Fed-batch

Useful in Antibiotic Fermentation

→ reactor is fed continuously (or intermittently)
reactor is emptied periodically

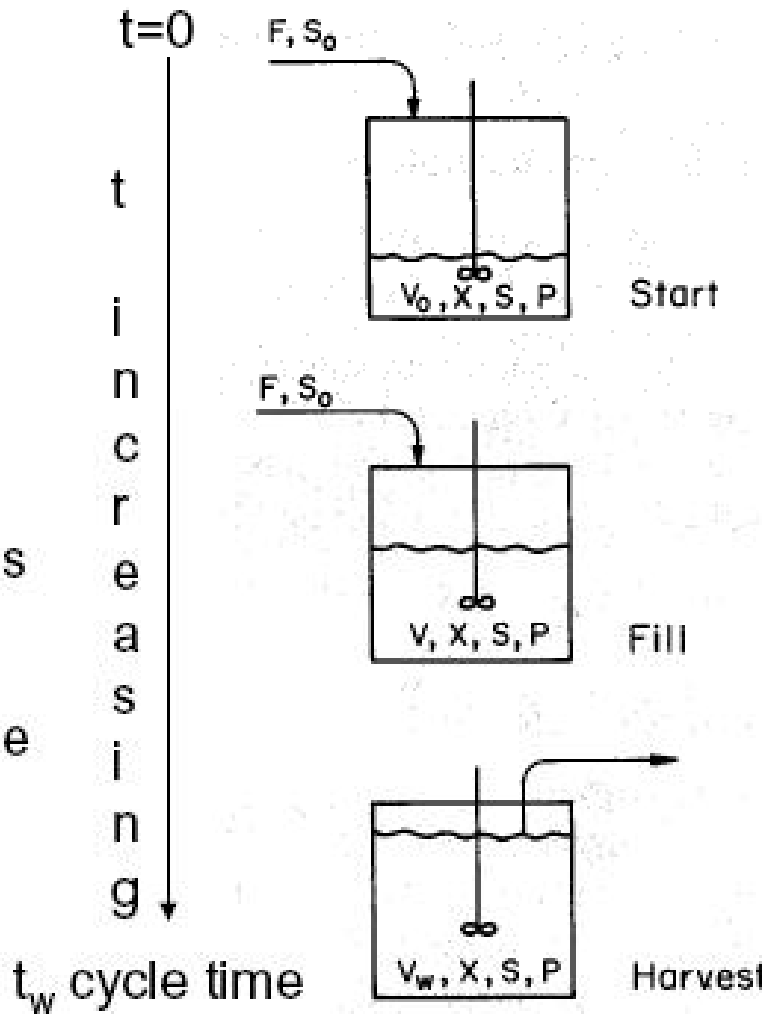
→ purpose is to maintain low substrate concentration, S

→ 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.



Fed-batch

Analysis of Fed-Batch Operation

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

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

($X_0 = 0$) (X is constant)

$$V\mu X = X\frac{dV}{dt} \quad \Rightarrow \quad \mu = \frac{1}{V}\frac{dV}{dt} = \frac{F}{V(t)} = D$$

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

Fed-batch

Analysis of Fed-Batch Operation (cont.)

X = cell mass concentration

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 $$\boxed{X_t = X_{to} + Y_{X/S} S_o F t} = (V_o + Ft) X_m$$

Cell conc. (X) is constant, but total cell mass (X_t) is increasing.

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 $= q_P$

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

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

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

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

Perfusion

- Chemostat with recycle
- High cell density, high productivity

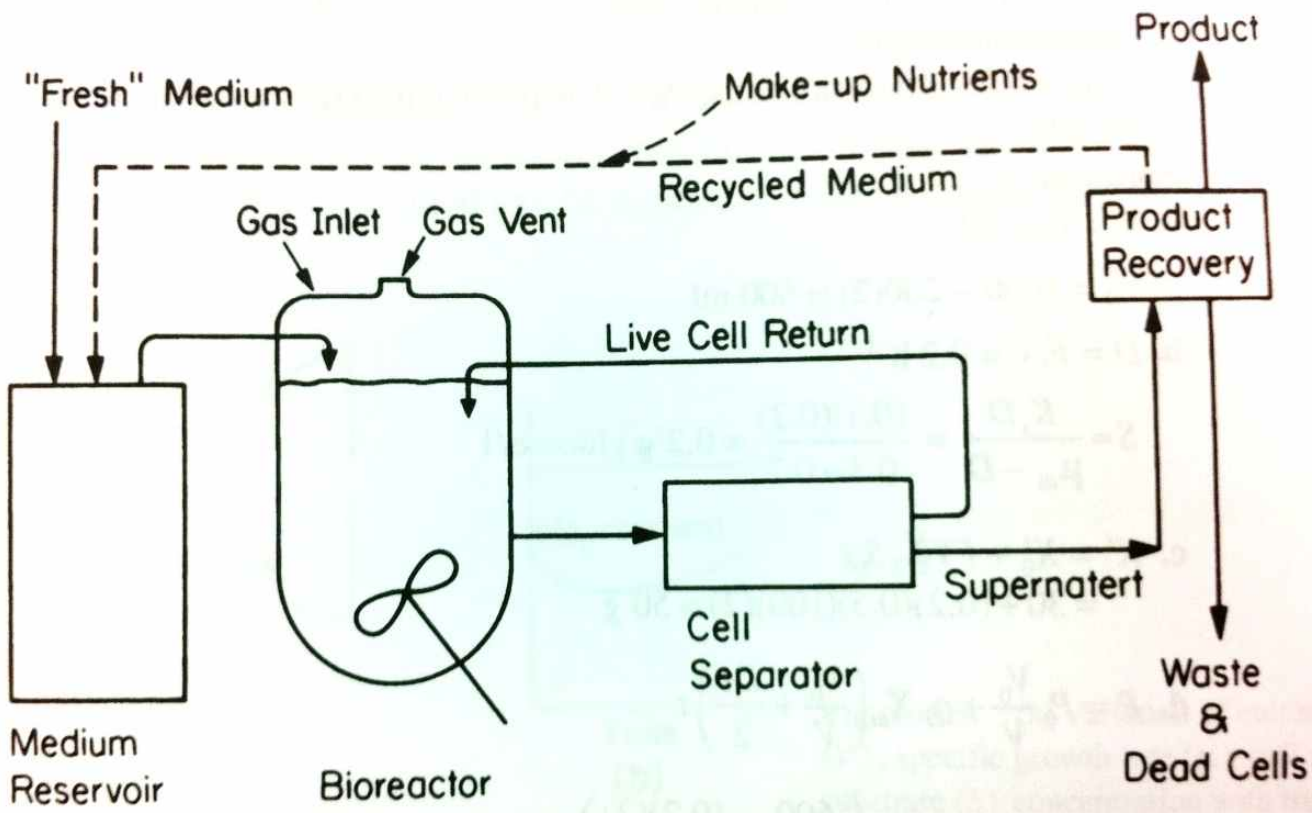


Fig. 9.10. Schematic of a perfusion system with external centrifugation and return of cells. Internal retention of cells is also possible. Return of spent medium is optional.

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 (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.

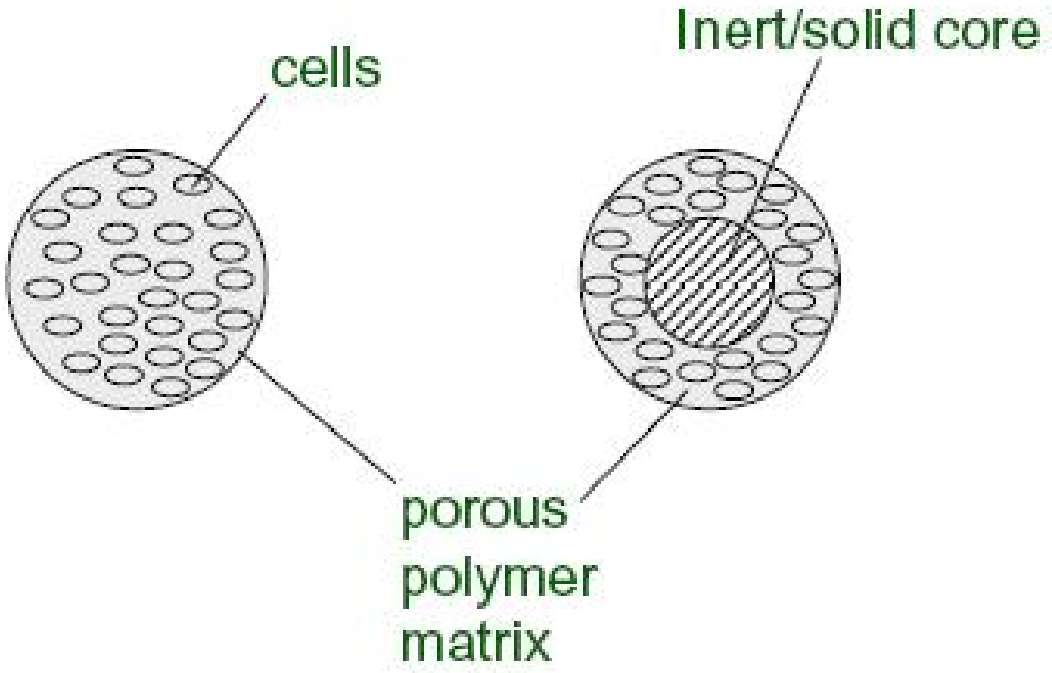
Methods of immobilization

Active Immobilization:

1. Entrapment in a Porous Matrix:

Polymeric Beads:

Polymers:
agar, alginate
κ-carrageenan
polyacrylamide
gelatin, collagen

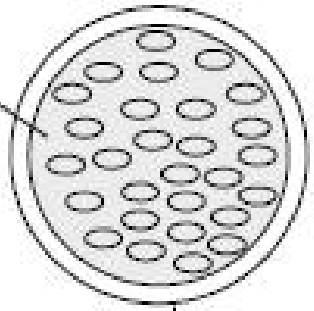


Methods of immobilization

Encapsulation:

hollow spherical particle

liquid core with cells

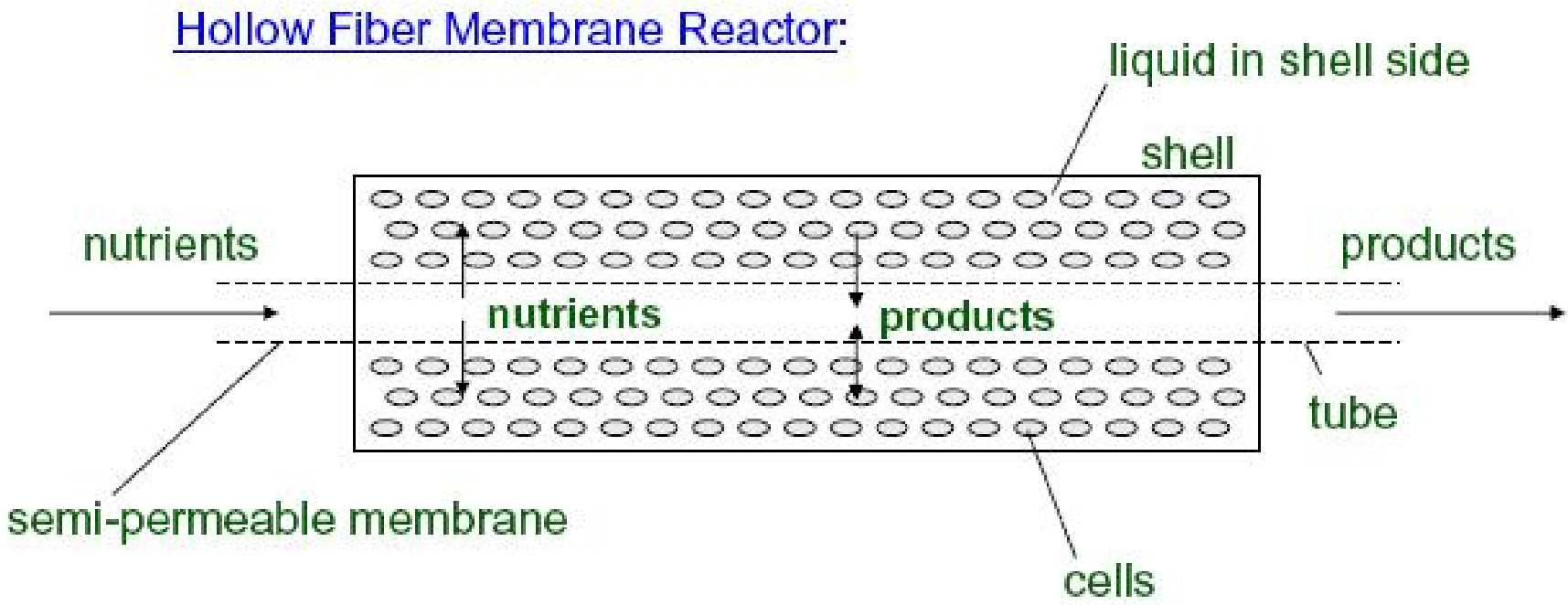


"less severe mass transfer limitations"

semipermeable membrane

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

Methods of immobilization

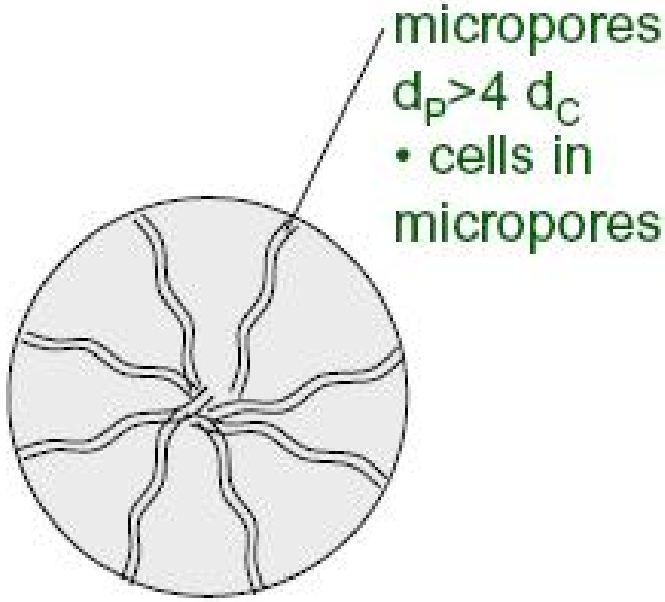


Methods of immobilization

2. Cell Binding to Inert Supports:

Micro-porous Supports:

"mass transfer limitations occur"

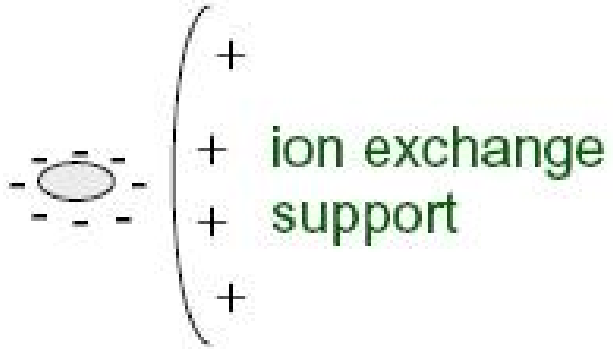


porous glass, porous silica, alumina ceramics, gelatin, activated carbon Wood chips, poly propylene ion-exchange resins (DEAE-Sephadex, CMC-), Sepharose

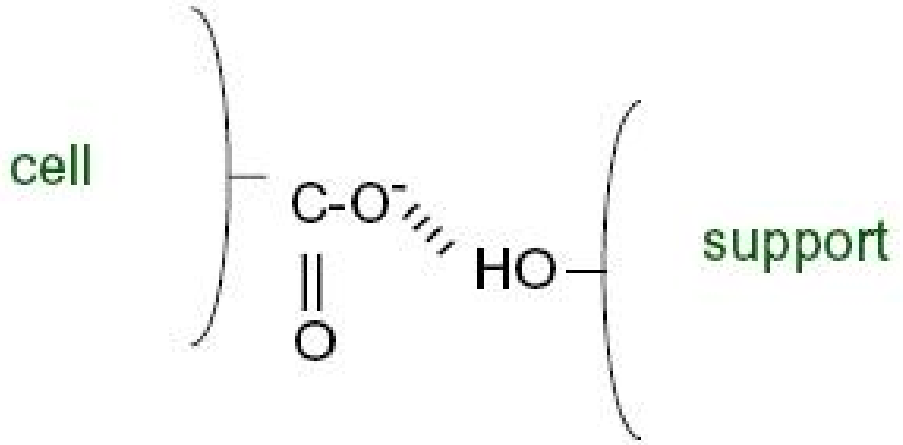
Methods of immobilization

Binding Forces:

Electrostatic Attraction



Hydrogen Bonding



Methods of immobilization

Binding Forces:

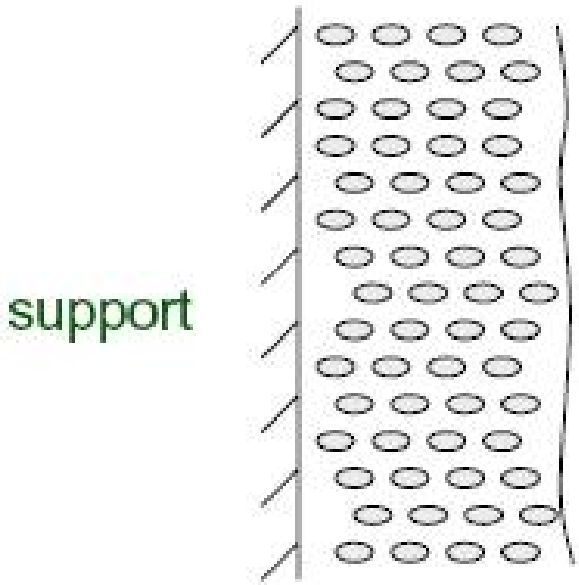
Covalent Bonding: (review enzyme covalent bonding)

Support materials: CMC-carbodiimide
support functional groups
-OH, -NH_2, -COOH

Binding to proteins on cell surface

Methods of immobilization

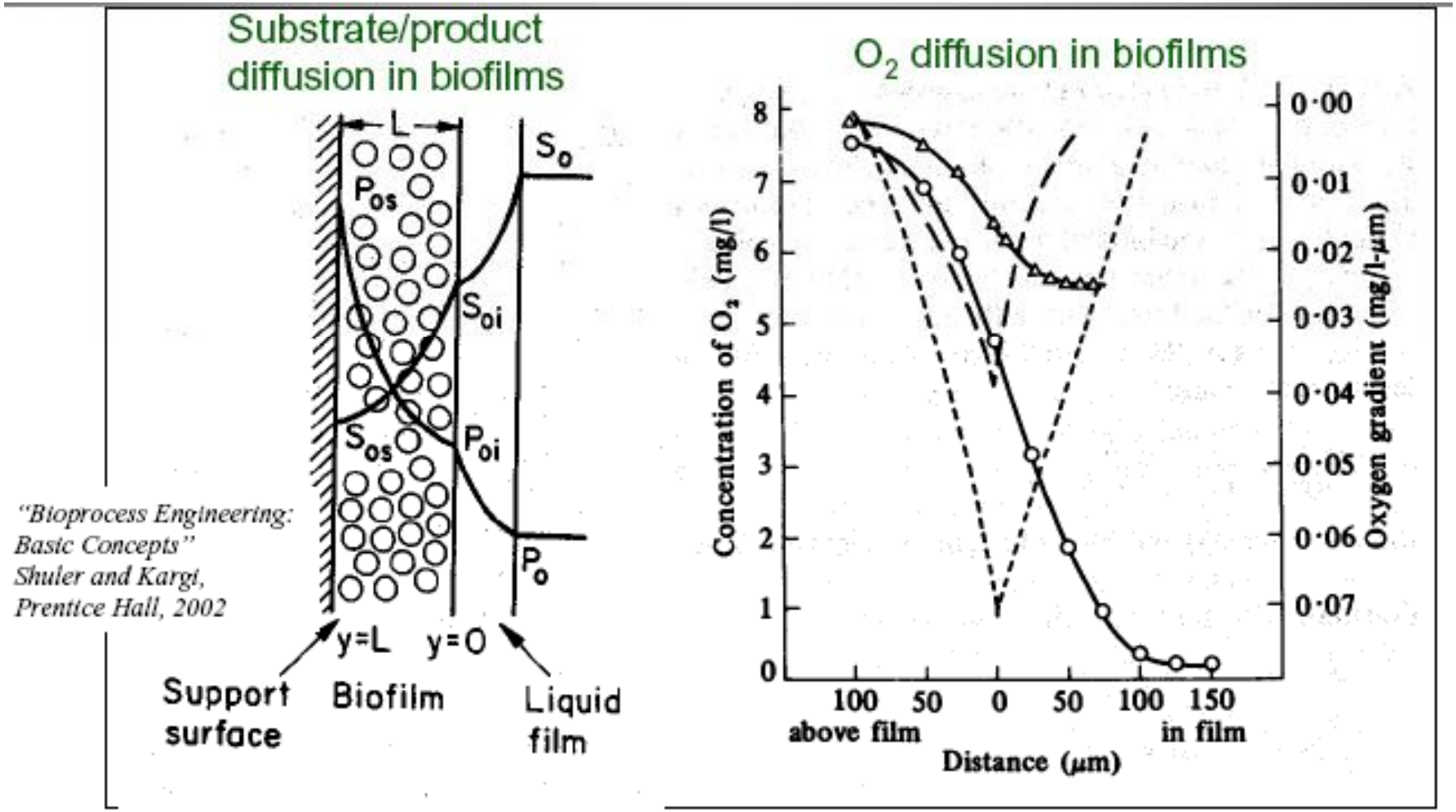
Passive Immobilization:



- wastewater treatment
- mold fermentations
- fouling of processing equipment

Biofilm
(biopolymer + polysaccharides)

Analysis of biofilm mass transfer



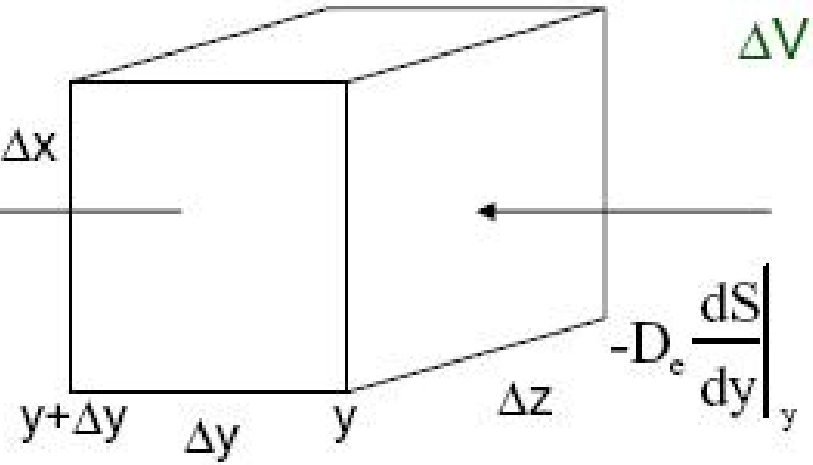
Analysis of biofilm mass transfer

Differential Substrate Balance:

Material volume in biofilm, $\Delta V = \Delta x\, \Delta y\, \Delta z$

Rate of diffusion out through the area $\Delta x\, \Delta z$

Rate of diffusion in through the area $\Delta x\, \Delta z$



$$-D_e \left.\frac{dS}{dy}\right|_{y+\Delta y} \Delta x\, \Delta z$$

$$-D_e \left.\frac{dS}{dy}\right|_{y} \Delta x\, \Delta z$$

Rate of substrate consumption in the volume $\Delta V = \Delta x\, \Delta y\, \Delta z$ (due to cell growth; or product formation)

$$\frac{1}{Y^M_{X/S}} \frac{\mu_{max}\, S}{K_S + S} X\, \Delta x\, \Delta y\, \Delta z$$

$$\frac{1}{Y_{P/S}} \frac{q_p\, S}{K_S + S} X\, \Delta x\, \Delta y\, \Delta z$$

Analysis of biofilm mass transfer

Differential Substrate Balance at Steady-State: (No production)

Rate of diffusion in through the area $\Delta x\,\Delta z$ − Rate of diffusion out through the area $\Delta x\,\Delta z$ − Rate of substrate consumption in the volume $\Delta V = \Delta x\,\Delta y\,\Delta z$ $= 0$

$$-D_e \left.\frac{dS}{dy}\right|_y \Delta x\,\Delta z - \left(-D_e \left.\frac{dS}{dy}\right|_{y+\Delta y} \Delta x\,\Delta z \right) - \frac{1}{Y^M_{X/S}} \frac{\mu_{max}\,S}{K_S + S} X\,\Delta x\,\Delta y\,\Delta z = 0$$

Divide through by $\Delta x\,\Delta y\,\Delta z$ and switch order of first 2 terms

$$\frac{D_e \left.\frac{dS}{dy}\right|_{y+\Delta y} - D_e \left.\frac{dS}{dy}\right|_y}{\Delta y} - \frac{1}{Y^M_{X/S}} \frac{\mu_{max}\,S}{K_S + S} X = 0$$

Analysis of biofilm mass transfer

$$D_e \frac{d^2S}{dy^2} = \frac{1}{Y^M_{X/S}} \frac{\mu_{max} S}{K_S + S} X \qquad \text{eqn 9.49}$$

Boundary Conditions

$S = S_{oi}$ at $y = 0$ (at the biofilm / liquid interface)

$\frac{dS}{dy} = 0,$ at $y = L$ (at the biofilm / support interface)

Dimensionless Substrate Balance at Steady-State:

$$\frac{d^2\bar{S}}{d\bar{y}^2} = \frac{\phi^2 \bar{S}}{1 + \beta \bar{S}} \qquad \text{eqn 9.51}$$

A numerical solution is required

where $\bar{S} = \frac{S}{S_o}, \quad \bar{y} = \frac{y}{L}, \quad \beta = \frac{S_o}{K_S},$

and $\phi = L\sqrt{\frac{\mu_{max} X}{Y^M_{X/S} D_e K_S}}$ "Thiele Modulus"

Analytical solution is possible for 0 order and 1st order kinetics

Boundary Conditions

$\bar{S} = 1$ at $\bar{y} = 0$ (at the biofilm / liquid interface)

$\frac{d\bar{S}}{d\bar{y}} = 0,$ at $\bar{y} = 1$ (at the biofilm / support interface)

Analysis of biofilm mass transfer

Zero Order Substrate Consumption Kinetics:

$$\frac{d^2\bar{S}}{d\bar{y}^2} = \frac{\phi^2\,\bar{S}}{1+\beta\bar{S}} \quad , \text{ for } \beta >> 1$$

$$\frac{d^2\bar{S}}{d\bar{y}^2} = \frac{\phi^2}{\beta} \quad \text{zero - order substrate consumption kinetics}$$

$$\frac{d\bar{S}}{d\bar{y}} = \frac{\phi^2}{\beta}\bar{y} + C_1$$

Boundary condition #2, at $\bar{y} = 1$, $\frac{d\bar{S}}{d\bar{y}} = 0$

$$0 = \frac{\phi^2}{\beta}(1) + C_1 \quad \Rightarrow \quad C_1 = -\frac{\phi^2}{\beta}$$

$$\frac{d\bar{S}}{d\bar{y}} = \frac{\phi^2}{\beta}\bar{y} - \frac{\phi^2}{\beta} \qquad \bar{S} = \frac{\phi^2}{2\beta}\bar{y}^2 - \frac{\phi^2}{\beta}y + C_2$$

Boundary condition #1, at $\bar{y} = 0$, $\bar{S} = 1$

$$1 = \frac{\phi^2}{\beta}(0^2) - \frac{\phi^2}{\beta}(0) + C_2 \quad \Rightarrow \quad C_2 = 1$$

$$\bar{S} = \frac{\phi^2}{2\beta}\bar{y}^2 - \frac{\phi^2}{\beta}\bar{y} + 1 \quad \text{or} \quad \boxed{\bar{S} = \frac{\phi^2}{\beta}\left(\frac{\bar{y}^2}{2} - \bar{y}\right) + 1}$$

Biofilm effectiveness

The effectiveness factor is calculated by dividing the rate of substrate diffusion into the biofilm by the maximum substrate consumption rate.

$$\text{Effectiveness Factor, } \eta = \frac{\text{Diffusion rate}}{\text{Max reaction rate}}$$

$$N_S A_S = -A_S D_e \left.\frac{dS}{dy}\right|_{y=0} = \eta \left(\frac{\mu_{max} S_o X}{Y^M_{X/S} (K_S + S_o)} \right) (A_S L)$$

Rate of substrate diffusion into biofilm through an area A_S at the surface at y = 0

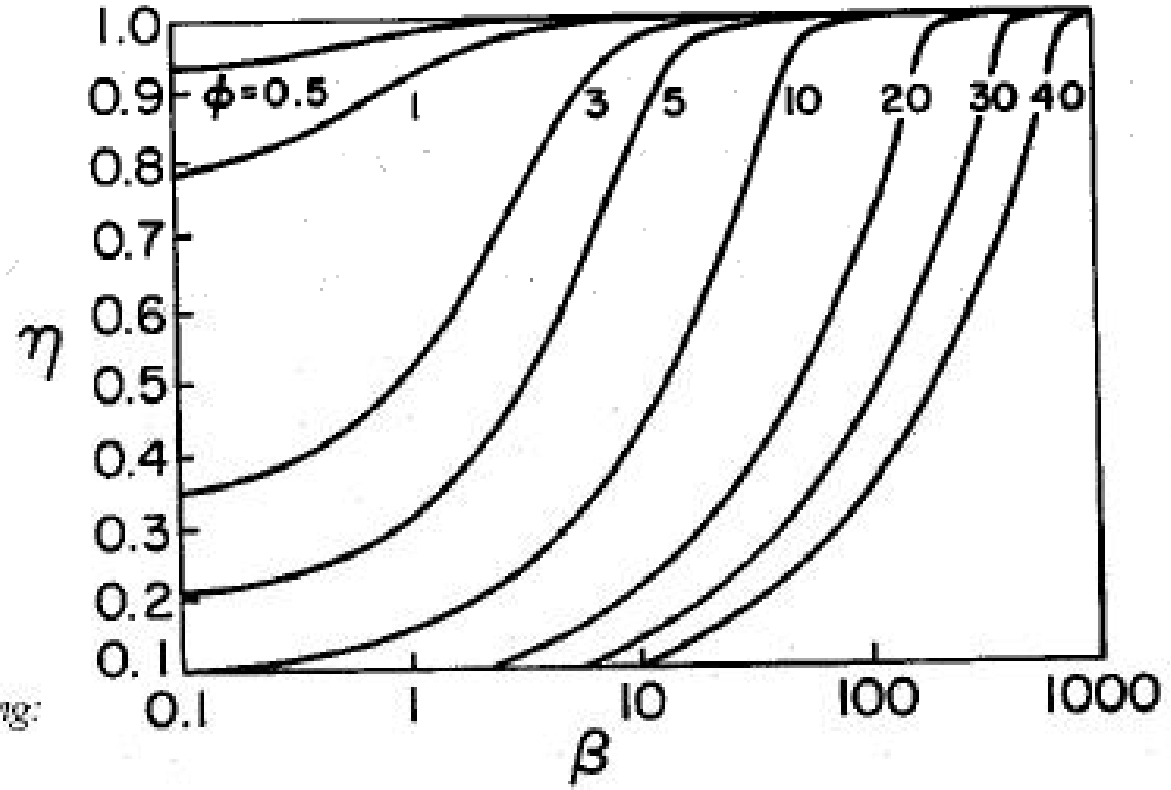
Volumetric rate of substrate consumption within the biofilm in a volume ($A_S L$)

Effectiveness factor

Thiele modulus = $\frac{\text{Rxn rate @ surface}}{\text{Diffusion rate}}$

Biofilm is most effective for $\beta >> 1$

η increases as ϕ decreases for any value of β



$= S_o/K_s$

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