Growth Processes in a Cascade of Bioreactors: Mathematical Models

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Mathematical modeling of continuous multiple bioreactors is complicated by two factors. First, the chemical environments differ in different reactors, so biomass receives an environmental shock when it is transferred between reactors. Second, biomass occurs as discrete cells, which of course are and remain segregated from one another, so an element of biomass that enters a reactor does not mix with the biomass already present. This differs from the behavior of an element of liquid which enters a reactor in that such an element quickly mixes with the liquid already present. The biomass in a bioreactor receiving biomass from an external source is therefore heterogeneous with respect to the history of environmental conditions and composition. This article shows how to construct a mathematical model of multiple bioreactor apparatus that accounts for these complications. It also describes simpler models that do not account for both of them.

Introduction

Continuous propagation of microbial or animal cells in apparatus employing multiple bioreactors with flows of culture between the reactors and with feed of sterile nutrient medium to more than the first reactor provides more flexibility in arranging the history of environmental circumstances seen by the cells than one has with a single CSTR apparatus. Because of this, one expects that the optimum performance of an apparatus employing multiple bioreactors will be significantly better than that of a single CSTR apparatus. Of course, the number of operating parameters that must be chosen is greater with the former type of apparatus, and so for purposes of optimization and control, it is highly desirable to have an accurate mathematical model of the growth process as it occurs in the multiple bioreactor apparatus. Two circumstances of the multiple bioreactor apparatus complicate the task of modeling growth processes in it, however.

The first of these is that the abiotic environments of cells in different reactors will be different, so cells will be subjected to sudden changes in their environments when they are transferred from one reactor to another. Since internal regulatory processes come into play when environmental circumstances change, biomass will be in a transient state of growth after it has passed from one reactor to another, and this will be true even when the whole apparatus is operating in a steady state. It was shown some time ago (Fredrickson et al., 1971) that one cannot expect mathematical models of transient growth processes that are *unstructured* to be successful, because unstructured models always predict that growth, nutrient uptake, and product formation rates respond instantaneously to changes in the cellular environment, something that cannot happen in reality. Various kinds of *structured* models have been proposed to account for the transient cellular phenomena that accompany changes in the cellular environment, and some such model should be used for apparatus employing multiple bioreactors.

The second complicating feature of an apparatus using multiple bioreactors with interreactor flows of culture is that some or all of the reactors will be receiving biomass from an external source or sources at all times during the growth process, not just when the reactors are inoculated. Structured mathematical models that have been used for growth processes have been developed for bioreactors that receive no biomass from external sources after they have been inocu-

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lated. Fredrickson (1992) and Miao and Kompala (1992) showed simultaneously that these models are not directly applicable to reactors that have external sources of biomass. This happens because biomass is *segregated.* That is, biomass that enters a reactor from some other reactor does not in general have the same internal state as the biomass already in the reactor, and the introduced biomass *retains its identity and does not mix* with the biomass in the reactor. Thus, the biomass in a bioreactor with one or more external sources of biomass is heterogeneous and is characterized by differing internal states. This is different from what happens in a chemical reactor. Liquid introduced into a CSTR chemical reactor quickly mixes with the liquid already present in the reactor and does not retain its identity, and the liquid in the reactor has a homogeneous or nearly homogeneous composition.

Fredrickson (1992) considered the general problem of how to apply structured models to a series of CSTR bioreactors. If streams flow countercurrently between the reactors, so that biomass is transferred not only from tank n to tank $n+1$, but also from tank $n+1$ to tank n, the only feasible way to handle the modeling problem appears to be to use a so-called *corpuscular* model (Roels, 1983). Such a model accounts for the discrete, cellular nature of biomass and so automatically takes biomass segregation into account. However, the equations of a corpuscular model are population balance equations and so are partial differential-integral equations. Efficient numerical methods of solving these kinds of equations for the chemically structured models (see below) that need to be used have not been available in the past, and it is only recently that some progress has been made at developing such methods. We shall not consider countercurrent flow situations in this article and so we shall not have to deal with partial differential-integral equations.

If there is only unidirectional transfer of biomass between reactors, *continuum* models, which do not take the corpuscular nature of biomass into account, can be used. These are the kinds of models that will be considered in this article. The simplest kind of continuum structured model, and the only kind to be considered here, is the *chemically* or *compositionally* structured continuum model. The elements of such a model are stated below. The equations of this kind of model for a homogeneous collection of biomass are first-order, nonlinear, ordinary differential equations.

Consider the simplest nontrivial case of a two-tank cascade of CSTRs, with inoculum added only to the first tank at startup. The biomass in the first tank is homogeneous in the sense that it is composed of and/or descended from cells that have seen the same history of environmental conditions but the biomass in the second tank is heterogeneous because it is composed of and/or descended from cells that passed from the first to the second tank at different times. This heterogeneity in the second tank is reflected in the nonuniform composition of the biomass in that tank; there is a *distribution* of compositions in that tank. A continuum, chemically structured model may be applied only to biomass that has a uniform composition, and, therefore, it may be applied only to infinitesimal fractions of biomass in the tank. Fredrickson (1992) showed that the ordinary differential equations of a chemically structured model become partial differential equations in a bioreactor with an external source of biomass. However, none of these equations are also integral equations, like the population balance equation of a corpuscular model.

The objectives of this article are as follows. First, cascades of two continuous bioreactors are often used in waste disposal processes but this arrangement is seldom if ever used in industrial situations employing pure cultures because of the practical difficulty of maintaining aseptic conditions in a pair of continuous reactors. If a rigorous model of growth of a pure culture in a cascade showed that there were decided advantages to be obtained by using a cascade, then that might be the stimulus needed to expend the development efforts required to overcome the difficulty mentioned. The first objective of this article is to provide part of the rigorous model for growth of a pure culture in a cascade.

Reconsideration of Fredrickson (1992) showed that the choices of independent variables made in it had been unfortunate, and that reformulation of the equations with different choices of independent variables would make solution of the equations easier. In addition, reconsideration provided several new insights into the problem. The second objective of this article is therefore to present the reformulated equations and state the new insights that have been obtained.

No numerical examples were given in the 1992 article, nor was anything useful said about how to solve the equations of the model. A third objective of this article is therefore to develop efficient numerical means of solving the sets of partial differential equations that describe the performance of a continuous, multiple CSTR bioreactor apparatus when segregation of biomass is taken into account.

The fourth objective of this article is to discover what effects segregation of biomass has on the performance of multiple CSTR bioreactor apparatus. This will be achieved by comparing the performance of the apparatus predicted when segregation is taken into account with the performance predicted when it is assumed that biomass is not segregated.

Investigations too numerous to cite have used unstructured models to predict the performance of continuous, multiple CSTR bioreactor apparatus. The fifth objective of this article will be to show how to construct models which are, in some sense, the unstructured versions of structured models, and then compare apparatus performance predicted by a structured model when biomass segregation is taken into account with the performance predicted by the unstructured model obtained from the structured model.

It is known that some chemically structured models and even unstructured models can predict multiple steady states for a single CSTR without an external source of biomass. If multiplicity is encountered in the first tank of a cascade, it will certainly be encountered in the second tank, but the multiplicity there may be more complicated. For example, for each steady state of tank 1, there might be several steady states of tank 2. A sixth objective of this article therefore to make some preliminary investigations of the multiplicity of steady states of a two-tank cascade of CSTRs.

The material to be presented falls naturally into two parts: namely, the development of the theory and the numerical methods to be used to solve the equations, and then the application of the theory and methods to provide numerical examples for the simplest nontrivial case of a two-tank bioreactor apparatus. The theory and numerical methods will be

presented in this article and the examples will be presented in a subsequent article.

Elements of a Chemically Structured Model for a Homogeneous Collection of Biomass

A chemically structured continuum model for a homogeneous collection of biomass—called a ''pseudoclone'' by Fredrickson (1992)—assumes that biomass has a spatially uniform chemical composition which changes as internal and interfacial biochemical reactions occur. The elements of a continuum, chemically structured model are statements of what are (1) the components of biomass; (2) the stoichiometries of the (internal) reactions between these components and also of the (interfacial) reactions between these components and substances present in the abiotic environment; and (3) the kinetics of the aforementioned reactions.

Let $z(t)$ be the state vector of a pseudoclone at time t. The elements of the state vector are the mass fractions of the various components of which the biomass is assumed to be composed. Similarly, let $s(t)$ be the state vector of the liquid abiotic environment of the pseudoclone at time t. The elements of this vector are the mass concentrations of the various nutrients, dissolved gases, and other substances in the liquid in contact with the pseudoclone. When the stoichiometry and kinetics of each of the internal and interfacial reactions that occur are specified, one will be able to calculate the net rates at which the masses of biomass components are being produced or consumed and the net rates at which masses of substances are being exchanged between the biomass and its abiotic environment. If we let $p(t)$ and $r(t)$ be vectors whose elements are the net rates of production of masses of the biomass components and the net rates of uptake of masses of abiotic substances, both per unit amount of total biomass of the pseudoclone, then knowledge of the stoichiometries and kinetics of the biochemical reactions will allow us to express these two vectors as functions of the two state vectors

$$p(t) = f[z(t), s(t); k], \tag{1}$$

$$r(t) = g[z(t), s(t); k]. \tag{2}$$

The vector-valued functions f and g are determined by the stoichiometries and kinetics of the reactions that occur and by the molecular weights of the substances involved, as explained by Fredrickson (1992). The specific growth rate $\mu(t)$ at any time is the net rate of increase of biomass per unit amount of biomass present at that time, and so one sees that it is the sum of the elements of the vector $p(t)$. If we let 1 be a constant vector all of whose elements are 1, then we can write

$$\mu(t) = 1^{\dagger}p(t) = 1^{\dagger}f[z(t), s(t)] \equiv h[z(t), s(t)]. \tag{3}$$

It is to be noted that one cannot specify the specific growth rate arbitrarily when a structured model is used; instead, the specific growth rate is determined by the stoichiometries and kinetics of the reactions assumed to occur. The elements of the vector k, which appears in these functions, are the stoichiometric coefficients of the components in the reactions

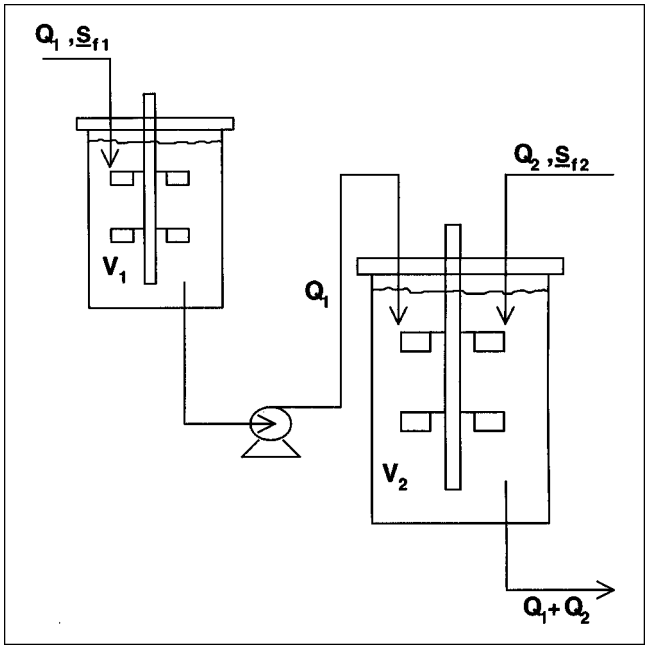

Figure 1. Bioreactor cascade arrangement considered in the text.

that occur, the rate parameters of the reactions, and the molecular weights of the components; it is the vector of model parameters. In what follows, we shall assume that the same model applies throughout the system to be studied and that the same model parameters apply everywhere in it, too, so that the vector k need not and will not be carried along in our notation. If, however, bioreactors were operated at different temperatures, for example, then the vector k would be needed in the notation because the differing temperatures would make the rate parameters of the reactions different in different reactors.

A chemically structured model follows what in the theory of stochastic processes is called the Markov hypothesis. The model's prediction for what happens in the increment of time following the current instant of time is determined by the state of the system being modeled at the current instant of time and is not affected by how that state was attained. Current behavior is dependent on the past history of the environmental conditions seen by the biomass, but the dependence is implicit, rather than explicit. Therefore, the equations that express the model are mathematically autonomous.

Application of a Chemically Structured Model to the First Tank of a Cascade of CSTRs

Figure 1 shows the two-tank bioreactor system to be considered in this article. In general, the tanks contain different volumes of culture V_1 and V_2 which, however, do not change with time. Volumetric flow rates are indicated by Qs and are assumed to be independent of time. The overflow from tank 1 carries biomass into tank 2, but the two other streams which enter these two tanks are assumed to contain no biomass; these two other streams are assumed also to have constant

compositions and flow rates. It is assumed that biomass and nutrient medium are present in both tanks at time 0, and that the biomass in both tanks has a uniform state because the tanks have been operated batchwise prior to time 0; the initial states in the tanks may be different, however. It is assumed finally that the liquid portions of the cultures in both tanks are perfectly mixed and that the transit time for transfer of culture from tank 1 to tank 2 is negligibly small.

For tank 1, the balances on biomass, components of biomass, and substances present in the abiotic portion of the culture are

$$V_1 \frac{dx_1^1}{dt} = -Q_1 x_1^1 + V_1 \mu_1^1 x_1^1, \tag{4}$$

$$V_1 \frac{d}{dt}\left(x_1^1 z_1^1\right) = -Q_1 x_1^1 z_1^1 + V_1 p_1^1 x_1^1, \tag{5}$$

$$V_1 \frac{ds_1}{dt} = Q_1(s_{1f} - s_1) - V_1 r_1^1 x_1^1, \tag{6}$$

respectively. In these equations, μ_1^1 is the specific growth rate of the biomass in tank 1, x_1^1 is the biomass in a unit volume of the culture in tank 1, the elements of the vector s_1 are the masses of the abiotic substances in a unit volume of the culture in tank 1, and the elements of the vector s_{1f} are the masses of the abiotic substances in a unit volume of the liquid fed to tank 1. The vector z_1^1 is the state vector of the biomass in tank 1.

It we use Eq. 4 to eliminate the time derivatives of x_1^1 from Eqs. 5 we obtain

$$\frac{dz_1^1}{dt} = p_1^1 - \mu_1^1 z_1^1. \tag{7}$$

This equation, which contains only *intrinsic variables* (Fredrickson, 1976) may be called the equation of change of the state of the biomass in tank 1. Equations 5 and 7 are not independent, and one can use either of these equations, of course, together with Eq. 4, to describe the changes in the amount and composition of the biomass in tank 1. We shall use Eq. 7 rather than Eq. 5.

The vectors p_1^1 and r_1^1 and the specific growth rate μ_1^1 are related to the state vectors z_1^1 and s_1^1 by

$$p_1^1 = f\left[z_1^1, s_1\right], \tag{8}$$

$$r_1^1 = g\left[z_1^1, s_1\right], \tag{9}$$

$$\mu_1^1 = h\left[z_1^1, s_1\right], \tag{10}$$

where it is understood that all of the vectors and the scalar are evaluated at the current time t. The superscript 1 on these quantities indicates that they are properties of a pseudoclone whose ancestral biomass was inoculated into tank 1. This notation is redundant for tank 1, but it is needed for tank 2, and, so, for the sake of consistency, the double index notation is used for tank 1, also.

Strictly speaking, the vectors denoted as s_1 in Eqs. 6 on the one hand and Eqs. 8 to 10 on the other are not the same because in Eq. 6, the elements of the vector are the masses per unit volume of *culture* whereas in Eqs. 8 to 10 they are masses per unit volume of the *liquid portion* of the culture. If the biovolume fraction of the culture is small compared to 1, and we shall assume that it is, the difference between the two kinds of concentrations is negligible and the vector s_1 which appears in Eqs. 6 is the same as the vector s_1 that appears in Eqs. 8 to 10. The corrections that must be applied when the biovolume fraction is not small compared to 1 are described by Monboquette (1987) and Fredrickson and Hu (1989).

Equations 4 and 6 to 10, together with initial conditions for the state vector and for the biomass and abiotic substances concentrations, constitute the mathematical description of the first tank provided by a chemically structured model. These equations may be written compactly as

$$\frac{dx_1^1}{dt} = -D_1 x_1^1 + h\left[z_1^1, s_1\right] x_1^1, \tag{11}$$

$$\frac{dz_1^1}{dt} = f\left[z_1^1, s_1\right] - h\left[z_1^1, s_1\right] z_1^1, \tag{12}$$

$$\frac{ds_1}{dt} = D_1(s_{1f} - s_1) - g\left[z_1^1, s_1\right] x_1^1, \tag{13}$$

in which we have defined the dilution rate of tank 1 by $D_1 \equiv Q_1/V_1$. This set of nonlinear ordinary differential equations of the first order is to be solved subject to appropriate initial conditions on the dependent variables of the equations.

Application of a Chemically Structured Model to the Second Tank of a Cascade of CSTRs: Biomass Segregation Accounted For

Fredrickson (1992) defined a pseudoclone as a collection of clones that have seen the same history of environmental conditions. All of the biomass in tank 1 is a single pseudoclone, but that is not true of the biomass in tank 2. That biomass is composed of (1) biomass which is descendent from the biomass that was inoculated into tank 2, and (2) biomass which is descendent from biomass that was transferred into tank 2 from 1. The first class of biomass is a single pseudoclone, and we shall denote its mass concentration in tank 2 by x_2^2, and this is, of course, a function of time. The biomass which is descendent from biomass transferred from tank 1 to tank 2 is not, however, a single pseudoclone, because biomass is transferred continuously. This biomass is, in fact, an infinite number of pseudoclones. The biomass in tank 2 is heterogeneous for two reasons, therefore, and one sees that it is heterogeneous even if no biomass is inoculated into tank 2 initially.

Consider a pseudoclone in tank 2 which is descendent from biomass that was inoculated into tank 1 at time 0. Suppose that its ancestral biomass entered tank 2 at time a_1, where $0 < a_1 < t$. The notation is used because the ancestral biomass had been in tank 1 for a time a_1, and so had age a_1 in tank 1, when it was transferred to tank 2. The transferred biomass initiated a new pseudoclone in tank 2, and at time t, the age of this pseudoclone in tank 2 is $a_2 = t - a_1$. A pseudoclone in tank 2 whose ancestral biomass was inoculated into tank 1 is therefore characterized by the current time t and by the two

ages a_1 and a_2, and these three times are related by

$$a_1 + a_2 = t. \tag{14}$$

The state vectors of all of the biomass in tank 1 and of the biomass in tank 2 that is descendent from biomass that was inoculated into tank 2 are functions only of time, but the state vector of a pseudoclone in tank 2 that is descendent from biomass that was inoculated into tank 1 depends on one of the ages a_1 or a_2 as well as on t. In fact, one could regard this state vector to be a function of any two of these time quantities, since when any two are specified, Eq. 14 determines the third. Fredrickson (1992) chose the independent variables for the state vector to be a_1 and a_2, but he chose the independent variables for the density of the distribution of biomass (see below) to be the time t and age a_2. For reasons to be explained later, we shall in this article take the independent variables for both the state vector and the density of the distribution of biomass to be time t and age a_1.

Define a density function $X_2^1(t, a_1)$ such that $X_2^1(t, a_1)\, da_1$ is the amount of biomass, per unit volume of culture in tank 2 and at time t whose ancestral biomass entered tank 2 from tank 1 at times between a_1 and $a_1 + da_1$. The total amount of biomass, per unit volume of culture in tank 2, and whose ancestral biomass entered tank 2 from tank 1 is therefore.

$$\int_0^t X_2^1(t, a_1)\, da_1$$

at time t, so that the total concentration of biomass in tank 2 at time t is

$$x_2(t) = x_2^2(t) + \int_0^t X_2^1(t, a_1)\, da_1. \tag{15}$$

The balance equations that describe the concentration and composition of the biomass in tank 2 that is descendent from biomass inoculated into that tank have exactly the same form as the corresponding equations for the biomass in tank 1, and are

$$\frac{dx_2^2}{dt} = -D_2 x_2^2 + h\left[z_2^2, s_2\right] x_2^2, \tag{16}$$

$$\frac{dz_2^2}{dt} = f\left[z_2^2, s_2\right] - h\left[z_2^2, s_2\right] z_2^2, \tag{17}$$

where z_2^2 and x_2^2 are functions only of time. The derivation of these equations is exactly the same as the derivation of Eqs. 11 and 12. We are not yet in a position to write the balance equations for the masses of abiotic substances in tank 2, because those contain terms for uptake by biomass descendent from biomass inoculated into tank 1, as well as from biomass inoculated into tank 2.

We can now derive the balance equation for the density function $X_2^1(t, a_1)$. Let a_1' and a_1'' be constants having the dimension of time and being such that $0 < a_1' < a_1'' < t$. Then, the balance equation on biomass of pseudoclones whose ancestral biomass entered tank 2 between times a_1' and a_1'' is

$$V_2 \frac{d}{dt} \int_{a_1'}^{a_1''} X_2^1 da_1 = -(Q_1 + Q_2) \int_{a_1'}^{a_1''} X_2^1 da_1 + V_2 \int_{a_1'}^{a_1''} \mu_2^1 X_2^1 da_1. \tag{18}$$

In this equation, it is to be understood that X_2^1 and μ_2^1 are functions of t and a_1; the latter quantity is the specific growth rate of a pseudoclone in tank 2 that is descendent from biomass inoculated into tank 1. Although biomass is transferred from tank 1 to tank 2, there is no term for this in Eq. 18 because it is a balance on a group of pseudoclones whose ancestral biomass entered tank 2 before the time for which the balance is made. Leibniz's rule for differentiating an integral can be used to rewrite Eq. 18 as

$$\int_{a_1'}^{a_1''} \left[V_2 \frac{\partial X_2^1}{\partial t} + (Q_1 + Q_2) X_2^1 - V_2 \mu_2^1 X_2^1 \right] da_1 = 0, \tag{19}$$

and, from this and appropriate mathematical arguments, it follows that

$$\frac{\partial X_2^1}{\partial t} = \left(-D_2 + \mu_2^1\right) X_2^1, \tag{20}$$

which is the required balance equation. In it, we have defined the dilution rate of tank 2 by $D_2 \equiv (Q_1 + Q_2)/V_2$. See, for example, Hildebrand (1962) for Leibniz's rule for differentiating an integral.

A boundary condition on the density function X_2^1 is needed, and it can be obtained by making a balance on *all* of the biomass in tank 2 that is descendent from biomass that was inoculated into tank 1. This balance equation is

$$V_2 \frac{d}{dt} \int_0^t X_2^1 da_1 = -(Q_1 + Q_2) \int_0^t X_2^1 da_1 + V_2 \int_0^t \mu_2^1 X_2^1 da_1 + Q_1 x_1^1. \tag{21}$$

The last term on the righthand side of this equation is the rate at which biomass is being transferred from tank 1 to tank 2 at the time for which the balance is made. Use of Leibniz's rule allows this to be rewritten as

$$\int_0^t \left[\frac{\partial X_2^1}{\partial t} + \left(D_2 - \mu_2^1\right) X_2^1 \right] da_1 = \phi D_2 x_1^1(t) - X_2^1(t, t). \tag{22}$$

By Eq. 20, the integral on the lefthand side of the equation is 0, so it follows that

$$X_2^1(t, t) = \phi D_2 x_1^1(t), \tag{23}$$

and this is the required boundary condition. In it, we have defined ϕ as the fraction of the total flow into tank 2 that comes from tank 1: $\phi \equiv Q_1/(Q_1 + Q_2)$.

The balance equations on the masses of biomass components in pseudoclones whose ancestral biomass entered tank

2 between times a_1' and a_1'' is

$$V_2\frac{d}{dt}\int_{a_1'}^{a_1''}X_2^1 z_2^1\,da_1=-(Q_1+Q_2)\int_{a_1'}^{a_1''}X_2^1 z_2^1\,da_1+\int_{a_1'}^{a_1''}p_2^1 X_2^1\,da_1, \tag{24}$$

where it is understood that z_2^1 and p_2^1 are functions of time t and age a_1. The same arguments used to arrive at Eq. 20 may be used to show from the present equation that

$$\frac{\partial}{\partial t}\left(X_2^1 z_2^1\right)=-D_2 X_2^1 z_2^1+p_2^1 X_2^1. \tag{25}$$

A set of boundary conditions on the elements of the state vector are needed and these can be obtained by making balances on biomass components in *all* of the biomass in tank 2 that is descendent from biomass inoculated into tank 1. This balance reads

$$V_2\frac{d}{dt}\int_0^t X_2^1 z_2^1\,da_1=-(Q_1+Q_2)\int_0^t X_2^1 z_2^1\,da_1 + V_2\int_0^t p_2^1 X_2^1\,da_1+Q_1 x_1^1 z_1^1, \tag{26}$$

and from this, with the use of Leibniz's rule and Eq. 23, we obtain the required boundary conditions as

$$z_2^1(t,t)=z_1^1(t). \tag{27}$$

A set of balance equations for the components of biomass that involve only intrinsic variables may be derived by combining Eqs. 20 and 25; the result of this is

$$\frac{\partial z_2^1}{\partial t}=p_2^1-\mu_2^1 z_2^1. \tag{28}$$

The balance equations for the components of the abiotic environment may now be written and they are

$$V_2\frac{ds_2}{dt}=Q_1 s_1+Q_2 s_{2f}-(Q_2+Q_2)s_2-V_2\left[r_2^2 x_2^2+\int_0^t r_2^1 X_2^1\,da_1\right] \tag{29}$$

where it is to be understood that r_2^2 depends on t but that r_2^1 depends on t and a_1.

The aforementioned dependences on time and age are implicit rather than explicit, and they arise because r_2^2 is an explicit function of the state vectors z_2^2 and s_2 given by

$$r_2^2=g\left[z_2^2,s_2\right] \tag{30}$$

and because r_2^1 and μ_2^1 are explicit functions of the state vectors z_2^1 and s_2 given by

$$r_2^1=g\left[z_2^1,s_2\right], \tag{31}$$

$$\mu_2^1=h\left[z_2^1,s_2\right]. \tag{32}$$

Similarly, the dependence of p_2^1 on t and a_1 arise because this vector is explicitly dependent on the state vectors z_2^1 and s_2

$$p_2^1=f\left[z_2^1,s_2\right]. \tag{33}$$

The differential equations that govern the growth process in tank 2 may now be written compactly as Eqs. 16 and 17 together with

$$\frac{\partial X_2^1}{\partial t}=-D_2 X_2^1+h\left[z_2^1,s_2\right]X_2^1, \tag{34}$$

$$\frac{\partial z_2^1}{\partial t}=f\left[z_2^1,s_2\right]-h\left[z_2^1,s_2\right]z_2^1, \tag{35}$$

$$\frac{ds_2}{dt}=D_2\left[\phi s_1+(1-\phi)s_{2f}-s_2\right] - g\left[z_2^2,s_2\right]x_2^2-\int_0^t g\left[z_2^1,s_2\right]X_2^1\,da_1. \tag{36}$$

In addition, the boundary conditions expressed by Eqs. 23 and 27 must be satisfied.

The parallelism of Eqs. 34 and 35 with Eqs. 16 and 17, and also with Eqs. 11 and 12, is obvious. Each pair of equations describes a single pseudoclone: Eqs. 11 and 12 describe the biomass in tank 1 which is a single pseudoclone; Eqs. 16 and 17 describe the biomass in tank 2 that is descendent from the biomass inoculated into that tank; and Eqs. 34 and 35 describe the biomass in tank 2 that is descendent from biomass transferred into tank 2 from tank 1 at time a_1. A difference is that Eqs. 34 and 35 are partial rather than ordinary differential equations, because biomass is transferred from tank 1 to tank 2 at different times.

An additional remark about the foregoing equations will be useful in what follows. In writing the equations we have assumed that the density function X_2^1 and the state vector z_2^1 are functions of the two variables (t,a_1). As pointed out earlier, we could have assumed that they were functions of (t,a_2) or (a_2,a_1), and, as will be shown, it is sometimes advantageous to make one of the latter assumptions. If we regard X_2^1 and z_2^1 to be functions of a different pair of variables, Eqs. 34 and 35 will be changed. One may use standard rules of calculus for change of variables to show that all possible cases are covered by Eqs. 37 and 38

$$\left(\frac{\partial X_2^1}{\partial t}\right)_{a_1}=\left(\frac{\partial X_2^1}{\partial t}\right)_{a_2}+\left(\frac{\partial X_2^1}{\partial a_2}\right)_t=\left(\frac{\partial X_2^1}{\partial a_2}\right)_{a_1} =-D_2 X_2^1+h\left[z_2^1,s_2\right]X_2^1, \tag{37}$$

$$\left(\frac{\partial z_2^1}{\partial t}\right)_{a_1}=\left(\frac{\partial z_2^1}{\partial t}\right)_{a_2}+\left(\frac{\partial z_2^1}{\partial a_2}\right)_t=\left(\frac{\partial z_2^1}{\partial a_2}\right)_{a_1} =f\left[z_2^1,s_2\right]-h\left[z_2^1,s_2\right]z_2^1. \tag{38}$$

In these equations we have adopted the notation for partial derivatives used in thermodynamics. That is, the quantity whose derivative is taken is assumed to be a function of the variable with respect to which differentiation is done and the

variable which is held constant during the differentiation, this latter variable being indicated by a subscript on the derivative. The equations must be consistent, of course, and the same pair of independent variables must be used on both sides of both of these equations.

Distribution of Composition States in the Second Tank of a Cascade of CSTR Bioreactors

As pointed out earlier, the biomass in tank 2 is heterogeneous even if no biomass was inoculated into that tank. This heterogeneity results from the continuous transfer of biomass from tank 1 to tank 2. The equations of the preceding section allow us to calculate the heterogeneity of the biomass in tank 2 insofar as its *age* is concerned. That is, at time t, $V_2 x_2^2(t)$ is the amount of biomass in tank 2 that is descendent from biomass inoculated into that tank and $V_2 X_2^1(t, a_1)da_1$ is the amount of biomass in tank 2 that is descendent from biomass that was transferred from tank 1 to tank 2 in the time interval a_1 to $a_1 + da_1$, where $0 < a_1 < t$.

Knowledge of the age heterogeneity of biomass can be used to calculate the means of the state vector $\boldsymbol{z}_2^1$ and the rate quantities p_2^1, r_2^1, and μ_2^1. The mean value of one of the vectors is defined by

$$\bar{\boldsymbol{v}}_2^1 \equiv \frac{\int_0^t \boldsymbol{v}_2^1 X_2^1 da_1}{\int_0^t X_2^1 da_1} = \frac{1}{x_2^1}\int_0^t \boldsymbol{v}_2^1 X_2^1 da_1, \tag{39}$$

where $\boldsymbol{v}$ is $\boldsymbol{z}$, p, or r. Because of Eq. 3, we have then

$$\bar{\mu}_2^1 \equiv \frac{\int_0^t \mu_2^1 X_2^1 da_1}{\int_0^t X_2^1 da_1} = \frac{1}{x_2^1}\int_0^t \mu_2^1 X_2^1 da_1, \tag{40}$$

also.

An important use to which these definitions can be put is to show that Eqs. 21, 26, and 29, the balance equations for biomass, biomass components, and abiotic substances, may be rewritten in terms of the foregoing mean quantities as

$$\frac{dx_2^1}{dt} = D_2\left(\phi x_1^1 - x_2^1\right) + \bar{\mu}_2^1 x_2^1, \tag{41}$$

$$\frac{d\left(x_2^1 \bar{z}_2^1\right)}{dt} = D_2\left(\phi x_1^1 z_1^1 - x_2^1 \bar{z}_2^1\right) + \bar{p}_2^1 x_2^1, \tag{42}$$

$$\frac{ds_2}{dt} = D_2\left[\phi s_1 + (1-\phi)s_{2f} - s_2\right] - r_2^2 x_2^2 - \bar{r}_2^1 x_2^1. \tag{43}$$

Combination of Eqs. 41 and 42 yields the additional equation

$$\frac{d\bar{z}_2^1}{dt} = \phi D_2 \frac{x_1^1}{x_2^1}\left(z_1^1 - \bar{z}_2^1\right) + \bar{p}_2^1 - \bar{\mu}_2^1 \bar{z}_2^1. \tag{44}$$

The total biomass in tank 2 is $x_2 = x_2^2 + x_2^1$ and Eqs. 16 and 41 show, therefore, that

$$\frac{dx_2}{dt} = D_2(\phi x_1 - x_2) + \mu_2^2 x_2^2 + \bar{\mu}_2^1 x_2^1. \tag{45}$$

In this equation, we have put $x_1 \equiv x_1^1$ so as to have a uniform notation for the total concentration of biomass in a tank.

We shall show an application of the foregoing equations in the next section, but before that there is another point to be made. That is that the distribution of ages is, in fact, an artificiality that we are forced to introduce in order to account for biomass segregation when we use a continuum model rather than a corpuscular model. The distribution of *ages* in the second tank is not the distribution of primary importance for helping us understand the behavior of the biomass in that tank. The important thing is the distribution of *composition states*, because it is that distribution that really determines the dynamics of the heterogeneous biomass in the second tank. Because of this, observations of how the distribution of ages changes as operating parameters change do not give us a complete picture of what is going on in the bioreactor.

The relation between the distributions of age and composition states can be found using some results from the theory of probability; see, for example, Papoulis (1965). When steady state has been achieved in both tanks, the solution of the problem is that the density of the distribution of the ith element of the state vector is related to the density of the distribution of ages by

$$\text{density of distribution of } \hat{z}_{2(i)}^1 = \left(\hat{x}_2^1\right)^{-1}\left\{\left|\frac{d\hat{z}_{2(i)}^1(a_2')}{da_2}\right|^{-1}\hat{X}_2^1(a_2') + \left|\frac{d\hat{z}_{2(i)}^1(a_2'')}{da_2}\right|^{-1}\hat{X}_2^1(a_2'') + \cdots\right\}. \tag{46}$$

In this equation, a caret over a quantity indicates the steady-state value of that quantity, $a_2 = t - a_1$ is the age in tank 2 of a pseudoclone that originated in tank 1, and it is understood that a_2', a_2'', ... are the ages for which the element of the state vector has the value at which its density function is evaluated. If the age-state relation is monotone, Eq. 46 has only one term.

Application of Chemically Structured Model to the Second Tank of a Cascade of CSTRs: Biomass Segregation Not Accounted For

In order to see what the effects of biomass segregation are we shall retain all features of the foregoing theory except we shall assume that biomass transferred from tank 1 to tank 2 mixes instantly and completely with the biomass already present in tank 2, so that the biomass in tank 2 is homogeneous at all times. Comparison of the solution of the equations of this unsegregated model with those of the segregated model given in the previous sections of the article will reveal what the effects of biomass segregation are. Since the biomass in the first tank is homogeneous whether or not one accounts

for segregation, the equations describing the first tank are still Eqs. 11–13.

When it is assumed that biomass transferred from tank 1 to tank 2 mixes instantly and completely with the biomass already present in tank 2, then the biomass in tank 2 will always be homogeneous with regard to composition, and the balance equations for biomass and the components of biomass in tank 2 are

$$\frac{dx_2}{dt} = D_2(\phi x_1 - x_2) + \mu_2 x_2, \tag{47}$$

$$\frac{d}{dt}(x_2 z_2) = D_2(\phi x_1 z_1 - x_2 z_2) + p_2 x_2. \tag{48}$$

There is now no need to place identifying superscripts on any of the quantities in the foregoing equations. Since the biomass in tank 2 is now homogeneous, the state vector z_2 will depend only on time and the specific growth rate μ_2, and the specific biomass component production rate vector p_2 will be (implicit) functions of time but not functions of any pseudoclone age. Substitution of Eq. 47 into Eq. 48 yields the analog of Eq. 28 as

$$\frac{dz_2}{dt} = D_2\phi\frac{x_1}{x_2}(z_1 - z_2) + p_2 - \mu_2 z_2. \tag{49}$$

This differs from Eq. 28 not only in that it is an ordinary rather than a partial differential equation, but also in that it contains the variables x_1 and x_2 that are not intrinsic. This last is a consequence of the neglect of biomass segregation.

The balance equations for the components of the abiotic environment are

$$\frac{ds_2}{dt} = D_2\left[\phi s_1 + (1-\phi)s_{2f} - s_2\right] - r_2 x_2. \tag{50}$$

The production rate vector p_2, the uptake rate vector r_2, and the specific growth rate μ_2 are implicit functions of time because they are explicit functions of the time-dependent state vectors z_2 and s_2 according to the equations

$$p_2 = f[z_2, s_2], \tag{51}$$

$$r_2 = g[z_2, s_2], \tag{52}$$

$$\mu_2 = h[z_2, s_2]. \tag{53}$$

In summary, the equations that govern growth processes in tank 2 when biomass segregation is not accounted for are

$$\frac{dx_2}{dt} = D_2(\phi x_1 - x_2) + h[z_2, s_2]x_2, \tag{54}$$

$$\frac{dz_2}{dt} = D_2\phi\frac{x_1}{x_2}(z_1 - z_2) + f[z_2, s_2] - h[z_2, s_2]z_2, \tag{55}$$

$$\frac{ds_2}{dt} = D_2\left[\phi s_1 + (1-\phi)s_{2f} - s_2\right] - g[z_2, s_2]x_2. \tag{56}$$

Initial conditions for these equations are needed, and they are that the biomass concentration and composition and the concentrations of the abiotic substances must be specified at time 0. When no biomass is inoculated into tank 2, the initial concentration of biomass in it is 0 and the initial composition of the biomass is unspecified. In this case, it must be true that

$$\lim_{t \downarrow 0} z_2(t) = z_{1o}, \tag{57}$$

where z_{1o} is the initial state vector in tank 1. This shows that the first term on the righthand side of Eq. 55 is the indeterminate form 0/0 at time 0. The limit of the derivative on the lefthand side may be found using l'Hôpital's theorem and Eqs. 54 and 57; the result is

$$2\lim_{t \downarrow 0}\frac{dz_2}{dt} = f[z_{1o}, s_{1o}] - h[z_{1o}, s_{1o}]z_{1o} + f[z_{1o}, s_{2o}] - h[z_{1o}, s_{2o}]z_{1o}. \tag{58}$$

Equations 54 to 56 should be compared with the versions of Eqs. 41, 44, and 43 for the case where $x_2^2 = 0$ so $x_2 = x_2^1$. These two sets of equations would be identical if the relations between the mean state vector and the mean rate quantities $\bar{p}_2^1$, $\bar{r}_2^1$, and $\bar{\mu}_2^1$ obeyed the relations

$$\bar{p}_2^1 = f\left[\bar{z}_2^1, s_2\right], \tag{59}$$

$$\bar{r}_2^1 = g\left[\bar{z}_2^1, s_2\right], \tag{60}$$

$$\bar{\mu}_2^1 = h\left[z_2^1, s_1\right]. \tag{61}$$

These equations will be true in the special case where the functions f and g are *linear* in the state vector z; that is, where

$$f(z, s) = A(s) + M(s)z, \tag{62}$$

$$g(z, s) = B(s) + N(s)z, \tag{63}$$

where the vectors A and B and the matrices M and N (M being square) are functions of s, as indicated, but not of z. When this is the case, we can calculate that

$$\bar{p}_2^1 = \left(x_2^1\right)^{-1}\int_0^t p_2^1 X_2^1 da_1 = \left(x_2^1\right)^{-1}\left[A\int_0^t X_2^1 da_1 + M\int_0^t z_2^1 X_2^1 da_1\right] = A + M\bar{z}_2^1 = f\left(\bar{z}_2^1, s_2\right),$$

with the same result following for $\bar{r}_2^1$. Equation 3 then shows that Eq. 61 will also be satisfied in this case. When the functions f and g are linear in the state vector z, therefore, the balance equations for total biomass, masses of the components of the biomass, and masses of the environmental substances obey *in the mean* the balance equations that govern these quantities when segregation of biomass is neglected, and so these latter balance equations become exact. This shows that when we are dealing with structured models that are linear in the sense that Eqs. 62 and 63 are satisfied, it is not

necessary to consider the heterogeneity of the biomass in tank 2 in order to find the concentration of biomass and the concentrations of the abiotic substances in the tank.

It must be kept in mind that linearity of a structured model is a *sufficient* condition for Eqs. 59 to 61 to be true, but we have not proved that it is a *necessary* condition. In fact, it is not a necessary condition, for there are situations where a nonlinear structured model that does not take account of biomass segregation predicts the same things for the second tank as the nonlinear structured model that does take segregation into account. This happens, for example, when the distribution of *states* is narrow in some sense. We shall find that such a narrow distribution of states is a possibility even when the distribution of ages is broad.

Application of Unstructured Model to the First and Second Tanks of a Cascade of CSTR Bioreactors

Unstructured models of growth assume that the current specific growth rate and the current specific nutrient uptake vector of biomass of organisms of a certain kind are determined by the current vector of concentrations of substances in the environment of the organisms. They assume that there is no dependence of these quantities on the state of the organism, so whatever composition structure the organisms have is irrelevant. It is for this reason that such models are called unstructured. Since composition structure is irrelevant, so is biomass heterogeneity caused by addition of biomass from external sources.

If we assume that an unstructured model applies to tanks 1 and 2 of a cascade of CSTR bioreactors, the balance equations for biomass and the components of the abiotic environment will be

$$\frac{dx_1}{dt} = -D_1 x_1 + \mu_{uns}(s_1)\, x_1, \tag{64}$$

$$\frac{ds_1}{dt} = D_1(s_{1f} - s_1) - r_{uns}(s_1)\, x_1. \tag{65}$$

for tank 1 and

$$\frac{dx_2}{dt} = D_2(\phi x_1 - x_2) + \mu_{uns}(s_2)\, x_2, \tag{66}$$

$$\frac{ds_2}{dt} = D_2\left[\phi s_1 + (1-\phi)s_{2f} - s_2\right] - r_{uns}(s_2)\, x_2, \tag{67}$$

for tank 2. These equations, or special cases of them, have appeared many times in the literature. In them, $\mu_{uns}(s)$ and $r_{uns}(s)$ denote the functional dependences of the specific growth rate and the specific nutrient uptake vector, respectively, on the environmental state vector.

In order to have a complete model one needs to have equations for how the specific growth rate and the specific nutrient uptake rate vector depend on the state of the organisms' environment. In actual practice, this is done by fitting equations to data taken on some balanced growth situation, often steady-state growth in a single chemostat. Here, we shall follow the same procedure and construct an unstructured model corresponding to a given structured model by requiring that the functions $\mu_{uns}(s)$ and $r_{uns}(s)$ be such that they make the same predictions about the biomass and abiotic environment concentrations for steady-state growth in a single CSTR fed with sterile nutrient medium that the structured model makes.

Equations 64 to 67, the functional dependences $\mu_{uns}(s)$ and $r_{uns}(s)$, and appropriate initial conditions for the biomass and abiotic environments in the two tanks form the mathematical description of the two-tank cascade provided by the unstructured model corresponding to a given structured model.

Steady-State Behavior of a Cascade of Two CSTR Bioreactors

If a stable steady-state situation is attained in tank 1 of the cascade, the equations describing the biomass concentration and the biomass and abiotic environment state vectors are obtained by setting all time derivatives equal to 0 in Eqs. 11 to 13. These equations apply to the unsegregated, as well as to the segregated model. If we use an unstructured model, the steady-state equations are Eqs. 64 and 65, with the time derivatives set equal to 0. All three kinds of models yield the same steady-state results for the biomass concentration and the concentrations of the abiotic substances for the first tank.

The steady-state in tank 2 is of greater interest. If we use a structured model but neglect biomass segregation, the equations describing the steady-state in tank 2 are Eqs. 54 to 55, with the time derivatives on the lefthand sides set equal to 0. Similarly, if we use an unstructured model, the steady-state equations for tank 2 will be Eqs. 66 and 67, with the time derivatives on their lefthand sides set equal to 0. Since these two sets of equations are different, the predictions of these two models for the steady-state behavior in tank 2 will be different.

We turn now to the equations of a structured model that account for biomass segregation. Assuming that biomass was inoculated into tank 2 and that pseudoclones descendent from this remain in the tank when steady state is achieved, the equations that describe this biomass will be Eqs. 16 and 17 with the time derivatives on their lefthand sides set equal to 0

$$0 = -D_2 \hat{x}_2^2 + h\left[\hat{z}_2^2, \hat{s}_2\right] \hat{x}_2^2, \tag{68}$$

$$\mathbf{0} = f\left[\hat{z}_2^2, \hat{s}_2\right] - h\left[\hat{z}_2^2, \hat{s}_2\right] \hat{z}_2^2. \tag{69}$$

The behavior in tank 2 of biomass descendent from biomass inoculated into tank 1 is more complicated because the biomass is not in steady-state growth, even though the whole system is operating in a steady state and the concentrations of abiotic substances in the second tank are not changing with time. In this circumstance, the density of the distribution of states and the state vector become functions of age a_2 only, so that $(\partial X_2^1/\partial t)_{a_2} = 0$ and $(\partial \mathbf{z}_2^1/\partial t)_{a_2} = \mathbf{0}$. Equations 37 and 38 then show that $(\partial X_2^1/\partial t)_{a_1} \neq 0$ and $(\partial \mathbf{z}_2^1/\partial t)_{a_1} \neq \mathbf{0}$, so that is it inconvenient to regard the density and the distribution to be functions of a_1 and t, as we have done up until now. We therefore assume that X_2^1 and $\mathbf{z}_2^1$ are functions of (t, a_2) rather than of (t, a_1), and Eqs. 37 and 38 then show that Eqs.

34 and 35 must be replaced by

$$\frac{\partial X_2^1}{\partial t} + \frac{\partial X_2^1}{\partial a_2} = -D_2 X_2^1 + h\left[z_2^1, s_2\right] X_2^1, \tag{70}$$

$$\frac{\partial z_2^1}{\partial t} + \frac{\partial z_2^1}{\partial a_2} = f\left[z_2^1, s_2\right] - h\left[z_2^1, s_2\right] z_2^1. \tag{71}$$

The boundary condition Eq. 23 now becomes

$$X_2^1(t,0) = \phi D_2 x_1(t). \tag{72}$$

Equations 70 and 72, but not Eq. 71 were given earlier by Fredrickson (1992). The advantage of these equations is that the partial time derivatives on their lefthand sides vanish in the steady state, and so the equations become the ordinary differential equations

$$\frac{d\hat{X}_2^1}{da_2} = -D_2 \hat{X}_2^1 + h\left[\hat{z}_2^1, \hat{s}_2\right] \hat{X}_2^1, \tag{73}$$

$$\frac{d\hat{z}_2^1}{da_2} = f\left[\hat{z}_2^1, \hat{s}_2\right] - h\left[\hat{z}_2^1, \hat{s}_2\right] \hat{z}_2^1, \tag{74}$$

and they are to be solved subject to the boundary conditions

$$\hat{X}_2^1(0) = \phi D_2 \hat{x}_1 \tag{75}$$

$$\hat{z}_2^1(0) = \hat{z}_1^1 \tag{76}$$

In these equations, a caret over a quantity indicates the steady-state value of the quantity.

The steady-state balance equation for the abiotic substances can now be written using Eq. 36; it is

$$\mathbf{0} = D_2\left[\phi \hat{s}_1 + (1-\phi) s_{2f} - \hat{s}_2\right] - g\left[\hat{z}_2^2, \hat{s}_2\right] \hat{x}_2^2 - \int_0^\infty g\left[\hat{z}_2^1, \hat{s}_2\right] X_2^1 da_2 \tag{77}$$

Similarly, the steady-state balance equation for total biomass in tank 2 is

$$0 = D_2\left(\phi \hat{x}_1 - \hat{x}_2\right) + \left(1^\dagger f\left[\hat{z}_2^2, \hat{s}_2\right]\right) \hat{x}_2^2 + \int_0^\infty \left(1^\dagger f\left[\hat{z}_2^1, \hat{s}_2\right]\right) \hat{X}_2^1 da_2. \tag{78}$$

The foregoing equations may be used to deduce that, if a steady state is achieved in tank 2, then biomass descendent from cells inoculated into that tank will have been washed from it and all of the biomass in the tank will be descendent from cells inoculated into tank 1.

Formal integration of Eq. 73 and application of the boundary condition (Eq. 75) gives the steady-state density of biomass distribution for biomass descended from cells inoculated into tank 1 as

$$\hat{X}_2^1(a_2) = \phi D_2 \hat{x}_1 \exp\left\{-\int_0^{a_2}\left[D_2 - \hat{\mu}_2^1(a_2')\right] da_2'\right\}, \tag{79}$$

where we have reverted to the notation $\hat{\mu}_2^1(a_2) = h[\hat{z}_2^1(a_2), \hat{s}_2]$ for the specific growth rate in tank 2. If a steady state is achieved in the second tank, the state of the biomass in it that is descendent from biomass introduced from tank 1 must stop changing and become constant for sufficiently large age a_2, say for $a_2 \geq \tau$, where $\tau > 0$ is the time required for the state of transferred biomass to adjust completely to the constant environment in tank 2. Then for $a_2 \geq \tau$, the steady-state density of the biomass concentration will be given by

$$\hat{X}_2^1(a_2) = \phi D_2 \hat{x}_1 C \exp\left\{-\left[D_2 - \hat{\mu}_2^1(\tau)\right](a_2 - \tau)\right\}, \tag{80}$$

where C and $\hat{\mu}_2^1(\tau)$ are positive constants.

In tank 2, the steady-state concentration of biomass descendent from cells inoculated into tank 1 is given by

$$\hat{x}_2^1 = \int_0^\infty \hat{X}_2^1(a_2)\, da_2. \tag{81}$$

Clearly, this biomass concentration must be finite, and in order for this to be so, Eqs. 80 and 81 show that it is neccesary that

$$D_2 > \hat{\mu}_2^1(\tau). \tag{82}$$

That is, if a steady state is achieved in tank 2, the specific growth rate of biomass descendent from cells inoculated into tank 1 that is fully adjusted to the constant environment in tank 2 must be less than the dilution rate imposed on the tank. Now biomass descendent from cells that were inoculated into tank 2 must be fully adjusted to conditions in that tank if and when a steady state is achieved in it, and since, by hypothesis, the model that applies to biomass inoculated into tank 1 also applies to biomass inoculated into tank 2, it follows that $h[\hat{z}_2^2, \hat{s}_2] = \mu_2^1(\tau)$. Hence

$$D_2 > h\left[\hat{z}_2^2, \hat{s}_2\right], \tag{83}$$

and Eq. 68 then shows that $\hat{x}_2^2 = 0$.

In the foregoing, we have assumed that the states achieved when biomasses of different initial states adjust to a constant environment are the same. It is conceivable that there are chemically structured models for which this is not so, where different initial states might lead to fully adjusted states that had different specific growth rates. Even if this were true, it would still seem that the conclusion that $\hat{x}_2^2 = 0$ would still be valid, for in order for it not to be valid, the specific growth rate of biomass in tank 2 descendent from cells inoculated into that tank would have to be *exactly* equal to D_2, and there is no obvious reason why such exact adjustment of the specific growth rate would occur.

These considerations show that, at steady state in tank 2, there is no very ''old'' biomass, whether descendent from cells inoculated into tank 1 or descendent from cells inoculated

into tank 2 in the tank; all of the very ''old'' biomass has been washed from the tank and replaced by biomass that came from tank 1, biomass that we could call ''young'' biomass.

Numerical Methods for Solving the Equations

Three modeling approaches for a bioreactor system consisting of two CSTRs connected in series have been presented in the preceding sections. The corresponding mathematical formulations are general in the sense that they can be applied to any biological process that can be represented mathematically by Eqs. 1 to 3. This generality was also preserved in the development of numerical algorithms which solve the steady-state and transient problems for each of the different kinds of models—segregated, unsegregated, and unstructured—that are used in the modeling approaches described.

The steady-state problem for either an unsegregated or an unstructured model consists of a system of nonlinear algebraic equations. As a result, in most cases, it is not possible to obtain the solution(s) of these equations analytically. In such cases, the solution to the problem is achieved with the use of the Newton-Raphson method.

However, the steady-state problem for the segregated model is more complicated. It consists of a set of nonlinear algebraic equations describing the steady state in the first tank (Eqs. 11 to 13 with the time derivatives set equal to zero), a set of nonlinear ordinary differential equations for the steady-state biomass and state distributions with respect to the age a_2 (Eqs. 73 and 74 subject to the boundary conditions given by Eqs. 75 and 76, respectively), and a set of nonlinear integro-algebraic equations (Eq. 77) describing the steady-state consumption of the substrate(s) in the second tank.

The steady-state solution for the first tank is found either analytically (whenever possible) or using the Newton-Raphson method. The boundary conditions for the biomass and state age distributions are then calculated from Eqs. 75 and 76, respectively. The equations for the second tank are subsequently solved with an iterative procedure consisting of the following steps:

Step 1. A set of values for the concentration(s) of the substrate(s) in the second tank is assumed.

Step 2. The ordinary differential equations (Eq. 74) for the age distribution of the states are integrated with the use of the Runge-Kutta 4th-order method (Young and Gregory, 1988), forward in a_2, using the set of values for the concentration(s) of the substrate(s) assumed in step 1 and the boundary conditions given by Eqs. 76.

Step 3. The age distributions of the specific growth rate and the specific rates of consumption of the substrate(s) are evaluated using the assumed set of concentration(s) of substrate(s) and the age distribution of the states calculated in step 2.

Step 4. The steady-state biomass age distribution is evaluated from Eq. 79, using the steady-state value for the first tank and the specific growth rate age distribution calculated in step 3.

Step 5. The righthand side of Eq. 77 is evaluated using the age distributions of the specific rates of consumption of the substrate(s) calculated in step 3, the steady-state solution for the first tank, the biomass age distribution calculated in step 4, and the set of values for the concentration(s) of the substrate(s) assumed in step 1. If the L_2 norm of the righthand side of Eq. 77 vector is smaller than ϵ ($\epsilon = 10^{-9}$), the iterative procedure stops since all of the steady-state equations are satisfied and convergence is achieved. However, if this convergence criterion is not met, a new estimate for the concentration(s) of the substrate(s) is obtained using the formulae of the secant method (Young and Gregory, 1988), and the procedure consisting of steps 2 to 5 is repeated until convergence is reached.

When an unsegregated or unstructured model is used, the transient problem for the two-tank cascade consists of a system of nonlinear ordinary differential equations. These equations are integrated in time using the Runge-Kutta 4th-order method.

When a segregated model is used, the mathematical formulation of the transient problem for the first tank leads to a set of nonlinear ordinary differential equations (Eqs. 11 to 13). Formulation for the second tank yields a set of nonlinear ordinary differential equations for the time evolution of the biomass and the biomass components which are direct descendents of the biomass initially inoculated into the second tank (Eqs. 16 and 17), a set of nonlinear partial differential equations for the biomass and biomass components which are descendents of biomass inoculated into the first tank (Eqs. 34 and 35 subject to the boundary conditions Eqs. 23 and 27, respectively), and a set of ordinary integro-differential equations describing substrate consumption in the second tank (Eq. 36). Notice that Eq. 36 is nonautonomous. Notice also that the partial differential equations do not contain derivatives with respect to the time spent in the first tank (a_1).

The equations for the first tank and for the biomass and biomass components which are descendents of biomass initially inoculated into the second tank are solved using the Runge-Kutta 4th-order method. For the equations of the second tank which depend on the time spent in the first tank (Eqs. 34 to 36), an age grid is created at each point t of the time integration, with the age grid step $\Delta a_1 = \Delta t$, where Δt is the timestep. Equations 34 to 36 are integrated in time with the Runge-Kutta 4th-order method using the solution from the previous point in time ($t - \Delta t$). The values of the biomass and biomass component age distributions at age $a_1 = t$ are evaluated at each point in time using the boundary conditions (Eqs. 23 and 27) and the solution for the first tank at time t.

Discussion and Conclusions

The biomass in the second tank of a bioreactor cascade is heterogeneous with respect to age and perhaps also with respect to origin. Equations describing the density of the distribution of pseudoclone biomass with respect to age of the pseudoclones whose ancestral biomass came from the first tank have been written in several ways, using all possible different combinations of independent variables. The equations using time and age in tank 1 are more convenient for transient calculations, but the equations using time and age in tank 2 are more convenient for steady-state calculations. Equations that assume the biomass in the second tank is homogeneous have been written as well. One set of these equations uses a structured model, but the other uses an unstruc-

tured model. Comparison of the results predicted by the latter two sets of equations with the results predicted by the first-described equations will be made in a subsequent article in order to show, among other things, what are the effects of biomass segregation in the second tank. However, it has been shown already in this article that, if the structured model used is linear in the state vector, segregation can be ignored for the purposes of calculating the biomass concentration and the concentrations of nutrients and products present in the abiotic environment.

One thing that occurred to us as we did this work was that the use of pseudoclone age is an artificial device that was adopted to handle biomass segregation when a continuum rather than a corpuscular model of growth and product formation is used. This device had obscured the fact that the really important heterogeneity in the second tank is the heterogeneity of states, not the heterogeneity of pseudoclone ages. Hence, a method for calculating the distribution of states from the known distribution of ages was worked out and is described in the article.

The concepts of this article are also applicable to a single bioreactor, even a single batch bioreactor, when environmental conditions in the reactor are heterogeneous. Since it is known that environmental heterogeneity exists in large bioreactors and since this is very likely involved in the notoriously difficult problem of bioreactor scale-up, a few detailed remarks on heterogeneity of a single reactor will be made.

Bailey and Ollis (1986) give a number of models for single nonideal bioreactors and one of them is a model for a bioreactor in which there is a ''dead volume.'' The media in the dead volume and the main volume are both assumed to be well-mixed, but they are assumed to have different compositions; also, medium is assumed to flow from the main volume to the dead volume and at the same rate from the dead volume to the main volume. These authors give an analysis of this model which, however, does not take account of biomass segregation and which also uses an unstructured model. In principle, one can use the concepts of this article to do a more rigorous analysis, but such an analysis is a nontrivial matter. If we use a continuum structured model, as in this article, handling the multiplicity of biomass histories poses a difficult problem. In a two-tank cascade, as considered above, the biomass in tank 1 has only one history and the biomass in tank 2 has at most two histories; either it is descended from biomass that was inoculated into tank 2 or else it is descended from biomass that was transferred from tank 1 into tank 2. However, in the dead volume model of a single bioreactor, the flow and counterflow between the compartments means that the biomasses in the volumes will have many more histories. In the dead volume, for example, we have to consider histories represented by

$$\begin{aligned} &D\\ &M \rightarrow D\\ &D \rightarrow M \rightarrow D\\ &M \rightarrow D \rightarrow M \rightarrow D\\ &\quad\vdots\\ &\quad\vdots \end{aligned}$$

where D and M represent dead and main volumes, respectively, and arrows represent transfers of biomass from one to the other. It would not be difficult to write down the equations describing this situation, but it would seem that the computations would very likely be very complex.

One can cut through this Gordian knot by using a corpuscular model, because such a model automatically accounts for biomass segregation. Thus, the equations that describe the distributions of particle states in the two compartments of the bioreactor are

$$\frac{\partial F_M}{\partial t} + \nabla\cdot(r_M F_M) = 2\int_\Omega \gamma_M p_M F_M \, d\Omega - \left(\gamma_M + \frac{1}{1-\varphi} D\right) F_M - \frac{\rho}{1-\varphi} D(F_D - F_M), \quad (84)$$

$$\frac{\partial F_D}{\partial t} + \nabla\cdot(r_D F_D) = 2\int_\Omega \gamma_D p_D F_D \, d\Omega - \gamma_D F_D - \frac{\rho}{\varphi} D(F_M - F_D), \quad (85)$$

in which the symbols F, r, γ, p, and ∇ are as described by Fredrickson (1992), Ω is the domain of biomass composition states involved, ρ is the ratio of the intercompartmental volumetric flow rate to the volumetric flow rate through the reactor, and φ is the fraction of bioreactor liquid volume that is ''dead.'' In addition to these equations there are two vector equations which are the balance equations for masses of abiotic substances in the two parts of the bioreactor. It is, of course, much easier to write these equations than to use them to solve the problem at hand. Nevertheless, recent development of numerical methods for solving population balance equations make it seem that it might soon be possible to use them to predict the effects of bioreactor inhomogeneity (Mantzaris et al., 1998). The motivation for using corpuscular models is usually stated to be that only such models can handle the quantitative phenomena associated with the existence and occurrence of cell cycles. It is interesting to note that the inhomogeneous bioreactor is an example of a practical situation demanding use of a corpuscular model even though cell cycles are here only a matter of incidental interest.

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