CHE 361
Bioprocess Engineering

Lecture 4: Enzyme Kinetics & Enzymatic Reactors

Outline

- Determining Rate Parameters
- Complex Enzymatic Kinetics
  - ✓ Allosteric enzymes & Inhibited kinetics
  - ✓ Effects of pH and temperature
- Enzymatic Reactors
  - ✓ Batch Reactor
  - ✓ Continuous Stirred Tank Reactor
- Transport Limitations
  - ✓ Multiphase enzymatic reactions
  - ✓ Diffusion limitations in immobilized systems

Michaelis-Menten Kinetics

$$S + E \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

k_{cat} – turnover number

K_m – Michaelis constant

$V_m = k_{cat}[E_t]$

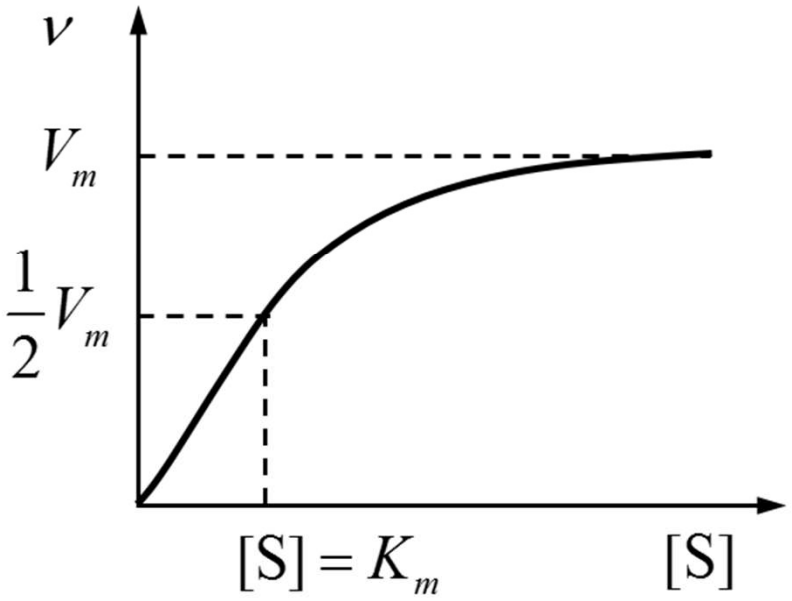
$$k_{cat} \equiv k_3$$

$$K_m = (k_2 + k_3)/k_1$$

*** The rate of product formation:**

$$\nu = \frac{d[P]}{dt} = k_{cat}[ES]$$

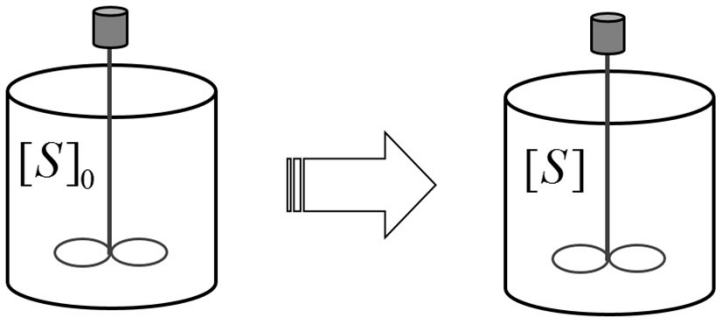
$$\nu = \frac{d[P]}{dt} = \frac{V_m[S]}{K_m + [S]}$$

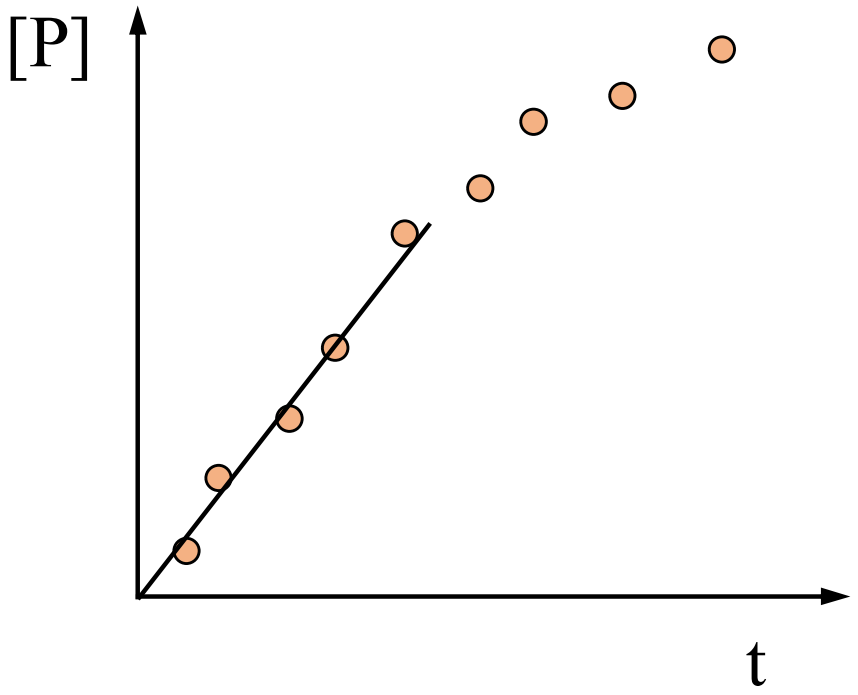


Determining Rate Parameters

- **Experimental data are typically obtained in *initial-rate experiments* in *batch reactors***

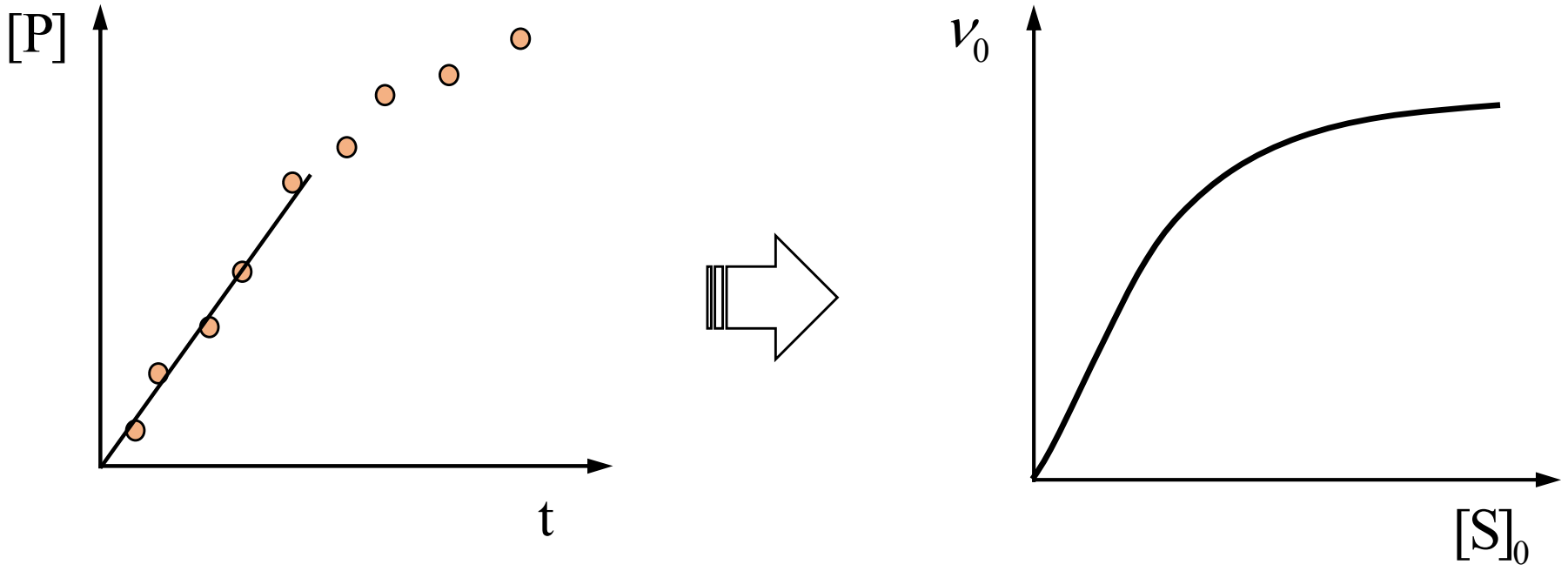
$$\nu_0 = \left.\frac{d[\mathrm{P}]}{dt}\right|_{t=0} = -\left.\frac{d[\mathrm{S}]}{dt}\right|_{t=0}$$





Determining Rate Parameters (Cont'd)

- Many experiments with different initial substrate concentrations to generate many pairs of ν_0 vs. $[S]_0$ data

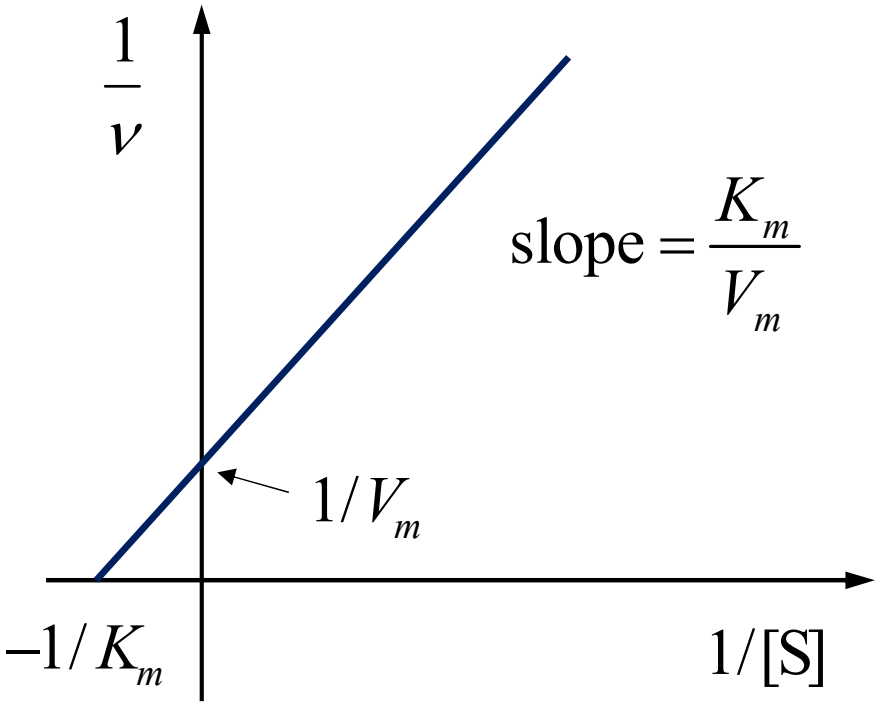


Lineweaver-Burk Plot

*** The Lineweaver-Burk plot is a double-reciprocal plot**

*** Note that errors for data points obtained at low substrate concentrations influence the slope and the intercept more then data points obtained at high substrate concentrations (because of the reciprocal character of the plot)*

$$\frac{1}{v} = \frac{1}{V_m} + \frac{K_m}{V_m}\frac{1}{[S]}$$

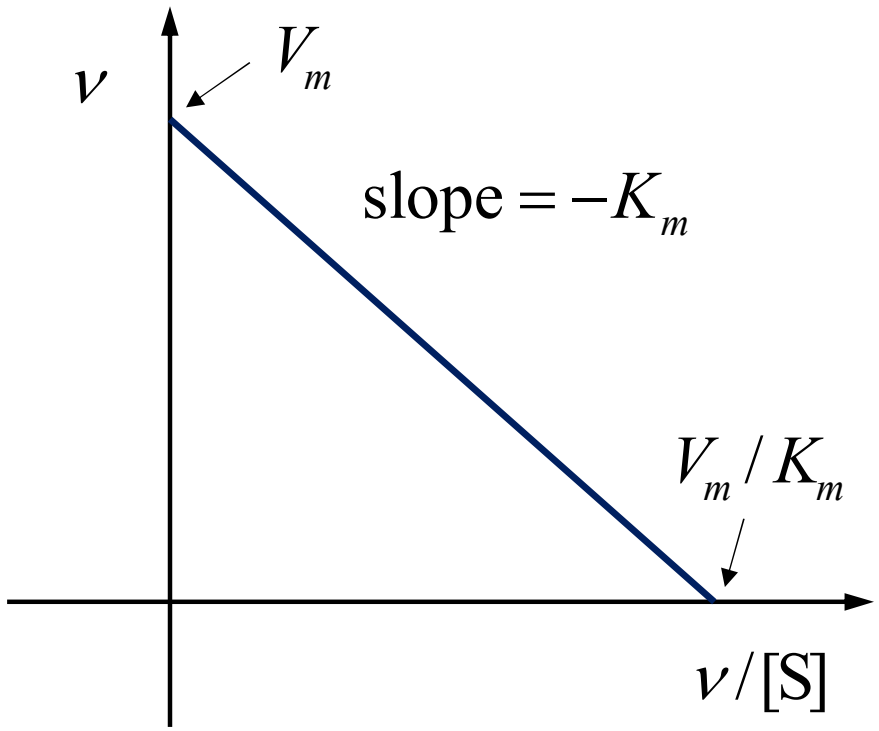


Eadie-Hofstee Plot

*** The Eadie-Hofstee plot is obtained by rearrangement of the Michaelis-Menten equation**

*** This plot can be subject to large errors (because both coordinates contain v); a good method for estimating K_m, NOT V_m*

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

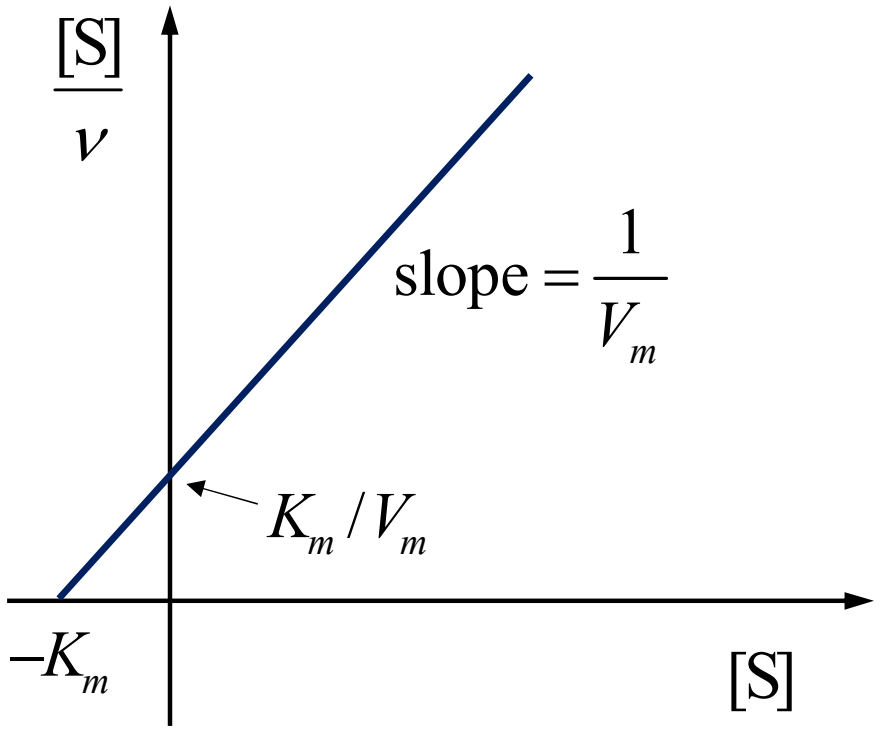


Hanes-Woolf Plot

*** The Hanes-Woolf plot is obtained by rearrangement of the Michaelis-Menten equation**

*** This plot can be used to determine V_m more accurately*

$$\frac{[S]}{v} = \frac{K_m}{V_m} + \frac{1}{V_m}[S]$$

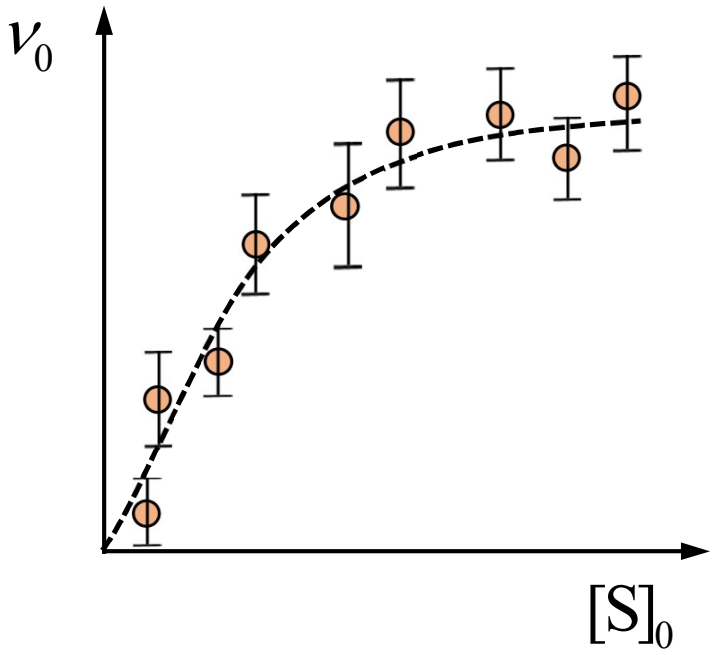


Non-Linear Regression

*** This type of analysis allows for multiple parameter estimation by minimizing the difference between the experimental data and values calculated using Michaelis-Menten equation**

*** The best-fit curve is often assumed to be the one that minimizes the sum of squared residuals*

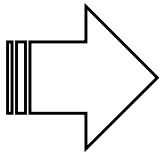
$$Minimize \sum \left(v_{exp} - \frac{V_m[\mathrm{S}]}{K_m + [\mathrm{S}]} \right)^2$$

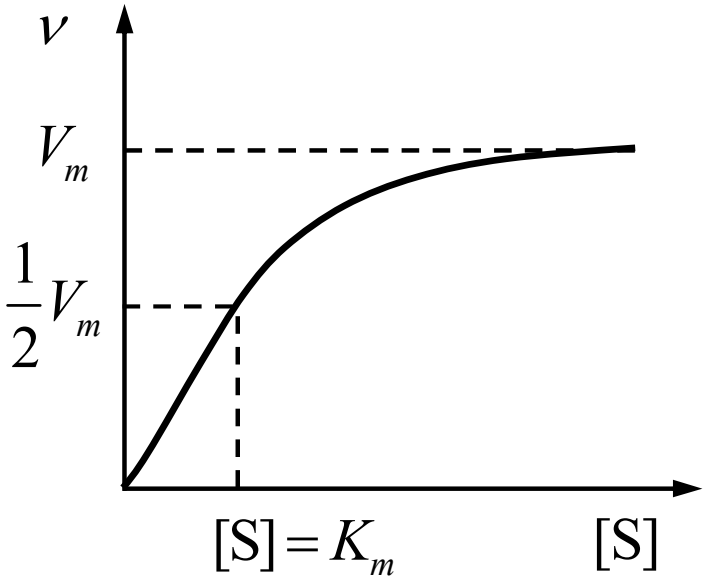


Determining Rate Parameters: Summary

- The **Lineweaver-Burk plot** can lead to *large errors at low substrate concentrations*
- The **Eadie-Hofstee plot** can lead to *large errors for estimated* V_m
- The **Hanes-Woolf** plot can be used *to estimate* V_m *more accurately*
- The **Nonlinear Regression** *might require weighted least squares analysis*, when the dependent variable does not have constant variance

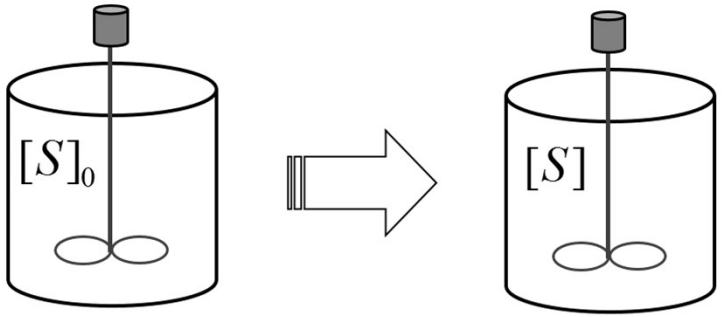
* If data points are relatively error free and the maximum rate was evidently achieved, V_m and K_m can be directly extracted from the data



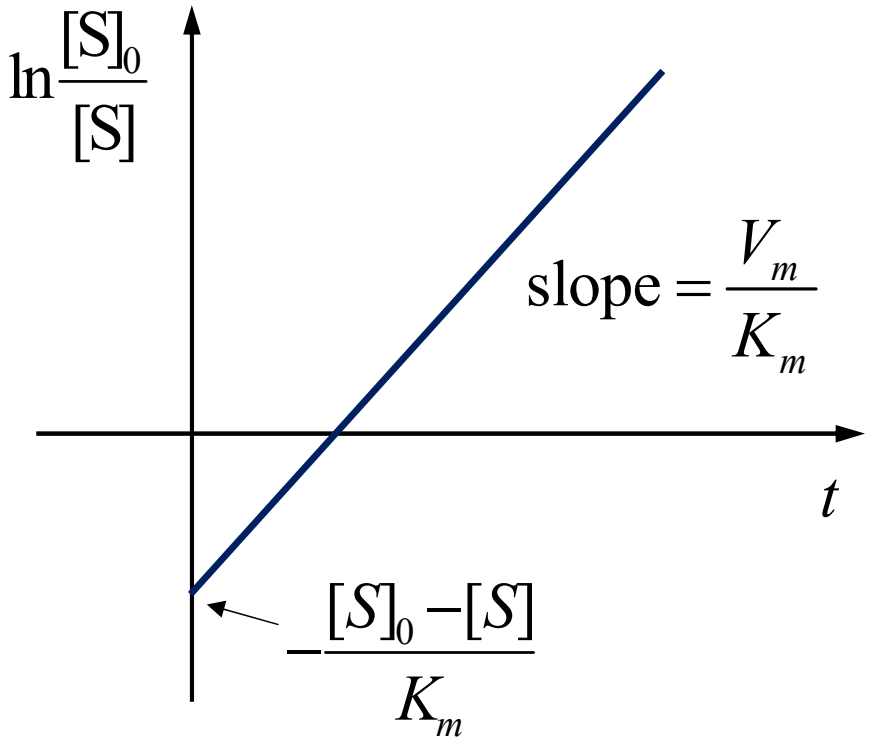


Parameter Estimation from Batch Kinetics

*** Batch reactor solution for the Michaelis-Menten kinetics:**



$$\ln\frac{[S]_0}{[S]} = \frac{V_m}{K_m}t - \frac{[S]_0 - [S]}{K_m}$$



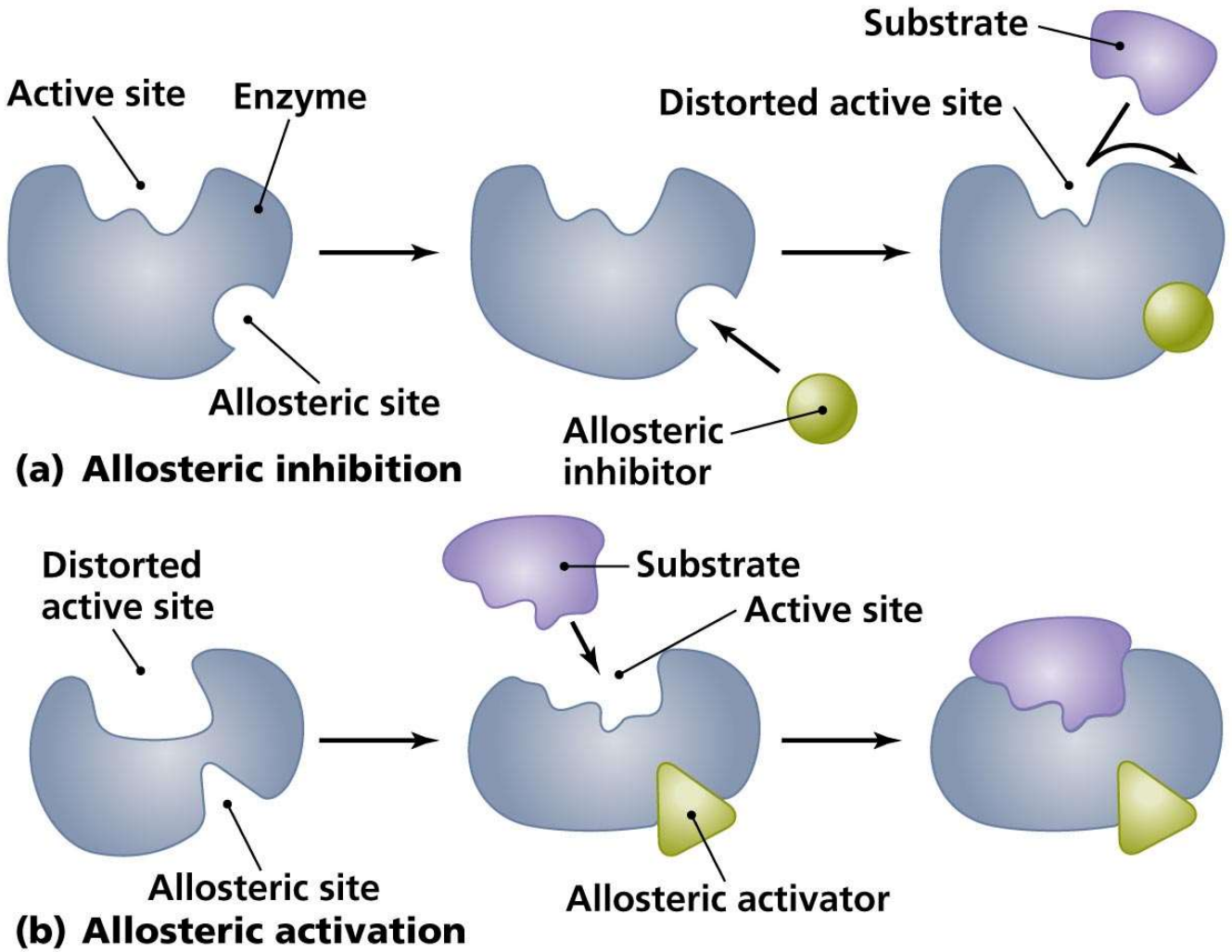
Complex Enzymatic Kinetics

- Michaelis-Menten kinetics is the simplest mechanism
- Many enzymes have multiple building sites resulting in complex kinetic expressions
- Complex kinetics is required for robust regulation of cellular functions:
  - ✓ In case of overproduction, it may be necessary to slow down the rate of an enzymatic reaction
  - ✓ It may be necessary to rapidly activate an enzyme to higher production rate in response to intracellular signaling
- In industry, previously isolated enzymes are often used outside of the cell, in a bioreactor

Allosteric (Cooperative) Enzymes

- **Allosteric enzymes have multiple substrate-binding sites**
- **They change their conformational ensemble upon binding of an *effector***
- **Binding of the *effector* results in an apparent change in binding affinity at a different ligand binding site**
- **Allosteric behavior is common in regulation, in control mechanisms such as *feedback* and *feedforward loops***

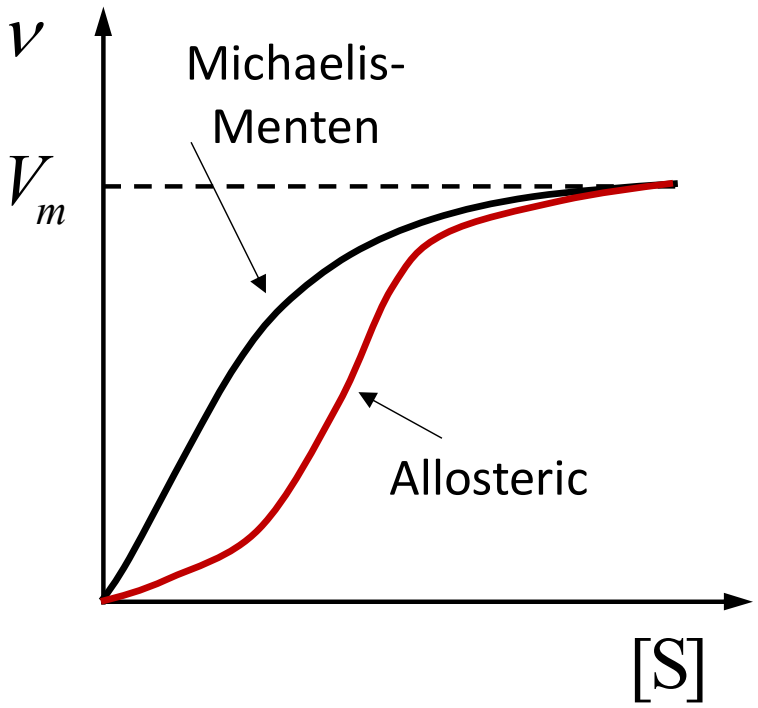
Allosteric Regulation Mechanism



Hill Equation

*** The Hill equation was originally formulated by Archibald Hill in 1910 to describe the sigmoidal O_2 binding curve of *hemoglobin***

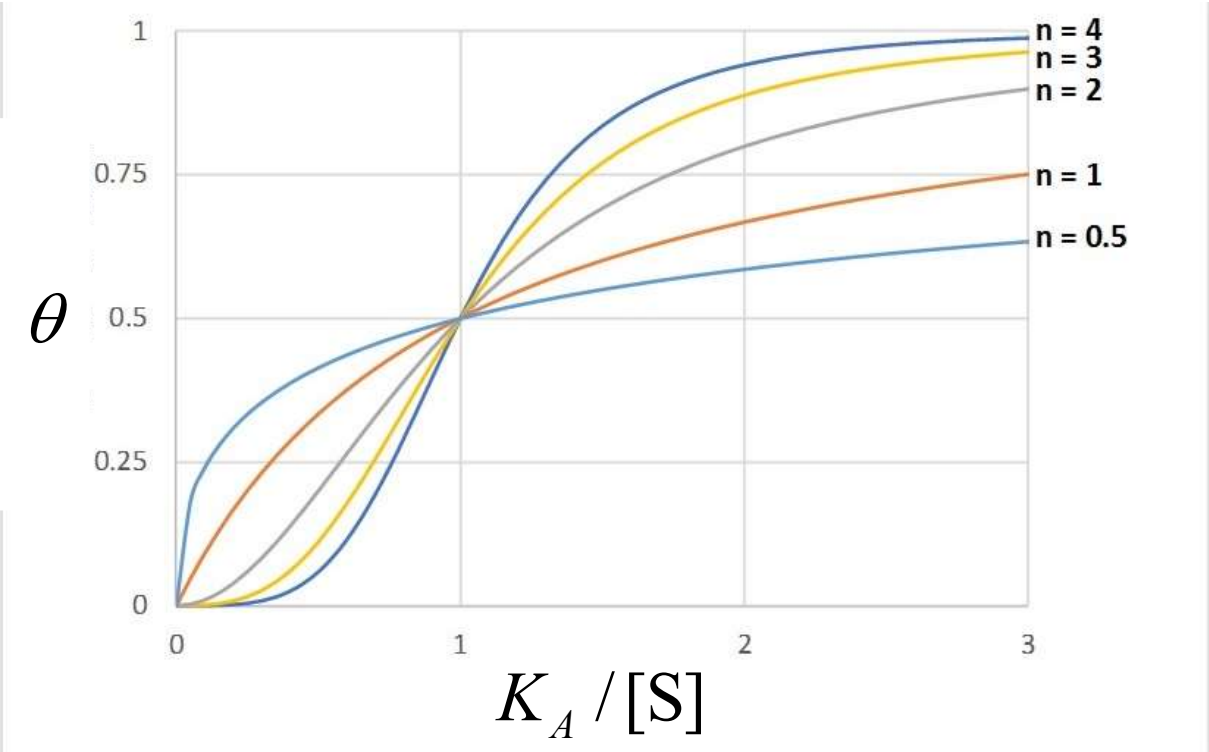
$$v = \frac{d[\mathrm{P}]}{dt} = \frac{V_m[\mathrm{S}]^n}{K''_m + [\mathrm{S}]^n}$$



Hill Equation (Cont'd)

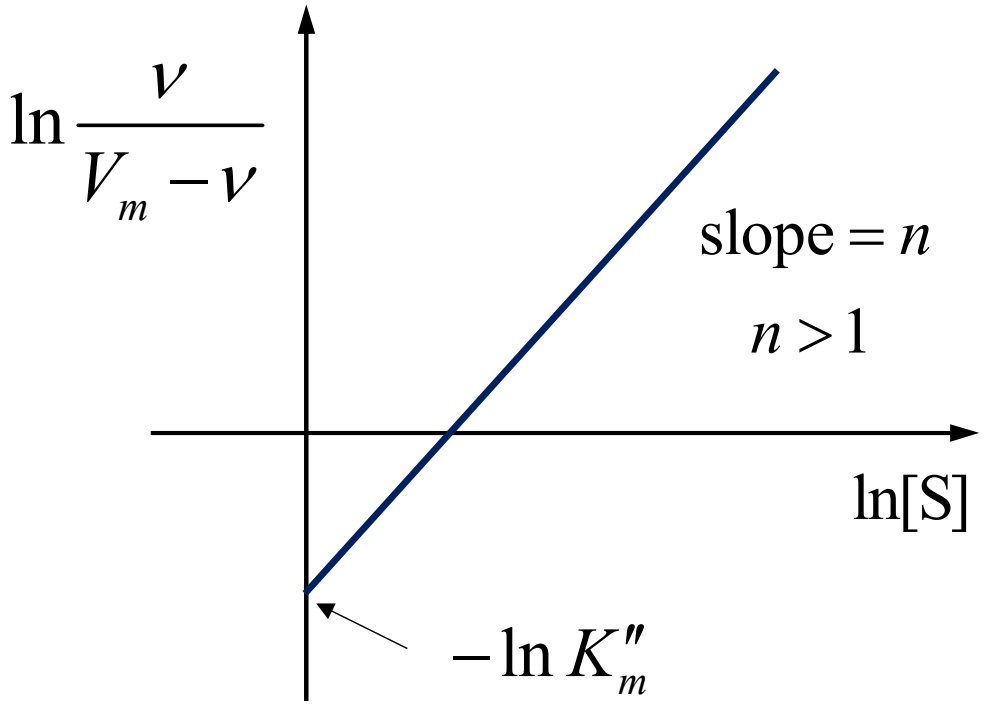
$$\nu = \frac{d[\mathrm{P}]}{dt} = \frac{V_m[\mathrm{S}]^n}{K_m'' + [\mathrm{S}]^n}$$

$$\frac{\nu}{V_m} = \theta = \frac{[\mathrm{S}]^n}{K_m'' + [\mathrm{S}]^n} = \frac{1}{\left(\frac{K_A}{[\mathrm{S}]}\right)^n + 1}$$



Cooperativity Coefficient Estimation

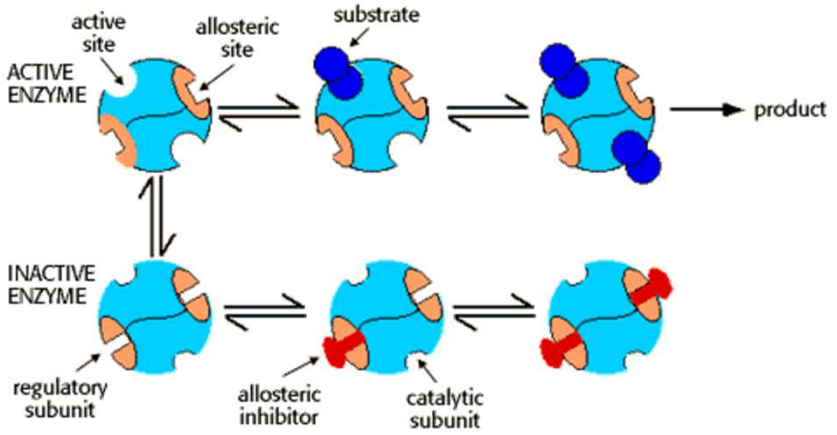
$$\nu = \frac{V_m [\mathrm{S}]^n}{K_m'' + [\mathrm{S}]^n} \quad \Rightarrow \quad \ln \frac{\nu}{V_m - \nu} = n \ln[\mathrm{S}] - \ln K_m''$$



Inhibited Enzyme Kinetics

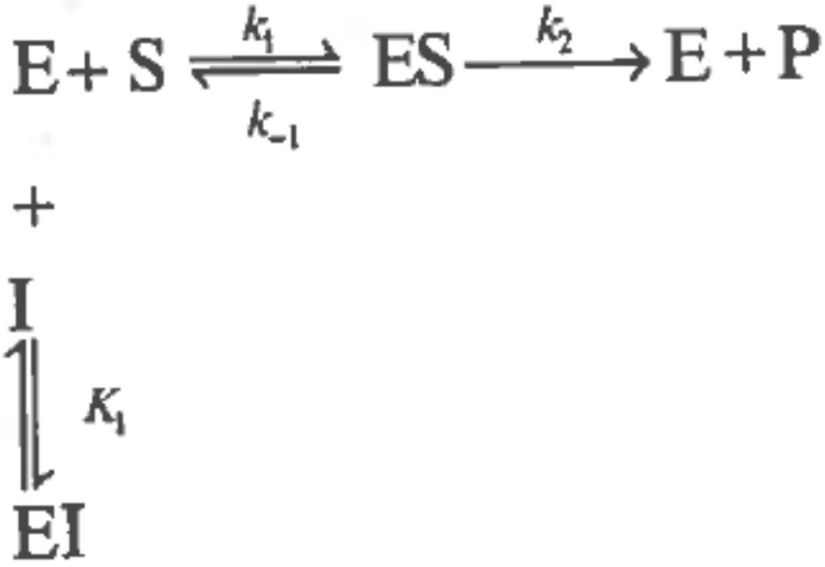
- **Enzyme inhibitors bind to enzymes and reduce their activity**
- **Inhibition may be irreversible or reversible**
- **Irreversible inhibitors such as heavy metals (e.g., lead, cadmium, mercury) form a stable complex with an enzyme (can only be reversed using certain chelating agents, e.g., EDTA)**
- **Reversible inhibitors dissociate more easily**
- **There are *competitive, noncompetitive,* and *uncompetitive inhibitors*; there is also *substrate inhibition***

An example of noncompetitive (allosteric) inhibition:



Competitive Inhibition

*** Inhibitor is a substrate analog that competes with substrate molecules for the active site of the enzyme**



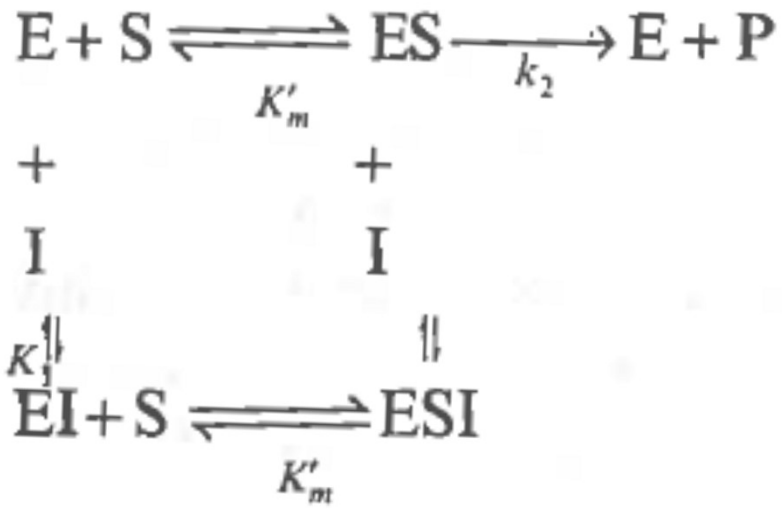
$$\nu = \frac{V_m[\mathrm{S}]}{K_m\left(1+\frac{[\mathrm{I}]}{K_I}\right)+[\mathrm{S}]}$$

$$K_m = \frac{[\mathrm{E}][\mathrm{S}]}{[\mathrm{ES}]}$$

$$K_I = \frac{[\mathrm{E}][\mathrm{I}]}{[\mathrm{EI}]}$$

Non-Competitive Inhibition

- **Non-competitive inhibitors are NOT substrate analogs**
- **Inhibitors bind on sites other than the active sites**
- **Non-competitive inhibitors could be considered as allosteric inhibitors**

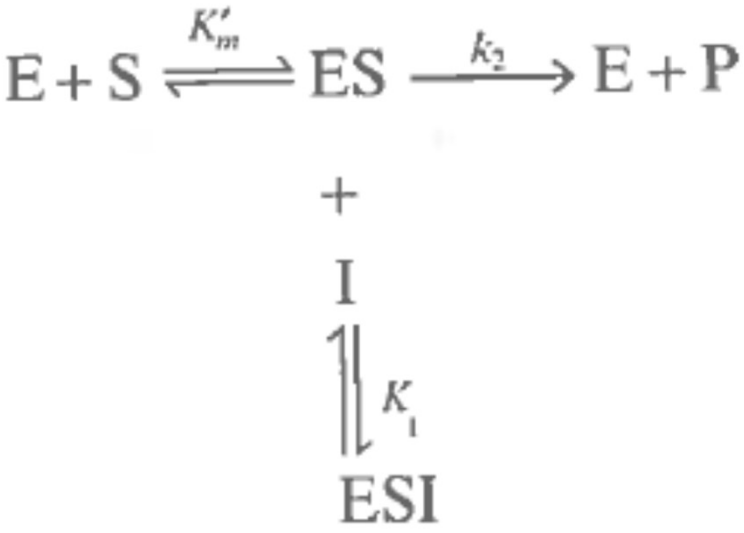


$$v = \frac{V_m}{\left(1 + \frac{[\mathrm{I}]}{K_I}\right)\left(1 + \frac{K_m}{[\mathrm{S}]}\right)}$$

$$K_I = \frac{[\mathrm{E}][\mathrm{I}]}{[\mathrm{EI}]} = \frac{[\mathrm{ES}][\mathrm{I}]}{[\mathrm{ESI}]}$$

Uncompetitive Inhibition

- **Uncompetitive inhibitors bind to the ES complex ONLY**
- **Uncompetitive inhibitors have no affinity for the enzyme itself**

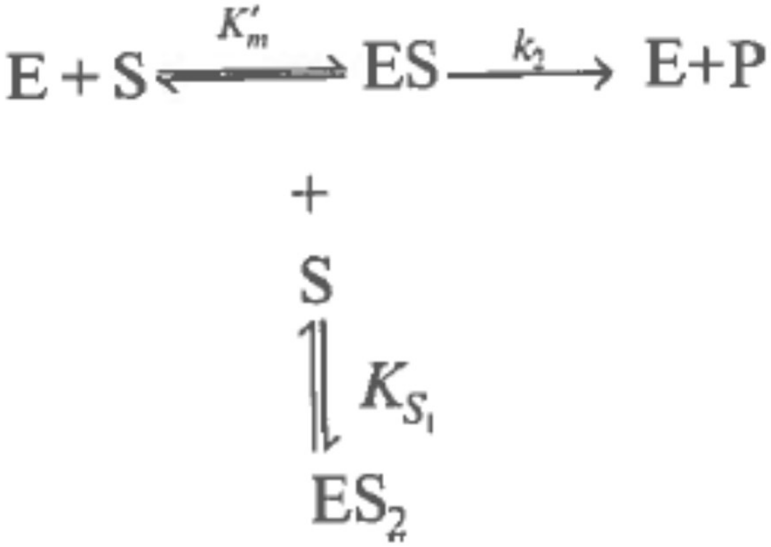


$$v = \frac{V_m[\mathrm{S}]}{K_m + [\mathrm{S}]\left(1 + \frac{[\mathrm{I}]}{K_I}\right)}$$

$$K_I = \frac{[\mathrm{ES}][\mathrm{I}]}{[\mathrm{ESI}]}$$

Substrate Inhibition

*** High substrate concentrations may cause inhibition in some enzymatic reactions**



$$v = \frac{V_m[\mathrm{S}]}{K_m + [\mathrm{S}] + \frac{[S]^2}{K_{SI}}}$$

$$K_{SI} = \frac{[\mathrm{S}][\mathrm{ES}]}{[\mathrm{ES}_2]}$$

Substrate Inhibition (Cont'd)

$$\nu = \frac{V_m[\mathrm{S}]}{K_m + [\mathrm{S}] + \frac{[S]^2}{K_{SI}}}$$

At low substrate concentration:

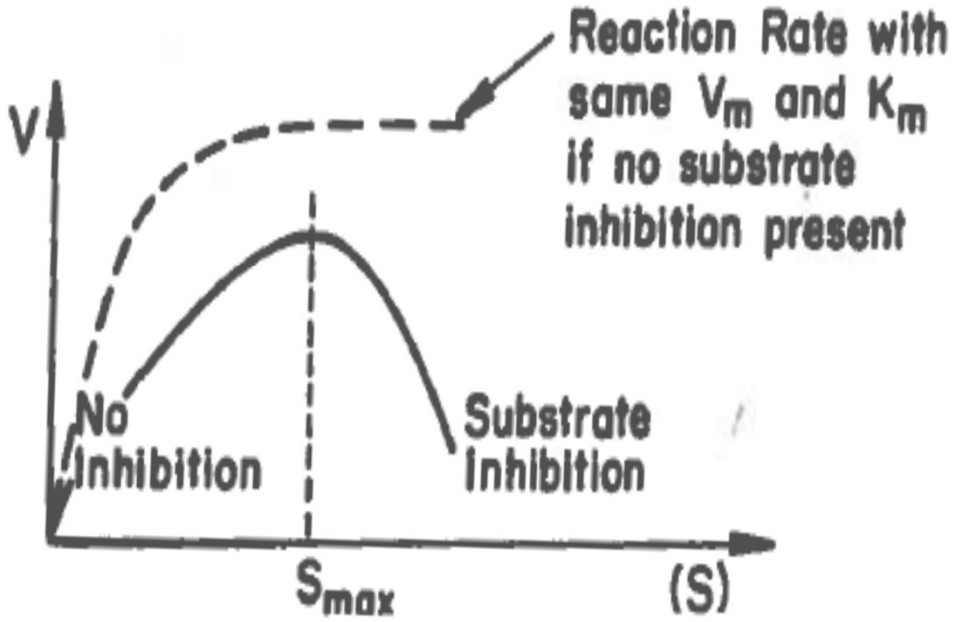
$$\frac{[S]^2}{K_{SI}} << 1 \quad \Rightarrow \quad \nu = \frac{V_m}{\left(1 + \frac{K_m}{[\mathrm{S}]}\right)} \quad \Rightarrow \quad \frac{1}{\nu} = \frac{K_m}{V_m}\frac{1}{[\mathrm{S}]} + \frac{1}{V_m}$$

At high substrate concentration:

$$\frac{K_m}{[\mathrm{S}]} << 1 \quad \Rightarrow \quad \nu = \frac{V_m}{\left(1 + \frac{[\mathrm{S}]}{K_{SI}}\right)} \quad \Rightarrow \quad \frac{1}{\nu} = \frac{1}{K_{SI}V_m}[\mathrm{S}] + \frac{1}{V_m}$$

Substrate Inhibition (Cont'd)

$$v = \frac{V_m[\mathrm{S}]}{K_m + [\mathrm{S}] + \frac{[S]^2}{K_{SI}}} \Rightarrow \frac{dv}{d[S]} = 0 \Rightarrow [\mathrm{S}]_{\max} = \sqrt{K_m K_{SI}}$$



Inhibited Enzyme Kinetics: Summary

a) Competitive:

$$v = \frac{V_m[\mathrm{S}]}{K_{m,app} + [\mathrm{S}]}$$

$$K_{m,app} = K_m\left(1 + \frac{[\mathrm{I}]}{K_I}\right)$$

c) Uncompetitive:

$$v = \frac{V_{m,app}[\mathrm{S}]}{K_{m,app} + [\mathrm{S}]}$$

$$V_{m,app} = \frac{V_m}{1 + [\mathrm{I}]/K_I} \qquad K_{m,app} = \frac{K_m}{1 + [\mathrm{I}]/K_I}$$

b) Non-competitive:

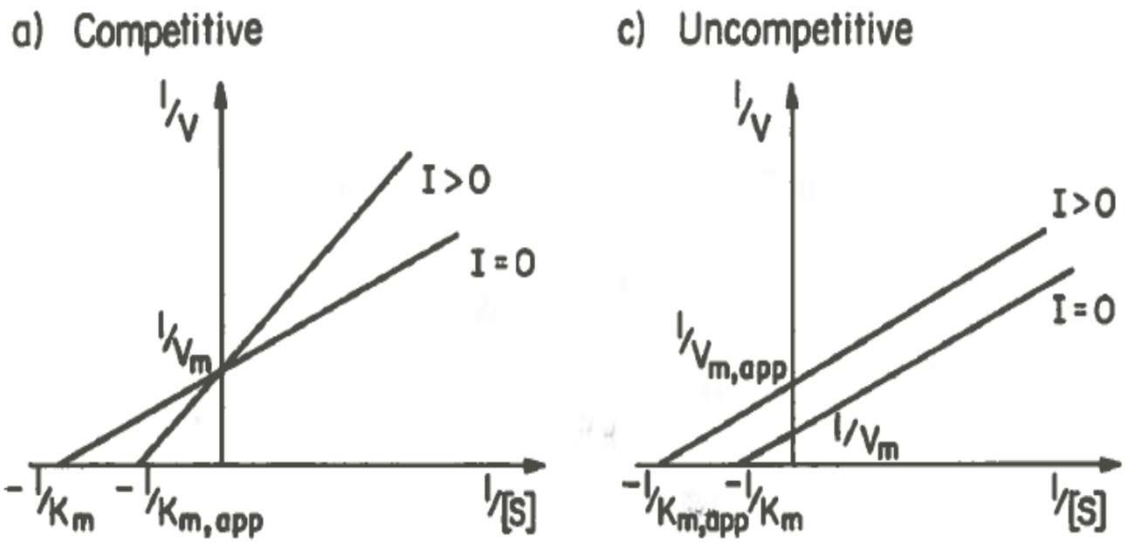
$$v = \frac{V_{m,app}}{1 + K_m/[\mathrm{S}]}$$

$$V_{m,app} = \frac{V_m}{\left(1 + \frac{[\mathrm{I}]}{K_I}\right)}$$

d) Substrate inhibition:

$$v = \frac{V_m[\mathrm{S}]}{K_m + [\mathrm{S}] + \frac{[S]^2}{K_{SI}}}$$

Inhibited Enzyme Kinetics: Summary



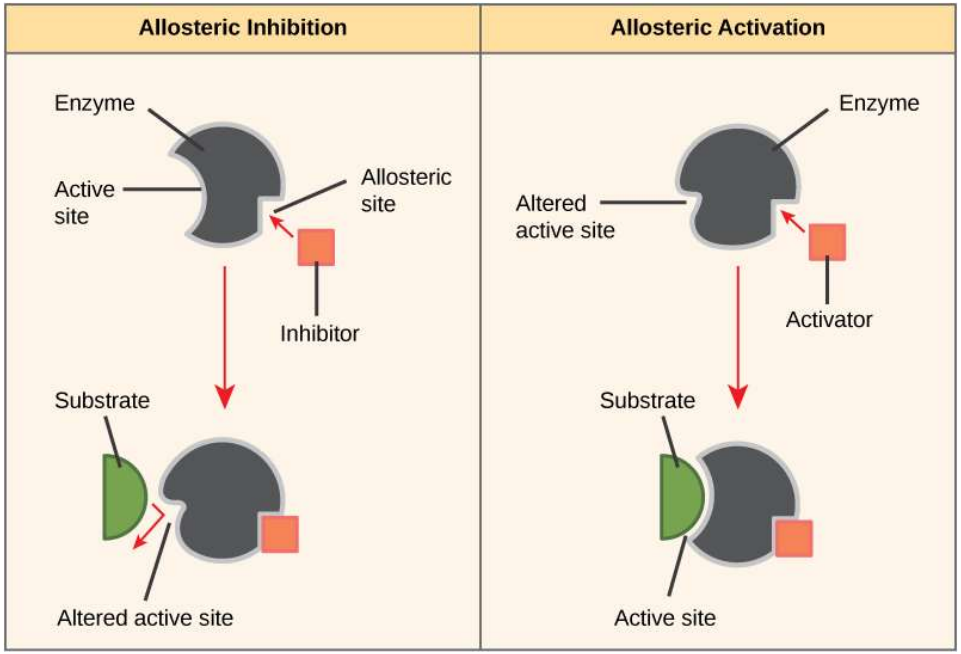
Enzyme Activation

- **Some molecules can bind to enzyme <u>increasing</u> the enzymatic activity**
- ***Enzyme activators* are usually involved in *allosteric enzymes* regulating metabolism**
- **The activation energy of the enzymatic reaction is reduced upon binding the activator to the enzyme molecule, increasing the reaction rate**

$$v = \frac{V_{m,app}[\mathrm{S}]}{K_{m,app} + [\mathrm{S}]}$$

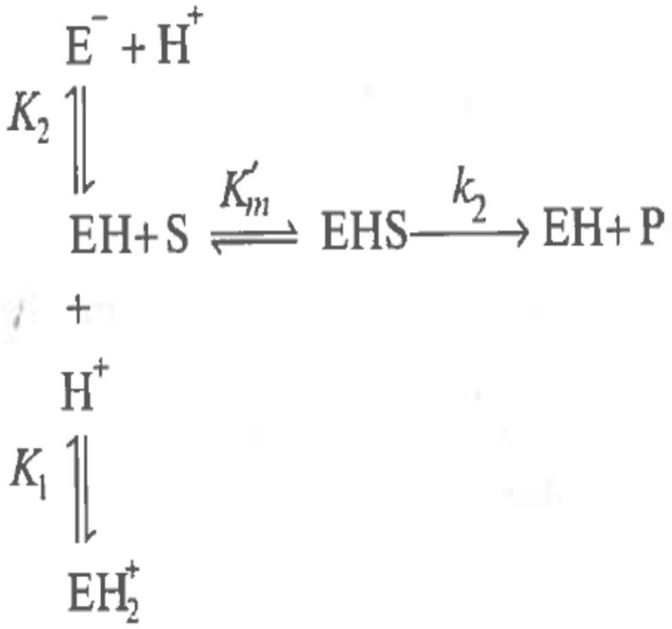
$$V_{m,app} = \frac{V_m}{1 + [\mathrm{A}]/K_A}$$

$$K_{m,app} = \frac{K_m}{1 + [\mathrm{A}]/K_A}$$



Effect of pH: Ionizing Enzymes

- **Certain enzymes have ionic groups on their active sites**
- **For enzymatic action, these groups have to be in a certain form (acid or base)**
- **pH variations affect the ionic group, changing the enzyme activity**



$$v = \frac{V_m[\mathrm{S}]}{K_{m,app} + [\mathrm{S}]} \qquad K_{m,app} = K_m\left(1 + \frac{K_2}{[\mathrm{H}^+]} + \frac{[\mathrm{H}^+]}{K_1}\right)$$

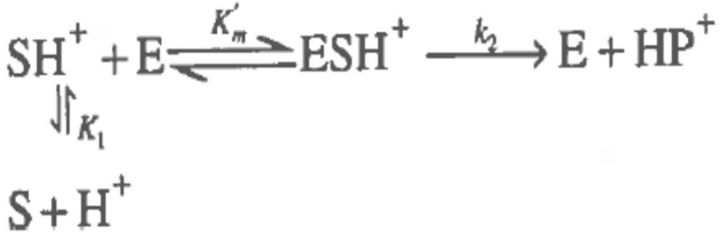
$$K_m = \frac{[\mathrm{EH}][\mathrm{S}]}{[\mathrm{EHS}]}$$

$$K_1 = \frac{[\mathrm{EH}][\mathrm{H}^+]}{[\mathrm{EH}_2^+]}$$

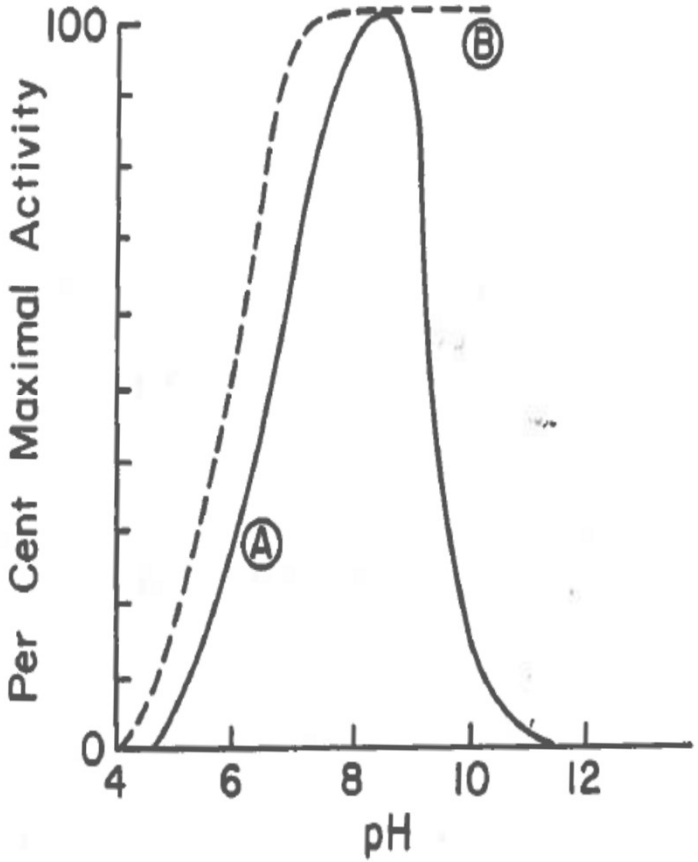
$$K_2 = \frac{[\mathrm{E}^-][\mathrm{H}^+]}{[\mathrm{EH}]}$$

Effect of pH: Ionizing Substrate

*** For ionizing substrate, the following scheme and rate expression apply:**



$$v = \frac{V_m[\mathrm{S}]}{K_m\left(1+\frac{K_1}{[\mathrm{H}^+]}\right)+[\mathrm{S}]}$$



Effect of Temperature

- **The rate of enzyme-catalyzed reactions increases with temperature, but only to a certain extent**
- **Above certain temperature enzyme activity decreases because of denaturation**

$$S + E \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_3} E + P$$

*** Activation:**

$$\nu = k_{cat}[E] = A\exp\left(-\frac{E_a}{R_g T}\right) \quad \Rightarrow \quad \ln\nu = -\frac{E_a}{R_g}\frac{1}{T} + \ln A$$

Effect of Temperature (Cont'd)

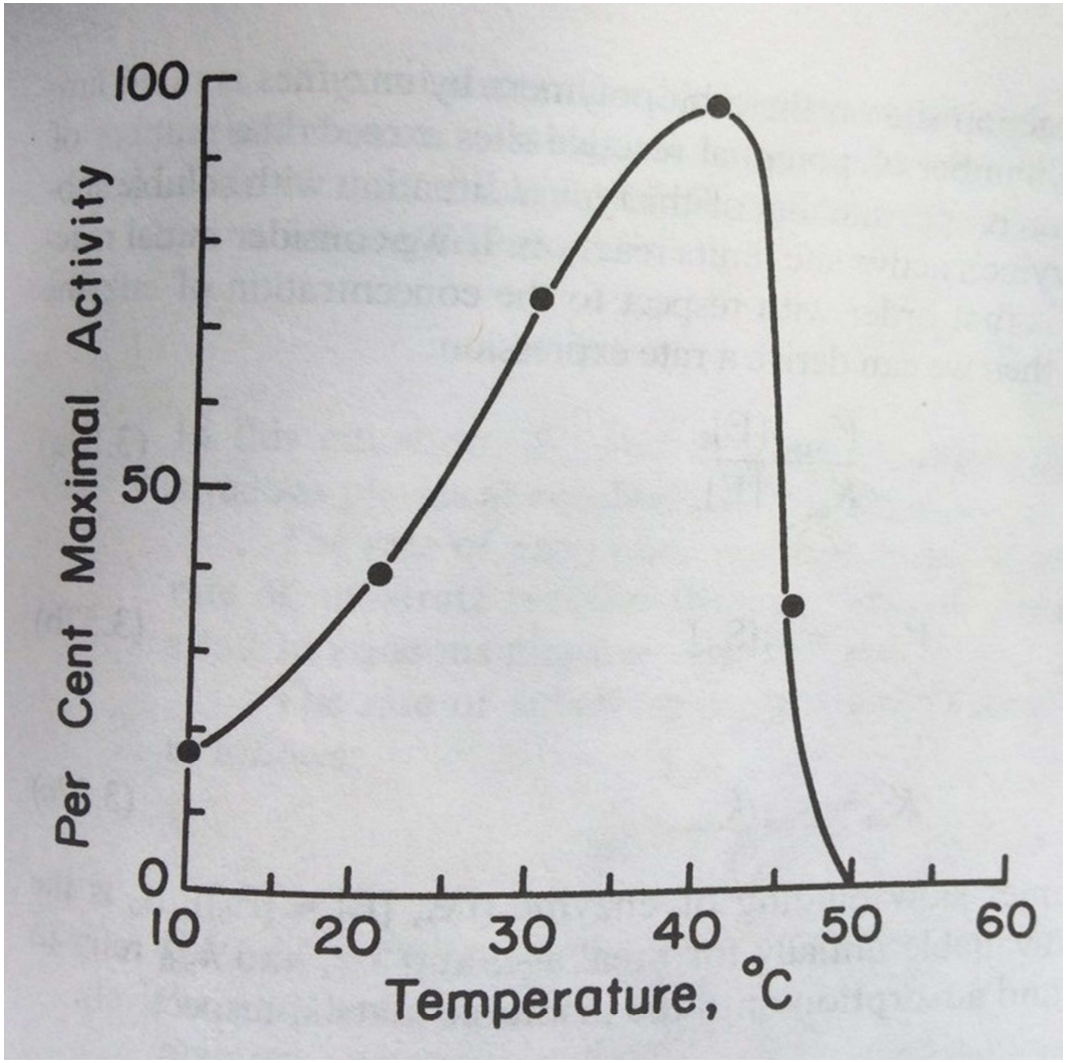
* **Deactivation:**

$$-\frac{d[\mathrm{E}]}{dt} = k_d[\mathrm{E}]$$

$$[\mathrm{E}] = [\mathrm{E}]_0 e^{-k_d t}$$

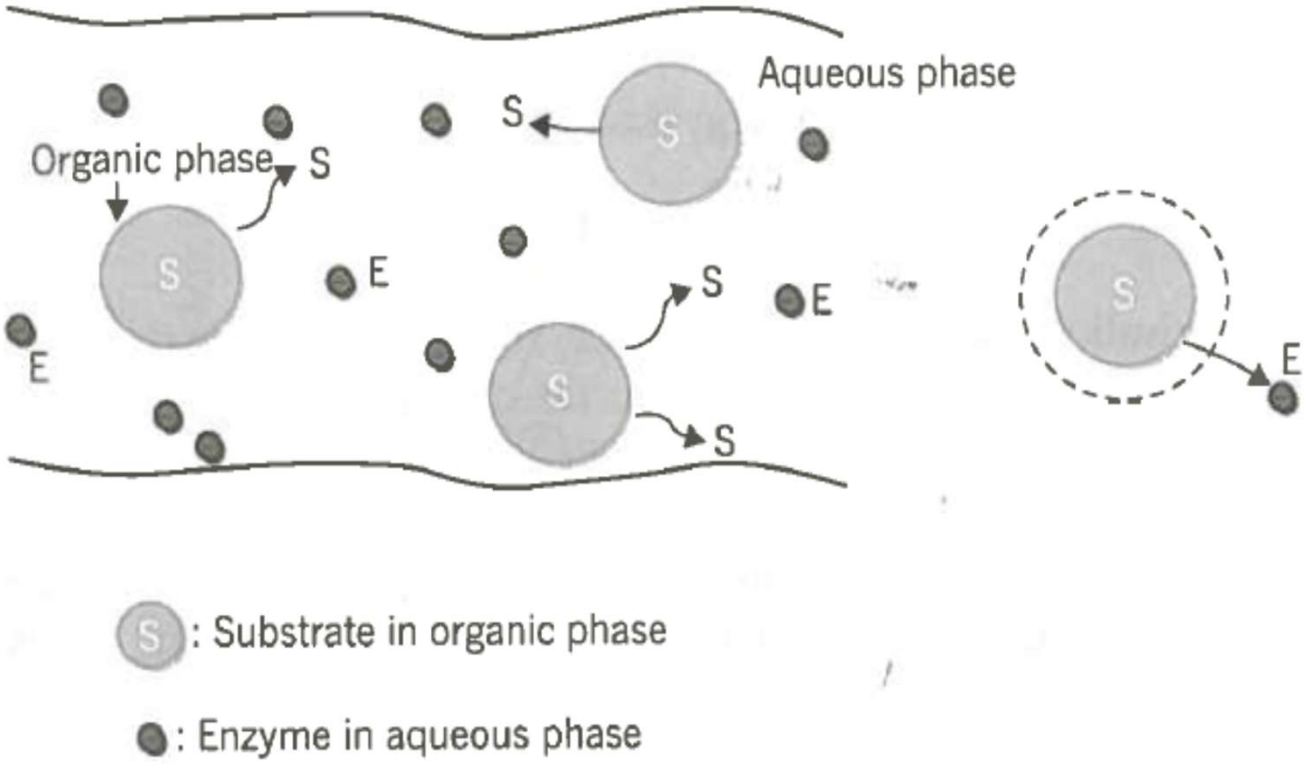
$$\nu = A e^{-E_a/RT}[\mathrm{E}]_0 e^{-k_d t}$$

$$k_d = A_d \exp\left(-\frac{E_d}{R_g T}\right)$$



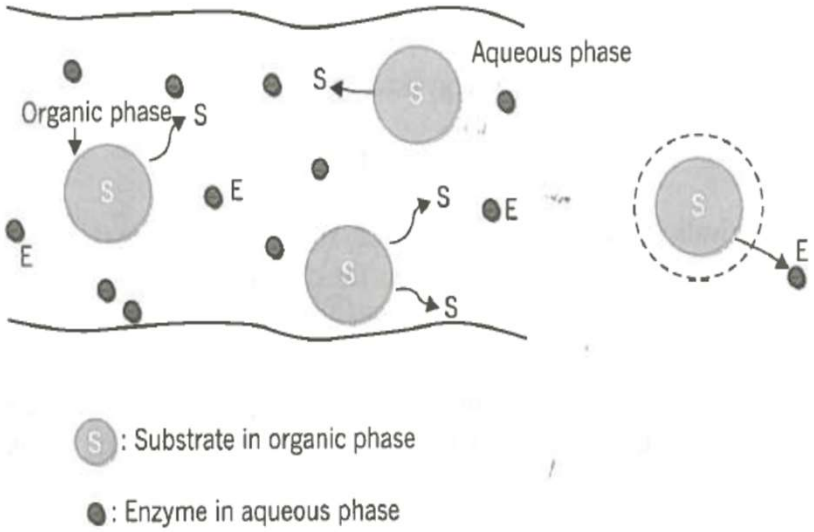
Transport Limitations: Multiphase Reactions

*** Enzymes function in aqueous media (cells contain 80% water), but some substrates are primarily soluble in organic phase**



Multiphase Enzymatic Reactions (Cont'd)

*** The main limiting factor is the rate of substrate transfer from the organic phase to the aqueous phase**



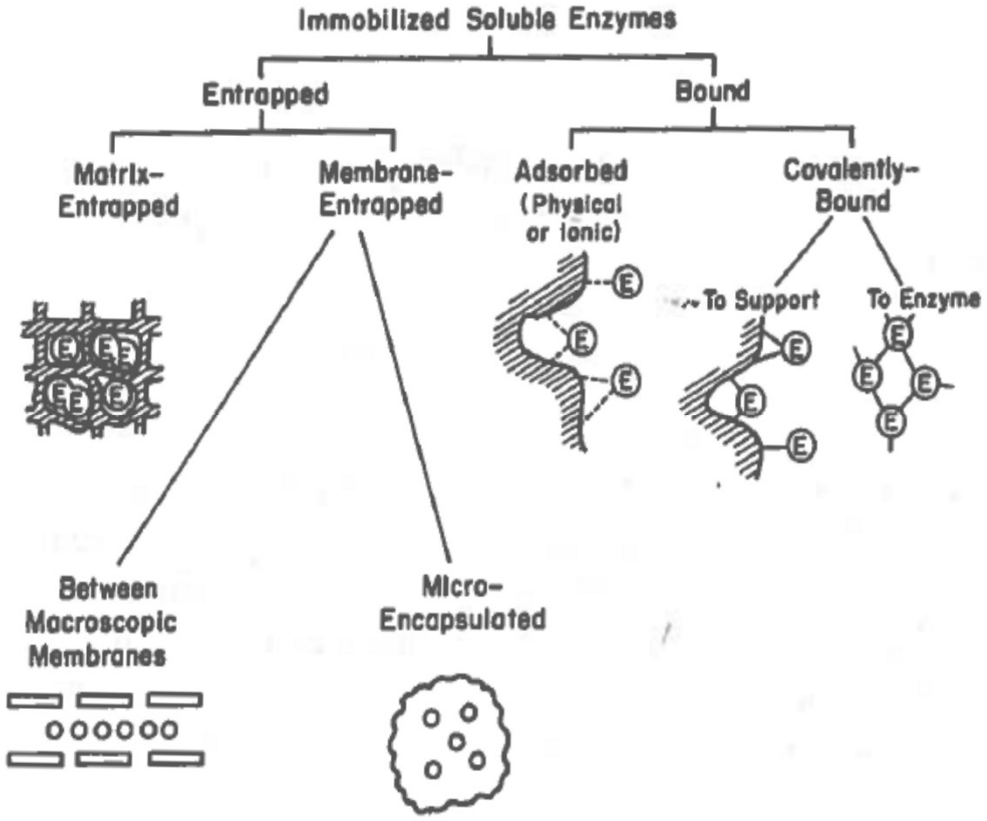
$$K_L a(S_0 / p - S_w) = v = \frac{V_m S_w}{K_s + S_w}$$

**** For severe mass-transfer limitation:**

$$S_w << S_0 / p \quad \Rightarrow \quad v = K_L a S_0 / p$$

Enzyme Immobilization

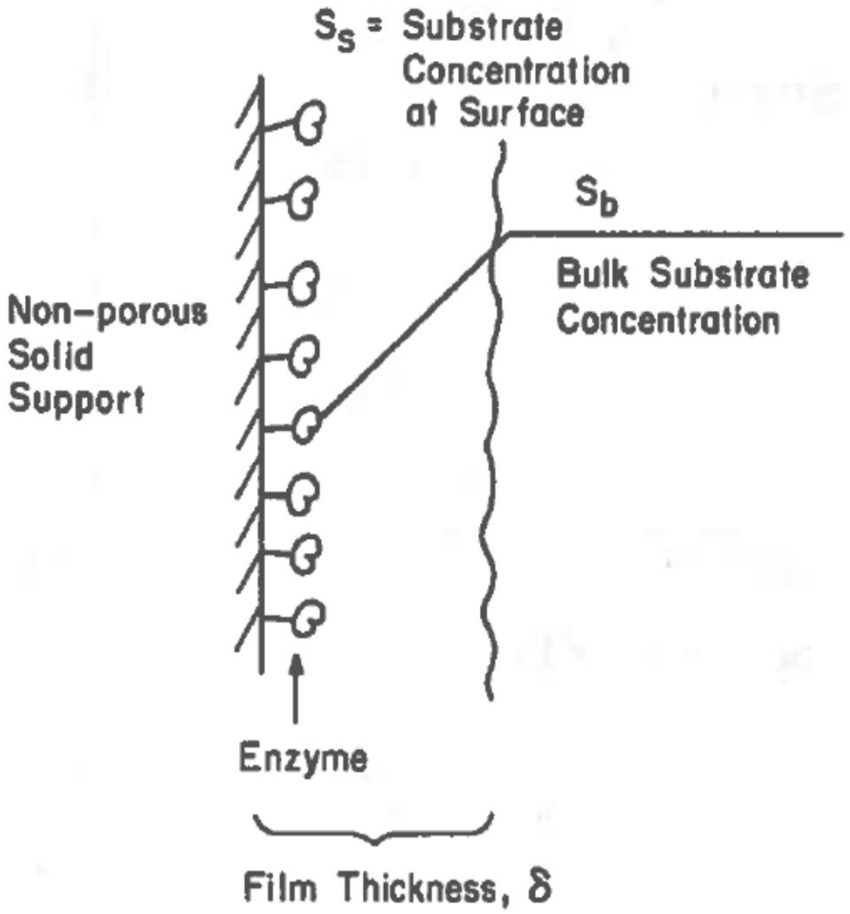
*** Immobilization allows for *enzyme re-utilization* and *elimination of enzyme recovery and purification*; also can improve enzyme activity**



Enzyme Immobilization (Cont'd)

- **Entrapment**
  - ✓ *Matrix entrapment:* usually polymeric materials, e.g., calcium alginate, agar, poly-acrylamid, collagen, κ-carrageenin
  - ✓ *Membrane entrapment:* usually hollow fibers made of nylon, cellulose, polysulfone, and poly-acrylate
  - ✓ *Microencapsulation:* microscopic hollow spheres made of polymer
  - ➢ **Disadvantages:** enzyme leakage, diffusional limitations, reduce activity and stability, lack of microenvironment control
- **Surface immobilization**
  - ✓ *Adsorption:* attached to a support (alumina, silica, porous glass, activated carbon, cellulose, ion-exchange resins) by physical forces, e.g., van der Waals
  - ✓ *Covalent binding:* amino, carboxyl, hydroxyl, and sulfhydryl groups
  - ➢ **Disadvantages:** desorption (for physical binding) and loss in activity and stability because of conformational changes (fro covalent binding)

Diffusional Limitations



Damköhler number:

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

*** Diffusion limited:**

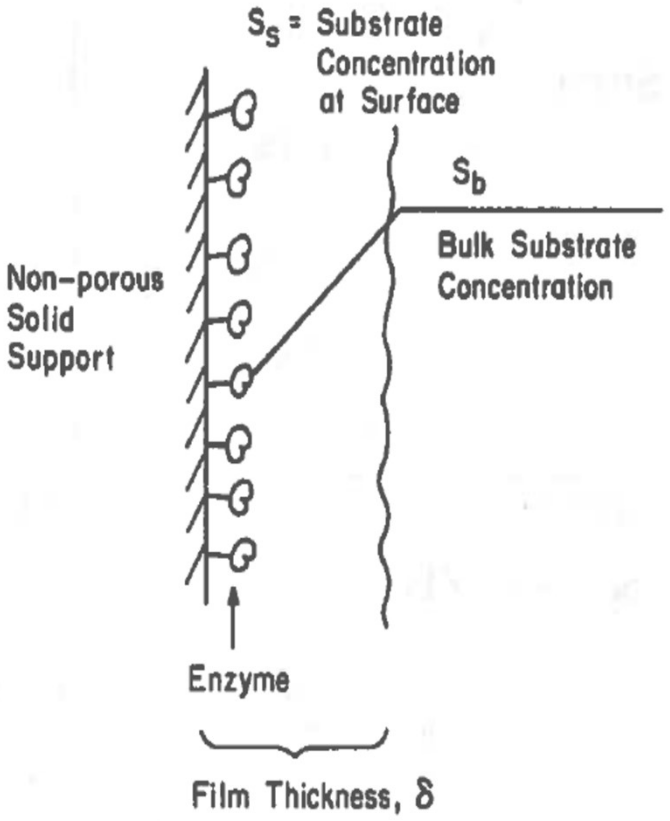
$$Da >> 1$$

**** Reaction limited:**

$$Da << 1$$

External Diffusional Limitations

* At steady state, the reaction rate is equal to the mass transfer rate:



$$J_S = k_L(S_b - S_s) = \frac{V_m S_s}{K_m + S_s}$$

* Fast reaction asymptote ($S_s \approx 0$, Da >> 1):

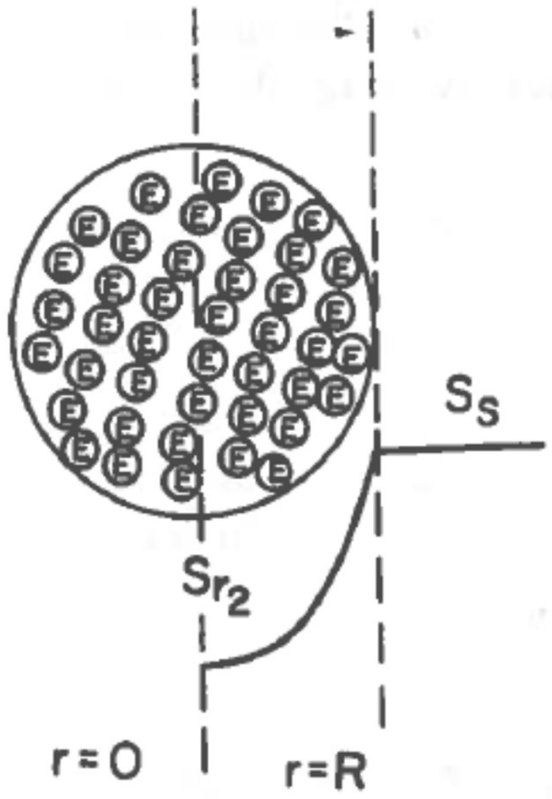
$$\nu = k_L S_b$$

** Slow reaction asymptote (Da << 1):

$$\nu = \frac{V_m S_b}{K_{m,app} + S_b} \qquad K_{m,app} = K_m\left(1 + \frac{V_m}{k_L(S_b + K_m)}\right)$$

Internal Diffusion Limitations

*** Enzyme immobilized in a spherical particle (porous matrix):**



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

Dimensionless form:

$$\frac{d^2s}{dx^2}+\frac{2}{x}\frac{ds}{dx}=\phi^2\frac{s}{1+s/\beta} \qquad \beta=\frac{K_m}{S_s}$$

Thiele modulus:

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

Effectiveness Factor

*** The rate of substrate consumption is equal to the substrate flux into the particle:**

$$r_s = J_s\Big|_{r=R} = 4\pi R^2 D_e \left.\frac{dS}{dr}\right|_{r=R}$$

Effectiveness factor:

$$\eta = \frac{\text{reaction rate within the particle}}{\text{maximum reaction rate}}$$

$$\eta = \frac{4\pi R^2 D_e \left.\dfrac{dS}{dr}\right|_{r=R}}{\dfrac{V_m'' S_s}{K_m + S_s}}$$

Asymptotic cases:

**** zero-order reaction:***

$$\beta \to 0$$

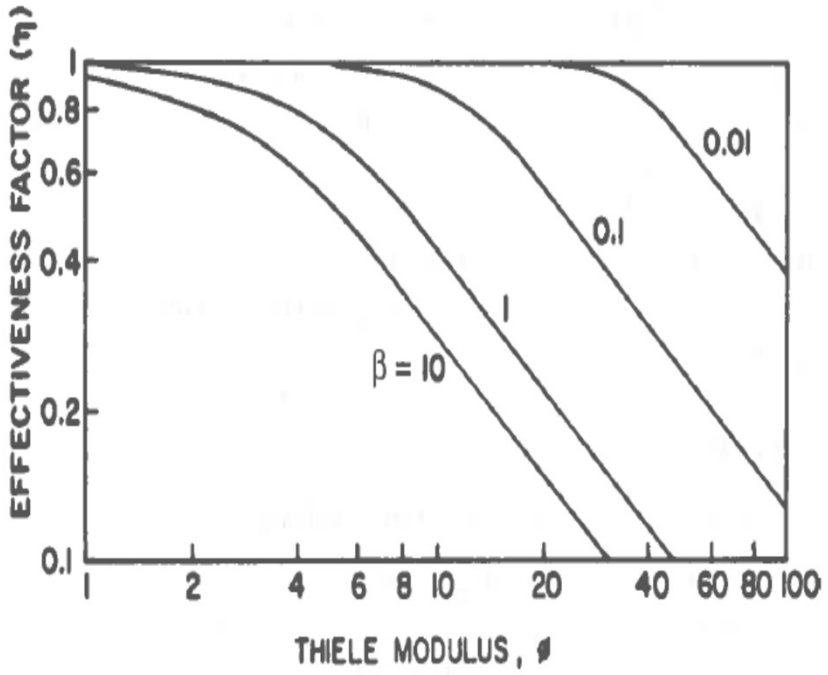
$$\eta \approx 1 \quad \text{for} \quad 1 < \phi < 100$$

**** 1st-order reaction:***

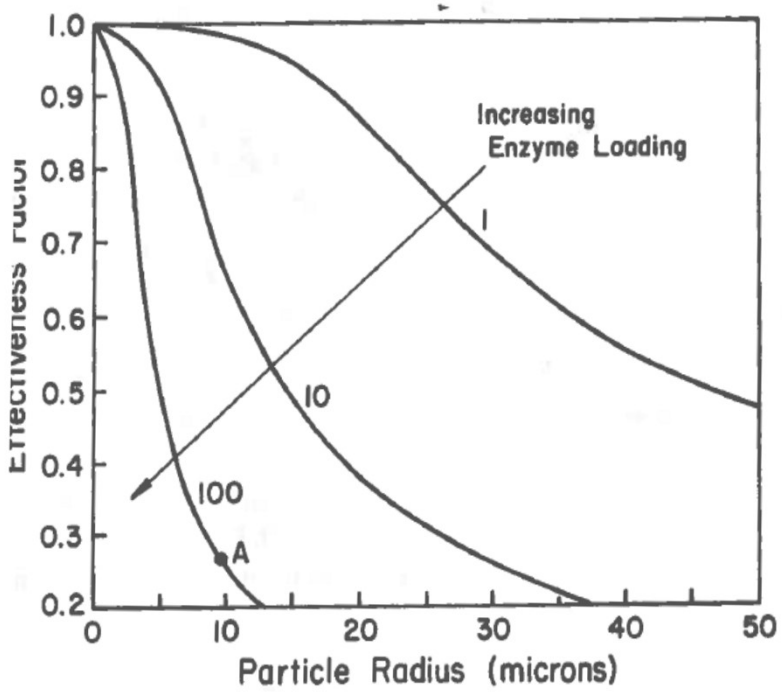
$$\beta >> S \quad \Rightarrow \quad \eta = \frac{3}{\phi}\left[\frac{1}{\tanh\phi} - \frac{1}{\phi}\right]$$

Effectiveness Factor (Cont'd)

Effectiveness factor dependence on Thiele modulus



Effectiveness factor dependence on particle radius



Batch Reactor

Batch Reactor – a <u>closed</u> stirred tank with a heating/cooling system operated in a <u>transient</u> (unsteady) mode. Sizes vary from < 1L to > 10,000 L; typically made from stainless steel or glass. Liquids and solids are charged via connections on the top. Gases are discharged through connections on the top, while liquids are discharged from the bottom.

<u>Advantages</u>:

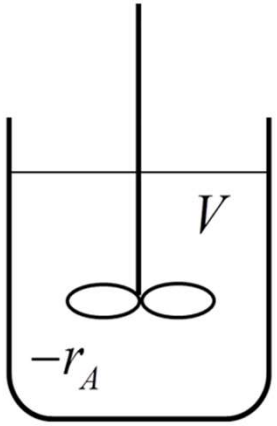
- Simple design with no inlets or outlets
- Inexpensive construction
- No spatial gradients
- Excellent for product development and high value/low volume production

<u>Disadvantages</u>:

- High labor (charge/discharge) cost
- Products variability (from batch to batch)

Batch Reactor: Design Equation

Assuming an **ideal Batch Reactor**, i.e., that the mixing is perfect and, thus there are no concentration or temperature gradients in the reactor:



*** General mole balance:**

$$\frac{dN_S}{dt} = \cancel{F_{S,in}} - \cancel{F_{S,out}} + \int r_S dV$$

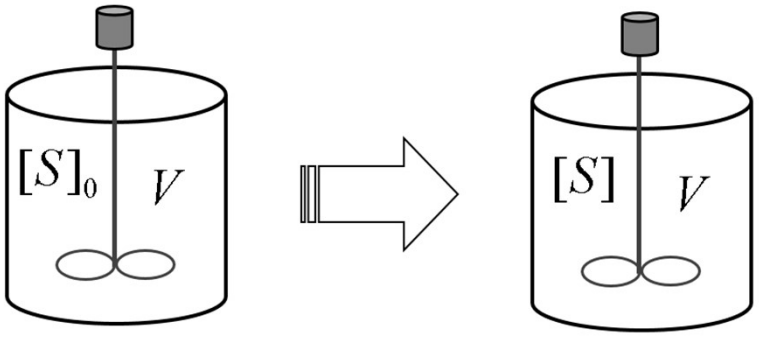
$$\frac{dN_S}{dt} = \int r_S dV$$

if well-mixed:

$$\int r_S dV = r_S \int dV = r_S V$$

$$\Rightarrow \quad \boxed{\frac{dN_S}{dt} = r_S V}$$

Enzymatic Reaction in a Batch Reactor

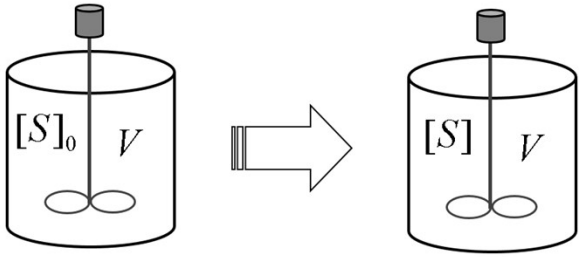


$$\frac{dN_S}{dt} = r_S V$$

$$-r_S = \frac{V_{\max}[\mathrm{S}]}{K_M + [\mathrm{S}]}$$

What would be the time required to achieve a certain substrate conversion?

Enzymatic Reaction in a Batch Reactor



$$\frac{dN_S}{dt} = r_S V \qquad -r_S = \frac{V_{\max}[\mathrm{S}]}{K_M + [\mathrm{S}]}$$

*** Design equation in terms of substrate concentration:**

$$\frac{dN_S}{dt} = r_S V \quad \Rightarrow \quad \frac{d(N_S/V)}{dt} = \frac{d[\mathrm{S}]}{dt} = r_S$$

**** Conversion definition:**

$$[S] = [S]_0(1-X)$$

***** Time required to achieve certain conversion:**

$$t = \frac{K_M}{V_{\max}} \ln \frac{1}{(1-X)} + \frac{[S]_0 X}{V_{\max}}$$

Continuous Stirred Tank Reactor

Continuous Stirred Tank Reactor **(CSTR)** – a stirred tank with inlet/outlet and a heating/cooling system operated in a continuous (typically steady-state) mode. Commonly used for gas-liquid and liquid-liquid reactions; excellent solution for enzymatic reactions and for bioreactors.

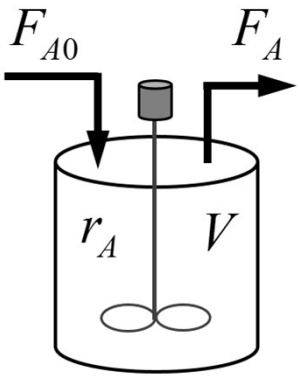
Advantages:

- Simple design & inexpensive construction
- No spatial gradients (for good mixing)
- Simple control (for steady state operation)
- Large scale (continuous) production

Disadvantages:

- Lowest conversion per unit volume
- Large reactor volume
- Imperfect mixing for larger reactors

Mole Balances in Terms of Concentration



$$\text{In} - \text{Out} + \text{Generation} = \text{Accumulation}$$

$$F_{S0} - F_S + \int r_S dV = \frac{dN_S}{dt}$$

***) Steady state, well mixed:**

$$F_{S0} - F_S = -\int r_S dV = -r_S V$$

****) In terms of concentration:**

$$\upsilon = \frac{F_i}{C_i} \Rightarrow V = \frac{F_{S0} - F_S}{-r_S} = \frac{\upsilon_0 S_0 - \upsilon S}{-r_S} \overset{\upsilon=const}{=} \frac{\upsilon_0 (S_0 - S)}{-r_S}$$

$$V = \frac{\upsilon_0 (S_0 - S)}{-r_S}$$

Example: BR vs. CSTR

The enzyme urease decomposes urea into products ammonia and carbon dioxide according to the following enzymatic reaction:

$$S + E \rightleftarrows E{\bullet}S \rightarrow E + P$$

$$-r_S = \frac{V_{max}[S]}{K_M + [S]}$$

k_{cat} – turnover number

K_M – Michaelis constant

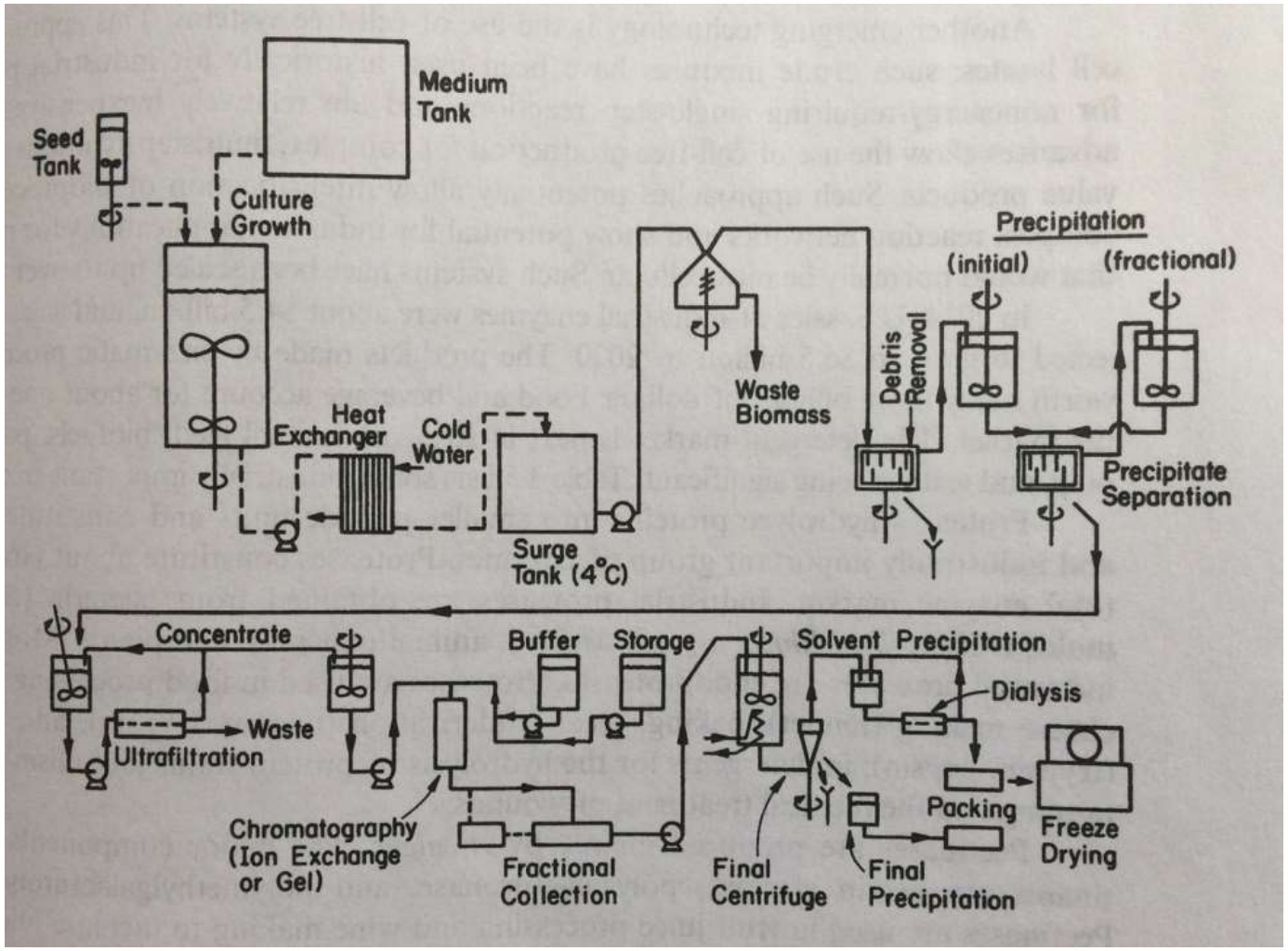
$V_{max} = k_{cat}[E_t]$

The known kinetic parameters are the Michaelis constant, K_M = 0.027 mol/L and the turnover number, k_{cat} = 0.266 mol/(g s).

1) Calculate time required to achieve 76% urea conversion in a batch reactor with a volume of 1 L. The enzyme amount is 1 mg. Initial urea concentration is 0.1 mol/L.
2) What would be the conversion of urea if this enzymatic reaction is carried out continuously in a CSTR with identical volume (1 L) and enzyme amount (1 mg)? The enzyme is immobilized inside the reactor, i.e. it cannot be washed out from the reactor. Aqueous solution of urea is fed to the reactor at the rate of 0.1 L/min; urea concentration in the feed solution is 0.1 mol/L.

Large-Scale Production

A flowsheet for the production of an extracellular enzyme:



Summary

- Enzymatic kinetics parameter estimation could be done by several methods
- Inhibitory integrations could lead to complex kinetics
- Effects of pH and temperature are very important
- Enzyme immobilization has several advantages but could lead to significant diffusion limitations
- The overall enzymatic catalysis phenomenon is a combination of transport and reaction processes