

## **Chapter 9**

# **Operating Considerations for Bioreactors**

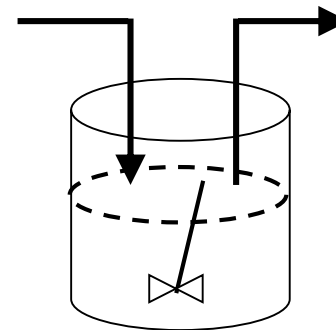
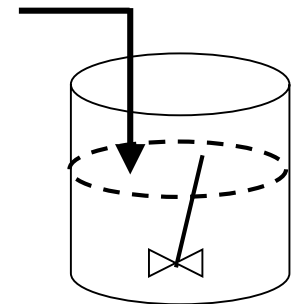
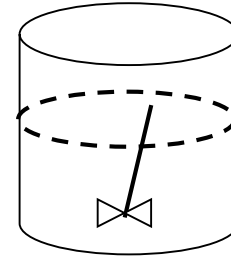


# 9. Operating Considerations for Bioreactors

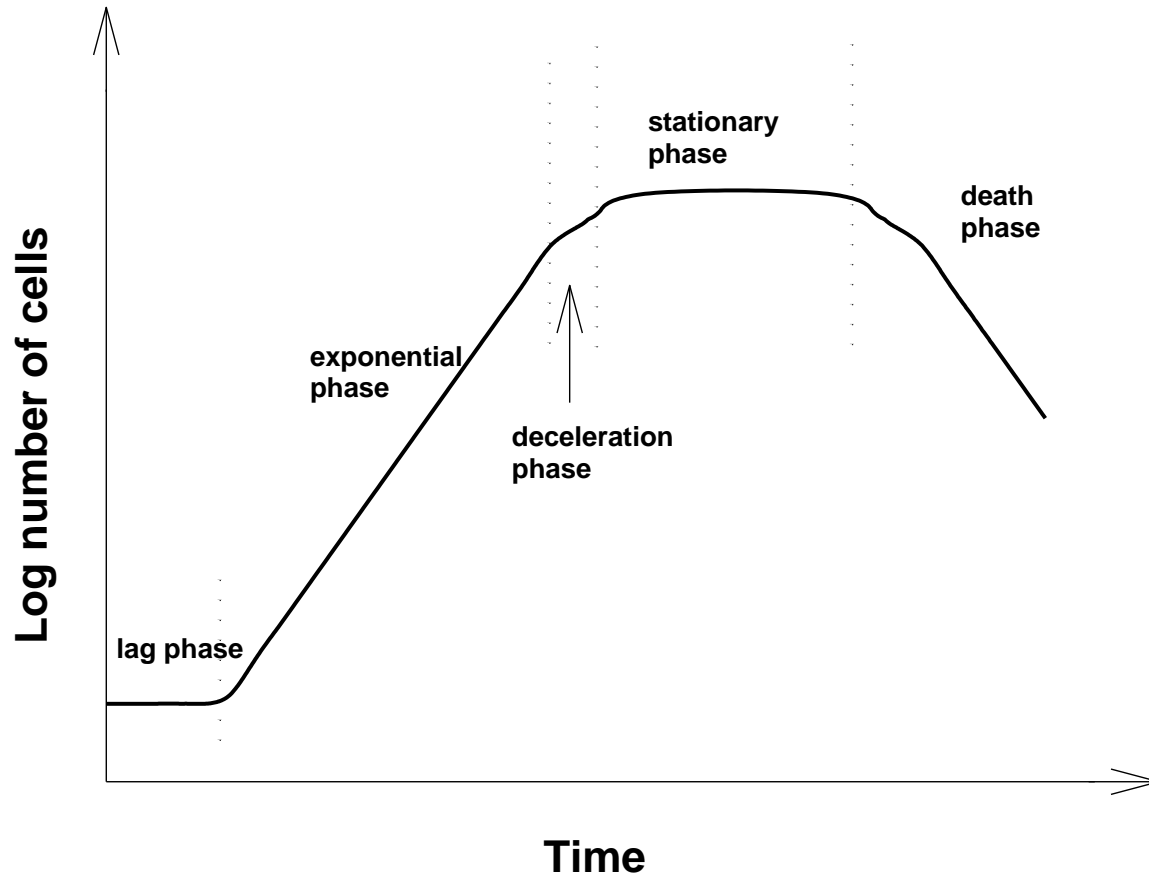
- 9.1. Introduction
- 9.2. Choosing the cultivation method
  - Between batch and continuous operations
- 9.3. Modifying batch and continuous reactors
  - 9.3.1. Chemostat with recycle
  - 9.3.2. Multistage chemostat systems
  - 9.3.3. Fed-batch operation
  - 9.3.4. Perfusion systems

# Cell Culture

- **Batch Culture**
  - No addition or removal
  - Simple and widely used
- **Fed-batch Culture**
  - Addition but no removal
- **Continuous Culture**
  - Addition and removal



# Cell Growth in Batch Culture



# Exponential Growth Phase

$$\frac{dX}{dt} = \mu X \text{ ----- (1)}$$

$$X = X_0 \text{ at } t = 0$$

$$\ln \frac{X}{X_0} = \mu t \text{ ----- (2)}$$

$$X = X_0 e^{\mu t} \text{ ----- (3)}$$

# Doubling Time ( $\tau_d$ )

$$X = X_0 e^{\mu t}$$

$$2X_0 = X_0 e^{\mu \tau_d}$$

$$\ln 2 = \mu \tau_d$$

$$\tau_d = \frac{\ln 2}{\mu} = \frac{0.693}{\mu} \text{ --- (4)}$$

# Exponential Period

$$(2) \longrightarrow \ln \frac{X_m}{X_0} = \mu \cdot t_e$$

$$t_e = \frac{1}{\mu} \ln \frac{X_m}{X_0} \text{ --- (5)}$$

where  $X_m$ : maximum cell concentration  
 $t_e$ : exponential period

# Batch Cycle Time ( $t_c$ )

$$t_c = t_e + t_l$$

$$\begin{aligned} &= \frac{1}{\mu} \ln \frac{X_m}{X_0} + t_l \text{ --- (6)} \\ \uparrow & \\ (5) & \end{aligned}$$

- $t_l$ : \* lag + harvesting + preparation  
\* varies with size of equipment and nature of the fermentation  
\* normally 3-10 h



# Productivity for Cell Mass

$$Productivity = \frac{X_m - X_0}{t_c} \left( = \frac{YS_F}{t_c} \right)$$

$$\begin{array}{c} \overline{\overline{=}} \\ \uparrow \\ (6) \end{array} \frac{YS_F}{\left( \frac{1}{\mu} \ln \frac{X_m}{X_0} + t_l \right)} \text{---} \text{---} \text{---} (7)$$

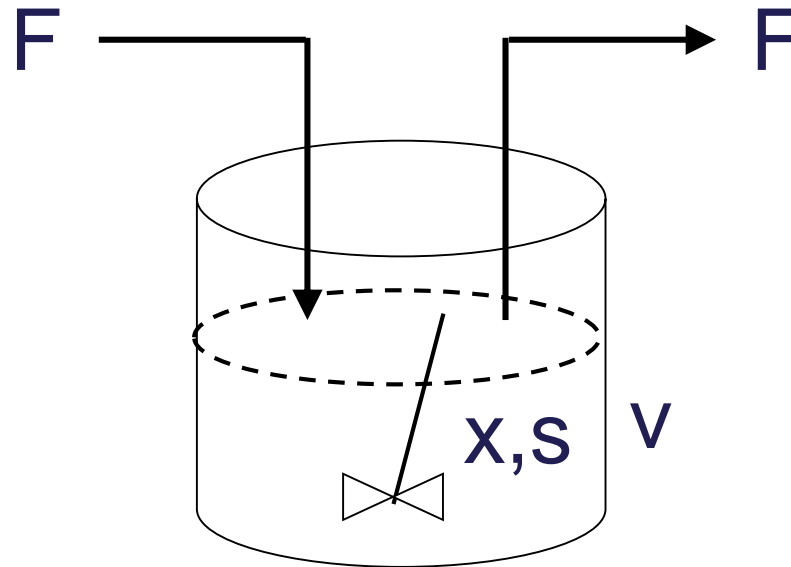
# Mass Balance

$$\text{Acc} = \text{In} - \text{Out} + \text{Gen} - \text{Con}$$

**Accumulation rate = Input rate – Output rate + Generation rate – Consumption rate**

$$\frac{d(\quad)}{dt} =$$

# Continuous Operation (Chemostat)



**$F$  (L/h) : Flow Rate**

**$V$  (L) : Working Volume**

**$X$  (g/L) : Cell Concentration**

**$S$  (g/L) : Substrate Concentration**

# Mass Balances

- cell

$$\frac{d(XV)}{dt} = 0 - XF + \mu XV - 0 \dots \dots (1)$$

- substrate

$$\frac{d(SV)}{dt} = S_F F - SF + 0 - \frac{1}{Y} \mu XV \dots \dots (2)$$

# Mass Balances

**At steady state**

$$0 = -XF + \mu XV \text{ ----- (3)}$$

$$0 = (S_F - S)F - \frac{1}{Y} \mu XV \text{ ----- (4)}$$

$$(3) \rightarrow \mu = F / V = D \text{ ----- (5)}$$

$$(4) \xrightarrow{\uparrow} X = \frac{Y}{\mu} (S_F - S) \frac{F}{V} \text{ ----- (6)}$$

$$(5) \quad = Y(S_F - S) \text{ ----- (7)}$$

# Concentrations at Steady State

## Monod Equation

$$\mu(= D) = \frac{\mu_m S}{K_S + S} \text{ --- (8)}$$

$$S = \frac{DK_S}{\mu_m - D} \text{ --- (9)}$$

(7)  $\rightarrow$

$\uparrow$   
(9)

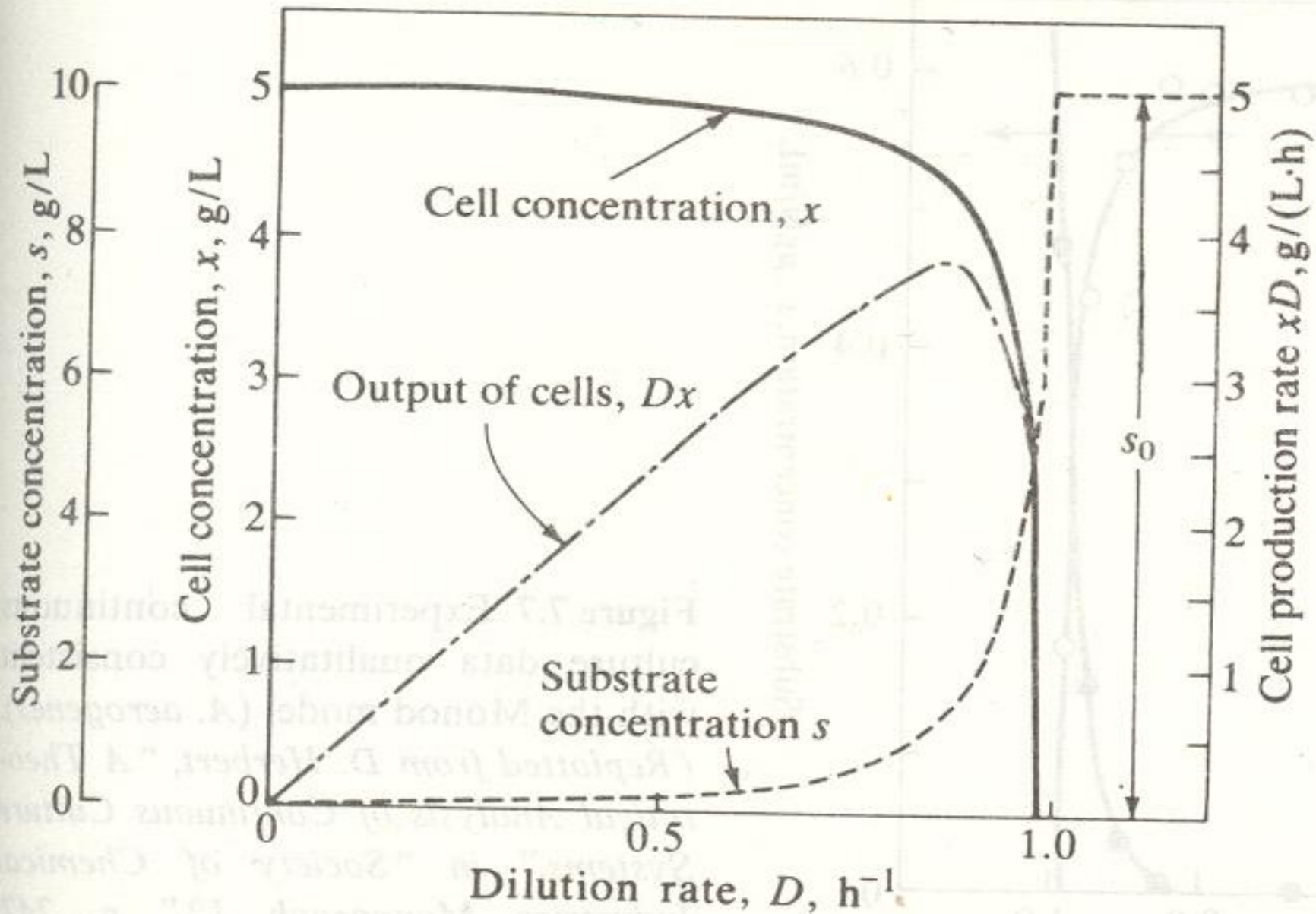
$$X = Y \left( S_F - \frac{DK_S}{\mu_m - D} \right) \text{ --- (10)}$$

# Productivity of Chemostat

$$\textit{Productivity} = \frac{X(\text{g / L}) \times F(\text{L / hr})}{V(\text{L})} = DX$$

$$DX = Y \left( S_F D - \frac{D^2 K_S}{\mu_m - D} \right) \text{--- (11)}$$

(10)





# To Maximize the Productivity

$$\frac{d(DX)}{dD} = 0$$

$$D_{opt} = \mu_m \left( 1 - \sqrt{\frac{K_S}{K_S + S_F}} \right) \text{--- (12)}$$

$$(10) \quad \begin{array}{c} \rightarrow \\ \uparrow \\ (12) \end{array} X_{opt} = Y \left( S_F + K_S - \sqrt{K_S (S_F + K_S)} \right) \text{--- (13)}$$

$$D_{opt} X_{opt} = Y \mu_m \left( 1 - \sqrt{\frac{K_S}{K_S + S_F}} \right) \left( S_F + K_S - \sqrt{K_S (S_F + K_S)} \right) \text{--- (14)}$$

# Maximum Productivity

$$D_{opt}X_{opt} = Y\mu_m \left( 1 - \sqrt{\frac{K_S}{K_S + S_F}} \right) (S_F + K_S - \sqrt{K_S(S_F + K_S)}) \quad (14)$$

**(usually  $S_F \gg K_S$ )**

$$= Y\mu_m (1 - 0)(S_F + 0 - 0)$$

$$= Y\mu_m S_F \text{ -----} (15)$$

# Comparison of Chemostat and Batch (or Fed-Batch) Cultures

$$\frac{\textit{Productivity}_{chemo}}{\textit{Productivity}_{batch}} = Y\mu_m S_F \div \left( \frac{YS_F}{\left( \frac{1}{\mu_b} \ln \frac{X_m}{X_0} + t_l \right)} \right)$$
$$= \mu_m \left( \frac{1}{\mu_b} \ln \frac{X_m}{X_0} + t_l \right) \text{--- (16)}$$

# Comparison of Chemostat and Batch (or Fed-Batch) Cultures

$$\frac{\textit{Productivity}_{chemo}}{\textit{Productivity}_{batch}} = \mu_m \left( \frac{1}{\mu_b} \ln \frac{X_m}{X_0} + t_l \right) \text{--- (16)}$$

In most commercial fermentations

$$\frac{X_m}{X_0} \approx 10 - 20 \quad \left( \ln \frac{X_m}{X_0} \approx 2.3 - 3.0 \right)$$

Continuous systems have a significant productivity advantage for primary products.

# Comparison of Chemostat and Batch (or Fed-Batch) Cultures

Ex) For an *E. coli* fermentation with

$$\frac{X_m}{X_0} = 20, \quad \mu_m = \mu_b = 1.0h^{-1}, \quad t_l = 5h$$

$$\frac{\text{Productivity}_{chemo}}{\text{Productivity}_{batch}} = 8$$

# Most commercial bioprocesses are batch. Why?

- Eq(16) applies only to growth-associated products.
  - Secondary product is only made at very low dilutions, far below those values optimal for biomass production.
- Genetic instability
- Sterility, operability, reliability
- Market economics
  - Batch systems provide much greater flexibility.

# Examples of Continuous Operation

- Production of single-cell protein (SCP)
- Waste treatment
- Some other large-volume growth-associated products