

CENTRE OF BIOTECHNOLOGY

REVISED SYLLABUS

Recommended by Academic Program Committee, IIDS

COURSE CURRICULA

Semester I	(24 credits)
Papers	(14 credits)
Paper 1: Analytical Techniques (BIT-501)	(3 credits)
Paper 2: Biochemistry (BIT-502)	(4 credits)
Paper 3: Genetics and Microbiology (BIT-503)	(3 credits)
Paper 4: Biostatistics and Computer Applications in Biotechnology (BIT-504)	(2 credits)
Laboratory Technique I (BIT-031)	(10 credits)
Semester II	(18 credits)
Papers	(12 credits)
Paper 1: Biophysical Chemistry (BIT-505)	(2 credits)
Paper 2: Cell Biology (BIT-506)	(3 credits)
Paper 3: Molecular Biology (BIT-507)	(3 credits)
Paper 4: Fermentation Technology (BIT-508)	(2 credits)

Laboratory Technique II (BIT-032)

(6 credits)

Semester III

(22 credits)

Papers

(12 credits)

Paper 1: Animal Cell Biotechnology (BIT-509)

(2 credits)

Paper 2: Genetic Engineering (BIT-510)

(3 credits)

Paper 3: Immunology (BIT-511)

(3 credits)

Paper 4: Plant Biotechnology (BIT-512)

(2 credits)

Laboratory Technique III (BIT-033)

(10 credits)

Semester IV

(16 credits)

Research dissertation (BIT031)

(16 credits)

Grand total of credits = 80

Semester-I Paper Code BIT-501

ANALYTICAL TECHNIQUES

(3 credits)

- 1. Basic techniques:** Buffer preparations; Cell disintegration; Dialysis and ultrafiltration.
- 2. Spectroscopy:** Principles and applications of UV-Visible, Fluorescence and Infra-Red spectroscopy.
- 3. Chromatography:** Principles and applications of Paper and Thin layer chromatography; Size exclusion, Ion exchange, Hydrophobic, Reverse phase and Affinity chromatography; HPLC and FPLC.
- 4. Electrophoresis:**
Theory and application of Polyacrylamide and Agarose gel electrophoresis; Different variants of polyacrylamide gel electrophoresis (PAGE) like native, SDS-PAGE, 2D-PAGE, Blotting Techniques: Southern, Western and Northern blotting.
- 5. Centrifugation:** Sedimentation, Analytical ultra-centrifugation, Preparative ultra-centrifugation: zonal and equilibrium density gradient ultracentrifugation.
- 6. Radioactivity:** Concept of radioactivity; Radioactivity counting methods with principles of different types of counters; Autoradiography.
- 7. Microscopy:** Phase contrast microscope, Fluorescence microscope, confocal microscope, Time lapse imaging, Electron microscopy: SEM & TEM.

Text books/References:

1. Principles and Techniques of Practical Biochemistry by Keith Wilson and John Walker (5th edition, 2000).
2. Physical Biochemistry, application to Biochemistry and Molecular Biology by David Freifelder (2nd edition, 1982).
3. Analytical Biochemistry by D. Holme and H. Peck (3rd edition, 1998). 4. Protein Purification: Principles and Practice by Robert K. Scopes (3rd edition, 1993).

1. **Chemical foundations of Biology:** Water-properties, pH, pKa, acids, bases, buffers; weak bonds, covalent bonds.
2. **Protein:** Physical and chemical properties of amino acids; Primary, secondary, tertiary and quaternary structure; Amino acid composition and primary structure analysis.
3. **Carbohydrates:** mono, di and polysaccharides; Structural and functional role; Glycoprotein and Glycolipid.
4. **Lipids:** Structure and properties of storage and membrane lipids; Lipoproteins; Structural organization of biological membrane.
5. **Nucleic acids:** Structure and properties of purines, pyrimidines, nucleosides, nucleotides, helical structure of DNA. Different forms of DNA. Denaturation and renaturation of DNA.
6. **Enzyme catalysis:** General principles of catalysis; Quantitation of enzyme activity and efficiency; Enzyme characterization and Michaelis-Menten kinetics; activation, inhibition and covalent modification; single substrate enzymes.
7. **Metabolic pathways:** Energy concepts and energy rich compounds; Glycolysis, gluconeogenesis, pentose phosphate pathway, citric acid cycle and oxidative phosphorylation; Fatty acid biosynthesis and oxidation.

Text books/References:

1. Biochemistry by Jeremy M. Berg, John L. Tymoczko and Lubert Stryer (6th edition, 2008).
2. Lehninger Principles of Biochemistry by David L. Nelson and Michael M. Cox (5th edition, 2009).
3. Biochemistry by V.Voet and J.G. Voet (4th edition Dec 2010).

GENETICS AND MICROBIOLOGY

Semester-I Paper Code: BIT-503

(3 credits)

- 1. Study of microorganisms:** General characteristics and salient features related to structure, function, physiology and significance of cyanobacteria, actinomycetes, fungi, yeast, viruses, rickettsia & mycoplasma. Ultrastructure of a bacterial cell: spore, cell wall, flagella, cell membrane, capsule, pili. Microbial growth: Batch, fed-batch, continuous kinetics, synchronous growth, yield constants, methods of growth estimation, stringent response, death of a bacterial cell.
- 2. Microbial systematics, Molecular Taxonomy, Basic microbiological techniques:** Microscopy, Pure culture, nutrition, enrichment, sterilization, disinfection, safety in the microbiological laboratory.
- 3. Study of ecophysiological, biochemical and nutritional aspects of phylogenetically diverse representative groups of organisms:** extremophiles - thermophiles, psychrophiles, halophiles, methanogens, archaeobacteria, Nitrogen fixing organisms and nitrogen fixing genes, Mycorrhiza: types and its functions
- 4. Microbial Ecology:** interactions among microbial populations, microbial interaction with animals, microbial interaction with plants
- 5. Diseases of humans:** Bacterial meningitis, Botulism, Poliomyelitis, African trypanosomiasis, salmonellosis, giardiasis, hepatitis and AIDS.

Text books/References:

- 1) Pleczar MJ Jr., Chan ECS and Kreig NR., Microbiology, 5th Edition, 1993
- 2) Lansing Prescott, John Harley and Donald Klein, Microbiology R.Y.Stanier, General Microbiology
- 3) D. Peter Snustad, Michael J. Simmons, John B. Jenkins, PRINCIPLES OF GENETICS

BIostatistics AND COMPUTER APPLICATION IN BIOTECHNOLOGY

Semester-1 Paper Code: BIT -504

(2 credits)

UNIT: I BIOSTATISTICS

Biostatistics: Introduction & scope; Data & Variable , Collection, Organization & presentation of data, Measures of Central Tendency (Mean, Median and Mode); Measures of Dispersion (Range , Mean Deviation from mean, median & mode, Standard deviation); Correlation & Regression ; Probability, Simple theorem of Probability.

UNIT: II COMPUTER APPLICATION

- a. Introduction to computer, major components, distinction between hardware and software, computer memory, hard-disk and CD-ROM, computer peripherals
- b. Internet: Introduction & Use
- c. Biological Database: Primary, Secondary and composite Database. Nucleotide Sequence Database, Protein sequence Database
- d. Sequence analysis: Type of sequence analysis, Pairwise sequence analysis, Multiple sequence analysis, Global sequence analysis, Local sequence analysis.
- e. Phylogenetic analysis

Text books/References

1. Introductory Probability and Statistical Application by Paul Meyer
2. Mathematical Statistics by Goel
3. Introductory Statistics, 6th Edition by Prem S. Mann, Wiley, 2006
4. Handbook of Computer Communication Standard vol.3, Stalling W. 5. Developing Bioinformatics Computer Skill by O'Reilly

LAB COURSE 1

- 1) To prepare acetate buffer and validate Henderson-Hasselbach equation.
- 2) Estimation of protein by Folin's method.
- 3) Estimation of protein by Biuret method
- 4) Estimation of Carbohydrates by Anthrone method
- 5) Detection of amino acids by ninhydrin method
- 6) Detection of aminoacids by paper chromatography
- 7) Detection of aminoacids by thin layer chromatography
- 8) Estimation of DNA by DPA method
- 9) Estimation of RNA by Orcinol method
- 10) Separation of proteins by SDS-PAGE
- 11) To determine protein sensitivity limit using Coomassie staining & Silver nitrate staining of protein
- 12) Separation of proteins by centrifugation
- 13) Enzyme purification and assay
- 14) Effect of pH and temperature on enzyme activity
- 15) Sterlization, disinfection, safety in microbiological laboratory
- 16) Preparation of media for growth of micro organisms
- 17) Isolation and maintenance of microorganism by plating, streaking and serial dilution.
- 18) Staining and enumeration of microorganisms.
- 19) Growth curve, measure of bacterial population.
- 20) BLAST

Semester-II Paper Code: BIT-505

BIOPHYSICAL CHEMISTRY

(2 credits)

- 1. Interaction in biological systems:** Intra and inter molecular forces, electrostatic interactions, hydrogen bonding, van der Waal interactions, hydrophobic interactions, disulfide bond.
- 2. Protein Structure:** Conformational properties of polypeptide, Ramachandran plot. Tertiary structure; concept of domain and fold, Quaternary structure; Oligomeric proteins and cooperativity.
- 3. Multiple equilibrium:** Titration of proteins to evaluate total and net charge; Scatchard and hill plots; Protein stability, denaturation, unfolding equilibrium; Kinetics and thermodynamics of protein folding; Protein refolding and aggregation; Effect of solvent and temperatures on the protein stability and folding.
- 4. Methods for the structure analysis:** Far-UV and near UV-Circular Dichroism (CD); Fluorescence, single molecule fluorescence spectroscopy, fluorescent probes ; Hydrogen-Deuterium (HD) exchange; Fourier-transform Infra-Red (FT-IR) spectroscopy; Mass spectrometry (ESI and MALDITOF); Nuclear magnetic resonance (NMR) spectroscopy; X-ray crystallography.

Text books/Reference:

1. Introduction to Protein Structure by Carl Branden and John Tooze (2nd edition, 1999)
2. Proteins structure and molecular properties by Thomas E. Creighton (2nd edition , 1992)
3. Principles of Physical Biochemistry by Kensal E. van Holde, Curtis Johnson and PuiShing Ho (2nd edition 2005).
4. Protein structure: A practical approach by Thomas E. Creighton (2nd edition, 1997)
5. Latest reviews from journals

CELL BIOLOGY

(3 credits)

1. Cell Structure and Methods in Cell Biology

Cell: structural and functional organization, Cell motility, Other sub cellular organelle like Nucleus, Endoplasmic reticulum, Golgi, Mitochondria, Lysosomes; Fractionation of sub cellular organelles, Principles and applications of the microscopy, Cell counting.

2. Bio-membrane structure and Function

Plasma Membrane: organization and properties, Dynamics transport across membrane, Cell signaling: Types of receptors (Intracellular and cell surface), signal transduction by membrane bound, cytosolic and nuclear receptors via various pathways

3. Endo-membrane System and Cellular Motility

General organization of protein transport within and outside the cell, Mechanisms of endocytosis and exocytosis, Protein sorting and secretion, Vesicular transport, Mechanism of intracellular digestion

4. Cell Dynamics

Cell dynamics, cytoskeleton and cell surface, Microfilaments: Structural organization, cell motility and cell shape; Microtubule: Structural and functional organization, cilia, flagella, centriole; Intermediate filaments, Cell-cell interactions and cell matrix interaction

5. Cell Cycle & Cell Death

Mitosis, Meiosis, Eukaryotic Cell cycle and its regulation, Apoptosis, Cancer biology - Mechanism of carcinogenesis, tumor suppressor genes and oncogene.

Text books/References

- 1) Alberts et al (2007). Molecular Biology of the Cell. Garland
- 2) Lodish et al (2004). Molecular Cell Biology. Freeman
- 3) Karp (2005). Cell and Molecular Biology. John Wiley
- 4) Lewin (2007). Genes IX. Jones and Barlett
- 5) Cooper (2007). The Cell: A molecular Approach. ASM Press

1. Replication in prokaryotes & eukaryotes

Replication initiation and its regulation, elongation and termination in prokaryotes and eukaryotes; Enzymes and accessory proteins; Fidelity; Replication of single stranded circular DNA

2. Repair & Recombination

Gene stability and DNA repair enzymes: Photoreactivation, Nucleotide excision repair, Mismatch correction, SOS repair;

3. Prokaryotic & Eukaryotic Transcription

Prokaryotic Transcription; Transcription unit; Promoters-Constitutive and Inducible; Operators; Regulatory elements: Attenuation, Positive and negative regulation, Transcriptional control in lambda phage; Operon concept- lac, trp, ara, his and gal operons; Initiation, Elongation and Termination steps of prokaryotic transcription; Transcript processing recognition, Promoters and enhancers, Transcription factors: TATA binding proteins (TBP) and TBP associated factors (TAF), Activators and repressors; Transcriptional and post-transcriptional gene silencing; Processing of hnRNA, tRNA, rRNA; 5'-Cap formation; 3'-end processing and polyadenylation; Splicing; RNA editing; Nuclear export of mRNA; mRNA stability; Catalytic RNA.

4. Translation & Transport

Translation machinery; Ribosomes; Steps of translation and its mechanism in prokaryotes and eukaryotes: Initiation, elongation and termination; Genetic codon and its properties; Co- and post translational modifications; Genetic code in mitochondria;

5. Mutation & Transposition

Text books/References:

1. Lewin's GeneX-10th Edition Jones and Bartlett Publishers 2010.
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

Semester-II; Paper Code: BIT-508

FERMENTATION BIOTECHNOLOGY

(2 credits)

1. Fermentation technology: fermentation media, Design, operation & applications of fermentors.
2. Isolation and screening of commercially important microbes, media formulation, Strain improvement; microbial growth kinetics, effect of environmental conditions on microbial growth.
3. Industrial production of: ethanol, citric acid, acetic, fumaric and gluconic acid, solvents (glycerol, acetone and butanol), antibiotics (penicillin, streptomycin, tetracycline), amino acids (lysine & glutamic acid), pectolytic enzymes (Pectinases, Invertase, proteases and lipases) and Vitamins (Vit B₁₂, Riboflavin)
4. Immobilization of enzymes (or whole cells): immobilization techniques, carriers used, and characteristics of free vs. immobilization enzymes. Design & operation of immobilized enzyme reactors, applications of immobilized enzymes.
5. Downstream processing: removal of microbial cells & solid matters, foam separation, precipitation, membrane filtration, centrifugation, cell disruptions, liquid-liquid extraction, chromatography, membrane process & crystallization.

Text books/ References:

1. P F Stanbury, S. Hall, A. Whitaker. Principles of Fermentation Technology, Second Edition. Publisher Butterworth-Heinemann
2. Crueger, W. and Crueger, A. Biotechnology: A Textbook of Industrial Microbiology. PanimaPublsihers
3. AH Patel. A text book of Industrial Microbiology by, Macmillan Publishers India

Semester-II; Paper Code: BIT-032

LAB COURSE 11

(6 credits)

- 1) Protein concentration determination by using molar extinction coefficient.
- 2) Protein conformational studies by Fluorescence and CD.
- 3) Thermal unfolding of protein and calculation of thermodynamic parameters from temperature scanning
- 4) Effect of solvent conditions on thermal stability of protein.
- 5) Microscopy: a) simple, b) compound c) phase contrast microscopes.
- 6) Study of Permanent Slides
- 7) Haemocytometer: calibration and measurement of biological samples.
- 8) Electron microscopy: Demonstration and good photographs for interpretation.
- 9) Isolation of organelles
- 10) Blood smear identification of leucocytes by Giemsa stain
- 11) To study dye binding property using different proteins
- 12) Isolation of genomic DNA from plants/bacteria/fungus.
- 13) Quantification and purity determination of isolated genomic DNA by UV spectrophotometry and agarose gel electrophoresis.
- 14) Preparation of competent cells
- 15) Transformation of plasmid DNA (pBR 322/pUC 18/pUC 19) into DH 5 α strain of *E.coli*, calculation of transformation efficiency.
- 16) Isolation of plasmid DNA by alkaline lysis and phenol method.
- 17) Restriction digestion
- 18) Ligation of foreign DNA into cloning/expression vector
- 19) Polymerase chain reaction
- 20) RFLP analysis of the PCR product

Semester-III; Paper Code: BIT-509

ANIMAL CELL BIOTECHNOLOGY

(3 credits)

Animal Tissue Culture

Biology of cultured cells. Primary cultures. Nutrient requirements of mammalian cells. Media for culturing cells. Established cell lines. Suspension culture techniques. Generation of immortal cell lines. Cell separation techniques. Large scale production of cells using bioreactor and microcarrier. Tumor cells. Characterization and maintenance of cells, karyotyping, cryopreservation and revival, Detection and prevention of contaminants in cell cultures. Concepts of tissue engineering and role of scaffold. siRNA and microRNA.

Embryo Transfer and Related Techniques

Introduction of superovulation. Embryo collection and evaluation. Embryo spitting. Embryo sexing. Embryo transfer. In vitro fertilization. Embryo cloning. Production of transgenic animals and gene farming.

Applications of Tissue Culture

Commercial applications of animal tissue culture: Tissue culture as a screening system. Cytotoxicity and diagnostic tests. Development and preparation of vaccines against infecting organisms, mammalian cloning. Gene therapy: somatic and germ line gene therapy.

Text books/ References:

- 1) Animal Cell Culture Technique, Ed. Martin, Clynes. Springer, 1998
- 2) Animal Cell Culture-Practical Approach, 3rd Edition, Ed. John R.W. Masters, Oxford University Press, 2000
- 3) Stem Cells, C.S.Potten, Elsevier, 2006
- 4) Stem Cell Biology and Gene Therapy, Peter J. Quesenberry, 1st Edition, Willy – Less, 1998

1. Basic Principles of Gene cloning

2. Vehicles for Gene Cloning: Plasmids and Bacteriophages

3. Purification of DNA from Living Cells,

Preparation of total cell DNA, Plasmid DNA & bacteriophage DNA

4. Manipulation of purified DNA

The range of DNA manipulative enzyme, Enzyme for cutting DNA- restriction endonucleases, Ligation –Joining DNA molecules together

5. Introduction of DNA into Living Cells

Transformation- the uptake of DNA by Bacterial cells,

Identification of recombinants

Introduction of phage DNA into bacterial cells

Identification of non-bacterial cells

6. Cloning Vectors for *E.coli*

Cloning vectors based on *E.coli* plasmids, M13 bacteriophage, lambda bacteriophage, Lambda and other high capacity vectors enable genomic libraries to be constructed, Vectors for other bacteria **Cloning**

7. Vectors for Eukaryotes

Vectors for yeast and other fungi, Cloning vectors for higher plants and animals

8. Obtaining a clone of a specific Gene

The problem of selection , Direct Selection, Identification of a clone from a gene library, Methods for clone identification

9. The polymerase chain reaction

The polymerase chain reaction in detail, problems with the error rate of Taq polymerase

10. The Application of Gene cloning

Studying Gene Location and structure, Gene Expression and function, studying Genomes, Production of protein cloned Genes, Gene Cloning and DNA Analysis in Medicine, Gene Cloning in Agariculture, Forensic Science.

Text books/References:

1. S.B. Primrose, R.M. Twyman and R.W.Old; Principles of Gene Manipulation. 6th Edition, S.B. University Press, 2001.
2. J. Sambrook and D.W. Russel; Molecular Cloning: A Laboratory Manual, Vols 1-3, CSHL, 2001.
3. Brown TA, Genomes, 3rd ed. Garland Science 2006.
4. Technical Literature from Stratagene, Promega, Novagene, New England Biolab etc.

IMMUNOLOGY

(3 credits)

1. Introduction to immune system

Immune system overview, innate and acquired immune system, Components of immune system, Antigens, Structure and function of antibody, Monoclonal antibodies, Immunological Methods, Techniques in humoral and cellular immunology, Antigen-antibody interactions, B and T cell receptors and coreceptors

2. Immunoglobulin and T-cell receptor genes

Organization of Ig gene loci, Molecular mechanisms of generation of antibody diversity, Expression of Ig genes, Regulation of Ig gene transcription, Antibody engineering, Organization of TCR gene loci, Generation of TCR diversity

3. Generation and regulation of immune responses

Antigen processing and presentation, MHC-restriction, Cytokines, T Cell Maturation, activation and differentiation, B Cell Generation, Activation and differentiation, Clonal selection and immunological memory, Complement system, Regulation of immune responses, Immunological tolerance

4. Immunological Disorders

Primary and secondary immunodeficiencies, Autoimmune disorders, Hypersensitive reactions.

5. Immune system in human health

Immune response to infectious diseases and malignancy, Concept of immunotherapy, Vaccines.

Text books/References:

- 1) Kuby (2006). Immunology. Freeman
- 2) Abbas et al (2007). Cellular and Molecular Immunology. Saunders
- 3) Benjamin et al (2003). Immunology – A Short Course. Wiley-Liss
- 4) Roitt (2003). Essential Immunology. Blackwell

PLANT BIOTECHNOLOGY

(2 credits)

1. Plant Tissue Culture

Historical perspective; Totipotency; Organogenesis; Somatic embryogenesis; Artificial seed production; Micropropagation; Somaclonal variation; Androgenesis and its applications in genetics and plant breeding; Germplasm conservation.

2. Protoplast culture and Somatic Hybridization

Protoplast isolation; Culture and its applications; somatic hybridizations and applications;

3. Agrobiology

Agrobacterium-plant interaction; Virulence; Ti and Ri plasmids; Opines and their significance; T-DNA transfer; Disarming the Ti plasmid.

4. Genetic Transformation *Agrobacterium* mediated gene delivery; Cointegrate and binary vectors; Direct gene transfer- PEG-mediated, electroporation, particle bombardment and alternative methods; Screening markers; Chloroplast transformation.

5. Molecular Mapping & Marker Assisted Selection (MAS)

Quantitative and qualitative traits; MAS for genes of agronomic importance, e.g. insect resistance, grain quality and grain yield; Gene mapping and cloning; QTL mapping and cloning.

6. Plants as Biofactories

Concept of biofactories; Fermentation and production of industrial enzymes; vitamins and antibiotics and other biomolecules; Secondary metabolite production; Production of pharmaceutically important compounds; Bioenergy generation.

Text books/References:

1. H. S. Chawla. Plant Biotechnology A Practical Approach Science Publishers, USA
2. Plant Biotechnology: Methods in Tissue Culture and Gene Transfer Book Description, Orient Longman Publishers
3. S.S. Bhojwani, M.K. Razdan - Plant Tissue Culture: Theory and Practice. Elsevier Science **Semester-**

1. III;Paper Code: BIT-033

LAB COURSE 111

(11 credits)

- 1) Immunoblotting and Dot blot assays
- 2) Protein profiling of total blood serum & ammonium sulphate purified fraction using SDS PAGE
- 3) Staining of cell cultures and observations under microscope
- 4) Growth studies. Cell count, protein estimation
- 5) Preparation of tissue culture media
- 6) Micropropagation Techniques – Stem or nodal culture
- 7) Callus induction and growth measurement
- 8) Suspension culture techniques
- 9) Restriction digestion of λ -DNA with Hind III endonuclease.
- 10) Determination of molecular wt. of fragments obtained after Hind III digestion of λ DNA.
- 11) Restriction digestion of vector (gel analysis) and insert with suitable restriction endonuclease.
- 12) Ligation of foreign DNA into linearized plasmid (pBR 322/pUC 18/pUC 19/any expression vector) and its transformation into DH 5 α strain of *E.coli*.
- 13) Recombinant screening by blue-white selection and confirmation of recombinant construct by agarose gel electrophoresis.
- 14) Plasmid isolation and confirming recombinant by PCR and RE digestion
- 15) Purification of protein on Ni-NTA column and analysis of purification by SDS-PAGE.

Semester-IV; Research Dissertation: BIT-031

(16 credits)

Final year student (1V SEM) will do research work on defined topic within Centre of Biotechnology, University of Allahabad for a duration of one semester under the guidance of Centre faculty.