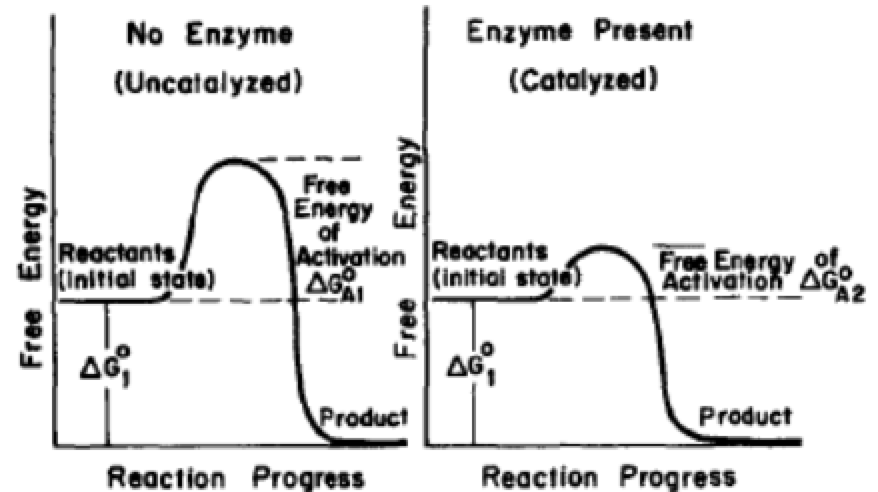
Comments on Enzymes (Chapter 3)

Overview Enzymes

Enzymes are biocatalysts – lower activity energy of a reaction by binding with the substrate



- Proteins, glycoproteins, or RNA molecules with high molecular weights (15,000 to several million)
- Some proteins require a nonprotein group as a cofactor

Require optimal conditions (pH, temperature, ionic strength) for maximum activity

Can be used in suspension or in immobilized form

- Immobilization provides enzyme reutilization w/o recovery & purification
- May result in diffusion limitations, enzyme instability, loss of activity, and/or a shift in optimal conditions (pH, ionic strength)

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Issue with enzyme "concentration"

We talk about enzyme concentration but that is a complicated subject

- Only in highly purified concentrations can be talk about [E] as mol/L or g/L
- In crude preparations concentration is in terms of "units"
  - "Unit" an amount of enzyme that gives a prescribed amount of activity under specified conditions
    - For example, one unit would form one μmol product per minute @ specified pH & temperature with substrate concentration much greater than K_m
  - "Specific activity" is number units per amount of total protein
    - Concentrating the crude preparation could increase the specific activity
    - Only include enzyme that is still catalytically active – concentrating can denature enzyme & make it inactive

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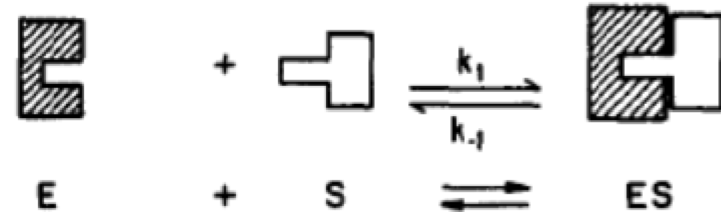
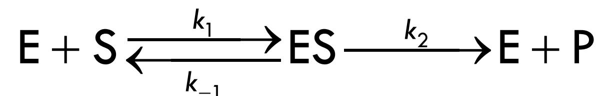
Enzyme pathways & kinetics

Simplest model is the "lock & key" model



"Saturation" kinetic model formulated Henri and Michaelis & Menten…



$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \mathrm{ES} \xrightarrow{k_2} \mathrm{E} + \mathrm{P}$$

Product balance…

$$v \equiv \frac{d[\mathrm{P}]}{dt} = k_2 [\mathrm{ES}]$$

Enzyme balance…

$$\frac{d[\mathrm{ES}]}{dt} = k_1 [\mathrm{E}][\mathrm{S}] - k_{-1}[\mathrm{ES}] - k_2 [\mathrm{ES}] \quad \text{and} \quad [\mathrm{E}] = [\mathrm{E}_0] - [\mathrm{ES}]$$

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Enzyme pathways & kinetics

Assume rapid equilibrium for the first step…

$$K'_m = \frac{k_{-1}}{k_1} = \frac{[\mathrm{E}][\mathrm{S}]}{[\mathrm{ES}]}$$

From the enzyme balance ($[\mathrm{E}] = [\mathrm{E}_0] - [\mathrm{ES}]$)…

$$K'_m = \frac{\left([\mathrm{E}_0] - [\mathrm{ES}]\right)[\mathrm{S}]}{[\mathrm{ES}]} \quad \Rightarrow \quad [\mathrm{ES}] = \frac{[\mathrm{E}_0][\mathrm{S}]}{K'_m + [\mathrm{S}]}$$

From the product balance…

$$v \equiv \frac{d[\mathrm{P}]}{dt} = k_2[\mathrm{ES}] = k_2 \frac{[\mathrm{E}_0][\mathrm{S}]}{K'_m + [\mathrm{S}]} = \frac{V_m[\mathrm{S}]}{K'_m + [\mathrm{S}]}$$

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Enzyme pathways & kinetics

More general, assume quasi-steady state…

$$\frac{d[\mathrm{ES}]}{dt} \approx 0 = k_1[\mathrm{E}][\mathrm{S}] - k_{-1}[\mathrm{ES}] - k_2[\mathrm{ES}] \quad \Rightarrow \quad [\mathrm{ES}] = \frac{k_1[\mathrm{E}][\mathrm{S}]}{k_{-1}+k_2}$$

From the enzyme balance ($[\mathrm{E}] = [\mathrm{E}_0] - [\mathrm{ES}]$)…

$$[\mathrm{ES}] = \frac{k_1\left([\mathrm{E}_0]-[\mathrm{ES}]\right)[\mathrm{S}]}{k_2+k_{-1}} \quad \Rightarrow \quad [\mathrm{ES}] = \frac{[\mathrm{E}_0][\mathrm{S}]}{\left(\frac{k_2+k_{-1}}{k_1}\right)+[\mathrm{S}]}$$

From the product balance…

$$v \equiv \frac{d[\mathrm{P}]}{dt} = k_2[\mathrm{ES}] = k_2\frac{[\mathrm{E}_0][\mathrm{S}]}{\left(\frac{k_2+k_{-1}}{k_1}\right)+[\mathrm{S}]} = \frac{V_m[\mathrm{S}]}{K_m+[\mathrm{S}]} \quad \Rightarrow \quad \begin{cases} V_m = k_2[\mathrm{E}_0] \\ K_m = \dfrac{k_2+k_{-1}}{k_1} \end{cases}$$

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Determining rate parameters for Michaelis-Menten kinetics

Model parameters K_m & V_m to relate v & [S]

$$v = \frac{V_m [S]}{K_m + [S]}$$

Appropriate for K_m from low concentrations	Appropriate for V_m from high concentrations
$\frac{1}{v} = \left(\frac{K_m}{V_m}\right) \cdot \frac{1}{[S]} + \left(\frac{1}{V_m}\right)$	$\left(\frac{[S]}{v}\right) = \left(\frac{K_m}{V_m}\right) + \left(\frac{1}{V_m}\right) \cdot [S]$

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More Complicated Enzyme Kinetics

Allosteric enzymes – Have more than one binding site

$$v = -\frac{d[S]}{dt} = \frac{V_m [S]^n}{K_m'' + [S]^n}$$

- n is the cooperativity coefficient; n > 1, positive cooperativity
- Putting into straight-line form…

$$v = \frac{V_m [S]^n}{K_m'' + [S]^n} \quad \Rightarrow \quad \frac{v}{V_m - v} = \frac{[S]^n}{K_m''}$$

$$\ln\left(\frac{v}{V_m - v}\right) = n \ln([S]) - \ln(K_m'')$$

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More Complicated Enzyme Kinetics – Inhibition

Competition for the enzymes

- Competitive
  - Inhibitor binds with enzyme but is not on a path to create product
- Noncompetitive
  - Binds with sites other than those used by the substrate.
  - Other reagents needed to block the inhibitor binding.
- Uncompetitive
  - Binds to the ES complex.
  - Can slow down the overall reaction
- Substrate inhibition
  - Substrate can bind to ES to form a non-productive complex form
  - Seen at large substrate concentrations

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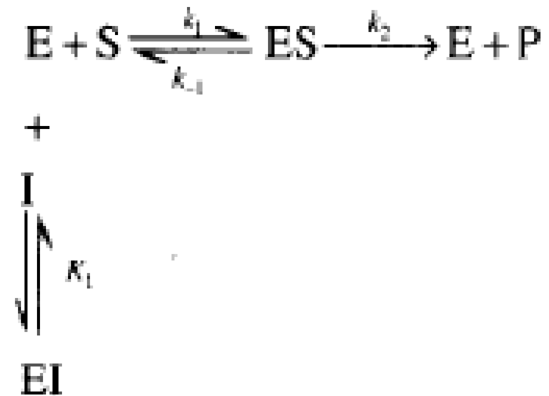
More Complicated Enzyme Kinetics – Inhibition

Competitive inhibition



- Competes with the binding of the enzyme but does not lead to product formation. Effect can be overcome with high substrate concentrations

$$v = \frac{V_m [S]}{K'_m \left(1 + \frac{[I]}{K_I}\right) + [S]} = \frac{V_m [S]}{K'_{m,app} + [S]} \quad \Rightarrow \quad K'_{m,app} = K'_m \left(1 + \frac{[I]}{K_I}\right)$$

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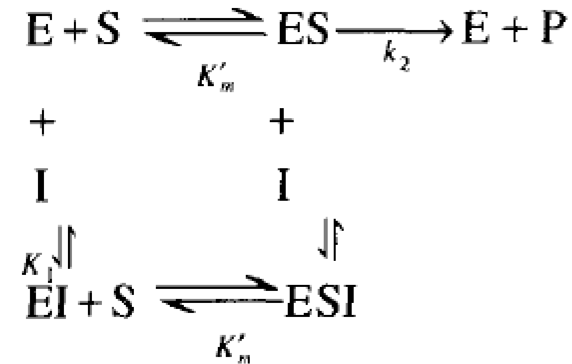
More Complicated Enzyme Kinetics – Inhibition

Noncompetitive inhibition



- Binds with sites other than those used by the substrate. Other reagents needed to block the inhibitor binding.

$$v = \frac{V_m}{\left(1+\frac{[I]}{K_I}\right)\left(1+\frac{K'_m}{[S]}\right)} = \frac{V_{m,app}[S]}{\left(K'_{m,app}+[S]\right)} \quad \Rightarrow \quad V_{m,app} = \frac{V_m}{\left(1+\frac{[I]}{K_I}\right)}$$

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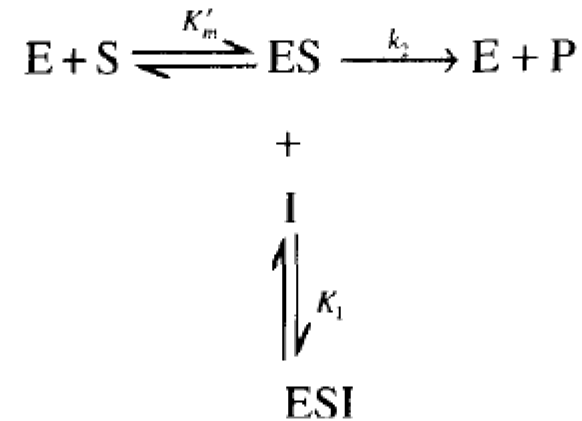
More Complicated Enzyme Kinetics – Inhibition

Uncompetitive inhibition



- Binds to the ES complex. Reduction of Vm more apparent then the reduction of K'_m

$$v = \frac{\dfrac{V_m}{1+[I]/K_I}}{\dfrac{K'_m}{1+[I]/K_I}+[S]} = \frac{V_{m,app}[S]}{K'_{m,app}+[S]} \Rightarrow \begin{cases} V_{m,app} = \dfrac{V_m}{1+[I]/K_I} \\ K'_{m,app} = \dfrac{K'_m}{1+[I]/K_I} \end{cases}$$

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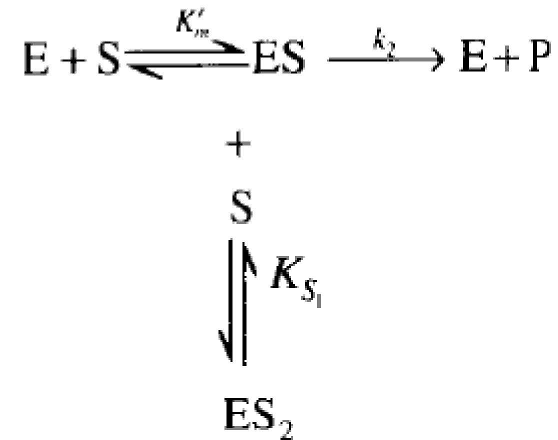
More Complicated Enzyme Kinetics – Inhibition

Substrate inhibition



- Low substrate concentrations do not show inhibition, only at larger values

$$v = \frac{V_m[S]}{K'_m + [S] + \dfrac{[S]^2}{K_{SI}}} \Rightarrow \begin{cases} v \approx \dfrac{V_m[S]}{K'_m + [S]} & \text{when } [S]^2 / K_{SI} << 1 \\ v \approx \dfrac{V_m}{1 + \dfrac{[S]}{K_{SI}}} & \text{when } K'_m / [S] << 1 \end{cases}$$

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Comparison of inhibited enzyme kinetics

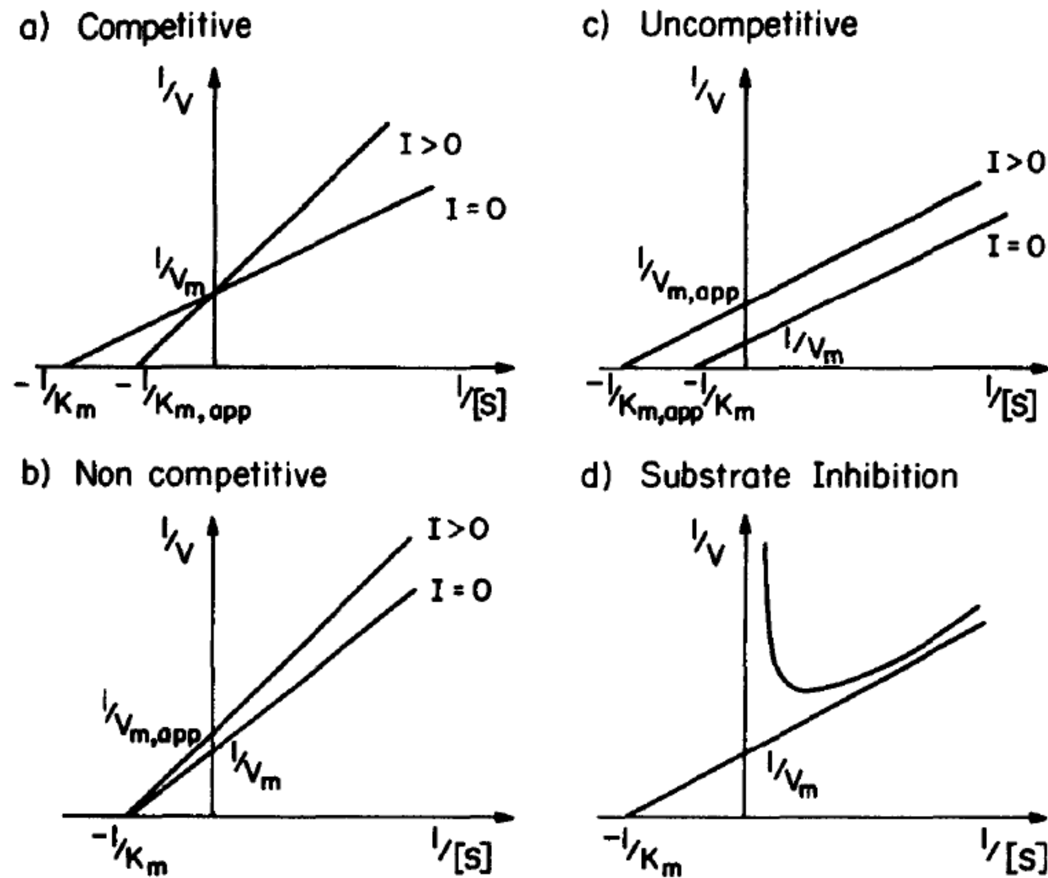

Figure 3.10. Different forms of inhibited enzyme kinetics.

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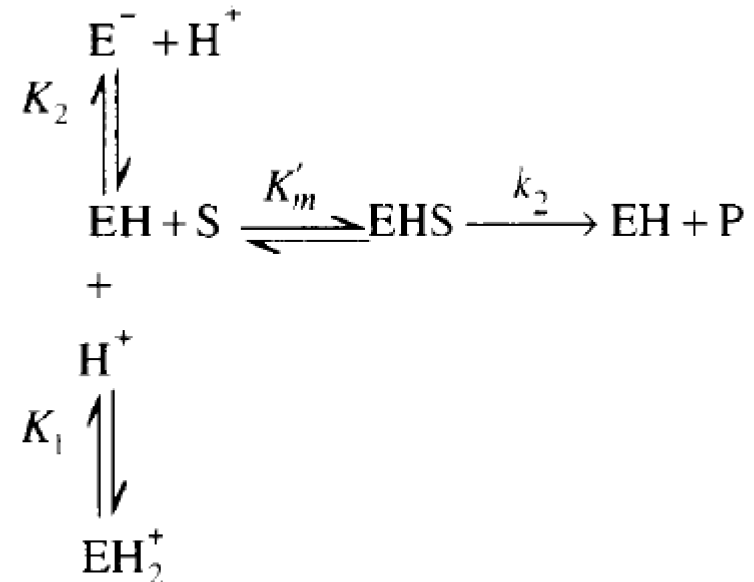
Effect of pH

Some enzymes have ionic groups on their active sites

- May change the activity of the site
- May change the overall shape of the enzyme



- Low substrate concentrations do not show inhibition, only at larger values

$$v = \frac{V_m[S]}{K'_m\left(1+\frac{K_2}{[H^+]}+\frac{[H^+]}{K_1}\right)+[S]} = \frac{V_m[S]}{K'_{m,app}+[S]} \quad \Rightarrow \quad K'_{m,app} = K'_m\left(1+\frac{K_2}{[H^+]}+\frac{[H^+]}{K_1}\right)$$

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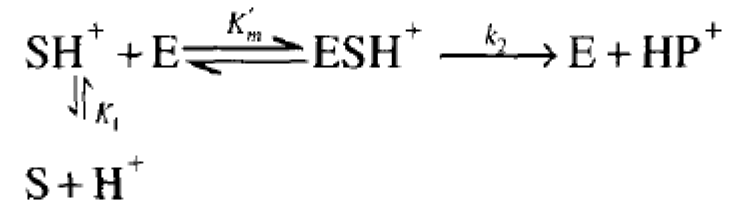
Effect of pH

Some substrates may be ionized



$$v = \frac{V_m[S]}{K'_m\left(1+\frac{K_1}{[H^+]}\right)+[S]} = \frac{V_m[S]}{K'_{m,app}+[S]} \quad \Rightarrow \quad K'_{m,app} = K'_m\left(1+\frac{K_1}{[H^+]}\right)$$

Optimum pH values usually determined experimentally

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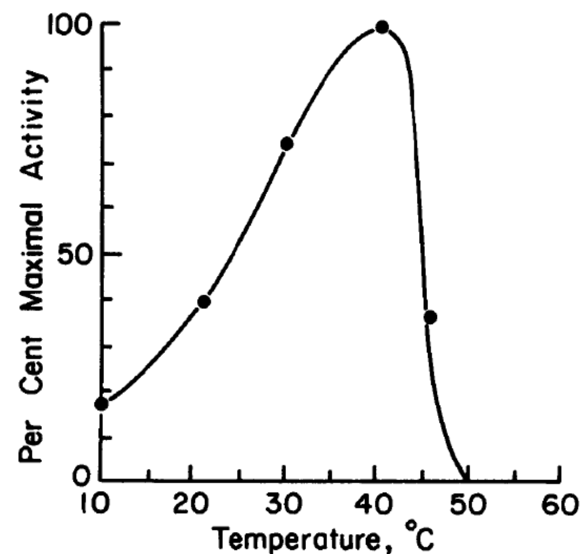
Temperature effects

Two competing effects

- Increasing temperature usually will increase the rate coefficients

$$V_m = k_2 [E_0] \quad \text{and} \quad k_2 = A \exp(-E_a / RT)$$

- However, increased temperature could denature the enzyme & lower its activity
  - There is a time dependency to this denaturing. Figure 3.15 shows inactivity after being exposed to higher temperatures for 10 minutes.



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Insoluble substrate

Mass transfer limited. More potential reactive sites than enzyme molecules

$$v = \frac{V_{max,S}\left[E\right]}{K_{eq} + \left[E\right]} \quad \Rightarrow \quad \begin{cases} V_{max,S} = k_2\left[S_0\right] \\ K_{eq} = \dfrac{k_{desorb}}{k_{adsorb}} \end{cases}$$

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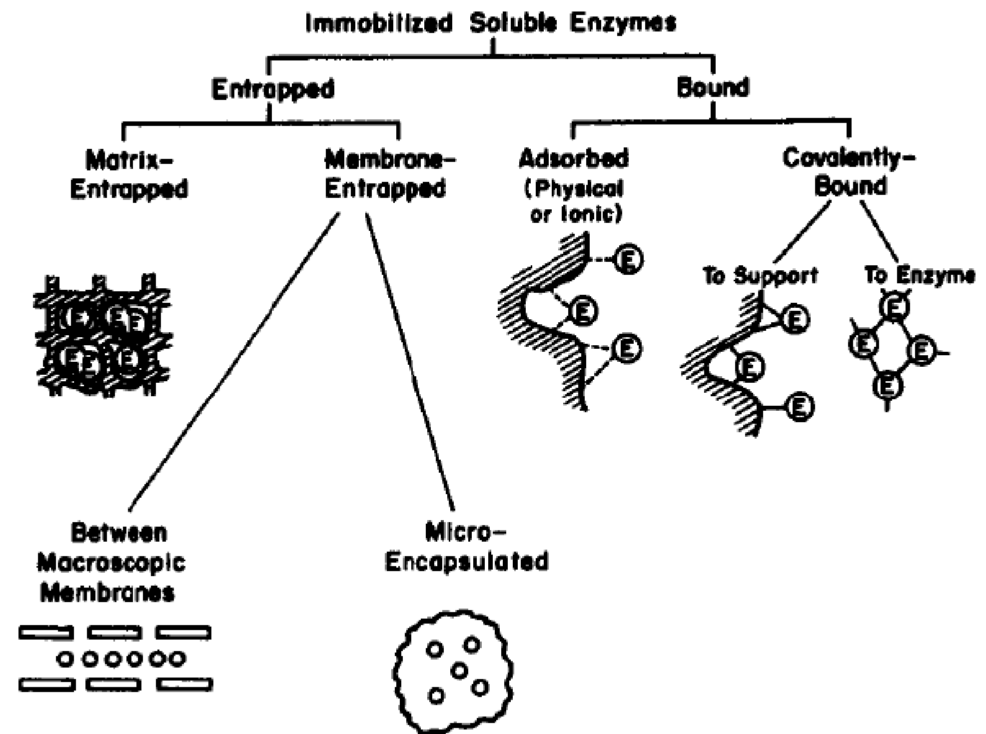
Immobilized enzyme systems

Restrict the enzymes within the reactor & not allowing them to leave with the product

- Enzyme reutilization without enzyme recovery & purification processes
- Product purity is usually improved
- Minimize effluent handling problems

Methods

- Surface immobilization
- Entrapment



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Immobilized enzyme systems

TABLE 3.4 Effect of Immobilization Methods on the Retention of Enzymatic Activity of Aminoacylase

Support	Method	Observed activity (units)	Enzyme activity immobilized (%)
Polyacrylamide	Entrapment	526	52.6
Nylon	Encapsulation	360	36.0
DEAE-cellulose	Ionic binding	668	55.2
DEAE-Sephadex A-50	Ionic binding	680	56.2
CM-Sephadex C-50	Ionic binding	0	0
Iodoacetyl cellulose	Covalent binding	472	39.0
CNBr-activated Sephadex	Covalent binding	12	1.0
AE-cellulose	Cross-linked with glutaraldehyde	8	0.6

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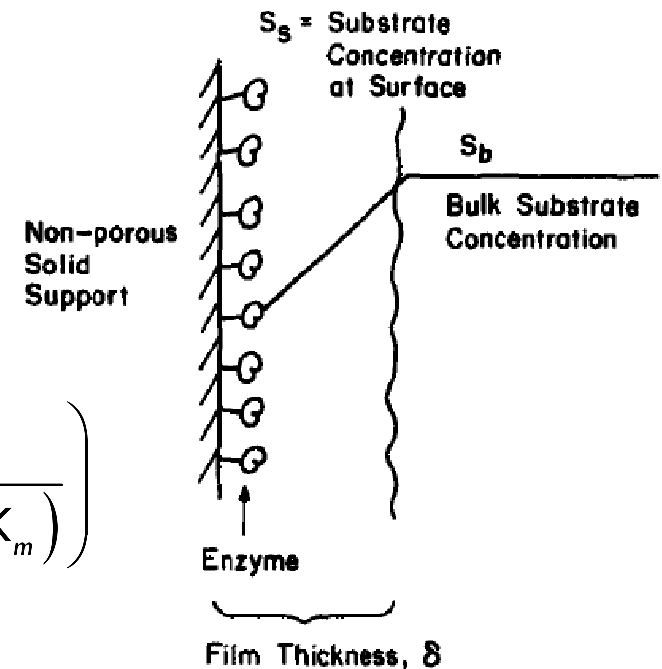
Immobilized enzyme systems – surface bound

Diffusional limitations may be significant

- Damköhler number…

$$N_{Da} = \frac{\text{Maximum rate of reaction}}{\text{Maximum rate of diffusion}} = \frac{V_{m'}}{k_L \left[S_b\right]}$$



- When reaction limited (N_{Da} << 1)

$$v \approx \frac{V'_m \left[S_b\right]}{K_{m,app} + \left[S_b\right]} \quad \Rightarrow \quad K_{m,app} = K_{m,app}\left(1 + \frac{V'_m}{k_L\left(\left[S_b\right] + K_m\right)}\right)$$

when diffusion limited (N_{Da} >> 1)

$$v \approx k_L \left[S_b\right]$$

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Immobilized enzyme systems – porous matrix

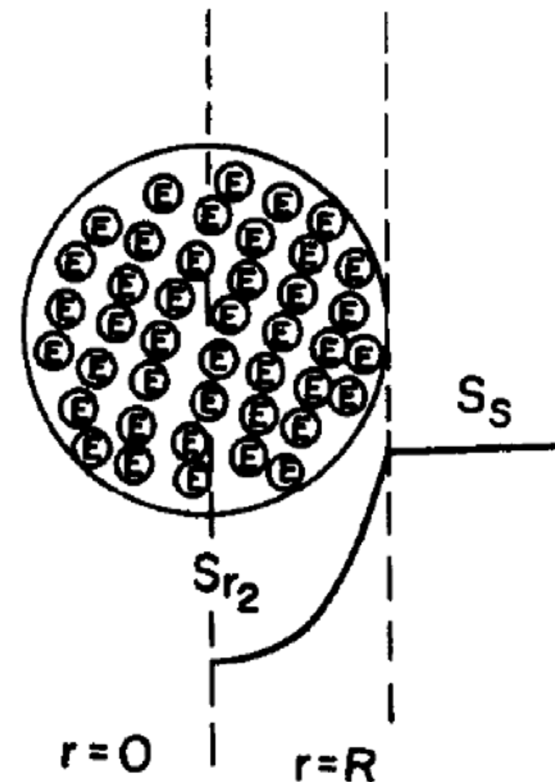
Now the substrate must diffuse through the pathways within the pore

- Rate of substrate consumption is equal to the rate across the boundary of the support particle

$$v = r_S = \eta \frac{V_m''[S_S]}{K_m + [S_S]}$$

where η is the effectiveness factor

$$\eta = \frac{3}{\phi}\left[\frac{1}{\tanh\phi} - \frac{1}{\phi}\right] \quad \text{and} \quad \phi = R\sqrt{\frac{V_m''' / K_m}{D_{eff}}}$$



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Summary

Kinetics of enzyme systems well described by Michaels-Menton equation form

- Modification of intrinsic parameters for various inhibition effects
- Based on quasi-steady state assumptions

Inhibition effects

- Competitive inhibition
- Noncompetitive inhibition
- Uncompetitive inhibition
- Substrate inhibition

Other effects

- pH
- Temperature
- Enzyme concentration

Enzyme immobilization

- Surface immobilization
- Entrapment

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