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The Effect of Hydrogen Ion on the Steady-State Multiplicity of Substrate-Inhibited Enzymatic Reactions

II. Transient Behavior

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Abstract

In this paper we concentrate our attention on the stability and transient behavior of the isothermal system (CSTR) with a substrate-inhibited enzyme reaction producing hydrogen ions. Our investigation covers the region of multiple steady states uncovered previously (1) (ordinary hysteresis and isola). We investigate the local stability characteristics of the different steady states, the effect of the initial condition on the transient behavior and the response of the system to feed disturbances of various magnitudes and durations.

Index Entries: Hydrogen ion effect, on substrate-inhibited enzyme reactions; substrate inhibition, and hydrogen ion effect; enzyme reactions, hydrogen ion effect on; steady-state multiplicity, of enzyme reactions; hysteresis; isola.

Introduction

In a previous paper (*1*) we have concentrated our attention on the phenomenon of multiplicity of the steady state in open reactive enzyme systems. For the case of substrate-inhibited reactions accompanied by hydrogen ion production we have

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uncovered a new type of hysteresis consisting of a closed curve on the multiplicity diagram that is disconnected from the rest of the multiplicity curve; this closed curve is called "isola" (*3*). In the present paper we investigate the transient behavior for this case in order to elucidate the factors that affect the dynamics of such a system; the specific system considered is an isothermal one (CSTR). The phenomenon of multiplicity investigated in the previous paper (*1*), together with the study of the transient behavior reported in the present paper, are quite important for the start-up and control of the system. This occurs because, if the optimum steady state of the system is in the multiplicity region, then the startup behavior, together with the transient behavior of the system, will decide which steady state the system will eventually reach. Also, if the system is operating at such an optimum steady state, then any disturbance may shift it to one of the other steady states, such that when the disturbance is removed, the system does not return to its original steady state. This type of behavior makes it necessary to find the region of stability for such a steady state in order to identify the maximum allowable disturbance, and hence to be able to design suitable control loops (*2–7*). *The mathematical model equations used to describe the isothermal system (CSTR) can also represent a perfectly mixed cell with all the mass transfer resistance concentrated at the wall of the cell, and for this case the hysteresis and the transient behavior are important for an understanding of the abrupt changes in biological systems caused by their responses to small changes in the environmental conditions (8–10).*

Kinetic Rate Equation for a Substrate-Inhibited Enzyme Reaction Producing Hydrogen Ions

We consider the general case where the product of the reaction is a fully ionized acid, the enzyme is sensitive to hydrogen ion concentration, and at the same time inhibited by excess substrate:

$$S \xrightarrow{\text{inh.}\ E} P_1 + P_2^- + H^+ \qquad (1)$$

The derivation of the rate of reaction equation is given in ref. (*1*) for both competitive and non-competitive inhibition mechanisms. The rate of reaction can be represented by the following function (*1*),

$$r(s, h) = \frac{V_m S}{S + \left(\dfrac{1 + h^2 + \delta \cdot h}{h}\right) + \alpha_I S^2} \qquad (2)$$

Where,

$$s = [S]/K_s \quad h = [H]/K_h, \ \delta = K_h/K_h'$$
$$\alpha_I = K_s/K_I \quad \text{(for the noncompetitive case)},$$
$$\alpha_I = K_s^2/K_I \quad \text{(for the competitive case)}$$

Formulation of the Unsteady State Material Balance Equations

We consider a reactor of volume V_c where an enzyme of concentration $\bar{E}$ catalyzes the formation of a fully ionized product P_2 [Equation (1)]. Material balance equations must be formulated for hydrogen ions and hydroxyl ions, as well as the substrate. The unsteady state material balance equation for hydogen and hydroxyl ions are,

$$q[\mathrm{H}]_f = q[\mathrm{H}] - (V_c \bar{E}) r([\mathrm{S}], [\mathrm{H}]) + R_w + V_c \, d[\mathrm{H}]/dt$$

and,

$$q[\mathrm{OH}]_f = q[\mathrm{OH}] + \mathrm{R}_w + V_c \, d[\mathrm{OH}]/dt \tag{4}$$

combining Eqs. (3) and (4) to eliminate R_w we obtain the following equation,

$$q([\mathrm{H}]_f - [\mathrm{H}]) - q([\mathrm{OH}]_f - [\mathrm{OH}]) = (V_c \bar{E}) r([\mathrm{S}]$$
$$[\mathrm{H}] + V_c \, d[\mathrm{H}]/dt + V_c \, d[\mathrm{OH}]/dt \tag{5}$$

we assume that H^+ and OH^- ions are essentially at equilibrium, hence,

$$[\mathrm{H}][\mathrm{OH}] = K_w \tag{6}$$

We use Eq. (6) to eliminate [OH] from Eq. (5) and we define the following dimensionless parameters.

$$B_s = V_m V_c \bar{E}/K_s q \quad B_h = V_m V_c E/K_h q$$
$$\gamma = K_w/K_h \quad \tau = V_c/q$$

Thus we obtain the following dimensionless unsteady-state mass balance equation for the hydrogen ion concentration,

$$\tau dh/dt = h^2/h^2 - \gamma[(h_f - h) - \gamma(1/h_f - 1/h) + B_h r'(s,h)] \tag{7}$$

Where,

$$r'(s,h) = r(s,h)/V_m$$

Similarly, mass balance on the substrated gives,

$$\tau ds/\mathrm{dt} = s_f - s - B_s r'(s,h) \tag{8}$$

Steady State

Under steady-state conditions, Eqs. (7) and (8) become

$$s_f - s = B_s r'(s,h) \tag{9}$$

and,

$$h_f - h - \gamma(1/h_f - 1/h) = -B_h r'(s,h) \tag{10}$$

Equations (9 and 10) can be solved by the method explained in ref. (*1*) and the relations between the various parameters (s_f, B_s, B_h, h_f, ...) and the state variables (s, h) can thus be obtained. The detailed steady-state analysis is

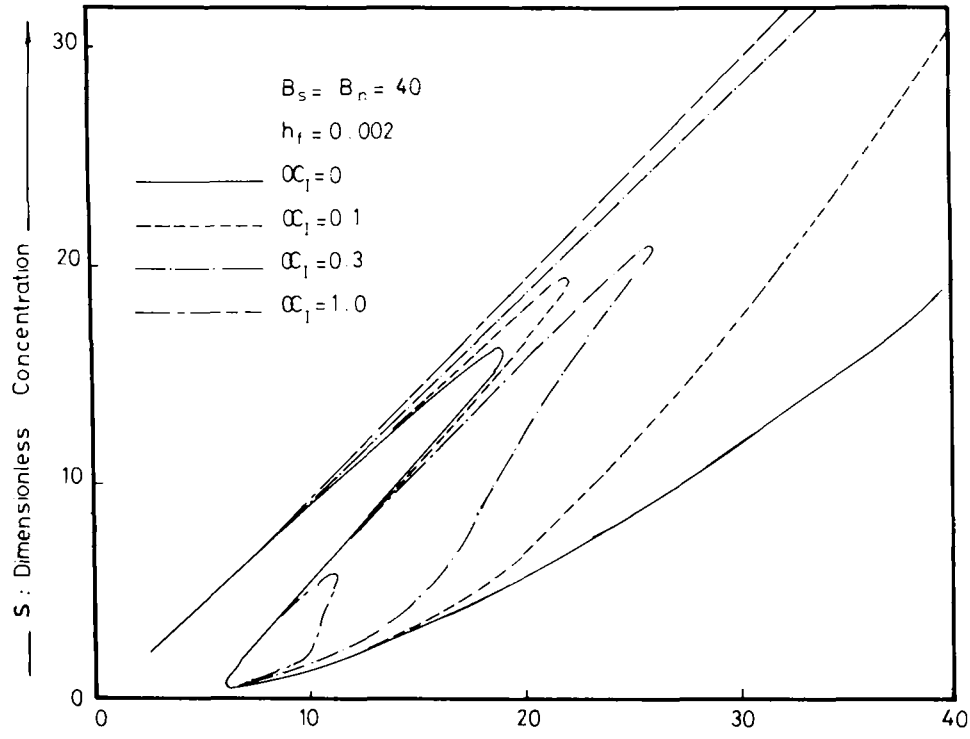

Fig. 1. Effect of α_I on the hysteresis, low value of $B(B = B_s = B_h = 40)$.

given elsewhere (*3, 13*); however, in this part of the present paper we offer only a sample of the steady-state results. The results are shown in Figs. (1–3), these results show the effect of the inhibition parameter α_I under different conditions. For four values of $\alpha_I = 0, 0.1, 0.3, 1.0$. The value of $\alpha_I = 0$ corresponds to simple hydrogen ion production without substrate inhibition. It is observed from Figs. 1–3 that as the value of α_I increases, the tail of the S-shaped hysteresis curve is shifted upwards until an "Isola" is formed at a certain value of α_I. The critical value of α_I (at which the isola is formed) increases as B increases. Further increase in α_I above the critical value causes the "Isola" to shrink until it completely disappears and the hysteresis becomes a single curve of unique steady states.

Unsteady State

The transient behavior of the system is obtained from the numerical solution of the unsteady state equations (*7, 8*) for given initial conditions.

Phase Plane Analysis Figure 4 shows how the system approaches its final steady state from different initial conditions for the same values of the parameters. The trajectories are drawn on a semilogarithmic scale, which relates the hydorgen ion and substrate concentrations. It is clear from Fig. 4 that there is a set of initial

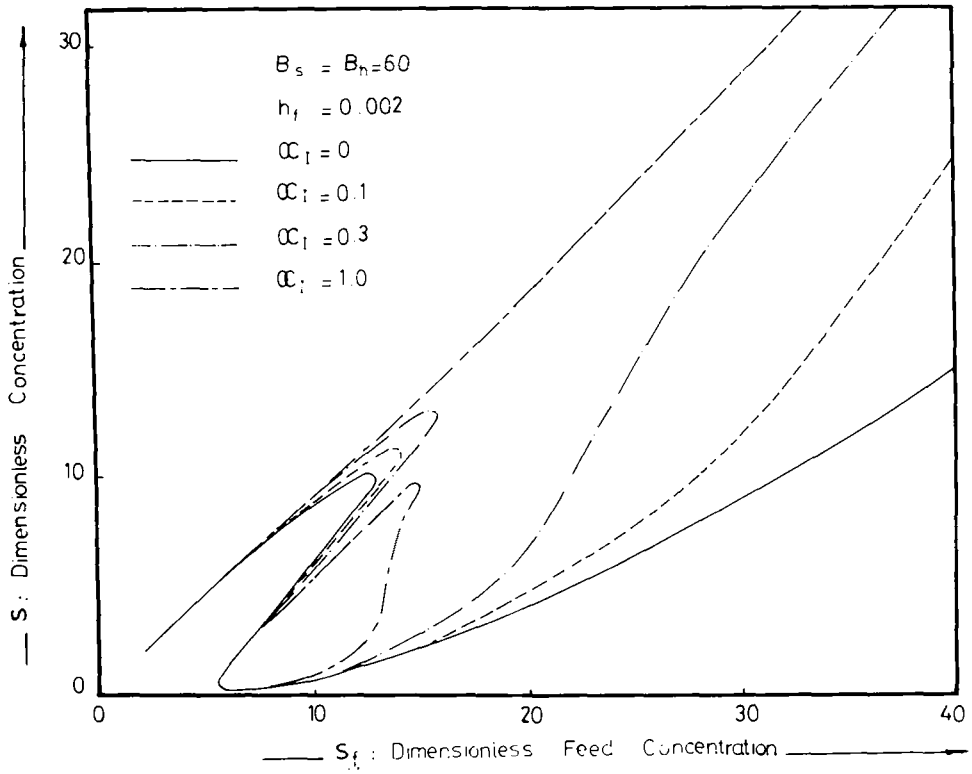

Fig. 2. Effect of α_I on the hysteresis, intermediate value of $B(B = B_s = B_h = 60)$.

conditions that leads the system to the high conversion steady state (steady state 2), while the other set of initial conditions leads to the low conversion steady state (steady state 1). The separatix is the dashed line A–B.

Local Stability Analysis Linearization of the unsteady-state material balance equations (*7*, *8*) in the neighborhood of the steady state and following the well-known local stability analysis for lumped parameter systems (*12*, *13*), we obtain the following two conditions for the stability of the steady states,

(1) $g_{11} + g_{22} < 0$
(2) $g_{11}g_{22} - g_{12}g_{21} > 0$

Where,

$$g_{11} = -1 - B_s \cdot \partial \dot{r}/\partial_s$$
$$g_{12} = -B_s \partial \dot{r}/\partial h$$
$$g_{21} = B_h \partial \dot{r}/\partial s$$
$$g_{22} = -1 - \gamma/h^2 + B_h \cdot \partial \dot{r}/\partial h$$

A sample of the local stability analysis results is given in Table 1. A detailed local stability analysis is given elsewhere (*13*). Table 1 gives the values of the eigenvalues, λ_1, λ_2 for the different steady states considered. It is observed that in

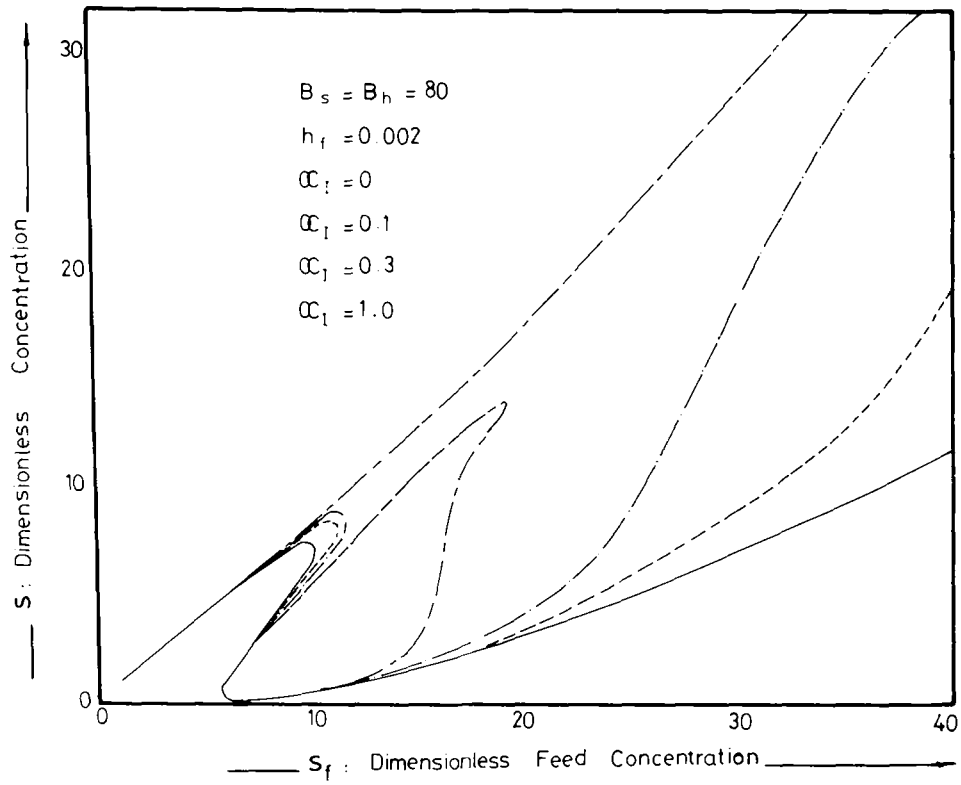

Fig. 3. Effect of α_I on the hysteresis, high value of $B(B = B_s = B_h = 80)$.

the case of multiple steady states the middle one is always unstable, while the other two steady states are always stable. Unique steady states are always stable.

Response of the System to Various Input Stimuli

It is important to investigate the response of the system when subjected to a sudden change in the feed concentration. The duration of the change will affect the transient behavior as shown in Figs. 5–10 (the parameters are the same as the parameters in Fig. 4).

Figures 5–7 show the response of the system after a step-input stimulus in the dimensionless feed concentration from 10 to 24. Figure 5 shows how the system attains the uniquely stable steady state corresponding to $s_f = 24$. Figure 6 shows that if the input stimulus is applied for a duration of $t/\tau = 7.0$, and is then removed, the system will not recover its original steady state, but will rather tend to go the high-conversion stable steady state. Figure 7 shows that if the input stimulus is applied for a duration of $t/\tau = 5.0$, and then removed, th e system will recover its original steady state. In general, for this case the system recovers its original steady state if the dimensionless duration of the disturbance, t/τ, is less than 6.2. Figures 8–10 show the response of the system after a step input stimulus in the di-

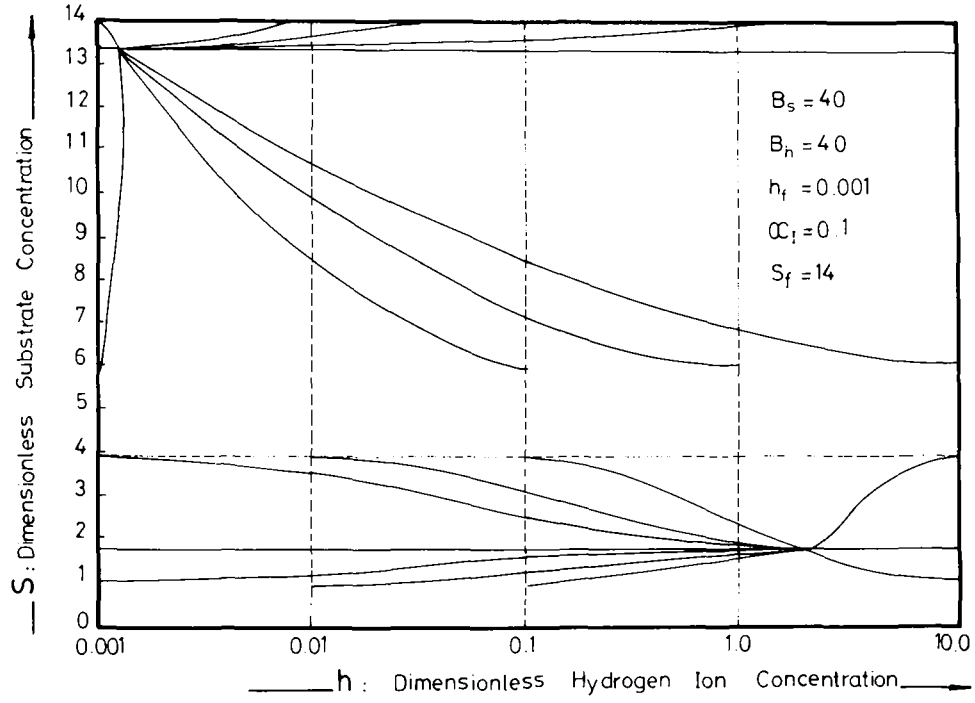

Fig. 4. Effect of initial conditions on the transient behavior of the system during startup.

Table 1
Local Stability Analysis: The Eigenvalues for Different Values of the Parameters

Values of the parameters							
B_s	B_h	h_i	α_I	S_f	λ_1	λ_2	Stability
100	100	0.002	0.1	2	−1998.7	−1.25	Stable
				10	−16.8	−0.99	Stable
				18	−7.7	−0.925	Stable
				26	−4.6	−1.00	Stable
60	60	0.002	0.1	2	−2178.3	−1.6	Stable
				10	−10.21	−1.01	Stable
				10	−0.8	+211.2	Unstable
				10	−853.1	−1.6	Stable
				18	−4.61	−1.13	Stable
				26	−2.5	−1.01	Stable
80	80	0.002	0.1	2	−2085.6	−1.1	Stable
				10	−13.5	−1.11	Stable
				10	−0.6	+271.8	Unstable
				10	−297.9	−1.35	Stable
				18	−6.07	−1.00	Stable
				26	−3.62	−1.95	Stable

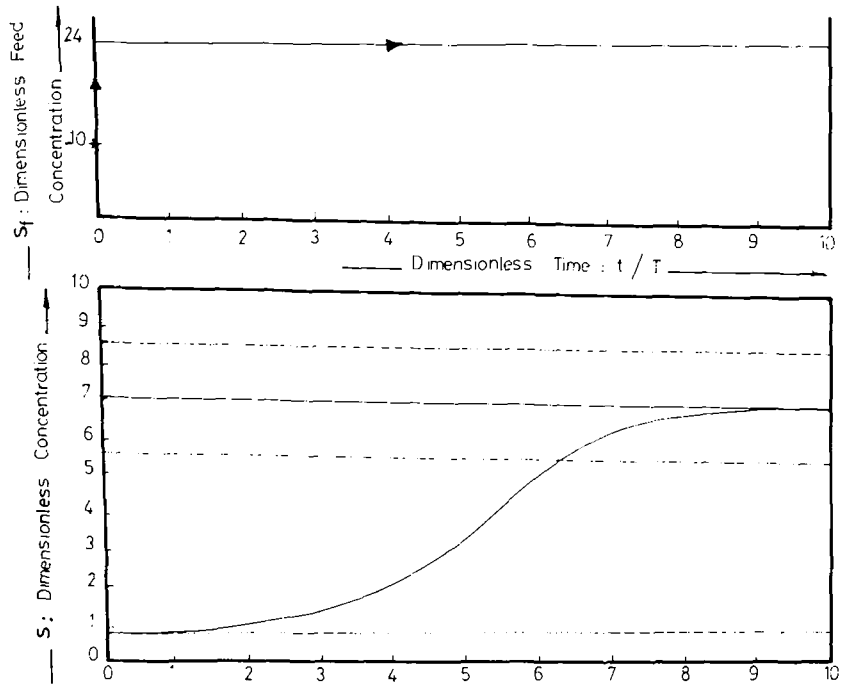

Fig. 5. Response of the system to a step input stimulus in s_f from 10 up to 24.

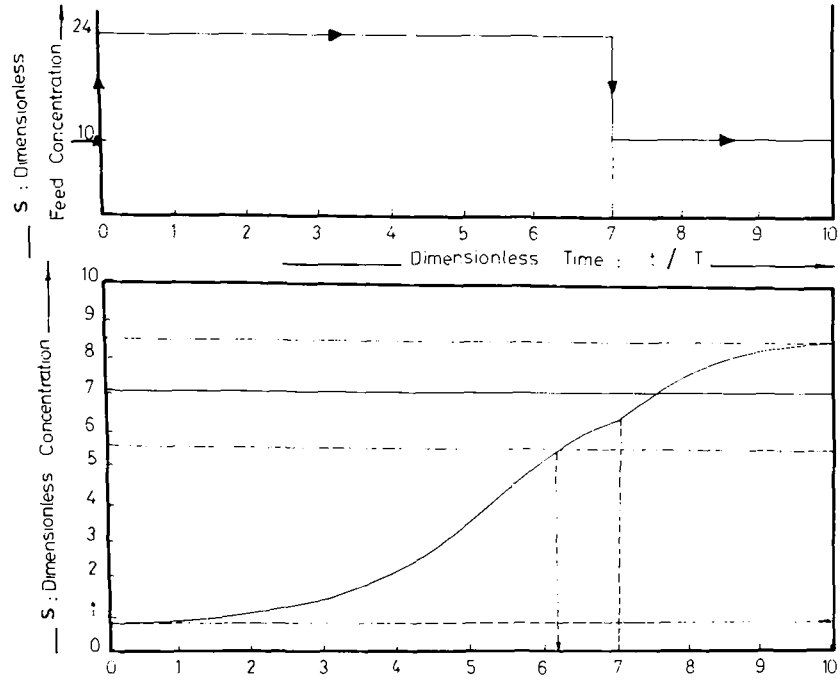

Fig. 6. Response of the system to a square pulse in s_f of duration $t/\tau = 7.0$.

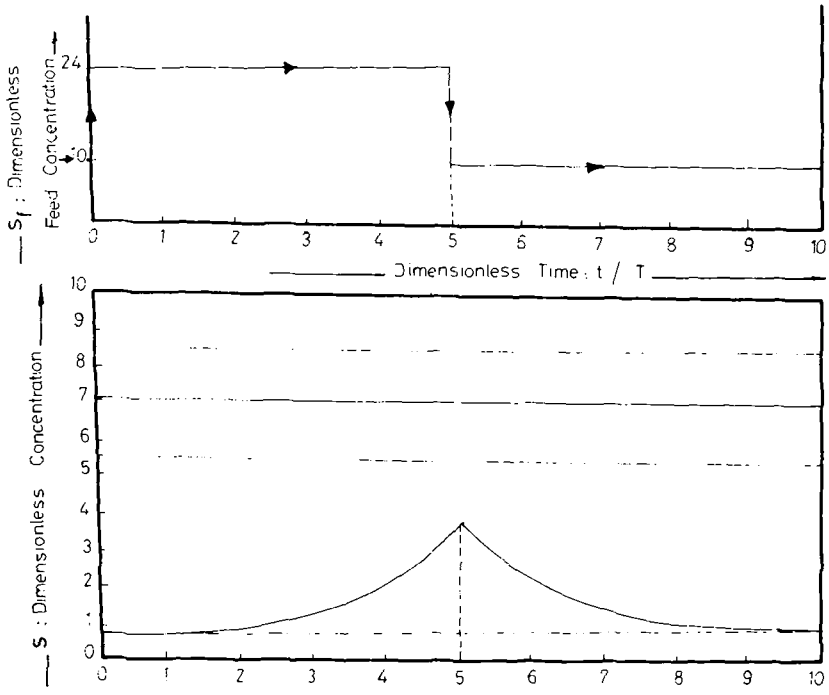

Fig. 7. Response of the system to a square pulse in s_f of duration $t/\tau = 5.0$.

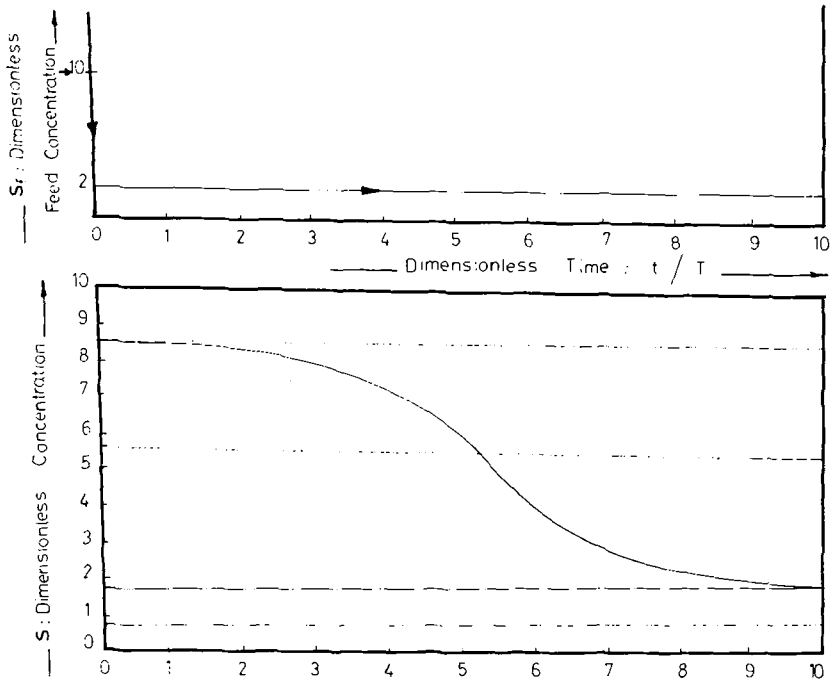

Fig. 8. Response of the system to a step stimulus in s_f from 10 to 2.

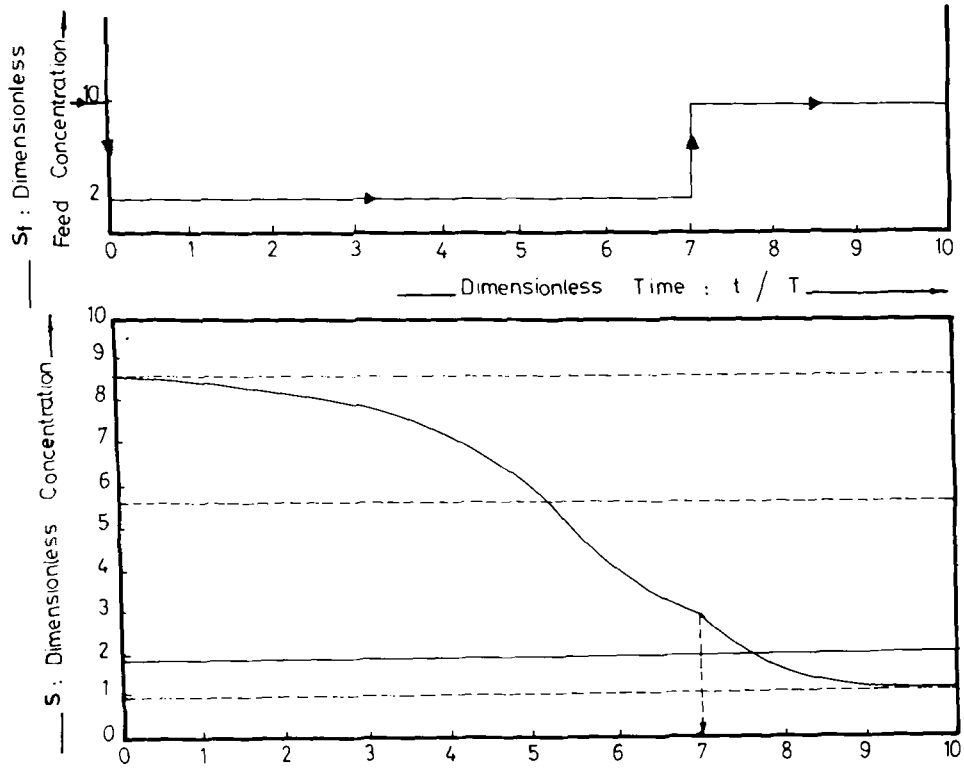

Fig. 9. Response of the system to a square pulse in s_f of duration $t/\tau = 7.0$.

mensionless feed concentration from 10 to 2. In this case the system will recover its original steady state if t/τ is less than 5.2. If t/τ is greater than 5.2, the system will move to the low-conversion steady state after the removal of the disturbance (Figs. 9 and 10).

It is clear from the above results that the size of the disturbance and its duration both affect the reversibility of the state of the system after the removal of the disturbance. If the system is operating at the high conversion (low concentration) steady state and then the feed concentration is increased, the concentration of the substrate in the system increases consequently. If the duration of the disturbance is long enough, the enzyme is inhibited and the system does not return to its original steady state when the disturbance is removed. If the system is operating at the low-concentration (high substrate concentration) inhibited steady state, then the decrease in the feed concentration causes the substrate concentration in the system to decrease and if the duration of the disturbance is long enough the concentration of the substrate decreases to the extent that the enzyme is activated and when the disturbance is removed the system does not return to its original steady state, but it goes to the high conversion steady state.

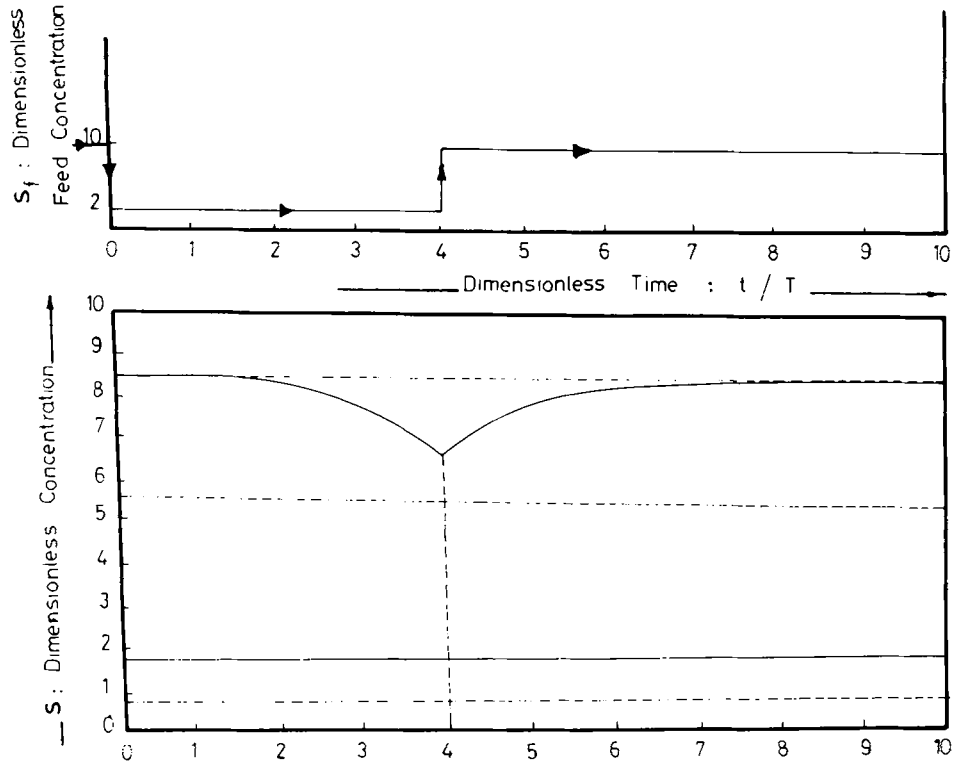

Fig. 10. Response of the system to a square pulse in s_f of duration $t/\tau = 4.0$.

Conclusions

The phenomenon of multiplicity in enzyme reactors has an important effect on the transient behavior of the reactor for both startup and response to feed disturbances. In the region of multiplicity, the duration and magnitude of the feed disturbance strongly affect the productivity of the reactor after the removal of the disturbance.

Notations

B_s	dimensionless parameter = $V_m V_c \bar{E}/K_s q$
B_h	*dimensionless parameter* = $V_m V_c \bar{E}/K_h q$
E_t	total concentration of enzyme active sites, gmol/g enzyme.
$\bar{E}$	concentration of enzyme in the reactor, g enzyme/L
[H]	hydrogen ion concentration in the reactor, g-mol/L
h	dimensionless hydrogen ion concentration = $[H]/K_h$

k rate constant for the reaction on the active site, s^{-1}
K_h dissociation constant for the formation of the inactive enzyme form, g-mol/L
K_h' dissociation constant for the formation of the enzyme-active form, g-mol/L
K_s dissociation constant for the formation of the enzyme complex (ES), g-mol/L
K_I dissociation constant for the formation of the inhibition complex (ES_2), g-mol/L
[OH] hydroxyl-ion concentration in the reactor, g-mol/L
q volumetric flow rate, L/s
r rate of reaction, g-mol/s g-enzyme
r' dimensionsless rate of reaction = r/V_m
R_w rate of water formation, g-mol/s L
[S] substrate concentration in the reactor, g-mol/L
s dimensionless substrate concentration = $[S]/K_s$
t time, s
V_m = kE_t, g-mol/s g-enzyme
V_c = reactor volume, L

Greek Letters

α_I dimensionless inhibition constant = K_s/K_I, K_s^2/K_I
δ dimensionless parameter = K_h/K_h
γ dimensionless parameter = K_w/K_h^2
τ residence time = V_c/q, s

Subscripts

f feed conditions
h hydrogen ions
I inhibition
s substrate

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