6

Cell Kinetics and Fermenter Design

6.1 INTRODUCTION

Understanding the growth kinetics of microbial, animal, or plant cells is important for the design and operation of fermentation systems employing them. Cell kinetics deals with the rate of cell growth and how it is affected by various chemical and physical conditions.

Unlike enzyme kinetics discussed in Chapter 2, cell kinetics is the result of numerous complicated networks of biochemical and chemical reactions and transport phenomena, which involves multiple phases and multicomponent systems. During the course of growth, the heterogeneous mixture of young and old cells is continuously changing and adapting itself in the media environment which is also continuously changing in physical and chemical conditions. As a result, accurate mathematical modeling of growth kinetics is impossible to achieve. Even with such a realistic model, this approach is usually useless because the model may contain many parameters which are impossible to determine.

Therefore, we must make assumptions to be able to arrive at simple models which are useful for fermenter design and performance predictions. Various models can be developed based on the assumptions concerning cell components and population as shown in Table 6.1 (Tsuchiya et al., 1966). The simplest model is the unstructured, distributed model which is based on the following two assumptions:

1. Cells can be represented by a single component, such as cell mass, cell number, or the concentration of protein, DNA, or RNA. This is true for balanced growth, since a doubling of cell mass for balanced growth is accompanied by a doubling of all other measurable properties of the cell population.

2. The population of cellular mass is distributed uniformly throughout the culture. The cell suspension can be regarded as a homogeneous solution. The heterogeneous nature of cells can be ignored. The cell concentration can be expressed as dry weight per unit volume.
Table 6.1 Various Models for Cell Kinetics

<table>
<thead>
<tr>
<th>Population</th>
<th>Cell Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distributed</td>
<td>Cells are represented by a single component, which is uniformly distributed throughout the culture</td>
</tr>
<tr>
<td>Segregated</td>
<td>Cells are represented by a single component, but they form a heterogeneous mixture</td>
</tr>
<tr>
<td></td>
<td>Multiple cell components, uniformly distributed throughout the culture interact with each other</td>
</tr>
<tr>
<td></td>
<td>Cells are composed of multiple components and form a heterogeneous mixture</td>
</tr>
</tbody>
</table>

Besides the assumptions for the cells, the medium is formulated so that only one component may be limiting the reaction rate. All other components are present at sufficiently high concentrations, so that minor changes do not significantly affect the reaction rate. Fermenters are also controlled so that environmental parameters such as pH, temperature, and dissolved oxygen concentration are maintained at a constant level.

In this chapter, cell kinetic equations are derived from the unstructured, distributed model, and those equations are applied for the analysis and design of ideal fermenters. More realistic models which consider the multiplicity of cell components, structured model, are introduced at the end of the chapter.

6.2 DEFINITIONS

First, let us define the terminologies we use for microbial growth. If we mention the cell concentration without any specification, it can have many different meanings. It can be the number of cells, the wet cell weight, or the dry cell weight per unit volume. In this text, the following nomenclature is adopted:

- $C_X$: Cell concentration, dry cell weight per unit volume
- $C_N$: Cell number density, number of cells per unit volume
- $\rho$: Cell density, wet cell weight per unit volume of cell mass

Accordingly, the growth rate can be defined in several different ways, such as:

- $\frac{dC_X}{dt}$: Change of dry cell concentration with time
- $r_X$: Growth rate of cells on a dry weight basis
- $\frac{dC_N}{dt}$: Change of cell number density with time and growth rate of cells on a number basis
- $\delta$: Division rate of cells on a number basis, $d \log_2 C_N / dt$
It appears that $dC_x/dt$ and $r_x$ are always the same, but this is not true. The former is the change of the cell concentration in a fermenter, which may include the effect of the input and output flow rates, cell recycling, and other operating conditions of a fermenter. The latter is the actual growth rate of the cells. The two quantities are the same only for batch operation.

The growth rate based on the number of cells and that based on cell weight are not necessarily the same because the average size of the cells may vary considerably from one phase to another. When the mass of an individual cell increases without division, the growth rate based on cell weight increases, while that based on the number of cells stays the same. However, during the exponential growth period, which is the phase that we are most interested in from an engineer’s point of view, the growth rate based on the cell number and that based on cell weight can be assumed to be proportional to each other.

Sometimes, the growth rate can be confused with the division rate, which is defined as the rate of cell division per unit time. If all of the cells in a vessel at time $t = 0$ ($C_N = C_{N_0}$) have divided once after a certain period of time, the cell population will have increased to $C_{N_0} \times 2$. If cells are divided $n$ times after the time $t$, the total number of cells will be

$$C_N = C_{N_0} \times 2^N \quad (6.1)$$

and the average division rate is

$$\bar{\delta} = \frac{n}{t} \quad (6.2)$$

Since $N = \log_2 C_N - \log_2 C_{N_0}$ according to Eq. (6.1), the average division rate is

$$\bar{\delta} = \frac{1}{t} \left( \log_2 C_N - \log_2 C_{N_0} \right) \quad (6.3)$$

and the division rate at time $t$ is

$$\delta = \frac{d \log_2 C_N}{dt} \quad (6.4)$$

Therefore, the growth rate defined as the change of cell number with time is the slope of the $C_N$ versus $t$ curve, while the division rate is the slope of the $\log_2 C_N$ versus $t$ curve. As explained later, the division rate is constant during the exponential growth period, while the growth rate is not. Therefore, these two terms should not be confused with each other.

### 6.3 GROWTH CYCLE FOR BATCH CULTIVATION

If you inoculate unicellular microorganisms into a fresh sterilized medium and measure the cell number density with respect to time
and plot it, you may find that there are six phases of growth and death, as shown in Figure 6.1. They are:

1. **Lag phase**: A period of time when the change of cell number is zero.

2. **Accelerated growth phase**: The cell number starts to increase and the division rate increases to reach a maximum.

3. **Exponential growth phase**: The cell number increases exponentially as the cells start to divide. The growth rate is increasing during this phase, but the division rate which is proportional to $\frac{d\ln C_N}{dt}$, is constant at its maximum value, as illustrated in Figure 6.1.

4. **Decelerated growth phase**: After the growth rate reaches a maximum, it is followed by the deceleration of both growth rate and the division rate.

5. **Stationary phase**: The cell population will reach a maximum value and will not increase any further.

6. **Death phase**: After nutrients available for the cells are depleted, cells will start to die and the number of viable cells will decrease.

6.3.1 Lag Phase

The lag phase (or initial stationary, or latent) is an initial period of cultivation during which the change of cell number is zero or negligible. Even though the cell number does not increase, the cells may grow in size during this period.

The length of this lag period depends on many factors such as the type and age of the microorganisms, the size of the inoculum, and culture conditions.

The lag usually occurs because the cells must adjust to the new medium before growth can begin. If microorganisms are inoculated from a medium with a low nutrient concentration to a medium with a high concentration, the length of the lag period is usually long. This is because the cells must produce the enzymes necessary for the metabolization of the available nutrients. If they are moved from a high to a low nutrient concentration, there is usually no lag phase.

Another important factor affecting the length of the lag phase is the size of the inoculum. If a small amount of cells are inoculated into a large volume, they will have a long lag phase. For large-scale operation of the cell culture, it is our objective to make this lag phase as short as possible. Therefore, to inoculate a large fermenter, we need to have a series of progressively larger seed tanks to minimize the effect of the lag phase.
At the end of the lag phase, when growth begins, the division rate increases gradually and reaches a maximum value in the exponential growth period, as shown by the rising inflection at B in Figure 6.1. This transitional period is commonly called the accelerated growth phase and is often included as a part of the lag phase.

6.3.2 Exponential Growth Phase

In unicellular organisms, the progressive doubling of cell number results in a continually increasing rate of growth in the population. A bacterial culture undergoing balanced growth mimics a first-order autocatalytic chemical reaction (Carberry, 1976; Levenspiel, 1972). Therefore, the rate of the cell population increase at any particular time is proportional to the number density \( C_N \) of bacteria present at that time:

\[
\frac{r_N}{dt} = \mu C_N
\]
where the constant $\mu$ is known as the specific growth rate [hr$^{-1}$]. The specific growth rate should not be confused with the growth rate, which has different units and meaning. The growth rate is the change of the cell number density with time, while the specific growth rate is

$$
\mu = \frac{1}{C_N} \frac{dC_N}{dt} = \frac{d\ln C_N}{dt}
$$

(6.6)

which is the change of the natural log of the cell number density with time. Comparing Eq. (6.4) and Eq. (6.6) shows that

$$
\mu = \frac{d\ln C_N}{dt} = \ln 2 \left( \frac{d\log C_N}{dt} \right) = \delta \ln 2
$$

(6.7)

Therefore, the specific growth rate $\mu$ is equal to $\ln 2$ times of the division rate, $\delta$.

If $\mu$ is constant with time during the exponential growth period, Eq. (6.5) can be integrated from time $t_1$ to $t$ as

$$
\int_{C_{N_0}}^{C_N} \frac{dC_N}{C_N} = \int_{t_0}^{t} \mu dt
$$

(6.8)

to give

$$
C_N = C_{N_0} \exp \left[ \mu (t - t_0) \right]
$$

(6.9)

where $C_{N_0}$ is the cell number concentration at $t_1$ when the exponential growth starts. Eq. (6.9) shows the increase of the number of cells exponentially with respect to time.

The time required to double the population, called the doubling time $(t_d)$, can be estimated from Eq. (6.9) by setting $C_N = 2C_{N_0}$ and $t_1 = 0$ and solving for $t$:

$$
t_d = \frac{\ln 2}{\mu} = \frac{1}{\delta}
$$

(6.10)

The doubling time is inversely proportional to the specific growth rate and is equal to the reciprocal of the division rate.

---

1 This equation has the same form as the rate equation for an enzyme catalyzed reaction, the Michaelis-Menten equation:

$$
r_p = \frac{r_{\text{max}} C_S}{K_M + C_S}
$$

and also as the Langmuir adsorption isotherm:

$$
\theta = \frac{C_A}{K + C_A}
$$

where $\theta$ is the fraction of the solid surface covered by gas molecules and $K$ is the reciprocal of the adsorption equilibrium constant.
6.3.3 Factors Affecting the Specific Growth Rate

Substrate Concentration: One of the most widely employed expressions for the effect of substrate concentration on \( \mu \) is the Monod equation, which is an empirical expression based on the form of equation normally associated with enzyme kinetics or gas adsorption:

\[
\mu = \frac{\mu_{\text{max}} C_S}{K_S + C_S}
\]  

(6.11)

where \( C_S \) is the concentration of the limiting substrate in the medium and \( K_S \) is a system coefficient. This relationship is shown graphically in Figure 6.2. The value of \( K_S \) is equal to the concentration of nutrient when the specific growth rate is half of its maximum value (\( \mu_{\text{max}} \)).

While the Monod equation is an oversimplification of the complicated mechanism of cell growth, it often adequately describes fermentation kinetics when the concentrations of those components which inhibit the growth are low.

According to the Monod equation, further increase in the nutrient concentration after \( \mu \) reaches \( \mu_{\text{max}} \) does not affect the specific growth rate, as shown in Figure 6.2. However, it has been observed that the specific growth rate decreases as the substrate concentration is increased beyond a certain level.

![Fig. 6.2 Dependece of the specific growth rate on the concentration of the growth limiting nutrient: \( \mu_{\text{max}} = 0.935 \text{ hr}^{-1}, K_S = 0.22 \times 10^{-4} \text{ mol/L.} \)](image)

Several other models have been proposed to improve the Monod equation. They are:

\[
\mu = \frac{\mu_{\text{max}} C_S}{K_{11} + C_S + (C_{12} C_S)^2}
\]  

(6.12)
If several substances can limit the growth of a microorganism, the following model can be employed

\[ \mu = \mu_{\text{max}} \frac{C_1}{K_1 + C_1} \frac{C_2}{K_2 + C_2} \ldots \]  

(6.16)

If the limiting nutrient is the energy source for the culture, a certain amount of substrate can be used for purposes other than growth. Some models include a term, \( k_e \), which accounts for the maintenance of cells as

\[ \mu = \frac{\mu_{\text{max}} C_s}{K_s + C_s} - k_e \]  

(6.17)

When \( C_s \) is so low that the first term of the right-hand side of Eq. (6.17) is less than or equal to \( k_e \), the specific growth rate is equal to zero. These alternative models give a better fit for the growth of certain microorganisms, but their algebraic solutions are more difficult than for the Monod equation.

**Product Concentration:** As cells grow they produce metabolic by-products which can accumulate in the medium. The growth of microorganisms is usually inhibited by these products, whose effect can be added to the Monod equation as follows:

\[ \mu = \mu_{\text{max}} \left( \frac{C_s}{K_s + C_s} \right) \left( \frac{K_p}{K_p + C_p} \right) \]  

(6.18)

or

\[ \mu = \mu_{\text{max}} \left( \frac{C_s}{K_s + C_s} \right) \left( 1 - \frac{C_p}{C_{\text{pm}}} \right)'' \]  

(6.19)

The preceding equations both describe the product inhibition fairly well. The term \( C_{\text{pm}} \) is the maximum product concentration above which cells cannot grow due to product inhibition.

**Other Conditions:** The specific growth rate of microorganisms is also affected by medium pH, temperature, and oxygen supply. The optimum pH and temperature differ from one microorganism to another.
6.3.4 Stationary and Death Phase

The growth of microbial populations is normally limited either by the exhaustion of available nutrients or by the accumulation of toxic products of metabolism. As a consequence, the rate of growth declines and growth eventually stops. At this point a culture is said to be in the stationary phase. The transition between the exponential phase and the stationary phase involves a period of unbalanced growth during which the various cellular components are synthesized at unequal rates. Consequently, cells in the stationary phase have a chemical composition different from that of cells in the exponential phase.

The stationary phase is usually followed by a death phase in which the organisms in the population die. Death occurs either because of the depletion of the cellular reserves of energy, or the accumulation of toxic products. Like growth, death is an exponential function. In some cases, the organisms not only die but also disintegrate, a process called lysis.

6.4 STIRRED-TANK FERMENTER

As defined in Chapter 2, a bioreactor is a device within which biochemical transformations are caused by the action of enzymes or living cells. The bioreactor is frequently called a fermenter whether the transformation is carried out by living cells or in vivo cellular components (enzymes). However, in this text, we call the bioreactor employing living cells a fermenter to distinguish it from the bioreactor which employs enzymes, the enzyme reactor. In laboratories, cells are usually cultivated in Erlenmeyer flasks on a shaker. The gentle shaking in a shake flask is very effective to suspend the cells, to enhance the oxygenation through the liquid surface, and also to aid the mass transfer of nutrients without damaging the structure of cells.

For a large-scale operation, the stirred-tank fermenter (STF) is the most widely used design in industrial fermentation. It can be employed for both aerobic or anaerobic fermentation of a wide range of cells including microbial, animal, and plant cells. Figure 6.3 shows a diagram of a fermenter used for penicillin production (Aiba et al., 1973). The mixing intensity can be varied widely by choosing a suitable impeller type and by varying agitating speeds. The mechanical agitation and aeration are effective for the suspension of cells, oxygenation, mixing of the medium, and heat transfer. The STF can be also used for high viscosity media. It was one of the first large-scale fermenters developed in the pharmaceutical industries. Its performance and characteristics are extensively studied. Since a stirred-tank fermenter is usually built with stainless steel and operated in mild operating conditions, the life expectancy of the fermenter is also long.
The disadvantage of the STF stems from its advantages. The agitator is effective in mixing the fermenter content, but it consumes a large amount of power and can damage a shear-sensitive cell system such as mammalian or plant cell culture. Fluid shear in mixing is produced by velocity gradients from tangential and radial velocity components of fluids leaving the impeller region. The velocity profile of a regular flat-bladed, disk turbine is parabolic in shape, with the highest observed velocity occurring at the centerline of each blade. Moving away from the centerline, the resultant velocity decreases by 85 percent at one blade-width distance above or below, creating a high shear region (Bowen, 1986). As the blade width-to-diameter ratio increases, the velocity profile becomes a more blunt, less parabolic shape, which yields a lower amount of shear due to a more gradual velocity gradient. Therefore, by increasing the blade width, a STF can be employed successfully in cultivating animal cells (Feder and Tolbert, 1983) or plant cells (Hooker et al., 1990).

![Fig. 6.3 Cutaway diagram of a fermenter used for penicillin production.](image)

Many bench-scale fermenters are made of glass with a stainless steel top plate. As mentioned earlier, larger fermenters are made of stainless steel (Type 316). The height-to-diameter ratio of the vessels is either two-to-one or three-to-one. It is usually agitated with two or three turbine impellers. The impeller shaft enters the vessel either from the top or the bottom of the vessel through a bearing housing and mechanical seal assembly. The impeller diameter to tank diameter ratio \((D_I/D_T)\) is generally 0.3 to 0.4. In the case of a two-impeller system, the distance between the first impeller and the bottom of the vessel and between the two impellers is 1.5 impeller diameters. The distance is reduced to one impeller diameter in the case of a three-impeller system. Four equally spaced baffles are usually installed to prevent a vortex formation which reduces the mixing efficiency. The width of the baffle is usually about one tenth of the tank diameter. For aerobic fermentation, a single orifice sparger or ring sparger is used to aerate the fermenter. The sparger is located between the bottom impeller and the bottom of the vessel. The pH in a fermenter can be maintained by employing either a buffer solution
or a pH controller. The temperature is controlled by heating or cooling as the system requires.

### 6.4.1 Batch or Plug-Flow Fermenter

An ideal stirred fermenter is assumed to be well mixed so that the contents are uniform in composition at all times. Another ideal fermenter is the plug-flow fermenter, the analysis of which is analogous to the ideal batch fermenter.

In a **tubular-flow fermenter**, nutrients and microorganisms enter one end of a cylindrical tube and the cells grow while they pass through. Since the long tube and lack of stirring device prevents complete mixing of the fluid, the properties of the flowing stream will vary in both longitudinal and radial direction. However, the variation in the radial direction is small compared to that in the longitudinal direction. The ideal tubular-flow fermenter without radial variations is called a **plug-flow fermenter** (PFF).

In reality, the PFF fermenter is hard to be found. However, the packed-bed fermenter and multi-staged fermenter can be approximated as PFF. Even though the steady-state PFF is operated in a continuous mode, the cell concentration of an ideal batch fermenter after time \( t \) will be the same as that of a steady-state PFF at the longitudinal location where the residence time \( i \) is equal to \( t \) (Figure 6.4). Therefore, the following analysis applies for both the ideal batch fermenter and steady-state PFF.

If liquid medium is inoculated with a seed culture, the cell will start to grow exponentially after the lag phase. The change of the cell concentration in a batch fermenter is equal to the growth rate of cells in it:

\[
\frac{dC}{dt} = r_x = \mu C_x
\]  

(6.20)

To derive the performance equation of a batch fermentation, we need to integrate Eq. (6.20) to obtain:

\[
\int_{C_x}^{C_x} \frac{dC_x}{C_x_0} \frac{r_x}{r_x} = \int_{C_{x_0}}^{C_x} \frac{dC_x}{\mu C_x} = \int_{t_0}^{t} dt = t - t_0
\]  

(6.21)

It should be noted that Eq. (6.21) only applies when \( r_x \) is larger than zero. Therefore, \( t_1 \) in Eq. (6.21) is not the time that the culture was inoculated, but the time that the cells start to grow, which is the beginning of the accelerated growth phase.

According to Eq. (6.21), the batch growth time \( t - t_0 \) is the area under the \( 1/r_x \) versus \( C_x \) curve between \( C_{x_0} \) and \( C_x \) as shown in Figure 6.5. The solid line in Figure 6.5 was calculated with the Monod equation and the shaded area is equal to \( t - t_0 \). The batch growth time is rarely estimated by this graphical method since the \( C_x \) versus \( t \)
The curve is a more straightforward way to determine it. However, the graphical representation is useful in comparing the performance of the various fermenter configurations, which is discussed later. At this time just note that the curve is U shaped, which is characteristic of auto catalytic reactions:

\[ \frac{C_X}{C_{S_0}} = \frac{C_{X_0}}{C_{S_0}} \text{ at } t = t_0 \]

\[ C_S = C_{S_0} \]

(a)

Fig. 6.4 Schematic diagram of (a) batch stirred-tank fermenter and plug-flow fermenter

\[ S + X \rightarrow X + X \]

The rate for an autocatalytic reaction is slow at the start because the concentration of X is low. It increases as cells multiply and reaches a maximum rate. As the substrate is depleted and the toxic products accumulate, the rate decreases to a low value.

If Monod kinetics adequately represents the growth rate during the exponential period, we can substitute Eq. (6.11) into Eq. (6.21) to obtain

\[ \int_{C_{X_0}}^{C_X} \frac{(K_S + C_S) dC_X}{\mu_{\text{max}} C_S C_X} = \int_{t_0}^{t} dt \]  \hspace{1cm} (6.22)

Eq. (6.22) can be integrated if we know the relationship between \( C_S \) and \( C_X \). It has been observed frequently that the amount of cell mass produced is proportional to the amount of a limiting substrate consumed. The growth yield \( (Y_{X/S}) \) is defined as

\[ Y_{X/S} = \frac{\Delta C_X}{-\Delta C_S} = \frac{C_X - C_{X_0}}{-(C_S - C_{S_0})} \]  \hspace{1cm} (6.23)

Substitution of Eq. (6.23) into Eq. (6.22) and integration of the resultant equation gives a relationship which shows how the cell concentration changes with respect to time:

\[ (t - t_0) \mu_{\text{max}} = \left( \frac{K_S Y_{X/S}}{C_{X_0} + C_{S_0} Y_{X/S}} + 1 \right) \ln \frac{C_X}{C_{X_0}} + \frac{K_S Y_{X/S}}{C_{X_0} + C_{S_0} Y_{X/S}} \ln \frac{C_{S_0}}{C_S} \]  \hspace{1cm} (6.24)
Fig. 6.5 A graphical representation of the batch growth time $t - t_1$ (shaded area). The solid line represents the Monod model with $\mu_{\text{max}} = 0.935 \text{ hr}^{-1}$, $K_S = 0.71 \text{ g/L}$, $Y_{X/S} = 0.6$, $C_{X_0} = 1$ g/L, and $C_{S_0} = 10$ g/L.

The Monod kinetic parameters, $\mu_{\text{max}}$ and $K_S$, cannot be estimated with a series of batch runs as easily as the Michaelis-Menten parameters for an enzyme reaction. In the case of an enzyme reaction, the initial rate of reaction can be measured as a function of substrate concentration in batch runs. However, in the case of cell cultivation, the initial rate of reaction in a batch run is always zero due to the presence of a lag phase, during which Monod kinetics does not apply. It should be noted that even though the Monod equation has the same form as the Michaelis-Menten equation, the rate equation is different. In the Michaelis-Menten equation,

$$\frac{dC_p}{dt} = \frac{r_{\text{max}}C_S}{K_M + C_S} \quad (6.25)$$

while in the Monod equation,

$$\frac{dC_X}{dt} = \frac{\mu_{\text{max}}C_SC_X}{K_S + C_S} \quad (6.26)$$

There is a $C_X$ term in the Monod equation which is not present in the Michaelis-Menten equation.

**Example 6.1**

The growth rate of *E. coli* be expressed by Monod kinetics with the parameters of $\mu_{\text{max}} = 0.935 \text{ hr}^{-1}$ and $K_S = 0.71 \text{ g/L}$ (Monod, 1949). Assume that the cell yield $Y_{X/S}$ is 0.6 g dry cells per g substrate. If $C_{X_0}$ is 1 g/L and $C_{S_1} = 10$ g/L when the cells start to grow exponentially, at $t_0 = 0$, show how $\ln C_X$, $C_X$, $C_S$, $d \ln C_X/dt$, and $dC_X/dt$ change with respect to time.
You can solve Eqs. (6.23) and (6.24) to calculate the change of $C_X$ and $C_S$ with respect to time, which involves the solution of a nonlinear equation. Alternatively, the Advanced Continuous Simulation Language (ACSL, 1975) can be used to solve Eqs. (6.22) and (6.23).

<table>
<thead>
<tr>
<th>Table 6.1</th>
<th>ACSL Program for Example 6.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONSTANT MUMAX .. = 0.935, KS = 0.71, YXS = 0.6, ...</td>
<td></td>
</tr>
<tr>
<td>CX2 = 1., CS2 = 10, TSTOP = 10</td>
<td></td>
</tr>
<tr>
<td>CINTERVAL CINT = 0.1</td>
<td>$ 'COMMUNICATION INTERVAL'</td>
</tr>
<tr>
<td>VARIABLE T = 0.0</td>
<td></td>
</tr>
<tr>
<td>CX = INTEG(MUMAX<em>CS</em>CX/(KS+CS), CX2)</td>
<td></td>
</tr>
<tr>
<td>CS = CS2 - (CX-CX2)/YXS</td>
<td></td>
</tr>
<tr>
<td>LNCX = ALOG(CX)</td>
<td></td>
</tr>
<tr>
<td>DLNCX = MUMAX*CS/(KS+CS)</td>
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</tr>
<tr>
<td>DCXDT = MUMAX<em>CS</em>CX/(KS+CS)</td>
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</tr>
<tr>
<td>TERMT(CS.LE.0) END</td>
<td></td>
</tr>
<tr>
<td>END</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 6.6 Solution to Example 6.1.

Table 6.1 shows the ACSL program and Figure 6.6 shows the results. Comparison of Figure 6.6 with Figure 6.1 shows that Monod kinetics can predict the cell growth from the start of the exponential growth phase to the stationary phase.

6.4.2 Ideal Continuous Stirred-tank Fermenter

Microbial populations can be maintained in a state of exponential growth over a long period of time by using a system of continuous culture. Figure 6.7 shows the block diagram for a continuous stirred-tank fermenter (CSTF). The growth chamber is connected to a
reservoir of sterile medium. Once growth has been initiated, fresh medium is continuously supplied from the reservoir.

\[ \frac{dC_X}{dt} = \frac{V dC_X}{dt} \]

where \( r_X \) is the rate of cell growth in the fermenter and \( dC_X/dt \) represents the change of cell concentration in the fermenter with time.

For the steady-state operation of a CSTF, the change of cell concentration with time is equal to zero \( (dC_X/dt = 0) \) since the microorganisms in the vessel grow just fast enough to replace those lost through the outlet stream, and Eq. (6.27) becomes

\[ \tau_m = \frac{V}{F} = \frac{C_X - C_{Xi}}{r_X} \]

Eq. (6.28) shows that the required residence time is equal to \( C_X - C_{Xi} \) times \( 1/r_X \), which is equal to the area of the rectangle of width \( C_X - C_{Xi} \) and height \( 1/r_X \) on the \( 1/r_X \) versus \( C_X \) curve.
Figure 6.8 shows the $1/r_X$ versus $C_X$ curve. The shaded rectangular area in the figure is equal to the residence time in a CSTF when the inlet stream is sterile. This graphical illustration of the residence time can aid us in comparing the effectiveness of fermenter systems. The shorter the residence time in reaching a certain cell concentration, the more effective the fermenter. The optimum operation of fermenters based on this graphical illustration is discussed in the next section.

If the input stream is sterile ($C_{X_i'} = 0$), and the cells in a CSTF are growing exponentially ($r_X = \mu C_X$), Eq. (6.28) becomes

$$\tau_m = \frac{1}{\mu} = \frac{1}{D} \quad \text{(6.29)}$$

where $D$ is known as dilution rate and is equal to the reciprocal of the residence time ($\tau_m$). Therefore, for the steady-state CSTF with sterile feed, the specific growth rate is equal to the dilution rate. In other words, the specific growth rate of a microorganism can be controlled by changing the medium flow rate. If the growth rate can be expressed by Monod equation, then

$$D = \mu = \frac{1}{\tau_m} = \frac{\mu_{\text{max}} C_S}{K_S + C_S} \quad \text{(6.30)}$$

From Eq. (6.30), $C_S$ can be calculated with a known residence time and the Monod kinetic parameters as:

$$C_S = \frac{K_S}{\tau_m \mu_{\text{max}} - 1} \quad \text{(6.31)}$$

![Fig. 6.8 A graphical representation of the estimation of residence time for the CSTF. The line represents the Monod model with $\mu_{\text{max}} = 0.935 \text{ hr}^{-1}$, $K_S = 0.71 \text{ g/L}$, $Y_{X/S} = 0.6$, $C_{S_i} = 10 \text{ g/L}$, and $C_{X_i'} = 0$.](image)

It should be noted, however, that the preceding expression is only valid when $\tau_m \mu_{\text{max}} > 1$. If $\tau_m \mu_{\text{max}} < 1$, the growth rate of the cells is less than the rate of cells leaving with the outlet stream.
Consequently, all of the cells in the fermenter will be washed out and Eq. (6.31) is invalid.

If the growth yield \( Y_{X/S} \) is constant, then

\[
C_X = Y_{X/S} \left( C_{Si} - C_S \right) \quad (6.32)
\]

Substituting Eq. (6.31) into Eq. (6.32) yields the correlation for \( C_X \) as

\[
C_X = Y_{X/S} \left( C_{Si} - \frac{K_S}{\tau_m \mu_{\text{max}} - 1} \right) \quad (6.33)
\]

\[
P = C_{Pi} + Y_{P/S} \left( C_{Si} - \frac{K_S}{\tau_m \mu_{\text{max}} - 1} \right) \quad (6.34)
\]

Again, Eqs. (6.33) and (6.34) are valid only when \( \tau_m \mu_{\text{max}} > 1 \)

In this section, we set a material balance for the microbial concentration and derive various equations for the CSTF. The same equations can be also obtained by setting material balances for the substrate concentration and product concentration.

### 6.4.3. Evaluation of Monod Kinetic Parameters

The equality of the specific growth rate and the dilution rate of the steady-state CSTF shown in Eq. (6.30) is helpful in studying the effects of various components of the medium on the specific growth rate. By measuring the steady-state substrate concentration at various flow rates, various kinetic models can be tested and the value of the kinetic parameters can be estimated. By rearranging Eq. (6.30), a linear relationship can be obtained as follows:

\[
\frac{1}{\mu} = \frac{K_S}{\mu_{\text{max}} C_S} + \frac{1}{\mu_{\text{max}}} \quad (6.35)
\]

where \( \mu \) is equal to the dilution rate (D) for a chemostat. If a certain microorganism follows Monod kinetics, the plot of \( 1/\mu \) versus \( 1/C_S \) yields the values of \( \mu_{\text{max}} \) and \( K_S \) by reading the intercept and the slope of the straight line. This plot is the same as the Lineweaver-Burk plot for Michaelis-Menten kinetics. It has the advantage that it shows the relationship between the independent variable (\( C_S \)) and dependent variable (\( \mu \)). However, \( 1/\mu \) approaches infinity as the substrate concentration decreases. This gives undue weight to measurements made at low substrate concentrations and insufficient weight to measurements at high substrate concentrations.

Eq. (6.30) can be rearranged to give the following linear relationships, which can be employed instead of Eq. (6.35) for a better estimation of the parameters in certain cases.
However, the limitation of this approach to determine the kinetic parameters is in the difficulty of running a CSTF. For batch runs, we can even use shaker flasks to make multiple runs with many different conditions at the same time. The batch run in a stirred fermenter is not difficult to carry out, either. Since there is no input and output connections except the air supply and the length of a run is short, the danger of contamination of the fermenter is not serious.

For CSTF runs, we need to have nutrient and product reservoirs which are connected to the fermenter aseptically. The rate of input and output stream needs to be controlled precisely. Sometimes, the control of the outlet flowrate can be difficult due to the foaming or plugging by large cell aggregates. Since the length of the run should last several days or even weeks to reach a steady state and also to vary the dilution rates, there is always a high risk for the fermenter to be contaminated. Frequently, it is difficult to reach a steady state because of the cell's mutation and adaptation to new environment.

Furthermore, since most large-scale fermentations are carried out in batch mode, the kinetic parameters determined by the chemostat study should be able to predict the growth in a batch fermenter. However, due to the significantly different environments of batch and continuous fermenters, the kinetic model developed from the CSTF runs may fail to predict the growth behavior of a batch fermenter. Nevertheless, the verification of a kinetic model and the evaluation of kinetic parameters by running chemostat is the most reliable method because of its constant medium environment.

The data from batch runs can be used to determine the kinetic parameters, even though this is not a highly recommended procedure. The specific growth rate during a batch run can be estimated by measuring the slope of the cell concentration versus time curve at the various points. The substrate concentration needs to be measured at the same points where the slope is read. Then, the plots according to Eqs. (6.35), (6.36), (6.37) can be constructed to determine the kinetic parameters. However, the parameter values obtained in this method need to be checked carefully whether they are in the reasonable range for the cells tested.

**Example 6.2**

A chemostat study was performed with yeast. The medium flow rate was varied and the steady-state concentration of cells and glucose in the fermenter were measured and recorded. The inlet concentration
of glucose was set at 100 g/L. The volume of the fermenter contents was 500 mL. The inlet stream was sterile.

<table>
<thead>
<tr>
<th>Flow rate (F, mL/hr)</th>
<th>Cell Cone. (C_x, g/L)</th>
<th>Substrate Cone. (C_s, g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td>5.97</td>
<td>0.5</td>
</tr>
<tr>
<td>50</td>
<td>5.94</td>
<td>1.0</td>
</tr>
<tr>
<td>71</td>
<td>5.88</td>
<td>2.0</td>
</tr>
<tr>
<td>91</td>
<td>5.76</td>
<td>4.0</td>
</tr>
<tr>
<td>200</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

a. Find the rate equation for cell growth.

b. What should be the range of the flow rate to prevent washout of the cells?

**Solution:**

a. Let's assume that the growth rate can be expressed by Monod kinetics. If this assumption is reasonable, the plot of $1/\mu$ versus $1/C_s$ will result in a straight line according to Eq. (6.35).

The dilution rate for the chemostat is

$$D = \frac{F}{V}$$

![Graph](image)

**Fig. 6.9** The plot of $1/D$ versus $1/C_s$ for Example 6.2.

The plot of $1/D$ versus $1/C_s$ is shown in Figure 6.9 which shows a straight line with intercept

$$\frac{1}{\mu_{max}} = 3.8$$
and slope

$$\frac{K_S}{\mu_{max}} = 5.2$$

Therefore, $\mu_{max} = 0.26$ hr$^{-1}$, and $K_S = 1.37$ g/L. The rate equation of cell growth is

$$r_x = \frac{0.26C_SC_X}{1.37 + C_S}$$

b. To prevent washout of the cells, the cell concentration should be maintained so that it will be greater than zero. Therefore, from Eq. (6.33).

$$C_X = Y_X/S(C_S - \frac{K_S}{\tau_m\mu_{max} - 1}) > 0$$

Solving the preceding equation for $\tau_m$ yields

$$\tau_m = \frac{V}{F} = \frac{K_S + C_S}{C_S; \mu_{max}}$$

Therefore,

$$F = \frac{VC_S;\mu_{max}}{K_S + C_S} = \frac{0.5(100)(0.26)}{1.37 + 100} = 1.128 \text{ L/hr}$$

### 6.4.4 Productivity of CSTF

Normally, the productivity of the fermenter is expressed as the amount of a product produced per unit time and volume. If the inlet stream is sterile ($C_{X_i} = 0$), the productivity of cell mass is equal to $C_X/\tau_m$, which is equal to the slope of the straight line OAB of the $C_X$ vs. $\tau_m$ curve, as shown in Figure 6.10. The productivity at point A is equal to that at point B. At point A, the cell concentration of the outlet stream is low but the residence time is short, therefore, more medium can pass through. On the other hand, at point B the cell concentration of the outlet stream is high, but the residence time is long, so a smaller amount of medium passes through. Point A is an unstable region because it is very close to the washout point D, and because a small fluctuation in the residence time can bring about a large change in the cell concentration. As the slope of the line increases, the productivity increases, and the length of AB decreases. The slope of the line will have its maximum value when it is tangent to the $C_X$ curve. Therefore, the value of the maximum productivity is equal to the slope of line OC. The maximum productivity will be attained at the point D.
The operating condition for the maximum productivity of the CSTF can be estimated graphically by using \( \frac{1}{r_X} \) versus \( C_X \) curve. The maximum productivity can be attained when the residence time is the minimum. Since the residence time is equal to the area of the rectangle of width \( C_X \) and height \( \frac{1}{r_X} \) on the \( \frac{1}{r_X} \) versus \( C_X \) curve, it is the minimum when the \( \frac{1}{r_X} \) is the minimum, as shown in Figure 6.11.

![Graph 6.10](image1)

**Fig. 6.10** The change of the concentrations of cells and substrate as a function of the residence time. Productivity is equal to the slope of the straight line OAB. The curve is drawn by using the Monod model with \( \mu_{\text{max}} = 0.935 \, \text{hr}^{-1} \), \( K_s = 0.71 \, \text{g/L} \), \( Y_{X/S} = 0.6 \), and \( C_{S_i} = 10 \, \text{g/L} \).

![Graph 6.11](image2)

**Fig. 6.11** A graphical illustration of the CSTF with maximum productivity. The solid line represents the Monod model with \( \mu_{\text{max}} = 0.935 \, \text{hr}^{-1} \), \( K_s = 0.71 \, \text{g/L} \), \( Y_{X/S} = 0.6 \), \( C_{S_i} = 10 \, \text{g/L} \), and \( C_{X_i} = 0 \).

It would be interesting to derive the equations for the cell concentration and residence time at this maximum cell productivity. The cell productivity for a steady-state CSTF with sterile feed is

\[
\frac{C_X}{\tau_m} = r_X = \frac{\mu_{\text{max}} C_s C_X}{K_s + C_S}
\]
The productivity is maximum when \( dr_x/dC_x = 0 \). After substituting \( C_s = C_{S_i} - C_x Y_{X/S} \) into the preceding equation, differentiating with respect to \( C_x \), and setting the resultant equation to zero, we obtain the optimum cell concentration for the maximum productivity as

\[
C_{X,opt} = Y_{X/S} C_{S_i} \frac{\alpha}{\alpha + 1}
\]  

(6.39)

where

\[
\alpha = \sqrt{\frac{K_s + C_{S_i}}{K_s}}
\]  

(6.40)

Since

\[
C_s - C_{S_i}/Y_{X/S}
\]

\[
C_{S,opt} = \frac{C_{S_i}}{\alpha + 1}
\]  

(6.41)

Substituting Eq. (6.41) into Eq. (6.38) for \( C_s \) yields the optimum residence time:

\[
\tau_{m,opt} = \frac{\alpha}{\mu_{max}(\alpha - 1)}
\]  

(6.42)

### 6.4.5 Comparison of Batch and CSTF

As discussed earlier, the residence time required for a batch or steady-state PFF to reach a certain level of cell concentration is

\[
\tau_b = t_0 + \int_{C_{X_0}}^{C_x} \frac{dC_x}{r_x}
\]  

(6-43)

where \( t_0 \) is the time required to reach an exponential growth phase. The area under the \( 1/r_x \) versus \( C_x \) curve between \( C_X \) and \( C_{X_i} \) is equal to \( t_b - t_{1,r} \), as shown in Figure 6.5.

On the other hand, the residence time for the CSTF is expressed as Eq. (6.28) which is equal to the area of the rectangle of width \( C_X - C_{X_i} \) and height \( 1/r_x \).

Since the \( 1/r_x \) versus \( C_x \) curve is U shaped, we can make the following conclusions for single fermenter.

1. The most productive fermenter system is a CSTF operated at the cell concentration at which value of \( 1/r_x \) is minimum, as shown in Figure 6.12(a), because it requires the smallest residence time.

2. If the final cell concentration to be reached is in the stationary phase, the batch fermenter is a better choice than the CSTF because the residence time required for the batch as shown in Figure 6.12(b) is smaller than that for the CSTF.
Fig. 6.12 Graphical illustration of the residence time required (shaded area) for the (a) CSTF and (b) Batch fermenter.

Fig. 6.13 Graphical illustration of the total residence time required (shaded area) when two fermenters are connected in series: (a) CSTF and PFF, and (b) two CSTFs.

6.5 MULTIPLE FERMENTERS CONNECTED IN SERIES

A question arises frequently whether it may be more efficient to use multiple fermenters connected in series instead of one large fermenter. Choosing the optimum fermenter system for maximum productivity depends on the shape of the $1/r_X$ versus $C_X$ curve and the process requirement, such as the final conversion.

In the $1/r_X$ versus $C_X$ curve, if the final cell concentration is less than $C_{X,\text{opt}}$, one fermenter is better than two fermenters connected in series, because two CSTFs connected in series require more residence time than one CSTF does. However, if the final cell concentration is much larger than $C_{X,\text{opt}}$, the best combination of two fermenters for a minimum total residence time is a CSTF operated at $C_{X,\text{opt}}$ followed by a PFF, as shown in (Figure 6.13a). A CSTF operated at $C_{X,\text{opt}}$ followed by another CSTF connected in series is also better than one CSTF (Figure 6.13b).

6.5.1 CSTF and PFF in Series

Figure 6.14 shows the schematic diagram of the two fermenters connected in series, CSTF followed by PFF. The result of the material
balance for the first fermenter is the same as the single CSTF which we have already developed. If the input stream is sterile \((C_{Xi} = 0)\), the concentrations of the substrate, cell and product can be calculated from Eqs. (6.31), (6.33), and (6.34), as follows:

\[
C_{S1} = \frac{K_S}{\tau_m \mu_{max} - 1} \tag{6.44}
\]

\[
C_{X1} = Y_{X/S} \left(C_{Si} - \frac{K_S}{\tau_m \mu_{max} - 1} \right) \tag{6.45}
\]

\[
C_{P1} = C_{P1} + Y_{p/s} \left(C_{Si} - \frac{K_S}{\tau_m \mu_{max} - 1} \right) \tag{6.46}
\]

For the second PFF, the residence time can be estimated by

\[
\tau_{p2} = \int_{C_{x1}}^{C_{x2}} \frac{dC_X}{r_X} = \int_{C_{x1}}^{C_{x2}} \frac{(K_S + C_S)dC_X}{\mu_{max}C_SC_X} \tag{6.47}
\]

Since growth yield can be expressed as

\[
Y_{X/S} = \frac{C_{X2} - C_{X1}}{C_{S1} - C_{S2}} \tag{6.48}
\]

Integration of Eq. (6.47) after the substitution of Eq. (6.48) will result,

\[
\tau_{p2} \mu_{max} = \left(\frac{K_S Y_{X/S}}{C_{X1} + C_{S1} Y_{X/S}} + 1\right) \ln \frac{C_{X2}}{C_{X1}} + \frac{K_S Y_{X/S}}{C_{X1} + C_{S1} Y_{X/S}} \ln \frac{C_{S1}}{C_{S2}} \tag{6.49}
\]

If the final cell concentration \((C_{X2})\) is known, the final substrate concentration \((C_{S2})\) can be calculated from Eq. (6.48). The residence time of the second fermenter can then be calculated using Eq. (6.49). If the residence time of the second fermenter is known,
Eqs. (6.48) and (6.49) have to be solved simultaneously to estimate the cell and substrate concentrations. Another approach is to integrate Eq. (6.47) numerically until the given \( \tau_{p_2} \) value is reached.

### 6.5.2 Multiple CS TFs in Series

If the final cell concentration is larger than \( C_{X_{opt}} \), the best combination of two fermenters for a minimum total residence time is a CSTF operated at \( C_{X_{opt}} \) followed by a PFF, as explained already. However, the cultivation of microorganisms in the PFF is limited to several experimental cases, such as the tubular loop batch fermenter (Russell et al., 1974) and scraped tubular fermenter (Moo-Young et al., 1979). Furthermore, the growth kinetics in a PFF can be significantly different from that in a CSTF.

Another more practical approach is to use multiple CSTFs in series, since a CSTF operated at \( C_{X_{opt}} \) followed another CSTF connected in series is also better than one CSTF.

Hill and Robinson (1989) derived an equation to predict the minimum possible total residence time to achieve any desired substrate conversion. They found that three optimally designed CSTFs connected in series provided close to the minimum possible residence time for any desired substrate conversion.

Figure 6.15 shows the schematic diagram of the multiple CSTFs connected in series. For the \( n \)th steady-state CSTF, the material balance for the microorganisms can be written as

\[
F(C_{X_{n-1}} - C_{X_n}) + V_n R_{X_n} = 0 \tag{6.50}
\]

Fig. 6.15 Schematic diagram of the multiple CSTFs connected in series.

\[
r_{X_n} = \frac{\mu_{max} C_{Sn} C_{X_n}}{K_S + C_{Sn}} \tag{6.51}
\]

Growth yield can be expressed as

\[
\gamma_{X/S} = \frac{C_{X_n} - C_{X_{n-1}}}{C_{S_{n-1}} - C_{S_n}} \tag{6.52}
\]
By solving Eqs. (6.50), (6.51), and (6.52) simultaneously, we can calculate either dilution rate with the known cell concentration, or vice versa.

The estimation of the cell or substrate concentration with the known dilution rate can be done easily by using graphical technique as shown in Figure 6.1 (Luedeking, 1967). From Eq. (6.50), the dilution rate of the first reactor when the inlet stream is sterile is

$$D_1 = \frac{F}{V_1} = \frac{r_{X_1}}{C_{X_1}}$$

which can be represented by the slope of the straight line connecting the origin and \((C_{X_1}, S_{X_1})\) in Figure 6.16. Similarly, for the second fermenter

$$D_2 = \frac{F}{V_2} = \frac{r_{X_2}}{C_{X_2} - C_{X_1}}$$

which is the slope of the line connecting \((C_{X_1}, r_{X_1})\) and \((C_{X_2}, r_{X_2})\).

Therefore, by knowing the dilution rate of each fermenter, you can estimate the cell concentration of each fermenter, or vice versa.

**Example 6.3**

Suppose you have a microorganism that obeys the Monod equation:

$$\frac{dC_x}{dt} = \frac{\mu_{max} C_S C_x}{K_S + C_S}$$

where \(\mu_{max} = 0.7 \text{ hr}^{-1}\) and \(K_S = 5 \text{ g/L}\). The cell yield (\(Y_{X/S}\)) is 0.65. You want to cultivate this microorganism in either one fermenter or two in series. The flow rate and the substrate concentration of the inlet stream should be 500 L/hr and 85 g/L, respectively. The substrate concentration of the outlet stream must be 5 g/L.
a. If you use one CSTF, what should be the size of the fermenter? What is the cell concentration of the outlet stream?

b. If you use two CSTFs in series, what sizes of the two fermenters will be most productive? What are the concentration of cells and substrate in the outlet stream of the first fermenter?

c. What is the best combination of fermenter types and volumes if you use two fermenters in series?

**Solution:**

a. For a single steady-state CSTF with a sterile feed, the dilution rate is equal to specific growth rate:

\[
D = \frac{F}{V} = \frac{\mu_{\text{max}} C_S}{K_S + C_S} = \frac{0.7(5)}{5 + 5} = 0.35
\]

\[
V = \frac{F}{D} = \frac{500}{0.35} = 1,429 \text{ L}
\]

The cell concentration of the outlet stream is

\[
C_X = Y_{X/S} (C_{S_i} - C_s) = 0.65 (85 - 5) = 52 \text{ g/L}
\]

b. For two CSTFs in series, the first fermenter must be operated at \(C_{X_1, \text{opt}}\) and \(C_{S_1, \text{opt}}\)

Therefore, from Eq. (6.39) through Eq. (6.42),

\[
\alpha = \frac{K_S + C_S}{K_S} = \sqrt{\frac{5 + 85}{5}} = 4.2
\]

\[
C_{X_1} = C_{X_\text{opt}} = Y_{X/S} C_{S_i} \frac{\alpha}{\alpha + 1} = 0.65(85) \frac{4.2}{4.2 + 1} = 45 \text{ g/L}
\]

\[
C_{S_1} = C_{S_\text{opt}} = \frac{C_{S_i}}{\alpha + 1} = \frac{85}{4.2 + 1} = 1.6 \text{ g/L}
\]

\[
\tau_{\text{m1}} = \tau_{\text{mopt}} = \frac{\alpha}{\mu_{\text{max}}(\alpha - 1)} = \frac{4.2}{0.7(4.2 - 1)} = 1.9 \text{ hr}
\]
For the second fermenter, from Eq. (6.50),

\[ F(C_{X_1} - C_{X_2}) + \frac{V_2\mu_{\text{max}}C_{S_2}C_{X_2}}{K_s + C_{S_2}} = 0 \]

By rearranging the preceding equation for \( V_2 \),

\[ V_2 = \frac{F(C_{X_2} - C_{X_1})}{\mu_{\text{max}}C_{S_2}C_{X_1} / (K_s + C_{S_2})} = \frac{500(52 - 45)}{0.7(5)(52)/(5 + 5)} = 192 \text{ L} \]

The total volume of the two CSTFs is

\[ V = V_1 + V_2 = 950 + 192 = 1,142 \text{ L} \]

which is 20 percent smaller than the volume required when we use a single CSTF.

c. The best combination is a CSTF operated at the maximum rate followed by a PFF. The volume of the first fermenter is 950 L as calculated in part (b).

For the second PFF, from Eq. (6.54)

\[ \tau_{p_2} = \frac{1}{\mu_{\text{max}}} \left( \frac{K_sY_{X/S}}{C_{X_1} + C_{S_1}Y_{X/S}} + 1 \right) \ln \frac{C_{X_2}}{C_{X_1}} + \frac{K_sY_{X/S}}{C_{X_1} + C_{S_1} + C_{S_1}Y_{X/S}} \ln \frac{C_{S_2}}{C_{S_2}} \]

\[ = \frac{1}{0.7} \left[ \ln \frac{45}{45 + 16(0.65)} + \frac{5(0.65)}{45} + \frac{5(0.65)}{45 + 16(0.65)} \ln \frac{16}{5} \right] = 0.32 \]

Therefore,

\[ V_2 \tau_{p_2} F = 0.32 \times 500 = 160 \text{ L} \]

The total volume of the CSTF and PFF is

\[ V = V_1 + V_2 = 950 + 160 = 1,110 \text{ L} \]

which is 22 percent smaller than the volume required when we use a single CSTF. The additional saving by employing the second PFF instead of a second CSTF is not significant in this case.

### 6.6 CELL RECYCLING

For the continuous operation of a PFF or CSTF, cells are discharged with the outlet stream which limits the productivity of fermenters. The productivity can be improved by recycling the cells from the outlet stream to the fermenter.
6.6.1 PFF with Cell Recycling

A PFF requires the initial presence of microorganisms in the inlet stream as a batch fermenter requires initial inoculum. The most economical way to provide cells in the inlet stream is to recycle the part of the outlet stream back to the inlet with or without a cell separation device. Figure 6.17 shows the schematic diagram of a PFF with cell recycling. Unlike the CSTF, the PFF does not require the cell separator in order to recycle, though its presence increases the productivity of the fermenter slightly as will be shown later. The performance equation of the PFF with Monod kinetics can be written as:

\[
\frac{V}{(1+R)F} = \frac{\tau_p}{1+R} = \int_{C_x}^{C_{xf}} \frac{dC_x}{r_x} = \int_{C_x}^{C_{xf}} \frac{(K_s + C_s)dC_x}{\mu_{max}C_sC_x}
\]

(6.55)

where \(\tau_p\) is the residence time based on the flow rate of the overall system. The actual residence time in the fermenter is larger than \(\tau_p\) due to the increase of the flow rate by the recycling.

If the growth yield is constant,

\[
C_s = C'_s - \frac{1}{Y_{X/S}} (C_X - C'_X)
\]

(6.56)

Substituting Eq. (6.56) into Eq. (6.55) for \(C_s\) and integrating it will result:

\[
\frac{\tau_p \mu_{max}}{1+R} = \left( \frac{K_s Y_{X/S}}{C_x + C_s Y_{X/S}} + 1 \right) \ln \frac{C_{Xf}'}{C_X'} + \frac{K_s Y_{X/S}}{C_X' + C_s Y_{X/S}} \ln \frac{C_s'}{C_s}
\]

(6.57)

where \(C_X'\) and \(C_s'\) can be estimated from the cell and substrate balance at the mixing point of the inlet and the recycle stream as

\[
C_X' = \frac{C_{xi} + RC_{X_i}}{1+R}
\]

(6.58)

\[
C_s' = \frac{C_{si} + RC_{S_i}}{1+R}
\]

(6.59)
The cell concentrations of the outlet stream, can be estimated from the overall cell balance

\[ C_{Xf} = \frac{1}{\beta} [C_{Xf} + \gamma_{X/S}(C_{Sf} - C_{Si})] \]  

(6.60)

The cell concentrations of the recycle stream, can be estimated from the cell balance over the filter as

\[ C_{XR} = \frac{1 + R + \beta}{R} C_{Xf} \]  

(6.61)

where \( \beta \) is the bleeding rate which is defined as

\[ \beta = \frac{B}{F} \]  

(6.62)

Figure 6.18 shows the effect of the recycle rate (R) on the residence time of a PFF system with recycling. Note that \( \tau \) is calculated based on the inlet flowrate that is the true residence time for the fermenter system. The actual \( \tau \) in the PFF is unimportant because it decreases with the increase of the recycle rate. When \( \beta = 1 \), the bleeding rate is equal to the flow rate and the flow rate of filtrate stream \( L \) is zero, therefore, the recycle stream is not filtered. The residence time will be infinite if \( R \) is zero and decreases sharply as \( R \) is increased until it reaches the point the decrease is gradual. In this specific case, the optimum recycle ratio may be somewhere around 0.2.

\[ \tau_p = \frac{V}{F} \]

Fig. 6.18 The effect of the recycle rate (R) and the bleeding rate \((\beta = B/F)\) on the residence time \((\tau = V/F \text{ hr})\) of a PFF system with recycling. The curve is drawn by using the Monod model with, \( \mu_{\text{max}} = 0.935 \text{ hr}^{-1}, K_S = 0.71 \text{ g/L}, Y_{X/S} = 0.6, C_{Si} = 10 \text{ g/L}, C_{Sf} = 1.3 \text{ g/L}, \) and \( C_{Xf} = 0 \).
Another curve in Figure 6.18 is for $\beta = B/F = 1.8$. The required residence time can be reduced by concentrating the recycle stream 25 to 40% when $R$ is between 0.2 and 1.0. When $R \leq 1.2$, the part of curve is noted as a dotted line because it may be difficult to reduce the recycle ratio below 0.2 when $\beta = 0.8$. For example, in order to maintain $R = 0.1$, The amount of $1.3F$ needs to be recycled and concentrated to $0.1F$, which may be difficult depending on the cell concentration of the outlet. The higher the concentration factor in a filter unit is, the higher the danger of the filter failure can be expected.

The analysis in this section and the next can be also applied for a cell settler as a cell separator. The outlet stream of the cell settler will be equal to $F = B + L$ and its concentration will be $(B/F)C_{xf} = \beta C_{sf}$.

![Diagram](image)

Fig. 6.19 Schematic diagram of CSTF with cell recycling.

### 6.6.2 CSTF with Cell Recycling

The cellular productivity in a CSTF increases with an increase in the dilution rate and reaches a maximum value. If the dilution rate is increased beyond the maximum point, the productivity will be decreased abruptly and the cells will start to be washed out because the rate of cell generation is less than that of cell loss from the outlet stream. Therefore, the productivity of the fermenter is limited due to the loss of cells with the outlet stream. One way to improve the reactor productivity is to recycle the cell by separating the cells from the product stream using a cross-flow filter unit (Figure 6.19).

The high cell concentration maintained using cell recycling will increase the cellular productivity since the growth rate is proportional to the cell concentration. However, there must be a limit in the increase of the cellular productivity with increased cell concentration because in a high cell concentration environment, the nutrient-transfer rate will be decreased due to overcrowding and aggregation of cells. The maintenance of the extremely high cell concentration is also not practical because the filter unit will fail more frequently at the higher cell concentrations.
If all cells are recycled back into the fermenter, the cell concentration will increase continuously with time and a steady state will never be reached. Therefore, to operate a CSTF with recycling in a steady-state mode, we need to have a bleeding stream, as shown in Figure 6.19. The material balance for cells in the fermenter with a cell recycling unit is

\[
FC_{x_i} - BC_x + V \mu C_X = V \frac{dC_x}{dt} \quad (6.63)
\]

It should be noted that actual flow rates of the streams going in and out of the filter unit do not matter as far as overall material balance is concerned. For a steady-state CSTF with cell recycling and a sterile feed,

\[
\beta D = \frac{\beta}{\tau_m} = \mu \quad (6.64)
\]

Now, \( \beta D \) instead of \( D \) is equal to the specific growth rate. When \( \beta = 1 \), cells are not recycled, therefore, \( D = \mu \).

If the growth rate can be expressed by Monod kinetics, substitution of Eq. (6.11) into Eq. (6.64) and rearrangement for \( C_S \) yields

\[
C_S = \frac{\beta K_S}{\tau_m \mu_{\text{max}} - \beta} \quad (6.65)
\]

which is valid when \( \tau_m \mu_{\text{max}} > \beta \). The cell concentration in the fermenter can be calculated from the value of \( C_S \) as

\[
C_X = \frac{Y_{X/S}}{\beta} (C_{S_i} - C_S) \quad (6.66)
\]
Figure 6.20 shows the effect of bleeding ratio on the cell productivity for the Monod model. As $\beta$ is reduced from 1 (no recycling) to 0.5, the cell productivity is doubled.

### 6.7 ALTERNATIVE FERMENTERS

Many alternative fermenters have been proposed and tested. These fermenters were designed to improve either the disadvantages of the stirred tank fermenter-high power consumption and shear damage, or to meet a specific requirement of a certain fermentation process, such as better aeration, effective heat removal, cell separation or retention, immobilization of cells, the reduction of equipment and operating costs for inexpensive bulk products, and unusually large designs.

Fermenters are usually classified based on their vessel type such as tank, column, or loop fermenters. The tank and column fermenters are both constructed as cylindrical vessels. They can be distinguished based on their height-to-diameter ratio ($H/D$) as (Schiigerl, 1982):

\[
H/D < 3 \text{ for the tank and} \\
H/D > 3 \text{ for the column fermenter}
\]

A loop fermenter is a tank or column fermenter with a liquid circulation loop, which can be a central draft tube or an external loop.

### Table 6.2 Classifications of Fermenters

<table>
<thead>
<tr>
<th>Vessel Type</th>
<th>Compressed Air</th>
<th>Internal Moving Parts</th>
<th>External Pumping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tank</td>
<td>—</td>
<td>stirred-tank</td>
<td>—</td>
</tr>
<tr>
<td>Column</td>
<td>bubble column</td>
<td>multistage</td>
<td>sieve tray</td>
</tr>
<tr>
<td></td>
<td>tapered column</td>
<td>(or cascade)</td>
<td>packed-bed</td>
</tr>
<tr>
<td>Loop</td>
<td>air-lift</td>
<td>propeller loop</td>
<td>jet loop</td>
</tr>
<tr>
<td></td>
<td>pressure cycle</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Another way to classify fermenters is based on how the fermenter contents are mixed: by compressed air, by a mechanical internal moving part, or by external liquid pumping. Representative fermenters in each category are listed in Table 6.2 and the advantages and disadvantages of three basic fermenter types are listed in Table 6.3.

### 6.7.1 Column Fermenter

The most simple fermenter is the bubble column fermenter (or tower fermenter), which is usually composed of a long cylindrical vessel
with a sparging device at the bottom [Figure 6.21 (a)-(c)]. The fermenter contents are mixed by the rising bubbles which also provide the oxygen needs of the cells. Since it does not have any moving parts, it is energy efficient with respect to the amount of oxygen transfer per unit energy input. As the cells settle, high cell concentrations can be maintained in the lower portion of the column without any separation device.

<table>
<thead>
<tr>
<th>Type</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stirred-tank</td>
<td>1. Flexible and adaptable</td>
<td>1. High power consumption</td>
</tr>
<tr>
<td></td>
<td>2. Wide range of mixing intensity</td>
<td>2. Damage shear sensitive cells</td>
</tr>
<tr>
<td></td>
<td>3. Can handle high viscosity media</td>
<td>3. High equipment costs</td>
</tr>
<tr>
<td>Bubble Column</td>
<td>1. No moving parts</td>
<td>1. Poor mixing</td>
</tr>
<tr>
<td></td>
<td>2. Simple</td>
<td>2. Excessive foaming</td>
</tr>
<tr>
<td></td>
<td>3. Low equipment costs</td>
<td>3. Limited to low viscosity system</td>
</tr>
<tr>
<td></td>
<td>4. High cell concentration</td>
<td></td>
</tr>
<tr>
<td>Air-lift</td>
<td>1. No moving parts</td>
<td>1. Poor mixing</td>
</tr>
<tr>
<td></td>
<td>2. Simple</td>
<td>2. Excessive foaming</td>
</tr>
<tr>
<td></td>
<td>3. High gas absorption efficiency</td>
<td>3. Limited to low viscosity system</td>
</tr>
<tr>
<td></td>
<td>4. Good heat transfer</td>
<td></td>
</tr>
</tbody>
</table>

However, the bubble column fermenter is usually limited to aerobic fermentations and the rising bubbles may not provide adequate mixing for optimal growth. Only the lower part of the column can be maintained with high cell concentrations, which leads to the rapid initial fermentation followed by a slower one involving less desirable substrates. As the cell concentration increases in a fermenter, high air-flow rates are required to maintain the cell suspension and mixing. However, the increased air-flow rate can cause excessive foaming and high retention of air bubbles in the column which decreases the productivity of the fermenter. As bubbles rise in the column, they can coalesce rapidly leading to a decrease in the oxygen-transfer rate. Therefore, column fermenters are inflexible and limited to a relatively narrow range of operating conditions.

To overcome the weaknesses of the column fermenter, several alternative designs have been proposed. A tapered column fermenter [Figure 6.21 (b)] can maintain a high air-flow rate per unit area at the lower section of the fermenter where the cell concentration is high.
Several sieve plates can be installed in the column [Figure 6.21 (c)] for the effective gas-liquid contact and the breakup of the coalesced bubbles. To enhance the mixing without internal moving parts, the fermentation broth can be pumped out and recirculated by using an external liquid pump [Figure 6.21 (d)(e)].

Fig. 6.21 Column fermenters: (a) bubble column, (b) tapered bubble column, (c) sieve-tray bubble column, (d) sieve-tray column with external pumping, and (e) packed-bed column with external pumping.

6.7.2 Loop Fermenter

A loop fermenter is a tank or column fermenter with a liquid circulation loop, which can be a central draft tube or external loop. Depending on how the liquid circulation is induced, it can be classified into three different types: air-lift, stirred loop, and jet loop (Figure 6.22).

The liquid circulation of the air-lift fermenter is induced by sparged air which creates a density difference between the bubble-rich part of the liquid in the riser and the denser bubble-depleted part of the liquid in the downcomer as shown in Figure 6.22 (a). The liquid circulation and mixing can be enhanced by circulating liquid externally using a pump as shown in Figure 6.22 (b). However, adding the pump diminishes the real advantages of an air-lift fermenter for being simple and energy efficient.

The ICI pressure cycle fermenter (Imperial Chemical Industries Ltd., England) is an air-lift fermenter with an outer loop, which was developed for the aerobic fermentation requiring heat removal such as the single-cell protein production from methanol. Medium and air are introduced into the upper and lower parts of the loop as shown in Figure 6.22 (c). The air serves two purposes: It provides the oxygen needed for the growth of the microorganisms and the rising air creates natural circulation of the liquid in the fermenter through the loop. A heat exchanger to cool the liquid medium is installed in the loop. It was claimed that the fermenter gives a high rate of oxygen absorption per unit of volume, that it uses a high proportion of
oxygen in the air passed through the fermenter, and that the high
circulation of the fermentation liquor provides good mixing
(Technical Brochure, ICI Ltd.).

6.8 STRUCTURED MODEL

So far, the kinetic models described in this chapter have been
unstructured, distributed models based on assumptions that cells can
be represented by a single component, such as cell mass or cell
number per unit volume and the population of the cellular mass is
distributed uniformly throughout the culture. These models do not
recognize the change in the composition of cells during growth.
Unstructured, distributed models such as Monod’s equation can
satisfactorily predict growth behavior of many situations. However,
they cannot account for lag phases, sequential uptake of substrates,
or changes in mean cell size during the growth cycle of batch culture.

Structured models recognize the multiplicity of cell components
and their interactions. Many different models have been proposed
based on the assumptions made for cell components and their
interactions. More comprehensive review of these models can be
found in Tsuchiya et al. (1966) and Harder and Roels (1982). In this
section, general equations describing structured models are derived
and a simple structured model is introduced.

6.8.1 General Structured Model

Let’s define a system as an individual cell or multiple cells. The
system does not contain any of the abiotic phase of culture. Instead, it
is the biotic phase only, which possesses mass \( m \) on a dry basis and
specific volume \( \hat{v} \). Let’s assume that there are \( c \) components in
the cell and the mass of the \( j \)th component per unit volume of system is
\( \hat{C}_{X_j} \). It is also assumed that there exist kinetic rate expressions for \( p \)
reactions occurring in the system and the rate of the \( j \)th component
formed from the \( i \)th reaction per unit volume of system is \( \dot{r}_{X_{ji}} \).

Then, during batch cultivation, the change of the \( j \)th component in
the system with respect to time can be expressed as (Fredrickson,
1976):

\[
\frac{d(m\hat{v}\hat{C}_{X_j})}{dt} = m\hat{v} \sum_{i=1}^{p} \dot{r}_{X_{ji}} \quad (6.67)
\]

If we assume that the specific volume \( \hat{v} \) is constant with time,
Eq. (6.67) can be rearranged to

\[ 2 \text{ Caused or produced by living beings.} \]
Fig. 6.22 Loop fermenters: (a) air-lift, (b) air-lift with external pumping (c) IC1 pressure cycle.

\[
\frac{d\hat{C}_x}{dt} = \sum_{i=1}^{p} \frac{1}{m} \frac{dm}{dt} \hat{C}_{x,i}
\]

(6.68)

where the second term of the right-hand side of Eq. (6.68) represents the dilution of intracellular components by the growth of biomaterial. It should be noted that all variables denoted by circumflexes in the preceding equations are intracellular properties. Since the structural models recognize the multiplicity of cell components and their interactions in the cell, it makes more sense to express the model with intrinsic variables.

Since \( \hat{C}_x = \sum_{j=1}^{n} \hat{C}_{x,j} \) the summation of Eq. (6.68) for all \( c \) components yields

\[
\frac{d\hat{C}_x}{dt} = \sum_{j=1}^{c} \sum_{i=1}^{p} \frac{m}{m} \hat{r}_{x_{i,j}} - \frac{1}{m} \frac{dm}{dt} \hat{C}_x
\]

(6.69)

where \( \hat{C}_x \) is the mass of biomaterial per unit volume of the biotic system that is equal to \( 1/\hat{v} \), which was assumed to be constant. Therefore, Eq. (6.69) is simplified to

\[
\frac{1}{m} \frac{dm}{dt} = \frac{1}{\hat{C}_x} \sum_{j=1}^{c} \sum_{i=1}^{p} \hat{r}_{x_{i,j}}
\]

(6.70)

which gives the relationship between the rate of cell growth and the kinetic rate expressions of all reactions occurring in cells.

The concentration terms \( m \) the preceding equations can be expressed as mass per unit culture volume \( V \) instead of that per biotic system volume \( m \_v \). The two different definitions of concentration are related as
\[ \hat{C}_{X_i} = \frac{V}{m\hat{V}} C_{X_i} \]  

(6.71)

Note that the concentration based on the culture volume is no longer denoted with a circumflex. Substituting Eq. (6.71) into Eq. (6.68) and simplifying for the constant \( V \) yields

\[ \frac{dC_{X_i}}{dt} = \frac{m\hat{V}}{V} \sum_{i=1}^{p} \hat{r}_{X_{i,i}} \]  

(6.72)

It should be noted that even though concentration terms are expressed based on the total culture volume, kinetic parameters still remain on a biotic phase basis in the formulation.

### 6.8.2 Two-Compartment Model

One of the simplest structured models is the two-compartment model proposed by Williams (1967) and is based on the following assumptions:

1. The cell comprises two basic compartments: a synthetic portion A such as precursor molecules and RNA, and a structural portion B such as protein and DNA.

\[ \hat{C}_{X} = \hat{C}_{X_A} + \hat{C}_{X_B} \]  

(6.73)

where \( \hat{C}_{X} \) is equal to \( 1 \) \( V \), and assumed to be constant,

\[ \frac{d\hat{C}_{X}}{dt} = 0 \]  

(6.74)

2. The synthetic portion A is fed by uptake from a substrate \( S \) and the structural portion B is in turn fed from the synthetic portion as:

\[ S \rightarrow A \rightarrow B \]  

(6.75)

3. The rate of the first reaction in Eq. (6.75) is proportional to the product of substrate and cell concentrations as:

\[ \hat{r}_{X_{A,A}} = k_1 \bar{C}_S \hat{C}_{X} \]  

(6.76)

where \( \bar{C}_S \) is the mass of substrate per unit abiotic volume, \( V-m\hat{V} \). The rate of the second reaction in Eq. (6.75) is proportional to the product of the concentrations of the synthetic portion and the structural portion as:

\[ \hat{r}_{X_{B,A}} = -r_{X_{2,A}} = k_2 \bar{C}_{X_A} \hat{C}_{X_B} \]  

(6.77)

---

3 The original Williams model (1967) was modified according to the suggestion made by Fredrickson (1976).
If we write Eq. (6.68) for each component, and substitute Eqs. (6.76) and (6.77) for the reaction rates, we obtain

$$\frac{d\hat{C}_{X_A}}{dt} = k_1\hat{C}_S\hat{C}_X - k_2\hat{C}_{X_A}\hat{C}_{X_B} - \frac{1}{m}\frac{dm}{dt}\hat{C}_{X_A} \tag{6.78}$$

$$\frac{d\hat{C}_{X_B}}{dt} = k_2\hat{C}_{X_A}\hat{C}_{X_B} - \frac{1}{m}\frac{dm}{dt}\hat{C}_{X_A} \tag{6.79}$$

The change of the substrate concentration is

$$\frac{d\hat{C}_S}{dt} = -\frac{k_1}{Y_{X/S}}\hat{C}_S\hat{C}_X \tag{6.80}$$

where $Y_{X/S}$ is the yield constant.

Following, Eqs. (6.73), (6.74), (6.78), (6.79), and (6.80) can be solved simultaneously to obtain the change of $\hat{C}_X, \hat{C}_{X_A}, \hat{C}_{X_B}, \hat{C}_S$, and $m$.

1. Cell division occurs if and only if the structural portion $(m\hat{v}\hat{C}_{X_B})$ has doubled its initial value, and a dividing cell apportions each component equally to its two daughter cells. Therefore, the total number of cells in the system will be proportional to the structural portion as

$$n \propto m\hat{v}\hat{C}_{X_B} \tag{6.81}$$

The average mass of a cell is equal to the total cell mass divided by total cell number. Therefore,

$$\text{Average mass of cell} = \frac{m}{n} \propto \frac{m\hat{v}\hat{C}_X}{m\hat{v}\hat{C}_{X_B}} = \frac{\hat{C}_X}{\hat{C}_{X_B}} \tag{6.82}$$

As a result, this model can also predict the change of the average cell size with respect to time, which is not possible with the Monod model. Eqs. (6.73), (6.78), (6.79), and (6.80) can be expressed with the concentrations in terms of mass per unit culture volume as

$$C_X = C_{X_A} + C_{X_B} \tag{6.83}$$

$$\frac{dC_{X_A}}{dt} = \left(\frac{V}{V-m\hat{v}}\right)k_1C_SC_X - \left(\frac{V}{m\hat{v}}\right)k_2C_{X_A}C_{X_B} \tag{6.84}$$

$$\frac{dC_{X_B}}{dt} = \left(\frac{V}{m\hat{v}}\right)k_2C_{X_A}C_{X_B} \tag{6.85}$$

$$\frac{dC_S}{dt} = -\frac{1}{Y_{X/S}}\left(\frac{V}{V-m\hat{v}}\right)k_2C_SC_X \tag{6.86}$$
where \( C_X = (m \dot{v}/V) \hat{C}_X, C_{X_A} = (m \dot{v}/V) \hat{C}_{X_A}, C_{X_B} = (m \dot{v}/V) \hat{C}_{X_B} \),

and

\[
C_S = \frac{(V - m \dot{v})}{V} \bar{C}_S
\]

The mass \( m \) is related to \( C_X \) as

\[
m = C_X V \tag{6.87}
\]

Now, the total number of cells is proportional to \( C_{X_B} \) and the average mass of a cell to \( C_X/C_{X_B} \).

Figure 6.23 illustrates simulation curves of a batch culture, which show the changes of mass \((C_X/C_{X_{\text{max}}})\), number \((C_X/C_{X_{B\text{max}}})\), and size of cells \((C_X/C_{X_B})\), and the substrate concentration \((C_S/C_{S0})\) in dimensionless form. This curve shows the following features:

1. During a lag phase, cells grows in size but not in number.
2. Cells are largest during the exponential phase.
3. The total cell mass reaches its asymptote before cell number.
4. Cells no longer grow or divide during a stationary phase

![Simulation curves](image)

**Fig. 6.23** Simulation results of a batch culture using the two compartment model proposed by Williams (1967), which show the changes of mass, number, and size of cells, and the substrate concentration in dimensionless form. Parameters: \( C_{S0} = 1 \text{ g/L}, C_{X_{A0}} = 0, C_{X_{B0}} = 0.05 \text{ g/L}, k_1 = 1.25 \text{ L/g hr}, k_2 = 0.0002 \text{ L/g hr}, \dot{v} = 0.0002 \text{ L/g} \)

Even though Williams's model provides many features that unstructured models are unable to predict, it requires only two parameters, which is the same number of parameters required for Monod kinetics.
6.9 NOMENCLATURE

\[ B \quad \text{flow rate of bleeding stream, } m^3/s \]
\[ C \quad \text{concentration, mass per unit volume of culture, } kg/m^3 \]
\[ \hat{C} \quad \text{intracellular concentration, mass per unit volume of biotic phase, } kg/m^3 \]
\[ \bar{C} \quad \text{extracellular concentration, mass per unit volume of abiotic phase, } kg/m \]
\[ C_1, C_2 \quad \text{constants} \]
\[ C_N \quad \text{cell number density, number of cells/m} \]
\[ D \quad \text{dilution rate, } s^{-1} \]
\[ F \quad \text{flow rate, } m^3/s \]
\[ K_S \quad \text{system coefficient for the Monod kinetics, } kg/m \]
\[ L \quad \text{flow rate of filtrate stream, } m^3/s \]
\[ \bar{m} \quad \text{average mass of cell in a system, } kg \]
\[ m \quad \text{mass of biotic phase, } kg \]
\[ n \quad \text{number of cells} \]
\[ r \quad \text{rate of growth per unit volume, } kg/m^3s \]
\[ \bar{r}_{i,j} \quad \text{rate of } j\text{th component formed from } i\text{th reaction per unit volume of a system, intracellular property, } kg/m^3s \]
\[ r_{i,j} \quad \text{rate of } j\text{th component formed from } i\text{th reaction per unit volume of a system, extracellular property, } kg/m^3s \]
\[ t \quad \text{time, } s \]
\[ t_d \quad \text{doubling time, } s \]
\[ \hat{V} \quad \text{specific volume of a system containing only biotic phase, } m^3/kg \]
\[ V \quad \text{working volume of fermenter, } m^3 \]
\[ Y \quad \text{yield constant} \]
\[ \beta \quad \text{bleeding ratio, defined as } B/F \]
\[ \bar{\delta} \quad \text{average cell division rate, } s^{-1} \]
\[ \mu \quad \text{specific growth rate, } s^{-1} \text{ or } kg/m^3s \]
\[ \rho \quad \text{density, } kg/m^3 \]
\[ \tau \quad \text{residence time, } s \]

SUBSCRIPT

\[ b \quad \text{batch fermenter} \]
\[ i \quad \text{input stream} \]
mixed fermenter

cell in number basis

cell in dry weight basis

product

plug-flow fermenter

substrate

6.10 PROBLEMS

6.1 Derive the relationship giving the change with respect to time of the cell concentration in a batch fermenter, Eq. (6.24).

6.2 Aiba et al. (1968) reported the results of a chemostat study on the growth of a specific strain of baker’s yeast as shown in the following table. The inlet stream of the chemostat did not contain any cells or products.

<table>
<thead>
<tr>
<th>Dilution Rate D, hr(^{-1})</th>
<th>Inlet cone. (g/L)</th>
<th>Steady-state concentration (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose (C_{S_{in}})</td>
<td>Glucose (C_S)</td>
</tr>
<tr>
<td>0.084</td>
<td>21.5</td>
<td>0.054</td>
</tr>
<tr>
<td>0.100</td>
<td>10.9</td>
<td>0.079</td>
</tr>
<tr>
<td>0.160</td>
<td>21.2</td>
<td>0.138</td>
</tr>
<tr>
<td>0.198</td>
<td>20.7</td>
<td>0.186</td>
</tr>
<tr>
<td>0.242</td>
<td>10.8</td>
<td>0.226</td>
</tr>
</tbody>
</table>

   a. Find the rate equation for cell growth.

   b. Find the rate equation for product (ethanol) formation.

6.3 Andrews (1968) proposed the following model for the growth of microorganisms utilizing inhibitory substrates.

\[
\mu = \frac{\mu_{\text{max}}}{1 + \frac{K_S}{C_S} + \frac{C_S}{K_I}}
\]

Assume that a chemostat study was performed with a microorganism. The volume of the fermenter content was 1 L. The inlet stream was sterile. The flow rate and inlet substrate concentration were varied and the steady-state concentration of glucose in the fermenter was measured and recorded as shown in the table (the data are arbitrary).

   a. Determine the kinetic parameters \((\mu_{\text{max}}, K_S, K_I)\) of this microorganism.

   b. If the cell yield, \(Y_{X/S}\), is 0.46 g/g, what is the steady-state cell concentration when the flow rate is 0.20 L/h?
c. Andrews concluded in his paper that the primary result of substrate inhibition in a continuous culture may be process instability. Explain what might happen if you suddenly increase the substrate concentration from 30 to 60 g/L and why.

<table>
<thead>
<tr>
<th>Flow rate L/hr</th>
<th>Inlet glucose cone. $G_{si}$ g/L</th>
<th>Steady-state glucose cone. $C_s$ g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.20</td>
<td>30</td>
<td>0.5</td>
</tr>
<tr>
<td>0.25</td>
<td>30</td>
<td>0.7</td>
</tr>
<tr>
<td>0.35</td>
<td>30</td>
<td>1.1</td>
</tr>
<tr>
<td>0.50</td>
<td>30</td>
<td>1.6</td>
</tr>
<tr>
<td>0.70</td>
<td>30</td>
<td>3.3</td>
</tr>
<tr>
<td>0.80</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>0.50</td>
<td>60</td>
<td>30</td>
</tr>
<tr>
<td>0.60</td>
<td>60</td>
<td>22</td>
</tr>
<tr>
<td>0.70</td>
<td>60</td>
<td>15</td>
</tr>
</tbody>
</table>

6.4 Rate equations for the cells (yeast), substrate in the ethanol fermentation process are given (glucose), and product as follows:

$$
r_X = \frac{dC_X}{dt} = \mu_{\text{max}} \left( \frac{C_S}{K_S + C_S} \right) \left( 1 - \frac{C_P}{C_{pm}} \right)^n C_X
$$

$$
r_S = \frac{dC_S}{dt} = -\frac{1}{Y_{X/P}} \frac{dC_X}{dt}
$$

$$
r_P = \frac{dC_P}{dt} = -\frac{1}{Y_{X/P}} \frac{dC_X}{dt}
$$

where $K_S = 1.6$ g/L, $\mu_{\text{max}} = 0.24$ hr$^{-1}$, $Y_{X/P} = 0.06$, $Y_{X/S} = 0.06$, $C_{pm} = 100$ g/L, $C_{p0} = 0$, $C_{xo} = 0.1$ g/L, and $n = 2$.

a. Calculate the change of $C_X$, $C_P$, and $C_S$ as a function of time when $C_S = 001$ g/L.

b. Show the effect of the initial substrate concentration on the $C_X$ versus $t$ curve.

c. Show the effect of the maximum growth rate ($\mu_{\text{max}}$) on the $C_X$ versus $t$ curve ($C_{S1} = 100 \text{ g/L}$).

6.5 The growth rate of *E. coli* in synthetic medium can be expressed by Monod kinetics as

$$
r_X = \frac{0.935C_SC_X}{0.71 + C_S} \text{ [g/L hr]}
$$
where $C_S$ is the concentration of a limiting substrate, glucose. You are going to cultivate *E. coli* in a steady-state CSTF (working volume: 10 L) with a flow rate of 7 L/hr. The initial substrate concentration is 10 g/L and the cell yield constant ($Y_{X/S}$) is 0.6. The feed stream is sterile.

a. What will be the doubling time and the division rate of the cells in the CSTF?

b. What will be the cell and substrate concentrations of the outlet stream?

c. If you connect one more 10/L CSTF to the first one, what will be the cell and substrate concentrations in the second fermenter?

d. If you increase the flow rate from 7 to 10 L/hr for these two fermenters connected in series, what will happen and why? Make a recommendation to avoid the problem if there is any.

6.6 Suppose that the growth rate of a microorganism can be expressed as the following equation:

$$r_X = \mu_{\text{max}} \left[ 1 - \exp\left(-\frac{C_S}{K_S}\right) \right] C_X$$

where $\mu_{\text{max}} = 1.365 \text{ hr}^{-1}$ and $K_S = 6.8 \text{ g/L}$. The cell yield $Y_{X/S}$ is found to be 0.45.

a. If you cultivate this microorganism in a 10 L CSTF with the flow rate of 2.8 L/hr, what will be the steady-state cell concentration of the outlet stream? The substrate concentration of the inlet stream is 13 g/L. The inlet stream is sterile.

b. Explain the difference between this model and Monod model by using $\mu$ versus $C_S$ graph.

6.7 Herbert et al. (1956) reported that the growth kinetics of *Aerobacter cloacae* in a chemically defined medium (glycerol as a limiting substrate) could be expressed by Monod kinetics as follows:

$$r_X = \frac{dC_X}{dt} = \frac{\mu_{\text{max}} C_S C_X}{K_S + C_S}$$

where $\mu_{\text{max}} = 0.85 \text{ hr}^{-1}$ and $K_S = 1.23 \times 10^{-2} \text{ g/L}$. The yield was found to be 0.53 g dry weight of organism/g glycerol used.

You are a biochemical engineer who has been assigned the task of designing the most effective continuous fermentation system to grow the microorganism (*Aerobacter cloacae*) with glycerol as its limiting substrate. For the following three questions, the concentration of glycerol in the feed stream and that of glycerol in the outlet stream should be 3 g/L and 0.1 g/L, respectively.
a. Since you have learned that the $1/r_X$ versus $C_S$ curve for Monod kinetics has a U shape, you have recommended that the most effective system would be the combination of a continuous stirred-tank fermenter (CSTF) and a plug-flow fermenter (PFF). You were quite sure of this because the substrate concentration in the outlet stream has to be so low. However, your boss is insisting that the use of second PFF in addition to the first CSTF will not improve the productivity very much. Who is right? Prove whether you are right or wrong by drawing the $1/r_X$ versus $C_S$ curve for this microorganism. Does it have a U shape? Discuss why you are right or wrong. (If you are right, think about how you can nicely correct your boss's wrong idea. If you are wrong, it will teach you that you have to be careful not to make a quick conclusion without adequate analysis.)

b. Recommend the best fermenter system (fermenter type and volume) which can handle 100 L/hr of feed stream. The best fermenter system is defined as that which can produce the maximum amount of cells per unit time and volume.

c. If $K_S = 1.23$ g/L instead of $1.23 \times 10^{-2}$ g/L, what is the best fermenter system (fermenter type, volume) which can handle 100 L/hr in the feed stream. Draw the block diagram of the fermenter system with the concentrations of the substrate and the cells in the inlet and outlet streams of each fermenter. How is this case different from the case of part (a) and why?

6.8 Suppose you have an organism that obeys the Monod equation:

$$\frac{dC_X}{dt} = \frac{\mu_{\text{max}}C_SC_X}{K_S + C_S}$$

where $\mu_{\text{max}} = 0.5$ hr$^{-1}$ and $K_S = 1$ g/L.

The organism is being cultivated in a steady-state CSTF, where $F = 100$ L/hr, $C_{S_i} = 50$ g/L, and $Y_{X/S} = 0.5$.

a. What size vessel will give the maximum total rate of cell production?

b. What are the substrate and cell concentrations of the optimum fermenter in part (a)?

c. If the exiting flow from the fermenter in part (a) is fed to a second fermenter (CSTF), what should be the size of the second fermenter to reduce the substrate concentration to 1 g/L?

d. If the exiting flow from the first fermenter in part (a) is fed to a second fermenter whose size is the same as the first, what will be the cell and substrate concentrations leaving the second fermenter?
6.9 You are going to cultivate yeast, *Saccharomyces cerevisiae*, by using a 10 m³-fermenter your company already owns. You want to find out the amount of ethanol the fermenter can produce. Therefore, a chemostat study was carried out and the Monod kinetic parameters for the microorganism grown in the glucose medium at 30°C, pH 4.8, were found to be: \( K_S = 0.0025 \text{ g/L} \) and \( \mu_{\text{max}} = 0.25 \text{ h}^{-1} \). The ethanol yield (\( Y_{P/S} \)) is 0.44 (g/g) and cell yield (\( Y_{X/S} \)) is 0.019 (g/g). The inlet substrate concentration is 50 g/L.

a. What flow rate will give the maximum total ethanol production in the continuous fermenter and what is the maximum ethanol production rate?

b. If you want to convert 95 percent of the incoming substrate, what must the ethanol production rate be for the continuous fermenter?

c. If you have two 5 m³-fermenters instead of one 10 m³-fermenter, what is your recommendation for the use of these fermenters to convert 95 percent of the incoming substrate? Would you recommend connecting two fermenters in series to improve the productivity? Why or why not?

6.10 You are a biochemical engineer in a pharmaceutical company. Your company is a major producer of penicillin. Currently, what kind of fermenter is your company using for penicillin production? Why?

Your boss asked you to study the possibility of using an air-lift fermenter as a replacement since it has many advantages. What is your recommendation?

6.11 Consider an organism with the following data for a 1-L chemostat, using an inlet substrate concentration \( C_S \) of 30 g/L:

<table>
<thead>
<tr>
<th>Flow rate (mL/hr)</th>
<th>Substrate Concentration (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( F )</td>
<td>( C_S )</td>
</tr>
<tr>
<td>27.5</td>
<td>10.0</td>
</tr>
<tr>
<td>24.2</td>
<td>5.56</td>
</tr>
<tr>
<td>22.1</td>
<td>3.70</td>
</tr>
<tr>
<td>18.8</td>
<td>2.32</td>
</tr>
</tbody>
</table>

a. In a continuous, perfectly mixed vessel at steady state with no cell death, if inlet substrate concentration \( C_S \) now equals 25.0 g/L and cell concentration \( C_X \) is 0.0 g/L, what dilution rate \( D \) will give the maximum total rate of cell production? What are the outlet cell and substrate concentrations at this dilution rate?
b. Using the preceding organism, your boss would like you to design a continuous reactor system with an inlet flow and substrate concentration of 250 L/hr and 25 g/L, respectively, which will produce an overall yield of product of 2100 kg/yr, given an operating time of 300 day/yr at 24 hr/day. Assume no cells or product in the system inlet. Would you recommend a single fermenter of two fermenters in series? Design a system which will meet production constraints while minimizing total fermenter volume. Report reactor volumes and effluent cell, substrate, and product concentrations for the proposed fermenter(s).

[Contributed by Brian S. Hooker, TriState University.]

6.12 A strain of yeast is being cultivated in a 30-L CSTF with a cell recycling system (cell settler) as shown in the following figure. The cell settler was designed so that the cell concentration of its outlet stream is 30 percent of that of its inlet stream, whereas the substrate concentrations of the two streams are the same. The growth rate of the cells can be represented by the Monod kinetics with the parameters: $K_s = 0.05$ g/L, $\mu_{\text{max}} = 0.3$ h$^{-1}$, and $Y_{X/S} = 0.025$. Calculate the steady-state substrate and cell concentrations in the fermenter. The inlet substrate concentration is 100 g/L and the flow rate is 20 L/hr. The feed stream is sterile.

![Cell settler diagram]

6.13 You need to cultivate hypothetical microbial cells with the Monod kinetic parameter values of $\mu_{\text{max}} = 5.0$ hr$^{-1}$ and $K_s = 20$ g/L. The cell yield ($Y_{X/S}$) is 0.4 and the substrate concentration is 30 g/L. The required substrate conversion is 97%. Estimate the residence time required for the following fermenter configurations:

a. Plug-flow fermenter with the inlet cell concentration of 0.5 g/L.

b. Plug-flow fermenter with a recycle stream (no cell separator) with the flow rate of 0.4F. The inlet cell concentration is 0.5 g/L. How your answer will be changed if you reduce the inlet cell concentration to zero?
c. One steady-state CSTF with a recycle stream (no cell separator) with the flow rate of 0.4F. The inlet stream is sterile.

6.14 By using the structured model proposed by Ramkrishna et al. (1967), show the change of the concentrations [in g dry weight/L] of G-mass, H-mass, and inhibitor, and the fraction of G-mass with time during a batch cultivation of a microorganism which has the following parameters and initial conditions:

\[
\begin{align*}
\mu & = 0.5 \text{ hr}^{-1} \\
\mu' & = 2.5 \text{ hr}^{-1} \\
K_S & = 0.2 \text{ g/L} \\
K'_S & = 0.1 \text{ g/L} \\
K & = 150 \text{ L/g hr} \\
K' & = 70 \text{ L/g hr} \\
K_G & = 3.0 \times 10^{-5} \text{ g/L} \\
K'_G & = 0.5 \times 10^{-5} \text{ g/L} \\
\alpha_S & = 8
\end{align*}
\]

Compare your simulation result with Figure 14 of the paper by Ramkrishna et al. (1967). If you showed the change of the cell concentrations in g dry weight/liter, the shape of the curves are quite different from those in the paper. What are the differences? Explain. Do the parameter values predict realistic growth curves? What new features can this model predict which the Monod model cannot?

6.11 REFERENCES


