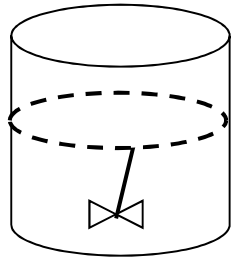
Chapter 9

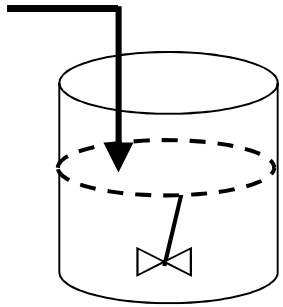
Operating Considerations for Bioreactors

Culture methods

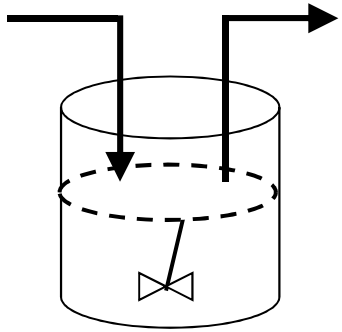
- **Batch Culture**
  - No addition or removal
  - Simple and widely used



- **Fed-batch Culture**
  - Addition but no removal



- **Continuous Culture**
  - Addition and removal



Choosing culture methods

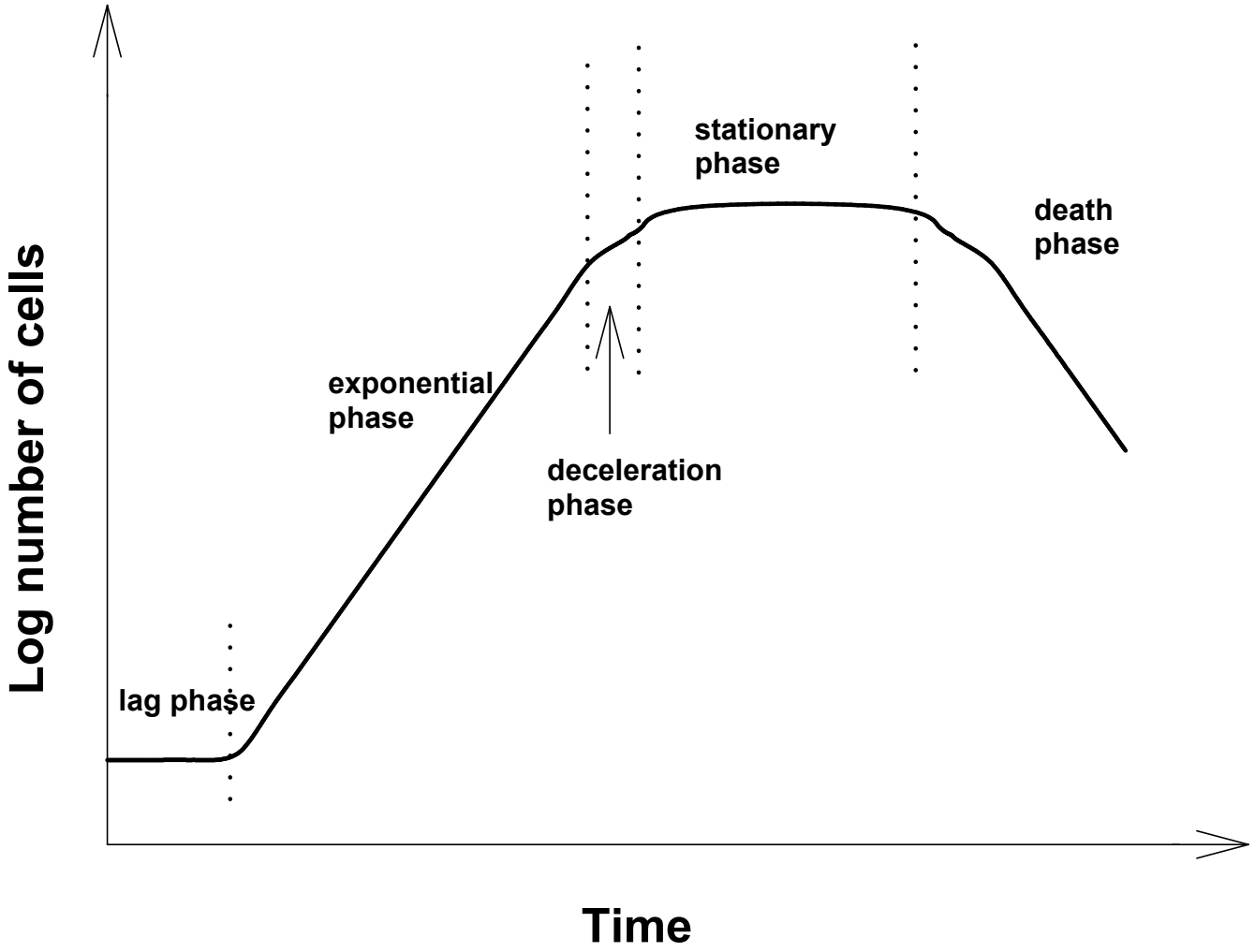
The Choice of Bioreactor Affects Many Aspects of Bioprocessing.

1. Product concentration and purity
2. Degree of substrate conversion
3. Yields of cells and products
4. Capitol cost in a process (>50% total capital expenses)

Further Considerations in Choosing a Bioreactor.

1. Biocatalyst. (immobilized or suspended)
2. Separations and purification processes

Cell growth in batch culture



Exponential growth phase

$$\frac{dX}{dt} = \mu X$$

$$X = Xo \quad at \quad t = 0$$

$$\ln \frac{X}{X_0} = \mu t$$

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

Doubling time (t_d)

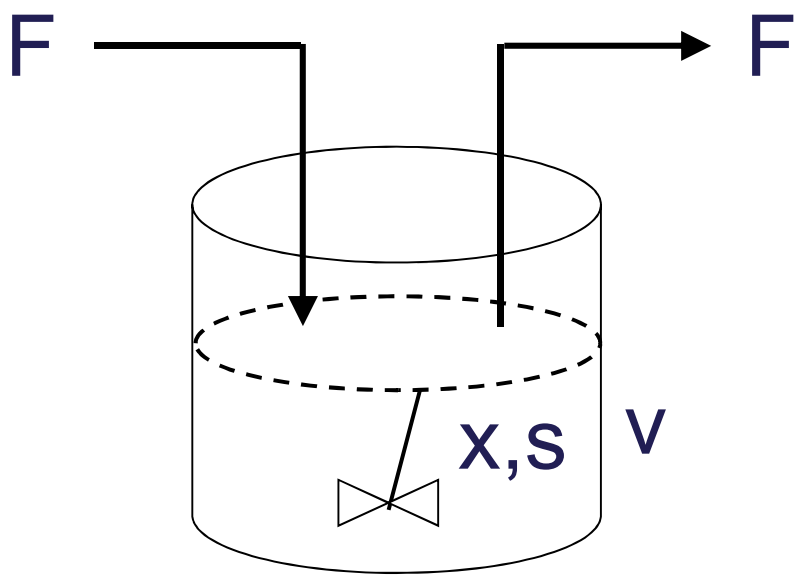
$$X = X_0 e^{\mu t}$$

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

$$\ln 2 = \mu t_d$$

$$t_d = \frac{\ln 2}{\mu} = \frac{0.693}{\mu}$$

Continuous culture (Chemostat)



F (L/h) : Flow Rate
V (L) : Working Volume
X (g/L) : Cell Concentration
S (g/L) : Substrate Concentration

Chemostat : Substrate mass balances

$$FS_o - FS - V_R \mu X \frac{1}{Y^M_{X/S}} - V_R q_p X \frac{1}{Y_{P/S}} = V_R \frac{dS}{dt}$$

S = bioreactor and outlet substrate concentration (g/L)

S_o = inlet substrate concentration (g/L)

$Y^M_{X/S}$ = maximum cell yield coefficient (g cells / g substrate)

$Y_{P/S}$ = product yield coefficient (g product / g substrate)

q_p = specific rate of extracellular product formation $\left(\frac{g\ P}{g\ cells \bullet hr}\right)$

From Chap 6

Cell concentration in chemostat

For the simple case of no product formation ($q_p=0$), steady-state ($dS/dt=0$), and no endogenous metabolism, $k_d=0$.

$$D(S_o - S) = \frac{\mu X}{Y^M_{X/S}}$$

at steady-state, $\mu = D$, and solving for X,

$$X = Y^M_{X/S}(S_o - S)$$

or

$$X = Y^M_{X/S}\left(S_o - \frac{K_S D}{\mu_{max} - D}\right)$$

From Chap 6

Cell productivity of chemostat

Cell production rate in CSTR [g/h] = FX

Pr_X = productivity for cell production = DX = FX / V

Pr_P = productivity for product formation = DP

The dilution rate (D) which maximizes productivity is found by taking dPr/dD = 0 and solving for D ($D_{optimum}$).

For example, $D_{optimum}$ for X with $k_d = 0$ and $q_P = 0$

$$X = Y^{M}_{X/S}\left(S_o - \frac{K_S\, D}{\mu_{max} - D}\right) \quad \Rightarrow \quad DX = Y^{M}_{X/S} D\left(S_o - \frac{K_S\, D}{\mu_{max} - D}\right)$$

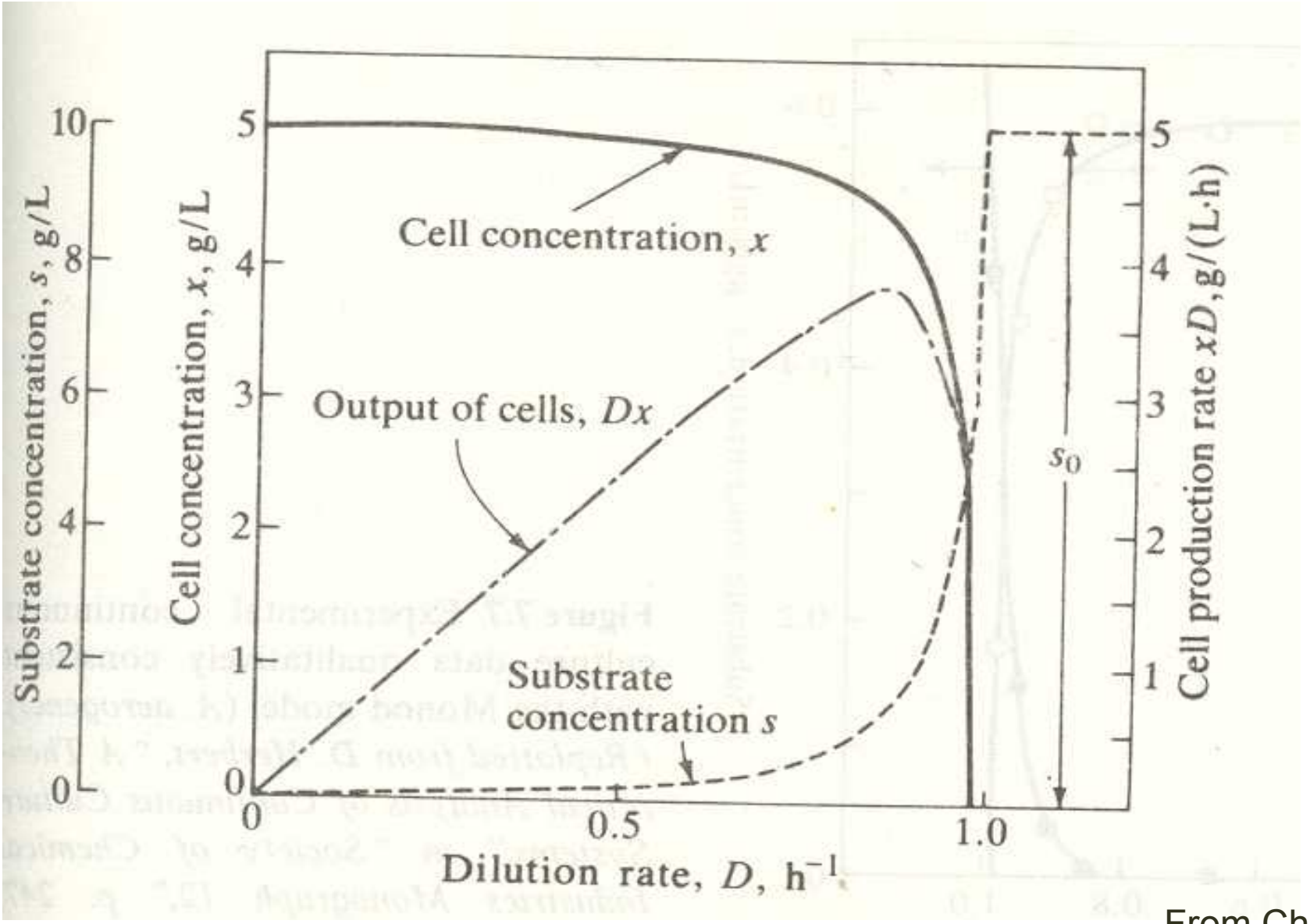
take $\frac{d(DX)}{dD} = 0$ and solve for D (D_{opt})

$$D_{opt} = \mu_{max}\left(1 - \sqrt{\frac{K_S}{K_S + S_o}}\right)$$

K_S is usually << S
so $D_{opt} \sim \mu_{max}$ (washout point)

From Chap 6

Chemostat



From Chap 6

Batch or continuous culture ?

These choices represent extremes in bioreactor choices

Productivity →for cell mass or growth-associated products

Batch Culture: assume $k_d = 0$ and $q_p = 0$

r_b = rate of cell mass production in 1 batch cycle

$$r_b = \frac{X_m - X_o}{t_c} = \frac{Y^M_{X/S} S_o}{t_c}$$

$$t_c = \text{batch cycle time} = \frac{1}{\mu_{max}} \ln \frac{X_m}{X_o} + t_l$$

Exponential growth time

Lag time
Harvest &
Preparation

Batch or continuous culture ?

Continuous Culture: assume $k_d = 0$ and $q_p = 0$

r_c = rate of cell mass production in continuous culture

$$r_c = D_{opt} X_{opt} = r_{c,\,max}$$

$$\text{set } \frac{dDX}{dD} = 0 \quad \Rightarrow \quad D_{opt} = \mu_{max} \left(1 - \sqrt{\frac{K_S}{K_S + S_o}}\right)$$

$$X_{opt} = Y^M_{X/S} \left(S_o - \frac{K_S D_{opt}}{\mu_{max} - D_{opt}}\right) = Y^M_{X/S} \left(S_o + K_S - \sqrt{K_S (S_o + K_S)}\right)$$

$$D_{opt} X_{opt} = Y^M_{X/S} \mu_{max} \left(1 - \sqrt{\frac{K_S}{K_S + S_o}}\right) \left(S_o + K_S - \sqrt{K_S (S_o + K_S)}\right)$$

$$\approx \boxed{Y^M_{X/S} \mu_{max} S_o} \text{ when } K_S \ll S_o$$

Batch or continuous culture ?

Comparing Rates in Batch and Continuous Culture

$$\frac{r_c}{r_b} = \frac{Y^M_{X/S}\,\mu_{max}\,S_o}{Y^M_{X/S}\,S_o \Big/ \left(\frac{1}{\mu_{max}}\ln\frac{X_m}{X_o} + t_l\right)} = \ln\frac{X_m}{X_o} + t_l\mu_{max}$$

A commercial fermentation with

$$\frac{X_m}{X_o} = 20,\ t_l = 5\ \text{hr, and}\ \mu_{max} = 1.0\ \text{hr}^{-1}$$

$$\frac{r_c}{r_b} = 8 \Rightarrow$$

Continuous culture method is ~ 10 times more productive for primary products (biomass & growth associated products)

Batch or continuous culture ?

Why is it that most commercial bioprocess is Batch??

1. Secondary Product Productivity → is > in batch culture

   (SPs require very low concentrations of S, $S << S_{opt}$)

2. Genetic Instability → makes continuous culture less productive

   (revertants are formed and can out-compete highly selected and and productive strains in continuous culture.)

3. Operability and Reliability

   (sterility and equipment reliability > for batch culture)

4. Market Economics

   (Batch is flexible → can product many products per year)

Batch or continuous culture ?

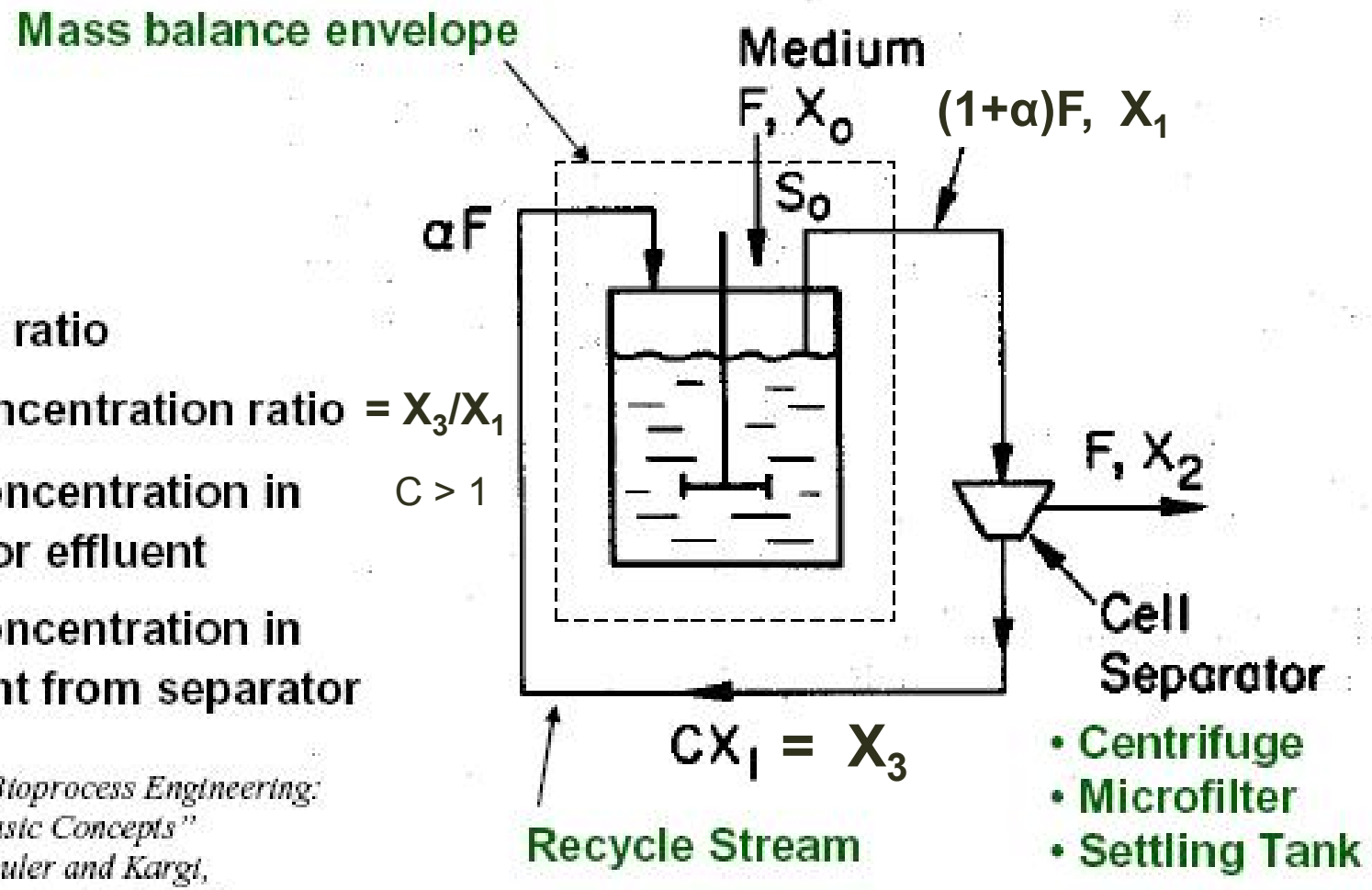
Most Bioprocesses are Based on Batch Culture

(In terms of number, mostly for secondary, high value products)

High Volume Bioprocesses are Based on Continuous Culture

(mostly for large volume, lower value, growth associated products -- ethanol production, waste treatment, single-cell protein production)

Modified bioreactors : Chemostat with recycle



α = recycle ratio

C = cell concentration ratio = X_3/X_1

X_1 = cell concentration in reactor effluent

C > 1

X_2 = cell concentration in effluent from separator

"Bioprocess Engineering: Basic Concepts"
Shuler and Kargi,
Prentice Hall, 2002

Chemostat with recycle

To keep the cell concentration higher than the normal steady-state level, cells in the effluent can be recycled back to the reactor.

Advantages of Cell Recycle

1. Increase productivity for biomass production
2. Increase stability by dampening perturbations of input stream properties

Chemostat with recycle : Cell balance

X_3

$$FX_o + \alpha FCX_1 - (1+\alpha)FX_1 + V_R \mu X_1 = V_R \frac{dX_1}{dt}$$

$$\text{at steady - state } \left(\frac{dX_1}{dt} = 0\right) \text{ and sterile feed } (X_o = 0)$$

$$\alpha FCX_1 - (1+\alpha)FX_1 + V_R \mu X_1 = 0$$

$\div V$ and solving for μ

$$\mu = [1+\alpha(1-C)]D \quad \cdots\cdots\cdots (1)$$

$$\text{Since } C > 1 \text{ and } \alpha(1-C) < 0, \text{ then } \mu < D$$

A chemostat can be operated at dilution rates higher than the specific growth rate when cell recycle is used

Chemostat with recycle

$$\mu = [1 + \alpha(1 - C)]D$$

Monod Equation, $\mu = \dfrac{\mu_{max}\, S}{K_S + S}$

Substitute Monod Eqn. into above, solve for S

$$S = \frac{K_S D(1 + \alpha(1 - C))}{\mu_{max} - D(1 + \alpha(1 - C))} \quad \cdots\cdots (2)$$

Chemostat with recycle : substrate balance

$$FS_o + \alpha FS - (1+\alpha) FS - V_R \frac{\mu X_1}{Y^M_{X/S}} = V_R \frac{dS}{dt}$$

at steady state

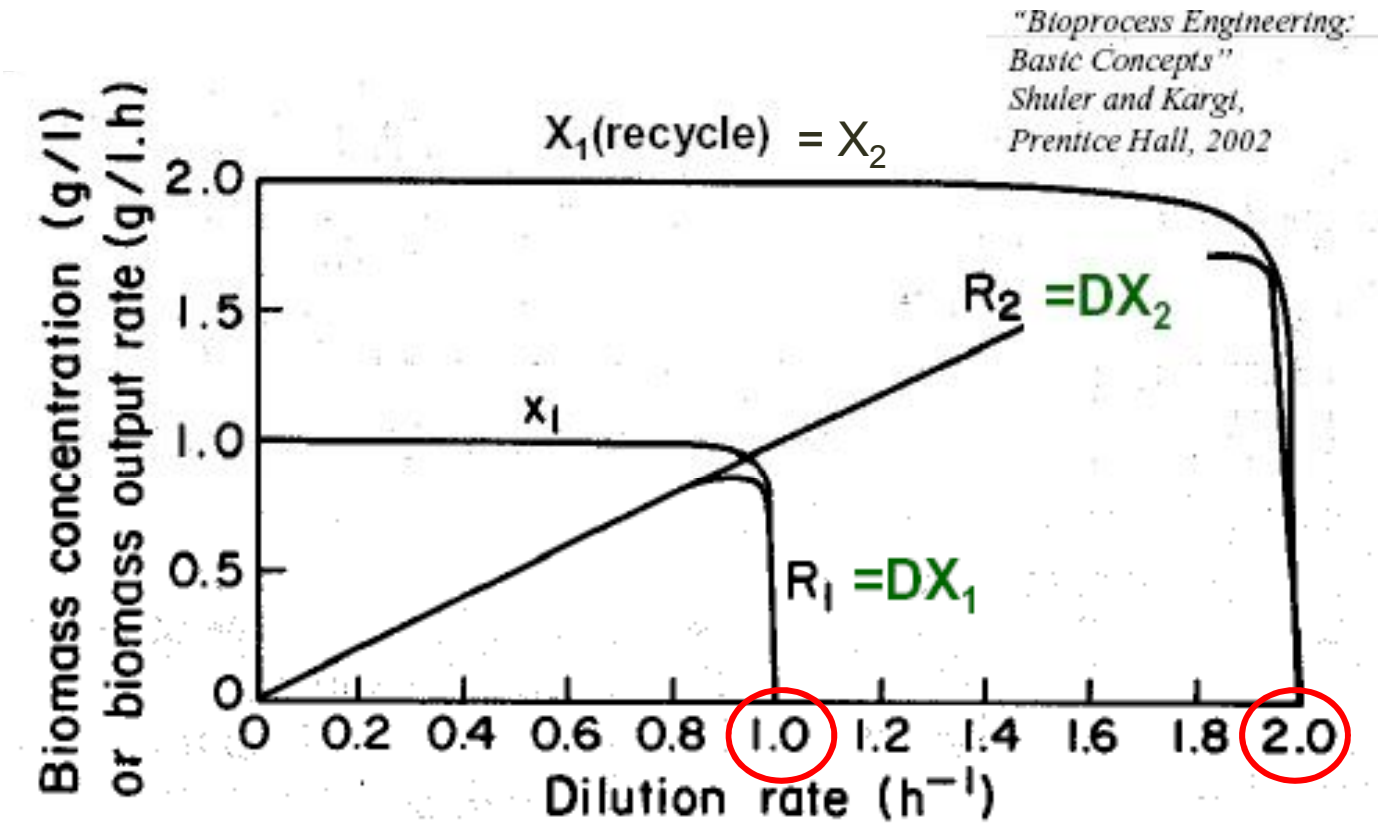
$$FS_o + \alpha FS - (1+\alpha) FS - V_R \frac{\mu X_1}{Y^M_{X/S}} = 0$$

and solving for X

$$X_1 = \frac{D}{\mu} Y^M_{X/S} (S_o - S); \qquad \frac{D}{\mu} = \frac{1}{[1+\alpha(1-C)]}$$ ••• (3) ← from eqn (1)

$$X_1 = \frac{Y^M_{X/S} (S_o - S)}{[1+\alpha(1-C)]} \overset{(2)}{=} \frac{Y^M_{X/S}}{[1+\alpha(1-C)]} \left[S_o - \frac{K_s D (1+\alpha(1-C))}{\mu_{max} - D(1+\alpha(1-C))} \right]$$

Chemostat with recycle : comparison



← from eqn (2)

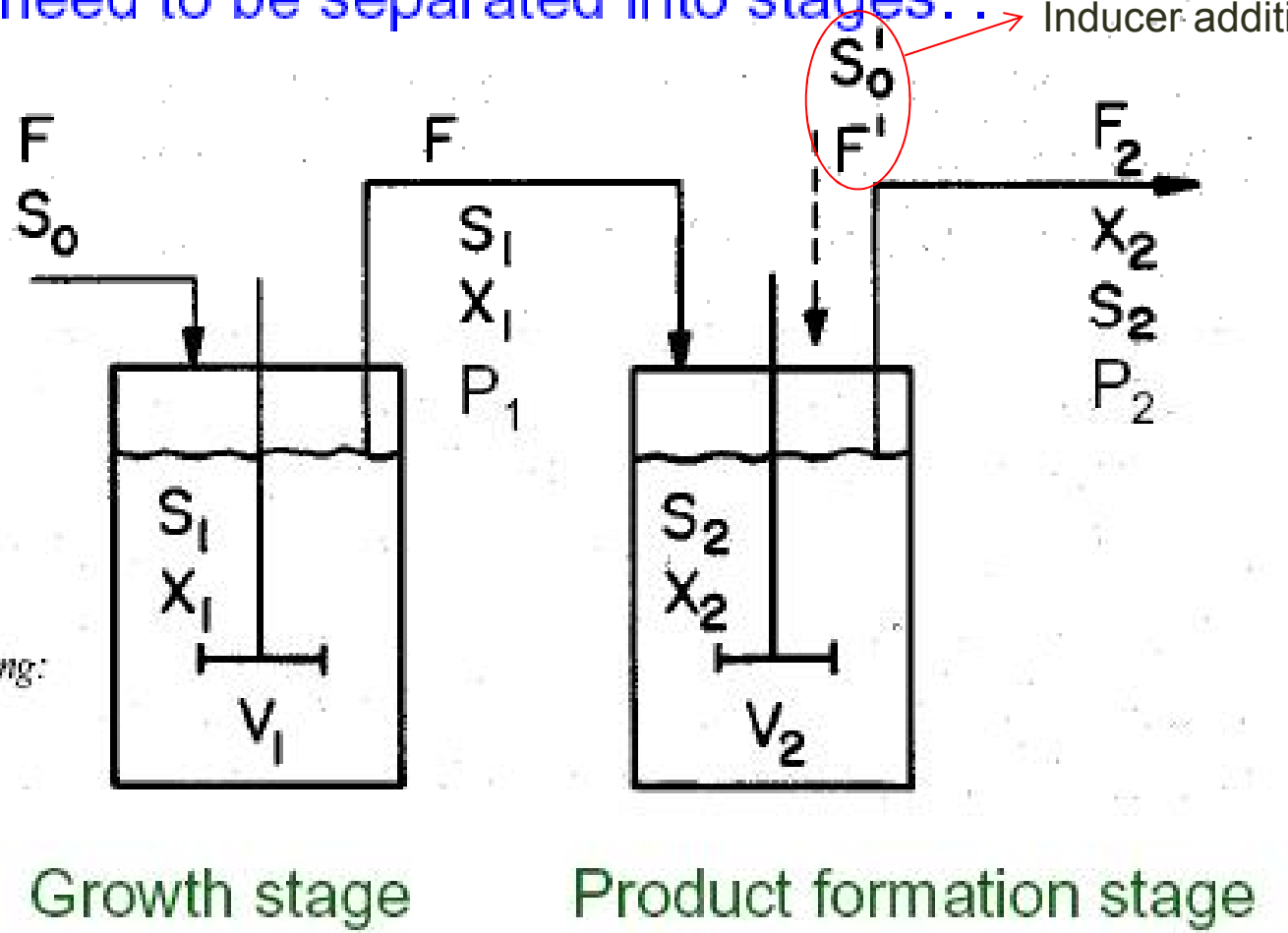
α=0.5, C=2.0, μ_{max}=1.0 hr^{-1}, K_S=0.01 g/L, $Y^M_{X/S}$=0.5

X_1 = cell concentration in reactor effluent with no recycle

X_1(recycle) = cell concentration in effluent with recycle

Multiple chemostat

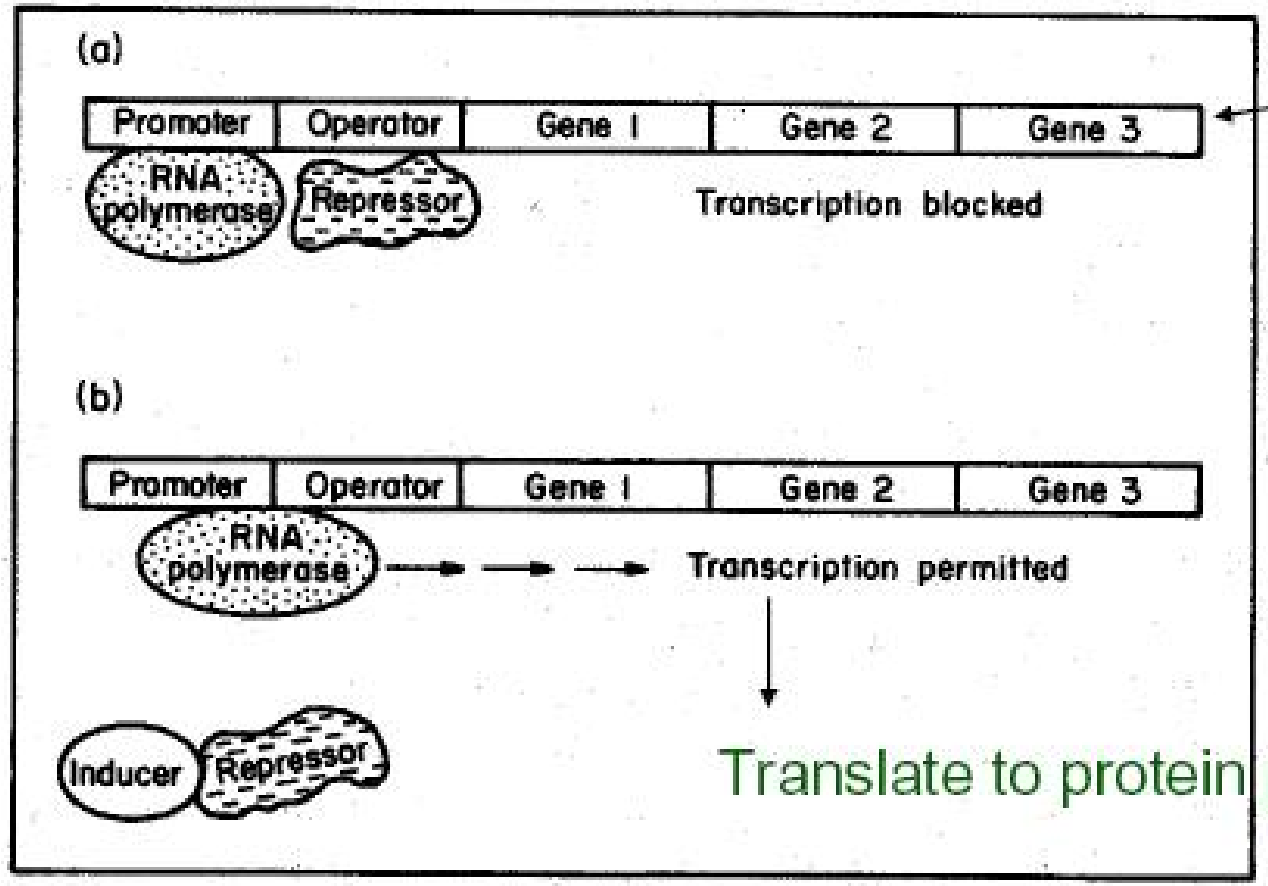
Applicable to fermentations in which growth and product formation need to be separated into stages: .



"Bioprocess Engineering: Basic Concepts"
Shuler and Kargi,
Prentice Hall, 2002

Multiple chemostat

1. Genetically Engineered Cells:



Recombinant DNA

Translate to protein product

"Bioprocess Engineering: Basic Concepts"
Shuler and Kargi, Prentice Hall, 2002

Multiple chemostat

Features of Genetically Engineered Cells:

→ have inserted recombinant DNA (plasmids) which allow for the production of a desired protein product.

→ GE cells grow more slowly than original non-modified strain (due to the extra metabolic burden of producing product).

→ Genetic Instability causes the GE culture to (slowly) lose ability to produce product. The non-plasmid carrying cells or the cells with mutation in the plasmid (revertants) grow faster.

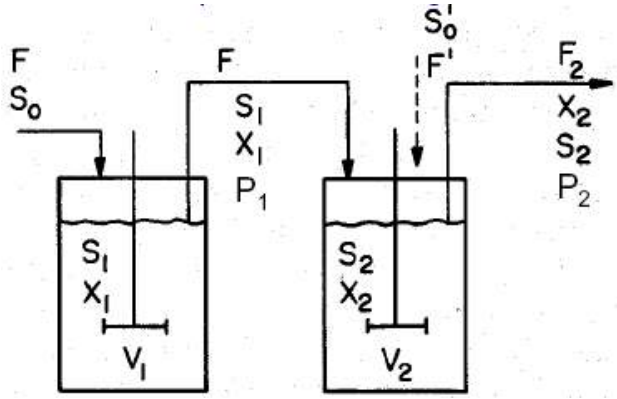
Multiple chemostat

Genetically Engineered Cells (cont.):

In the first stage, only cell growth occurs and no *inducer* is added for product formation. The GE cells grow at the maximum rate and are not out-competed in the first chemostat by *revertant* cells. When cell concentrations are high, an inducer is added in the latter (or last) chemostat to produce product at a very high rate.

Multiple chemostat

2-Stage Chemostat System Analysis



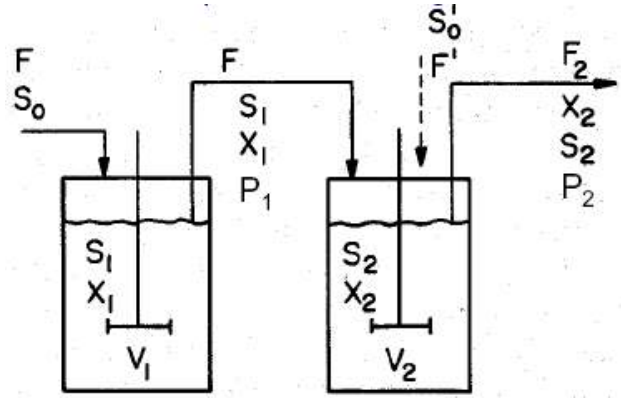
Stage 1 - cell growth conditions, $k_d=0$, $q_p=0$, steady-state

$$\mu_1 = \frac{\mu_{max} S_1}{K_S + S_1} = D_1 \quad \text{from biomass balance}$$

$$\text{rearranging, } \boxed{S_1 = \frac{K_S D_1}{\mu_{max} - D_1}} \quad \text{where } D_1 = \frac{F}{V_1}$$

$$\boxed{X_1 = Y^M_{X/S}(S_o - S_1)} \quad \text{from substrate balance}$$

Multiple chemostat



2-Stage Chemostat System Analysis

Stage 2 - product formation conditions, $k_d=0$, $F'=0$, steady-state

$$FX_1 - FX_2 + V_2\mu_2X_2 = V_2\frac{dX_2}{dt} = 0 \quad \text{biomass balance}$$

$$\mu_2 = \frac{\mu_{max}\,S_2}{K_S + S_2} = D_2\left(1 - \frac{X_1}{X_2}\right) \quad \text{where } D_2 = \frac{F}{V_2}$$

$$FS_1 - FS_2 - V_2\frac{\mu_2X_2}{Y^M_{X/S}} - V_2\frac{q_PX_2}{Y_{P/S}} = V_2\frac{dS_2}{dt} = 0 \quad \text{substrate balance}$$

$$FP_1 - FP_2 + V_2q_PX_2 = V_2\frac{dP_2}{dt} = 0 \quad \text{product balance}$$

Multiple chemostat

2-Stage Chemostat System Analysis

$P_2(t) = ?$

Stage 2 - product formation conditions, $k_d=0$, $F'=0$, steady-state

$$\mu_2 = \frac{\mu_{max} S_2}{K_S + S_2} = D_2 \left(1 - \frac{X_1}{X_2}\right)$$ biomass balance

$$S_2 = S_1 - \left(\frac{\mu_2 X_2}{D_2 Y^M_{X/S}} + \frac{q_p X_2}{D_2 Y_{P/S}} \right)$$ substrate balance

2 equations, 2 unknowns (S_2, X_2)

$$FP_1 - FP_2 + V_2 q_p X_2 = V_2 \frac{dP_2}{dt} = 0$$ product balance

use X_2 in product balance to solve for P_2