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A Mathematical Model for the Continuous Culture of Microorganisms Utilizing Inhibitory Substrates

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Summary

A mathematical model is presented for both batch and continuous cultures of microorganisms utilizing inhibitory substrates. The key feature of the model is the use of a inhibition function to relate substrate concentration and specific growth rate. Simulation studies show that the primary result of inhibition by substrate in a batch culture is an increase in the lag time whereas in continuous culture inhibition by substrate may result in process instability. The model should be of value in investigations of the stability of biological processes used for the treatment of certain industrial wastes such as those containing phenols, thiocyanates, nitrates, ammonia, volatile acids, etc., which are known to be inhibitory to many of the organisms metabolizing them.

INTRODUCTION

Current continuous culture theory is primarily based on the relationship between limiting substrate concentration and growth rate as originally proposed by Monod.[1] Although several other relationships have been suggested,[2–4] most investigators have found that the Monod relationship provides a reasonable fit for continuous culture data even for mixed culture systems such as those used in biological processes for waste treatment.

However, as has been pointed out by Powell,[5] the Monod relationship cannot be valid for those substrates which limit growth at low concentrations and are inhibitory to the organism at higher concentrations. Common examples are the inhibition of *Nitrobacter* by nitrite and *Nitrosomonas* by ammonia. Indeed, the Monod relationship may be only a special case of a more general relationship since even glucose can inhibit growth if present in a high enough concentration. In conventional continuous cultures, operated near steady

state, inhibitory concentrations of substrate would rarely be present. However, inhibitory concentrations could occur during startup operations or under transient conditions resulting from changes in process loading. A model incorporating the inhibitory effect of these higher concentrations would be of special value in investigations of the stability of biological processes used for the treatment of certain industrial wastes such as those containing phenols, thiocyanates, nitrates, ammonia, volatile acids, etc., which are known to be inhibitory to many of the organisms metabolizing them. The model would also be useful in selecting the appropriate startup procedures for processes utilizing such substrates.

Analytical solutions of the nonlinear differential equations representing the dynamic behaviour of microbial systems are usually unavailable. However, these equations can be solved using the analog computer or one of the digital-analog simulation programs.[6,7] The solutions presented in this paper were obtained using PACTOLUS[8] on the IBM 360 computer.

GENERAL THEORY

Inhibition Function

Little information is available concerning the functional relationship between an inhibitory substrate and the specific growth rate of the organism utilizing such a substrate. In order to illustrate the dynamic behavior of microorganisms utilizing an inhibitory substrate a function such as that proposed by Haldane[9] for the inhibition of enzymes by high substrate concentration will be used. Although there is no theoretical basis for the use of this function for microorganisms, it should be pointed out that the Monod relationship, which is empirical, is similar in form to the Michaelis-Menton expression upon which the Haldane function is based. Also, Boon and Laudelout,[10] working with *Nitrobacter winogradskyi*, have shown that the rate of nitrite oxidation can be related to nitrite concentration by this type of inhibition function.

This inhibition function may be expressed as:

$$\mu = \frac{\hat{\mu}}{1 + K_s/S + S/K_i} \tag{1}$$

where μ = specific growth rate, time^{-1}; $\hat{\mu}$ = maximum specific growth rate in the absence of inhibition, time^{-1}; S = limiting substrate concentration, mass/volume; K_s = saturation constant, numerically equals lowest concentration of substrate at which the specific growth rate is equal to one-half the maximum specific growth rate in the absence of inhibition, mass/volume; K_i = inhibition constant, numerically equals the highest substrate concentration at which the specific growth rate is equal to one-half the maximum specific growth rate in the absence of inhibition, mass/volume.

In the usual continuous culture, operated near steady state, substrate concentrations are low and the term S/K_i is therefore much less than the term K_s/S even for low values of K_i. Under these conditions the inhibition function reduces to the Monod function. In batch cultures, even for the higher values of K_i, the term S/K_i may be significant because of the higher substrate concentrations present during the early stages of growth.

The inhibition functions for several values of K_i are shown in Figure 1. It will be noted that even when K_s and K_i are well separated there is a considerable reduction in the maximum specific growth rate attainable as compared to the case without inhibition. The maximum rate attainable may be obtained by setting the first derivative of eq. (1) equal to zero and can be expressed as:

$$\hat{\mu}_m = \frac{\hat{\mu}}{1 + 2(K_s/K_i)^{0.5}} \tag{2}$$

where $\hat{\mu}_m$ = maximum specific growth rate attainable in the presence of inhibition, time^{-1}.

The substrate concentration at this growth rate is:

$$S_m = (K_s K_i)^{0.5} \tag{3}$$

where S_m = substrate concentration at maximum specific growth rate attainable in the presence of inhibition, mass/volume.

Figure 1 also illustrates that there are two possible substrate concentrations for each value of the specific growth rate except at the maximum attainable specific growth rate. As will be shown later, the higher substrate concentration can represent an unstable situation in continuous culture.

A more detailed treatment of the properties of this function is given by Dixon and Webb[11] with techniques which may be used to evaluate the function parameters from experimental data.

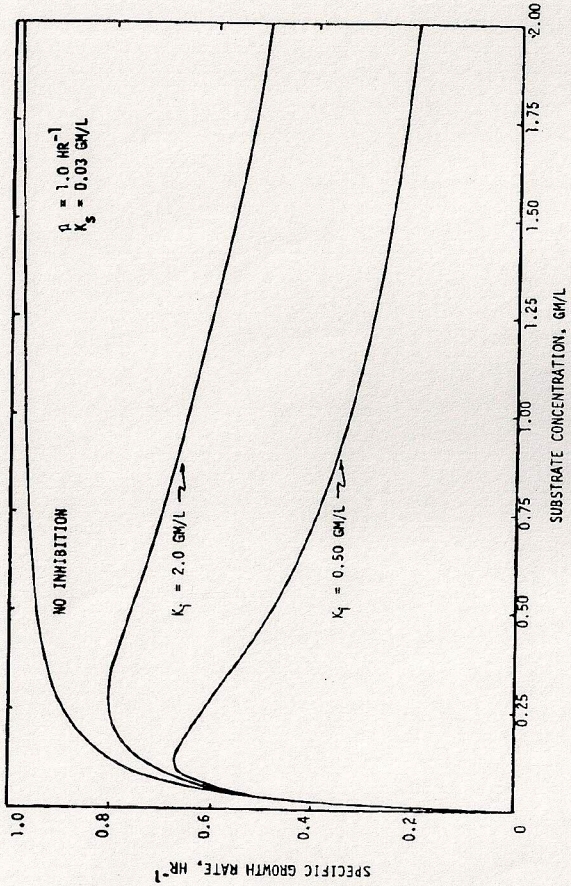

Fig. 1. Substrate inhibition function.

Batch Culture

The basic differential equations, incorporating the inhibition function, for organism growth and substrate utilization in batch culture are given below. Since the primary purpose of this paper is to illustrate the effect of the inhibition function on the dynamics of microbial processes, the model has been kept as simple as possible by assuming that there is no lag phase, organism death, endogenous respiration, substrate used for maintenance energy, or inhibition by products.

$$\frac{dX}{dt} = \left[\frac{\hat{\mu}}{1 + K_s/S + S/K_i}\right]X \tag{4}$$

$$\frac{dS}{dt} = -\frac{1}{Y}\left[\frac{\hat{\mu}}{1 + K_s/S + S/K_i}\right]X \tag{5}$$

where t = time; Y = yield coefficient, mass organisms produced/mass substrate utilized.

The block diagram for the solution of these equations using PACTOLUS is given in Figure 2. In developing the block diagram it was more convenient to use the inhibition function in the form:

$$\mu = \hat{\mu}\left[\frac{S}{(S)^2/K_i + S + K_s}\right] \tag{6}$$

Continuous Culture

The differential equations for continuous culture may be developed by applying material balances to substrate and organisms in the usual manner. The same assumptions are made as for the batch culture system in order to keep the model as simple as possible. For a complete mixing, continuous-flow reactor the basic equations are:

$$\frac{dX_1}{dt} = \frac{X_0}{\theta} - \frac{X_1}{\theta} + \left[\frac{\hat{\mu}}{1 + K_s/S_1 + S_1/K_i}\right]X_1 \tag{7}$$

$$\frac{dS_1}{dt} = \frac{S_0}{\theta} - \frac{S_1}{\theta} - \frac{1}{Y}\left[\frac{\hat{\mu}}{1 + K_s/S_1 + S_1/K_i}\right]X_1 \tag{8}$$

where θ = mean residence time in the reactor; X_0 = organism concentration in the influent to the reactor, mass/volume; X_1 = organ-

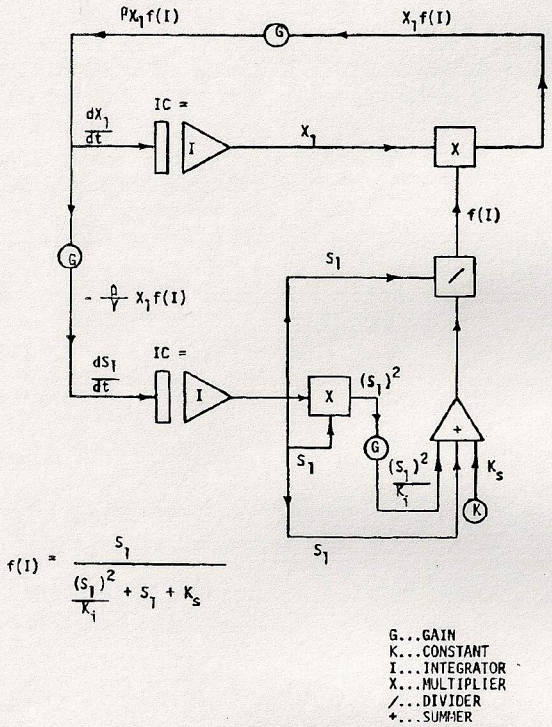

Fig. 2. Block diagram for a batch culture with substrate inhibition.

ism concentration in the effluent from the reactor, mass/volume; S_0 = substrate concentration in the influent to the reactor, mass/volume; S_1 = substrate concentration in the effluent from the reactor, mass/volume.

The block diagram for the solution of these equations using PACTOLUS is given in Figure 3.

At steady state, dX_1/dt and dS_1/dt are equal to zero and eqs. (7) and (8) may be solved for the steady-state values of substrate and organism concentration in the reactor effluent. These are:

$$S_1 = \frac{K_i(\hat{\mu}\theta - 1) + [(K_i)^2(\hat{\mu}\theta - 1)^2 - 4K_sK_i]^{0.5}}{2} \quad (9)$$

$$X_1 = Y(S_0 - S_1) \quad (10)$$

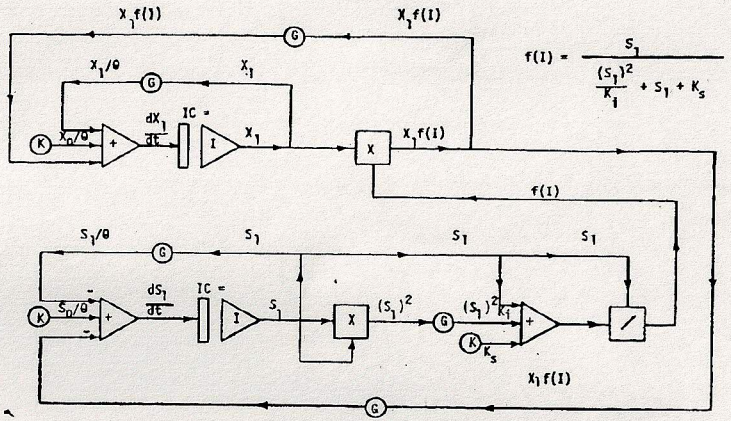

Fig. 3. Block diagram for a continuous culture with substrate inhibition.

Two values of substrate and organism concentration are possible for each residence time since the substrate equation is a quadratic. As previously mentioned, the higher substrate concentration (and associated organism concentration) represent an unstable situation in continuous culture. Plots of substrate concentration versus residence time for different values of the inhibition constant are shown in Figure 4. It will be noted that the residence time at which "washout" occurs increases as the substrate becomes more inhibitory (lower values of K_i). The "washout" residence time may be calculated as:

$$\theta w = (1/\hat{\mu})[1 + 2(K_s/K_i)^{0.5}] \quad (11)$$

where θw = "washout" residence time.

SIMULATION STUDIES

Batch Culture

The effect of substrate inhibition on organism growth in batch cultures is illustrated in Figure 5 for several values of the inhibition constant. The initial innoculum (X_i) has been chosen as 0.005 g/l and the initial substrate concentration (S_i) as 10.0 g/l. With the more inhibitory substrates (low K_i) the model indicates a pro-

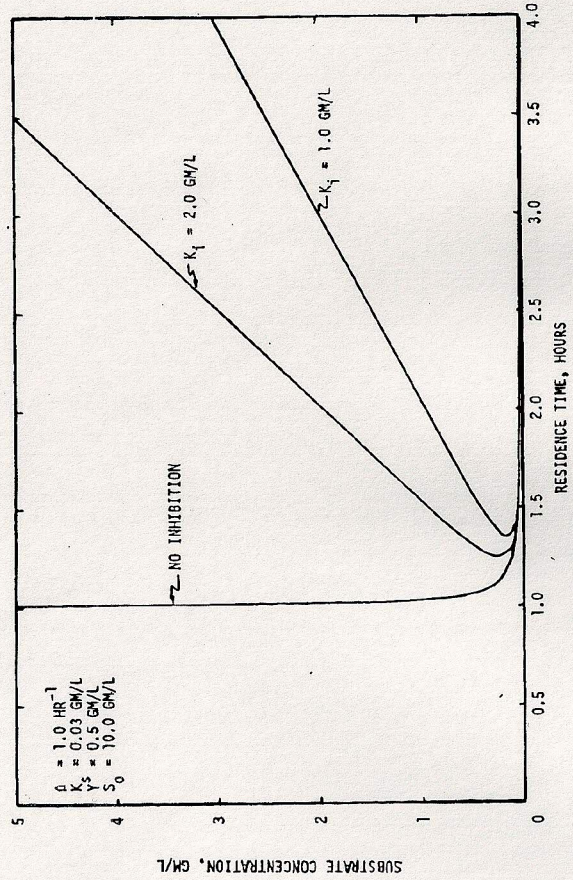

Fig. 4. Substrate concentration at steady state for a continuous culture utilizing an inhibitory substrate.

nounced "lag" phase. This may assist in explaining the long lag periods sometimes observed in batch cultures since it is common practice to use media containing 1–2% (10–20 g/l) substrate in preparing batch cultures. Even with a high value of K_i this concentration of substrate could result in a significant lag phase.

This prolonged lag phase may be avoided by using a larger innoculum as illustrated in Figure 6. With a sufficiently large innoculum the lag may not be noticeable. When a large innoculum is not available, as in the startup of most industrial waste treatment processes, it is common practice to prepare a so-called "acclimated seed" by mixing domestic sewage with the waste to be treated. In addition to furnishing an innoculum, this also has the effect of diluting the waste, thereby decreasing the concentration of the inhibitory substrate. After the substrate has been metabolized the organisms are allowed to settle, a portion of the supernatent removed, and another portion of waste added. This procedure is continued until the organism population is sufficiently high to permit operation as a continuous-flow system without dilution of the incoming waste stream. Simulation of this procedure is illustrated in Figure 7 where an original organism innoculum of 0.005 g/l is exposed to a substrate concentration of 2.0 g/l with the substrate concentration being increased in 2.0 g/l steps after reduction in each case to 0.02 g/l. It can be seen that only 12.5 hr are required to obtain an organism concentration of 5 g/l using this procedure as compared to the 36 hr required if the original innoculum was exposed to the full 10 g/l of substrate at the beginning. An even more significant improvement in the time requirement would be obtained for those organisms with lower maximum specific growth rates.

Continuous Culture

The primary result of inhibition by substrate in a batch culture is an increase in the lag time. However, in the absence of organism death, the substrate will eventually be metabolized. Such is not the case in continuous cultures where high concentrations of inhibitory substrates may result in process instability with "washout" of the organisms. Figure 1 and the "steady-state" equations for substrate and organism concentration in the reactor indicate that there are two possible values of substrate and organism concentration for each specific growth rate and therefore each residence time in the reactor.

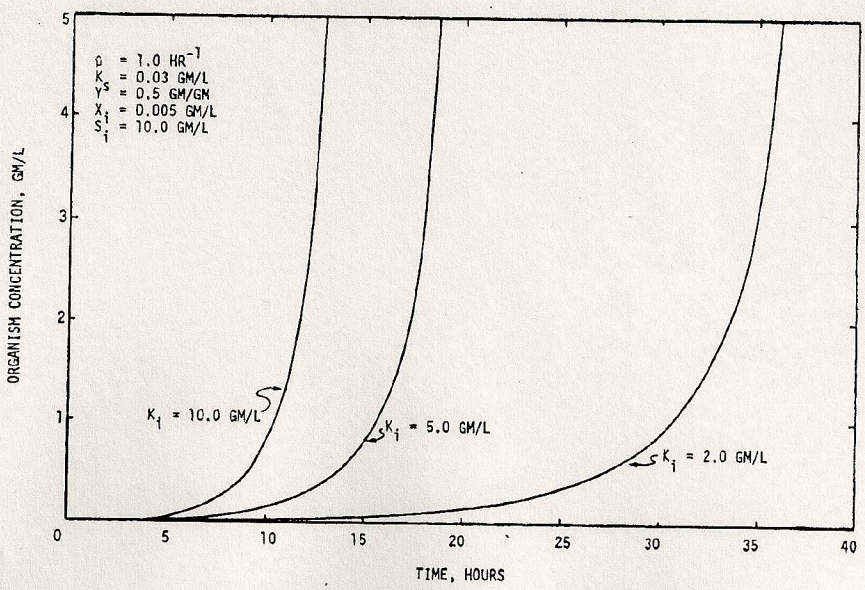

Fig. 5. Effect of K_i on organism growth in batch culture.

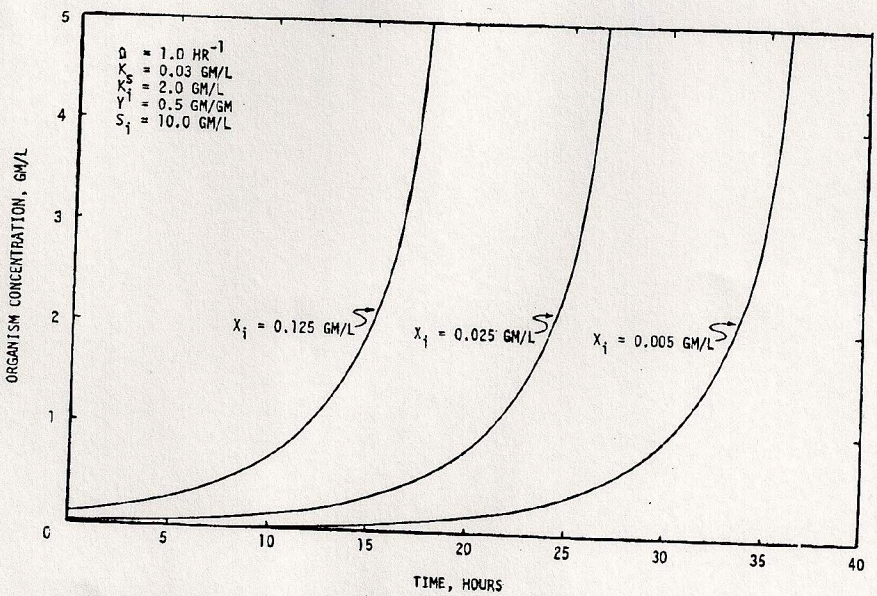

Fig. 6. Effect of initial innoculum on organism growth in batch culture.

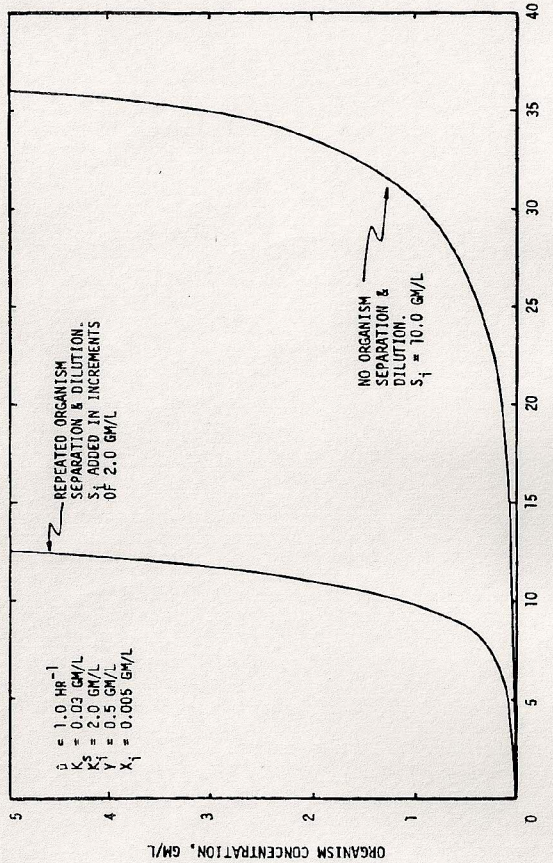

Fig. 7. Preparation of "acclimated seed" for inhibitory substrates by repeated organism separation and dilution.

The unstable nature of the "steady state" at the higher substrate concentration is demonstrated in Figure 8 where it is assumed that the substrate concentration (and associated organism concentration) is at the higher "steady-state" value for an influent substrate concentration (S_0) of 5.0 g/l and a residence time (θ) of 3.0 hr. When S_0 is increased to 5.2 g/l organism washout occurs and the effluent substrate concentration (S_1) approaches S_0 as a limit. When S_0 is decreased to 4.8 g/l the process recovers with a decrease in S_1 and increase in X_1. The unstable nature of the process at the higher "steady-state" value can also be illustrated by a phase plane plot using the techniques presented by Koga and Humphrey.[12]

The model also indicates that process failure may occur during start-up of a reactor if insufficient innoculum is present. The effect of two different values of initial organism concentration (X_1) on the startup of a continuous-flow reactor from a batch reactor is shown in Figure 9. At time 0+ the reactor is abruptly converted from a batch reactor to a continuous-flow reactor with a residence time (θ) of 3 hr and S_0 = 5.0 g/l. The process fails when X_i = 0.10 g/l but recovers when X_i = 0.50 g/l. Failure could be avoided by gradually increasing S_0 to its final value of 5 g/l (ramp forcing of S_0) and maintaining θ constant at 3 hr or by gradually increasing flow into the reactor (ramp forcing of θ) until θ reaches its final value of 3 hr and maintaining S_0 constant at 5 g/l.

During continuous-flow operation failure may occur due to step changes in S_0. The effect of step forcing S_0 from 5 to 20 g/l is shown in Figure 10. Process failure will occur at progressively lower values for the step forcing of S_0 as the residence time in the reactor is decreased. Failure may be avoided, as during startup, by using ramp forcing of S_0. As an example, the ramp forcing of S_0 from 5 to 20 g/l in one hour is also shown in Figure 10. Under these conditions the process recovers.

DISCUSSION AND CONCLUSIONS

The mathematical model presented assists in explaining the long lag phases sometimes involved in the development of batch cultures using inhibitory substrates and demonstrates the possible instability of continuous-flow biological processes using such substrates. The key feature of the model is the use of an inhibition function to relate

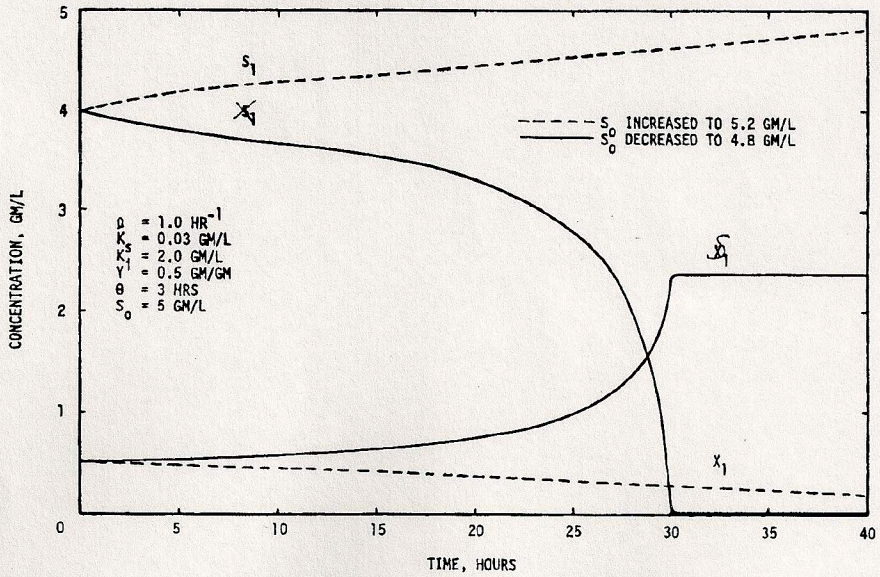

Fig. 8. Unstable nature of the second "steady state" for a continuous culture utilizing an inhibitory substrate.

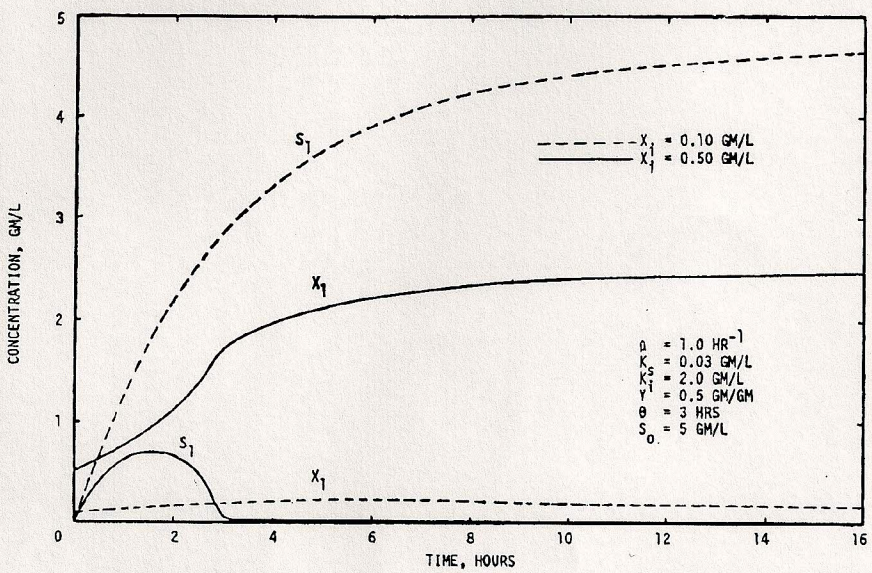

Fig. 9. Effect of initial innoculum on startup of a continuous culture utilizing an inhibitory substrate.

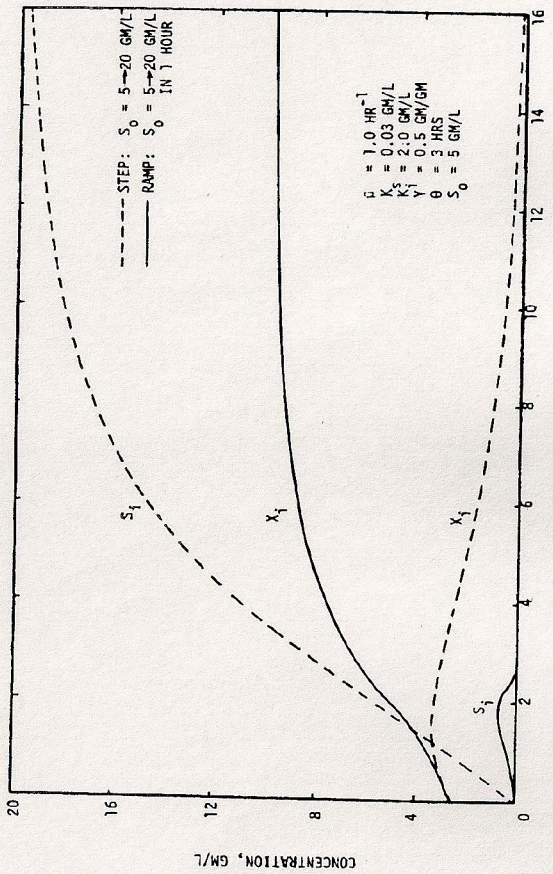

Fig. 10. Effect of step and ramp forcing of S_0 for a continuous culture utilizing an inhibitory substrate.

substrate concentration and specific growth rate. Undoubtedly, this function, as has the Monod function, will require modification as experimental data are obtained. However, the model presented should serve as a valuable framework for these modifications. The model is currently being changed to incorporate the effects of (*1*) organism death, (*2*) utilization of substrate for maintenance energy, (*3*) inhibition by products, and (*4*) delay of organism response to changes in substrate concentration. The general effect of these changes would be to decrease process stability. Possible factors which could increase process stability are mutation and adaptation.

The model should be of value in the design and operation of continuous-flow biological processes utilizing the more inhibitory substrates. Operational changes currently being investigated are (*1*) organism separation and recycle, (*2*) multistage operation with separate substrate supply to each reactor, and (*3*) recycle without organism separation in a fixed-film reactor. The general effect of these modifications should be to increase process stability.

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