Chapter 9

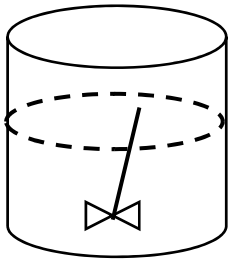
Operating Considerations for Bioreactors

9. Operating Considerations for Bioreactors

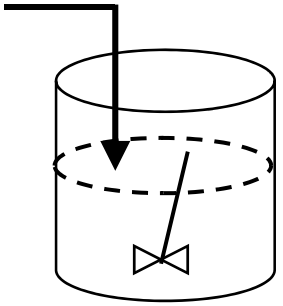
- 9.1. Introduction
- 9.2. Choosing the cultivation method
  - Between batch and continuous operations
- 9.3. Modifying batch and continuous reactors
  - 9.3.1. Chemostat with recycle
  - 9.3.2. Multistage chemostat systems
  - 9.3.3. Fed-batch operation
  - 9.3.4. Perfusion systems

Cell Culture

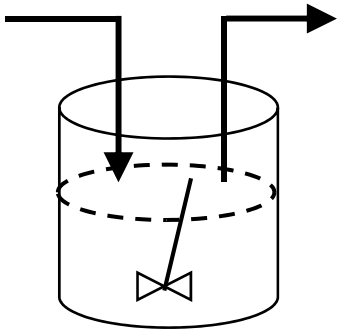
- Batch Culture
  - No addition or removal
  - Simple and widely used



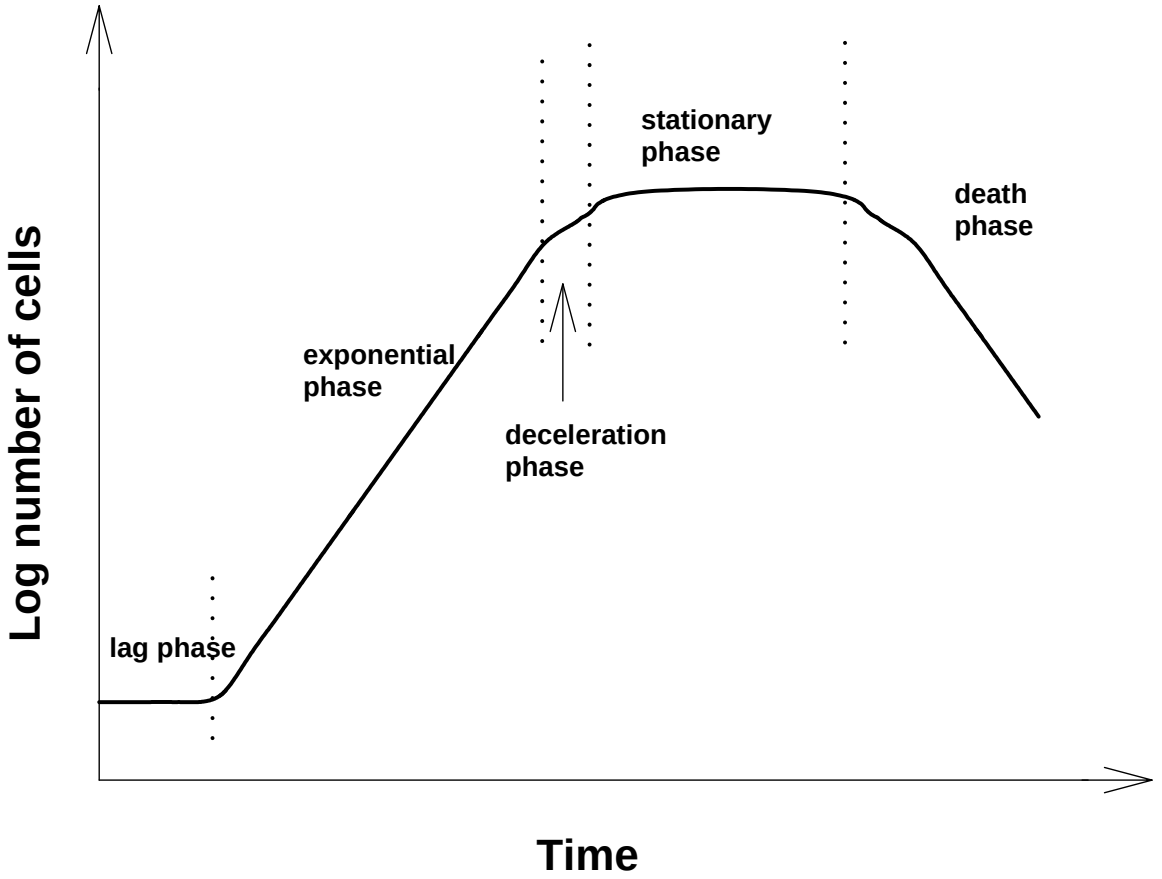
- Fed-batch Culture
  - Addition but no removal



- Continuous Culture
  - Addition and removal



Cell Growth in Batch Culture



Exponential Growth Phase

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

X = Xo at t = 0

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

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

Doubling Time (τ_d)

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

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

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

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

Exponential Period

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

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

where X_m: maximum cell concentration
t_e : exponential period

Batch Cycle Time (t_c)

$$t_c = t_e + t_l$$

$$= \frac{1}{\mu} \ln \frac{X_m}{X_0} + t_l \quad ---(6)$$

(5)

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

Productivity for Cell Mass

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

$$= \frac{YS_F}{\left(\frac{1}{\mu} \ln \frac{X_m}{X_0} + t_l \right)} \quad ---(7)$$

(6)

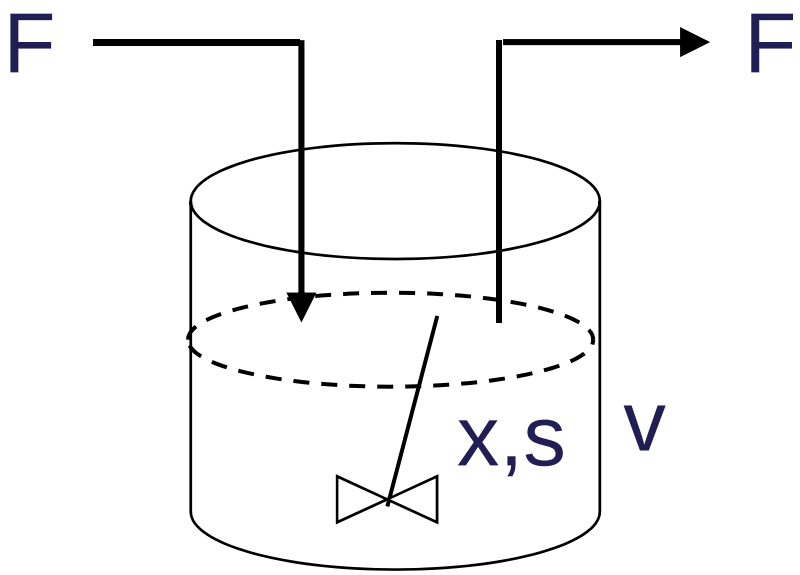
Mass Balance

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

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

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

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

$$\frac{d\left(XV\right)}{dt} = 0 - XF + \mu XV - 0 \text{.....(1)}$$

- substrate

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

Mass Balances

At steady state

$$0 = -XF + \mu XV \quad ----(3)$$

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

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

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

(5) $= Y(S_F - S)$ ---(7)

Concentrations at Steady State

Monod Equation

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

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

(7) → (9)

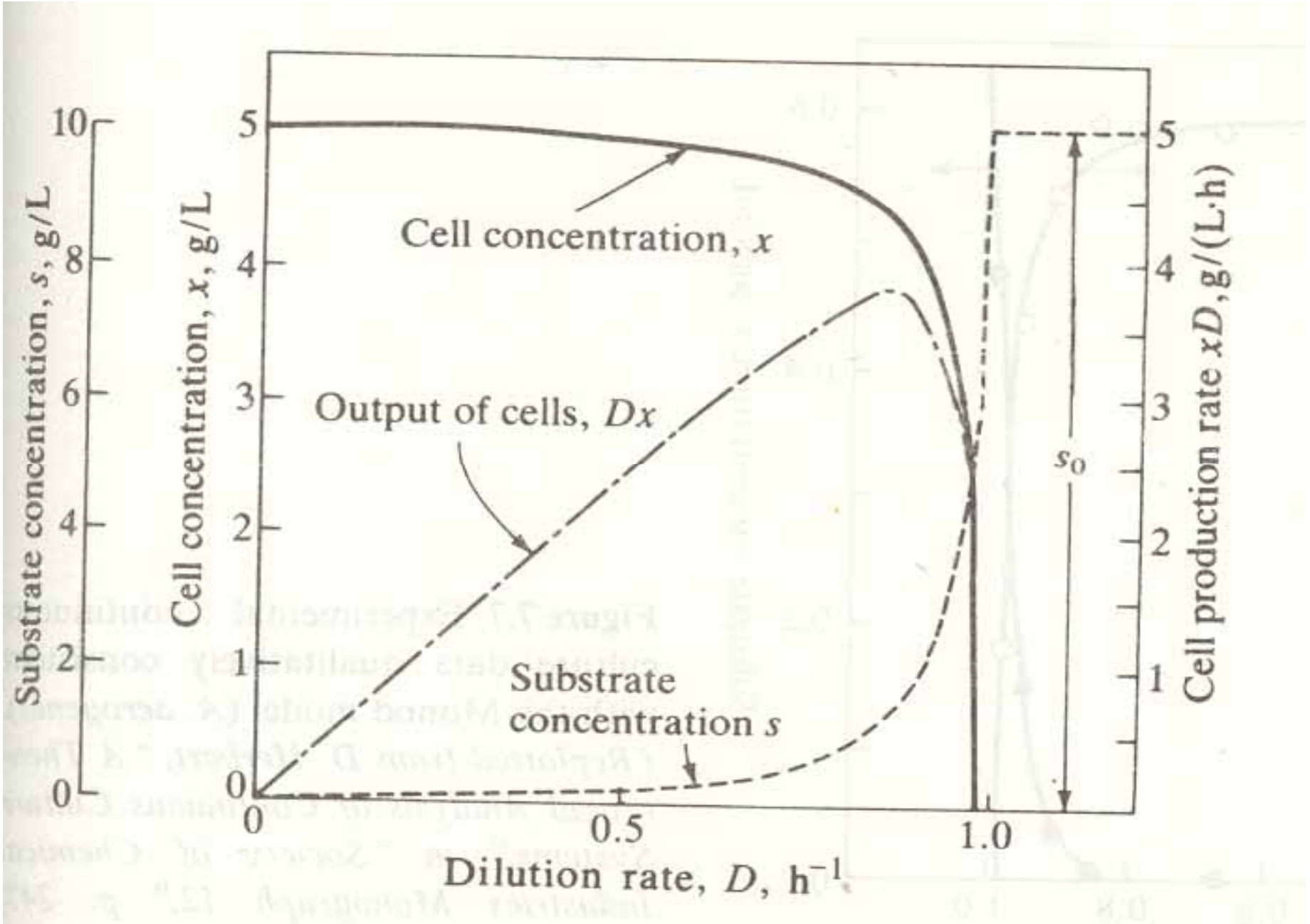
$$X=Y(S_F-\frac{DK_S}{\mu_m-D})---(10)$$

Productivity of Chemostat

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

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

(10)


Substrate concentration, s, g/L
Cell concentration, x, g/L
Cell production rate xD, g/(L·h)
Cell concentration, x
Output of cells, Dx
Substrate concentration s
s_0
Dilution rate, D, h^{-1}
0
0.5
1.0
0
1
2
3
4
5
6
8
10

To Maximize the Productivity

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

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

(10) → $$X_{opt} = Y\left(S_F + K_S - \sqrt{K_S(S_F + K_S)}\right) --(13)$$

(12) ↑

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

Maximum Productivity

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

(usually $S_F >> K_s$)

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

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

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

$$\frac{Productivity_{chemo}}{Productivity_{batch}} = Y\mu_m S_F \div \frac{YS_F}{\left(\frac{1}{\mu_b}\ln\frac{X_m}{X_0} + t_l\right)}$$

$$= \mu_m\left(\frac{1}{\mu_b}\ln\frac{X_m}{X_0} + t_l\right)^{-1} \quad (16)$$

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

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

In most commercial fermentations

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

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

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

$$\frac{Productivity_{chemo}}{Productivity_{batch}} = 8$$

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 vales 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