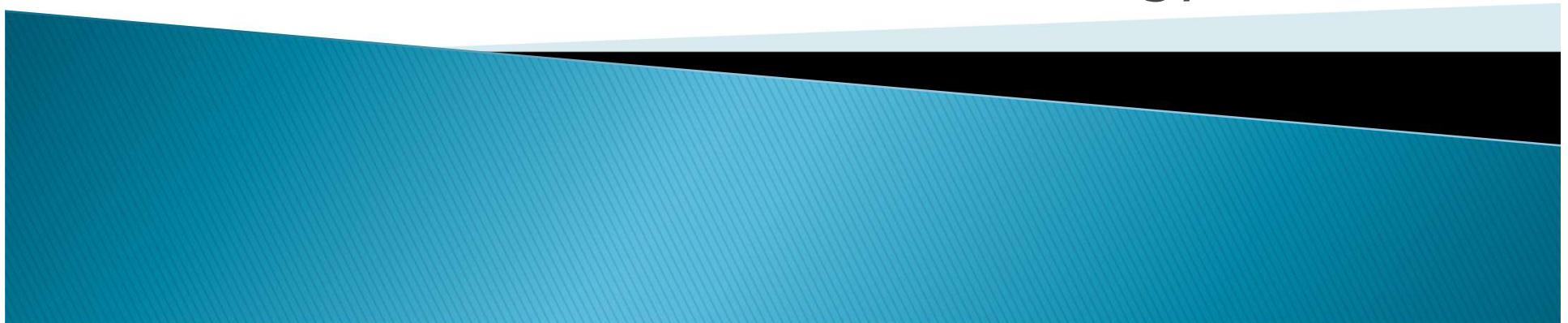


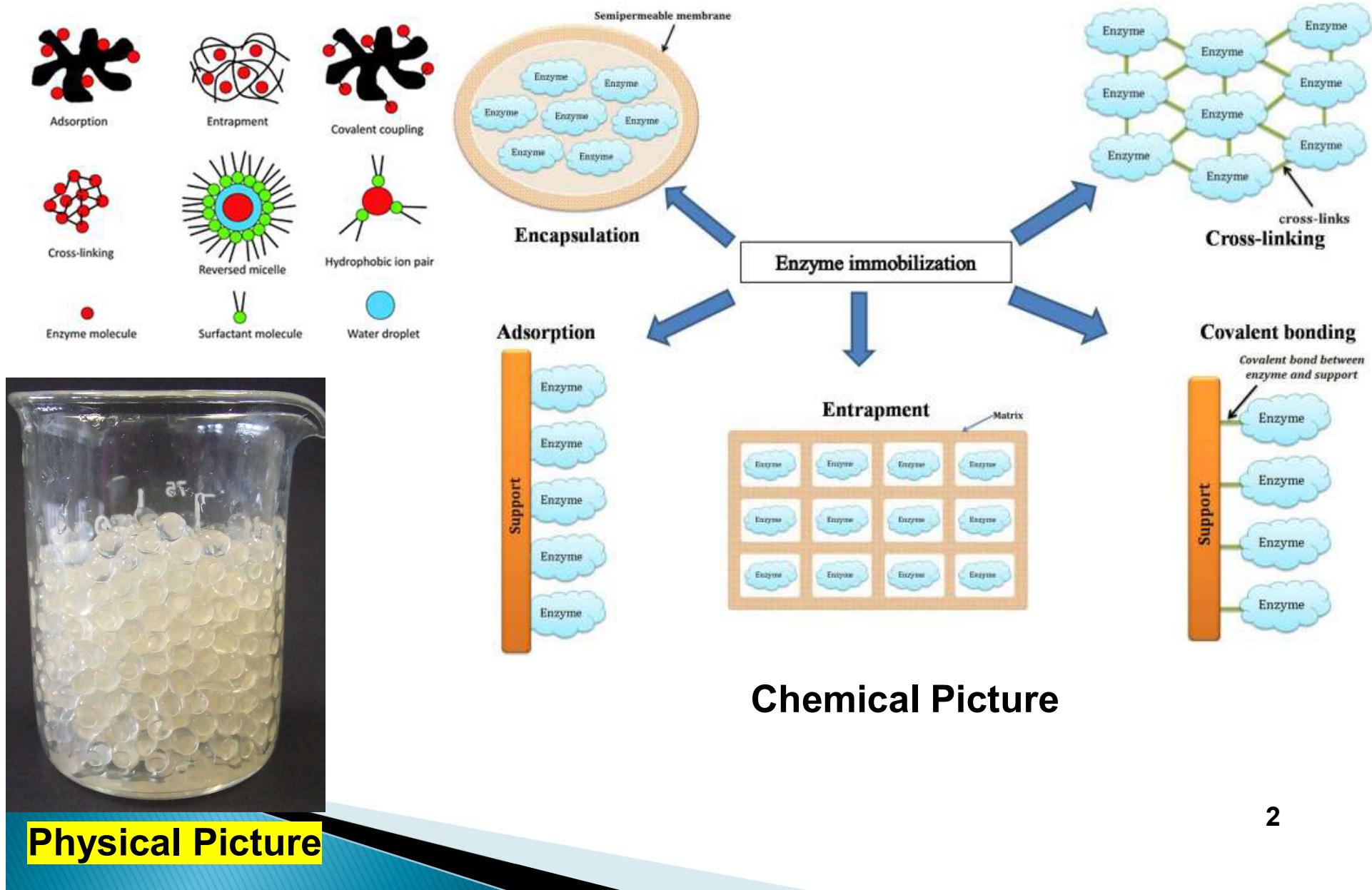
CH40001 Biochemical Engineering

Chapter 4. Immobilized Enzymes

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Enzyme Immobilization Methods



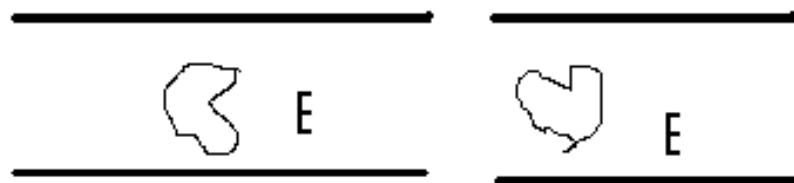
Application of Immobilized Enzymes

- Enzyme drugs encapsulated in gel hollow fiber or micro capsule membrane.

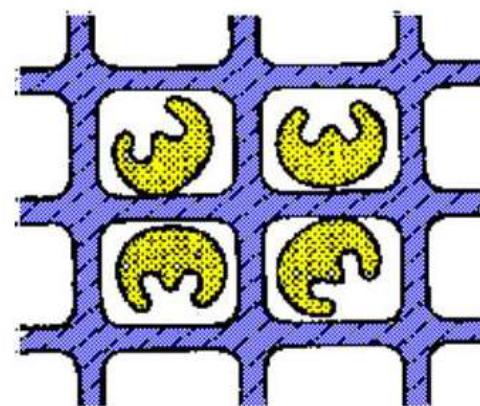
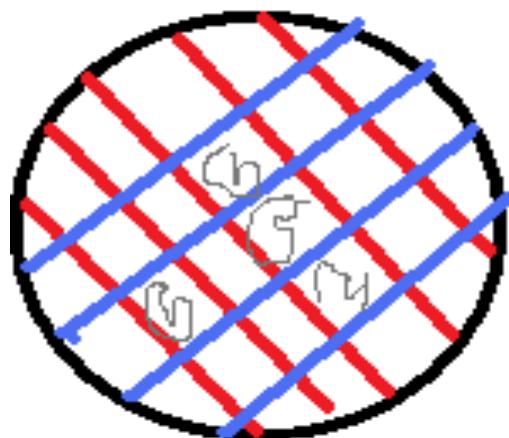
Advantages: (i) enzymes not susceptible to antibody attack; (ii) easy separation of enzymes from reaction mixture, allowing easy purification of products; (iii) re-use of enzymes across production cycles, which significantly reduces product costs.
- Disadvantage: increased mass transfer resistances and decreased efficiency of substrate utilization.

e.g. ; encapsulated enzymes, digestive enzymes, steroids.

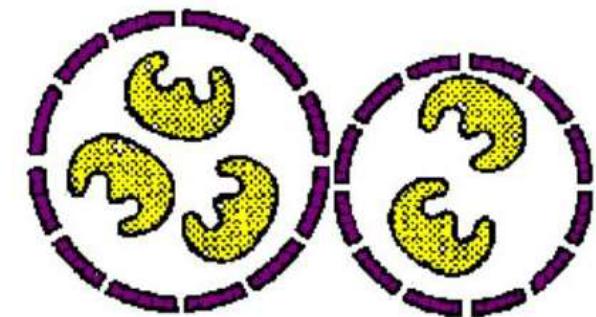
Physical entrapments of enzymes



Enzymes trapped in
hollow fiber membrane



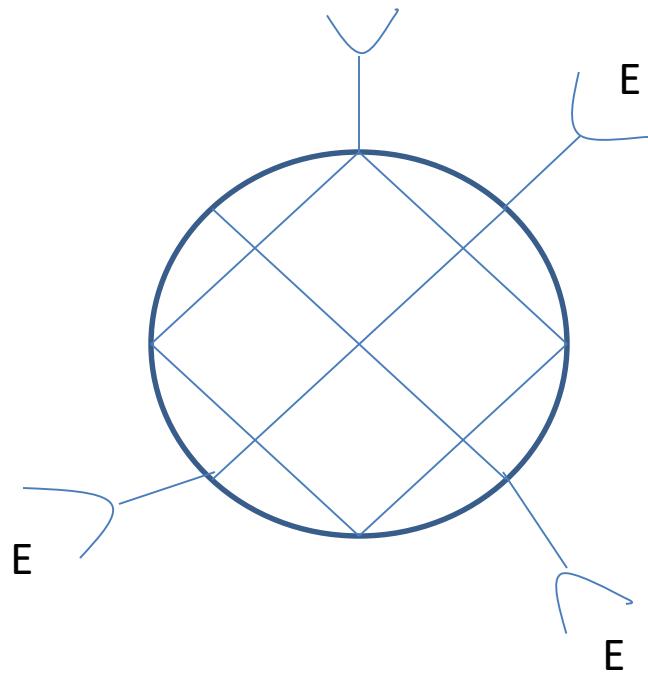
entrapped in a matrix



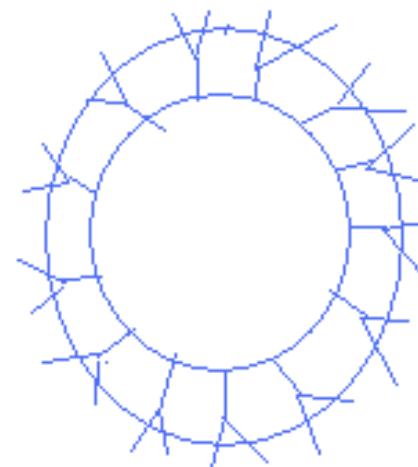
entrapped in droplets

Enzyme entrapment within insoluble gel matrix

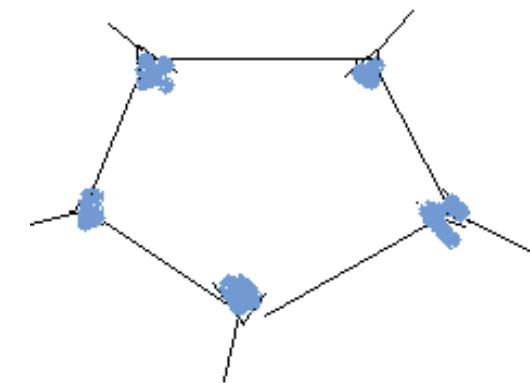
Enzyme Immobilization by Chemical Methods



Enzyme covalently
attached to water-
insoluble matrix

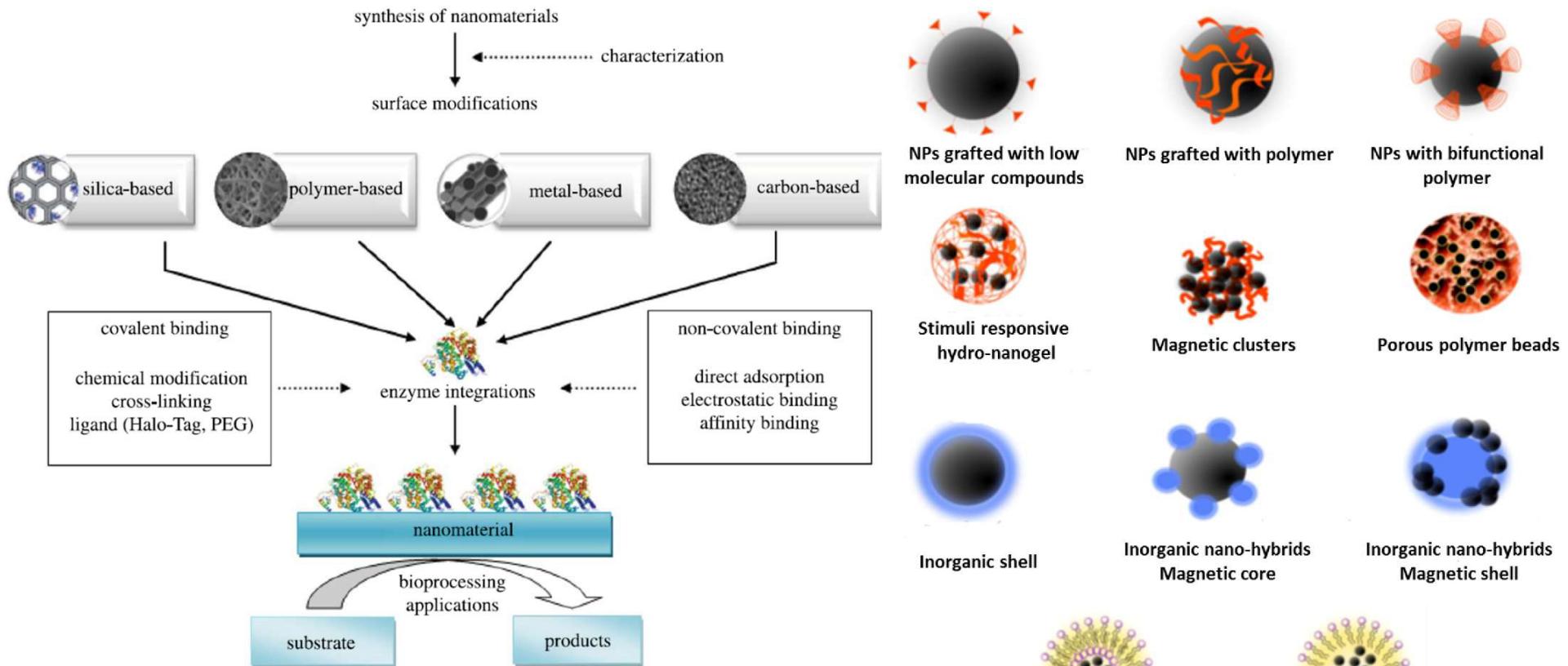


Cross linked
enzyme
using
functional
reagent.



Cross linked
enzyme
matrix

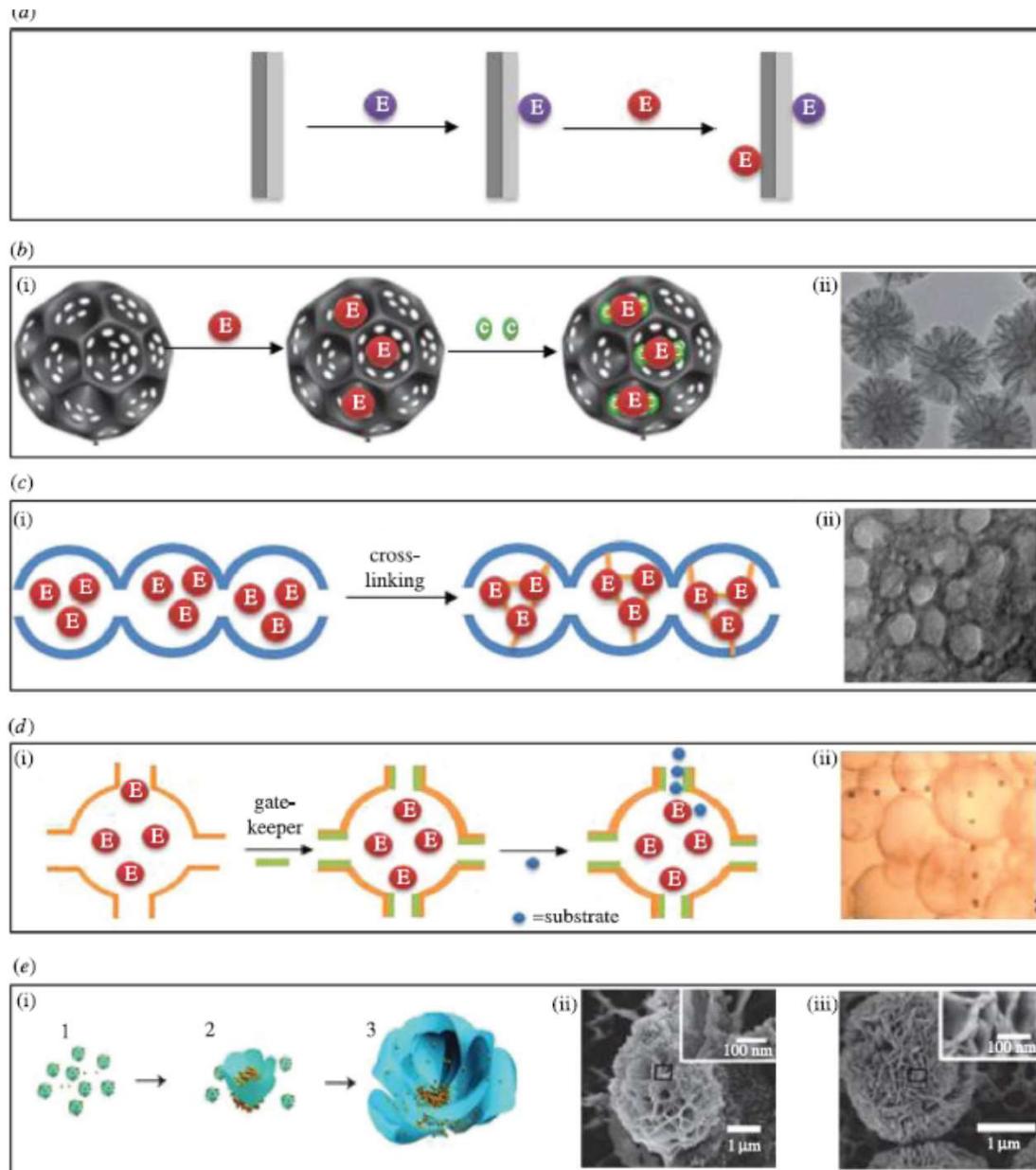
Scaling down the immobilization to nano-scale to minimize mass-transfer resistance



Misson M, Zhang H, Jin B. 2015 Nano-biocatalyst advancements and bioprocessing applications.

J. R. Soc. Interface 12: 20140891.

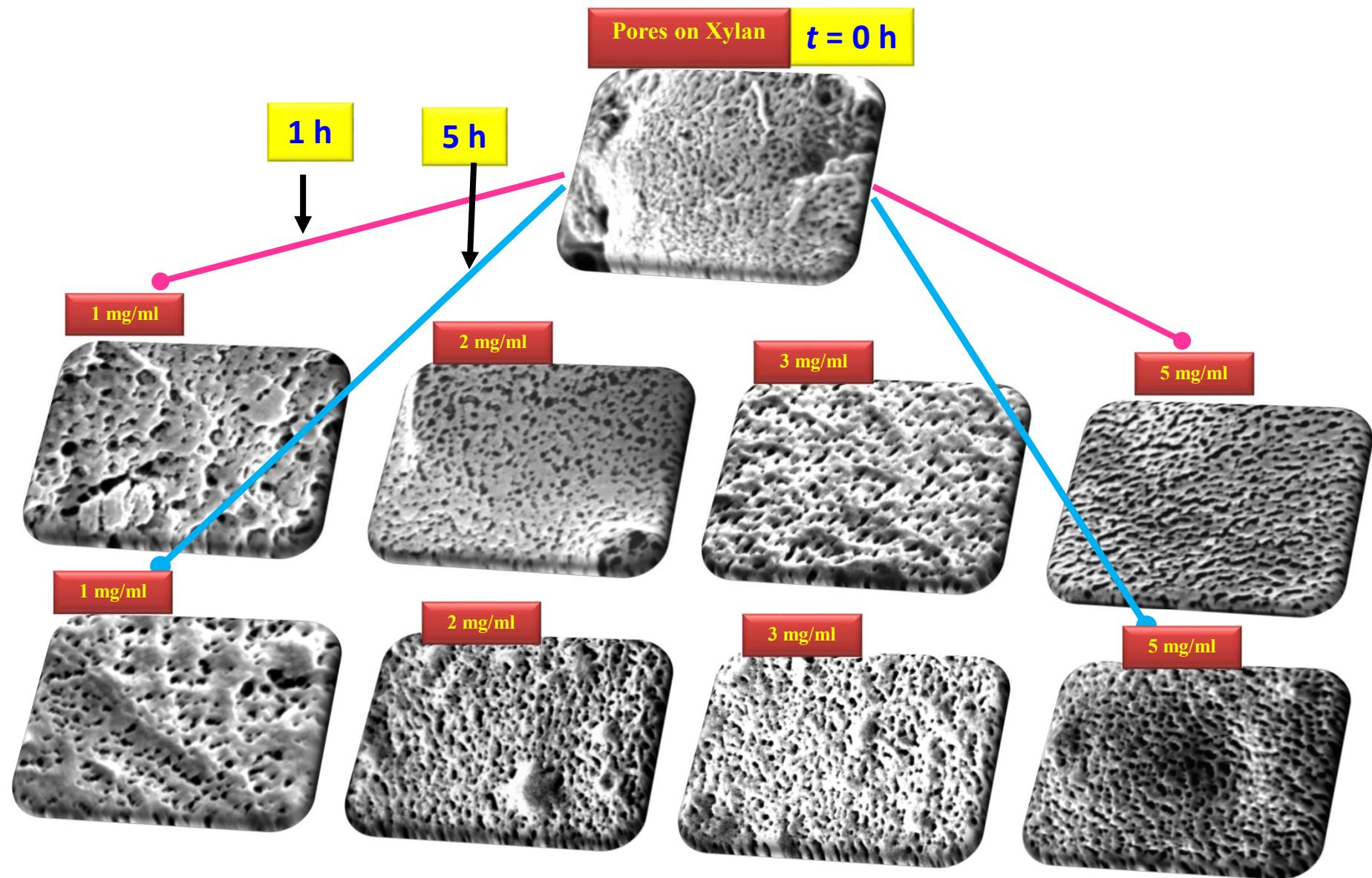
<http://dx.doi.org/10.1098/rsif.2014.0891>



Misson M, Zhang H, Jin B. 2015
Nano-biocatalyst advancements
and bioprocessing applications. J.
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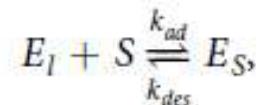
Figure 2. Nanocarriers with unique physico-chemical properties. (a) Side-by-side hybrid NFs promote immobilization of two enzymes to perform simultaneous reactions. (b) Schematic illustration of dendrimer-like nanoporous silica for the co-immobilization of enzyme with cofactors or other biomolecules (i), TEM image of dendrimer-like nanopores silica (ii) [29]. (c) Schematic illustration (i) and TEM image [30] (ii) of ship-in-a-bottle pore structures to retain and stabilize enzymes inside the nanocages. (d) Schematic illustration (i) and optical micrograph [31] (ii) of nanocages with substrate-diffusion gatekeepers to prevent enzyme leaching. (e) Schematic diagram (i) and SEM images of the formation of BSA-incorporated $\text{Cu}_3(\text{PO}_4)_2 \cdot 3\text{H}_2\text{O}$ nanoflowers (spheres in nanoflowers' core as protein molecules) at 12 h (ii) and 3 days (iii) in enhancing enzyme activity and stability [32]. (Online version in colour.)

Dynamics of Pore-scale Enzyme Adsorption



Non-equilibrium Enzyme Adsorption

The reversible enzyme adsorption-desorption is represented by



where S represents the available sites for adsorption of enzyme on the solid substrate ($=[\Theta_{max}] - [E_s]$), $\Theta_{max} (= \Omega_{max} \sum_{i=12}^n [X_i])$ represents the maximum adsorption sites on the substrate, E_s is the adsorbed enzyme concentration, E_l is the free liquid phase enzyme concentration, $[X_i]$ is the xylan concentration of chain length i , and $\sum_{i=12}^n [X_i]$ represents the total hemicellulose concentration in the solid phase at any time t . The rate of non-equilibrium adsorption of the enzyme on the solid surface is given by

$$\frac{d[E_s]}{dt} = k_{ads}[E_l](\Omega_{max} \sum_{i=12}^n [X_i] - [E_s]) - k_{des}[E_s].$$

<https://www.nature.com/articles/srep38173>

At equilibrium: $d[E_s]/dt = 0$

Langmuir Equilibrium Adsorption Isotherm

Nobel Prize in Chemistry, 1932

$$[E_s^*] = \frac{\sigma_{ad} \Omega_{\max} [C_s] [E_l^*]}{1 + \sigma_{ad} [E_l^*]},$$

$$\frac{[C_s]}{[E_s^*]} = \frac{1}{\sigma_{ad} \Omega_{\max}} \frac{1}{[E_l^*]} + \frac{1}{\Omega_{\max}},$$

where C_s is the solid concentration (mg/ml), E_s^* and E_l^* are the equilibrium concentrations of the free enzymes in the solid and liquid phases (mg/ml), respectively, σ_{ad} ($= k_{ads}/k_{des}$) is the adsorption equilibrium constant (ml/mg), k_{ads} (ml/mg/min) and k_{des} (min⁻¹) are the adsorption and desorption rate constants, respectively, and Ω_{\max} is the maximum enzyme adsorption on the solid substrate (mg of enzyme/mg of substrate).

Enzyme Adsorption to Hemicellulose

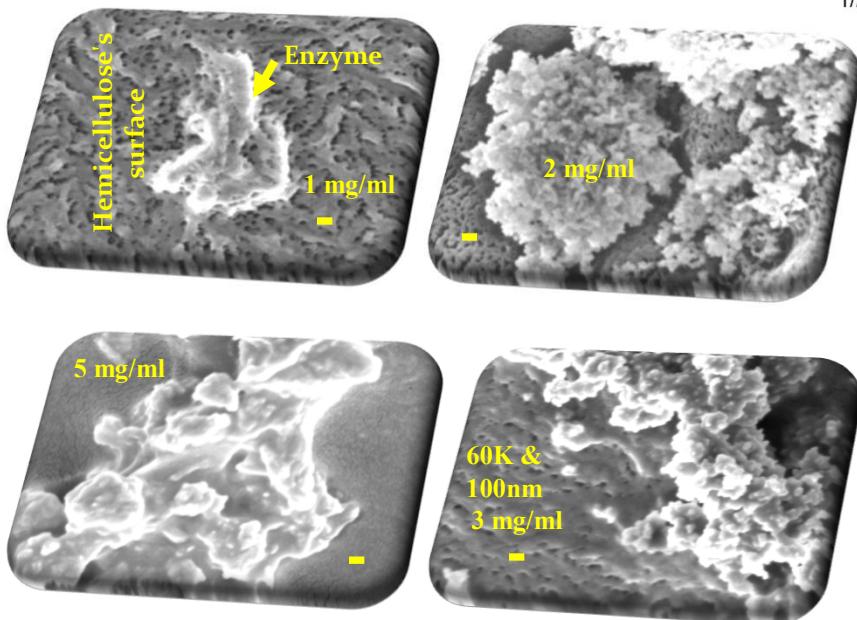
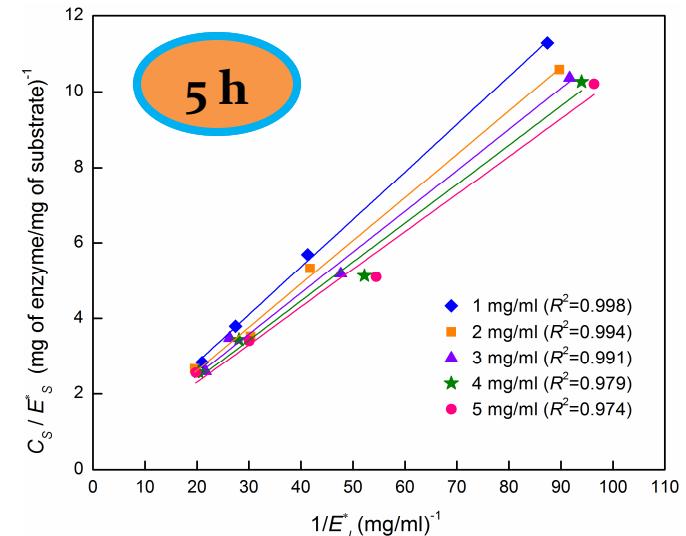
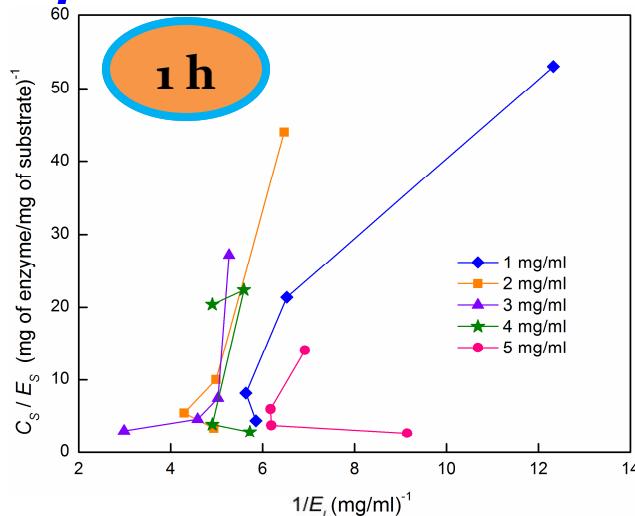
Equilibrium or non-equilibrium?

$C_s = 1-5 \text{ mg/ml}$

$E_o/C_s = 0.1-0.4$

NaAc buffer sol. (pH 5.0)

Endoxylanse (*T. longibrachiatum*),
4°C, 1-8 h



Langmuir
adsorption
isotherm model¹:

$$[E_s^*] = \frac{\sigma_{ad} \Omega_{\max} [C_s] [E_l^*]}{1 + \sigma_{ad} [E_l^*]}$$

C_s (mg/ml)	Ω_{\max} (mg/mg)	σ_{ad} (ml/mg)
1	2.985	3.018
2	3.030	3.000
3	3.012	3.046
4	3.049	3.065
5	3.077	3.095

Non-equilibrium adsorption of enzyme < 5 h.
Equilibrium adsorption of the Langmuir-type
at 5 h.

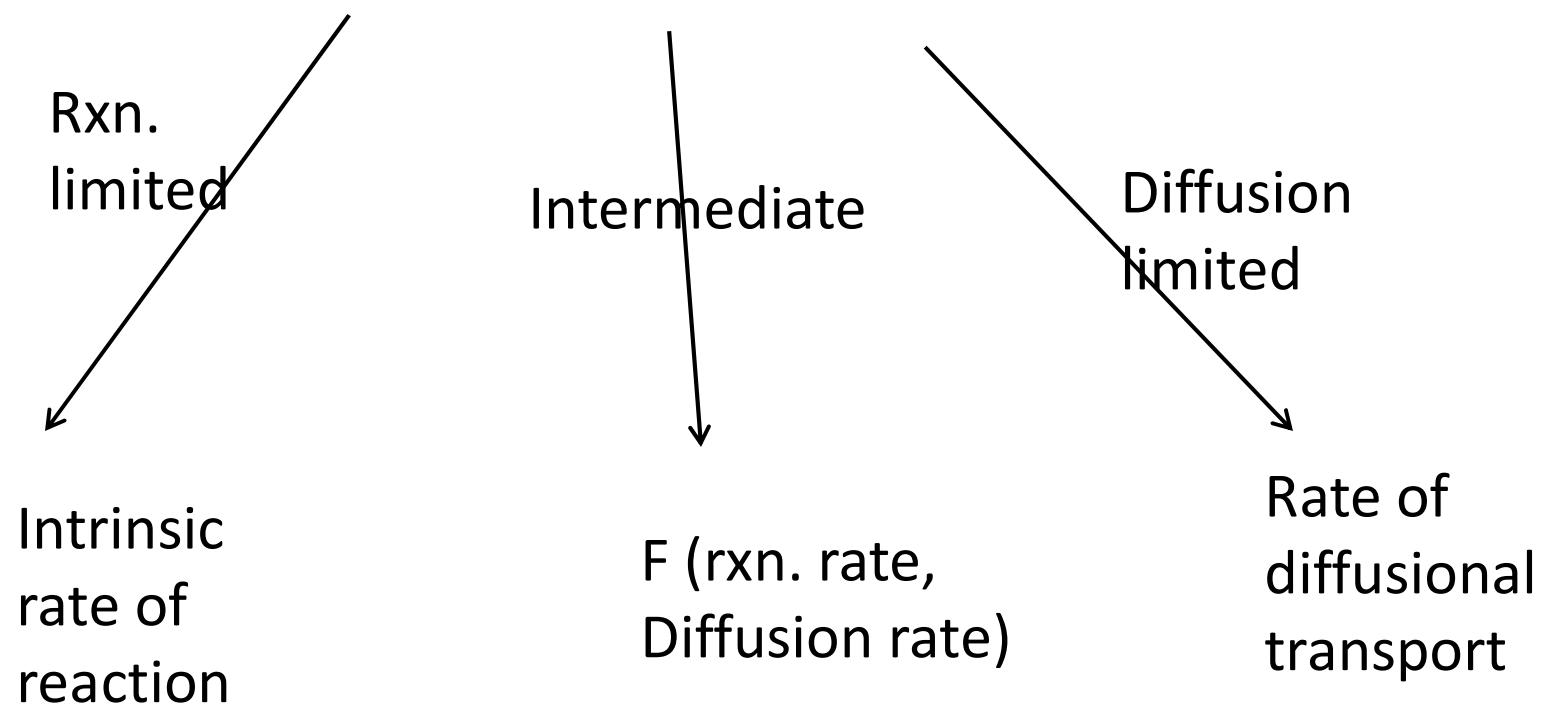
¹Zhang and Lynd, 2004. *Biotechnol. Bioeng.* 88, 797-824

Immobilized Enzyme kinetics (in presence of mass transfer resistances)

Biological reactions

- 1) Homogeneous
- 2) Heterogeneous
- 3) Pseudo homogeneous
- 4) Coupled Homogeneous -Heterogeneous

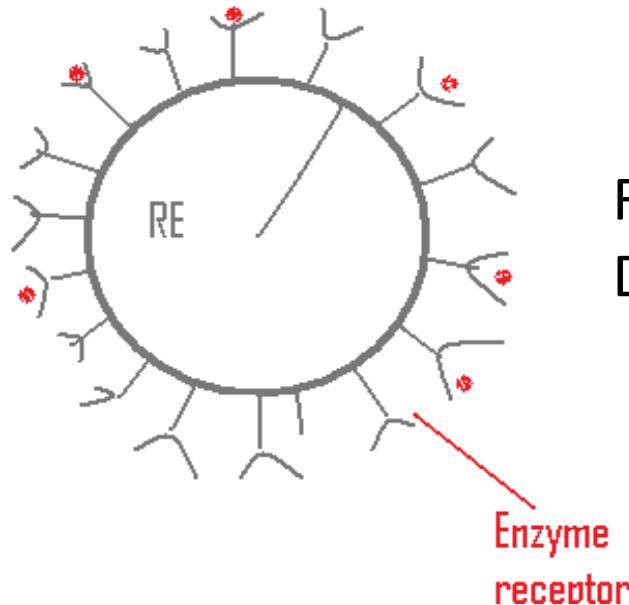
Observed rate of reaction



Enzyme covalently attached to water insoluble matrix acts as receptors (C_E).

Substrate dissolved in water plasma (C_S).

Assumption: no convection effects.



R_E = radius of enzyme matrix
 D_S = diffusivity of enzyme in the liquid

Reaction

Reactive binding between substrate and enzyme occurs at the surface of the matrix $R_S = k_1 C_S$, where $k_1 = R_{max}/K_M$, (for dilute solution of substrate i.e. $C_S \ll K_M$)

$$R_S = \frac{R_{\max} C_S}{K_M + C_S}$$

- Dilute solution of C_s : $C_s \ll K_M$, $R_S = k_1 C_S$, where $k_1 = R_{\max} / K_M$
- Concentrated solution: $C_s \gg K_M$, $R_S = R_{\max}$ (zero order)

Dilute solutions of substrates

$$R_s = k_1 C_s$$

Reaction diffusion model

Governing Eqn.: $\frac{D_s}{r^2} \frac{d}{dr} \left(r^2 \frac{dC_s}{dr} \right) = 0$

B.C.'s: $r = R_E, -4\pi R_E^2 D_s \left(\frac{dC_s}{dr} \right) = k_1 C_s$

$$r \rightarrow \infty, C_s = C_{s0}$$

$$\frac{d}{dr} \left(r^2 \frac{dC_s}{dr} \right) = 0,$$

$$C_s - C_{s0} = \frac{a_1}{r},$$

$$r^2 \frac{dC_s}{dr} = a_1,$$

$$a_1 = \frac{k_1 C_{s0}}{k_1 + 4\pi D_s R_E}$$

$$\frac{dC_s}{dr} = \frac{a_1}{r^2},$$

$$C_s = C_{s0} \left(1 - \frac{k_1 R_E / r}{k_1 + 4\pi D_s R_E} \right)$$

$$C_s = -\frac{a_1}{r} + a_2,$$

- Observed flux=observed rate of enzyme substrate reaction):

$$-4\pi R_E^2 D_S \left(\frac{dC_S}{dr} \right)_{r=R_E} = -4\pi D_S a_1$$

$$\begin{aligned} &= \frac{4\pi D_S k_1 R_E}{k_1 + 4\pi D_S R_E} C_{s0} \\ &= k_{\text{obs}} C_{s0} \end{aligned}$$

where $k_{\text{obs}} = \frac{4\pi D_S k_1 R_E}{k_1 + 4\pi D_S R_E}$

$$\frac{1}{k_{\text{obs}}} = \frac{1}{k_1} + \frac{1}{4\pi D_S R_E}$$

- Resistances in series
- Conclusion : reaction is controlled by both reaction and mass transfer.

- Case 2: Concentrated solution of substrates,
- Rxn rate = $R_{\max} = k_{\max}$,

(Governing Eqn :)

$$\frac{D_s}{r^2} \frac{d}{dr} \left(r^2 \frac{dC_s}{dr} \right) = 0$$

Boundary Conditions:

$$r = R_E : -4\pi R_E^2 D_s \left(\frac{dC_s}{dr} \right) = k_{\max}$$

$$r \rightarrow \infty, C_s = C_{s0}$$

- solving the equation, we get

$$C_s = C_{s0} - \frac{a_1}{r}$$

$$u \sin g, b c's, a_1 = \frac{k_{\max}}{4\pi D_s},$$

$$C_s = C_{s0} - \frac{k_{\max}}{4\pi D_s}$$

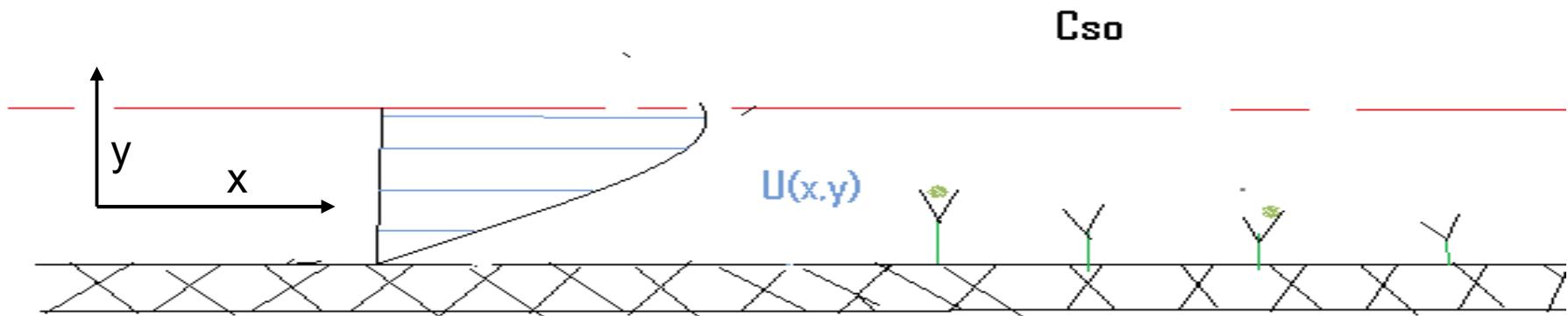
- Observed flux:

$$-4\pi R_E^2 D_S \left(\frac{dC_S}{dr} \right)_{r=R_E} = 4\pi D_S a_1 = k_{\max}$$

$$k_{\text{obs}} = k_{\max}$$

- No diffusion resistance hence system is reaction controlled.

Interphase mass transfer between enzyme receptors and substrate dissolved in moving fluid



Enzyme substrate binding occurs at the interface of the matrix and the solution following first order kinetics (i.e. dilute solution of substrate):
 $-R'' = k''C_s (x,y=0)$.

- (1) Solution: use Convection Diffusion Reaction (CDR) equation and solve.
- (2) use mass transfer coefficient concept.

- CDR equation:

$$D_s \frac{\partial^2 C_s}{\partial x^2} + D_s \frac{\partial^2 C_s}{\partial y^2} = u_x \frac{\partial C_s}{\partial x}$$

B.C's

$$y = 0, N_y = \frac{\partial C_s}{\partial y} = -k'' C_s$$

Other B.C's are same as in boundary layer problem

Method using mass transfer coefficient

$$N_y(y=0) = k_f(C_{S0} - C_s(at, y=0)) = k'' C_s(at, y=0)$$

- Using eqn 4,

$$k_f C_{S0} = (k'' + k_f) C_S \text{ (at } y = 0\text{)}$$

$$C_S \text{ (at } y = 0\text{)} = \frac{k_f C_{S0}}{k_f + k''}$$

$$R'' = k'' C_S \text{ (at } y = 0\text{)} = \frac{k_f k'' C_{S0}}{k_f + k''}$$

$$k_{obs} = \frac{k_f k''}{k_f + k''}$$

$$\frac{1}{K_{obs}} = \frac{1}{k_f} + \frac{1}{k''}$$

Resistances in series

- Time scales in the system

$$t_m = \frac{L}{k_f}; t_R = \frac{L}{k''}$$

$$Da = \frac{t_m}{t_R} = \frac{k''}{k_f}$$

$$-R'' = \frac{k''}{1+Da} C_{S0}$$

1) If $Da \ll 1$, reaction is very slow as compared to mass transfer
= Reaction controlled.

$$-R'' = k'' C_{S0}$$

2) If $Da \gg 1$ reaction is very fast as compared to mass transfer
= mass transfer controlled.

$$-R'' = k_F C_{S0}$$

Mass transfer coefficients for laminar flows

- Flat plate

$$\frac{k_{\text{obs}} Z}{D_m} = .323 \text{Re}^{0.5} \text{Sc}^{.333}$$

- Circular pipe

$$\frac{k_f D}{D_m} = 1.86 \text{Re}^{0.5} \text{Sc}^{.333}$$

- Forced convection around solid spheres:

$$\frac{k_f D}{D_m} = 2 + 0.6 \text{Re}^{0.5} \text{Sc}^{.333}$$

- Falling film

$$\frac{k_{\text{obs}} Z}{D_m} = .69 \text{Re}^{0.5} \text{Sc}^{.333}$$

Transport and reaction to enzyme encapsulated in tissue matrix: Intraphasic Mass Transfer

- Chemical reaction:

Heterogeneous reaction that occurs within a tissue or porous matrix containing immobilized enzymes are called “intraphasic” although the reaction occurs at the interphase between fluid and cell surface or at extracellular matrix the phases within the tissues is considered to be “pseudo homogeneous “if the reaction is macroscopically uniform.

- **reaction and diffusion occur parallelly .**
- if reaction is too fast as compared to diffusion the concentration at some places becomes zero because now its diffusion controlled.

- For drugs , biomolecules, therapeutic molecules, if the diffusion is too slow reaction rate could decrease significantly

$$D_{\text{eff}} \nabla^2 C = -R_v$$

$$\bar{R}_v =$$

**Macroscopic
rxn rate**

$$\bar{R}_v = \frac{1}{V} \int_V R_r dv$$

$$= - \frac{1}{V} \int_V D_{\text{eff}} \nabla^2 C dv$$

$$= - \frac{1}{V} \int_S D_{\text{eff}} n \cdot \nabla C ds$$

$$\eta = \frac{\bar{R}_v}{\bar{R}_v(C_s)}$$

$$C = C_s, \text{on}, s$$

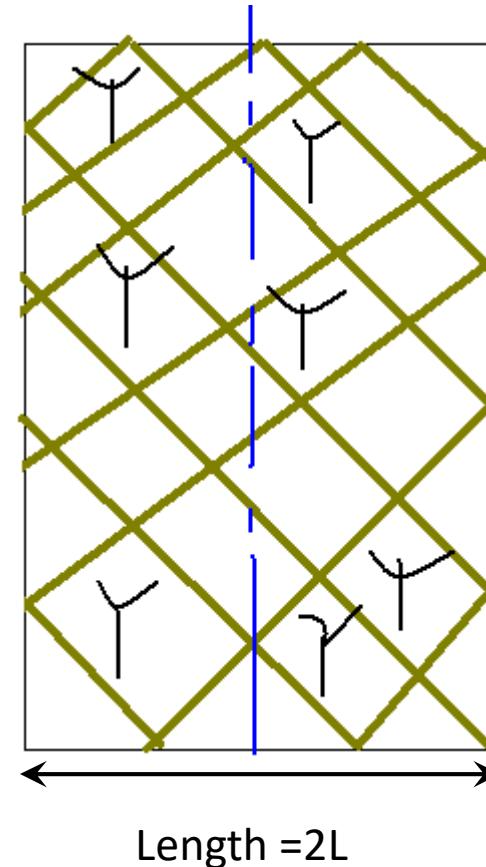
Intraphase Mass Transfer and Reaction in Tissue gel matrix (Rectangular Slab)

$$-R_V = k_V C$$

$$D_{\text{eff}} \nabla^2 C = k_V C$$

$$\text{BC1: } x = \pm L, C = k_{\text{av}} C_0$$

$$\text{B.C.2 : } x = 0, \frac{dC}{dx} = 0$$



Non dimensionalisation of Model Equation

$$\phi^2 = \frac{k_v L^2}{D_{\text{eff}}} = \frac{t_D}{t_R}$$

$$\hat{x} = \frac{x}{L}, \theta = \frac{C}{k_w C_0}$$

Dimensionless model

$$\frac{d^2\theta}{d\hat{x}^2} = \phi^2 \theta$$

$$\hat{x} = \pm 1, \theta = 1$$

$$\hat{x} = 0, \frac{d\theta}{d\hat{x}} = 0$$

Solution

$$\theta = A e^{\phi \hat{x}} + B e^{-\phi \hat{x}}$$

$$\theta = A(e^{\phi \hat{x}} + e^{-\phi \hat{x}})$$

$$= 2A \cosh(\phi \hat{x})$$

$$A = \frac{1}{2 \cosh(\phi)}$$

$$\theta = \frac{C}{k_{av} C_0} = \frac{\cosh(\phi \hat{x})}{\cosh(\phi)}$$

Calculation of η :

$$-R_v = \frac{1}{V} \int_v R_v dv$$

$$= \frac{1}{2L} \int_{-L}^L k_v c dx$$

$$= \frac{k_v}{L} \int_0^L C dx$$

$$= k_v k_{av} C_0 \int_0^1 \theta dx$$

$$= \frac{k_v k_{av} C_0}{\cosh(\phi)} \int_0^1 \cosh(\phi \hat{x}) d\hat{x}$$

$$= \frac{k_v k_{av} C_0 \tanh(\phi)}{\phi}$$

$$-R_V(c_s) = k_v k_{av} C_0$$

$$\eta = \frac{R_v}{R_V(c_s)} = \frac{\tanh(\phi)}{\phi}$$

Intraphase Mass Transfer and Reaction in Tissue gel matrix (Sphere)

$$\frac{D_{\text{eff}}}{r^2} \frac{d}{dr} \left(r^2 \frac{dc}{dr} \right) = k_v c$$

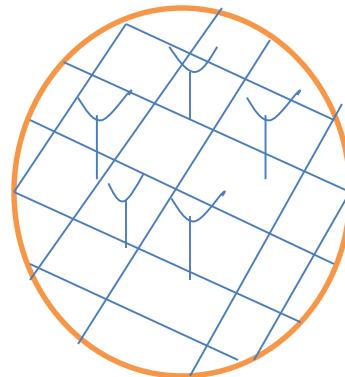
$$r = 0, \frac{dc}{dr} = 0$$

$$r = R_M, c = k_{av} c_0$$

$$\xi = \frac{r}{R_M}$$

$$\frac{1}{\xi^2} \frac{d}{d\xi} \left(\xi^2 \frac{dc}{d\xi} \right) = \phi^2 c$$

$$c = \frac{f}{\xi}$$



$$\frac{dc}{d\xi} = \frac{-f}{\xi^2} + \frac{1}{\xi} \frac{df}{d\xi}$$

$$\frac{d^2 f}{d\xi^2} = \phi^2 f$$

$$\frac{df}{d\xi} = \frac{f}{\xi} \text{ at } \xi = 0,$$

$$f = k_{av} c_0 \text{ at } \xi = 1.$$

$$f = A \sinh(\phi \xi) + B \cosh(\phi \xi)$$

$$f = A \sinh(\phi \xi) + B \cosh(\phi \xi)$$

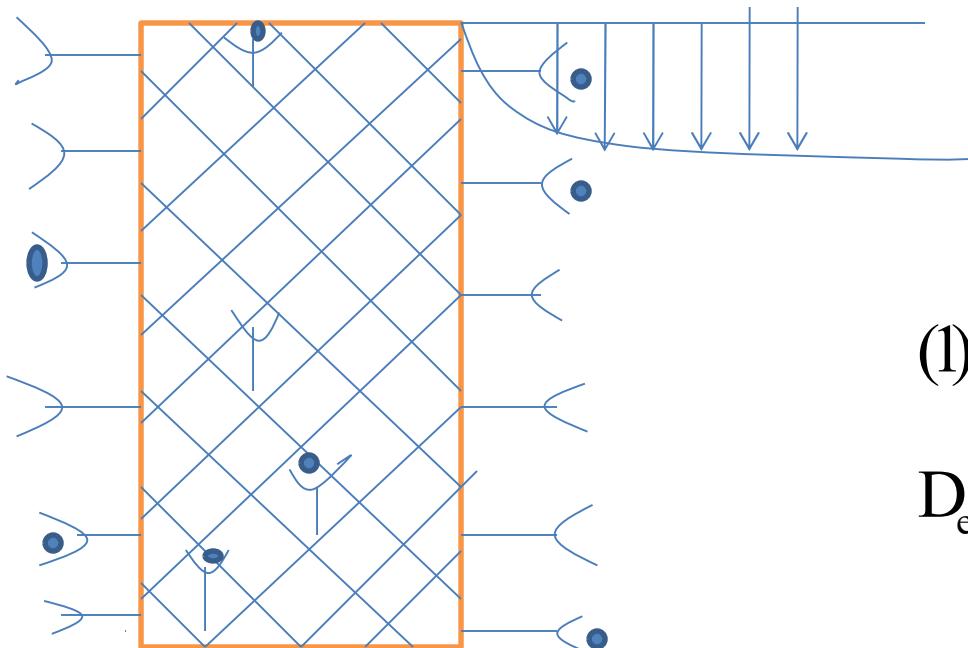
Using the B.C.'s we get

$$c = K_{av} c_0 \frac{\sinh(\phi \xi)}{\xi \sinh(\phi)}$$

$$\eta = \frac{\int_0^R cr^2 dr}{\frac{R^3}{3} K_{av} c_0} = \frac{3 \int_0^1 \sinh(\phi \xi) \xi d\xi}{\sinh(\phi)}$$

$$= \frac{3}{\phi} \left(\frac{1}{\tanh(\phi)} - \frac{1}{\phi} \right)$$

Gel/Tissue matrix with receptors on surface as well as encapsulated within



$$D_{\text{eff}} \frac{d^2 c}{dx^2} = k_v c$$

$$(1) x = \pm l,$$

$$D_{\text{eff}} \frac{dc}{dx} = k_f (c_0 - c(x = \pm l)) - k_s c(x = \pm L)$$

$$(2) \text{ at } x = 0, \frac{dc}{dx} = 0$$

- Dimensionless Variables

$$\theta = \frac{c}{c_0 k_{av}}, \hat{x} = \frac{x}{L}, \phi^2 = \frac{k_v L^2}{D_{eff}}$$

- Dimensionless Eqns:

$$\frac{d^2 \theta}{d \hat{x}^2} = \phi^2 \theta$$

$$(1) \hat{x} = 0, \frac{d\theta}{d\hat{x}} = 0$$

$$(2) \hat{x} = \pm 1, \frac{d\theta}{d\hat{x}} = Bi \{1 - \theta(x = \pm 1)\}$$

$$\theta = A \cosh(\phi \hat{x}) + B \sinh(\phi \hat{x})$$

$$Bi = \frac{k_f L}{D_{eff} k_{av}} = \frac{L^2 / D_{eff}}{\frac{L k_{av}}{k_f}} = \frac{t_{int \text{raphase.m.t}}}{t_{int \text{erphase.m.t}}}$$

$$B = 0,$$

$$\theta = A \cosh(\phi \hat{x}),$$

$$A \phi \sinh(\phi) = Bi(1 - A \cosh(\phi))$$

$$A = \frac{Bi}{\phi \sinh(\phi) + A \cosh(\phi)}$$

$$\theta = \frac{Bi \cosh(\phi \hat{x})}{\phi \sinh(\phi) + Bi \cosh(\phi)}$$

$$= \frac{Bi \sinh(\phi)}{\phi(\phi \sinh(\phi) + Bi \cosh(\phi))}$$

$$= k_v k_{av} c_0 \frac{\tanh(\phi)}{\phi \left(\frac{\phi}{Bi} \tanh(\phi) + 1 \right)}$$

$$-R_v(c_s) = k_v k_{av} c_0$$

$$= k_v k_{av} c_0 \int_0^1 \frac{Bi \cosh(\phi \hat{x}) d\hat{x}}{\phi \sinh(\phi) + Bi \cosh(\phi)}$$

$$\eta = \frac{-R_v}{-R_v(c_s)} = \frac{\tanh(\phi)}{\phi \left(\frac{\phi}{Bi} \tanh(\phi) + 1 \right)}$$

Definition of observed Thiele modulus

- $\Phi = \text{observed Thiele modulus} = \eta\phi^2$

$$\begin{aligned}\Phi &= \frac{R_v}{R_v(c_s)} \frac{k_v L^2}{D_{\text{eff}}} \\ &= \frac{R_v}{k_v k_{av} c_0} \frac{k_v L^2}{D_{\text{eff}}} \\ &= \frac{R_v L^2}{D_{\text{eff}} k_{av} c_0} \\ &= \frac{\text{observed reaction rate}}{\text{int raphase diffusion rate}}\end{aligned}$$

Generalized expression for η

$$\eta = \frac{\tanh(\phi)}{\phi}$$
$$\phi^2 = \frac{k_v L^2}{D_{\text{eff}}}$$

and $L = \frac{W}{2}$ for rectangular geometry of width w

$$\begin{aligned}&= \frac{R}{2} \text{ for cylinder} \\ &= \frac{R}{3} \text{ for sphere}\end{aligned}$$

Examination of limiting cases

for reaction limited reactions in tissueed matrix where

$$Bi \gg 1 \text{ & } \phi^2 \ll 1$$

$$\eta = \frac{\tanh(\phi)}{\phi} \approx \frac{\phi}{\phi} = 1$$

$$\Phi = \eta \phi^2 = \phi^2$$

if $\Phi \ll 1 \Rightarrow$ intraphase diffusion rate \gg observed reaction rate

\therefore diffusion not limiting $R_v = k_v k_{av} c_0$

- For diffusion limited reactions: $\text{Bi} \gg 1 \quad \phi^2 \gg 1$

$$\eta = \frac{\tanh(\phi)}{\phi} = \frac{1}{\phi} \quad (\because \text{for } \phi > 3, \tanh(\phi) \approx 1)$$

$$\Phi = \eta \phi^2$$

$$= \phi$$

$\because \phi^2 \gg 1, \Phi \gg 1$ (significant diffusion limited)

now for this case, observed reaction rate \gg diff rate.

$$\Phi = \phi$$

$$\frac{R_{v,obs} L^2}{D_{eff} k_{av} c_0} = L \sqrt{\frac{k_v}{D_{eff}}}$$

$$R_{v,obs} = \frac{\sqrt{k_v D_{eff}}}{L} k_{av} c_0$$

Intermediate case with finite Bi

$$\Phi = \eta \phi^2$$

$$= \frac{\tanh(\phi)}{\phi \left(\frac{\phi \tanh(\phi)}{\text{Bi}} + 1 \right)} \phi^2$$

$$= \frac{\phi \tanh(\phi)}{\left(\frac{\phi \tanh(\phi)}{\text{Bi}} + 1 \right)}$$

Bi is finite, $\phi > 3$

now for $\phi > 3$, $\tanh(\phi) = 1$

$$\Phi = \frac{Bi\phi}{Bi + \phi}$$

$$\frac{1}{\Phi} = \frac{1}{Bi} + \frac{1}{\phi}$$

substituting for each

$$\Phi = \frac{\frac{k_f L}{D_{eff} k_{av}} \cdot \sqrt{\frac{k_v}{D_{eff}} L}}{\frac{k_f L}{D_{eff} k_{av}} + \sqrt{\frac{k_v}{D_{eff}} L}}$$

$$R_v = \frac{k_f \sqrt{k_v D_{eff}} L}{k_f + k_{av} \sqrt{k_v D_{eff}}}$$

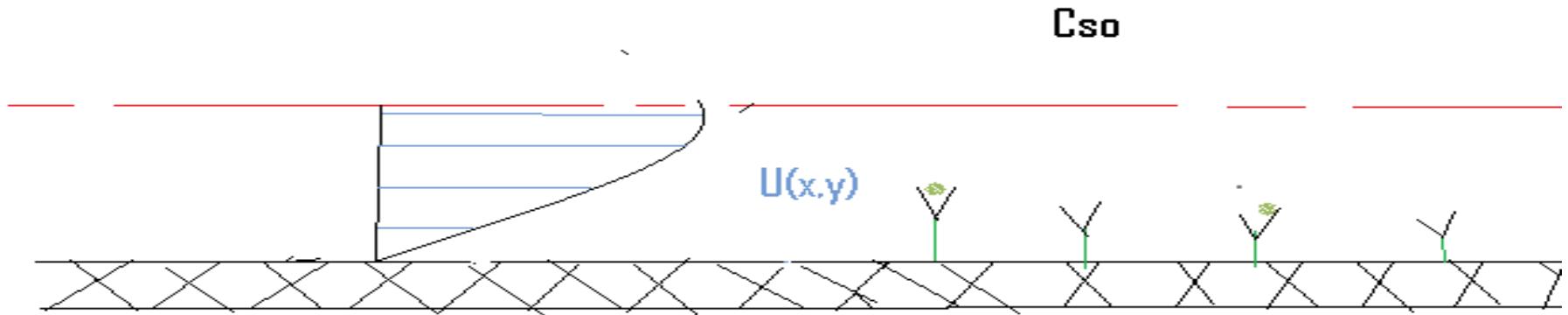
special case , (discussed before)

for $Bi \ll 1$, $\eta = Bi / \phi^2$

$$\therefore \Phi = \eta \phi^2 = Bi$$

$$R_v = \frac{D_{eff} k_{av} c_0 Bi}{L^2}$$

Derivations of models using Michaelis Menten Kinetics instead of first order kinetics (in case of interphase resistance)



$$N_y(y=0) = k_f(c_0 - c(y=0)) = \frac{R_{\max} "c(y=0)}{k_M + c(y=0)} \quad \theta = \frac{c(y=0)}{c_0},$$

$$1 - \theta = \frac{D a \theta}{1 + \beta \theta}$$

$$(1 - \theta)(1 + \beta \theta) = D a \theta$$

$$1 - \theta + \beta \theta - \beta \theta^2 = D a \theta$$

$$\beta = \frac{c_0}{k_M},$$

$$D a = \frac{R_{\max} "}{k_M k_f}$$

$$\beta\theta^2 + (\text{Da} - \beta + 1)\theta - 1 = 0$$

$$\theta = \frac{(\beta - \text{Da} - 1) + \sqrt{(\text{Da} - \beta + 1)^2 + 4\beta}}{2\beta}$$

$$\eta = \frac{\text{Rate}}{\text{Rate}(c_0)} = \frac{c(y=0)}{k_M + c(y=0)} \cdot \frac{K_M + c_0}{c_0} = \frac{\theta(1 + \beta)}{1 + \beta\theta}$$

now substitute,

$$\theta = \frac{(\beta - \text{Da} - 1) + \sqrt{(\text{Da} - \beta + 1)^2 + 4\beta}}{2\beta}$$

to obtain $\eta = \eta(\beta, \text{Da})$

limiting cases:

(1) $\beta \rightarrow 0$ (1st order)

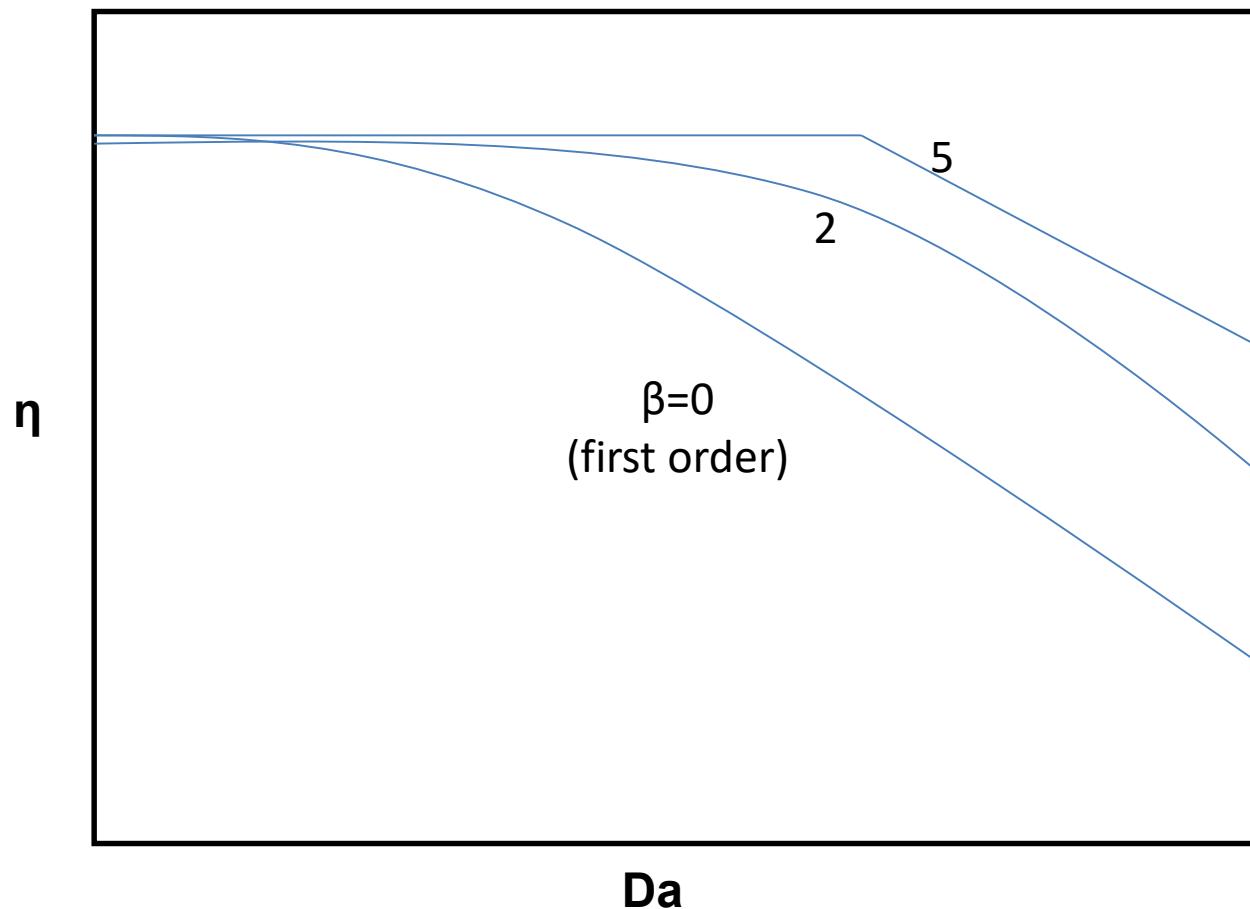
$$\theta \rightarrow \frac{1}{1 + Da} \quad (\text{use the quadratic form to obtain this}).$$

$$\eta = \frac{1}{1 + Da} \leftarrow (\text{check with model for 1}^{\text{st}} \text{ Rxn})$$

(2) $\beta \rightarrow \infty$ (zeroth order Rxn)

$\eta \rightarrow 1$ (\therefore rate is independent of Conc.)

(check with model for zeroth order Rxn)



Conclusion: the effect of M-M kinetics is to reduce the significance of fluid phase mass transfer effects until surface conc. declines below K_M