Exam. Code: 0909
Sub. Code: 33368

2076

B.E. (Biotechnology) Fifth Semester

BIO-511: Enzyme Engineering and Technology

Time allowed: 3 Hours | Max. Marks: 50

NOTE: Attempt five questions in all, including Question No. I which is compulsory and selecting two questions from each Section. State clearly your assumptions.

x-x-x

Q. 1) Write briefly: *(2× 5 =10)*

a) In case of immobilized enzymes, the equation $V = K_L (S_b - S_s) = \frac{V_{max} \cdot S}{K_m + S + S^2/K_s}$ represents _____.

b) Define extracellular and extracellular enzyme with examples?

c) Define V_{max} and K_m? What is the turnover number?

d) Define effectiveness factor for immobilized enzyme? What do you mean by the total effectiveness factor for the system is close to unity?

e) Derive a performance equation for plug flow reactor.

SECTION – A

Q. 2) Derive the rate expression (V) for reaction scheme given by King-Altman's method,

$$E + S \leftrightarrow ES \rightarrow E + P$$
$$E + I \leftrightarrow EI_1$$
$$EI_1 + I \leftrightarrow EI_2$$

(10)

Q. 3. a) The protein catalyzes the reaction:

$$2H_2O_2 \text{ ----> } 2H_2O + O_2$$

And has a K_m 25×10^{-3} and turn over number 4.0×10^7 s^{-1}. Calculate the initial velocity of this reaction if the total enzyme concentration is 0.016×10^{-6} and the initial substrate concentration is 4.32×10^{-4}M. Calculate V_{max} for this enzyme.

b) Derive a rate of expression (V) for substrate inhibition kinetics and show that at maximum reaction rate of substrate concentration is

$$S_{max} = \sqrt{K_m \times K_S}$$

c) Derive the rate of expression (V) for different type of enzyme inhibitions. *(4, 3, 3)*

Q. 4. a) The enzymatic hydrolization of fish oil extract from crude oil has been carried out using lipase. One of the desired products is docosahexaenic. For the enzyme $V_{max} = 0.00266$ µmol/min. and K_m 0.052 M. The initial concentration of substrate 0.1M .Calculate the time needed to convert 80% substrate conversion .the reaction is carried out under isothermal conditions. If the enzyme is used as deactivates with half life 4.4hrs, what is the time required for 90% substrate conversion?

b) If Enzyme follows the deactivation kinetics, what will be effect of V_{max} and K_m, explain?

c) Find out degree of inhibition caused by competitive enzyme inhibition when [S] = K_m and [I] = ½ K_I

(5, 3, 2)

P.T.O.

SECTION - B

Q. 5) Name the various methods in Block diagram. Discuss the entrapment method in details. Write advantages and disadvantages of entrapment method. How will you eliminate the enzyme leakage and diffusion problem during immobilization? *(10)*

Q. 6. a) Invertase catalysis the reaction:

$$C_{12}H_{22}O_{11} + H_2O \rightarrow C_6H_{12}O_6 + C_6H_{12}O_6$$

(Sucrose) (Glucose) (Fructose)

Invertase from *Aspergillus oryzae* is immobilized in porous resin particle of diameter 1.6 mm. the effective diffusivity of sucrose in the resin is 1.3×10^{-11} m^2 s^{-1}. At a sucrose concentration of 0.85 kg m^{-3}, K_m and V_{max} for immobilized enzyme is 3.5 kg m^{-3} and 0.12 kg s^{-1} m^{-3} respectively. The observed reaction rate for free enzyme found to be 12.5 kg s^{-1} m^{-3}.

i) Calculate effectiveness factor.

ii) Determine the zero order reaction constant for immobilized invertase.

b) Derive the equation for effectiveness of an immobilized enzyme, assume that rate of substrate consumption can be expressed as zero order kinetics. *(5, 5)*

Q. 7 a) An enzymatic reaction follows M-M kinetics with V_{max} = 15 mmol m^{-3} s^{-1} and K_m = 5 mM. The reaction was carried out in batch reactor. Starting with an initial substrate concentration of 0.5 M, Calculate the time required for 50% conversion of the substrate?

b) Enzymatic isomerization glucose to fructose can be expressed by reaction mechanism:

$$E + S \leftrightarrow ES \leftrightarrow E + P$$

The kinetic parameter is:

$$\frac{V_{m,s}}{K_{m,s}} = 0.128 \quad , \quad \frac{V_{m,p}}{K_p} = 0.098 \quad , \quad \frac{1}{K_{m,s}} = 0.383 \quad , \quad \frac{1}{K_p} = 0.25$$

If the feed (glucose) concentration is 1.0 kg mole/liter and desired conversion is 40%. Calculate the productivity in above reaction mechanism in CSTR. *(4, 6)*

x-x-x