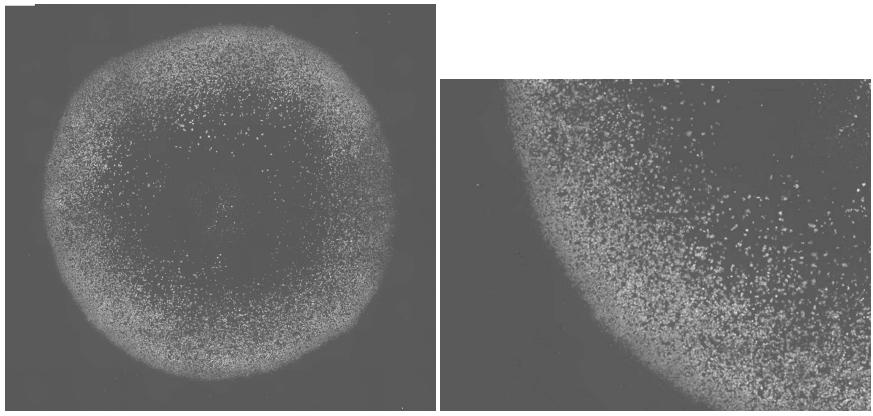

Figure 10.5: Cell infection by a virus. The cells are uniformly distributed, and the virus is placed initially in the center and diffuses outward. The cells fluoresce after they become infected. The dark inner core shows dead cells.

large monomer droplets and initiate polymerization, but the total surface area of the micelle phase is usually orders of magnitude larger than the surface area of the large monomer droplets and the polymerization in the droplets can be neglected.[1]

10.2.3 Biological Cells

Reactions involving living cells are ubiquitous in nature and, today, also in the bioprocess industries. Improving our understanding of and control over these reactions has large implications for human health. Consider the interactions of cells and viruses, for example. Viral infection of cells is responsible for maladies such as the common cold, influenza, chickenpox, cold sores, Ebola hemorrhagic fever, AIDS, avian influenza, and SARS. Figure 10.5 shows images of cell infection by a virus. The cells are uniformly distributed, and the virus is placed initially in the center and diffuses outward. The cells fluoresce after they become infected. The dark inner core shows the cells killed by the

[1] Alternatively, in *dispersion* polymerization, the monomer droplets are made much smaller, usually by exposing the monomer phase to high shear rates, and the small monomer droplets serve as the locus of polymerization without the presence of any micellar phase.

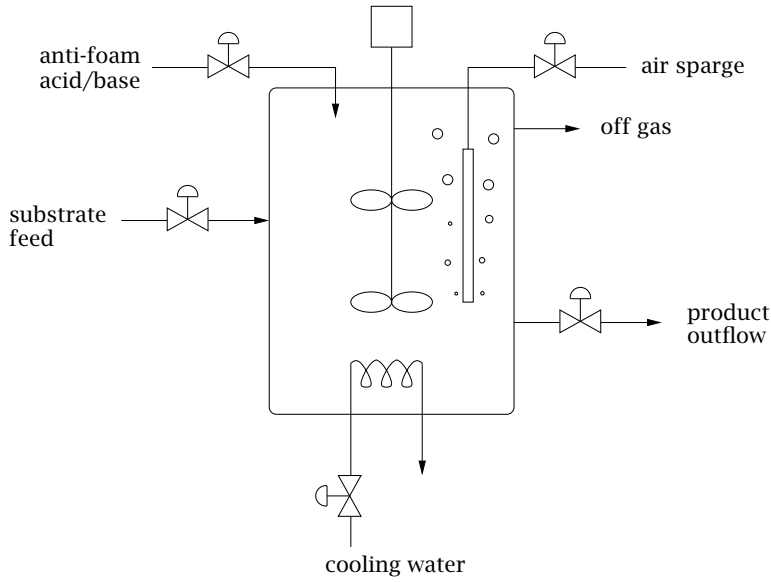

Figure 10.6: Basic fermentation system.

virus. Modeling the distribution of cells as a function of time since infection and interaction with the signaling molecules released into their environment enables quantitative predictions that can be tested and verified with experimental measurement [5].

Living cells are also used to manufacture many important antibiotics and other pharmaceuticals. Figure 10.6 displays a simple schematic of a fermentation system used for antibiotic manufacture. The cells can grow exponentially quickly so that although there are no cells in the feed, they can establish a nonzero steady state in the CSTR, commonly known as a chemostat in the bioprocess industries. The classic paper [3] was one of the first to lay out the fundamentals for modeling the dynamic behavior of these kinds of cell populations. We further develop the modeling of chemostats later in the chapter.

10.3 Population Balance

Given this brief overview of applications, we next develop the evolution equation for the particle size distribution, known as the population balance. We first treat deterministic models with a single size coordinate and single source of nucleation of new particles at a single (zero) size.

concentration, R, is constant giving

$$B^0(t) = k_{mm} R m(t)$$

in which k_{mm} is the mass transfer coefficient for the radical entry into micelles. Finally, the monomer balance is given by

$$\frac{dM}{dt} = \frac{1}{\tau}(M_f - M) - v_p \int_0^\infty k_p i \phi f(L,t) L^3 dL$$

which accounts for the flow streams and the consumption of monomer due to the polymerization taking place inside the particles.

Here we see already the fairly complicated set of equations required to track the particle composition and size distribution, as well as the continuous phase species. Again, the interactions between the micelle balance and the population balance lead to complex dynamic behavior such as sustained oscillations. See [16] for details on how to relax the many simplifying assumptions made here. □

Example 10.4: Fermentation model

Write down a population balance for the cells and the continuous phase balances for substrate and product to model fermentation in a CSTR.

Solution

In the bioprocess literature, a *segregated* model is one that explicitly models the population of cells, i.e., includes a population balance. A *structured* model is one that requires more than one chemical species to describe the state of the cell. We choose cell mass to be the internal coordinate describing the cell population.[4] The rate of change of a given cell's mass, $\dot{m}$, due to the metabolic reactions is called the cell growth rate, denoted μ. Cell growth rate is usually normalized by the cell mass, so it has units of inverse time, and we have $\dot{m} = \mu m$. The population balance is then

$$\frac{\partial f(m,t)}{\partial t} = -\frac{\partial(\mu m f(m,t))}{\partial m} + B - D - \frac{1}{\tau} f \tag{10.17}$$

in which B accounts for production of new cells, usually by cell division, which produces two new cells of roughly half the mass of the mother cell. The death term D accounts for losses of cells of size m by, for

[4] Cell mass alone may be inadequate to predict cell division; cell age may also be used if that is a better predictor of cell division.

example, cell death and cell division. Note that, for convenience, the outflow term is treated separately from the other death terms. The continuous phase balances consist of the substrate(s), S, that the cells consume for cell growth, and the product(s), P, secreted by the cells as side products of their metabolic processes. The cell growth yield, $\mathcal{Y}$, is the ratio of cell mass increase to substrate mass consumed. In the segregated models, the cell growth rate and growth yield may be functions of any of the continuous phase concentrations and the cell's mass. A typical balance would be [17]

$$\frac{dS}{dt} = \frac{1}{\tau}(S_f - S) - \int_0^\infty f(m,t)\frac{\mu(S,m)m}{\mathcal{Y}(S,m)}dm$$

The formation rate of some products is often well correlated with the cell growth rate. These are the so-called growth-associated products such as enzymes and proteins. Many secondary metabolites, such as antibiotics, are nongrowth-associated products, and they form at a relatively constant rate, even if the cell growth rate is zero. A typical product formation rate expression accounting for both forms is

$$q_p(S,m) = \alpha\mu(S,m) + \beta$$

and the product balance would be

$$\frac{dP}{dt} = -\frac{1}{\tau}P + \int_0^\infty f(m,t)q_p(S,m)m\,dm$$

□

10.5 Nonsegregated Fermentation Model

Given the complexity of determining B and D, and the metabolic functions μ, $\mathcal{Y}$, and q_p for cell lines and products of interest, fermentor models are often simplified further to make them more tractable. The first common simplification is to ignore the distribution of cells and lump all cells together in a single species, biomass. Although this simplification does violence to the known biology, we shall see that these models still provide insight and useful predictions of aspects of fermentor behavior. Given our previous starting point, we can define total biomass as

$$X := \int_0^\infty f(m,t)m\,dm$$

If we assume that μ is only a weak function of cell mass (otherwise this model simplification is not accurate), we can integrate the population balance as follows. Multiply the population balance, Equation 10.17, by m and integrate over all cell mass. The left-hand side becomes

$$\int_0^\infty \frac{\partial f(m,t)}{\partial t} m \, dm = \frac{d}{dt} \int_0^\infty f(m,t) m \, dm = \frac{dX}{dt}$$

Using integration by parts, the first term on the right-hand side of the population balance becomes

$$-\int_0^\infty \frac{\partial(\mu m f(m,t))}{\partial m} m \, dm = -\, m^2 \mu f \Big|_0^\infty + \int_0^\infty f(m,t) \mu m \, dm$$

The integrand vanishes at the two limits, and μ can be taken outside the integral giving

$$-\int_0^\infty \frac{\partial(\mu m f(m,t))}{\partial m} m \, dm = \mu X$$

The cell division terms cancel in the integration over B and D because cell mass is conserved on cell division. If cell death is negligible, then these terms disappear completely. The final result, neglecting cell death, is that the population balance reduces to the following total biomass balance

$$\frac{dX}{dt} = \mu(S)X - \frac{1}{\tau}X$$

The substrate and product balances can be simplified if we assume that the yield and product formation rate do not vary appreciably over the cell population. Taking these terms outside the integrals gives

$$\frac{dS}{dt} = \frac{1}{\tau}(S_f - S) - \frac{\mu(S)}{y(S)}X$$
$$\frac{dP}{dt} = -\frac{1}{\tau}P + q_p(S)X$$

We next discuss the functional form of the cell growth rate and its dependence on the substrate.

Substrate limited growth. Many different cell growth expressions have been found useful [1, 17]

$$\text{Monod equation:} \quad \mu = \frac{\mu_m S}{K_s + S}$$

$$\text{Blackman equation:} \quad \mu = \begin{cases} \mu = \mu_m & S \geq 2K_s \\ \mu = \frac{\mu_m S}{2K_s} & S < 2K_s \end{cases}$$

$$\text{Tessier equation:} \quad \mu = \mu_m (1 - e^{-K_s S})$$

$$\text{Moser equation:} \quad \mu = \frac{\mu_m S^n}{K_s + S^n}$$

$$\text{Contois equation:} \quad \mu = \frac{\mu_m S}{K_{sx} X + S}$$

We recognize the Monod equation [12] for cell growth rate as the simplest form of the Langmuir adsorption isotherm and the resulting Hougen-Watson kinetics for reaction rates on catalyst surfaces discussed in Chapter 5. When multiple substrates, $S_1, S_2, \ldots$, affect cell growth, a simple model for overall growth rate is to take the smallest rate as the limiting growth rate

$$\mu = \min_j \mu(S_j)$$

Growth inhibitors. At high substrate or product concentrations, cell growth is inhibited. Common functional forms for this inhibition are the following:

$$\text{substrate inhibition:} \quad \mu = \frac{\mu_m S}{K_s + S + K_1 S^2}$$

$$\text{product inhibition:} \quad \mu = \frac{\mu_m S}{K_s \left(1 + \frac{P}{K_p}\right) + S}$$

Reactor behavior. Assuming Monod kinetics for cell growth, the combined biomass and substrate mass balances are

$$\begin{aligned} \frac{dX}{dt} &= \left(-D + \frac{\mu_m S}{K_s + S} \right) X \\ \frac{dS}{dt} &= D(S_f - S) - \frac{1}{y} \left(\frac{\mu_m S}{K_s + S} \right) X \end{aligned} \tag{10.18}$$

in which we have used dilution rate, $D = 1/\tau$, instead of residence time for the outflow terms.[5] Since there is no product inhibition in

[5] There should be no confusion with the D in the population balance death term in this section.

this model, the product balance is not required to solve the biomass and substrate balances. We first analyze the steady state of this model. Setting the time derivatives to zero, we notice first from the biomass balance that $X_s = 0$ is a steady state, and substituting this result into the substrate balance gives $S_s = S_f$. We can find a second steady state by setting the bracketed term to zero in the biomass equation and solving for S_s, which gives $S_s = DK_s/(\mu_m - D)$. Substituting this result into the substrate balance and solving yields $X_s = \gamma(S_f - S_s) = \gamma(S_f - DK_s/(\mu_m - D))$. So we see that there are two steady states for all values of parameters:

$$\begin{aligned} X_{s1} &= 0 & S_{s1} &= S_f \\ X_{s2} &= \gamma(S_f - S_{s2}) & S_{s2} &= \frac{DK_s}{\mu_m - D} \end{aligned} \tag{10.19}$$

Consider the dilution rate to be the parameter of interest, and notice that the second steady state makes physical sense only if $D < D_c$. Otherwise X_s is negative and $S_s > S_f$. We can solve $S_{s2} = S_f$ to find this critical value of dilution rate and obtain

$$D_c = \frac{\mu_m S_f}{K_s + S_f}$$

For high dilution rate, $D > D_c$, we have only one physically meaningful steady state in which $X_s = 0$ and $S_s = S_f$. In this parameter regime, the dilution rate is too large for the system to support any biomass and any initial biomass simply washes out of the reactor. This steady state is known as the "washout" steady state. For low dilution rate $D < D_c$, there are two possible steady states, the washout steady state, and a steady state with positive biomass production and substrate consumption. Here we have another classic case of steady-state multiplicity as studied in Chapter 6. Exercise 10.7 asks you to show that the washout steady state is stable for $D > D_c$ and unstable for $D < D_c$. The second, nontrivial steady state has the opposite stability; it is unstable for $D > D_c$ and stable for $D < D_c$. These results are shown in Figure 10.12 for a range of dilution rates. The other parameter values used to prepare the figure are

$$\mu_m = 1 \qquad K_s = 1 \qquad S_f = 5 \qquad \gamma = 1$$

Finally, increasing dilution rate further, we notice that there is a singularity in S_{s2} at $D = \mu_m$, and the substrate steady state changes sign and

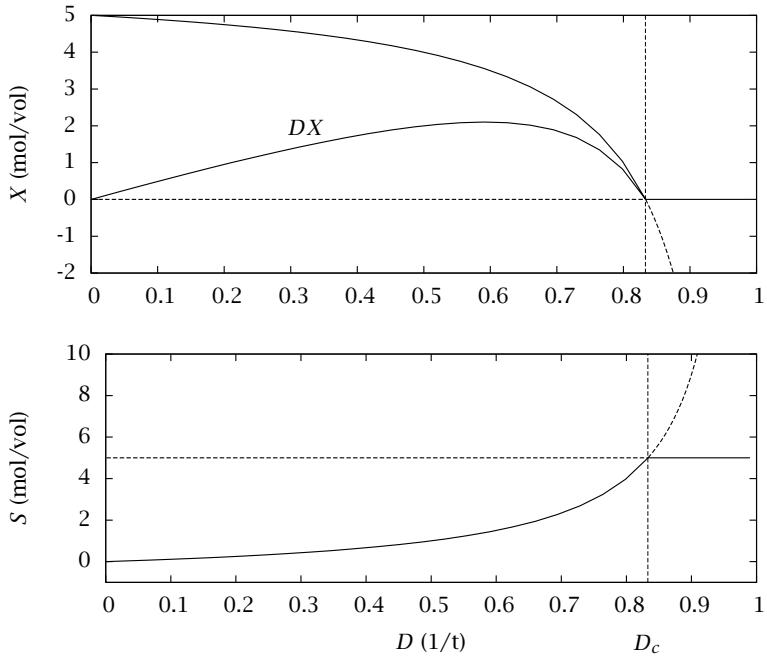

Figure 10.12: The two steady-state biomass and substrate concentrations versus dilution rate; stable (solid), unstable (dashed). Stability changes at $D = D_c$. Also shown is total biomass production rate, DX, for the stable steady state.

becomes negative and the biomass becomes positive. Although this steady state is then also stable, it is not physically meaningful because of the negative substrate concentration (see also Exercise 10.7).

Notice that the steady-state production rate of biomass is given by the product DX_s, which has units of mass per volume of reactor per time. This quantity is also plotted in Figure 10.7. Notice that it has an optimum, which can be found by differentiating DX_{s2} with respect to D and setting to zero. The result is

$$D^0 = \mu_m \left(1 - \sqrt{\frac{K_s}{K_s + S_f}}\right) \tag{10.20}$$

A low dilution rate gives a high reactor biomass *concentration* but little

biomass *outflow* from the reactor.[6] Operating at a high dilution rate leads to washout and zero biomass production. An optimum naturally exists between these operational extremes.

10.6 Stochastic Models of Nucleation and Growth

Turning attention back to the general topic of modeling particulate reactors, we can also consider the phenomena of particle nucleation and growth entirely from a stochastic perspective. As we saw in the discussion of stochastic kinetics in Chapter 4, the stochastic perspective provides valuable understanding of certain experimental observations, such as dispersion (spreading) in the particle size distribution with time. We also can derive the population balance of the previous sections starting with the stochastic equations and taking the limit of large numbers and small sizes of the solute molecules compared to the small numbers and large sizes of the growing crystals.

10.6.1 Modeling Particle Growth and Dissolution

We start with a simple experiment to make the discussion concrete. Imagine we have a single, pure-solid particle of some initial size in a well-stirred supersaturated solution of solute molecules with some initial supersaturation. This experiment is easy to conceptualize and also easy to perform in the laboratory. We assume that the particle's crystal structure and geometric shape are not important variables needed to describe the growth rate of the particle. This assumption is valid for certain kinds of particles. Because we have a single particle experiencing only growth, the particle size distribution is arbitrarily narrow at this single size. The supersaturation in the solution phase is the driving force for particle growth. If the particle were to grow large enough that it removes enough solute from the solution phase, then the solution phase approaches saturation and the driving force for further growth drops to zero. The equilibrium state for this simple system is a single particle of a size larger than the initial size, coexisting with a saturated solution phase. Similarly, if the solution phase is initially undersaturated, then the particle dissolves, releasing solute back to the solution phase until either the particle dissolves completely, or the solution phase reaches saturation.

[6] Recall that one sells the mass leaving the reactor, not the reactor concentration.