Mass Balances, Energy Balances and Parameter Estimation

In this chapter, we will present the general methodology to develop mass and enthalpy balances for biological wastewater treatment processes. We will also show how to obtain the model parameters from experimental data. A general assumption that we will make in this chapter and throughout this book is that the reactors will always be considered perfectly mixed.

3.1 MASS BALANCES

Mass balances have the general form:

\[
\text{Accumulation} = \text{Input} - \text{Output} + \text{Generation} - \text{Consumption} \quad (3.1)
\]

This general equation takes different forms depending on whether we are considering a batch or a continuous reactor.

3.1.1 Mass Balances in Batch Reactors

In a batch reactor, we have no input and output terms, so the general mass balance can be written simply as:

\[
\text{Accumulation} = \text{Generation} - \text{Consumption} \quad (3.2)
\]
For example, consider a batch reactor where an organic substrate is spiked at time 0 and is consumed by microorganisms under aerobic conditions (Figure 3.1).

Let us suppose, for example, that the substrate is glucose. We have seen in Chapter 2 that the stoichiometry of microbial growth on glucose can be written as follows:

\[
\begin{align*}
\text{C}_6\text{H}_{12}\text{O}_6 & + (6 - 7.96Y_{X/S})\text{O}_2 + 1.59Y_{X/S}\text{NH}_3 \rightarrow \\
1.59Y_{X/S}\text{C}_2\text{H}_7\text{O}_2\text{N} & + (6 - 7.96Y_{X/S})\text{CO}_2 + (6 - 3.18Y_{X/S})\text{H}_2\text{O}
\end{align*}
\]

The most important species for which we want to write mass balances are biomass, substrate (i.e. glucose), nitrogen and oxygen. First of all, we have to agree on the units we will use for the various species in the mass balances. Here we decide to express the concentrations of all the species in kg/m³. We assume Monod kinetics (Chapter 2) for biomass growth, i.e.:

\[
r_X = \frac{\mu_{\text{max}} S}{K_S + S} X
\]

The use of this rate equation for biomass growth means that we are assuming that all the other substrates are not rate limiting and in excess. In case other substrates may be limiting (a frequent case is oxygen, if the aeration rate is not enough to maintain oxygen concentration to non-limiting values), the approach described in Section 2.1.2.1 needs to be used (e.g. Equation 2.55, if oxygen concentration is also rate limiting).

For endogenous metabolism, we assume a first-order dependence on biomass concentration (Chapter 2):

\[
r_{\text{end}} = -bX
\]
Biomass balance:
Biomass accumulated = biomass generated – biomass consumed
Biomass is generated by growth and consumed by endogenous metabolism, so
\[
\frac{dX}{dt} \left( \frac{\text{kg biomass}}{m^3 \cdot \text{day}} \right) = r_X + r_{\text{end}} = \left( \frac{\mu_{\text{max}} S}{K_S + S} - b \right) X \tag{3.3}
\]

Substrate balance:
Substrate accumulated = substrate generated – substrate consumed
There is no substrate generation in this process and substrate is consumed by biomass growth:
\[
\frac{dS}{dt} \left( \frac{\text{kg substrate}}{m^3 \cdot \text{day}} \right) = r_S = -\frac{r_X}{Y_{X/S}} = -\frac{\mu_{\text{max}} S}{K_S + S} \frac{X}{Y_{X/S}} \tag{3.4}
\]

Where in this case, the units of the growth yield, \( Y_{X/S} \), will be kg biomass/kg substrate (glucose).

Ammonia balance:
Ammonia accumulated = ammonia generated – ammonia removed
Ammonia is generated by endogenous metabolism and is removed by biomass growth:
\[
\frac{d(NH_3)}{dt} \left( \frac{\text{kg N}}{m^3 \cdot \text{day}} \right) = -0.12 \cdot r_{\text{end}} - 0.12 \cdot r_X = -\left( \frac{\mu_{\text{max}} S}{K_S + S} - b \right) X \cdot 0.12 \tag{3.5}
\]

Here we have assumed that 12% of the microorganisms’ dry weight is composed of nitrogen (this follows from the empirical formula of the microorganisms \( C_5H_7O_2N \)).

Oxygen balance:
Here we are interested in the oxygen balance in the liquid phase.
Oxygen accumulated = Oxygen in + oxygen generated – oxygen removed
Oxygen enters the liquid phase due to transfer from the gas phase, and it is removed by biomass growth and endogenous metabolism. There is no oxygen generated.
\[
\frac{dC_{O_2}}{dt} \left( \frac{\text{kg oxygen}}{m^3 \cdot \text{day}} \right) = -\frac{\mu_{\text{max}} S}{K_S + S} \left( \frac{1.07}{Y_{X/S}} - 1.42 \right) X - 1.42bX \tag{3.6}
\]
\[
+ k_L a \left( C^*_O - C_{O_2} \right)
\]
In this equation, the term \( \left( \mu_{\text{max}} S/K_s + S \right) \cdot \left[ (1.07/Y_{X/S}) - 1.42 \right] X + 1.42 bX = \) OUR represents the oxygen uptake rate (OUR) by the microorganisms per unit volume of the reactor (in Equation 3.6, it is taken with the negative sign because it causes the dissolved oxygen concentration to decrease), while the term \( k_1 a(C_{\text{O}_2} - C_{\text{O}_2}) \) represents the rate of oxygen transfer from the gas to the liquid phase.

Assuming that the parameters \( \mu_{\text{max}}, K_s, b \) and \( Y_{X/S} \) are known, the mass balances above allow for the calculation of the biomass, substrate, ammonia and oxygen profiles over time.

Often in wastewater treatment processes, the feed is not composed of a single carbon source, and in this case, the substrate can be characterised only by its chemical oxygen demand (COD). Therefore, in this case, the units for the substrate will be kg COD/m³ (instead of kg substrate/m³) and the units for the growth yield \( Y_{X/S} \) will have to be kg biomass/kg COD. Also, the substrate might not be (entirely) readily biodegradable, but (part of it) might be slowly biodegradable. The slowly biodegradable substrate \( X_s \) needs to be hydrolysed before being metabolised and here we will assume the rate equation for hydrolysis seen in Chapter 2. The mass balances for a batch reactor where the carbon source is measured as COD are shown below, where the mass balances for the slowly biodegradable substrate \( X_s \) are also shown:

\[
\frac{dX_s}{dt} \left( \frac{\text{kgCOD}}{\text{m}^3 \cdot \text{day}} \right) = r_{\text{hydr}} = -k_h \frac{X_s}{K_s + X_s} X \tag{3.7}
\]

\[
\frac{dS}{dt} \left( \frac{\text{kgCOD}}{\text{m}^3 \cdot \text{day}} \right) = r_s - r_{\text{hydr}} = k_h \frac{X_s}{K_s + X_s} X - \frac{\mu_{\text{max}} S}{K_s + S} \frac{X}{Y_{X/S}} \tag{3.8}
\]

\[
\frac{d(N\text{H}_3)}{dt} \left( \frac{\text{kg N}}{\text{m}^3 \cdot \text{day}} \right) = -\left( \frac{\mu_{\text{max}} S}{K_s + S} - b \right) X \cdot 0.12 \tag{3.9}
\]

\[
\frac{dX}{dt} \left( \frac{\text{kg biomass}}{\text{m}^3 \cdot \text{day}} \right) = r_X + r_{\text{end}} = \left( \frac{\mu_{\text{max}} S}{K_s + S} - b \right) X \tag{3.10}
\]

\[
\frac{dC_{\text{O}_2}}{dt} \left( \frac{\text{kg oxygen}}{\text{m}^3 \cdot \text{day}} \right) = -\frac{\mu_{\text{max}} S}{K_s + S} \cdot X \cdot \left( \frac{1}{Y_{X/S}} - 1.42 \right) - 1.42 bX + k_1 a(C_{\text{O}_2}^* - C_{\text{O}_2}) \tag{3.11}
\]
Where the OUR by the microorganisms is:

\[
\text{OUR} \left( \frac{\text{kg oxygen}}{\text{m}^3 \cdot \text{day}} \right) = \frac{\mu_{\text{max}} S}{K_S + S} \cdot \left( \frac{1}{Y_{X/S}} \right) - 1.42 + 1.42bX \]  
(3.12)

If nitrate is used as electron acceptor instead of oxygen (anoxic conditions), the nitrate balance in batch tests is the following:

\[
\frac{d\text{NO}_3}{dt} \left( \frac{\text{kg N} - \text{NO}_3}{\text{m}^3 \cdot \text{day}} \right) = -\frac{\mu_{\text{max}} S}{K_S + S} \cdot \left( \frac{1}{Y_{X/S}} \right) - 1.42 \frac{bX}{2.86} 
\]  
(3.13)

Note that the nitrate consumption rate is equal to the oxygen consumption rate (assuming the same values of the parameters) divided by the factor 2.86, as explained in Chapter 2.

For another example, let us consider anaerobic digestion carried out in a batch reactor. For simplicity, we assume that glucose is the substrate. We assume that glucose is converted into acetic acid and hydrogen by fermentative microorganisms. Acetic acid is then converted into methane by acetoclastic methanogens and hydrogen is converted into methane by hydrogenotrophic methanogens. A scheme of a batch reactor for anaerobic digestion is shown in Figure 3.2.

![Figure 3.2 Scheme of a batch anaerobic digester and of the model of the anaerobic digestion of glucose used here.](image-url)
Mass balances for batch anaerobic digestion are more complicated than for aerobic systems because in anaerobic processes, we are also interested in calculating the composition of the gas phase, which in general is not of interest for aerobic systems. Also, anaerobic digestion inevitably requires multiple species of microorganisms and mass balances need to be written for each of them.

We will assume that glucose and acetic acid are present only in the liquid phase, while hydrogen, methane and carbon dioxide are present in both phases.

The mass balances for the various components in a batch reactor are written below.

Fermentative microorganisms ($X_{GLU}$) convert glucose into acetic acid and hydrogen. These microorganisms grow on glucose and are removed by endogenous metabolism.

$$\frac{dX_{GLU}}{dt} \left( \frac{\text{kg microorganisms}}{\text{m}^3.\text{day}} \right) = r_{X_{GLU}} + r_{\text{endGLU}}$$

$$= \left( \mu_{\text{maxGLU}} \frac{\text{GLU}}{K_{SGLU} + \text{GLU}} - b_{GLU} \right) X_{GLU}$$

(3.14)

Acetoclastic methanogens ($X_{AC}$). These microorganisms grow on acetic acid and are removed by endogenous metabolism.

$$\frac{dX_{AC}}{dt} \left( \frac{\text{kg microorganisms}}{\text{m}^3.\text{day}} \right) = r_{X_{AC}} + r_{\text{endAC}}$$

$$= \left( \mu_{\text{maxAC}} \frac{\text{AC}}{K_{SAC} + \text{AC}} - b_{AC} \right) X_{AC}$$

(3.15)

Hydrogenotrophic methanogens ($X_{H2}$). These microorganisms grow on acetic acid and are removed by endogenous metabolism.

$$\frac{dX_{H2}}{dt} \left( \frac{\text{kg microorganisms}}{\text{m}^3.\text{day}} \right) = r_{X_{H2}} + r_{\text{endH2}}$$

$$= \left( \mu_{\text{maxH2}} \frac{\text{H2}}{K_{SCH2} + \text{H2}} - b_{H2} \right) X_{H2}$$

(3.16)
Inert biomass. These are inactive microorganisms produced by the endogenous metabolism of \(X_{\text{GLU}}\), \(X_{\text{Ac}}\) and \(X_{\text{H2}}\).

\[
\frac{dX_{\text{inert}}}{dt} \left( \frac{\text{kg microorganisms}}{\text{m}^3 \cdot \text{day}} \right) = -r_{\text{endGLU}} - r_{\text{endAc}} - r_{\text{endH2}} \\
= b_{\text{GLU}} X_{\text{GLU}} + b_{\text{Ac}} X_{\text{Ac}} + b_{\text{H2}} X_{\text{H2}} \tag{3.17}
\]

Glucose (GLU). Glucose is removed by fermentative microorganisms.

\[
\frac{d\text{GLU}}{dt} = -\frac{r_{\text{XGlu}}}{Y_{X/\text{SGlu}}} \tag{3.18}
\]

Acetic acid. Acetic acid is produced by fermentative microorganisms that grow on glucose and is removed by acetoclastic methanogens.

\[
\frac{d\text{Ac}}{dt} = \left( \frac{0.67}{Y_{X/\text{SGlu}}} - 0.88 \right) r_{\text{XGlu}} - \frac{r_{\text{XAc}}}{Y_{X/\text{SAc}}} \tag{3.19}
\]

Hydrogen in the liquid phase. Hydrogen in the liquid phase is produced by fermentative microorganisms, is removed by hydrogenotrophic microorganisms and transfers to or from the gas phase.

\[
\frac{d\text{H}_2}{dt} = \left( \frac{0.044}{Y_{X/\text{SGlu}}} - 0.058 \right) r_{\text{XGlu}} - \frac{r_{\text{XH2}}}{Y_{X/\text{SH2}}} + k_L a \left( k_{\text{eqH2}} \cdot p_{\text{H2}} - \text{H}_2 \right) \tag{3.20}
\]

Methane in the liquid phase. Methane in the liquid phase is produced by acetoclastic and hydrogenotrophic microorganisms, and transfers to and from the gas phase.

\[
\frac{d\text{CH}_4}{dt} = \left( \frac{0.267}{Y_{X/\text{SAc}}} - 0.354 \right) r_{\text{XAc}} + \left( \frac{2}{Y_{X/\text{SH2}}} - 0.352 \right) r_{\text{XH2}} + k_L a \left( k_{\text{eqCH4}} \cdot p_{\text{CH4}} - \text{CH}_4 \right) \tag{3.21}
\]

Carbon dioxide in the liquid phase. Carbon dioxide in the liquid phase is produced by fermentative microorganisms and acetoclastic methanogens, is removed by hydrogenotrophic methanogens, and transfers to and from the gas phase.
In Equation 3.22, the term
\[
\frac{dX}{dt} = \left[ \frac{0.49}{Y_{X/\text{Glu}}} - 0.65 \right] r_{\text{Glu}} + \left[ \frac{0.73}{Y_{X/\text{Ac}}} - 0.97 \right] r_{\text{Ac}}
\]
accounts for the fact that only a fraction of the generated carbon dioxide is present as such in the liquid phase, and part of it is present as other forms of carbonic acid (undissociated carbonic acid, bicarbonate, carbonate), which we have assumed to be in equilibrium (the equilibrium is function of pH as seen in Chapter 2).

Hydrogen in the head space. It transfers to and from the liquid phase, and is removed by the outlet flow of the gas phase.
\[
\frac{dp_{\text{H}_2}}{dt} = -k_1a\left( k_{eq\text{H}_2} \cdot p_{\text{H}_2} - \text{H}_2 \right) \frac{V_{\text{liquid}}}{\rho_{\text{H}_2}} \frac{p_{\text{tot}}}{V_{\text{head space}}} - Q_{\text{gas}} \frac{p_{\text{H}_2}}{V_{\text{head space}}} \quad (3.23)
\]

Methane in the head space. It transfers to and from the liquid phase, and is removed by the outlet flow of the gas phase.
\[
\frac{dp_{\text{CH}_4}}{dt} = -k_1a\left( k_{eq\text{CH}_4} \cdot p_{\text{CH}_4} - \text{CH}_4 \right) \frac{V_{\text{liquid}}}{\rho_{\text{CH}_4}} \frac{p_{\text{tot}}}{V_{\text{head space}}} - Q_{\text{gas}} \frac{p_{\text{CH}_4}}{V_{\text{head space}}} \quad (3.24)
\]

Carbon dioxide in the head space. It transfers to and from the liquid phase, and is removed by the outlet flow of the gas phase.
\[
\frac{dp_{\text{CO}_2}}{dt} = -k_1a\left( k_{eq\text{CO}_2} \cdot p_{\text{CO}_2} - \text{CO}_2 \right) \frac{V_{\text{liquid}}}{\rho_{\text{CO}_2}} \frac{p_{\text{tot}}}{V_{\text{head space}}} - Q_{\text{gas}} \frac{p_{\text{CO}_2}}{V_{\text{head space}}} \quad (3.25)
\]
Gas flow rate from the head space. The gas flow rate from the head space is equal to the sum of the gases leaving the headspace.

\[
\frac{Q_{\text{gas}}}{V_{\text{liquid}}} = \frac{k_{L_a} \left( k_{eq\text{CO}_2} \cdot p_{\text{CO}_2} - \text{CO}_2 \right)}{\rho_{\text{CO}_2}} - \frac{k_{L_a} \left( k_{eq\text{H}_2} \cdot p_{\text{H}_2} - \text{H}_2 \right)}{\rho_{\text{H}_2}} - \frac{k_{L_a} \left( k_{eq\text{CH}_4} \cdot p_{\text{CH}_4} - \text{CH}_4 \right)}{\rho_{\text{CH}_4}} \left( 1 - \frac{p_{\text{swat}}}{p_{\text{tot}}} \right)
\]

Assuming the kinetic parameters and physical properties of the system are known, Equations 3.14 through 3.26 can be solved to give the time profiles of all the variables in a batch anaerobic reactor.

**Example 3.1**

A batch reactor is fed with wastewater at 500 mgCOD/l. The initial concentration of the microorganisms is 100 mg/l. Assume the substrate in the wastewater is all readily biodegradable. Calculate the initial rate of the following processes:

- Substrate removal;
- Net micro-organism production;
- Oxygen consumption by the microorganisms

Kinetic parameters:

\[
\mu_{\text{max}} = 4 \text{ day}^{-1}
\]

\[
b = 0.1 \text{ day}^{-1}
\]

\[
k_{\text{hydr}} = 3 \text{ kg COD/kg biomass.day}
\]

\[
K_X = 0.2 \text{ kg COD/kg biomass}
\]

\[
K_S = 0.004 \text{ kg COD/m}^3
\]

\[
Y_{X/S} = 0.2 \text{ biomass/kg COD}
\]

**Solution**

The initial rates can be calculated using Equations 3.8, 3.10 and 3.11, the last without including the \( k_{L_a} \) term, since the rate of oxygen

\[
\]
consumption by the microorganisms is required and not the overall rate of decrease in the oxygen concentration (which depends also on the rate of oxygen supply, i.e. on the $k_{l,a}$).

$$\frac{dS}{dt} \left( \frac{\text{kg COD}}{\text{m}^3 \cdot \text{day}} \right) = -\frac{4 \text{ day}^{-1} \cdot 0.500 \text{ kg COD}}{0.004 \text{ kg COD} \text{ m}^3 + 0.500 \text{ kg COD} \text{ m}^3} \cdot 0.1 \text{ kg biomass} \cdot \frac{\text{kg COD}}{0.2 \text{ kg biomass}}$$

$$= -1.98 \frac{\text{kg COD}}{\text{m}^3 \cdot \text{day}}$$

$$\frac{dX}{dt} \left( \frac{\text{kg biomass}}{\text{m}^3 \cdot \text{day}} \right)$$

$$= \left( \frac{4 \text{ day}^{-1} \cdot 0.500 \text{ kg COD}}{0.004 \text{ kg COD} \text{ m}^3 + 0.500 \text{ kg COD} \text{ m}^3} - 0.1 \text{ day}^{-1} \right) \cdot 0.1 \frac{\text{kg biomass}}{\text{day}}$$

$$= 0.39 \frac{\text{kg biomass}}{\text{m}^3 \cdot \text{day}}$$

$$r_{O_2\text{biomass}} \left( \frac{\text{kg oxygen}}{\text{m}^3 \cdot \text{day}} \right)$$

$$= -\frac{4 \text{ day}^{-1} \cdot 0.500 \text{ kg COD}}{0.004 \text{ kg COD} \text{ m}^3 + 0.500 \text{ kg COD} \text{ m}^3} \cdot 0.1 \frac{\text{kg biomass}}{\text{day}} \cdot \left[ \frac{1}{0.2 \frac{\text{kg biomass}}{\text{kg COD}}} - 1.42 \right]$$

$$= -1.43 \frac{\text{kg oxygen}}{\text{m}^3 \cdot \text{day}}$$
Note that the rate of oxygen consumption could also have been calculated immediately using the COD balance from the rate of substrate removal and biomass formation (converted into COD):

\[
r_{\text{O}_2\text{biomass}} \left( \frac{\text{kg oxygen}}{\text{m}^3\cdot\text{day}} \right) = -\left( 1.98 \frac{\text{kg COD}}{\text{m}^3\cdot\text{day}} - 0.39 \frac{\text{kg biomass}}{\text{m}^3\cdot\text{day}} - 1.42 \frac{\text{kg COD}}{\text{kg biomass}} \right) \\
= -1.43 \frac{\text{kg oxygen}}{\text{m}^3\cdot\text{day}}
\]

**Example 3.2**

A batch reactor is inoculated with only nitrifying microorganisms at a concentration of 20 mg/l. The initial concentration of ammonia is 10 mg N-NH₃/l. Calculate the initial rates of:

- Ammonia removal;
- Nitrate production;
- Net micro-organism production;
- Oxygen consumption

\[\mu_{\text{maxA}} = 0.8 \text{ day}^{-1}\]
\[b_A = 0.05 \text{ day}^{-1}\]
\[K_{SA} = 0.001 \text{ kg N/m}^3\]
\[Y_{X_{\text{A}ANO}_3} = 0.15 \text{ kg biomass/kg N – NO}_3\]

**Solution**

The calculation follows immediately from mass balances and from the stoichiometry and kinetics for nitrifying microorganisms seen in Chapter 2.
The growth rate of nitrifying microorganisms is given by:

\[ r_{XA} = \frac{\mu_{max} \cdot \Delta}{K_{SA} + \Delta} \cdot X_A \]

\[ = \frac{0.8 \text{ day}^{-1} \cdot 0.01 \frac{\text{kg N-NH}_3}{\text{m}^3}}{0.001 \frac{\text{kg N-NH}_3}{\text{m}^3} + 0.01 \frac{\text{kg N-NH}_3}{\text{m}^3}} \cdot 0.02 \frac{\text{kg biomass}}{\text{m}^3} \]

\[ = 0.015 \frac{\text{kg biomass}}{\text{m}^3 \cdot \text{day}} \]

And the net rate of microorganisms production is:

\[ \frac{dX_A}{dt} = r_{XA} - b_A X_A = 0.015 \frac{\text{kg biomass}}{\text{m}^3 \cdot \text{day}} - 0.05 \text{ day}^{-1} \cdot 0.02 \frac{\text{kg biomass}}{\text{m}^3} \]

\[ = 0.014 \frac{\text{kg biomass}}{\text{m}^3 \cdot \text{day}} \]

\[ \frac{d\text{NH}_3}{dt} = -\left( \frac{1}{Y_{XA/NO3}} + 0.12 \right) r_{XA} \]

\[ = -\left( \frac{1 \frac{\text{kg N-NH}_3}{\text{kg biomass}}}{0.15} + 0.12 \right) 0.015 \frac{\text{kg biomass}}{\text{m}^3 \cdot \text{day}} \]

\[ = -0.102 \frac{\text{kg N-NH}_3}{\text{m}^3 \cdot \text{day}} \]
\[
\frac{d\text{NO}_3}{dt} \left( \frac{\text{kg N} - \text{NO}_3}{\text{m}^3 \cdot \text{day}} \right) = \frac{1}{Y_{X\text{A}/\text{NO}_3}} r_{X\text{A}} \\
= \frac{1}{0.15} \frac{\text{kg biomass}}{\text{kg N} - \text{NO}_3} \cdot 0.015 \frac{\text{kg biomass}}{\text{m}^3 \cdot \text{day}} \\
= 0.100 \frac{\text{kg N} - \text{NO}_3}{\text{m}^3 \cdot \text{day}}
\]

\[
\frac{r_{O_2}}{\left( \frac{\text{kg oxygen}}{\text{m}^3 \cdot \text{day}} \right)} \\
= -\left( \frac{4.54}{Y_{X\text{A}/\text{NO}_3}} - 0.04 \right) r_{X\text{A}} \\
= -\left( \frac{4.54}{0.15} \frac{\text{kg oxygen}}{\text{kg N} - \text{NO}_3} - 0.04 \frac{\text{kg oxygen}}{\text{biomass}} \right) \cdot 0.015 \frac{\text{kg biomass}}{\text{m}^3 \cdot \text{day}} \\
= -0.45 \frac{\text{kg oxygen}}{\text{m}^3 \cdot \text{day}}
\]

**Example 3.3**

Calculate the time profiles of substrate, ammonia, biomass and oxygen concentration for a biological reactor fed with glucose as the only carbon source. Assume an initial concentration of glucose equal to 1 g/l and an initial biomass concentration equal to 0.1 g/l. Assume oxygen is transferred with a \( k\text{L} a \) equal to 100 day\(^{-1} \) and the saturation concentration of oxygen in water is 9 mg/l.

Kinetic parameters:

\[
\mu_{\text{max}} = 6 \text{ day}^{-1} \\
b = 0.2 \text{ day}^{-1} \\
Y_{X/S} = 0.3 \text{ kg biomass/kg substrate}
\]
Solution

The solution comes from the integration of Equations 3.3 through 3.6 and is reported in Figure 3.3. Substrate concentration drops at an increasing rate as biomass concentration increases. After the substrate is removed completely, biomass concentration starts to decrease slowly due to endogenous metabolism. Ammonia concentration decreases during biomass growth and increases very slowly during endogenous metabolism because ammonia is released due to biomass decay. The OUR increases during substrate removal because biomass grows and removes the substrate at an increasing rate, and

FIGURE 3.3 Example 3.3. Profiles of substrate, biomass, ammonia, OUR and dissolved oxygen during an aerobic batch test.
then, after the substrate is removed completely from the medium, the OUR falls sharply and it is only due to endogenous metabolism. Correspondingly with the OUR profile, the dissolved oxygen concentration decreases when the substrate is present and increases rapidly when the substrate is removed completely.

3.1.2 Mass Balances in Continuous Reactors

Assume the biological process is carried out in a continuous reactor, such as the one shown in Figure 3.4.

Substrate and ammonia are continuously fed to the reactor at a concentration $S_0$ and $NH_3_0$, and the feed flow rate is $Q$. In the reactor, substrate and ammonia are consumed and biomass is produced. The outlet stream has the same flow rate of the feed, $Q$, and the same composition of the reactor, due to the assumption of perfect mixing. The mass balances have the general form:

\[
\text{Accumulation} = \text{Input} - \text{Output} + \text{Generation} - \text{Consumption}
\]

After the initial startup phase, continuous reactors reach steady state (the accumulation terms becomes equal to 0), and the steady-state mass balance can be written as:

\[
\text{Input} + \text{Generation} = \text{Output} + \text{Consumption}
\]

Assuming glucose as a substrate, we have the following mass balances.

**Biomass:**

There is no biomass in the feed (no input term), biomass is generated by growth and consumed by endogenous metabolism, and is present in the output stream.

![FIGURE 3.4 Scheme of a continuous reactor (no microorganisms in the feed) where microorganisms remove the substrate under aerobic conditions.](image-url)
\[
\frac{d(XV)}{dt} \left( \frac{\text{kg biomass}}{\text{day}} \right) = r_X V + r_{\text{end}} V - Q_X
\]
\[
= \left( \frac{\mu_{\text{max}} S}{K_S + S} - b \right) XV - Q_X
\]

(3.27)

And at steady state, where \( \frac{d(XV)}{dt} = 0 \):
\[
\left( \frac{\mu_{\text{max}} S}{K_S + S} - b \right) XV = Q_X
\]

(3.28)

Substrate:
Substrate is present in the feed and in the output stream, is not generated and is removed by biomass growth.
\[
\frac{d(SV)}{dt} \left( \frac{\text{kg substrate}}{\text{day}} \right) = QS_0 + r_S V - QS = QS_0 - \frac{\mu_{\text{max}} S}{K_S + S} \frac{XV}{Y_{X/S}} - QS
\]

(3.29)

And at steady state:
\[
QS_0 = \frac{\mu_{\text{max}} S}{K_S + S} \frac{XV}{Y_{X/S}} + QS
\]

(3.30)

Ammonia:
Ammonia is present in the feed and in the output stream, is generated by endogenous metabolism and removed by biomass growth.
\[
\frac{d(NH_3 V)}{dt} \left( \frac{\text{kg N}}{\text{day}} \right) = QNH_{30} - QNH_3 - 0.12 \cdot r_{\text{end}} V - 0.12 \cdot r_X V
\]
\[
= QNH_{30} - QNH_3 - \left( \frac{\mu_{\text{max}} S}{K_S + S} - b \right) XV \cdot 0.12
\]

(3.31)

And at steady state:
\[
QNH_{30} = QNH_3 + \left( \frac{\mu_{\text{max}} S}{K_S + S} - b \right) XV \cdot 0.12
\]

(3.32)

Oxygen:
Oxygen is present in the feed, is transferred from the gas phase (input), is not generated, is consumed by the biomass and is present in the output stream.
\[
\frac{d(O_2V)}{dt} \left( \frac{\text{kg oxygen}}{\text{day}} \right) = QC_{O2} \cdot \frac{\mu_{\text{max}} S}{K_S + S} \left( \frac{1}{Y_{X/S}} \right) XV - 1.42bXV + k_l a \left(C_{O2}^* - C_{O2}\right)V - QC_{O2}
\]  
\[\text{(3.33)}\]

And at steady state:

\[
QC_{O2} + k_l a \left(C_{O2}^* - C_{O2}\right)V = \frac{\mu_{\text{max}} S}{K_S + S} \left( \frac{1}{Y_{X/S}} \right) XV + 1.42bXV + QC_{O2}
\]  
\[\text{(3.34)}\]

Assuming the kinetic parameters, the volume of the reactor and the flow rate \(Q\) are known, Equations 3.28, 3.30, 3.32 and 3.34 can be solved simultaneously to calculate the steady state of the biological reactor, i.e. the values of the substrate, biomass, ammonia and oxygen concentration at steady state.

### 3.2 ENTHALPY BALANCES

The general form of enthalpy balances corresponds with the general form of mass balances and can be written as:

\[
\text{Enthalpy accumulated} = \text{Enthalpy in} - \text{Enthalpy out} + \text{Enthalpy added to the system} - \text{Enthalpy removed from the system}
\]
\[\text{(3.35)}\]

#### 3.2.1 Enthalpy Balances for Batch Systems

In a purely batch system, we do not have in and out terms, and therefore, the enthalpy balance can be written as:

\[
\text{Enthalpy accumulated} = \text{Enthalpy added to the system} - \text{Enthalpy removed from the system}
\]
\[\text{(3.36)}\]

However, in biological reactions, we never have a completely batch process because even when we do not have any liquid inlet and outlet streams, we may have an inlet gas stream and we always have an outlet gas stream.

For example, let us consider an aerobic adiabatic batch process, where a carbon source is being removed by microorganisms. The reactor is filled with a liquid phase that contains the substrate, the nitrogen source and the
inoculum of microorganisms. Oxygen is supplied by sparging the reactor with the chosen gas, air or pure oxygen. In this case, even though the process can be considered batch as far as the liquid phase is concerned, we do have an inlet stream, i.e. the gas phase that is used to provide oxygen, and an outlet stream, i.e. the gas phase that contains the carbon dioxide generated by the reaction. Since the process is adiabatic, there is no enthalpy added or removed from the system. In the following, we will ignore, for simplicity, the contribution to the enthalpy balance of the inlet gas stream (the contribution of dissolved oxygen, however, will be included in the enthalpy of the liquid phase). However, it is important to include in the enthalpy balance the contribution of the outlet gas stream because it includes carbon dioxide, which is a product of the reaction. In this example, we will ignore any water vapour that may be present in the outlet gas stream (otherwise, its enthalpy should also be taken into account).

With these assumptions, the generic form of the enthalpy balance is:

\[
\text{Enthalpy accumulated} = - \text{Enthalpy out} \quad (3.37)
\]

Since biological reactions always take place in the water phase and the liquid phase can always be considered a dilute solution with its physical properties equal to the properties of water, the total enthalpy of the liquid phase can be written as \( V \rho H \) where \( H \) (J/kg) is the enthalpy of the liquid phase per unit mass and the density of the liquid phase can be considered constant. The volume of the liquid phase can also be considered constant because there are no inlet or outlet liquid streams and the change in volume due to the biological reactions and to the dissolution of oxygen can be ignored. Therefore, the enthalpy accumulated, i.e. the rate of enthalpy change, is given by \( V \rho (dH/dt) \). The enthalpy leaving the system is due to the carbon dioxide generated by the reaction that leaves the system with the gas phase. For simplicity, here we ignore the solubility of carbon dioxide and assume that all the carbon dioxide generated by the reaction evolves as a gas. Therefore, the term ‘enthalpy out’ can be written as \( r_{CO2} VH_{CO2} \), where \( r_{CO2} \) (kg/m\(^3\).day) is the rate of carbon dioxide generation per unit volume of the biological reactor.

Therefore, with all these assumptions, the enthalpy balance for the biological process is:

\[
V \rho \frac{dH}{dt} = -r_{CO2} VH_{CO2} \quad \Rightarrow \quad \rho \frac{dH}{dt} = -r_{CO2} H_{CO2} \quad (3.38)
\]
$H$ is the enthalpy of the liquid phase per unit mass and can be written as:

$$H = \frac{M_S H_S + M_{O_2} H_{O_2} + M_{NH_3} H_{NH_3} + M_X H_X + M_{H_2O} H_{H_2O}}{M_{tot}}$$ (3.39)

Where $M$ is the mass (kg) of the various substances, the subscript $S$ stands for substrate, $O_2$ for oxygen, $NH_3$ for ammonia, $X$ for biomass and $H_2O$ for water. The mass of the various substances can be expressed as a function of their respective concentrations, $S$, $C_{O_2}$, $C_{NH_3}$, $X$, $C_{H_2O}$ (kg/m$^3$):

$$M_S = S \cdot V; \quad M_{O_2} = C_{O_2} \cdot V; \quad M_{NH_3} = C_{NH_3} \cdot V;$$

$$M_X = X \cdot V; \quad M_{H_2O} = C_{H_2O} V; \quad M_{tot} = \rho V$$ (3.40)

Therefore, the derivative of the specific enthalpy $H$ can be expressed as:

$$\frac{dH}{dt} \left( \frac{J}{\text{kg.day}} \right) = \frac{1}{\rho V} \left[ H_S \frac{dS}{dt} + H_{O_2} \frac{dC_{O_2}}{dt} + H_{NH_3} \frac{dC_{NH_3}}{dt} + H_X \frac{dX}{dt} + H_{H_2O} \frac{dC_{H_2O}}{dt} + S \frac{dH_S}{dt} + C_{O_2} \frac{dH_{O_2}}{dt} + C_{NH_3} \frac{dH_{NH_3}}{dt} + X \frac{dH_X}{dt} + \rho \frac{dH_{H_2O}}{dt} \right]$$ (3.41)

From Chapter 2, assuming that the specific heat is independent on temperature (in the temperature range considered here), the derivative of the specific enthalpy for a generic species $A$ is:
\[
\frac{dH_A}{dt} = c_{pa} \frac{dT}{dt}
\]

And therefore the various terms \(dH_S/dt\), \(dH_{O_2}/dt\) and so on can be written as \(dH_S/dt = c_{ps}(dT/dt)\), \(dH_{O_2}/dt = c_{PO2liq}(dT/dt)\), etc.

Therefore, the derivative of the specific enthalpy of the liquid phase can be rewritten as:

\[
\frac{dH}{dt} \left( \frac{J}{kg\cdot day} \right) = \frac{1}{\rho} \left[ H_S \frac{dS}{dt} + H_{O_2} \frac{dC_{O_2}}{dt} + H_{NH_3} \frac{dC_{NH_3}}{dt} + H_X \frac{dX}{dt} + H_{H_2O} \frac{dC_{H_2O}}{dt} + S_{cps} \frac{dT}{dt} + C_{O_2} c_{PO2} \frac{dT}{dt} + C_{NH_3} c_{PNH_3} \frac{dT}{dt} + X c_{PX} \frac{dT}{dt} + C_{H_2O} c_{PH_2O} \frac{dT}{dt} \right]
\]

This expression can be simplified if we assume that since we are in a dilute solution and water is by far the main component in it:

\[
S_{cps} + C_{O_2} c_{PO2} + C_{NH_3} c_{PNH_3} + X c_{PX} + C_{H_2O} c_{PH_2O} = \rho \cdot c_{PH_2O}
\]

And so we obtain:

\[
\frac{dH}{dt} \left( \frac{J}{kg\cdot day} \right) = \frac{1}{\rho} \left[ H_S \frac{dS}{dt} + H_{O_2} \frac{dC_{O_2}}{dt} + H_{NH_3} \frac{dC_{NH_3}}{dt} + H_X \frac{dX}{dt} + H_{H_2O} \frac{dC_{H_2O}}{dt} + \rho c_{PH_2O} \frac{dT}{dt} \right]
\]

And, therefore, the enthalpy balance for the adiabatic batch system can be rewritten as:

\[
\rho c_{PH_2O} \frac{dT}{dt} = - H_S \frac{dS}{dt} - H_{O_2} \frac{dC_{O_2}}{dt} - H_{NH_3} \frac{dC_{NH_3}}{dt} - H_X \frac{dX}{dt} - H_{H_2O} \frac{dC_{H_2O}}{dt} - r_{CO2} H_{CO2}
\]
In order to calculate the temperature change in the system, we need to be able to calculate the various terms $dS/dt$, $dC_{O_2}/dt$, etc. These terms depend on the rate and stoichiometry of the biological process. If, for example, the substrate is glucose, the enthalpy balance can be written as:

$$
\rho c_p \frac{dT}{dt} = -H_S \frac{-r_X}{Y_{X/S}} - H_{O_2} \left( -r_X \cdot \left( \frac{1.07}{Y_{X/S}} - 1.42 \right) X - 1.42bX + k_1 a (C_{O_2} - C_{O_2}) \right) - H_{NH_3} \left( -0.12 \cdot r_{end} - 0.12 \cdot r_X \right) - H_X \left( r_X + r_{end} \right) - H_{H_2O} \left( \frac{0.6}{Y_{X/S}} - 0.28 \right) r_X - 0.32 r_{end} - H_{CO_2} \left( \frac{1.47}{Y_{X/S}} - 1.95 \right) r_X - 1.95 r_{end}
$$

From Equation 3.47, the change in the reactor temperature as the batch reaction proceeds can be calculated from the rate of biomass growth and oxygen transfer, and from the stoichiometry of the growth reaction and of the endogenous metabolism.

If the batch reactor is not adiabatic, then we need to include the term for the heat transfer to or from the external environment, i.e.:

$$
\text{Enthalpy accumulated} = \text{Enthalpy added to the system} - \text{Enthalpy removed from the system} \quad (3.48)
$$

Let us assume that enthalpy is added or removed from the reactor through a jacket, where a heating or cooling fluid flows. The enthalpy added to (or removed from) the system per unit time depends on the overall heat transfer coefficient, as described in Chapter 2:

$$
\dot{Q} = UA \Delta T
$$

where $\Delta T$ is the temperature difference between the fluid in the reactor and the fluid in the jacket (here we are assuming that the jacket is perfectly mixed, i.e. that the temperature in the jacket is uniform and equal to the outlet temperature of the jacket fluid).
Therefore, referring to the batch process we have considered so far, with the addition of heat addition/removal via the jacket, the enthalpy balance can be written as:

\[ V \rho \frac{dH}{dt} = -r_{\text{CO}_2} VH_{\text{CO}_2} - UA \Delta T \Rightarrow \rho \frac{dH}{dt} = -r_{\text{CO}_2} H_{\text{CO}_2} - \frac{UA(T - T_j)}{V} \]  (3.49)

which becomes, with the assumptions made above:

\[ \rho c_{\text{pH}_2\text{O}} \frac{dT}{dt} = -H_S \frac{dS}{dt} - H_{\text{O}_2} \frac{dC_{\text{O}_2}}{dt} - H_{\text{NH}_3} \frac{dC_{\text{NH}_3}}{dt} \]

\[ -H_X \frac{dX}{dt} - H_{\text{H}_2\text{O}} \frac{dC_{\text{H}_2\text{O}}}{dt} - r_{\text{CO}_2} H_{\text{CO}_2} - \frac{UA(T - T_j)}{V} \]  (3.50)

If we assume, as we have done previously, that the substrate is glucose, the temperature profile in the batch reactor is given by the equation below:

\[ \rho c_p \frac{dT}{dt} = -H_S \frac{-r_X}{Y_{X/S}} \]

\[ -H_{\text{O}_2} \left(-r_X \cdot \left( \frac{1.07}{Y_{X/S}} - 1.42 \right) X - 1.42 b X + k_l a \left( C'_{\text{O}_2} - C_{\text{O}_2} \right) \right) \]

\[ -H_{\text{NH}_3} \left(-0.12 \cdot r_{\text{end}} - 0.12 \cdot r_X \right) - H_X \left( r_X + r_{\text{end}} \right) \]  (3.51)

\[ -H_{\text{H}_2\text{O}} \left( \frac{0.6}{Y_{X/S}} - 0.28 \right) r_X - 0.32 r_{\text{end}} \]

\[ -H_{\text{CO}_2} \left( \frac{1.47}{Y_{X/S}} - 195 \right) r_X - 1.95 r_{\text{end}} \] \[ - \frac{UA(T - T_j)}{V} \]

Assuming that the heat transfer coefficient \( U \) and the geometry of the reactor (\( A \) and \( V \)) are known, this equation can be integrated to calculate the temperature profile in the reactor \( T(t) \) if the temperature in the jacket \( T_j \) is known and constant. In practice, the temperature in the jacket can be maintained constant by using a control loop that adjusts the flow rate of the fluid in the jacket. However, in the general case, the temperature in the jacket can be calculated by means of an enthalpy balance for the fluid in the jacket.
For the fluid in the jacket, we need the general enthalpy balance for a continuous flow system, i.e.:

\[
\text{Enthalpy accumulated} = \text{Enthalpy in} - \text{Enthalpy out} \\
+ \text{Enthalpy added to the system} - \text{Enthalpy removed from the system}
\]  

(3.35)

For the fluid in the jacket, Equation 3.35 can be written as

\[
V_J \rho_J \frac{dH_J}{dt} = w_J c_{pJ} (T_{J\text{IN}} - 25) - w_J c_{pJ} (T_J - 25) + UA (T - T_J)
\]  

(3.52)

Equation 3.52 becomes, assuming that the specific heat for the fluid in the jacket is independent of temperature in the considered temperature range:

\[
\frac{dT_J}{dt} = \frac{w_J c_{pJ} (T_{J\text{IN}} - T_J) + UA (T - T_J)}{c_{pJ} V_J \rho_J}
\]  

(3.53)

This equation gives the temperature profile for the fluid in the jacket and can be integrated together with the enthalpy balance for the fluid in the reactor. In summary, if we have a jacketed batch reactor, the temperature profile in the reactor and in the jacket can be obtained by writing the enthalpy balances both in the reactor and in the jacket. They result in a system of two differential equations in the two unknowns \(T\) and \(T_J\), Equations 3.51 and 3.53, which can be solved with the appropriate initial conditions for the two variables.

**Example 3.4**

a) Calculate the temperature profile for the batch reaction in Example 3.3, assuming adiabatic conditions;

b) Calculate the temperature profile for the same batch reaction, but assuming an initial glucose concentration of 10 g/l. A higher \(k_{L,a}\) is required in this case, so assume a \(k_{L,a}\) value equal to 6000 day\(^{-1}\);

c) For the case with an initial substrate concentration equal to 10 g/l, assume that the reactor is cooled with a jacket with inlet water at a temperature of 10°C. Assume that the reactor has a cylindrical shape, volume of 10 m\(^3\) and a diameter of 2 m. Assume that the jacket has a void space, where water flows, of 3 cm. Assume that the overall heat transfer coefficient is 100 W/m\(^2\)/K. Calculate the flow rate of the cooling fluid that is required to limit the temperature increase of the reactor to 10°C.
Solution

The temperature profiles for cases (a) and (b) are obtained from the integration of the enthalpy balance, Equation 3.47. The only difference between cases (a) and (b) is the initial substrate concentration.

In the enthalpy balances, \( r_X \) and \( r_{\text{end}} \) are given by the usual equations:

\[
r_X = \frac{\mu_{\text{max}} S}{K_s + S} X \quad r_{\text{end}} = -bX
\]

with the parameter values given in Example 3.3.

The first step is to calculate the profiles of biomass, substrate and oxygen with time, and then the corresponding values of \( r_X \) and \( r_{\text{end}} \) at each time step. Since we are assuming that the kinetic parameters are independent of temperature, the profiles are the same as those obtained in Example 3.3.

The next step is to calculate the enthalpy terms \( H_S \), \( H_{O_2} \), and so on. The enthalpies of the various species are expressed by the general formula, in which we need to pay attention to express all the terms as J/kg, instead of J/mol, because the rates are expressed as kg and not mol (note that for the dissolved species and for biomass the specific heat has been taken equal to the value for water):

\[
H_A(T) = \Delta H_{\text{fa}}(25^\circ\text{C}) + \left[ \lambda_{\text{phase change}}(25^\circ\text{C}) \right] + c_{\text{PA}}(T - 25)
\]

For glucose:

\[
H_S(T) = \Delta H_{\text{fGLU}}(25^\circ\text{C}) + \lambda_{\text{dissolutionGLU}}(25^\circ\text{C}) + c_{\text{PH2O}}(T - 25)
\]

\[
= -7.1 \cdot 10^6 + 6.1 \cdot 10^4 + 4186(T - 25)
\]

For oxygen:

\[
H_{O_2}(T) = \Delta H_{\text{fO2}}(25^\circ\text{C}) + \lambda_{\text{dissolutionO2}}(25^\circ\text{C}) + c_{\text{PH2O}}(T - 25)
\]

\[
= 0 - 4.6 \cdot 10^5 + 4186(T - 25)
\]

For ammonia:

\[
H_{O_2}(T) = \Delta H_{\text{fNH3}}(25^\circ\text{C}) + c_{\text{PH2O}}(T - 25)
\]

\[
= -4.8 \cdot 10^6 + 4186(T - 25)
\]
For biomass:

\[ H_x(T) \left( \frac{J}{kg} \right) = \Delta H_{fx}(25^\circ C) + c_{H_2O}(T - 25) \]

\[ = -6.8 \cdot 10^6 + 4186(T - 25) \]

For water:

\[ H_{H_2O}(T) \left( \frac{J}{kg} \right) = \Delta H_{H_2O}(25^\circ C) + c_{H_2O}(T - 25) \]

\[ = -1.6 \cdot 10^7 + 4186(T - 25) \]

For carbon dioxide:

\[ H_{CO_2}(T) \left( \frac{J}{kg} \right) = \Delta H_{CO_2}(25^\circ C) + c_{CO_2gas}(T - 25) \]

\[ = -8.9 \cdot 10^6 + 910(T - 25) \]

After defining the various enthalpy terms, the problem can be solved by numerical integration of Equation 3.47, which can be done easily in Microsoft Excel as described in the Appendices (of course, any other mathematical software can also be used to solve this equation).

The obtained profiles are reported in Figure 3.5.

FIGURE 3.5 Example 3.4. Calculated temperature profiles for a batch reactor during the removal of the substrate.
As expected, the temperature increases during the biological process because biological reactions are exothermic and the temperature increase is much larger for the higher initial substrate concentration. The temperature profiles show a sharp change in slope when the substrate is removed completely. This is due to the fact that the rate of endogenous metabolism is much lower than the growth rate and, therefore, the rate of heat generation is much lower and the temperature increases at a much lower, almost insignificant, rate. The final temperature is below 25°C when the initial substrate concentration is 1 g/l, but it is higher than 45°C when the initial substrate is 10 g/l. In the latter case, cooling is probably required in order to maintain the reactor at the desired temperature, and this is shown in part c).

c) If heat is removed via a jacket, the temperature profile in the reactor is given by Equations 3.51 and 3.53, which need to be solved simultaneously. In order to solve the equations, we need to calculate the area of the jacketed area $A$ and the volume of the jacket $V_J$. We know the volume of the reactor and its diameter, from which we calculate the height:

$$V = \frac{\pi D^2}{4} H \quad \Rightarrow \quad H = \frac{4V}{\pi D^2} = 3.2 \text{ m}$$

Therefore, the jacketed area $A$ is, assuming that all the surface of the reactor is jacketed:

$$A = \frac{\pi D^2}{4} + \pi DH = 23.1 \text{ m}^2$$

And the volume of the jacket can be reasonably approximated by multiplying the jacketed area by the jacket gap:

$$V_J = A \cdot 0.03 = 0.69 \text{ m}^3$$

With these values, Equations 3.51 and 3.53 can be solved for different values of the flow rate of the cooling fluid in the jacket, obtaining the profiles for the reactor temperature shown in Figure 3.6. A flow rate of the cooling fluid of at least 80,000 kg/day is required in order to keep the maximum temperature increase in the reactor to within 10°C.

Note that with a fixed value of the flow rate of the cooling fluid, the reactor temperature will inevitably be variable, because the rate of heat generation changes with time. Therefore, after the substrate is removed entirely, the reactor temperature will start decreasing
because the rate of heat removal will be higher than the rate of heat generation. If we wanted to keep the reactor temperature really constant, we should use a control loop that regulates the cooling fluid flow rate as a function of the reactor temperature. The control loop would increase the flow rate of the cooling fluid when the reactor temperature tends to increase, i.e. when the substrate is removed rapidly, and would decrease it when the rate of heat generation is lower, for example, after the substrate has been removed completely and the only process occurring is endogenous metabolism.

Figure 3.6 also compares the temperature profile in the reactor and in the jacket when the cooling fluid flow rate is 80,000 kg/day.
3.2.2 Enthalpy Balances for Continuous Systems

In a continuous process at steady state, i.e. with no accumulation of enthalpy, the generic enthalpy balance can be written as

\[
\text{Enthalpy in} + \text{Enthalpy added to the system} = \text{Enthalpy out} - \text{Enthalpy removed from the system} \quad (3.54)
\]

If the reactor is adiabatic, there is no heat transfer to or from the external environment, and therefore, the generic form of the heat balance is

\[
\text{Enthalpy in} = \text{Enthalpy out} \quad (3.37)
\]

Let us consider a continuous adiabatic biological reactor where a substrate \( S \) is removed by microorganisms. The feed contains the substrate and ammonia in water, while the effluent of the reactor will contain the residual substrate and ammonia, the biomass, dissolved oxygen and carbon dioxide. Air or pure oxygen is supplied to the reactor to maintain the oxygen concentration in the reactor to the desired value. Similar to what we have done for the enthalpy balances on the batch reactor, we ignore the enthalpy content of the inlet gas, and we assume that all the produced carbon dioxide evolves from the reactor as a gas.

With these assumptions, we have:

\[
\text{Enthalpy in} = Q\left( S_0 H_S(T_{\text{feed}}) + NH_{30} H_{NH3}(T_{\text{feed}}) + H_2OH_{H_2O}(T_{\text{feed}}) \right) \quad (3.55)
\]

and

\[
\text{Enthalpy out} = Q\left( SH_S(T) + NH_3 H_{NH3}(T) + XH_X(T) \right.
\]
\[+ H_2OH_{H_2O}(T) + C_{O2} H_{O2}(T) + r_{CO2} H_{CO2}(T) V \quad (3.56)
\]

Therefore, the enthalpy balance for this system is:

\[
Q\left( S_0 H_S(T_{\text{feed}}) + NH_{30} H_{NH3}(T_{\text{feed}}) + H_2OH_{H_2O}(T_{\text{feed}}) \right)
= Q\left( SH_S(T) + NH_3 H_{NH3}(T) + XH_X(T) \right.
\]
\[+ H_2OH_{H_2O}(T) + C_{O2} H_{O2}(T) + r_{CO2} H_{CO2}(T) V \quad (3.57)
\]

If the concentration of all the variables at steady state is known, Equation 3.57 can be solved to calculate the temperature in a continuous adiabatic biological reactor.
If the reactor is not adiabatic, but heat is exchanged, for example, using a cooling fluid in the jacket, the general form of the enthalpy balance has to be used and the enthalpy balances becomes:

\[
Q \left( S_0 H_5 (T_{\text{feed}}) + NH_3 H_{NH3} (T_{\text{feed}}) + H_2 OH_{H2O} (T_{\text{feed}}) \right) \\
= Q \left( S_0 H_5 (T) + NH_3 H_{NH3} (T) + XH_X (T) + H_2 OH_{H2O} (T) \\
+ CO_2 H_{O2} (T) \right) + r_{CO2} H_{CO2} (T) V + UA (T - T_j)
\] (3.58)

The jacket temperature \( T_j \) can be calculated through an enthalpy balance on the jacket fluid:

\[
\text{Enthalpy in} + \text{Enthalpy added to the system} = \text{Enthalpy out} \quad (3.59)
\]

Equation 3.59 corresponds to

\[
w_\ell c_{p\ell} (T_{\text{IN}} - T_{\text{REF}}) + UA (T - T_j) = w_\ell c_{p\ell} (T_j - T_{\text{REF}}) \quad (3.60)
\]

which becomes

\[
w_\ell c_{p\ell} (T_j - T_{\text{IN}}) = UA (T - T_j) \quad (3.61)
\]

So, assuming the heat transfer area \( A \), the jacket flow rate \( W_j \), the inlet temperature of the cooling fluid \( T_j \) and the heat transfer coefficient \( U \) are known, the steady-state temperatures of the reactor and of the jacket can be calculated by solving the system of the two Equations 3.59 and 3.61 with the two unknowns \( T \) and \( T_j \).

**Example 3.5**

Consider a continuous biological reactor where microorganisms grow aerobically on glucose as the only carbon source. Assume an inlet concentration of glucose of 10 g/l and a mass transfer coefficient for oxygen equal to 6000 day\(^{-1}\). Assume an inlet temperature of the feed of 20°C. In the enthalpy balances, ignore the contribution of dissolved ammonia.

a) Calculate the concentrations of glucose, oxygen and biomass in the reactor and the reactor temperature as function of the residence time, assuming adiabatic operation;
b) Calculate the reactor temperature as a function of the heat transfer coefficient $U$ for a residence time of 0.2 days and assuming the reactor is jacketed, with a flow rate of the cooling fluid of 50,000 kg/day and with inlet temperature of the jacket fluid of 5°C. Assume that the reactor has a volume of 10 m$^3$ and the geometrical dimensions of Example 3.4;

c) Repeat the calculations of part (b) assuming that the reactor volume is 100 m$^3$, with the geometrical dimensions in the same ratio as in Example 3.4. Assume the jacket fluid has a flow rate of 500,000 kg/day.

**Solution**

a) The first step is to calculate the steady-state concentrations of glucose, dissolved oxygen and biomass as a function of the residence time. This can be done by solving the mass balances for glucose, biomass and oxygen as described in Section 3.1. The results are shown in Figure 3.7. Glucose concentration is very close to zero for all the values of the residence time, above a certain minimum value for which there is no removal (and there is washout of the microorganisms). Biomass concentration is zero if the residence time is below the minimum value; it rapidly increases when the minimum residence time is reached and then it slowly decreases, due to endogenous metabolism, as the residence time increases further. The

![FIGURE 3.7 Example 3.5. Steady-state concentrations of substrate, biomass and dissolved oxygen in a continuous biological reactor as a function of the residence time.](image-url)
steady-state concentration of dissolved oxygen increases as the residence time increases above the minimum value because the overall rate of oxygen transfer is proportional to $k_LaV$ and $V$ increases as the residence time increases (while $k_La$ is assumed to remain constant for any value of the reactor volume).

The temperature profile as a function of the residence time can be calculated by solving the steady-state enthalpy balance, Equation 3.57, using the values of the concentration just calculated and the values of the specific enthalpies as a function of the temperature reported in Example 3.4. The obtained temperature profile is shown in Figure 3.8. There is a significant increase in the reactor temperature, which reaches a value higher than 45°C. For all the values of the residence time above the minimum required, the temperature of the reactor is affected only slightly by the residence time. This is due to the fact that when the substrate is removed completely, the heat is generated only by the endogenous metabolism, and this phenomenon has a slow rate.

b) If the reactor is jacketed, the temperatures in the reactor and in the jacket are given by the simultaneous solutions of Equations 3.59 and 3.61. The results are shown in Figure 3.9, as a function of the overall heat transfer coefficient. Clearly, by increasing the heat transfer coefficient, the temperature in the reactor decreases because more heat is removed from the system.

![FIGURE 3.8  Example 3.5. Steady-state temperature profile in a continuous adiabatic biological reactor.](image-url)
c) If the reactor volume is 100 m$^3$, we need to calculate the diameter and height of the reactor taking into account that their ratio is the same as that in the previous case. In that case, the ratio between height and diameter was

$$\frac{H}{D} = \frac{3.2}{2} = 1.6$$

Therefore:

$$V = \frac{\pi D^2}{4} H = \frac{1.6\pi D^3}{4} \Rightarrow D = 4.3 \text{m}, \quad H = 6.8 \text{m}$$

This gives a heat transfer area equal to:

$$A = \frac{\pi D^2}{4} + \pi DH = 106.6 \text{m}^2$$

The enthalpy balances for the reactor and the jacket can now be solved with the new values of $A$, $V$, and $W_j$. The plot of the temperature in the reactor as a function of the heat transfer coefficient $U$ is shown in Figure 3.10. For a given value of $U$, the temperature in the reactor is higher for the larger reactor. This is because the $A/V$ ratio is lower for the larger reactor, and therefore, the heat removal rate per unit volume of reactor decreases as the size increases. This shows that in maximising heat transfer, smaller reactors are better than larger ones.
In order to simulate and design a biological wastewater treatment plant, the values of the kinetic parameters and of the growth yield have to be known. They are usually determined from experimental data.

Parameter fitting to the experimental data can be done using two approaches: linear and nonlinear regression. Linear regression requires linearisation of the model equations, while nonlinear regression requires direct comparison of the nonlinear equation with the experimental data. Regardless of the use of linear or nonlinear regression, the general procedure to determine the model parameters that best fit the experimental data can be summarised as follows:

- Generate a set of experimental data. The experimental data can be obtained in batch or continuous experiments;
- Write the mass balances for the relevant species in the (batch or continuous) experiments. In writing the mass balances, the model parameters, which are so far unknown, will appear;
- Compare the profiles generated by the model with the experimental data. The profiles generated by the model will be dependent on the values of the model parameters. The optimum set of model parameters is the one that gives profiles which are as close to the experimental data as possible;
Experiments aimed at determining the kinetic parameters of biomass growth on a substrate can be done in many different ways and there is no set rule on which way is preferable. In the following, a few typical experiments that can be used to determine the model parameters are described. The data shown in this section are used only to explain the procedures and to show which experimental data can be expected in the various tests, and therefore, they do not represent real experiments.

3.3.1 Estimation of the Endogenous Metabolism Coefficient \( b \) by Batch Tests

Let us assume we want to measure the coefficient \( b \) for heterotrophic microorganisms. The easiest procedure is to take the microorganisms out of the biological reactor and carry out a batch test measuring the OUR in the absence of any external carbon source. In this case, the only contribution to the OUR is endogenous metabolism and the OUR is expressed as:

\[
\text{OUR} \left( \frac{\text{kg} \text{O}_2}{\text{m}^3 \text{day}} \right) = 1.42 b X
\]  

(3.62)

So one very simple approach to measure \( b \) is to measure the OUR of the biomass sample in the absence of external substrate and then calculate \( b \) from:

\[
b \left( \text{day}^{-1} \right) = \frac{\text{OUR}}{1.42 X}
\]  

(3.63)

This procedure requires only one determination of OUR and the measurement of the biomass concentration \( X \). This procedure is very easy to implement, but the main limitation is that the measurement of \( X \) is often not straightforward. Even though \( X \) can be approximated in many cases by the volatile suspended solids (VSS), it is important to observe that VSS might give an overestimation of \( X \), since not all the VSS are necessarily composed of active biomass.

A more rigorous method to measure \( b \) is to measure the OUR profile over time, starting from a condition where no external substrate is present. This method does not require any information on the initial biomass concentration. The OUR profile during this extended-time experiment is still given by Equation 3.62, but in this case, the biomass concentration during the test decreases according to the equation:

\[
\frac{dX}{dt} = -bX
\]  

(3.64)
which becomes:

$$X = X_0 e^{-bt}$$  \hspace{1cm} (3.65)

And by substituting it in Equation 3.62, we obtain:

$$\text{OUR} = 1.42bX_0 e^{-bt}$$  \hspace{1cm} (3.66)

If we want to use the linear-regression procedure, we have to linearise Equation 3.66. Since the initial OUR, $\text{OUR}_0$, is given by:

$$\text{OUR}_0 = 1.42bX_0$$  \hspace{1cm} (3.67)

we have:

$$\text{OUR} = \text{OUR}_0 e^{-bt}$$  \hspace{1cm} (3.68)

and:

$$\ln(\text{OUR}) = \ln(\text{OUR}_0) - bt$$  \hspace{1cm} (3.69)

Therefore, according to Equation 3.69, if we plot $\ln(\text{OUR})$ versus time, we should obtain a straight line with a negative slope, from which we obtain $b$. Figure 3.11 shows a typical OUR plot and the linearisation procedure to obtain $b$. The value of $b$ obtained in this case is 0.2019 day$^{-1}$.

Alternatively, Equation 3.66 can be used in a nonlinear-regression procedure. According to this procedure, the OUR is calculated as a function of time for given values of the parameters $b$ and $X_0$. The calculated OUR values are compared with the experimental data and the optimum values of the parameters $b$ and $X_0$ are those which minimise the difference between the model and the experimental data. Figure 3.12 shows the plot obtained with the nonlinear-regression procedure. The nonlinear procedure gives an optimum value of $b$ equal to 0.203 day$^{-1}$, which is almost identical to the $b$ value calculated with the linear-regression procedure. However, in general, the values obtained with the linear and nonlinear regression will be different, even though usually not largely.

### 3.3.2 Estimation of Kinetic Parameters on a Readily Biodegradable Substrate by Batch Tests

In this section, two typical procedures to measure the kinetic parameters for biomass growth on a readily biodegradable substrate are shown. The procedures are quite similar and are both based on the measurement of the dissolved oxygen concentration.
FIGURE 3.11 OUR profiles in the absence of external substrate for the determination of parameter $b$. Top: OUR data. Bottom: linearization of $\ln(\text{OUR})$ to calculate $b$.

FIGURE 3.12 Fitting of the OUR profile using the nonlinear regression for the calculation of the parameter $b$. OUR data are the same as in Figure 3.11.
3.3.2.1 Intermittent Aeration Procedure

The experimental procedure can be summarised as follows:

1. Biomass acclimated to the substrate is used, for example, the biomass may be taken from a continuous reactor where the same substrate was used in the feed;

2. Biomass is initially aerated, using diffusers, in the absence of any carbon source;

3. The readily biodegradable substrate is spiked in the reactor at a known concentration. Aeration with diffusers continues until the end of the test.

During the whole length of the test, the OUR is measured at regular intervals, both before and after the addition of the substrate, until the substrate is removed completely from the liquid phase. In order to measure the OUR, aeration is stopped at regular intervals. When aeration is stopped, the oxygen concentration decreases and the slope of the decrease is measured. During this phase, when the oxygen concentration is decreasing, there is typically still some mass transfer of oxygen from the atmosphere to the liquid phase. Therefore, even when the aeration with diffusers is interrupted, the $k_{L}a$ for the oxygen transfer from the gas phase to the liquid phase will not be zero, although it will be much lower than when the aeration is on with the diffusers. Therefore, the rate of oxygen consumption by the microorganisms, OUR, when aeration with diffusers is off, is given by:

$$\text{OUR} = \text{slope} + k_{L}a \cdot \left( C_{O_{2}}^\ast - C_{O_{2}} \right)$$  \hspace{1cm} (3.70)$$

Where ‘slope’ is the slope of the oxygen concentration versus time curve when aeration with diffusers is off, $k_{L}a$ is the mass transfer coefficient for oxygen when aeration with diffusers is off, $C_{O_{2}}^\ast$ is the saturation concentration of oxygen and $C_{O_{2}}$ is the concentration of oxygen in the liquid phase when aeration is off. Obviously $C_{O_{2}}$ is decreasing when aeration is off, but a good approximation is to use a constant value equal to the average value of the oxygen concentration during the period when aeration is off. In a good experimental setup, the term $k_{L}a \cdot \left( C_{O_{2}}^\ast - C_{O_{2}} \right)$ is much lower than the ‘slope’ term, and may be neglected in some cases.

When enough points have been collected to calculate the slope, and therefore, the OUR, aeration with diffusers is started again, so that the
oxygen concentration comes back up. This procedure of setting the aeration on and off is repeated at regular intervals during the test so that the evolution of the OUR versus time can be obtained.

A typical profile obtained in these tests is shown in Figure 3.13. Before the addition of the external substrate, the OUR is very low because it is only due to the endogenous metabolism. Immediately after the addition of the external substrate, the OUR shows a sharp increase and then increases it further due to growth on the substrate. When the substrate is removed completely, the OUR shows a sharp drop because the only metabolism is again endogenous metabolism.

In order to obtain the model parameters from the experimental data, we need to write the equations that describe the OUR evolution versus time as a function of the model parameters. This equation is:

\[
\text{OUR} \left( \frac{\text{kg oxygen}}{\text{m}^3 \cdot \text{day}} \right) = \mu_{\text{max}} \frac{S}{K_S + S} \cdot X \cdot \left( \frac{1}{Y_{X/S}} - 1.42 \right) + 1.42bX
\]

In order to determine the values of the parameters \(\mu_{\text{max}}, K_S, b, Y_{X/S}\), Equation 3.12 needs to be compared with the experimental data, trying to find a set of parameters that minimises the difference between the model and the data. This can be done using either linear or nonlinear regression. An important observation is that the value of the parameter \(K_S\) for readily biodegradable substrates is usually very difficult to determine with these tests and indeed with any types of tests. The reason for this is that \(K_S\) for

![FIGURE 3.13 Typical OUR profile in a batch test with readily biodegradable substrate.](image-url)
readily biodegradable substrates is usually very low and is usually \(<<S\) during most of the respirometric tests. Therefore, \(K_S\) can be usually taken from the literature, for example, a reasonable value can be assumed as 0.004 kg COD/m\(^3\).

The linear-regression procedure is presented first. A limitation of the linear procedure is that it only allows for the determination of the parameter \(\mu_{\text{max}} - b\), from which the value of \(\mu_{\text{max}}\) can be calculated if the value of \(b\) is known. Assuming that the substrate is in excess, i.e. \(S >> K_S\), Equation 3.12 can be rewritten as:

\[
\text{OUR} \left( \frac{\text{kg oxygen}}{\text{m}^3 \cdot \text{day}} \right) = \mu_{\text{max}} \cdot X \cdot \left( \frac{1}{Y_{X/S}} - 1.42 \right) + 1.42bX
\]

\[
= \left[ \mu_{\text{max}} \left( \frac{1}{Y_{X/S}} - 1.42 \right) + 1.42b \right] X
\]

(3.71)

The biomass concentration during the test can be expressed as:

\[
\frac{dX}{dt} = (\mu_{\text{max}} - b) X \Rightarrow X = X_0 e^{(\mu_{\text{max}} - b)t}
\]

(3.72)

where \(X_0\) is the biomass concentration at the time the external substrate is added, which, for the linear-regression procedure, is considered equal to time 0. Combining Equations 3.71 and 3.72, we obtain:

\[
\text{OUR} = \left[ \mu_{\text{max}} \left( \frac{1}{Y_{X/S}} - 1.42 \right) + 1.42b \right] X_0 e^{(\mu_{\text{max}} - b)t}
\]

(3.73)

At the time of the addition of the substrate, the value of OUR is \(\text{OUR}_0\), given by:

\[
\text{OUR}_0 = \left[ \mu_{\text{max}} \left( \frac{1}{Y_{X/S}} - 1.42 \right) + 1.42b \right] X_0
\]

(3.74)

Therefore, we have:

\[
\frac{\text{OUR}}{\text{OUR}_0} = e^{(\mu_{\text{max}} - b)t} \Rightarrow \ln(\text{OUR}) = \ln(\text{OUR}_0) + (\mu_{\text{max}} - b) \cdot t
\]

(3.75)

Therefore, a plot of \(\ln(\text{OUR})\) versus time should give a straight line with a slope equal to \(\mu_{\text{max}} - b\). The linearisation of the experimental data of Figure 3.13 is shown in Figure 3.14.
From the slope of the regression line, we obtain:

\[ \mu_{\text{max}} - b = 7.1 \text{ day}^{-1} \]

The linear procedure is simple and only requires measurement of the OUR after the external substrate addition. It does not require any information on the endogenous metabolism, or on the initial biomass or substrate concentration. However, the main limitation of the linear-regression procedure is that it only allows determination of the difference \( \mu_{\text{max}} - b \).

If all the parameters that describe microbial growth need to be determined from an experiment such as the one described above, then the nonlinear-regression procedure needs to be used. The nonlinear procedure uses all the data from the experiment and allows the simultaneous estimation of the parameters \( \mu_{\text{max}}, b, \) and \( Y \). However, the nonlinear procedure requires knowledge of the initial values of the substrate and the biomass concentration. If not known, the initial biomass concentration can be treated as an additional parameter and estimated on the experimental data, but this would add some additional uncertainty to the fitting procedure.

In using the nonlinear procedure, the OUR simulated by the model (Equation 3.12), needs to be calculated over the whole length of the test, from before the endogenous phase to the end of the test. Obviously during the test, the substrate and biomass concentration, which determine the OUR values, change. Therefore, the values of the variables \( S \) and \( X \) in Equation 3.12 need to be calculated as a function of time during the OUR test. The variables \( S \) and \( X \) during the experiment can be calculated by
integrating the respective mass balance Equations 3.8 and 3.10 (note that in Equation 3.8, the term due to the slowly biodegradable substrate will be ignored in this case). Integration of differential equations is shown in Appendix B.

The results of the fitting with the nonlinear procedure are shown in Figure 3.15. In the fitting, the following values of the initial substrate and biomass concentrations were used: $S_0 = 0.3 \text{ kgCOD/m}^3$ and $X_0 = 0.05 \text{ kg biomass/m}^3$. The obtained values of the parameters are reported below:

$$\mu_{\text{max}} = 8.1 \text{ day}^{-1}$$

$$b = 0.2 \text{ day}^{-1}$$

$$Y_{X/S} = 0.28 \text{ kg biom/kgCOD}$$

It is worth noting that the values of the difference $\mu_{\text{max}} - b$ are similar but not the same as the value obtained on the same data with the linear procedure.

3.3.2.2 Continuous Aeration Procedure

Similarly to the intermittent aeration procedure, this procedure is based on the measurement of dissolved oxygen and requires a biomass that is already acclimated to the readily biodegradable substrate(s). The micro-organisms are put in the reactor in the absence of the external substrate and aerated
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continuously, either by means of diffusers or by mechanical aeration. The concentration of dissolved oxygen is measured continuously for the whole length of the test. We assume that $k_La$ and the concentration of oxygen at saturation are both known. The initial biomass concentration is also known (e.g. from a measurement of VSS). Initially, the oxygen concentration will be slightly lower than the saturation value because the microorganisms are consuming oxygen due to endogenous metabolism. After having recorded the initial concentration of oxygen for a short period of time (typically a few minutes), the external substrate is spiked at a known concentration. After the spiking of the external substrate, the oxygen concentration starts decreasing because OUR by the microorganisms increases. When the substrate is removed entirely, the oxygen concentration will rise again because of the decrease in the microbial OUR. Figure 3.16 shows a typical oxygen profile obtained from this procedure.

The model parameters can be calculated from the dissolved oxygen profile using nonlinear regression. The equations that describe the microorganisms’ substrate and oxygen profiles during this test are Equations 3.8 (ignoring the slowly biodegradable substrate) and (3.10 and 3.11). The equations need to be integrated to calculate the profiles of $X$, $S$ and $O_2$. These profiles, and in particular the $O_2$ profile, depend on the values of the parameters $b$, $\mu_{\text{max}}$, $K_S$ and $Y_{X/S}$. The optimum values of the parameters are the ones that make the $O_2$ curve as close to the experimental data as possible. As discussed for the intermittent aeration procedure, the value of $K_S$ for readily biodegradable substrates is usually very low and very difficult

![Dissolved oxygen profile](image)

**FIGURE 3.16** Example of the dissolved oxygen profile that can be obtained with the continuous aeration procedure.
to determine, and therefore, it can often be assumed from the literature, for example, \( K_S = 0.004 \) kgCOD/m\(^3\).

Figure 3.17 shows an example of fitting the model to the dissolved oxygen profile of Figure 3.9. In this case, the results of the fitting were \( \mu_{\text{max}} = 5.08 \) day\(^{-1}\), \( Y_{X/S} = 0.30 \) kg biomass/kg COD and \( b = 0.23 \) day\(^{-1}\). \( K_S \) was fixed at 0.004 kgCOD/m\(^3\) and the initial biomass concentration was 0.1 kg biomass/m\(^3\).

3.3.3 Estimation of Kinetic Parameters with a Real Wastewater by Batch Tests

In case the substrate is made of a real wastewater, in general, it can be assumed that the substrate will be composed of both readily and slowly biodegradable substrates. In this case, therefore, the kinetic model will have to include, in addition to growth and endogenous metabolism, also the hydrolysis of the slowly biodegradable substrates. The equations that describe the kinetics have been reported in Section 3.1, and the parameters that have to be determined are \( k_h \), \( K_x \), \( Y_{X/S} \), \( \mu_{\text{max}} \) and \( b \). The parameter \( K_S \) can be estimated based on the literature. Similar to what was shown in the previous sections, OUR data can be used to estimate the parameter values, but since the number of parameters to be fitted is quite large, one single experiment might not be enough for a reliable estimation of all the parameters. Better results and more reliable estimations are obtained if multiple experiments are run under different conditions, with the same type of biomass and of wastewater, and are fitted with the same parameters.
Figure 3.18 shows typical OUR profiles that can be expected in experiments with a real wastewater. The tests represent typical results for high and low initial biomass concentration, or, better, for high and low $X_0/S_0$ ratio, since it is the ratio between the initial concentrations of biomass and substrate that determines the shape of the OUR curve. Note that it is possible to observe a significant increase in the OUR only in the test at low initial $X_0/S_0$, because if the $X_0/S_0$ ratio is high, there is not enough substrate to observe an appreciable biomass growth. In Figure 3.18, the presence of a slowly biodegradable COD fraction is shown by the fact that the OUR drops more gradually than in the case of purely readily biodegradable COD. This can be seen by comparing the final part of the OUR

![Graph showing typical OUR profiles with real wastewater with two different values of the initial biomass concentration, top: high (0.4 g/l) and bottom: low (0.04 g/l). Initial substrate concentration in both tests: 200 mgCOD/l.](image)
profiles in Figure 3.18 with the final part of the OUR profile in Figure 3.13, where the substrate was entirely readily biodegradable).

In order to find the values of the parameters that best fit the experimental data, Equations 3.7, 3.8 and 3.10 need to be integrated and the resulting OUR values need to be calculated from Equation 3.12. Then, the values of the parameters need to be adjusted to fit the experimental data, using nonlinear regression, as discussed in Appendix C. Note that in this type of tests, an important parameter that needs to be determined on the basis of the experimental data is the fraction of the influent COD that is slowly biodegradable \( (X_{S0}) \). We assume that the total COD of the wastewater at the start of the test is known \( (X_{S0} + S_0) \), so that either \( X_{S0} \) or \( S_0 \) can be adjusted to fit the experimental data and the other one can be calculated as the difference. By using nonlinear regression, the following values of the parameters have been obtained and the model results are compared with the experimental data in Figure 3.19:

\[
\mu_{\text{max}} = 4.8 \text{ day}^{-1} \\
b = 0.3 \text{ day}^{-1} \\
k_h = 4.5 \text{ kgCOD/kg biomass/day} \\
K_x = 0.088 \text{ kg COD/kg biomass} \\
Y_{X/S} = 0.32 \text{ kg biomass/kg glucose}
\]

### 3.3.4 Estimation of Kinetic Parameters on Readily Biodegradable Substrates by Continuous Reactors

All the previous examples use batch tests to determine the model parameters. Instead of batch tests, continuous reactors can also be used. The method described in this section allows the calculation of \( \mu_{\text{max}} \), \( K_s \), and \( Y_{X/S} \) using data from various runs of a continuous reactor, with measurement of residual substrate concentration and biomass concentration at steady state. For example, consider the determination of kinetic parameters for a readily biodegradable substrate. We can feed this substrate to a continuous reactor without recycle where biomass grows and the substrate is removed. At steady state, the mass balances for substrate and biomass are shown below:

\[
QS_0 = \frac{\mu_{\text{max}} S X}{K_s + S Y_{X/S}} V + QS
\]
We define the dilution rate as:

\[ D(\text{day}^{-1}) = \frac{Q}{V} \]  \hspace{1cm} (3.75)

The biomass balance can, therefore, be rewritten as:

\[ \frac{\mu_{\text{max}} S}{K_S + S} = D + b \quad \Rightarrow \quad \frac{1}{S} = \frac{1}{D + b} - \frac{K_S}{\mu_{\text{max}}} \]  \hspace{1cm} (3.76)
and substituting into the substrate balance, the substrate balance can be rearranged as:

\[
\frac{(S_0 - S)}{X} = \frac{1}{Y_{X/S}} + \frac{b}{Y_{X/S}} \frac{1}{D}
\]  

(3.77)

Therefore, in order to obtain the model parameters from chemostat experiments, a series of runs at different residence time, i.e. different dilution rate \( D \), needs to be carried out and the steady state has to be achieved in all the runs. The values of the substrate \((S)\) and biomass concentration \((X)\) in the reactor at steady state need to be measured. Then the following plots need to be generated:

\[
\frac{(S_0 - S)}{X} \text{ vs } \frac{1}{D}
\]

(3.78)

\[
\frac{1}{S} \text{ vs } \frac{1}{D + b}
\]

(3.79)

The first plot will give the values of \( b \) and \( Y_{X/S} \) from Equation 3.77, while once \( b \) is known, the second plot will give the values of \( \mu_{\text{max}} \) and \( K_s \) from Equation 3.76.

The procedure is illustrated in Figures 3.20 through 3.22. Figure 3.20 shows typical profiles of biomass and substrate in a chemostat experiment at different dilution rates. Figure 3.21 shows the linearised plots according to Equation 3.77 and Figure 3.22 shows the linearisation of Equation 3.76. From Figure 3.21, the following values of the parameters can be calculated:

\[
Y_{X/S} = 0.196 \frac{\text{kg biomass}}{\text{kg COD}}
\]

\[
b = 0.295 \text{ day}^{-1}
\]

And from Figure 3.22:

\[
\mu_{\text{max}} = 4.57 \text{ day}^{-1}
\]

\[
K_s = 0.0026 \frac{\text{kg COD}}{\text{m}^3}
\]

3.3.5 Estimation of Kinetic Parameters under Anoxic Conditions

The determination, or estimation, of the parameters \( \mu_{\text{max}}, K_s, b \) and \( Y_{X/S} \) under anoxic conditions, i.e. when nitrate is used, instead of oxygen, as electron acceptor, can be done using experimental procedures that are
absolutely analogous to the ones described in previous sections for aerobic experiments. The only difference is that the experiments have to be designed and carried out so that there is no oxygen available for the microorganisms, which will then use nitrate. Therefore, as for aerobic experiments, the kinetic parameters can be determined by measuring only the OUR, under anoxic conditions, the parameters can be determined just from the nitrate profile.

For example, Figure 3.23 shows the use of nonlinear regression to estimate the value of parameter $b$ from the nitrate profile in an experiment.
with microorganisms and no external substrate addition. The nitrate profile in Figure 3.23 is described by Equation 3.13, with \( S = 0 \), and by doing nonlinear regression using this equation, we obtain:

\[
b = 0.21 \text{ day}^{-1}
\]

Figure 3.24 shows the nitrate profile in a typical experiment with external substrate and the comparison with the best-fit model, given by Equation 3.13. In this case, the fitted parameters have been \( \mu_{\text{max}} \) and \( Y_{X/S} \). The value of \( b \) has been taken equal to the value obtained from Figure 3.23 (\( b = 0.21 \text{ day}^{-1} \))
and the value of $K_s$ has been assumed to be 0.004 kgCOD/m$^3$. In this case, the values of the parameters obtained are:

$$\mu_{\text{max}} = 4.9 \text{ day}^{-1}$$

$$Y_{X/S} = 0.44 \frac{\text{kg biomass}}{\text{kg COD}}$$

3.3.6 Estimation of Kinetic Parameters for Anaerobic Microorganisms

The estimation of parameters under anaerobic conditions is usually more complicated than under aerobic conditions because the anaerobic degradation of the organic matter requires the coexistence of various
microbial populations, each of them with its own kinetic parameters to be determined. In principle, however, parameter estimation under anaerobic conditions can be done using the same tools as under aerobic conditions, using nonlinear regression to find the values of the parameters that make the model to correspond as close to the experimental data as possible. For example, let us consider the simplest case where the substrate is glucose, and there are three populations of microorganisms: fermentative, which convert glucose into acetic acid and hydrogen, and acetoclastic methanogens and hydrogenotrophic methanogens, which convert acetic acid and hydrogen, respectively, into methane. Assuming we do a batch test, by spiking a certain concentration of glucose at time 0, we should be able to measure the following variables during the test: glucose and acetic acid in the liquid phase, and hydrogen and methane produced. The amount of hydrogen and methane produced can be calculated by measuring the volume of gas produced and the composition of the liquid phase. Figure 3.25 shows the typical profiles that are to be expected in this type of test.

In order to do the parameter fitting, we have to consider that the mass balances for this system are described by Equations 3.14–3.26. From these equations, the profiles of glucose, acetic acid, and produced hydrogen and methane can be calculated. Then, the optimum values of the kinetic parameters can be estimated as the values that make the model predictions to correspond as close to the experimental data as possible. For example,
with the data in Figure 3.25, the best-fit model is shown in Figure 3.26. The calculated results in Figure 3.26 have been obtained by using fixed values, from the literature, of the following parameters:

\[
K_{SGU} = 0.02 \text{ kg/m}^3
\]

\[
b_{GLU} = 0.3 \text{ day}^{-1}
\]

\[
K_{SAC} = 0.14 \text{ kg/m}^3
\]

\[
b_{AC} = 0.2 \text{ day}^{-1}
\]

\[
K_{SH2} = 1.6E-5 \text{ kg/m}^3
\]

\[
b_{H2} = 0.3 \text{ day}^{-1}
\]

The values of the following parameters have been adjusted to fit the experimental data:

\[
\mu_{maxGLU} = 2.46 \text{ day}^{-1}
\]

\[
Y_{X/SGU} = 0.1 \text{ kg } X_{GLU} / \text{ kg GLU}
\]
Note the large number of parameters that have been necessary to fit. In particular, it has been necessary also to fit the initial concentrations of the microorganisms involved, $X_{0GLU}$, $X_{0Ac}$ and $X_{0H2}$. An important limitation of this procedure, common to many other cases of nonlinear regression, is that similar values of the calculated profiles, in agreement with the experimental data, can be calculated with different combinations of parameters. In this case, for example, it is likely that different combinations of $\mu_{max}$, $Y_{X/S}$ and $X_0$ may give equally good fitting of the experimental data. If this is the case, in order to have a more robust fitting, it is recommended to run multiple tests, under different conditions (e.g. different substrate/biomass ratios) and then simultaneously fit all the data with the same set of parameters.

3.4 KEY POINTS

- Mass balances have the general form:
  \[
  \text{Accumulation} = \text{input} - \text{output} + \text{generation} - \text{consumption};
  \]

- We can write mass balances for each of the relevant species in biological wastewater treatment processes, for example, substrate, ammonia, oxygen and biomass. In writing mass balances, it is important to decide which units to use, for example, whether to express the...
carbon source as substrate or as COD, and be consistent in their use. Also, in writing mass balances, it is important to specify the type of system we are considering, for example, whether it is a batch reactor or a continuous-flow reactor;

- Enthalpy balances have the general form;
- Enthalpy accumulated = Enthalpy in – Enthalpy out + Enthalpy added to the system – Enthalpy removed from the system;
- In writing the enthalpy balances, the specific enthalpies (as \( \text{J/mol} \) or \( \text{J/kg} \)) of all the species entering and leaving the system need to be considered. In enthalpy balances, we need to consider whether the system is batch or continuous and whether it is adiabatic or there is heat transfer with the environment or with an external cooling medium;
- The values of the parameters in the kinetic models are usually to be determined from experimental data or to be taken from the literature (where somebody has determined them for us);
- In the experimental determination of the model parameters, the general procedure is the following: with reference to the experimental setup, write the mass balances for the species of interest using the kinetic models with the unknown parameters; carry out the experiment(s), measuring at least some of the variables that appear in the mass balances; compare the values of the variables simulated by the model with their experimental values, determining the values of the model parameters that minimise the difference between the simulated and the experimental data. In order to minimise the uncertainty in parameter estimation, it is recommended that multiple tests be carried out under the same or different conditions and that all the tests be fitted with the same set of parameters.

Questions and Problems

3.1 A perfectly mixed aerobic continuous reactor without recycle (chemostat) is fed with wastewater with a COD concentration of 300 mgCOD/l, at a flow rate of 100 ml/hr. The COD is entirely soluble and the only processes occurring are microbial growth and endogenous metabolism. It can be assumed that the products of
endogenous metabolism are only carbon dioxide and water, with no generation of inert products. The effluent of the reactor has a soluble COD of 20 mgCOD/l and a concentration of microorganisms of 50 mg/l. What is the oxygen consumption rate (g/day) in the reactor?

3.2 Consider the reactor of problem 3.1. It is desired to run the reactor with the same wastewater but using nitrate, instead of oxygen, as electron acceptor. To do so, the reactor is sealed to prevent air from coming in, and sodium nitrate (NaNO₃) is added to the feed. Assuming that the growth yield on the COD while using nitrate is the same as that while using oxygen, what is the concentration of sodium nitrate which needs to be added to the feed?