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NBT403

(Following Paper ID and Roll No. to be filled in your Answer Book)

PAPER ID : 154413

Roll No.

B. Tech.

(SEM. IV) THEORY EXAMINATION, 2014-15

ENZYME ENGINEERING

Time : **3** Hours] [Total Marks : **100**

Note : Attempt all questions, All questions carry equal marks.

1 Attempt any four parts of the following : [5x4=20]

(a) Define modulators, coenzyme and cofactor.

(b) Explain enzyme engineering with its applications.

(c) How does an active site differ from a regulatory site ?

(d) Let us consider the enzyme reaction:

$$E + S \underset{k'_1}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} E + P$$

Given that $k_1 = 1 \times 10^9$, $k'_1 = 1 \times 10^6$ and $k_2 = 1 \times 10^2$

(I) Calculate the MM constant for the reaction.

(II) Find out the dissociation constant for enzyme substrate.

(e) What is K_{cat} ? Define K_m and V_{max} and explain its significance.

(f) Explain Fisher's and Koshland's hypothesis for monosubstrate reaction.

2 Attempt and two parts of the following : [10×2=20]

(a) How are enzymes classified based on the reaction they catalyse Explain with suitable examples.

(b) Discuss the effect of pH, temperature and substrate concentration on enzyme action.

(c) Calculate the velocity of enzyme catalyzed reaction at 3×10^{-6} M substrate concentration. When the velocity of the reaction is 40 μ mol/min at substrate concentration of 1×10^{-4} M The k_m value is 3×10^{-5}.

3 Attempt any two parts of the following : [10×2=20]

(a) What is the role of selection of sources in the isolation of enzymes ? Discuss the different purification techniques.

(b) Explain the direct and linked assay with suitable example in detail.

(c) Consider the following data of an enzyme catalyzed reaction:

Substrate Concentration (in M)	Velocity ($innmolesL^{-1}$ min^{-1})
1.11×10^{-6}	20
4.99×10^{-6}	48
1.00×10^{-5}	60
2.63×10^{-4}	79
1.00×10^{-2}	80

(I) Calculate V_{max} and K_m of the reaction.

(II) Calculate the velocity of the reaction when the substrate concentration is 3.33×10^{-6} M.

(III) Calculate the velocity of the reaction at substrate concentration of 4.99×10^{-6} M, if the enzyme concentration is doubled.

4 Attempt any two parts of the following : [10×2=20]

(a) Discuss the stability and diffusion effect of immobilized enzyme.

(b) Explain adsorption and entrapment method of immobilization. Discuss the application of immobilized enzyme.

(c) What is allosteric enzyme? Explain Monod-Wyman-Changeux (MWC) model or symmetrical model to explain the "cooperative binding".

5 Attempt any two parts of the following : [10×2=20]

(a) How can we use K_m and V_{max} values to distinguish different types of inhibition ?

(b) What is the principle behind biosensors ? Explain potentiometric biosensor in detail.

(c) Describe the different components to design an enzyme reactor. Explain Packed Bed Reactor and Fluidized Bed Reactor.