Exam. Code: 0909
Sub. Code: 6308

2123

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

x-x-x

1) Write briefly: *(1×10 =10)*

a) What do you mean by optimum temperature and pH?
b) Define enzyme activity and specific enzyme activity?
c) Define extracellular enzyme? Give two examples.
d) Write down the formula for the calculation of amylase activity.
e) What are the functions of protease and lipase enzyme?
f) What is the turnover number?
g) What do you mean by half life of enzyme
h) Define effectiveness factor for immobilized enzyme?
i) Define substrate inhibition?
j) What is the Hanes –Woolf plot?

SECTION - A

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

Fats → Fatty acid + glycerol

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

b) Find out degree of inhibition caused by competitive enzyme inhibition when $[S]=K_m$ and $[I]=½ K_I$.

c) Explain effect of substrate and enzyme concentration on enzyme activity. *(4, 3, 3)*

3. a) Derive the rate expression (V) for reaction scheme given by King-Altman's method.

$$E + S \leftrightarrow ES \rightarrow E + P$$
$$E + I \leftrightarrow EI_1$$

b) An enzymatic reaction has a simple Michaelis-Menten mechanism:

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

K_1 and K_{-1} are very fast, K_2 = 100 1/sec and $K_M = 10^{-4}$ M at 280 K while K_2 = 200 1/sec, and $K_M = 1.5\times10^{-4}$ M at 300 K. (i) For [S] = 0.1 M and $[E_0] = 10^{-5}$ 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? *(4, 6)*

4. a) An enzyme catalyzed reaction ($K_m = 2.7\times10^{-3}$ M) is inhibited by a competitive inhibitor I ($K_I = 3.1\times10^{-5}$). 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) Describe the type of enzyme inhibitions and compare V_{max} and K_m with controlled enzyme.

c) An enzyme with a K_m of 1×10^{-3} M was assayed using an initial substrate concentration of 3×10^{-5} M. After 2 min, 5 percent of the substrate was converted. What is the maximum velocity of this reaction? *(4, 4, 2)*

P.T.O.

SECTION - B

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

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

The kinetic parameter is:

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

If the feed (glucose) concentration is 1.0 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)

6.a) Inversion of sucrose by invertase follows substrate inhibition kinetics. Immobilized invertase

Preparation to be used in CSTR and following data are given

S_o=100 moles/lit., S_{max}= 20 moles/lit., K_m=8moles/lit, V_{max}=4.45×10^{-3}moles.$lit^{-1}sec^{-1}$

Calculate the feed rate to get maximum productivity, if the reactor volume 0.001 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)

7. a) Name the various methods in Block diagram. Discuss entrapment method and write a procedure for enzyme immobilization by sodium alginate method.

b) 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.

(4, 6)

x-x-x