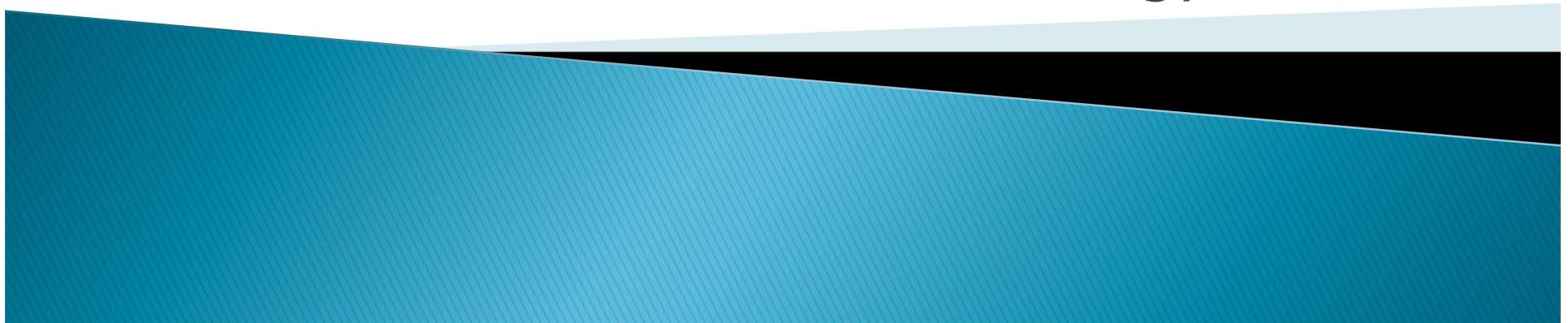


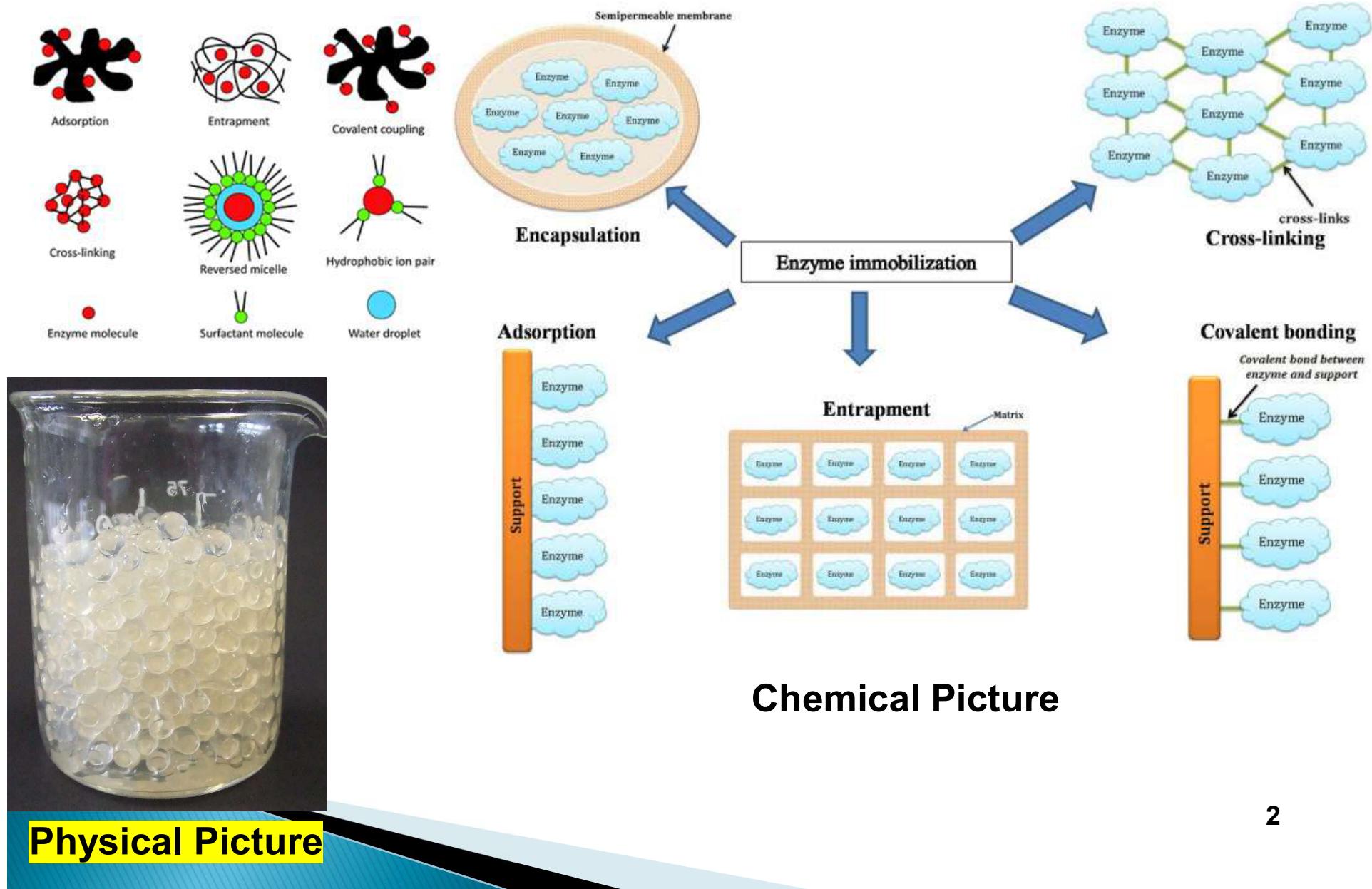
CH40001 Biochemical Engineering

Chapter 4. Immobilized Enzymes

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Indian Institute of Technology



Enzyme Immobilization Methods



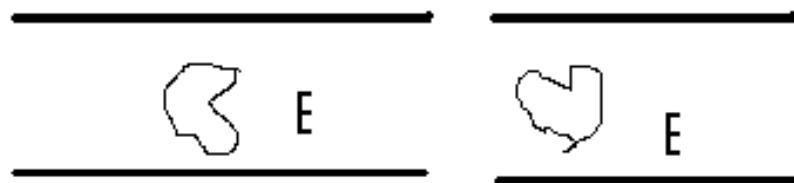
Application of Immobilized Enzymes

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

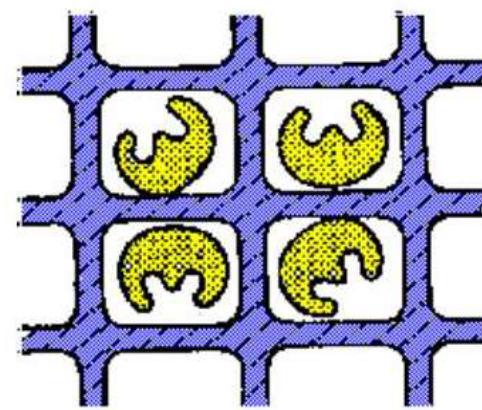
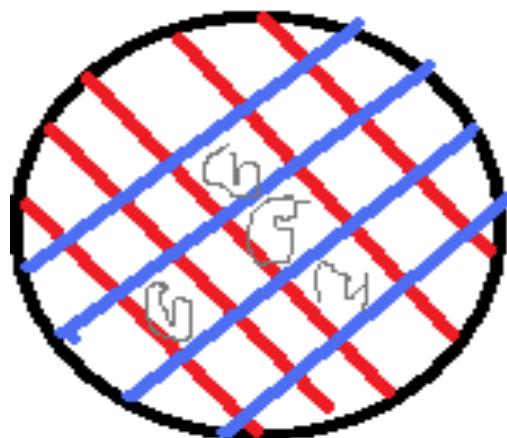
Advantages: (i) enzymes not susceptible to antibody attack; (ii) easy separation of enzymes from reaction mixture, allowing easy purification of products; (iii) re-use of enzymes across production cycles, which significantly reduces product costs.
- Disadvantage: increased mass transfer resistances and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

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

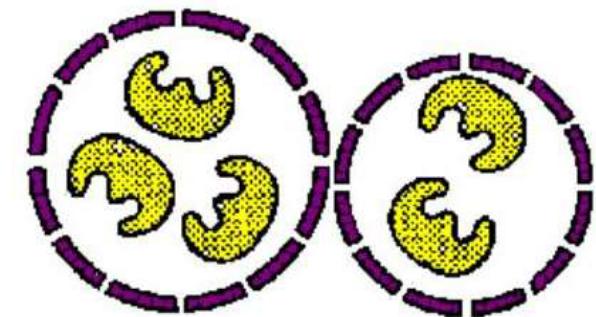
Physical entrapments of enzymes



Enzymes trapped in
hollow fiber membrane



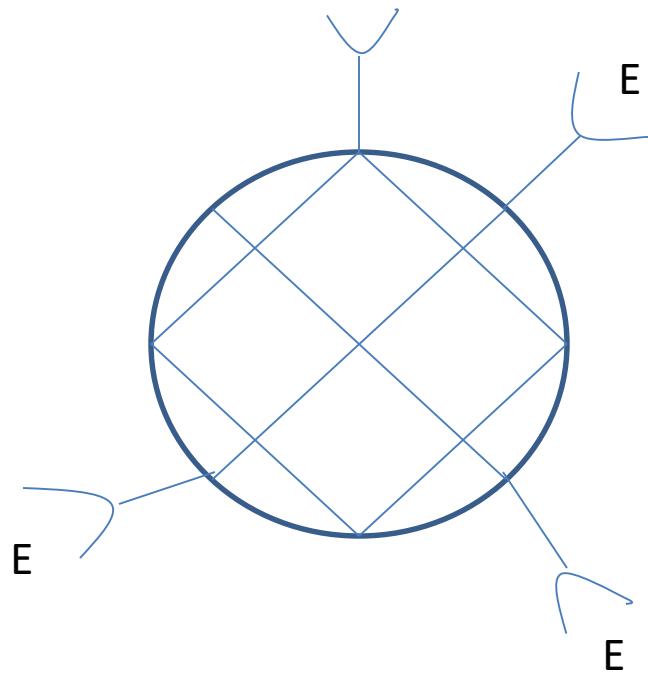
entrapped in a matrix



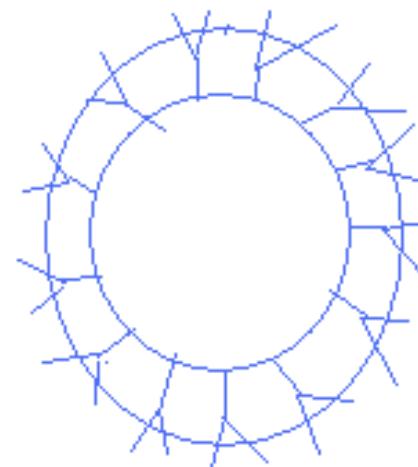
entrapped in droplets

Enzyme entrapment within insoluble gel matrix

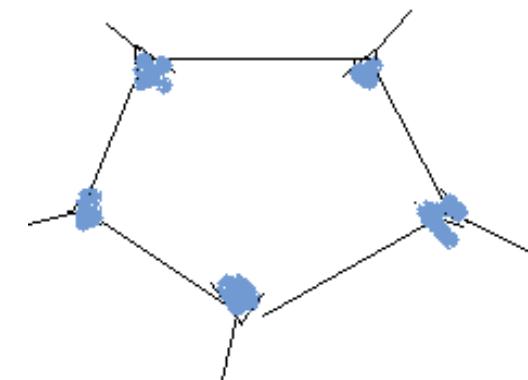
Enzyme Immobilization by Chemical Methods



Enzyme covalently attached to water-insoluble matrix

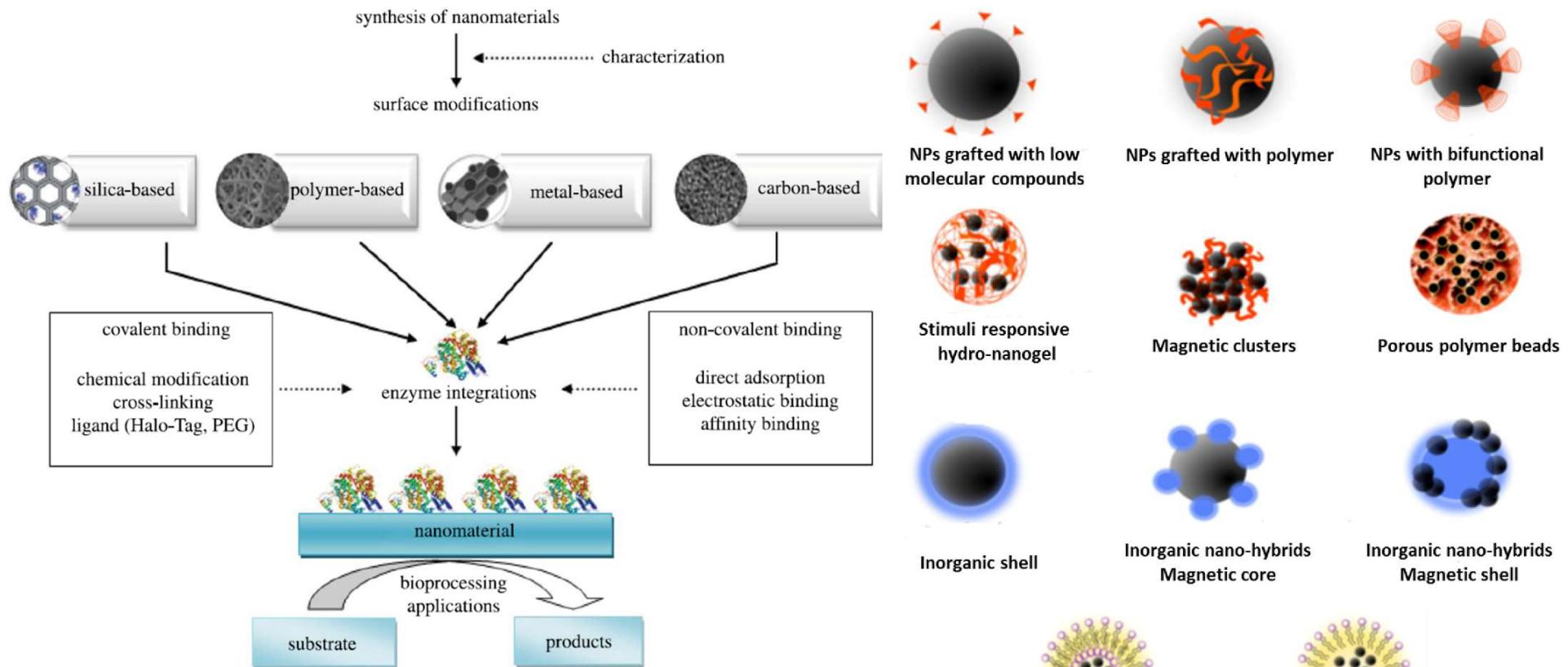


Cross linked enzyme using functional reagent.



Cross linked enzyme matrix

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

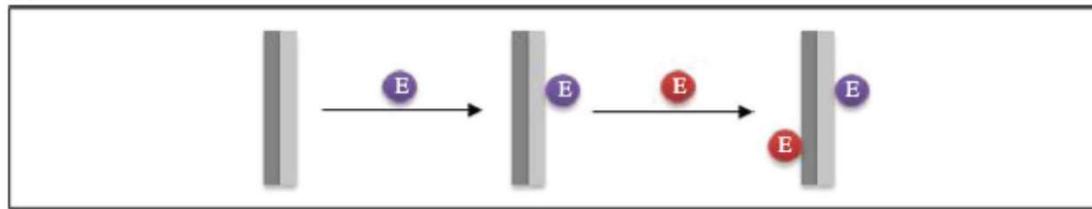


Misson M, Zhang H, Jin B. 2015 Nano-biocatalyst advancements and bioprocessing applications.

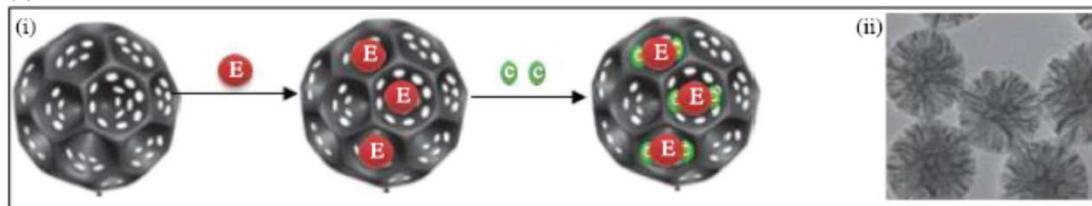
J. R. Soc. Interface 12: 20140891.

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

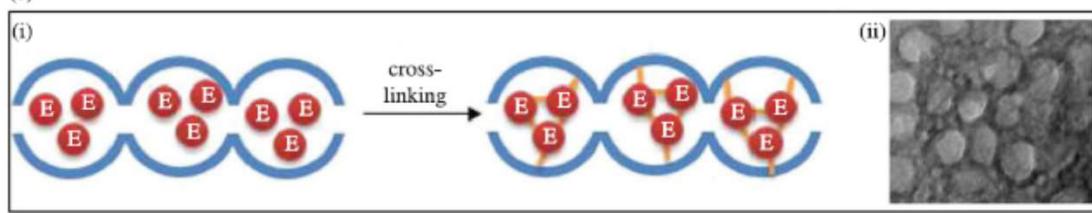
(a)



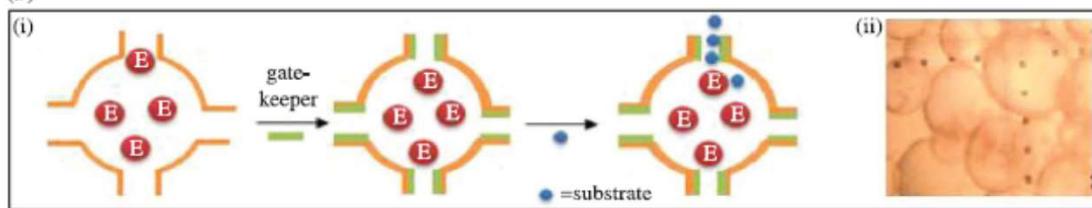
(b)



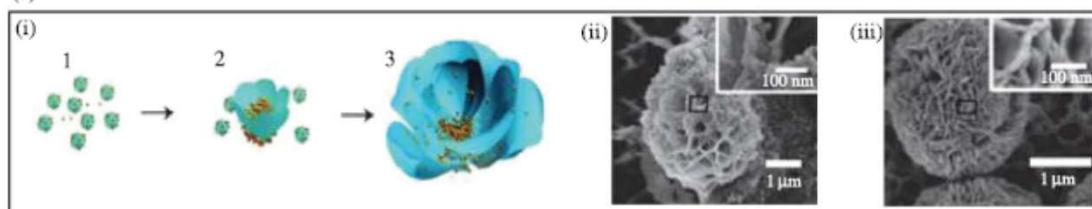
(c)



(d)



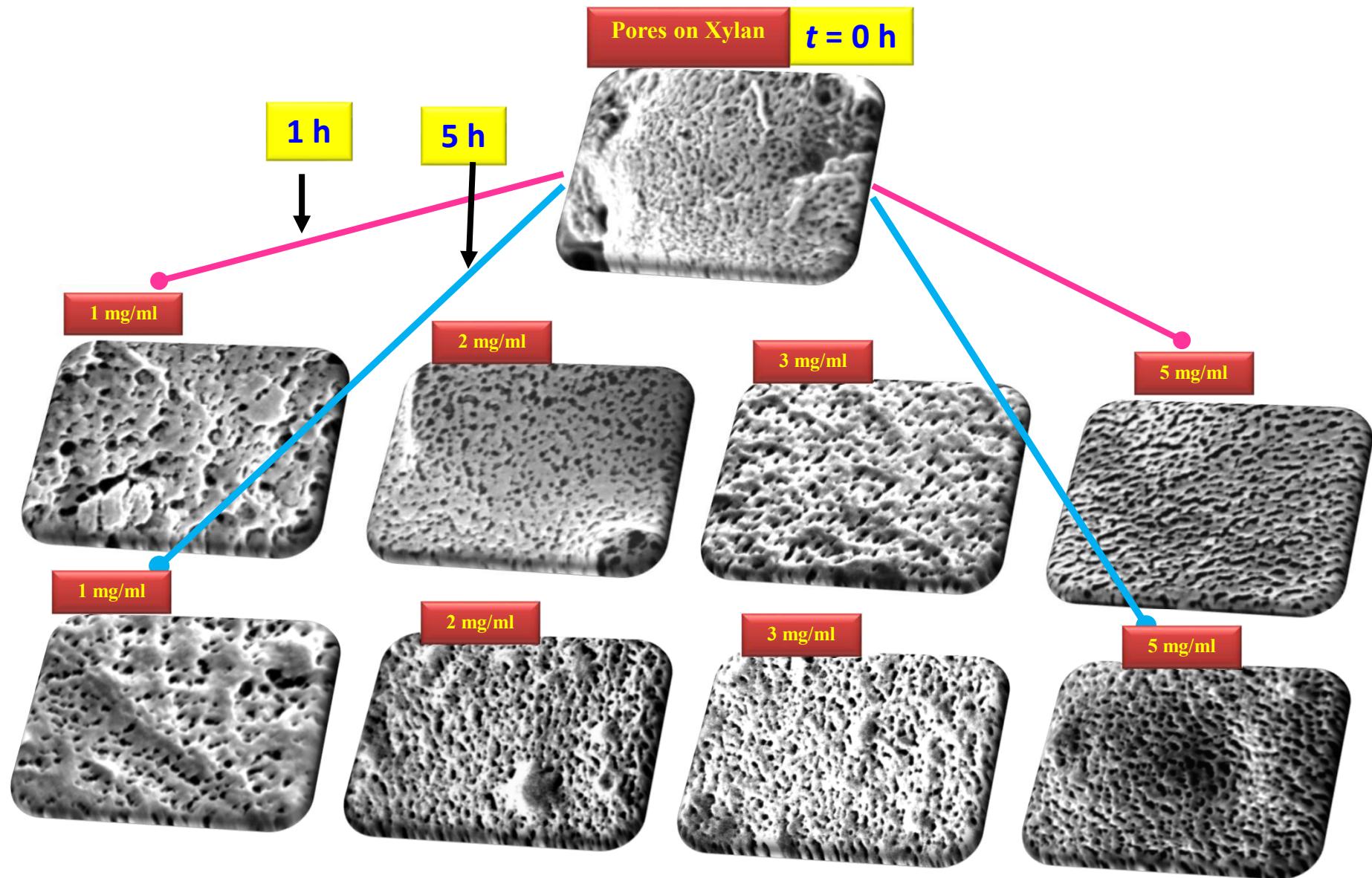
(e)



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>

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.)

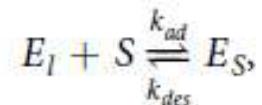
Dynamics of Pore-scale Enzyme Adsorption



Magnifications: 100K, Scale bar: 100 nm

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

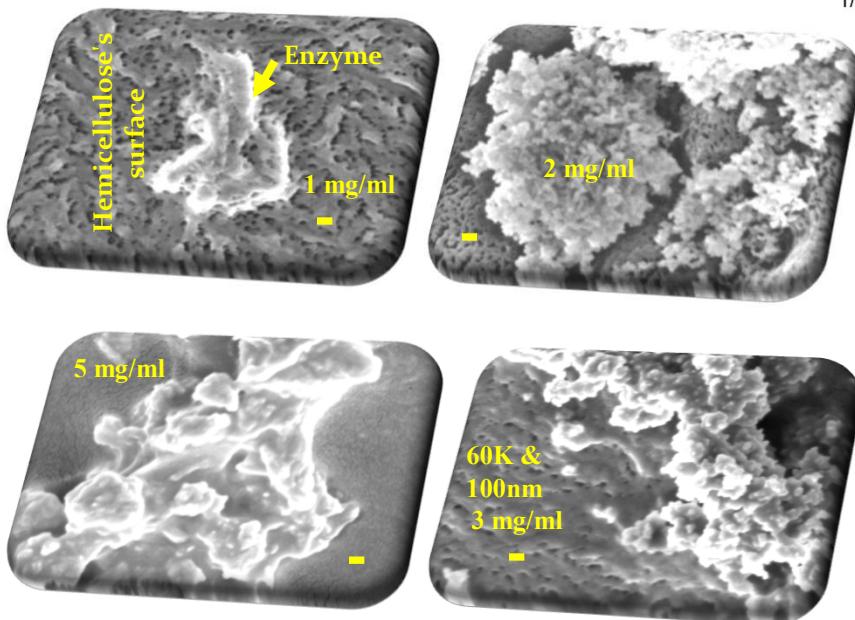
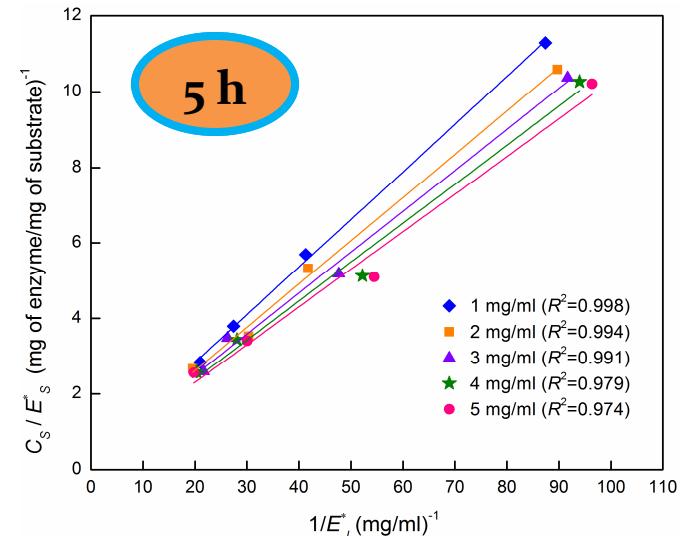
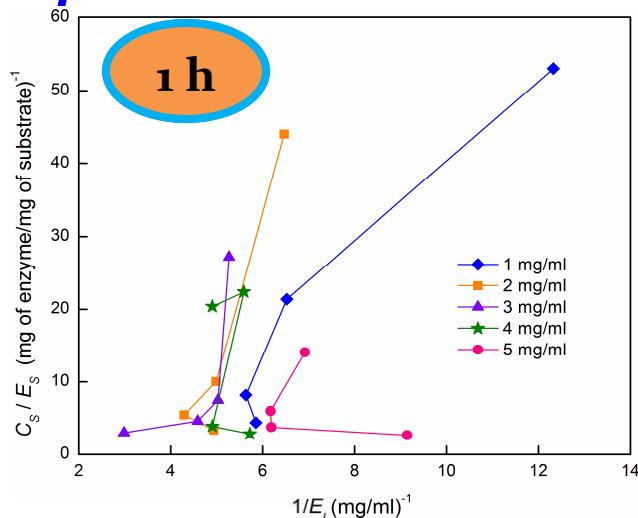
Equilibrium or non-equilibrium?

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

$E_o/C_s = 0.1-0.4$

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.

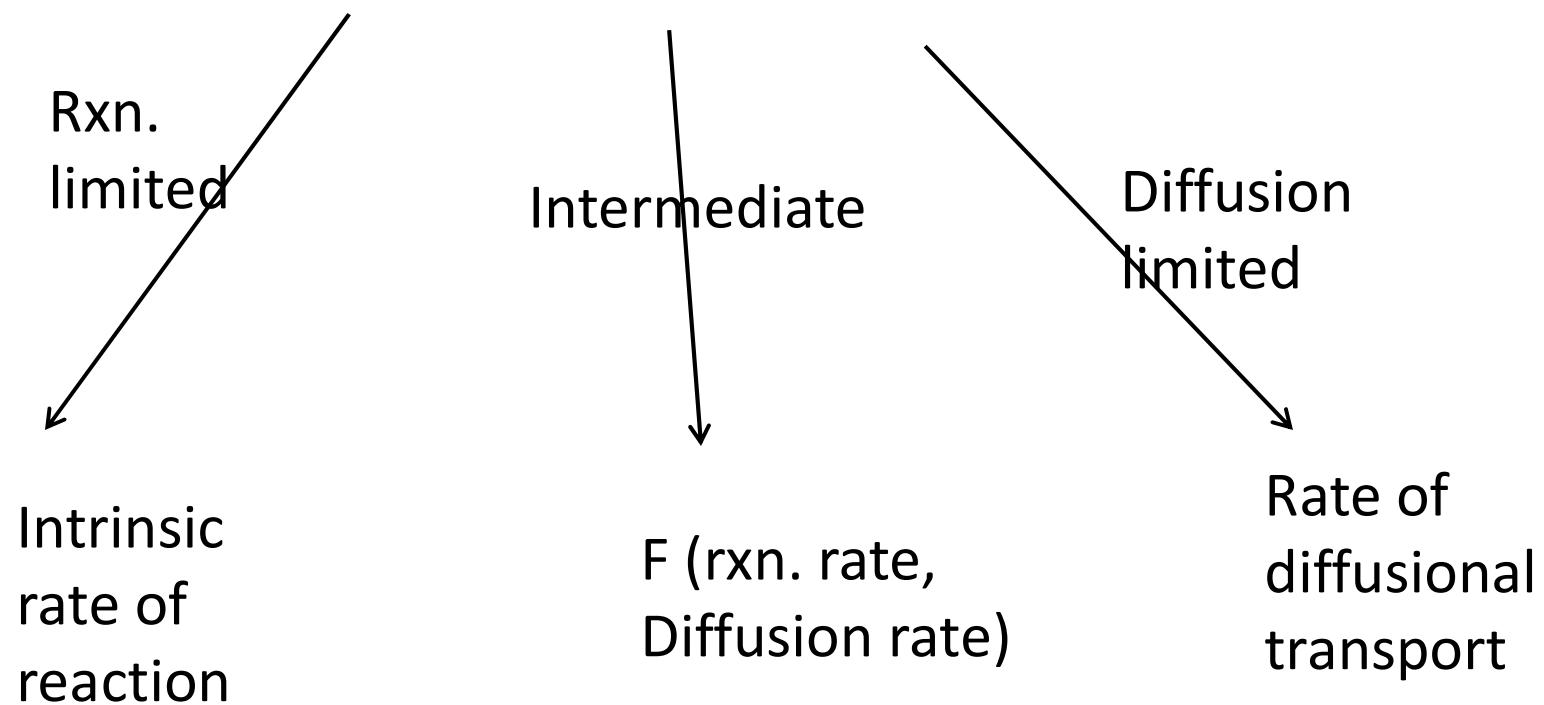
¹Zhang and Lynd, 2004. *Biotechnol. Bioeng.* 88, 797-824

Immobilized Enzyme kinetics (in presence of mass transfer resistances)

Biological reactions

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

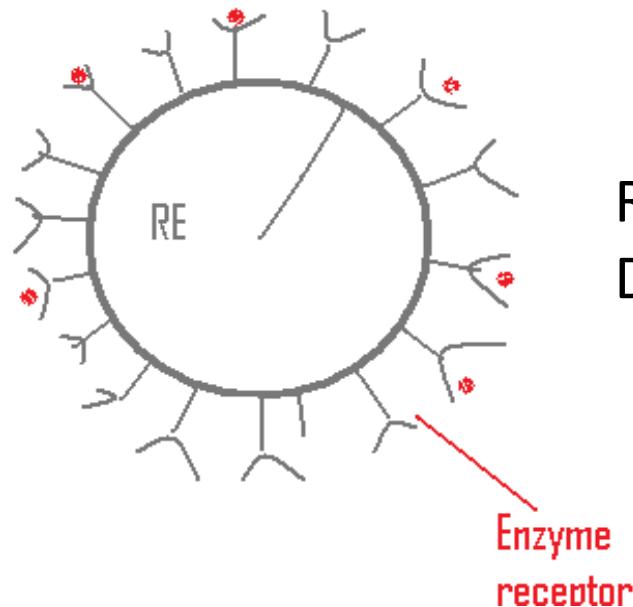
Observed rate of reaction



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):

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

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

$$= k_{obs} C_{s0}$$

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

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

- 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

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

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

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

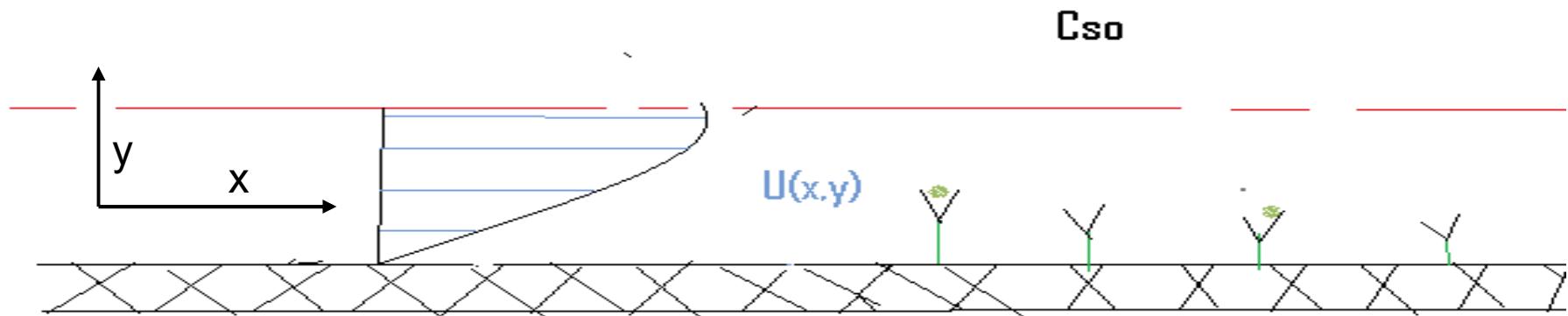
- Observed flux:

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

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

- 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,

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

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

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

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

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

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

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

- Circular pipe

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

- Forced convection around solid spheres:

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

- Falling film

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

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**

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

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

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

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

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

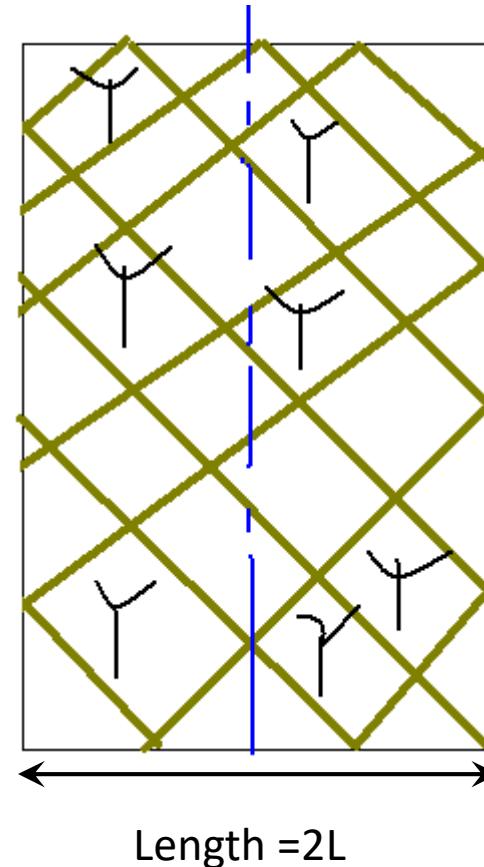
Intraphase Mass Transfer and Reaction in Tissue gel matrix (Rectangular Slab)

$$-R_V = k_V C$$

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

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

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



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)

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

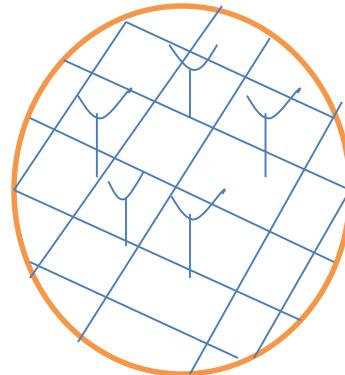
$$r = 0, \frac{dc}{dr} = 0$$

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

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

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

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



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

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

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

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

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

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

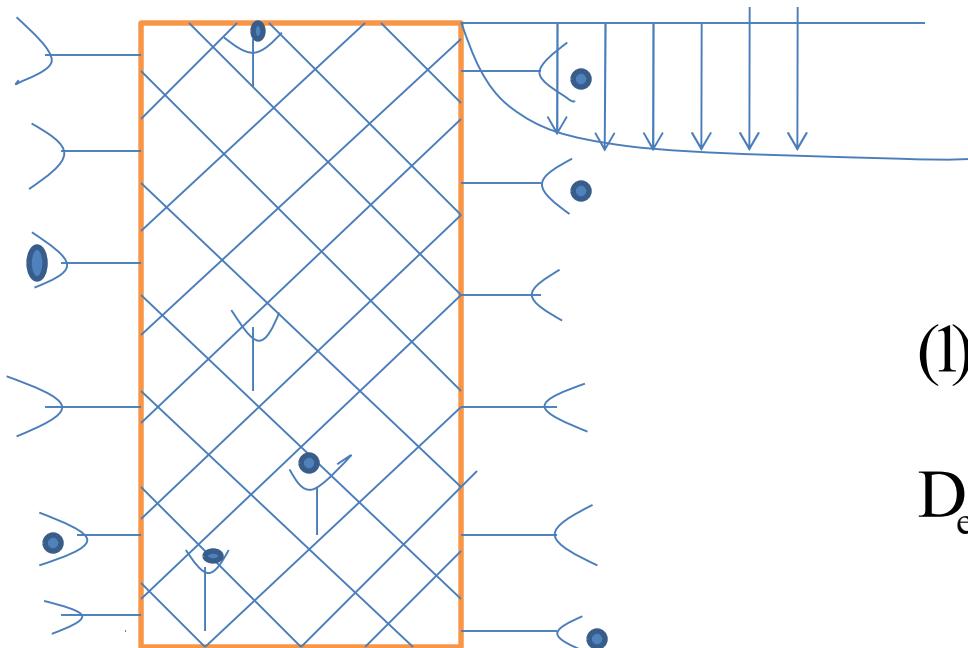
Using the B.C.'s we get

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

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

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

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

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

- Dimensionless Eqns:

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

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

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

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

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

$$B = 0,$$

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

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

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

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

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

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

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

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

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

Definition of observed Thiele modulus

- Φ = 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: $Bi >> 1 \quad \phi^2 >> 1$

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

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

$$= \phi$$

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

now for this case, observed reaction rate $>>$ 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

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

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

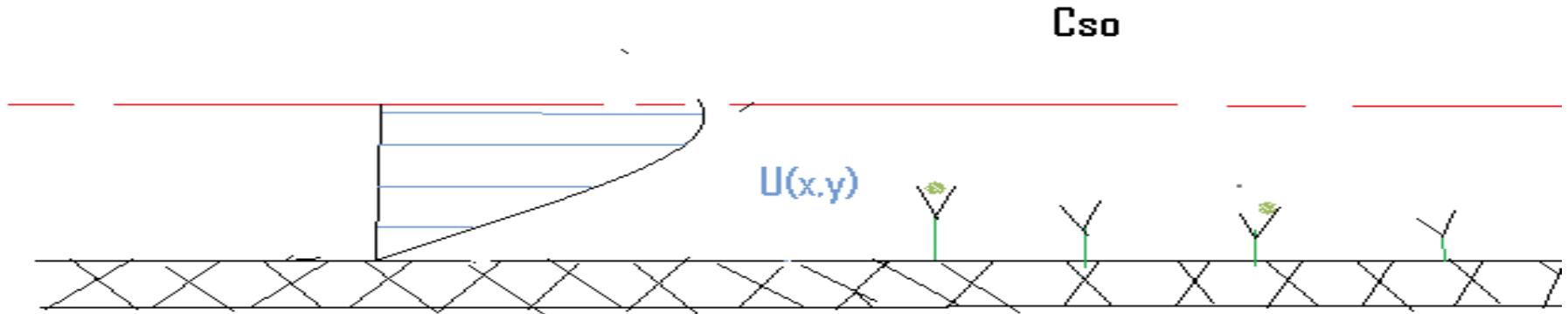
special case , (discussed before)

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)



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

$$1 - \theta = \frac{Da\theta}{1 + \beta\theta}$$

$$(1 - \theta)(1 + \beta\theta) = Da\theta$$

$$1 - \theta + \beta\theta - \beta\theta^2 = Da\theta$$

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

$$Da = \frac{R_{\max} "}{k_M k_f}$$

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

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

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

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)

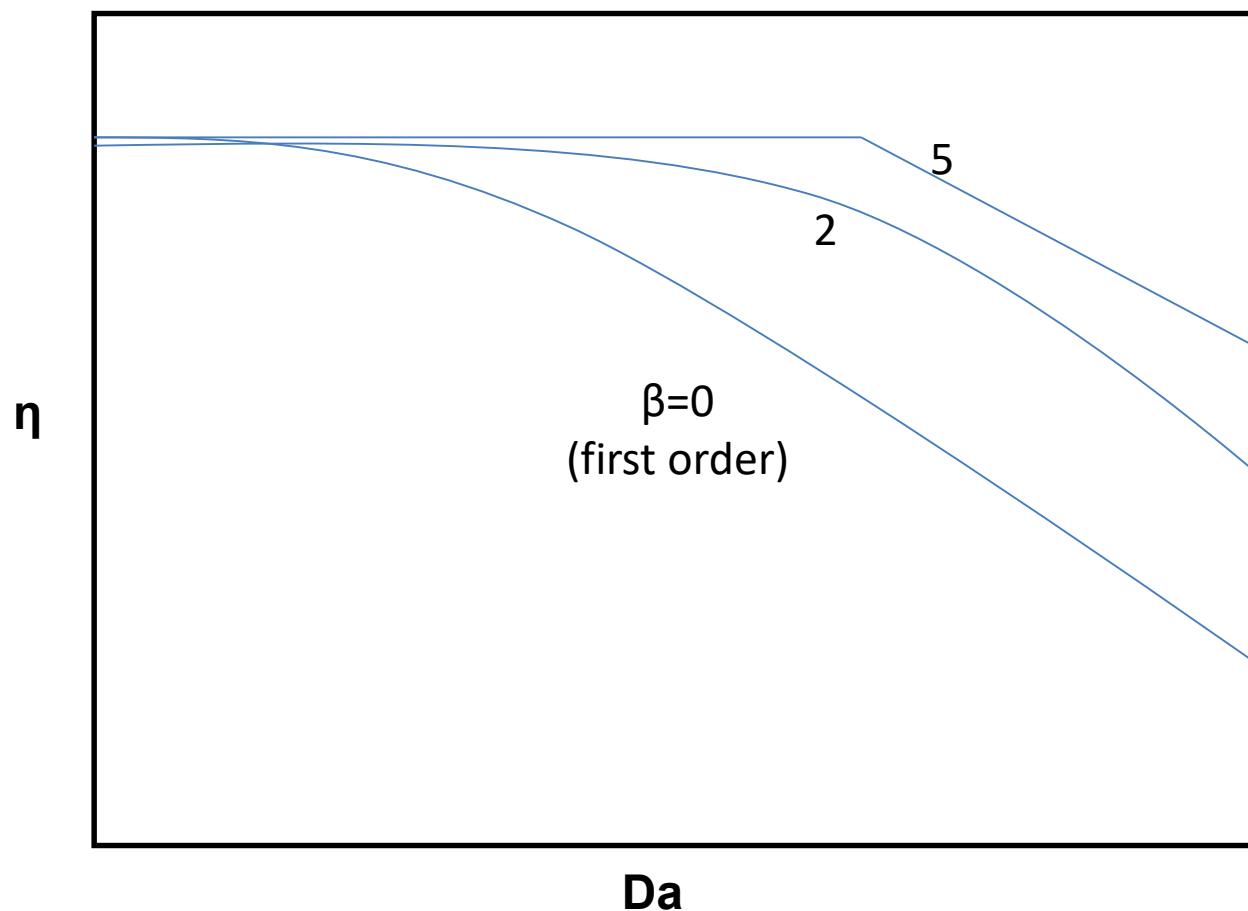
$$\theta \rightarrow \frac{1}{1 + Da} \quad (\text{use the quadratic form to obtain this}).$$

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

(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

CH40001 Biochemical Engineering

Chapter 5. Microbial Growth Models

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Algal Cultivation



Pictures of Bubble Column Algal Photobioreactors

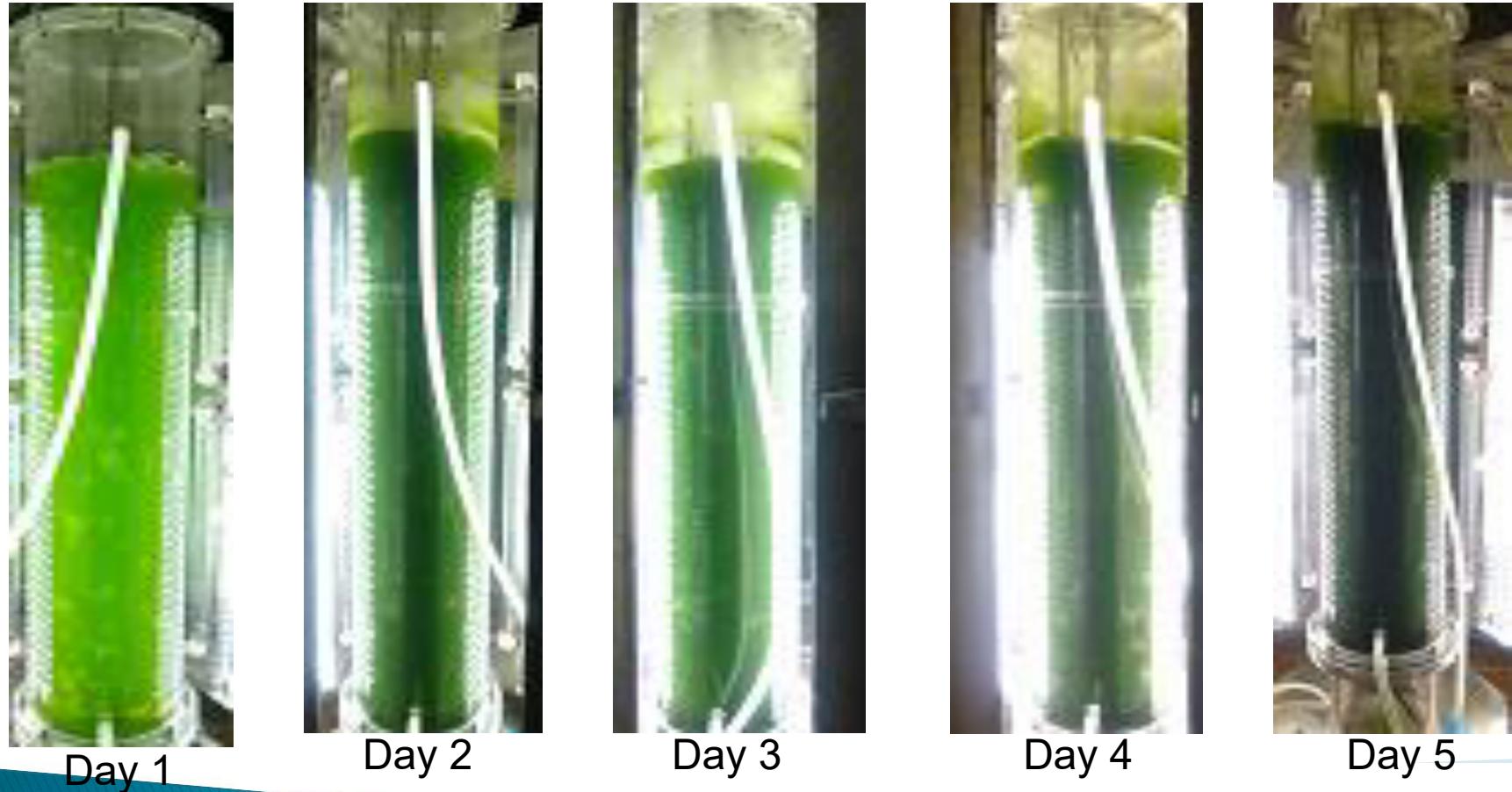


Fig 1: Growth of algae with time in bubble column pilot plant photobioreactor in mixotrophic conditions at atmospheric CO_2 concentrations

General reaction resulting in cell growth

- When cells are grown on ammonia :



$CH_a O_b N_c$ is the elemental composition of the cell.

$CH_l O_m$ is the elemental composition of the carbon source.

$CH_p O_q N_r$ is the elemental composition of the elemental products.

- In the process of cell growth,
 - ATP (“energy currency” of the cell),
 - NADPH (employed for cellular electron transport)are generated based on the oxidation of the carbon source $CH_l O_m$.

MICROBIAL GROWTH

- ▶ Cell Cycle – Various events that occur during the growth of a single cell from its inception till its time of division into daughter cells are referred to as the cell cycle.
 1. **M-phase** : Nucleus division (mitosis) occurs.
 2. **Inter-phase** : Daughter cells formed from cell division(mitosis) enter G phase.
 3. **G₁-phase** : High rate of biosynthesis.
 4. **S-phase** : DNA synthesis occurs till the DNA content of the cell has doubled.
 5. **G₂-phase**: Initiation of mitosis.
(next: repeat the sequence from step 1)
- ▶ Nomenclature:
 r_X : rate of cell growth (volumetric rate of increase of cell concentration X)
X : Cell concentration (usually dry cell weight per volume)
 μ : specific rate = r_X/X

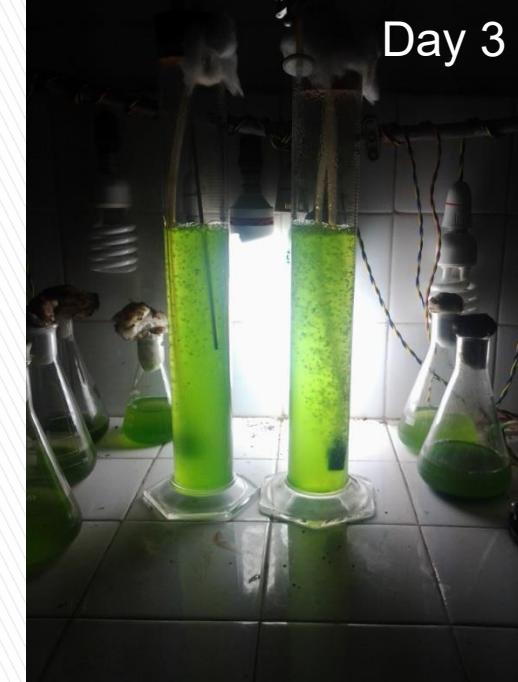
Day 1



Day 2



Day 3



Multiple Substrates = Acetic Acid + Carbon dioxide,

Mixotrophic Growth (= Autotrophic + Heterotrophic)

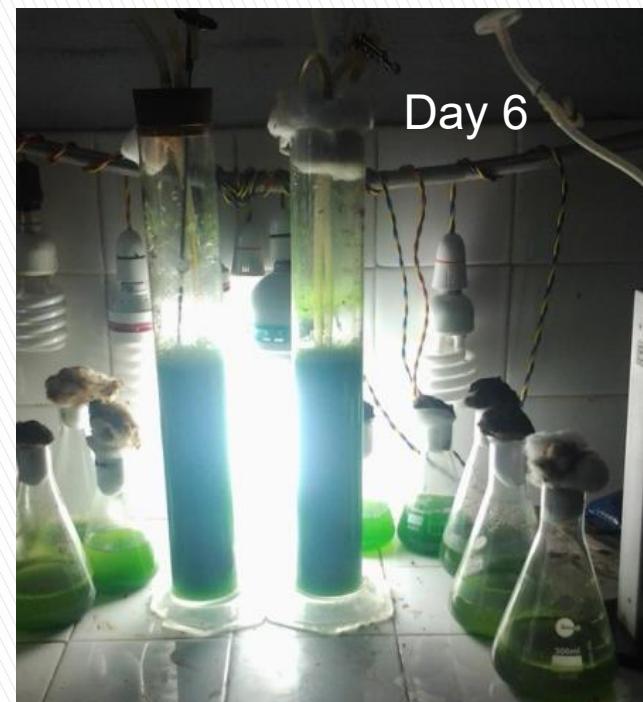
Day 4



Day 5



Day 6



Types of Growth Kinetic Models

- ▶ Structured model: recognizing the components in cells in response to the environment.
- ▶ Unstructured model: assuming fixed cell composition (balanced-growth).
- ▶ Segregation model: segregating the culture into individual units (cells) that may differ from each other.
- ▶ Nonsegregation model: cells are the same in the culture.

Unstructured Models

- Unstructured model: assuming fixed cell composition.

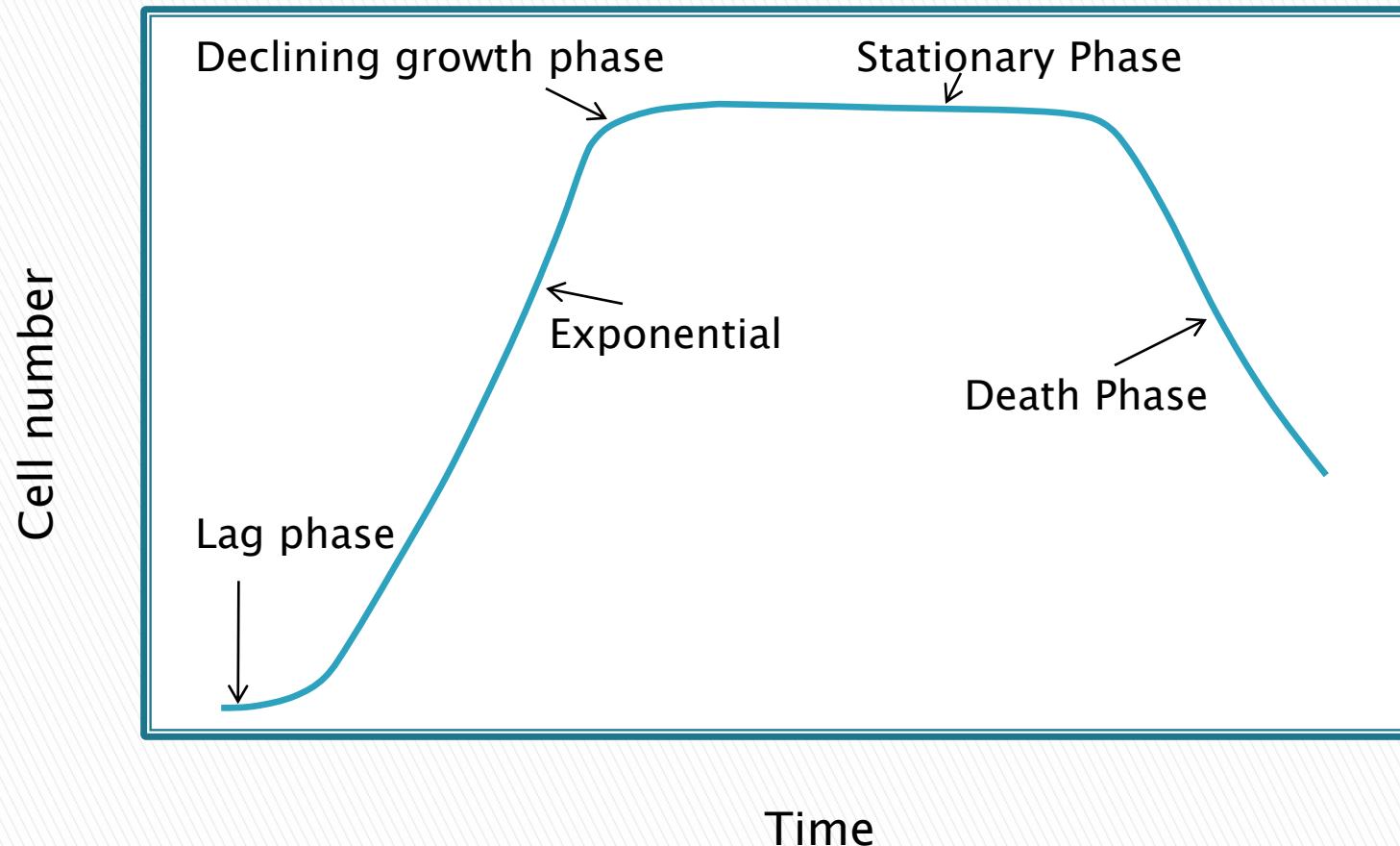
Applicable to balanced-growth condition:

- exponential growth phase in batch culture
- single-stage, steady state continuous culture
- cell response is fast compared to external changes
- the magnitude of the external changes is not too large (e.g. 10%-20% variation from initial conditions).

- Nonsegregation model: assuming all cells are the same in the culture.

Satisfactory under most circumstances.

Typical Microbial growth in a batch reactor



Malthusian Model for Cell Growth

- ▶ During exponential phase in a batch reactor,

$$\frac{dX}{dt} = \mu X \quad \mu = \text{growth rate of cells}$$

Initial Condition: $X = X_0, t = t_{lag}$

$$X = X_0 e^{\mu(t - t_{lag})}$$

$$\text{or, } \ln\left(\frac{X}{X_0}\right) = \mu(t - t_{lag})$$

$$t_d = \frac{\ln 2}{\mu} \quad (\text{Doubling time})$$

$$\nu = \frac{1}{N} \frac{dN}{dt} \quad \text{or,} \quad \mu = \frac{1}{X} \frac{dX}{dt}$$

Monod Growth model

- Monod Growth Equation: unstructured and non-segregation model

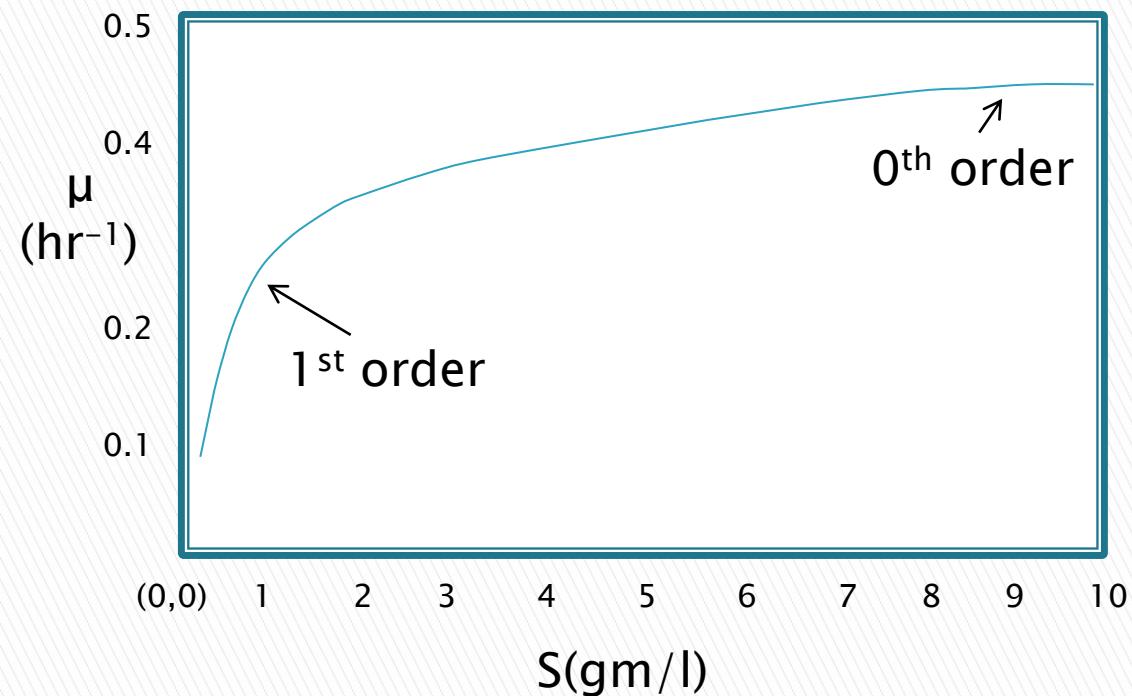
Assumption:

- a single enzyme system with Michaelis-Menten kinetics is responsible for uptake of substrate S, and the amount of that enzyme or its activity is sufficient low to be growth-rate limiting.
- the relationship of specific growth rate to substrate concentration assumes the form of saturation kinetics.
- a single chemical species is growth-rate limiting while changes in other nutrient concentrations have no effect.

Applicable when growth is slow and population density is low.

iii. Monod model (J Monod, Ann. Inst. Pasteur: 79, 390 (1950))

$$\mu = \frac{\mu_{\max} S}{K_S + S} \quad (\text{specific growth rate is substrate dependent})$$



$$\frac{dX}{dt} = \mu X = \frac{\mu_{\max} S X}{K_S + S}$$

μ has a Michaelis-Menton kinetics

$$\mu = \frac{\mu_{\max} S}{K_S} \quad (1^{\text{st}} \text{ order}) \text{ for } S \ll K_S$$

$$= \mu_{\max} \quad (0^{\text{th}} \text{ order}) \text{ for } S \gg K_S$$

Values of μ_{\max} and K_s for various organisms and substrates (at optimum temperature)

Organism &Growth Temperature	Limiting Nutrient	μ_{\max} (hr ⁻¹)	K_s (mg/lit)
Escherichia Coli (37°C)	Glucose	0.8–0.14	2–4
Escherichia Coli (37°C)	Glycerol	0.87	2
Escherichia Coli (37°C)	Lactose	0.8	20
Sacromyces Cerevisiae (30°C)	Glucose	0.5–0.6	25
Candida Tropicallis (30°C)	Glucose	0.5	25–75
Klebsiella Aerogenes	Glycerol	0.85	9

Values of activation energy for various microorganisms

Organism	Temp($^{\circ}$ C)	E_A , Kcal/mole
Aspergillus Nidulans	20-37	14
E. Coli	23-37	13.1
Klebsiella Aerogenes	20-40	14.2
Psychrophillic Pseudomonad	2-12	23.8

Models for cell-growth

i. Malthusian Model:

$$r_X = \mu X = \frac{dX}{dt} \text{ (for batch reactor)}$$

$$X = X_0 e^{\mu(t-t_{lag})}$$

Shortcoming: predicts unlimited growth.

ii. Logistic model:

To overcome this shortcoming, Verhulst (1844) and Pearl & Reed (1920) proposed the addition of a cell-concentration dependent second term:

$$r_X = kX(1 - \beta X)$$

For a batch system,

$$\frac{dX}{dt} = kX(1 - \beta X) \text{ with } X = X_0 \text{ at } t = 0.$$

$$\Rightarrow X = \frac{X_0 e^{kt}}{1 - \beta X_0 (1 - e^{kt})} \text{ (logistic eqn)}$$

iv. Modified Monod Model:

It is found experimentally the rate of growth decreases at high values of initial substrate concentration S_0 .

$$\mu = \frac{\mu_{\max} S}{K_S + K_S S_0 + S}$$

v. Konak Model(1974):

$$\frac{d\mu}{dS} = k(\mu_{\max} - \mu)^p$$

where p, k are adjustable parameters.

when $p=1$,

$$\mu = \mu_{\max} (1 - e^{ks}) \quad \text{Tiessier equation}$$

for $p \neq 1$,

$$\mu_{\max}^{1-p} - (\mu_{\max} - \mu)^{1-p} = (1 - p)kS$$

Above eqn. \rightarrow Monod model for $p=2$

$$\mu = \frac{\mu_{\max} S}{\frac{\mu_{\max}}{k} + S}$$

Other Types of Growth Kinetics

► Substrate Inhibition

$$\mu = \frac{\mu_{\max} S}{K_S + S + S^2 / K_I}$$

$$\frac{dX}{dt} = \mu X = \frac{\mu_{\max} S X}{K_S + S + S^2 / K_I}$$

Here, K_I is the Haldane or substrate inhibition coefficient

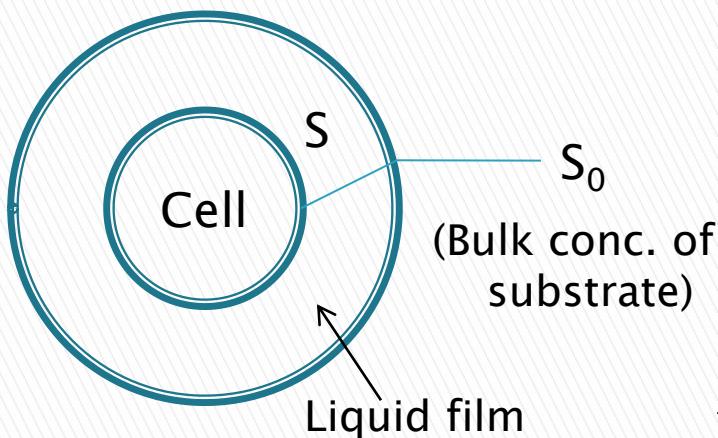
► Product Inhibition

$$\mu = \frac{\mu_{\max} S}{K_S + S} \left[1 - \left(\frac{[P]}{[P^*]} \right)^n \right],$$

Where $[P]$ is the ethanol conc., n is a determinable constant, $[P]^*$ is the critical ethanol conc. above which cells cease to grow (~ 10 gm/lit for *S. cerevisiae*).

Coupling of Mass Transfer & Monod Kinetics

$$\text{Rate of substrate transport} = k_L a' \left(\frac{X}{\rho_{cell}} \right) (S_0 - S)$$



$$\text{Rate of substrate uptake} = qX = \frac{q_{\max}SX}{K_s + S}$$

Equating these two rates:

$$\frac{q_{\max}SX}{K_s + S} = k_L a' \left(\frac{X}{\rho_{cell}} \right) (S_0 - S)$$

$$\text{Noting that, } q = \frac{q_{\max} S}{K_s + S} \Rightarrow S = \frac{q K_s}{q_{\max} - q}$$

The above equation could be solved for S_0 in terms of q and transport properties as

$$S_0 = \frac{q K_s}{q_{\max} - q} + \frac{q \rho_{cell}}{k_L a'}$$

Note : $Y_{X/S}$ = yield of $X/S = \frac{\mu}{q}$

$$S_0 = \frac{\mu K_s}{\mu_{\max} - \mu} + \frac{\mu \rho_{cell}}{k_L a' Y_{X/S}}$$

The last equation is a quadratic which could be solved for μ if determinant > 0 .

$$\Rightarrow 4S_0 \left(\frac{\mu_{\max} \rho_{cell}}{k_L a' Y_{X/S}} \right) < \left[S_0 + K_s + \left(\frac{\mu_{\max} \rho_{cell}}{k_L a' Y_{X/S}} \right) \right]^2$$

When the above condition is satisfied ,

μ (after binomial expansion and considering the first term)

$$\mu_{MT} = \mu_{\max} \frac{S_0}{K_s + S_0 + \frac{\mu_{\max} \rho_{cell}}{k_L a' Y_{X/S}}} = \mu_{\max} \frac{S_0}{K_{app} + S_0}$$

$$\text{where, } K_{app} = K_s + \frac{\mu_{\max} \rho_{cell}}{k_L a' Y_{X/S}}$$

In the absence of mass transfer resistance (Monod model)

$$\mu_M = \mu_{\max} \frac{S_0}{K_s + S_0}$$

Now, $\because K_{app} > K_s$

$$\therefore \mu_M > \mu_{MT}$$

Growth of Fungal Colony

- ▶ Fungi growth often show a constant rate of increase of radius of the mold colony, which could be expressed as

$$\frac{dr}{dt} = K, \text{ where } K \text{ is a constant.}$$

At any instant 't', the volume of the colony is given by (for *cylindrical mold*)

$$X = \pi r^2 h \rho$$

$$\frac{dX}{dt} = 2\pi r h \rho \frac{dr}{dt} = 2\pi \sqrt{\frac{X}{\pi h \rho}} h \rho K = 2\sqrt{\pi h \rho X} K$$

At $t = 0, X = X_0$,

$$\text{Integrating, } X = (\lambda t + X_0^{1/2})^2 \quad \text{Where, } \lambda = K\sqrt{\pi h \rho}$$

► Spherical Mold:

$$X = \frac{4}{3} \pi r^3 \rho$$

$$\frac{dX}{dt} = 4\pi r^2 \rho \frac{dr}{dt} = \frac{4}{3} \pi \rho K \left(\frac{3X}{4\pi \rho} \right)^{2/3}$$

At $t = 0, X = X_0$,

Integrating, $X = \left(\frac{\gamma t}{3} + X_0^{1/3} \right)^3$ Where, $\gamma = K(36\pi\rho)^{1/3}$

Diffusion of oxygen/nutrients in the fungal pellet

$$\frac{1}{r} \frac{d}{dr} \left(r \frac{dC_{O_2}}{dr} \right) = \frac{R^2 \left(\frac{v_{\max}}{K_M D_{eff}} \right) C_{O_2}}{1 + \beta C_{O_2}} \quad (\text{dimensionless equation})$$

If, $C_{O_2} \gg K_M$ (nutrients present in abundance)

$$\frac{1}{r} \frac{d}{dr} \left(r \frac{dC_{O_2}}{dr} \right) = \frac{R^2 v_{\max}}{S_0 D_{eff}}$$

with B.C.s $C_{O_2}(\bar{r} = 1) = 1, \frac{dC_{O_2}}{d\bar{r}}(\bar{r} = 0) = 0.$

Solution:
$$C_{O_2} = 1 - \frac{R^2 v_{\max}}{6S_0 D_{eff}} \left[1 - \left(\frac{r}{R} \right)^2 \right]$$

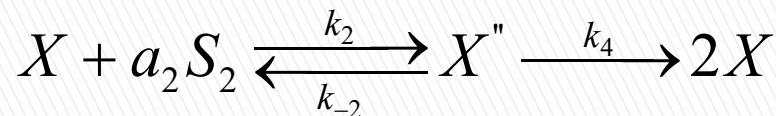
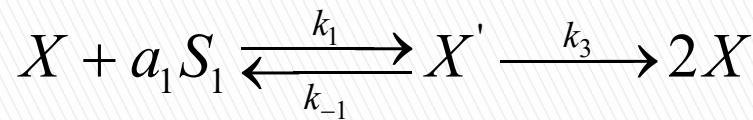
- ▶ Please note the number 6 must be replaced by 4 in all equations on this slide.
- ▶ At critical radius (R_{crit}), when the center of the pellet is depleted of oxygen, growth stops.

When $R = R_{crit}$, $C_{O_2} = 0$ at $r = 0$

$$\frac{R_{crit}^2 v_{\max}}{6S_0 D_{eff}} = 1 \quad \Rightarrow \quad R_{crit} = \sqrt{\frac{6S_0 D_{eff}}{v_{\max}}}$$

Multiple Substrates and Models

- ▶ Multiple Substrates:



Where, $Y_{X/S_1} = \frac{1}{a_1}$, $Y_{X/S_2} = \frac{1}{a_2}$

Balance Equations:

$$\frac{dX'}{dt} = k_1 X S_1 - k_{-1} X' - k_3 X'$$

$$\frac{dX''}{dt} = k_2 X S_2 - k_{-2} X'' - k_4 X''$$

Constraint Equation: $X_T = X' + X''$

Pseudo-steady state approximation: $\frac{dX'}{dt} = 0, \frac{dX''}{dt} = 0.$

$$\frac{dX}{dt} = \frac{dX_T}{dt} = k_3 X' + k_4 X''$$

$$\therefore \frac{dX'}{dt} = 0 \Rightarrow X' = \frac{k_1 X S_1}{k_{-1} + k_3}$$

$$\frac{dX''}{dt} = 0 \Rightarrow X'' = \frac{k_2 X S_2}{k_{-2} + k_4}$$

$$X_T = X \left[1 + \frac{k_1 S_1}{k_{-1} + k_3} + \frac{k_2 S_2}{k_{-2} + k_4} \right]$$

$$\begin{aligned}
\frac{dX_T}{dt} &= k_3 X' + k_4 X'' = X \left[\frac{k_3 k_1 S_1}{k_{-1} + k_3} + \frac{k_2 k_4 S_2}{k_{-2} + k_4} \right] \\
&= X_T \left[1 + \frac{k_1 S_1}{k_{-1} + k_3} + \frac{k_2 S_2}{k_{-2} + k_4} \right]^{-1} \left[\frac{k_3 k_1 S_1}{k_{-1} + k_3} + \frac{k_2 k_4 S_2}{k_{-2} + k_4} \right] \\
&= \mu X_T
\end{aligned}$$

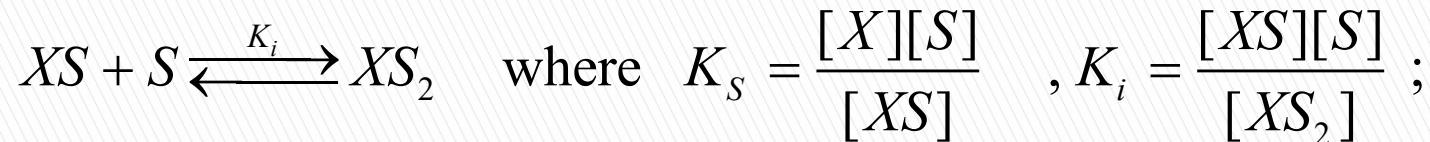
$$\therefore \mu = \frac{\mu_{\max 1} S_1}{K_1 + S_1 + \alpha_2 S_2} + \frac{\mu_{\max 2} S_2}{K_2 + S_2 + \alpha_1 S_1}$$

$$\text{where, } \alpha_2 = \left(\frac{k_2}{k_1} \right) \left(\frac{k_{-1} + k_3}{k_{-2} + k_4} \right) = \frac{K_1}{K_2} , \alpha_1 = \frac{1}{\alpha_2}$$

$$\text{and, } K_1 = \frac{k_{-1} + k_3}{k_1} , K_2 = \frac{k_{-2} + k_4}{k_2} ,$$

$$\mu_{\max 1} = k_3 , \mu_{\max 2} = k_4$$

Effect of Inhibitory Substrates



$$\frac{d[X_T]}{dt} = K[XS] = \frac{K}{K_S} [X][S]$$

$$\begin{aligned} [X_T] &= [X] + [XS] + [XS_2] \\ &= [X] + \frac{[X][S]}{K_S} + \frac{[X][S]^2}{K_i K_S} \\ &= [X] \left(1 + \frac{[S]}{K_S} + \frac{[S]^2}{K_i K_S} \right) \end{aligned}$$

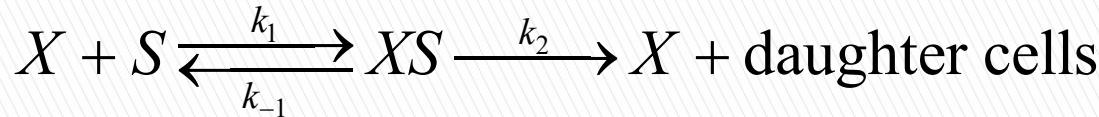
$$\frac{d[X_T]}{dt} = \frac{K}{K_S} [X][S] = \frac{K}{K_S} \frac{[S]}{\left(1 + \frac{[S]}{K_S} + \frac{[S]^2}{K_i K_S}\right)} [X_T]$$

$$\mu = \frac{1}{[X_T]} \frac{d[X_T]}{dt} = \frac{\mu_{\max} [S]}{\left(1 + \frac{[S]}{K_S} + \frac{[S]^2}{K_i K_S}\right)}$$

This rate of growth reaches a maximum value at $S = S_{crit}$ beyond which it declines. To find the maximum specific growth:

$$\frac{d\mu}{dS} = 0 \quad \Rightarrow \quad S_{crit} = \sqrt{K_i K_S}$$

Allosteric Inhibition



Same Procedure as previous case,

$$\frac{d[X_T]}{dt} = k_2[XS] + \beta k_2[SXS]$$

$$\mu = \mu_{\max} \frac{S \left(1 + \frac{\beta S}{K_M} \right)}{K_M + S + \frac{S}{K'_M}}$$

$$\text{where, } K_M = \frac{k_{-1} + k_2}{k_1} \quad \& \quad K'_M = \frac{k_{-3} + \beta k_2}{k_3}$$

CH40001 Biochemical Engineering

Chapter 6.

(a) Fermentation

(b) Design and Analysis of

Bioreactors (Fermenters)

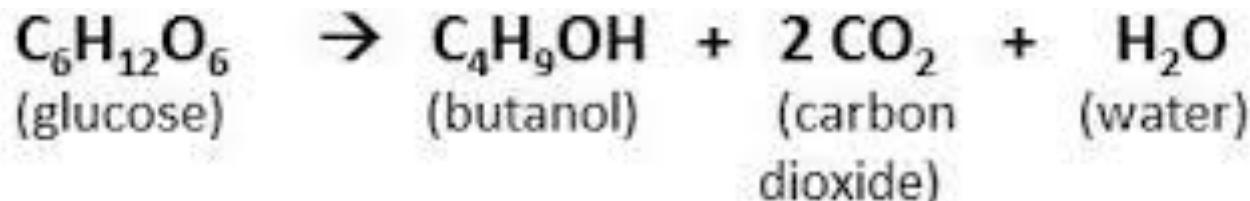
Saikat Chakraborty

Department of Chemical Engineering

Indian Institute of Technology

FERMENTATION OF SUGARS

anhydrous glucose $C_6H_{12}O_6$	\longrightarrow	ethanol 2 C_2H_5OH	+	carbon dioxide 2 CO_2
FW 180 1 gram		46(x2) 0.51 grams		44 (x2) 0.49 grams
glucose monohydrate $C_6H_{12}O_6 \cdot H_2O$	\longrightarrow	ethanol 2 C_2H_5OH	+	carbon dioxide 2 CO_2 + water H_2O
FW 198 1 gram		46 (x2) 0.46 grams		44 (x2) 0.44 grams + 18 0.091 grams
sucrose $C_{12}H_{22}O_{11}$	+	water H_2O	\longrightarrow	ethanol 4 C_2H_5OH + carbon dioxide 4 CO_2
FW 342 1 gram		18 0.053 grams		46 (x4) 0.54 grams + 44 (x4) 0.51 grams



BIOLOGICAL FERMENTATION

~~(using yeasts or bacteria)~~

- ▶ It is a Chemical transformation of Fermentable Sugars, especially Glucose, which gives Valuable Products such as Fructose, Ethanol, numerous Organic Acids and other by-products through Biochemical conversion by a certain microorganism such as **yeasts and bacteria**.
- ▶ The Degradation of Carbohydrates by Microorganisms is followed by Glucolytic or Embden-Myerhof-Parnas (EMP) Pathways.
- ▶ In this, Carbohydrates are reduced to Pyruvate with the aid of Nicotinamide Adenine Dinucleotide (NADH) and Ethanol is the end Product.
- ▶ Depending on the Biomass, Process Conditions, Process Economy and Cost, Fermentation can be carried out as:

► **Types of Biological Fermentation**

- **SHF** : In this Process, Enzyme Production, Enzymatic Hydrolysis and Fermentation Is performed Separately in Separate Vessels. The Process Is called Separate Hydrolysis and Fermentation.
- **DMC** : Direct Microbial Conversion combines all three major processes (Enzyme Production, Hydrolysis and Fermentation) in one step.
- **SHF** : Simultaneous Saccharification (i.e., hydrolysis) and Fermentation combines Hydrolysis of the Substrate and Fermentation in one step. It reduces the Cellulase Inhibition, which in turn Increases Sugar Production Rates, Yields and Concentrations.

SF is very advantageous in comparison with other processes mentioned above.

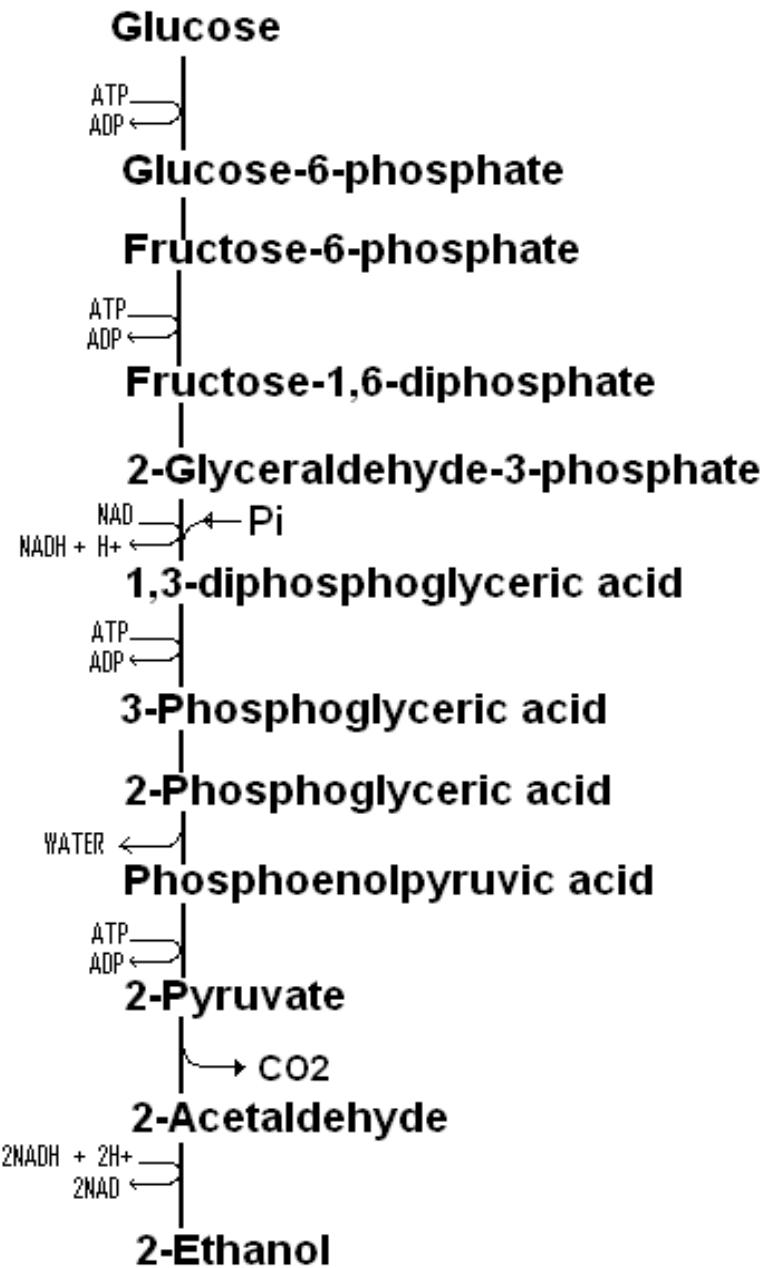
Process is discussed below.

SIMULTANEOUS SEDIMENTATION AND FERMENTATION (SSF)

- The process was first illustrated in 1977 and it gives high Yield and rapid Rates of Conversion.
- droplets released by Cell-free Enzyme (Candy/peach) are immediately converted to the end product by the microorganism.
- Since the Optimum Temperature for both Saccharification and Fermentation is different, 70°C is often used for such as *Rhizopus* enzymes and 50°C for *Aspergillus* enzymes.

- ▶ **Most of the time, it's a good idea to dilute the carrier solution or dilute with 15 ml of water in a test tube.**
- ▶ **Traditional immunotherapy, shock waves, combination of immunotherapy and radiotherapy.**
- ▶ **After the first DLI, Recovery time X-ray and Antibiotic with good supportive care, good diet, good tolerance for treatment.**
- ▶ **The outcome of the immunotherapy, *Cell transfer* are:**
 - 1. Graft versus Host Disease (GVHD), in which the foreign genetic elements are present - bone and**
 - 2. DLI in which the foreign genetic elements are integrated into the host chromosome.**
- ▶ **In most of the X-ray is suggested giving the cell transfer when it is combined to X-ray - DLI (2011).**
- ▶ **It can prevent DLI and the risk of rejection (GVHD) and**

- ▶ In **Prokaryotes** glucose is converted to glyceraldehyde-3-phosphate which can then be converted to pyruvate with NADH oxidation.
- ▶ The pyruvate is then converted to ethanol via an alcohol dehydrogenase by the sequential action of **Pyruvate decarboxylase** and **Alcohol dehydrogenase**.
- ▶ In the scheme, a number of NADH oxidation steps are required to produce NADH for reduction.



The following diagram shows the pathway:

Starch Fermentation

- ▶ In Starch Fermentation enzymes, is used as an enzyme, obtained from *Azogloea* species, *Aspergillus* species and *Penicillium* species.
- ▶ The products are glucose, maltose, maltotriose, maltotetraose, maltopentaose and maltose.
- ▶ Yeast strains *S. cerevisiae* Y120, Aro 2300 and AroCC 20000 conditioned as yeast fermenting for the yeast ethanol production which can convert both glucose and maltose.
- ▶ Maltase present in the Yeast converts Maltose into Glucose. Another enzyme Zymase present in the yeast, then converts Glucose into Ethanol and Carbon dioxide.



Enzymes are used in the co-culture fermentation with *Zymomonas* / *maltozase* on the filamentous bacteria, galactose, glucose, manose, maltose, maltotriose, maltotetraose, maltopentaose, maltose and cellulose.

Modeling and Design of Biological Fermentation

- ▶ Design of Chemostats (continuous stirred bioreactors) in which simultaneous growth of yeast/bacteria and fermentation of glucose occur
- ▶ Stability of Chemostats



Models for Growth of *Saccharomyces cerevisiae* and *Zymomonas* metabolizing Glucose Fermentation in a Batch Reactor

Monod Model ($\mu = \mu_{max} \frac{S}{k_s + S}$, $X = \frac{\mu}{Y_{x/s}}$, $Z = \frac{1}{Y_{x/s}}$)

$$\frac{dX}{dt} = \mu X = \frac{\mu_{max} S X}{k_s + S}$$

expressing X in terms of S from above eqn.

$$\frac{dS}{dt} = -\frac{1}{Y_{x/s}} \frac{\mu_{max} S X}{k_s + S}$$

$$\frac{dS}{dt} = -\frac{\mu_{max}}{Y_{x/s}} \frac{X_0 + Y_{x/s} \{S_0 - S\}}{k_s + S} S$$

with initial conditions: $X(0)=X_0$, $S(0)=S_0$

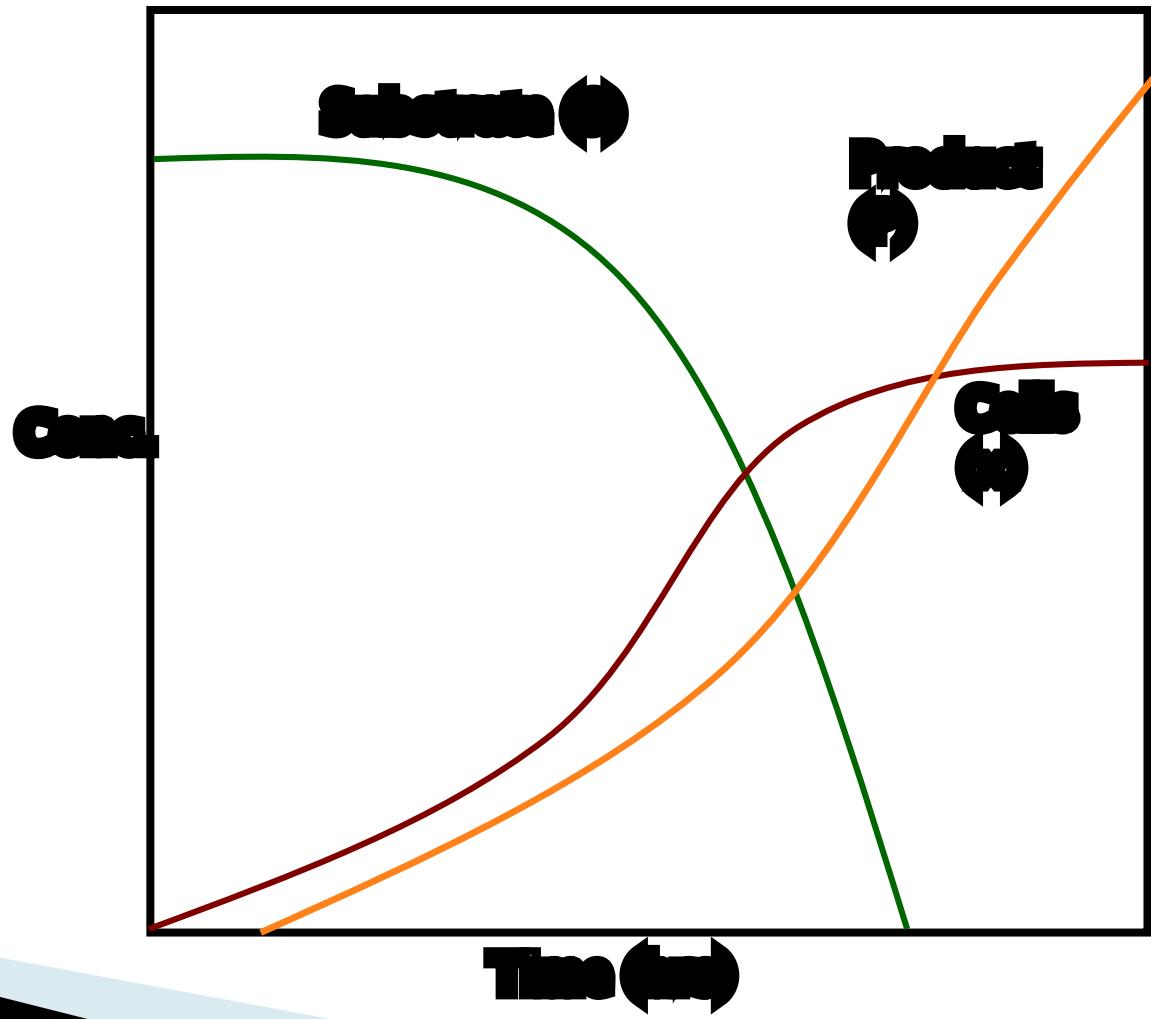
Using above eqns we get the invariance

$$\frac{d}{dt}(X + Y_{x/s}S) = 0$$

$$X + Y_{x/s}S = X_0 + Y_{x/s}S_0$$

on integration , $[X_0 + Y_{x/s} \{S_0 + k_s\}] \ln\left(\frac{X_0 + Y_{x/s} \{S_0 - S\}}{X_0}\right)$

$$-k_s Y_{x/s} \ln\left(\frac{S}{S_0}\right) = \mu_{\max} (X_0 + Y_{x/s} S_0) t$$



Death of cells in batch culture:

$$\frac{dX_v}{dt} = \mu X_v - k X_v$$

$$\frac{dX_d}{dt} = k X_v$$

$$\frac{dX_T}{dt} = \frac{d}{dt}(X_v + X_d) = \mu X_v$$

$$X_v = X_{v0} \exp[(\mu - k)t]$$

$$X_T = X_{T0} \left(1 + \frac{\mu}{\mu - k} \exp[(\mu - k)t]\right)$$

$$\text{if } \mu = k, X_T = X_{T0} + X_{v0} \mu t$$

Other Types of Growth Kinetics

► Substrate Inhibition

$$\mu = \frac{\mu_{\max} S}{K_S + S + S^2 / K_I}$$

$$\frac{dX}{dt} = \mu X = \frac{\mu_{\max} S X}{K_S + S + S^2 / K_I}$$

Here, K_I is the Haldane or substrate inhibition coefficient

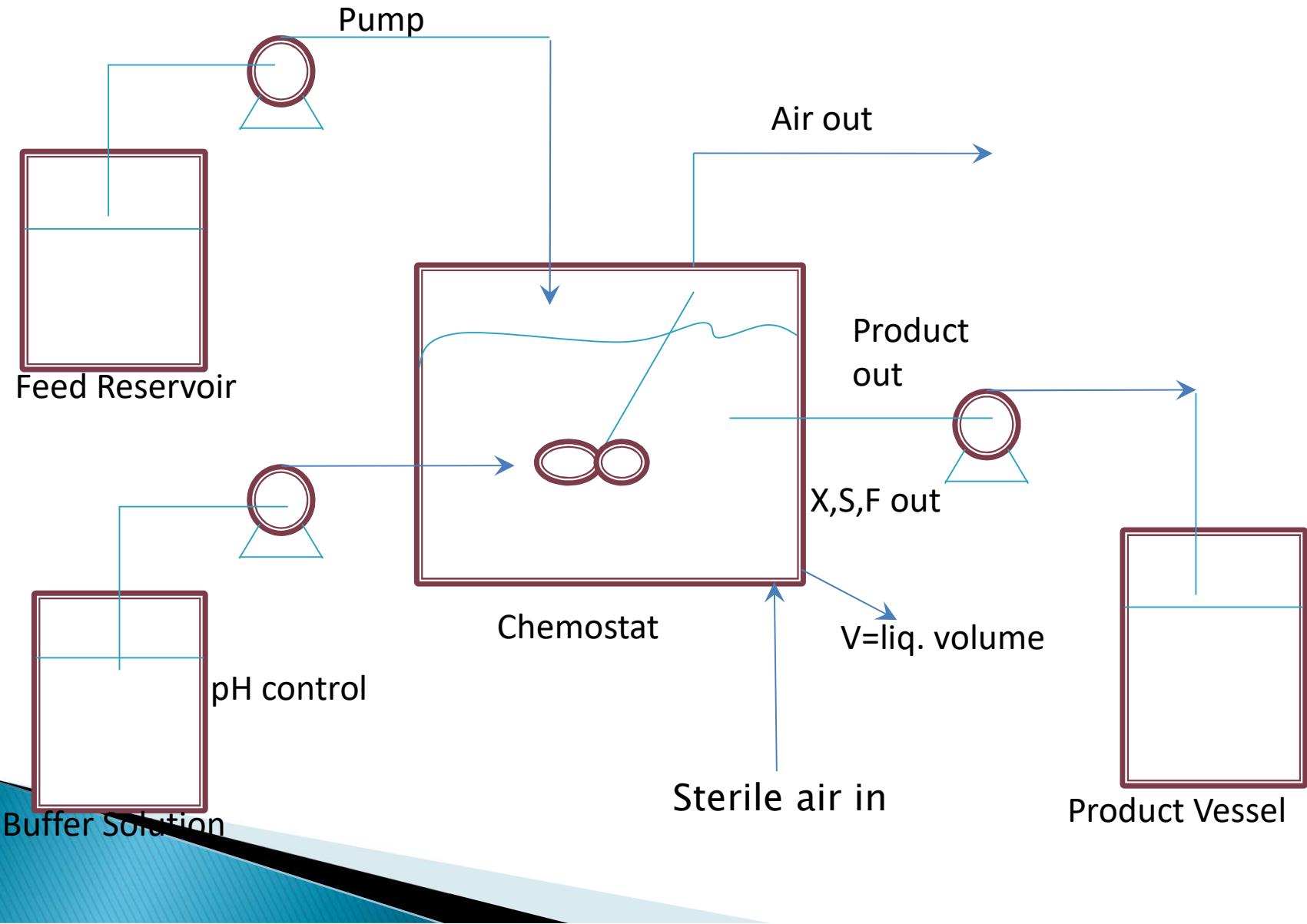
► Product Inhibition

$$\mu = \frac{\mu_{\max} S}{K_S + S} \left[1 - \left(\frac{[P]}{[P^*]} \right)^n \right],$$

Where $[P]$ is the ethanol conc., n is a determinable constant, $[P]^*$ is the critical ethanol conc. above which cells cease to grow (~ 10 gm/lit for *S. cerevisiae*).



A Chemostat and Its accessories



Design of Chemostats (continuous stirred tank bioreactors)

$$\frac{dV}{dt} = F_{in} - F_{out}$$

Total volume balance.

$$\frac{dXV}{dt} = F_{in}X_0 - F_{out}X + \mu XV$$

Mass balance for cells

$$\frac{dSV}{dt} = F_{in}S_0 - F_{out}S - \frac{1}{Y_{x/s}} \mu XV$$

Mass balance
for substrates

if $F_{in} = F_{out} = F \Rightarrow V = \text{const.}$

$$\frac{dX}{dt} = \frac{F}{V} (X_0 - X) + \mu X$$

$$\frac{dS}{dt} = \frac{F}{V} (S_0 - S) - \frac{1}{Y_{x/s}} \mu X.$$



$$\frac{F}{V} = D(\text{dilution rate})[\text{s}^{-1}] = \frac{1}{\tau} \text{ (inverse of residence time)}$$

$$\frac{dX}{dt} = D(X_0 - X) + \mu X \quad (1)$$

$$\frac{dS}{dt} = D(S_0 - S) - \frac{1}{Y_{x/s}} \mu X \quad (2)$$

$$\mu = \frac{\mu_{\max} S}{k_s + S} \quad \text{:Monod Growth Kinetics.}$$

If death of microorganisms (yeast/bacteria) is included in the model, μ in eqs. (1) must be replaced by $(\mu - k)$, where k is the 1st order 'death constant' for the micro-organism.



at steady state from eq (1)

$$D \ X_0 = (D - \mu) X$$

for sterile feed , $X_0 = 0$ and $D = \mu$

i.e., $\frac{\mu_{\max} S_{ss}}{k_s + S_{ss}} = D \Rightarrow S_{ss} = \frac{Dk_s}{\mu_{\max} - D}$

provided $X_{ss} \neq 0$ and $\mu_{\max} > D$

from eqn. (2)

$$D(S_0 - S_{ss}) - \frac{1}{Y_{x/s}} \mu X_{ss} = 0$$

noting $D = \mu$ for $X_0 = 0$

$$X_{ss} = Y_{x/s} \left(S_0 - \frac{D k_s}{\mu_{max} - D} \right)$$

when $S_{ss} = S_0$, $D_{max} = \frac{\mu_{max} S_0}{k_s + S_0}$

As $D \rightarrow \mu_{\max}$ $X_{ss} \rightarrow \infty$

\therefore solutions are possible for $D > \mu_{\max}$ and $D < \mu_{\max}$

for $X_{ss} \neq 0$, $D = \mu \Rightarrow D < \mu_{\max}$

for $X_{ss} = 0$, $D > \mu_{\max}$

steady state solutions :

$$(1) D < \mu_{\max} : \quad X_{ss} = Y_{x/s} \left(S_0 - \frac{Dk_s}{\mu_{\max} - D} \right)$$

$$S_{ss} = \frac{Dk_s}{\mu_{max} - D} \text{ provided } S_0 > \frac{Dk_s}{\mu_{max} - D}$$

if $S_0 < \frac{Dk_s}{\mu_{max} - D}$, X_{ss} & S_{ss} are not in the feasible domain.
(i.e they are negative)

$$\text{and } S_{ss} = S_0$$

$$X_{ss} = 0$$

$$(2) D < \mu_{max} : \quad X_{ss} = 0 \\ S_{ss} = S_0$$

Summary of chemostat behavior

$$\frac{dX}{dt} = D(X_0 - X) + \mu X$$

$$\frac{dS}{dt} = D(S_0 - S) - \frac{1}{Y_{x/s}} \mu X$$

$$\mu = \frac{\mu_{\max} S}{k_s + S}$$



for sterile feed ($X_0 = 0$):

D	$S_0 > \frac{Dk_s}{\mu_{\max} - D}$	$S_0 < \frac{Dk_s}{\mu_{\max} - D}$
$D < \mu_{\max}$	$S_{ss} = \frac{Dk_s}{\mu_{\max} - D}, X_{ss} = Y_{x/s}(S_0 - S)$	$S_{ss} = S_0, X_{ss} = 0$
$D > \mu_{\max}$	$S_{ss} = S_0, X_{ss} = 0$	$S_{ss} = S_0, X_{ss} = 0$



Stability of Chemostats during Fermentation

Liapunov method of linear stability analysis of chemical reactor
(Bilous and Amundson, 1955)

the unsteady state mass balance eqn.

For a CSTR could be written vectorially as

$$\frac{d\underline{C}}{dt} = \underline{f}(\underline{C}, \underline{p})$$

steady state solution may be obtained from:

$$\underline{f}(\underline{C}_{ss}, \underline{p}) = 0$$

\underline{x} = deviations from steady state

$$\underline{x}(t) = \underline{C}(t) - \underline{C}_{ss}$$

\underline{C} = conc. vector

$$= \begin{pmatrix} S \\ X \end{pmatrix}$$

\underline{p} = parameter vector

$$= \begin{pmatrix} D \\ Y_{x/s} \\ k_s \\ \mu_{max} \\ S_0 \end{pmatrix}$$

$$\underline{C}(t) = \underline{C}_{ss} + \underline{x}(t)$$

$$\frac{d\underline{x}}{dt} = \underline{f}(\underline{C}_{ss} + \underline{x}, \underline{p})$$

If $g(\underline{C}_{ss} + \underline{x})$ is scalar,

Taylor series expansion of $g(\underline{C}_{ss} + \underline{x})$ is given as

$$g(\underline{C}_{ss} + \underline{x}) = g(\underline{C}_{ss}) + \frac{\partial g}{\partial \underline{C}_{ss}} \underline{x} + \frac{\partial^2 g}{\partial \underline{C}_{ss}^2} \left(\frac{\underline{x}^2}{2!} \right) + \text{higher order terms.}$$



For reactors:

Taylor series expansion (about steady state \underline{C}_{ss})

$$\underline{f}(\underline{C}_{ss} + \underline{x}, \underline{p}) = \underline{f}(\underline{C}_{ss}, \underline{p}) + \underline{\underline{A}} \underline{x} + \text{h.o.t}$$

$\uparrow \quad \uparrow \quad \uparrow \quad \uparrow$
 $n \times 1 \quad n \times 1 \quad n \times n \quad n \times 1$

$$\underline{f}(\underline{C}_{ss} + \underline{x}, \underline{p}) \approx \underline{\underline{A}} \underline{x}$$

($\because f(\underline{C}_{ss}, \underline{p}) = 0$ & magnitude of higher order terms $\ll |\underline{\underline{A}} \underline{x}|$ iff $|\underline{x}|$ is small.

n =number of species.

=number of mass balance equations

\therefore if $|\underline{x}|$ is small i.e $|\underline{x}| \ll |\underline{C}_{ss}|$,

$$\frac{d\underline{x}}{dt} = \underline{f}(\underline{C}_{ss} + \underline{x}, \underline{p}) = \underline{\underline{A}}\underline{x}$$

where a_{ij} are the elements of $\underline{\underline{A}}$ evaluated at steady state.

the solution of

$$\frac{d\underline{x}}{dt} = \underline{\underline{A}}\underline{x} \text{ with initial conditions } \underline{x} = \underline{x}_0$$

$$\underline{x}(t) = \sum_{i=1}^n \alpha_i \underline{\beta}_i e^{\lambda_i t}$$

$$a_{ij} = \frac{\partial f_i(\underline{C}_{ss}, \underline{p})}{\partial C_j}$$

where $\underline{\beta}_i$ and λ_i are corresponding eigenvalues of $\underline{\underline{A}}$.

the eigen vectors of $\underline{\underline{A}}$ are obtained by solving

$\det(\underline{\underline{A}} - \lambda \underline{\underline{I}}) = 0$ where $\underline{\underline{I}}$ is the $n \times n$ square matrix. the eigen vectors

$\underline{\beta}_i$ is obtained by solving

$$(\underline{\underline{A}} - \lambda_i \underline{\underline{I}}) \underline{\beta}_i = 0 \quad \text{where } i=1,2,\dots,n$$

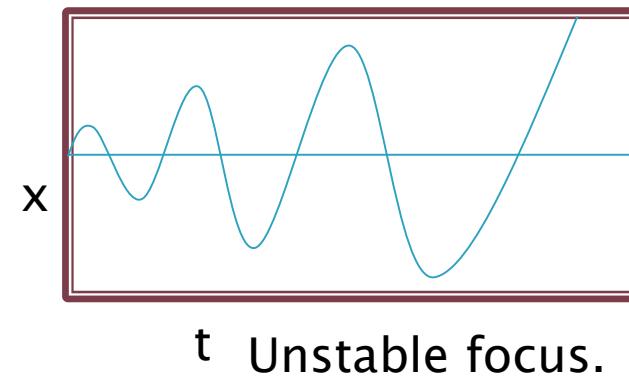
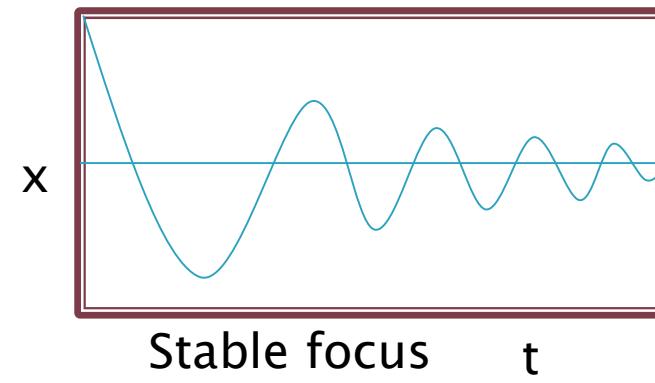
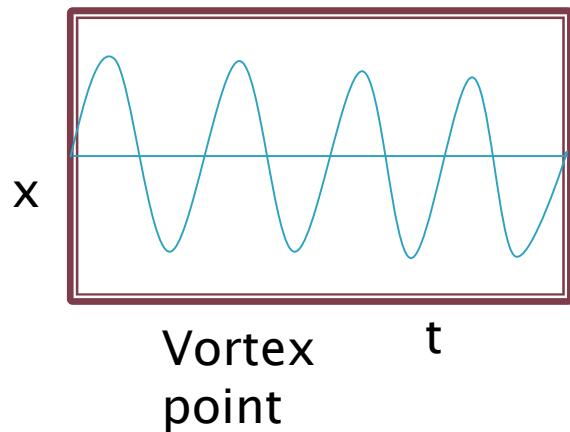
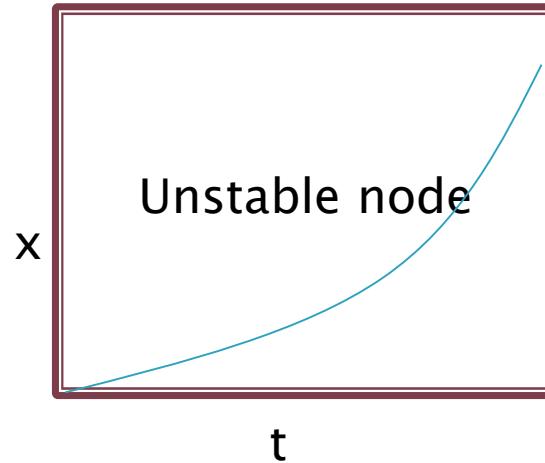
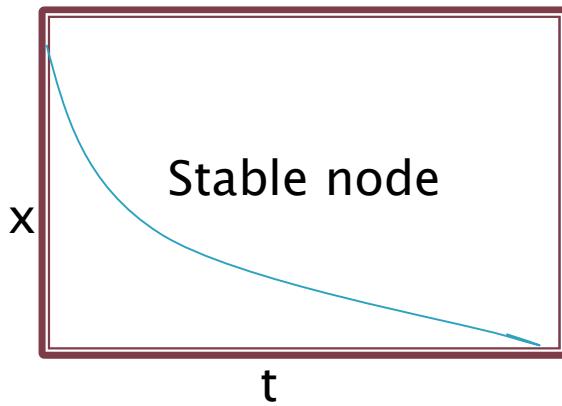
and α_i are such that the initial condition is satisfied by solving.

$$\sum_{i=1}^n \alpha_i \underline{\beta}_i = \underline{x}_0$$

Note: there are n eigen values for $n \times n$ non singular $\underline{\underline{A}}$ and n corresponding eigen vectors.

in general $\lambda = a_i \pm b_i i$.

- 1) if all λ_i 's have $a_i < 0$ and $b_i = 0$ for all i , then the steady state is locally stable.
- 2) if even one λ_i has $a_i > 0$ and $b_i = 0$ then the steady state is unstable.
- 3) if $a_i = 0$ and $b_i \neq 0$, sustained oscillations.
- 4) if $a_i < 0$ and $b_i \neq 0$, damped oscillations.
- 5) if $a_i > 0$ and $b_i \neq 0$, undamped oscillation.



To check stability we need not compute all eigenvalues

to determine if their real parts are positive or negative.

$$|\underline{A} - \lambda \underline{I}| = 0 \text{ where } \underline{A} \text{ is } n \times n,$$

$$\lambda^n + B_1 \lambda^{n-1} + B_2 \lambda^{n-2} + \dots + B_{n-1} \lambda + B_n = 0$$

Hurwitz criteria: all roots of an eqn. will have negative real roots iff

$$B_1 > 0$$

$$\det \begin{pmatrix} B_1 & B_3 \\ 1 & B_2 \end{pmatrix} > 0$$

$$\det \begin{pmatrix} B_1 & B_3 & B_5 & \dots \\ 1 & B_2 & B_4 & \dots \\ 0 & B_1 & B_3 & \dots \\ \vdots & \vdots & \vdots & \ddots \\ & & & B_n \end{pmatrix} > 0$$

Stability of a chemostat :

$$\frac{dx}{dt} = D(X_0 - X) + \frac{\mu_{\max} S}{k_s + S} X$$

$$\frac{dy}{dt} = D(S_0 - S) - \frac{1}{Y_{x/s}} \frac{\mu_{\max} S}{k_s + S} X$$

$$f_1(X, S) = D(X_0 - X) + \frac{\mu_{\max} S}{k_s + S} X$$

$$f_2(X, S) = D(S_0 - S) - \frac{1}{Y_{x/s}} \frac{\mu_{\max} S}{k_s + S} X$$



$$A = \begin{pmatrix} \frac{\partial f_1}{\partial X} & \frac{\partial f_1}{\partial S} \\ \frac{\partial f_2}{\partial X} & \frac{\partial f_2}{\partial S} \end{pmatrix} = \begin{pmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{pmatrix}$$

$$\frac{\partial f_1}{\partial X} = -D + \mu$$

$$\frac{\partial f_1}{\partial S} = X \frac{\partial \mu}{\partial S}$$

$$\frac{\partial f_2}{\partial X} = -\frac{\mu}{Y_{x/s}}$$

$$\frac{\partial f_2}{\partial S} = -D - \frac{X}{Y_{x/s}} \frac{\partial \mu}{\partial S}$$



$$\left| \begin{matrix} A & -\lambda I \\ \hline \hline \end{matrix} \right| = 0$$

$$\begin{pmatrix} a_{11} - \lambda & a_{12} \\ a_{21} & a_{22} - \lambda \end{pmatrix} = 0$$

$$(a_{11} - \lambda)(a_{22} - \lambda) - a_{12}a_{21} = 0$$

$$\text{or } \lambda^2 - (a_{11} + a_{22})\lambda + (a_{11}a_{22} - a_{12}a_{21}) = 0$$

$$\therefore B_1 = -(a_{11} + a_{22})$$

$$B_2 = (a_{11}a_{22} - a_{12}a_{21})$$

$$B_3 = 0$$

\Rightarrow for both eigenvalues to have negative real parts.

$$B_1 > 0$$

$$\&\& \det \begin{pmatrix} B_1 & B_3 \\ 1 & B_2 \end{pmatrix} > 0$$

i.e $B_1 B_2 > 0$

i.e $B_2 > 0$

criteria : $B_1 > 0 \ \&\& B_2 > 0$

$$\Rightarrow (a_{11} + a_{22}) < 0 \ \&\& (a_{11}a_{22} - a_{12}a_{21}) > 0$$

for Monod Growth Model:

$$\mu = \frac{\mu_{\max} S}{k_s + S}$$

$$\frac{\partial \mu}{\partial S} = \frac{\mu_{\max} k_s}{(k_s + S)^2} > 0 \text{ for all } S$$

from previous analysis for $S_0 < \frac{Dk_s}{\mu_{\max} - D}$

the reaction does not take off.

so, we consider the case: $S_0 > \frac{Dk_s}{\mu_{\max} - D}$

with $X_0 = 0$ (sterile feed).

$$\Rightarrow \text{for } D < \mu_{\max} \text{ where } S_{ss} = \frac{Dk_s}{\mu_{\max} - D}$$

$$A = \begin{pmatrix} 0 & X_{ss} \left(\frac{d\mu}{ds} \right) \\ -\frac{D}{Y_{x/s}} & -\left(\frac{X_{ss}}{Y_{x/s}} \left(\frac{\partial \mu}{\partial S} \right)_{ss} + D \right) \end{pmatrix}$$

note $a_{11} = 0$, $a_{22} < 0 \therefore B_1 > 0$

$$a_{12} = X_s \left(\frac{d\mu}{ds} \right)_{ss} > 0, \quad a_{21} = \frac{-D}{\mu_{\max} - D}$$

$-a_{12}a_{21} > 0 \therefore \text{Stable Steady State}$

for $D > \mu_{\max}$ $S_{ss} = S_0$ and $X_{ss} = 0$.

$$A = \begin{pmatrix} -D + \frac{\mu_{\max} S_0}{k_s + S_0} & 0 \\ -\frac{\mu_{\max} S_0}{k_s + S_0} & -D \end{pmatrix}$$

$$a_{11} + a_{22} = -2D + \frac{\mu_{\max} S_0}{k_s + S_0} < 0 \quad (\because D > \mu_{\max})$$

$$a_{11}a_{22} - a_{12}a_{21} = D(D - \frac{\mu_{\max} S_0}{k_s + S_0}) > 0$$

\therefore both criteria satisfied \therefore both λ_i 's have a_i 's < 0

• Stable Steady State.