Exam.Code:0909
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1078

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. 1 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 bio catalyst? Give four examples.
b) What do you understand by competitive enzyme inhibition?
c) What are cofactors? How are they useful?
d) Define substrate inhibition.
e) What is the enzyme's turnover number?
f) Why we immobilize the enzyme?
g) Write three general properties of enzymes.
h) What do you understand by V_{max} and K_m value of enzymes?
i) Write the formula of Thiele modulus (Φ).
j) Write two application of amylase and protease enzyme.

SECTION-A

Q.2 A) Discuss various advantages of enzymes as compared to conventional chemical catalysts.

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

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

$$E + I \leftrightarrow EI_1$$

$$EI_1 + I \leftrightarrow EI_2$$

C) Explain allosteric enzyme and hill coefficient. *(2, 5, 3)*

Q.3. A) After 8 minutes in batch reaction substrate (S_0 = 1.0 moles) is 80% converted, after 11 minutes conversion is 90%. Find V_{max} and K_m.

B) 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?

C) Explain allosteric enzyme and hill coefficient. *(3, 5, 2)*

Q.4 A) 15 micrograms of an enzyme of molecular weight 30,000 working at V_{max} catalyzes the conversion of 60 micromole of substrate into product in 3 min. What is the enzyme's turnover number (in sec^{-1})?

P.T.O.

B) An enzyme has a K_m of 4.7×10^{-5}M. If the V_{max} of the preparation is 22 μ moles liter^{-1} min^{-1}, what velocity would be observed in the presence of 2.0×10^{-4}M substrate and 5.0×10^{-5}M of:

i) A competitive inhibitor,

ii) Noncompetitive inhibitor

iii) An uncompetitive inhibitor

If K_I for all these cases is 3.0×10^{-4}M?

iv) What is the degree of inhibition in all cases?

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

SECTION-B

Q.5) Discuss the various techniques for enzyme immobilization. Loss of biocatalyst activity during immobilization, explain? How will you eliminate the enzyme leakage and diffusion limitation during Immobilization? *(10)*

Q. 6. A) Invertase is immobilized in ion-exchange resin of diameter 1.0 mm. the immobilized beads are packed into small column reactor and sucrose solution at a concentration of 16 g mol m^{-3} is pumped rapidly through the bed. In another reactor an identical quantity of free enzyme is mixed into same volume of sucrose solution. Assume that the kinetic parameters for free enzyme are:

$$K_m = 8.8 \text{ g mol m}^{-3} \text{ and } V_{max} = 3.19 \text{ g mol m}^{-3} \text{ s}^{-1}.$$

The effective diffusivity of sucrose in ion exchange resin is 2×10^{-10} m^{2}s^{-1}. Assume that the rate of sucrose utilization to be zero order and rate of constant 8.33 g mol m^{-3}s^{-1}. What should be rate of reaction for immobilized system?

B) An enzyme which hydrolyzes the cellobiose to glucose, β-glycosidase is immobilized in sodium alginate gel sphere (*2.5* mm in diameter). Assume that the zero order reaction occurs at every point within the sphere with $K_0 = 0.0795$ mol/s-m^3 and cellobiose moves through the sphere by molecular diffusion with $D_C = 0.6 \times 10^{-5}$ cm^2/s (cellobiose in gel). Calculate the effectiveness factor of the immobilized enzyme when the cellobiose concentration in bulk solution is 10 mol / m^3.

C) Write down substrate balance equation in immobilized enzyme beads? *(5, 3, 2)*

Q.7 a) Design the performance equation for Batch, CSTR, and PFR if the systems follow the competitive enzyme inhibition kinetics.

b) 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:

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

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)? *(5, 5)*

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