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EFFECT OF FEEDBACK INHIBITION ON THE BEHAVIOR OF MULTIENZYME MONOLINEAR REACTION CHAINS

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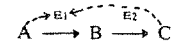
The multiplicity characteristics of the simple two-enzyme reaction chain

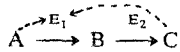


are investigated numerically for simple lumped and distributed reaction systems. The quasi-steady-state response of the concentration within each of the two systems to changes in environmental concentration is generated for a range of kinetic parameters. The effect of product inhibition in suppressing the region of multiple steady-state behavior is followed quantitatively.

INTRODUCTION

Enzyme catalysis is associated with multiple steady-state phenomena and the consequent hysteresis and discontinuous behavior, whenever the reaction is autocatalyzed by the product (1) or inhibited by excess substrate (2). In the case of substrate inhibited enzymes, previous studies dealt with the dependence of the multiplicity behavior on system heterogeneity (3) and flow conditions (4) in well-stirred and tubular reactors, and on the spatial distribution of activity, convective, diffusive, and kinetic parameters in active enzyme membranes (5). Memory loops associated with product autocatalysis coupled to substrate inhibition in open reactive systems have been also considered (6).

The present paper presents a steady-state analysis of the characteristics of the simple monolinear three-component reaction chain



featuring coupled substrate and feedback product inhibition. The internal concentration responses to quasi steady-state changes in environmental substrate concentration are investigated for two model systems. These

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include a simple cell model, lumping the mass transfer resistances in a spherical shell encapsulating a homogeneous solution of the two enzymes, and a membrane model, where the diffusive resistance and the activity of the two enzymes are uniformly distributed across the membrane.

REACTION KINETICS

Enzyme E_1, catalyzing the first step of the considered two consecutive reactions scheme, is subject to both substrate inhibition and final product regulation. The rate of substrate transformation is thus given by

$$r_A = \frac{K_1}{1+(1/K_A C_A)(1+K_{AI}C_A^2+K_{CI}C_C)} \tag{1}$$

The second step is assumed to follow Michaelis–Menten kinetics; hence

$$r_B = \frac{K_2}{1+(1/K_B C_B)} \tag{2}$$

LUMPED ENZYME ACTIVITY AND MASS-TRANSFER RESISTANCES

Considering a thin membrane shell encapsulating a homogeneous solution of the two enzymes and immersed in a large volume of a solution of the reacting species, the intracellular steady-state concentration of the various components will correspond to the equality of the rates of intracellular reaction and mass exchange with the surrounding environment. Lumping the membrane diffusive resistance and that of the convective films on both sides into an overall mass transfer coefficient U, assumed equal for all species, the dynamic equilibrium mole balances around the cell become

$$(\bar{C}_A - C_A)/r_A = (\bar{C}_B - C_B)/r_B = (C_c - \bar{C}_c)/r_B = R/3U \tag{3}$$

Defining a reference concentration C_{ref}, Eq. (1), (2), and (3) may be put into dimensionless form and combined to give

$$X_A = F(X_B) = (\bar{X}_A + \bar{X}_B) - X_B - \phi_{BC}^2/(1+a_B/X_B) \tag{4}$$

$$X_C = X_f - (X_A + X_B) \tag{5}$$

$$X_B = \frac{\phi_{AC}^2}{1+a_A/F(X_B)+a_{AI}F(X_B)+a_{CI}[X_f - F(X_B)+X_B]/F(X_B)} + \bar{X}_B - \phi_{BC}^2/(1+a_B/X_B) \tag{6}$$

For a fixed set of bulk conditions, kinetic, and mass transfer parameters, the nonlinear algebraic equation (6) can be solved by any iterative algorithm to obtain either one or three real values of X_B. These may be subsequently substituted in Eqs. (4) and (5) to obtain either one or three corresponding values of X_A and X_c, respectively.

DISTRIBUTED ACTIVITY AND DIFFUSIVE RESISTANCE

The concentration distribution inside a membrane of thickness δ, with uniformly distributed activity of E_1 and E_2, and immersed in a solution of the reacting species, will be considered next. Assuming that the three reaction species have the same diffusion coefficient in the membrane, the differential steady state component mole balances are given by the set of nonlinear second-order differential equations:

$$\left(\frac{d^2C_A}{dL^2}\right)\Big/ r_A = \left(\frac{d^2C_B}{dL^2}\right)\Big/ (r_B - r_A) = \left(\frac{d^2C_c}{dL^2}\right)\Big/ (-r_B) = 1/D \tag{7}$$

Combining the system of equations (7) with (1) and (2) and putting into dimensionless form gives

$$\frac{d^2X_A}{dw^2} = \frac{\phi_{AM}^2}{1 + a_A/X_A + a_{AI}X_A + a_{CI}(X_c/X_A)} \tag{8}$$

$$\frac{d^2X_B}{dw^2} = \frac{\phi_{BM}^2}{1 + a_B/X_B} - \frac{\phi_{AM}^2}{1 + a_A/X_A + a_{AI}X_A + a_{CI}(X_c/X_A)} \tag{9}$$

$$\frac{d^2X_C}{dw^2} = -\frac{\phi_{BM}^2}{1 + a_B/X_B} \tag{10}$$

Assuming that all three components have the same convective film coefficient, k, and that the bulk concentrations are equal on both sides of the membrane, the six boundary conditions of Eq. (7) become

$$[(dX_A/dw)/(\bar{X}_A - X_A)]_{L=\delta} = Sh = -[(dX_A/dw)/(\bar{X}_A - X_A)]_{L=0} \tag{11}$$

$$[(dX_B/dw)/(\bar{X}_B - X_B)]_{w=1} = Sh = -[(dX_B/dw)/(\bar{X}_B - X_B)]_{w=0} \tag{12}$$

$$[(dX_c/dw)/(\bar{X}_c - X_c)]_{w=1} = Sh = -[(dX_c/dw)/(\bar{X}_c - X_c)]_{w=0} \tag{13}$$

The system of equations (8)–(13) has been solved numerically using the collocation method (7). Four internal collocation points were used to generate the intramembrane concentration profiles.

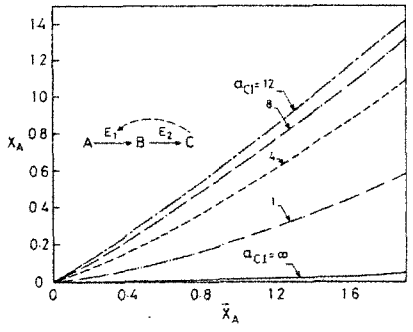

FIG. 1. Effect of feedback regulation on the static behavior of substrate concentration, $a_{AI} = 0$.

RESULTS

The dependence of the internal concentration on the bulk concentration of A has been obtained for fixed values of bulk concentrations of B and C ($\bar{X}_B = 0.4$ and $\bar{X}_C = 0.2$). All mass transfer and kinetic parameters (e.g., ϕ^2, Sh, a_A, a_B) have been also fixed, except a_{AI} and a_{CI}, which were assigned different values to show the effect of substrate and product inhibition. Results obtained for the cell system are presented in Figs. 1–7, and those pertaining to the membrane system are given in Figs. 8 and 9.

Figures 1–3 show that as the regulatory effect of C on E_1 increases, in the absence of inhibition by excess substrate, the intracellular concentration of A increases, while those of B and C decrease. As $a_{CI} \to \infty$, the intracellular concentrations of B and C reach equilibrium values, determined by the bulk concentrations of B and C. Figure 4 shows the static response when E_1 is inhibited by excess A without any product regulation effect. Up to three steady-states may exist for one and the same bulk concentration, which is typical of substrate inhibited kinetics. It was also found that as a_{AI} increases, the region of catastrophic behavior appears at lower bulk concentrations of A.

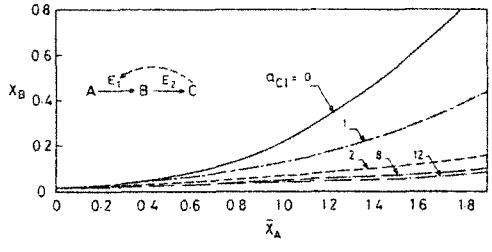

FIG. 2. Intermediate product concentration response, $a_{AI} = 0$.

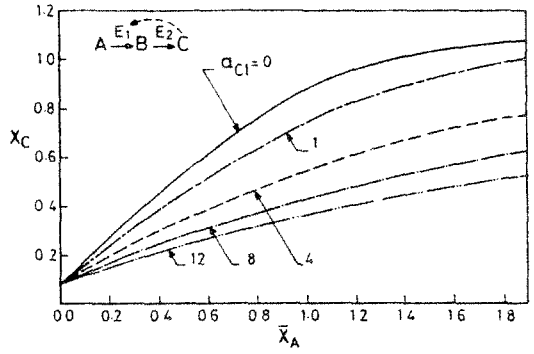

FIG. 3. Final product concentration response, $a_{AI} = 0$.

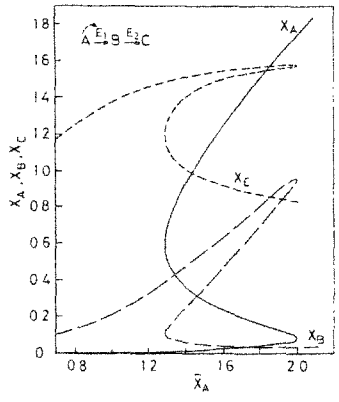

FIG. 4. Concentration responses in the presence of substrate inhibition, $a_{AI} = 10$, $a_{CI} = 0$.

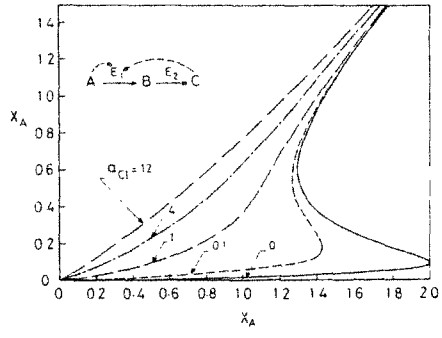

FIG. 5. Effect of a_{CI} on the response of A, $a_{AI} = 10$.

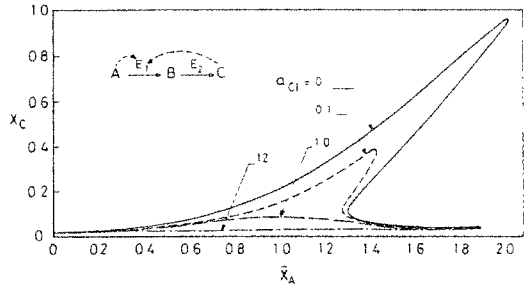

FIG. 6. Effect of a_{CI} on the response of B, $a_{AI} = 10$.

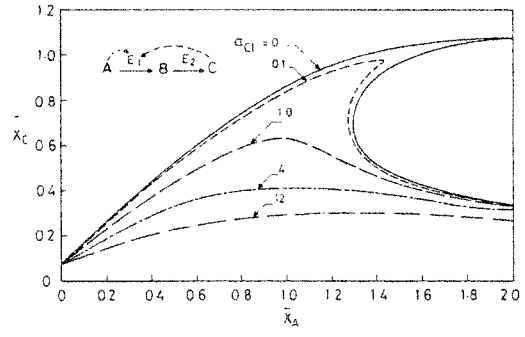

FIG. 7. Effect of a_{CI} on the response of C, $a_{AI} = 10$.

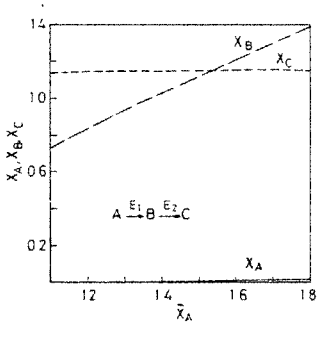

FIG. 8. Concentration responses inside the membrane at $W = 0.125$, in the absence of inhibitory effects, $a_{AI} = 0$, $A_{CI} = 0$.

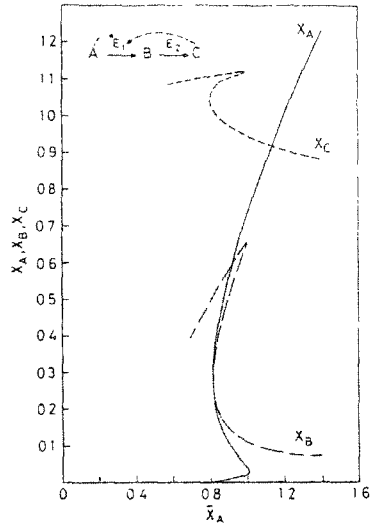

FIG. 9. Memory loops inside the membrane at $w = 0.125$, $a_{AI} = 10$, $a_{CI} = 0.1$.

Figures 5, 6, and 7 demonstrate the effect of changing the feedback regulation effect in the presence of substrate inhibition. The limiting case $a_{CI} = 0$ is also shown. It is seen that as a_{CI} increases, the range of multiplicity becomes smaller, till the hysteresis loop disappears altogether.

The results obtained for the distributed model are in general qualitative agreement with those obtained for the cell model. The internal concentration varies, however, along the depth inside the membrane. Figure 8 gives the static response for the case where both reaction steps follow Michaelis–Menten kinetics. Figure 9 depicts the memory loops obtained at an internal point for the more general case $a_{AI} \neq 0$ and $a_{CI} \neq 0$. In this case also, increasing the sensitivity to inhibition by C suppresses the catastrophic behavior imparted by substrate inhibition.

CONCLUSIONS

The static characteristics of simple systems featuring coupling between mass transfer and a two-step enzyme reaction have been investigated. The nonlinear algebraic equations describing the encapsulated two-enzyme system and the nonlinear two-point boundary value differential equations describing the distributed system have been solved numerically. A maximum of three steady states was observed over a range of parameters. The middle conversion steady state was found to be always unstable.

The general trend of the static response is the same for both systems. The multiplicity effect is nevertheless more pronounced in the lumped

system. In the absence of inhibition by excess substrate, the effect of feedback regulation is to raise the internal concentration of A and to lower those of B and C. In the presence of substrate inhibition, the effect of feedback regulation is to shrink the region of appearance of memory loops.

NOMENCLATURE

a_A	dimensionless constant $= 1/K_A C_{ref}$
a_{AI}	dimensionless substrate inhibition parameter $= K_{AI} \cdot C_{ref}/K_A$
a_B	dimensionless constant $= 1/K_B C_{ref}$
a_{CI}	dimensionless regulation parameter, K_{CI}/K_A
$\bar{C}_{A,B,C}$	bulk concentration (mol $\cdot$ cm^{-3})
$C_{A,B,C}$	local concentration of individual species A, B, C (mol $\cdot$ cm^{-3})
C_{ref}	reference concentration (mol cm^{-3})
D	diffusion coefficient (cm^2 s^{-1})
K_1	maximum rate for the step catalyzed by E_1 (mol cm^{-3} s^{-1})
K_2	maximum rate for the step catalyzed by E_2 (mol cm^{-3} s^{-1})
K_A	Michaelis–Menten constant for A (mol^{-1} cm^3)
K_B	Michaelis–Menten constant for B (mol^{-1} cm^3)
K_{AI}	substrate inhibition parameter (mol^{-2} cm^6)
K_{CI}	product regulation parameter (mol^{-1} cm^3)
k	external film transfer coefficient (cm s^{-1})
L	depth across the membrane (cm)
R	radius of the spherical shell (cm)
$r_{A,B}$	rate of reaction of species A, B (mol cm^{-3} s^{-1})
Sh	Sherwood number $= k\delta/D$
U	overall mass transfer coefficient (cm $\cdot$ s^{-1})
W	dimensionless distance $= L/\delta$
X	dimensionless concentration $= C/C_{ref}$
$\bar{X}$	dimensionless bulk concentration $= C/C_{ref}$
X_f	$(X_A + X_B + X_C)$
δ	membrane thickness (cm)
ϕ_{AC}	cell Thiele modulus for $A = (RK_1/3UC_{ref})^{1/2}$
ϕ_{AM}	membrane Thiele modulus for $A = (K_1\delta^2/DC_{ref})^{1/2}$
ϕ_{BC}	cell Thiele modulus for $B = (RK_2/3UC_{ref})^{1/2}$
ϕ_{BM}	membrane Thiele modulus for $B = (K_2\delta^2/DC_{ref})^{1/2}$

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