In the following sections we consider a somewhat more advanced model for bacterial growth in a chemostat.

4.2 BACTERIAL GROWTH IN A CHEMOSTAT[2]

In experiments on the growth of microorganisms under various laboratory conditions, it is usually necessary to keep a stock supply of the strain being studied. Rather than use some dormant form, such as spores or cysts, which would require time to produce active cultures, a convenient alternative is to maintain a continuous culture from which actively growing cells can be harvested at any time.

To set up this sort of culture, it is necessary to devise a means of replenishing the supply of nutrients as they are being consumed and at the same time maintain some convenient population levels of the bacteria or other organism in the culture. This is usually done in a device called a *chemostat,* shown in Figure 4.2.

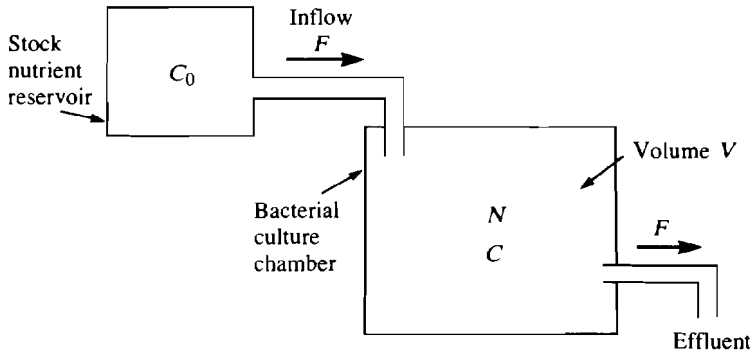

Figure 4.2 *The chemostat is a device for harvesting bacteria. Stock nutrient of concentration* C_0 *enters the bacterial culture chamber with inflow rate* F. *There is an equal rate of efflux, so that the volume* V *is constant.*

A stock solution of nutrient is pumped at some fixed rate into a growth chamber where the bacteria are being cultivated. An outflow valve allows the growth medium to leave at the same rate, so that the volume of the culture remains constant.

Our task is to design the system so that

1. The flow rate will not be so great that it causes the whole culture to be washed out and eliminated.

2. Portions of this material were adapted from the author's recollection of lectures given by L. A. Segel to students at the Weizmann Institute. It has also appeared recently in Segel (1984).

2. The nutrient replenishment is sufficiently rapid so that the culture continues to grow normally.

We are able to choose the appropriate stock nutrient concentration, the flow rate, and the size of the growth chamber.

In this example the purpose of the model will be twofold. First, the progression of steps culminating in precise mathematical statements will enhance our understanding of the chemostat. Second, the model itself will guide us in making appropriate choices for such parameters as flow rates, nutrient stock concentration, and so on.

4.3 FORMULATING A MODEL

A First Attempt

Since a number of factors must be considered in keeping track of the bacterial population and its food supply, we must take great care in assembling the equations. Our first step is to identify quantities that govern the chemostat operation. Such a list appears in Table 4.1, along with assigned symbols and dimensions.

Table 4.1 ***Chemostat Parameters***

Quantity	*Symbol*	*Dimensions*
Nutrient concentration in growth chamber	C	Mass/volume
Nutrient concentration in reservoir	C_0	Mass/volume
Bacterial population density	N	Number/volume
Yield constant	$Y = 1/\alpha$	(See problem 6)
Volume of growth chamber	V	Volume
Intake/output flow rate	F	Volume/time

We also keep track of assumptions made in the model; here are a few to begin with:

1. The culture chamber is kept well stirred, and there are no spatial variations in concentrations of nutrient or bacteria. (We can describe the events using ordinary differential equations with time as the only independent variable.)

At this point we write a preliminary equation for the bacterial population density N. From Fig. 4.2 it can be seen that the way N changes inside the culture chamber depends on the balance between the number of bacteria formed as the culture reproduces and the number that flow out of the tank. A first attempt at writing this in an equation might be,

$$\frac{dN}{dt} = KN - FN \qquad (11)$$

rate of change of bacteria = reproduction − outflow

where K is the reproduction rate of the bacteria, as before.

To go further, more assumptions must be made; typically we could simplify the problem by supposing that

2. Although the nutrient medium may contain a number of components, we can focus attention on a single growth-limiting nutrient whose concentration will determine the rate of growth of the culture.
3. The growth rate of the population depends on nutrient availability, so that $K = K(C)$. This assumption will be made more specific later, when we choose a more realistic version of this concentration dependence than that of simple proportionality.

Next we write an equation for changes in C, the nutrient level in the growth chamber. Here again there are several influences tending to increase or decrease concentration: inflow of stock supply and depletion by bacteria, as well as outflow of nutrients in the effluent. Let us assume that

4. Nutrient depletion occurs continuously as a result of reproduction, so that the rule we specified for culture growth and that for nutrient depletion are essentially going to be the same as before. Here α has the same meaning as in equation (6b).

Our attempt to write the equation for rate of change of nutrient might result in the following:

(*wrong*):
$$\frac{dC}{dt} = -\alpha K(C)N - FC + FC_0 \qquad (12)$$

minus for depletion during growth; minus for depletion due to outflow; plus due to replenishment from stock solution

Corrected Version

Equations (11) and (12) are not quite correct, so we now have to uncover mistakes made in writing them. A convenient way of achieving this is by comparing the *dimensions* of terms appearing in an equation. These have to match, clearly, since it would be meaningless to equate quantities not measured in similar units. (For example 10 msec^{-1} can never equal 10 lb.)

By writing the exact dimensions of each term in the equations, we get

(*wrong*): $$\frac{dN}{dt} = \underbrace{K(C)}N - FN$$

Dimensions: $$\frac{\text{number}}{\text{volume} \times \text{time}} = \frac{1}{\text{time}}\frac{\text{number}}{\text{volume}} - \frac{\text{volume}}{\text{time}}\frac{\text{number}}{\text{volume}}$$

From this we see that

1. $K(C)$, the growth rate, must have dimensions of 1/time.
2. The second term on the RHS is incorrect because it has an extra volume dimension that cannot be reconciled with the rest of the equation.

By considering dimensions, we have uncovered an inconsistency in the term FN of equation (11). A way of correcting this problem would be to divide FN by a quantity bearing dimensions of volume. Since the only such parameter available is V, we are led to consider FN/V as the appropriate correction. Notice that FN is the *number* of bacteria that leave per minute, and FN/V is thus the effective *density* of bacteria that leave per minute.

A similar analysis applied to equation (12) reveals that the terms FC and FC_0 should be divided by V (see problem 6). After correcting by the same procedure, we arrive at the following two corrected versions of equations (11) and (12):

$$\frac{dN}{dt} = K(C)N - \frac{FN}{V}, \tag{13a}$$

$$\frac{dC}{dt} = -\alpha K(C)N - \frac{FC}{V} + \frac{FC_0}{V}, \tag{13b}$$

As we have now seen, the analysis of dimensions is often helpful in detecting errors in this stage of modeling. However, the fact that an equation is dimensionally consistent does not always imply that it is correct from physical principles. In problems such as the chemostat, where substances are being transported from one compartment to another, a good starting point for writing an equation is the physical principle that *mass is conserved*. An equivalent conservation statement is that *the number of particles is conserved*. Thus, noting that

NV = number of bacteria in the chamber,

CV = mass nutrient in the chamber,

we obtain a mass balance of the two species by writing

$$\frac{d(NV)}{dt} = K(C)NV - FN, \tag{14a}$$

$$\frac{d(CV)}{dt} = -\alpha K(C)NV - FC + FC_0, \tag{14b}$$

(problem 9). Division by the constant V then leads to the correct set of equations (13a, b).

For further practice at formulating differential-equation models from word problems an excellent source is Henderson West (1983) and other references in the same volume.

4.4 A SATURATING NUTRIENT CONSUMPTION RATE

To add a degree of realism to the model we could at this point incorporate the fact that bacterial growth rates may depend on nutrient availability. For low nutrient abundance, growth rate typically increases with increasing nutrient concentrations. Eventually, when an excess of nutrient is available, its uptake rate and the resultant reproductive rate of the organisms does not continue to increase indefinitely. An appropriate assumption would thus be one that incorporates the effect of a *saturating* dependence. That is, we will assume that

5. The rate of growth increases with nutrient availability only up to some limiting value. (The individual bacterium can only consume nutrient and reproduce at some limited rate.)

One type of mechanism that incorporates this effect is Michaelis-Menten kinetics,

$$K(C) = \frac{K_{\max} C}{K_n + C}, \tag{15}$$

shown in Figure 4.3. Chapter 7 will give a detailed discussion of the molecular events underlying saturating kinetics. For now, it will suffice to note that $K_{\max}$ represents an upper bound for $K(C)$ and that for $C = K_n$, $K(C) = \frac{1}{2}K_{\max}$.

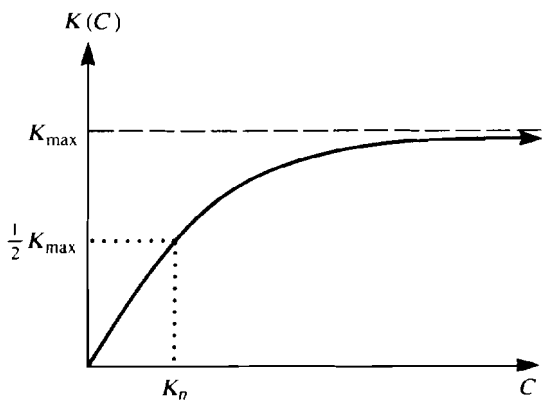

***Figure 4.3** Michaelis-Menten kinetics: Bacterial growth rate and nutrient consumption* K(C) *is assumed to be a saturating function of nutrient concentration. See equation (15).*

Our model equations can now be summarized as follows:

$$\frac{dN}{dt} = \left(\frac{K_{\max} C}{K_n + C}\right) N - \frac{FN}{V} \tag{16a}$$

$$\frac{dC}{dt} = -\alpha\left(\frac{K_{\max} C}{K_n + C}\right) N - \frac{FC}{V} + \frac{FC_0}{V}. \tag{16b}$$

In understanding these statements we draw a distinction between quantities that are variables, such as N and C and those that are parameters. There is little we can do to control the former *directly*, as they undergo changes in response to their inherent dynamics. However, we may be able to select values of certain parameters (such as F, C_0, and V) that will influence the process. (Other parameters such as $K_{\max}$ and K_n depend on the types of bacteria and nutrient medium selected in the experiment.)

It is of interest to determine what happens as certain combinations of parameters are varied over a range of values. Conceivably, an increase in some quantities could just compensate for a decrease in others so that, qualitatively, the system as a whole remains the same. Thus, while a total of six parameters appear in equations (16a,b) the chemostat may indeed have fewer than six *degrees of freedom*. This idea can be made more precise through further *dimensional analysis* of the equations in order to rewrite the model in terms of dimensionless quantities.

4.5 DIMENSIONAL ANALYSIS OF THE EQUATIONS

As shown in Table 4.1, quantities measured in an experiment such as that of the chemostat are specified in terms of certain conventional units. These are, to a great extent, arbitrary. For example a bacterial density of 10^5 cells per liter can be written in any one of the following equivalent ways:

$$\begin{aligned} N &= 10^5 \text{ cells/liter}, \\ &= 1 \text{ (unit of } 10^5 \text{ cells)/liter}, \\ &= 100 \text{ cells/milliliter}, \\ &= N^* \hat{N}. \end{aligned}$$

Here we have distinctly separated the measured quantity into two parts: a number N^*, which has no dimensions, and a quantity $\hat{N}$, which represents the units of measurement and carries the physical dimensions. The values 10^5, 1, 100, and N^* all refer to the same observation but in terms of different scales. As time evolves, N and N^* might change, but $\hat{N}$ is a constant, reflecting the fact that the scale of measurement does not change.

All of the original variables can be expressed similarly, as follows:

measured quantity	=	scalar multiple	×	unit carrying dimensions,
N	=	N^*	×	$\hat{N}$,
C	=	C^*	×	$\hat{C}$,
t	=	t^*	×	τ.

We shall see presently that advantage is gained by expressing the equations in terms of such *dimensionless quantities* as N^*, C^*, and t^*. To do so, we first substitute the expressions $N^*\hat{N}$, $C^*\hat{C}$, $t^*\tau$ for N, C, and t respectively in equations (16a,b) and then exploit the fact that $\hat{N}$, $\hat{C}$, and τ are time-independent constants. We obtain

$$\frac{d(N^*\hat{N})}{d(t^*\tau)} = \left(\frac{K_{\max}C^*\hat{C}}{K_n + C^*\hat{C}}\right)N^*\hat{N} - \frac{F}{V}(N^*\hat{N}), \tag{17a}$$

$$\frac{d(C^*\hat{C})}{d(t^*\tau)} = -\alpha\left(\frac{K_{\max}C^*\hat{C}}{K_n + C^*\hat{C}}\right)N^*\hat{N} - \frac{FC^*\hat{C}}{V} + \frac{FC_0}{V}. \tag{17b}$$

Now multiply both sides by τ, divide by $\hat{N}$ or $\hat{C}$, and group constant terms together. The result is

$$\frac{dN^*}{dt^*} = \tau K_{\max}\left(\frac{C^*}{K_n/\hat{C} + C^*}\right)N^* - \frac{\tau F}{V}N^*, \tag{18a}$$

$$\frac{dC^*}{dt^*} = \left(\frac{-\alpha\tau K_{\max}\hat{N}}{\hat{C}}\right)\left(\frac{C^*}{K_n/\hat{C} + C^*}\right)N^* - \frac{\tau F}{V}C^* + \frac{\tau FC_0}{V\hat{C}}. \tag{18b}$$

By making judicious choices for the measuring scales $\hat{N}$, τ, and $\hat{C}$, which are as yet unspecified, we will be able to make the equations look much simpler and contain fewer parameters. Equations (18a,b) suggest a number of scales that are inherent to the chemostat problem. Notice what happens when we choose

$$\tau = \frac{V}{F}, \qquad \hat{C} = K_n, \qquad \hat{N} = \frac{K_n}{\alpha\tau K_{\max}}.$$

The equations now can be written in the following form, in which we have dropped the stars for notational convenience.

$$\frac{dN}{dt} = \alpha_1\left(\frac{C}{1 + C}\right)N - N, \tag{19a}$$

$$\frac{dC}{dt} = -\left(\frac{C}{1 + C}\right)N - C + \alpha_2. \tag{19b}$$

The equations contain two dimensionless parameters, α_1 and α_2, in place of the original six (K_n, $K_{\max}$, F, V, C_0, and α). These are related by the following equations:

$$\alpha_1 = (\tau K_{\max}) = \frac{VK_{\max}}{F},$$

$$\alpha_2 = \frac{\tau FC_0}{V\hat{C}} = \frac{C_0}{K_n}.$$

In problem 8 we discuss the physical meaning of the scales τ, $\hat{C}$, and $\hat{N}$ and of the new dimensionless quantities that appear here.

We have arrived at a dimensionless form of the chemostat model, given by equations (19a,b). Not only are these equations simpler; they are more revealing. By the above we see that only two parameters affect the chemostat. No other choice of τ, $\hat{C}$, and $\hat{N}$ yields less than two parameters (see problem 10). Thus the chemostat has two degrees of freedom.

Equations (19a,b) are nonlinear because of the term $NC/(1 + C)$. Generally this means that there is little hope of finding explicit analytic solutions for $N(t)$ and $C(t)$. However, we can still explore the nature of special classes of solutions, just as we did in the nonlinear difference-equation models. Since we are interested in maintaining a continuous culture in which bacteria and nutrients are present at some fixed densities, we will next determine whether equations (19a,b) admit a steady-state solution of this type.

4.6 STEADY-STATE SOLUTIONS

A steady state is a situation in which the system does not appear to undergo any change. To be more precise, the values of *state variables,* such as bacterial density and nutrient concentration within the chemostat, would be constant at steady state even though individual nutrient particles continue to enter, leave, or be consumed. Setting derivatives equal to zero,

$$\frac{dN}{dt} = 0, \tag{20a}$$

$$\frac{dC}{dt} = 0, \tag{20b}$$

we observe that the quantities on the RHS of equations (19a,b) must be zero at steady state:

$$F(\bar{N}, \bar{C}) = \alpha_1\left(\frac{\bar{C}}{1 + \bar{C}}\right)\bar{N} - \bar{N} = 0, \tag{21a}$$

$$G(\bar{N}, \bar{C}) = -\left(\frac{\bar{C}}{1 + \bar{C}}\right)\bar{N} - \bar{C} + \alpha_2 = 0. \tag{21b}$$

This condition gives two algebraic equations that are readily solved explicitly for $\bar{N}$ and $\bar{C}$.

From (21a) we see that

$$\text{either } \bar{N} = 0 \tag{22a}$$

$$\text{or } \frac{\bar{C}}{1 + \bar{C}} = \frac{1}{\alpha_1}. \tag{22b}$$

After some simplification, (22b) becomes $\bar{C} = 1/(\alpha_1 - 1)$. From equation (21b), if $\bar{N} = 0$ we get $\bar{C} = \alpha_2$; on the other hand, if $\bar{N} \neq 0$, we get

$$\left(\frac{\bar{C}}{1 + \bar{C}}\right)\bar{N} = (\alpha_2 - \bar{C}). \tag{23}$$

Using (22b), we get

$$\bar{N} = \frac{1 + \bar{C}}{\bar{C}}(\alpha_2 - \bar{C}) = \alpha_1(\alpha_2 - \bar{C}). \tag{24}$$

Combining the information in equations (23) and (24) leads to the conclusion that there are two steady states:

$$(\bar{N}_1, \bar{C}_1) = \left(\alpha_1\left(\alpha_2 - \frac{1}{\alpha_1 - 1}\right), \frac{1}{\alpha_1 - 1}\right). \tag{25a}$$

$$(\bar{N}_2, \bar{C}_2) = (0, \alpha_2). \tag{25b}$$

The second solution, $(\bar{N}_2, \bar{C}_2)$, represents a situation that is not of interest to the experimentalists: no bacteria are left, and the nutrient is at the same concentration as the stock solution (remember the meaning of α_2 and the concentration scale to which it refers). The first solution (25a) looks more inspiring, but note that it does not always exist biologically. This depends on the magnitudes of the terms α_1 and α_2. Clearly, if $\alpha_1 < 1$, we get negative values. Since population densities and concentrations must always be positive, negative values would be meaningless in the biological context. The conclusion is that α_1 and α_2 must be such that $\alpha_1 > 1$ and $\alpha_2 > 1/(\alpha_1 - 1)$. In problem 8 we reach certain conclusions about how to adjust the original parameters of the chemostat to satisfy these constraints.

4.7 STABILITY AND LINEARIZATION

Thus far we have arrived at two steady-state solutions that satisfy equations (19a, b). In realistic situations there are always small random disturbances. Thus it is of interest to determine whether such deviations from steady state will lead to drastic changes or will be damped out.

By posing these questions we return once more to stability, a concept that was intimately explored in the context of difference-equation models. In this section we retrace the steps that were carried out in Section 2.7 to reach essentially identical conclusions, namely that, *close to the steady state, the problem can be approximated by a linear one.*

Let us look at a more general setting and take our system of ordinary differential equations to be

$$\frac{dX}{dt} = F(X, Y), \tag{26a}$$

$$\frac{dY}{dt} = G(X, Y), \tag{26b}$$

where F and G are nonlinear functions. We assume that $\bar{X}$ and $\bar{Y}$ are steady-state solutions, i.e., they satisfy

$$F(\bar{X}, \bar{Y}) = G(\bar{X}, \bar{Y}) = 0. \tag{27}$$

Now consider the close-to-steady-state solutions

$$X(t) = \bar{X} + x(t), \tag{28a}$$

$$Y(t) = \bar{Y} + y(t). \tag{28b}$$

Frequently these are called *perturbations* of the steady state. Substituting, we arrive at

$$\frac{d}{dt}(\bar{X} + x) = F(\bar{X} + x, \bar{Y} + y), \tag{29a}$$

$$\frac{d}{dt}(\bar{Y} + y) = G(\bar{X} + x, \bar{Y} + y). \tag{29b}$$

On the left-hand side (LHS) we expand the derivatives and notice that by definition $d\bar{X}/dt = 0$ and $d\bar{Y}/dt = 0$. On the right-hand side (RHS) we now expand F and G in a Taylor series about the point $(\bar{X}, \bar{Y})$, remembering that these are functions of two variables (see Chapter 2 for a more detailed discussion). The result is

$$\frac{dx}{dt} = F(\bar{X}, \bar{Y}) + F_x(\bar{X}, \bar{Y})x + F_y(\bar{X}, \bar{Y})y + \text{terms of order } x^2, y^2, xy, \text{ and higher,} \tag{30a}$$

$$\frac{dy}{dt} = G(\bar{X}, \bar{Y}) + G_x(\bar{X}, \bar{Y})x + G_y(\bar{X}, \bar{Y})y + \text{terms of order } x^2, y^2, xy, \text{ and higher.} \tag{30b}$$

where $F_x(\bar{X}, \bar{Y})$ is $\partial F/\partial x$ evaluated at $(\bar{X}, \bar{Y})$, and similarly for F_y, G_x, G_y and other terms.

Again by definition, $F(\bar{X}, \bar{Y}) = 0 = G(\bar{X}, \bar{Y})$, so we are left with

$$\frac{dx}{dt} = a_{11}x + a_{12}y, \tag{31a}$$

$$\frac{dy}{dt} = a_{21}x + a_{22}y, \tag{31b}$$

where the matrix of coefficients

$$\mathbf{A} = \begin{pmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{pmatrix} = \begin{pmatrix} F_x & F_y \\ G_x & G_y \end{pmatrix}_{(\bar{X}, \bar{Y})}. \tag{32}$$

is the Jacobian of the system of equations (26a,b). See Section 2.7 for definition.

To ultimately determine the question of stability, we are thus led to the question of how solutions to equation (31a,b) behave. We shall spend some time on this topic in the next sections. The methods and conclusions bear a strong relation to those we use for systems of difference equations.

4.8 LINEAR ORDINARY DIFFERENTIAL EQUATIONS: A BRIEF REVIEW

In this section we rapidly survey the minimal mathematical background required for analysis of ordinary differential equations (ODEs) such as those encountered in this chapter. For a broader review this section could be supplemented with material from any standard text on ODEs. (See references for suggested sources.)