Enzyme immobilization

Immobilization - Definition

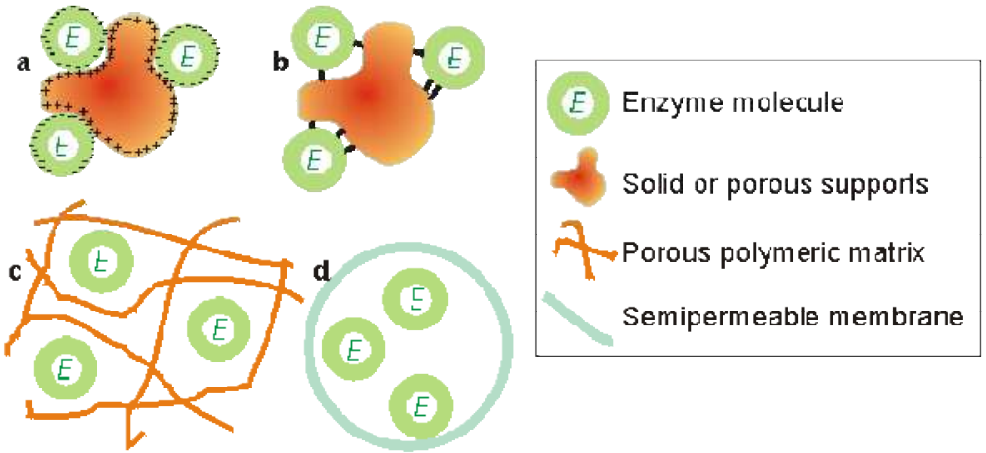
The containment of enzyme solution within a confined space for the purpose of retaining and re-using enzyme in processing equipment. There are many advantages that accompany immobilized enzymes and many methods for immobilization.

Advantages

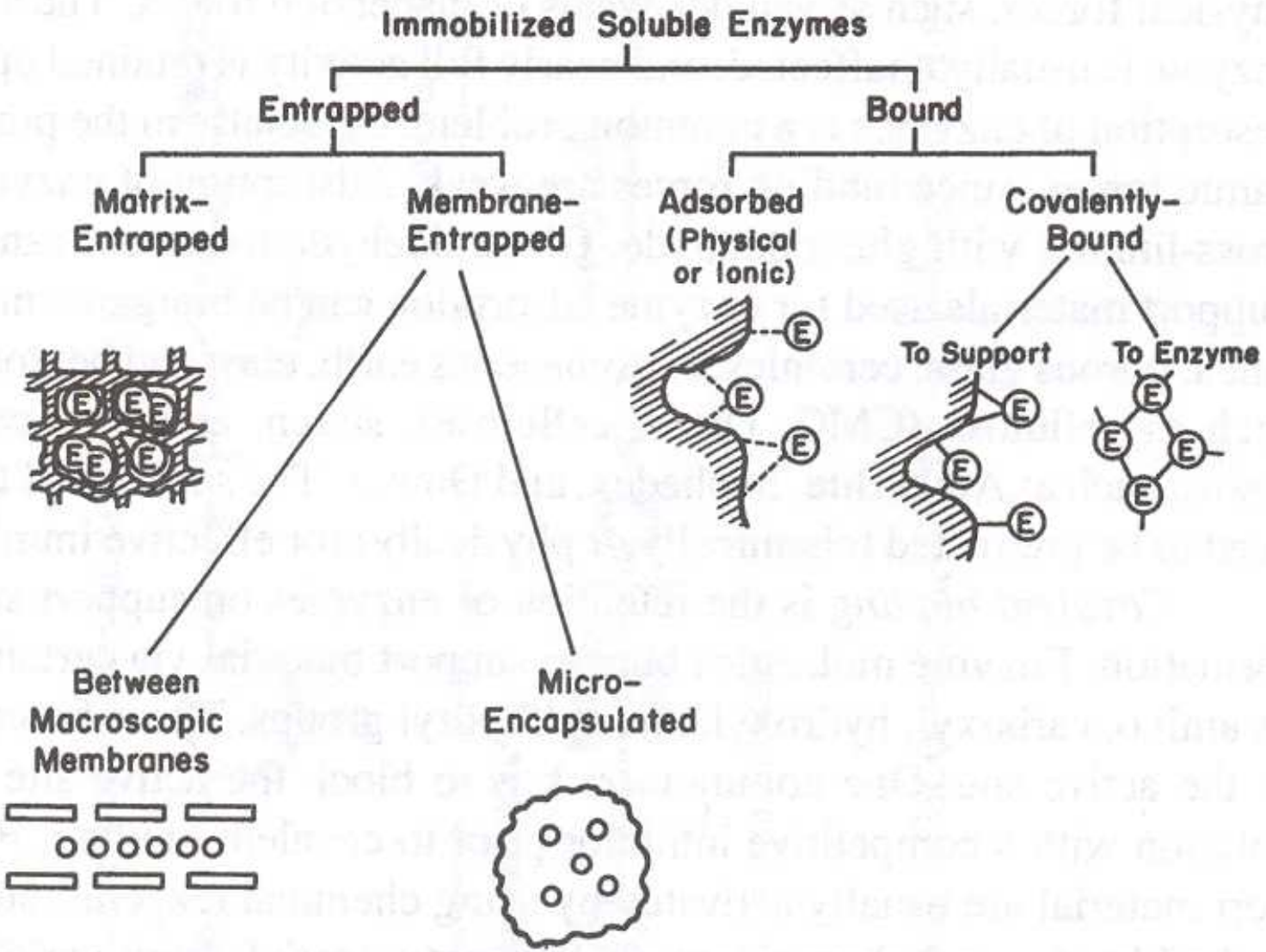
1. Reduce costs of operation compared to *free enzyme systems* where additional separation and purification steps are needed.

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



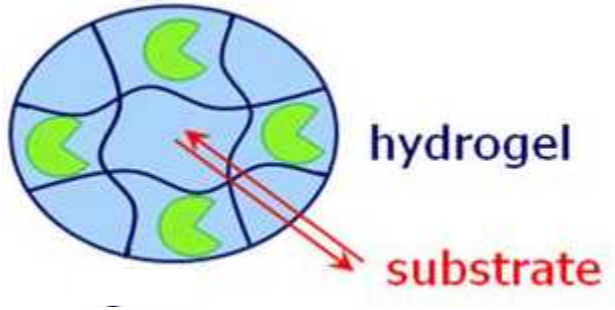
Matrix entrapment of enzymes

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

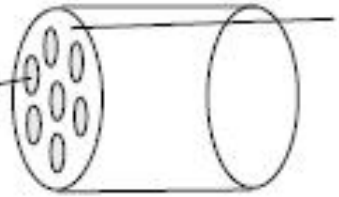
Enzymes solution may be confined between thin semi-permeable membranes. Membrane materials include;

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

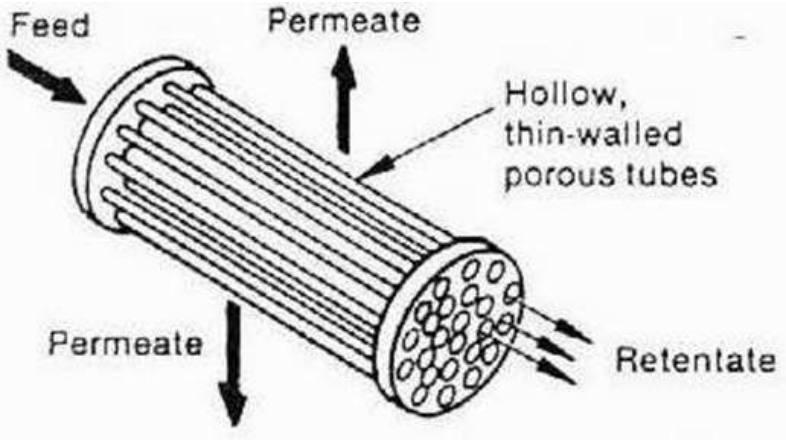
Membrane Configurations

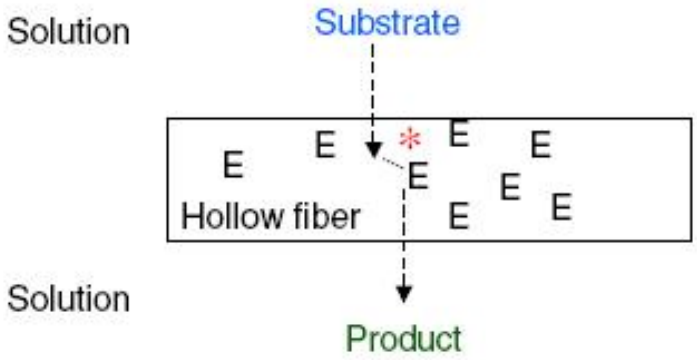
Hollow fiber configuration is a popular arrangement for separating enzyme from substrate and product solution.

Hollow fibers containing a stationary enzyme solution



Mobile fluid outside fiber tubes containing substrate and products





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)*

Thiol

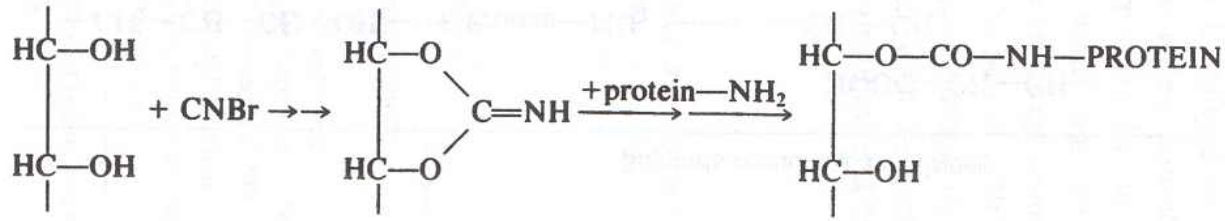
Active site of enzyme must not participate in covalent bonding. Enzyme inhibitors are added to enzyme solution during covalent bonding treatment.

Surface immobilization : support bonding

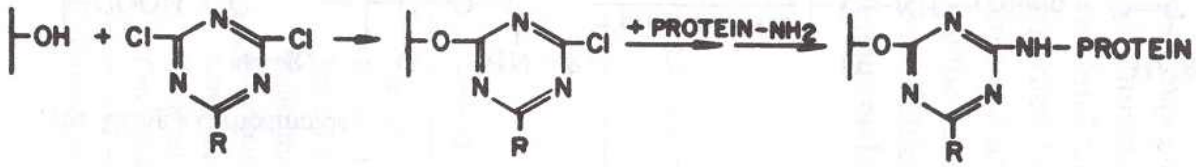
TABLE 3.3 Methods of Covalent Binding of Enzymes to Supports

Supports with —OH

(a) Using cyanogen bromide

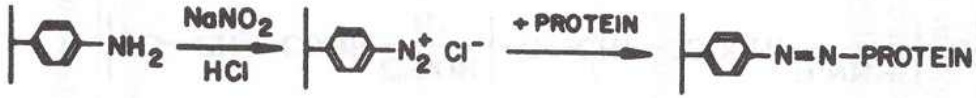


(b) Using S-triazine derivatives

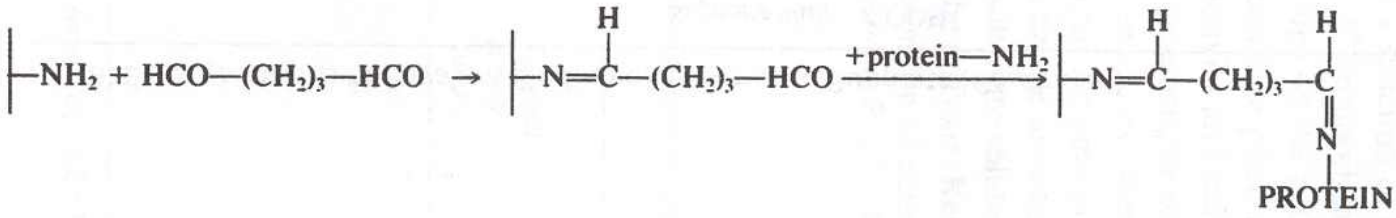


Supports with —NH_2

(a) By diazotization



(b) Using glutaraldehyde



Surface immobilization : support bonding

TABLE 3.3 Methods of Covalent Binding of Enzymes to Supports (*Continued*)

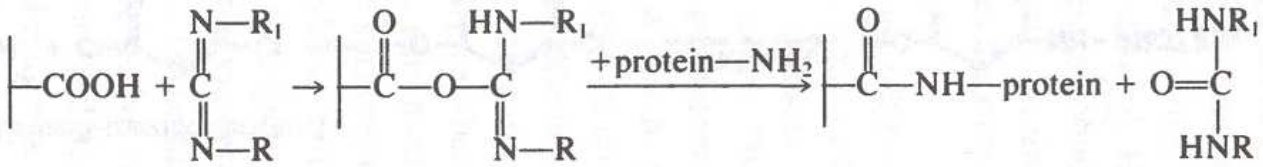
Supports with —COOH

(a) Via azide derivative

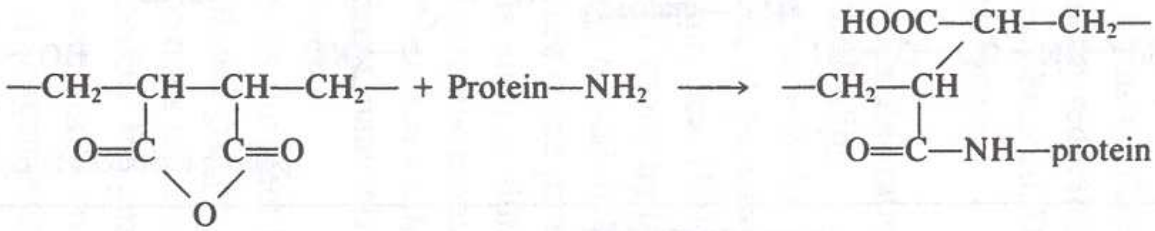
1) $\text{—O—CH}_2\text{—COOH} \xrightarrow[\text{H}^+]{\text{CH}_3\text{OH}} \text{—O—CH}_2\text{—COOCH}_3 \xrightarrow{\text{H}_2\text{NNH}_2} \text{—O—CH}_2\text{—CO—NH—NH}_2$

2) $\text{—O—CH}_2\text{—CO—NH—NH}_2 \xrightarrow[\text{HCl}]{\text{NaNO}_2} \text{—O—CH}_2\text{—CON}_3 \xrightarrow{+\text{protein—NH}_2} \text{—O—CH}_2\text{—CO—NH—PROTEIN}$

(b) Using a carbodiimide



Supports containing anhydrides



With permission, from D. I. C. Wang et al., *Fermentation and Enzyme Technology*, John Wiley & Sons, New York, 1979.

Diffusional limitations : immobilized enzyme systems

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.

Dämkohler Number

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

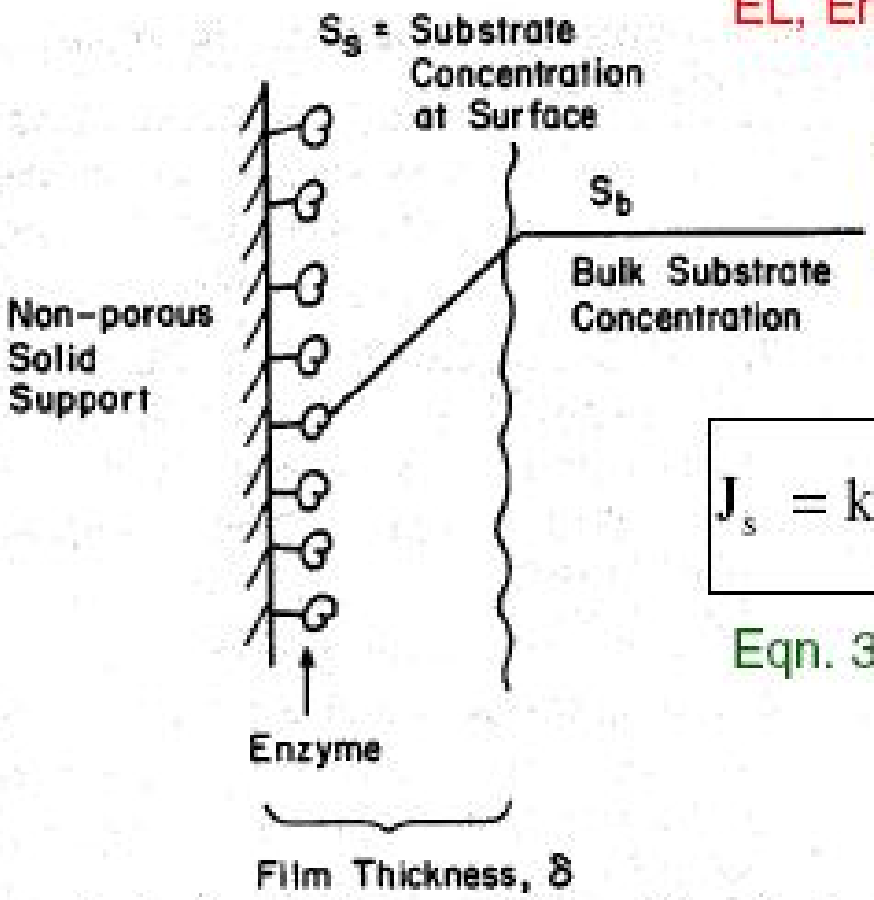
If Da>>1, diffusion rate is limiting the observed rate
If Da<<1, reaction rate is limiting.

K_L = mass transfer coeff.

Diffusional effects on surface-bound enzymes on non-porous supports

S_s = ?

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



$$V_m' = \frac{V_m}{[E_o]} * EL \ (mole/s*cm^2)$$

Diffusion rate = reaction rate

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

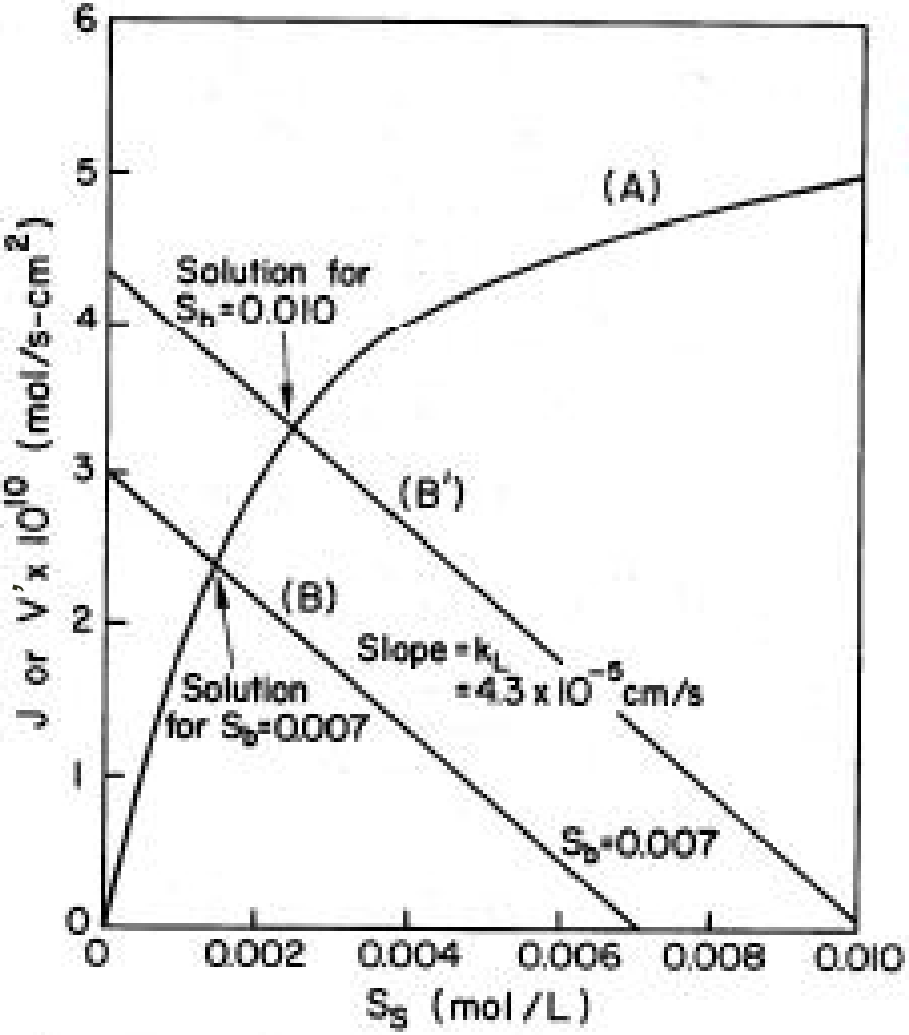
Eqn. 3.53 at steady state

Figure 3.17. profile in a li enzymes.

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

Diffusional effects on surface-bound enzymes on non-porous supports

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



Graphical Solution to Eqn. 3.53

(A) is reaction kinetics

(B) is mass transfer rate

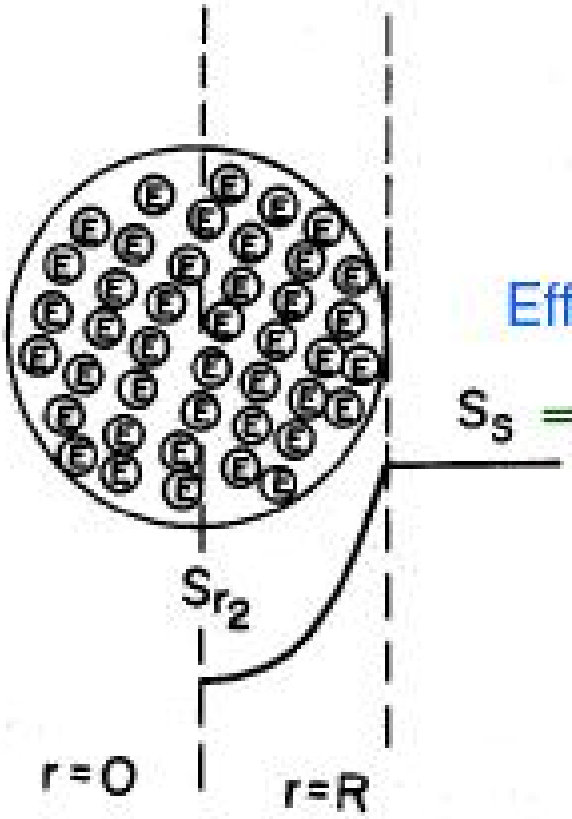
Intersection is solution for $[S_s]$

Figure 3.18 is useful for observing effects of

- stirring rate (k_L)
- changes in $[S_b]$
- changes in enzyme loading

Diffusional effects on enzymes immobilized in a porous matrix

Enzymes within a porous matrix



Substrate Mass Balance Equation

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

Diffusion rate = reaction rate

Effective diffusivity

mole/(s•cm³ support)

$S_s = S_b$; negligible film resistance

[S] = f(r) = ?

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

Boundary Conditions

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

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

Diffusional effects on enzymes immobilized in a porous matrix

Dimensionless Substrate Mass Balance Equation

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

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

Boundary Conditions

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

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

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

$$= \frac{\text{Max. rxn rate}}{\text{Diffusion rate}}$$

rxn rate at surface

Large Φ → Diffusional limitation

Effectiveness of immobilized enzymes

At steady state,

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

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

Sphere surface area

$$r_s = \eta \frac{V_m'' [S_s]}{K_m + [S_s]} \quad ; \text{ reaction rate}$$

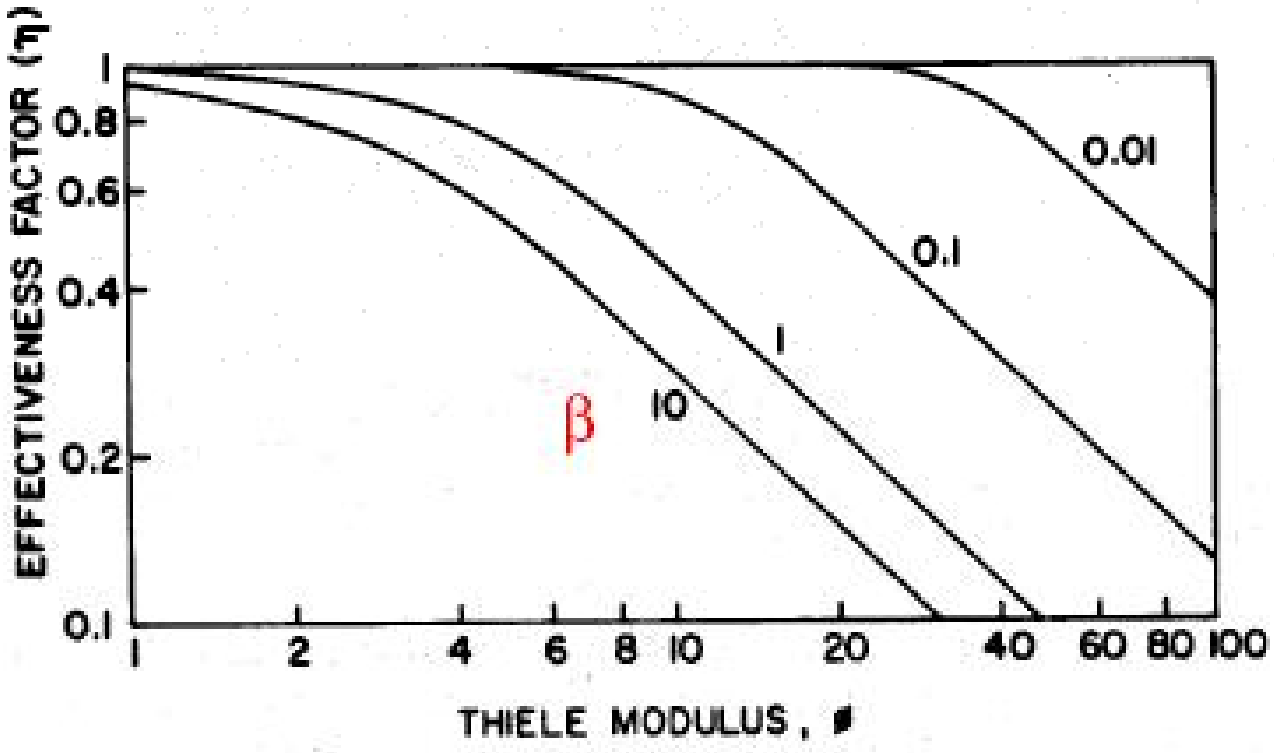
No diffusional limitation

$\eta = 1$, no diffusion limitations

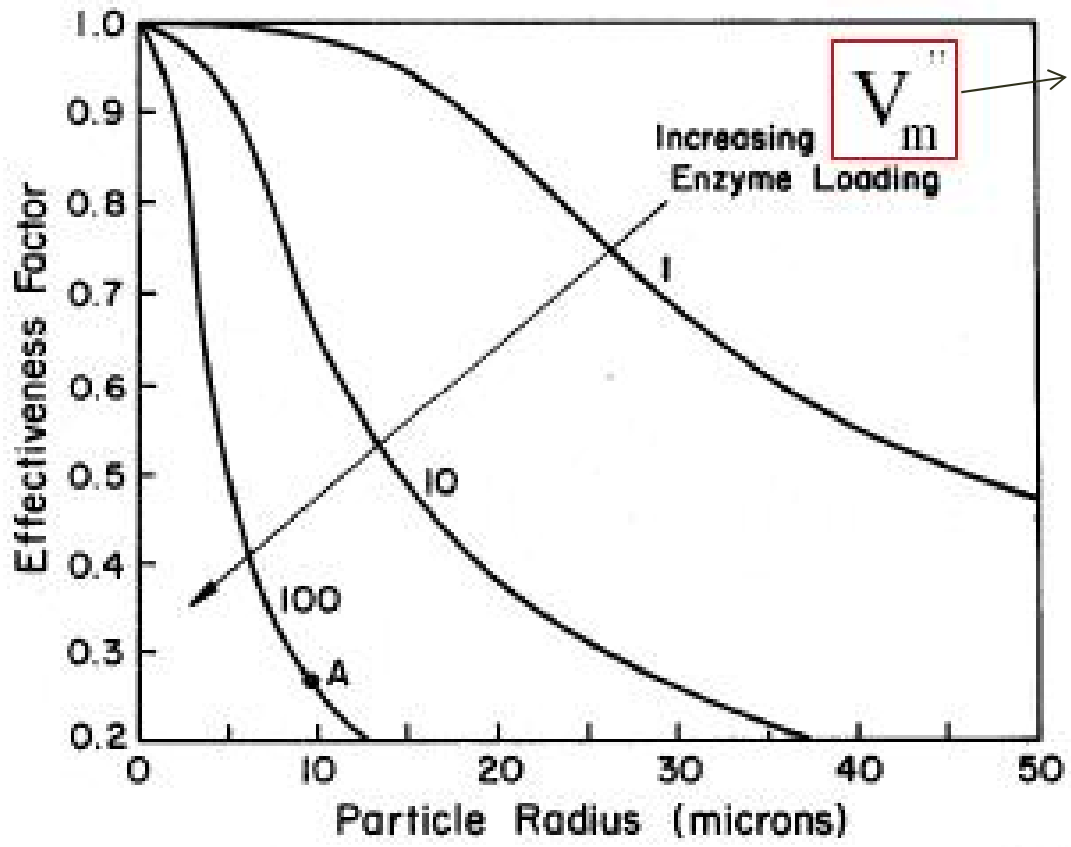
$\eta < 1$, diffusion limits reaction rate

effectiveness factor

Effectiveness factor versus Thiele modulus



Effectiveness factor versus particle radius / enzyme loading

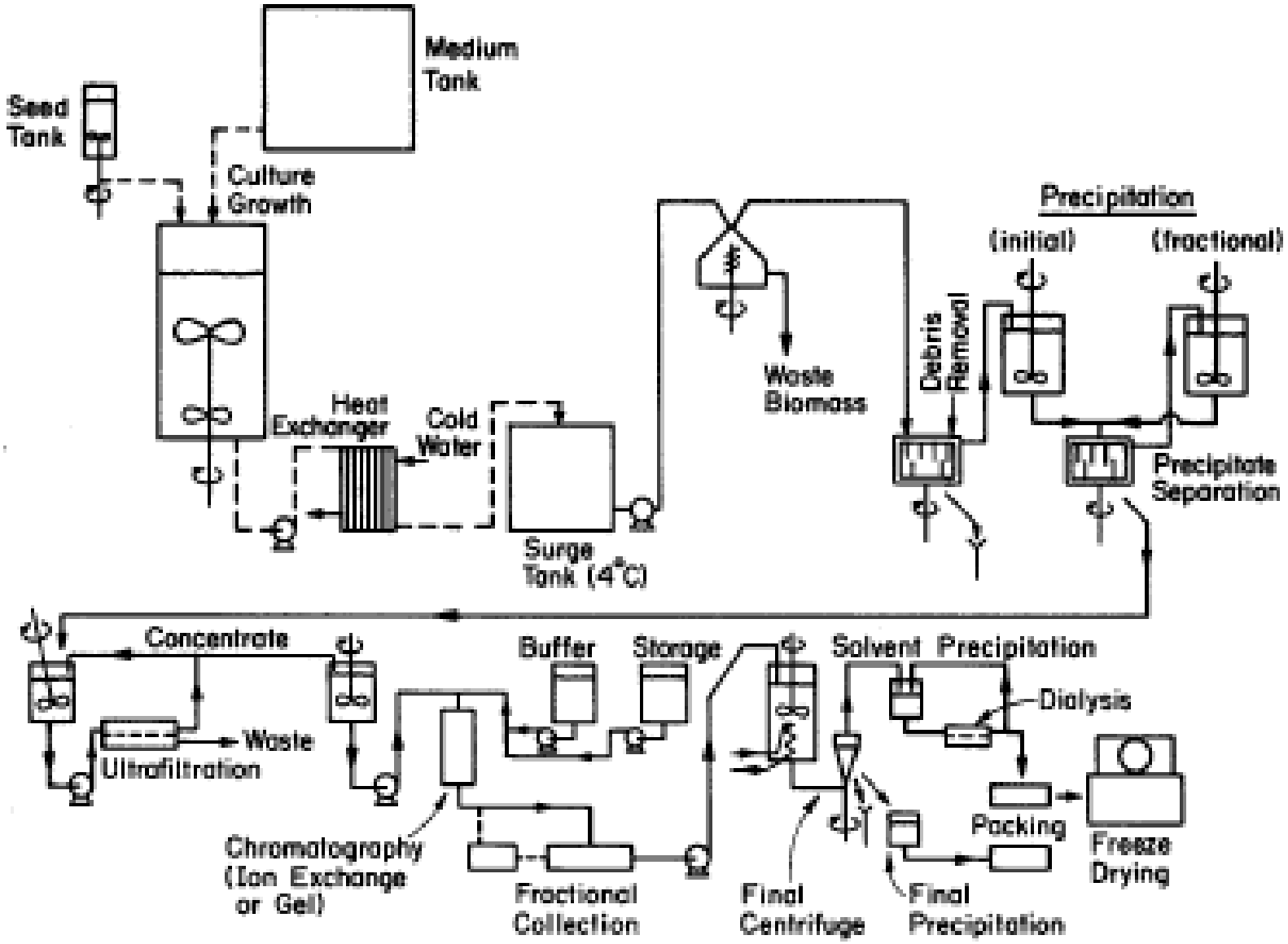


V_m per unit vol. of matrix

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

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

Production of industrial (extracellular) enzymes



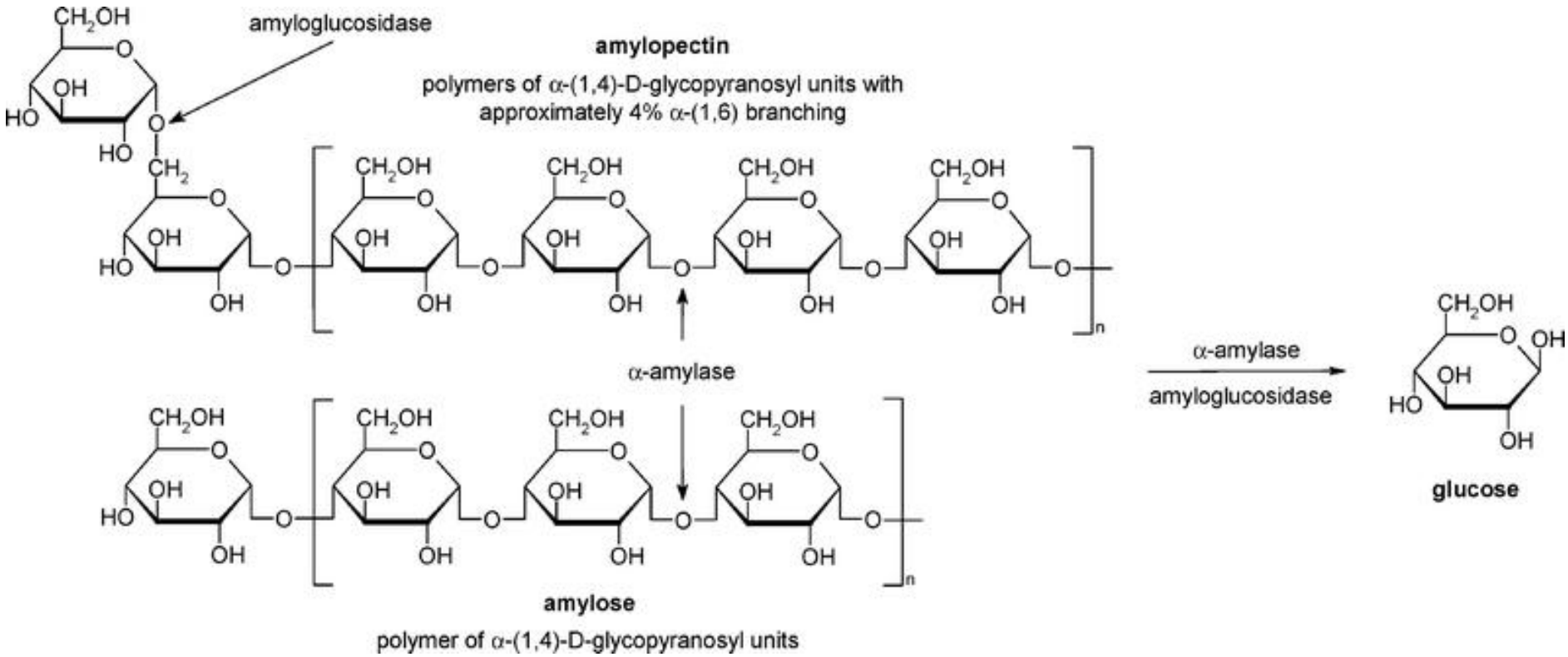
Major industrial enzymes

TABLE 3.6 Some Industrially Important Enzymes

Name	Example of 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/recombinant *E. coli*	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
Lignases	Fungal	Biopulping of wood for paper 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

Amylase

Used for starch (amylose + amylopectin) hydrolysis and glucose production



Medical use 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

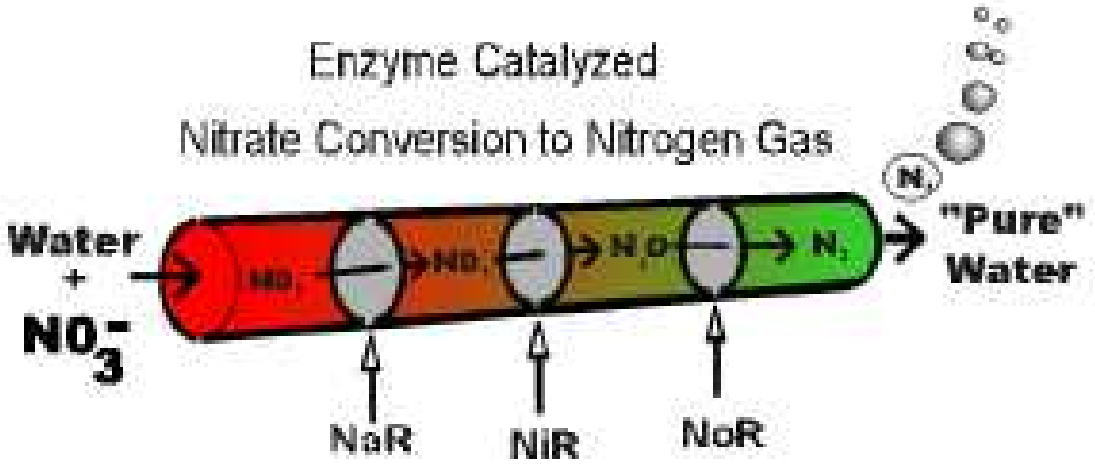
Asparaginase - an anticancer drug (cancer cells need asparagine)

Glucose oxidase - blood levels; glucose $\rightarrow$ gluconic acid + H_2O_2

Tissue Plasminogen Activator (TPA) - dissolves blood clots

Enzyme and biosensor

EzNET System for Eliminating Nitrate Pollution



Enzymes:

NaR=NitrateReductase; NiR=NitriteReductase

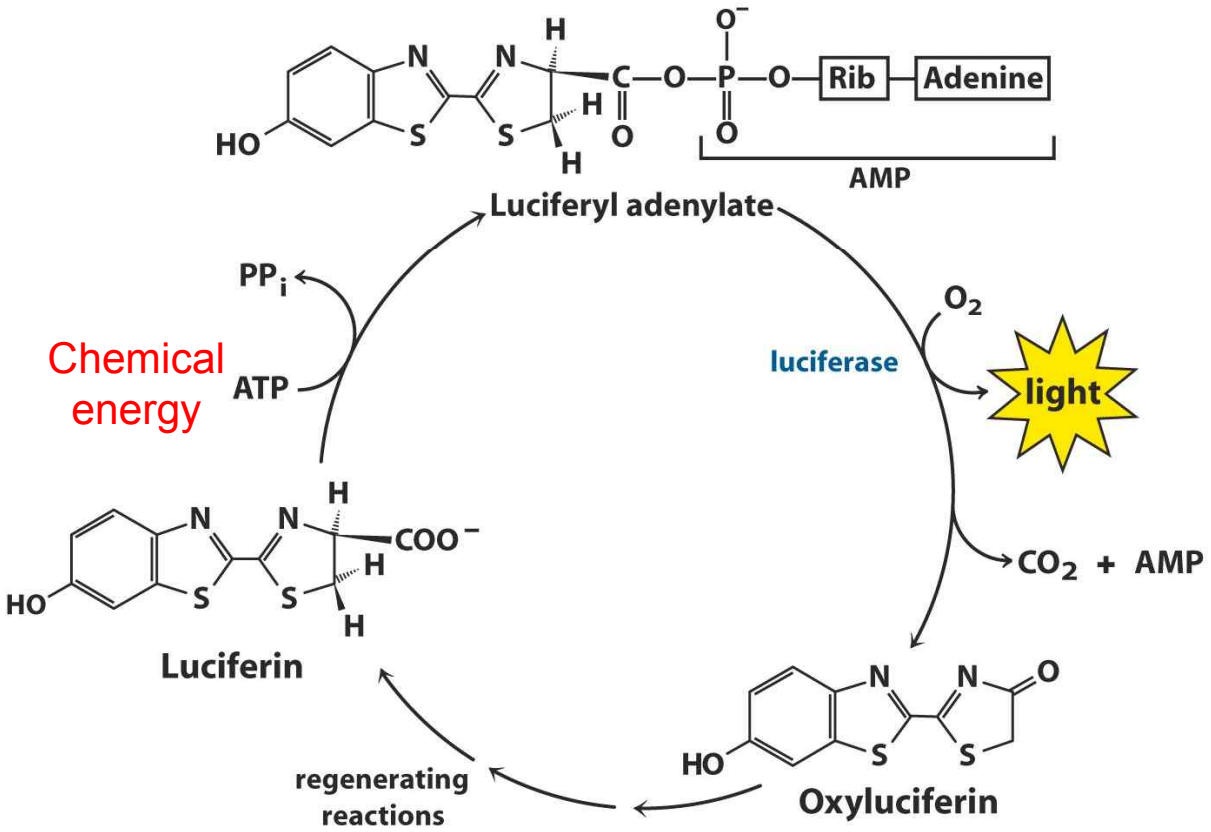
NoR=NitrousOxideReductase

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.

Bioluminescence of Firefly

- Conversion of chemical energy to light energy using ATP

firefly



Recombinant luciferase