Exam.Code:1033
Sub. Code: 7570

2054

M.E. (Bio-Technology) Second Semester
MEBIO-203: Enzyme Engineering

Time allowed: 3 Hours **Max. Marks: 50**

NOTE: Attempt five questions in all, including Question No. 1 which is compulsory and selecting two questions from each Section. State clearly your assumptions.

x-x-x

1) Write briefly:
   - **a)** Define enzyme activity and specific enzyme activity?
   - **b)** What are the cofactors? How are they useful?
   - **c)** Write down the formula for the calculation of amylase activity.
   - **d)** According the Michaelis-Menten equation, what is the V/V_{max} ratio when [S] = 3 K_m?
   - **e)** Define Enzyme immobilization? List two advantages and disadvantages of immobilization.

(5x2)

SECTION – A

2. a) Derive the rate of expression (V) for different type of enzyme inhibitions.
   - **b)** Explain effect of substrate and enzyme concentration on enzyme activity.
   - **c)** 15 micrograms of an enzyme of molecular weight 30,000 working at V_{max} catalyzes the conversion of 60 micromole of substrate into product in 3 min. What is the enzyme's turnover number (in sec^{-1})?
   - **d)** The equilibrium constant for the given reaction is **5** for given reaction scheme. Suppose we have a mixture of

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

[S] = 2×10^{-4} M and [P] = 3×10^{-4} M.

What initial velocity will the reaction start towards equilibrium? If

$K_m^S = 3\times10^{-5}$ M, $V_{max}^S = 2$ μ moles.$lit^{-1}min.^{-1}$, $V_{max}^P = 4$ μ moles.$lit^{-1}min.^{-1}$. *(3, 2, 2, 3)*

3) A carboxypeptidase was found to have K_m= 2 μM and k_{cat} = 150 s^{-1} for substrate A.

- **(a)** What is the initial rate of reaction for [A] = 5 μM and [E_0] = 0.01 μM?
- **(b)** The presence of 5 mM of a competitive inhibitor decreased the initial rate by a factor of 2. What is the value of K_I?
- **(c)** A competing substrate B is added to part (a). It's K_m = 10 μM and k_{cat} = 100 s^{-1}. Calculate V_B/V_A. *(2, 5, 3)*

4. a) Define substrate inhibitions? Derive a rate of expression (V) for substrate inhibition kinetics and show that at maximum reaction rate of substrate concentration is

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

- **b)** Defined Biocatalyst and what are differences between Biocatalyst and Chemical catalyst?
- **c)** Show diagrammatically the role of enzyme in lowering the activation energy barrier.
- **d)** After 8 minutes in batch reaction substrate (S_0 = 1.0 moles) is 80% converted, after 11 minutes conversion is 90%. Find V_{max} and K_m. *(3, 2, 2, 3)*

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SECTION - B

5 a) Discuss various idealized enzyme reactor systems. Discuss which you justified to be the best?

b) Enzyme (E) catalyzes the transformation of substrate (S) to produce product (P) as follows:

$$A \xrightarrow{Enzyme} P, \quad v = \frac{200\ E_0 S}{2 + S}$$

If we introduce enzyme (E_0 = 0.001 mol/lit.) and substrate (S_0 = 10 mol/lit.) into a batch reactor and let the reaction proceed, find the time needed for the concentration of substrate to drop 0.025 mol/lit. *(5, 5)*

6 a) An enzyme which hydrolyzes the cellobiose to glucose, β-glycosidase is immobilized in sodium alginate gel sphere (*2.5* mm in diameter).Assume that the zero order reaction occurs at every point within the sphere with $K_0 = 0.0795$ mol/s-m^3 and cellobiose moves through the sphere by molecular diffusion with $D_C = 0.6 \times 10^{-5}$ cm^2/s (cellobiose in gel). Calculate the effectiveness factor of the immobilized enzyme when the cellobiose concentration in bulk solution is 10 mol/m^3.

b) Immobilized lactose is used to hydrolyze lactose in dairy waste to glucose and galactose. Enzyme is immobilized in resin particles and packed into at 0.05 m^3 Plug – flow column. The total effectiveness factor for the system is close to unity; K_m for the immobilized enzyme is 1.32 kg m^{-3}; V_{max} is 45 kg m^{-3} h^{-1}. The lactose concentration in the feed stream is 9.5 kg m^{-3}; a substrate conversion is 98% is required. At what flow rate should the reactor be operated?

c) The isomerisation of 5×10^{-2} mol·dm^{-1} bulk concentration of glucose to fructose is conducted at 313°K in a batch reactor using immobilised glucose isomerase. The reaction exhibits reversible Michaelis-Menten kinetics and is characterised by K_m value of 2×10^{-3} mol·dm^{-1}. The determined effectiveness factor η of 0.7 reveals an appreciable contribution of mass transport to the measured reaction rate. Calculate the substrate concentration at the solid-liquid interface under these conditions. *(3, 3, 4)*

7) Write a critical review on "Nano flower or Metal organic frameworks (MOFs) for efficient immobilized enzyme". *(10)*

x-x-x