POOL OF DISCIPLINE SPECIFIC ELECTIVES (DSE) COURSES

SEM IV

DISCIPLINE SPECIFIC ELECTIVES (DSE-5): Bioenergetics and Enzymology
Zoo-DSE-5

CREDIT DISTRIBUTION, ELIGIBILITY AND PRE-REQUISITES OF THE COURSE

Course title & Code	Credits	Credit distribution of the course			Eligibility criteria	Pre-requisite of the course	Department offering the course
		Lecture	Tutorial	Practical			
Bioenergetics and Enzymology Zoo-DSE-5	04	03	Nil	01	Passed Class XII with Biology/ Biotechnology	Basic knowledge of Biochemistry	Zoology

Learning Objectives

The learning objectives of this course are as follows:

- to develop a holistic understanding of the complex enzymatic reactions occurring within body through lectures, practical and laboratory exercises, assignments, seminars and visit to research Institutes.
- to appreciate the basic laws of thermodynamics; free energy, and equilibrium to acquire the knowledge to introspect and understand the core concepts of biochemistry
- to build upon undergraduate-level knowledge of biochemical principles with specific emphasis on concepts of transfer of energy in different metabolic pathways.
- to learn about the basic tools used over and over in biological reactions.

Learning Outcomes

By studying this course, students will be able to

- differentiate between the "high energy" biomolecules with respect to their hydrolysis and group transfers.
- appreciate the energy stored in reduced organic compounds that can be used to reduce cofactors such as NAD^+ and FAD, which serve as universal electron carriers.
- Increase the understanding of the function of electron-transport chain in mitochondria and the chemi-osmotic theory involved in ATP synthesis.
- explain the thermodynamic basic principles for energy transformation in biological membranes.

- use spectroscopic and other physical analytical methods to use membrane proteins and biological redox processes.

SYLLABUS OF DSE-5

UNIT- 1: Principles of Biophysical Chemistry **5 hrs**

Concept of pH, buffers, Principles of thermodynamics: free-energy, entropy, enthalpy, chemical bonds and stabilizing interactions: van der Waals, electrostatic, hydrogen bonding and hydrophobic interactions.

UNIT- 2: Bioenergetics: **9 hrs**

Concept of free energy, standard free energy, determination of ΔG for a reaction. Relationship between equilibrium constant and standard free energy change, biological standard state & standard free energy change in coupled reactions. Biological oxidation-reduction reactions, redox potentials, relation between standard reduction potentials and free energy change.

High energy phosphate compounds- introduction, phosphate group transfer, free energy of hydrolysis of ATP and sugar phosphates along with reasons for high ΔG.

Transfer of energy: Electron Transport Chain, Bioenergetics of the liver.

UNIT- 3: Kinetics of enzyme action **10 hrs**

Concept of ES complex, Derivation of Michaelis-Menten equation for uni-substrate reactions. Different plots for the determination of Km and V_{max} and their physiological significances. Importance of K_{cat}/K_m. Kinetics of zero and first order reactions.

Classification of multi substrate reactions with example of each class. Ping Pong random and ordered BiBi mechanisms. Use of initial velocity, inhibition and exchange studies to differentiate between multi substrate reaction mechanisms.

Reversible (glutamine synthase and phosphorylase) and irreversible (proteases) inhibition. Competitive, non-competitive, uncompetitive, linear-mixed type inhibitions and their kinetics, Suicide inhibitor.

UNIT- 4: Mechanism of Enzyme Action **8 hrs**

Cofactor dependency, pH, temperature and ionic strength dependency; Acid-base catalysis, covalent catalysis, proximity, orientation effect. Strain and distortion theory. Chemical modification of active site groups. Mechanism of action of chymotrypsin.

UNIT V: Enzyme Regulation **7 hrs**

Feedback inhibition and feed forward stimulation; Allosteric enzymes: qualitative description of "concerted" & "sequential" models for allosteric enzymes;
Half site reactivity, Flip-flop mechanism, positive and negative co-operativity.

UNIT VI: Multi-enzyme system: **6 hrs**

Occurrence, isolation and their properties: Mechanism of action and regulation of pyruvate dehydrogenase & fatty acid synthase complexes. Enzyme-enzyme interaction, multiple forms of enzymes with special reference to lactate dehydrogenase.

Practical **(30 hrs)**

(Laboratory periods: 15 classes of 2 hours each)

1. Titration of a weak acid using a pH meter, preparation of buffers
2. Verification of Beer-Lambert's law and determination of absorption coefficients.
3. Preparation of cytochrome C from goat/chicken heart and distinguish between different cytochromes in ETC using absorbance spectra.
4. Isolation of NAD from brewer's yeast. Calculate Gibbs' Free Energy for electron flowfrom reduced NADH to Oxygen.
5. Assay of enzyme activity and specific activity, e.g. acid phosphatase, alkaline phosphates, SGOT, SGPT.
6. Determination of K_m and V_{max} using Lineweaver-Burk graph. (Dry experiment).
7. Enzyme inhibition - calculation of Ki for competitive inhibition. (Dry experiment)
8. Perform complex energy calculations that can be applied to biological systems. (Dryexperiment)

Essential/recommended readings

1. Lehninger by D. Nelson, and M. Cox, (2017) "The principles of Biochemistry", 7 th edition, M.W.H. Freeman and Company, New York.
2. D. M. Greenberg, (2014) "Metabolic Pathways", 3rd edition, Academic Press, Elsevier Science & Technology Books,
3. David G. Nicholls and Stuart J. Ferguson (2013) "Bioenergetics 4", Academic Press.
4. L. Stryer, (2012) "Biochemistry", 7 th edition, W.H. Freeman and Company, New York.

Suggestive readings

1 J. M. Berg, J. L. Tymoczko, L. Stryer (2007) "Biochemistry", 6th edition, W. H. Freeman andCompany, New York, NY, 2007.

2 D.J. Voet, J.G. Voet, C.W. Pratt, (2008) "Principles of Biochemistry" 3rd edition, John Wiley & Sons, Inc.

NOTE: Examination scheme and mode shall be as prescribed by the Examination Branch, University of Delhi, from time to time.