Chapter 3: Enzymes

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Presentation Outline: Lectures 4 and 5

- Introduction to Enzymes
- Kinetics of Enzyme-Catalyzed Reactions
- Effects of Environmental Conditions on Kinetics
- Inhibition of Enzyme Catalyzed Reactions
- Immobilized Enzyme Systems
- Industrial Uses of Enzymes

Introduction to Enzymes (3.1 and 3.2)

Constituents of Enzymes

Protein molecule(s)

Co-factors

Co-enzymes

TABLE 3.2 Cofactors (Metal Ions) and Coenzymes of Some Enzymes

	Coenzyme	*Entity transferred*
Zn^{2+}		
Alcohol dehydrogenase	Nicotinamide adenine dinucleotide	Hydrogen atoms (electrons)
Carbonic anhydrase	Nicotinamide adenine dinucleotide phosphate	Hydrogen atoms (electrons)
Carboxypeptidase	Flavin mononucleotide	Hydrogen atoms (electrons)
Mg^{2+}	Flavin adenine dinucleotide	Hydrogen atoms (electrons)
Phosphohydrolases	Coenzyme Q	Hydrogen atoms (electrons)
Phosphotransferases	Thiamin pyrophosphate	Aldehydes
Mn^{2+}	Coenzyme A	Acyl groups
Arginase	Lipoamide	Acyl groups
Phosphotransferases	Cobamide coenzymes	Alkyl groups
Fe^{2+} or Fe^{3+}	Biocytin	Carbon dioxide
Cytochromes	Pyridoxal phosphate	Amino groups
Peroxidase	Tetrahydrofolate coenzymes	Methyl, methylene, formyl or formimino groups
Catalase		
Ferredoxin		
Cu^{2+} (Cu^{+})		
Tyrosinase		
Cytochrome oxidase		
K^{+}		
Pyruvate kinase (also requires Mg^{2+})		
Na^{+}		
Plasma membrane ATPase (also requires K^{+} and Mg^{2+})		

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Introduction to Enzymes (3.1)

Naming of Enzymes

- *adding suffix -ase to*
  - *→ substrate converted (e,g, urease)*
  - *→ reaction catalyzed (e,g, dehydrogenase)*

Enzymes Facts

- *over 2,000 known enzymes*
- *more efficient than chemical catalyses*
- *high molecular weight ($15{,}000 < MW < 10^6$ Daltons)*

Major Classes of Enzymes (Table 3.1)

Oxidoreductases
- *oxidation – reduction reactions*

Transferases
- *transfer of whole functional groups (e.g. NH_2 group)*

Hydrolases
- *Hydrolysis reactions involving various functional groups*

Lyases
- *Additions to double bonds*

Isomerases
- *oxidation – reduction reactions*

Ligases
- *formation of bonds with ATP cleavage*

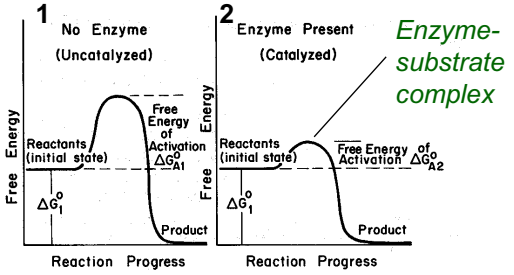
How Enzymes Work (3.2)

Lower the activation energy of enzyme-substrate complex

$$\frac{rate_2}{rate_1} = \frac{e^{\left(-\frac{\Delta G^o_{A2}}{RT}\right)}}{e^{\left(-\frac{\Delta G^o_{A1}}{RT}\right)}} = 10^8$$

for $\Delta G^o_{A1} = 18$ kcal / mole

$\Delta G^o_{A2} = 7$ kcal / mole



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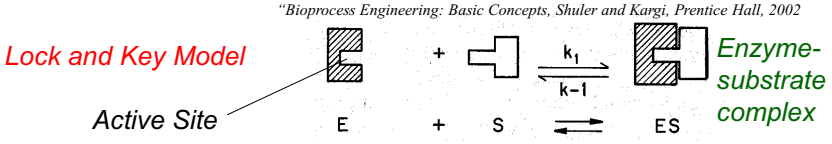
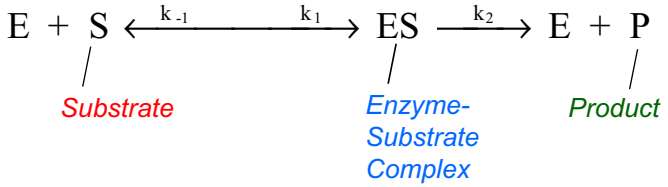


Enzyme Kinetics (3.3)

Michaelis-Menten Kinetics



Relate product reaction rate to measurable quantities

Enzyme Kinetics (3.3)

Mass Balance Equations

Product Rate $$\upsilon = \frac{d[P]}{dt} = k_2[ES]$$

ES Rate $$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES]$$

Enzyme Balance $$[E] = [E_o] - [ES]$$

Michaelis-Menten Kinetics (eqn. 3.8)

Rapid Equilibrium Assumption (formation of ES is rapid)

Equilibrium Constant (relates [ES] to [E] , [S]

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

Combine K'_m equation with E balance equation

$$[ES] = \frac{[E_o][S]}{K'_m + [S]}$$

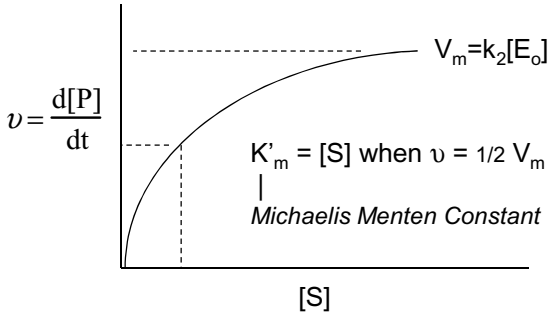
Product Rate Eqn. - Michaelis-Menten Equation

$$\upsilon = k_2[ES] = \frac{k_2[E_o][S]}{K'_m + [S]} = \frac{V_m[S]}{K'_m + [S]}$$

maximum rate

Michaelis-Menten Kinetics (cont.)

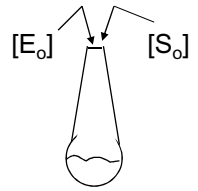
Saturation Kinetics



- *at high [S] → $\upsilon=V_m$ (constant) - why? → all active sites on E filled with S*
- *if K'_m is small → S has high affinity for E*

Experimental Determination of V_m and K'_m

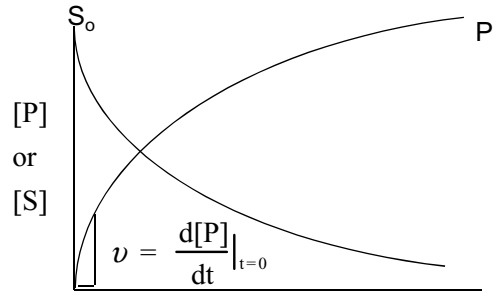
Saturation Kinetics



Batch Reactor

measure

t	[S]	[P] .



→ *determine rate (slope) from measured data*

Plotting Experimental Data

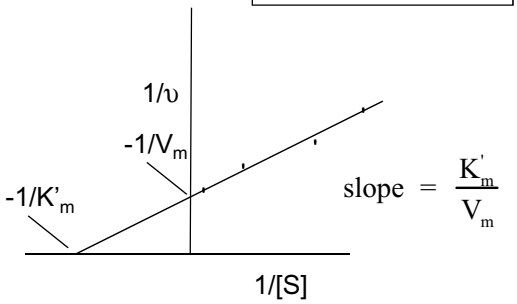
Double-Reciprocal Plot (*Lineweaver-Burk Plot, eqn. 3.13*)

rearrange $v = \frac{V_m[S]}{K'_m + [S]}$

$$\boxed{\frac{1}{v} = \frac{1}{V_m} + \frac{K'_m}{V_m}\frac{1}{[S]}}$$

Limitation: K'_m not determined accurately.

Data at low [S] Influence Regression too much.

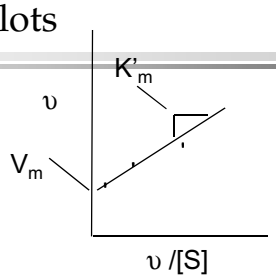


slope $= \frac{K'_m}{V_m}$

Other Types of Plots

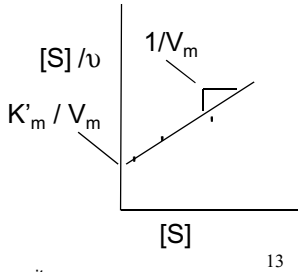
Eadie-Hofstee Plot (eqn. 3.14)

$$\upsilon = V_m + K'_m \frac{\upsilon}{[S]}$$



Hanes-Woolf Plot (eqn. 3.15)

$$\frac{[S]}{\upsilon} = \frac{K'_m}{V_m} + \frac{1}{V_m}[S]$$



Complex Enzyme Kinetics: Inhibition

A) Competitive Inhibition (eqn. 3.20 - 3.23)

$$\upsilon = \frac{V_m [S]}{K'_m\left[1 + \frac{[I]}{K_I}\right] + [S]}$$

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

$+$

$I \rightarrow$ Inhibitor binds to active site on enzyme

$\updownarrow K_I$

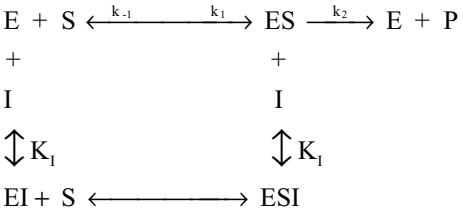
$EI \rightarrow$ Enzyme / Inhibitor complex

- $K'_{m,app}$ → *net effect of I is to increase K'm*
- *overcome inhibition by increasing [S]*

Complex Enzyme Kinetics: Inhibition

B) Noncompetitive Inhibition (eqn. 3.24 - 3.27)

$$v = \frac{V_m [S]}{\left[1 + \frac{[I]}{K_I}\right](K'_m + [S])}$$



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

→ *net effect of I is to reduce V_m*

- *overcome inhibition by blocking inhibitor*

More Inhibition Kinetics (eqn. 3.28 - 3.38)

C) Uncompetitive Inhibition
D) Substrate Inhibition

- *have similar mechanisms by inhibiting on ES*

Summary of Inhibition Kinetics

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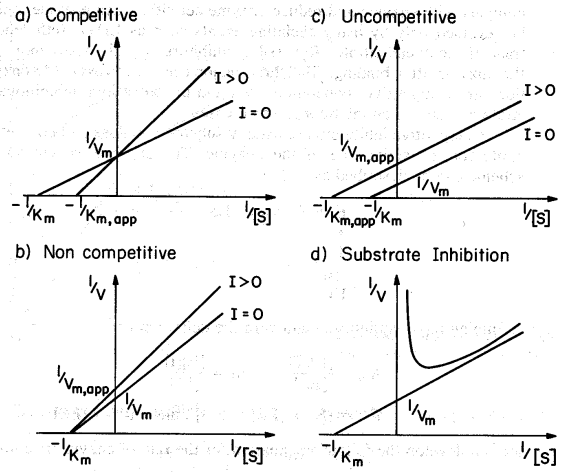

Figure 3.10. Different forms of inhibited enzyme kinetics.

Temperature Effects on Enzyme Kinetics

The rate of enzyme conversion of substrate will increase with temperature up to an optimum. Above this temperature, enzyme activity will decrease as enzyme denatures (Tertiary structure lost). Figure 3.15 shows a typical response.

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Temperature activation

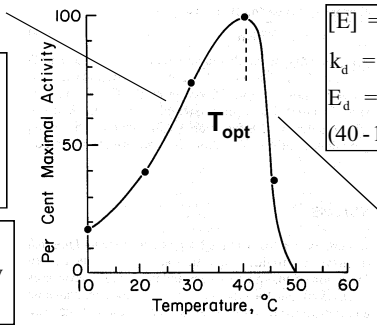
$V_m = k_2 [E_o]$, $T<T_{opt}$

$= k_2 [E]$, $T>T_{opt}$

[E] is concentration of active enzyme

$k_2 = A\, e^{-E_a/RT}$

E_a = activation energy (4 - 20 kcal / mole)



$[E] = [E_o]\, e^{-k_d t}$

$k_d = A_d\, e^{-E_d/RT}$

E_d = deactivation energy (40 - 130 kcal / mole)

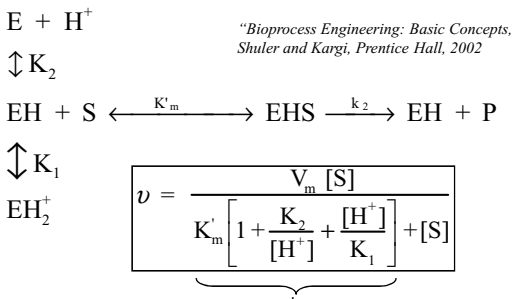
Temperature deactivation

pH Effects on Enzyme Kinetics (eqn. 3.40 - 3.44)

Enzymes are active only over a small pH range

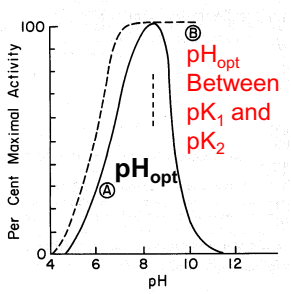
Reasons

1. Tertiary structure is pH-dependent
2. Active site functional group charges are pH-dependent



$$\upsilon = \frac{V_m [S]}{K'_m \left[1 + \frac{K_2}{[H^+]} + \frac{[H^+]}{K_1}\right] + [S]}$$

$K'_{m,app} \rightarrow \infty$ at high and low $[H^+]$



Immobilized Enzyme Systems

Immobilization - Definition

The containment of enzyme solution within a confined space for the purpose of retaining and re-using enzyme in processing equipment. There are many advantages that accompany immobilized enzymes and many methods for immobilization.

Immobilized Enzyme Systems

Advantages

1. Reduce costs of operation compared to *free enzyme systems* where additional separation and purification steps are needed.
2. Some immobilization methods can increase enzyme activity.
3. A model system to study enzyme action in *membrane-bound enzymes* that occur in the cell.

Disadvantages

1. Many immobilized enzymes exhibit lower activity compared to free enzymes.
2. More expensive to prepare than free enzymes.
3. Mass transfer limitations due to immobilization methods.

Methods of Enzyme Immobilization

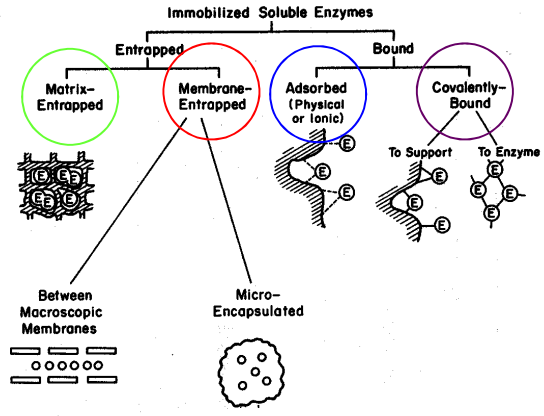


Figure 3.16. Major immobilization methods.

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Matrix Entrapment of Enzymes

Matrix Entrapment

The enzyme solution is mixed with a *polymeric fluid* that solidifies into various forms, depending on application (usually small beads). The polymeric material is *semi-permeable*. Large molecular weight enzymes can not diffuse out, but smaller substrate and product molecules can.

Matrices for Entrapment

- *Ca-alginate*
- *Agar*
- *Polyacrylamide*
- *Collagen*

Membrane Entrapment

Membrane Materials

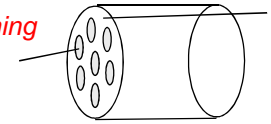
Enzymes solution may be confined between thin semi-permeable membranes. Membrane materials include;

- *Nylon*
- *Polysulfone*
- *Cellulose*
- *Polyacrylate*

Membrane Configurations

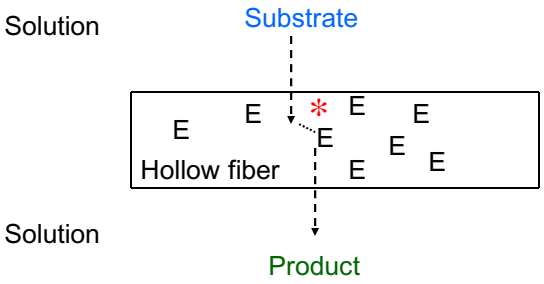
Hollow fiber configuration is a popular arrangement for separating enzyme from substrate and product solution.

Hollow fibers containing a stationary enzyme solution



Mobile fluid outside fiber tubes containing substrate and products

Membrane Entrapment: Diffusion Processes



Surface Immobilization: Adsorption

Adsorption: Attachment of enzymes to stationary solids by *weak physical forces* (van der Waals or dispersion forces). Active site is normally unaffected and nearly *full activity* is observed. Desorption of enzymes is a common problem.

Solid Support Materials:

- *Alumina*
- *Porous Glass*
- *Diatomaceous Earth*
- *Cellulose Materials*
- *Ion Exchange Resin*
- *Silica*
- *Ceramics*
- *Clay*
- *Activated Carbon*
- *Starch*

Surface Immobilization: Covalent Bonding

Covalent Bonding: The retention of enzyme on support surfaces by covalent bonding between *functional groups* on the enzyme and those on the support surface.

Functional Groups on Enzymes:

- *Amino (protein-NH_2)*
- *Hydroxyl (protein-OH)*
- *Carboxyl (protein-COOH)*
- *Sulfhydryl (Protein-SH)*

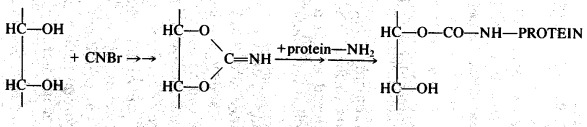
Active site of enzyme must not participate in covalent bonding. Enzyme inhibitors are added to enzyme solution during covalent bonding treatment.

Surface Immobilization: Support Bonding

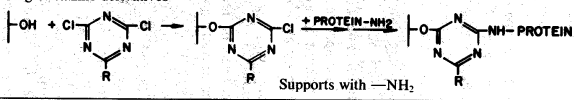
TABLE 3.3 Methods of Covalent Binding of Enzymes to Supports

Supports with —OH

(a) Using cyanogen bromide

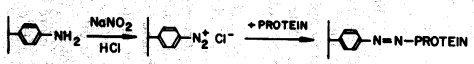


(b) Using *S*-triazine derivatives

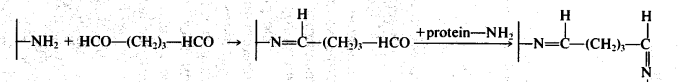


Supports with —NH_2

(a) By diazotization



(b) Using glutaraldehyde



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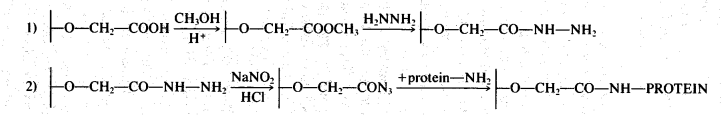
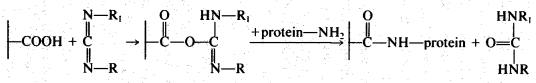
Surface Immobilization: Support Bonding

Supports with —COOH

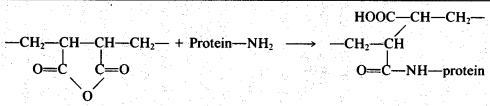
(a) Via azide derivative



(b) Using a carbodiimide



Supports containing anhydrides



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Diffusional Limitations: Immobilized Enzyme Systems (section 3.4.2)

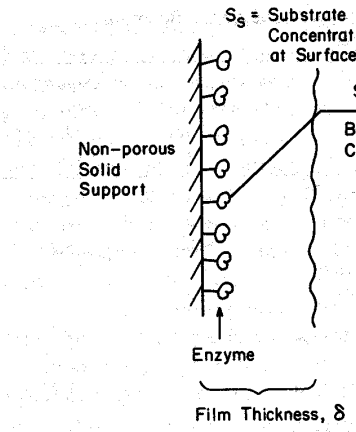
Diffusional limitations are observed to various degrees in all immobilized enzyme systems. This occurs because substrate must diffuse from the bulk solution up to the surface of the immobilized enzyme prior to reaction. The rate of diffusion relative to enzyme reaction rate determines whether limitations on intrinsic enzyme kinetics is observed or not.

Damkohler Number

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

If Da>>1, diffusion rate is limiting the observed rate
If Da<<1, reaction rate is limiting.

Diffusional Effects on Surface-Bound Enzymes on Non-porous Supports



EL, Enzyme Loading (mg enzyme/cm^2)

$$V_m' = \frac{V_m}{[E_0]} \cdot EL \quad (mol/sec\,cm^2)$$

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

Eqn. 3.53

Figure 3.17. profile in a li enzymes.

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Diffusional Effects on Surface-Bound Enzymes on Non-porous Supports (cont.)

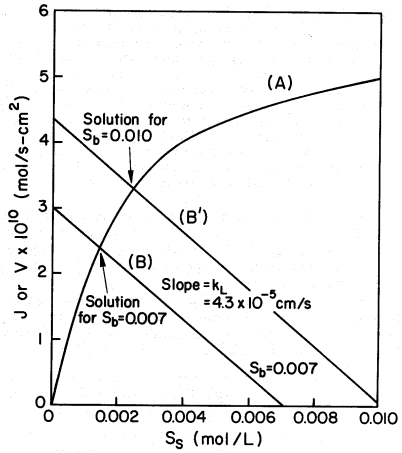

Figure 3.18. Graphical solution for amount of reaction per unit surface area for

Graphical Solution to Eqn. 3.53

(A) is reaction kinetics

(B) is mass transfer rate

Intersection is solution for [S_s]

Figure 3.18 is useful for observing effects of
- stirring rate (k_L)
- changes in [S_b]
- changes in enzyme loading

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Diffusional Effects in Enzymes Immobilized in a Porous Matrix

Enzymes within a porous matrix

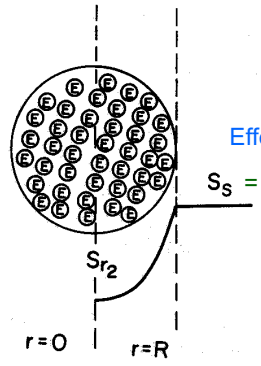

Figure 3.19. profile in a po containing imi it is assumed limitation exis surface conce

Substrate Mass Balance Equation

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

Effective diffusivity

mole/(s•cm^3 support)

$S_S = S_b$; negligible film resistance

Boundary Conditions

$$\text{at } r = R,\ [S] = [S_s]$$

$$\text{ar } r = 0,\ \frac{d[S]}{dr} = 0$$

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Diffusional Effects in Enzymes Immobilized in a Porous Matrix

Dimensional Substrate Mass Balance Equation

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

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

Boundary Conditions

$$\text{at } \bar{r}=1,\ \bar{S}=1$$

$$\text{ar } \bar{r}=0,\ \frac{d\bar{S}}{dr}=0$$

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

Effectiveness of Immobilized Enzymes

Rate of reaction within matrix (r_s) is equal to
the rate of diffusion through matrix surface (N_s)

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

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

η = 1, no diffusion limitations
η < 1, diffusion limits reaction rate

effectiveness factor

Effectiveness Factor and Thiele Modulus/Michaelis Constant

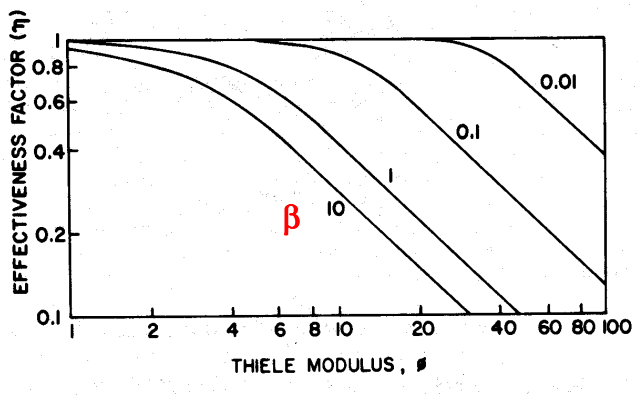

Figure 3.20. Theoretical relationship between the effectiveness factor η and

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Effectiveness Factor and Particle Radius/Enzyme Loading

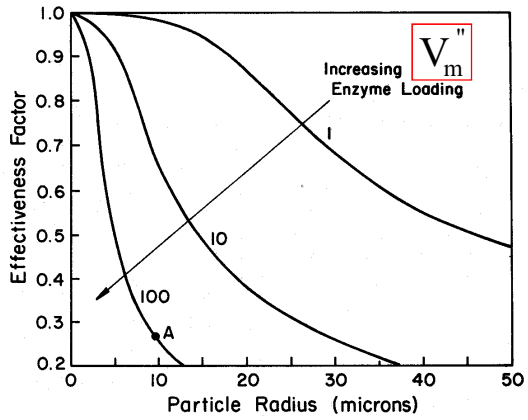


Figure 3.21. The effectiveness factor decreases with increases in enzyme loading

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

Overview of Industrial and Medicinal Enzymes

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

Major Industrial Enzymes

Table 3.6

Table 3.6 Some Industrially Important Enzymes

Name	Source	Application
Amylase	*Bacillus subtilis, Aspergillus niger*	Starch hydrolysis, glucose production
Glucoamylase	*A. niger, Rhizopus niveus, Endomycopsis*	Saccharification of starch, glucose production
Trypsin	Animal pancreas	Meat tenderizer, beer haze removal
Papain	Papaya	Digestive aid, meat tenderizer, medical applications
Pepsin	Animal stomach	Digestive aid, meat tenderizer
Rennet	Calf stomach	Cheese manufacturing
Glucose isomerase	*Flavobacterium arborescens, Bacillus coagulans, Lactobacillus brevis*	Isomerization of glucose to fructose
Penicillinase	*B. subtilis*	Degradation of penicillin
Glucose oxidase	*A. niger*	Glucose → gluconic acid, dried-egg manufacture
Lipases	*Rhizopus*, pancreas	Hydrolysis of lipids, flavoring and digestive aid
Invertase	*S. cerevisiae*	Hydrolysis of sucrose for further fermentation
Pectinase	*A. oryzae, A. niger, A. flavus*	Clarification of fruit juices, hydrolysis of pectin
Cellulase	*Trichoderma viride*	Cellulose hydrolysis

Production Statistics of Industrial Enzymes, (1990)

Enzyme	Type	World Market ($)	Market Share (%)
Proteases	alkaline (detergents)	100 MM	25.0
	other alkaline	24 MM	6.0
	neutral	48 MM	12.0
	animal rennet	26 MM	6.5
	microbial rennet	14 MM	3.5
	trypsins	12 MM	3.0
	other acid proteases	12 MM	3.0

Production Statistics of Industrial Enzymes (cont., 1990)

Enzyme	World Market ($)	Market Share (%)
α-amylases	20 MM	5.0
β-amylases	52 MM	13.0
Glucose isomerase (soft drinks)	24 MM	6.0
Pectinase (Juice/Wine Making)	12 MM	3.0
Lipase (Soaps/detergents, cheese..)	12 MM	3.0
All Others	44 MM	11.0

"Typical" Production of Industrial Enzymes

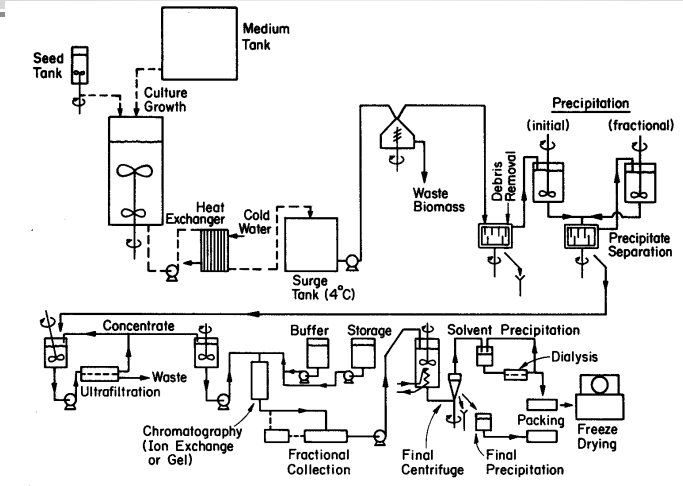


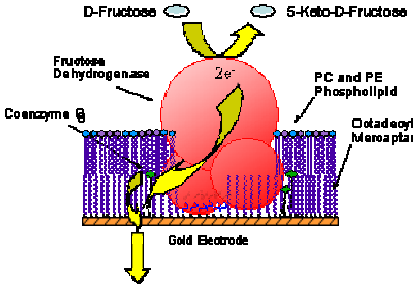
Figure 3.25. A flowsheet for the production of an extracellular enzyme.

Medicinal Uses of Enzymes

Used for Diagnosis and Therapy

Trypsin and Streptokinase - as antiinflammatory agents
Lysozyme - as an antibiotic for gram-positive cells
Urokinase - as an agent to dissolve blood clots
Asparaginase - an anticancer drug (cancer cells need asparagine)
Glucose oxidase - blood levels; glucose $\rightarrow$ gluconic acid + H_2O_2
Tissue Plasminogen Activator (TPA) - dissolves blood clots

Enzymes and Biosensors



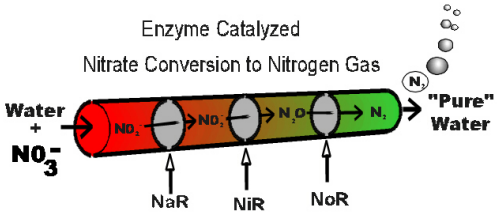
Membrane-bound redox enzymes constitute a large and important class of enzymes. The cell membrane provides the scaffolding upon which these enzymes arrange into systems for multi-step catalytic processes. The reconstruction of portions of this redox catalytic machinery, interfaced to an electrical circuit, leads to novel sensing devices.

Enzymes and Biosensors

EzNET System for Eliminating Nitrate Pollution

Enzyme Catalyzed

Nitrate Conversion to Nitrogen Gas



Enzymes:

NaR=NitrateReductase; NiR=NitriteReductase

NoR=NitrousOxideReductase

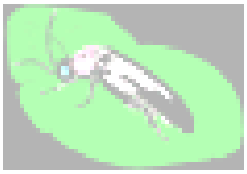
These enzymes are immobilized on "beads" with an electron-carrying dye. In this formulation, the reduction of nitrate to environmentally safe nitrogen gas is driven by a low voltage direct current.

Enzyme Engineering

Bioluminescence a Biomarker for Toxicity of HPV Chemicals and in Drug Development

Eileen Kim, Ph.D. student, and Cambrex Corporation

62 kDa molecular weight oxygenase
Yellow green light emission at 560 nm
Quantum yield: 88 photon/cycle
Light output proportional to [ATP]



Firefly Luciferase

luciferin + luciferase + ATP ⟶
luciferyl adenylate-luciferase + pyrophosphate

luciferyl adenylate-luciferase + O2 ⟶
oxyluciferin + luciferase + AMP + light

Inhibition of Luciferase

Strong inhibition of Luciferase by chloroform, a High Production Volume (HPV) chemical. This inhibition limits the assay applications. It is desired to engineer a Luciferase that is not inhibited by HPV chemicals

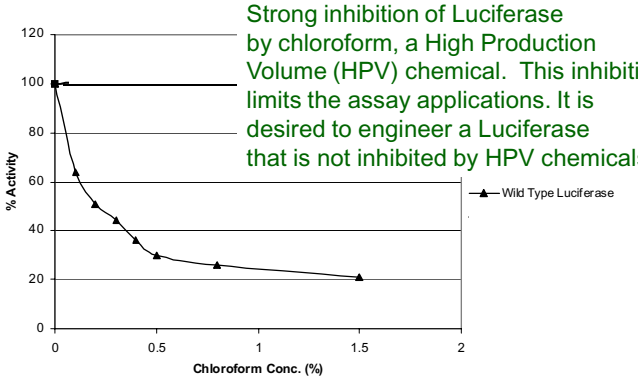
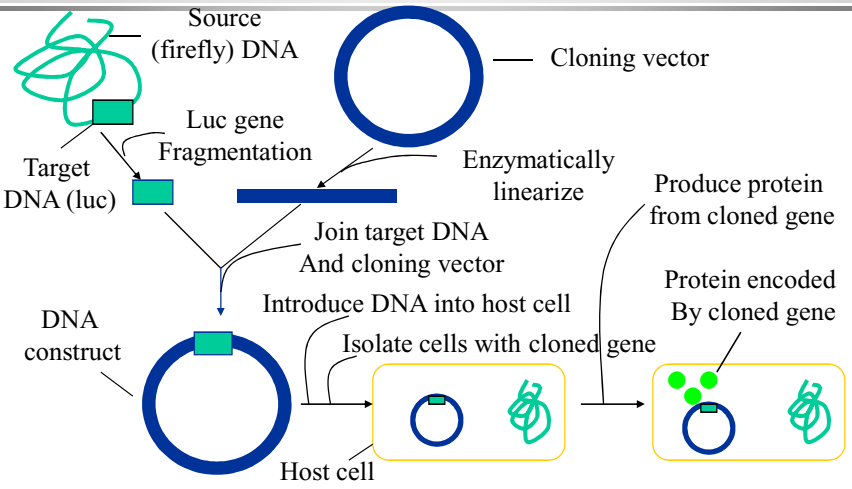


Figure 2. Inhibition of Luciferase Activity by increasing the concentration of Chloroform

Kim et al., AIChE Annual Meeting Presentation Record, November 16, 2003, San Francisco, CA

Recombinant Luciferase



Inhibition of Luciferase

Inhibition by chloroform is much reduced in the mutant Luciferase compared to the wild type.

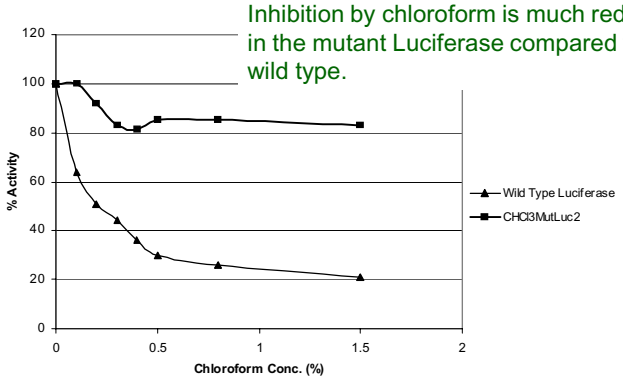


Figure 2. Inhibition of Luciferase Activity by increasing the concentration of Chloroform

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