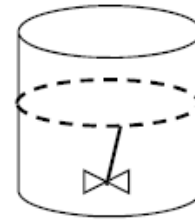


# Ch. 9 현탁배양과 고정화배양을 위한 생물반응기 운전에 대한 고찰

# Culture methods

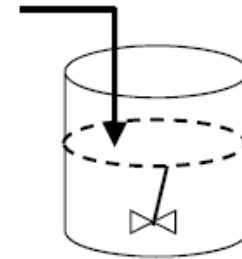
## ■ Batch Culture

- No addition or removal
- Simple and widely used



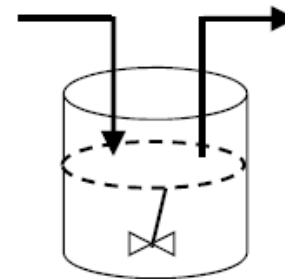
## ■ Fed-batch Culture

- 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. Capital cost in a process (>50% total capital expenses)

Further Considerations in Choosing a Bioreactor.

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

## 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

$$r_b = \frac{X_m - X}{t_c} = \frac{Y_{X/S}^M S_o}{t_c}$$

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

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

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

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

$$X_{\text{opt}} = Y_{X/S}^M \left( S_o - \frac{K_s D_{\text{opt}}}{\mu_{\text{max}} - D_{\text{opt}}} \right) = Y_{X/S}^M \left( S_o + K_s - \sqrt{K_s (S_o + K_s)} \right)$$

$$\begin{aligned} D_{\text{opt}} X_{\text{opt}} &= Y_{X/S}^M \mu_{\text{max}} \left( 1 - \sqrt{\frac{K_s}{K_s + S_o}} \right) \left( S_o + K_s - \sqrt{K_s (S_o + K_s)} \right) \\ &\approx Y_{X/S}^M \mu_{\text{max}} S_o \text{ when } K_s \ll S_o \end{aligned}$$

# Batch or continuous culture ?

## Comparing Rates in Batch and Continuous Culture

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

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,  $S \ll S_{opt}$ )
2. Genetic Instability → makes continuous culture less productive  
(revertants are formed and can out-compete highly selected 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

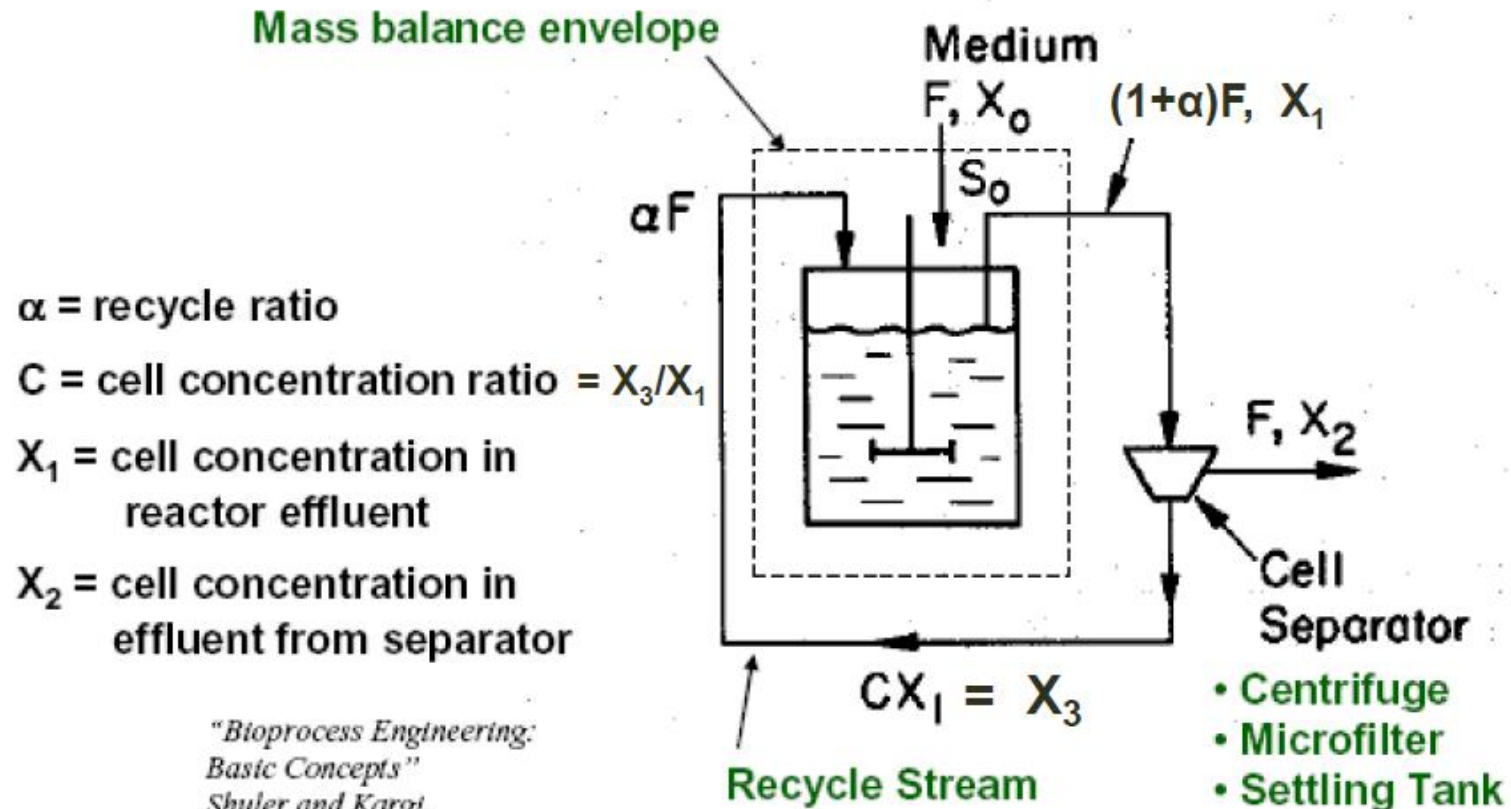
(In terms of number, mostly for secondary, high value products)

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



$\alpha$  = recycle ratio

$C$  = cell concentration ratio =  $X_3/X_1$

$X_1$  = cell concentration in reactor effluent

$X_2$  = 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

$$FX_0 + \alpha FCX_1 - (1 + \alpha)FX_1 + V_R \mu X_1 = V_R \frac{dX_1}{dt}$$

at steady - state ( $\frac{dX_1}{dt} = 0$ ) and sterile feed ( $X_0 = 0$ )

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

and solving for  $\mu$

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

Since  $C > 1$  and  $\alpha(1 - C) < 0$ , 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

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

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

Substitute Monod Eqn. into above, solve for S

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



## Chemostat with recycle :

### substrate balance

$$FS_0 + \alpha FS - (1+\alpha) FS - V_R \frac{\mu X_1}{Y_{X/S}^M} = V_R \frac{dS}{dt}$$

at steady state

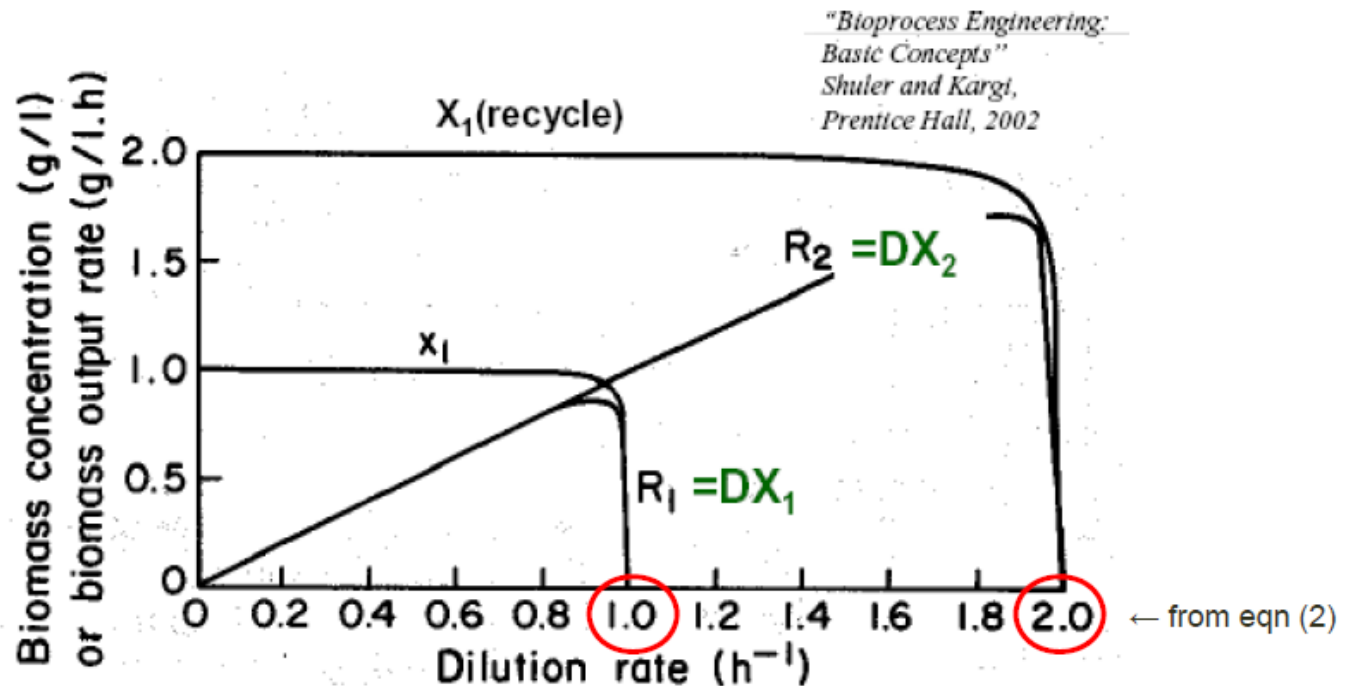
$$FS_0 + \alpha FS - (1+\alpha) FS - V_R \frac{\mu X_1}{Y_{X/S}^M} = 0$$

and solving for X

$$X_1 = \frac{D}{\mu} Y_{X/S}^M (S_0 - S); \quad \frac{D}{\mu} = \frac{1}{[1 + \alpha(1-C)]} \dots (2) \leftarrow \text{from eqn (1)}$$

$$X_1 = \frac{Y_{X/S}^M (S_0 - S)}{[1 + \alpha(1-C)]} = \frac{Y_{X/S}^M}{[1 + \alpha(1-C)]} \left[ S_0 - \frac{K_s D (1 + \alpha(1-C))}{\mu_{\max} - D(1 + \alpha(1-C))} \right]$$

# Chemostat with recycle : comparison



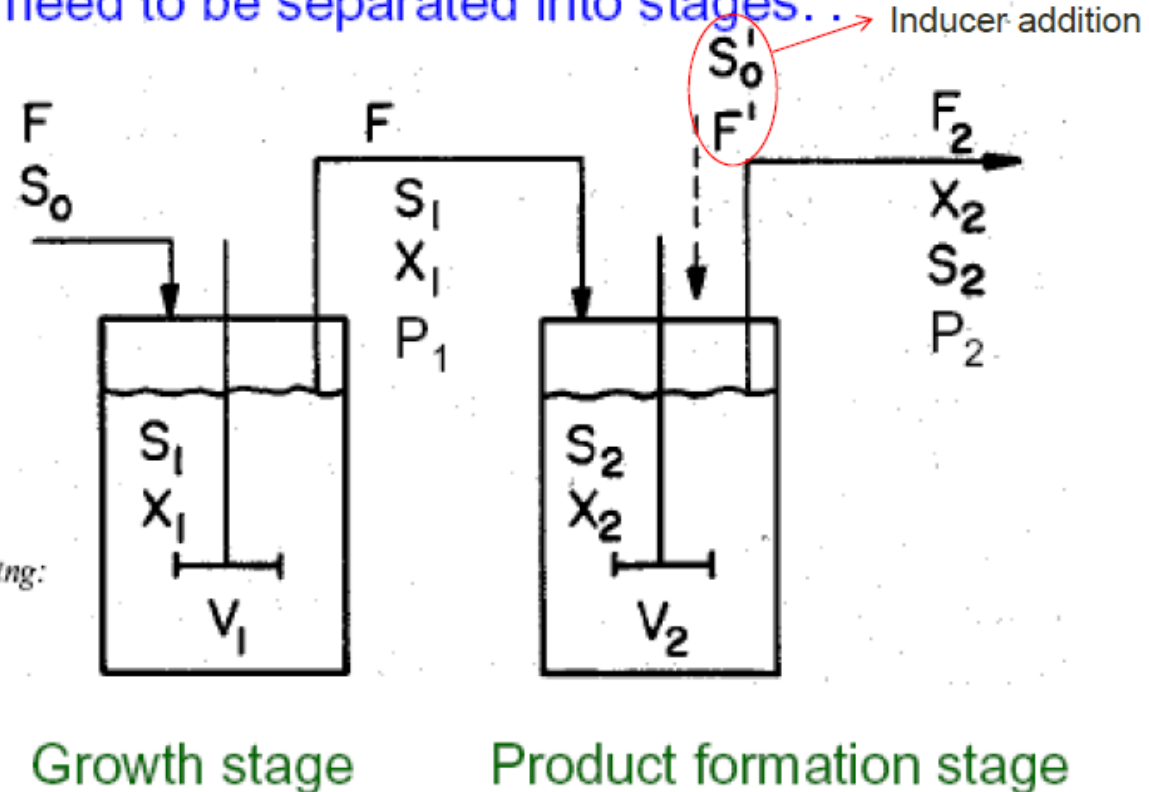
$$\alpha=0.5, C=2.0, \mu_{\max}=1.0 \text{ hr}^{-1}, K_S=0.01 \text{ g/L}, Y_{X/S}^M=0.5$$

$X_1$  = cell concentration in reactor effluent with no recycle

$X_1(\text{recycle})$  = 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

### 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

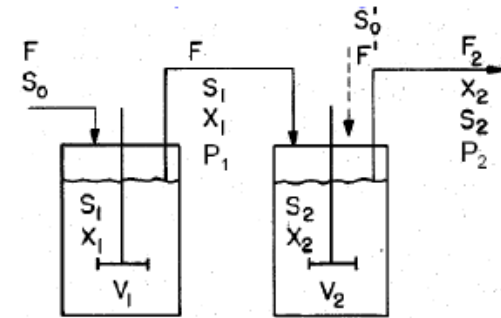
$$\mu_1 = \frac{\mu_{\max} S_1}{K_s + S_1} = D_1 \quad \text{from biomass balance}$$

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

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



# Multiple chemostat



## 2-Stage Chemostat System Analysis

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

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

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

$$FS_1 - FS_2 - V_2\frac{\mu_2 X_2}{Y_{X/S}^M} - V_2\frac{q_p X_2}{Y_{P/S}} = V_2\frac{dS_2}{dt} = 0 \quad \text{substrate balance}$$

$$FP_1 - FP_2 + V_2q_p X_2 = V_2\frac{dP_2}{dt} = 0 \quad \text{product balance}$$

# Multiple chemostat

## 2-Stage Chemostat System Analysis

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

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

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

2 equations, 2 unknowns ( $S_2, X_2$ )

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

use  $X_2$  in product balance to solve for  $P_2$



# Fed-batch

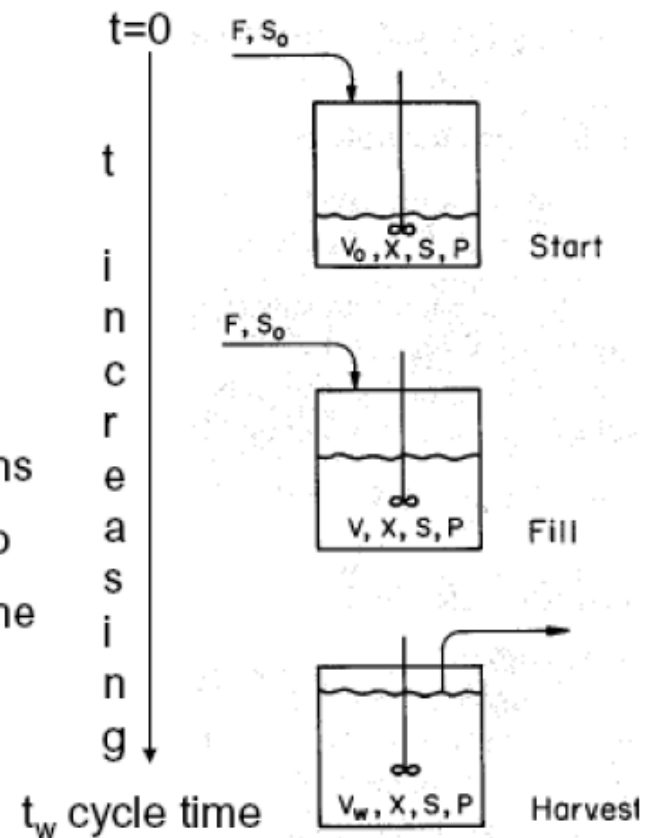
## Useful in Antibiotic Fermentation

- reactor is fed continuously (or intermittently)  
reactor is emptied periodically
- purpose is to maintain low substrate concentration,  $S$
- useful in overcoming *substrate inhibition* or *catabolic repression*, so that product formation increases.

# Fed-batch

Before  $t = 0$ , almost all of the substrate,  $S_o$ , in the initial volume,  $V_o$ , is converted to biomass,  $X_m$ , with little product formation ( $X = X_m \approx Y_{X/S} S_o$ ) and  $P \approx 0$ .

At  $t=0$ , feed is started at a low flow rate such that substrate is utilized as fast as it enters the reactor. Therefore,  $S$  remains very low in the reactor and  $X$  continues to maintain at  $\approx Y_{X/S} S_o$  over time. The volume increases with time in the reactor and Product formation continues.



## Feeding Strategies

- Predetermined feeding strategies: constant feeding, stepwise increased feeding, and exponential feeding
- Simple indirect feedback methods: pH-stat, DO-stat
- Exponential feeding combined with pH-stat
- Feeding according to carbon source uptake or demand: using  $\text{CO}_2$  evolution rate (CER) and automatic on-line monitoring system



# Fed-batch

## Analysis of Fed-Batch Operation

Volume:  $\frac{dV}{dt} = F \Rightarrow V = V_o + Ft$

Biomass:  $\cancel{FX_o^0} + V\mu X = \frac{d(XV)}{dt} = V\cancel{\frac{dX}{dt}^0} + X\frac{dV}{dt}$

$$V\mu X = X\frac{dV}{dt} \Rightarrow \mu = \frac{1}{V} \frac{dV}{dt} = \frac{F}{V} = D$$

$$\mu = \frac{F}{V} = \frac{F}{V_o + Ft} = \frac{D_o}{1 + D_o t}$$



# Fed-batch

## Analysis of Fed-Batch Operation (cont.)

Total Biomass:  $X_t$  (g cells) vs time

$$\frac{dX}{dt} = 0 \quad \text{or} \quad \frac{d\left(\frac{X_t}{V}\right)}{dt} = \frac{V\left(\frac{dX_t}{dt}\right) - X_t\left(\frac{dV}{dt}\right)}{V^2} = 0$$

rearranging  $\frac{dX_t}{dt} = \frac{X_t}{V} \frac{dV}{dt} = X_m F = Y_{X/S} S_o F$

integrating  $X_t = X_{to} + Y_{X/S} S_o F t$

## Fed-batch

### Analysis of Fed-Batch Operation (cont.)

Product Formation: total product,  $P_t = PV$

For many secondary products, the specific rate of product formation is a constant  $= q_p$

$$\frac{dP_t}{dt} = q_p X_t = q_p (V_o + Ft) X_m$$

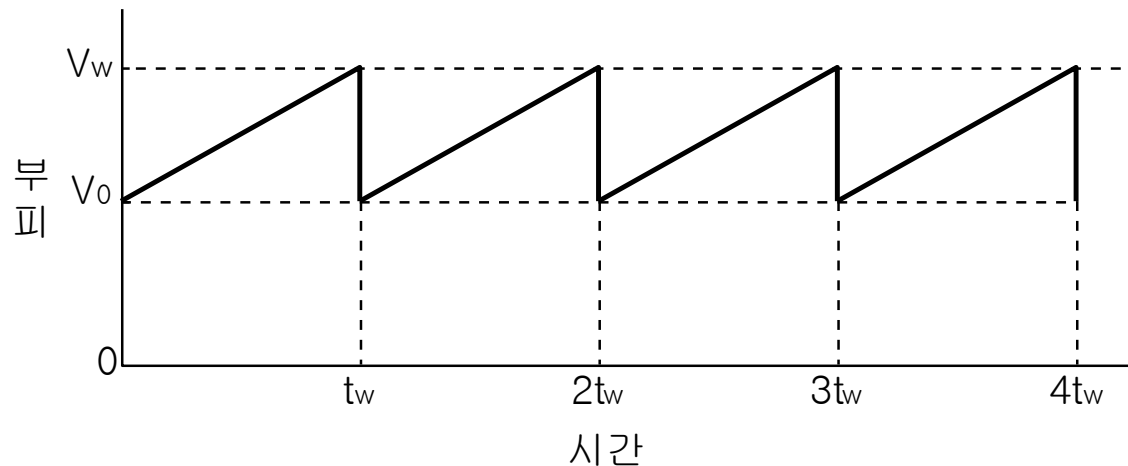
$$\text{integrating, } P_t = P_{to} + q_p X_m (V_o + \frac{Ft}{2})t$$

$$\text{or } P = \frac{P_o V_o}{V} + q_p X_m (\frac{V_o}{V} + \frac{Dt}{2})t$$

$$\text{or } P = \frac{P_o V_o}{(V_o + Ft)} + q_p X_m (\frac{V_o}{(V_o + Ft)} + \frac{Ft}{2(V_o + Ft)})t$$

## 반복유가식배양 (repeated fed-batch culture)

- 배지부피가  $V_w$ 에 도달하게 되면 일정 부분의 배지를 제거하여 잔류 배지의 부피가  $V_0$ 가 되게 함
- 간격(interval)을 두고 배지의 일부를 제거
- 배지의 부피가 주기적으로 변화





# Perfusion

- Chemostat with recycle
- High cell density, high productivity

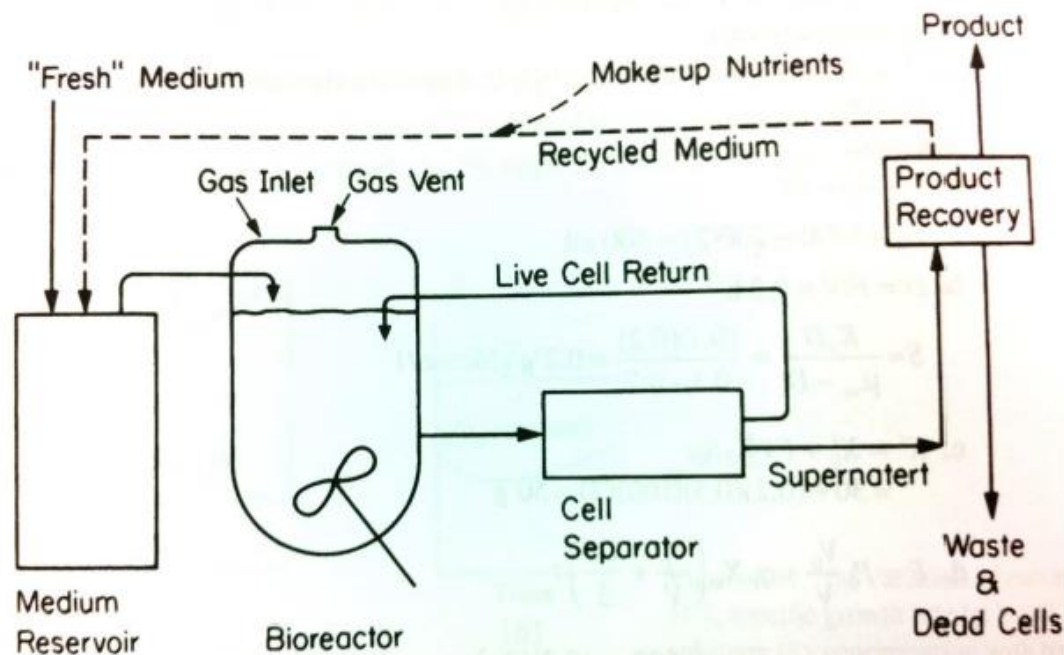


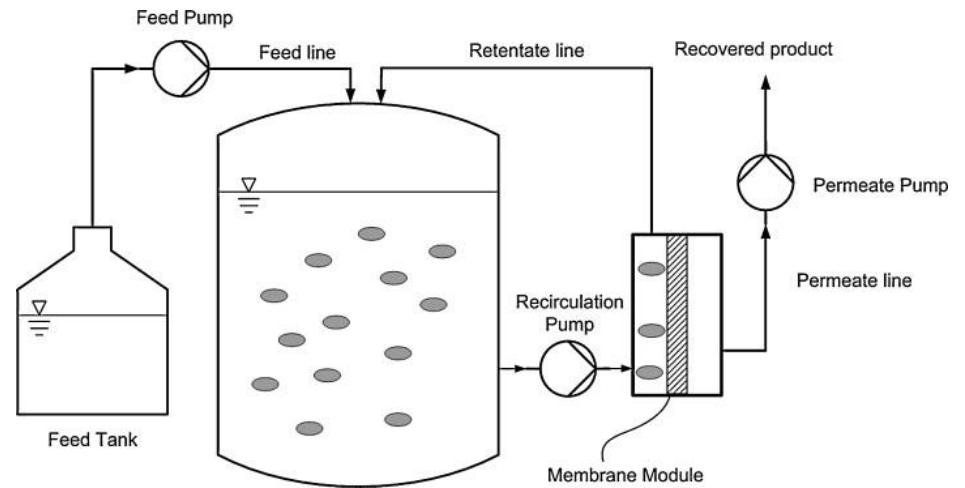
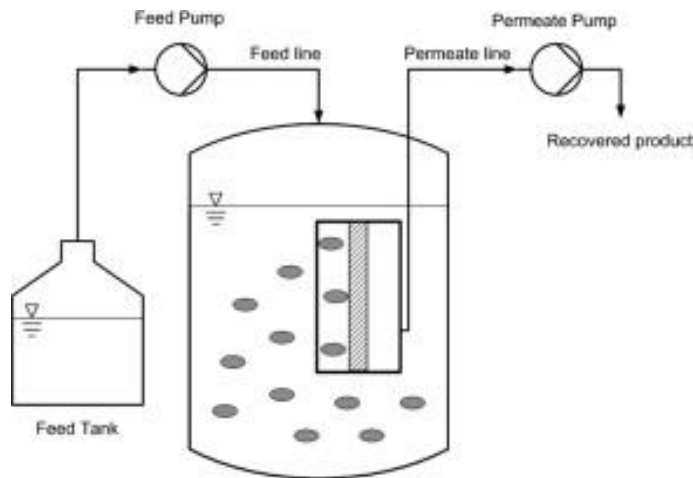
Fig. 9.10. Schematic of a perfusion system with external centrifugation and return of cells. Internal retention of cells is also possible. Return of spent medium is optional.

## 막 생물반응기

- 세포분리를 위한 막 생물반응기: 폐수처리, 막 카트리지는 통기조 안에 설치, 막 오염(fouling)이 주된 문제 (강한 교반이나 통기, 주기적인 역세척으로 부분적 제거)
- 생성물 제거를 위한 막 생물반응기: 에탄올, 아세톤-부탄올 발효, 동물세포배양 (lactate, 암모늄 제거)
- 기질의 조절적 공급을 위한 막 생물반응기: 막에 의해 생물층과 비생물층 분리, 기질은 생물층으로 공급, 생성물은 동시에 제거

# 막 생물반응기

- 내부막 생물반응기
- 외부막 생물반응기



# Immobilized cell system

Restriction of cell mobility within a confined space

## Potential Advantages:

1. Provides high cell concentrations per unit of reactor volume.
2. Eliminates the need for costly cell recovery and recycle.
3. May allow very high volumetric productivities.
4. May provide higher product yields, genetic stability, and shear damage protection.
5. May provide favorable microenvironments such as cell-cell contact, nutrient-product gradients, and pH gradients resulting in higher yields.



# Immobilized cell system

## Potential Disadvantages/Problems:

1. If cells are growing (as opposed to being in stationary phase) and/or evolve gas ( $\text{CO}_2$ ), physical disruption of immobilization matrix could result.
2. Products must be excreted from the cell to be recovered easily.
3. Mass transfer limitations may occur as in immobilized enzyme systems.



# Methods of immobilization

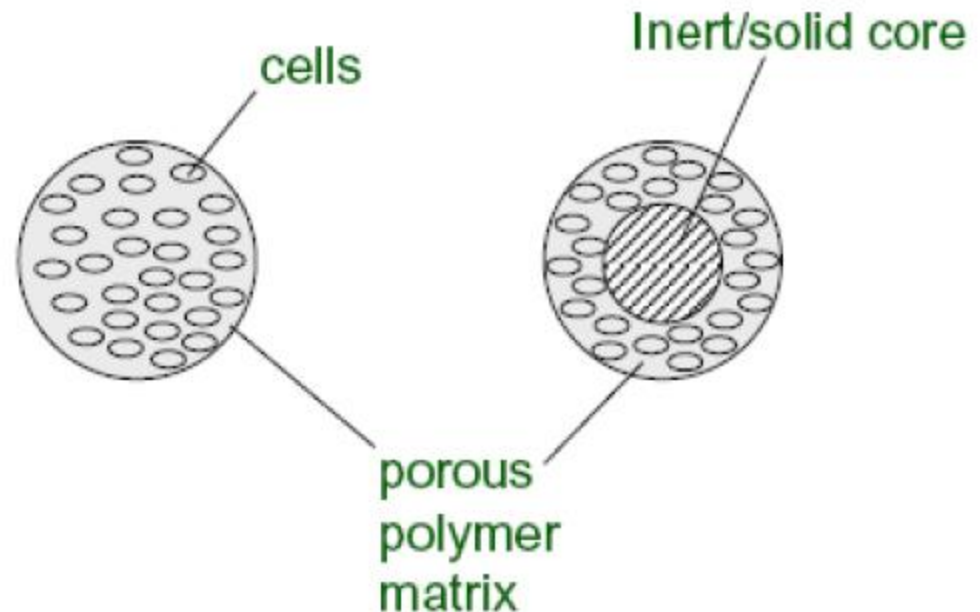
## Active Immobilization:

### 1. Entrapment in a Porous Matrix:

#### Polymeric Beads:

#### *Polymers:*

agar, alginate  
κ-carrageenan  
polyacrylamide  
gelatin, collagen



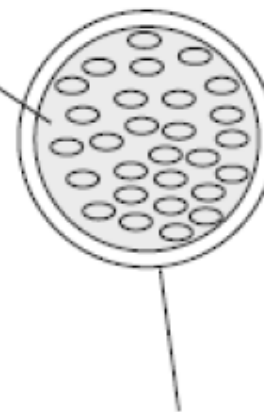
# Methods of immobilization

## Encapsulation:

hollow spherical particle

liquid core with cells

“less severe mass transfer limitations”



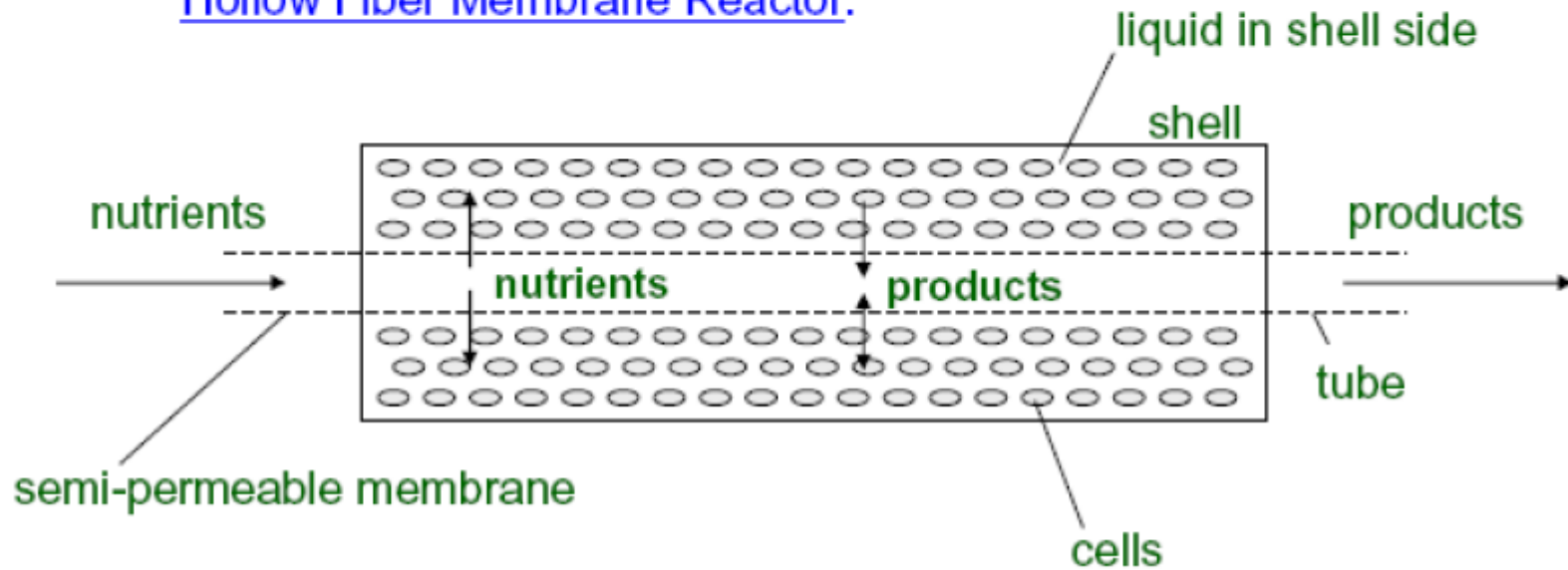
semipermeable membrane

## *Membrane:*

nylon, collodion,  
polystyrene,  
polylysine-alginate hydrogel  
Cellulose acetate-ethyl acetate

# Methods of immobilization

## Hollow Fiber Membrane Reactor:

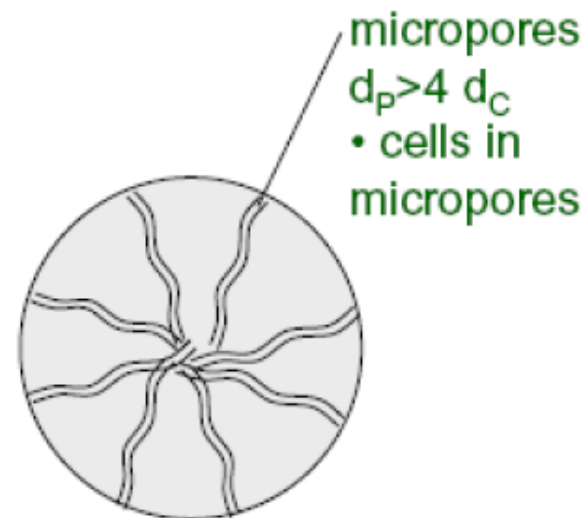


# Methods of immobilization

## 2. Cell Binding to Inert Supports:

### Micro-porous Supports:

“mass transfer limitations occur”

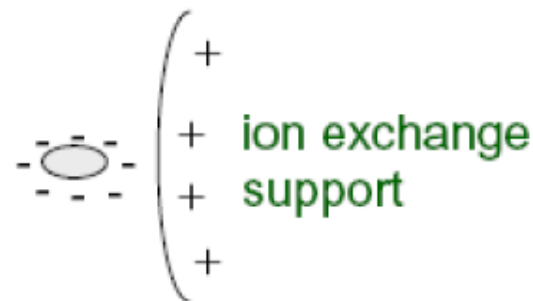


porous glass, porous silica, alumina  
ceramics, gelatin, activated carbon  
Wood chips, poly propylene ion-exchange resins  
(DEAE-Sephadex, CMC-), Sepharose

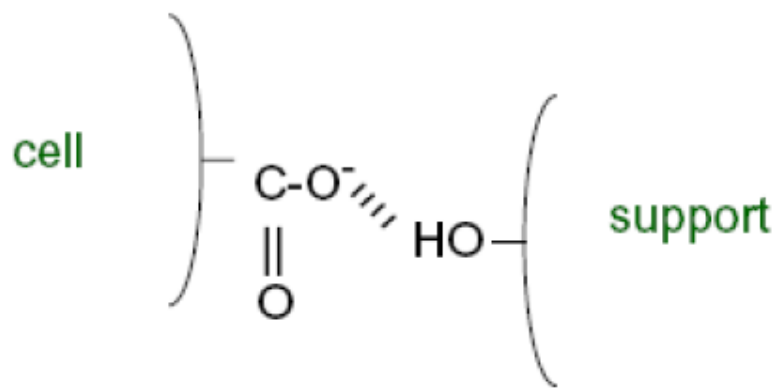
# Methods of immobilization

Binding Forces:

Electrostatic Attraction



Hydrogen Bonding





# Methods of immobilization

Binding Forces:

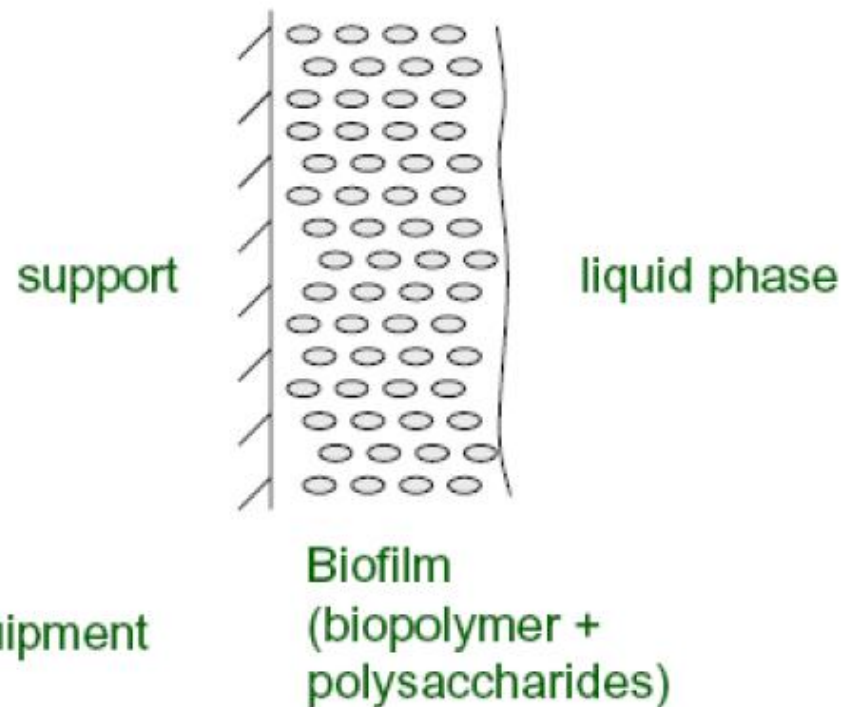
Covalent Bonding: (review enzyme covalent bonding)

Support materials: CMC-carbodiimide  
support functional groups  
-OH, -NH<sub>2</sub>, -COOH

Binding to proteins on cell surface

# Methods of immobilization

Passive Immobilization:



- wastewater treatment
- mold fermentations
- fouling of processing equipment

## 고상발효 (solid-state fermentation, SSF)

- 낮은 수분 함량(40–80%)에서 고체기질의 발효
- 전형적 액중 발효: 수분 95% 이상
- 젖은 농산물 기질(쌀, 밀, 보리, 옥수수, 콩 등) 위에 세포외 효소 생산 사상곰팡이 발효
- 아시아에서 템페, 된장, 간장 발효
- 장점: 발효 반죽이나 반응기의 작은 부피에서 오는 시설비, 운전비 절감, 낮은 수분함량으로 인한 낮은 오염 가능성, 생성물 분리 용이
- 단점: 교반이 잘되지 않아 배지 불균일, 발효 반죽 내의 pH, DO 제어 문제