Growth Processes in Bioreactors with External Sources of Biomass: Application of Structured, Continuum Models

A. G. Fredrickson
Dept. of Chemical Engineering and Materials Science, Institute for Advanced Studies in Biological Process Technology, University of Minnesota, Minneapolis 55455 and St. Paul 55108, MN

Batch, fed-batch, and single-stage CSTR bioreactors operate without external sources of biomass after they have been inoculated. However, other kinds of bioreactors, such as the second and subsequent reactors in a cascade of CSTRs, operate with continuous introduction of biomass from one or more external sources. The biomass in a bioreactor with an external source is not homogeneous with respect to past history of environmental conditions, and growth of this biomass is not balanced (steady-state) growth even when the bioreactor operates in steady state. So-called unstructured models of growth, which assume biomass to lack any internal structure or to have an invariant internal state, can give only a first approximation to the growth rate behavior of the biomass in a bioreactor with an external source of biomass. Structured models, which endow biomass with a changeable internal structure, are required to obtain more accurate predictions of growth rate behavior in such reactors. Introduction of structure is not sufficient for improved accuracy, however, and the fact that biomasses from different sources are and remain segregated from one another must also be accounted for by any structured growth model used. This article presents, among others, the systems of different equations that result from application of the notions to the reactors of a cascade of CSTRs.

Introduction

Any kind of a bioreactor requires inoculation with biomass from an external source to get it started, but several important kinds of bioreactors operate without external sources of biomass after they have been inoculated. The most obvious example is the batch bioreactor, but fed-batch and single CSTR (chemostat) bioreactors fed with sterile nutrient medium also operate without external sources of biomass after they have been inoculated. One might argue that these kinds of bioreactors do have external sources of biomass, since it is well known that biofilms often form on the walls of such reactors and that the fluid culture in the reactor is re-inoculated with biomass from such films. This article, however, deals only with ideal kinds of bioreactors, so complications caused by biofilm formation, spatially inhomogeneous cultures, and so on will not be considered. The term "external source of biomass" will then refer to biomass introduced into a bioreactor from some other reactor, after the bioreactor under consideration has been inoculated. The biomass of the inoculum will be called "initial biomass."

Several kinds of bioreactors operating with continuous introduction of biomass from one or more external source or sources have been described in the literature, and some of these are used in practice. If two or more CSTRs are connected in series, then the second, third, and so on, CSTRs in such a cascade will have biomass introduced into them from the previous reactor in the cascade. A single CSTR operating with cell recycle is also a bioreactor operating with a single external source of biomass, provided that the cell separation and recycle scheme used alters the physiological state of the recycled biomass. Recently, Lovitt and Wimpenny (1981) have described the *gradostat* apparatus. This is a sequence of two or more CSTRs, in which two fluid streams flow countercurrently from stage to stage. Sterile nutrient media of different compositions are fed at the two ends of the sequence, and cultures of different

physiological states are removed simultaneously from the ends. Evidently, all of the reactors of a gradostat have at least one external source of biomass.

The population in a reactor operating without an external source of biomass is a collection of clones of its initial biomass, while that in a reactor with an external source is a mixture of clones from different sources. Consider the various stages of a cascade of CSTRs. The population in the first stage is homogenous, in the sense that its population is a collection of clones of its initial biomass. Assuming that the reactor is well-mixed, all of these clones have seen the same history of environmental conditions. On the other hand, the second-stage population is not homogenous in the same sense, because its population is a mixture of clones of its initial biomass and of clones of the initial biomass of the first stage. Moreover, because biomass is continually being transferred from the first stage to the second and the chemical environments in different reactors are different, clones of the initial biomass of the first stage that are in the second stage have different past histories of environmental circumstances since their ancestral cells passed from the first to the second stage at different times. This will be true even when both reactors are well-mixed, and when steady states have been achieved in both. Continuing with this train of thought, we see that the population in the third stage of a cascade of CSTRs will be even more inhomogenous with respect to the two factors of ultimate origin and history of environmental conditions than that in the second stage, and that this trend of increasing heterogeneity will continue to increase with stage number in the cascade.

Kinetic and material balance equations purporting to describe the operation of well-mixed bioreactors with external sources of biomass have appeared in many research articles, review articles, monographs, and textbooks. As far as I am aware, all of these works have used models whose cells are assumed not to have internal structure that changes in response to environmental changes; in effect, the models used treat the biomass as having an invariant physiological state. Such models have been called *unstructured* models of growth (Tsuchiya et al., 1966; Fredrickson et al., 1970). Obviously, unstructured models cannot take into account the heterogeneity of ultimate origin and environmental history that necessarily occur in bioreactors with external sources of biomass. Moreover, it has been shown to be appropriate to use unstructured models in situations that are *balanced growth* (Fredrickson et al., 1971), but the situation in a bioreactor having an external source of biomass is not balanced growth, even when the reactor as a whole is operating in a steady state. The reason that it is not balanced growth is that when biomass passes from one bioreactor into another, it is exposed suddenly to a new chemical environment, and regulatory processes within the biomass begin to adjust the physiological state of the biomass to be appropriate for the new environment. Adjustment cannot occur instantaneously; thus, introduced biomass will be in a state of transient, unbalanced growth for a significant length of time after introduction.

Evidently, unstructured growth models cannot give a rigorous description of growth in a bioreactor having one or more external sources of biomass, and structured models are required for that purpose. But just introduction of structure into the model is not enough. We must also take account of the phenomenon of *segregation*, which means that if we mix cultures containing biomasses of different states, *the biomasses do not coalesce and form biomass of a single new state, but instead, they remain segregated as biomasses of different states.* I am not aware of a source that has made an actual and correct application of a structured model to a bioreactor with an external source of biomass. For example, the formulation of structured models that I presented earlier (Fredrickson, 1976) dealt only with their application to bioreactors having no external sources of biomass, and Bailey and Ollis give in their textbook (1986, p. 409) a balance equation for structured biomass that must be modified if there is an external source of biomass, as in the second or later stages of a CSTR cascade.

The purpose of this article is therefore to show how to apply structured models and to take account of segregation to describe growth processes in a bioreactor having an external source of biomass. The main emphasis will be on continuum rather than corpuscular (Roels, 1983) structured models of growth processes, as the difficulties associated with the latter class of models seem to preclude their practical application at the present time.

Use of Structured, Corpuscular Models

Roels (1983) used the term "corpuscular" to describe growth models that take account of the fact that a cell population is divided into discrete "corpuscles," which remain segregated from one another. He used the term "continuum" for models that do not take this division into account. Earlier, Tsuchiya et al. (1966) had called the two kinds of models "segregated" and "distributed," and later Fredrickson et al. (1970) and Ramkrishna (1979) referred to them as "segregated" and "nonsegregated," although Ramkrishna also used the term "lumped" as an alternative to "nonsegregated." I now prefer Roels' term "corpuscular" to "segregated," because to be biologically correct, every model, whether corpuscular or continuum (in the sense of Roels), should reflect the fact that biomass is segregated. I shall also use Roels' term "continuum model," and in his sense, in spite of the fact that I have some reservations about it.

Structured, corpuscular models automatically take segregation into account, and therefore, such a model is, in principle, the right kind of model to use to describe growth processes in bioreactors having external sources of biomass. As an example, consider a well-mixed bioreactor, which is fed with several streams containing biomasses. A so-called population balance equation can be written for the biomass in the reactor, and this balance is nothing more than an accounting for the various processes, physical as well as biological, that change the number of cells in the reactor that have their internal state confined to an infinitesimal volume of state space. A population balance is: rate of accumulation = net rate of gain by growth processes − rate of loss by cell division + rate of gain by division of cells of different state + rate of gain from external sources − rate of loss by washout. In writing the term representing rate of gain from an external source of biomass, it is correct to take this to be just the rate at which numbers of cells of the given state are carried from the source under consideration into the bioreactor on which the population balance is made. This is so because once a cell from an external source has been carried into a bioreactor, we cannot, by definition, distinguish it from other cells in the reactor that have the same state.

Fredrickson et al. (1967) wrote a population balance equation for a well mixed bioreactor that does not have an external source of biomass. This balance equation assumed that the state and so the structure of a cell was specified by a vector, y, of single-cell properties. If we rewrite this equation for a well mixed bioreactor with one or more external sources of biomass, it becomes:

$$\frac{\partial F}{\partial t} = -\nabla \cdot [rF] - \gamma F + 2\int_v \gamma p F dv' + \sum_k D_k F_k - DF, \quad (1)$$

where the term on the lefthand side is the rate of accumulation, the first term on the righthand side is the net rate of gain by growth, and so on. In this equation, F is a density function defined such that Fdv gives the number of cells, per unit volume of culture in the reactor, that have states y in the element dv of state space, ∇ is a divergence operator appropriate for the state space at hand, r is the single-cell growth rate vector, γdt is the fraction of cells of a given state that divide in a time interval of length dt, p gives the probability that fission of a mother cell with a state in dv' will produce a daughter cell with a state in dv, $D_k = q_k/V$, q_k is the volumetric flow rate at which culture from the kth external source of biomass enters the bioreactor, V is the reactor volume, F_k is the density function of the biomass in the kth external source, and D is the sum of all the D_k.

Equation 1 and its generalizations, which account for transitions between recognizable discrete stages of the cell cycle, handle external sources of biomass correctly; therefore, such equations can serve as the starting point for modeling growth processes in bioreactors having such sources. Equation 1 and the generalizations noted are attractive also because the notion that cells go through a cell cycle is built into them, and thus, as experimental work unravels the details of what happens during the cell cycle of an organism, the information provided can be incorporated into more and more detailed and accurate models of the organism's growth and activities.

There are, however, several severe difficulties associated with attempts to model the behavior of any kind of bioreactor with structured, corpuscular models. These have been discussed thoroughly by Ramkrishna (1979, 1985), and so it is not necessary to give statements of the difficulties here. One of their consequences is that most workers concerned with modeling growth and related phenomena have chosen to use structured, continuum models rather than structured, corpuscular models. Such models have nothing to say about how properties of a cell population are distributed, and they cannot handle cell cycle phenomena in asynchronously dividing populations either. However, they can accommodate transient growth phenomena to some extent, and there has been much interest in them in recent years. Hence, I shall consider here the application of structured, continuum models to bioreactors with external sources of biomass.

Concepts of a Pseudoclone and Its Ages

A *pseudoclone* is a collection of clones, each descendant from a cell in the inoculum to a given bioreactor, and each of which experiences the same history of environmental conditions during some defined interval of time. The term "pseudoclone" is used since the biomass involved in it is not the descendant of a single cell. The term "clone" applies to all cells descendant from a single cell whether or not they have experienced the same history of environmental conditions, but experience of a common history of environmental conditions is an essential part of the definition of the term "pseudoclone." Because of this difference, a different term might be more appropriate for what I have called a "pseudoclone," but I have been unable to think of such a term. If the history of environmental conditions can be broken down into a succession of continuous changes during n finite intervals of time, with these intervals being separated by $n-1$ instants of time in which discontinuous changes of environmental conditions occur, then n *ages* are associated with the pseudoclone, and these are the time intervals during which environmental conditions change continuously. Evidently, the sum of these ages is the defined interval of time mentioned in the opening sentence of this paragraph.

In the case of a cascade of CSTRs or a gradostat apparatus, where the bioreactors can be considered to be well mixed and where transfers from one reactor to another can be considered to occur instantaneously, the ages of a pseudoclone present in a bioreactor at time t after start-up of the apparatus consist of the time elapsed, since the pseudoclone entered the bioreactor plus the times that it spent in other bioreactors of the apparatus. In a cascade apparatus, the age at which a pseudoclone is transferred from one reactor to another is subject to the laws of probability, but the direction of transfer is determinate. (To be exact, we should say that a *portion* of a pseudoclone is transferred and the nontransferred portion becomes a different pseudoclone, since after transfer the two portions experience different environmental conditions.) In a gradostat, age at transfer is likewise subject to the laws of probability, but so is the direction of transfer. Because of the indeterminate direction of transfer, the gradostat apparatus cannot be handled by the methods to be described below, and the only way I can see to use structured models for this apparatus is to make the models corpuscular as well.

It will be assumed in the following developments that all pseudoclones are from the same species, and moreover that they have the same genetic constitution. In terms of the structured model used for biomass growth, this means that the same model and the same model parameters will apply to biomass from all sources.

Differential Equations Governing Concentrations and Age Distributions of Biomasses of Pseudoclones in a Cascade of Well Mixed CSTRs

Consider a cascade of well mixed CSTRs of equal volume, with sterile nutrient medium being introduced into reactor 1, and culture being transferred from reactor 1 to reactor 2, from reactor 2 to reactor 3, and so on. Assume that the volume fraction of biomass is not large in any reactor, so that the corrections for finite biomass volume fraction discussed by Monbouquette (1987) and Fredrickson and Hu (1989) are not needed. Assume that a structured, continuum model describes the growth, nutrient consumption and product formation of biomass in the apparatus. The problem at hand is to derive the differential equations and associated initial and boundary conditions that describe the concentrations and age distributions of biomasses of pseudoclones in reactors 1, 2, 3, and so

on. Since we will be dealing with pseudoclones, segregation of biomass will automatically be taken into account.

It will suffice for our purposes to derive the equations for the third reactor in a cascade. The third reactor is far enough along in the cascade for the equations to exhibit their general features, but not so far along as to make them excessively complicated. If a cascade of interest has only two CSTRs, then the equations describing the apparatus will be somewhat simpler than the ones given here.

The biomass in the third reactor of a cascade is not homogenous, but, in general, will contain pseudoclones that originated in the first and second reactors as well as in itself. "Originated" means was inoculated into the reactor named and so was present at the start-up of the cascade at time $t = 0$. Hence, if we let $x_i(t)$ be the total concentration of biomass in reactor i at time t, and $x_i^j(t)$ be the concentration in reactor i at time t of biomass that originated in reactor $j (j \le i)$, then for reactor 3:

$$x_3(t) = x_3^3(t) + x_3^2(t) + x_3^1(t). \tag{2}$$

Evidently, there is only one pseudoclone in reactor 3 that originated in that reactor, and the age of that pseudoclone is $a_3 = t$. The notation used here and subsequently is that a_i is the time spent in the ith reactor, if the pseudoclone is in a reactor downstream from the ith one, or the time elapsed since it entered the ith reactor if it is in the ith reactor. Hence, the concentration of biomass of this pseudoclone satisfies the differential equation:

$$\frac{dx_3^3}{dt} = -Dx_3^3 + \mu_3^3 x_3^3 \tag{3}$$

and the initial condition:

$$x_3^3(0) = x_3(0), \tag{4}$$

where $x_3(0)$ is the biomass concentration of the inoculum in reactor 3. In Eq. 3, there is no term for input of biomass of the pseudoclone into reactor 3, because there is no such input. Also, μ_3^3 is the specific growth rate of the pseudoclone, and it is a function of time, or of age a_3 (but not both t and a_3, since for a particular pseudoclone t and a_3 cannot be varied independently; instead $da_3/dt = 1$), as well as of the state of the abiotic part of the culture in the third reactor at time t. In what follows, we shall assume that μ_3^3 is a function of t and of the state of the abiotic part of the culture at time t. The specific growth rate is a function of time, because the state of the pseudoclone is a function of time.

Consider now pseudoclones originating in reactor 2 that are present in reactor 3 at time t. Evidently, there are an infinity of these, and the biomass of such pseudoclones is not homogeneous but has a distribution of ages a_3 ranging from 0 to t. However, the times spent by a particular pseudoclone in reactors 2 and 3 must be the current elapsed time since start-up: $a_2 + a_3 = t$. Let a function $X_3^2(t,a_3)$ be defined such that $X_3^2(t,a_3)da_3$ is the concentration of biomass of pseudoclones originating in reactor 2 that have been in reactor 3 for a time between a_3 and $a_3 + da_3$ at time t. This function is the non-normalized density of the distibution of ages in reactor 3 of pseudoclones which originated in reactor 2. Then we can write:

$$x_3^2(t) = \int_0^t X_3^2(t,a_3)da_3 = \int_0^t X_3^2(t,t-a_2)da_2. \tag{5}$$

The second version of Eq. 5 arises because if t and a_2 are given for the pseudoclone, then a_3 is given also, and is $a_3 = t - a_2$.

By a balance on pseudoclonal biomass of age between $a_{3,1}$ and $a_{3,2}$, where $a_{3,1}$ and $a_{3,2}$ are *fixed* ages, made over a time interval t to $t + dt$, we have that:

$$d\int_{a_{3,1}}^{a_{3,2}} X_3^2(t,a_3)da_3 = X_3^2(t,a_{3,1} - dt)dt - X_3^2(t,a_{3,2} - dt)dt + dt\int_{a_{3,1}}^{a_{3,2}} (\mu_3^2 - D)X_3^2(t,a_3)da_3.$$

From this, we can show that the density function $X_3^2(t,a_3)$ must satisfy the partial differential equation:

$$\frac{\partial X_3^2}{\partial t} + \frac{\partial X_3^2}{\partial a_3} = (\mu_3^2 - D)X_3^2, \tag{6}$$

where μ_3^2 is the specific growth rate of a pseudoclone in reactor 3 that originated in reactor 2. This specific growth rate is a function of age a_3 and of current time (or of ages a_3 and a_2, since $a_2 + a_3 = t$) as well as of the state of the abiotic part of the culture in the third reactor at the current time. In what follows, we shall take μ_3^2 to be a function t and a_3 and of the state of the abiotic part of the culture. Although pseudoclones originating in reactor 2 are washed in to reactor 3, no term for this source occurs in Eq. 6. This is because the equation holds for $a_3 > 0$, and pseudoclonal biomass originating in reactor 2 has age $a_3 = 0$ when it first appears in reactor 3. By balance on pseudoclonal biomass of age 0 to da_3, we obtain:

$$X_3^2(t,0) = Dx_2^2(t), \tag{7}$$

which is the boundary condition that must be satisfied by Eq. 6. The quantity $x_2^2(t)$ is the concentration in reactor 2 of the pseudoclone that originated in that reactor. There is only one such, and its age a_2 at time t is $a_2 = t$.

The initial condition for Eq. 6 is provided by the fact that, at time $t = 0$, reactor 3 contains no biomass of a pseudoclone originating in reactor 2. In mathematical terms, therefore, we have that:

$$X_3^2(0,a_3) = 0 \quad \forall\ a_3 \ge 0. \tag{8a}$$

In turn, this means that, at time $t > 0$, reactor 3 contains no biomass of a pseudoclone originating in reactor 2 that is as old as t. Thus, we have the useful relation:

$$X_3^2(t,t) = 0 \quad \forall t \ge 0. \tag{8b}$$

If each term of Eq. 6 is integrated on a_3 from 0 to t and use is made of Eqs. 5, 7 and 8b, one obtains:

$$\frac{dx_3^2}{dt} = D(x_2^2 - x_3^2) + \int_0^t \mu_3^2 X_3^2 da_3. \tag{9}$$

This is a balance on biomass of all pseudoclones in reactor 3 originating in reactor 2, and it is the analog of Eq. 3, which is a balance on biomass of the pseudoclone in reactor 3 originating in reactor 3. The initial condition for $x_3^2(t)$ follows from Eqs. 5 and 8a, and is:

$$x_3^2(0) = 0. \tag{10}$$

Consider finally pseudoclones originating in reactor 1 that are present in reactor 3 at time t. Evidently, there is an infinity of these, and three ages, a_1, a_2, and a_3, are associated with each. Here, a_1 was the age of the pseudoclone when it was transferred from reactor 1 to reactor 2, a_2 was its age (in reactor 2) when it was transferred from reactor 2 to reactor 3 at time $a_1 + a_2$, and a_3 is its age in reactor 3 at time t: $a_1 + a_2 + a_3 = t$.

Define a function $X_3^1(t,a_3,a_2)$ such that $X_3^1(t,a_3,a_2)\, da_3 da_2$ is the concentration of biomass of pseudoclones originating in reactor 1 that were in reactor 2 for a time between a_2 and $a_2 + da_2$ and have been in reactor 3 for a time between a_3 and $a_3 + da_3$ at time t. This function is the nonnormalized density of the joint distribution of ages in reactors 2 and 3 of pseudoclones which originated in reactor 1. If desired, one can calculate the joint distributions of ages in reactors 1 and 3 or 1 and 2, if the joint distribution in reactors 2 and 3 is known, because the three ages must sum to the current time, t. It follows then that:

$$x_3^1(t) = \int_0^t da_2 \int_0^{t-a_2} X_3^1(t,a_3,a_2)\, da_3 = \int_0^t da_3 \int_0^{t-a_3} X_3^1(t,a_3,a_2)\, da_2$$

$$= \int_0^t da_3 \int_0^{t-a_3} X_3^1(t,a_3,t-a_3-a_1)\, da_1 = \ldots . \tag{11}$$

The double integrals in the first line of Eq. 11 are over the domain in $a_2 - a_3$ space defined by $a_2 \geq 0$, $a_3 \geq 0$, and $a_2 + a_3 \leq t$.

By another balance on pseudoclonal biomass of age between fixed limits, we can show that the density function $X_3^1(t,a_3,a_2)$ must satisfy the partial differential equation:

$$\frac{\partial X_3^1}{\partial t} + \frac{\partial X_3^1}{\partial a_3} = (\mu_3^1 - D) X_3^1, \tag{12}$$

where μ_3^1 is the specific growth rate of a pseudocline in reactor 3 that originated in reactor 1. This specific growth is a function of the current time, and the two ages a_3 and a_2 (or of the three ages a_3, a_2, and a_1, since these sum to t) as well as the current state of the abiotic part of the culture in the third reactor. In what follows, we shall take μ_3^1 to be a function of t, a_3, and a_2 and of the current state of the abiotic part of the culture. In spite of the fact that X_3^1 depends on a_2 as well as on a_3 and t, there is no derivative $\partial X_3^1/\partial a_2$ in Eq. 12 because, to put it figuratively, the clock that measures a_2 stops running and the clock that measures a_3 starts running when the pseudoclone is transferred into reactor 3 from reactor 2.

By balance on pseudoclonal biomass of age 0 to da_3, we obtain the boundary condition:

$$X_3^1(t,0,a_2) = D X_2^1(t,a_2), \tag{13}$$

where $X_2^1(t,a_2)da_2$ is the concentration of pseudoclonal biomass originating in reactor 1 and present in reactor 2 that has age between a_2 and $a_2 + da_2$ at time t.

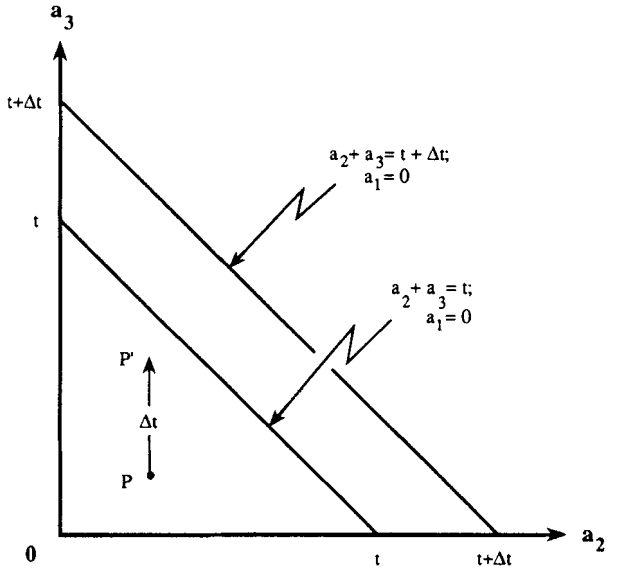

Figure 1. Time-age diagram for pseudoclones originating in the first reactor of a cascade of CSTRs and currently present in the third reactor of the cascade.

Since at time $t = 0$ reactor 3 contains no biomass of a pseudoclone that originated in reactor 1 and passed through reactor 2, the initial condition for Eq. 12 is:

$$X_3^1(0,a_3,a_2) = 0 \quad \forall a_2,\ a_3 \geq 0. \tag{14a}$$

We may deduce also that:

$$X_3^1(t,t-a_2,a_2) = 0 \quad \forall t \geq a_2 \geq 0. \tag{14b}$$

The time-age relations from which the foregoing result follows can be understood most readily by portraying them on a diagram on which a_3 is plotted against a_2, as in Figure 1. Here, only ages confined to the triangular domain $a_2 \geq 0$, $a_3 \geq 0$, and $a_2 + a_3 \leq t \geq 0$ are of concern to us. Any point within or on the boundary of this domain represents the three ages of a pseudoclone at the indicated time. Insofar as a_1 is concerned, a_1 is equal to t if the point is at the origin, but it is equal to 0 if the point lies on the hypotenuse of the triangle. As time increases, the hypotenuse moves outward parallel to itself, and during time interval t to $t + \Delta t$, the point P representing the ages of a pseudoclone originating in reactor 1 that is in reactor 3 at time t moves vertically upward by an amount Δt to P', because its ages a_1 and a_2 do not change but its age a_3 increases by Δt. Equation 14b states that X_3^1 is zero along the hypotenuse of the triangle, and the reason for this is simply that since no biomass of a pseudoclone originating in reactor 1 was present in reactors 2 and 3 initially, there has been insufficient time at time t to produce in reactor 3 a pseudoclone with such origin and having ages $a_1 = 0$, $a_2 + a_3 = t$.

Although $X_3^1 = 0$ along the hypotenuse of the triangular domain of Figure 1, it does not follow that X_3^1 is only infinitesimally greater than 0 for points falling below and to the

left of the hypotenuse by an infinitesimal amount. In fact, if we plotted X_3^1 on a vertical axis perpendicular to the $a_2 - a_3$ plane, this would show the distribution of biomass of the pseudoclones originating in reactor 1 and present in reactor 3 at an instant of time, and this distribution might well achieve a maximum value along a line only infinitesimally removed from the hypotenuse of the triangular domain.

If each term of Eq. 12 is integrated over the triangular domain in Figure 1 and use is made of Eqs. 11, 13 and 14b, one obtains:

$$\frac{dx_3^1}{dt} = D(x_2^1 - x_3^1) + \int_0^t da_2 \int_0^{t-a_3} \mu_3^1 X_3^1 da_3$$

$$= D(x_2^1 - x_3^1) + \int_0^t da_3 \int_0^{t-a_2} \mu_3^1 X_3^1 da_2. \quad (15)$$

This is a balance on biomass of all pseudoclones in reactor 3 which originated in reactor 1, and it is the analog of Eqs. 3 and 9. The initial condition for $x_3^1(t)$ follows from Eqs. 11 and 14a, and is:

$$x_3^1(0) = 0. \quad (16)$$

A balance for total biomass in reactor 3 may be obtained from Eqs. 3, 9, and 15 using Eq. 2 and the corresponding equation $x_2(t) = x_2^2(t) + x_2^1(t)$ for reactor 2; this gives:

$$\frac{dx_3}{dt} = D(x_2 - x_3) + \mu_3^3 x_3^3 + \int_0^t \mu_3^2 X_3^2 da_3 + \int_0^t da_2 \int_0^{t-a_2} \mu_3^1 X_3^1 da_3, \quad (17)$$

for which the initial condition is:

$$x_3(0) = x_3^3(0). \quad (18)$$

Equation 17 displays the effects on the growth of biomass of the heterogeneity with respect to origin and of age of the pseudoclones in reactor 3. There is of course another aspect of heterogeneity—a pseudoclone is not a homogenous collection of biomass, but continuum models cannot handle that kind of heterogeneity. If, as assumed when an unstructured model is used, the specific growth rates that appear in Eq. 17 happened to depend only on current conditions in the abiotic part of the culture in reactor 3, then $\mu_3^3 = \mu_3^2 = \mu_3^1 = \mu_3$ and Eq. 17 would reduce to a form that has appeared many times in the literature:

$$\frac{dx_3}{dt} = D(x_2 - x_3) + \mu_3 x_3. \quad (19)$$

Of course, this gives no hint of the heterogeneity of the biomass in reactor 3.

Not all of the equations for the third reactor in a cascade given above need to be considered when one is trying to calculate what goes on in that reactor. The essential equations are the balance differential equations (Eqs. 3, 6, and 12), together with their associated initial conditions (Eqs. 4, 8a and 14a), and the boundary conditions (Eqs. 7 and 13) for the two partial differential equations (Eqs. 6 and 12). These boundary conditions require knowledge of the state of things in the second reactor of the cascade. To determine these, a set of essential equations must be written for that reactor. These will be two balance equations: one an ordinary differential equation (for x_2^2) and the other a partial differential equation (for X_2^1), and their associated initial conditions and the boundary condition for the partial differential equation. The latter boundary condition will require knowledge of the state of things in the first reactor, and to determine these, an ordinary differential equation (for x_1^1) and its associated boundary condition will be required. If there are more than three reactors in the cascade, sets of essential equations must be written for each of them, and of course, the numbers of essential equations will increase with reactor number, a reflection of the increasing heterogeneity of the populations in each successive downstream reactor.

Evidently, these sets of essential equations do not have to be solved simultaneously but rather can be solved successively. Thus, one would start with the equations for the first reactor, solve these, proceed to the equations for the second reactor, and repeat the calculations stage by stage until all reactors have been considered.

Equations of Change of the Abiotic Portion of the Culture in One of the Reactors of a Cascade of CSTR Bioreactors

A population of microorganisms or cells and its abiotic environment evolve together in time, and one must know at what rates growth of the population removes nutrients from the environment and secretes products of growth into it. Let r_i^j be the vector of specific rates of uptake or excretion of environmental substances by a pseudoclone which originated in the jth reactor and which is present in the ith reactor ($j \le i$). I adopt the convention that an element of r_i^j for a specific environmental substance is positive if that substance is taken up by the pseudoclone, whereas it is negative if the substance is produced and excreted into the medium by the pseudoclone. For convenience, I shall speak of r_i^j as the specific rate of nutrients uptake vector, but it should be remembered that the same symbol refers also to product formation. Like specific growth rate, the specific rate of nutrients uptake by a pseudoclone depends on time and the age or ages of the pseudoclone. For example, r_3^1 can be taken to be a function of (t,a_3,a_2) or (a_3,a_2,a_1), but in what follows, I shall take it to be a function of (t,a_3,a_2); the same convention will be followed for specific nutrients uptake rate vectors for other pseudoclones.

Rather than try to write material balance equations on environmental substances present in the abiotic portion of the culture in an arbitrary reactor of a cascade, I will follow the plan adopted previously and write the equations for the third reactor of a cascade. It will then be clear how to write the equations for any reactor in the cascade. For the third reactor, the required balance equation is:

$$\frac{ds_3}{dt} = D(s_2 - s_3) + K_L a(\Delta s)_{M,3} - r_3^3 x_3^3 - \int_0^t r_3^2 X_3^2 da_3 - \int_0^t da_2 \int_0^{t-a_2} r_3^1 X_3^1 da_3, \quad (20)$$

where s_2 and s_3 are the vectors of concentrations of environmental substances present in the steams leaving the second and third reactors of the cascade, $K_L a$ is the diagonal matrix whose elements are the coefficients for mass transfer of the various environmental substances from the gas phase to the liquid phase in the reactor, and $(\Delta s)_{M,3}$ is the vector of appropriate mean driving forces for transfer of masses from the gas to the liquid phase in the reactor. For environmental substances that are not dissolved gases, one can set the corresponding elements of the mass-transfer matrix equal to zero, or one can set the corresponding elements of the driving force vector equal to zero.

Steady-State Versions of the Equations

The situations described in the preceding two sections will be somewhat simplified if biomass is inoculated only into the first reactor of a cascade $[x^j_i(0) = 0 \ \forall i,j>1]$. The populations in the downstream reactors will still be heterogeneous, but now the heterogeneity is just heterogeneity of age; there is no heterogeneity with respect to reactor of origin.

If a cascade apparatus approaches a steady-state condition as time since start-up increases indefinitely, it seems likely that the populations in the apparatus will approach the condition described in the previous paragraph, even when all of the reactors were inoculated with biomass. The reason is that as Eq. 20 shows, the various pseudoclones in a reactor have to compete for the nutrients present therein, and in this intraspecific competition, pseudoclones originating in the first reactor have an advantage over pseudoclones originating in downstream reactors: there is always a source of pseudoclones which originated in the first reactor, provided, of course, that this reactor is not operated at such a high dilution rate that the population is washed out of it. If an unstructured model applied, specific growth rates of various pseudoclones in the ith reactor would all be the same ($\mu^i_i = \mu^{i-1}_i = \mu^{i-2}_i$, and so on) because all the pseudoclones experience the same environmental conditons and, when an unstructured model applies, all have the same internal state. The theory of pure and simple competition (see, for example, Fredrickson and Stephanopoulos, 1981) then shows that all pseudoclones except for those originating in the first reactor will be washed from the cascade, if sufficient time is allowed. However, when stuctured models are used, it is conceivable that under appropriate conditions, the pseudoclone originating in a given reactor will be able to compete successfully with pseudoclones originating in previous reactors of the cascade.

Assuming that the cascade apparatus achieves a steady state and that only pseudoclones originating in the first reactor are present in this state, we find from the steady-state versions of equations such as Eqs. 12 and 13 that the steady-state, nonnormalized age distribution functions are given by:

$$\hat{X}^1_2(a_2) = D\hat{x}^1_1 e^{-Da_2} \exp\left\{\int_0^{a_2} \hat{\mu}^1_2 da_2\right\}, \tag{21a}$$

$$\hat{X}^1_3(a_3,a_2) = D\hat{X}^1_2(a_2) e^{-Da_3} \exp\left\{\int_0^{a_3} \hat{\mu}^1_3 da_3\right\}, \tag{21b}$$

and so on, where a caret over a quantity indicates a steady-state value. In addition, the steady-state versions of the balance equations on the substances present in the abiotic portions of the culture become:

$$D(\hat{s}_1 - \hat{s}_2) + K_L a(\Delta\hat{s})_{M,2} = D\hat{x}^1_1 \int_0^\infty \hat{r}^1_2 e^{-Da_2} \exp\left\{\int_0^{a_2} \hat{\mu}^1_2 da_2\right\} da_2, \tag{22a}$$

$$D(\hat{s}_2 - \hat{s}_3) + K_L a(\Delta\hat{s})_{M,3} = D^2\hat{x}^1_1 \int_0^\infty da_2 \int_0^\infty \hat{r}^1_3 e^{-(Da_2 + Da_3)} \exp\left\{\int_0^{a_2} \hat{\mu}^1_2 da_2 + \int_0^{a_3} \hat{\mu}^1_3 da_3\right\} da_3, \tag{22b}$$

and so on.

Equations 22a and 22b are one set of key equations for calculating steady-state conditions in a cascade apparatus. If we knew how the steady-state specific growth rates and nutrients uptake rates of pseudoclones originating in reactor 1 and present in reactor i depended on pseudoclone ages and the composition of the abiotic portion of the culture in reactor i, then Eqs. 22 would allow us to calculate what that composition is, provided that we knew the composition of the abiotic portion of the culture in reactor $i-1$ and the steady-state biomass concentration in reactor 1. The solution of the calculation would depend on the dilution rate used, of course, and there would be a limit on the value of this parameter beyond which steady states could not be achieved.

The calculation described in the previous paragraph requires knowledge of how the specific growth and nutrient uptake rates depend on the ages of a pseudoclone as well on how they depend on the composition of the pseudoclone's environment. This is where a structured, continuum model enters into our considerations. We shall show next how such a model can be used to provide the necessary information.

Equations of State Change of a Pseudoclone

The equations written above are incomplete as they do not tell us how the various μ's and r's depend on time and ages, nor is it clear from these equations how the concentrations (s's) in the abiotic part of the cultures affect the μ's and r's. The missing piece in the conceptual apparatus is provided by the structured, continuum model that describes the growth, nutrient uptake, and product formation kinetics of biomass. The nature and form of the equations that give the dependences of μ's and r's on time, ages, and concentrations in the abiotic portions of cultures will depend on the specific kind of model chosen to describe state and change of state of biomass. Unstructured models are the trivial case. With them, there is no need to describe state because it is assumed to be invariant, so specific growth rate and specific rates of nutrients uptake are functions only of the concentrations of substances present in the abiotic portions of cultures. These simplifications do not apply when structured models are used.

We shall consider here only those structured models for which the state of a pseudoclone is described by a *vector* of state variables. If we are dealing with a pseudoclone that originated in the jth reactor but is in the ith reactor ($i \geq j$) at the

time under consideration, then we shall denote the state vector of this pseudoclone by z_i^j. If $j=i$, z_i^j can be regarded to be a function of t or a_i, but not both, since for a particular pseudoclone, t and a_i increase simultaneously and at the same rate: $da_i/dt = 1$. Similarly, if $j<i$, z_i^j can be regarded to a function of $(t, a_{i-1}, \ldots, a_j)$ or $(a_i, a_{i-1}, \ldots, a_j)$. It will be convenient to choose the state vector to depend on the set of ages, so that, for example, we shall regard z_3^3 to be a function of (a_3), z_3^2 to be a function of (a_3, a_2), and z_3^1 to be a function of (a_3, a_2, a_1). It should be noted that this is a different convention than the one used for the μ's and the r's. If $j < i$, our convention for these quantities is that they are functions of $(t, a_i, a_{i-1}, \ldots, a_{j+1})$. The difference arises because the state vector z can only refer to a single pseudoclone, whereas μ and r can refer to different pseudoclones.

The mathematical expression of a structured model with state determined by a vector is a set of equations of change of the state vector, and these will be of the form:

$$\frac{\partial}{\partial a_i} z_i^j = f\,[z_i^j, s_i(t); k] \tag{23}$$

where f is a specified, vector-valued function of: (1) the state vector of the pseudoclone at the current time ($t = a_i + a_{i-1} + \ldots + a_j$ if the pseudoclone originated in the jth reactor); (2) the state vector of the abiotic environment in the ith vessel at the current time; and (3) a vector of model parameters, k. Since the model is to be applicable to all pseudoclones, the function f and the vector of model parameters k are the same for all pseudoclones, and therefore, it is unnecessary to place pseudoclone-identifying indices qn these vectors. The foregoing equation states that the rate of change of the current state of the pseudoclone's biomass is determined by the current state of that biomass and by the current state of the abiotic environment of the biomass. Structured models describable by Eq. 23 are thus mathematically *autonomous*. Equation 23 is to be solved subject to the boundary condition:

$$z_i^j(0, a_{i-1}, \ldots, a_j) = z_{i-1}^j\,(a_{i-1}, \ldots, a_j) \tag{24}$$

if $i > j$. If $i=j$, then the value of z_i^j when $a_i = 0$ is just the state vector of the initial biomass inoculated into the ith reactor.

With structured models of the kind under consideration, one does not specify the specific growth rate and specific nutrients uptake rate vector; instead, these are determined by the model and by the states of the pseudoclone and its abiotic environment. In particular, the model will determine a scalar-valued function g and a vector-valued function h such that:

$$\mu_i^j(t, a_i, a_{i-1}, \ldots, a_{j+1}) = g[(z_i^j\,(a_i, a_{i-1}, \ldots, a_{j+1}, t - a_i - \ldots - a_{j+1}), s_i(t); k] \tag{25}$$

and

$$r_i^j(t, a_i, a_{i-1}, \ldots, a_{j+1}) = h[z_i^j\,(a_i, a_{i-1}, \ldots, a_{j+1}, t - a_i - \ldots - a_{j+1}), s_i(t); k] \tag{26}$$

for $i > j$. It must be remembered here that we have adhered to the conventions that μ's and r's are functions of $(t, a_i, a_{i-1}, \ldots, a_{j+1})$, whereas z's are functions of $(t, a_i, a_{i-1}, \ldots, a_j)$. Also, as with the previously-defined function f, the functions g and h are the same for all pseudoclones. Thus, the specific growth and nutrients uptake rates of a pseudoclone are determined by its current state and by the current state of its abiotic environment.

As a specific example of a structured model of the generic kind describable by the foregoing equations, consider a model that has composition structure only and in effect, views biomass as a continuum having a spatially uniform, albeit changeable, composition. The structured models described by Roels (1983, Chap. 10) and the "compartmental" and "metabolic" structured models described by Bailey and Ollis (1986, Sec. 7.4), are of this type. [In my view, application of the term "compartmental" to these kinds of models is unfortunate; it would be better to reserve that term for structured models where the "compartments" are of variable composition as well as of size. Such models are now being used to describe, for example, the kinetics of protein synthesis and secretion by animal cells (Sambanis et al., 1991; Bibila and Flickinger, 1991a,b).]

In models having composition structure only, biomass is considered to be composed of S_b different chemical substances which react with each other and with the S_a components of the abiotic environment to produce the phenomena of nutrient uptake, growth and product formation. The total number of biotic and abiotic substances involved in the model is thus $S = S_b + S_a$. Components of the biomass (biotic substances) will be numbered 1, 2, . . ., S_b and components of the abiotic environment will be numbered $S_b + 1$, $S_b + 2$, . . ., $S_b + S_a$. Substances that are present in both biomass and its abiotic environment will be numbered twice: one number among those for biotic substances and the other among those for abiotic substances. It is supposed that R reactions occur and that the rates of these reactions, per unit volume of biomass, are determined by the current state (composition, temperature, and so on) of the biomass and the current state of the abiotic environment of the biomass. Specification of the stoichiometry and kinetics of these reactions, along with the division of the biomass into components, are thus the principal elements of the model.

Let $A = [\alpha_{rs}]$ be the $R \times S$ matrix of stoichiometric coefficients α_{rs}: α_{rs} is the stoichiometric coefficient of the sth substance in the rth reaction. Reactions, for which α_{rs} is zero for all abiotic substances, represent reactions that occur entirely within the biomass; they may be called *internal reactions*. Reactions which involve both biotic and abiotic substances may be called *transfer reactions*. It is assumed that there is no reaction for which α_{rs} is zero for all biotic substances; that is, it is assumed that there are no *external reactions*—those involving abiotic substances only. If external reactions needed to be accounted for in particular cases, they should be considered separately, and source and sink terms resulting from their occurrence must be added to the balance equations on the abiotic portions of cultures, such as Eq. 20. The reason that external reactions must be treated separately from internal and transfer reactions is that the rates of the former reactions are independent of the biomass concentrations in a culture and dependent instead only on the concentrations in the abiotic portion of the culture.

In addition to the stoichiometric matrix A, let R be a (col-

umn) vector whose R elements are the rates of the reactions, measured in equivalents per unit time per unit volume of biomass. Finally, let M be a diagonal $S \times S$ matrix whose elements are the molecular weights of the biotic and abiotic substances.

It will be convenient to partition the stoichiometric matrix A as:

$$A = [A_b | A_a] \tag{27}$$

and the matrix of molecular weights M as:

$$M = \begin{bmatrix} M_b & | & 0 \\ - & \cdot & - \\ 0 & | & M_a \end{bmatrix}. \tag{28}$$

Here, A_b is the $R \times S_b$ matrix of stoichiometric coefficients of the biotic substances, A_a is the $R \times S_a$ matrix of stoichiometric coefficients of the abiotic substances, M_b is the $S_b \times S_b$ diagonal matrix of molecular weights of the biotic substances, and M_a is the $S_a \times S_a$ diagonal matrix of molecular weights of the abiotic substances. With these definitions, one sees that the net rates of production of *masses* of the biotic substances, per unit volume of biomass, is the vector $M_b A_b{}^T R$, whereas the net rates of production of masses of the abiotic substances, per unit volume of biomass, is the vector $M_a A_a{}^T R$. In these expressions, T denotes the transpose of a matrix. The net rates of production of biotic substances of all kinds and of abiotic substances of all kinds are $\mathbf{1}_b{}^T M_b A_b{}^T R$ and $\mathbf{1}_a{}^T M_a A_a{}^T R$, respectively, where $\mathbf{1}_b$ and $\mathbf{1}_a$ are column vectors whose S_b and S_a elements, respectively, are all 1's. Since mass is neither produced nor destroyed by chemical reactions it follows that:

$$\mathbf{1}_b^T M_b A_b^T R + \mathbf{1}_a^T M_a A_a^T R = 0. \tag{29}$$

Consider a pseudoclone which originated in reactor 1, entered reactor 3 at time $a_1 + a_2$, and at time t ($\geq a_1 + a_2$) is in reactor 3. Let m_3^1 be the current mass of this pseudoclone in reactor 3, let z_3^1 be the vector whose S_b elements are the masses of the biotic substances per unit volume of the pseudoclone's biomass, and let v be the specific volume of the biomass. Mass balances on the biotic substances present in the pseudoclone's biomass then yield the vector equation:

$$\frac{\partial}{\partial a_3}[m_3^1 v z_3^1] = m_3^1 v M_b A_b^T R_3^1 - D m_3^1 v z_3^1. \tag{30}$$

In writing this equation, we have put identifying indices on the vector of reaction rates, since these depend on the state of the particular pseudoclone being considered. No such indices are needed on M_b and A_b, since these are simply constant matrices whose elements are the same for all pseudoclones. Rearrangement of the foregoing equation under the reasonable simplifying assumption that the specific volume of the biomass is constant gives:

$$\frac{\partial}{\partial a_3} z_3^1 = M_b A_b^T R_3^1 - \mu_3^1 z_3^1, \tag{31}$$

where

$$\mu_3^1 \equiv D + \frac{d}{da_3} \ln m_3^1. \tag{32}$$

That the sum on the righthand side of Eq. 32 is indeed the specific growth rate of the pseudoclone—the net rate of production of biomass of the pseudoclone per unit amount of its biomass present—may be seen by multiplying each term of Eq. 31 by the row vector $\mathbf{1}_b^T$. Since $\mathbf{1}_b^T z_3^1$ is the sum of the elements of z_3^1 and since this in turn is equal to v^{-1}, we see that the indicated procedure yields:

$$\frac{\partial}{\partial a_3}\left(\frac{1}{v}\right) = 0 = \mathbf{1}_b^T M_b A_b^T R_3^1 - \frac{1}{v}\mu_3^1$$

so

$$\mu_3^1 = v \mathbf{1}_b^T M_b A_b^T R_3^1, \tag{33}$$

and the quantity on the right is indeed the net rate of production of biomass of the pseudoclone per unit amount of pseudoclone biomass present.

Since $M_a A_a^T R_3^1$ is the vector whose elements are the net rates of production of abiotic substances per unit volume of biomass, we see that the specific nutrients uptake rate vector $\mathbf{r}_3^1$ is given by:

$$r_3^1 = v \mathbf{M}_a (-A_a)^T R_3^1. \tag{34}$$

Moreover, from Eqs. 29, 33 and 34 it follows that:

$$\mu_3^1 = \mathbf{1}_a^T r_3^1, \tag{35}$$

so that μ_3^1 is just the sum of the specific rates of uptake of the nutrients.

Evidently, Eq. 31 is a specific version of the generic equation of state change (Eq. 23); Eq. 33 is a specific version of generic Eq. 25 that gives μ's as functions of the states of a pseudoclone and its abiotic environment; and Eq. 34 is a specific version of generic Eq. 26 that gives r's as functions of the states of a pseudoclone and its abiotic environment. The generic version of Eq. 35 was not given, but some such conservation equation must be applicable to any model employing a vector description of internal state.

The composition-structured model just described is not the only one that is a special case of the generic structured model described by Eqs. 23, 25 and 26. For example, some kinds of compartmental models, where the composition of the compartments as well as their size change, might be special cases of the generic structured model given. In addition, the so-called cybernetic models that are being developed by Ramkrishna and his coworkers (see, for example, Baloo and Ramkrishna, 1991) have composition structure as in the special model described above, but in addition to composition variables they also have so-called cybernetic variables. Since the cybernetic variables turn out to be functions of the composition variables, it appears that such models also are special versions of the generic model described by Eqs. 23, 25 and 26.

Computational Problems

Suppose that we are given a structured, continuum model of the generic kind described by Eqs. 23, 25 and 26, and that all the parameters of this model are known. The problem at hand is then how to use this model to predict the time courses of biomass and medium concentrations in the successive stages of a cascade of CSTRs. As mentioned earlier, this problem can be solved successively, so that one first solves the equations for the first reactor in the cascade to find out what happens in it, and then one uses these results in solving the equations for the second reactor in the cascade, and so on.

The problem posed by the first reactor is that of a single-stage CSTR to which a structured, continuum model applies. This problem has been solved for many different models; for an early solution and comparison with unstructured models see Pickett (1982), and for other, more recent, solutions see Roels (1983, Chap. 10), Bailey and Ollis (1986, Sec. 7.4); and for solutions of cybernetic models see Baloo and Ramkrishna (1992).

In general, the equations that must be solved for the first stage are:

$$\frac{dx_1^1}{dt} = -Dx_1^1 + \mu_1^1 x_1^1, \tag{36a}$$

$$\frac{ds_1}{dt} = D(s_0 - s_1) + K_L a(\Delta s)_{M,1} - r_1^1 x_1^1, \tag{36b}$$

$$\frac{d}{da_1} z_1^1 = \frac{d}{dt} z_1^1 = f(z_1^1, s_1; k), \tag{36c}$$

where

$$\mu_1^1(t) = g[z_1^1(t), s_1(t); k], \tag{36d}$$

and

$$r_1^1(t) = h[z_1^1(t), s_1(t); k]. \tag{36e}$$

In addition, there will be initial conditions for x_1^1, s_1, and z_1^1. The differential equations involved are all first-order ordinary differential equations and, in fact, autonomous ordinary differential equations. This happens because the population in the first stage is homogeneous and constitutes a single pseudoclone whose age a_1 is equal to the time elapsed since the start-up of the apparatus.

Partial differential equations are encountered when we come to the second reactor of the cascade, because the population in it is not homogeneous, but contains the pseudoclone descendent from the reactor's inoculum as well as pseudoclones introduced from the first reactor. If we restrict ourselves to the simplest case where only the first reactor is provided with inoculum, then the equations describing the dynamics of the second reactor are:

$$\frac{\partial X_2^1}{\partial t} + \frac{\partial X_2^1}{\partial a_2} = (\mu_2^1 - D) X_2^1, \tag{37a}$$

$$\frac{ds_2}{dt} = D(s_1 - s_2) + K_L a(\Delta s)_{M,2} - \int_0^t r_2^1 X_2^1 da_2, \tag{37b}$$

$$\frac{\partial}{\partial a_2} z_2^1 = f(z_2^1; s_2; k), \tag{37c}$$

where

$$\mu_2^1(t, a_2) = g[z_2^1(a_2, t - a_2), s_2(t); k], \tag{37d}$$

and

$$r_2^1(t, a_2) = h[z_2^1(a_2, t - a_2), s_2(t); k]. \tag{37e}$$

The partial differential equation for X_2^1 is subject to the boundary condition:

$$X_2^1(t, 0) = Dx_1^1(t) \tag{37f}$$

and the partial differential equation for z_2^1 is subject to the boundary condition:

$$z_2^1(0, a_1) = z_1^1(a_1) = z_1^1(t). \tag{37g}$$

Appropriate initial conditions must also be satisfied by X_2^1 and s_2.

Evidently, Eqs. 37 pose a computational problem that is substantially more complicated than the one posed by Eqs. 36, since Eqs. 37 involve partial differential equations as well as the solutions of Eqs. 36, which, of course, are time-dependent. The steady-state versions of Eqs. 37 again produce only ordinary differential equations, however. This is because, when a steady state of the cascade apparatus is achieved, the state of the biomass transferred from reactor 1 to reactor 2 will no longer depend on time (or age a_1), and so X_2^1 and z_2^1 become functions only of age a_2.

The steady-state solutions of Eqs. 37a and 37b are Eqs. 21a and 22a, respectively. Equation 37c is now an ordinary differential equation which is autonomous, since s_2 is independent of time in the steady state. Hence, a trial-and-error solution would involve: (1) assuming the steady-state vector s_2; (2) solving Eq. 37c and using Eqs. 37d and 37e to find how μ_2^1 and r_2^1 vary with a_2, and then using these variations to do the integrals in Eq. 22a. The calculated results will allow one to check the assumed vector s_2.

When the third reactor of a three-stage cascade is considered, a partial differential equation cannot be avoided, even when the cascade operates in a steady state. This is because the biomass in the second reactor is not homogenous, and so pseudoclones passing from the second to the third reactor exhibit a distribution of ages and so states. Thus, state vector z_3^1 is a function of a_2 as well as of a_3, and so its equation of change is a partial differential equation that has to satisfy the boundary condition $z_3^1(0, a_2) = z_2^1(a_2)$.

Discussion and Conclusions

The material presented in the preceding paragraphs will show what an enormous simplification is made when one assumes that an unstructured continuum model will describe growth, nutrient uptake, and product formation kinetics in a bioreactor with an external source of biomass. Biologically, this assumption means that we ignore the very considerable heterogeneity of the biomass in such a reactor, as well as all of the biological consequences that result from that heterogeneity. Mathemat-

ically and computationally, the assumption allows us to replace coupled sets of first-order partial differential and ordinary differential equations with coupled sets of first-order ordinary differential equations. The latter equations lead to computational problems that are solved readily by well-known numerical methods; computational methods need to be worked out for the former equations and they may well prove to be quite complicated.

Will the use of structured, continuum models provide predictions of growth, nutrient uptake, and product formation phenomena in bioreactors with external sources of biomass that are significantly more accurate than predictions made using unstructured models? This is an important question because, if only modest improvements in accuracy result from use of a structured model, there would be little point in using it instead of a simpler unstructured model. I expect that the degree of improvement provided by structured models will be case-dependent and will be greatest for those cases where: (1) the environmental shock on passing from one reactor to another is large; (2) the kinetic expressions in the structured model are highly nonlinear. The second expectation arises because the nonlinearity mentioned makes the dynamics of growth, nutrient uptake, and product formation more sensitive to changes in the mean internal state of the population, and one of the primary effects of addition of biomass from an external source to biomass in a reactor is to alter the mean internal state of the biomass in it. What needs to be done here is to apply a number of structured, continuum models that have been presented in the literature to situations of, say, a two-stage cascade of CSTRs and compare the predictions of the models to those of appropriate unstructured models applied to the same reactor configuration. Situations that might be suitable for such comparisons include the growth with product inhibition situation studied by Bonomi and Fredrickson (1979), the production of periplasmic protein in recombinant yeast studied by Davis et al. (1990), and many others, where the use of cascades has been suggested to be advantageous.

Finally, it should be pointed out that the experimental use of a two-stage chemostat cascade offers the possibility of observing transient responses of biomass to step changes, up or down, in nutrient availability. So-called "shift-up" and "shift-down" experiments are usually carried out in batch or single chemostat apparatus, and in both, a true step change in the concentration of a nutrient—concentration goes suddenly from an initial value that has prevailed for a long time to a different value that then prevails for subsequent times—cannot be made. But a step change in nutrient availability is exactly what is seen by a pseudoclone introduced into the second stage of a two-stage, steady-state chemostat cascade. Since the direction and amount of the step change are controllable by addition of concentrated or dilute sterile nutrient medium to the second stage, the two-stage cascade is potentially very useful as a means of doing controlled shift-up and shift-down experiments. Some means of differentiating between biomasses of different ages would be required, of course, but modern experimental techniques should be equal to the challenge posed.

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Notation

A = stoichiometric matrix partitioned into A_a and A_b by Eq. 27
$A_a(A_b)$ = stoichiometric matrix for abiotic (biotic) substances
a_i = age of pseudoclone in ith bioreactor of a cascade
D = dilution rate (reciprocal of mean holding time) of a CSTR
D_k = q_k/V
f = vector-valued function defined by Eq. 23
F = density of distribution of states in structured, corpuscular model
g = scalar-valued function defined by Eq. 25
h = vector-valued function defined by Eq. 26
K_La = matrix of volumetric mass transfer coefficients for a CSTR
k = vector of model parameters
M = diagonal matrix of component molecular weights; partitioned into M_a and M_b by Eq. 28
$M_a(M_b)$ = diagonal matrix of molecular weights of abiotic (biotic) substances
m_i^j = biomass of pseudoclone originating in jth reactor and currently in ith reactor
p = partioning function in structured, corpuscular model
q_k = volumetric flow rate of culture into bioreactor from kth external source of biomass
r = vector of single-cell growth rates in structured, corpuscular model
r_i^j = vector of specific nutrients uptake rates for pseudoclone originating in jth reactor and currently in ith reactor
R = vector of reaction rates
R = number of reactions
S = $S_a + S_b$
$S_a(S_b)$ = number of abiotic (biotic) substances
s_i = vector of concentrations of substances in abiotic portion of culture in ith reactor
T = as a superscript, denotes transpose of a matrix or vector
t = time
v = volume of state space in structured, corpuscular model
v = specific volume of biomass
V = volume of culture in a bioreactor
X_i^j = nonnormalized density of distribution of ages of biomass of a pseudoclone originating in jth reactor and currently present in ith reactor
x_i = total concentration of biomass in culture in ith reactor
x_i^j = concentration in ith reactor of biomass of all pseudoclones originating in jth reactor
z_i^j = state vector of pseudoclone originating in jth reactor and currently in ith reactor
α_{rs} = stoichiometric coefficient of sth substance in rth reaction
$(\Delta s)_{M,i}$ = vector of mean driving forces for mass transfer of dissolved gases in ith reactor
γ = time-specific probability of cell division in structured, corpuscular model
μ_i = specific growth rate of biomass in ith reactor when unstructured model is used
μ_i^j = specific growth rate of pseudoclone originating in jth reactor and currently present in ith reactor
$\hat{}$ = steady-state value of quantity underneath

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