

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: (1×10 =10)
- According to the Michaelis-Menten equation, what is the V/V_{\max} ratio when $[S] = 3 K_m$?
 - Define extracellular enzyme? Give two examples.
 - List two advantages and disadvantages of immobilization.
 - Name three starch hydrolyzing enzymes.
 - What is the turnover number?
 - Define effectiveness factor for immobilized enzyme?
 - What is the Hanes – Woolf plot?
 - Define enzyme activity and specific enzyme activity?
 - What is the half life of enzyme?
 - What are the functions of protease and lipase enzymes?

SECTION – A

2. a) Derive the rate of expression (V) for different types of enzyme inhibitions.
b) Explain the effect of substrate and enzyme concentration on enzyme activity.
c) Find out Degree of inhibition (DOI) caused by uncompetitive inhibition when $[S] = \frac{1}{2} K_m$ and $[I] = K_i$
d) The equilibrium constant for the given reaction is 5 for the given reaction scheme. Suppose we have a mixture of

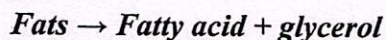


$$[S] = 2 \times 10^{-4} \text{ M and } [P] = 3 \times 10^{-4} \text{ M.}$$

What initial velocity will the reaction start towards equilibrium? If

$$K_m^S = 3 \times 10^{-5} \text{ M, } V_{\max}^S = 2 \mu \text{ moles.lit}^{-1}\text{min.}^{-1}, V_{\max}^P = 4 \mu \text{ moles.lit}^{-1}\text{min.}^{-1}. (3, 2, 2, 3)$$

3. a) Lipase is being investigated as an additive to laundry detergent for removal of stains from fabric. The general reaction is ---



The Michaelis constant for pancreatic lipase is 5 mM. At 60 °C, lipase is subjected to deactivation with a half life of 8 min. Fat hydrolysis under specific conditions which simulates a top-loading washing machine. The initial fat concentration is 45 mM and maximum reaction rate of hydrolysis is $0.07 \text{ mmol l}^{-1} \text{ s}^{-1}$. How long does it take for the enzyme to hydrolyse 80% of the fat present?

- b) If an enzyme follows the deactivation kinetics, show that only V_{\max} , not K_m is affected.
c) One microgram of a pure enzyme (MW=73000) catalyzed a reaction at a rate of $0.3 \mu \text{ moles/min.}$ under optimum conditions. Calculate the turnover number. (5, 3, 2)

(2)

- 4) A carboxypeptidase was found to have $K_m = 2 \mu\text{M}$ and $k_{\text{cat}} = 150 \text{ s}^{-1}$ for substrate A.
- What is the initial rate of reaction for $[A] = 5 \mu\text{M}$ and $[E_0] = 0.01 \mu\text{M}$?
 - 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 ?
 - A competing substrate B is added to part (a). It's $K_m = 10 \mu\text{M}$ and $k_{\text{cat}} = 100 \text{ s}^{-1}$. Calculate V_B/V_A . (2, 5, 3)

SECTION - B

5. a) Enzymatic isomerization glucose to fructose can be expressed by reaction mechanism:



The kinetic parameter is:

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

If the feed (glucose) concentration is 1.0 kg mole/liter and desired conversion is 50%. Calculate the productivity in above rate expression in CSTR.

- 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 $45 \text{ kg m}^{-3} \text{ h}^{-1}$. 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? (6, 4)
6. a) The isomerisation of $5 \times 10^{-2} \text{ mol} \cdot \text{dm}^{-3}$ 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 $2 \times 10^{-3} \text{ mol} \cdot \text{dm}^{-3}$. 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.
- Discuss external and internal mass transfer in immobilized enzyme. (5, 5)
- 7) Write a critical review on “Nano flower for efficient immobilized enzyme” (10)