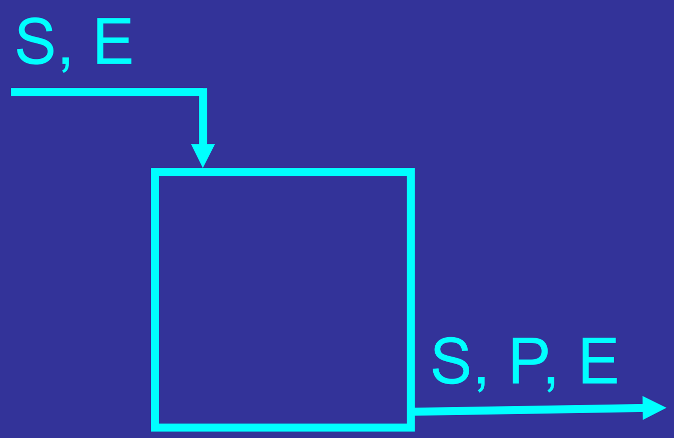
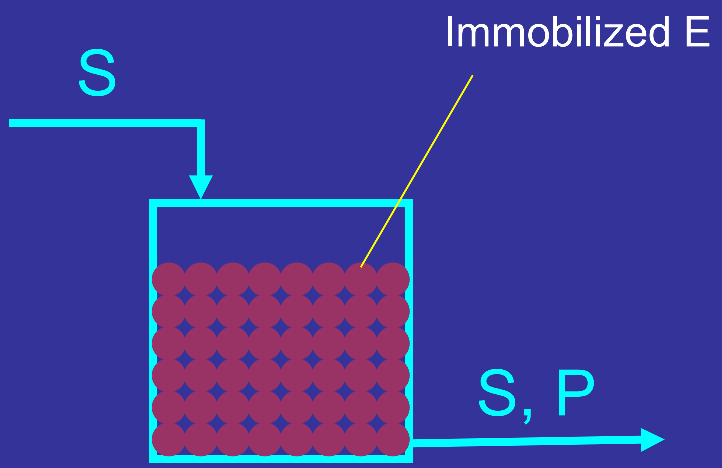
I. Immobilization of Enzymes

1. Motivation

Consider the following two designs for a continuous enzyme process S → P



Process I



Process II

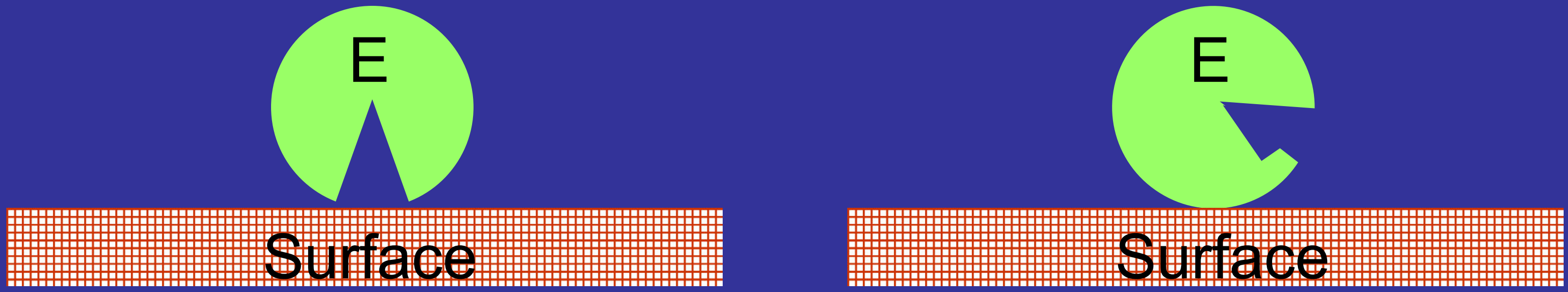
Advantages of Process II

- Enzyme is not lost after process and might therefore be used for greater duration.
- Enzyme does not need to be separated from products. (Conversely, products do not need to be separated from enzyme.)
- Higher enzyme concentrations are attainable without sacrificing these advantages.
- Some enzymes are more stable if they are immobilized. (Many native enzymes are “immobilized” in the cell.)

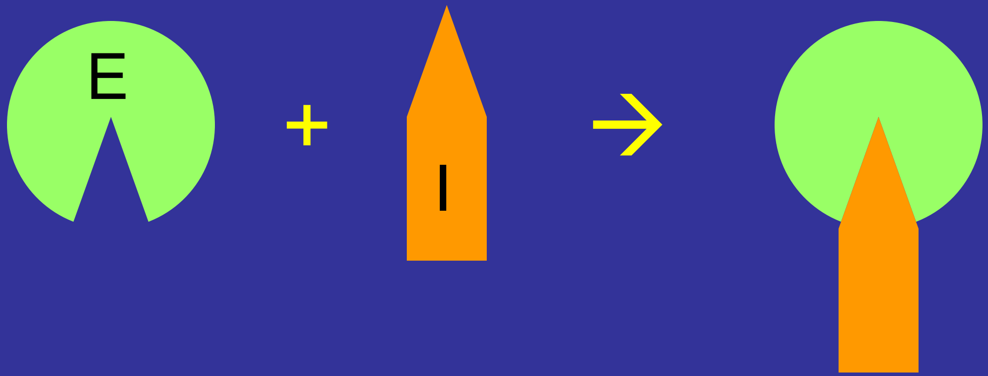
Disadvantages of Process II

- An additional process (and cost) is associated with enzyme immobilization.
- Enzymes may leak from immobilized state.
- Diffusional limitations. Substrate(s) and product(s) must transfer across a boundary to get to/from active site.
- Can be difficult to control environment immediately affecting enzyme and its activity.
- Some enzymes are less stable if they are immobilized. (Structural change)

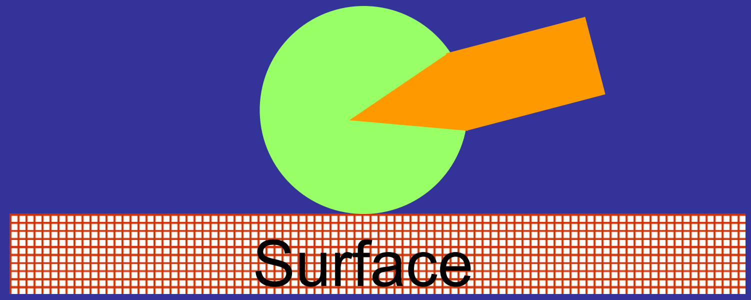
How to reduce structural changes and prevent binding at activity site:



a. Mix enzyme with competitive inhibitor



b. Now immobilize



c. Wash the competitive inhibitor away

2. External Mass Transfer Effects

a. General Derivation

- Enzymes are immobilized on surface of uncharged, nonporous flat plate.
- Entire surface is uniformly accessible to substrate in adjacent fluid.

Consider S → P as immobilized enzyme reaction

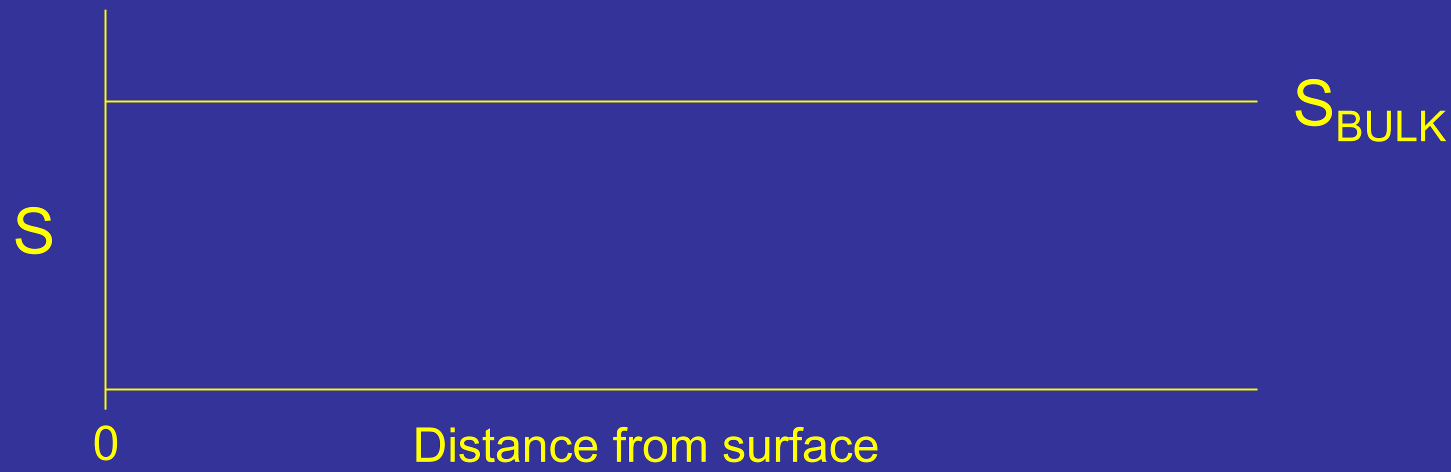
Case I – Enzyme reaction is very slow

Surface concentration (S_{SURF}) is identical to bulk concentration (S_{BULK})

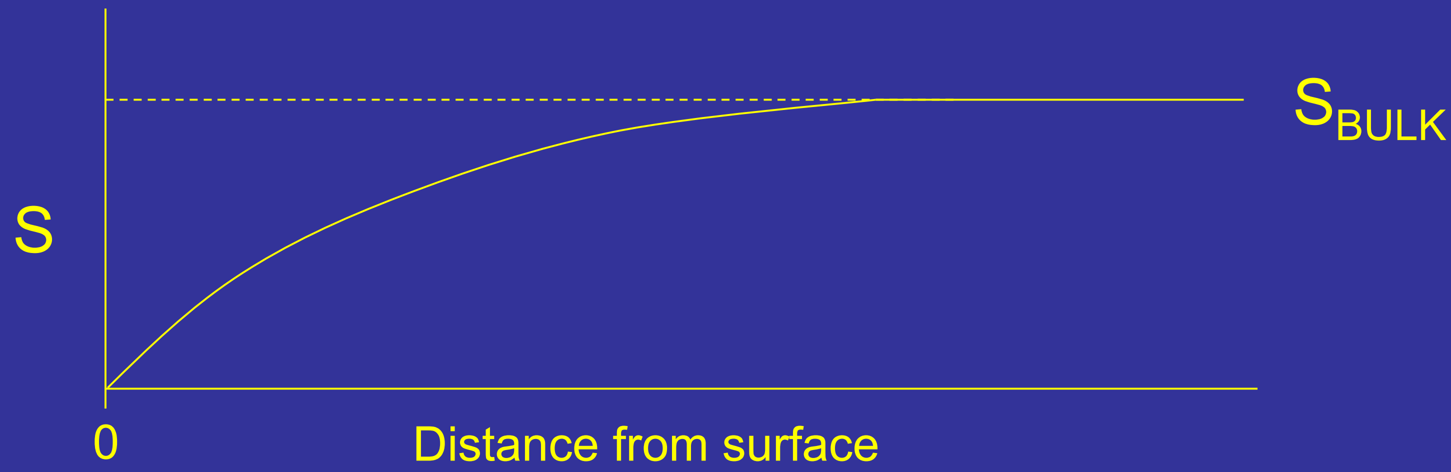
Case II – Enzyme reaction is very fast

Surface concentration (S_{SURF}) is zero

Case I – Enzyme reaction is very slow



Case II – Enzyme reaction is very fast



Case I – Enzyme reaction is very slow

Rate of reaction is limited by enzyme and its intrinsic reaction rate

Case II – Enzyme reaction is very fast

Rate of reaction is limited by rate of mass transfer

At steady-state, substrate and product will not accumulate at the surface, and the rate of reaction is equal to the rate of mass transfer…

rate of reaction = rate of mass transfer

$$\frac{V_{MAX}' S_{SURF}}{K_M + S_{SURF}} = k_L(S_{BULK} - S_{SURF})$$

$$\frac{V_{MAX}' S_{SURF}}{K_M + S_{SURF}} = k_L(S_{BULK} - S_{SURF})$$

<u>Comments</u>

This equation applies to Michaelis-Menten kinetics. If reaction has different kinetics, use different expression for "rate of reaction".

Maximum surface rate of reaction = V_{MAX}' (occurs when S_{SURF} is large)

$V_{MAX}' \propto E_{SURF}$

Maximum rate of mass transfer = $k_L S_{BULK}$ (occurs when $S_{SURF} = 0$)

For convenience define a dimensionless group

Damkohler Number

$$Da = \frac{\text{Max Rate of Reaction}}{\text{Max Rate of Mass Transfer}}$$

$$Da = \frac{V_{MAX}'}{k_L S_{BULK}}$$

If Da >> 1 system is limited by mass transfer

"mass transfer limited regime"

If Da ~ 1 mass transfer and reaction rate are of similar magnitude

If Da << 1 system is limited by enzyme kinetics

"reaction limited regime"

$$\frac{V_{MAX}' S_{SURF}}{K_M + S_{SURF}} = k_L (S_{BULK} - S_{SURF})$$

Unknown: S_{SURF}

Parameters: S_{BULK}, k_L, V_{MAX}', K_M

Introduce dimensionless parameters:

$$x = \frac{S_{SURF}}{S_{BULK}} \qquad \alpha = \frac{K_M}{S_{BULK}}$$

κ (kappa) in many books;
others define $\beta = S_{BULK}/K_M$

$$\frac{V_{MAX}'S_{SURF}}{K_M + S_{SURF}} = k_L(S_{BULK} - S_{SURF})$$

$$xS_{BULK} = S_{SURF} \qquad \alpha S_{BULK} = K_M$$

Then Reaction Rate = $\dfrac{V_{MAX}'xS_{BULK}}{\alpha S_{BULK} + xS_{BULK}}$

$$\frac{V_{MAX}'x}{\alpha + x}$$

Then Mass Transfer Rate = $k_L(S_{BULK} - xS_{BULK})$

$$k_L S_{BULK}\,(1 - x)$$

So….

$$\frac{V_{MAX}'x}{\alpha + x} = k_L S_{BULK} (1 - x)$$

Remember….

$$Da = \frac{V_{MAX}'}{k_L S_{BULK}}$$

Thus….

$$\frac{x}{\alpha + x} = \frac{(1 - x)}{Da}$$

Write as a quadratic….

$$x^2 + Dax + \alpha x - x - \alpha = 0$$

$$x^2 + \beta x - \alpha = 0$$

Where….

$$\beta = Da + \alpha - 1$$

$$x^2 + \beta x - \alpha = 0$$

Solve Quadratic….

$$x = \frac{\beta}{2}\left(-1 \pm \sqrt{1 + 4\alpha/\beta^2}\right)$$

Select sign so that $x \geq 0$

Note that when $\beta = 0$

$$x = \sqrt{\alpha}$$

b. Mass Transfer-Limited Regime

$$x = \frac{\beta}{2}\left(-1 \pm \sqrt{1 + 4\alpha/\beta^2}\right) \qquad \beta = Da + \alpha - 1$$

$$x = \frac{S_{SURF}}{S_{BULK}} \qquad \alpha = \frac{K_M}{S_{BULK}} \qquad Da = \frac{V_{MAX}'}{k_L S_{BULK}}$$

As $V_{MAX}' \rightarrow \infty$ $\quad Da \rightarrow \infty \quad \beta \rightarrow \infty \quad x \rightarrow 0$

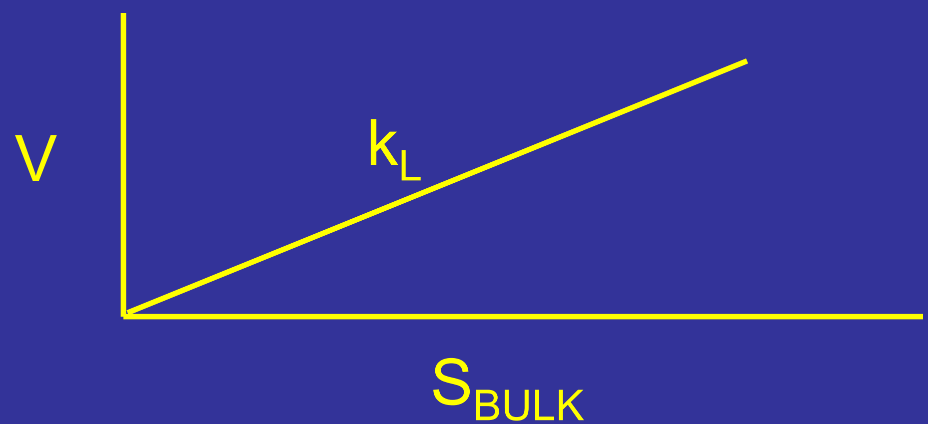
$$S_{SURF} \rightarrow 0$$

As $V_{MAX}' \rightarrow \infty \quad S_{SURF} \rightarrow 0$

The faster the enzyme, the lower the surface substrate concentration. If the enzyme is very fast, then…

$$\text{Reaction Rate} = k_L(S_{BULK} - S_{SURF}), \quad S_{SURF} \rightarrow 0$$

$$\text{Reaction Rate} = k_L S_{BULK}$$



Implications of Reaction Rate = $k_L S_{BULK}$ (Mass Transfer Limited Regime)

As long as reaction is fast enough…

- Reaction rate is independent of V_{MAX}'
- Reaction rate is independent of K_M
- Reaction rate is independent of presence of inhibitors which affect K_M and V_{MAX}'
- Reaction rate is independent of (suboptimal) temperature and pH, which affect K_M and V_{MAX}'

Mass transfer disguises the true kinetic behavior of enzymes.

Studies aimed at determining activity/denaturation in immobilised systems should be conducted in reaction-limited regime.

Experimentally, to operate in reaction-limited regime requires high fluid flow rates to minimize mass transfer resistance. High fluid flow rates can be problematic for shear sensitive enzymes.

May be difficult to achieve reaction-limited regime for very fast enzymes.

c. Reaction-Limited Regime

$$\frac{x}{\alpha + x} = \frac{(1 - x)}{Da}$$

As $V_{MAX}' \rightarrow 0$ $Da \rightarrow 0$ $x \rightarrow 1$ $S_{SURF} \rightarrow S_{BULK}$

$$\text{Reaction Rate} = \frac{V_{MAX}'x}{\alpha + x}$$

$$\text{Reaction Rate } (x = 1) = \frac{V_{MAX}'}{\alpha + 1}$$

d. External Effectiveness Factor

As noted previously, one way to determine the regime is by the Damkohler number.

Another way is by the External Effectiveness Factor….

External Effectiveness Factor = η_E =

$$\frac{\text{Observed Reaction Rate}}{\text{Reaction Rate without Mass Transfer Limitation}}$$

$$\frac{\text{Observed Reaction Rate}}{\text{Reaction Rate without Mass Transfer Limitations}}$$

$$= \frac{\dfrac{V_{MAX}'x}{\alpha + x}}{\dfrac{V_{MAX}'}{\alpha + 1}}$$

$$\boxed{\eta_E = \frac{x(\alpha + 1)}{(\alpha + x)}}$$

$$\eta_E = \frac{x(\alpha + 1)}{(\alpha + x)}$$

η_E is a measure of the influence of external mass transfer resistance on the observed reaction rate.

if $\eta_E \ll 1$, then mass transfer resistance is restricting the supply of substrate to the surface.

Example Problem 3.3 (see pp. 85-86)

$V_{MAX}' = (6 \times 10^{-6}\ \text{mol/s·mg})(1 \times 10^{-4}\ \text{mg/cm}^2)$
$= 6 \times 10^{-10}\ \text{mol/cm}^2\text{·s}$
$= \underline{6 \times 10^{-4}\ \mu\text{mol/cm}^2\text{·s}}$

$K_M = 2 \times 10^{-3}\ \text{mol/L} = \underline{2\ \mu\text{mol/cm}^3}$

$k_L = \underline{4.3 \times 10^{-5}\ \text{cm/s}}$

Find S_{SURF}, V and η_E when …..

A) $S_{BULK} = 7\ \mu mol/cm^3\ (3.5 \times K_M)$

$$Da = \frac{V_{MAX}'}{k_L S_{BULK}} = \frac{(6 \times 10^{-4})}{(4.3 \times 10^{-5})(7)} = 1.993$$

$$\alpha = \frac{K_M}{S_{BULK}} = \frac{(2)}{(7)} = 0.2857 \qquad \beta = Da + \alpha - 1 = 1.279$$

$$x = \frac{\beta}{2}\left(-1 \pm \sqrt{1 + 4\alpha/\beta^2}\right) = 0.1940$$

$$S_{SURF} = xS_{BULK} = (0.1940)(7.0) = \underline{\mathbf{1.36\ \mu mol/cm^3}}$$

$$V_{TRUE} = \frac{V_{MAX}'x}{\alpha + x} = \frac{(6 \times 10^{-4})(0.1940)}{(0.2857 + 0.1940)} = \underline{\mathbf{2.43 \times 10^{-4}\ \mu mol/cm^2 s}}$$

$$\eta_E = \frac{x(\alpha + 1)}{\alpha + x} = \frac{(0.1940)(1.2857)}{(0.2857 + 0.1940)} = \underline{\mathbf{0.52}}$$

B) $S_{BULK} = 14\ \mu mol/cm^3$

$$Da = \frac{V_{MAX}'}{k_L S_{BULK}} = \frac{(6 \times 10^{-4})}{(4.3 \times 10^{-5})(14)} = 0.997$$

$$\alpha = \frac{K_M}{S_{BULK}} = \frac{(2)}{(14)} = 0.1429 \qquad \beta = Da + \alpha - 1 = 0.1395$$

$$x = \frac{\beta}{2}\left(-1 \pm \sqrt{1 + 4\alpha/\beta^2}\right) = 0.3146$$

$$S_{SURF} = xS_{BULK} = (0.3146)(14.0) = \underline{\mathbf{4.40\ \mu mol/cm^3}}$$

$$V_{TRUE} = \frac{V_{MAX}'x}{\alpha + x} = \frac{(6 \times 10^{-4})(0.3146)}{(0.1429 + 0.3146)} = \underline{\mathbf{4.13 \times 10^{-4}\ \mu mol/cm^2 s}}$$

$$\eta_E = \frac{x(\alpha + 1)}{\alpha + x} = \frac{(0.3146)(1.1429)}{(0.1429 + 0.3146)} = \underline{\mathbf{0.79}}$$

C) $V_{MAX}' = 12 \times 10^{-4}$ μmol/cm²s (S_{BULK} = 7 mmol/cm³)

$$Da = \frac{V_{MAX}'}{k_L S_{BULK}} = \frac{(12 \times 10^{-4})}{(4.3 \times 10^{-5})(7)} = 3.986$$

$$\alpha = \frac{K_M}{S_{BULK}} = \frac{(2)}{(7)} = 0.2857$$

$$\beta = Da + \alpha - 1 = 3.272$$

$$x = \frac{\beta}{2}\left(-1 \pm \sqrt{1 + 4\alpha/\beta^2}\right) = 0.0851$$

$$S_{SURF} = xS_{BULK} = (0.0851)(7.0) = \underline{\mathbf{0.60\ \mu mol/cm^3}}$$

$$V_{TRUE} = \frac{V_{MAX}'x}{\alpha + x} = \frac{(12 \times 10^{-4})(0.0851)}{(0.2857 + 0.0851)} = \underline{\mathbf{2.75 \times 10^{-4}\ \mu mol/cm^2 s}}$$

$$\eta_E = \frac{x(\alpha + 1)}{\alpha + x} = \frac{(0.0851)(1.2857)}{(0.2857 + 0.0851)} = \underline{\mathbf{0.30}}$$

D) S_{BULK} = 7 μmol/cm^3 but k_L is 8.6 × 10^{-5} cm/s

$$Da = \frac{V_{MAX}'}{k_L S_{BULK}} = \frac{(6 \times 10^{-4})}{(8.6 \times 10^{-5})(7)} = 0.997$$

$\alpha = 0.2857$

$\beta = 0.2824$

$x = 0.4116$

$$S_{SURF} = xS_{BULK} = (0.4116)(7.0) = \underline{\mathbf{2.88\ \mu mol/cm^3}}$$

$$V_{TRUE} = \frac{V_{MAX}'x}{\alpha + x} = \frac{(6 \times 10^{-4})(0.4116)}{(0.2857 + 0.4116)} = \underline{\mathbf{3.54 \times 10^{-4}\ \mu mol/cm^2 s}}$$

$$\eta_E = \frac{x\,(\alpha + 1)}{\alpha + x} = \frac{(0.4116)(1.2857)}{(0.2857 + 0.4115)} = \underline{\mathbf{0.76}}$$

E) $V_{MAX}' = 24 \times 10^{-4}$ μmol/cm^{2}s (S_{BULK} = 7 mmol/cm^3)

$$Da = \frac{V_{MAX}'}{k_L S_{BULK}} = \frac{(24 \times 10^{-4})}{(4.3 \times 10^{-5})(7)} = 7.97$$

$\alpha = 0.2857$

$\beta = 7.2591$

$x = 0.0391$

S_{SURF} = **0.27 μmol/cm^3**

V_{TRUE} = **2.89 × 10^{-4} μmol/cm^{2}s**

η_E = **0.15**

Summary (units and exponents left out)

	S_{BULK}	V_{MAX}'	k_L	S_{SURF}	V_{TRUE}	η_E
A	7	6	0.43	1.36	2.43	0.52
B	14	6	0.43	4.40	4.13	0.79
C	7	12	0.43	0.60	2.75	0.30
D	7	6	0.86	2.88	3.54	0.76
E	7	24	0.43	0.27	2.89	0.15

Compared to example A, increasing the enzyme level or activity is not effective because system is already in mass transfer limited regime. ($Da>1$)