GUJARAT TECHNOLOGICAL UNIVERSITY

BRANCH NAME: Biotechnology (04)
SUBJECT NAME: Enzymes & Proteins
SUBJECT CODE: 2170401
B.E. 7th SEMESTER

Type of course: B.E. (Biotechnology

Prerequisite: Basic concepts of Biochemistry and Molecular Biology

Rationale: It is one of the core subjects of Biotechnology. It involves understanding of nature, properties, kinetics, types and mechanisms of enzyme action. It also involves various levels of protein organization, protein folding, protein evolution, designing of novel protein with desired function and study of disease related proteins.

Teaching and Examination Scheme:

Teaching Scheme			Credits	Examination Marks						Total Marks
L	T	P	C	Theory Marks			Practical Marks			
				ESE (E)	PA (M)		ESE (V)		PA (I)	
					PA	ALA	ESE	OEP		
4	**0**	**3**	**7**	**70**	**20**	**10**	**20**	**10**	**20**	**150**

Content:

Sr. No.	Content	Total Hrs	% Weightage
1	**Introduction to Enzymes:** Classification of enzymes. Mechanisms of enzyme action; concept of active site and energetics of enzyme substrate complex formation; specificity of enzyme action; principles of catalysis – collision theory, transition state theory; role of entropy in catalysis.	**7**	**10.41**
2	**Kinetics of Enzyme Action:** Kinetics of single substrate reactions; estimation of Michelis – Menten parameters, multi substrate reactions- mechanisms and kinetics; turnover number; types of inhibition & models –substrate, product. Allosteric regulation of enzymes, Monod Changeux Wyman model, pH and temperature effect on enzymes & deactivation kinetics.	**9**	**14.58**
3	**Enzyme Immobilization:** Physical and chemical techniques for enzyme immobilization – adsorption, matrix entrapment, encapsulation, cross-linking, covalent binding etc., - examples, advantages and disadvantages. Analysis of Film and Pore Diffusion Effects on Kinetics of immobilized Enzyme Reactions; Formulation of dimensionless group and calculation of Effectiveness Factors.	**8**	**12.50**
4	**Purification & Characterization of Enzymes from Natural Sources:** Production and purification of crude enzyme extracts from plant, animal and microbial sources; methods of characterization of enzymes; development of enzymatic assays. Commercial application of enzymes in food, pharmaceutical and other industries; Enzymes for analytical and diagnostic applications.	**8**	**12.50**

5	**Genomics & Proteomics:** Genome sequences- Introduction, determination of Gene sequences, Determination of amino acid sequences and protein structures, A survey of protein structures and functions, Protein folding patterns, Modular structure of proteins, Protein evolution, Integration and control of protein function.	**7**	**10.41**
6	**Chemical Structure & Activity of Proteins:** The polypeptide chain and protein conformation, The amino acids, Protein main chain conformation, Side chain conformation, Stabilization of the native state, Spectroscopic methods of characterizing proteins in solution, Protein structure determination, Protein-ligand interaction, Catalysis by enzyme, Conformational change, Control of protein activity, Control of protein function: allosteric regulation.	**9**	**14.58**
7	**Evolution of Protein Structure and Function** Introduction to secondary, tertiary & quaternary structure of proteins, Classification of protein folding patterns, Structural relationships among homologous proteins, Evolution of globins, Evolution of DNA-binding domains of dehydrogenases, Evolution of visual pigments and related molecules, Evolution of new functions in proteins, Classification of protein functions.	**7**	**10.41**
8	**Protein Engineering, Folding, Prediction, Design & Proteins with Partners:** The significance of protein engineering, Protein folding – including Thermodynamics and kinetics-key concepts, The effect of denaturants on rates of folding and unfolding: Chevron plots, The molten globule, Folding funnels, Protein misfolding and GroEL-GroES chaperone protein, Idea of Protein design. General properties of protein-protein interfaces, Multi-subunit protein, Protein-DNA interactions, Overview of Proteins in Disease.	**9**	**14.58**

Suggested Specification table with Marks (Theory):

Distribution of Theory Marks					
R Level	**U Level**	**A Level**	**N Level**	**E Level**	**C Level**
21	28	7	7	7	--

Legends: R: Remembrance; U: Understanding; A: Application, N: Analyze and E: Evaluate C: Create and above Levels (Revised Bloom's Taxonomy)

Note: This specification table shall be treated as a general guideline for students and teachers. The actual distribution of marks in the question paper may vary slightly from above table.

Reference Books:

1. T. Palmer, ENZYMES, First Edn., EW Press
2. Arther M. Lesk , Introduction to Protein science: Architecture, Function and Genomics, OXFORD University Press, Second Edition.
3. Buchholz-Kasche, Biocatalysts and Enzyme Technology, WILEY-VCH, First Ed.
4. W. Aehle, Enzymes in Industry, WILEY-VCH, First Edn.
5. Harvey W. Blanch, Douglas S. Clark, "Biochemical Engineering", Marcel Dekker, Inc
6. James. E. Bailey & David F. Ollis, "Biochemical Engineering Fundamentals", McGraw-Hill.
7. Wiseman, "Enzyme Biotechnology", Ellis Horwood Pub.
8. James M. Lee, "Biochemical Engineering", PHI, USA.
9. Moody PCE, and A J WILKINSON, "Protein Engineering", IRL Press, Oxford, 1990
10. Creighton TE, Proteins, Freeman WH, Second Edition 1993.
11. Branden C, Tooze R, "Introduction of Protein Structure", Garland, 1993.

Course Outcome:

After learning the course the students should be able to:

1. Develop fundamental understanding of Enzymes & Proteins
2. Understand the kinetics and mechanism of enzyme action
3. Understand the levels of protein organization
4. Understand protein folding and protein evolution
5. Understand the designing of novel protein with desired function
6. Understand the involvement of proteins in diseases.

List of Experiments:

1. Isolation and purification of Amylase from Bacterial Species.
2. Assay of acid phosphatase or alkaline phosphatase
3. Enzyme curve amylase and Substrate saturation kinetics of amylase
4. Obtain temperature optima of enzyme α-amylase
5. Thermo stability of enzyme α-amylase.
6. Assay the enzyme activity of invertase
7. The effect of pH on amylase activity
8. The effect of inhibitor($HgCl_2$) on amylase
9. Immobilize enzyme (invertase) by gel-entrapment method
10. To perform SDS page of given protein sample.
11. To perform Western Blotting.
12. To perform 2D page Electrophoresis.
13. To study the effect of Chemical agents on Protein Denaturation –Renaturation Kinetics.
14. To study the effect of Physical agents on Protein Denaturation –Renaturation Kinetics.

Open Ended Problem:

Students are free to select any project related to Enzymes & Proteins based on its application in the field of Biology/Biotechnology. Some of the suggested projects are:

- Comparative study of various chemical denaturants on protein structure.
- Comparative study of various physical denaturants on protein structure.
- Role of Osmolytes in protein denaturation-renaturation kinetics.

Major Equipment:

The major equipment required for experimentation include Colorimeter, Spectrophotometer, Digital Balance, Digital pH meter, Water bath, Centrifuge, Micro-pipette, etc.

List of Open Source Software/learning website:

Students can refer to video lectures available on the websites including NPTEL. Students can refer to the CDs which are available with some reference books. Students can develop their own flow-sheets for demonstration of various protein – protein interactions, integration and control of protein functions within the biological system.

ACTIVE LEARNING ASSIGNMENTS: Preparation of power-point slides, which include videos, animations, pictures, graphics for better understanding theory and practical work – The faculty will allocate chapters/ parts of chapters to groups of students so that the entire syllabus to be covered. The power-point slides should be put up on the web-site of the College/ Institute, along with the names of the students of the group, the name of the faculty, Department and College on the first slide. The best three works should submit to GTU.