

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)

- Define extracellular and intracellular enzyme?
- What are the functions of amylase, protease and lipase enzyme?
- Name the various methods of 'Enzyme Immobilization' in Block diagram
- According the Michaelis-Menten equation, what is the V/V_{\max} ratio when $[S] = 3 K_m$?
- 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 1.5×10^{-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 μ moles/min. under optimum conditions. Calculate the turnover number.

(4,4,2)

Q. 3. a) An enzyme catalyzed reaction ($K_m = 2.7 \times 10^{-3}$ M) is inhibited by a competitive inhibitor I ($K_i = 3.1 \times 10^{-5}$). Suppose that the substrate concentration is 3.6×10^{-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 $2.5 \text{ m mol/m}^3\text{-s}$, $K_m = 8.9 \text{ mM}$. 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).

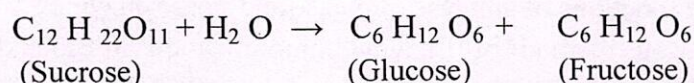
(7, 3)

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:



Invertase from *Aspergillus oryzae* is immobilized in porous resin particle of diameter 1.8 mm. the effective diffusivity of sucrose in the resin is $1.5 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$. 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 $0.12 \text{ kg s}^{-1} \text{ m}^{-3}$ respectively. The observed reaction rate for free enzyme found to be $16.5 \text{ kg s}^{-1} \text{ m}^{-3}$.

- 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 $K_0 = 0.0795 \text{ mol/s-m}^3$ and cellobiose moves through the sphere by molecular diffusion with $D_C = 0.6 \times 10^{-5} \text{ cm}^2/\text{s}$ (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