Immobilized Cell System

- **Immobilization Advantages**
  - Provide high cell concentration
  - Reuse cell
  - Eliminate washout problem at high dilution rate and cell recovery
  - May provide favorable microenvironmental conditions for cells
    - (i.e., cell–cell contact, nutrient–product gradients, pH gradients)
  - May improve genetic stability
  - Protect against shear damage
  - Can perform multi-step biosynthesis reactions that are not practical purified immobilized enzyme preparation.
- **Disadvantages**
  - Diffusional limitation are important.
  - Growth and gas evoluation may lead to significant mechanical disruption of the immobilizing matrix.

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Immobilization Methods

- **Active immobilization of cells:**

  entrapment or **binding** of cells by physical or chemical forces

- **Passive Immobilization:**

  Biological Films

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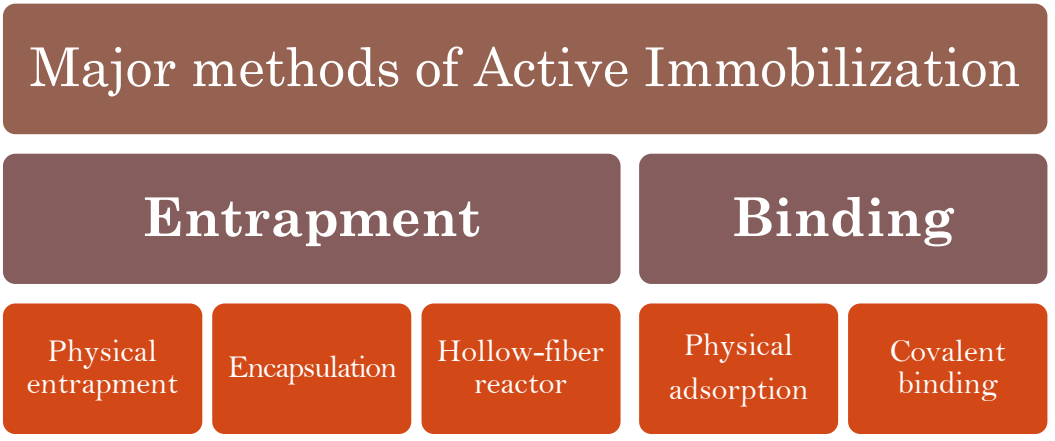
Immobilization Methods
Active immobilization

- **Active immobilization of cells:**
  entrapment or **binding** of cells by physical or chemical forces.



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Active Immobilization

- **Entrapment**
  - **Physical entrapment** within porous matrices is the most widely used method of cell immobilization.
    - Matrices used for cells immobilization:
      - porous polymers (agar, alginate, k-carrageenan, polyacrylamide, chitosan, gelatin, collagen)
        - **should be porous enough to allow the transport of substrates and products in and out of the bead**
      - porous metal screens
      - polyurethane, silica gel, polystyrene, and cellulose triacetate.

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Active Immobilization

- **Entrapment**
  - **Encapsulation** is another method of cell entrapment.
    - Microcapsules are hollow, spherical particles bound by semipermeable membranes.
    - Cells are entrapped within the hollow capsule volume.
    - The transport of nutrients and products in and out of the capsule takes place through the capsule membrane.

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Active Immobilization

- **Entrapment**
  - **Hollow-fiber reactor**
    - Mass-transfer analog of the shell-and-tube heat exchanger in which the tubes are made of semipermeable membranes.
    - Cells are inoculated on the shell side and are allowed to grow in place.
    - The nutrient solution is pumped through the insides of the tubes.
    - Nutrients diffuse through the membrane and are utilized by the cells, and metabolic products diffuse back into the flowing nutrient stream.

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Active Immobilization

- **Binding:** physical adsorption or covalent binding.
  - **Physical Adsorption**
    - **Major advantage:** direct contact between nutrient and support materials.
    - Adsorption is a simple, inexpensive method of cell immobilization.
    - However, limited cell loadings and rather weak binding forces reduce the attractiveness of this method.
    - Hydrodynamic shear around adsorbed cells should be very mild to avoid the removal of cells from support surfaces.

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Active Immobilization

- **Binding:** physical adsorption or covalent binding.
  - **Covalent binding**
    - The most widely used method for enzyme immobilization, but it is not as widely used for cell immobilization.
    - Functional groups on cell and support material surfaces are not usually suitable for covalent binding.
    - Covalent binding forces are stronger than adsorption forces, resulting in more stable binding. However, with growing cells, large numbers of cell progeny must be lost.
    - Support materials with desired functional groups are rather limited.

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Immobilization Methods
Passive immobilization

- **Passive Immobilization: Biological Films**
  - ***Biological films*** are the multilayer growth of cells on solid support surfaces.
  - The support material can be inert or biologically active.
  - Biofilm formation is common in natural and industrial fermentation systems, such as biological waste-water treatment and mold fermentations.
  - The interaction among cells and the binding forces between the cell and support material may be very complicated.

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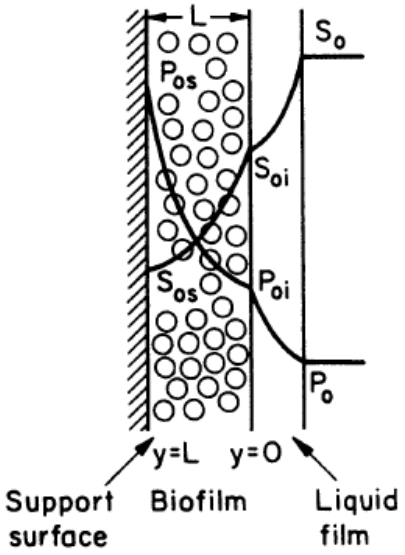
Schematic Representation of a Biofilm



In the presence of diffusion limitation, the rate of substrate consumption or flux is expressed in terms of the effectiveness factor.

$$N_s = \eta\left(\frac{r_m S_0}{K_s + S_0}\right)L$$

N_S = substrate flux into the biofilm (mg S/cm^2 h)
L = biofilm thickness or the characteristic length of the support particle ($L = V_P/A_P$)
η = effectiveness factor
$r_m = \mu_m X/Y_{X/S}$ (g subs/cm^3 h)

In the absence of diffusion limitations, $\eta \cong 1$
In the presence of diffusion limitations, $\eta < 1$.

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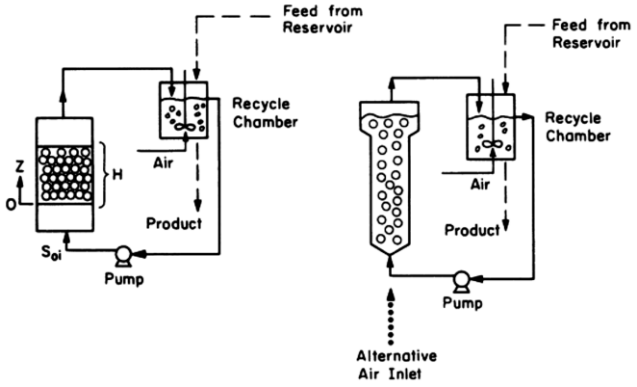
Packed-bed and Fluidized-bed Biofilm or Immobilized cell Bioreactors



- When the fluid recirculation rate is high, the system approaches CFSTR behavior.
- When the fluid recirculation rate is low or even zero (some waste-treatment systems), the system must be treated as a PFR.

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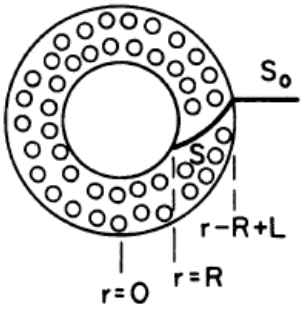
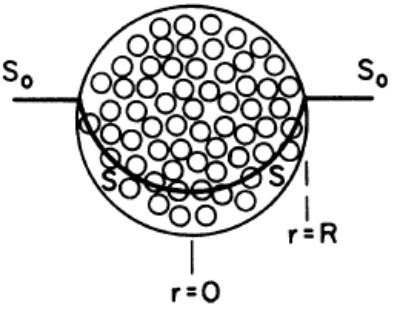
Spherical Support Particles



(a) Microbial film on inert spherical support particle



(b) Spherical microbial floc

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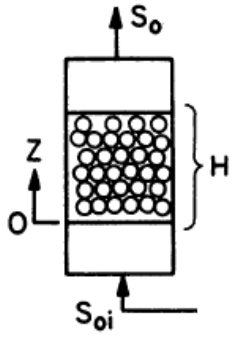
Packed-bed with Low Fluid Recirculation

- Material balance on the rate limiting substrate over a differential element

$$-F\,dS_0 = N_S aA\,dz \quad \text{OR} \quad -F\frac{dS_0}{dz} = \eta\frac{r_m S_0}{K_s + S_0}LaA$$

S_0 = bulk liquid-phase substrate concentration (mg S/cm^3) and is a function of height
F = liquid nutrient flow rate (cm^3/h)
a = biofilm or support particle surface area per unit reactor volume (cm^2/cm^3),
A = cross-sectional area of the bed (cm^2)



- Integration yields

$$K_s \ln\frac{S_{0i}}{S_0} + (S_{0i} - S_0) = \frac{\eta r_m LaA}{F}H$$

Example 9.4

Glucose is converted to ethanol by immobilized *S. cerevisiae* cells entrapped in Ca-alginate beads in a packed column. The specific rate of ethanol production is q_P = 0.2 g ethanol/g cell-h, and the average dry-weight cell concentration in the bed is $\bar{X}$ = 25 g/l bed. Assume that growth is negligible (i.e., almost all glucose is converted to ethanol) and the bead size is sufficiently small that $\eta \cong 1$. The feed flow rate is F = 400 l/h, and glucose concentration in the feed is S_{0i} = 100 g glucose/l. The diameter of the column is 1 m, and the product yield coefficient is
$Y_{P/S} \approx 0.49$ g ethanol/g glucose.

a. Write a material balance on the glucose concentration over a differential height of the column and integrate it to determine $S = S(z)$ at steady state.
b. Determine the column height for 98% glucose conversion at the exit of the column.
c. Determine the ethanol concentration in the effluent.

Example 9.4 – Solution

a. A material balance on the glucose concentration over a differential height of the column (dz) yields

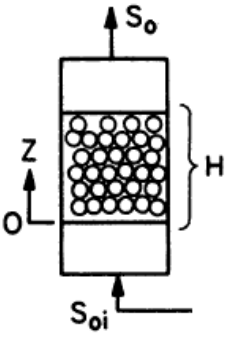
$$-F\,dS_0 = \frac{q_P\overline{X}}{Y_{P/S}}dV = \frac{q_P\overline{X}}{Y_{P/S}}A\,dz$$

S_o = the bulk liquid-phase substrate concentration (mg S/cm^3) and is a function of height

F = liquid nutrient flow rate (cm^3/h)

A = cross-sectional area of the bed (cm^2)

dz = differential height of an element of the column (cm)



- Integration yields

$$-F\int_{S_{0i}}^{S_n} dS_0 = \frac{q_P\overline{X}}{Y_{P/S}}A\int_0^H dz$$

$$S_{0i} - S_0 = \frac{q_P\overline{X}}{Y_{P/S}}\frac{A}{F}H$$

Example 9.4 – Solution

b. Determine the column height for 98% glucose conversion at the exit of the column.

S_o = 0.02(100) = 2 g glucose/l.

Substituting the given values into the equation $S_{0i} - S_0 = \frac{q_P\overline{X}}{Y_{P/S}}\frac{A}{F}H$ yields

$$(100-2) = \frac{(0.2)(25)}{0.49}\frac{(\pi/4)(10)^2}{400}H$$

$$H = 49\text{ dm} = 4.9\text{ m}$$

c. Determine the ethanol concentration in the effluent.

$P = Y_{P/S}(S_{oi} - S_o) = 0.49(98) = 48$ g/l.