

OBJECTIVES : To enable the students to :

- Classify enzymes and describe their general effects and regulation
- Understand the process of enzyme catalysis and various factors affecting it.
- Identify methods of enzyme purification
- Describe important biochemical techniques used for the cleansing and characterization of proteins including enzyme kinetic examinations .
- Apply the knowledge of enzyme to Industrial and Clinical processes.

COURSE :

UNIT I : INTRODUCTION - History, general characteristics, nomenclature, IUB enzyme classification (rationale, overview and specific examples) . Significance of numbering system. Definition with examples of holoenzyme, apoenzyme, coenzyme, cofactor, activator, inhibitors, active site (identification of groups excluded) metallo-enzymes, units of enzyme activity, isoenzymes, monomeric enzymes, oligomeric enzymes and multi-enzymes complexes . Enzyme specificity.

UNIT II : ENZYME KINETICS – Factors affecting enzyme activity : enzyme concentration, substrate concentration, pH and temperature. Derivation of Michaelis – Menten equation for uni-substrate reactions. K_m and its significance. Line Weaver-Burk plot and its limitations. Importance of K_{cat} / K_m . Bi-substrate reactions-brief Reversible and irreversible inhibition, competitive, non-competitive and uncompetitive inhibitions, determination of K_m & V_{max} in presence and absence of inhibitor.

UNIT III : ENZYME CATALYSIS : Nature of non-enzymatic catalysis, Measurement and expression of enzyme activity-enzyme assays. Definition of IU, katal, enzyme turn over number and specific activity.

Role cofactors in enzyme catalysis : NAD/NADP, FMN/FAD, coenzyme A , biocytin, cobamide, lipoamide, TPP, pyridoxal phosphate, tetrahydrofolate. Mechanism of enzyme action : Acid-base catalysis, electrostatic catalysis & metalion catalysis. Regulation of enzyme activity, covalent modulation. distortion Mechanism of action of trypsin, carboxypeptidase, rinbonuclease and lysozyme.

UNIT IV : Enzyme Purification – Methods for isolation, purification and characterization of enzymes. Allosteric enzymes (Ribozyme, Abzyme) – The role of enzymes – AT case

UNIT V : INDUSTRIAL AND CLINICAL APPLICATION OF ENZYMES- Immobilization of enzyme and their industrial applications. Production of glucose from starch, cellulose and dextran; use of lactase in dairy industry; production of glucose-fructose syrup from sucrose; use of proteases in food, detergent and leather industry; medical application of enzymes ; use of glucose oxides in enzyme electrodes.

REFERENCES :

1. Price, N.C&Stevens, L (II Ed) Fundamentals of Enzymology. Oxford University Press, Chennai.
2. Whitkar, J.R.(1972) Principles of Enzymology for Food Science, M.Dekker Publishers, New York.
3. Stryer, I(III Ed) Biochemistry. W.H.Freeman & Co. San Francisco.

ST.JOSEPH'S COLLEGE FOR WOMEN (AUTONOMOUS), VISAKHAPATNAM
IV SEMESTER
BCH 4851 (1)
w.e.f 2008-2011(V batch)

BIOCHEMISTRY
ENZYMOLGY
PRACTICAL – II B

TIME: 4 Hrs/Week
Max. Marks: 50

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COURSE :

Enzyme Assays

- a. Salivary amylase using starch as substrate
- b. Effect of temperature on enzyme activity and determination of activation energy.
- c. Effect of pH on enzyme activity and determination of optimum pH.
- d. Effect of enzyme concentration on enzyme activity.
- e. Effect of substrate concentration on enzyme activity and determination of K_m constant.
- f. Assay of acid phosphatase from potatoes.
- g. Assay of urease from (horse gram)
- h. Determination of proteolytic activity of trypsin.

REFERENCES:

1. Plummer, D.T. (1979) An Introduction to Practical Biochemistry, Tata MC Graw Hill Book Co., Bombay.
2. Oser B.L.(1961) Hawk's Physiological Chemistry, Tata MC Graw Hill Book Co. Bombay.
3. Burtis, C.A & Ashwood, E.R (Eds)(V Edn) Tietz Fundamentals of clinical Biochemistry . WBSaunders & Co. New York.