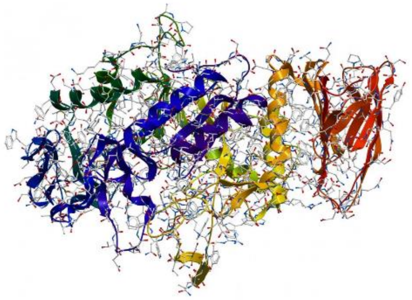


CEN-CHE 422
ENZYME ENGINEERING

KINETICS OF IMMOBILIZED ENZYMES

(Heterogeneous System)

Kinetics (k, K_m, r_{max})
Mass transfer (k_La, k_L, D_e)

k, K_m, r_{max} = kinetics constants

k_La, k_L = mass transfer coefficents (externall mass transfer)

D_e= effective diffusion coefficient (intraparticle diffusion)

Reaction rate:

$$r = \frac{r_{\max} C_S}{K_m + C_S}$$ mol/cm³ s

$$r' = \frac{r'_{\max} C_S}{K_m + C_S}$$ mol/cm² s

Mass transfer rate in boundary condition

$$N = k_L a\left(C_{So} - C_{SS}\right)$$ mol/cm³ s

$$N = k_L\left(C_{So} - C_{SS}\right)$$ mol/cm² s

Diffusion rate in pellet: (Fick's Law)

$$N = -D_e \frac{dC_S}{dr}$$ mol/cm² s

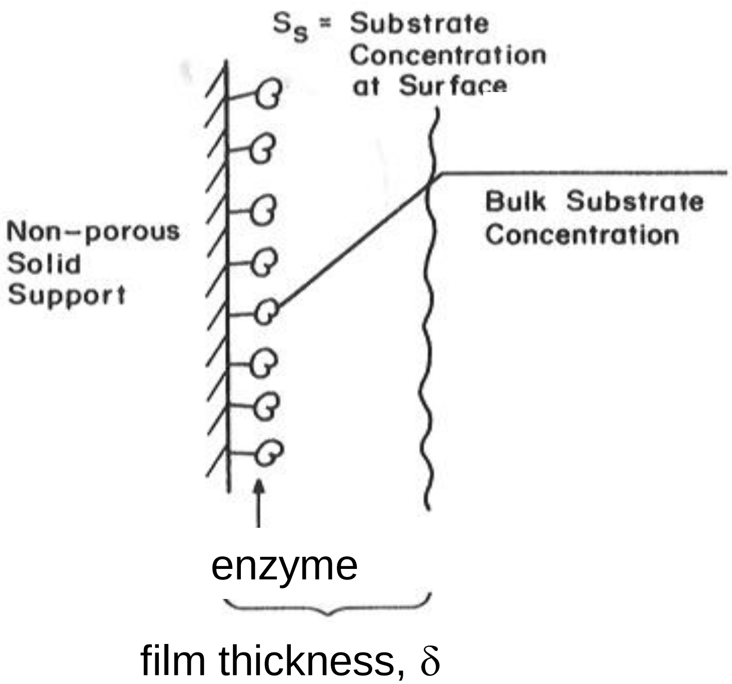
Mass Transfer Resistances

- ✓ **External mass transfer resistances**
- ✓ **Intraparticle diffusion resistances**

- ✓ *Nature of the support material (porous/nonporous)*
- ✓ Hydrodynamical conditions surrounding the support material
- ✓ Distribution of the enzyme inside or on the surface of the support material

Mass Transfer Effects on Surface-Bound Enzymes on Non-porous Pellets

(external mass transfer)



Process:

1) Transfer of substrate to the pellet surface

consecutive phenomena

2) Enzyme reaction on the pellet surface

The slowest step limits the whole process rate

(overall reaction rate)

Damköhler number (Da)

$$N = k_L a(C_{So} - C_{SS})$$

$$r = \frac{r_{\max} C_S}{K_m + C_S}$$

$$Da = \frac{maximum\ reaction\ rate}{maximum\ mass\ transfer\ rate} = \frac{r'_{max}}{k_L C_{So}}$$

where:

C_{So} = substrate bulk concentration (mol/cm^3)

k_L = mass transfer coefficient (cm/s)

r'_{max} = maximum reaction rate (mol/cm^2 s)

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

$$Da = \frac{maximum\ reaction\ rate}{maximum\ mass\ transfer\ rate} = \frac{r'_{max}}{k_L C_{So}}$$

Da<<1	**Slow reaction rate;** reaction rate is the limiting step
Da≈1	The diffusion and reaction resistances are comparable
Da>>1	**Slow mass transfer rate;** mass transfer rate is the limiting step

If reaction rate is limiting (Da <<1), observed rate:

$$r = \frac{r_{\max} C_{SS}}{K_m + C_{SS}} \qquad C_{So} = C_{SS} \qquad \boxed{r' = \frac{r'_{\max} C_{So}}{K_m + C_{So}}}$$

If mass transfer is limiting (Da >>1), observed rate :

$$N = k_L \left(C_{So} - C_{SS} \right) \qquad C_{SS} \rightarrow 0 \qquad \boxed{N = k_L C_{So}}$$

If there is no limiting step, observed rate:

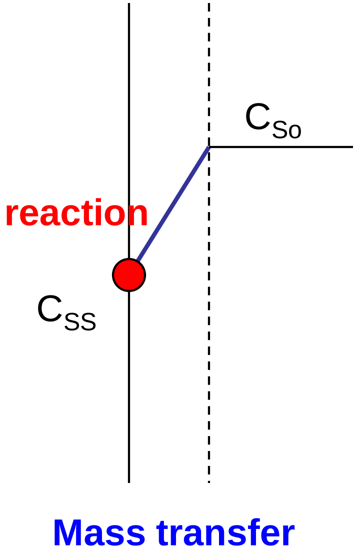
$$r' = \frac{r'_{\max} C_{SS}}{K_m + C_{SS}} \qquad \textit{or} \qquad N = k_L \left(C_{So} - C_{SS} \right)$$

C_{So}= bulk concentration ✔

C_{SS}= surface concentration????

Assumption: There is no (substrate) accumulation on the pellet surface (steady state)

Rate of reaction within pellet (r’) is equal to the rate of mass transfer through pellet surface (N)



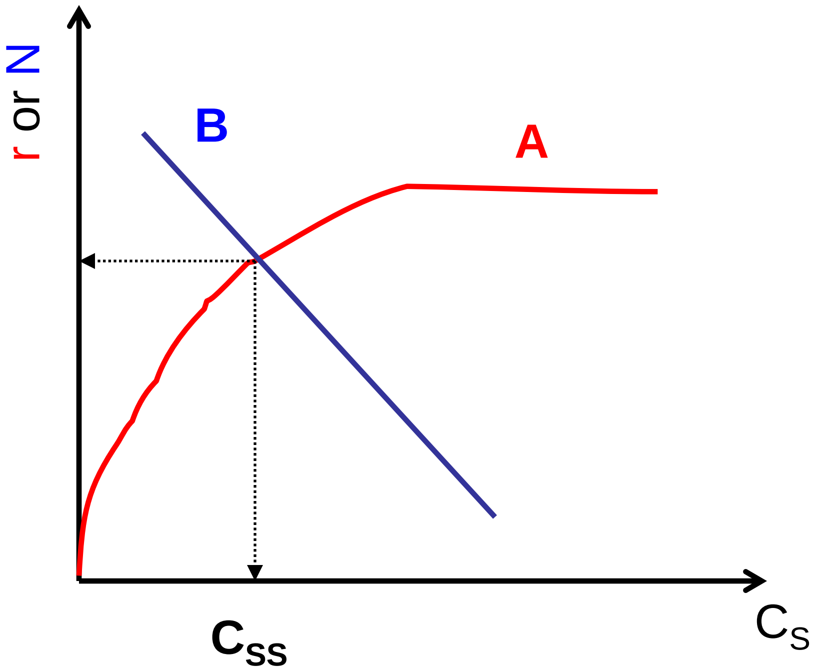
Reaction rate= Mass transfer rate

$$\boxed{k_L(C_{So} - C_{SS}) = \frac{r'_{\max} C_{SS}}{K_m + C_{SS}}}$$

C_{SS} is calculated

r'_{max} = maximum reaction rate per pellet outer surface area (mol/cm^2 s)

Graphical solution



A: reaction kinetics

B: mass transfer rate

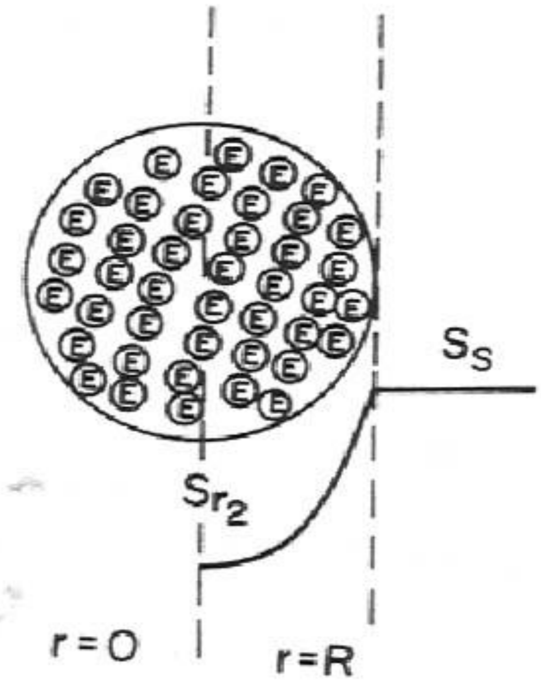
Intersection is the solution for Css

C_{SS} and reaction rate (r or N) are read on the graph

$$k_L(C_{So} - C_{SS}) = \frac{r'_{\max} C_{SS}}{K_m + C_{SS}}$$

Diffusional Effects in Enzymes Immobilized in a Porous Matrix

(intraparticle diffusion)



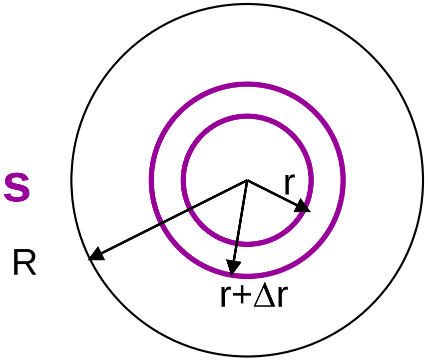
Steady state concentration profile within the pellet?

Assumptions:

- enzyme is uniformly distributed in a spherical support particle;
- the reaction kinetics are expressed by Michaelis–Menten kinetics,
- there is no partitioning of the substrate between the exterior and interior of the support

Concentration and reaction rate change within the pellet

Substrate mass balance in a volume element with the radius r and thickness of Δr



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

Substrate in through system boundaries - **Substrate out through system boundaries** - **Substrate consumed within system** = **Substrate accumulated within system**

$$NA|_r \qquad NA|_{r+\Delta r} \qquad r_T \Delta V$$

At steady state (0)

the diffusion coefficient

***unit*=(mol/m^2 s) x m^2=mol/s**

***unit*=(mol/m^3 s) x m^3=mol/s**

$$N = -D_e \frac{dC_S}{dr}$$

$$A = 4\Pi r^2$$

$$\Delta V = 4\Pi r^2 \Delta r$$

$$\boxed{D_e \left[\frac{d^2 C_S}{dr^2} + \frac{2}{r} \frac{dC_S}{dr} \right] = \frac{r_{\max} C_S}{K_m + C_S}}$$

Variation of C_S with r

Effective diffusion coefficient (diffusivity)

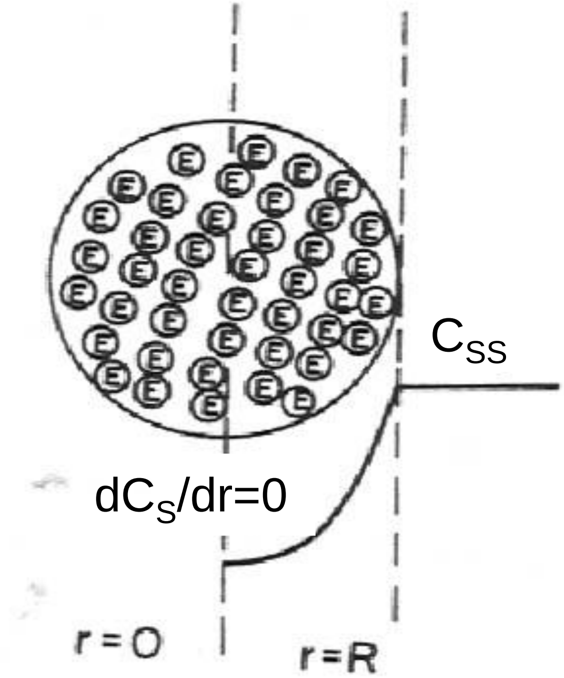
$$\boxed{D_e = \frac{D_{AB}\,\varepsilon}{\tau}}$$

DAB is the diffusion coefficient in gas or liquid filling the pores,
ε is the porosity available for the transport (dimensionless),
τ is the tortuosity (dimensionless).

$$D_e\left[\frac{d^2C_S}{dr^2}+\frac{2}{r}\frac{dC_S}{dr}\right]=\frac{r_{max}C_S}{K_m+C_S}$$

BC1: r=R $C_S=C_{SS}$

BC2: r=0 $dC_S/dr=0$



The solution in dimensionless form:

$\beta=K_m/C_{SS}$ Dimensionless MM constant

$$\left[\frac{d^2\bar{C}_S}{d\bar{r}^2}+\frac{2}{\bar{r}}\frac{d\bar{C}_S}{d\bar{r}}\right]=\frac{R^2r_{max}}{C_{SS}D_e}\left(\frac{\bar{C}_S}{\bar{C}_S+\beta}\right)$$

or

$$\boxed{\left[\frac{d^2\bar{C}_S}{d\bar{r}^2}+\frac{2}{\bar{r}}\frac{d\bar{C}_S}{d\bar{r}}\right]=\varphi^2\left(\frac{\bar{C}_S}{1+\frac{\bar{C}_S}{\beta}}\right)}$$

$$\Rightarrow \quad \boxed{\varphi=R\sqrt{\frac{r_{max}/K_m}{D_e}}}$$

Thiele Modulus

$$\boxed{\left[\frac{d^2\overline{C_S}}{d\overline{r}^2}+\frac{2}{\overline{r}}\frac{d\overline{C_S}}{d\overline{r}}\right]=\varphi^2\left(\frac{\overline{C_S}}{1+\frac{\overline{C_S}}{\beta}}\right)}$$

This equation gives the substrate profile within the pellet with the boundary conditions of $\underline{r}=1$ $\underline{C}_S=1$
$\underline{r}=0$ $d\underline{C}_S/dt=0$

Reaction rate under the the diffusion limiting conditions is expressed in terms of effectiveness factor (η)

$$\eta = \frac{reaction\,rate\,with\,diffusion\,limitation}{reaction\,rate\,without\,diffusion\,limitation}$$

$$\eta = \frac{r_{observed}}{r_{intrinsic}} = \frac{r}{\frac{r_{max}\,C_{ss}}{K_m + C_{ss}}} \quad \Rightarrow \quad \boxed{r = \eta \frac{r_{\max} C_{SS}}{K_m + C_{SS}}}$$

η $\Rightarrow$ a measure of the extent of diffusion limitation

$\eta<1$ $\Rightarrow$ diffusion limits reaction rate.

$\eta\approx1$ $\Rightarrow$ no diffusion limitations

η $\Rightarrow$ function of ϕ and β

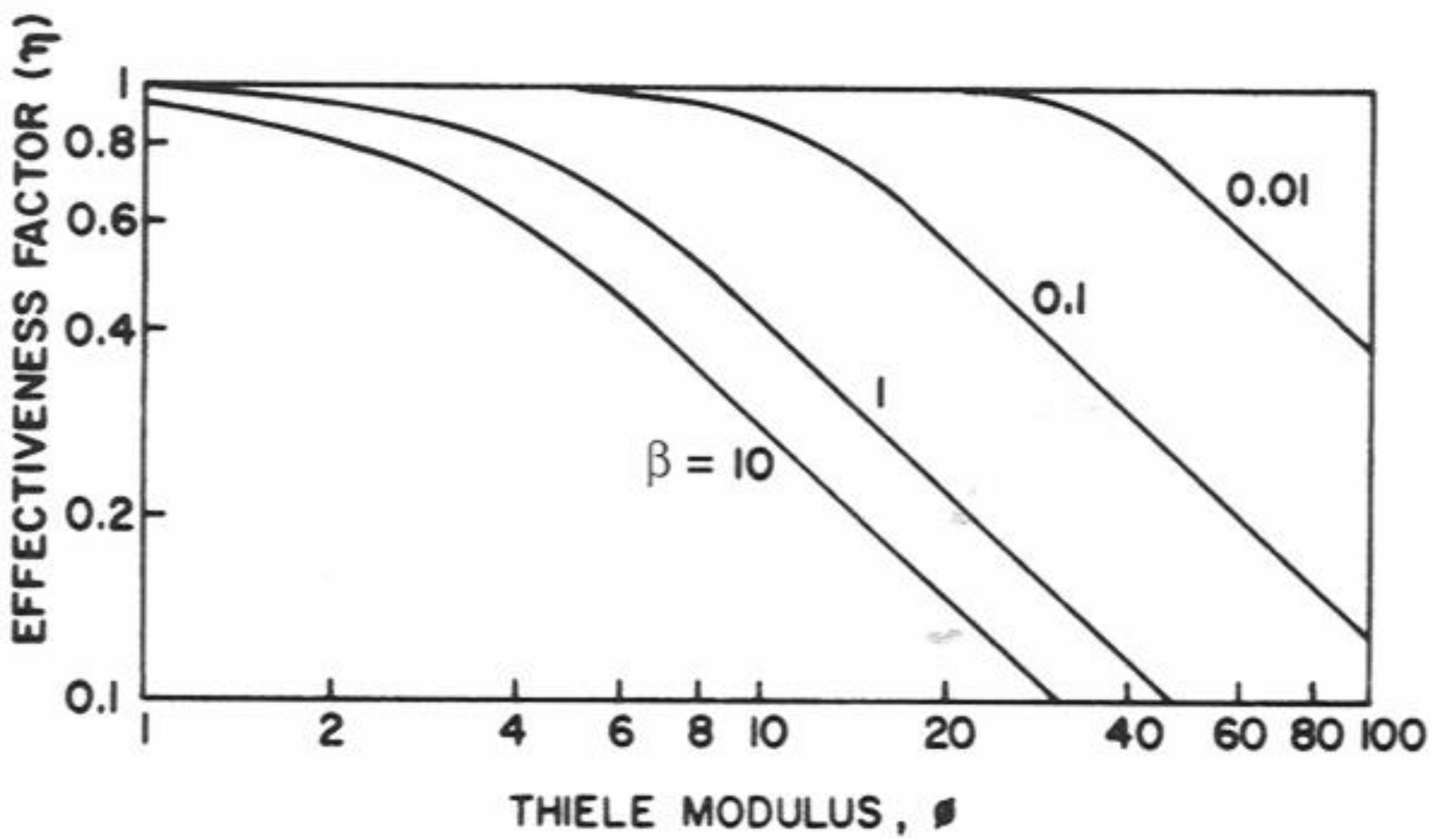


Figure 3.20. Theoretical relationship between the effectiveness factor η and first-order Thiele modulus, ϕ, for a spherical porous immobilized particle for various values of β, where β is the dimensionless Michaelis constant. (With permission, from D. I. C. Wang et al., *Fermentation and Enzyme Technology*, John Wiley & Sons, Inc., New York, 1979, p. 329.)

$\beta = K_m / C_{SS}$

- ✓ *For zero order reaction:* ($C_{SS} > K_m$; $\beta \to 0$; $r = r_{max}$)
  For $1 < \phi < 100$ $\eta = 1$

- ✓ *For first order reaction* ($K_m > C_{SS}$; $\beta \to \infty$; $r = (r_{max}/K_m) C_{SS}$ and $\eta = f(\phi, \beta)$. For high ϕ values

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

- ✓ *For MM kinetics* $\eta = f(\phi, \beta)$ and η is read using the graph

If r_{max} ve K_m are not known ϕ cannot be calculated???

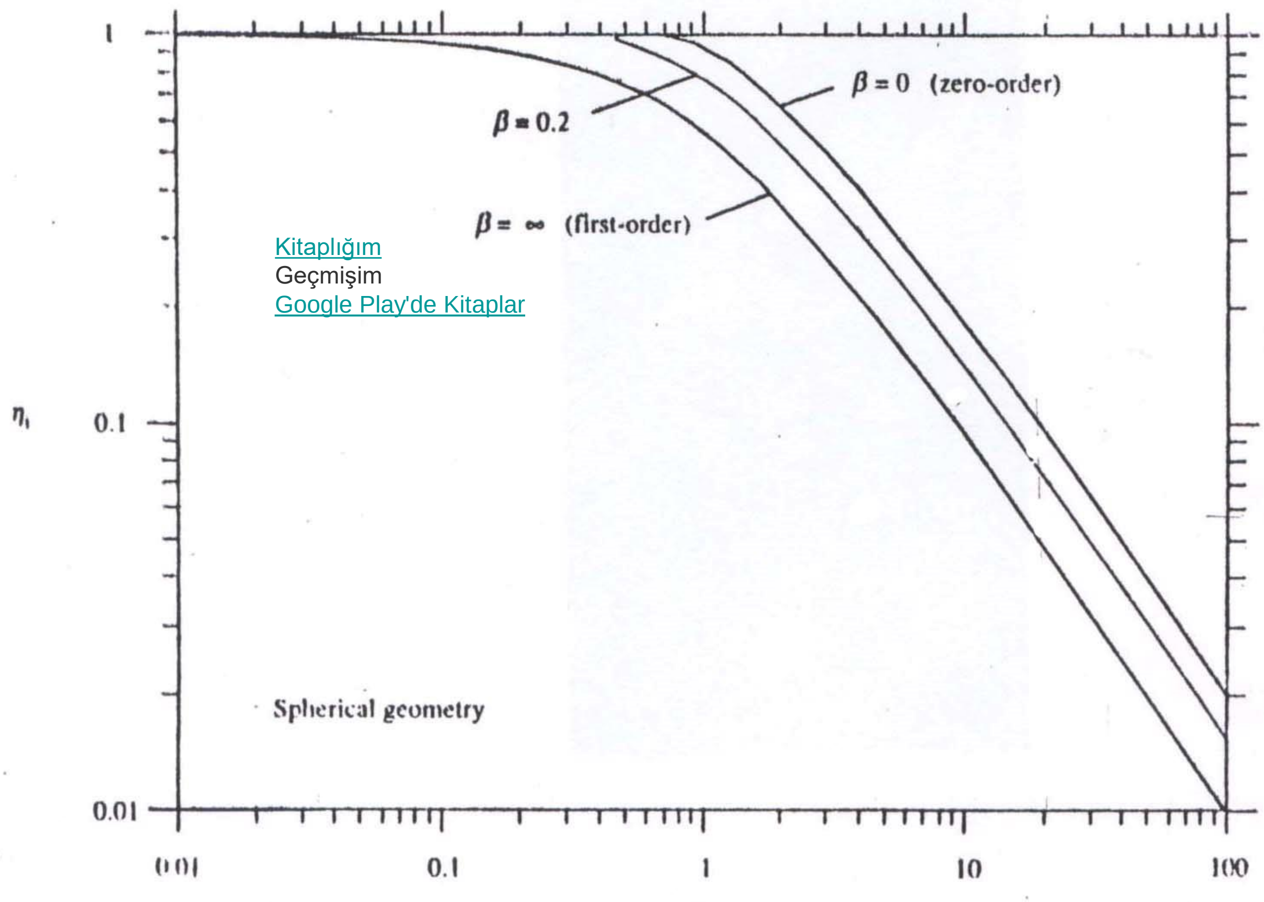
Observed Thiele Modulus

$\eta=f(\Phi,\beta)$

experimental

$$\boxed{\Phi_{obs} = \frac{r_{obs}}{D_e C_{So}}\left(\frac{R}{3}\right)^2}$$

Doran PM, Bioprocess Engineering Principles, Academic Press, 2013



$$\eta = \frac{r_{observed}}{r_{intrinsic\ (real)}}$$

$$\boxed{r = \eta \frac{r_{max} C_{ss}}{K_m + C_{ss}}}$$

Calculated value

Observed rate with the effect of diffusion limitation

$\eta < 1$

$\eta = 1$

the extent of diffusion limitation