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.

Flow rate F, mℓ/hr	Cell Concentration C_X, g/ℓ	Substrate Concentration C_S, g/ℓ
31	5.97	0.5
50	5.94	1.0
71	5.88	2.0
91	5.76	4.0
200	0	100

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

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

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

$$r_X = \frac{0.26\,C_S\,C_X}{1.37 + C_S}$$

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)

$$C_X = Y_{X/S}\left(C_{S_i} - \frac{K_S}{\tau_m \mu_{max} - 1}\right) > 0$$

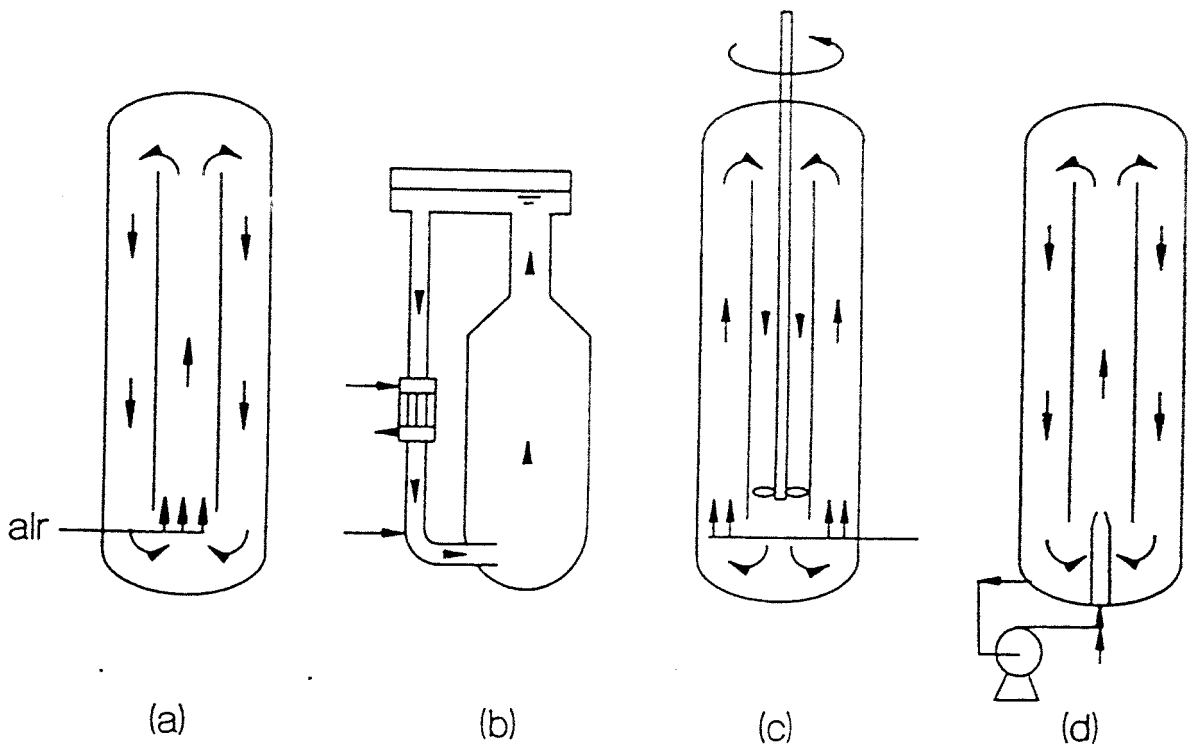


Figure 6.20 Loop fermenters: (a) air-lift, (b) ICI pressure cycle, (c) stirred loop, and (d) jet loop.

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 6.19(c)] for the effective gas-liquid contact and the breakup of the coalesced bubbles. The cylindrical column can be divided into multiple stages which are equipped with stirrers [Figure 6.19(d)]. This configuration will be analogous to the stirred-tank fermenter connected in series as explained in a earlier section. To enhance the mixing without internal moving parts, the fermentation broth can be pumped out and recirculated by using an external liquid pump [Figure 6.19(e) and (f)].

6.8.2 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 6.20).

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 6.20(a).

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 6.20(b). 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.).

The liquid circulation and mixing can be enhanced by installing a propeller or by circulating liquid externally using a pump as shown in Figure 6.20(c) and (d). However, adding the propeller or pump diminishes the real advantages of an air-lift fermenter for being simple and energy efficient.

Nomenclature

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

SUBSCRIPT

b	batch fermenter
i	input stream
m	mixed fermenter
n	cell in number basis
X	cell in dry weight basis
P	product
p	plug-flow fermenter

S substrate

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.

Dilution Rate D, hr^{-1}	Inlet Glucose Conc. C_{S_i}, g/ℓ	Steady-state Glucose Conc. C_S, g/ℓ	Steady-state Ethanol Conc. C_P, g/ℓ	Steady-state Cell Conc. C_X, g/ℓ
0.084	21.5	0.054	7.97	2.00
0.100	10.9	0.079	4.70	1.20
0.160	21.2	0.138	8.57	2.40
0.198	20.7	0.186	8.44	2.33
0.242	10.8	0.226	4.51	1.25

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.

$$\mu = \frac{\mu_{max}}{1 + \frac{K_S}{C_S} + \frac{C_S}{K_I}}$$

Assume that a chemostat study was performed with a microorganism. The volume of the fermenter content was $1\,\ell$. 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 follows (the data are arbitrary):

Flow Rate F, ℓ/hr	Inlet Glucose Concentration C_{S_i}, g/ℓ	Steady-state Glucose Concentration C_S, g/ℓ
0.20	30	0.5
0.25	30	0.7
0.35	30	1.1
0.50	30	1.6
0.70	30	3.3
0.80	30	10
0.50	60	30
0.60	60	22
0.70	60	15

a. Determine the kinetic parameters (μ_{max}, K_S, and 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.

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

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

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

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

where $K_S = 1.6$ g/ℓ, $\mu_{max} = 0.24\ \text{hr}^{-1}$, $Y_{X/P} = 0.16$, $Y_{X/S} = 0.06$, $C_{P_m} = 100$ g/ℓ, $C_{P_0} = 0$, $C_{X_0} = 0.1$ g/ℓ, and $n = 2$.

a. Calculate the change of C_X, C_P, and C_S as a function of time when $C_{S_0} = 100$ 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 (μ_{max}) on the C_X versus t curve ($C_{S_0} = 100\,\text{g}/\ell$).

6.5 Derive Eqs. (6.39), (6.41), and (6.42).

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

$$r_X = \frac{0.935 C_S C_X}{0.71 + C_S} \qquad [\text{g}/\ell\,\text{hr}]$$

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: $10\,\ell$) with a flow rate of $7\,\ell$/hr. The initial substrate concentration is $10\,\text{g}/\ell$ 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 $10\,\ell$/hr for these two fermenters connected in series, what will happen and why? Make a recommendation to avoid the problem if there is any.

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

$$r_X = \mu_{max}(1 - e^{-C_S/K_S})C_X$$

where $\mu_{max} = 0.365\,\text{hr}^{-1}$ and $K_S = 6.8\,\text{g}/\ell$. The cell yield $Y_{X/S}$ is found to be 0.45.

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

b. Explain the difference between this model and Monod model by using μ versus C_S graph.

6.8 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:

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

where $\mu_{max} = 0.85 hr^{-1}$ and $K_S = 1.23 \times 10^{-2}$ 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 you 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 1.23×10^{-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?

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

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

where $\mu_{max} = 0.5\ hr^{-1}$ and $K_S = 2$ g/ℓ.

The organism is being cultivated in a steady-state CSTF, where $F = 100\ \ell$/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?

6.10 You are going to cultivate yeast, *Saccharomyces cerevisiae*, by using a 10 m^3-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: $K_S = 0.025$ g/ℓ and $\mu_{max} = 0.25\ h^{-1}$. 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 m^3-fermenters instead of one 10 m^3-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?

6.11 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?

6.12 Consider an organism with the following data for a 1-ℓ chemostat, using

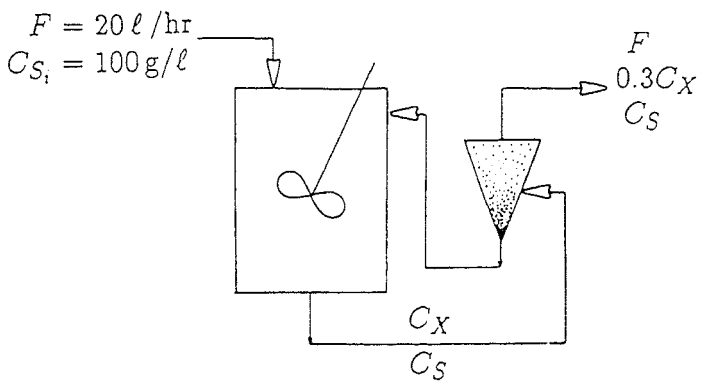
an inlet substrate concentration C_{S_i} of 30 g/ℓ:

Flowrate F (mℓ/hr)	Concentration Substrate C_S (g/ℓ)	Cell C_X (g/ℓ)	Product C_P (g/ℓ)
27.5	10.0	12.0	1.04
24.2	5.56	14.7	1.27
22.1	3.70	15.8	1.37
18.8	2.32	16.7	1.44

a. In a continuous, perfectly mixed vessel at steady state with no cell death, if inlet substrate concentration C_{S_i} now equals 25.0 g/ℓ and cell concentration C_{X_i} 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.*]

6.13 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, $\mu_{max} = 0.3\ \text{h}^{-1}$, 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.



6.14 A plug-flow fermenter is to be used to cultivate microbial cells. It has been determined that the fermenter efficiency can be improved by recycling a portion of the product stream so that it returns to the entrance for an additional pass through the fermenter. The recycle rate (R) is defined as

$$R = \frac{\text{volume of fluid returned to entrance}}{\text{volume leaving the system}}$$

a. Show that an optimal recycle rate must satisfy

$$\ln \frac{1 + R(1 - X_{Sf})}{R(1 - X_{Sf})} = \frac{R + 1}{R[1 + R(1 - X_{Sf})]}$$

where X_{Sf} is the fraction of a limiting substrate S that is converted to cell mass. The optimal recycle rate corresponds to the minimum-sized reactor needed to attain a desired level of conversion.

b. Determine the recycle ratio needed to minimize reactor size for fractional conversion of $X_{Sf} = 0.995$.

6.15 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:

$$\mu = 0.5\,\mathrm{hr}^{-1} \qquad a'_S = 2$$
$$\mu' = 2.5\,\mathrm{hr}^{-1} \qquad a_T = 0$$
$$K_S = 0.2\,\mathrm{g}/\ell \qquad a'_T = 0.2 \times 10^{-4}$$
$$K'_S = 0.1\,\mathrm{g}/\ell \qquad a_{T_1} = 0.0267$$
$$K = 150\,\ell/\mathrm{ghr} \qquad a'_{T_1} = 0$$
$$K' = 70\,\ell/\mathrm{ghr} \qquad C_{S_0} = 10\,\mathrm{g}/\ell$$
$$K_G = 3.0 \times 10^{-5}\,\mathrm{g}/\ell \qquad C_{T_0} = 0\,\mathrm{g}/\ell$$
$$K'_G = 0.5 \times 10^{-5}\,\mathrm{g}/\ell \qquad C_{X_{H0}} = 8.0 \times 10^{-5}\,\mathrm{g}/\ell$$
$$a_S = 8 \qquad C_{X_{G0}} = 1.0 \times 10^{-6}\,\mathrm{g}/\ell$$

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?

Chapter 7

Genetic Engineering

The central tool for the new biotechnology is the recombinant DNA technique.[1] It allows direct manipulation of genetic material of individual cells. By inserting foreign genetic information into fast-growing microorganisms, we can produce foreign gene products (proteins) with higher rates and yields that have not been possible with any other cellular systems. This technology is also known as genetic engineering because it involves the manipulation of genetic materials.[2] In this chapter, basic principles involved in recombinant DNA technology and problems involved in cultivating the genetically engineered cells are briefly described.

7.1 DNA and RNA

Deoxyribonucleic acid (DNA) is the most important molecule in living cells and contains all of the information that specifies the cell. DNA and ribonucleic acid (RNA) are macromolecules that are linear polymers built up from simple subunits, nucleotides.[3] The monomeric unit, *nucleotide*, has the following three components (Figure 7.1):

1. A cyclic five-carbon (pentose) sugar: deoxyribose for DNA, and ribose for RNA.
2. A nitrogenous base of either purine or pyrimidine derivation, covalently attached to the 1′-carbon atom of the sugar by an N-glycosylic bond as shown in Figure 7.1.
   a. The purines: adenine (A) and guanine (G).
   b. The pyrimidines: cytosine (C), thymine (T) for DNA only, and uracil (U) for RNA only.
3. A phosphate attached to the 5′ carbon of the sugar by phosphoester linkage.

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[1] Read Chapter 1 for a general introduction to the new biotechnology.

[2] The name *genetic engineering* should not mislead the readers that it is a field of engineering; it is a field of biological science.

[3] Macromolecules: a polymer, especially one composed of more than 100 repeated monomers.

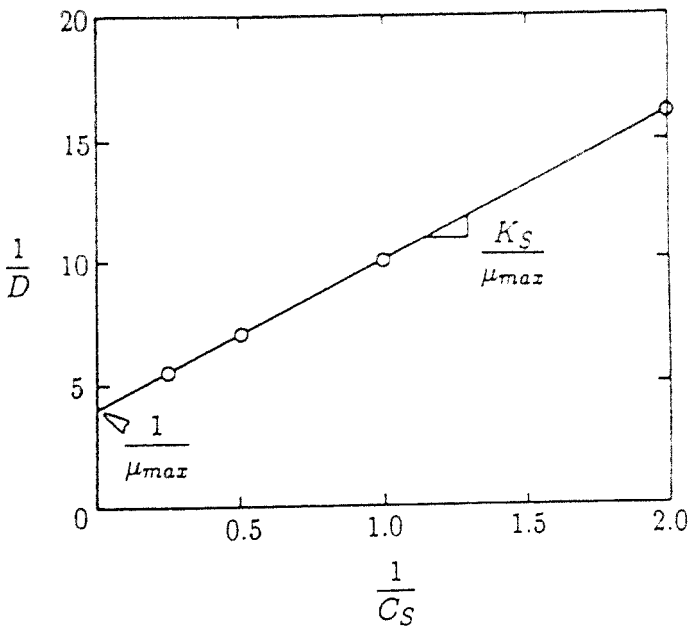


Figure 6.9 The plot of $1/D$ versus $1/C_S$ for Example 6.2.

Solving the preceding equation for τ_m yields

$$\tau_m = \frac{V}{F} > \frac{K_S + C_{S_i}}{C_{S_i}\mu_{max}}$$

Therefore,

$$F < \frac{VC_{S_i}\mu_{max}}{K_S + C_{S_i}} = \frac{0.5(100)(0.26)}{1.37 + 100} = 0.128\,\ell/\text{hr}$$

6.5.3 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/τ_m, which is equal to the slope of the straight line $\overline{OAB}$ of the C_X vs. τ_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

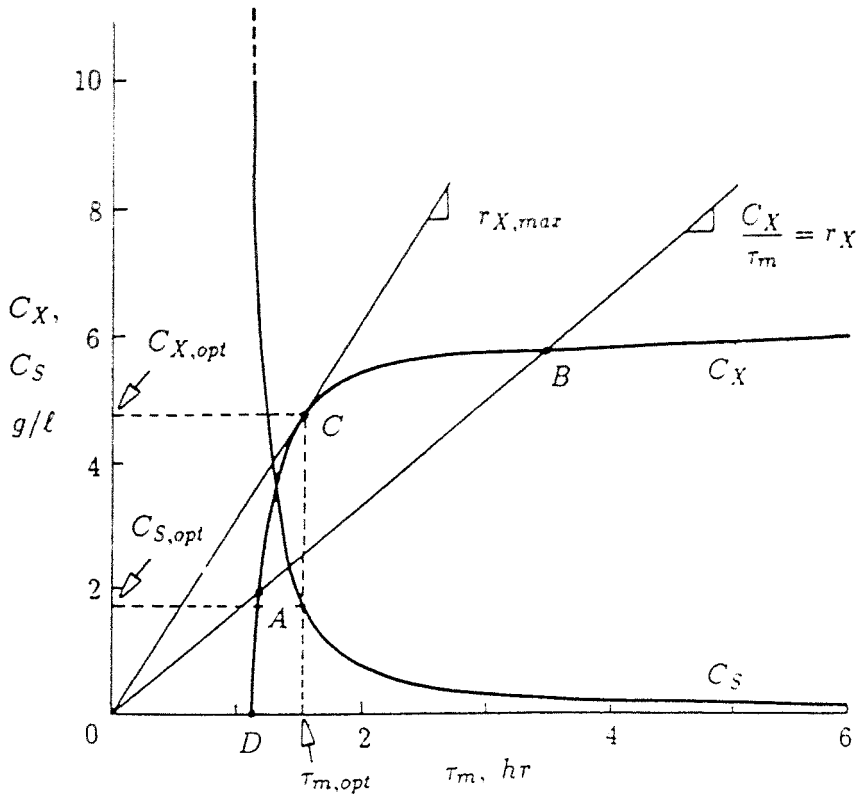


Figure 6.10 The change of the concentrations of cells and substrate as a function of the residence time. Productivity is equal to the slope of the straight line $\overline{OAB}$. The curve is drawn by using the Monod model with $\mu_{max} = 0.935$, $K_S = 0.71\,g/\ell$, $Y_{X/S} = 0.6$, $C_{S_i} = 10\,g/\ell$.

the cell concentration. As the slope of the line increases, the productivity increases, and the length of $\overline{AB}$ 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 $\overline{OC}$. The maximum productivity will be attained when $\tau_m = (\tau_m)_{opt.}$ and $C_X = (C_X)_{opt.}$ as shown in Figure 6.10.

The operating condition for the maximum productivity of the CSTF can be estimated graphically by using $1/r_X$ 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 $1/r_X$ on the $1/r_X$ versus C_X curve, it is the minimum when the $1/r_X$ is the minimum, as shown in Figure 6.11.

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

$$\frac{C_X}{\tau_m} = r_X = \frac{\mu_{max} C_S C_X}{K_S + C_S} \tag{6.38}$$

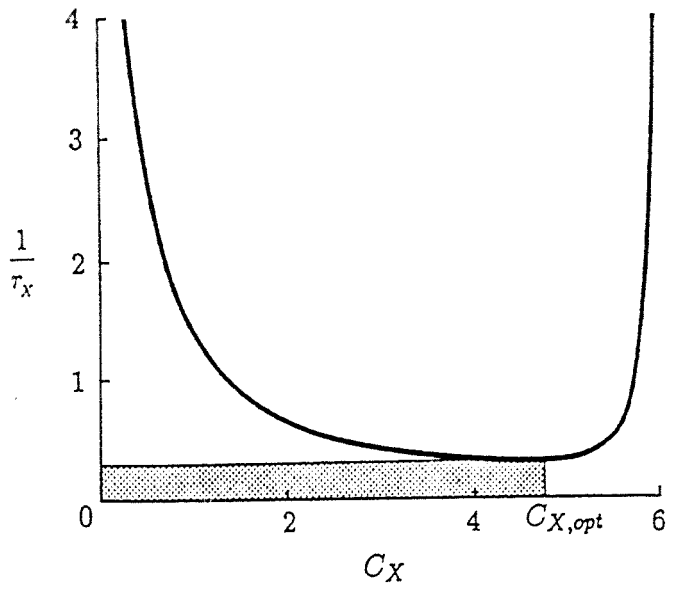


Figure 6.11 A graphical illustration of the CSTF with maximum productivity. The solid line represents the Monod model with $\mu_{max} = 0.935$, $K_S = 0.71\,g/\ell$, $Y_{X/S} = 0.6$, $C_{S_i} = 10\,g/\ell$, $C_{X_i} = 0$.

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

$$C_{X,opt} = Y_{X/S}C_{S_i}\frac{\alpha}{\alpha+1} \tag{6.39}$$

where

$$\alpha = \sqrt{\frac{K_S + C_{S_i}}{K_S}} \tag{6.40}$$

Since $C_S = C_{S_i} - C_X/Y_{X/S}$,

$$C_{S,opt} = \frac{C_{S_i}}{\alpha+1} \tag{6.41}$$

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

$$\tau_{m,opt} = \frac{\alpha}{\mu_{max}(\alpha-1)} \tag{6.42}$$

6.5.4 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

$$\tau_b = t_0 + \int_{C_{X_0}}^{C_X} \frac{dC_X}{r_X} \tag{6.43}$$

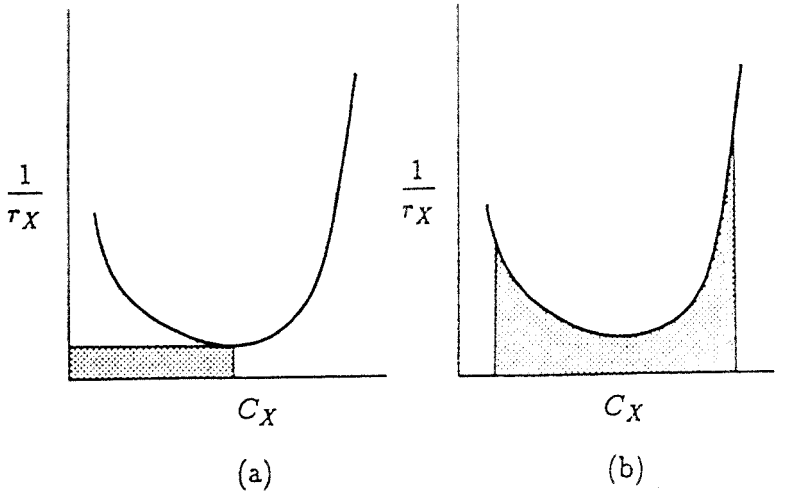


Figure 6.12 Graphical illustration of the residence time required (shaded area) for the (a) CSTF and (b) Batch fermenter.

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_0} and C_X is equal to $\tau_b - t_0$., 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 6.12a, 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 6.12b is smaller than that for the CSTF.

6.6 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 $C_{X,opt}$, one fermenter is better than two fermenters connected in series, because two CSTFs connected in series require more residence time than one

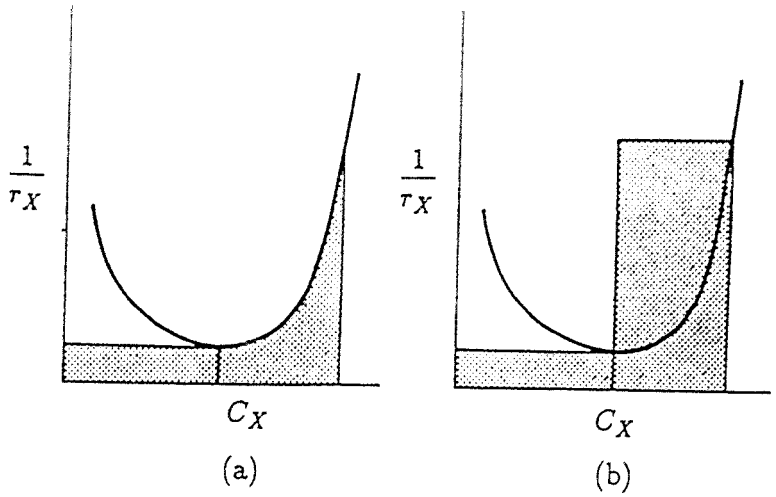


Figure 6.13 Graphical illustration of the total residence time required (shaded area) when two fermenters are connected in series: (a) CSTF and PFF, and (b) two CSTFs.

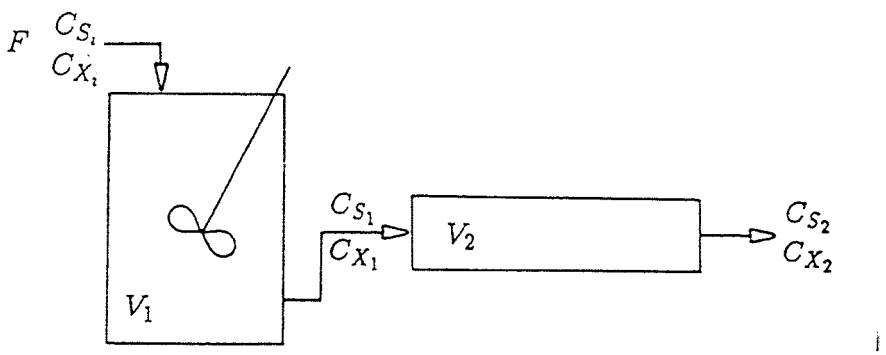


Figure 6.14 Schematic diagram of the two fermenters, CSTF and PFF, connected in series.

CSTF does. However, if the final cell concentration is much 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 shown in (Figure 6.13a). A CSTF operated at $C_{X,opt}$ followed by another CSTF connected in series is also better than one CSTF (Figure 6.13b).

6.6.1 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 ($C_{X_i} = 0$), the concentrations of the substrate,

cell and product can be calculated from Eqs. (6.31), (6.33), and (6.34), as follows:

$$C_{S_1} = \frac{K_S}{\tau_{m1}\mu_{max} - 1} \tag{6.42}$$

$$C_{X_1} = Y_{X/S}\left(C_{S_i} - \frac{K_S}{\tau_{m1}\mu_{max} - 1}\right) \tag{6.44}$$

$$C_{P_1} = C_{P_i} + Y_{P/S}\left(C_{S_i} - \frac{K_S}{\tau_{m1}\mu_{max} - 1}\right) \tag{6.45}$$

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

$$\tau_{p2} = \int_{C_{X_1}}^{C_{X_2}} \frac{dC_X}{r_X} = \int_{C_{X_1}}^{C_{X_2}} \frac{(K_S + C_S)dC_X}{\mu_{max}C_S C_X} \tag{6.46}$$

Since growth yield can be expressed as

$$Y_{X/S} = \frac{C_{X_2} - C_{X_1}}{C_{S_1} - C_{S_2}} \tag{6.47}$$

Integration of Eq. (6.46) after the substitution of Eq. (6.47) will result,

$$\tau_{p2}\mu_{max} = \left(\frac{K_S Y_{X/S}}{C_{X_1} + C_{S_1}Y_{X/S}} + 1\right)\ln\frac{C_{X_2}}{C_{X_1}} + \frac{K_S Y_{X/S}}{C_{X_1} + C_{S_1}Y_{X/S}}\ln\frac{C_{S_1}}{C_{S_2}} \tag{6.48}$$

If the final cell concentration (C_{X_2}) is known, the final substrate concentration (C_{S_2}) can be calculated from Eq. (6.47). The residence time of the second fermenter can then be calculated using Eq. (6.48). If the residence time of the second fermenter is known, Eqs. (6.47) and (6.48) have to be solved simultaneously to estimate the cell and substrate concentrations. Another approach is to integrate Eq. (6.46) numerically until the given τ_{p2} value is reached.

6.6.2 Multiple CSTFs 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

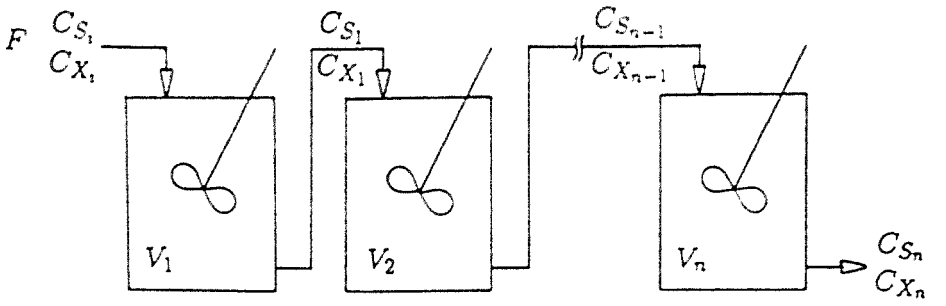


Figure 6.15 Schematic diagram of the multiple CSTFs connected in series.

is also better than one CSTF. Figure 6.15 shows the schematic diagram of the multiple CSTFs connected in series. For the nth steady-state CSTF, the material balance for the microorganisms can be written as

$$F(C_{X_{n-1}} - C_{X_n}) + V_n r_{X_n} = 0 \tag{6.49}$$

where

$$r_{X_n} = \frac{\mu_{max} C_{S_n} C_{X_n}}{K_S + C_{S_n}} \tag{6.50}$$

Growth yield can be expressed as

$$Y_{X/S} = \frac{C_{X_n} - C_{X_{n-1}}}{C_{S_{n-1}} - C_{S_n}} \tag{6.51}$$

By solving Eqs. (6.49), (6.50), and (6.51) 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 6.16 (Luedeking, 1967). From Eq. (6.49), the dilution rate of the first reactor when the inlet stream is sterile is

$$D_1 = \frac{F}{V_1} = \frac{r_{X_1}}{C_{X_1}} \tag{6.52}$$

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

$$D_2 = \frac{F}{V_2} = \frac{r_{X_2}}{C_{X_2} - C_{X_1}} \tag{6.53}$$

which is the slope of the line connecting $(C_{X_1}, 0)$ and (C_{X_1}, r_{X_1}). Therefore, by knowing the dilution rate of each fermenter, you can estimate the cell concentration of each fermenter, or vice versa.

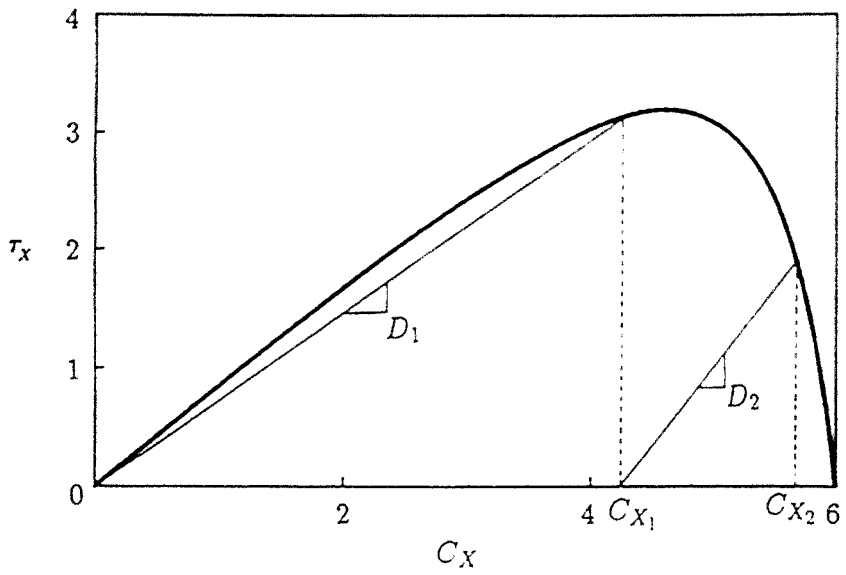


Figure 6.16 Graphical solution for a two-stage continuous fermentation. The line represents the Monod model with $\mu_{max} = 0.935$, $K_S = 0.71\,g/\ell$, $Y_{X/S} = 0.6$, $C_{S_i} = 10\,g/\ell$, $C_{X_i} = 0$.

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 $\mu_{max} = 0.7\,hr^{-1}$ and $K_S = 5\,g/\ell$. 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 $500\,\ell/hr$ and $85\,g/\ell$, respectively. The substrate concentration of the outlet stream must be $5\,g/\ell$.

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:

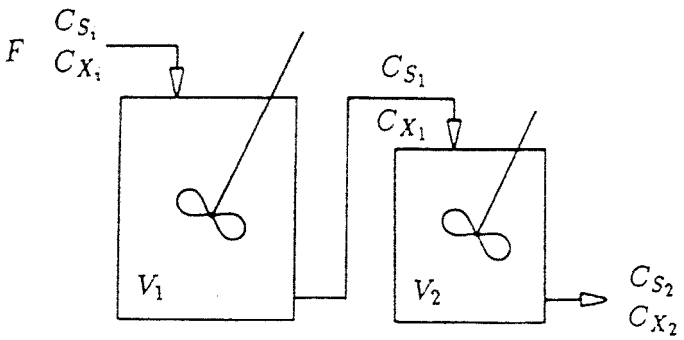
$$D = \frac{F}{V} = \frac{\mu_{max} C_S}{K_S + C_S} = \frac{0.7(5)}{5+5} = 0.35$$

$$V = \frac{F}{D} = \frac{500}{0.35} = 1,429\,\ell$$

The cell concentration of the outlet stream is

$$C_X = Y_{X/S}(C_{S_i} - C_S) = 0.65(85 - 5) = 52\,\text{g}/\ell$$

b. For two CSTFs in series, the first fermenter must be operated at $C_{X,opt}$ and $C_{S,opt}$.



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

$$\alpha = \sqrt{\frac{K_S + C_{S_i}}{K_S}} = \sqrt{\frac{5 + 85}{5}} = 4.2$$

$$C_{X_1} = C_{X,opt} = Y_{X/S}C_{S_i}\frac{\alpha}{\alpha + 1} = 0.65(85)\frac{4.2}{4.2 + 1} = 45\,\text{g}/\ell$$

$$C_{S_1} = C_{S,opt} = \frac{C_{S_i}}{\alpha + 1} = \frac{85}{4.2 + 1} = 16\,\text{g}/\ell$$

$$\tau_{m1} = \tau_{m,opt} = \frac{\alpha}{\mu_{max}(\alpha - 1)} = \frac{4.2}{0.7(4.2 - 1)} = 1.9\,\text{hr}$$

$$V_1 = \tau_{m1}F = 1.9(500) = 950\,\ell$$

For the second fermenter, from Eq. (6.49),

$$F(C_{X_1} - C_{X_2}) + \frac{V_2\mu_{max}C_{S_2}C_{X_2}}{K_S + C_{S_2}} = 0$$

By rearranging the preceding equation for V_2,

$$V_2 = \frac{F(C_{X_2} - C_{X_1})}{\mu_{max}C_{S_2}C_{X_2}/(K_S + C_{S_2})} = \frac{500(52 - 45)}{0.7(5)(52)/(5 + 5)} = 192\,\ell$$

The total volume of the two CSTFs is

$$V = V_1 + V_2 = 950 + 192 = 1,142\,\ell$$

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. (6.48)

$$\tau_{p2} = \frac{1}{\mu_{max}}\left[\left(\frac{K_S Y_{X/S}}{C_{X_1} + C_{S_1} Y_{X/S}} + 1\right)\ln\frac{C_{X_2}}{C_{X_1}} + \frac{K_S Y_{X/S}}{C_{X_1} + C_{S_1} Y_{X/S}}\ln\frac{C_{S_1}}{C_{S_2}}\right]$$

$$= \frac{1}{0.7}\left[\left(\frac{5(0.65)}{45 + 16(0.65)} + 1\right)\ln\frac{52}{45} + \frac{5(0.65)}{45 + 16(0.65)}\ln\frac{16}{5}\right] = 0.32$$

Therefore,

$$V_2 = \tau_{p2} F = 0.32(500) = 160\,\ell$$

The total volume of the CSTF and PFF is

$$V = V_1 + V_2 = 950 + 160 = 1,110\,\ell$$

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.

6.7 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 6.17).

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.

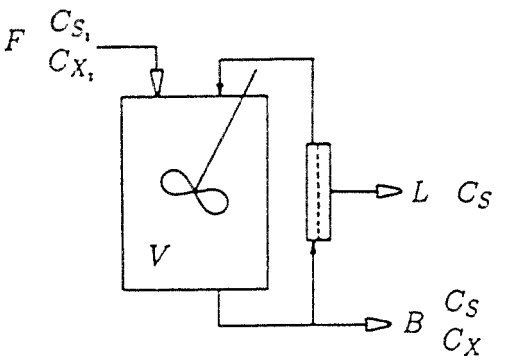


Figure 6.17 Schematic diagram of CSTF with cell recycling.

Therefore, to operate a CSTF with recycling in a steady-state mode, we need to have a bleeding stream, as shown in Figure 6.17. The material balance for cells in the fermenter with a cell recycling unit is

$$FC_{X_i} - BC_X + V\,\mu\,C_X = V\frac{dC_X}{dt} \tag{6.54}$$

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,

$$\beta D = \frac{\beta}{\tau_m} = \mu \tag{6.55}$$

where β, the bleeding ratio, is defined as

$$\beta = \frac{B}{F} \tag{6.56}$$

Now, βD 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. (6.55) and rearrangement for C_S yields

$$C_S = \frac{\beta K_S}{\tau_m \mu_{max} - \beta} \tag{6.57}$$

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

$$C_X = \frac{Y_{X/S}}{\beta}(C_{S_i} - C_S) \tag{6.58}$$

Figure 6.18 shows the effect of bleeding ratio on the cell concentration and productivity. As β is reduced from 1 to 0.5, the cell concentration and productivity is doubled.

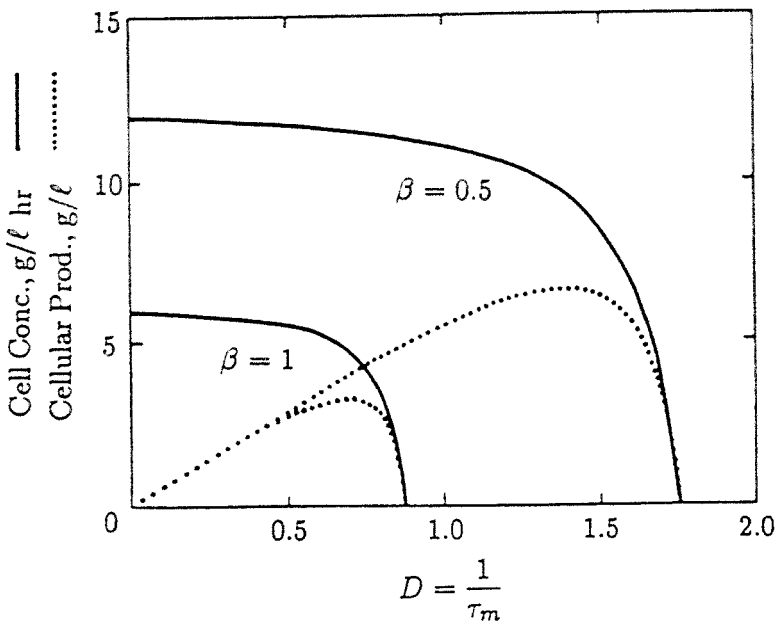


Figure 6.18 The effect of bleeding ratio on the cellular productivity.

6.8 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 (Schügerl, 1982):

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

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.

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 6.3 and the advantages and disadvantages of three basic fermenter types are listed in Table 6.4.

Table 6.3
Classifications of Fermenters

Vessel Type	Primary Source of Mixing		
	Compressed Air	*Internal Moving Parts*	*External Pumping*
Tank	-	stirred-tank	-
Column	bubble column tapered column	multistage (or cascade)	sieve tray packed-bed
Loop	air-lift pressure cycle	propeller loop	jet loop

Table 6.4
Advantages and Disadvantages of Three Basic Fermenter Configurations

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

6.8.1 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 sparg-

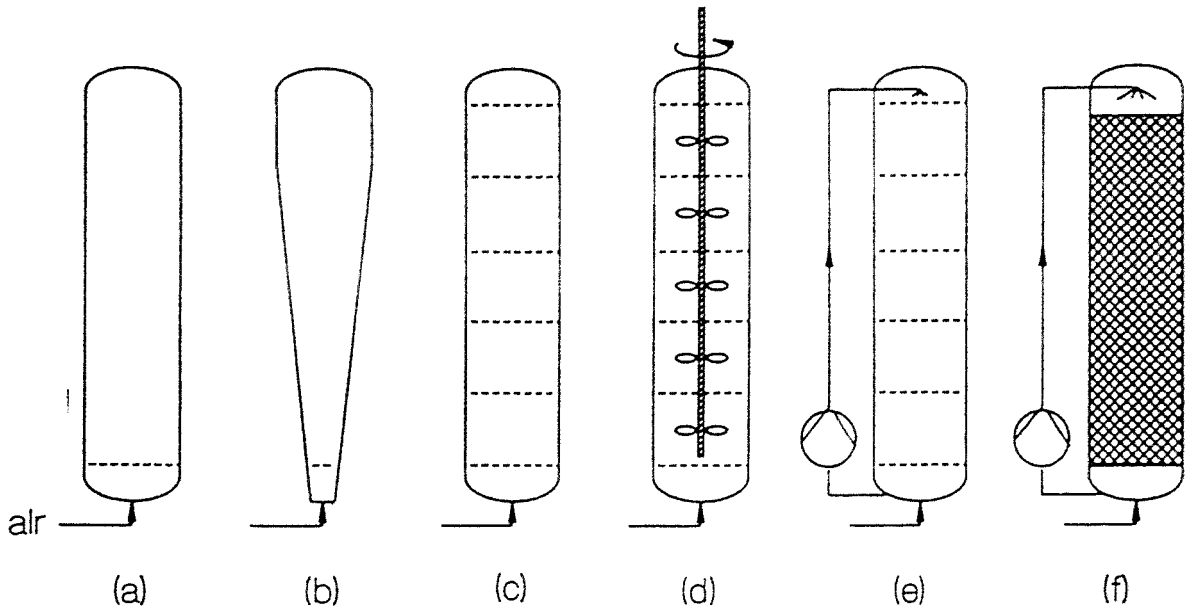


Figure 6.19 Column fermenters: (a) bubble column, (b) tapered bubble column, (c) sieve-tray bubble column, (d) multistage stirred column, (e) sieve-tray column with external pumping, and (f) packed-bed column with external pumping.

ing device at the bottom [Figure 6.19(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.

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 6.19(b)]