Department of Biochemical Engineering & Biotechnology
Major Test (I Semester 2006 – 07)
BEL 403: Enzyme Engineering & Technology

M.M. : 25 + 15 **Time : Two hours**

(Part A)

. 1. Outline the role of enzymes in (a) textile processing and (b) detergents, indicating the catalytie functions involved their in.

(4)

2. An enzyme is immobilized on the surface of a non-porous matrix and used in a Packed bed reactor. Assuming that first order kinetics applies to the reaction, derive an expression for the maximum reaction velocity that accounts for the external film diffusion in the reactor.

(4)

3. A soluble enzyme following M.-M. kinetics (K_m = 8.9 mM, V_m=2.5 mmoles.m^{-3}.s^{-1}) is used to carry out a biotransformation in a batch stirred reactor. If S_0 = 12 mM and the half life of the enzyme is 4.5 hours, calculate the time required to achievc 90% conversion of the substrate.

(5)

4. Enzymatic isomerization of glucose to fructose can be kinetically expressed by the rate exprcssion:

$$v = \frac{0.128\,(G) - 0.098\,(F)}{0.096 + 0.383\,(G) + 0.25\,(F)}$$

where [G] and [F] represent the concentrations of glucose and fructose respectively. If the feed substrate concentration is 1.0 M and the desired conversion is 40 %, compare the productivity of the above reaction if carried out in CSTR / PFR of the same volume and enzyme loading.

(5)

5. A packed bed reactor containing immobilized glucose isomerase in the form of thin chips (av. Surface area= 1.12×10^{-4} m^2) is operated continuously with a substrate (1.0M glucose) feed of viscosity (μ) 0.601×10^{-3} N.sec.m^{-2} and density (ρ) 1074 Kg.m^{-3}. The Molecular diffusivity of 1.0 M glucose is 1.18×10^{-9} m^2. S^{-1} and the voidage of the reactor is determined as 0.605. At a feed superficial mass velocity of 0.041Kg.m^{-2}.s^{-1}, the reaetor output is 0.16 Kg.mol. m^{-3}.h^{-1}. From these data, compute the external film diffusion coefficient (k_L) and the substrate concentration gradient between bulk and the particle surface. The IME packing density is 0.173×10^3 kg.m^{-3} and a_m = 5.9 m^2. Kg^{-1}. Given:

For $0.0016 < N_{Re} < 55$ & $0.35 < \varepsilon < 0.75$;

$J_D = [k_L/G]\,[\mu/\rho D]^{2/3} = 1.09/\varepsilon\,(N_{Re})^{-2/3}$.

(7)

(Part B)

1. Initial studies of an esterase using a racemic mixture as substrate revealed that the L-enantiomer was the true substrate, as it was completely converted into product whereas the D-enantiomer could be recovered unchanged at the end of the reaetion. On the basis of this result the kinetics of the reaction were analyzed assuming that the D-enantiomer had no effect on the enzyme, and a Michaelis constant for the L-enantiomer was estimated to be 2mM. Susbequent work made it clear that it would have been more reasonable to assume that the D-enantiomer was a competitive inhibitor with K_I equal to the K_M value of the L-enantiomer. How should the original K_M estimate be revised to take aecount of this information? (Hint: For calculation purposes, assume [D-enantiomer] = [L-enantiomer]).

(5)

2. The following rate constants were measured for a hydrolase: $k_1 = 6x10^7$ /M.sec, $k_{-1} = 1x10^2$ /sec and $k_2 = 4x10^2$ /sec. After how much time does the hydrolysis reaction obey the Michaelis-Menten equation, given the substrate concentration of 100 μM and total enzyme concentration of 500 nM? (Hint: For calculation purposes, it is often assumed that any measurement approaching 0.99 can approximated as 1.00).

(5)

3. Explain, in a concise quantitative manner, the Levinthal's paradox for protein folding. Describe the major theory for overcoming the Levinthal's paradox for conformational search of proteins (Brevity with accuracy will be rewarded, extraneous information will lead to negative points).

(3 + 2 = 5)