Immobilized Enzymes

Basics:

- What is an Enzyme????
- What is substrate???
- How Substrate and Enzyme interaction takes place???
- What are applications of these Enzymes???
- Why do we need to do immobilization of Enzymes???

Introduction:

- **Immobilization** is the process of confining or restricting the mobility of enzyme in a fixed space like support or matrix
- That is restricting the movement of enzymes in the free space
- The support or matrix allows the exchange of substrate or promoter or inhibitor via diffusion
- Immobilization increases the stability of the enzyme

Advantages of enzyme immobilization:

- Increase in the stability of enzyme
- Recyclable
- Increase in product purity
- Can be used in continuous process
- Applicable in industrial and medical use
- Minimize effluent and related disposal problems

Applications of immobilized enzyme system:

- Industry: antibiotics, beverages, amino acids etc.
- Biomedical: treatment, diagnosis and drug delivery
- Food industry: production of concentrated syrups
- Waste water management: sewage and industrial effluents

Industrial applications of immobilized enzymes:

Enzyme (and microorganism)	*Application*
Aminoacylase	Optical resolution of DL-amino acids (commercial synthesis of L-amino acids)
Glucose isomerase	Isomerization of glucose to fructose (production of high fructose corn syrup; 8 million tons)
Penicillin amidase	Production of 6-aminopenicilloic acid; 7500 tons (manufacture of semisynthetic antibiotics)
β-Galactosidase	Hydrolysis of lactose to galactose and glucose (treatment of milk and whey)
Lipase	Interesterification of fats
Nitrile hydratase	Production of acrylamide from acrylonitrile; 15,000 tons
L-Aspartate β-decarboxylase (*Pseudomonas dacunhae*)	Production of L-alanine
Aspartase (*Escherichia coli*)	Production of L-aspartic acid
Fumarase (*Brevibacterium ammoniagenes*)	Production of L-malic acid
Aspartic amino transferase (*Escherichia coli*)	Production of L-phenylalanine

Douglas et al., 1995

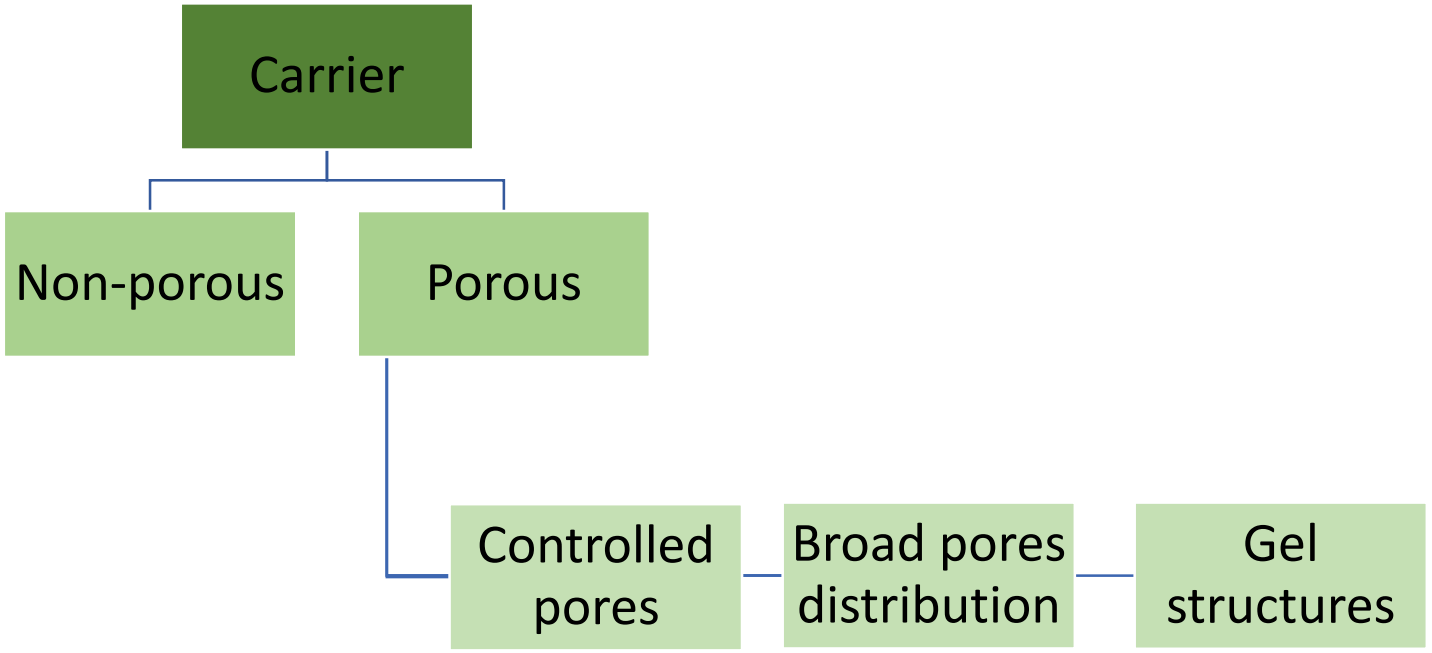
Industrial applications immobilized enzymes:

Enzyme type	Enzyme	applications
Oxidoreductase	catalase	sterilization of milk
	glucose oxidase	removal of glucose from food
	lipoxidase	bleach in white bread
	peroxidase	paper manufacturing
Hydrolase	α and β amylase	brewing
	cellulase	wine making
	glucoamylase	starch processing
	penicillin amidase	antibiotics
	keratinase	leather manufacturing
Lyase	fumerate hydratase	malic acid
Isomerase	glucose isomerase	fructose syrup production

Classification of solid matrices:

- **Organic:**
  - Natural Polymer:
    - Polysaccharides: cellulose, dextran's, agar, agarose, chitin, alginate
    - Proteins: collagen, albumin
    - Carbon
  - Synthetic Polymer:
    - Polystyrene
    - Other polymers: poly acrylate polymethacrylates, polyacrylamide, polyamides, vinyl, and allyl-polymers
- **Inorganic:**
  - Natural: bentonite, silica
  - Processed: glass, metals, controlled pore metal oxides

Carrier/ matrics morphology and configuration:



Non-porous carriers:

- **Advantages:**
  - Reduced diffusion effects that is substrate in solution can be reacted with the enzymes with reduced difficulty

- **Disadvantages:**
  - Availability of less surface area

Porous carriers:

- **Advantages:**
  - Availability of high surface area for immobilization
  - Internal surface bonding protects enzymes from the turbulence of external environment
  - A charged surface opposite to that of substrate may enhance the enzyme substrate reaction by attracting the substrate
  - Large enzymes/ cells loading is possible (since more surface area more loading is possible)

- **Disadvantages:**
  - Larger enzymes or substrate can not penetrate the smaller pores
  - If the surface charge will be same as substrate then substrate may never contact with enzyme
  - Broad pore distribution may reduce substrate enzyme interaction
  - Entrapped or encapsulated enzymes may get sever limitations by diffusional effects

Desired characteristics of solid matrix/ carriers:

- **Chemical Durability** with respect to reactor environment
- **High available surface area** for enzyme attachment
- **Mechanical strength and dimensional stability** to protect enzyme structure
- **Microbial resistant** to avoid destruction of enzyme and the carrier
- **Thermal stability** of the carrier
- **Carrier shape and size**
- **Regeneration** of carrier

Properties suggesting carrier applications:

Carrier	General application	Conditions suggesting type of application
Collagen, cellulose acetate	Polymer hydrolysis and collection of product free of substrate	Membrane to separate substrate and products (protein hydrolysis)
Controlled pore glass or silica	Continuous plug flow reactors at pH below 7.0	Dimensional stability, low pressure drops, durable below 7.0
Controlled pore alumina	Continuous plug flow reactors at pH 5–11	Dimensional stability, low pressure drop, durable above pH 5.0
Controlled pore titania	Continuous plug flow reactors at pH 3–9	Dimensional stability, low pressure drops, durable at pH 3–9

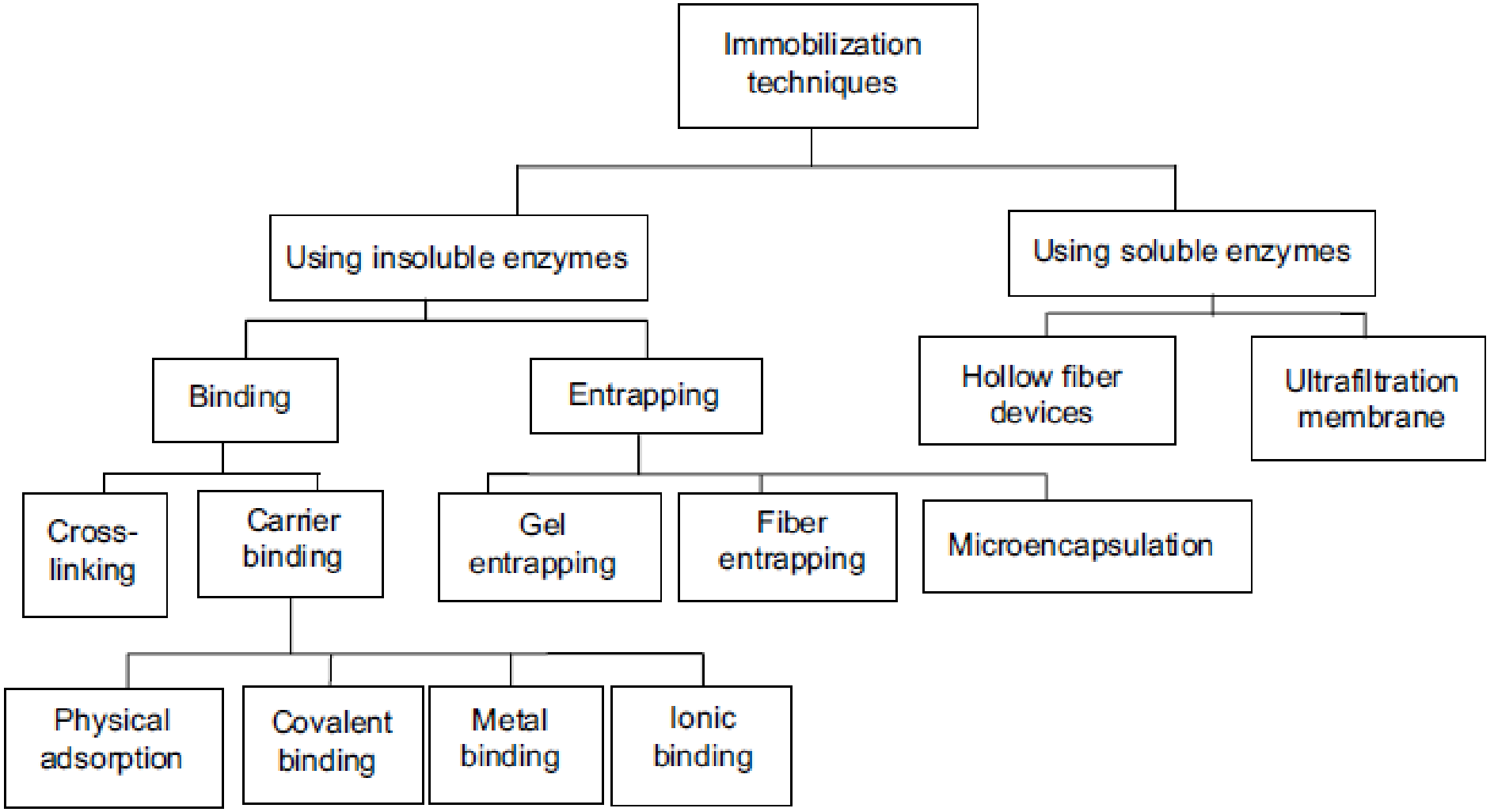
Carrier optimization:

- Pore diameter

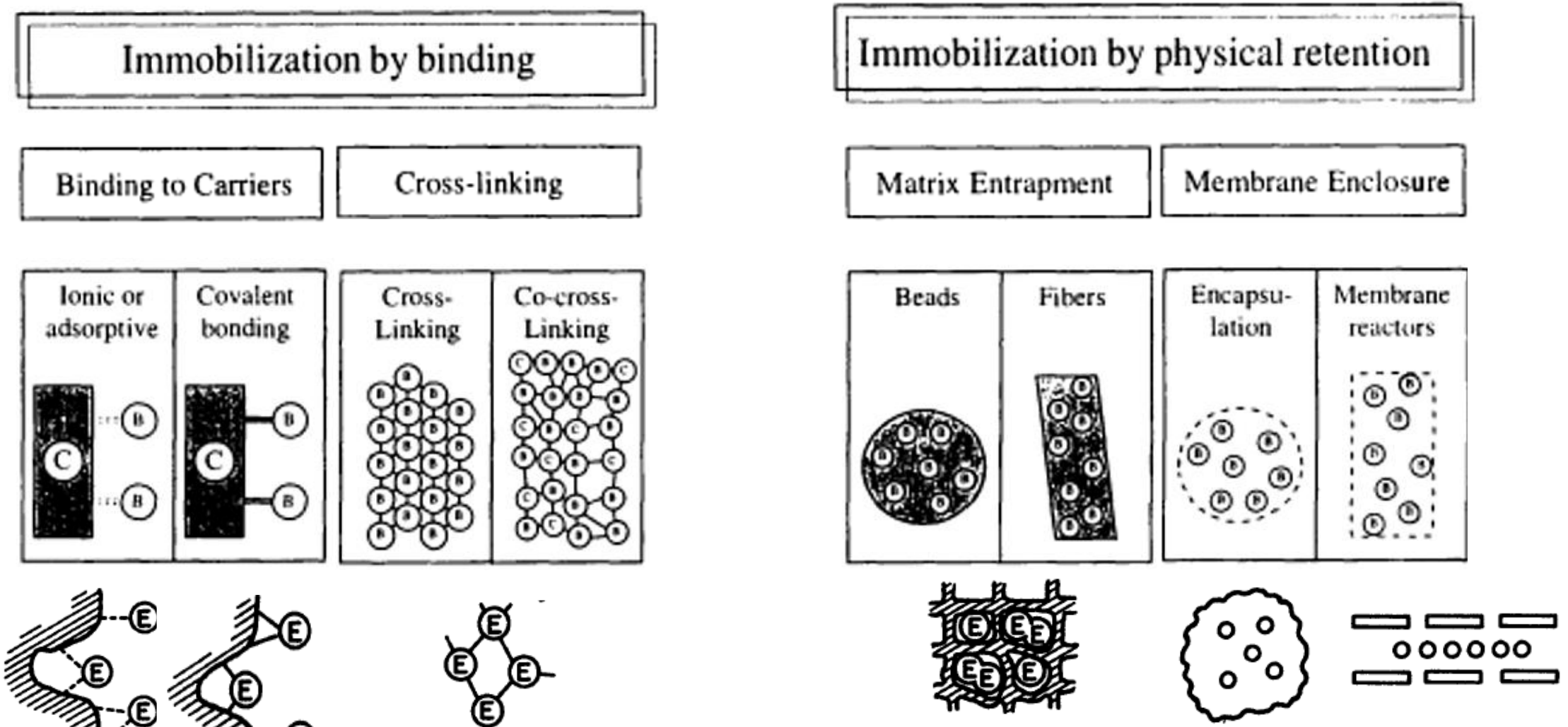
- If the major dimension of enzyme unit cell > major dimension of substrate, then pore diameter should be chosen wrt enzyme diameter

- If the major dimension of enzyme unit cell < major dimension of substrate, then pore diameter should be chosen wrt substrate diameter

Immobilization Techniques:



Immobilization Techniques:

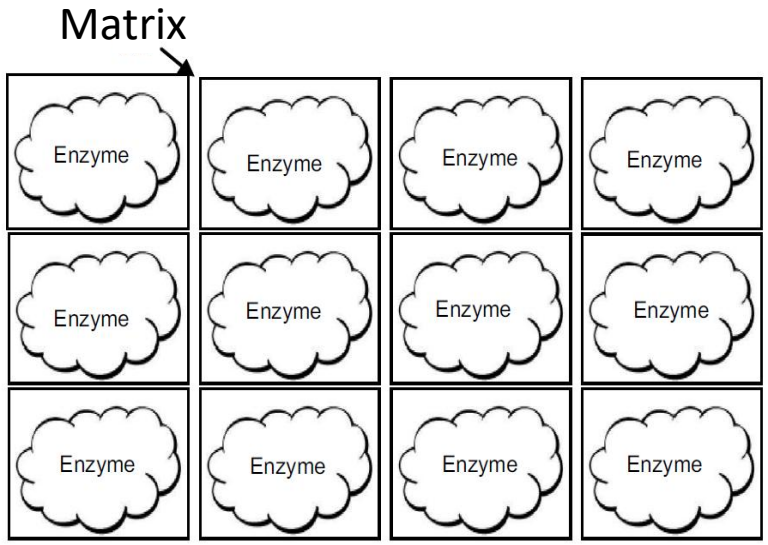


Matrix Entrapment :

- Enzyme solution is mixed with polymetric fluid and then solidifies into various forms depending on its applications (usually small beads)

- The polymetric material is semipermeable. The large molecular weight enzyme can not diffuse out but smaller substrate and product molecule can.

- Matrixes for entrapment: Ca-alginate, agar, k-carrageenin, polyacrylamide and collagen



Matrix Entrapment :

- **Methods of Entrapment:**

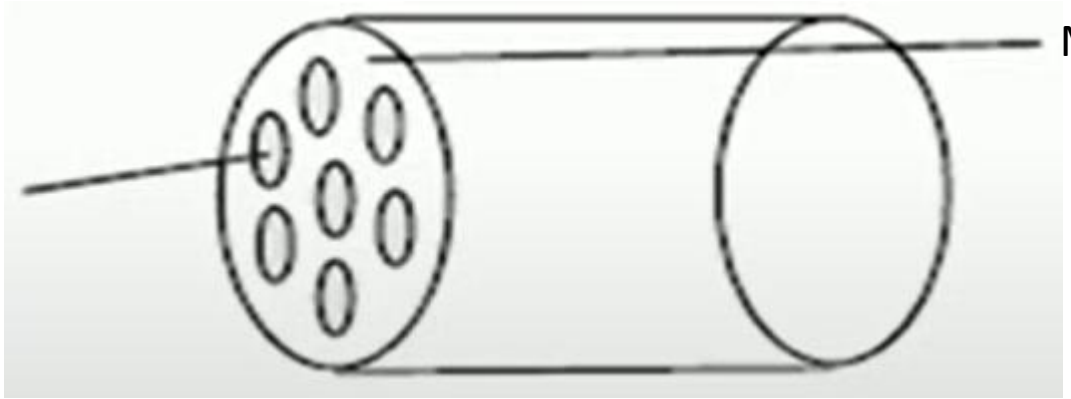
  - Inclusion in gel: enzymes get trapped inside the gel
  - Inclusion in fiber: enzymes supported on matrix made of fiber material
  - Inclusion in microcapsules: enzymes entrapment in microcapsules formed by monomer mixture such as polyamide or calcium alginate

Membrane Entrapment :

- Hollow fiber units have been used to entrap an enzyme solution between thin semi permeable membrane

- Material used in membranes: nylon, cellulose, polysulfone, polyacrylate

Hollow fibers containing stationery enzyme solution

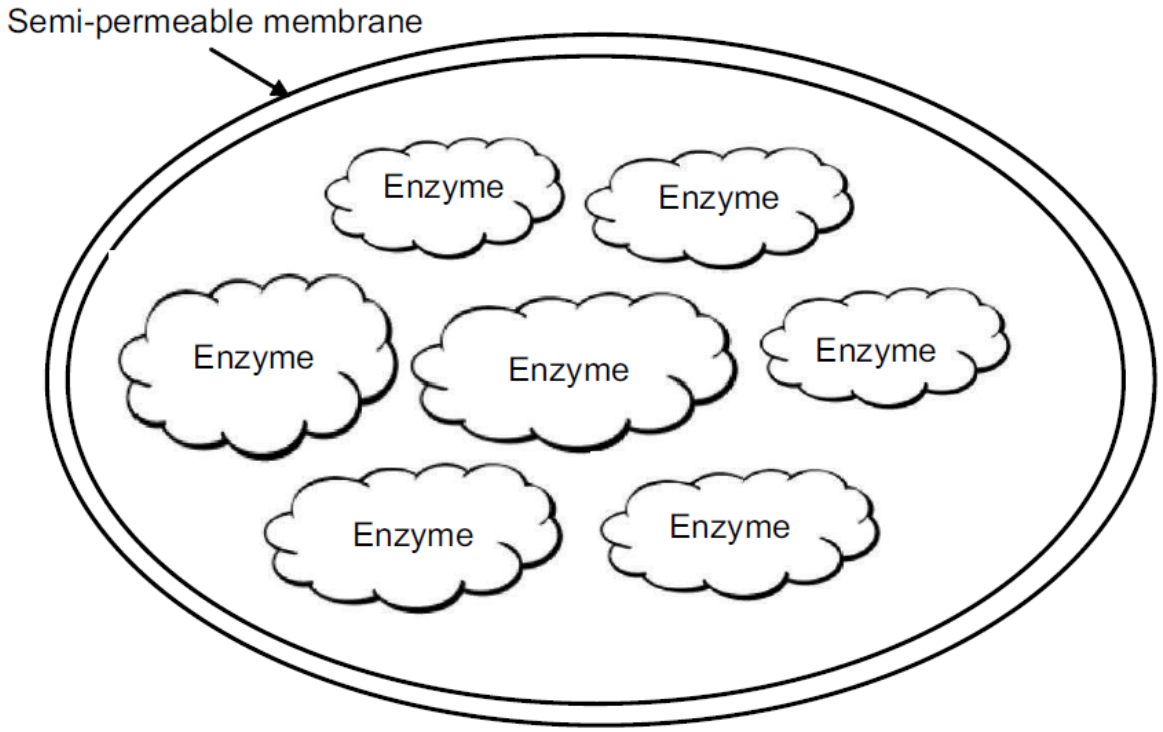


Mobile fluid outside fiber tubes containing substrate and product

Microencapsulation:

- Encapsulation is done by enclosing the enzymes in a membrane capsules.

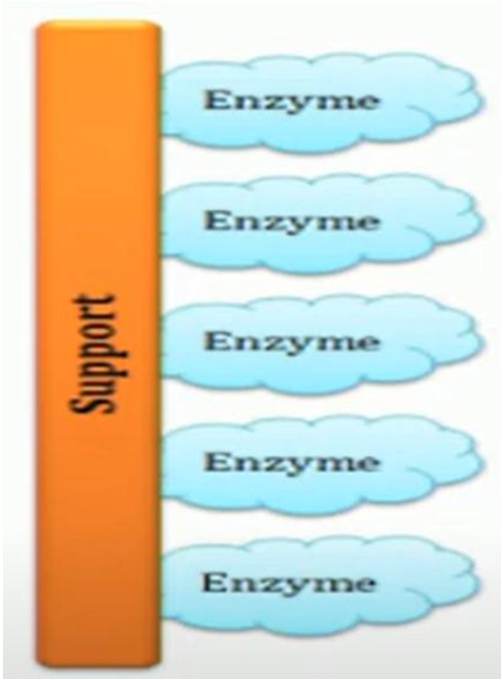
- Effectiveness depends on the stability of enzymes inside the capsules.



Entrapment Technique:

- **Advantages:**
  - Highly stable
  - Easy handling
  - No chemical modification
- **Disadvantages:**
  - Enzyme deactivation may be possible
  - Leakage of enzyme through large size pores

Adsorption Technique:



- Adsorption technique is a physical phenomena.
- The attachment of enzymes on support occurs by weak van der Waals force.
- Active site of enzyme should not take part in the immobilization attachment.
- Desorption of enzyme is a common problem in the presence of hydrodynamic force, since binding forces are very weak.
- Solid support materials: alumina, silica, porous glass, ceramics, diatomaceous earth, clay and bentonite, starch, activated carbon, ion exchange resins(amberlite)

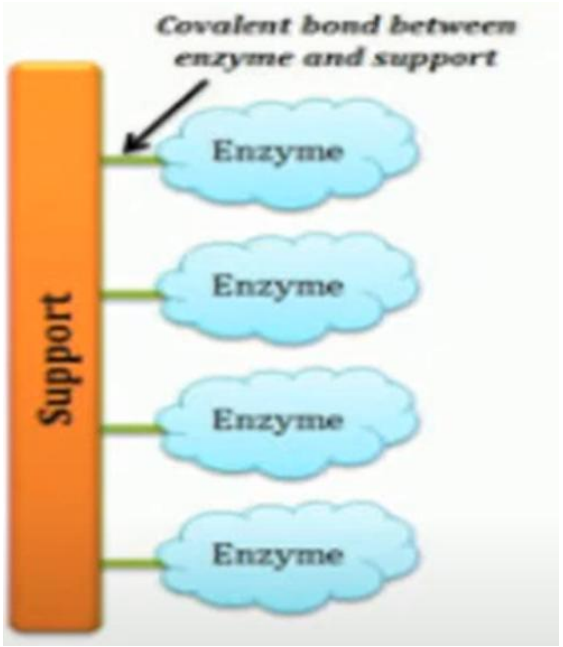
Adsorption Technique:

- **Advantages:**
  - Simple and economical
  - Limited loss of enzyme
  - Reversible process
- **Disadvantages:**
  - Weak binding
  - Low surface area for binding
  - Sensitive to pH and temperature

Covalent Bonding:

- Enzyme attachment on the support surface occurs by covalent bond formation between functional groups of enzyme and those on support surface.

- Different functional groups of support material:
  - Amino
  - Carboxyl
  - Hydroxyl
  - Sulfhydryl

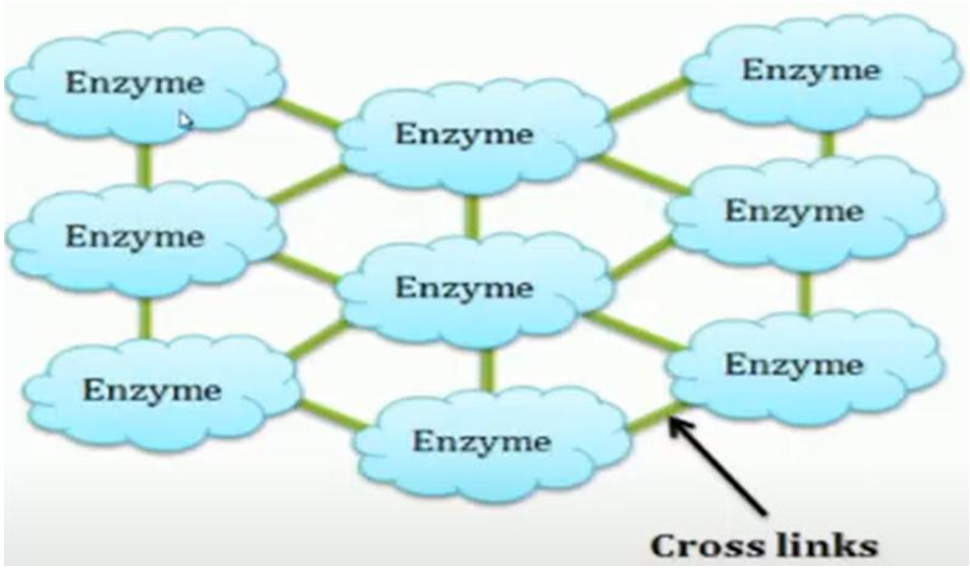


Covalent Bonding:

- **Advantages:**
  - Highly stable
  - No leakage
  - Wide range of carrier matrix available
- **Disadvantages:**
  - Costly and complicated
  - Low enzyme activity
  - High risk of modification of active sites

Cross linking:

- Enzymes are directly linked by covalent bonds between various groups
- There is no involvement of matrix or support unlike other methods
- Cheap but not used generally with pure enzymes



Cross linking:

- **Advantages:**
  - No leakage
  - Highest stability
  - Strongly bound
  - No hinderance to active sites
- **Disadvantages:**
  - Complicated and expensive
  - Less possibility of active site modification

Comparison of different immobilization techniques:

Properties	Adsorption	Covalent binding	Entrapment	Membrane encapsulation
Binding force	Variable	Strong	Weak	Strong
Cost	Low	High	Moderate	High
Preparation	Simple	Difficult	Difficult	Simple
Enzyme leakage	Yes	No	Yes	No
Applicability	Wide	Selective	Wide	Very wide
Operational problems	High	Low	High	High
Effect of the solid matrix	Yes	Yes	Yes	No
Diffusional problem	No	No	Yes	Yes
Microbial protection	No	No	Yes	Yes

Characterization of immobilized enzymes:

❖ Activity:

- Measured in international units (IU)
- IU is the macromolecules of substrate converted per min per gram of immobilized enzymes

Activity is sensitive to:

- Initial substrate concentration
- Concentration of immobilized enzymes
- Temperature
- pH
- Reaction time
- Agitation
- Flowrate
- Physical dimensions of the carrier

Characterization of immobilized enzymes:

❖ Bound protein:

- Amount of protein bound to the carrier
- Unit: mg of protein per gram of carrier or solid matrix

❖ Specific activity of bound protein:

- Micro molecules of substrate converted per min per mg of bound protein
- Provides idea of effectiveness of the immobilization procedure

Characterization of immobilized enzymes:

❖ **Coupling yield:**

- An indicator of the activity of immobilized enzymes

$$\text{Coupling yield} = \frac{\text{Overall activity of immobilized enzyme}}{\text{Overall activity of the initial enzyme solution immoblized}} \times 100$$

Kinetics of enzyme catalyzed reactions using immobilized enzymes

Immobilized enzyme system:

❖ **Immobilized enzyme system includes:**

- Insoluble immobilized enzyme
- Soluble substrate
- Product

❖ **Mass transfer resistance is an important factor in immobilized enzyme system**

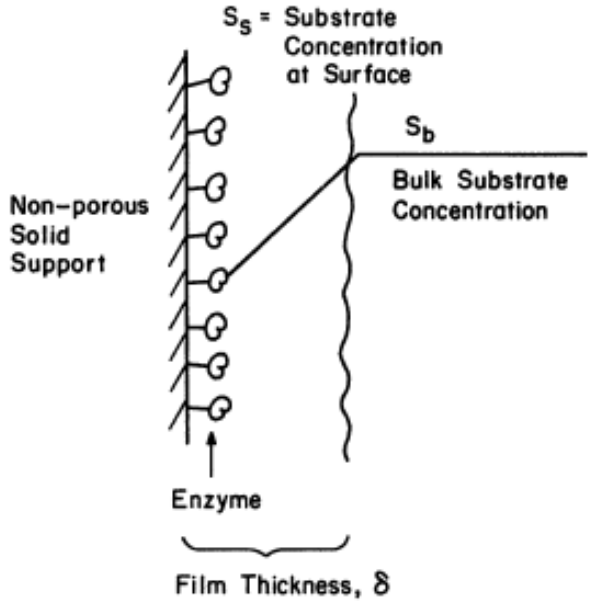
Mass transfer resistance occurs due to large particle size of immobilized systems or due to the inclusion of enzymes in polymetric matrix

❖**Mass transfer resistance is absent in the free enzyme system**

Transportation of substrate in Immobilized enzyme system:

❖**Different processes involved in heterogeneous reaction:**

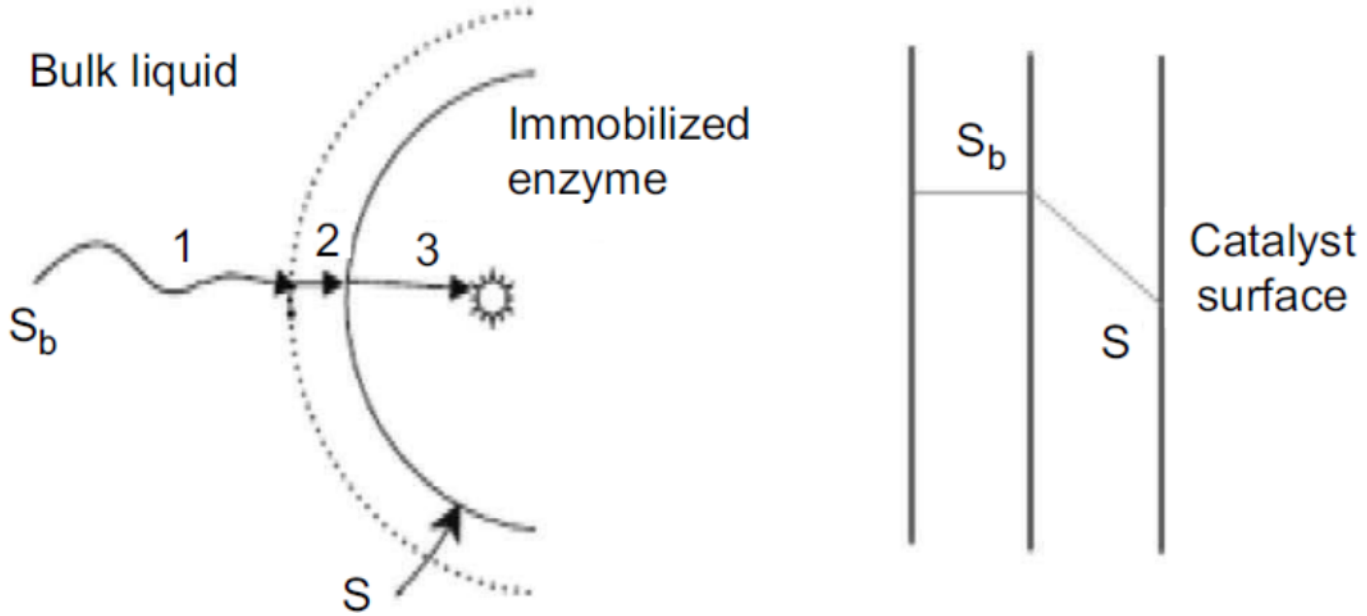
- Diffusion of substrate into the matrix
- Adsorption of substrate
- Reaction
- Desorption of product and unreacted substrate
- Diffusion of product and unreacted substrate



Transportation of substrate in Immobilized enzyme system:

❖**Hypothetical path of substrate from liquid to the reaction site in an immobilized reaction system:**

- Transfer from bulk liquid to relatively unmixed liquid layer surrounding the immobilized enzymes
- Diffusion through relatively unmixed liquid layer
- Diffusion from the surface of the particle to the active site of the enzyme in an inner support



Steps 1 and 2 are the external mass transfer resistance. Step 3 is the intra-particle/internal mass transfer resistance

Transportation of substrate in Immobilized enzyme system:

❖**The diffusion problem of the substrate used in the immobilization of an enzyme can be classified as**

- **External mass-transfer resistance:** In the transport of substrates towards the surface, and products away

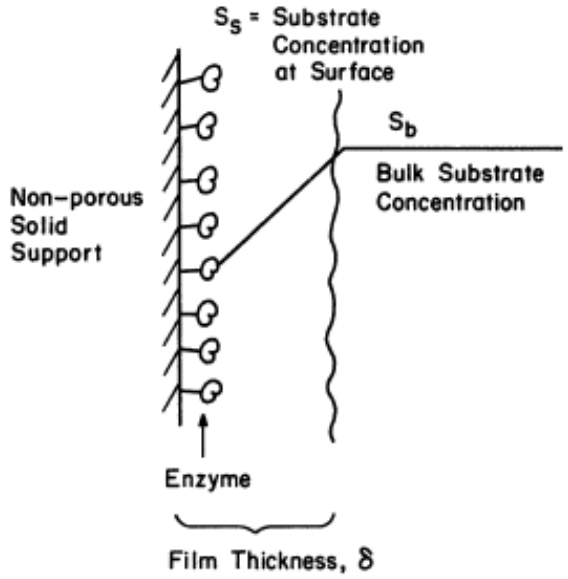
- **Internal mass-transfer resistance:** In the transport of the substrates and products, within the pores of immobilised enzyme particles

External mass transfer resistance:

Diffusion Effects in Surface-bound Enzymes on Nonporous Support Materials

- Enzyme are evenly distributed on the surface of a nonporous support material.

$$E+S \rightleftharpoons ES \xrightarrow{k_2} P + E$$



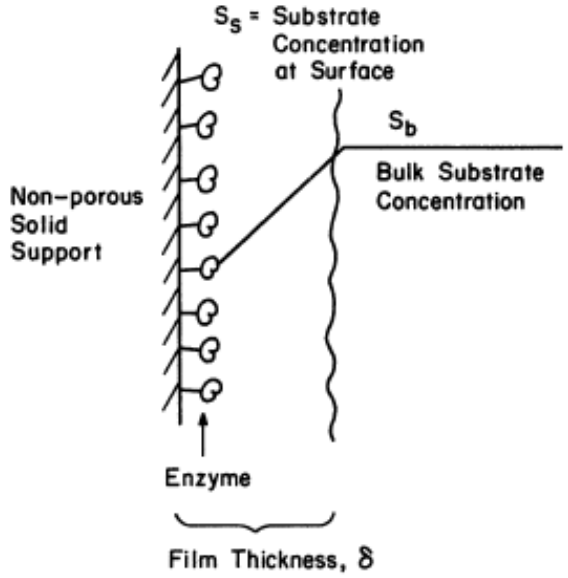
Assume the enzyme catalyzed reaction rate follows Michaelis-Menten type kinetics.

Ss: substrate concentration at the surface;
Sb: substrate concentration in bulk solution.

External mass transfer resistance:

Diffusion Effects in Surface-bound Enzymes on Nonporous Support Materials

- **Assume**
- Enzyme are evenly distributed on the surface of a nonporous support material.
- All enzyme molecules are equally active.
- Substrate diffuses through a thin liquid film surrounding the support surface to reach the reactive surface.
- The process of immobilization has not altered the enzyme structure and the intrinsic parameters (Vm, Km) are unaltered



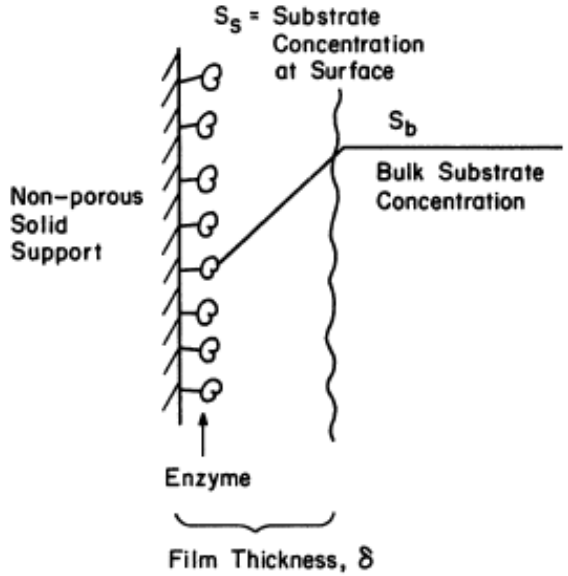
External mass transfer resistance:

Diffusion Effects in Surface-bound Enzymes on Nonporous Support Materials

The external diffusion rate J_S (g/cm²-s):

$$J_S = k_L([S_b]-[S_S])$$

k_L is the liquid mass transfer coefficient (cm/s),
Ss/Sb substrate concentration (gm/cm³)



If the product formation rate is :

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

V_m' the maximum reaction rate per unit surface area (g/cm²-s)

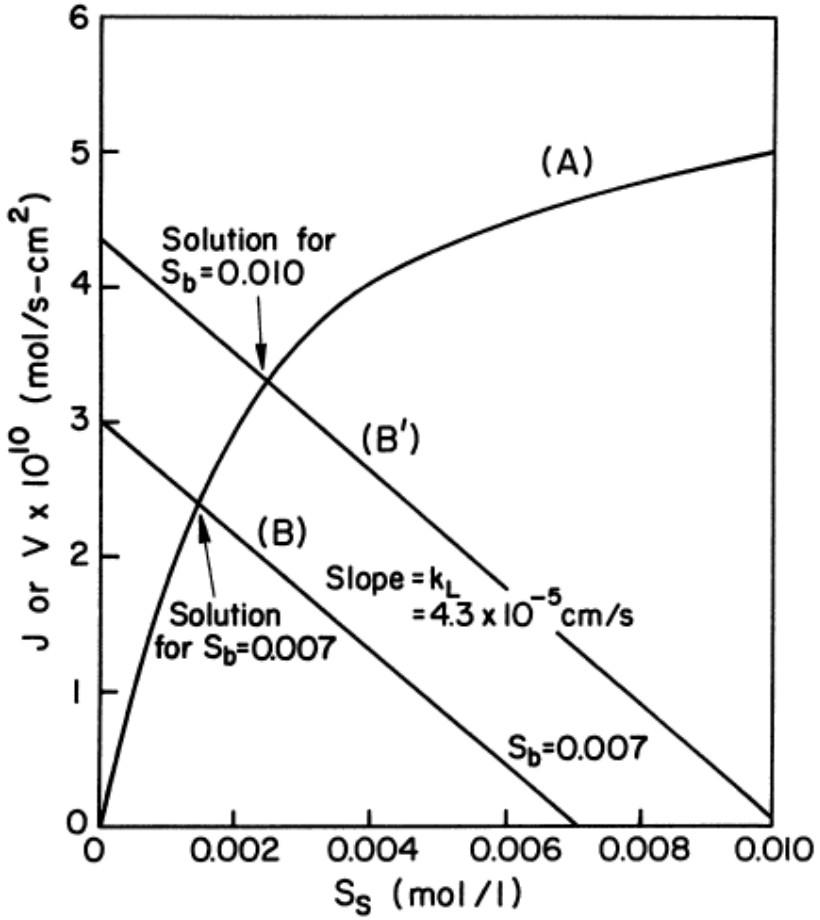
At steady state, the reaction rate is equal to the mass-transfer rate:

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

External mass transfer resistance:

Diffusion Effects in Surface-bound Enzymes on Nonporous Support Materials

- This equation is quadratic in [Ss], the substrate concentration at the surface.
- It can be solved analytically, but the solution is cumbersome.
- Curve A results from a knowledge of the intrinsic solution-based kinetic parameters and the surface loading of enzyme (right side of eq.).
- Line B is the mass transfer equation (left side of eq.).
- The intersection of the two lines is the reaction rate, v.
- The responses for two different bulk substrate concentrations are shown in figure.



Graphical solution for reaction rate per unit of surface area for enzyme immobilized on a non-porous support

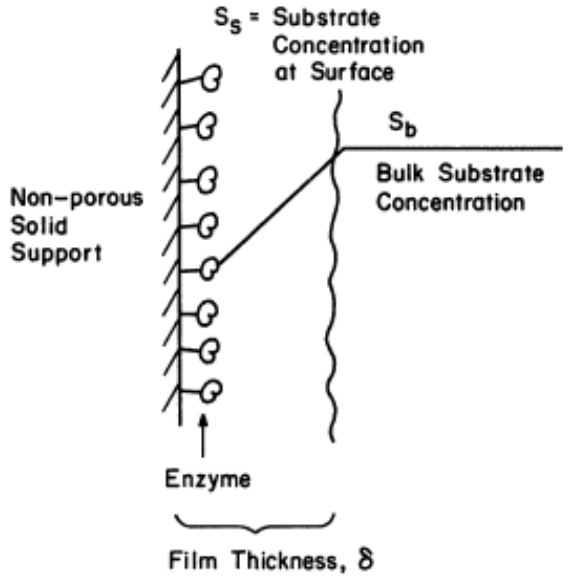
External mass transfer resistance:

Diffusion Effects in Surface-bound Enzymes on Nonporous Support Materials

When the system is strongly external diffusion (liquid film mass-transfer) limited, [Ss]≈0, the reaction is rapid compared to mass transfer

- The overall reaction rate is equal to the rate:

$$v = k_L[S_b]$$



The system behaves as pseudo first order.

The rate is a linear function of bulk substrate concentration.

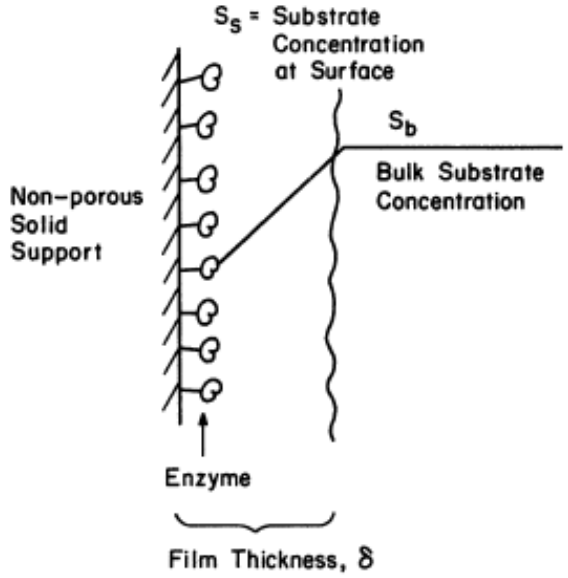
External mass transfer resistance:

Diffusion Effects in Surface-bound Enzymes on Nonporous Support Materials

When the system is strongly reaction limited, [Sb] ≈ [Ss]

the overall reaction rate is equal to the rate:

$$v = \frac{V_m'[S_b]}{K_{m,app} + [S_b]}$$



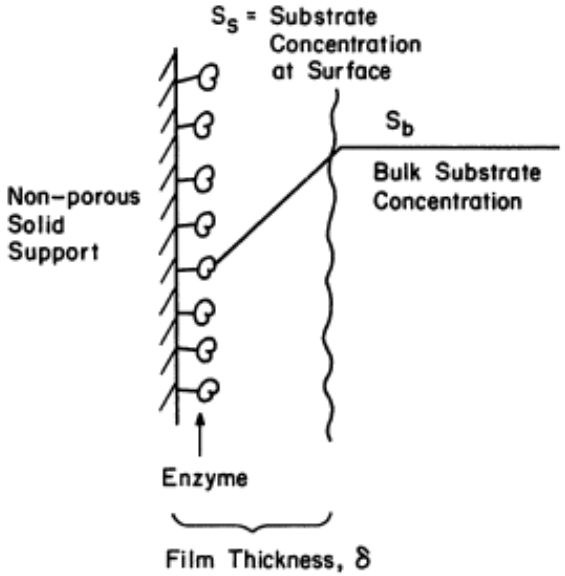
Where with appropriate assumptions,

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

$K_{m,app}$ is increased compared to Km.
It is a function of mixing speed and S_b and determined experimentally.

External mass transfer resistance: (Alternatively)

- In case the enzyme is immobilized only on the external surface of a solid matrix, then mass transport is to be considered from the bulk solution to the surface of the solid matrix and reaction occurs at that position. This is known as external mass-transfer resistance.

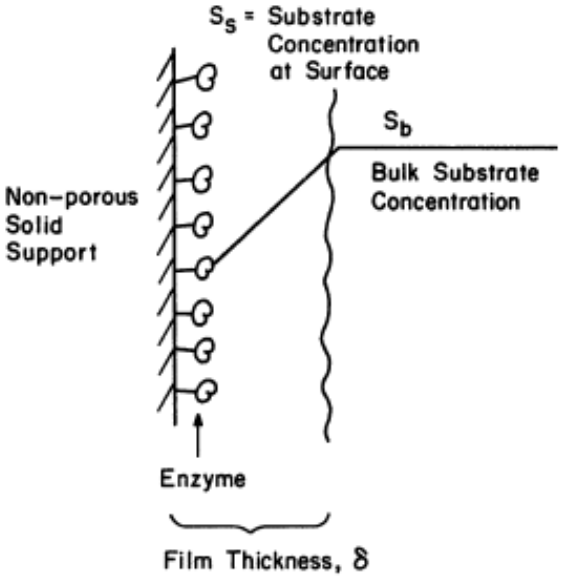


- The rate of mass transfer from the bulk solution to the surface is given by

$$N_S = k_a a_m (S_b - S_s)$$

- where ka is the mass-transfer coefficient,
  am is the surface area per unit volume,
  Sb is the bulk-phase substrate concentration,
  and Ss is the substrate concentration at the surface of the solid matrices.

External mass transfer resistance:



- The Michaelis–Menten equation may be written as

$$v = \frac{v_{max} S_s}{k_m + S_s}$$

- At steady-state (SS) conditions,

Mass-transfer rate = Rate of reaction at the surface of the solid matrix

where the internal diffusion limitations are not significant

$$k_a a_m (S_b - S_s) = \frac{v_{max} S_s}{k_m + S_s} = v$$

This shows the relationship between substrate concentration in the bulk of liquid solution and that at the surface of an immobilized enzymes

External mass transfer resistance:

- The may be written as

$$S_s = S_b - \frac{v}{k_a a_m} = \frac{(S_b k_a a_m - v)}{k_a a_m}$$

- From Michaelis-Menten equation

$$\frac{1}{v} = \frac{1}{v_{max}} + \frac{k_m}{v_{max}} \frac{1}{S_s}$$

- Using Ss value

$$\frac{1}{v} = \frac{1}{v_{max}} + \frac{k_m}{v_{max}} \frac{k_a a_m}{(S_b k_a a_m - v)}$$

$$\left[\frac{1}{v} = \frac{1}{v_{max}} + \left(\frac{k_m}{v_{max}} \right) \left(\frac{1}{S_b} \right) \frac{1}{\left(1 - \frac{v}{S_b k_a a_m} \right)} \right]$$

- Observed kinetics is different from intrinsic kinetics because of mass transfer limitations

Effectiveness factor and Damköhler number:

- The influence of mass transfer on the overall reaction process is expressed using the effectiveness factor

$$\eta = \frac{\text{Observed rate of reaction}}{\text{Rate of reaction in case of no mass-transfer resistance, i.e., } S_s = S_b}$$

- The observed reaction rate using Michaelis–Menten equation may be written as:

$$v_{obs} = \frac{v_{max} S_s}{k_m + S_s}$$

- The reaction rate with no mass-transfer limitation is

$$v_{S_s \to S_b} = \frac{v_{max} S_b}{k_m + S_b}$$

- Then,

$$\eta = \frac{v_{obs}}{v_{S_s \to S_b}}$$

Effectiveness factor and Damköhler number:

$$\eta = \frac{v_{max} S_s (k_m + S_b)}{(k_m + S_s) v_{max} S_b} \qquad \eta = \frac{S_s (k_m + S_b)}{S_b (k_m + S_s)}$$

- Using dimensionless parameters,

$$x = \frac{S_s}{S_b} \qquad K = \frac{k_m}{S_b}$$

- We get,

$$\eta = \frac{x/(k+x)}{1/(k+1)} = \frac{x(k+1)}{(k+x)}$$

- The Damköhler number (Da) is a dimensionless number and may be represented as

$$Da = \frac{\text{Maximum rate of reaction}}{\text{Maximum rate of mass transfer}} = \frac{v_{max}}{k_m a_m S_b}$$

Effectiveness factor and Damköhler number correlation:

- At steady state condition,

$$k_a a_m (S_b - S_s) = \frac{v_{max} S_s}{k_m + S_s}$$

$$k_a a_m S_b \left(1 - \frac{S_s}{S_b}\right) = \frac{v_{max} \frac{S_s}{S_b}}{\frac{k_m}{S_b} + \frac{S_s}{S_b}}$$

- Using, $x = \frac{S_s}{S_b}$ $K = \frac{k_m}{S_b}$

$$\frac{1-x}{Da} = \frac{x}{K+x}$$

- We know that,

$$\eta = \frac{x/(k+x)}{1/(k+1)} = \frac{x(k+1)}{(k+x)}$$

$$\eta = \frac{x}{(K+x)}(k+1) = \frac{(1-x)(K+1)}{Da}$$

$$\frac{1-x}{Da} = \frac{\eta}{k+1}$$

Significance of effectiveness factor:

- Here, if $\eta < 1$ indicates the effect of increasing mass-transfer resistance and reduction in observed activity.
- Actual rate of reaction will be less as compared to whatever substrate is available for the reaction

- Here, if X =1, surface concentration will be equal to bulk concentration.
- Then, $\eta = 1$ which indicates there is no mass transfer limitation.
- This means that a heterogeneous system will become a homogeneous system.

- Here, if X ~ 0, then the mass transfer rate is very slow compared to the reaction rate.

$$\eta = \frac{1+k}{Da}$$

Significance of Damköhler number :

- When **Da >> 1**, then the reaction rate is much greater than the mass transfer rate and the overall reaction is controlled by the mass transfer rate (X ~ 0)

- So the reaction kinetics may be represented as, $\bar{v} = k_m a_m S_b$ $\eta = \frac{1+k}{Da}$

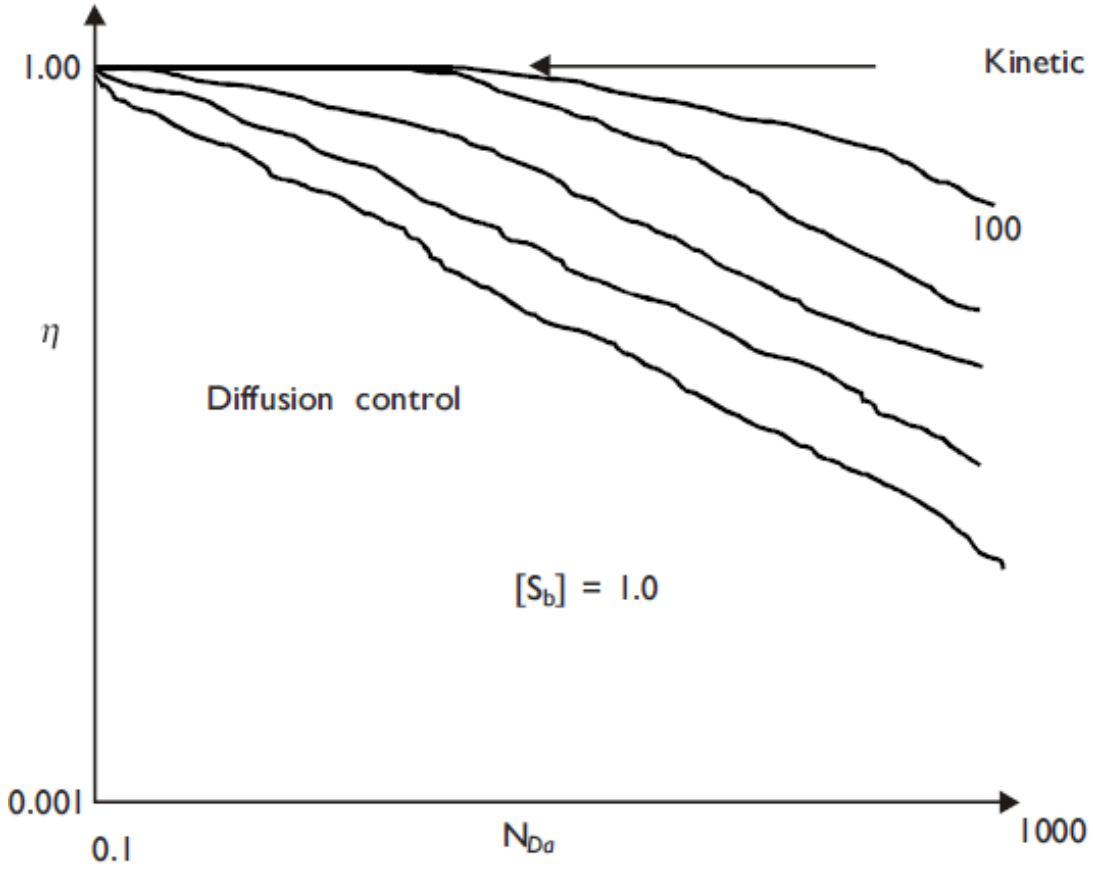
- As long as Da is very large, the observed rate of reaction, $\bar{v}$ is first order with respect to the bulk substrate concentration and is independent of the intrinsic rate parameters Vmax and km.

- When **Da << 1**, then the mass transfer rate is much greater than the reaction rate, and the overall reaction is controlled by the enzymatic reaction rate (X =1, η = 1)

- So the reaction kinetics may be represented as

$$\bar{v} = \frac{v_{max} S_b}{k_m + S_b}$$

Effectiveness factor and Damköhler number:



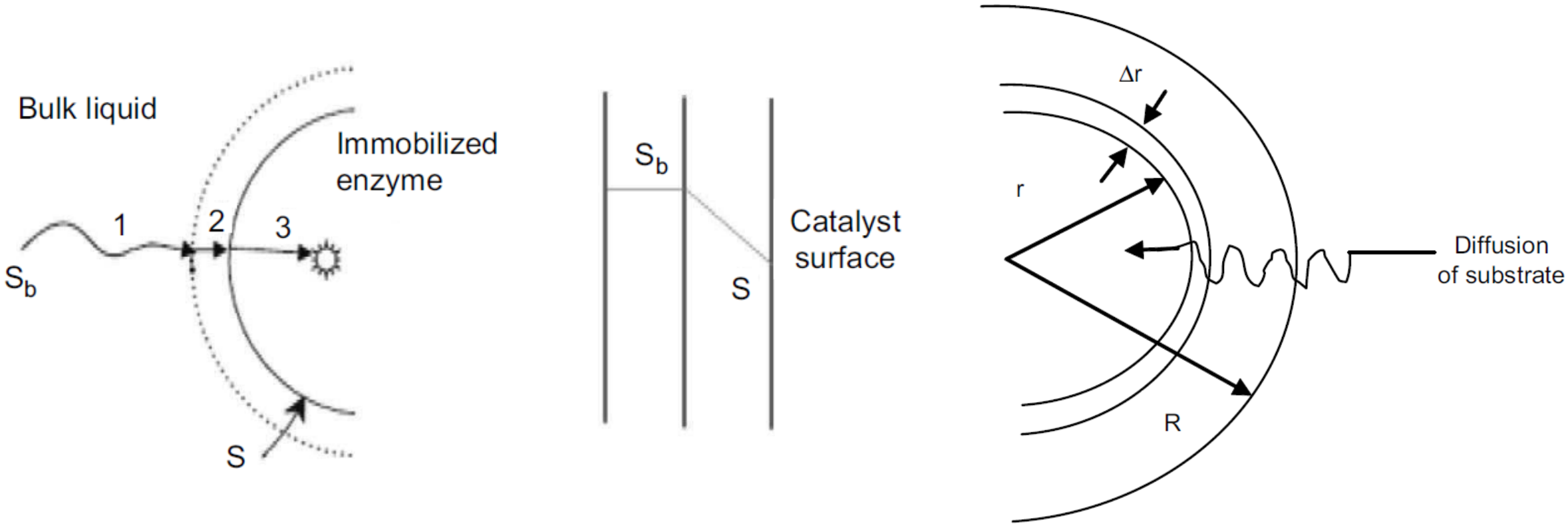
Effective factors vs. Damkohler number

Effect of internal mass transfer:

- Considering enzymes immobilized in spherical particles (porous bio-catalyst)

- **Assumption:**
  - The particle is isothermal.
  - Mass transfer takes place by **diffusion only**.
  - **Fick's law** can be used to describe diffusion using constant **effective diffusivity**.
  - The particle is homogeneous.
  - The particle is in a **steady state**.
  - Variation of substrate concentration with a **single spatial variable**.

Effect of internal mass transfer:



Internal mass transfer in a spherical particle

Diffusion of substrate inside the spherical particle

Steps 1 and 2 are the external mass transfer resistance. Step 3 is the intra-particle/internal mass transfer resistance

Effect of internal mass transfer:

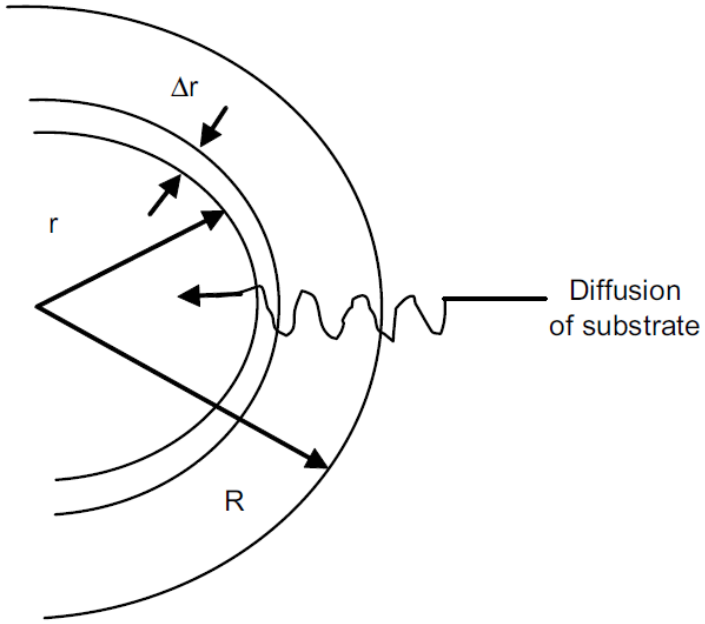
- Assuming a thin spherical shell of thickness Δr located at radius r from the center
- The steady-state shell mass balance for a shell on substrate S may be written as

$$\text{Rate of diffusion in input} = \left(D_{es} \frac{dS}{dr} 4\pi r^2 \right)\Big|_{r+\Delta r}$$

$$\text{Rate of diffusion in output} = \left(D_{es} \frac{dS}{dr} 4\pi r^2 \right)\Big|_{r}$$

$$\text{Rate of generation} = 0$$

$$\text{Rate of substrate degradation} = (-r_S) 4\pi r^2 \Delta r$$



- At a steady state, the rate of accumulation is 0.
- Here Des is the effective diffusivity of the substrate, S is the concentration of the substrate in the particle, and r is the distance.

Effect of internal mass transfer:

- The substrate balance (material balance) may be written as follows:

Rate of input + Rate of generation = Rate of output + Rate of consumption + Rate of accumulation

- For steady-state shell mass balance

$$\left(D_{es}\frac{dS}{dr}4\pi r^2\right)\Big|_{r+\Delta r}+0=\left(D_{es}\frac{dS}{dr}4\pi r^2\right)\Big|_{r}+(-r_S)4\pi r^2\Delta r+0$$

- Rearranging the above equation we get; Steady-state shell mass balance

$$\left(D_{es}\frac{dS}{dr}4\pi r^2\right)\Big|_{r+\Delta r}-\left(D_{es}\frac{dS}{dr}4\pi r^2\right)\Big|_{r}=(-r_S)4\pi r^2\Delta r$$

- Dividing both terms by $4\pi\Delta r$ gives

$$\frac{\left(D_{es}\frac{dS}{dr}r^2\right)\Big|_{r+\Delta r}-\left(D_{es}\frac{dS}{dr}r^2\right)\Big|_{r}}{\Delta r}=(-r_S)r^2$$

Effect of internal mass transfer:

- Taking limit $\Delta r \to 0$

$$\lim_{\Delta r \to 0} \frac{\left(D_{es} \frac{dS}{dr} r^2 \right)\Big|_{r+\Delta r} - \left(D_{es} \frac{dS}{dr} r^2 \right)\Big|_{r}}{\Delta r} = (-r_S) r^2$$

- At steady-state shell mass balance,

$$\frac{d}{dr}\left(D_{es} \frac{dS}{dr} r^2 \right) = (-r_S) r^2$$

- Differentiating,

$$D_{es}\left(\frac{d^2S}{dr^2} r^2 + 2r \frac{dS}{dr} \right) = (-r_S) r^2$$

$$\boxed{D_{es}\left(\frac{d^2S}{dr^2} + \frac{2}{r}\frac{dS}{dr} \right) = (-r_S)}$$

Represents diffusion and reaction in a spherical biocatalyst

Effect of internal mass transfer:

- Steady-state shell mass balance,

$$D_{es}\left(\frac{d^2S}{dr^2}+\frac{2}{r}\frac{dS}{dr}\right)=(-r_S)$$

$$\left(\frac{d^2S}{dr^2}+\frac{2}{r}\frac{dS}{dr}\right)=\frac{(-r_S)}{D_{es}}$$

- **The boundary condition** for the above equation is as follows:
- At r = R, S = Sb, where Sb is the bulk substrate concentration

$$r=0, \qquad \frac{dS}{dr}=0$$ (Rate of mass transfer)

- Assuming non-dimensional parameters

$$\bar{S}=\frac{S}{S_b} \qquad \bar{r}=\frac{r}{R}$$

Effect of internal mass transfer:

- Replacing S and r with non-dimensional parameters and we get,

$$\frac{S_b}{R^2}\frac{d^2\bar{S}}{d\bar{r}^2}+\frac{2S_b}{R^2\bar{r}}\frac{d\bar{S}}{d\bar{r}}=\frac{(-r_S)}{D_{es}}$$

$$\frac{d^2\bar{S}}{d\bar{r}^2}+\frac{2}{\bar{r}}\frac{d\bar{S}}{d\bar{r}}=\frac{R^2(-r_S)}{D_{es}S_b}$$

- Now, boundary condition for this equation is:

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

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

- If the reaction follows M-M kinetics, then

$$\frac{d^2\bar{S}}{d\bar{r}^2}+\frac{2}{\bar{r}}\frac{d\bar{S}}{d\bar{r}}=\frac{R^2\dfrac{v_{max}S}{k_m+S}}{D_{es}S_b}$$

Effect of internal mass transfer:

$$\mathrm{RHS} = \frac{R^2 \dfrac{v_{\max} S}{k_m + S}}{D_{es} S_b}$$

$$= \frac{R^2 v_{\max}}{D_{es} k_m} \left[\frac{\dfrac{S}{S_b}}{1 + \dfrac{S}{k_m}} \right]$$

$$\mathrm{RHS} = \frac{R^2 v_{\max}}{D_{es} k_m} \left[\frac{\bar{S}}{1 + \left\{ \dfrac{S/S_b}{k_m/S_b} \right\}} \right] = \frac{R^2 v_{\max}}{D_{es} k_m} \left[\frac{\bar{S}}{1 + \left(\dfrac{\bar{S}}{\beta} \right)} \right] = 9\varphi^2 \left[\frac{\bar{S}}{1 + \left(\dfrac{\bar{S}}{\beta} \right)} \right]$$

where β = km/Sb is the saturation parameter or Dimensionless Michaelis constant

Effect of internal mass transfer:

- ϕ is called the **Thiele modulus**

$$\varphi^2 = \left(\frac{V_p}{A_p}\right)^2 \frac{v_{max}}{D_{es} k_m} = \frac{\text{Maximum reaction rate}}{\text{Maximum mass-transfer rate (intra particulate)}}$$

- where Vp and Ap are the volume and area of the particle, respectively
- For spherical geometry,

$$\varphi^2 = \left(\frac{\frac{4}{3}\pi R^3}{4\pi R^2}\right)^2 \frac{v_{max}}{D_{es} k_m} = \frac{R^2}{9} \frac{v_{max}}{D_{es} k_m}$$

$$9\varphi^2 = \frac{R^2 v_{max}}{D_{es} k_m}$$

- To determine the effect of mass-transfer resistance on the rate of reaction, the **effectiveness factor** of an immobilized enzyme (η) can be defined as

$$\eta = \frac{\text{Observable reaction rate}}{\text{Reaction rate in case of no mass-transfer resistance}} = \frac{\text{Performance of heterogeneous system}}{\text{Performance of homogeneous system}}$$

Effectiveness factor and Thiele modulus (intra particle mass transfer):

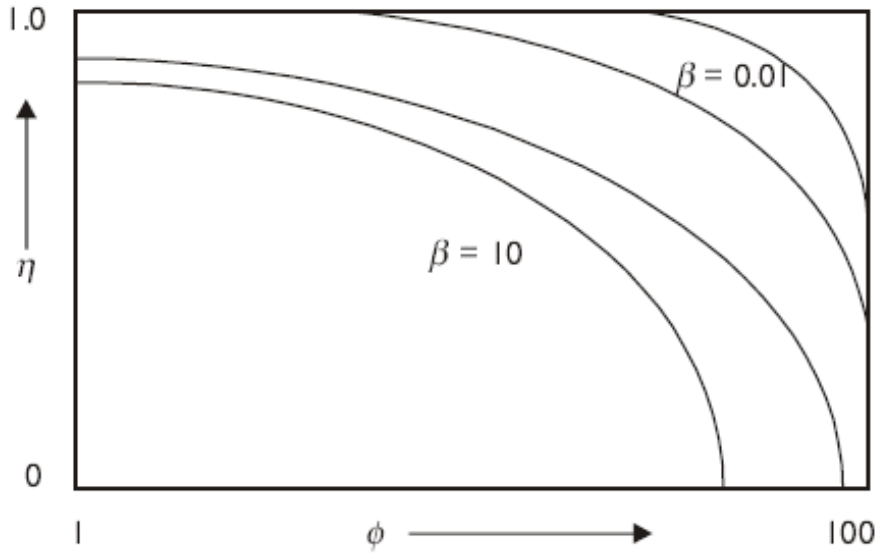
- Effectiveness factor is the ratio of the reaction rate with diffusion limitation to the reaction rate with no diffusion limitation.
- Or, the value of effectiveness factor is a measure of the extent of diffusion limitation.

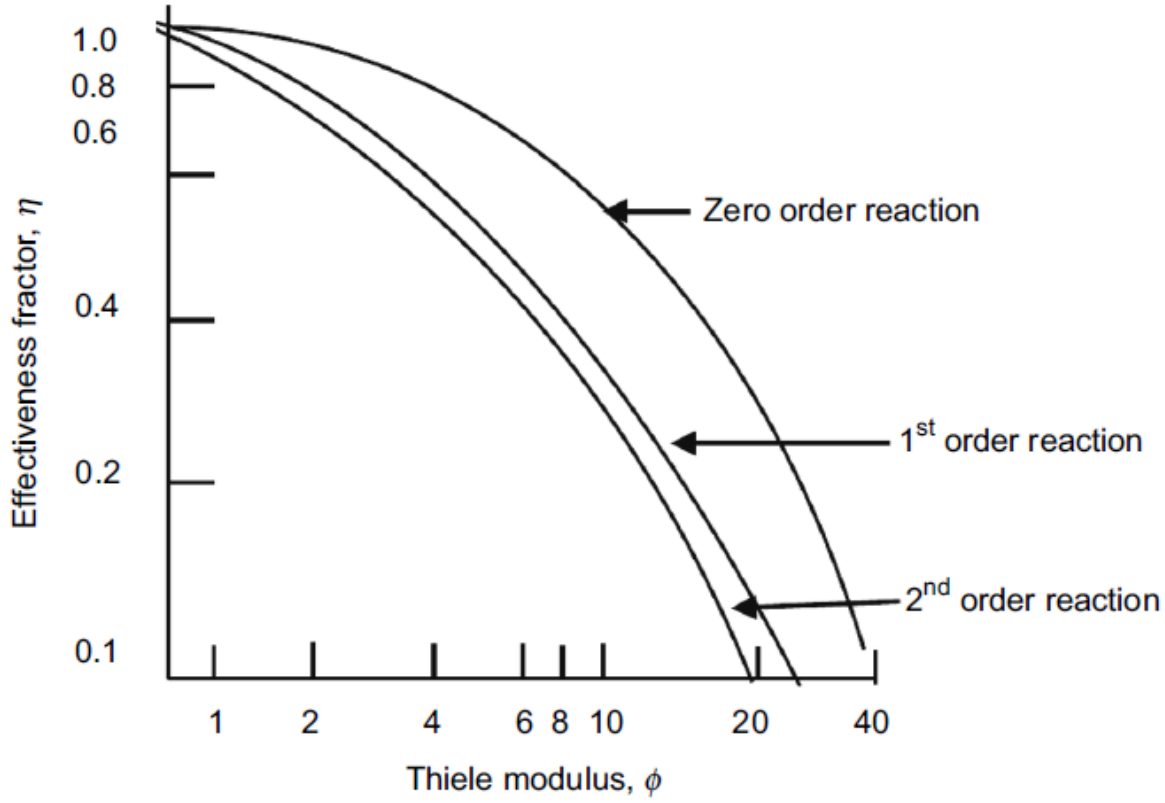
- For **$\eta < 1$**, the conversion is diffusion limited
- For **$\eta \sim 1$,** the conversion is limited by the reaction rate and diffusion limitations are negligible
- $\eta = f(\phi, \beta)$

- For zero order reaction rate ($\beta \rightarrow 0$), $\eta \sim 1$.
- For first order reaction ($\beta \rightarrow \infty$), $\eta = f(\phi, \beta)$ and η is approximated to the following equation for high values of ϕ

$$\eta = \frac{3}{\varphi}\left[\frac{1}{\sinh\varphi} - \frac{1}{\varphi}\right]$$

Effectiveness factor and Thiele modulus (intra particle mass transfer):





Correlation between Thiele modulus and Effectiveness factor

Effectiveness factor and Thiele modulus:

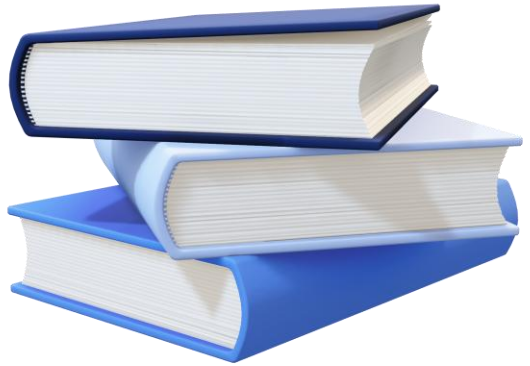
- It is difficult to determine the kinetic parameters such as k_m and v_{max} in many practical cases.
- To overcome this problem, the observable Thiele modulus (Φ) is considered, which is known as the Weisz criteria.

Φ	η	Limiting regime	Mass-transfer significance
<0.3	≈ 1	Reaction	Mass-transfer resistance negligible
>3	$\propto 1/\Phi$	Diffusion	Mass resistance significance

- It is an intrinsic kinetic parameter

$$\Phi = \left(\frac{V_p}{A_p}\right)^2 \frac{(-r_S)_{obs}}{D_{es} S_b}$$

Review:



- Immobilized enzymes
  - Advantages/Disadvantages
  - Applications
  - Different type of matrices/support
  - Different immobilization techniques

- Kinetics of immobilized enzymes
  - Internal mass transfer limitations: Effectiveness factor, Damkohler number
  - External mass transfer limitations: Effectiveness factor, Thiele Modulus

Problem statement 1:

D. Thornton and co-workers studied the hydrolysis of sucrose at pH = 4.5 and 25°C using crude invertase obtained from baker's yeast in free and immobilized form. The following initial velocity data were obtained with 408 units of crude enzyme (1 unit = quantity of enzyme hydrolyzing 1 mmol of sucrose/min when incubated with 0.29 M sucrose in a buffer at pH 4.5 and 25°C).

- Determine the Km and Vm for this reaction using both free and immobilized enzyme.
- Do the data indicate any diffusion limitations in the immobilized enzyme preparation?

V_0 (mmol hydrolyzed/l-min)		
Free enzyme	Immobilized enzyme	S_0 (mol/l)
0.083	0.056	0.010
0.143	0.098	0.020
0.188	0.127	0.030
0.222	0.149	0.040
0.250	0.168	0.050
0.330	0.227	0.100
0.408	0.290	0.290

Solution for problem statement 1:

Double-reciprocal plot (Lineweaver–Burk plot)

- Double-reciprocal form

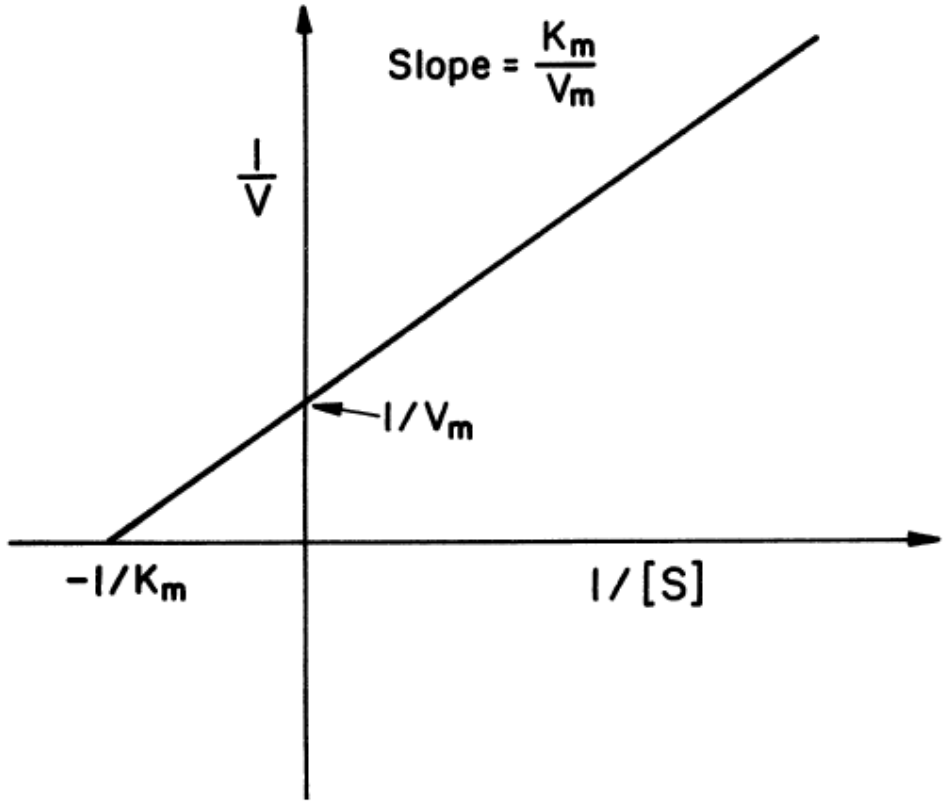
$$\frac{1}{v} = \frac{1}{V_m} + \frac{K_m}{V_m}\frac{1}{[S]}$$

where Km = k-1/k1, which is the dissociation constant of the ES complex

The maximum forward velocity of the reaction is Vm

where $V_m = k_2[E_0]$.

A plot of 1/v versus 1/[S] yields a linear line with a slope of Km/Vm and y-axis intercept of 1/Vm,



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

Solution for problem statement 1:

From a double-reciprocal plot of 1/v versus 1/S

for **free enzyme:**

-1/Km = -20 and Km = 0.05 M. 1/Vm = 2 and

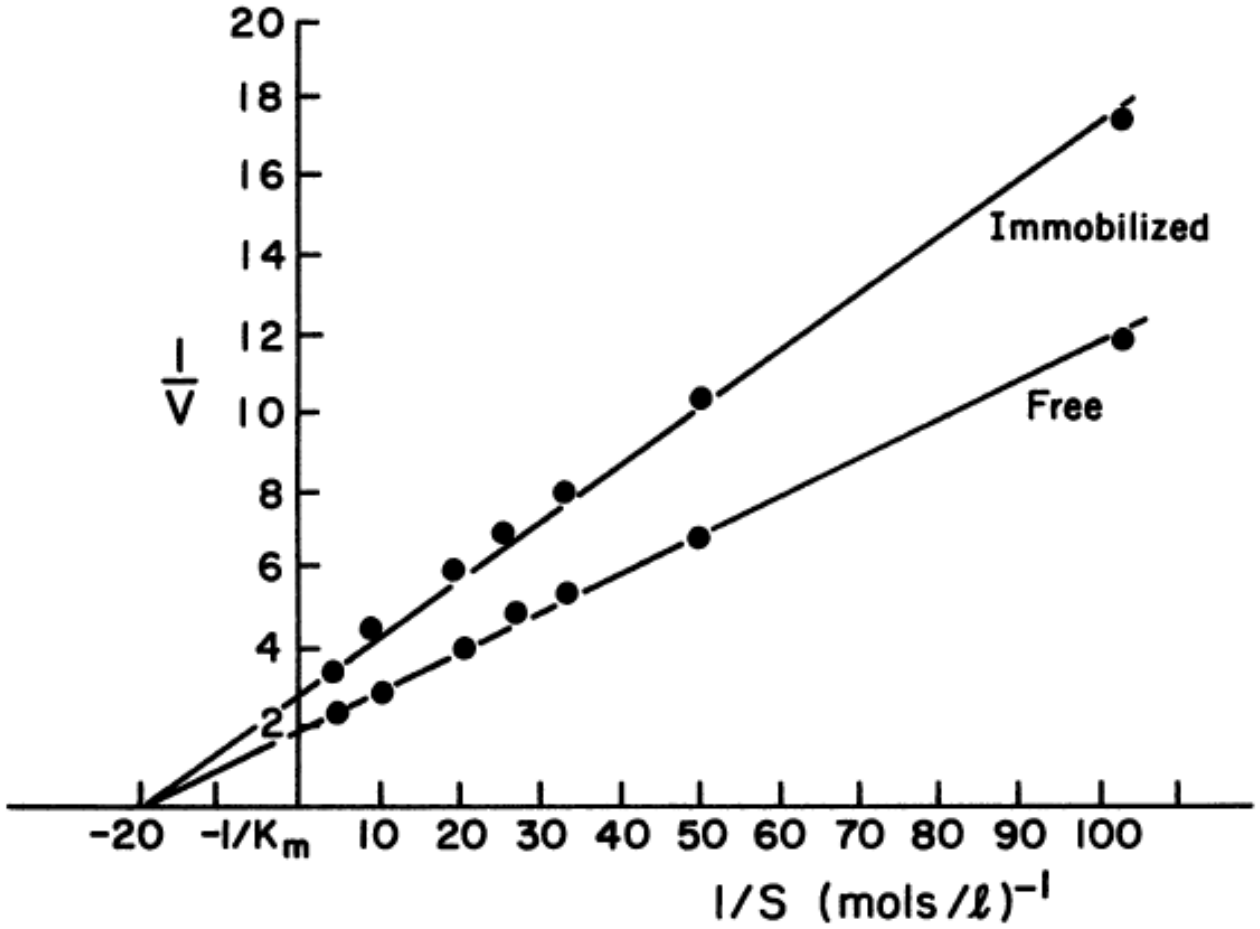
Vm = 0.5 mmol/l min.

From a double-reciprocal plot of 1/v versus 1/S

for the **immobilized enzyme:**

-1/Km = -20 and Km = 0.05 M. 1/Vm = 3

and Vm = 0.33 mmol/l-min



Since the Km values for free and immobilized enzymes are the same, there is no diffusion limitation

Problem statement 2:

Immobilization of baby hamster kidney cells is done in alginate beads. The average particle diameter is 5 mm. The rate of oxygen consumption at a bulk concentration of 8×10^{-3} kg O_2/m^3 is 8.4×10^{-5} kg/s m^3 catalyst. The effective diffusivity of oxygen in the beads is 1.88×10^{-9} m^2/s. The oxygen concentration at the surface of the catalyst is assumed to be equal to the bulk concentration. It is assumed that oxygen uptake follows zero-order kinetics. Do you think internal mass-transfer effects are significant?

Solution for problem statement 2:

The observable Thiele modulus may be written as

$$\Phi = \left(\frac{R}{3}\right)^2 \frac{(-r_{O_2})_{obs}}{D_{es}\, S_b}$$

$$R = \frac{5\times10^{-3}\,\mathrm{m}}{2} = 2.5\times10^{-3}\,\mathrm{m}$$

$$\Phi = \left(\frac{2.5\times10^{-3}\,\mathrm{m}}{3}\right)^2 \frac{8.4\times10^{-5}\,\mathrm{kg/s\,m^3}}{(1.88\times10^{-9}\,\mathrm{m^2/s})(8\times10^{-3}\,\mathrm{kg/m^3})} = 3.88$$

From the Weisz criteria ($\Phi > 3$), the internal mass-transfer effects are significant.

Problem statement 3:

Glucose is converted to fructose by using immobilized glucose isomerase. Find the height of the immobilized enzymes column?

Following data are given:

Diameter of the column D_T = 5 cm

Particle size 30/40 mesh (average diameter (dp) = 0.71 mm)

Feed rate F = 500 mL/h

Glucose concentration in feed at 60°C is 500 g/L.

Glucose conversion (C.E.) efficiency is 60%.

Feed viscosity μ = 3.6 c.p. at 60°C

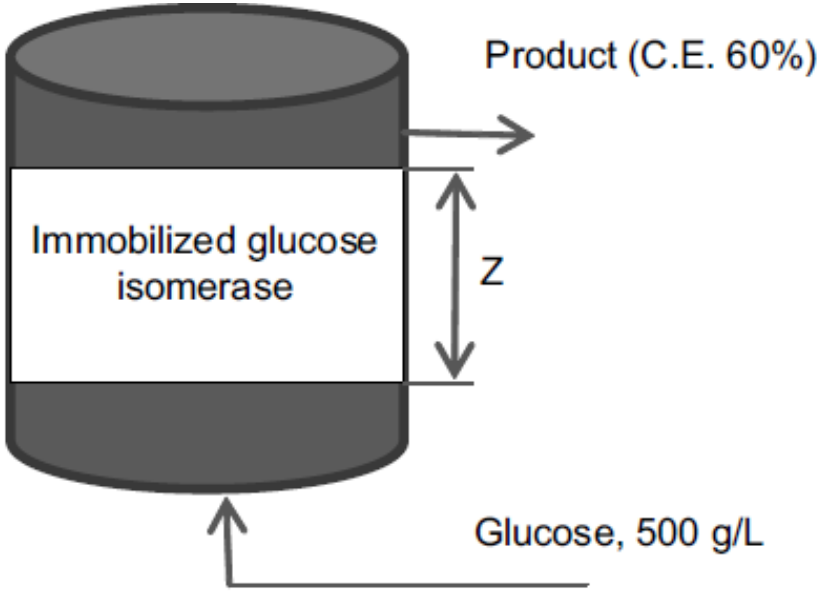
Feed density r = 1.23 g/mL at 60°C

Substrate diffusivity D = 0.21×10^{-5} cm^2/s at 60°C

Void fraction e = 0.35

Solution for problem statement 3:

We assume that Z is the height of the column, ε is the void fraction, a_v is the ratio of the particle surface area to volume, Y_2 is the mole fraction of the substrate in the product, Y_1 is the mole fraction of the substrate in the feed, and D_T is the diameter of the column.



Solution for problem statement 3:

Satterfield has suggested an expression for the column height as follows:

$$Z = \frac{\varepsilon (Re)^{\frac{2}{3}} (Sc)^{\frac{2}{3}}}{1.09 a_v} \ln\left(\frac{Y_1}{Y_2}\right)$$

$$\text{where the Reynolds number } Re = \frac{D_T u \rho}{\mu}$$

$$\text{Velocity of the fluid } (u) = \frac{\text{Volumetric feed flow rate}}{\text{Cross-sectional area of the column}}$$

$$\text{Volumetric feed flow rate} = 500 \frac{\text{mL}}{\text{h}} = 0.139 \frac{\text{mL}}{\text{s}}$$

$$\text{Cross-sectional area of the column} = \pi\left(\frac{D_T}{2}\right)^2 = \pi(2.5)^2 \text{ cm}^2$$

Solution for problem statement 3:

$$u = \frac{0.139 \frac{\text{mL}}{\text{s}}}{\pi (2.5)^2 \text{cm}^2} = 7.079 \times 10^{-3} \frac{\text{cm}}{\text{s}}$$

$$Re = \frac{(5)(7.079 \times 10^{-3})(1.23)}{3.6} = 0.0121$$

$$\text{Scmidth number} = Sc = \frac{\mu}{D\rho} = \frac{3.6}{0.21 \times 10^{-5} \times 1.23} = 1.3937 \times 10^{6}$$

$$a_v = \frac{\text{Surface area of particle}}{\text{Volume of particle}} = \frac{4\pi \left(\frac{d_p}{2}\right)^2}{\frac{4}{3}\pi \left(\frac{d_p}{2}\right)^3} = \frac{6}{d_p} \quad = \frac{6}{0.71\,\text{mm}} = \frac{6}{0.071\,\text{cm}} = 84.50\,\text{cm}^{-1}$$

Solution for problem statement 3:

- All substrate present at the entrance

$$Y_1 = \frac{\text{Mole of glucose}}{\text{Total moles of all constituents}} = \frac{\frac{500}{180}}{\frac{500}{180}} = 1$$

- 60 % conversion efficiency

$$Y_2 = \frac{\text{Mole of glucose}}{\text{Total moles of all constituents}} = \frac{\frac{500 \times (1 - 0.60)}{180}}{\frac{500}{180}} = \frac{\frac{500 \times 0.40}{180}}{\frac{500}{180}} = 0.40$$

Putting all the known values in the proposed equation, we get:

$$Z = \frac{0.35(0.0121)^{\frac{2}{3}}(1.3937 \times 10^6)^{\frac{2}{3}}}{1.09 \times 84.50\ \text{cm}^{-1}} \ln\left(\frac{1}{0.40}\right) = 2.29\ \text{cm}$$