

# DEVI AHILYA VISHVA VIDYALAYA, INDORE

## M.Sc. Biotechnology (Two Year Course)

w.e.f. from 2011-2012

<b>Semester – I</b>		<b>Th. +CCE</b>	<b>Total</b>
I	Biochemistry	85+15	100
II	Cell and Development Biology	85+15	100
III	Microbiology	85+15	100
IV	Biostatistics & Bioinformatics	85+15	100
V	Practical – I (Paper I & II)		50
VI	Practical – II (Paper III & IV)		50
			<b>500</b>
<b>Semester – II</b>			
I	Molecular Biology	85+15	100
II	Bacterial Genetics and Genetic Engineering	85+15	100
III	Immunology	85+15	100
IV	Analytical Techniques	85+15	100
V	Practical – I (Paper I & II)		50
VI	Practical – II (Paper III & IV)		50
			<b>500</b>
<b>Semester – III</b>			
I	Enzyme Technology	85+15	100
II	Food Biotechnology	85+15	100
III	Environmental Biotechnology	85+15	100
IV	Plant Biotechnology	85+15	100
V	Practical – I (Paper I & II)		50
VI	Practical – II (Paper III & IV)		50
			<b>500</b>
<b>Semester – IV</b>			
I	Bioprocess Technology	85+15	100
II	Genomics, Proteomics, IPR & Biosafety	85+15	100
III	Animal Biotechnology	85+15	100
VI	Project		100
V	Practical – I (Paper I)		50
VI	Practical – II (Paper II & III)		50
			<b>500</b>
	<b>Total</b>		<b>2000</b>

## **SEMESTER – I**

### **Paper -I**

## **BIOCHEMISTRY**

### **Unit I**

Amino acids – structure and functional group properties; Peptides and covalent structure of proteins; Elucidation of primary and higher order structures; Evolution of protein structure; Structure-function relationships in model proteins like ribonuclease A, myoglobin, hemoglobin, chymotrypsin etc.; Tools to characterize expressed proteins.

### **Unit II**

Enzyme catalysis – general principles of catalysis; Quantitation of enzyme activity and efficiency; Enzyme characterization and Michaelis-Menten kinetics; Relevance of enzymes in metabolic regulation, activation, inhibition and covalent modification; Single substrate enzymes

### **Unit III**

Sugars - mono, di, and polysaccharides; Suitability in the context of their different functions- cellular structure, energy storage, signaling; Glycosylation of other biomolecules - glycoproteins and glycolipids; Lipids - structure and properties of important members of storage and membrane lipids; their organization; lipoproteins.

### **Unit IV**

Biomembrane organization - sidedness and function; Membrane bound proteins - structure, properties and function; phase transitions in lipids, polysaccharides, molecular shapes and conformation, comparison between different membrane models, diffusion and permeability, carrier transport, ion transport, active and passive transport, ion pumps, water transport, use of liposomes for membrane models and drug delivery systems.

### **Unit V**

Bioenergetics-basic principles; Equilibria and concept of free energy; Coupled processes; Glycolytic pathway; Krebs's cycle; Oxidative phosphorylation; Photosynthesis; Elucidation of metabolic pathways; Logic and integration of central metabolism; entry/exit of various biomolecules from central pathways; Principles of metabolic regulation; Regulatory steps; Signals and second messengers.

### **Texts/References:**

1. V.Voet and J.G.Voet, Biochemistry, 3<sup>rd</sup> edition, John Wiley, New York, 2004.
2. A.L. Lehninger, Principles of Biochemistry, 4<sup>th</sup> edition, W.H Freeman and

Company, 2004.

3. L. Stryer, Biochemistry, 5<sup>th</sup> edition, W.H. Freeman and Company, 2002.

SEMESTER - I

SEMESTER - I

## PAPER- II

### CELL AND DEVELOPMENTAL BIOLOGY

#### Unit I

**Cell Theory & Methods of Study** : Structure of Prokaryotic and Eukaryotic cells  
Microscope and its modifications – Light, phase contrast and interference, Fluorescence, Confocal, Electron (TEM and SEM), Electron tunneling and Atomic Force Microscopy, etc.

**Membrane Structure and Function** : Structural models; Composition and dynamics; Transport of ions and macromolecules; Pumps, carriers and channels; Endo- and Exocytosis; Membrane carbohydrates and their significance in cellular recognition; Cellular junctions and adhesions; Structure and functional significance of plasmodesmata. Cellular responses to environmental signals in plants and animals.

#### Unit II

**Organelles** : Nucleus – Structure and function of nuclear envelope, lamina and nucleolus; Macromolecular trafficking; Chromatin organization and packaging; Cell cycle and control mechanisms; Mitochondria – structure, organization of respiratory chain complexes, ATP synthase, Structure-function relationship; Mitochondrial DNA and male sterility; Origin and evolution; Chloroplast– Structurefunction relationship; Chloroplast DNA and its significance; Chloroplast biogenesis; Origin and evolution. Sub cellular fractionation and criteria of functional integrity.

#### Unit III

**Endo-membrane System and Cellular Motility** : Structure and function of microbodies, Golgi apparatus, Lysosomes and Endoplasmic Reticulum; Organization and role of microtubules and microfilaments; Cell shape and motility; Actinbinding proteins and their significance; Muscle organization and function; Molecular motors; Intermediate filaments; Extracellular matrix in plants and animals.

#### Unit IV

**Cellular Movements and Pattern Formation** : Laying of body axis planes; Differentiation of germ layers; Cellular polarity; Model plants like Fucus and Volvox; Maternal gene effects; Zygotic gene effects; Homeotic gene effects in Drosophila; Embryogenesis and early pattern formation in plants; Cell lineages and developmental control genes in Caenorhabditis.

## Unit V

**Differentiation of Specialized Cells :** Stem cell differentiation; Blood cell formation; Fibroblasts and their differentiation; Differentiation of cancerous cells and role of proto-oncogenes; Phase changes in Salmonella; Mating cell types in yeast; Surface antigen changes in Trypanosomes; Heterocyst differentiation in Anabaena; Sex determination in Drosophila.

**Plant Meristem Organization and Differentiation :** Organization of Shoot Apical Meristem(SAM); Organization of Root Apical Meristem(RAM); Pollen germination and pollen tube guidance; Phloem differentiation; Self-incompatibility and its genetic control; Embryo and endosperm development; Heterosis and apomixes.

### Texts/References:

1. Lodish *et al.*, Molecular cell Biology, 4th Edition, W.H. Freeman & Company, 2000.
2. Smith & Wood, Cell Biology, 2nd Edition, Chapman & Hall, London, 1996.
3. Watson *et al.*, Molecular Biology of the gene, 5th Edition, Pearson Prentice Hall. USA, 2003.
4. B. M. Turner, Chromatin & Gene regulation, 1st Edition, Wiley-Blackwell, 2002.
5. Benjamin Lewin, Gene IX, 9th Edition, Jones and Barlett Publishers, 2007.

SEMESTER – I

Paper -III

## MICROBIOLOGY

### Unit I

**Microbial Diversity & Systematics** : Classical and modern methods and concepts in classification of microorganisms; Classification of Bacteria according to Bergey's manual. Ultrastructure of Archaea (Methanococcus); Eubacteria (*E.coli*); Unicellular Eukaryotes (Yeast).

### Unit II

**Microbial Techniques** : Principles of microbial nutrition, Types of culture media. Theory and practice of sterilization. Cultivation of microorganisms : Pure culture and enrichment culture methods. Culture collection and maintenance of cultures.

### Unit III

**Microbial Growth** : Microbial growth: Batch, fed-batch, continuous culture, synchronous growth. Growth as affected by environmental factors like temperature, acidity, alkalinity, water availability and oxygen. Methods of growth estimation.

### Unit IV

**Microbial Interactions and Infection** : Host–Pathogen interactions; Mechanism of pathogenesis. Pathogenicity islands and their role in bacterial virulence. Types of toxins and their structure and mode of action.

### Unit V

**Virology and Mycology** : Structure and Classification of Bacterial, Plant, Animal viruses. Satellite viruses, Viroids, Virusoids. Structure, Classification and general features of fungi. Life cycle of *Penicillium* and *Saccharomyces*.

### Texts/References:

1. Pelczar MJ Jr., Chan ECS and Kreig NR., Microbiology, 5th Edition, Tata McGraw Hill, 1993.
2. Maloy SR, Cronan JE Jr., and Freifelder D, Microbial Genetics, Jones Bartlett Publishers, Sudbury, Massachusetts, 2006.
3. Crueger and A Crueger, (English Ed., TDW Brock); Biotechnology: A textbook of Industrial Microbiology, Sinaeur Associates, 1990.

4. G Reed, Prescott and Dunn's, Industrial Microbiology, 4th Edition, CBS Publishers, 1987.
5. M.T. Madigan and J.M. Martinko, Biology of Microorganisms, 11<sup>th</sup> Edition, Pearson Prentice Hall, USA, 2006.

SEMESTER – I

Paper-IV

## **BIOSTATISTICS AND BIOINFORMATICS**

### **Unit I**

Fundamental concepts in applied probability; Exploratory data analysis and statistical inference; Probability and analysis of one and two way samples; discrete and continuous probability models; Expectation and variance; Central limit theorem; Inference; Hypothesis; Critical region and error probabilities; Tests for proportion; Equality of proportions; equality of means of normal populations (variance known, variance unknown); Chi-square test for independence; P-value of the statistic; Confidence limits; Introduction to one way and two-way analysis of variance; Data transformations

### **Unit II**

Elements of programming languages - C and PERL; Data base concept; Database management system; Database browsing and Data retrieval; Sequence database and genome database; Data Structures and Databases; Databases such as GeneBank; EMBL; DDBJ; Swissplot; PIR; MIPS; TIGR; Hovergen; TAIR; PlasmoDB; ECDC; Searching for sequence database like FASTA and Blast algorithm.

### **Unit III**

Cluster analysis; Phylogenetic clustering by simple matching coefficients; Sequence Comparison; Sequence pattern; Regular expression based pattern; Theory of profiles and their use in sequence analysis; Markov models; Concept of HMMS; Baum-Welch algorithm; Use of profile HMM for protein family classification; Pattern recognition methods

### **Unit IV**

Goals of a Microarray experiment; Normalization of Microarray data; Detecting differential gene expression; Principle component analysis; Clustering of microarray data; Structure determination by X-ray crystallography; NMR spectroscopy; PDB (Protein Data Bank) and NDB (Nucleic Acid Data Bank); File formats for storage and dissemination of molecular structure.

### **Unit V**

Methods for modeling; Homology modeling; Threading and protein structure prediction; Structure-structure comparison of macromolecules with reference to proteins; Force fields; Molecular energy minimization; Monte Carlo and molecular dynamics simulation

**Texts/References:**

1. Wayne W. Daniel, Biostatistics : A foundation for Analysis in the Health Sciences, 8th Edition, Wiley, 2004.
2. Prem S. Mann, Introductory Statistics, 6th Edition, Wiley, 2006.
3. John A. Rice, Mathematical Statistics and Data Analysis, 3<sup>rd</sup> Edition, John A. Rice, Duxbury Press, 2006.
4. Campbell and Heyer, Discovering Genomics, Proteomics, & Bioinformatics, 2nd Edition, Benjamin Cummings, 2002.
5. Cynthia Gibas and Per Jambeck, Developing Bioinformatics Computer Skill, 1st Edition, O'Reilly Publication, 2001.

**LAB ON BIOCHEMISTRY AND CELL BIOLOGY**

1. To prepare an Acetic-NaAcetate Buffer system and validate the Henderson-Hasselbach equation.
2. To determine an unknown protein concentration by plotting a standard graph of BSA using UV-Vis Spectrophotometer and validating the Beer- Lambert's Law.
3. Titration of Amino Acids and separation of aliphatic, aromatic and polar amino acids by TLC.
4. To determine an unknown sugar concentration by Nelson Somogyii's and DNS method.
5. Determination of enzyme activity and studying the effect of temperature, pH, enzyme concentration substrate concentration on enzyme activity.
6. Isolation of biomolecules from natural sources.
7. Microscopy: Bright field, phase contrast and fluorescence microscopy.
8. Microtomy.
9. Subcellular fractionation and marker enzymes.
10. Histochemical techniques.
11. Mitosis and Meiosis.

**LAB ON MICROBIOLOGY, BIOSTATISTICS AND BIOINFORMATICS**

1. Sterilization, disinfection, safety in microbiological laboratory.

2. Preparation of media for growth of various microorganisms.
3. Identification and culturing of various microorganisms.
4. Staining and enumeration of microorganisms.
5. Growth curve, measure of bacterial population by turbidometry and studying the effect of temperature, pH, carbon and nitrogen.
6. Isolation and identification of fungus.
7. Isolation of bacteriophage.
8. Introduction to MSEXCEL-Use of worksheet to enter data, edit data, copy data, move data.
9. Use of in-built statistical functions for computations of Mean, S.D., Correlation, regression coefficients etc.
10. Use of bar diagram, histogram, scatter plots, etc. graphical tools in EXCEL for presentation of data.
11. Introduction to SYSTAT package.
12. Searching PubMed , Introduction to NCBI, NCBI data bases, BLAST BLASTn, BLASTp, PSI-BLAST, Sequence manipulation Suite, Multiple sequence alignment, Primer designing, Phylogenetic Analysis.
13. Protein Modeling, Protein structure Analysis, Docking, Ligplot interactions.

## SEMESTER – II

### Paper-I

## MOLECULAR BIOLOGY

### Unit – I

**Genome organization** : Organization of bacterial genome; Structure of eukaryotic chromosomes; Role of nuclear matrix in chromosome organization and function; Matrix binding proteins; Heterochromatin and Euchromatin; DNA reassociation kinetics (Cot curve analysis); Repetitive and unique sequences; Satellite DNA; DNA melting and buoyant density; Nucleosome phasing; DNase I hypersensitive regions; DNA methylation & Imprinting.

### Unit II

**DNA Structure; Replication; Repair & Recombination** : Structure of DNA - A-,B-, Z- and triplex DNA; Measurement of properties-Spectrophotometric, CD, AFM and Electron microscope analysis of DNA structure; Replication initiation, elongation and termination in prokaryotes and eukaryotes; Enzymes and accessory proteins; Fidelity; Replication of single stranded circular DNA; Gene stability and DNA repair- enzymes; Photoreactivation; Nucleotide excision repair; Mismatch correction; SOS repair; Recombination: Homologous and non-homologous; Site specific recombination; Chi sequences in prokaryotes; Gene targeting; Gene disruption; FLP/FRT and Cre/Lox recombination.

### Unit III

**Prokaryotic & Eukaryotic Transcription** : Prokaryotic Transcription; Transcription unit; Promoters- Constitutive and Inducible; Operators; Regulatory elements; Initiation; Attenuation; Termination-Rho-dependent and independent; Anti-termination; Transcriptional regulation-Positive and negative; Operon concept-lac, trp, ara, his, and gal operons; Transcriptional control in lambda phage; Transcript processing; Processing of tRNA and rRNA

Eukaryotic transcription and regulation; RNA polymerase structure and assembly; RNA polymerase I, II, III; Eukaryotic promoters and enhancers; General Transcription factors; TATA binding proteins (TBP) and TBP associated factors (TAF); Activators and repressors; Transcriptional and post-transcriptional gene silencing

### Unit IV

**Post Transcriptional Modifications** : Processing of hnRNA, tRNA, rRNA; 5'-Cap formation; 3'-end processing and polyadenylation; Splicing; RNA editing; Nuclear export of mRNA; mRNA stability; Catalytic RNA.

**Translation & Transport** : Translation machinery; Ribosomes; Composition and assembly; Universal genetic code; Degeneracy of codons; Termination codons; Isoaccepting tRNA;

Wobble hypothesis; Mechanism of initiation, elongation and termination; Co- and post-translational modifications; Genetic code in mitochondria; Transport of proteins and molecular chaperones; Protein stability; Protein turnover and degradation

## **Unit V**

**Bacterial mutants and mutations :** Isolation; Useful phenotypes (auxotrophic, conditional, lethal, resistant); Mutation rate; Types of mutations(base pair changes; frameshift; insertions; deletions; tandem duplication); Reversion vs. suppression; Mutagenic agents; Mechanisms of mutagenesis; Assay of mutagenic agents (Ames test)

**Genetic variation :** Mutations; kinds of mutation; agents of mutation; genome polymorphism; uses of polymorphism.

### **Text/References:**

1. Benjamin Lewin, Gene IX, 9th Edition, Jones and Barlett Publishers, 2007.
2. J.D. Watson, N.H. Hopkins, J.W Roberts, J. A. Seitz & A.M. Weiner; Molecular Biology of the Gene, 6th Edition, Benjamin Cummings Publishing Company Inc, 2007.
3. Alberts et al; Molecular Biology of the Cell, 4th edition, Garland, 2002.
5. Glick BR & Pasternak JJ, Molecular Biotechnology, 3rd Edition, ASM Press, 1998.

SEMESTER - II

## PAPER-II

### BACTERIAL GENETICS AND GENETIC ENGINEERING

#### Unit I

**Gene transfer in bacteria** : History; Transduction – generalized and specialized; Conjugation – F, F', Hfr; F transfer; Hfr-mediated chromosome transfer; Transformation – natural and artificial transformation; Merodiploid generation; Gene mapping; Transposable genetic elements; Insertion sequences; Composite and Complex transposons; Replicative and non-replicative transposition; Genetic analysis using transposons.

#### Unit II

**Bacteriophages and Plasmids** : Bacteriophage–structure; Assay; Lambda phage – genetic map, lysogenic and lytic cycles; Gene regulation; Filamentous phages such as M13; Plasmids – natural plasmids; their properties and phenotypes; Plasmid biology - copy number and its control; Incompatibility; Plasmid survival strategies; Antibiotic resistance markers on plasmids (mechanism of action and resistance); Genetic analysis using phage and plasmid

**Restriction-modification systems** : History; Types of systems and their characteristics; Methylation dependent restriction systems; applications.

#### Unit III

**Basics Concepts OF Genetic Engineering** : Restriction Enzymes; DNA ligase, Klenow enzyme, T4 DNA polymerase, Polynucleotide kinase, Alkaline phosphatase; Cohesive and blunt end ligation; Linkers; Adaptors; Homopolymeric tailing; Labeling of DNA: Nick translation, Random priming, Radioactive and non-radioactive probes, Hybridization techniques: Northern, Southern and Colony hybridization, Fluorescence in situ hybridization; Chromatin Immunoprecipitation; DNA-Protein Interactions-Electromobility shift assay; DNaseI footprinting; Methyl interference assay

#### Unit IV

**Cloning Vectors** : Plasmids; Bacteriophages; M13 mp vectors; PUC19 and Bluescript vectors, Phagemids; Lambda vectors; Insertion and Replacement vectors; EMBL; Cosmids; Artificial chromosome vectors (YACs; BACs); Animal Virus derived vectors-SV-40; vaccinia/baculo & retroviral vectors; Expression vectors; pMal; GST; pET-based vectors; Protein purification; His-tag; GST-tag; MBP-tag etc.; Intein-based vectors; Inclusion bodies; Methodologies to reduce formation of inclusion bodies; Baculovirus and pichia vectors system, Plant based vectors, Ti and Ri as vectors, Yeast vectors, Shuttle vectors

## Unit V

**Cloning Methodologies** : Insertion of Foreign DNA into Host Cells; Transformation; Construction of libraries; Isolation of mRNA and total RNA; cDNA and genomic libraries; cDNA and genomic cloning; Expression cloning; Jumping and hopping libraries; Southwestern and Farwestern cloning; Protein-protein interactive cloning and Yeast two hybrid system; Phage display; Principles in maximizing gene expression

### Texts/References:

1. S.R. Maloy, J.E. Cronan, D. Friefelder, Microbial Genetics, 2<sup>nd</sup> Edition, Jones and Bartlett Publishers, 1994.
2. N. Trun and J. Trempy, Fundamental Bacterial Genetics, Blackwell publishing, 2004.
5. Hartl L D and Jones B, Analysis of genes and genomes, 3rd Edition, Jones and Bartlett Publishers, 1994.
6. S.B. Primrose, R.M. Twyman and R.W.Old; Principles of Gene Manipulation. 6th Edition, S.B.University Press, 2001.
7. J. Sambrook and D.W. Russel; Molecular Cloning: A Laboratory Manual, Vols 1-3, CSHL, 2001.
8. Brown TA, Genomes, 3rd ed. Garland Science 2006
9. Campbell AM & Heyer LJ, Discovering Genomics, Proteomics and Bioinformatics, 2nd Edition. Benjamin Cummings 2007
10. Primrose S & Twyman R, Principles of Gene Manipulation and Genomics, 7th Edition, Blackwell, 2006.

SEMESTER – II

Paper-III

## IMMUNOLOGY

### Unit I

#### **Immunology- fundamental concepts and anatomy of the immune system :**

Components of innate and acquired immunity; Phagocytosis; Complement and Inflammatory responses; Haematopoiesis; Organs and cells of the immune system- primary and secondary lymphoid organs; Lymphatic system; Lymphocyte circulation; Lymphocyte homing; Mucosal and Cutaneous associated Lymphoid tissue.(MALT&CALT).

### Unit II

**Immunogens, Immunoglobulins and Immune response :** Immuno-chemistry of Antigens - immunogenicity, antigenic determinants, haptens, Toxins-Toxoids, Hapten-carrier system; Role and properties of adjuvants. Immunoglobulins-basic structure, classes & subclasses of immunoglobulins.; Multigene organization of immunoglobulin genes; B-cell receptor; Immunoglobulin superfamily; Basis of self – non-self discrimination; B cell maturation, activation and differentiation; Generation of antibody diversity; T-cell maturation, activation and differentiation and T-cell receptors; Functional T Cell Subsets; Cell-mediated immune responses, ADCC. Complement pathways.

### Unit III

**Antigen-antibody interactions :** Antigen – Antibody interaction, affinity, cross reactivity, specificity. Precipitation, agglutination and complement mediated immune Reactions.

Immuno assays: RIA, ELISA, Western blotting, ELISPOT assay, immunofluorescence, and immunoelectron microscopy.

### Unit IV

**Vaccinology :** Active and passive immunization; Live, killed, attenuated, sub unit vaccines; Vaccine technology- Role and properties of adjuvants, recombinant DNA and protein based vaccines, plant-based vaccines, reverse vaccinology; Peptide vaccines, conjugate vaccines.

### Unit V

**Clinical Immunology :** Major Histocompatibility Complex and HLA typing. Hypersensitivity – Type I, II, III and IV; Autoimmunity; Types of autoimmune diseases. Transplantation – Immunological basis of graft rejection; Clinical transplantation and immunosuppressive therapy; Tumor immunology – Tumor antigens; Immune response to tumors and tumor evasion of the immune system. Cancer cells and Immunodeficiencies.

**Texts/References:**

1. Kuby, RA Goldsby, Thomas J. Kindt, Barbara, A. Osborne Immunology, 6th Edition, Freeman, 2002.
2. Brostoff J, Seaddin JK, Male D, Roitt IM., Clinical Immunology, 6<sup>th</sup> Edition, Gower Medical Publishing, 2002.
3. Janeway et al., Immunobiology, 4th Edition, Current Biology publications., 1999.
4. Paul, Fundamental of Immunology, 4th edition, Lippencott Raven, 1999.
5. Goding, Monoclonal antibodies, Academic Press. 1985.

SEMESTER - II

PAPER- IV

## ANALYTICAL TECHNIQUES

### Unit I

**Basic Techniques :** Buffers; Methods of cell disintegration; Enzyme assays and controls; Detergents and membrane proteins; Dialysis, Ultrafiltration and other membrane techniques

**Spectroscopy Techniques :** UV, Visible and Raman Spectroscopy; Theory and application of Circular Dichroism; Fluorescence; MS, NMR, PMR, ESR and Plasma Emission spectroscopy

### Unit II

**Chromatography Techniques :** TLC and Paper chromatography; Chromatographic methods for macromolecule separation - Gel permeation, Ion exchange, Hydrophobic, Reverse-phase and Affinity chromatography; HPLC and FPLC; Criteria of protein purity

**Electrophoretic techniques :** Theory and application of Polyacrylamide and Agarose gel electrophoresis; Capillary electrophoresis; 2D Electrophoresis; Disc gel electrophoresis; Gradient electrophoresis; Pulsed field gel electrophoresis

### Unit III

**Centrifugation :** Basic principles; Mathematics & theory (RCF, Sedimentation coefficient etc); Types of centrifuge - Microcentrifuge, High speed & Ultracentrifuges; Preparative centrifugation; Differential & density gradient centrifugation; Applications (Isolation of cell components); Analytical centrifugation; Determination of molecular weight by sedimentation velocity & sedimentation equilibrium methods

### Unit IV

**Radioactivity :** Radioactive & stable isotopes; Pattern and rate of radioactive decay; Units of radioactivity; Measurement of radioactivity; Geiger-Muller counter; Solid & Liquid scintillation counters (Basic principle, instrumentation & technique); Brief idea of radiation dosimetry; Cerenkov radiation; Autoradiography; Measurement of stable isotopes; Falling drop method; Applications of isotopes in biochemistry; Radiotracer techniques; Distribution studies; Isotope dilution technique; Metabolic studies; Clinical application; Radioimmunoassay

### Unit V

**Advanced Techniques :** Protein crystallization; Theory and methods; API-electrospray and MADI-TOF; Mass spectrometry; Enzyme and cell immobilization techniques; DNA & Peptide Synthesis.

### **Texts/References:**

1. Freifelder D., Physical Biochemistry, Application to Biochemistry and Molecular Biology, 2nd Edition, W.H. Freeman & Company, San Francisco, 1982.
2. Keith Wilson and John Walker, Principles and Techniques of Practical Biochemistry, 5th Edition, Cambridge University Press, 2000.
3. D. Holme & H. Peck, Analytical Biochemistry, 3rd Edition, Longman, 1998.
4. R. Scopes, Protein Purification - Principles & Practices, 3rd Edition, Springer Verlag, 1994.
5. Selected readings from Methods in Enzymology, Academic Press.

### **Lab on Molecular Biology and Genetic Engineering**

1. Isolation of bacterial genomic DNA.
2. Plasmid DNA isolation and DNA quantitation: Plasmid minipreps
3. Restriction digestion.
4. Preparation of competent cells.
5. Agarose gel electrophoresis.
6. Restriction Enzyme digestion of DNA
7. Purification of DNA from an agarose gel
8. DNA Ligation
9. Transformation of E.coli with standard plasmids, Calculation of transformation efficiency
10. Cloning of genomic DNA in standard plasmid vectors
11. Confirmation of the insert, Miniprep of recombinant plasmid DNA Restriction mapping
12. Transformation of yeast *Saccharomyces cerevisiae*

### **Lab on Immunology and Analytical techniques**

1. Selection of animals, Preparation of antigens, Immunization and methods of bleeding, Serum separation, Storage.
2. Antibody titre by ELISA method.
3. Double diffusion, Immuno-electrophoresis and Radial Immuno diffusion.

4. Complement fixation test.
5. Isolation and purification of IgG from serum.
6. SDS-PAGE, Immunoblotting, Dot blot assays.
7. Blood smear identification of leucocytes by Giemsa stain.
8. Separation of leucocytes by dextran method.
9. Demonstration of Phagocytosis of latex beads.
10. Separation of mononuclear cells by Ficoll-Hypaque.
11. Lymphoproliferation by mitogen / antigen induced.
12. Immunodiagnostics using commercial kits.
13. Purification and Separation Techniques - Ammonium Sulfate precipitation Ion-exchange Chromatography, Gel Filtration Affinity Chromatography

## SEMESTER – III

### Paper – I

#### ENZYME TECHNOLOGY

##### Unit 1

Introduction to enzymology and historical developments in enzymology.

Enzyme classification, IUBMB enzyme classification.

Enzyme Activity: Techniques of enzyme isolation, Principle and techniques of enzyme assay, factors affecting enzyme activity.

##### Unit 2

Intracellular localization of enzymes

Mechanism of Enzyme Action : Investigation of active site

Enzyme activators, co-enzymes and co-factors in enzyme catalysis

Purification of enzyme : Techniques of separation and purification, test of homogeneity.

##### Unit 3

Enzyme Kinetics, Bioenergetics and Catalysis

Single substrate kinetics : Equilibrium and steady state kinetics, significance of  $K_m$ ,  $V_{max}$  &  $K_{cat}$ .

Multisubstrate reaction kinetics : General rate equation, ordered, random order and ping-pong mechanisms

##### Unit 4

Enzyme inhibition and its kinetics: Reversible and irreversible inhibition, competitive, non-competitive and uncompetitive, mixed, partial and substrate inhibition.

Thermal kinetics : Effect of temperature on reaction rate, enzyme stability, Arrhenius equation and activation energy.

##### Unit 5

Allosteric enzymes and sigmoidal kinetics : Co-operativity, MWC & KNF models, enzyme memory and pneumonical enzymes.

Isoenzymes, multienzyme complex and multifunctional enzymes, and their physiological significance.

Biosensors ; Enzymes as analytical reagents.

Ribozymes and catalytic antibodies.

##### References

1. Enzymes : Dixon & Webb
2. Biological Chemistry : Mahler & Cordes
3. Principles of Biochemistry : Lehninger
4. Methods in Enzymology : Relevant volumes
5. Enzymes : Boyer
6. Handbook of Enzymes : Dr. Anil Kumar
7. Enzymes: Biochemistry, Biotechnology, Clinical Chemistry : Trevor Palmer

## **SEMESTER – III**

### **Paper – II**

## **FOOD BIOTECHNOLOGY**

### **Unit I:**

**Introduction to Food Processing:** Biotechnology in relation to the food industry, nutritive value of food, and types of microorganisms associated with food, its sources, types and behavior in foods.

### **Unit II:**

**Food Preservation:** Bioprocessing of meat, fisheries, vegetables, dairy products, enzymes and chemicals used in food processing, New Preservation Technologies.

### **Unit III:**

**Food Spoilage & Food Borne Diseases:** Microbial spoilage of food, Food -borne infections & intoxications.

### **Unit IV:**

**Fermented Food Products:** Dairy products, non-beverage plant products, beverages and related products of baking. Microbes as food, Probiotics, prebiotics, single cell proteins, single cell oil.

### **Unit V:**

**Quality Control:** Microbial analysis of food. Quality control, Food Hygiene, Food Regulations and Standards

## **TEXT BOOKS**

1. Roger A., Gordan B., and John T., Food Biotechnology, 1989.
2. Frazier, Food Microbiology.
3. G. Reed, Prescott and Dunn's Microbiology, CBS Publishers,
4. Introductory Food Microbiology, Author – H.A. Modi.

## **SEMESTER – III**

### **Paper – III**

#### **ENVIRONMENTAL BIOTECHNOLOGY**

##### **Unit I**

Environment: basic concept and issues,  
Pollution: Types of pollution, methods for measurement of pollution,  
Methodology for environment management – the problem solving approach, its limitation.

##### **Unit II**

Air and Water pollution: Air pollution and its control through Biotechnology, Water as scarce natural resources, Need for water management, Measurement of water pollution, Source of water pollution.  
Waste water treatment: physical, chemical and biological treatment processes,  
Microbiology of waste water treatment,

##### **Unit III**

Aerobic process: Activated sludge, Oxidation ditches, Trickling filter, Towers, Rotating disc, Rotating drums, and Oxidation ponds. Anaerobic digestion, Anaerobic filters, Up flow anaerobic sludge blanket reactor.  
Treatment schemes for waste water of dairy, distillery, tannery, sugar and antibiotic industries.

##### **Unit IV:**

Microbiological degradation of xenobiotic in Environment. Ecological consideration, decay behavior & degradative plasmid. Hydrocarbons, Oil pollution, Surfactants, Pesticides.

##### **Unit V:**

Bioremediation of contaminated soils and waste land, Biopesticides in integrated pest management, Soil waste source and management (composting, vermiculture, methane production).  
Global environmental problems, Ozone depletion, UV-B, Green house effect, Acid rain, their impact and Biotechnological approaches for management.

## SEMESTER – III

### Paper – IV

#### PLANT BIOTECHNOLOGY

##### Unit - I

**Introduction to cell and Tissue Culture :** Tissue culture media (composition and preparation), tissue culture as a technique to produce novel plants and hybrids. Initiation and maintenance of callus and suspension culture; single cell clones. Organogenesis; somatic embryogenesis; transfer and establishment of whole plants in soil. Shoot-tip culture: rapid clonal propagation and production of virus-free plants. Embryo culture and embryo rescue. Protoplast isolation, culture and fusion; selection of hybrid cells and regeneration of hybrid plants; symmetric and asymmetric hybrids, cybrids. Anther, pollen and ovary culture for production of haploid plants and homozygous lines.

##### Unit - II

**Plant transformation Technology:** basis of tumor formation, hairy root, features of Ti and Ri plasmids, mechanisms of DNA transfer, role of virulence genes, use of Ti and Ri as vectors, binary vectors, use of 35S and other promoters, genetic markers, use of reporter genes with introns, use of scaffold attachment regions methods of nuclear transformation, viral vectors and their application, multiple gene transfers, Vectors-less or direct DNA transfer, particle bombardment, electroporation, microinjection, transformation of monocots. Transgene stability and gene silencing.

**Chloroplast transformation:** Vectors, advantages.

##### Unit - III

**Application of plant Transformation for productivity and performance:** Herbicide resistance, insect resistance, virus resistance, disease resistance, nematode resistance, abiotic stress, post-harvest losses, long shelf life of fruits and flowers, male sterile lines, bar and barnase systems.

##### Unit - IV

**Metabolic Engineering and Industrial Products :** Plant secondary metabolites, control mechanisms and manipulation of phenylpropanoid pathway, shikimate pathway; alkaloids, polyhydroxybutyrate, therapeutic proteins, lysosomal enzymes, antibodies, edible vaccines, purification strategies, oleosin partitioning technology.

##### Unit - V

**Molecular Marker aided-Breeding:** Basic techniques or rDNA techniques RFLP maps, linkage analysis, RAPD markers, STS, microsatellites, SCAR (sequence characterized amplified regions), SSCP (single strand conformational polymorphism), AFLP, QTL, map based cloning, molecular marker assisted selection. Cryopreservation, slow growth and DNA Banking for germplasm conservation.

### **Texts/References:**

1. J. Hammond, P. McGarvey and V. Yusibov (Eds.): Plant Biotechnology. Springer verlag, 2000.
2. T-J. Fu, G. Singh, and W.R. Curtis (Eds.); Plant Cell and Tissue Culture for the Production of Food Ingredients. Kluwer Academic/Plenum Press. 1999.
3. H. S. Chawla: Biotechnology in Crop Improvement. International Book Distributing Company. 1998.
4. R.J. Henry: Practical Application of Plant Molecular Biology. Chapman and Hall. 1996.
5. P.K. Gupta : Elements of Biotechnology. Rastogi and Co. Meerut. 1996.
6. S.S. Bhojwani and M.K. Razdan: Plant tissue culture: Theory and practice, a revised edition (1996)

### **Lab on Plant Biotechnology**

1. Preparation of media.
2. Surface sterilization.
3. Organ Culture.
4. Callus propagation, organogenesis, transfer of plants to Soil.
5. Protoplast isolation and culture.
6. Anther culture, production of Haploids.
7. Cytological examination of regenerated plants.
8. Agro bacterium culture, selection of transformants, reporter gene (GUS) assays.
9. Preparation of tissue culture medium and membrane filtration.

## SEMESTER – IV

### Paper – I

#### BIOPROCESS TECHNOLOGY

##### Unit I

**Basic principle of Bioprocess Technology :** Isolation, screening and maintenance of industrially important microbes; Microbial growth and death kinetics (an example from each group, particularly with reference to industrially useful microorganisms); Strain improvement for increased yield and other desirable characteristics.

##### Unit II

**Upstream and Downstream processing :** Media formulation; Sterilization; Aeration and agitation in bioprocess; Scale up and scale down process.

Bioseparation - filtration, centrifugation, sedimentation, flocculation; Cell disruption; Liquid-liquid extraction; Purification by chromatographic techniques; Reverse osmosis and ultra filtration; Drying; Crystallization; Storage and packaging; Treatment of effluent and its disposal.

##### Unit III

**Concepts of basic mode of fermentation processes:** Bioreactor designs; Types of fermentation and fermenters; Concepts of basic modes of fermentation - Batch, fed batch and continuous; Conventional fermentation v/s biotransformation; Solid substrate, surface and submerged fermentation; Measurement and control of bioprocess parameters.

##### Unit IV

**Microbial Technology – I:** Microbial processes-production of primary(ethanol, organic acid – lactic acid; amino acids – glutamic acid and lysine; vitaminB<sub>12</sub>) and secondary metabolites (antibiotics – penicillin and streptomycin).

##### Unit V

**Microbial Technology – II:** Industrial production, recovery, stability and formulation of bacterial and fungal enzymes-amylase, protease, Enzyme and whole cell Immobilization of and their industrial applications.

Use of microbes in mineral beneficiation and oil recovery, Single Cell Proteins, Bioinsecticides, Biofertilizers.

**Texts/ References:**

1. Jackson AT., Bioprocess Engineering in Biotechnology, Prentice Hall, Engelwood Cliffs, 1991.
2. Shuler ML and Kargi F., Bioprocess Engineering: Basic concepts, 2<sup>nd</sup> Edition, Prentice Hall, Engelwood Cliffs, 2002.
3. Stanbury RF and Whitaker A., Principles of Fermentation Technology, Pergamon press, Oxford, 1997.
4. Baily JE and Ollis DF., Biochemical Engineering fundamentals, 2<sup>nd</sup> Edition, McGraw-Hill Book Co., New York, 1986.
5. Aiba S, Humphrey AE and Millis NF, Biochemical Engineering, 2<sup>nd</sup> Edition, University of Tokyo press, Tokyo, 1973.
6. Comprehensive Biotechnology: The Principles, Applications and Regulations of Biotechnology in Industry, Agriculture and Medicine, Vol 1, 2, 3 and 4. Young M.M., Reed Elsevier India Private Ltd, India, 2004.
7. Mansi EMTEL, Bryle CFA. Fermentation Microbiology and Biotechnology, 2nd Edition, Taylor & Francis Ltd, UK, 2007.

## SEMESTER – IV

### Paper II

#### GENOMICS, PROTEOMICS, IPR & BIOSAFTEY

##### Unit I

**Introduction** : DNA sequencing principles and Sequencing methods; Enzymatic DNA sequencing; Chemical sequencing of DNA; Automated DNA sequencing; RNA sequencing; Chemical Synthesis of oligonucleotides; Recognition of coding and non-coding sequences gene annotation, EST's and SNP's..

##### Unit II

**Tools for genome analysis**:-RFLP, DNA fingerprinting, RAPD, , Linkage and Pedigree analysis-physical and genetic mapping, Primer design; PCR: its types and applications, Site Specific Mutagenesis

**Gene silencing techniques**; Introduction to siRNA; siRNA technology; Micro RNA; Construction of siRNA vectors; Principle and application of gene silencing; Gene knockouts and Gene Therapy; Creation of knockout mice; Disease model; Somatic and germ-line therapy- in vivo and ex-vivo; Suicide gene therapy; Gene replacement; Gene targeting; Transgenics; cDNA and intragenic arrays.

##### Unit III

**Proteomics** : Protein analysis (includes measurement of concentration, aminoacid composition, N-terminal sequencing); 2-D electrophoresis of proteins; Microscale solution isoelectricfocusing; Peptide fingerprinting; LC/MS-MS for identification of proteins and modified proteins; MALDI-TOF; SAGE.

**Functional genomics and proteomics**: Analysis of microarray data; Protein and peptide microarray-based technology; PCR-directed protein *in situ* arrays; Structural proteomics

##### Unit IV

**Introduction to Intellectual Property**: Types of IP: Patents, Trademarks, Copyright & Related Rights, Industrial Design, Traditional Knowledge, Protection of GMOs, IP as a factor in R&D; IPs of relevance to Biotechnology and few Case Studies.

Types of patent application: Ordinary, PCT, Conventional, Divisional and Patent of Addition; Patent databases.

## Unit V

**Biosafety:** Introduction; Historical Background; Introduction to Biological Safety Cabinets; Primary Containment for Biohazards; Biosafety Levels: Biosafety Levels of Specific Microorganisms; Recommended Biosafety Levels for Infectious Agents and Infected Animals; Biosafety guidelines- Government of India; Definition of GMOs & LMOs; Roles of Institutional Biosafety Committee, RCGM, GEAC etc. for GMO applications in food and agriculture.

### Texts/References:

1. Voet D, Voet JG & Pratt CW, Fundamentals of Biochemistry, 2<sup>nd</sup> Edition. Wiley 2006
2. Brown TA, Genomes, 3<sup>rd</sup> Edition. Garland Science 2006
3. Campbell AM & Heyer LJ, Discovering Genomics, Proteomics and Bioinformatics, 2<sup>nd</sup> Edition. Benjamin Cummings 2007
4. Primrose S & Twyman R, Principles of Gene Manipulation and Genomics, 7th Edition, Blackwell, 2006.
5. Glick BR & Pasternak JJ, Molecular Biotechnology, 3rd Edition, ASM Press, 1998.
6. BAREACT, Indian Patent Act 1970 Acts & Rules, Universal Law Publishing Co. Pvt. Ltd., 2007
7. Kankanala C., Genetic Patent Law & Strategy, 1st Edition, Manupatra Information Solution Pvt. Ltd., 2007

### Important Links:

<http://www.w3.org/IPR/>

<http://www.wipo.int/portal/index.html.en>

[http://www.ipr.co.uk/IP\\_conventions/patent\\_cooperation\\_treaty.html](http://www.ipr.co.uk/IP_conventions/patent_cooperation_treaty.html)

[www.patentoffice.nic.in](http://www.patentoffice.nic.in)

[www.iprlawindia.org/](http://www.iprlawindia.org/) - 31k - Cached - Similar page

<http://www.cbd.int/biosafety/background.shtml>

<http://www.cdc.gov/OD/ohs/symp5/jyrtext.htm>

<http://web.princeton.edu/sites/ehs/biosafety/biosafetypage/section3.html>

## **SEMESTER – IV**

### **Paper III**

#### **ANIMAL BIOTECHNOLOGY**

##### **Unit – I**

Structure and organization of animal cell.

Equipment and materials for animal cell culture technology.

Introduction to the balanced salt solutions and simple growth medium, Brief discussion on the chemical, physical and metabolic functions of different constituents of culture medium. Role of carbon dioxide. Role of serum and supplements. Serum and protein free defined media and their application.

##### **Unit – II**

Measurement of viability and cytotoxicity.

Biology and characterization of the cultured cells, measuring parameters of growth.

Basic techniques of mammalian cell culture *in vitro*; disaggregation of tissue and primary culture, maintenance of cell culture; cell separation. Primary and established cell line cultures.

##### **Unit – III**

Scaling-up of animal cell culture.

Cell synchronization. Cell fusion.

Cell cloning and micromanipulation.

Cell transformation. Somatic cell genetics.

##### **Unit - IV**

Organ and histotypic cultures.

Three dimensional culture and tissue engineering.

Measurement of cell death.

Apoptosis.

##### **Unit – V**

Transfection of mammalian cells.

Application of animal cell culture: Production of biopharmaceuticals.

Cell culture based vaccines.

Stem cell cultures, embryonic stem cells and their applications.

Transgenic animals.

**Texts/References:**

1. Culture of Animal cells, (3<sup>rd</sup> Edition), Freshney, Wiley-Liss.
2. Animal Cell Culture – Practical Approach, Ed. John R.W. Masters, OXFORD.
3. Cell Growth and Division: A Practical Approach. Ed. R. Basega, IRL Press.
4. Cell Culture Lab Fax. Eds. M Butler and M. Dawson, Bios Scientific Publications Ltd. Oxford.
5. Animal Cell Culture Techniques. Ed. Marin Clynes, Springer.
6. Methods in Cell Biology, Vol. 57, Animal Cell Culture Methods. Ed. Jenni P Mather and David Barnes. Academic Press.

### **Lab on Bioprocess Technology, Genomics, Proteomics, IPR & Biosafety**

1. Isolation and screening of industrially important microorganisms.
2. Determination of thermal death point and thermal death time of microorganisms.
3. Production of microbial products in bioreactors.
4. Assay of antibiotics production.
5. Studying the kinetics of enzymatic reaction by microorganisms.
6. Production and purification of various enzymes from microbes.
7. Comparative studies of Ethanol production using different substrates.
8. Microbial production and downstream processing of an enzyme, e.g. amylase.
9. Various immobilization techniques of cells/enzymes, use of alginate for cell immobilization.
10. PCR amplification gene and analysis by agarose gel electrophoresis.
11. Polymerase Chain reaction, using standard 16srRNA eubacterial primers.
12. RFLP analysis of the PCR product.
13. Plasmid isolation and confirming recombinant by PCR and RE digestion.
14. Southern hybridization of *B. subtilis* genome with probe and non-radioactive detection.

### **Lab on Animal Biotechnology**

1. Preparation of single cell suspension from spleen and thymus.
2. Cell counting and cell viability.
3. Macrophage monolayer from PEC, and measurement of phagocytic activity.
4. Trypsinization of monolayer and sub-culturing.
5. Cryopreservation and thawing.
6. Measurement of doubling time.
7. Role of serum in cell culture.
8. Preparation of metaphase chromosomes from cultured cells.
9. Isolation of DNA and demonstration of apoptosis and DNA laddering.
10. MTT assay for cell viability and growth.
11. Cell fusion with PEG.

