Exam.Code:1033
Sub. Code: 35400

2055

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

Q. 1) Write briefly: *(5×2)*

a) Define extracellular and intracellular enzyme?

b) What are the functions of amylase, protease and lipase enzyme?

c) Name the various methods of 'Enzyme Immobilization' in Block diagram

d) According the Michaelis-Menten equation, what is the V/V_{max} ratio when [S] = 3 K_m?

e) Effect of substrate and enzyme concentration.

SECTION – A

Q. 2. a) Urease enzyme hydrolyzed urea at [S] = 0.03 mmol/L with a K_m value of around 0.06 mmol/L. The initial velocity observed was $1.5x10^{-3}$ mmol/L.min^{-1}. Calculate the initial velocity of the enzymatic reaction when using [S] =0.12 mmol/L.

b) Discuss type of enzyme inhibition Derive the rate of expression (V) for different type of enzyme inhibitions.

c) One microgram of a pure enzyme (MW=73000) catalyzed a reaction at a rate of 0.3 μmoles/min. under optimum conditions. Calculate the turnover number.

(4,4,2)

Q. 3. a) An enzyme catalyzed reaction ($K_m = 2.7x10^{-3}$ M) is inhibited by a competitive inhibitor **I** ($K_I = 3.1x10^{-5}$). Suppose that the substrate concentration is $3.6x10^{-4}$. How much the inhibitor is needed for 65% inhibition? How much does the substrate concentration have to be increased to reduce the inhibition to 25%?

b) Defined Biocatalyst and what are differences between Biocatalyst and Chemical catalyst?

c) 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. *(5, 2, 3)*

Q. 4. a) An enzyme is used to produce a compound that is further used to manufacture sunscreen lotion. V_{max} for enzyme 2.5 m mol/m^3-s, K_m = 8.9 mM. The initial concentration of substrate is 12 mM. If the reaction is being carried out under isothermal conditions, what batch reaction time is required for 90% substrate conversion and if the enzyme used in deactivates with half life of 4.4 hr, what is the batch reaction time required to achieve 90% substrate conversion?

b) The velocity of enzymatic reaction at 35 °C is twice as great as the velocity at 25 °C. Calculate activation energy (E_a). *(7, 3)*

P.T.O.

SECTION – B

Q. 5) Write a critical review on metal organic frameworks (MOFs) for enzyme immobilization and applications. *(10)*

Q. 6 a) Invertase catalysis the reaction:

$$\underset{\text{(Sucrose)}}{C_{12}H_{22}O_{11}} + H_2O \rightarrow \underset{\text{(Glucose)}}{C_6H_{12}O_6} + \underset{\text{(Fructose)}}{C_6H_{12}O_6}$$

Invertase from *Aspergillus oryzae* is immobilized in porous resin particle of diameter 1.8 mm. the effective diffusivity of sucrose in the resin is 1.5×10^{-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 16.5 kg s^{-1} m^{-3}.

i) Calculate effectiveness factor.

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

b) Discuss various idealized enzyme reactor systems. Discuss which you justified to be the best? *(5, 5)*

Q. 7 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) Derive the equation for effectiveness of an immobilized enzyme, assume that rate of substrate consumption can be expressed as zero order kinetics. *(4, 6)*

x-x-x