Exam.Code:1033
Sub. Code: 7570

2063

M.E. (Bio-Technology) Second Semester

MEBIO-203: Enzyme Engineering

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.

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1) Write briefly: *(1×10 =10)*

a) According the Michaelis-Menten equation, what is the V/V_{max} ratio when [S] = 3 K_m?
b) Define extracellular enzyme? Give two examples.
c) List two advantages and disadvantages of immobilization.
d) Name three starch hydrolyzing enzyme.
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) What is the half life of enzyme?
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) Explain effect of substrate and enzyme concentration on enzyme activity.
c) Find out Degree of inhibition (DOI) caused by uncompetitive inhibition when [S] = ½ K_m and [I] = K_I
d) The equilibrium constant for the given reaction is **5** for given reaction scheme. Suppose we have a mixture of

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

[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, $V_{max}^S = 2$ μ moles.lit^{-I}min.$^{-1}$, $V_{max}^P = 4$ μ moles.lit^{-1}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 ---

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) If Enzyme follows the deactivation kinetics, Show that only V_{max}, not K_m affected.
c) One microgram of a pure enzyme (MW=73000) catalyzed a reaction at a rate of 0.3 μmoles/min. under optimum conditions. Calculate the turnover number. *(5, 3, 2)*

P.T.O.

4) A carboxypeptidase was found to have K_m= 2 μM and k_{cat} = 150 s^{-1} for substrate A.

(a) What is the initial rate of reaction for [A] = 5 μM and [E_0] = 0.01 μM?

(b) 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?

(c) A competing substrate B is added to part (a). It's K_m = 10 μM and k_{cat} = 100 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:

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

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 50%. Calculate the productivity in above rate expression in CSTR.

b) 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 kg m^{-3} 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 × 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.

b) Discuss external and internal mass transfer in immobilized enzyme. *(5, 5)*

7) Write a critical review on "Nano flower for efficient immobilized enzyme" *(10)*

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