Exam. Code: 0909
Sub. Code: 6308

2074

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

Q.1) Write briefly: *(1×10 =10)*

a) Define competitive enzyme inhibition?
b) Define extracellular enzyme? Give two examples.
c) Why enzyme activity reduced after immobilization in few cases?
d) What do you mean by optimum temperature and pH?
e) What is the turnover number?
f) Define effectiveness factor for immobilized enzyme?
g) What is the Hanes –Woolf plot?
h) Define enzyme activity and specific enzyme activity?
i) Write down the formula for the calculation of amylase activity.
j) 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.

b) 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?

c) Find out degree of inhibition caused by competitive enzyme inhibition when [S] = K_m and [I] = ½ K_I *(3, 5, 2)*

Q.3. a) 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. 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?

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 ($K_m = 2.7\times10^{-3}$ M) is inhibited by a competitive inhibitor I (K_I = 3.1×10^{-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 1×10^{-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?

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)*

P.T.O.

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_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) 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)

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:

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

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)

x-x-x