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

I. Steady-State Considerations

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Abstract

The phenomenon of multiplicity is investigated for an isothermal continu ous stirred tank reactor (CSTR) in which an enzyme reaction producing hydrogen ions is taking place. The activity of the enzyme considered is sensitive to the hydrogen ion concentration and is inhibited by excess substrate. The enzyme is bound and the washout phenomenon is negligible.

The investigation has uncovered a new type of hysteresis, consisting of a closed curve on the multiplicity diagram, and is disconnected from the rest of the multiplicity curve. This phenomenon has also been observed and analyzed by Uppal et al. (*1*) in their investigation of the non-isothermal, non-adiabatic (CSTR), and they have termed those closed curves "Isolas." In the present paper we have elucidated the physical reason for the occurrence of those "Isolas" for the enzymatic reaction under consideration. We have also investigated the effect of different parameters on the appearance, disappearance, and size of the "Isolas."

Index Entries: Hydrogen ion, effect on immobilized enzyme reactions; immobilized enzyme reactions, hydrogen ion effect on; enzyme reactions, hydrogen ion effect on immobilized; multiplicity, of immobilized enzyme reactions; steady-state immobilized enzyme reactions, hydrogen ion effect on; isolas, and steady-state enzyme reactions.

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Introduction

Great attention has been given in the last 20 yr to the multiplicity phenomenon in open reacting systems and its associated hysteresis (i.e., short-term memory) phenomenon. This phenomenon means simply that, for a fixed input to the system under consideration, the steady state of the system is not uniquely determined by the system's parameters. The specific steady state of the system in this case depends upon the previous history of the system (i.e., for the same physico-chemical parameters and feed conditions, the reaction may attain different steady states depending upon its previous history). The multiplicity results from the coupling between a number of processes taking place within the system, one of these processes at least is a non-monotonic function of the state of the system. For a substrate-inhibited enzyme reaction, the dependence of the rate of reaction upon substrate concentration is a nonmonotonic function and multiplicity of the steady state for such a reaction when it takes place in an open system [(CSTR), perfectly mixed cell . . . etc.)] is known to occur (*2–7*). When the kinetics of the reaction obey a Michaelis-Menten (M-M) expression, then multiplicity of the steady state does not occur because the rate of reaction, which is the nonlinear process, is a monotonic function of the state of the system (*8–10*). However, multiplicity of the steady state is possible with M-M kinetics when the reaction produces hydrogen ions and the enzyme reaction rate is sensitive to the hydrogen ion concentration (e.g., the hydrolysis of an ester by an enzyme such as chymotripsin) (*11–13*). This results from the dependence of the enzyme reaction rate on the hydrogen ion concentration being a nonmonotonic function. When the enzyme is inhibited by excess substrate and the enzyme reaction produces hydrogen ions simultaneously, then the multiplicity phenomenon becomes more complex. This complexity is found because the rate of reaction depends upon two of the state variables of the system (i.e., substrate concentration and hydrogen ion concentration) and the dependence of the rate of reaction on each of these variables (with the other kept constant) is a nonmonotonic function (an example of such an enzyme is acetylocholinesterase). The purpose of this paper is to investigate the complex multiplicity phenomenon for this last case. The system considered is an isothermal (CSTR), however, the same equations can also represent a perfectly mixed cell with all the mass transfer resistance concentrated at the wall of the cell.

Steady-State Model

Consider a constant-feed isothermal continuously stirred tank reactor (CSTR) in which an enzyme reaction takes place in the liquid phase (*14, 15*).

We will treat the general case, in which the product of the reaction is a fully ionized acid and the enzyme is sensitive to hydrogen ion concentration and is inhibited by excess substrate,

$$S \xrightarrow[\text{inhibition}]{E_1} P_1 + P_2^- + H^+ \qquad (1)$$

One possible rate expression for such a case is (See Appendix):

$$r(\mathrm{S,H}) = \frac{V_m[\mathrm{S}]}{[\mathrm{S}] + \{K_s(K_h + [\mathrm{H}] + [\mathrm{H}]^2/K'_h)/[\mathrm{H}]\} + [\mathrm{S}]^2/K_i} \tag{2}$$

where [S] is the substrate concentration, [H] is the hydrogen ion concentration, V_m is the maximum reaction rate per unit mass of enzyme, the K values are equilibrium constants for the enzyme, and K_i is an inhibition constant.

If the active reactor volume is V_R and the enzyme concentration is $\bar{E}$(g enzyme/active reactor volume), and the volumetric feed flow rate is q, then the material balance on the hydrogen ions gives,

$$q([\mathrm{H}]_f - [\mathrm{H}]) = -(V_R\bar{E})r(\mathrm{S,H}) + R_w \tag{3}$$

where $[\mathrm{H}]_f$ is the concentration of hydrogen ions in the feed and R_w is the rate of hydrogen ion consumption to form water molecules.

Similar material balance on the OH^- ions gives,

$$q([\mathrm{OH}]_f - [\mathrm{OH}]) = R_w \tag{4}$$

Combining Eq. (3) and (4) to eliminate R_w, we obtain,

$$q([\mathrm{H}]_f - [\mathrm{H}]) - ([\mathrm{OH}]_f - [\mathrm{OH}]) = -(V_R\bar{E})r(\mathrm{S,H}) \tag{5}$$

We assume that H^+ and OH^- ions are at equilibrium,

$$H^+ + OH^- \rightleftharpoons H_2O$$

and

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

where K_w is the equilibrium dissociation constant for water. Substitution form Eq. (6) into Eq. (5) gives the following mass balance equation for the hydrogen ions,

$$q([\mathrm{H}]_f - [\mathrm{H}]) - K_w(1/[\mathrm{H}]_f - 1/[\mathrm{H}]) = -(V_R\bar{E})r(\mathrm{S,H}) \tag{7}$$

We introduce the following dimensionless variables and groups,

$$s = [\mathrm{S}]/K_s \quad h = [\mathrm{H}]/K_h \quad B_s = V_mV_R\bar{E}/K_sq \quad B_h = V_mV_R\bar{E}/K_hq$$
$$\gamma = K_w/K_h^2 \quad \delta = K_h/K'_h$$

Equation (7) can be written in the dimensionless form,

$$(h_f - h) - \gamma[(1/h_f) - (1/h)] + B_hr'(s,h) = 0 \tag{8}$$

where,

$$r'(s,h) = \frac{s}{s + [(1 + h + \delta h^2)/h] + \alpha_I s^2} \tag{9}$$

is called the dimensionless reaction rate and $\alpha_I = K_s/K_i$ is called the dimensionless substrate-inhibition parameter.

Similarly, for the steady-state substrate mass balance, we obtain:

$$s_f - s = B_s r'(s,h) \tag{10}$$

Method of Solution

Equations (8) and (10) are nonlinear algebraic equations and may possess multiple solutions for certain combinations of the parameters. The equations must be solved a large number of times in order to provide the kind of parametric study we are aiming at. To simplify our task we try to decouple the two equations in order to be able to reduce the problem to that of solving a single nonlinear algebraic equation. This is achieved by multiplying Eq. (10) by B_h and Eq. (8) by B_s and adding the two resulting equations to obtain,

$$B_h(s_f - s) + B_s(h_f - h) - \gamma B_s[(1/h_f) - (1/h)] = 0 \tag{11}$$

Equation (11) can be arranged to obtain (s) as a function of h.

$$s = s_f + (B_s/B_h)\{(h_f - h) - \gamma[(1/h_f) - (1/h)]\} \equiv f(h) \tag{12}$$

Therefore, Eq. (8) reduces to:

$$(h_f - h) - \gamma[(1/h_f) - (1/h)] = -B_h r[f(h), h] \tag{13}$$

Thus the problem reduces to the solution of Eq. (13) to obtain h, then the corresponding s is obtained from Eq. (12).

Results and Discussion

We consider three cases with respect to the kinetics of the reaction. We concentrate our attention on the steady-state behavior of the system: the unsteady state and its stability characteristics are discussed elsewhere (*6*, *13*, *19*).

Michaelis-Menten Kinetics (No Substrate Inhibition) with Hydrogen Ion Production

In this case the dimensionless rate of reaction term reduces to:

$$r(s,h) = \frac{s}{s + [(1 + h + \delta h^2)/h]} \qquad \text{i.e., } \alpha_I = 0 \tag{14}$$

Under these conditions, multiplicity of the steady state is possible; Fig. 1 shows the hysteresis (short-term memory) loops for such a case. The figure also shows the effect of varying h_f on the hysteresis loop. It is evident from the figure that, as h_f increases, the range of multiplicity decreases and is shifted toward lower values of s_f. With regard to conversion, if we compare the two cases of $h_f = 0.0025$ and $h_f = 0.005$, we notice that for the higher branch (corresponding in this case to lower rate of reaction) the conversion increases as h_f increases, while for the intermediate and

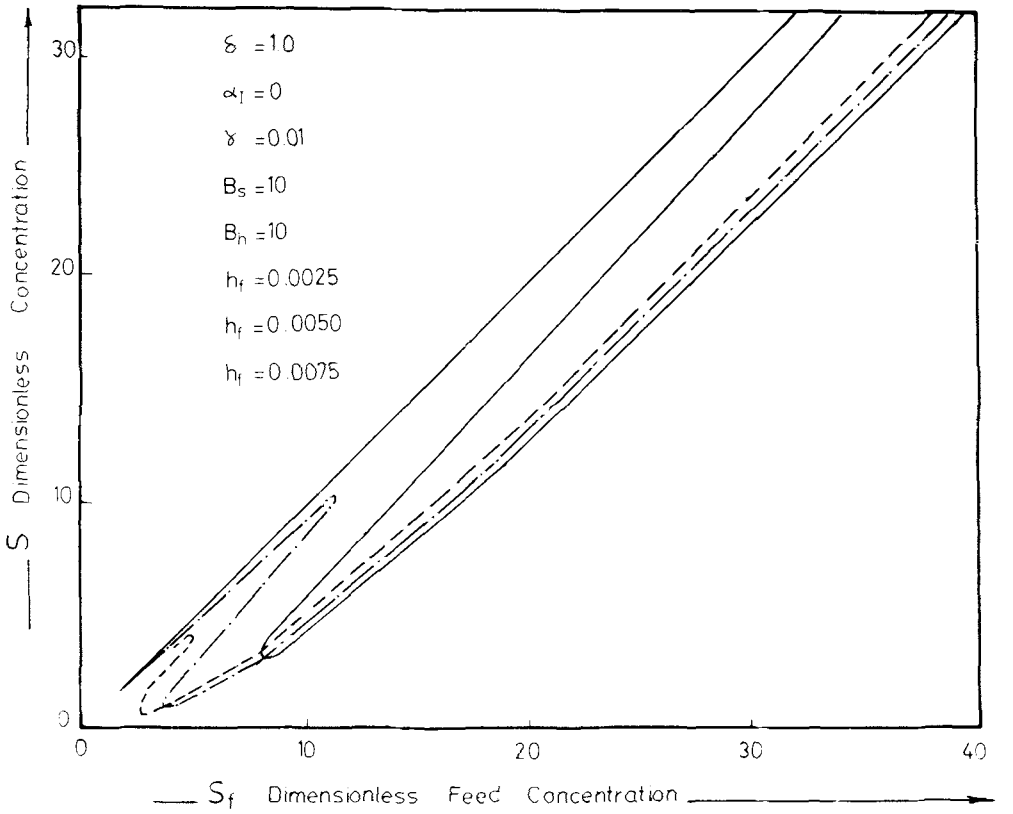


Fig. 1. Effect of h_f on the hysteresis loop. Hydrogen ion production without substrate inhibition. Single region of multiplicity ($\delta = 1$).

the lower branches the conversion decreases as h_f increases. These observations, of course, cannot be generalized since this effect depends upon the range of values of s_f, h_f, and the resulting s,h inside the reactor, and on whether they correspond to points before or after the maxima on the activity–pH curve. The effect of B_s is shown in Fig. 2; it is clear that as B_s increases there is a slight shift of the multiplicity region towards higher values of s_f and the range of multiplicity is negligibly affected.

In Fig. 3, it is noticed that as B_h increases there is a remarkable reduction in the multiplicity range and a large shift to lower values of s_f.

It must be clear that each hysteresis curve is a continuum of steady-state solutions representing the steady-state response of the reactor to slow variations in the feed conditions.

A more detailed parametric investigation of this case is given elsewhere (*13*).

Substrate-Inhibition Kinetics Without Hydrogen Ion Production

This is a well-known and extensively studied case that is known to give rise to multiplicity of the steady states (*3–7*).

In this case the dimensionless rate of reaction is given by,

$$r(s,h) = s/1 + s + \alpha_I s^2 \tag{15}$$

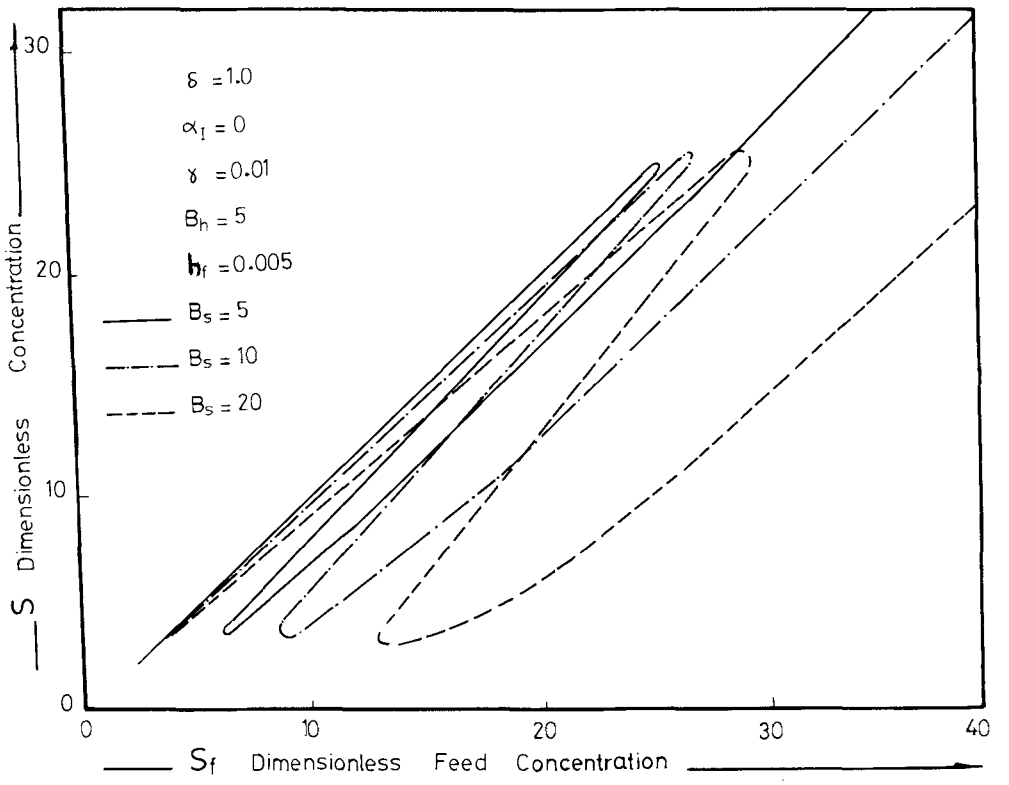


Fig. 2. Effect of B_s on the hysteresis loop. Hydrogen ion production without substrate inhibition. Single region of multiplicity ($\delta = 1$).

The General Case of the Combined Effect of Substrate-Inhibition and Hydrogen Ion Production

In this case the phenomenon of multiplicity becomes more complicated because of the appearance of the "isolas," which are closed curves on the multiplicity diagram (s–s_f diagram) that are disconnected from the rest of the multiplicity curve.

The phenomenon of "isolas," in this case, occurs because the rate of reaction depends upon two state variables simultaneously, i.e., substrate and H^+ ion concentrations. The dependence of the rate of reaction on either of the two variables (while the other is kept constant) is nonmonotonic.

Therefore, the dependence of the rate of reaction on both variables when they are both changing is expected to be quite complex. We have also to notice that as s decreases h increases because h is a product of the reaction. Thus the rate of reaction may decrease or increase by the change of s_f depending upon the local conditions inside the reactor s,h and their combined effect on the rate of reaction. Therefore it is possible for the different branches of the multiplicity curve to bend in opposite directions with the change of s_f or any other parameter such as α_I, B_s, or B_h, thus approaching each other, causing maxima and minima in the branches, and ultimately meeting each other to form an "isola." It should be very clear that these isolas are not limit cycles; each isola is a closed curve representing a continuum of steady-state solutions for various values of the substrate feed concentration.

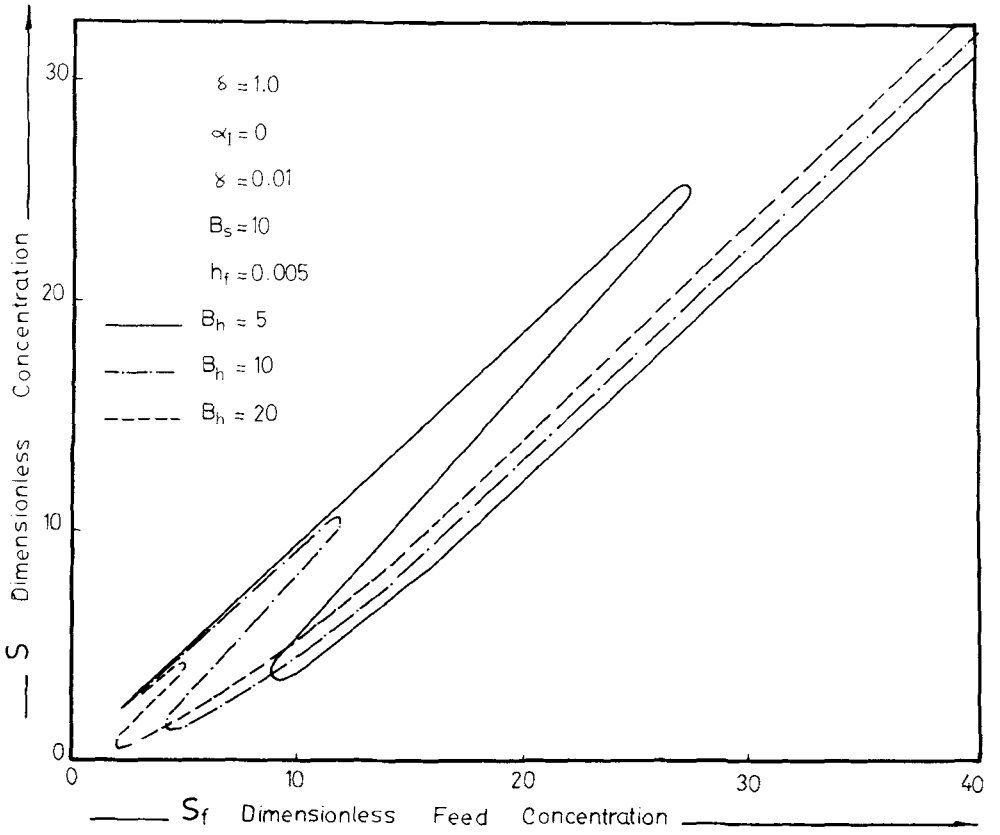


Fig. 3. Effect of B_h on the hysteresis loop. Hydrogen ion production without substrate-inhibition. Single region of multiplicity ($\delta = 1$).

Effect of α_I. The formation of the isola is shown in Fig. 4 for the special case of $\delta = 1.0$, and where all the system parameters are kept constant except α_I, which is increased from $\alpha_I = 0$ to $\alpha_I = 1.0$. It is important here to discuss the significance of the parameter δ before we proceed further. The parameter δ represents the ratio between the dissociation constant (K_h) for the formation of the inactive form of the enzyme E^{-2} and the dissociation constant (K'_h) for the formation of the active enzyme form $\bar{E}$ (see Appendix). The parameter δ is thus a measure for the sharpness of the maximum of the pH-activity curve; at $\delta = 1.0$ the maxima is a point, and for some enzyme systems, δ approach very closely the value 1.0 (*18*), as δ decreases the maximum becomes flatter and the enzyme activity increases (*17*, *18*).

For the case under consideration, i.e., $\delta = 1.0$, a single multiplicity region is observed, as shown in Figs. 4 and 5 for $\alpha_I = 0$ and 0.1. The formation of the isola takes place through the bending in opposite directions of the different branches of the hysteresis loop as shown in Fig. 4, where we notice that, as α_I increases, the lower branch bends upwards, while the middle branch bends downwards till the isola is formed for $\alpha_I = 0.3$. Further increase in α_I causes the isola to shrink till it disappears at high values, and with its disappearance the multiplicity of the steady state disappears.

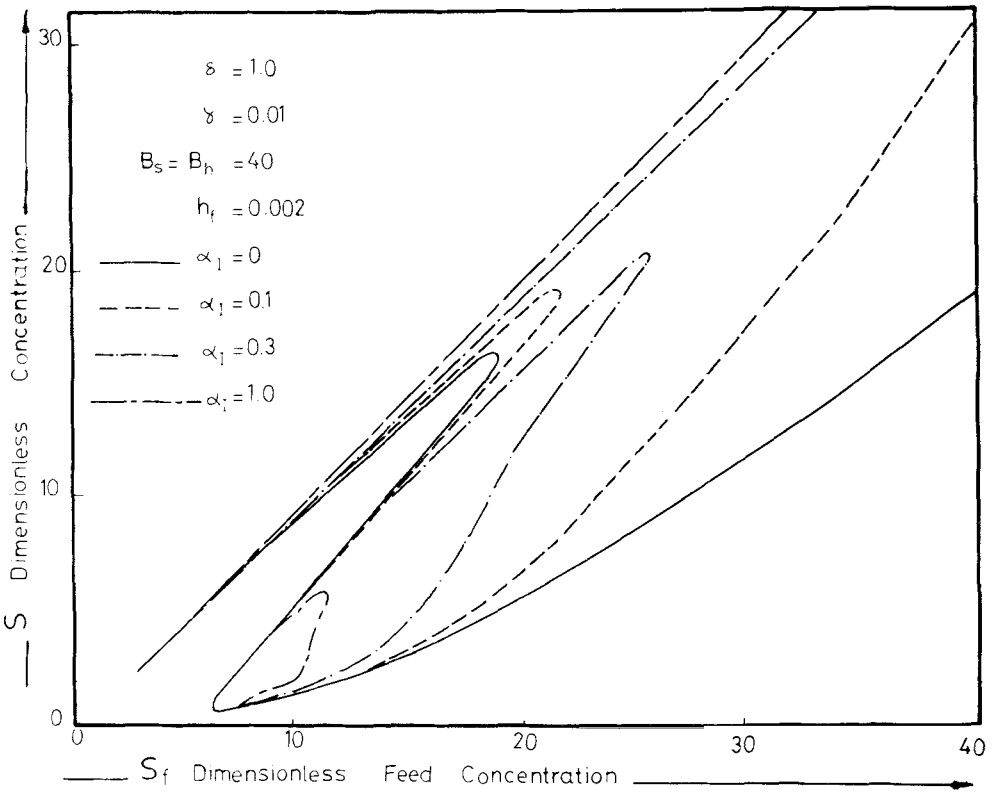


Fig. 4. The combined effect of substrate inhibition and hydrogen ion production. Effect of α_I on the appearance and disappearance of the isola for $\delta = 1$.

Effect of B. The effect of B_s, B_h (for the special case of $B_h = B_s = B$, $\delta = 1.0$) on isolas at constant α_I is opposite to the effect of α_I. The isolas increase in size as B increases and the bent branches that form the isola open up to form an ordinary hysteresis loop at high values of B. This effect is shown in Figs. 5–7.

In Fig. 5 for $B = 40$, $\alpha_I = 0.1$, an isola of considerable size is observed. As B increases, the lower branch bends downwards and the middle branch bends upwards (the isola opens up), and eventually for high values of B the isola disappears leaving an ordinary hysteresis loop.

In Fig. 6, the isola exists for all values of B considered (because $\alpha_I = 0.3$, which is higher than that in Fig. 5) and the size of the isola increases as B increases.

Figure 7 shows a case of $\alpha_I = 1.0$; for this case the isola also exists for all values of B, but it is much smaller than the isolas in Figs. 5 and 6, and the rate of increase in its size with the increase in B is rather slow.

Effect of δ. For cases with $\delta < 1.0$, the situation is quite different from the cases with $\delta = 1.0$, for $\delta < 1.0$, two distinct regions of multiplicity are observed, together with a narrow region of five steady states (see Fig. 8, for $\delta < 0.1$). Also for these cases the mechanism of the formation of the isola is quite different.

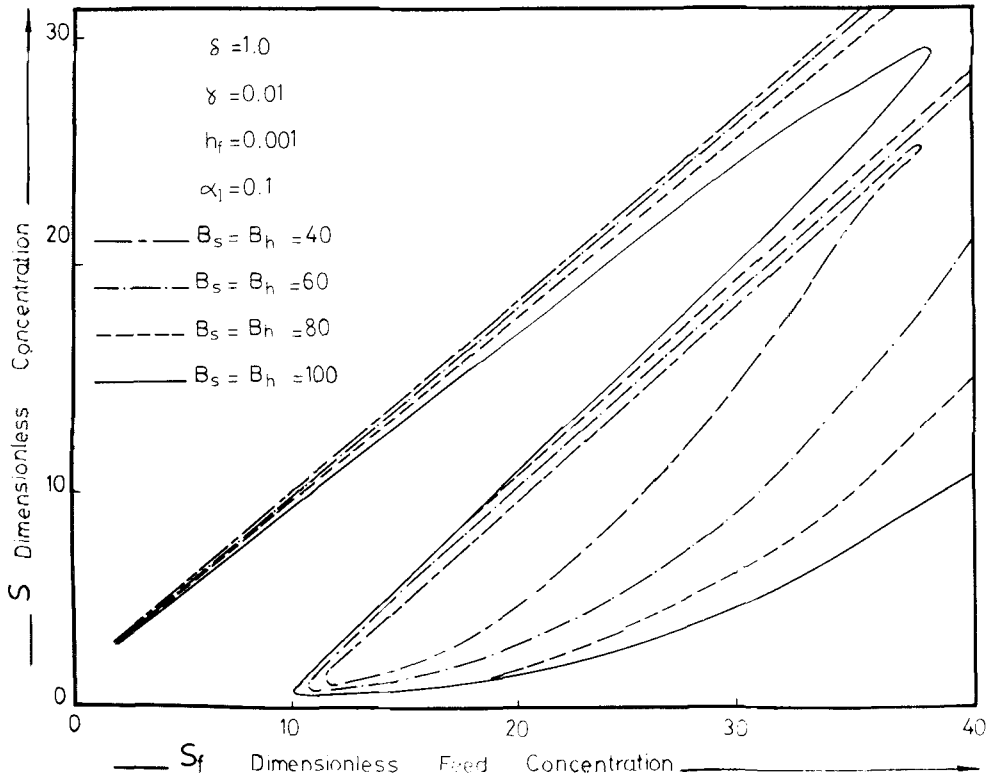
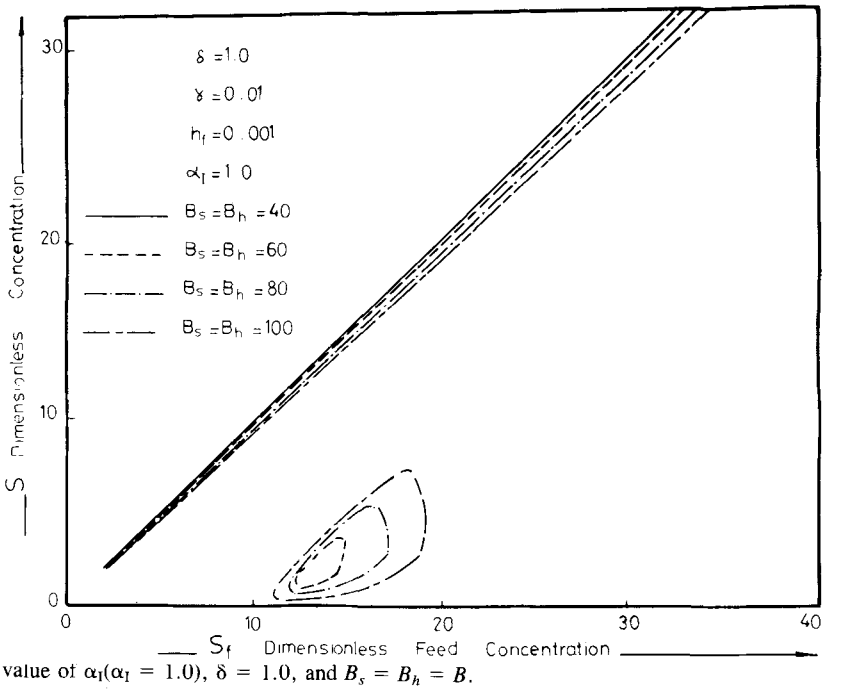


Fig. 5. The combined effect of substrate inhibition and hydrogen ion production. Effect of B_s, B_h on the appearance, disappearance, and size of the isola. A case of low value of $\alpha_I(\alpha_I = 0.1)$, $\delta = 1.0$ and $B_s = B_h = B$.

As shown in Figs. 8 and 9, the increase in α_I causes the first multiplicity region to grow in size and to move toward higher values of the feed substrate concentration, while the increase in α_I causes the second multiplicity region to grow in size and to move toward lower values of the feed substrate concentration. This movement of the two multiplicity regions in opposite directions continues with the increase in α_I until the two regions merge together at $\alpha_I = 0.7$, forming an isola with a narrow region of five steady states, as shown in Fig. 9. A further increase in α_I causes the isola to shrink and the region of five steady states to disappear (Fig. 9).

Conclusions

An open reactive enzyme system with substrate-inhibited enzyme and hydrogen ion production was investigated. The combined effect of substrate-inhibition and hydrogen ion production gives rise to a new multiplicity phenomenon, i.e., isolas that are closed, disconnected curves on the multiplicity diagram. The significance



value of $\alpha_I(\alpha_I = 1.0)$, $\delta = 1.0$, and $B_s = B_h = B$.

Fig. 6. The combined effect of substrate inhibition and hydrogen ion production. Effect of B_s, B_h on the appearance, disappearance, and size of the isola. A case of intermediate value of $\alpha_I(\alpha_I = 0.3)$, $\delta = 1.0$, and $B_s = B_h = B$.

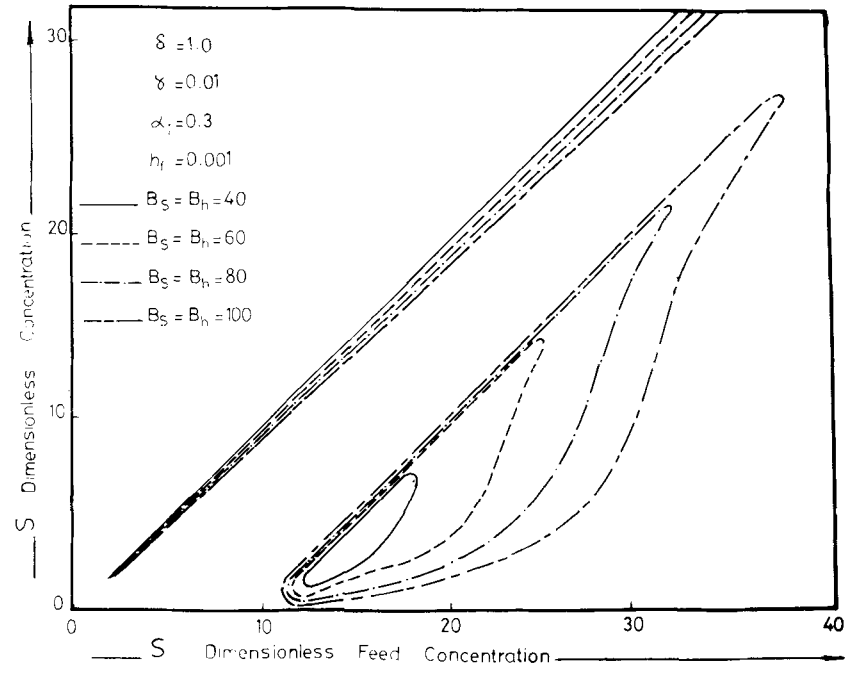


Fig. 7. The combined effect of substrate inhibition and hydrogen ion production. The effect of B_s, B_h on the appearance, disappearance, and size of the isola. A case of high value of $\alpha_I(\alpha_I = 1.0)$, $\delta = 1.0$, and $B_s = B_h = B$.

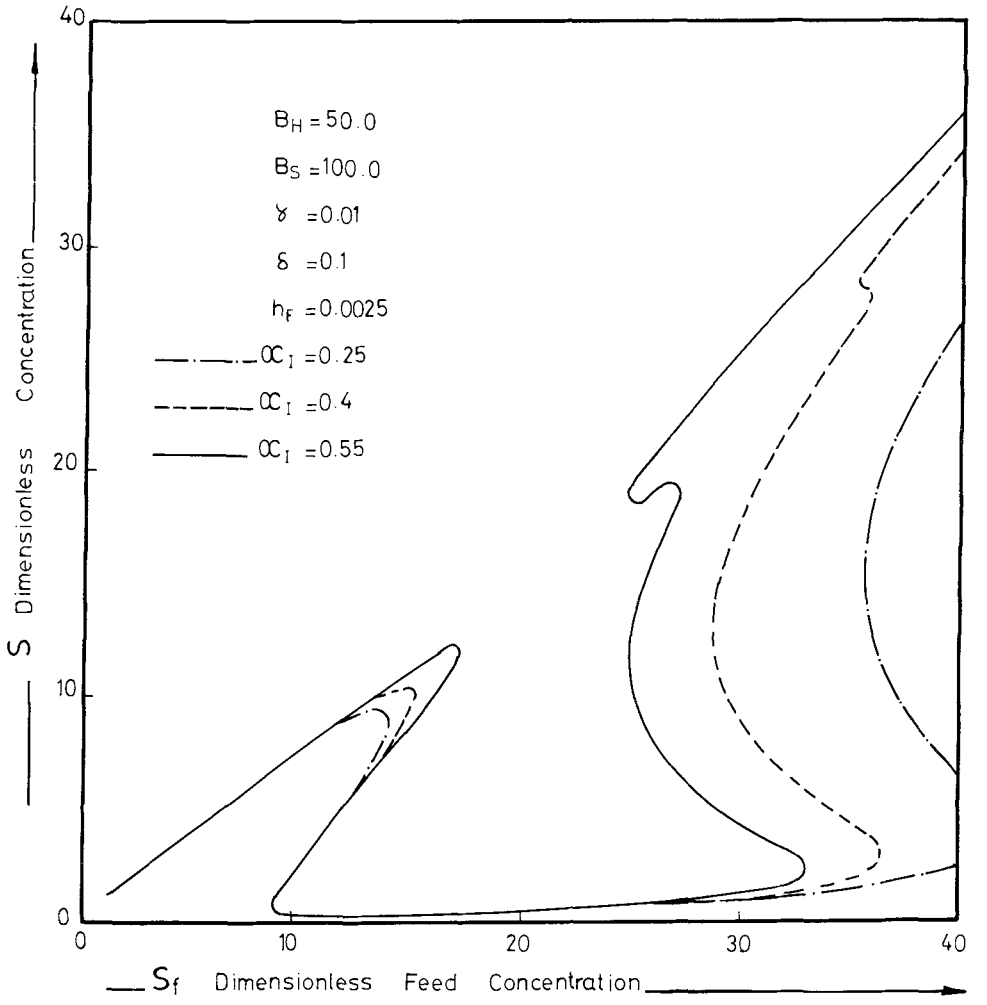


Fig. 8. The effect of α_I on the hysteresis phenomenon for a case of $\delta < 1.0$ ($\delta = 0.1$)

of these isolas, from the chemical reactor theory point of view, is that they represent unattainable steady states through the ordinary pseudo-steady-state variation of the feed conditions of the reactor. They can be attained only through a special procedure. Also their existence complicates the dynamic behavior of the system. The special procedure to be used to attain those steady states and the dynamic behavior and stability of those steady states will be the subject of the second part of this investigation. Some of the steady states on those isolas are stable and some are unstable (*19*).

We should also mention here that we discussed the significance of the phenomenon from the chemical reactor theory point of view only; its significance with respect to biological science for multicellular arrays may be more far reaching.

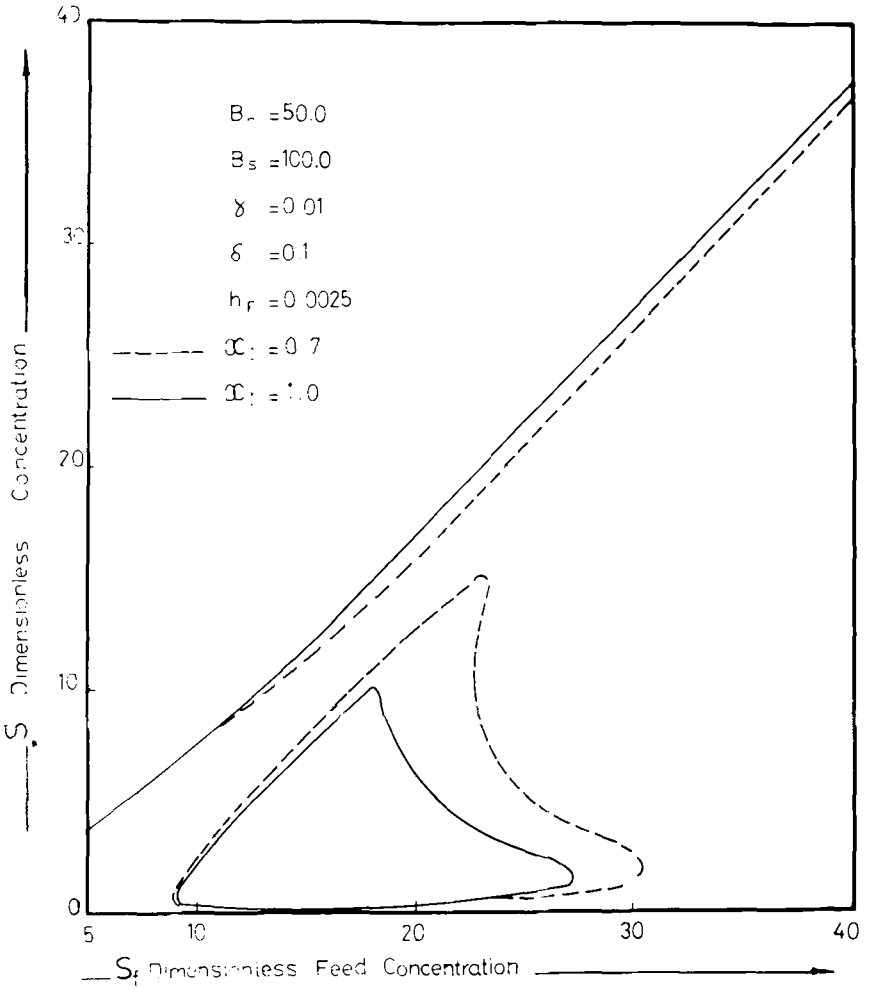


Fig. 9. The effect of α_I on the formation of isola and size of isola for $\delta = 0.1$

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Appendix

Derivation of the rate of reaction equation [Eq. (2)]:

Consider an enzyme, E that catalyzes the reaction,

$$S \xrightarrow{E} P + H^{+}$$

Where P is a fully ionized acid producing hydrogen ions, the activity of the enzyme E is sensitive to hydrogen ion concentration, and in addition the enzyme is inhibited by excess substrate.

One possible mechanism for the effect of hydrogen ion concentration on the activity of the enzyme is as follows (*17*),

$$E \rightleftharpoons E^{-} + H^{+} \tag{A.1}$$

$$E^{-} \rightleftharpoons E^{-2} + H^{+} \tag{A.2}$$

where E^{-} is the active form of the enzyme and E and E^{-2} are both inactive.

For the substrate inhibition we can use the following noncompetitive mechanism (the competitive mechanism gives the same functional form of the rate equation obtained here),

$$E + S \rightleftharpoons ES \tag{A.3}$$
$$ES + S \rightleftharpoons ES_2 \tag{A.4}$$

$$ES \longrightarrow P + E \tag{A.5}$$

From the reaction step (A.5) the rate of reaction is given by,

$$r = k'[ES] \tag{A.6}$$

If steps (A.1)–(A.4) are much faster than step (A.5), we can assume steps (A.1)–(A.4) to be at equilibrium, with equilibrium constants defined as follows (*17*),

$$K'_h = [E^-][H^+]/[E] \tag{A.7}$$
$$K_h = [E^{-2}][H^+]/[E^-] \tag{A.8}$$
$$K_s = [E^-][S]/[ES] \tag{A.9}$$
$$K_i = [E^-][S]/[ES_2] \tag{A.10}$$

The total concentration of active sites is denoted E_t (g-mol/g enzyme), and we can relate the different states of enzyme active sites by the conservation relation,

$$E_t = E + E^- + E^{-2} + ES + ES_2 \tag{A.11}$$

Substitution from Eqs. (A.7)–(A.10) into Eq. (A.11) gives,

$$E^- = \frac{E_t}{1 + ([H]/K'_h) + (K_h/[H]) + ([S]/K_s + ([S]^2/K_sK_i)} \tag{A.12}$$

From Eqs. (A.6) and (A.9) we obtain the rate of reaction as a function of the concentration of the active form of the enzyme [E$^-$] and the substrate concentration [S],

$$r = (k'/K_s)\,[E^-][S] \tag{A.13}$$

By substitution from Eq. (A.12) into Eq. (A.13), we obtain the rate of reaction,

$$r = \frac{V_m[S]}{[S] + \{[K_s(K_h + [H] + [H]^2)/K'_h]/[H]\} + ([S]^2/K_i)} \tag{2}$$

where, $V_m = k'E_t$

Note: If we use a competitive inhibition mechanism the resulting rate equation is,

$$r = \frac{V_m[S]}{[S] + \{K_s[K_h + [H] + ([H]^2/K'_h)]/[H]\} + [(K_s/K_i)][S]^2} \tag{A.14}$$

From Eqs. (2) and (A.14) we notice that, for both cases, K_i is mathematically independent of [H$^-$], which does not mean that the inhibition is physically independent of [H$^-$].