# QUIZ CE205

## Bioprocess Engineering – Part 2

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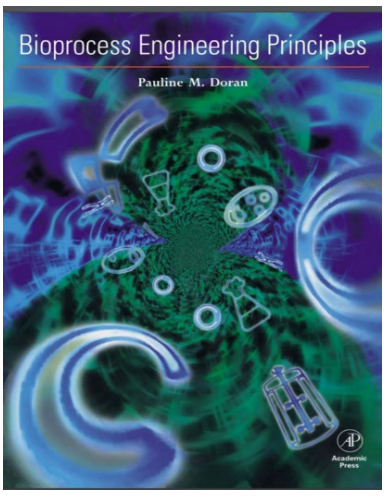
**Note**




Part 2 of our series of problems on bioprocess engineering is entirely derived from Pauline M. Doran's 1997 textbook *Bioprocess Engineering Principles*. Problems were reproduced with the permission of Elsevier Limited, Radarweg 29, 1043 NX Amsterdam, The Netherlands. This seminal text is, in my opinion, the best introductory treaty on biochemical engineering available on the market. I've included an Amazon link to the second edition on the website.

## PROBLEMS

### Problem 1.1 (Modified from Doran, 1997, w/ permission)

An enzyme is used to produce a compound used in the manufacturing of sunscreen lotion. $\mu_{max}$ for the enzyme is 2.75 mmol/(m³·s) and $K_M$ is 9.3 mmol/L. The initial concentration of substrate is 15 mmol/L. Plot the time required for batch reaction as a function of substrate conversion.

### Problem 1.2

The enzyme of the previous example deactivates with a half-life of 4.08 hr. Determine the batch reaction time required to achieve 90% conversion while considering enzyme deactivation.

**A)** $t_b$ = 4.11 h
**B)** $t_b$ = 5.38 h
**C)** $t_b$ = 6.36 h
**D)** $t_b$ = 7.04 h

### Problem 2.1 (Doran, 1997, w/ permission)

A strain of *Escherichia coli* has been genetically engineered to produce human protein. A batch culture is started by inoculating 12 g of cells into a 100-liter bubble column fermenter containing 10 g/L of glucose. The culture does not exhibit a lag phase. The maximum specific growth rate of the cells is 0.9 $h^{-1}$; the biomass yield from glucose is 0.575 g $g^{-1}$. Estimate the time needed to reach stationary phase.

**A)** $t_b$ = 1.81 h
**B)** $t_b$ = 2.54 h
**C)** $t_b$ = 3.11 h
**D)** $t_b$ = 4.32 h

### Problem 2.2

What will be the final cell density if the fermentation is stopped after only 70% of the substrate is consumed?

**A)** $C_{S,1}$ = 3.08 g/L
**B)** $C_{S,1}$ = 4.16 g/L
**C)** $C_{S,1}$ = 5.34 g/L
**D)** $C_{S,1}$ = 6.08 g/L

## ▶ Problem 3 (Doran, 1997, w/ permission)

6-Aminopenicillanic acid used to produce semi-synthetic penicillins is prepared by enzymatic hydrolysis of fermentation-derived penicillin G. Penicillin-G acylase immobilized in alginate is being considered for the process. The immobilized enzyme particles are sufficiently small so that mass transfer does not affect the reaction rate. The starting concentration of penicillin-G is 10% (w/v); because of the high cost of the substrate, 99% conversion is required. Under these conditions, enzymatic conversion of penicillin-G can be considered a first-order reaction. It has not been defined whether a batch, CSTR, or plug flow reactor would be most suitable. The downtime between batch reactions is expected to be 20 h. For the batch and CSTR reactors, the reaction rate constant is $0.8\times10^{-4}$ $s^{-1}$; in the PFR, the packing density of enzyme beads can be up to four times greater than in the other reactors. Determine the volume of the batch, CSTR, and plug flow reactors to accommodate this operation.

| 1. Batch reactor volume: | 2. CSTR volume: | 3. PFR volume: |
|---|---|---|
| **A)** $V$ = 16.5 m³ | **A)** $V$ = 102 m³ | **A)** $V$ = 0.596 m³ |
| **B)** $V$ = 29.9 m³ | **B)** $V$ = 157 m³ | **B)** $V$ = 0.944 m³ |
| **C)** $V$ = 36.2 m³ | **C)** $V$ = 203 m³ | **C)** $V$ = 1.83 m³ |
| **D)** $V$ = 43.3 m³ | **D)** $V$ = 244 m³ | **D)** $V$ = 3.61 m³ |

## ▶ Problem 4 (Doran, 1997, w/ permission)

*Pseudomonas methylotrophus* is used to produce single cell protein from methanol in a 1000-m³ pressure-cycle airlift fermenter. The biomass yield from substrate is 0.41 g $g^{-1}$, $K_S$ is 0.7 mg/L, and the maximum specific growth rate is 0.44 $h^{-1}$. The medium contains 4% (w/v) methanol. A substrate conversion of 98% is desirable. The reactor may be operated in either batch or continuous mode. If operated in batch, an inoculum of 0.01% (w/v) is used and the downtime between batches is 20 h. If continuous operations are used at steady state, a downtime of 25 days is expected per year. Neglecting maintenance requirements, compare the annual biomass production achieved using batch and continuous reactors.

**A)** The annual production from batch culture is about 6.5 times greater than the production from a continuous reactor.

**B)** The annual production from batch culture is about 12.7 times greater than the production from a continuous reactor.

**C)** The annual production from a continuous reactor is about 6.5 times greater than the production from batch culture.

**D)** The annual production from a continuous reactor is about 12.7 times greater than the production from batch culture.

## ▶ Problem 5 (Doran, 1997, w/ permission)

*Nicotiana tabacum* cells are cultured to high density for production of polysaccharide gum. The reactor used is a stirred tank that initially contains 100 liters of medium. The maximum specific growth rate of the culture is 0.18 $day^{-1}$ and the yield of biomass from substrate is 0.5 g $g^{-1}$. The concentration of growth-limiting substrate in the medium is 3% (w/v). The reactor is inoculated with 1.5 g/L of cells and operated in batch until the substrate is virtually exhausted; medium flow is then started at a rate of 4 L $day^{-1}$. Fed-batch operation is carried out for 40 days under quasi-steady-state conditions. True or false?

**1.( )** The batch culture time is greater than 12 days.

**2.( )** The final biomass concentration after the batch culture period is greater than 19 g/L.

**3.( )** The final mass of cells in the reactor is greater than 5.0 kg.

**4.( )** The fermenter is available 275 days per year with a downtime between runs of 24 h. Accordingly, the plant cell biomass produced annually is greater than 16 kg.

## ➔ Problem 6.1 (Doran, 1997, w/ permission)

Aspartase enzyme is used industrially for the manufacture of aspartic acid, a component of low-calorie sweetener. Fumaric acid ($C_4H_4O_4$) and ammonia are converted to aspartic acid ($C_4H_7O_4N$) according to the reaction

$$C_4H_4O_4 + NH_3 \rightleftarrows C_4H_7O_4N$$

Under investigation is a process using aspartase in intact *Bacillus cadaveris* cells. In the substrate range of interest, the conversion can be described using Michaelis-Menten kinetics with $K_S$ = 4.0 g/L. The substrate solution contains 15% (w/v) ammonium fumarate; enzyme is added in the form of lyophilized cells and the reaction is stopped when 85% of the substrate is converted. At 32°C, $\mu_{max}$ for the enzyme is 5.9 g $L^{-1}h^{-1}$ and its half-life is 10.5 days. At 37°C, $\mu_{max}$ increases to 8.5 g $L^{-1}h^{-1}$, but the half-life is reduced to 2.3 days. What temperature would you recommend?

**α)** The process should be carried out at 32°C.
**β)** The process should be carried out at 37°C.
**γ)** There is not enough information.

## ➔ Problem 6.2

The average downtime between batch reactions is 28 h. At the temperature chosen in the previous problem, calculate the reactor volume required to produce 5000 tonnes of aspartic acid per year.

**A)** $V$ = 51.2 m³
**B)** $V$ = 103 m³
**C)** $V$ = 149 m³
**D)** $V$ = 204 m³

## ▶ Problem 7 (Doran, 1997, w/ permission)

An *Enterobacter aerogenes* auxotroph capable of overproducing threonine has been isolated. The kinetic and yield parameters for this organism are investigated using a 2-liter chemostat fed with medium containing 10 g/L glucose. Steady-state cell and substrate concentrations are measured at a range of reactor flow rates.

| Flow Rate (L/h) | Cell Concentration (g/L) | Substrate Conc. (g/L) |
|---|---|---|
| 1 | 3.15 | 0.01 |
| 1.4 | 3.22 | 0.038 |
| 1.6 | 3.27 | 0.071 |
| 1.7 | 3.26 | 0.066 |
| 1.8 | 3.21 | 0.095 |
| 1.9 | 3.1 | 0.477 |

Determine the maximum specific growth rate, the saturation constant, the maintenance coefficient, and the true biomass yield from glucose for this culture. Use the Langmuir model. True or false?

**1.( )** The maximum specific growth rate ($\mu_{max}$) is greater than 0.8 $h^{-1}$.
**2.( )** The saturation constant ($K_S$) is greater than 0.03 g/L.
**3.( )** The maintenance coefficient ($m_S$) is greater than 0.2 g $g^{-1}$ $h^{-1}$.
**4.( )** The true biomass yield from glucose ($Y_{X/S}$) is greater than 0.25 g/g.

## ▶ Problem 8 (Doran, 1997, w/ permission)

*Lactobacillus casei* is propagated under essentially anaerobic conditions to provide a starter culture for manufacture of Swiss cheese. The culture produces lactic acid as a byproduct of energy metabolism. The system has the following characteristics:

| |
|---|
| Biomass yield from substrate, $Y_{X/S}$ = 0.23 kg kg$^{-1}$ |
| Saturation constant, $K_S$ = 0.15 kg/m$^3$ |
| Maximum growth rate, $\mu_{\max}$ = 0.35 h$^{-1}$ |
| Maintenace coefficient, $m_S$ = 0.135 kg kg$^{-1}$ h$^{-1}$ |

A stirred fermenter is operated in fed-batch mode under quasi-steady-state conditions with a feed flow rate of 4 m$^3$/h and feed substrate concentration of 80 kg/m$^3$. After 6 h, the liquid volume is 40 m$^3$. True or false?

**1.( )** The initial culture volume was greater than 20 m$^3$.

**2.( )** The concentration of substrate at quasi-steady-state conditions is greater than 0.05 kg/m$^3$.

**3.( )** The concentration of cells at quasi-steady-state conditions is greater than 15.0 kg/m$^3$.

**4.( )** The mass of cells produced during 6 h of fed-batch operation is greater than 300 kg.

## ▶ Problem 9 (Doran, 1997, w/ permission)

A two-stage chemostat system is used for production of secondary metabolite. The volume of each reactor is 0.5 m$^3$; the flow rate of feed is 50 L/h. Mycelial growth occurs in the first reactor; the second reactor is used for product synthesis. The concentration of substrate in the feed is 10 g/L. Kinetic and yield parameters for the organism are given below.

| |
|---|
| True biomass yield from substrate $Y_{X/S}$ = 0.5 kg kg$^{-1}$ |
| Saturation constant $K_S$ = 1.0 kg/m$^3$ |
| Maximum growth rate, $\mu_{\max}$ = 0.12 h$^{-1}$ |
| Maintenance constant, $m_S$ = 0.025 kg kg$^{-1}$ h$^{-1}$ |
| Rate of product formation not linked to energy metabolism, $q_P$ = 0.16 kg kg$^{-1}$ h$^{-1}$ |
| True product yield from substrate $Y_{P/S}$ = 0.85 kg kg$^{-1}$ |

Assume that product synthesis is negligible in the first reactor and growth is negligible in the second reactor. True or false?

**1.( )** The substrate concentration entering the second reactor is greater than 6.0 kg/m$^3$.

**2.( )** The cell concentration entering the second reactor is greater than 2.6 kg/m$^3$.

**3.( )** The overall substrate conversion for the two reactors is greater than 90%.

**4.( )** The final concentration of product is greater than 3.2 kg/m$^3$.

## ▶ Problem 10 (Doran, 1997, w/ permission)

An enzyme is used to convert substrate to a commercial product in a 1600-L batch reactor. $\mu_{\max}$ for the enzyme is 0.9 g/(L·hr); $K_S$ is 1.5 g/L. The substrate concentration at the start of the reaction is 3 g/L. According to the reaction stoichiometry, conversion of 1 g of substrate produces 1.2 g of product. The cost of operating the reactor including labor, maintenance, energy, and other utilities is estimated at $4800 per day. The cost of recovering the product depends on the substrate conversion achieved and the resulting concentration of product in the final reaction mixture. For conversions between 70% and 100%, the cost of downstream processing can be approximated using the equation $Q = 155 - 0.33X$, where $Q$ is the cost in $ per kg of product treated and $X$ is the percentage substrate conversion. Product losses during processing are negligible. The market price for the product is $750/kg. Currently, the enzyme reactor is operated with 75% substrate conversion; however, it was proposed that the substrate conversion be increased to 90%. Estimate the effect that this will have on the economics of the process.

## ▶ Problem 11 (Modified from Doran, 1997, w/ permission)

Liquid medium at a flow rate of 1.6 $m^3/h$ is to be sterilized by heat exchange with steam in a continuous sterilizer. The medium contains bacterial spores at a concentration of $8.68\times10^{11}$ $m^{-3}$. Values of the activation energy and Arrhenius constant for thermal destruction of these contaminants are 254 kJ g $mol^{-1}$ and $5.5\times10^{39}$ $h^{-1}$, respectively. A contamination risk of one organism surviving every 60 days of operation is considered acceptable. The sterilizer pipe has an inner diameter of 0.14 m and the length of the holding section is 28 m. The density of the medium is 1000 kg/$m^3$ and the viscosity is 4.0 kg $m^{-1}$ $h^{-1}$. What sterilizing temperature is required? Use the experimental curve in Figure 1.

**A)** $T$ = 32°C
**B)** $T$ = 48°C
**C)** $T$ = 61°C
**D)** $T$ = 81°C

## ➜ Problem 12.1 (Doran, 1997, w/ permission)

A 15-$m^3$ chemostat is operated with a dilution rate of 0.1 $h^{-1}$. A continuous sterilizer with steam injection and flash cooling delivers sterilized medium to the fermenter. Medium in the holding section of the sterilizer is maintained at 130°C. The concentration of contaminants in the raw medium is $10^5$ mL; an acceptable contamination risk is one organism every 3 months. The Arrhenius constant and activation energy for thermal death are estimated as $7.5\times10^{39}$ $h^{-1}$ and 288.5 kJ g $mol^{-1}$, respectively. The inner diameter of the sterilizer pipe is 12 cm. At 130°C, the liquid density is 1000 kg/$m^3$ and the viscosity is 4 kg $m^{-1}$ $h^{-1}$. Assuming perfect plug flow, determine the length of the holding section.

**A)** $L$ = 2.34 m
**B)** $L$ = 6.31 m
**C)** $L$ = 14.2 m
**D)** $L$ = 18.1 m

## ➜ Problem 12.2

What length is required if axial dispersion effects are taken into account? Use the experimental curve in Figure 1.

## ➜ Problem 12.3

If the sterilizer is constructed with the length determined in Part 1 and operated at 130°C as planned, estimate the frequency of fermenter contamination.

**A)** One contaminant enters the fermenter every 28 minutes.
**B)** One contaminant enters the fermenter every 41 minutes.
**C)** One contaminant enters the fermenter every 67 minutes.
**D)** One contaminant enters the fermenter every 84 minutes.

## ADDITIONAL INFORMATION

**Figure 1** Correlation for determining the axial dispersion coefficient in turbulent pipe flow. $Re$ is the Reynolds number, $D$ is the pipe diameter, $u$ is the average linear fluid velocity, $\rho$ is fluid density, $\mu$ is fluid viscosity, and $\mathcal{D}_z$ is the axial dispersion coefficient.

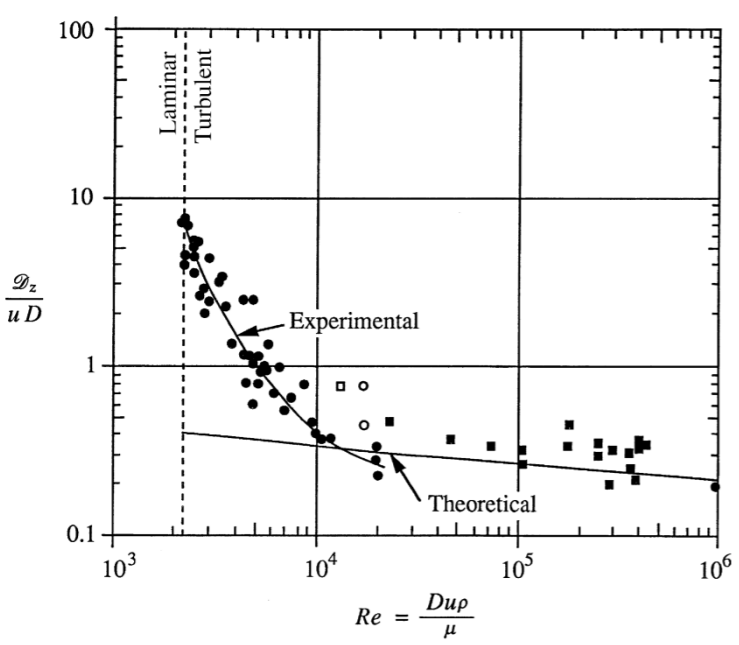
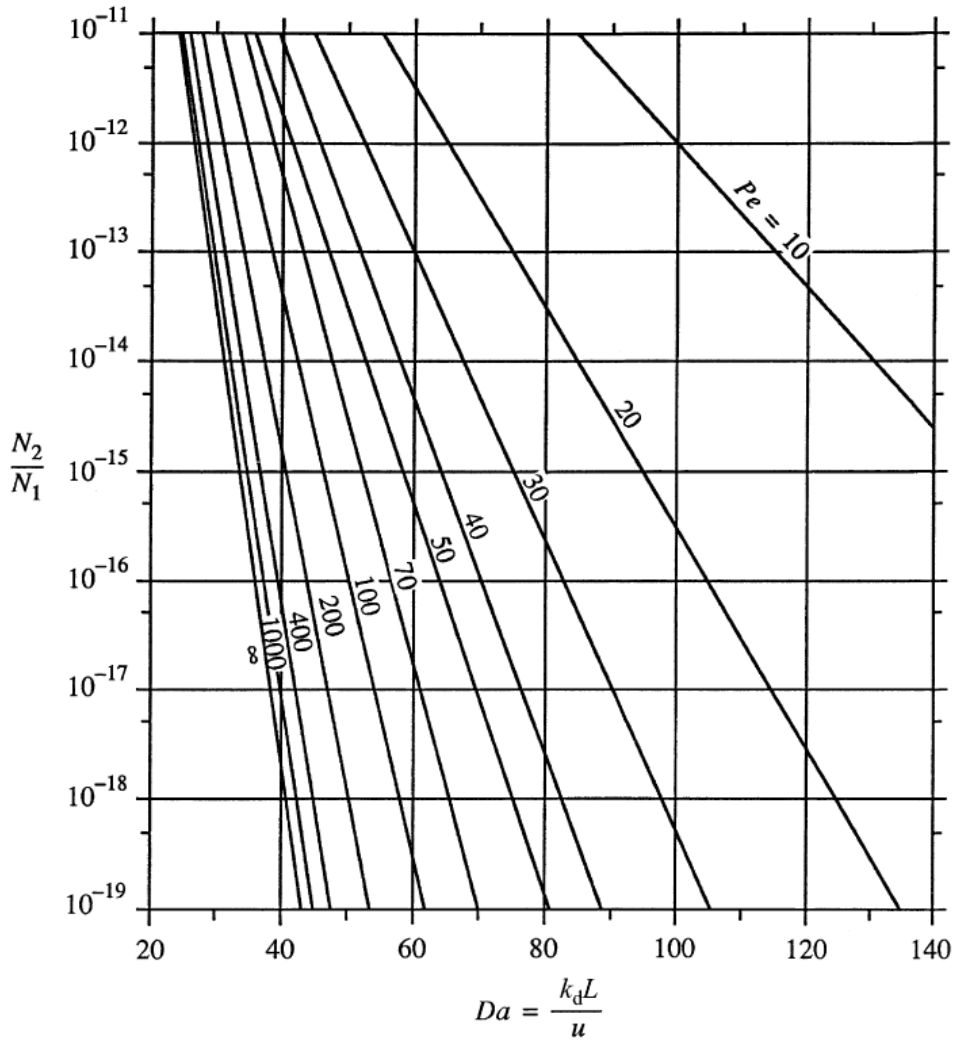


**Figure 2** Thermal destruction of contaminating organisms as a function of Peclet number $Pe$ and Damköhler number $Da$. $N_1$ is the number of viable cells entering the holding section of the sterilizer; $N_2$is the number of cells leaving.




**Equations**

**Eq. 1** → *Batch reaction time for a stirred batch enzyme reactor*

$$t_b = \frac{K_M}{\mu_{\max}} \ln\left(\frac{C_{S,0}}{C_{S,1}}\right) + \frac{C_{S,0} - C_{S,1}}{\mu_{\max}}$$

**where** $K_M$ is the Michaelis constant, $\mu_{\max}$ is the maximal growth rate, $C_{S,0}$ is the initial substrate concentration, and $C_{S,1}$ is the final substrate concentration.

**Eq. 2** → *Batch reaction time for a stirred batch enzyme reactor with enzyme deactivation*

$$t_b = -\frac{1}{k_d} \ln\left\{1 - k_d\left[\frac{K_M}{\mu_{\max,0}} \ln\left(\frac{C_{S,0}}{C_{S,1}}\right) + \frac{C_{S,0} - C_{S,1}}{\mu_{\max,0}}\right]\right\}$$

**where** $K_M$ is the Michaelis constant, $\mu_{\max,0}$ is the maximal growth rate before deactivation occurs, $k_d$ is the first-order deactivation rate constant, $C_{S,0}$ is the initial substrate concentration, and $C_{S,1}$ is the final substrate concentration.

**Eq. 3** → *Batch reaction time for cell culture in a batch reactor*

$$t_b = \frac{1}{\mu_{\max}} \ln\left[1 + \frac{C_{S,0} - C_{S,1}}{\left(\frac{1}{Y_{X/S}} + \frac{q_P}{\mu_{\max} Y_{P/S}} + \frac{m_S}{\mu_{\max}}\right) C_{X,0}}\right]$$

**where** $\mu_{\max}$ is the maximum growth rate, $Y_{X/S}$ is the true biomass yield from substrate, $q_P$ is the specific rate of product formation not directly linked with energy metabolism, $Y_{P/S}$ is the true product yield from substrate, $m_S$ is the maintenance coefficient, $C_{X,0}$ is the initial cell concentration, $C_{S,0}$ is the initial substrate concentration, and $C_{S,1}$ is the final substrate concentration. If no product is formed or if production is directly linked with energy metabolism, the equation simplifies to

$$t_b = \frac{1}{\mu_{\max}} \ln \left[ 1 + \frac{C_{S,0} - C_{S,1}}{\left( \frac{1}{Y_{X/S}} + \frac{m_S}{\mu_{\max}} \right) C_{X,0}} \right]$$

If maintenance requirements can be neglected, the equation is further simplified to

$$t_b = \frac{1}{\mu_{\max}} \ln \left[ 1 + \frac{Y_{X/S}}{C_{X,0}} \left( C_{S,0} - C_{S,1} \right) \right]$$

**Eq. 4** → *Variation of substrate concentration in a fed-batch reactor*

$$\frac{dC_S}{dt} = D\left( C_{S,0} - C_{S,1} \right) - \left( \frac{\mu}{Y_{X/S}} + \frac{q_P}{Y_{P/S}} + m_S \right) C_X$$

**where** $D$ is the dilution rate, $C_{S,0}$ is the initial substrate concentration, $C_{S,1}$ is the final substrate concentration, $\mu$ is growth rate, $Y_{X/S}$ is the true biomass yield from substrate, $q_P$ is the specific rate of product formation not directly linked with energy metabolism, $Y_{P/S}$ is the true product yield from substrate, $m_S$ is the maintenance coefficient, and $C_X$ is the cell concentration.

**Eq. 5** → *Cell mass in a fed-batch reactor*

$$X_1 = X_0 + Y_{X/S} C_{S,0} F t_{FB}$$

**where** $X_0$ is the initial cell mass, $Y_{X/S}$ is the true biomass yield from substrate, $F$ is the feed rate, and $t_{FB}$ is the fed-batch time after commencement of feeding.

**Eq. 6** → *Cell concentration in a CSTR*

$$C_X = \frac{D\left( C_{S,0} - C_{S,1} \right)}{\frac{D}{Y_{X/S}} + \frac{q_P}{Y_{P/S}} + m_S}$$

**where** $D$ is the dilution rate, $C_{S,0}$ is the initial substrate concentration, $C_{S,1}$ is the final substrate concentration, $Y_{X/S}$ is the true biomass yield from substrate, $q_P$ is the specific rate of product formation not directly linked with energy metabolism, $Y_{P/S}$ is the true product yield from substrate, and $m_S$ is the maintenance coefficient. If there is no product synthesis or if production is directly linked with energy metabolism, the equation simplifies to

$$C_X = \frac{D\left( C_{S,0} - C_{S,1} \right)}{\frac{D}{Y_{X/S}} + m_S}$$

If maintenance effects are ignored, the equation becomes simply

$$C_X = \left( C_{S,0} - C_{S,1} \right) Y_{X/S}$$

**Eq. 7** → *Steady-state product concentration as a function of biomass concentration*

$$C_{P,1} = C_{P,0} + \frac{q_P C_X}{D}$$

**where** $C_{P,0}$ is the initial product concentration, $q_P$ is the specific rate of product formation, $C_X$ is the biomass concentration, and $D$ is dilution rate.

# SOLUTIONS

## P.1 ➡ *Solution*

**Part 1:** As a first step, we convert the units of $\mu_{\max}$ to mmol/h,

$$\mu_{\max} = 2.75 \frac{\text{mmol}}{\text{m}^3 \cdot \text{sec}} \times \frac{3600}{1} \frac{\text{sec}}{\text{hr}} \times \frac{1}{1000} \frac{\text{m}^3}{\text{L}} = 9.9 \text{ mmol/h}$$

The batch reaction time required to reduce the substrate concentration from $C_{S,0}$ to $C_{S,1}$ is given by equation 1,

$$t_b = \frac{K_M}{\mu_{\max}} \ln\left(\frac{C_{S,0}}{C_{S,1}}\right) + \frac{C_{S,0} - C_{S,1}}{\mu_{\max}} \quad \text{(I)}$$

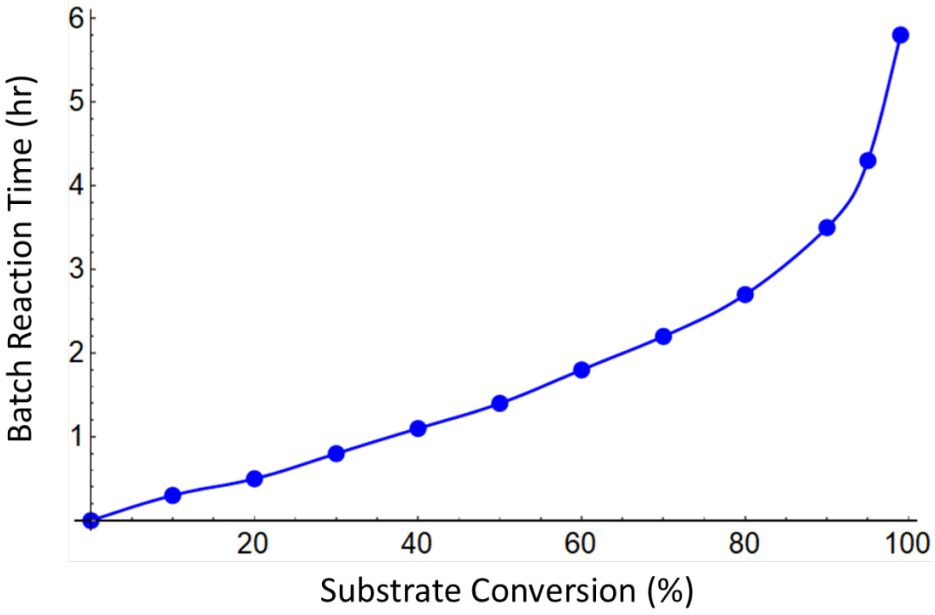
For example, with a substrate conversion of 10%, we get $C_{S,1}$ = 13.5 mmol/L. Thus,

$$t_b = \frac{9.3}{9.9} \ln\left(\frac{15}{13.5}\right) + \frac{15 - 13.5}{9.9} = 0.250 \text{ h}$$

Values of $t_b$ for other conversions tabulated below.

| Substrate Conversion (%) | $C_{S,1}$ (mM) | $t_b$ (hr) |
|---|---|---|
| 0 | 15 | 0.0 |
| 10 | 13.5 | 0.3 |
| 20 | 12 | 0.5 |
| 30 | 10.5 | 0.8 |
| 40 | 9 | 1.1 |
| 50 | 7.5 | 1.4 |
| 60 | 6 | 1.8 |
| 70 | 4.5 | 2.2 |
| 80 | 3 | 2.7 |
| 90 | 1.5 | 3.5 |
| 95 | 0.75 | 4.3 |
| 99 | 0.15 | 5.8 |

We aim for a plot of batch reaction time ($t_b$, the red column) versus substrate conversion (the blue column), as shown in continuation.




The data and graph show that 50% conversion calls for a batch reaction time of 1.4 hours. Achieving a conversion of 90% requires a $t_b$ of 3.5 hours, and 99% conversion calls for a nearly 6-hour-long operation.

**Part 2:** The batch reaction time with enzyme deactivation is given by equation 2,

$$t_b = -\frac{1}{k_d} \ln\left\{1 - k_d \left[\frac{K_M}{\mu_{\max,0}} \ln\left(\frac{C_{S,0}}{C_{S,1}}\right) + \frac{C_{S,0} - C_{S,1}}{\mu_{\max,0}}\right]\right\}$$

where $k_d$ is the first-order deactivation rate constant, which can be estimated from the enzyme's half-life,

$$k_d = \frac{\ln 2}{t_{1/2}} = \frac{0.693}{4.08} = 0.170 \text{ h}^{-1}$$

Substituting in the equation for $t_b$, we have, noting that $C_{S,1}$ = 1.5 mM for 90% conversion,

$$t_b = -\frac{1}{0.17} \ln\left\{1 - 0.17 \times \left[\frac{9.3}{9.9} \ln\left(\frac{15}{1.5}\right) + \frac{15 - 1.5}{9.9}\right]\right\} = \boxed{5.38 \text{ h}}$$

This represents an increase of nearly 54% relatively to the time required to achieve the desired conversion with no enzyme deactivation.

♦ The correct answer is **B.**

## P.2 ➡ *Solution*

**Part 1:** The initial concentration is $C_{X,0}$ = 12 g/100 L = 0.12 g/L. Assume that the stationary phase is reached when $C_{S,1}$ = 0. The batch culture time can be determined with equation 3,

$$t_b = \frac{1}{\mu_{\max}} \ln\left[1 + \frac{Y_{X/S}}{C_{X,0}}\left(C_{S,0} - C_{S,1}\right)\right] = \frac{1}{0.9} \ln\left[1 + \frac{0.575}{0.12} \times (10 - 0)\right] = \boxed{4.32\,\text{h}}$$

The culture should reach stationary phase within approximately 4 hours and 20 minutes.

♦ The correct answer is **D.**

**Part 2:** If only 70% of the substrate is consumed, $C_{S,1} = 0.3C_{S,0} = 0.3 \times 10 =$ 3 g/L. Appealing to the same formula as before yields

$$t_b = \frac{1}{0.9} \ln\left[1 + \frac{0.575}{0.12} \times (10 - 3)\right] = 3.94\ \text{h}$$

The biomass density at this time follows as

$$C_{S,1} = C_{S,0} \exp\left(\mu_{\max} t_b\right) = 0.12 \times \exp\left(0.9 \times 3.94\right) = \boxed{4.16\ \text{g/L}}$$

♦ The correct answer is **B.**

## P.3 ➡ *Solution*

**Part 1**: We have $C_{S,0}$ = 10% (w/v) = 10 g per 100 mL = 100 g/L = 100 kg/m³ and $C_{S,1}$ = 0.01 × 100 = 1 kg/m³. The equation for the rate of change of substrate in a batch reactor is

$$\frac{d\left(VC_S\right)}{dt} = -k_1 C_S V$$

where $V$ is the reactor volume and $k_1$ is the reaction rate constant. As $V$ can be considered constant in a batch reactor, this term can be taken outside of the derivative in the left-hand side and cancelled, giving

$$\frac{dC_S}{dt} = -k_1 C_S$$

We then separate variables and integrate to obtain

$$\frac{dC_S}{dt} = -k_1 C_S \rightarrow \frac{dC_S}{C_S} = -k_1 dt$$

$$\therefore \int \frac{dC_S}{C_S} = -\int k_1 dt$$

$$\therefore \ln C_S = -k_1 t + K$$

where $K$ is a constant. In view of the initial condition $C_S = C_{S,0}$ at $t$ = 0, it is easy to see that $K = \ln C_{S,0}$. Thus,

$$\ln C_S = -k_1 t + \ln C_{S,0}$$

$$\therefore \ln \frac{C_S}{C_{S,0}} = -k_1 t$$

$$\therefore t = -\frac{\ln\left(C_S / C_{S,0}\right)}{k_1}$$

Substituting $C_S = C_{S,1}$ = 1 kg/m³, $C_{S,0}$ = 100 kg/m³, and $k_1 = 0.8 \times 10^{-4}$ s$^{-1}$ gives

$$t = -\frac{\ln\left(1/100\right)}{0.8 \times 10^{-4} \times 3600} = 16.0\ \text{h}$$

A factor of 3600 was included to convert seconds to hours. If the downtime between batches is $t_{dn}$ = 20 h, the total time required to process each batch is calculated as

$$t_T = t_b + t_{dn} = 16.0 + 20 = 36 \text{ h}$$

Therefore, in one year, the number of batches carried out is

$$\text{Number of batches} = \frac{365 \text{ d} \times \frac{24}{1} \frac{\text{h}}{\text{d}}}{36 \text{ h per batch}} = 243$$

To treat 400 tonnes of penicillin G annually, the mass of penicillin G yielded per batch must be

$$\text{Mass of penicillin G treated per batch} = \frac{400,000}{243} = 1650 \text{ kg}$$

As the concentration of penicillin G added to the reactor is 100 kg/m$^3$, the reactor volume is calculated to be

$$V = \frac{1650 \text{ kg}}{100 \text{ kg} \cdot \text{m}^{-3}} = \boxed{16.5 \text{ m}^3}$$

The batch reactor volume required to produce the desired amount of penicillin is 16.5 m³.

♦ The correct answer is **A.**

**Part 2**: For a CSTR operated under steady state conditions, $F_0 = F_1 = F$, $V$ is constant, and $dC_S/dt = 0$. Applying a mass balance equation for a first-order reaction such as the present one yields

$$0 = FC_{S,0} - FC_S - k_1 C_S V$$

Manipulating the equation brings to

$$\frac{F}{V}\left(C_{S,0} - C_S\right) - k_1 V = 0 \rightarrow \frac{F}{V} = \frac{k_1 C_S}{C_{S,0} - C_S}$$

$$\therefore \frac{F}{V} = \frac{\left(0.8 \times 10^{-4}\right) \times 1.0}{100 - 1.0} = 8.08 \times 10^{-7} \text{ s}^{-1}$$

The flow rate of penicillin G into the CSTR is 400 tonnes per year. Given the concentration of substrate in the feed stream $C_{S,0}$ = 100 kg/m³, the total volumetric flow rate of the feed stream $F$ is

$$F = \frac{400,000 \frac{\text{kg}}{\text{year}} \times \frac{1}{365} \frac{\text{year}}{\text{d}} \times \frac{1}{24} \frac{\text{d}}{\text{h}} \times \frac{1}{3600} \frac{\text{h}}{\text{s}}}{100 \frac{\text{kg}}{\text{m}^3}} = 1.27 \times 10^{-4} \text{ m}^3/\text{s}$$

Substituting this result into the equation for $F/V$, the reactor volume is calculated to be

$$\frac{F}{V} = 8.08 \times 10^{-7} \rightarrow V = \frac{F}{8.08 \times 10^{-7}}$$

$$\therefore V = \frac{1.27 \times 10^{-4}}{8.08 \times 10^{-7}} = \boxed{157 \text{ m}^3}$$

The CSTR volume required to execute the operation is 157 m³.

♦ The correct answer is **B.**

**Part 3**: For the PFTR, if the density of the enzyme beads is four times greater than in the other reactors, we write $k_1$ = 4 × (0.8×10$^{-4}$) = 3.2×10$^{-4}$ s$^{-1}$. The differential equation for change in substrate concentration with position in the reactor for first-order kinetics is

$$u \frac{dC_S}{dz} = -k_1 C_S$$

where $u$ is the superficial velocity from the column and $z$ is the distance from the feed point. Separating variables and integrating, we obtain

$$u \frac{dC_S}{dz} = -k_1 C_S \rightarrow \frac{dC_S}{C_S} = -\frac{k_1}{u} dz$$

$$\therefore \int \frac{dC_S}{C_S} = \int -\frac{k_1}{u} dz$$

$$\therefore \ln C_S = -\frac{k_1}{u} z + K$$

The boundary condition is $C_S = C_{S,0}$ at $z = 0$. Applying this condition to the result above gives $K = \ln C_S$. Thus,

$$\ln C_S = -\frac{k_1}{u} z + \ln C_{S,0}$$

$$\therefore \ln \frac{C_S}{C_{S,0}} = -\frac{k_1}{u} z$$

At the end of the PFTR, $z = L$ and $C_S = C_{S,1}$, with the result that

$$\ln \frac{C_{S,1}}{C_{S,0}} = -\frac{k_1}{u} L$$

Applying the definition of reactor residence time, $\tau$, the relation above becomes

$$\ln \frac{C_{S,1}}{C_{S,0}} = -k_1 \tau$$

Solving for $\tau$ and substituting,

$$\ln \frac{C_{S,1}}{C_{S,0}} = -k_1 \tau \rightarrow \tau = -\frac{\ln\left(C_{S,1}/C_{S,0}\right)}{k_1}$$

$$\therefore \tau = -\frac{\ln\left(1/100\right)}{\left(3.2\times10^{-4}\right)\times 3600} = 4.0 \text{ h}$$

The factor 3600 was included to give the result in hours, not seconds. Note that this is ¼ of the value obtained for the batch reaction time $t_b$, as expected from the analogous kinetic characteristics of batch and PFTR reactors and the 4 × higher value of $k_1$ in the PFTR. As calculated for the CSTR, $F = 1.27\times10^{-4}$ m³/s. From the definition of $\tau$, it follows that

$$V = \tau F \rightarrow V = 4.0 \text{ h} \times \frac{3600}{1.0} \frac{\text{s}}{\text{h}} \times 1.27\times10^{-4} \frac{\text{m}^3}{\text{s}} = \boxed{1.83 \text{ m}^3}$$

♦ The correct answer is **C**.

## P.4 ➡ *Solution*

The initial substrate concentration is $C_{S,0}$ = 4% (w/v) = 4 g per 100 mL = 40 g/L = 40 kg/m³, and the final substrate concentration is $C_{S,1}$ = 0.02×40 = 0.8 kg/m³. For the batch reactor, $C_{X,0}$ = 0.01% (w/v) = 0.1 kg/m³. The batch culture time is given by equation 3,

$$t_b = \frac{1}{\mu_{\max}} \ln\left[1 + \frac{Y_{X/S}}{C_{X,0}}\left(C_{S,0} - C_{S,1}\right)\right] = \frac{1}{0.44} \times \ln\left[1 + \frac{0.41}{0.1} \times \left(40 - 0.8\right)\right] = 11.6 \text{ h}$$

The biomass density at this time follows as

$$C_{X,1} = C_{X,0} \exp\left(\mu_{\max} t_b\right) = 0.1 \times \exp\left(0.44 \times 11.6\right) = 16.5 \text{ kg/m}^3$$

The mass of cells produced per batch is

$$X = \left(C_{X,1} - C_{X,0}\right) V = \left(16.5 - 0.1\right) \times 1000 = 16{,}400 \text{ kg}$$

If the downtime between batches is 20 h, the total time required to process a single batch is determined as

$$t_T = t_b + t_{\text{dn}} = 11.6 + 20 = 31.6 \text{ h}$$

The number of batches processed in one year follows as

$$\text{Number of batches} = \frac{365 \times 24}{31.6} = 277$$

Therefore, the total annual biomass production of a batch reactor is 16,400 × 277 = 4.54×10$^6$ kg, or 4540 tonnes. Consider now a continuous reactor. In this case, the steady-state cell concentration is given by

$$C_X = \left(C_{S,0} - C_{S,1}\right) Y_{X/S} = \left(40 - 0.8\right) \times 0.41 = 16.1 \text{ kg/m}^3$$

The dilution rate $D$ that corresponds to $C_{S,1}$ = 0.8 kg/m³ is determined next,

$$D = \mu = \frac{\mu_{\max} C_{S,1}}{K_S + C_{S,1}} = \frac{0.44 \times 0.8}{\left(0.7 \times 1000 \times \dfrac{1}{10^6}\right) + 0.8} = 0.440 \text{ h}^{-1}$$

In the denominator of the equation above, factors 1000 and 1/10$^6$ were included to convert $K_S$ from mg/L to kg/m³. From the definition of dilution rate, the volumetric feed into the reactor is calculated as

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

$$\therefore F = 0.440 \times 1000 = 440 \text{ m}^3/\text{h}$$

This corresponds to a biomass production $F \times C_X$ = 440 × 16.1 = 7080 kg/h. The number of days per year available for continuous reactor operation is (365 − 25) = 340 d, or, equivalently, 340 × 24 = 8160 h. Accordingly, the total biomass produced per year is 7080 × 8160 = 5.78×10$^7$ kg, or 57,800 tonnes. This is 12.7 times the annual production from batch culture under the same conditions.

♦ The correct answer is **D**.

## P.5 ➡ *Solution*

**1.True.** The initial substrate concentration $C_{S,0}$ = 3% (w/v) = 3 g per 100 mL = 30 g/L. The batch culture time to achieve $C_{S,1}$ = 0 is determined with equation 3,

$$t_b = \frac{1}{\mu_{\max}} \ln\left[1 + \frac{Y_{X/S}}{C_{X,0}}\left(C_{S,0} - C_{S,1}\right)\right] = \frac{1}{0.18} \ln\left[1 + \frac{0.5}{1.5} \times \left(30 - 0\right)\right] = \boxed{13.3 \text{ d}}$$

The batch culture time is about 13 days and 7 hours.

**2.False.** The biomass density at 13.3 days is

$$C_{X,1} = C_{X,0} \exp\left(\mu_{\max} t_b\right) = 1.5 \exp\left(0.18 \times 13.3\right) = \boxed{16.4 \text{ g/L}}$$

**3.False.** The mass of cells at the start of fed-batch operation is equal to the final batch cell concentration multiplied by the initial medium volume,

$$X_0 = C_{X,1} V = 16.4 \times 100 = 1640 \text{ g}$$

The final mass of cells after 40 days of fed-batch culture is given by equation 5,

$$X_1 = X_0 + Y_{X/S} C_{S,0} F\, t_{FB} = 1640 + 0.5 \times 30 \times 4 \times 40 = 4040 \text{ g} = \boxed{4.04 \text{ kg}}$$

**4.True.** The mass of cells produced in each reactor run is equal to the final biomass minus the biomass used for inoculation,

$$\text{Biomass produced per run} = 4040 - 1.5 \frac{\text{g}}{\text{L}} \times 100 \text{ L} = 3890 \text{ g} = 3.89 \text{ kg}$$

The total reaction time is

$$t_T = t_b + t_{\text{fb}} + t_{\text{dn}}$$

where $t_b$ = 13.3 d is the reaction time, $t_{\text{fb}}$ = 40 d is the fed-batch operation time, and $t_{\text{dn}}$ = 24 h = 1 d is the fed-batch operation time. Thus,

$$t_T = 13.3 + 40 + 1 = 54.3 \text{ d}$$

In one year, the number of runs carried out is

$$\text{Number of runs} = \frac{275\ \text{d}}{54.3\ \text{d/run}} = 5.06 \approx 5$$

The total biomass produced annually is equal to the biomass produced per run multiplied by the number of runs per year,

$$\text{Biomass produced per year} = 3.89 \times 5 = \boxed{19.5\ \text{kg}}$$

## P.6 ➡ *Solution*

**Part 1:** The initial concentration of substrate is $C_{S,0}$ = 15% (w/v) = 15 g per 100 mL = 150 g/L. For 85% conversion, the final substrate concentration $C_{S,1}$ = 0.15×150 = 22.5 g/L. The deactivation rate constant at 32°C is

$$k_d = \frac{\ln 2}{t_{1/2}} = \frac{0.693}{10.5\ \text{day}} \times \frac{1}{24}\frac{\text{day}}{\text{h}} = 2.75 \times 10^{-3}\ \text{h}^{-1}$$

For an enzyme subject to deactivation, the batch reaction time is given by equation 2,

$$t_b = -\frac{1}{k_d}\ln\left\{1 - k_d\left[\frac{K_S}{\mu_{\max,0}}\ln\left(\frac{C_{S,0}}{C_{S,1}}\right) + \frac{C_{S,0} - C_{S,1}}{\mu_{\max,0}}\right]\right\}$$

$$\therefore t_b = -\frac{1}{2.75\times10^{-3}}\ln\left\{1 - 2.75\times10^{-3}\left[\frac{4.0}{5.9}\ln\left(\frac{150}{22.5}\right) + \frac{150-22.5}{5.9}\right]\right\} = 23.6\ \text{h}$$

At 37°C, the deactivation rate constant is

$$k_d = \frac{\ln 2}{t_{1/2}} = \frac{0.693}{2.3\ \text{day}} \times \frac{1}{24}\frac{\text{day}}{\text{h}} = 0.0126\,\text{h}^{-1}$$

The batch reaction time follows as

$$t_b = -\frac{1}{0.0126}\ln\left\{1 - 0.0126\left[\frac{4.0}{8.5}\ln\left(\frac{150}{22.5}\right) + \frac{150-22.5}{8.5}\right]\right\} = 17.7\ \text{h}$$

As the batch reaction time is lower at 37°C, we surmise that the recommended temperature is 37°C.

♦ The correct answer is **β**.

**Part 2:** Given the downtime $t_{dn}$ = 28 h, the total batch reaction time at 37°C is

$$t_T = t_b + t_{dn} = 17.7 + 28 = 45.7\ \text{h}$$

In one year, the number of batches carried out is

$$\text{No. of batches} = \frac{365\ \text{days} \times \dfrac{24}{1.0}\dfrac{\text{hour}}{\text{day}}}{45.7\ \text{h per batch}} = 192$$

In each batch, the mass of ammonium fumarate converted is 0.85×150 = 127.5 g/L multiplied by the reactor volume $V$. Therefore, the mass of substrates converted is 127.5$V$ g = 0.1275$V$ kg, where $V$ has units of liters. From the reaction stoichiometry, as the molecular masses of ammonium fumarate is very close to that of aspartic acid, the mass of aspartic acid produced is also 0.1275$V$ kg. After one year or 192 batches, the mass of aspartic acid produced is 0.1275$V$ × 192 = 24.5$V$ kg. Now, 5000 tonnes of aspartic acid correspond to $5\times10^6$ kg. To reach this target level, the reactor volume should be

$$24.5V = 5\times10^6$$

$$\therefore V = 204{,}000\ \text{L} = \boxed{204\ \text{m}^3}$$

♦ The correct answer is **D**.

## P.7 ➡ *Solution*

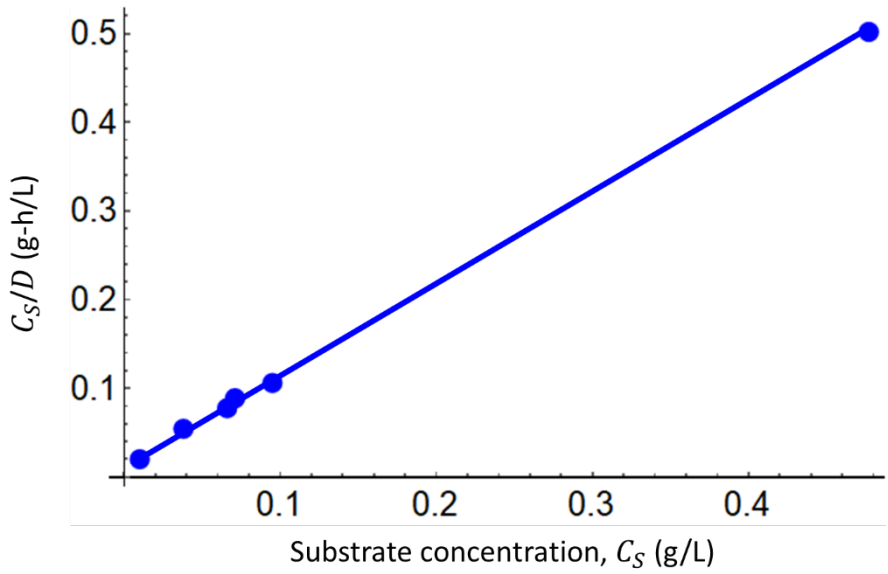
Using a Langmuir plot, which is represented by the equation

$$\frac{C_S}{D} = \frac{K_S}{\mu_{\max}} + \frac{C_S}{\mu_{\max}}$$

the values of $\mu_{\max}$ and $K_S$ can be determined from the slope and intercept of a graph of $C_S/D$ versus $C_S$. From the definition of dilution rate, $D = F/V$, values of $D$ are evaluated from the experimental flow rates using $V$ = 2 L = 2000 mL. The pertaining data are tabulated below.

| Flow Rate (L/h) | Dilution Rate ($h^{-1}$) | Substrate Conc. (g/L) | $C_S/D$ (g $L^{-1}$ h) |
|---|---|---|---|
| 1 | 0.5 | 0.010 | 0.0200 |
| 1.4 | 0.7 | 0.038 | 0.0543 |
| 1.6 | 0.8 | 0.071 | 0.0888 |
| 1.7 | 0.85 | 0.066 | 0.0776 |
| 1.8 | 0.9 | 0.095 | 0.106 |
| 1.9 | 0.95 | 0.477 | 0.502 |

We are looking for a plot of $C_S/D$, the red column, versus $C_S$, the blue column, as shown in continuation.




Fitting the data to a linear equation – for example, by using Mathematica's *LinearModelFit* function – we find that the data is represented by a line of the form $y$ = 1.0273$x$ + 0.01184. Comparing this with the equation for a Langmuir plot, we see that $1/\mu_{\max}$ = 1.0273 and, accordingly,

$$\frac{1}{\mu_{\max}} = 1.0273 \rightarrow \boxed{\mu_{\max} = 0.973 \text{ h}^{-1}}$$

In addition, the intercept of the line equals $K_S/\mu_{\max}$, whence we find the saturation constant as

$$\frac{K_S}{\mu_{\max}} = 0.01184 \rightarrow K_S = 0.01184 \times 0.973 = \boxed{0.0115 \text{ g/L}}$$

The next step is to establish the true biomass yield from glucose, $Y_{X/S}$, and the maintenance constant, $m_S$. To do so, we make use of the equation

$$\frac{1}{Y'_{X/S}} = \frac{1}{Y_{X/S}} + \frac{m_S}{D}$$

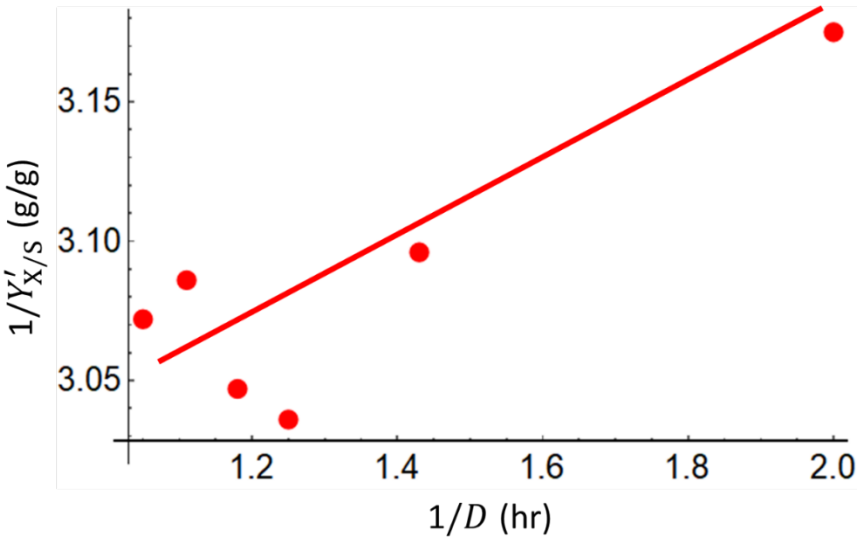
which implies that a plot of $1/Y'_{X/S}$ versus $1/D$ should produce a straight line. In a chemostat with sterile feed, the observed biomass yield from substrate, $Y'_{X/S}$, is given by

$$Y'_{X/S} = \frac{C_X}{C_{S,0} - C_{S,1}}$$

The data are tabulated below.

| Flow Rate (L/h) | $D$ ($h^{-1}$) | $1/D$ (h) | Cell Conc. (g/L) | Subst. Conc. (g/L) | $Y'xs$ (g/g) | $1/Y'xs$ (g/g) |
|---|---|---|---|---|---|---|
| 1 | 0.5 | 2.00 | 3.15 | 0.010 | 3.15/(10 - 0.01) = 0.315 | 3.175 |
| 1.4 | 0.7 | 1.43 | 3.22 | 0.038 | 3.22/(10 - 0.038) = 0.323 | 3.096 |
| 1.6 | 0.8 | 1.25 | 3.27 | 0.071 | 0.329 | 3.036 |
| 1.7 | 0.85 | 1.18 | 3.26 | 0.066 | 0.328 | 3.047 |
| 1.8 | 0.9 | 1.11 | 3.21 | 0.095 | 0.324 | 3.086 |
| 1.9 | 0.95 | 1.05 | 3.10 | 0.477 | 0.326 | 3.072 |

We are looking for a plot of $1/Y'_{X/S}$, the red column, versus $1/D$, the blue column, as follows.




The scatter in the plot is typical of measured values of $1/Y'_{X/S}$. Fitting the data to a line should yield $y = 0.1212x + 2.923$. Comparing this with the general form presented earlier, it is easy to see that the maintenance coefficient is

$$\boxed{m_s = 0.121 \text{ g g}^{-1}\text{h}^{-1}}$$

In addition, we have, from the intercept of the line,

$$\frac{1}{Y_{X/S}} = 2.923 \rightarrow \boxed{Y_{X/S} = 0.342 \text{ g g}^{-1}}$$

♦ Statements **1** and **4** are true, whereas statements **2** and **3** are false.

## P.8 ➡ *Solution*

**1.False**. Applying a volume balance to the system, we can easily obtain $V_0$,

$$V - V_0 = Ft \rightarrow V_0 = V - Ft$$
$$\therefore V_0 = 40 - 4.0 \times 6 = \boxed{16 \text{ m}^3}$$

**2.True**. From the definition of dilution rate, after 6 h of fed-batch operation when $V$ = 40 m³,

$$F = DV \rightarrow D = \frac{F}{V}$$
$$\therefore D = \frac{4}{40.0} = 0.1 \text{ h}^{-1}$$

The quasi-steady state substrate concentration is then

$$C_S = \frac{DK_S}{\mu_{\max} - D} = \frac{0.10 \times 0.15}{0.35 - 0.10} = \boxed{0.06 \text{ kg/m}^3}$$

**3.False**. Taking maintenance substrate requirements into account, for a specific rate of product formation $q_p$ = 0, a substrate concentration balance yields

$$\frac{dC_S}{dt} = D\left(C_{S,0} - C_{S,1}\right) - \left(\frac{\mu}{Y_{X/S}} + m_S\right)C_X$$

at quasi-steady-state conditions, we posit that $dC_S/dt \approx 0$, $\mu \approx D$, and $C_{S,1} \ll C_{S,0}$, reducing the equation above to

$$0 = DC_{S,0} - \left( \frac{D}{Y_{X/S}} + m_S \right) C_X$$

Solving for $C_X$ gives

$$C_X = \frac{DC_{S,0}}{\frac{D}{Y_{X/S}} + m_S}$$

which is one of the forms of equation 6. The quasi-steady state concentration of cells is then

$$C_X = \frac{DC_{S,0}}{\frac{D}{Y_{X/S}} + m_S} = \frac{0.1 \times 80}{\frac{0.1}{0.23} + 0.135} = \boxed{14.0 \text{ kg/m}^3}$$

**4.True**. After 6 h fed-batch operation, the mass of cells is

$$X_1 = C_X V_1 = 14.0 \times 40 = 560 \text{ kg}$$

At the start of fed-batch operation when the liquid volume is 16 m³, if operation is at quasi-steady state, the cell concentration = 14.0 kg/m³ and

$$X_0 = C_X V_0 = 14.0 \times 16 = 224 \text{ kg}$$

Therefore, the mass of cells produced during 6 hours of fed-batch operation is 560 – 224 = 336 kg.

## P.9 ➡ *Solution*

**1.False**. The dilution rate, which is the same for both reactors, is calculated as

$$D = \frac{F}{V} = \frac{50 \text{ L/h} \times \frac{1}{1000} \frac{\text{m}^3}{\text{L}}}{0.5 \text{ m}^3} = 0.1 \text{ h}^{-1}$$

The cell and substrate concentrations entering the second reactor are the same as those leaving the first reactor. The substrate concentration is given by the Monod equation,

$$C_S = \frac{DK_S}{\mu_{\max} - D} = \frac{0.10 \times 1.0}{0.12 - 0.10} = \boxed{5.0 \text{ kg/m}^3}$$

**2.False**. Observing that maintenance requirements are significant, the cell concentration is given by equation 6 in the form

$$C_X = \frac{D\left(C_{S,0} - C_{S,1}\right)}{\frac{D}{Y_{X/S}} + m_S} = \frac{0.10 \times \left(10 - 5.0\right)}{\frac{0.10}{0.5} + 0.025} = \boxed{2.22 \text{ kg/m}^3}$$

**3.True**. As growth is negligible in the second reactor, $C_X = C_{X,0}$ = 2.22 kg/m³. The substrate concentration is determined by applying a mass balance to the reactor and solving for $C_{S,1}$ with $\mu$ = 0,

$$FC_{S,0} - FC_{S,1} - \left( \frac{\cancel{\mu}}{Y_{X/S}} + \frac{q_P}{Y_{P/S}} + m_S \right) C_X V = 0$$

$$\therefore C_{S,1} = C_{S,0} - \left( \frac{q_P}{Y_{P/S}} + m_S \right) C_X \frac{V}{F}$$

$$\therefore C_{S,1} = 5.0 - \left( \frac{0.16}{0.85} + 0.025 \right) \times 2.2 \times \frac{0.5}{\left( 50 \times \frac{1}{1000} \right)} = 0.309 \text{ kg/m}^3$$

Accordingly, the substrate conversion for the two reactors is

$$\text{Overall substrate conversion} = \frac{C_{S,0} - C_{S,1}}{C_{S,0}} = \frac{10 - 0.309}{10} = \boxed{96.9\%}$$

**4.True**. Since product is not formed in the first reactor, $C_{P,0} = 0$ in the second reactor. The product concentration is determined from equation 7, namely

$$C_{P,1} = \frac{q_P C_X}{D} = \frac{0.16 \times 2.22}{0.10} = \boxed{3.55 \text{ kg/m}^3}$$

## P.10 ➡ *Solution*

For 75% conversion, we can write $C_{S,1} = 0.25C_{S,0}$. The batch reaction time is evaluated using equation 1,

$$t_b = \frac{K_S}{\mu_{\max}} \ln\left(\frac{C_{S,0}}{C_{S,1}}\right) + \frac{C_{S,0} - C_{S,1}}{\mu_{\max}}$$

$$\therefore t_b = \frac{1.5}{0.9} \ln\left(\frac{3.0}{0.25 \times 3.0}\right) + \frac{3.0 - 0.25 \times 3.0}{0.9} = 4.81 \text{ h}$$

The operating cost is then

$$\text{Operating cost} = 4.81 \text{ h} \times \frac{1}{24} \frac{\text{day}}{\text{h}} \times \$4800 \, \text{day}^{-1} = \$962$$

The cost of downstream processing per kg of product is

$$Q = 155 - 0.33X = 155 - 0.33 \times 75 = \$130/\text{kg}$$

The mass of product formed is determined from the mass of substrate consumed, which is equal to the change in substrate concentration multiplied by the volume $V$ of the reactor,

$$\text{Mass of substrate consumed} = \left(C_{S,0} - C_{S,1}\right)V = \left(3.0 - 0.25 \times 3.0\right) \times 1600 = 3600 \text{ g}$$

Since 1.2 g of product is formed per g of substrate consumed,

$$\text{Mass of product formed} = 1.2 \times 3600 = 4320 \text{ g} = 4.32 \text{ kg}$$

so that

$$\text{Downstream processing cost} = \$130 \text{ kg}^{-1} \times 4.32 \text{ kg} = \$562$$

The revenue from sale of the product is

$$\text{Revenue} = \$750 \text{ kg}^{-1} \times 4.32 \, \text{kg} = \$3240$$

Therefore, the cost benefit at 75% substrate conversion is

$$\text{Cost benefit} = \text{Revenue} - \text{Operating cost} - \text{Downstream processing cost}$$

$$\therefore \text{Cost benefit} = \$3240 - \$962 - \$562 = \$1720$$

We now proceed to the the calculations for 90% conversion. In this case, $C_{S,1} = 0.1C_{S,0}$. The batch reaction time is calculated as

$$t_b = \frac{K_S}{\mu_{\max}} \ln\left(\frac{C_{S,0}}{C_{S,1}}\right) + \frac{C_{S,0} - C_{S,1}}{\mu_{\max}}$$

$$\therefore t_b = \frac{1.5}{0.9} \ln\left(\frac{3.0}{0.1 \times 3.0}\right) + \frac{3.0 - 0.1 \times 3.0}{0.9} = 6.84 \text{ h}$$

At 90% conversion, the operating cost is raised due to the increased reaction time and equals

$$\text{Operating cost} = 6.84 \text{ h} \times \frac{1}{24} \frac{\text{day}}{\text{h}} \times \$4800 \, \text{day}^{-1} = \$1370$$

The cost of downstream processing per kg of product is

$$Q = 155 - 0.33X = 155 - 0.33 \times 90 = \$125/\text{kg}$$

The mass of substrate consumed is now

$$\text{Mass of substrate consumed} = \left(C_{S,0} - C_{S,1}\right)V = \left(3.0 - 0.1 \times 3.0\right) \times 1600 = 4320 \text{ g}$$

and the mass of product formed becomes

$$\text{Mass of product formed} = 1.2 \times 4320 = 5180 \text{ g} = 5.18 \text{ kg}$$

The associated downstream processing cost is

$$\text{Downstream processing cost} = \$125\,\text{kg}^{-1} \times 5.18\,\text{kg} = \$648$$

The sales revenue is

$$\text{Revenue} = \$750 \text{ kg}^{-1} \times 5.18 \text{ kg} = \$3890$$

Lastly, the cost benefit at 90% substrate conversion is

$$\text{Cost benefit} = \text{Revenue} - \text{Operating cost} - \text{Downstream processing cost}$$

$$\therefore \text{Cost benefit} = \$3890 - \$1370 - \$648 = \$1870$$

The gain per batch from increasing the conversion from 75 to 90% conversion is 1870 – 1720 = $150. That is, the conversion increase proposed would raise the cost benefit by 8.7%, and hence may be worth pursuing.

## P.11 ➡ *Solution*

The desired level of cell destruction is evaluated using a basis of 60 days. Ignoring any cell death in the heating and cooling sections, the number of cells entering the holding section over 60 days is

$$N_1 = 1.6 \frac{\text{m}^3}{\text{h}} \times \left( 8.68 \times 10^{11} \frac{1}{\text{m}^3} \right) \times \left( \frac{24}{1} \frac{\text{h}}{\text{day}} \times 60 \text{ days} \right) = 2.0 \times 10^{15}$$

$N_2$, the acceptable number of cells leaving during this period, is 1. Accordingly,

$$\frac{N_2}{N_1} = \frac{1}{2.0 \times 10^{15}} = 5.0 \times 10^{-16}$$

The linear velocity $u$ in the sterilizer is equal to the volumetric flow rate divided by the cross-sectional area of the pipe,

$$u = \frac{1.6}{\left( \frac{\pi \times 0.14^2}{4} \right)} = 104 \text{ m/h}$$

The Reynolds number is determined next,

$$\text{Re} = \frac{\rho u d}{\mu} = \frac{1000 \times 104 \times 0.14}{4.0} = 3640$$

Mapping this Reynolds number onto Figure 1 (and using the experimental curve as we were told), we read $\mathcal{D}_z/ud \approx 2.0$. The axial dispersion coefficient is then

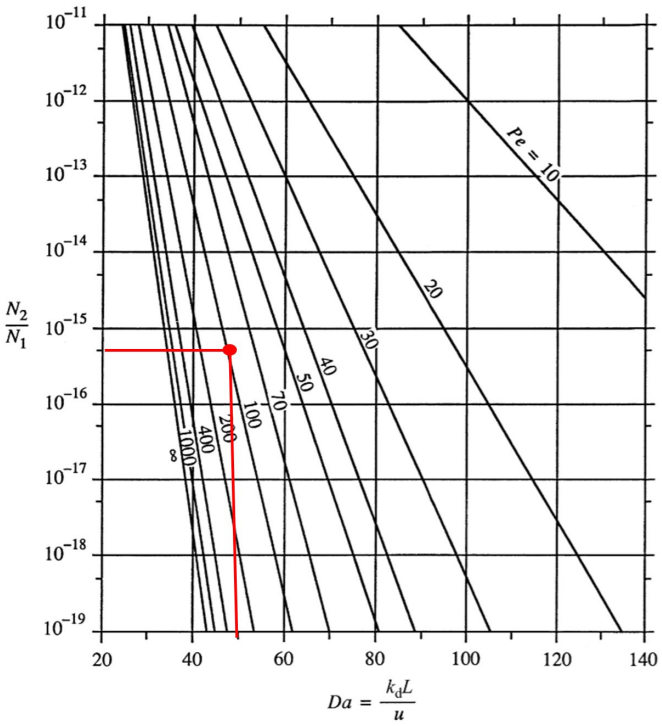
$$\frac{D_z}{ud} = 2.0 \rightarrow D_z = 2.0ud$$

$$\therefore D_z = 2.0 \times 104 \times 0.14 = 29.1 \text{ m}^2/\text{h}$$

The Peclet number follows as

$$Pe = \frac{uL}{D_z} = \frac{104 \times 28}{29.1} = 100$$

Using Figure 1, we can determine the value of the death constant $k_d$ for the desired level of cell destruction. For $N_2/N_1 = 5.0 \times 10^{-16}$ and $Pe = 100$, the corresponding Damköhler number is read as $Da \approx 50$; see below.




Using the definition of $Da$, we can establish the death constant,

$$Da = \frac{k_d L}{u} \rightarrow k_d = \frac{Da \times u}{L}$$

$$\therefore k_d = \frac{50 \times 104}{28} = 186 \text{ h}^{-1}$$

The sterilization temperature can be determined with the Arrhenius equation, namely

$$k_d = A \exp\left(-E_d / RT\right)$$

Taking logarithms and solving for $T$ gives

$$T = -\frac{E_d / R}{\ln\left(k_d / A\right)}$$

The activation energy is $E_d$ = 254 kJ g mol$^{-1}$ = 254×10$^3$ J g mol$^{-1}$ , and constant $A$ = 5.5×10$^{39}$. Substituting in the relation above, we obtain

$$T = -\frac{254 \times 10^3 / 8.314}{\ln\left[186 / \left(5.5 \times 10^{39}\right)\right]} = 354 \text{ K} = \boxed{81^\circ \text{C}}$$

The sterilization should be performed at a temperature of 81 degrees Celsius.

♦ The correct answer is **D**.

## P.12 ➡ *Solution*

**Part 1:** The medium volumetric flow rate is $F = DV$ = 0.1×15 = 1.5 m³/h. The linear velocity $u$ in the holding section of the sterilizer is determined by dividing $F$ by the cross-sectional area $A = \pi \times 0.06^2$ of the pipe,

$$u = \frac{F}{A} = \frac{1.5}{\pi \times 0.06^2} = 133 \text{ m/h}$$

The specific death constant is determined next. Given the Arrhenius constant = 7.5×10$^{39}$ h$^{-1}$ and the activation energy $E_d$ = 2.885×10$^5$ J-g/mol, we have

$$k_d = A \exp\left(-E_d / RT\right) = 7.5 \times 10^{39} \times \exp\left[-2.885 \times 10^5 / \left(8.314 \times 403.15\right)\right] = 313 \text{ h}^{-1}$$

Within a period of 3 months (= 90 d), the number of cells $N_1$ entering the sterilizer is equal to the medium volumetric flow rate $F$ multiplied by the cell concentration and the time,

$$N_1 = 1.5 \frac{\text{m}^3}{\text{h}} \times \left(10^5 \frac{1}{\text{mL}} \times \frac{10^6}{1} \frac{\text{mL}}{\text{m}^3}\right) \times \left(90 \text{ d} \times \frac{24}{1} \frac{\text{h}}{\text{d}}\right) = 3.24 \times 10^{14}$$

Within the same 3-month period, the acceptable number of cells remaining at the end of the sterilization treatment is $N_2 = 1$. Accordingly,

$$\frac{N_2}{N_1} = \frac{1}{3.24\times10^{14}} = 3.09\times10^{-15}$$

For perfect plug flow with no axial dispersion, the sterilization time is given by

$$t_{\text{hd}} = \frac{\ln\left(N_1/N_2\right)}{k_d} = \frac{\ln\left(3.24\times10^{14}/1\right)}{302} = 0.107 \text{ h}$$

To allow the medium to remain for this period of time in the holding section of the sterilizer pipe, the length of pipe required is equal to the linear velocity of the medium $u$ multiplied by $t_{\text{hd}}$,

$$L = ut_{\text{hd}} = 133\times0.107 = \boxed{14.2 \text{ m}}$$

♦ The correct answer is **C**.

**Part 2:** The Reynolds number with a pipe diameter $d$ = 0.12 m is calculated as

$$\text{Re} = \frac{\rho ud}{\mu} = \frac{1000\times133\times0.12}{4.0} = 3990$$

The value of $\mathcal{D}_z/ud$ corresponding to the Reynolds number obtained above can be read from the theoretical curve in Figure 1 and equals about 1.5. The axial dispersion coefficient is then

$$\frac{D_z}{ud} = 1.5 \rightarrow D_z = 1.5\times133\times0.12 = 23.9 \text{ m}^2/\text{h}$$

The Peclet number is computed as

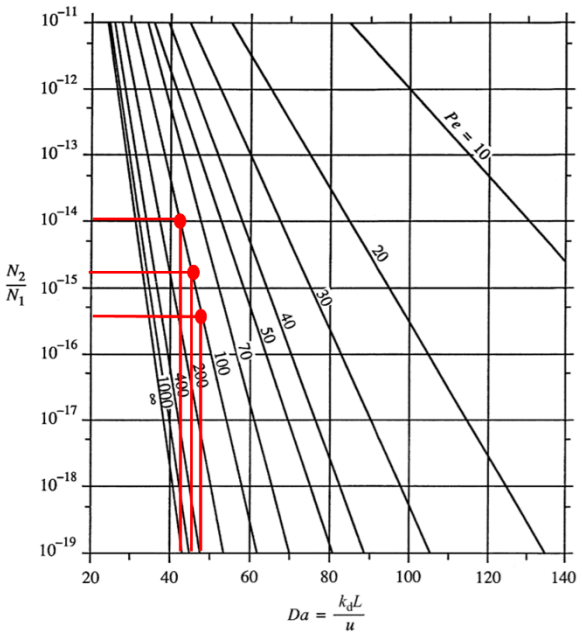
$$\text{Pe} = \frac{uL}{D_z} = \frac{133\times L}{23.9} = 5.56L \quad \text{(I)}$$

where $L$ has units of m. Similarly, an expression for the Damköhler number $Da$ is

$$\text{Da} = \frac{k_d L}{u} = \frac{313\times L}{133} = 2.35L \quad \text{(II)}$$

The design problem can be solved from trial-and-error methods and Figure 2. As a first guess, try $L$ = 20 m. The values of $Pe$ and $Da$ are calculated from equations (I) and (II), respectively, and the corresponding $N_2/N_1$ is read from Figure 2, Depending on how this value compares with the target of $3.09\times10^{-15}$, the value of $L$ is adjusted until the results for $N_2/N_1$ coincide. The calculations are summarized below.

| $L$ (m) | $Pe$ (Eq. I) | $Da$ (Eq. II) | $N_2/N_1$ (Fig. 2) |
|---|---|---|---|
| 20 | 111 | 47 | $4\times10^{-16}$ |
| 18 | 100 | 42 | $1\times10^{-14}$ |
| 19 | 106 | 45 | $2\times10^{-15}$ |



The last value of $N_2/N_1$ is as close to $3.09\times10^{-15}$ as practicable from the resolution of Figure 2. Therefore, the required length of pipe in the holding section is about 19 m, or 34% longer than that determined for ideal plug flow.

**Part 3:** For $L$ = 14.2 m, equations (I) and (II) developed in the previous part give $Pe$ = 5.56 × 14.2 = 79 and $Da$ = 2.35 × 14.2 = 33.4. Entering these values into Figure 2, we read $N_2/N_1 \approx 6.0\times10^{-12}$. Thus, $N_1/N_2 = 1/(6.0\times10^{-12}) = 1.67\times10^{11}$. As $N_2$ = 1, $N_1 = 1.67\times10^{11}$, i.e., one contaminant enters the fermenter for every $1.67\times10^{11}$ cells that enter the sterilizer. For $F$ = 1.5 m³/h and an input contaminant concentration of $10^5$ mL$^{-1}$, the time required for $1.67\times10^{11}$ contaminants to enter the sterilizer is calculated as

$$\text{Time} = \frac{1.67\times10^{11}}{1.5\ \frac{\text{m}^3}{\text{h}}\times\left(10^5\ \frac{1}{\text{mL}}\times\frac{10^6}{1}\ \frac{\text{mL}}{\text{m}^3}\right)\times\frac{1}{60}\ \frac{\text{h}}{\text{min}}} = 66.8\ \text{min}$$

Therefore, contaminants enter the fermenter at a rate of about one every 67 minutes.

♦ The correct answer is **C**.

## ANSWER SUMMARY

| | | |
|---|---|---|
| **Problem 1** | **1.1** | Open-ended pb. |
| | **1.2** | B |
| **Problem 2** | **2.1** | D |
| | **2.2** | B |
| **Problem 3** | **3.1** | A |
| | **3.2** | B |
| | **3.3** | C |
| **Problem 4** | | D |
| **Problem 5** | | T/F |
| **Problem 6** | **6.1** | β |
| | **6.2** | D |
| **Problem 7** | | T/F |
| **Problem 8** | | T/F |
| **Problem 9** | | T/F |
| **Problem 10** | | Open-ended pb. |
| **Problem 11** | | D |
| **Problem 12** | **12.1** | C |
| | **12.2** | Open-ended pb. |
| | **12.3** | C |

## REFERENCES


- DORAN, P. (1997). *Bioprocess Engineering Principles*. 1st edition. Waltham: Academic Press.
- DORAN, P. (2013). *Bioprocess Engineering Principles*. 2nd edition. Waltham: Academic Press.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