SEMESTER – II

ENZYMES & ANALYTICAL TECHNIQUES

THEORY

Programme: B.Sc. **Max. Hours: 60**
Course Code: U20/BIC/DSC/201 **Hours per week: 4**
Course Type: DSC – 2 **Max. Marks: 100**
No. of credits: 4

Course objective:

Acquire knowledge about enzymes, thermodynamics and various important techniques of separation.

Course Outcomes:

CO1: Understand in detail about enzymes including nomenclature, mechanism of enzyme action and kinetics.

CO2: Thorough knowledge on the influence of thermodynamics related to biochemical reactions.

CO3: Learn the separation techniques like chromatography, electrophoresis and centrifugation.

CO4: Analyze the principle and applications of spectrophotometry and radioactivity.

MODULE I: ENZYMES (15 Hrs)

Introduction to biocatalysis, Difference between chemical and biological catalysis.
Nomenclature and classification of enzymes. Characteristic features of enzymes.
Introduction to the principles of activation energy, transition state, Active site
Outline of mechanism of enzyme action – lock and key model, induced fit model.
Factors affecting catalysis – substrate concentration, pH, temperature, activators and inhibitors (competitive and non-competitive types). Michaelis constant (Km) and its significance. Line Weaver – Burk plots, Isoenzymes, zymogen activation – activation of trypsinogen and chymotrypsinogen, allosteric enzymes (elementary treatment). Ribozyme, abzyme (definitions only).

MODULE II: BIOENERGETICS (15 Hrs)

Energy transformations in the living system, First and second laws of Thermodynamics; Concept of enthalpy, entropy, free energy, exergonic and endergonic reactions. Helmholtz &Gibbs free energy. Relationship between standard free energy change and equilibrium constant.
High energy compounds and their role.Ultrastructure of mitochondria – $F_0 F_1$ ATPase; Oxidative phosphorylation- mitochondrial electron transport chain and carriers involved, sites of ATP production.
Outline of mechanism and Theories of oxidative phosphorylation:

- Chemical coupling hypothesis
- Conformational coupling hypothesis
- Chemiosmotic coupling hypothesis

Oxidation of extramitochondrial NADH – Shuttle systems,

- Malate Aspartate shuttle
- Glycerol 3 phosphate shuttle

ATP yield and P/O ratio.Inhibitors and uncouplers of oxidative phosphorylation.

MODULE III: SEPARATION METHODS (15 Hrs)

Chromatography - Principles and applications of separation methods like paper, thin layer,
Ion exchange, gel-filtration, Affinity Chromatography.
Electrophoresis –Principles and applications of paper, agarose and polyacrylamide - native and SDS gel electrophoresis.
Centrifugation – Principles and applications of centrifugation techniques – Differential, density gradient.
Dialysis.

MODULE IV: SPECTROSCOPIC&RADIO ISOTOPIC TECHNIQUES (15 Hrs)

Colorimetry and Spectrophotometry – Laws of light absorption- Beer Lambert's law, UV and Visible absorption spectra, molar extinction coefficient.
Biochemical applications of spectrophotometer.
Principles of fluorimetry& Flame photometry.
Nature of radioactivity; Radio isotopes; types of radioactive decay; β and γ emitters; Units of Radioactivity, Half-life. Uses of radioisotopes in biology.

Reference Books:

1. Lehninger: Principles of Biochemistry (2013) 6th ed.,Nelson, D.L. and Cox, M.M.W.H. Freeman and Company (New York).

2. Trevor Palmer: Enzymes (Biochemistry, Biotechnology, Clinical Chemistry), (2001) Horwood Publishing, ISBN 1-898563-78-0.

3. Wilson &Walke: Principles & Biochemical Techniques of Practical Biochemistry,(2000) Cambridge University Press. (Fifth Edition).

4. Upadhyaya&Upadhyaya. Biophysical Chemistry (2009) Himalayan Publishers.

5. Mathews: Biochemistry –3rd edition. Pearson Education Limited. (2003). ISBN: 81-297-0215-0.

ENZYMES & ANALYTICAL TECHNIQUES

MODEL QUESTION PAPER

THEORY

Course Code: U20/BIC/DSC/201 **Max. Marks : 60**
Credits: 4 **Time : 2 Hrs**

SECTION – A

I. Answer the following **4 x 10 = 40 M**

1. Write down the factors affecting the enzyme catalysis.
   OR
2. Give the classification of enzymes with suitable examples.

3. Explain the laws of Thermodynamics**.**
   OR
4. Illustrate with a flowchart how energy is obtained from NADH

5. Write the principle & application of Ion exchange chromatography
   OR
6. Write the principle & application of Affinity chromatography

7. Explain the principles on which Colorimeter & Spectrophotometer work. List the applications of UV Visible spectroscopy.
   OR
8. What are the types of Isotopes? Enumerate the uses of Radioisotopes

SECTION – B

II. Answer any <u>FOUR</u> **4 x 5 = 20 M**

9. Allosteric Enzymes
10. High Energy Compounds
11. Gel Filtration Chromatography
12. Units of Radioactivity
13. Molar Extinction Coefficient
14. Half Life