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Exam.Code:1033
Sub. Code: 7872

1059

M.E. (Bio-Technology) Second Semester
MEBIO-203: Enzyme Engineering

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.*

x-x-x

Q.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) 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) Define substrate inhibitions? Derive a rate of expression (V) for substrate inhibition kinetics and show that at maximum reaction rate of substrate concentration is

$$S_{max} = \sqrt{K_m \times K_S}$$

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 x 10^{-5} M and the K_M for this succinate is 4.4 x 10^{-6} M, what was the initial malonate concentration (K_I = 2.4 x 10^{-7} M)?

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. *(4, 4, 2)*

Q. 3. **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) Defined Biocatalyst and what are differences between Biocatalyst and Chemical catalyst?
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^{-1}min.$^{-1}$, $V_{max}^P = 4$ µ moles.lit^{-1}min.$^{-1}$. *(3, 2, 2, 3)*

P.T.O.

Q. 4. a) Aspartase enzyme is used in batch reaction for manufacture of aspartic acid, a component of low calorie sweetener. In the substrate range of interest the conversion can describe using michalis- menten kinetics with 4.0 g l^{-1}. The substrate solution contains 1.5 g l^{-1} ammonium fumarate, the reaction stop when 85% of substrate is converted. At 32°C, V_{max} for enzyme 5.9 g l^{-1} h^{-1} and its half life is 10.5 d. At 37°C V_{max} increases to 8.5 g l^{-1} h^{-1} but the half life is reduced to 2.3 d. Which operating temperature would you recommended?

b) The hydrolytic reaction between ATP → ADP is carried by ATPase enzyme, the following data given:

$$K_m = 1\times10^{-4}\ M,\quad K_2 = 1.67\times10^{-4}\ S^{-1},\ \text{Temperature} = 50°C,\quad pH = 4.6$$

If the initial substrate concentration S_0 =0.02 M .Calculate the enzyme required to achieve 10% conversion in 12 hrs. Assuming that enzyme follows simple Michaelis Mentan kinetics. *(5, 5)*

SECTION - B

Q. 5) Write a critical review on metal organic framework (MOF): A novel platform for enzyme immobilization and applications. *(10)*

Q. 6. a) 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?

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 mol /L V_{max} = 1.3 mol / L min. 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? *(5, 5)*

Q.7) 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. *(10)*

x-x-x