2}{(1 + S_1 + K_1 S_1^2)^2} \frac{S_2}{(1 + S_2 + K_2 S_2^2)} \quad (9a)$$

and

$$\frac{\partial\mu}{\partial S_2} = \frac{1 - K_2 S_2^2}{(1 + S_2 + K_2 S_2^2)^2} \frac{S_1}{(1 + S_1 + K_1 S_1^2)} \quad (9b)$$

the group

$$\left(\frac{\partial\mu}{\partial S_2} \right) \left(\frac{1}{Y_2} \right) + \left(\frac{\partial\mu}{\partial S_1} \right) \left(\frac{1}{Y_1} \right)$$

is always positive if $1 > K_1 S_1^2$ and $1 > K_2 S_2^2$. Please note that the case of $K_1 = K_2 = 0$ (no substrate inhibition) always satisfies $1 > K_1 S_1^2$ and $1 > K_2 S_2^2$. In this case, the steady state is stable because of the negative eigenvalue. With substrate inhibition, the steady state may be either stable or unstable. However, when Y_2 is a linear function of the second limiting substrate, the three roots of the characteristic equation are:

$$-D, \quad \frac{-A + \sqrt{A^2 - 4B}}{2}, \quad \frac{-A - \sqrt{A^2 - 4B}}{2} \quad (10)$$

Therefore, limit cycle is possible if $A = 0$ and $B > 0$ according to Hopf bifurcation theory.

(II) For trivial solution (washout):

The three eigenvalues are:

$$\lambda = -D, \quad -D, \quad \mu - D \quad (11)$$

It is a stable steady state when the dilution rate is greater than the specific growth rate ($D > \mu$), and an unstable node otherwise.

3. Result and discussion

Three cases, (I) both substrates without inhibition effect, (II) substrate inhibition for one substrate only, and (III) substrate inhibition for both limiting substrates, are discussed in the following results using numerical simulations according to the substrates with or without inhibition effect. The $S_1 - S_2$ phase plane is used to exam the possible classes of steady states and

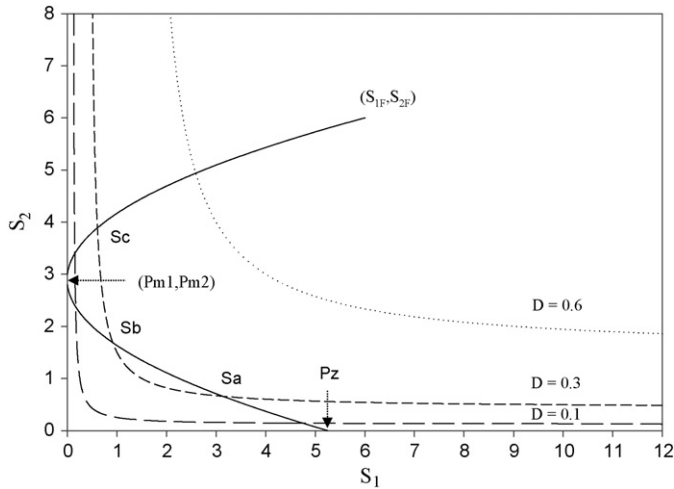


Fig. 1. Steady-state solutions from Eq. (5a) (dash line with different dilution rate) and Eq. (5b) (solid line) for both substrates without inhibition ($Y_1 = 8$, $a = 1$, $b = 5$, $S_{1F} = 6$, $S_{2F} = 6$ and $K_1 = K_2 = 0$).

bifurcation diagram commonly used is performed to classify the possible phase plots with dilution rate.

3.1. Case (I): both substrates without inhibition effect

Fig. 1 shows the steady-state solutions in $S_1 - S_2$ phase plane if both of the limiting substrates in the interactive model

follow the Monod models. The solid line is the feeding parabolic curve as presented in Eq. (5b) which can be moved by one of the operating factors, feeding concentration. The lines are growth hyperbola curves from Eq. (5a) with $K_1 = K_2 = 0$ which is decided by the other operating factor, dilution rate. For feeding curve, the characteristic of the parabolic curve with a right open end and the vertex $P_m (P_{m1}, P_{m2})$ is important to the appearance of the possible classes of steady states. The growth hyperbola curves (Fig. 1) with three dilution rates, $D = 0.1, 0.3$ and 0.6 , move upwards as the dilution rate increases. If the hyperbola curves are above the feed point (S_{1F}, S_{2F}) , a hyperbola curves and parabolic curve never intersect with each other. Therefore, only the trivial steady state exist, that is the washout condition. Critical dilution rate can be obtained where the growth curve passes the feeding concentration (S_{1F}, S_{2F}) . Except for trivial solution, there are three possible classes of steady states which are dependent upon the feeding concentration, i.e. the feeding parabolic curves. There is at most one non-washout steady state for whole range of dilution rates when P_{m2} (the coordinate of vertex of parabolic curve) is negative. This steady state is the highest one located on the upper half portion of the parabolic curve and always exists except the washout steady state. When P_{m2} is positive (i.e. $S_{2F} > a/b$ by Eq. (6a)), there could be either one or three steady states, determined by using the Eq. (5b) with $S_2 = 0$. This intersection of S_1 is noted as P_z (Fig. 1). When P_z is positive ($(Y_1/a)S_{1F} > S_{2F}$), i.e. the lower half portion of feeding parabolic curves across the first

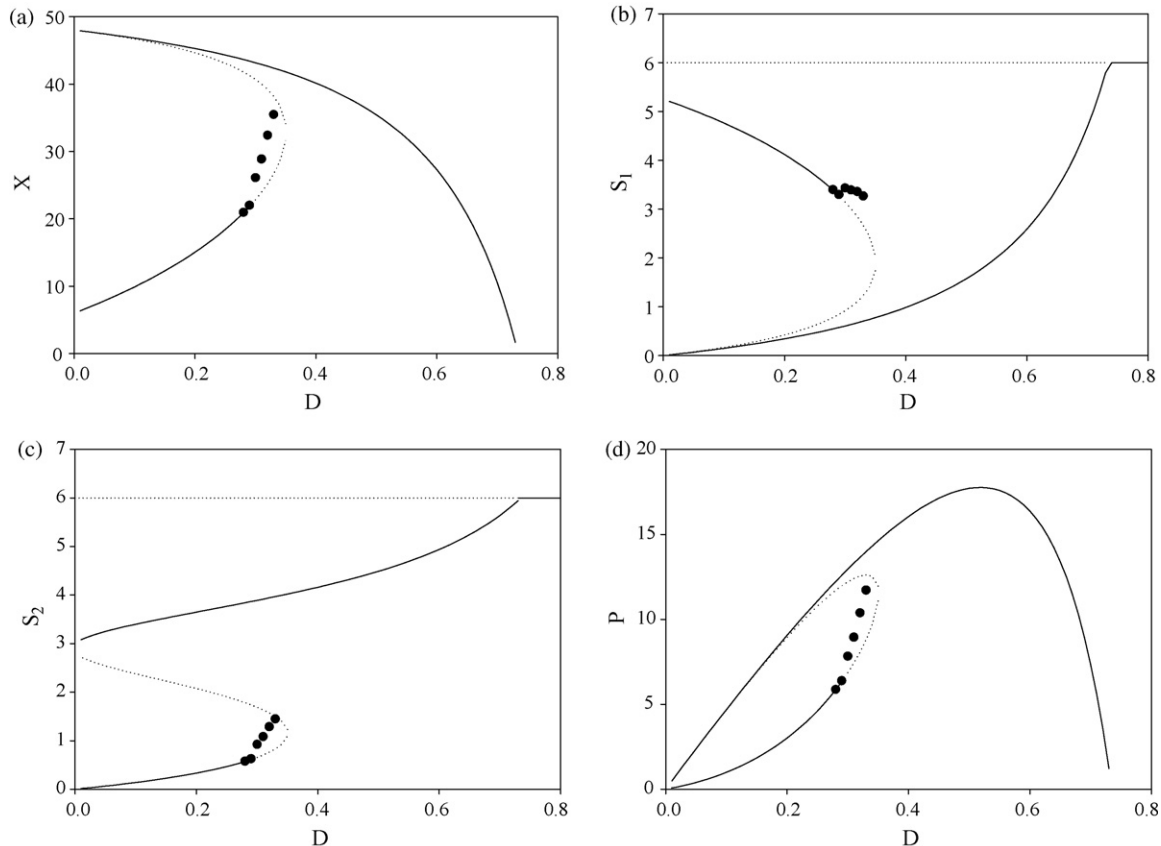


Fig. 2. (a) Bifurcation diagram of both substrates without inhibition for cell, (b) substrate 1, (c) substrate 2, and (d) productivity ($Y_1 = 8$, $a = 1$, $b = 5$, $S_{1F} = 6$, $S_{2F} = 6$ and $K_1 = K_2 = 0$).

quadrant, there are three steady-state solutions. Otherwise ($P_z < 0$), only one steady state can be obtained. In other words, if P_{m1} is positive (i.e. $S_{1F} > ((a + bS_{2F})^2/4bY_1)$ by Eq. (6a)), there are three steady-state solutions in the middle range of the dilution rate since P_z is always positive. It should be noticed that the lowest steady state only exist in the case of three steady-state solutions and in the lower range of the dilution rate.

For the purpose of illustration, the parameters ($Y_1 = 8$, $a = 1$, $b = 5$, $S_{1F} = 6$ and $S_{2F} = 6$) are chosen so that three non-trivial steady states exist and the vertex is in the second quadrant. The bifurcation diagram for cell and two limiting substrates in Fig. 1 with three steady states are shown in Fig. 2(a)–(c). The number of steady state when dilution rate approaches zero is three because of positive P_{m2} and P_z . In Fig. 2(c), the variation of three non-washout steady states (S_a , S_b and S_c) with dilution rate is shown with three regions of S_2 , 0–1.1 (S_a), 1.1–2.8 (S_b) and 3–6 (S_c). The highest steady state for S_2 (S_c) is always a stable attractor. The washout steady state is a saddle when S_c exists at the same time and becomes stable when S_c disappeared ($D > \mu$). This transition point is the critical operating dilution rate. Above the transition point, only one stable is exist, i.e. the washout steady state. S_b is always unstable (a saddle point) and Hopf bifurcation phenomenon appears in the lower steady state (S_a). S_a is a stable spiral steady state in lower dilution rate and is changed to unstable spiral when dilution rate increases to exceed bifurcation value. The limit cycle which is a circle in the three dimensional space appears in the transition. This stable limit cycle has an unstable spiral inside. When dilution rate increases, the average radius of the cycle becomes bigger. Up to maximum extend the cycle will break. Only one attracting steady state (S_c) in the state space is presented in higher dilution rate region and the maximum productivity which is the cell concentration multiplying by dilution rate is also in this region (Fig. 2(d)). The classification of possible phase plots is shown in Table 1 where eight different situations are classified.

3.2. Case (II): substrate inhibition for one substrate only

For the inhibition effect of substrate 1 only, the steady-state solution(s) formulated in Eqs. (5a) and (5b) with $K_2 = 0$ are shown in Fig. 3. The solid line is also the feeding parabolic curves as discussed in case (I). The others are growth curves

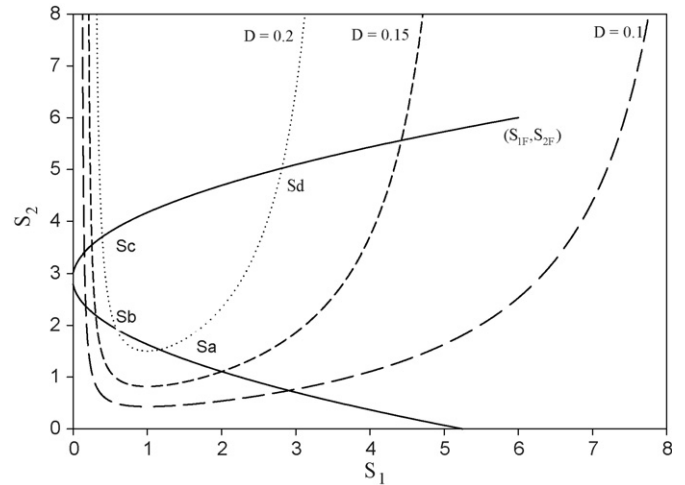


Fig. 3. Steady-state solutions from Eq. (5a) (dash line with different dilution rate) and Eq. (5b) (solid line) for only one substrate inhibition ($Y_1 = 8$, $a = 1$, $b = 5$, $S_{1F} = 6$, $S_{2F} = 6$, $K_1 = 1$ and $K_2 = 0$).

forming the U-shape curves. When the dilution rate increase, the U-shape moves upward resulting in the width of the shape decreases (Liu et al., 1993). The minimum of the U-shape curves in which the slope equals to zero can be obtained from the solutions of the first derivative of Eq. (5a) and $S_1 = \sqrt{K_1}$. Using the function substitute into Eq. (5a), one value of the concentration of substrate 2 can be obtained since $K_2 = 0$. Therefore, both sides of the minimum of the U-shape curves are monotonically increased to infinity which is limited by two vertical asymptotes. The steady-state solutions are the intersection points of the two curves, feeding and dilution rate curves. The number of non-washout steady state in this case is similar with case (I) except S_d shown in Fig. 3. Since substrate inhibition causes right hand side of U-shape dilution rate curves, the feeding parabolic curve intersects with U-shape, generating an additional and maximum steady state. Therefore, the maximum number of non-washout steady state is two when the vertex in the third or fourth quadrants ($P_{m2} < 0$). When the vertex is in the first or second quadrant ($P_{m2} > 0$), non-washout steady state is four (S_a , S_b , S_c and S_d) in the condition of $P_z > 0$ and two while P_z is below zero.

Table 1

The classification of possible phase plots with dilution rate as parameter for both substrates without inhibition effect

	Case							
	A	B	C	D	E	F	G	H
Region of dilution rate	0–0.144	0.144–0.292	0.292–0.33	0.33–0.35	0.35–0.52	0.52–0.572	0.572–0.735	0.735–0.8
Stable washout	0	0	0	0	0	0	0	1
Unstable washout	1	1	1	1	1	1	1	0
Stable node	2	1	1	1	1	0	1	0
Saddle	1	1	1	1	0	0	0	0
Stable spiral	0	1	0	0	0	1	0	0
Unstable spiral	0	0	1	1	0	0	0	0
Limit cycle	0	0	1	0	0	0	0	0
Total	4	4	5	4	2	2	2	1

$Y_1 = 8$, $a = 1$, $b = 5$, $S_{1F} = 6$, $S_{2F} = 6$ and $K_1 = K_2 = 0$.

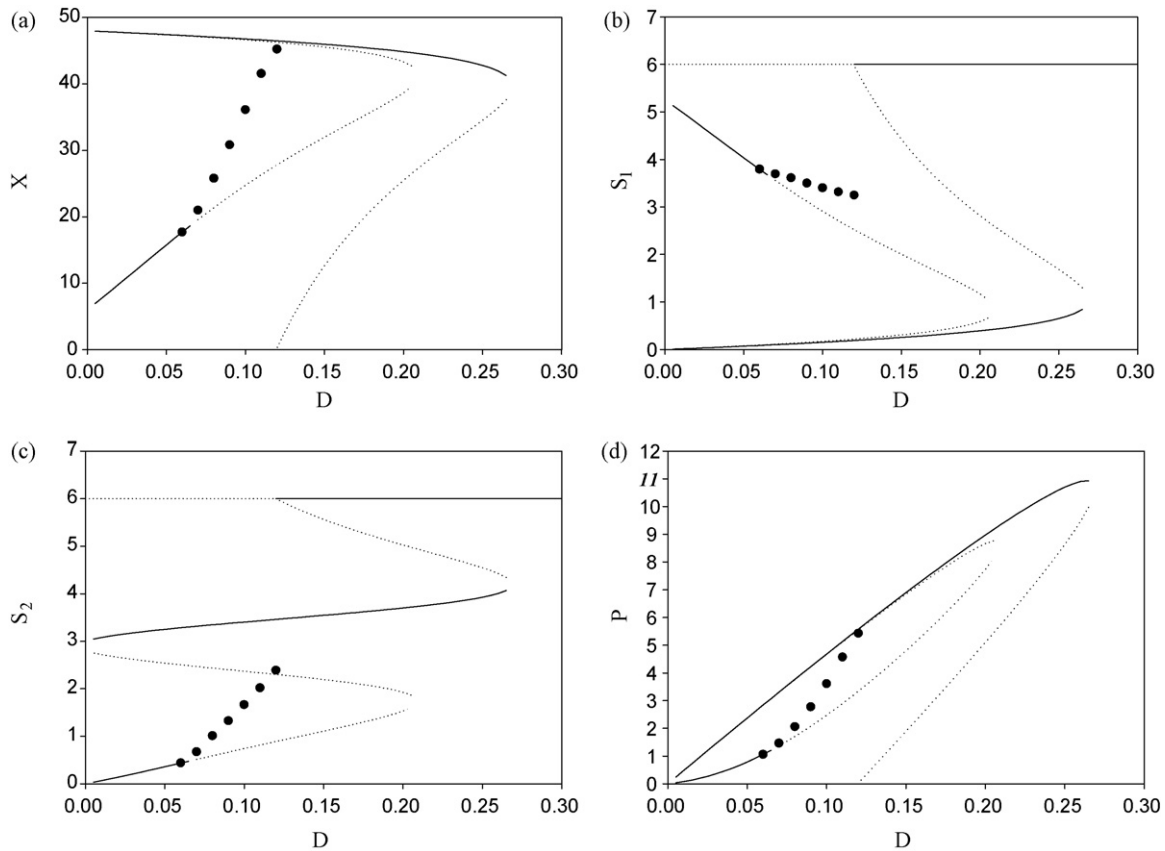


Fig. 4. (a) Bifurcation diagram of only one substrate inhibition for cell, (b) substrate 1, (c) substrate 2, and (d) productivity ($Y_1 = 8$, $a = 1$, $b = 5$, $S_{1F} = 6$, $S_{2F} = 6$, $K_1 = 1$ and $K_2 = 0$).

Comparing with case (I), the parameters are the same except additional parameter $K_1 = 1$.

The bifurcation diagram as a function of dilution rate is shown in Fig. 4(a)–(c) for cell and two limiting substrates, respectively. Since P_{m2} and P_z are positive, there are three non-washout steady states when dilution rate approaches to zero due to the selected parameters. The fourth steady state appears in the middle range of the dilution rate and disappears at the critical dilution rate ($D = \mu$). The variations of S_2 for the four non-washout steady states are indicated in Fig. 3(c) with four regions of S_2 , 0–1.7 (S_a), 1.7–2.8 (S_b), 3.0–4.2 (S_c) and 4.2–6.0 (S_d). The behaviors of three steady state S_a , S_b and S_c are similar

to case (I); S_c is a stable attractor, S_b is a saddle and S_a is changed from stable to unstable with increasing dilution rate. The stable limit cycle also appears in S_a and starts at the bifurcation value then breaks before S_d appears. S_d does not exist when dilution rate is lower since its steady-state value of (S_1, S_2) is greater than (S_{F1}, S_{F2}). It is always an unstable steady state. The washout steady state is unstable in the lower range of dilution rate, and becomes unstable as dilution rate increases resulting in the formation of the S_d . S_d is disappeared at the critical dilution rate ($D = \mu$). Productivity, defined by the product of cell concentration and dilution rate, is shown in Fig. 4(d) as a function of dilution rate. The maximum productivity is operated in the dilution rate D_m

Table 2
The classification of possible phase plots with dilution rate as parameter for only one substrate inhibition

	Case						
	A	B	C	D	E	F	G
Region of dilution rate	0–0.028	0.028–0.068	0.068–0.1	0.1–0.12	0.12–0.209	0.209–0.269	0.269–0.3
Stable washout	0	0	0	0	1	1	1
Unstable washout	1	1	1	1	0	0	0
Stable node	2	1	1	1	1	1	0
Saddle	1	1	1	1	2	1	0
Stable spiral	0	1	0	0	0	0	0
Unstable spiral	0	0	1	1	1	0	0
Limit cycle	0	0	1	0	0	0	0
Total	4	4	5	4	5	3	1

$Y_1 = 8$, $a = 1$, $b = 5$, $S_{1F} = 6$, $S_{2F} = 6$, $K_1 = 1$ and $K_2 = 0$.

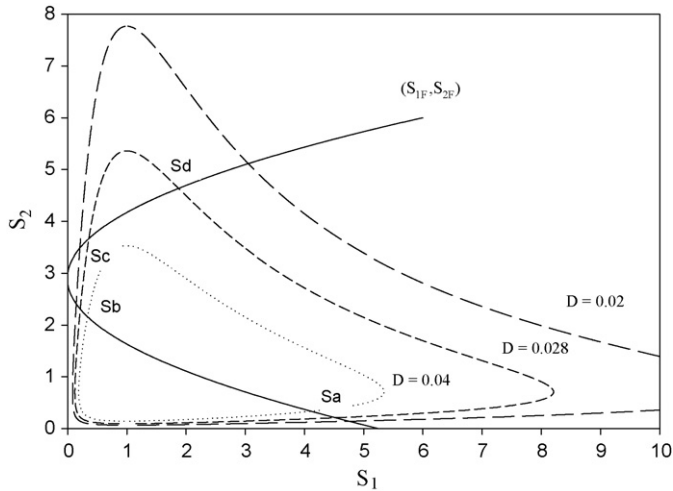


Fig. 5. Steady-state solutions from Eq. (5a) (dash line with different dilution rate) and Eq. (5b) (solid line) for both substrates with inhibition ($Y_1 = 8$, $a = 1$, $b = 5$, $S_{1F} = 6$, $S_{2F} = 6$, $K_1 = 1$ and $K_2 = 2$).

and the steady-state value of S_2 will be S_c (Fig. 3). Slightly increasing dilution rate at D_m will lead to the steady state at only one stable attractor-washout. The washout steady state is the condition of loss of all cells at steady state that means the productivity is zero. Therefore, an accurate flow-rate controller is needed to avoid the disturbance of dilution rate which is function of flow rate in operating bioreactor. The seven different classes of phase plots are presented in Table 2.

3.3. Case (III): substrate inhibition for both limiting substrates

When both of the limiting substrates in the interactive model produce an inhibition effect, the steady-state solution(s) is formulated in Eqs. (5a) and (5b) and shown in Fig. 5. Although the feeding parabolic curve (the solid line) is also the same as discussed in case (I), the growth curves become more complicated. The extreme of the curves which are the solutions of first derivative of Eq. (5a) can be obtained in $S_1 = \sqrt{K_1}$ as in case (II), but when using the function substitute into Eq. (5a), the concentration of substrate 2 can be obtained two values since $K_2 \neq 0$. Because S_1 and S_2 are symmetry in Eq. (5a), two extreme of the curves can be also obtained in $S_2 = \sqrt{K_2}$. Therefore, the closed-loop contour curve is expressed in Fig. 5 for the growth curves. When the dilution rate increases, the closed-loop contour curve reduces its size and finally approaches a point, where the dilution rate is the limiting dilution rate. The number of steady-state solutions which are the intersection points of feeding and dilution rate curves is similar as case (II). Therefore, the additional steady state is obtained when compared with case (I) and the maximum number of non-washout steady state is two when the vertex is located in the third and fourth quadrants. When the vertex is located in the first or second quadrant, there are four steady states S_a , S_b , S_c and S_d in the condition of $P_z > 0$ and two steady states while $P_z < 0$.

The parameters in the bifurcation diagram shown in Fig. 6(a)–(c) are the same as case (II); however, the K_2 is 2

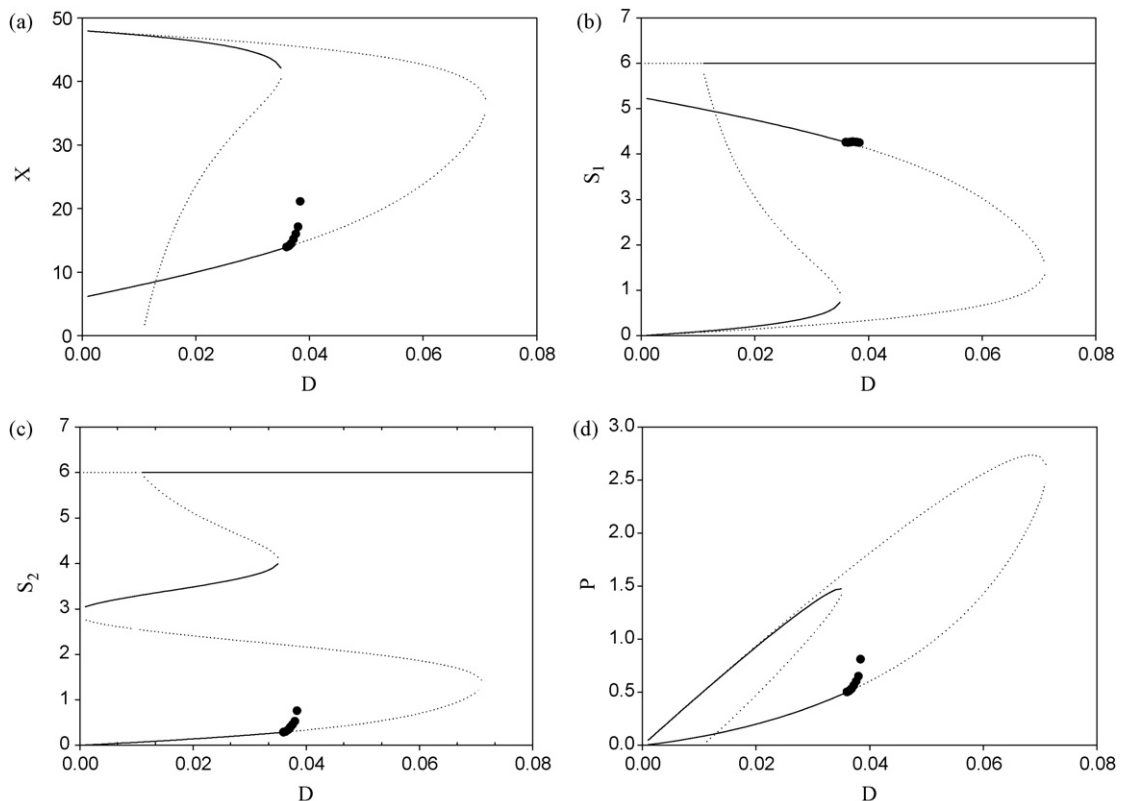


Fig. 6. (a) Bifurcation diagram of both substrates with inhibition for cell, (b) substrate 1, (c) substrate 2, and (d) productivity ($Y_1 = 8$, $a = 1$, $b = 5$, $S_{1F} = 6$, $S_{2F} = 6$, $K_1 = 1$ and $K_2 = 2$).

Table 3
The classification of possible phase plots with dilution rate as parameter for both limiting substrates with inhibition

	Case							
	A	B	C	D	E	F	G	H
Region of dilution rate	0–0.011	0.011–0.024	0.024–0.0352	0.0352–0.0368	0.0368–0.038	0.038–0.052	0.052–0.071	0.071–0.08
Stable washout	0	1	1	1	1	1	1	1
Unstable washout	1	0	0	0	0	0	0	0
Stable node	2	2	1	0	0	0	0	0
Unstable node	0	0	0	0	0	0	1	0
Saddle	1	2	2	1	1	1	1	0
Stable spiral	0	0	1	1	0	0	0	0
Unstable spiral	0	0	0	0	1	1	0	0
Limit cycle	0	0	0	0	1	0	0	0
Total	4	5	5	3	4	3	3	1

$Y_1 = 8$, $a = 1$, $b = 5$, $S_{1F} = 6$, $S_{2F} = 6$, $K_1 = 1$ and $K_2 = 2$.

instead. The stability of four steady states is similar to case (II) but the limit cycle is produced after S_d is disappeared. For productivity versus dilution rate (Fig. 6(d)), the difference of dilution rate between the maximum productivity and the limit cycle is very closure. It also needs to accurately control the dilution rate in order to obtain a maximum production due to hysteresis effect. The eight regions have been classified for different phase plots and are corrected in Table 3.

4. Conclusion

A typical plot to explain the effect of double substrates with one variable yield factor is given by using specific or specified values. The position of vertex (Eq. (6a)) and the intersection of S_1 (Eq. (6b)) in $S_1 - S_2$ contour determine the maximum number of steady state and there are function of control variables S_{1F} and S_{2F} . In the case of both substrates without inhibition effect (case (I)), at most one non-washout steady state is obtained for whole range of dilution rate when P_{m2} (the coordinate of vertex of parabolic curve) is negative. When P_{m2} is positive, there are three steady-state solutions if P_z is positive and only one steady state if P_z is negative. In the case of one substrate with inhibition (case (II)), the number of non-washout steady state is similar to case (I) except there is an additional steady state. Therefore, the maximum number of non-washout steady state is two when $P_{m2} < 0$. If $P_{m2} > 0$, there are four steady states in the condition of $P_z > 0$ and two steady states while $P_z < 0$. In the case (III) with substrate inhibition for both limiting substrates, the maximum number of non-washout steady state is the same as case (II) although the growth curve becomes a closed loop.

After simulation with many parameters, the steady state S_c is always a stable one and the maximum productivity is also operated in this condition. S_b and S_d (in cases (II) and (III)) are unstable and S_a which is the lowest steady state could be change from a stable mode to an unstable mode while dilution rate increases. The limit cycle (sustained oscillation) could appear during the transition. Because of the presence of hysteresis effect in the cases of substrate inhibition with one or two substrate, accurate control in operating bioreactor is needed to avoid the disturbance of dilution rate for producing maximum productivity.

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雙限制基質交互作用下生物反應器之動態分析

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摘要

雖然目前常以單一限制基質來描述微生物成長，但微生物總是從環境中攝取多種基質以利生長。因此，將現存的模式延伸到多基質有其實際的必要性。本文以 Andrew 的基質抑制模式，來探討雙限制基質交互作用下生物反應器之動態行為。模式中的兩個產率因子，一選擇為常數而另一為基質濃度之線性函數。對於所討論的三種情況而言，包括雙限制基質均無抑制、單限制基質抑制及雙限制基質均有抑制現象，數值分析顯示這三種情況均可得類似的至少三個非零解。其中最高的穩態總是穩定且可產生最大產量，而最低的穩態隨著稀釋速率增加從穩定態變化到非穩定態，且在轉換過程中出現極限圓。另一個穩態總是不穩定。對於一或二個基質抑制的情況，產生了第四個穩態但總是不穩定。