BIOCHEMICAL REACTORS

MODULE

8

After studying this module, the student should be able to

* Develop the dynamic modeling equations for a two-state biochemical reactor
* Understand the concept of "washout"
* Understand the different types of steady-state and dynamic behavior exhibited by the Monod and Substrate Inhibition models
* Find the number of steady-state solutions and to determine the stability of each steady-state

The major sections of this module are:

M8.1   Background
M8.2   Modeling Equations
M8.3   Steady-state Solution
M8.4   Dynamic Behavior
M8.5   Linearization
M8.6   Phase-plane Analysis
M8.7   Understanding Multiple Steady-states
M8.8   Bifurcation Behavior

M8.1   BACKGROUND

Biochemical reactors are used to produce a large number of intermediate and final products, including pharmaceuticals, food, and beverages. Biochemical reactor models are similar to chemical reactor models, since the same type of material balances are per-
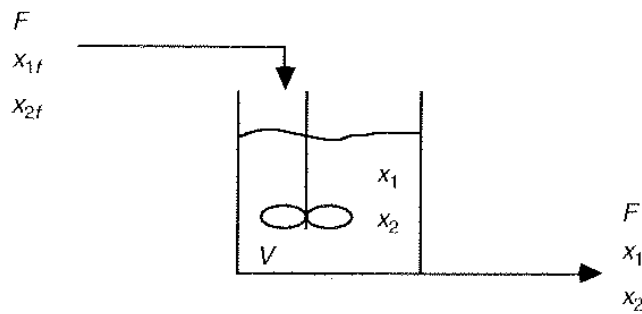
F
x_{1f}
x_{2f}

F
x_1
x_2 FIGURE M8.1   Biochemical reactor.

formed. In the simplest reactor we consider two components: biomass and substrate. The biomass consists of cells that consume the substrate. One example would be a wastewater treatment system, where the biomass is used to "eat" waste chemicals (substrate). Another example is fermentation, where cells consume sugar and produce alcohol.

Consider the schematic of a biochemical reactor shown in Figure M8.1.

In this module we assume that the reactor is perfectly mixed and that the volume is constant. We use the following notation:

$$x_1 = \text{biomass concentration} \quad = \frac{\text{mass of cells}}{\text{volume}}$$

$$x_2 = \text{substrate concentration} \quad = \frac{\text{mass of substrate}}{\text{volume}}$$

$$r_1 = \text{rate of cell generation} \quad = \frac{\text{mass of cells generated}}{\text{volume} \cdot \text{time}}$$

$$r_2 = \text{rate of substrate consumption} = \frac{\text{mass of substrate consumed}}{\text{volume} \cdot \text{time}}$$

$$F = \text{volumetric flowrate} \qquad = \text{volume/time}$$

Now we can write the material balances to describe the behavior of this system.

M8.2 MODELING EQUATIONS

The dynamic model is developed by writing material balances on the biomass (cells) and the substrate (feed source for the cells). Biomass grows by feeding on the substrate.

M8.2.1   Biomass Material Balance

We write the biomass material balance as:

rate of accumulation = in by flow − out by flow + generation

$$\frac{dVx_1}{dt} = Fx_{1f} - Fx_1 + Vr_1 \qquad (M8.1)$$

where x_{1f} is the concentration of biomass in the feed stream and F is the volumetric flowrate.

M8.2.2 Substrate Material Balance

The substrate material balance is written:

$$\text{rate of accumulation} = \text{in by flow} - \text{out by flow} - \text{consumption}$$

$$\frac{dVx_2}{dt} = Fx_{2f} - Fx_2 - Vr_2 \qquad (M8.2)$$

where x_{2f} is the concentration of substrate in the feed stream.

M8.2.3 Specific Growth Rate

The reaction rate (mass of cells generated/volume time) is normally written in the following form:

$$r_1 = \mu x_1 \qquad (M8.3)$$

where μ is the specific growth rate coefficient. We can think of μ as being similar to a first-order reaction rate constant; however, μ is not constant—it is a function of the substrate concentration as shown in Section M8.2.6. The units of μ are time^{-1}.

M8.2.4 Yield

There is a relationship between the rate of generation of biomass and the rate of consumption of substrate. Define Y as the yield, that is, the mass of cells produced per mass of substrate consumed:

$$Y = \frac{\text{mass of cells produced}}{\text{mass of substrate consumed}} = \frac{r_1}{r_2} \qquad (M8.4)$$

From (M8.4) we can write:

$$r_2 = \frac{r_1}{Y} \qquad (M8.5)$$

and substituting (M8.3) into (M8.5), we find:

$$r_2 = \frac{\mu x_1}{Y} \qquad (M8.6)$$

We assume in the subsequent analysis that Y is a constant.

M8.2.5 Dilution Rate

Assuming a constant volume reactor, we can write (M8.1) and (M8.2) as:

$$\frac{dx_1}{dt} = \frac{F}{V} x_{1f} - \frac{F}{V} x_1 + r_1 \qquad\qquad \text{(M8.7)}$$

$$\frac{dx_2}{dt} = \frac{F}{V} x_{2f} - \frac{F}{V} x_2 - r_2 \qquad\qquad \text{(M8.8)}$$

Defining F/V as D, the *dilution rate*, and using the rate expressions in (M8.3) and (M8.6), we find:

$$\frac{dx_1}{dt} = D x_{1f} - D x_1 + \mu x_1 \qquad\qquad \text{(M8.9)}$$

$$\frac{dx_2}{dt} = D x_{2f} - D x_2 - \frac{\mu x_1}{Y} \qquad\qquad \text{(M8.10)}$$

Generally, it is assumed that there is no biomass in the feed stream, so $x_{1f} = 0$. The bioreactor modeling equations are then normally written in the following form:

$$\frac{dx_1}{dt} = (\mu - D) x_1 \qquad\qquad \text{(M8.11)}$$

$$\frac{dx_2}{dt} = D (x_{2f} - x_2) - \frac{\mu x_1}{Y} \qquad\qquad \text{(M8.12)}$$

The dilution rate (D) is the same as the space velocity in the chemical reaction engineering literature. It is also the inverse of the reactor residence time and has units of time^{-1}.

The expressions for μ (specific growth rate) are developed in the following section.

M8.2.6   Growth Rate Expressions

The growth rate coefficient is usually not constant. A number of functional relationships between the growth rate coefficient and substrate concentration have been developed. The most common are (i) *Monod* and (ii) *Substrate inhibition*.

MONOD

The growth rate coefficient often varies in a hyperbolic fashion. The following form was proposed by Monod in 1942. Notice that μ is first-order at low x_2 and zero order at high x_2.

$$\mu = \frac{\mu_{max} x_2}{k_m + x_2} \qquad\qquad \text{(M8.13)}$$

Notice that μ is first-order at low x_2 and zero order at high x_2. That is, when x_2 is low:

$$\mu \approx \frac{\mu_{max}}{k_m} x_2$$

and when x_2 is high:

$$\mu \approx \mu_{max}$$

Since the reaction rate is:

$$r_1 = \mu x_1$$

this means that the Monod description is similar to a second-order (bimolecular) reaction when x_2 is low, since

$$r_1 \approx \frac{\mu_{max}}{k_m} x_2 x_1$$

and to a first-order reaction when x_2 is high, since

$$r_1 \approx \mu_{max} x_1$$

Equation (M8.13) is the same form as the Langmuir adsorption isotherm and the standard rate equation for enzyme-catalyzed reactions with a single substrate (Michaelis-Menten kinetics).

SUBSTRATE INHIBITION

Sometimes the growth rate coefficient increases at low substrate concentration, but decreases at high substrate concentration. The physical reason may be that the substrate has a toxic effect on the biomass cells at a higher concentration. This effect is called substrate inhibition and is represented by the following equation:
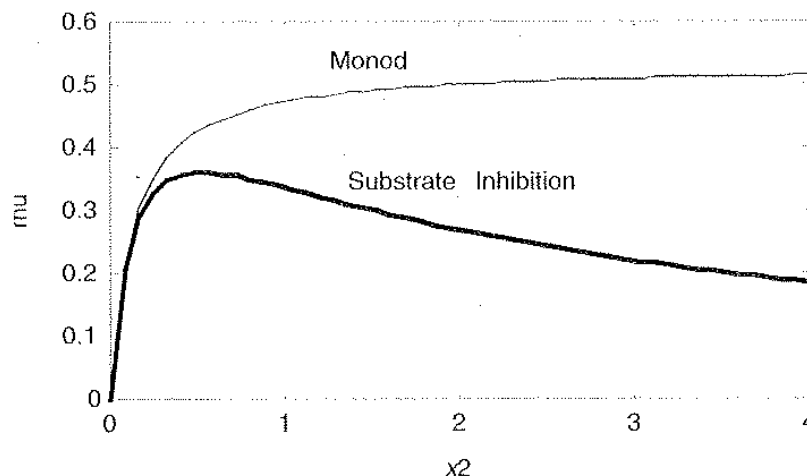
FIGURE M8.2   Comparison of Monod and substrate inhibition models for growth rate.

**TABLE M8.1   Parameters for Monod
and Substrate Inhibition Models**

Monod		Substrate Inhibition	
μ_{max}	= 0.53 hr^{-1}	μ_{max}	= 0.53 hr^{-1}
k_m	= 0.12 g/liter	k_m	= 0.12 g/liter
		k_1	= 0.4545 liter/g
Y	= 0.4	Y	= 0.4
x_{2fs}	= 4.0 g/liter	x_{2fs}	= 4.0 g/liter

$$\mu = \frac{\mu_{max}\, x_2}{k_m + x_2 + k_1\, x_2^2} \tag{M8.14}$$

Notice that the Monod equation is a special case of (M8.14), with $k_1 = 0$.

SPECIFIC GROWTH RATE RELATIONSHIPS

The characteristic relationships between substrate (x_2) and specific growth rate (μ) are quite different for Monod and substrate inhibition. The curves for μ as a function of x_2 for both models are compared in Figure M8.2. Notice that the substrate inhibition model exhibits a maximum in the growth rate curve, while Monod becomes zero-order at high substrate concentrations.

M8.3   STEADY-STATE SOLUTION

In this section, the MATLAB function fsolve will be used to solve for the steady-state values of the biomass and substrate concentrations. The numerical values used in our simulations are shown in Table M8.1.

We will study the following cases:

Case 1. Medium Dilution Rate, $D_s = 0.3$ hr^{-1}
Case 2. Low Dilution Rate, $D_s = 0.15$ hr^{-1}
Case 3. High Dilution Rate, $D_s = 0.45$ hr^{-1}

EXAMPLE M8.1   Case 1 Results (D = 0.3)

The function file bio_ss.m (Appendix 1) is set for Case 1 (D = 0.3) and the substrate inhibition model (k1 = 0.4545). The MATLAB function fsolve is used to solve for the steady-state values by entering the following in the command window (with an initial guess of x(1) = 1 and x(2) = 1):

```
» x = fsolve('bio_ss',[1;1])
```

The steady-state solution obtained is:

```
x =
    0.9951
    1.5122
```

Different initial guesses result in two other solutions for the substrate inhibition model. Also, the Monod model has two steady-state solutions. The reader should find the following results using `fsolve` and `bio_ss.m`, by entering different initial guesses.

Monod (2 steady-state solutions)

Equilibrium 1 $x_{1s} = 0$ $x_{2s} = 4.0$
Equilibrium 2 $x_{1s} = 1.5374$ $x_{2s} = 0.1565$

Substrate Inhibition (3 steady-state solutions)

Equilibrium 1 $x_{1s} = 0$ $x_{2s} = 4.0$
Equilibrium 2 $x_{1s} = 0.9951$ $x_{2s} = 1.5123$
Equilibrium 3 $x_{1s} = 1.5302$ $x_{2s} = 0.1745$

Notice that Equilibrium 3 on the SI model is almost identical to Equilibrium 2 for the Monod model. In this section we have discussed case 1 results ($D = 0.3$) only. Cases 2 and 3 will be discussed in Section M8.7.

In the next section we will analyze the dynamic behavior of this system, and in Section M8.7 we will show how multiple steady-state solutions arise.

M8.4   DYNAMIC BEHAVIOR

In the previous section we found that the Monod and substrate inhibition models had two and three steady-state solutions, respectively, for the Case 1 parameter values. In this section we perform simulations of the dynamic behavior of this system. A function file named `bio.m` is shown in Appendix 2.

M8.4.1   Case 1 (D = 0.3), Substrate Inhibition Model

The initial simulation is with the substrate inhibition parameters under Case 1 conditions ($D = 0.3$). The simulations for two different initial conditions are shown in Figure M8.3.

```
>> [t1,x1] = ode45('bio',0,30,[1;1]);

>> [t2,x2] = ode45('bio',0,30,[0.75;2]);
```
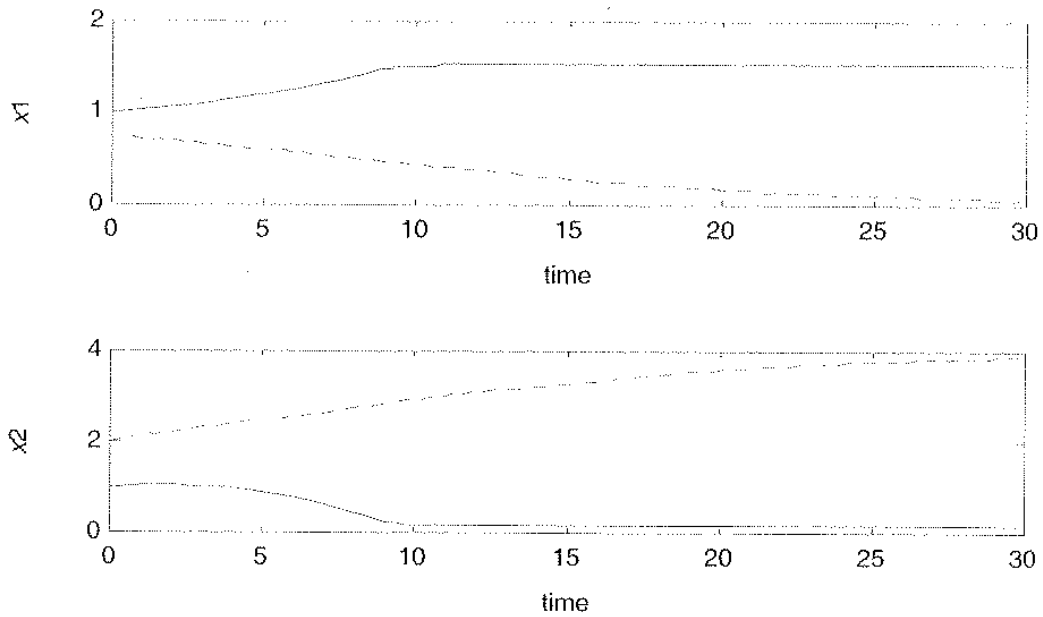
FIGURE M8.3   Substrate inhibition, Case 1. $x0 = [1,1]$ (solid), $x0 = [0.75,2]$ (dashed).

Although both initial conditions are reasonably close to the Equilibrium 2 solution found in section 3, one simulation converges to Equilibrium 1 (dashed line) while the other converges to Equilibrium 3 (solid line). We find in the next section that Equilibrium 2 is unstable. Further simulations will be performed and analyzed in the phase-plane (section 6).

M8.5   LINEARIZATION

In this section we find the linear state-space and transfer function models. So that there is no confusion in notation, we will use the following form:

$$\dot{z} = A z + B u$$
$$y = C z$$

where:

$$z_1 = x_1 - x_{1s}$$
$$z_2 = x_2 - x_{2s}$$

$$u_1 = D - D_s$$
$$u_2 = x_{2f} - x_{2fs}$$

The state-space matrices are:

$$A = \begin{bmatrix} \mu_s - D_s & x_{1s}\mu_s' \\ -\dfrac{\mu_s}{Y} & -D_s - \dfrac{\mu_s' x_{1s}}{Y} \end{bmatrix}$$

$$B = \begin{bmatrix} -x_{1s} & 0 \\ x_{2f} - x_{2fs} & D_s \end{bmatrix}$$

$$C = \begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix}$$

where it is assumed that both states are outputs. The notation μ_s' is used in the A matrix to represent the derivative of growth rate with respect to substrate concentration, evaluated at steady-state:

$$\mu_s' = \frac{\partial \mu_s}{\partial x_{2s}}$$

For the *Monod* model:

$$\mu_s' = \frac{\partial \mu_s}{\partial x_{2s}} = \frac{\mu_{max} k_m}{(k_m + x_{2s})^2} \tag{M8.15}$$

and for the *substrate inhibition* model:

$$\mu_s' = \frac{\partial \mu_s}{\partial x_{2s}} = \frac{\mu_{max}}{k_m + x_{2s} + k_1 x_{2s}^2} - \frac{\mu_{max} x_{2s}(1 + 2k_1 x_{2s})}{(k_m + x_{2s} + k_1 x_{2s}^2)^2}$$

$$\mu_s' = \frac{\mu_{max}(k_m + x_{2s} + k_1 x_{2s}^2) - \mu_{max} x_{2s}(1 + 2k_1 x_{2s})}{(k_m + x_{2s} + k_1 x_{2s}^2)^2} \tag{M8.16}$$

$$\mu_s' = \frac{\mu_{max}(k_m - k_1 x_{2s}^2)}{(k_m + x_{2s} + k_1 x_{2s}^2)^2}$$

M8.5.1   Substrate Inhibition Model

Here we analyze the substrate inhibition model under Case 1 conditions. A MATLAB m-file, `bio_jac.m` (Appendix 1), is used to generate the A matrix and the eigenvectors and eigenvalues.

EQUILIBRIUM POINT 1

The steady-state value (section 3) is $(x_{1s}, x_{2s}) = (0,4)$.
    The following command is entered:

```
»[jac,evec,lambda] = bio_jac([0;4])
```

where `jac` is the Jacobian (A matrix), `evec` is the eigenvector matrix and `lambda` are the eigenvalues.

```
jac =
    -0.1139          0
    -0.4652    -0.3000

evec =
         0     0.3714
    1.0000    -0.9285

lambda =
    -0.3000          0
         0    -0.1139
```

so,

$$\mathbf{A} = \begin{bmatrix} -0.1139 & 0 \\ -0.4652 & -0.300 \end{bmatrix}$$

$$\lambda_1 = -0.3 \qquad\qquad \xi_1 = \begin{bmatrix} 0 \\ 1 \end{bmatrix}$$

$$\lambda_2 = -0.1139 \qquad\qquad \xi_2 = \begin{bmatrix} 0.3714 \\ -0.9285 \end{bmatrix}$$

Since both eigenvalues are negative, the system is *stable* at equilibrium point 1, verifying the simulation results shown in Section M8.4.

EQUILIBRIUM POINT 2

The steady-state value is $(x_{1s}, x_{2s}) = (0.9951, 1.5122)$.

```
»[jac,evec,lambda] = bio_jac([0.9951;1.5122])

jac =
    0.0000    -0.0679
   -0.7500    -0.1302

evec =
    0.3714     0.2209
   -0.9285     0.9753

lambda =
    0.1698          0
         0    -0.3000
```

The positive eigenvalue (0.1698) indicates that equilibrium 2 is *unstable*.

EQUILIBRIUM POINT 3

The steady-state is $(x_{1s}, x_{2s}) = (1.5302, 0.1746)$.

```
»[jac,evec,lambda] = bio_jac([1.5302;0.1746])

jac =
    0.0000      0.9048
   -0.7500     -2.5619

evec =
    0.9492     -0.3714
   -0.3147      0.9285

lambda =
   -0.3000           0
        0     -2.2619
```
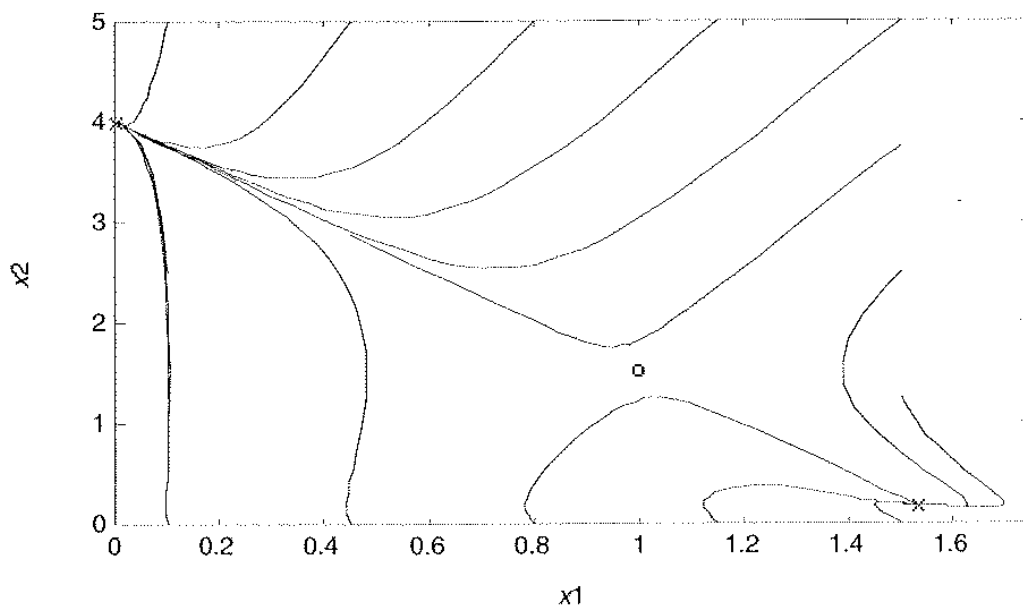
Both eigenvalues are negative, indicating that equilibrium point 3 is *stable*.

M8.6 PHASE-PLANE ANALYSIS

The m-file bio_phas_gen.m (Appendix 2) was used to generate the following phase-plane plot for the *substrate inhibition* model under Case 1 conditions (see Figure M8.4). Notice that all initial conditions converge to either the washout steady-state (trivial solu-

FIGURE M8.4 Phase-plane plot for *substrate inhibition* model, Case 1 conditions (x = stable steady-state, o = unstable steady-state).

tion, equilibrium 1) or equilibrium 3; while equilibrium 2 is a saddle point (unstable). These results are consistent with the stability analysis of Section M8.5.

A phase-plane plot for the Monod model was shown in Chapter 13.

M8.7   UNDERSTANDING MULTIPLE STEADY-STATES*

In this section we find analytically the steady-state solutions for the bioreactor model and determine their stability.

The steady-state solutions ($d_{x1}/dt = d_{x2}/dt = 0$) of (M8.11) and (M8.12) are:

$$0 = (\mu_s - D_s)\, x_{1s} \tag{M8.17}$$

$$0 = D_s\, (x_{2fs} - x_{2s}) - \frac{\mu_s x_{1s}}{Y} \tag{M8.18}$$

where the subscript s indicates steady-state.

There are two different types of solutions to (M8.17) and (M8.18). One is known as the trivial or "washout" solution. The other type is the nontrivial solution.

M8.7.1   Washout Condition

From (M8.17) and (M8.18) we can immediately see one solution, usually called the trivial solution.

$$x_{1s} = 0$$
$$x_{2s} = x_{2fs} \tag{M8.19}$$

This is also known as the washout condition, since the reactor concentrations are equal to the feed concentrations; that is, there is no "reaction." Since there is no biomass in the feed stream, then there is no biomass in the reactor under these conditions; all of the cells have been "washed out" of the reactor.

M8.7.2   Nontrivial Solutions

From (M8.17), assuming that $x_{1s} \neq 0$, then:

$$\mu_s = D_s \tag{M8.20}$$

which indicates that the specific growth rate is equal to the dilution rate, at steady-state.

From (M8.18) we find that:

$$D_s\, (x_{2fs} - x_{2s}) = \frac{\mu_s x_{1s}}{Y} \tag{M8.21}$$

and from (M8.20) and (M8.21):

$$x_{1s} = Y\, (x_{2fs} - x_{2s}) \tag{M8.22}$$

*This section contains a detailed analysis which the reader may wish to skip on a first reading.

We can solve for x_{2s}, by using the relationship for μ_s as a function of x_{2s} (either Monod or substrate inhibition), since we know that $\mu_s = D_s$ (from (M8.20)). Let $\mu_s(x_{2s})$ represent this general functionality. Then, we must solve:

$$\mu_s(x_{2s}) = D_s \qquad\qquad (M8.23)$$

for x_{2s}, then substitute this value into (M8.22) to solve for x_{1s}. The specific cases of Monod and substrate inhibition are shown in the subsections below.

MONOD

From (M8.13), the dilution rate at steady-state is

$$\mu_s = \frac{\mu_{max} x_{2s}}{k_m + x_{2s}} \qquad\qquad (M8.24)$$

Solving (M8.24) for x_{2s}, we find:

$$x_{2s} = \frac{k_m \mu_s}{\mu_{max} - \mu_s} \qquad\qquad (M8.25)$$

and since $\mu_s = D_s$

$$x_{2s} = \frac{k_m D_s}{\mu_{max} - D_s} \qquad\qquad (M8.26)$$

For (M8.26) to be feasible, we note that $D_s < \mu_{max}$. Actually, there is a more rigid requirement than that. From (M8.22) we note that the highest value that x_{2s} can be is x_{2fs}, otherwise x_{1s} will be less than zero. The maximum D_s in reality is then $\mu_s(x_{2fs})$, or (from (M8.22), letting $x_{2s} = x_{2fs}$):

$$D_s < \frac{\mu_{max} x_{2fs}}{k_m + x_{2fs}} \qquad (Monod) \qquad (M8.27)$$

We also see from (M8.26) that there is a single solution for x_{2s} as a function of D_s. This means that there is a total of *two* steady-state solutions for the Monod model, since there is also the washout (trivial) steady-state.

SUBSTRATE INHIBITION

We found in the previous subsection that there are two possible steady-states for the Monod model, for a given dilution rate. In this subsection we find the number of possible steady-states for the substrate inhibition model.

From (M8.14) at steady-state:

$$\mu_s = \frac{\mu_{max} x_{2s}}{k_m + x_{2s} + k_1 x_{2s}^2} \qquad\qquad (M8.28)$$

From (M8.28), we find that:

$$k_1 x_{2s}^2 + \left(1 - \frac{\mu_{max}}{\mu_s}\right) x_{2s} + k_m = 0 \tag{M8.29}$$

Since $\mu_s = D_s$ (M8.20), we substitute into (M8.29) to find:

$$k_1 x_{2s}^2 + \left(1 - \frac{\mu_{max}}{D_s}\right) x_{2s} + k_m = 0 \tag{M8.30}$$

Since (M8.30) is a quadratic equation, there will be two solutions for x_{2s}. This means that there are *three* steady-state solutions for substrate inhibition, since there is also the washout (trivial) steady-state.

We see from (M8.30) that for positive values of x_{2s} the coefficient multiplying x_{2s} must be negative. The implication is that μ_{max} must be greater than D_s (the same result as the Monod equation). This implication can be seen more clearly from the solution of the quadratic formula for (M8.30):

$$x_{2s} = \frac{-\left(1 - \frac{\mu_{max}}{D_s}\right) \pm \sqrt{\left(1 - \frac{\mu_{max}}{D_s}\right)^2 - 4k_1 k_m}}{2k_1} \tag{M8.31}$$

So, for solutions with physical significance:

$$\left(1 - \frac{\mu_{max}}{D_s}\right)^2 > 4k_1 k_m \tag{M8.32}$$

and $$\mu_{max} > D_s \tag{M8.33}$$

Because of (M8.33), we know that the term inside the brackets in (M8.32) is negative. For (M8.32) to be satisfied, then we know:

$$\left(1 - \frac{\mu_{max}}{D_s}\right) < -\sqrt{4k_1 k_m} \tag{M8.34}$$

which implies that:

$$D_s < \frac{\mu_{max}}{1 + 2\sqrt{k_1 k_m}} \qquad (substrate\ inhibition) \tag{M8.35}$$

We could have found the same result from viewing Figure M8.2. Notice that there is a peak in the μ_s curve, and again recall that $D_s = \mu_s$. The steady-state dilution rate, D_s cannot be above the peak in the x_{2s} versus μ_s curve. We can find the peak by finding $\partial \mu_s / \partial x_{2s} = 0$. From (M8.16):

$$\frac{\partial \mu_s}{\partial x_{2s}} = \frac{\mu_{max}(k_m - k_1 x_{2s}^2)}{(k_m + x_{2s} + k_1 x_{2s}^2)^2} = 0 \tag{M8.36}$$

We see from (M8.36) that $\partial\mu_s/\partial x_{2s} = 0$ if:

$$x_{2s} = \sqrt{\frac{k_m}{k_1}} \tag{M8.37}$$

We can substitute this result into (M8.28) to find:

$$\mu_s = \frac{\mu_{max}\sqrt{\frac{k_m}{k_1}}}{k_m + \sqrt{\frac{k_m}{k_1}} + k_1\frac{k_m}{k_1}} = \frac{\mu_{max}}{k_m\sqrt{\frac{k_1}{k_m}} + 1 + k_m\sqrt{\frac{k_1}{k_m}}} \tag{M8.38}$$

$$= \frac{\mu_{max}}{1 + 2k_m\sqrt{\frac{k_1}{k_m}}}$$

$$\mu_s = \frac{\mu_{max}}{1 + 2\sqrt{k_1 k_m}} \tag{M8.39}$$

so the maximum dilution rate (for the nontrivial steady-state) is:

$$D_s = \frac{\mu_{max}}{1 + 2\sqrt{k_1 k_m}}$$

which is the same result as (M8.35).

M8.7.3   Summary of Steady-State—Monod and Substrate Inhibition

WASHOUT (BOTH MONOD AND SUBSTRATE INHIBITION)

Both Monod and substrate inhibition models have a washout (trivial) steady-state:

$$x_{1s} = 0 \qquad x_{2s} = x_{2fs} \tag{M8.19}$$

NONTRIVIAL STEADY-STATE FOR MONOD

The nontrivial steady-state solutions for substrate and biomass are:

$$x_{2s} = \frac{k_m D_s}{\mu_{max} - D_s} \tag{M8.26}$$

$$x_{1s} = Y(x_{2fs} - x_{2s}) \tag{M8.22}$$

with the requirement that $D_s < \mu_{max}\, x_{2fs}/k_m + x_{2fs}$ (that is $D_s < \mu_s(x_{2fs})$)

NONTRIVIAL STEADY-STATES FOR SUBSTRATE INHIBITION

The two nontrivial steady-state solutions for substrate are:

$$x_{2s} = \frac{-\left(1 - \frac{\mu_{max}}{D_s}\right) + \sqrt{\left(1 - \frac{\mu_{max}}{D_s}\right)^2 - 4k_1k_m}}{2k_1}$$

(M8.31)

and the associated biomass concentration is:

$$x_{1s} = Y\left(x_{2fs} - x_{2s}\right)$$

(M8.25)

with the requirement for dilution rate:

$$D_s < \frac{\mu_{max}}{1 + 2\sqrt{k_1k_m}}$$

(M8.32)

M8.7.4   Stability of the Steady-States

The stability of each steady-state solution is determined from the eigenvalues of the Jacobian matrix (matrix A in the state-space form). For a two-state system we know that the eigenvalues are found by:

$$\det(\lambda I - A) = \lambda^2 - tr(A)\lambda + \det(A) = 0$$

(M8.40)

From Chapter 13 we know that the following conditions must be satisfied for stability of a second-order system:

$$tr(A) < 0$$

(M8.41)

$$\det(A) > 0$$

(M8.42)

That is, the eigenvalues (λ) will be negative if conditions (M8.41) and (M8.42) are satisfied. The Jacobian of the bioreactor modeling equations (M8.11 and M8.12) is:

$$A = \begin{bmatrix} \mu_s - D_s & x_{1s}\mu_s' \\ -\frac{\mu_s}{Y} & -D_s - \frac{\mu_s' x_{1s}}{Y} \end{bmatrix}$$

(M8.43)

where we have used the notation μ_s' to represent the derivative of growth rate with respect to substrate concentration, evaluated at steady-state:

$$\mu_s' = \frac{\partial \mu_s}{\partial x_{2s}}$$

(M8.44)

The trace and determinant of A are:

$$tr(A) = (\mu_s - D_s) - D_s - \frac{\mu_s' x_{1s}}{Y}$$

(M8.45)

$$\det(A) = -(\mu_s - D_s)\left(D_s + \frac{\mu_s' x_{1s}}{Y}\right) + \frac{x_{1s}\mu_s' \mu_s}{Y}$$

(M8.46)

We will use (M8.45), (M8.46), and the conditions shown in (M8.41) and (M8.42) to determine the stability of each steady-state.

STABILITY OF WASHOUT STEADY-STATE

Under washout conditions: $x_{2s} = x_{2fs}$ and $x_{1s} = 0$.

For stability of the washout steady-state, the following criteria then must be met. First, from the requirement that $\text{tr}(A) < 0$:

$$\mu_s - D_s - D_s < 0 \tag{M8.47}$$

and from the requirement that $\det(A) > 0$:

$$-(\mu_s - D_s)(D_s) > 0 \tag{M8.48}$$

From (M8.47) we see that the requirement for stability is then:

$$D_s > \frac{\mu_s}{2} \tag{M8.49}$$

while from (M8.48) the requirement for stability is:

$$D_s > \mu_s \tag{M8.50}$$

Notice that μ_s is evaluated at the substrate feed concentration for the washout condition. Perhaps the expression $\mu_s(x_{2fs})$ should be used to designate this relationship. Comparing (M8.49) and (M8.50), we see that (M8.50) is the more rigorous requirement for stability of the washout steady-state.

The growth rate expression for Monod kinetics is:

$$\mu_s = \mu_s(x_{2fs}) = \frac{\mu_{max} x_{2fs}}{k_m + x_{2fs}} \tag{M8.51}$$

while for substrate inhibition kinetics:

$$\mu_s = \mu_s(x_{2fs}) = \frac{\mu_{max} x_{2fs}}{k_m + x_{2fs} + k_1 x_{2fs}^2} \tag{M8.52}$$

Notice that $\mu_s(x_{2fs})$ is simply a shorthand expression for the specific growth rate evaluated at the substrate feed concentration. We must use (M8.50) along with either (M8.51) or (M8.52) to determine the stability of the washout steady-state. Notice that the washout steady-state will only be stable if D_s is high enough. We can think of D_s as a dynamic bifurcation parameter, because the stability of the washout steady-state will depend on the value of the dilution rate.

Stability of Washout Steady-State for Monod.   From (M8.50) and (M8.51), the washout steady-state will be stable if:

$$D_s > \frac{\mu_{max} x_{2fs}}{k_m + x_{2fs}} \tag{M8.53}$$

and unstable if:

$$D_s < \frac{\mu_{max} x_{2fs}}{k_m + x_{2fs}} \tag{M8.54}$$

Stability of Washout Steady-State for Substrate Inhibition.   From (M8.50) and (M8.52), the washout steady-state will be stable if:

$$D_s > \frac{\mu_{max} x_{2fs}}{k_m + x_{2fs} + k_1 x_{2fs}^2} \qquad \text{(M8.55)}$$

and unstable if:

$$D_s < \frac{\mu_{max} x_{2fs}}{k_m + x_{2fs} + k_1 x_{2fs}^2} \qquad \text{(M8.56)}$$

NONTRIVIAL STEADY-STATES

For the nontrivial steady-states, $D_s = \mu_s$. The stability requirements for the nontrivial steady-states are then:

$$-D_s - \frac{\mu_s' x_{1s}}{Y} < 0 \qquad \text{(M8.57)}$$

from the tr(A) specification, and

$$\frac{x_{1s} \mu_s' \mu_s}{Y} > 0 \qquad \text{(M8.58)}$$

from the det(A) specification. Since D_s (and therefore μ_s), x_{1s}, and Y are positive, (M8.57) and (M8.58) reduce to the requirement that:

$$\mu_s' > 0 \qquad \text{(M8.59)}$$

for stability.

Stability of Monod at the Nontrivial Steady-State.   From (M8.24):

$$\mu_s' = \frac{\mu_{max} k_m}{(k_m + x_{2s})^2} \qquad \text{(M8.60)}$$

We see immediately that μ_s' is always positive for the Monod model at the nontrivial steady-state; therefore, the nontrivial steady-state is always stable. Recall that $D_s < \mu_s(x_{2fs})$ for a nontrivial steady-state solution.

Stability of Substrate Inhibition at the Nontrivial Steady-States.   We can tell from the substrate inhibition curve in Figure M8.2 that a steady-state that is on the left side of the peak will be stable (since $\mu_s' > 0$), while a steady-state on the right side will be unstable (since $\mu_s' < 0$).

Numerically, from (M8.36):

$$\mu_s' = \frac{\mu_{max} (k_m - k_1 x_{2s}^2)}{(k_m + x_{2s} + k_1 x_{2s}^2)^2} \qquad \text{(M8.61)}$$

The x_{2s} that is on the left side of the peak, and is therefore *stable*, is (from (M8.31)):

$$x_{2s} = \frac{\left(\frac{\mu_{max}}{D_s} - 1\right) - \sqrt{\left(1 - \frac{\mu_{max}}{D_s}\right)^2 - 4k_1 k_m}}{2k_1}$$ (M8.62)

The x_{2s} that is on the right side of the peak, and is therefore *unstable*, is (from (M8.31)):

$$x_{2s} = \frac{\left(\frac{\mu_{max}}{D_s} - 1\right) + \sqrt{\left(1 - \frac{\mu_{max}}{D_s}\right)^2 - 4k_1 k_m}}{2k_1}$$ (M8.63)

Also, recall that $D_s < \mu_{max}/1 + 2\sqrt{k_1 k_m}$ (which is equivalent to requiring a real nontrivial solution).

M8.7.5   Case 1 ($D_s = 0.3$)

The reader should find the following results:

Monod

Equilibrium 1—washout	$x_{1s} = 0$	$x_{2s} = 4.0$	unstable
Equilibrium 2—nontrivial	$x_{1s} = 1.5374$	$x_{2s} = 0.1565$	stable

Substrate Inhibition

Equilibrium 1—washout	$x_{1s} = 0$	$x_{2s} = 4.0$	stable
Equilibrium 2—nontrivial	$x_{1s} = 0.9951$	$x_{2s} = 1.5123$	unstable (saddle point)
Equilibrium 3—nontrivial	$x_{1s} = 1.5302$	$x_{2s} = 0.1745$	stable

M8.7.6   Case 2

For a steady-state dilution rate of $D_s = 0.15$, the reader should find the following results:

Monod

Equilibrium 1—washout	$x_{1s} = 0$	$x_{2s} = 4.0$	unstable
Equilibrium 2—nontrivial	$x_{1s} = 1.5811$	$x_{2s} = 0.0474$	stable

Substrate Inhibition

Equilibrium 1—washout	$x_{1s} = 0$	$x_{2s} = 4.0$	unstable
Equilibrium 2—nontrivial	$x_{1s} = -0.6104$	$x_{2s} = 5.5261$	not feasible
Equilibrium 3—nontrivial	$x_{1s} = 1.5809$	$x_{2s} = 0.0478$	stable

Although there is a mathematical solution for equilibrium 2, it is not physically feasible, since it corresponds to a negative biomass concentration.

M8.7.7   Case 3

For a steady-state dilution rate of $D_s = 0.6$, the student should find the following results:

Monod

Equilibrium 1—washout	$x_{1s} = 0$	$x_{2s} = 4.0$	stable
Equilibrium 2—nontrivial	$x_{1s} = 2.0114$	$x_{2s} = -1.0286$	not feasible

Steady-state 2 is not feasible, because it corresponds to a negative substrate concentration.

Substrate Inhibition

Equilibrium 1—washout	$x_{1s} = 0$	$x_{2s} = 4.0$	stable
Equilibrium 2—nontrivial	$x_{1s} = 4.13 - 0.20j$	$x_{2s} = -0.13 + 0.50j$	not feasible
Equilibrium 3—nontrivial	$x_{1s} = 4.13 + 0.20j$	$x_{2s} = -0.13 - 0.50j$	not feasible

The second and third steady-states are not feasible because the concentrations for both the biomass and the substrate are complex.

There are some very interesting changes in the dynamic behavior of these models as we vary the dilution rate (again, we can think of dilution rate as a bifurcation parameter). Let us discuss this in order of the lowest dilution rate to the highest dilution rate.

LOW DILUTION RATE

Case 2 had the lowest dilution rate ($D_s = 0.15$). The Monod model has two steady-states—the washout steady-state is unstable and the other (nontrivial) steady-state is stable. This means that any set of initial conditions will eventually converge to the nontrivial steady-state, for the Monod model. The substrate inhibition model has only two feasible steady-states—the washout steady-state is unstable and the high conversion steady-state is stable.

The interesting result is that at low dilution rates, the substrate inhibition model behaves like the Monod model.

MEDIUM DILUTION RATE

Case 1 had the next highest dilution rate ($D_s = 0.30$). The Monod model has two steady-states—the washout steady-state is unstable and the other (nontrivial) steady-state is stable. This means that any set of initial conditions will eventually converge to the nontrivial steady-state, for the Monod model. The substrate inhibition model has three feasible steady-states. The washout (no conversion) steady-state is stable, the medium conversion steady-state is unstable and the high conversion (low x_{2s}) steady-state is stable. This means that any set of initial conditions will converge to one of the two stable steady-states. A phase-plane must be drawn to determine if a particular set of initial conditions will lead to washout.

HIGH DILUTION RATE

Case 3 had the highest dilution rate (D_s = 0.60). Both models had only one feasible steady-state, the washout steady-state, and it was stable. The student should be able to "sketch" phase planes for all three conditions for each of the models (Monod and substrate inhibition).

M8.8   BIFURCATION BEHAVIOR

The conditions for stability developed in Section M8.7 can be used to develop steady-state input-output diagrams for the numerical example presented in the previous sections.

M8.8.1   Diagram for the Monod Model

The diagram for the Monod model is shown in Figure M8.5. As calculated, the Monod model has two steady-states for dilution rates that are less than the specific growth rate under the feed conditions, $D_s < \mu_s(x_{2fs})$. The nontrivial steady-state is stable under those conditions, while the washout steady-state is unstable. For $D_s > \mu_s(x_{2fs})$ there is a single steady-state, the washout steady-state, and it is stable.

M8.8.2   Diagram for the Substrate Inhibition Model

The diagram for the substrate inhibition model is shown in Figure M8.6. At low dilution rates, where $D_s < \mu_s(x_{2fs})$, there are two steady-states (like the Monod model). The nontrivial steady-state is stable under those conditions, while the washout steady-state is unstable. For the intermediate dilution rate range, $\mu_s(x_{2fs}) < D_s < \mu_{max}/1 + 2\sqrt{k_1 k_m}$,
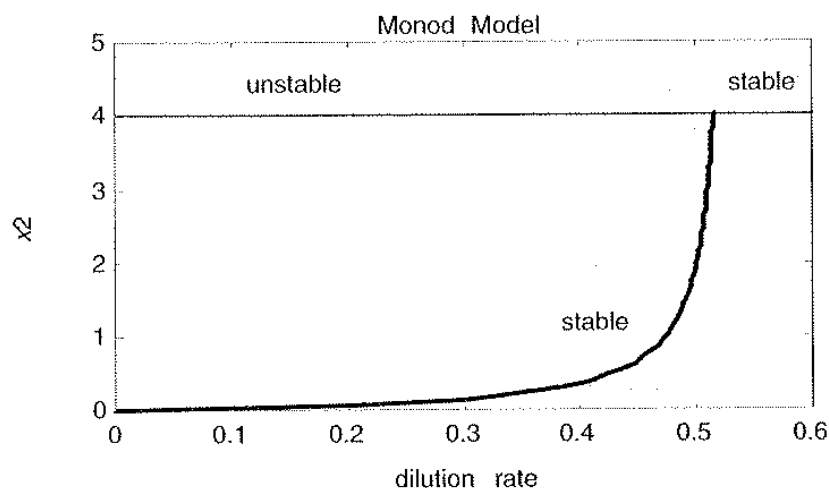

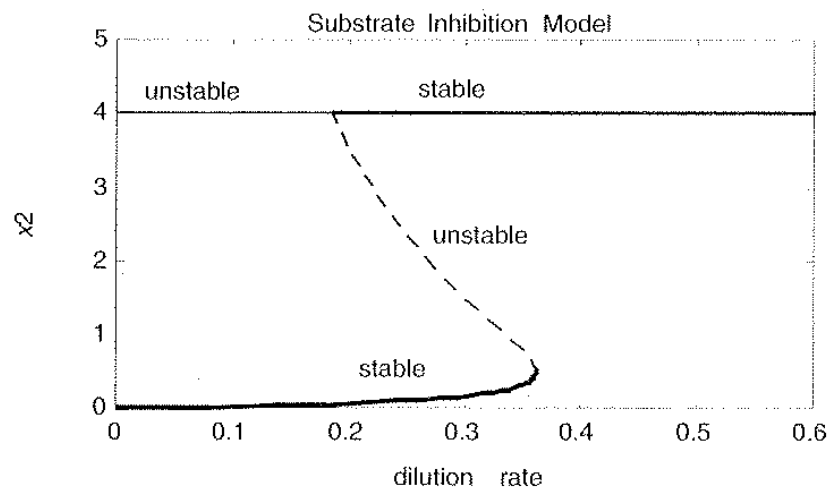**FIGURE M8.5**   Input-output diagram for the Monod model.

FIGURE M8.6   Input-output diagram for the substrate inhibition model.

there are three steady-states. Two of these are stable, while one is unstable. The stable steady-state that is attained will depend upon the initial conditions of the concentrations, or on the way that the process is started up. When the dilution rate meets the condition that $D_s > \mu_s(x_{2fs})$, there is a single steady-state, the washout steady-state, and it is stable.

M8.8.3   Hysteresis Behavior for the Substrate Inhibition Model

It is interesting to note that the way that the bioreactor is started up will determine the steady-state concentrations that the reactor achieves. Look at Figure M8.6. Notice that if we start at a very low dilution rate we will have only one stable steady-state, so the reactor must operate at that condition. If we slowly increase the dilution rate, we remain on the lower curve of Figure M8.6. When $D_s > \mu_{max}/1 + 2\sqrt{k_1 k_m}$ ($D_s = 0.36126$ for this example), the stable solution suddenly "leaps" to the upper stable steady-state (washout conditions). As we increase D_s further, we remain on the washout curve.

Now, assume that we are starting out at a high dilution rate along the upper curve, the washout conditions. As we slowly decrease the dilution rate, we remain on the washout curve until $D_s = \mu_s(x_{2fs})$, which is $D_s = 0.1861$ for this example. The stable steady-state then "jumps" down to the lower curve. As we continue to decrease the dilution rate further, we remain on the lower curve.

The type of behavior shown in Figure M8.6 is known as hysteresis and is exhibited by a number of processes, including exothermic chemical reactors and valves that "stick." The chemical reactor example is discussed further in Module 9.

The student should be able to show how the phase-plane behavior changes as a function of dilution rate, for the example shown in Figure M8.6.

SUMMARY

The modeling equations for a biochemical reactor were developed for Monod and substrate inhibition kinetics. We found that the Monod model normally has two steady-state solutions, while the substrate inhibition model normally has three steady-state solutions. At low dilution rates the substrate inhibition model behaves similarly to the Monod model, with a single stable steady-state. Washout will not be a problem at the low dilution rates.

At medium dilution rates the substrate inhibition model behaves quite differently from the Monod model. Depending on the initial conditions, the reactor will either converge to a high conversion or to washout conditions for the substrate inhibition model. It has not been discussed thus far, but if we wish to operate at an intermediate (unstable) conversion level, then feedback control must be used. Notice that the Monod model still has only one stable point, and there is no danger of wash-out.

At high dilution rates, both reactor models have only one feasible solution—washout. The flow is simply too high (residence time too low) for any cell growth.

FURTHER READING

An excellent source for an introduction to biochemical engineering is:

Bailey, J.E., & D.F. Ollis. (1986). *Biochemical Engineering Fundamentals*, 2nd ed. New York: McGraw-Hill.

STUDENT EXERCISES

1. In this module we developed the modeling equations assuming that no biomass is fed to the reactor. Analyze the system studied for the case where the biomass feed concentration is 2.5% of the substrate feed concentration (so $x_{1f} = 0.1$ for the numerical values used in this module).

   Is there still the possibility of a washout steady-state?

2. Modify bio_phas_gen.m and bio.m to perform a phase-plane analysis for cases 2 and 3 with the substrate inhibition model.

3. Data for specific growth rate coefficient as a function substrate concentration for a biochemical reactor are shown below:

x_2, g/liter	μ, hr^{-1}
0	0
0.1	0.38
0.25	0.54

0.5	0.63
0.75	0.66
1	0.68
1.5	0.70
3	0.73
5	0.74

a. Estimate the parameter values for a Monod model (k_m, μ_{max})

b. The production rate of cells (biomass) is $D*x1$. Find the steady-state value of the dilution rate that maximizes the production rate of cells. The substrate feed concentration is 5 g/liter.

c. Find the steady-state concentration of biomass and substrate at this dilution rate.

d. Find the linear state-space model at this dilution rate, with dilution rate and substrate feed concentration as the input variables. Also find the transfer function relating dilution rate to biomass concentration.

e. Simulate the responses (using the nonlinear dynamic model) of the concentrations of biomass and substrate to step increases and decreases of 10% in the dilution rate (changes are from the dilution rate found in b.). Compare these results with those of the linear system (remember to convert deviation variables back to physical variables).

4. In this module we have analyzed how the biomass and substrate concentrations change depending on the dilution rate. If the purpose of a particular biochemical reactor is to produce cells, then we are more concerned with the production rate of cells. The production rate is mass of cells produced per unit time:

$$\text{steady-state production rate of cells} = D_s*x_{1s}$$

For both the Monod and substrate inhibition models presented in this module, find the dilution rate that maximizes the production rate of cells. Analyze the stability of the reactor under this condition.

5. Consider a biochemical reactor where the consumption of substrate (x_2) promotes the growth of biomass (x_1) and formation of product (x_3). The three modeling equations are:

$$\frac{dx_1}{dt} = (\mu - D)x_1$$

$$\frac{dx_2}{dt} = D\left(x_{2f} - x_2\right) - \frac{\mu x_1}{Y}$$

$$\frac{dx_3}{dt} = -Dx_3 + [\alpha\mu + \beta]\, x_1$$

where the specific growth rate is a function of both the biomass concentration and the product concentration:

$$\mu = \frac{\mu_{max}\,(1 - P/P_m)\,x_2}{k_m + x_2 + k_1 x_2^2}$$

with the following parameter values:[1]

Variable	Value	Variable	Value
Y	0.4 g/g	α	2.2 g/g
β	0.2 hr^{-1}	μ_{max}	0.48 hr^{-1}
P_m	50 g/liter	k_m	1.2 g/liter
k_1	0.04545 liter/g	x_{2f}	20 g/liter
D	0.202 hr^{-1}	x_1	6 g/liter
x_2	5 g/liter	x_3	19.14 g/liter

a. Compare and contrast this model with that of the two-state model with substrate inhibition kinetics presented in this module.

b. Verify that the steady-state values for x_1, x_2, and x_3 presented in the table above are correct. For a steady-state input of $D = 0.202$ (and all of the other parameters constant), are there any additional solutions for the states (for example, the trivial solution?). Analyze the stability of all steady-state solutions obtained.

c. Perform dynamic simulations of the nonlinear model, with step changes of $\pm 10\%$ in the dilution rate. Discuss the results of your step changes (i.e., does an increase or decrease in D have a greater effect on the biomass concentration?). Compare your results with linear simulations.

APPENDIXES

1 Steady-State Biochemical Reactor Model, bio_ss.m

```
function f = bio_ss(x)
%
% b.w. bequette
% (c) 16 Nov 92
% revised 18 July 96
%
% find steady-states of bioreactor, using fsolve:
%
% x = fsolve('bio',x0)
%
```

[1]This model is from Chapter 4 of the following monograph: Henson, M.A., & D.E. Seborg (ed.). (1997). *Nonlinear Process Control*. Upper Saddle River, NJ: Prentice-Hall.

```
% where x0 is a vector of initial guesses
%
% x(1) = biomass
% x(2) = substrate
%
% biomass ("bugs") consumes the substrate
%
% D      = dilution rate (F/V, time^-1)
% Y      = yield biomass/substrate
% mu     = specific growth rate
% mumax  = parameter (both Monod and Substrate Inhibition)
% km     = parameter (both Monod and Substrate Inhibition)
% k1     = parameter (Substrate Inhibition only, k1 = 0 for Monod)
% sf     = substrate feed concentration
%
% the function vector consists of 2 equations
%
  f = zeros(2,1);
%
% parameter values
%
  D = 0.3;
  mumax = 0.53;
  Y = 0.4;
  km = 0.12;
  sf = 4.0;
  k1 = 0.4545;
%
% Substrate Inhibition expression for specific growth rate
%
  mu = mumax*x(2)/(km+x(2)+k1*x(2)*x(2));
%
% steady-state equations
% fsolve varies x(1) and x(2) to drive f(1) and f(2) to zero
%
  f(1) = (mu - D)*x(1);
  f(2) = (sf - x(2))*D - mu*x(1)/Y;
```

2   bio.m, Function File for Dynamic Simulation Using ode45

```
function xdot = bio(t,x)
%
% b.w. bequette
% (c) 18 July 96
%
% dynamic equations for bioreactor, integrated using ode45,
% using the following command
%
% [t,x] = ode45('bio',t0,tf,x0)
%
```

```
% where t0 is the initial time (usually 0), tf is
% the final time and x0 is the initial condition vector
% x0(1) = biomass initial condition
% x0(2) = substrate initial condition
%
% biomass ("bugs") consumes the substrate
%
% state variables
%
% x(1) = biomass
% x(2) = substrate
%
% D      = dilution rate (F/V, time^-1)
% Y      = yield biomass/substrate
% mu     = specific growth rate
% mumax  = parameter (both Monod and Substrate Inhibition)
% km     = parameter (both Monod and Substrate Inhibition)
% k1     = parameter (Substrate Inhibition only, k1 = 0 for Monod)
% sf     = substrate feed concentration
%
% the function vector consists of 2 equations
%
  f = zeros(2,1);
%
% parameter values
%
  D = 0.3;
  mumax = 0.53;
  Y = 0.4;
  km = 0.12;
  sf = 4.0;
  k1 = 0.4545;
%
% Substrate Inhibition expression for specific growth rate
%
  mu = mumax*x(2)/(km+x(2)+k1*x(2)*x(2));
%
% dynamic equations
%
  xdot(1) = (mu - D)*x(1);
  xdot(2) = (sf - x(2))*D - mu*x(1)/Y;
```

3   Phase-Plane Plot for Biochemical Reactor, `bio_phas_gen.m`

```
% bio_phas_gen.m
%
% b.w. bequette
% (c) 19 July 96
%
% generates phase-plane plots for the bioreactor
```

```
%
% set-up the axis limits
%
  axis([0 1.75 0 5]);
%
% stable and unstable points for substrate
% inhibition model
%
  x1u = [0.9951];
  x2u = [1.5122];
%
  x1s = [0;1.5302];
  x2s = [4;0.1746];
%
% place an 'x' on stable points
% place a 'o' on unstable points
%
  plot(x1u,x2u,'wo',x1s,x2s,'wx')
%
  hold on
%
% select different initial conditions
% x1 ranges from 0.1 to 1.5 (every 0.35)
% x2 ranges from 0 to 5 (every 1.25)
% total of 16 initial conditions
%
  x1init = [0.1 0.45 0.8 1.15 1.5 0.1 0.45 0.8 1.15 1.5];
  x2init = [ 0    0    0    0    0   5    5    5    5    5];
  x1inita = [1.5  1.5 1.5  0.1  0.1  0.1];
  x2inita = [1.25 2.5 3.75 1.25 2.5 3.75];
%
  x0 = [x1init x1inita;x2init x2inita];
%
% ncol = number of initial conditions
%
  [mrow,ncol] = size(x0);
%
% run simulations for each initial condition
%
  for i = 1:ncol;
%
        [t,x] = ode45('bio',0,30,[x0(:,i)]);
   %
        plot(x(:,1),x(:,2),'w')
   %
     end
%
  xlabel('x1')
  ylabel('x2')
  hold off
```

4 Direct Calculation of the Eigenvalues

Here we calculate the eigenvalues of the nontrivial solution:

$$\det(\lambda I - A) = \lambda^2 - \text{tr}(A)\lambda + \det(A) = 0 \tag{M8.40}$$

where the trace and determinant are

$$\text{tr}(A) = \mu_s - D_s - D_s - \frac{\mu_s' x_{1s}}{Y} \tag{M8.45}$$

$$\det(A) = -(\mu_s - D_s)\left(D_s + \frac{\mu_s' x_{1s}}{Y}\right) + \frac{x_{1s}\mu_s' \mu_s}{Y} \tag{M8.46}$$

For the nontrivial solution, $\mu_s = D_s$:

$$\text{tr}(A) = -D_s - \frac{\mu_s' x_{1s}}{Y} \tag{M8.A1}$$

$$\det(A) = \frac{x_{1s}\mu_s' \mu_s}{Y} \tag{M8.A2}$$

The roots of (M8.40) are:

$$\lambda = \frac{\text{tr}(A) \pm \sqrt{(\text{tr } A)^2 - 4\det A}}{2} \tag{M8.A3}$$

and since $D_s = \mu_s$:

$$\lambda = \frac{-\mu_s - \dfrac{x_s\mu_s'}{Y} \pm \sqrt{\left(-\mu_s - \dfrac{x_s\mu_s'}{Y}\right)^2 - \dfrac{4 x_s \mu_s'\mu_s}{Y}}}{2} \tag{M8.A4}$$

$$\lambda = \frac{-\mu_s - \dfrac{x_s\mu_s'}{Y} \pm \sqrt{\mu_s^2 + \dfrac{x_s^2\mu_s'^2}{Y^2} + \dfrac{2 x_s\mu_s'\mu_s}{Y} - \dfrac{4 x_s \mu_s'\mu_s}{Y}}}{2} \tag{M8.A5}$$

$$\lambda = \frac{-\mu_s - \dfrac{x_s\mu_s'}{Y} \pm \sqrt{\mu_s^2 + \dfrac{x_s^2\mu_s'^2}{Y^2} - \dfrac{2 x_s \mu_s'\mu_s}{Y}}}{2} \tag{M8.A6}$$

$$\lambda = \frac{-\mu_s - \dfrac{x_s\mu_s'}{Y} \pm \sqrt{\left(-\mu_s - \dfrac{x_s\mu_s'}{Y}\right)^2}}{2} \tag{M8.A7}$$

$$\lambda = \frac{-\mu_s - \dfrac{x_s\mu_s'}{Y} \pm \left(\mu_s - \dfrac{x_s\mu_s'}{Y}\right)}{2} \tag{M8.A8}$$

and our roots are:

$$\lambda_1 = -\mu_s \quad \text{and} \quad \lambda_2 = -\frac{x_s \mu_s'}{Y} \tag{M8.A9}$$

and since $\mu_s = D_s$, we are assured that one pole will always be negative. The second root will only be positive if μ_s' is negative. Since μ_s' is positive for the Monod model, the nontrivial solution is stable as long as the solution is feasible ($D_s < \mu_s(x_{2fs})$). The μ_s' can be either positive or negative for the substrate inhibition model, so a nontrivial steady-state may either be stable or unstable.