

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

**PAPER ID : 9589**

Roll No.

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**B.Tech.**

**(SEMESTER-IV) THEORY EXAMINATION, 2012-13**

**ENZYME ENGINEERING**

*Time : 3 Hours ]*

*[ Total Marks : 100*

**SECTION – A**

1. Attempt **all** question parts. **10 × 2 = 20**
- (a) How enzymes are classified ?
- (b) Explain with an example indirect assay of an enzymatic reaction.
- (c) Describe effect of substrate concentration on rate of enzymatic reaction.
- (d) Illustrate the difference between the types of enzyme inhibitors
- (e) Define specificity of an enzyme action.
- (f) Define Allosteric regulation of enzymes.
- (g) Define crossing over.
- (h) When alcohol dehydrogenase was immobilized on polyacrylamide,  $K_m$  for Butanol was 0.1 mM. When the enzyme was immobilized on a polymer of methacrylate and acrylamide,  $K_m$  for Butanol was found to be 0.025 mM. Explain the above (Assume all other conditions to be identical for both the cases)
- (i) Discuss briefly a few applications of immobilized enzymes.
- (j) Differentiate between multifunctional enzyme and multienzyme complex.



## SECTION – B

2. Attempt any **three** question parts :

**3 × 10 = 30**

- (a) Enumerate the immobilization of enzymes by following methods stating example for each
- (i) Adsorption (Physical method)
  - (ii) Covalent bonding (Chemical method)
- (b) Discuss about the Eadie-Hofstee and Lineweaver-Burk plot in case of competitive and un-competitive inhibition with help of mathematical model.
- (c) Penicillin is hydrolyzed and thereby rendered inactive by penicillinase (also known as  $\beta$ -lactamase), an enzyme present in some resistant strains of bacteria. The molar mass of this enzyme is 29,000 g/mole. The amount of penicillin hydrolyzed in 1 minute in a 10 mL solution containing  $10^{-9}$  g of purified enzyme was measured as function of the concentration of penicillin (in  $\mu\text{mol/L}$ ). Assume the concentration of penicillin does not change appreciably during the assay.

[Penicillin]	Amount Hydrolyzed (nmols)
1	0.11
3	0.25
5	0.34
10	0.45
30	0.58
50	0.61

- (i) Plot  $1/\text{rate}$  vs.  $1/[\text{S}]$  for these data.
  - (ii) What is  $K_M$  ?
  - (iii) What is  $V_{\text{max}}$  ?
  - (iv) What is the turnover number ?
- (d) Describe the analysis of Film and Pore Diffusion on Kinetics of Immobilized enzyme reactor.
- (e) Explain with a neat sketch of production of enzyme from Microbial sources.

### SECTION – C

Attempt **all** questions.

$5 \times 10 = 50$

3. Attempt any **two** parts.

$2 \times 5 = 10$

- (a) Michaelis-Menton equation is not sufficient to determine  $k_m$  value exactly. Why ?
- (b) What is the significance of  $k_m$  value ? Enumerate.
- (c) How will you estimate the parameters of M.M. Kinetics ?

4. Attempt any **one** part.

$1 \times 10 = 10$

(a) The Purification table of purification process is as follows.

S.No.	Procedure	Total protein (mg)	Activity(units)
i	Crude extract	20,000	4,000,000
ii	Precipitation (salt)	5,000	3,000,000
iii	Precipitation (pH)	4,000	1,000,000
iv	Ion exchange chromatography	200	800,000
v	Affinity	50	750,000
vi	Size-exclusion	45	675,000

- (i) From the above table, calculate specific activity of the enzyme solution after each purification procedure.
  - (ii) Which of the purification procedures used for this enzyme is most effective (i.e., gives the greatest relative increase in purity) ? Which one is least effective ?
  - (iii) Is there any indication based on the results shown in the table that the enzyme after step 6, is no pure ?
- (b) Explain the production & purification of enzyme from Animal sources.

5. Attempt any **one** part :

$1 \times 10 = 10$

- (a) The kinetics of an enzyme were analyzed in the absence and presence of inhibitors A and B.

(S) (mM)	$V_0$ (Moles/minute)		
	No inhibitor	5mMA	0.1 mM B
1.0	43	30	26
2.0	68	50	41
5.0	105	86	64
10.0	128	113	77
20.0	144	134	88

- (a) What type of inhibitors are A and B: Competitive or NonCompetitive ? (Use your graphs to answer this question).
- (b) In addition to calculating  $K_m$  and  $V_{(\text{maximum velocity})}$  in the absence of inhibitors, calculate the  $K_i$  constants for the inhibitors A and B.
- (b) Explain in detail about the Allosteric Regulation of enzymes.

6. Attempt any **one** part :

$1 \times 10 = 10$

- (a) Discuss the various operational strategies of immobilized enzyme reactors.
- (b) What do you understand by Operational Stability of immobilized enzyme (IE) ? Discuss the decay pattern of immobilized enzyme.

7. Attempt any **two** parts :

$2 \times 5 = 10$

- (a) Write a brief note on the stability of immobilized enzymes.
- (b) Enumerate the applications of enzymes as biosensors.
- (c) Write a note on bienzyme electrode systems with a suitable example.