For scanning

Exam. Code: 0909
Sub. Code: 6707

1129

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

(1×10 =10)

Q. 1) Write briefly:

a) Define enzyme activity and specific enzyme activity?
b) Write down the formula for the calculation of amylase activity.
c) Define K_m and significance for the estimation of K_m?
d) Define extracellular enzyme? Give two examples.
e) List two advantages and disadvantages of immobilization.
f) What are the functions of protease and lipase enzyme?
g) What is the turnover number?
h) Define effectiveness factor for immobilized enzyme?
i) What is the Hanes –Woolf plot?
j) Write down the formula for the calculation of Thiele Modulus (Θ).

SECTION – A

Q. 2. a) Defined Biocatalyst and what are differences between Biocatalyst and Chemical catalyst? Describe the Industrial applications of Biocatalyst.

b) Malonate, a competitive inhibitor of succinate dehydrogenase, was found to cause 95% Inhibition of the enzyme's activity. If the succinate (substrate) concentration for the enzyme was 3.5×10^{-5} M and the K_M for this succinate is 4.4×10^{-6} M, what was the initial malonate concentration ($K_I = 2.4 \times 10^{-7}$ M)? *(5, 5)*

Q. 2. a) Explain effect of substrate and enzyme concentration on enzyme activity.

b) 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

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? *(3, 3, 4)*

Q. 4. 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

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

What initial velocity will the reaction start towards equilibrium? If

$K_m^S = 3 \times 10^{-5}$ M, $V_{max}^S = 4$ μ moles.lit^{-1}min.$^{-1}$, $V_{max}^P = 6$ μ moles.lit^{-1}min.$^{-1}$.

b) Describe the type of enzyme inhibitions and compare V_{max} and K_m with controlled enzyme.

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

P.T.O.

SECTION - B

Q. 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:

$K_m = 0.25$ 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) Invertase catalysis the reaction:

$$\underset{\text{(Sucrose)}}{C_{12}H_{22}O_{11}} + H_2O \rightarrow \underset{\text{(Glucose)}}{C_6H_{12}O_6} + \underset{\text{(Fructose)}}{C_6H_{12}O_6}$$

Invertase from *Aspergillus oryzae* is immobilized in porous resin particle of diameter 1.6 mm. the effective diffusivity of sucrose in the resin is 1.3×10^{-11} m^2 s^{-1}. At a sucrose concentration of 0.85 kg m^{-3}, K_m and V_{max} for immobilized enzyme is 2.5 kg m^{-3} and 0.12 kg s^{-1} m^{-3} respectively. The observed reaction rate for free enzyme found to be 11.5 kg s^{-1} m^{-3}.

i) Calculate effectiveness factor.

ii) Determine the zero order reaction constant for immobilized invertase. *(4, 6)*

Q. 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) Derive the equation for effectiveness of an immobilized enzyme, assume that rate of substrate consumption can be expressed as zero order kinetics. *(5, 5)*

Q. 7 .a) Name the various methods in Block diagram. Discuss the entrapment method in details. Write advantages and disadvantages of entrapment method. How will you eliminate the enzyme leakage and diffusion problem during immobilization?

b) Design the performance equation for Batch reactor if the systems follow the enzyme deactivation kinetics. *(6, 4)*

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