U.S.N.										

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

Autonomous Institute Affiliated to VTU

June 2025 Semester End Main Examinations

Programme: B.E. **Semester: VI**

Branch: Biotechnology **Duration: 3 hrs.**

Course Code: 23BT6PCETK/ 22BT6PCETK/ 19BT6DCETK **Max Marks: 100**

Course: Enzyme Technology and Kinetics

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

		UNIT - I	*CO*	*PO*	Marks
1	a)	A new enzyme was isolated from a thermophilic bacterium found in deep-sea vents. Initial analysis showed that it catalyzes the removal of hydrogen from ethanol and transfers it to NAD^+, forming acetaldehyde and NADH. The research team is preparing to submit this enzyme for classification under the Enzyme Commission (EC) system. Based on the function, classify this enzyme according to the EC nomenclature system. Justify each digit of its EC number and provide its full class name.	*CO* 1	*PO1, PO2*	**04**
	b)	A team uses ammonium sulfate precipitation to concentrate a fungal enzyme and then performs gel filtration chromatography for further purification. After these steps, SDS-PAGE shows a single band. Name the purification stages applied and explain their working principle. What does the SDS-PAGE result imply?	*CO* 1	*PO1, PO2*	**08**
	c)	During the preparation of an enzyme, the activity is first measured from the initial tissue homogenate. The result is that 0.25 mL of the homogenate transforms 0.20 µmol of substrate in 4 minutes. The total volume of the homogenate is 150 mL, and its protein concentration is 5 mg/mL. After several steps of purification, the final volume of the homogenous enzyme solution is 1.0 mL, and its protein concentration is 3 mg/mL. A sample of 5 µL of the purified enzyme transforms 0.25 µmol of substrate in 5 minutes. Calculate the following: i) Specific activity of crude and purified enzyme ii) Degree of purification iii) Yield of enzyme activity (as a percentage)	*CO* 1	*PO1, PO2*	**08**
		OR			

<table>
<tr><td></td><td>2</td><td>a)</td><td>You are studying a thermophilic microorganism isolated from a hot spring. It produces a heat-stable enzyme (Enzyme Z) that degrades lignin into smaller aromatic compounds. You plan to purify and characterize this enzyme from the culture supernatant. Fortunately, a substrate analog bound to agarose beads is available and mimics lignin, allowing affinity purification.
i)Suggest any one advanced purification technique that would be applicable for Enzyme Z, and explain its working principle.
ii) After affinity purification, how will you confirm the presence and purity of Enzyme Z?
iii) You want to determine the pI of Enzyme Z. Describe how you would do this and interpret the result.
iv) Suppose SDS-PAGE shows a single 40 kDa band, but Western blot using an anti-ligninase antibody shows no signal. What are possible explanations?
v) You perform activity assays after thermal treatment at various temperatures. Activity remains stable up to 90°C, then drops. How would you interpret and utilize this property?</td><td>CO 2</td><td>PO1, PO2</td><td>10</td></tr>
<tr><td></td><td></td><td>b)</td><td>Explain the different types of enzyme specificities with suitable examples for each. Why is specificity important in enzyme-catalyzed reactions?</td><td>CO 2</td><td>PO1, PO2</td><td>10</td></tr>
<tr><td></td><td></td><td></td><td>UNIT - II</td><td></td><td></td><td></td></tr>
<tr><td></td><td>3</td><td>a)</td><td>Differentiate between allosteric and feedback regulation in enzymes with examples.</td><td>CO 2</td><td>PO2</td><td>04</td></tr>
<tr><td></td><td></td><td>b)</td><td>Differentiate between competitive, uncompetitive, and non-competitive inhibition.</td><td>CO 2</td><td>PO2</td><td>06</td></tr>
<tr><td></td><td></td><td>c)</td><td>An experiment was conducted to study the kinetics of a newly discovered enzyme. The initial reaction velocities (v_0) were measured at various substrate concentrations [S] as shown below:
<table>
<tr><th>Substrate, mol/L</th><th>Initial velocity, µmol/min</th></tr>
<tr><td>$2x10^{-3}$</td><td>105</td></tr>
<tr><td>$1x10^{-3}$</td><td>98</td></tr>
<tr><td>$5x10^{-4}$</td><td>87</td></tr>
<tr><td>$2x10^{-4}$</td><td>65</td></tr>
<tr><td>$1x10^{-4}$</td><td>50</td></tr>
<tr><td>$5x10^{-5}$</td><td>34</td></tr>
<tr><td>$2x10^{-5}$</td><td>19</td></tr>
<tr><td>$1x10^{-5}$</td><td>11</td></tr>
<tr><td>$5x10^{-6}$</td><td>6.2</td></tr>
</table>
(i) Draw a Lineweaver-Burke plot for this enzyme.
(ii) Determine K_M and V_{max} for this enzyme.</td><td>CO 2</td><td>PO2</td><td>10</td></tr>
<tr><td></td><td></td><td></td><td>OR</td><td></td><td></td><td></td></tr>
<tr><td></td><td>4</td><td>a)</td><td>Derive the Michaelis-Menten equation for a simple single substrate enzyme-catalyzed reaction.</td><td>CO 2</td><td>PO2</td><td>10</td></tr>
</table>

		b)	You perform initial velocity experiments at various substrate concentrations, both in the absence and presence of a competitive inhibitor and get the following results: (i) Plot the Lineweaver-Burk plot for both conditions. (ii) Identify the type of inhibition and explain its graphical characteristics. (iii) Determine Vmax and apparent Km from the plot.	*CO* 2	*PO2*	**10**

Substrate [S] (mM)	v (without inhibitor) µmol/min	v (with inhibitor) µmol/min
0.05	33.3	14.3
0.10	50.0	25.0
0.20	66.7	40.0
0.40	80.0	57.1
0.60	85.7	66.7

			UNIT - III			
	5	a)	A pharmaceutical company is designing inhibitors that mimic the transition state of an enzyme-catalyzed reaction. Explain how the enzyme active site, activation energy, and binding energy contribute to the specificity and catalytic efficiency of enzymes. Why is targeting the transition state effective?	*CO* 3	*PO1*	**08**
		b)	Explain metal ion catalysis taking Carbonic anhydrase as an example. Describe how Zn^{2+} facilitates this reaction.	*CO* 3	*PO1*	**08**
		c)	Lysozyme cleaves glycosidic bonds by distorting the sugar ring into a half-chair conformation. Explain the role of substrate strain and entropy effects in this process.	*CO* 3	*PO1*	**04**
			OR			
	6	a)	Ribonuclease A catalyzes RNA hydrolysis using acid-base catalysis. Describe the mechanism and the role of specific residues in this process. Why is this mechanism pH-sensitive?	*CO* 3	*PO1*	**10**
		b)	During digestion, chymotrypsin catalyzes peptide bond hydrolysis through covalent catalysis. Explain how the catalytic triad contributes to its function.	*CO* 3	*PO1*	**10**
			UNIT - IV			
	7	a)	You measure the kinetics of free and immobilized urease. Initial rates at different substrate concentrations are: Determine which system shows diffusional limitation and estimate Vmax.	*CO* 4	*PO1, PO7*	**10**

[S] (mM)	v (Free, µmol/min)	v (Immobilized, µmol/min)
5	0.32	0.18
10	0.50	0.28
20	0.67	0.35
50	0.83	0.45
100	0.90	0.48

		b)	Compare and contrast the following methods for immobilization of enzymes with suitable diagram. (i) Covalent binding (ii) Entrapment	*CO* 4	*PO1, PO7*	**10**
			OR			

<table>
<tr><td></td><td>8</td><td>a)</td><td>Illustrate the effect of solute diffusion on kinetics of immobilized enzyme.</td><td>CO 4</td><td>PO1, PO7</td><td>10</td></tr>
<tr><td></td><td></td><td>b)</td><td>Glucose isomerase is a key enzyme used in the production of high-fructose corn syrup (HFCS) by converting glucose into fructose. Due to its economic importance and repeated use in continuous processing, glucose isomerase is commonly immobilized on solid carriers like DEAE-cellulose, silica gel, or polyacrylamide and packed into column reactors.
The use of immobilized glucose isomerase has dramatically improved process efficiency, reduced enzyme cost per batch, and enabled continuous, automated operation with higher operational stability. The enzyme operates at 60°C and pH 7.5 and is reused for up to 1,000 hours in packed-bed reactors before activity drops significantly.
<table>
<tr><th>Run Time (hours)</th><th>Fructose Yield (%)</th></tr>
<tr><td>0</td><td>52</td></tr>
<tr><td>200</td><td>51</td></tr>
<tr><td>400</td><td>49</td></tr>
<tr><td>600</td><td>46</td></tr>
<tr><td>800</td><td>42</td></tr>
<tr><td>1000</td><td>38</td></tr>
</table>
(i)Explain why glucose isomerase is immobilized for HFCS production instead of using it in soluble form.
(ii) What could be the reasons for the decline in fructose yield over time despite constant process conditions? Suggest two strategies to address this issue.
(iii) In a glucose isomerase-based HFCS process, a technician accidentally changes the pH of the feed from 7.5 to 5.5. After several hours, fructose yield drops significantly.
What is the likely cause of this yield reduction? How can pH fluctuations affect immobilized enzyme performance?</td><td>CO 4</td><td>PO1, PO7</td><td>10</td></tr>
<tr><td></td><td></td><td></td><td>UNIT - V</td><td></td><td></td><td></td></tr>
<tr><td></td><td>9</td><td>a)</td><td>“The remarkable catalytic activity of enzymes in monophasic organic solution can be explained by the effect of specific properties”. Identify the parameters and discuss their effects on a biocatalytic reaction in a monophasic organic solvent system.</td><td>CO 5</td><td>PO1, PO3</td><td>10</td></tr>
<tr><td></td><td></td><td>b)</td><td>Give a schematic outline and explain the steps involved in the metagenome approach for isolation of novel biocatalysts.</td><td>CO 5</td><td>PO1, PO3</td><td>10</td></tr>
<tr><td></td><td></td><td></td><td>OR</td><td></td><td></td><td></td></tr>
<tr><td></td><td>10</td><td>a)</td><td>Differentiate between extremophiles and extremozymes. List the molecular adaptations of these enzymes to high temperatures. Explain these adaptations with any two suitable examples.</td><td>CO 5</td><td>PO1, PO3</td><td>10</td></tr>
<tr><td></td><td></td><td>b)</td><td>List any five reactions that are catalyzed by Ribozymes. Describe the characteristic features of hammerhead Ribozymes.</td><td>CO 5</td><td>PO1, PO3</td><td>10</td></tr>
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