Q. Code:650351

Reg. No. | | | | | | | | | | | | | |

B.E / B.TECH. DEGREE EXAMINATIONS, MAY 2023

Fourth Semester

BT18404 – ENZYME TECHNOLOGY AND BIOTRANSFORMATION

(Biotechnology)

(Regulation 2018/2018A)

TIME: 3 HOURS **MAX. MARKS: 100**

CO 1 Explain the concept of enzymatic reactions in biological systems.
CO 2 Identify the theoretical and practical aspects of enzyme kinetics in bioprocess applications.
CO 3 Compare various immobilization techniques to improve enzyme stability.
CO 4 Identify suitable techniques for production and purification of enzymes.
CO 5 Interpret enzymatic reaction mechanism and design artificial enzymes.

PART- A (10 x 2 = 20 Marks)

(Answer all Questions)

		CO	RBT LEVEL
1.	Explain the role of coenzymes and cofactors in enzymatic catalysis.	**1**	**2**
2.	Conclude the transition state stabilization has an influence over the enzymatic reaction and reaction rate.	**1**	**3**
3.	When an enzyme was immobilized on a solid matrix, K_m for a substrate was 0.1 mM, but for the same enzyme in free form, the K_m found to be 0.025 mM. Why there is decrease in K_m for immobilized enzyme (Assume all other conditions to be identical for both the cases).	**2**	**3**
4.	Write about Ping-Pong-Bi-Bi enzyme mechanism with an example.	**2**	**2**
5.	Assume you are working on immobilization of enzyme which is not having Tryptophan, Cysteine and Lysine in it. You are given an option to immobilize using covalent binding on a solid matrix, encapsulation, entrapment, gluteraldehyde based cross linking methods, which of these immobilization methods cannot be used, justify why?	**3**	**3**
6.	Differentiate immunosensor from enzymatic biosensors.	**3**	**3**
7.	Criticize the consequences that arises if the enzyme assay which you have developed is not measuring the initial velocity of the reaction.	**4**	**4**
8.	Illustrate how affinity chromatography is used for purification of enzyme.	**4**	**3**
9.	Justify why enzymatic synthesis in organic solvent is advantageous over the biocatalysis in pure aqueous media.	**5**	**4**
10.	Illustrate the role of catalytic traid in enzymatic catalysis.	**5**	**4**

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PART- B (5 x 14 = 70 Marks)

			Marks	CO	RBT LEVEL
11. (a)	**(i)**	Classify enzymes according to enzyme classification. Write their catalytic function and give an example for each class.	**(10)**	**1**	**2**
	(ii)	List the reaction sequence that happens once the substrate reaches the proximity of enzyme active site.	**(4)**	**1**	**2**
		(OR)			
(b)		Discuss about Arrhenius theory, how it can be applied for characterizing the enzymes and enzymatic reactions.	**(14)**	**1**	**2**
12. (a)	**(i)**	Derive the rate equation for homogeneous enzyme catalysis using Michaelis-Menten Kinetics.	**(10)**	**2**	**3**
	(ii)	How are the parameters of Michaelis Menten Kinetics estimated?	**(4)**	**2**	**3**
		(OR)			
(b)		Following data was obtained from enzyme assay at varying concentration of substrate.	**(14)**	**2**	**3**

S µM	0.5	1	1.5	2	2.5	3	4	5
V µM/s	1.00	1.54	1.88	2.11	2.27	2.40	2.58	2.70

Molecular mass of protein is 40 kDa, Protein Concentration taken for assay is 1 µg. Assume any missing data and calculate the catalytic efficiency of the enzyme.

13. (a)		Why immobilized enzymes are predominantly used rather than free enzymes in industrial scale enzymatic conversions? Outline various methods of enzyme immobilization with their major advantages and disadvantages.	**(14)**	**3**	**3**
		(OR)			
(b)		List the major components of biosensors? Explain the individual components, working principle of anyone biosensor with neat sketch.	**(14)**	**3**	**3**
14. (a)		An enzyme was purified through 4 chromatography/purification steps, following is the data obtained during each step for enzyme purification.	**(14)**	**4**	**3**

Step Purification	Volume (ml)	Protein (mg/ml)	Activity (U/ml)
Crude	760	0.8	854
Step 1	50	3.1	8456
Step 2	20	5.1	19584
Step 3	15	2.1	24516
Step 4	3	0.6	115470

Calculate total activity, specific activity and purification yield for each purification step. Represent it in the tabular form.

(OR)

(b)	Discuss about various enzyme purification methods which are applied for production and purification of enzymes. Criticize about problems associated with purification of such enzyme in industrial scale production.	**(14)**	**4**	**3**
15. (a)	Why enzymes are engineered to improve their stability and catalytic activity. Discuss about any one molecular biology method adopted for engineering enzyme.	**(14)**	**5**	**4**

(OR)

(b)	Support your justification by explaining the mechanism by which a chemical molecule that mimic the enzyme reaction center can catalysis a reaction.	**(14)**	**5**	**4**

PART- C (1 x 10 = 10 Marks)

(Q.No.16 is compulsory)

		Marks	CO	RBT LEVEL
16.	Design an enzymatic biosensor for detection of any body fluid which measures the metabolite and displays the digital output. Explain the components which you use for the designing and discuss their function.	**(10)**	**5**	**5**

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