# 3.4 Immobilized Enzyme Systems

The restriction of enzyme mobility in a fixed space is known as *enzyme immobilization*. Immobilization of enzymes provides important advantages, such as enzyme reutilization and elimination of enzyme recovery and purification processes, and may provide a better environment for enzyme activity. Since enzymes are expensive, catalyst reuse is critical for many processes. Since some of the intracellular enzymes are membrane bound, immobilized enzymes provide a model system to mimic and understand the action of some membrane-bound intracellular enzymes. Product purity is usually improved, and effluent handling problems are minimized by immobilization.

- Immobilization ~ enzyme reutilization
  ~ elimination of enzyme recovery and purification processes
  ~ may provides a better environment for enzyme activity

# 3.4.1 Immobilization Methods

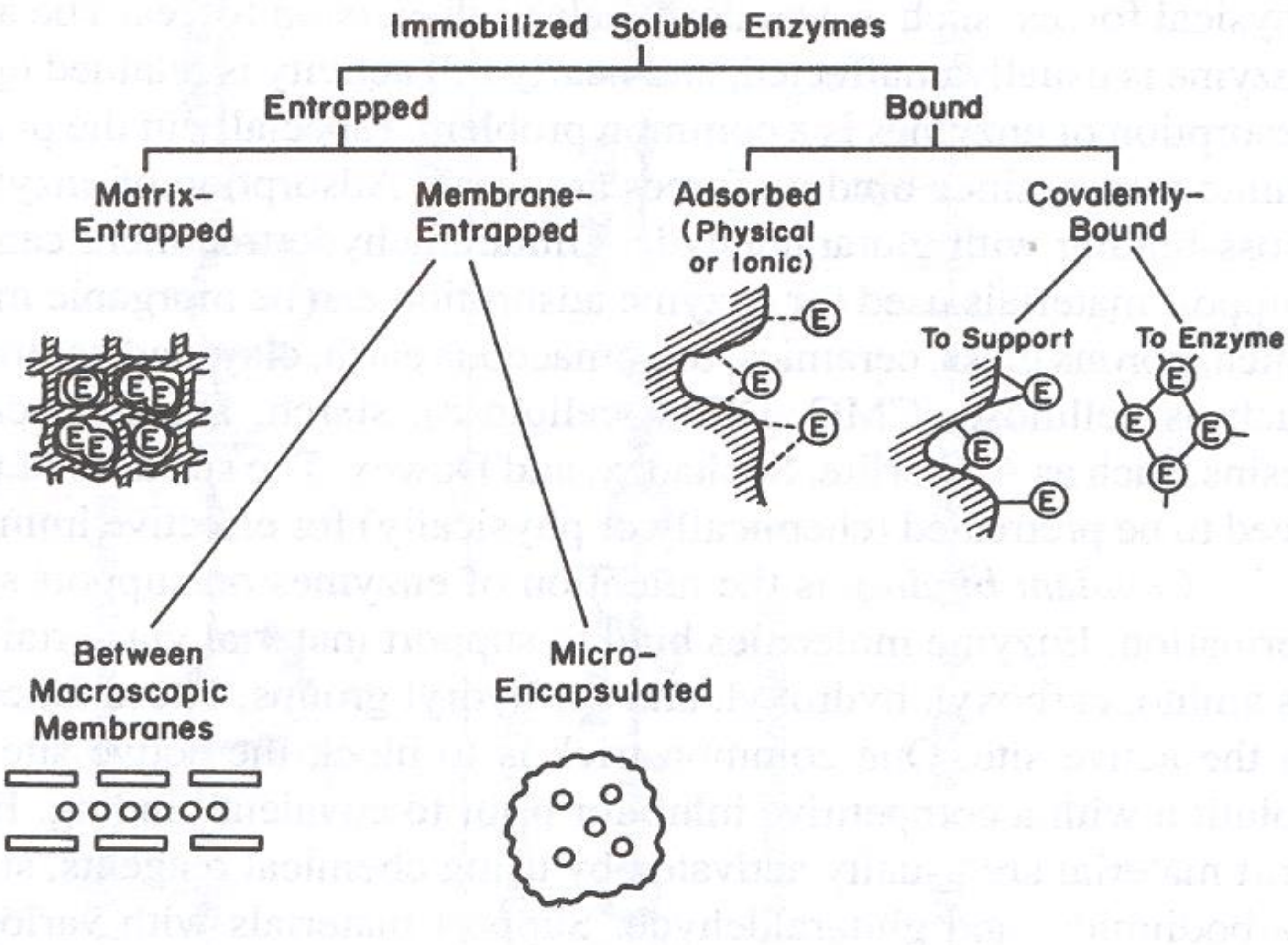



Figure 3.16. Major immobilization methods.

# 3.4.1.1 Entrapment

- Matrix-Entrapment
  - Matrix ~ Ca-alginate, agar, κ-carrageenin, polyacrylamide, and collagen

- Membrane-Entrapment
  - Hollow fiber units
    - Membrane: nylon, cellulose, polysulfone, and polyacrylate

# 3.4.1.2 Surface Immobilization

- Adsorption
  - Inorganic material ~ alumina, silica, porous glass, ceramics, diatomaceous earth, clay, bentonite
  - Organic material ~ cellulose, starch, activated carbon, ion-exchange resin
- Covalent binding

# 3.4.2 Diffusional Limitation

## Damkoehler Number (Da)

$$\mathrm{Da} = \frac{\text{maximum rate of reaction}}{\text{maximum rate of diffusion}} = \frac{V_m'}{k_L[S_b]}$$

where $[S_b]$ is substrate concentration in bulk liquid ($g/cm^3$)

$k_L$ is the mass-transfer coefficient (cm/s)

**If Da>>1, the diffusion rate is limiting.**
**If Da<<1, the reaction rate is limiting.**
**If Da is about 1, the diffusion and reaction rates are comparable.**

# Mass Transfer & Reaction

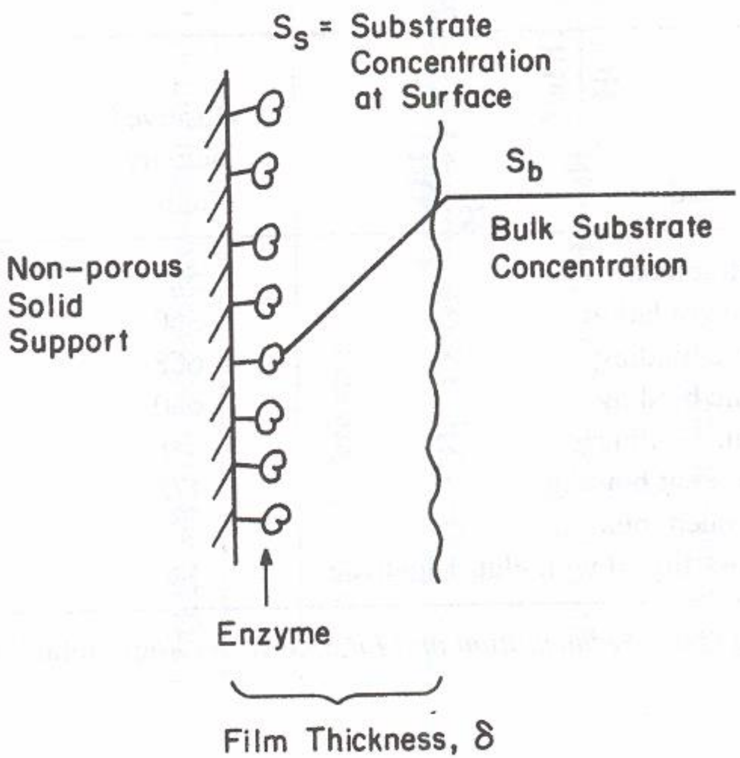
- Flux (g/cm²/sec) = $k_L \Delta S$
  - $k_L$ : mass transfer coefficient (cm/sec)
  - $\Delta S$ : concentration difference (g/cm³)

- Surface reaction rate (g/cm²/sec)

$$\frac{V_m'[S_s]}{K_m + [S_s]}$$

where $V_m'$ is the maximum reaction rate per unit of external surface area

# 3.4.2.1 Surface-Bound Enzyme




At steady state,

the reaction rate is equal to the mass-transfer rate:

$$J_s = k_L\left(\left[S_b\right]-\left[S_s\right]\right) = \frac{V_m'\left[S_s\right]}{K_m+\left[S_s\right]}$$

where $V_m'$ is the maximum reaction rate per unit of external surface area

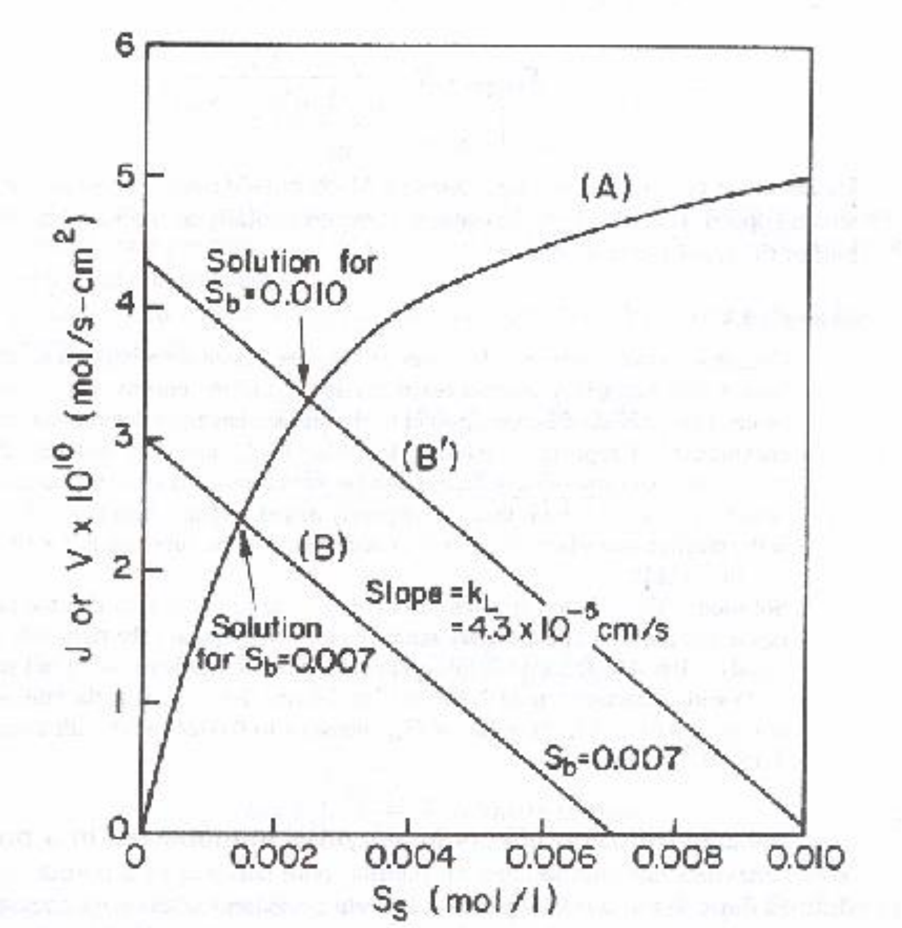
$k_L$ is the liquid mass-transfer coefficient.

# Solution for $S_s$

$$J_s = k_L\left([S_b]-[S_s]\right) = \frac{V_m'[S_s]}{K_m+[S_s]}$$

(i) Analytical solution
Above eq. is quadratic in $[S_s]$.

(ii) Graphical solution →



# Surface-Bound Enzyme

**[I]** When the system is strongly mass-transfer limited,

→ the reaction is rapid compared to mass transfer,

→ $[S_s] \approx 0$,

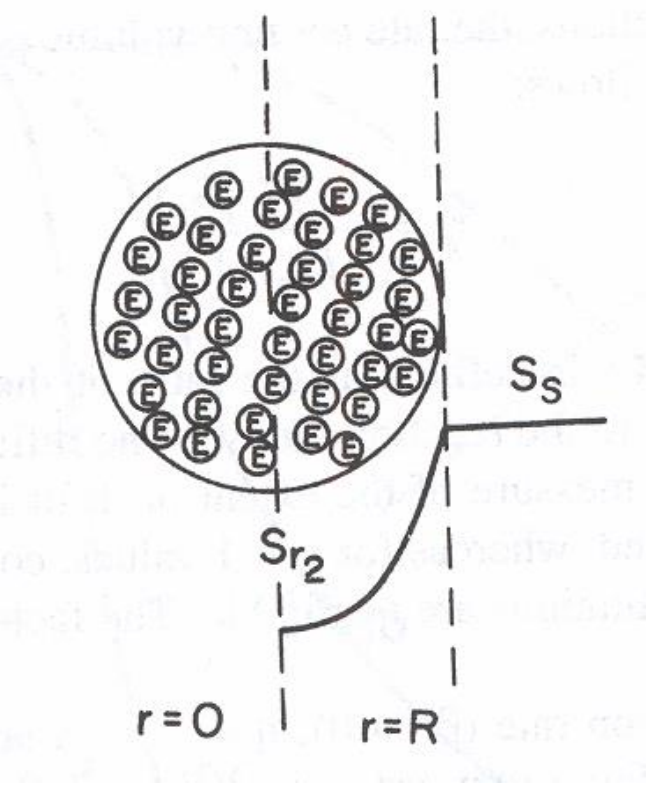
$$v \approx k_L[S_b], \qquad (\text{for Da} >> 1)$$

**[II]** When the system is reaction limited (Da << 1),

$$v = \frac{V'_m [S_b]}{K_{m,\text{app}} + [S_b]}$$

Under these circumstances, the apparent Michaelis–Menten "constant" is a function of stirring speed. Usually, $K_{m,\text{app}}$ is estimated experimentally as the value of $[S_b]$, giving one-half of the maximal reaction rate.

# 3.4.2.2 Immobilization in a Porous Matrix




**Assumption:**

- Enzyme is uniformly distributed.
- There is no partitioning of the substrate between the exterior and interior of the support.
- The reaction rate is expressed by M-M eq.

# Mass Balance Equation

- Mass Balance

  Accumulation rate

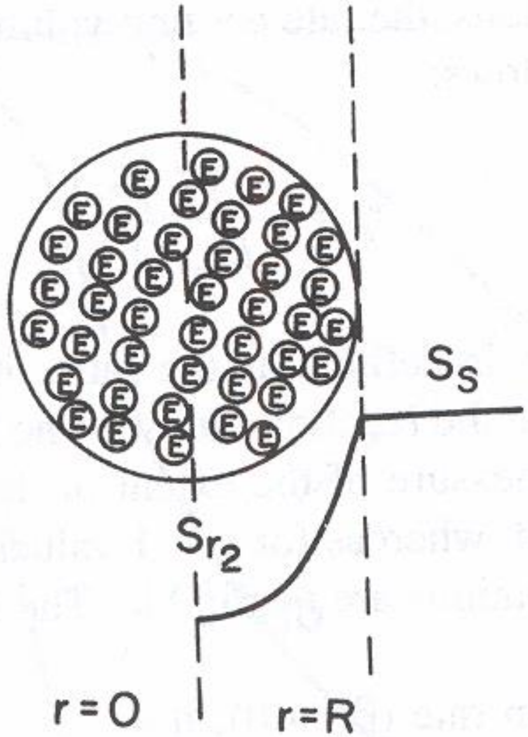
  = Input rate – Output rate + Generation rate

  – Consumption rate

- Fick's Law

  - $Flux_A = - D_{AB} \frac{dC_A}{dx}$

# 3.4.2.2 Immobilization in a Porous Matrix




$$D_e\left(\frac{d^2[S]}{dr^2}+\frac{2}{r}\frac{d[S]}{dr}\right)=\frac{V_m''[S]}{K_m+[S]}$$

**B. C.** $\begin{cases} [S]=[S_s] \text{ at } r=R \\ d[S]/dr=0 \text{ at } r=0 \end{cases}$

$V_m''$ is the maximum reaction rate per unit volume of support

$D_e$ is the effective diffusivity of substrate within the porous matrix

# Dimensionless Form

**Dimensionless Variables**

$$\bar{S} = \frac{[S]}{[S_s]}, \qquad \bar{r} = \frac{r}{R}, \qquad \beta = \frac{K_m}{[S_s]}$$

**Dimensionless Equations**

$$\frac{d^2\bar{S}}{d\bar{r}^2} + \frac{2}{\bar{r}}\frac{d\bar{S}}{d\bar{r}} = \frac{R^2 V_m''}{S_s D_e}\left(\frac{\bar{S}}{\bar{S}+\beta}\right)$$

$$\frac{d^2\bar{S}}{d\bar{r}^2} + \frac{2}{\bar{r}}\frac{d\bar{S}}{d\bar{r}} = \phi^2 \frac{\bar{S}}{1+\bar{S}/\beta}$$

where $\phi = R\sqrt{\frac{V_m''/K_m}{D_e}}$ = Thiele modulus

**B. C.**
$$\begin{cases} \bar{S} = 1 \text{ at } \bar{r} = 1 \\ d\bar{S}/d\bar{r} = 0 \text{ at } \bar{r} = 0 \end{cases}$$

# Effectiveness Factor

$$\eta = \frac{\textbf{Actual reaction rate (with diffusion limitation)}}{\textbf{Reaction rate in bulk solution (without diffusion limitation)}}$$

Actual reaction rate

$$r_s = N_s = 4\pi R^2 D_e \left. \frac{d[\mathrm{S}]}{dr} \right|_{r=R}$$

Reaction rate in bulk solution

$$\frac{V_m'' [\mathrm{S}_s]}{K_m + [\mathrm{S}_s]}$$

# Effectiveness Factor

$$r_s = \eta \frac{V_m'' [S_s]}{K_m + [S_s]}$$

$\eta < 1$ Diffusion limitation

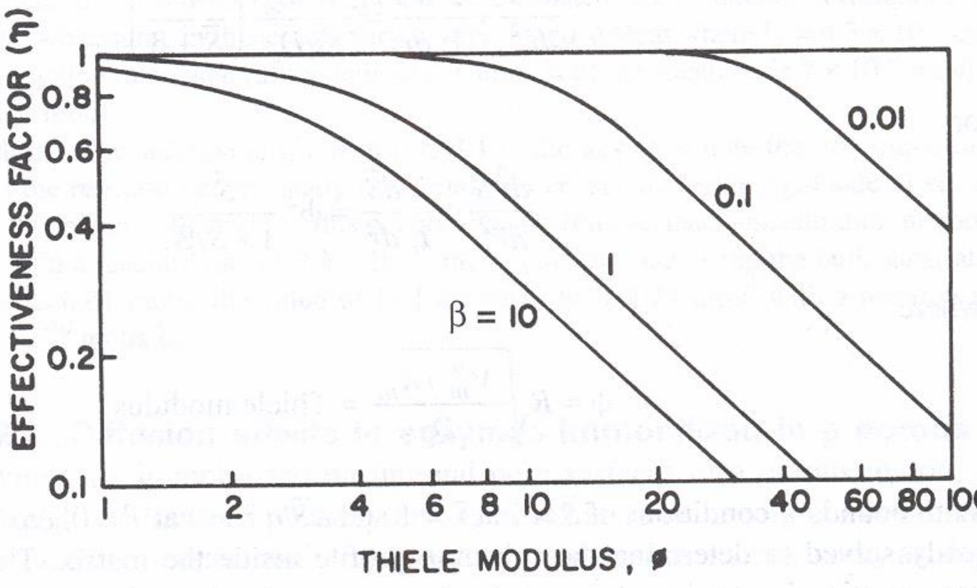
$\eta \approx 1$ Reaction rate limitation

# Effectiveness Factor

- For a zero-order reaction ( $\beta \rightarrow 0$),
  - $\eta \approx 1$ for a large range of $\phi$

- For a first-order reaction ( $\beta \rightarrow \infty$ ),

$$\eta(\phi,\beta) = \frac{3}{\phi}\left[\frac{1}{\tanh\phi} - \frac{1}{\phi}\right]$$



# Thiele Modulus

- $\Phi$ is a parameter which affects $\eta$.

$$\phi = R\sqrt{\frac{V_m'' / K_m}{D_e}} = \text{Thiele modulus}$$

- For the design of immobilized enzyme system,
  $V_m''$ and R are main variables, since $K_m$ and $D_e$ are fixed.

- High enzyme content (high $\phi$) → high $V_m''$, but low $\eta$
  Low enzyme content (low $\phi$) → low $V_m''$, but high $\eta$

# Effectiveness Factor