BE- 5th Sem ; Dec.-2022

Exam. Code: 0909
Sub. Code: 6308

2122

B.E. (Biotechnology) Fifth Semester

BIO-511: Enzyme Engineering and Technology

Time allowed: 3 Hours Max. Marks: 50

NOTE: *Attempt <u>five</u> 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) Define competitive enzyme inhibition?

b) Define extracellular enzyme? Give two examples.

c) Define enzyme immobilization and in few cases why the enzyme activity reduced after immobilization.

d) Write down the formula for the calculation of Thiele Modulus (Θ).

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

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

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

$$Fats \rightarrow Fatty\ acid + glycerol$$

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?

c) Find out degree of inhibition caused by competitive enzyme inhibition when [S] = K_m and [I] = ½ K_I *(4, 4, 2)*

3. a) Derive a rate of expression V for given reaction scheme:

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

The equilibrium constant for the given reaction is 5. Suppose we have a mixture of

[S] = 2×10^{-4} M and [P] = 3×10^{-4} M.

What initial velocity will the reaction start towards equilibrium? If

$K_m^S = 3\times10^{-5}$ M, $V_{max}^S = 2\ \mu$ moles.$lit^{-1}min.^{-1}$, $V_{max}^P = 4\ \mu$ moles.$lit^{-1}min.^{-1}$.

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. *(4, 3, 3)*

P.T.O.

4) Derive the rate expression (V) for reaction scheme given by King-Altman's method,

$$E + S \leftrightarrow ES \rightarrow E + P$$
$$E + I \leftrightarrow EI_1$$
$$EI_1 + I \leftrightarrow EI_2$$

(10)

SECTION - B

5. a) A substrate is converted to a product by the catalytic action of an enzyme. Assume that The Michaelis-Menten kinetics parameters for this reaction are:

$K_m = 0.03$ mol /L $\quad V_{max} = 1.3$ mol /L min.

i) What should be the size of steady-state CSTR to convert 95 percent to incoming substrate ($S_0 = 10$ mol/ L) with a flow rate of 10 L/ hr?

ii) What should be the size of the reactor if you employ a plug flow reactor instead of the CSTR in the part (i)?

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)*

6. a) Discuss External and Internal mass transfer in the immobilized enzyme.

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) Define enzyme immobilization. Write advantages and disadvantages of immobilization. Discuss the various methods of immobilization in details. *(10)*