

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. 1 which is compulsory and selecting two questions from each Section. State clearly your assumptions.

x-x-x

Q.1) Write briefly:

(1×10 =10)

- Define competitive enzyme inhibition?
- Define extracellular enzyme? Give two examples.
- Why enzyme activity reduced after immobilization in few cases?
- What do you mean by optimum temperature and pH?
- What is the turnover number?
- Define effectiveness factor for immobilized enzyme?
- What is the Hanes –Woolf plot?
- Define enzyme activity and specific enzyme activity?
- Write down the formula for the calculation of amylase activity.
- What are the functions of protease and lipase enzyme?

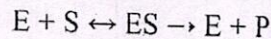
SECTION – A

Q.2. a) Derive the rate of expression (V) for different type of enzyme inhibitions.

- An enzyme is used to produce a compound that is further used to manufacture sunscreen lotion. V_{max} for enzyme $2.5 \text{ mol/m}^3\text{-s}$, $K_m = 8.9 \text{ mM}$. 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?
- Find out degree of inhibition caused by competitive enzyme inhibition when $[S] = K_m$ and $[I] = \frac{1}{2} K_i$

(3, 5, 2)

Q.3. a) An enzymatic reaction has a simple Michaelis-Menten mechanism:



K_1 and K_{-1} are very fast, $K_2 = 100 \text{ 1/sec}$ and $K_M = 10^{-4} \text{ M}$ at 280 K while $K_2 = 200 \text{ 1/sec}$, and $K_M = 1.5 \times 10^{-4} \text{ M}$ at 300 K. (i) For $[S] = 0.1 \text{ M}$ and $[E_0] = 10^{-5} \text{ M}$, what is v_0 at 280 K?

(ii) Calculate the activation energy for K_2 . (iii) What is K_{eq} at 280 K for E binding to S?

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

c) Explain effect of substrate and enzyme concentration on enzyme activity.

(6, 2, 2)

Q.4. a) An enzyme catalyzed reaction ($K_m = 2.7 \times 10^{-3} \text{ M}$) 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) An enzyme with a K_m of $1 \times 10^{-3} \text{ M}$ was assayed using an initial substrate concentration of $3 \times 10^{-5} \text{ M}$. After 2 min, 5 percent of the substrate was converted. What is the maximum velocity of this reaction?

c) Define substrate inhibitions? Derive a rate of expression (V) for substrate inhibition kinetics by King-Altman's method and show that at maximum reaction rate of substrate concentration is

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

(4, 2, 4)

(2)

SECTION - B

- Q. 5. a) Inversion of sucrose by invertase follows substrate inhibition kinetics. Immobilized invertase Preparation to be used in CSTR and following data are given
 $S_0=100$ moles/lit., $S_{\max}=20$ moles/lit., $K_m=8$ moles/lit., $V_{\max}=4.45 \times 10^{-3}$ moles.lit⁻¹sec⁻¹
 Calculate the feed rate to get maximum productivity, if the reactor volume 0.001 m^3 .
- b) The isomerisation of $5 \times 10^{-2} \text{ mol} \cdot \text{dm}^{-1}$ 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 $2 \times 10^{-3} \text{ mol} \cdot \text{dm}^{-1}$. 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. (4, 6)
- Q. 6. a) Name the various methods in Block diagram. Discuss entrapment method and write a procedure for enzyme immobilization by sodium alginate method.
- 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)
- Q.7a) 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 $1.0 \text{ kg mole/liter}$ and desired conversion is 40%. Calculate the productivity in above rate expression in CSTR.
- b) Design the performance equation for Batch reactor if the systems follow the enzyme deactivation kinetics. (7, 3)

x-x-x