U.S.N.										

B.M.S. College of Engineering, Bengaluru-560019

Autonomous Institute Affiliated to VTU

January / February 2025 Semester End Main Examinations

Programme: B.E. **Semester: VI**
Branch: Biotechnology **Duration: 3 hrs.**
Course Code: 22BT6PCETK **Max Marks: 100**
Course: ENZYME TECHNOLOGY & KINETICS

Instructions: 1. Answer any FIVE full questions, choosing one full question from each unit.
2. Missing data, if any, may be suitably assumed.

Important Note: Completing your answers, compulsorily draw diagonal cross lines on the remaining blank pages. Revealing of identification, appeal to evaluator will be treated as malpractice.

				CO	PO	Marks
			UNIT-I			
1	a)	A biochemist discovers and purifies a new enzyme, generating the following purification table		CO 1	PO2	10

Step	Procedure	Total protein (mg)	Activity (Units)
1	Crude extract	20,000	4,000,000
2	Precipitation (Salt)	5,000	3,000,000
3	Precipitation (pH)	4,000	1,000,000
4	Ion exchange chromatography	200	800,000
5	Affinity chromatography	50	750,000
6	Size exclusion chromatography	45	675,000

(i) Calculate the specific activity of the enzyme solution after each purification procedure.
(ii) Which of the purification procedures used for this enzyme is most effective and why?
(iii) Which of the purification procedures is least effective and why?
(iv) Is there any indication based on the results shown in the table that the enzyme after step 6 is now pure?
(v) What else could be done to estimate the purity of the enzyme preparation?

	b)	Classify the enzymes according to Enzyme Commission's system giving one example to each class.		CO 1	PO1	5
	c)	Develop a strategy for purification of enzymes based on molecular size		CO 1	PO1	5
		OR				

	2	a)	You have a crude lysate sample (CL) containing a mixture of six proteins (1, 2, 3, 4, 5, β-galactosidase), and your goal is to obtain purified β-galactosidase. Some characteristics of these proteins are shown in the table below:	***CO*** 1	***PO1***	**10**

Protein	Concentration of ammonium sulfate (AS) required for precipitation	Molecular Weight (kDa)	Isoelectric point (pI)
1	35%	40	4.2
2	25%	24	3.8
3	60%	6	5.5
4	85%	78	7.8
5	50%	58	8.5
β-galactosidase	47%	115	5.3

You begin your purification by performing an ammonium sulfate (AS) precipitation. You add the appropriate concentration of AS to your CL sample, incubate overnight at 4˚C, then centrifuge to generate a supernatant (AS-S) and pellet (AS-P).

i. What concentration of AS will you use to precipitate β-galactosidase?
ii. After addition of that concentration of AS and centrifugation, which protein(s) will be in the supernatant (AS-S)?
iii. Which protein(s) will be in the pellet (AS-P)?
iv. After resuspending the AS-P in column buffer, which method you use to desalt the protein sample.

One way to purify β-galactosidase away from any contaminating proteins in the AS-P sample would be to separate them based on their molecular weight.

v. What type of column separates on this basis?
vi. Which protein (from your AS-P) would elute first from this type of column?

Instead, you decide to use ion exchange chromatography to further purify β-galactosidase away from other proteins in your AS-P sample. You first run a cation exchange column equilibrated using column buffer with a pH of 4.7.

vii. What charge does the matrix of a cation exchange column have?
viii. At pH 4.7, which protein(s) from the AS-P stick to the anion exchange column?
ix. State how you would elute a protein bound to an anion exchange column, and explain how this elution method works in one or two sentences.

		b)	The molecular weight of an unspecified protein, at physiological conditions, is 70,000 Dalton, as determined by sedimentation equilibrium measurements and by gel filtration chromatography. The SDS-polyacrylamide gel electrophoresis (SDS PAGE) of the protein yields a single band corresponding to molecular weight of 70,000 Dalton. However, in the presence of the reducing agent, β-mercaptoethanol, the SDS PAGE shows two bands, corresponding to molecular weights of 30,000 and 20,000 Dalton. i. From these data, describe the native protein in terms of the number of subunits present, their molecular weight, stoichiometry of subunits, and the kinds of bonding (covalent, noncovalent) existing between the subunits. ii. You treat your protein with protease (e.g. trypsin), run the product of the proteolytic cleavage on the SDS PAGE, and discover that the SDS gel in the absence of β-mercaptoethanol still shows one band, but shows 4 bands in the presence of β-mercaptoethanol. How would you explain this result?	***CO*** 1	***PO1***	**05**
		c)	What are enzymes and what is their function? Describe any four specificities exhibited by the enzymes with one exampe for each.	***CO*** 1	***PO1***	**05**
			UNIT-II			
	3	a)	Two strains of Bacterium sweetans, A and B, use sucrose as a sole carbon source. The first step in the process of sucrose utilization is the passage of sucrose through a sucrose transporter protein in the membrane. The characteristics of the two transport proteins are as follows (assuming $[E]_{total}$ is the same in both) i. Assuming that the rate of sucrose uptake is the rate limiting step in growth, which strain will grow faster if the concentration of Sucrose is:10 mM? 100 mM? 1000 mM? ii. One strain was isolated from the soil and the other from the floor of an Ice Cream parlour. Explain which was likely to be which? Why?	***CO*** 2	***PO2***	**6**
		b)	Differentiate competitive and uncompetitive inhibition by using LB plots and other kinetic parameters.	***CO*** 2	***PO2***	**8**
		c)	"Certain proteins have "other shapes," or conformations, induced by the binding of ligands referred to as modulators. The conformational changes induced by the modulator(s) interconvert more-active and less-active forms of the protein". Substantiate the statement with a relevant example.	***CO*** 2	***PO2***	**6**

Strain	A	B
K_m	1000 mM	10 mM
V_{max}	1000 mmol/min	100 mmol/min

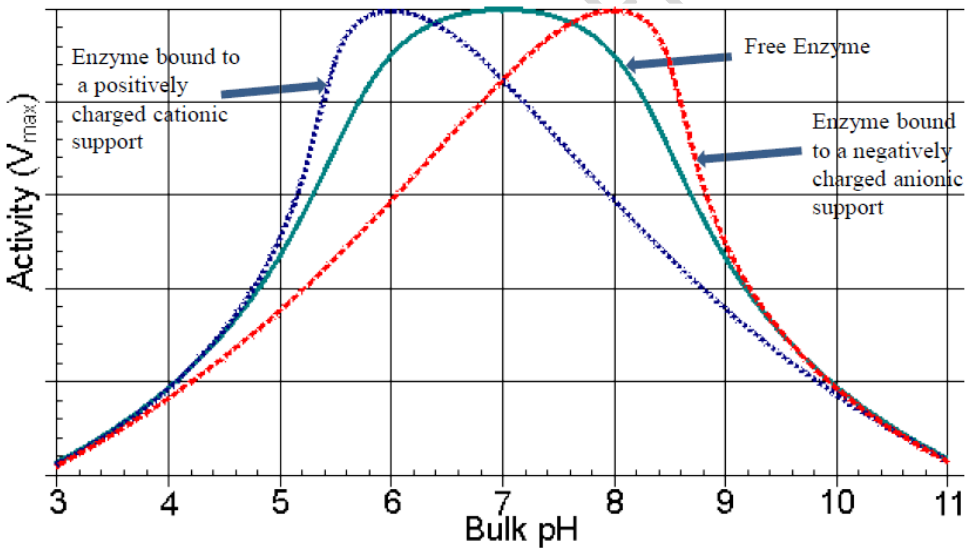
			OR			
	4	a)	Initial velocity data shown in the table were obtained for an enzyme deoxyribokinase, which catalyzes the following reaction. 2-Deoxyribose + ATP ⟶ ADP + 5-Phospho-2-deoxyribose Each assay at the indicated substrate concentration was initiated by adding enzyme to a final concentration of 0.01 nm. **Note:** The reaction conditions such as pH, buffer identity, amount of enzyme, and sample volumes were held constant for all sample).	***CO*** 2	***PO2***	**10**

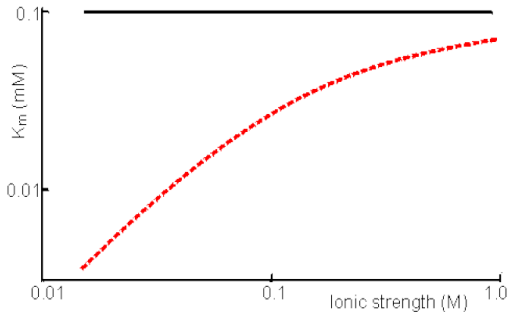
Sample No.	[2-Deoxy-ribose], µM	ADP Formation, Change in [ADP], mM/5 min
1	540	0.115×10^{-3}
2	1000	0.124×10^{-3}
3	1600	0.147×10^{-3}
4	2560	0.191×10^{-3}
5	5120	0.255×10^{-3}
6	6880	0.274×10^{-3}
7	15000	0.294×10^{-3}
8	20000	0.300×10^{-3}

i. Use MM plot and Lineweaver-Burk plot to graphically determine the K_m and V_{max}.

ii. Derive K_{cat} and the specificity constant using the K_m and V_{max} obtained from LB plot.

<table>
<tr><td></td><td></td><td>b)</td><td>An enzyme catalyzed reaction was found to be affected by an Inhibitor ‘X’. The following results were obtained at fixed total enzyme concentration.
<table><tr><td>[Substrate] (mmol l^{-1})</td><td colspan="2">Initial velocity (µmol l^{-1} min^{-1})</td></tr><tr><td></td><td>Uninhibited</td><td>Inhibited with ‘X’</td></tr><tr><td>5.0</td><td>147</td><td>100</td></tr><tr><td>6.67</td><td>182</td><td>122</td></tr><tr><td>10.0</td><td>233</td><td>156</td></tr><tr><td>20.0</td><td>323</td><td>222</td></tr><tr><td>40.0</td><td>400</td><td>278</td></tr></table>(i) Determine the type of inhibition.
(ii) Determine the K_m and V_{max} of the enzyme without and with inhibitor.
(iii)If K_I for this system is 2.9 mmol l^{-1}, calculate the inhibitor concentration present.</td><td>CO 2</td><td>PO2</td><td>10</td></tr>
<tr><td></td><td></td><td></td><td>UNIT -III</td><td></td><td></td><td></td></tr>
<tr><td>5</td><td>a)</td><td>Illustrate and compare the salient features of lock and key model with induced fit model of enzyme action. Add a note on non-productive binding.</td><td>CO 3</td><td>PO1</td><td>7</td></tr>
<tr><td></td><td>b)</td><td>An enzyme, a natural antibacterial agent found in tears and egg whites, cleaves the (β 1→4) glycosidic bond between NAM and NAG residues in peptidoglycan. Describe the mechanism of action of this enzyme.</td><td>CO 3</td><td>PO1</td><td>8</td></tr>
<tr><td></td><td>c)</td><td>Describe the salient features of active site of an enzyme.</td><td>CO 3</td><td>PO1</td><td>5</td></tr>
<tr><td></td><td></td><td></td><td>OR</td><td></td><td></td><td></td></tr>
<tr><td>6</td><td>a)</td><td>A serine protease that catalyzes the hydrolytic cleavage of peptide bonds is specific for peptide bonds adjacent to aromatic amino acid residues. The enzyme with a well understood mechanism, features general acid base catalysis, covalent catalysis, and transition-state stabilization. Illustrate and explain the mechanism of action of this proteolytic enzyme.</td><td>CO 3</td><td>PO1</td><td>7</td></tr>
<tr><td></td><td>b)</td><td>Define Coenzymes and explain the role of PLP as coenzyme.</td><td>CO 3</td><td>PO1</td><td>6</td></tr>
<tr><td></td><td>c)</td><td>Differentiate Metalloenzymes & Metal-activated enzymes. Explain the role of metal ions in enzyme catalysis with a specific example.</td><td>CO 3</td><td>PO1</td><td>7</td></tr>
<tr><td></td><td></td><td></td><td>UNIT-IV</td><td></td><td></td><td></td></tr>
<tr><td>7</td><td>a)</td><td>Biosensors are electronic monitoring devices that make use of an enzyme’s specificity and the technique of enzyme immobilization” Justify with an example.</td><td>CO 4</td><td>PO1</td><td>8</td></tr>
<tr><td></td><td>b)</td><td>Compare the methodology, merits and demerits of any two coupling and entrapment techniques of immobilization.</td><td>CO 4</td><td>PO1</td><td>8</td></tr>
<tr><td></td><td>c)</td><td>List the various economic advantages of enzyme immobilization.</td><td>CO 4</td><td>PO1</td><td>4</td></tr>
<tr><td></td><td></td><td></td><td>OR</td><td></td><td></td><td></td></tr>
</table>

	8	a)	Immobilization allows one to re-use the enzyme for an extended period of time and enables easier separation of the catalyst from the product. Whereas immobilization improves many properties of enzymes such as performance in organic solvents, pH tolerance, heat stability or the functional stability, the kinetic constants (e.g. K_m, V_{max}) of enzymes may be altered due to internal structural changes and restricted access to the active site. Give the plausible reasons for the following observations. (i) The apparent K_m for creatine kinase immobilized on DEAE-Cellulose is ten times smaller than that for the free enzyme. (ii) The apparent K_m for Ficain which acts on Benzoyl-L-arginine ethyl ester and immobilized on DEAE-cellulose is ten times greater than that for the free enzyme. Following is the schematic diagram of the variation in the profiles of activity of an enzyme, immobilized on the charged supports, with the pH of the solution.  (iii) Why there is apparent shifts in the behavior of the kinetic constants for the immobilized enzyme with respect to the solution pH? (iv) How do you produce a pH of 8 within the microenvironment?	***CO*** 4	***PO1***	**10**

		b)	The following diagram depicts the effect of immobilization and ionic strength on the Km of bromelain for its positively-charged substrate, N-α-benzoyl-L-arginine ethyl ester. The support is the negatively-charged poly anionic polymer, carboxymethyl cellulose. (------ for immobilized enzyme and ——— for soluble enzyme).  (i) Justify the fact that K_m increases with increase in the ionic strength. (ii) An enzyme with a pH optimum of 7.8 was bound in a positively charged support. Estimate the pH optimum of the immobilized enzyme at 330 K., given that the electronic potential was found to be 0.01 V. Assume that R = 8.314 J K^{-1} mol^{-1} and F = 96487 J V^{-}	***CO*** 4	***PO1***	**10**
			UNIT-V			
	9	a)	Explain the design and construction of novel enzymes by site directed mutagenesis using primer extension method.	***CO*** 5	***PO3***	**8**
		b)	Explain the surface-modified enzymes by PEG attachment. List the pros and cons of PEG modified enzymes.	***CO*** 5	***PO3***	**6**
		c)	List the advantages of biocatalytic transformations performed in organic media.	***CO*** 5	***PO1***	**6**
			OR			
	10	a)	Define artificial enzymes and explain how they are modified to work in organic media.	***CO*** 5	***PO3***	**8**
		b)	Illustrate and explain enzyme engineering cycle.	***CO*** 5	***PO3***	**6**
		c)	Describe the applications of biocatalyst obtained from thermophilic and hyperthermophilic microbes.	***CO*** 5	***PO1***	**6**

****