Enzyme-immobilization

Enzyme Engineering

Purpose of enzyme immobilization

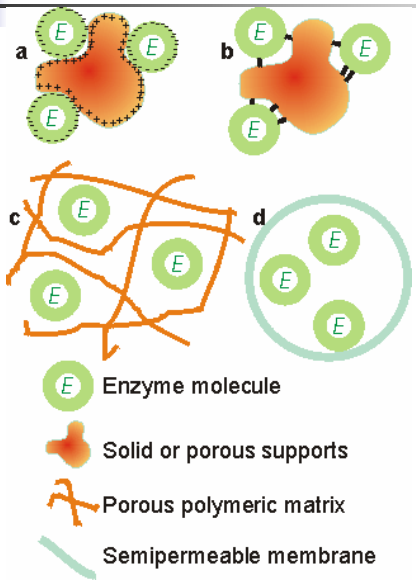
- Economic purpose
  - Enzymes can be reused
  - Recovery of product is easier
- Choice of immobilization

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-5O	Ionic binding	680	56.2
CM-Sephadex C-5O	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

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

Methods of immobilization



1. Absorption
2. Covalent linkage
3. Enzyme entrapment
4. Encapsulation of enzyme

1. Adsorption

- Simple method and high enzyme loading
- Incubating supporter with enzymes in an appropriate pH and ionic strength
- Driving force is hydrophobic intxn and salt bridge

% bound at	DEAE-Sephadex anion exchanger	CM-Sephadex cation exchanger
pH 2.5	0	100
pH 4.7	100	75
pH 7.0	100	34

Preparation of immobilised invertase by adsorption

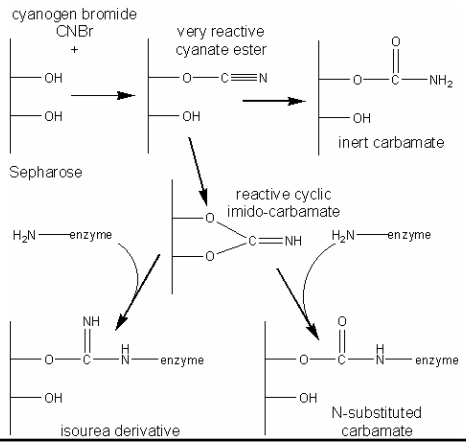
2. Covalent coupling

Residue	Content	Exposure	Reactivity	Stability of couple	Use
Aspartate	+	++	+	+	+
Arginine	+	++	-	±	-
Cysteine	-	±	++	-	-
Cystine	+	-	±	±	-
Glutamate	+	++	+	+	+
Histidine	±	++	+	+	+
Lysine	++	++	++	++	++
Methionine	-	-	±	-	-
Serine	++	+	±	+	±
Threonine	++	±	±	+	±
Tryptophan	-	-	-	±	-
Tyrosine	+	-	+	_+	+
C terminus	-	++	+	+	+
N terminus	-	++	++	++	+
Carbohydrate	- ~ ++	++	+	+	±
Others	- ~ ++	-	-	- ~ ++	-

2. Covalent coupling

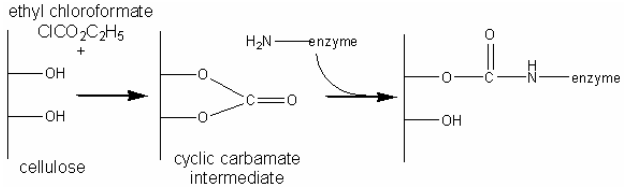
- Maximum 0.2 g enzyme/g matrix
- Very little leakage
- The most common methods in laboratory scale

1. CNBr method
   CNBr– activated Sepharose

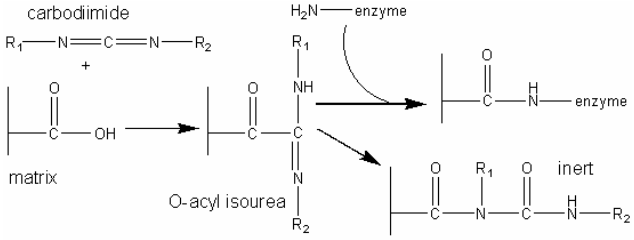


2. Covalent coupling

2. ethyl chloroformate : less toxic than CNBr

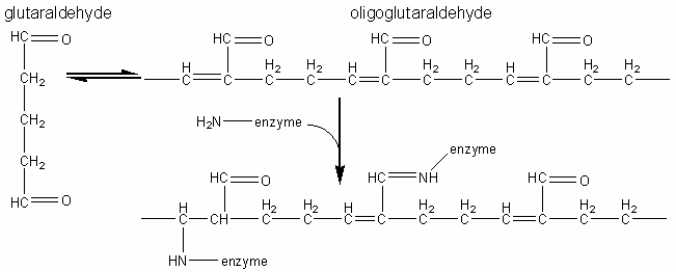


3. carbodiimide



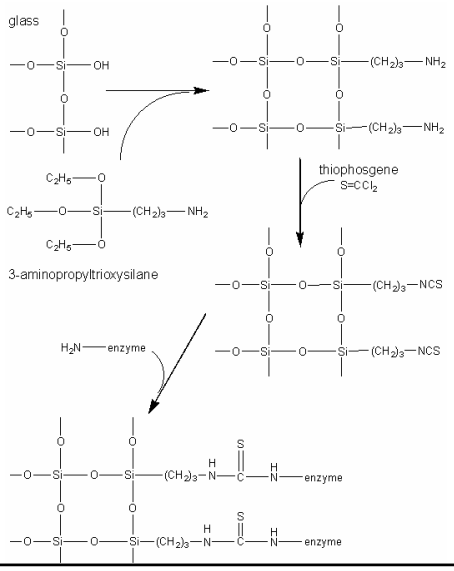
2. Covalent coupling

4. glutaraldehyde



2. Covalent coupling

5. 3-aminopropyltriethoxysilane



3. Entrapment

- More than 1 g enzyme/ 1 g gel
- Only for small substrate and product
- Enzyme can be covalently linked to acrylamide gel

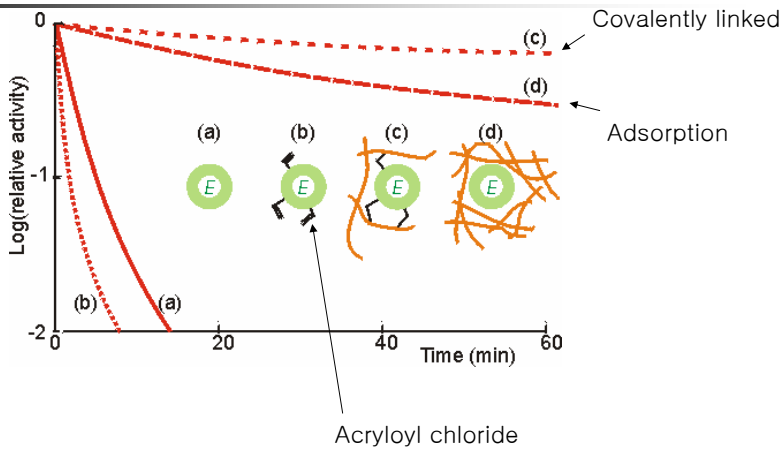
4. Membrane confinement

- Hollow fiber type of membrane can be used
- Although expensive, easy to use and convenient for any kinds of enzymes
- Liposome can be used for this purpose

Comparison between the methods

Characteristics	Adsorption	Covalent binding	Entrapment	Membrane confinement
Preparation	Simple	Difficult	Difficult	Simple
Cost	Low	High	Moderate	High
Binding force	Variable	Strong	Weak	Strong
Enzyme leakage	Yes	No	Yes	No
Applicability	Wide	Selective	Wide	Very wide
Running Problems	High	Low	High	High
Matrix effects	Yes	Yes	Yes	No
Large diffusional barriers	No	No	Yes	Yes
Microbial protection	No	No	Yes	Yes

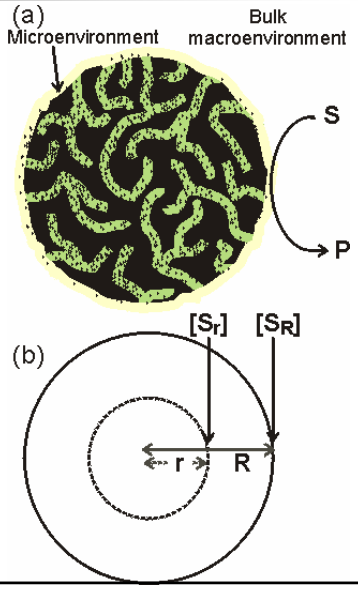
Immobilization enhances the stability of the enzyme



Activities of the chymotrypsin at 60 °C

Kinetics of immobilized enzyme

- Km and Vmax are changed
- Specificity can be changed (trypsin hydrolyze pepsinogen into 15 fragments in solution, but into 10 as immobilized form)



Kinetics of immobilized enzyme in nonporous solid support

- Assuming steady state,

$$J_s = k_L([S_0]-[S]) = \frac{V_{\max}[S]}{K_m+[S]}$$

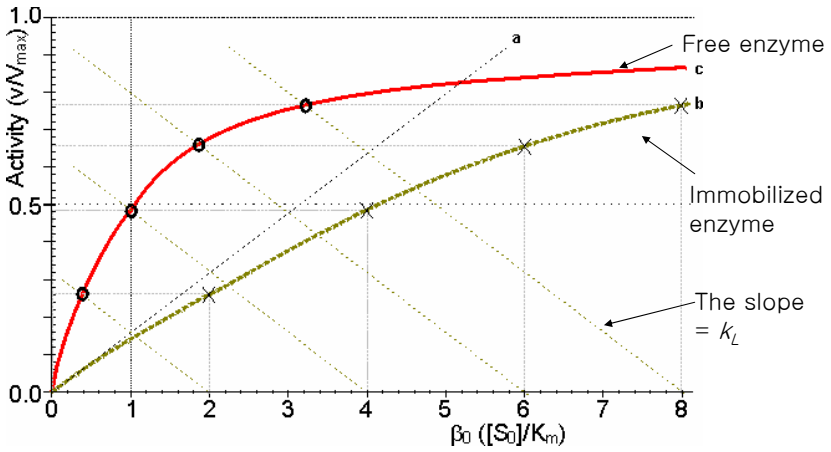
Michaelis-Menten eqn is defined in moles per unit time per unit area

- Defining dimensionless Damköhler number

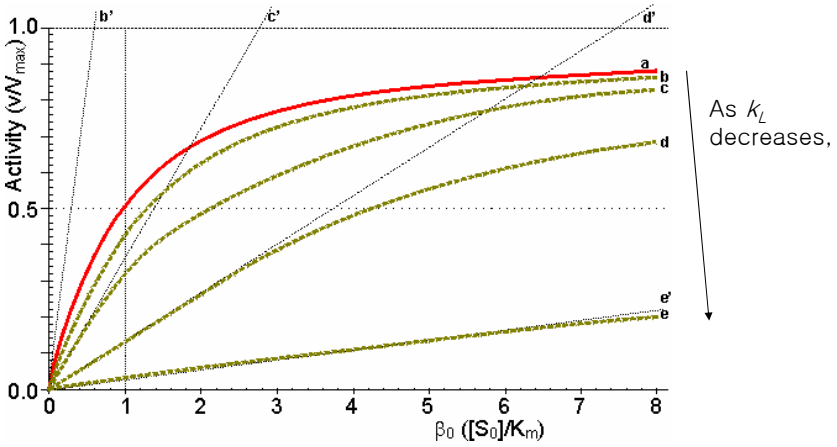
$$Da = \frac{V_{\max}}{k_L[S_0]}$$

Maximum reaction rate/maximum diffusion rate
If Da>>1, diffusion rare is limiting
If Da<<1, reaction rate is limiting

Kinetics of immobilized enzyme in nonporous solid support



Kinetics of immobilized enzyme in nonporous solid support



Kinetics of immobilized enzyme in porous matrix

- Assuming steady state,

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

Assuming that no external diffusion limitation

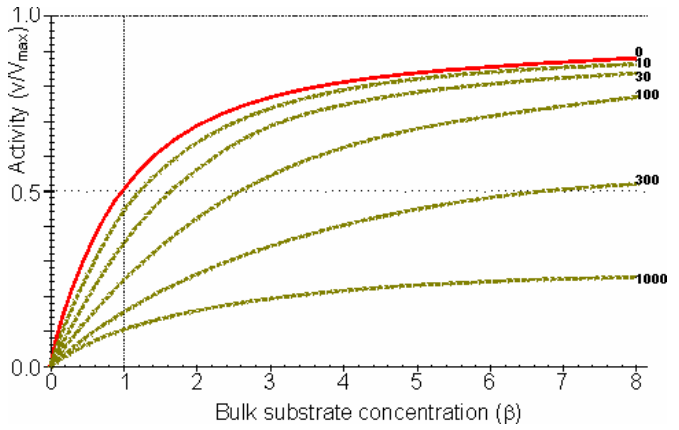
Michaelis-Menten eqn is defined in moles per unit time per unit volume

- Defining dimensionless numbers

$$\frac{d^2\bar{S}}{d\bar{r}^2} + \frac{2}{\bar{r}}\frac{d\bar{S}}{d\bar{r}} = \frac{R^2 V_m}{[S_0]D_e}\frac{\bar{S}}{\beta + \bar{S}} = \phi^2\frac{\bar{S}}{\beta + \bar{S}}$$

Where $\bar{S} = \frac{[S]}{[S_0]}, \bar{r} = \frac{r}{R}, \beta = \frac{K_m}{[S_0]}$

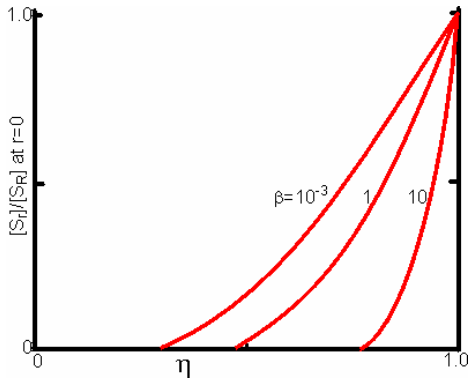
Kinetics of immobilized enzyme in porous matrix



As ϕ increases,

Kinetics of immobilized enzyme in porous matrix

η (effectiveness factor) is defined as the rate with diffusion limitation versus the rate w/o diffusion limitation



Kinetics of immobilized enzyme in porous matrix