1-34

Exam. Code: 0909 Sub. Code: 6707

1128

B.E. (Bio-Technology) 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.

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Q.1) Write briefly:

 $(1 \times 10 = 10)$

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- a) Define competitive enzyme inhibition?
- b) Define extracellular enzyme? Give two examples.
- c) Define enzyme immobilization and list two advantages of 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

- Q.2. a) Derive the rate of expression (V) for different type of enzyme inhibitions.
 - 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)?
 - c) At $E_0 = 200$ nM and substrate concentration = 40 μ M, the reaction velocity (V) of an enzyme is 9.6 μ MS⁻¹. Assuming K_{cat} to be 600 S⁻¹, what is the K_m? (4, 4, 2)
- Q.3. a) Defined Biocatalyst and what are differences between Biocatalyst and Chemical catalyst?

 Describe the Industrial applications in Food, Pharmaceutical and Medical Industry of Biocatalyst.
 - b) Show diagrammatically the role of enzyme in lowering the activation energy barrier.
 - c) Explain effect of substrate and enzyme concentration on enzyme activity. (6, 2, 2)
 - Q.4. a) Derive the rate expression (V) for reaction scheme given by King-Altman's method,

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

$$E + I \leftrightarrow EI_1$$

$$EI_1 + I \leftrightarrow EI_2$$

An enzyme is used to produce a compound that is further used to manufacture sunscreen lotion. V_{max} for enzyme 2.5 m mol/m³s, $K_m = 8.9$ 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? (5, 5)

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:

 $K_{\rm m} = 0.03 \; {\rm mol} \; / {\rm L}$ $V_{\rm max} = 1.3 \; {\rm mol} \; / {\rm L} \; {\rm min}.$

- i) What should be the size of steady-state CSTR to convert 95 percent to incoming substrate ($S_0 = 10 \text{ mol/ L}$) 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)
- Q. 6. a) Invertase catalysis the reaction:

 $C_{12} H_{22}O_{11} + H_2 O \rightarrow C_6 H_{12} O_6 + C_6 H_{12} O_6$ (Sucrose) (Glucose) (Fructose)

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 3.5 kg m $^{-3}$ and 0.12 kg s $^{-1}$ m $^{-3}$ respectively. The observed reaction rate for free enzyme found to be 12.5 kg s $^{-1}$ m $^{-3}$.

- i) Calculate effectiveness factor.
- ii) Determine the zero order reaction constant for immobilized invertase.
- 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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