# REVISED CURRICULUM M.Sc. BIOTECHNOLOGY COURSE

# (2010-2011 Onwards)

# INTERDISCIPLINARY BIOTECHNOLOGY UNIT ALIGARH MUSLIM UNIVERSITY, ALIGARH

# SEMESTER I

## Biochemistry

## 3 Credits

#### Unit I

Chemical basis of life; Composition of living matter; Water – properties; pH; ionization and hydrophobicity; Emergent properties of biomolecules in water; Biomolecular hierarchy; Macromolecules; Molecular assemblies; Structure-function relationships 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; lipoproteins

#### Unit IV

Biomembrane organization - sidedness and function; Membrane bound proteins - structure; properties and function; Transport phenomena; Nucleosides; nucleotides; nucleic acids - structure; diversity and function; sequencing; Brief overview of central dogma.

## Unit V

Bioenergetics-basic principles; Equilibria and concept of free energy; Coupled processes; Glycolytic pathway; Kreb'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; 3rd edition; John Wiley; New York; 2004.
- 2. A.L. Lehninger; Principles of Biochemistry; 5th edition; W.H Freeman and Company; 2004.
- 3. L. Stryer; Biochemistry; 5th edition; W.H. Freeman and Company; 2002.

## Analytical Techniques

**3 Credits** 

#### 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 Fransisco; 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.

## **Biostatistics & Computer Applications**

## 3 Credits

## 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 Miroarray 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

## Practicals:

Introduction to MSEXCEL-Use of worksheet to enter data; edit data; copy data; move data. Use of in-built statistical functions for computations of Mean; S.D.; Correlation; regression coefficients etc. Use of bar diagram; histogram; scatter plots; etc. graphical tools in EXCEL for presentation of data. Introduction to SYSTAT package. Searching PubMed ; Introduction to NCBI; NCBI data bases; BLAST BLASTn; BLASTp; PSI-BLAST; Sequence manipulation Suite; Multiple sequence alignment; Primer designing; Phylogenetic Analysis. Protein Modeling; Protein structure Analysis; Docking; Ligplot interactions.

#### 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; 3rd 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.

## Cellular & Molecular Biology

## 3 Credits

#### Unit I

Cell diversity: Chemical equilibrium and energetics; Cell theory; Cell organelles- endomembrane systems; Golgi apparatus; lysosomes; endoplasmic reticulum; nucleus and chromatin organization; Extracellular matrix - basal lamina; connective and other tissues; Cell-cell junctions; Cell wall- structural organization and functions; Cellular energy transactions- Role of mitochondria and chloroplast; Co- and post-translational modification of Proteins; Intracellular protein trafficking; Quality control in ER and Golgi; secretary pathway and vesicular trafficking; Import into mitochondria; chloroplast; peroxisome; lysosomes; Receptor-mediated endocytosis; Cytoskeleton- actin; myosin; microfilaments; microtubules and their dynamics; Intermediate filaments; Cell motility; Cilia and flagella; Motor proteins- kinesin and dynein; Differentiation of specialized cellsstem cells differentiation; blood cell formation.

#### Unit II

Basic techniques of cell biology; Light and electron microscopy; Confocal microscopy; atomic force microscopy; Sub-cellular fractionation; Culturing of metazoan cells; Protein-DNA Interactions; Footprinting and gel-shift asays; Yeast two hybrid and Phage display; Structure determination: Application of X-Rays; NMR; cryoelectron microscopy; RNA interference; hybridization techniques; Membrane transport- Passive and active transport; diffusion and osmosis; ion channels (gated & non-gated); Symport and Antiport; Uniport and Co-transport; Trans-epithelial transport; Transport of proteins and molecular chaperones.

#### Unit III

Cell cycle and cancer biology; Mitosis- role of cyclins; CDK; MPF and control of mitosis; Nuclear decondensation; Control of S-phase; Cell cycle control in mammalian cells; Check points and restriction points; Meiosis- asymmetric cell division; Gametogenesis and fertilization; Cell death and its regulation; Tumor cells and onset of cancer; Oncogenes; proto-oncogenes; Viral and cellular oncogenes; tumour suppressor genes from humans; Structure; function and mechanism of action of pRb and p53 tumour suppressor proteins; Types of cancer and metastasis; Genetic basis of cancer; Mutations as cause of cancer; Nonsense; missense and point mutations; Intragenic and Intergenic suppression; Frameshift mutations; Physical; chemical and biological mutagens; Carcinogens; Viruses and cancer; Cell signaling- surface receptors; Signal

transduction; Role of GPCR; Cytokine receptors; Receptor tyrosine kinase; MAPK pathways; Secondary messengers; Gene activation by cell surface receptors.

#### Unit IV

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; Overlapping genes; Split genes; Eukaryotic Gene structure; mobile genetic elements (transposons and retroposons) in Prokaryotes and Eukaryotes; Organelle DNAs; Organization and morphology of chromosomes; DNA replication- mechanism; enzymes and accessory proteins involved; control; Replication of single stranded circular DNA; gene stability and DNA repair enzymes; Photoreactivation; Nucleotide excision repair; mismatch correction; SOS repair; Homologous and non-homologous recombinations; Site specific recombinations; Chi sequences in prokaryotes; Gene targeting; Gene disruption; FLP/FRT and Cre/Lox recombination.

#### Unit V

Prokaryotic and Eukaryotic transcription; RNA polymerases; General and specific transcription factors; Regulatory elements- TATA box and TATA binding proteins; activators; repressors; Mechanisms of transcription regulation at initiation; elongation and Termination (Rho dependent and rho dependent); Attenuation and anti-termination; Regulation of transcription factor activity; Chromation remodelling and histone modification; Regulation of transcription in eukaryotes- regulatory sequences (Promoters and enhancers); Gene expression in bacteria; Operon Concept (lac; trp; ara; his operons); Transcriptional control in lambda phage; Pre mRNA Modifications- 5'- cap formation; 3'- end processing and polyadenylation; splicing; mRNA stability; Processing of hnRNA; RNA editing; Nuclear export of mRNA and its regulation; tRNA modification; cytoplasmic mechanism of post transcriptional control; Genetic code- degeneracy of codons: Wobble hypothesis: Genetic code in mitochondria: Translation- Procarvotic and eukaryotic translation; the translation machinery; Mechanism of initiation; elongation and Termination; regulation of translation; Oncogenes as transcriptional activators...

#### 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.
- 4. Lodish et al.; Molecular cell Biology; 4th Edition; W.H. Freeman & Company; 2000.
- 5. Smith & Wood; Cell Biology; 2nd Edition; Chapman & Hall; London; 1996.
- 6. B. M. Turner; Chromatin & Gene regulation; 1st Edition; Wiley-Blackwell; 2002.
- 7. Watson et al.; Molecular Biology of the gene; 5th Edition; Pearson Prentice Hall. USA; 2003

## Immunology

## 3 Credits

## Unit I

Immunology-fundamental concepts and anatomy of the immune system Components of innate and acquired immunity; Phagocytosis; Complement and Inflammatory responses; Haematopoesis; Organs and cells of the immune system- primary and secondary ymphoid organs; Lymphatic system; Lymphocyte circulation; Lymphocyte homing; Mucosal and Cutaneous associated Lymphoid tissue.(MALT&CALT); Mucosal Immunity; Antigens - immunogens; haptens; Major Histocompatibility Complex - MHC genes; MHC and immune responsiveness and disease susceptibility; HLA typing

#### Unit II

Immune responses generated by B and T lymphocytes Immunoglobulins-basic structure; classes & subclasses of immunoglobulins; antigenic determinants; Multigene organization of immunoglobulin genes; B-cell receptor; Immunoglobulin superfamily; Principles of cell signaling;Basis of self –non-self discrimination; Kinetics of immune response; memory; 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; Cytokines-properties; receptors and therapeutic uses; Antigen processing and presentation-endogenous antigens; exogenous antigens; non-peptide bacterial antigens and super-antigens; Cell-cell co-operation; Hapten-carrier system

#### Unit III

Antigen-antibody interactions Precipitation; agglutination and complement mediated immune reactions; Advanced immunological techniques - RIA; ELISA; Western blotting; ELISPOT assay; immunofluorescence; flow cytometry and immunoelectron microscopy; Surface plasma resonance; Biosenor assays for assessing ligand –receptor interaction; CMI techniques- lymphoproliferation assay; Mixed lymphocyte reaction; Cell Cytotoxicity assays; Apoptotosis; Microarrays; Transgenic mice; Gene knock outs

#### 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; Antibody genes and antibody engineering- chimeric and hybrid monoclonal antibodies; Catalytic antibodies and generation of immunoglobulin gene libraries.

## Unit V

Clinical Immunology Immunity to Infection : Bacteria; viral; fungal and parasitic infections (with examples from each group); Hypersensitivity – Type I-IV; Autoimmunity; Types of autoimmune diseases; Mechanism and role of CD4+ T cells; MHC and TCR in autoimmunity; Treatment 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 immunotherapy; Immunodeficiency-Primary immunodeficiencies; Acquired or secondary 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; 6th 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.

## **Microbiology & Industrial Applications**

#### 3 Credits

#### Unit I

Microbial Diversity & Systematics Classical and modern methods and concepts; Domain and Kingdom concepts in classification of microorganisms; Criteria for classification; Classification of Bacteria according to Bergey's manual; Molecular methods such as Denaturing Gradient Gel Electrophoresis (DGGE); Temperature Gradient Gel Electrophoresis (TGGE); Amplified rDNA Restriction Analysis and Terminal Restriction Fragment Length Polymorphism (T-RFLP) in assessing microbial diversity; 16S rDNA sequencing and Ribosomal Database Project.

#### Unit II

Microbial Growth & Physiology Ultrastructure of Archaea (Methanococcus); Eubacteria (E.coli); Unicellular Eukaryotes (Yeast) and viruses (Bacterial; Plant; Animal and Tumor viruses); Microbial growth: Batch; fed-batch; continuous kinetics; synchronous growth; yield constants; methods of growth estimation; stringent response; death of a bacterial cell. Microbial physiology: Physiological adoption and life style of Prokaryotes; Unicellular Eukaryotes and the Extremophiles (with classical example from each group)

#### Unit III

Microbial Interactions and Infection Host–Pathogen interactions; Microbes infecting humans; veterinary animals and plants; Pathogenicity islands and their role in bacterial virulence

#### Unit IV

Microbes and Environment Role of microorganisms in natural system and artificial system; Influence of Microbes on the Earth's Environment and Inhabitants; Ecological impacts of microbes; Symbiosis (Nitrogen fixation and ruminant symbiosis); Microbes and Nutrient cycles; Microbial communication system; Quorum sensing; Microbial fuel cells; Prebiotics and Probiotics; Vaccines

#### Unit V

Industrial Applications Basic principles in bioprocess technology; Media Formulation; Sterilization; Thermal death kinetics; Batch and continuous sterilization systems; Primary and secondary metabolites; Extracellular enzymes; Biotechnologically important intracellular products; exopolymers; Bioprocess control and monitoring variables such as temperature; agitation; pressure; pH Microbial processes-production; optimization;

screening; strain improvement; factors affecting down stream processing and recovery; Representative examples of ethanol; organic acids; antibiotics etc. Enzyme Technologyproduction; recovery; stability and formulation of bacterial and fungal enzymes-amylase; protease; penicillin acylase; glucose isomerase; Immobilised Enzyme and Cell based biotransformations-steroids; antibiotics; alkaloids; enzyme/cell electrodes.

## 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; 11th Edition; Pearson Prentice Hall; USA; 2006.

## Genetics & Genetic Engineering

## 3 Credits

#### Unit I

Basics Concepts:DNA Structure and properties; 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; DNasel footprinting;

## Unit II

Cloning Vectors and methodology; Plasmids; Bacteriophages; M13 mp vectors; PUC19 and Bluescript vectors; Phagemids; Lambda vectors; Cosmids; Artificial chromosome vectors (YACs; BACs); Animal Virus derived vectors-SV-40; Expression vectors; pMal; GST; pET-based vectors; Protein purification; His-tag; GST-tag; MBP-tag etc.; Inteinbased vectors; Inclusion bodies; Methodologies to reduce formation of inclusion bodies; Plant based vectors; Ti and Ri as vectors; Yeast vectors; Shuttle vectors. Cloning Methodologies Insertion of Foreign DNA into Host Cells; Transformation; Construction of libraries; Isolation of mRNA and total RNA; cDNA and genomic libraries; Southwestern and Far-western cloning; Protein-protein interactive cloning and Yeast two hybrid system; Phage display; Principles in maximizing gene expression

## Unit III

PCR the basics; application and sequencing: Primer design; Fidelity of thermostable enzymes; DNA polymerases; Types of PCR – multiplex; nested; reverse transcriptase; real time PCR; touchdown PCR; hot start PCR; colony PCR; cloning of PCR products; T-vectors; Proof reading enzymes; PCR in gene recombination; Deletion; addition; Overlap extension; Site specific mutagenesis; PCR in molecular diagnostics; Viral and bacterial detection; PCR based mutagenesis; Mutation detection: SSCP; RFLP; Oligo Ligation Assay (OLA): Sequencing methods; Enzymatic DNA sequencing; Chemical sequencing

of DNA; Automated DNA sequencing; RNA sequencing; Chemical Synthesis of oligonucleotides; Introduction of DNA into mammalian cells; Transfection techniques; 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 knock out mice; Suicide gene therapy; Gene replacement; Gene targeting;

#### Unit IV

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. Genetic variation Mutations; kinds of mutation; agents of mutation; genome polymorphism; uses of polymorphism. Gene mapping and human genome project Physical mapping; linkage and association Population genetics and evolution Phenotype; Gene frequency;

#### Unit V

Mendelian Genetics Introduction to human genetics; Background and history; Types of genetic diseases; Role of genetics in medicine; Human pedigrees; Patterns of single gene inheritance-autosomal recessive; Autosomal dominant; X linked inheritance; Hemoglobinopathies - Genetic disorders of hemoglobin and their diseases.

#### Text/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. Selected papers from scientific journals.
- 5. Technical Literature from Stratagene; Promega; Novagen; New England Biolab etc.
- 6. S.R. Maloy; J.E. Cronan; D. Friefelder; Microbial Genetics; 2nd Edition; Jones and Bartlett Publishers; 1994.
- 7. N. Trun and J. Trempy; Fundamental Bacterial Genetics; Blackwell publishing; 2004.
- 8. Strachan T and Read A P; Human molecular genetics; 3rd Edition Wiley Bios; 2006.
- 9. Mange E J and Mange A. P.; Human genetics; 2nd Edition; Sinauer Associates publications; 1999.
- 10. Hartl L D and Jones B; Analysis of genes and genomes; 3rd Edition; Jones and Bartlett Publishers; 1994.

## Proteins & Enzymes

## 3 Credits

#### Unit I

Peptides & proteins- Peptide bond conformation; dihedral angles; lonization behaviour of peptides; Peptide diversity in terms of size and composition; Peptides with biological activities; Primary: secondary and tertiary structure of proteins; Ramachandran plot; Fibrous and globular proteins; Forces stabilizing native protein conformation; Super-

secondary structure: quaternary structure; Prediction of secondary structure; Chemical modification of Proteins.

#### Unit II

Determination of protein structure- Sequence determination of proteins; N- and Cterminal amino acid analysis; Edman's degradation: classical and automated procedures; Use of mass spectrometry in primary structure determination; secondary structure determination with the help of CD; Tertiary structure study with the help of Xray diffraction

#### Unit III

Protein function- Protein ligand interactions; Qualitative and quantitative studies on cooperative and non-cooperative (Sigmoidal) binding of ligands; Hill equation; Sequential and concerted model for cooperative binding.

#### Unit IV

Protein denaturation and folding; Models of protein folding and association of proteins; Anfinsen's experiment: Thermodynamics of protein folding- Leventhal Paradox; Role of chaperons in folding; Protein misfolding disorders: Amlyloid fiber formation; Protein evolution.

#### Unit V

Enzymes- History; Features of enzyme catalyzed reaction; Properties and study of enzyme active sites; Kinetics of single and multi-substrate reactions; Enzyme inhibition-irreversible and reversible (competitive: noncompetitive and uncompetitive inhibition); Allosteric enzymes (sequential and concerted model).

Artificial enzymes- Synthetic and semi-synthetic enzymes: Catalytic antibodies; Molecular imprinting: Non-aqueous enzymology- Behaviour of enzymes in non aqueous media: application in synthesis and industry.

#### Texts/ References:

- 1. M.M. Cox and D.L.Nelson, Lehninger; Principles of Biochemistry;(2008) 5th edition; W.H Freeman and Company;
- 2. J.M. Berg, J.L. Tymoczko and L. Stryer; Biochemistry; 5th edition; (2007) W.H. Freeman and Company..
- 3. H.W. Blank & D.S, Clark; Applied Biocatalysis, Vol I (1991) Marcel Dekkar Inc. N.Y.
- 4. D. Whitford; Proteins, Structure and Function (2001) John Wiley & Sons Ltd.
- 5. R. Guerois and Lopez de la Paz; Protein Design Methods and Applicatios (2006) Humana Press

# SEMESTER III

## **Bioprocess Engineering & Technology**

## 3 Credits

## Unit I

Basic principle of Biochemical engineering 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

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; Fermentation economics; Fermentation media; Fermenter design-mechanically agitated; Pneumatic and hydrodynamic fermenters; Large scale animal and plant cell cultivation and air sterilization; Upstream processing: Media formulation; Sterilization; Aeration and agitation in bioprocess; Measurement and control of bioprocess parameters; Scale up and scale down process.

#### Unit III

Downstream processing 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 IV

Applications of enzymes in food processing Mechanism of enzyme function and reactions in process techniques; Enzymic bioconversions e.g. starch and sugar conversion processes; High-Fructose Corn Syrup; Interesterified fat; Hydrolyzed protein etc. and their downstream processing; baking by amylases; deoxygenation and desugaring by glucoses oxidase; beer mashing and chill proofing; cheese making by proteases and various other enzyme catalytic actions in food processing. Applications of Microbes in food process operations and production Fermented foods and beverages; Food ingredients and additives prepared by fermentation and their purification; fermentation as a method of preparing and preserving foods; Microbes and their use in pickling; producing colours and flavours; alcoholic beverages and other products; Process wastes-whey; molasses; starch substrates and other food wastes for bioconversion to useful products; Bacteriocins from lactic acid bacteria – Production and applications in food preservation.

#### Unit V

Enzyme kinetics; Two-substrate kinetics and pre-steady state kinetics; Allosteric enzymes; Enzyme mechanism; Enzyme inhibitors and active site determination Production; recovery and scaling up of enzymes and their role in food and other industries; Immobilization of enzymes and their industrial applications.

#### Texts/ References:

- 1. Jackson AT.; Bioprocess Engineering in Biotechnology; Prentice Hall; Engelwood Cliffs; 1991.
- 2. Shuler ML and Kargi F.; Bioprocess Engineering: Basic concepts; 2nd 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; 2nd Edition; McGraw-Hill Book Co.; New York; 1986.
- 5. Aiba S; Humphrey AE and Millis NF; Biochemical Engineering; 2nd 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.

## Immunotechnology & Molecular Virology

#### 3 Credits

## Unit I

Introduction to Immunotechnology Kinetics of immune response: memory; Principles of Immunization; Techniques for analysis of Immune response ; Antibody Related Techniques Immuno-chemistry of Antigens - immunogenecity: Antigenecity: haptens: Toxins-Toxoids: Hapten-carrier system; Genetic basis of immune response; Role and properties of adjuvants: Immune modulators; B cell epitopes; Hybridoma Rabbit: human; Antigen – Antibody interaction: affinity: cross reactivity: specificity: epitope mapping; Immuno assays: RIA: ELISA: Western blotting: ELISPOT assay: immunofluorescence: Surface plasma resonance: Biosensor assays for assessing ligand –receptor interaction New Generation Antibodies Multigene organization of immunoglobulin genes: Ab diversity; Antibody engineering; Phage display libraries; Antibodies as in vitro and in vivo probes

## Unit II

CMI and Imaging techniques CD nomenclature: Identification of immune Cells; Principle of Immunofluorescence Microscopy: Flurochromes; Staining techniques for live cell imaging and fixed cells; Flow cytometry: Instrumentation: Applications; Cell Functional Assays –lymphoproliferation: Cell Cytotoxicity: Mixed Lymphocyte Reaction: Apoptotosis: Cytokine expression; Cell cloning: Reporter Assays: In–situ gene expression techniques; Cell imaging Techniques- In vitro and In vivo; Immuno-electron microscopy; In vivo cell tracking techniques; Microarrays; Transgenic mice: gene knock outs

## Unit III

Classification of animal and plant viruses; Satellite viruses; Viroids; Virusoids etc.; Diseases causes by animal viruses and plant viruses; Economic loss due to important viruses : Genome organization of animal viruses; Replication of RNA viruses; Replication of DNA viruses; Structure of animal viruses and plant viruses;

Genome organization of DNA and RNA plant viruses; Replication of DNA and RNA plant viruses

## Unit IV

Methods to diagnose animal virus infections: Electron microscopy: Tissue culture growth of viruses : Virus quantitation assays: Viral serology: ELISA: neutralization assays; Molecular methods: hybridization: PCR: real time PCR: sequencing: microarray : gene silencing and antiviral assays

#### Unit V

Methods to study plant viruses; Infectivity assays – Sap transmission: insect vector transmission: agroinfection (using Agrobacterium); serological methods: immunelectrophoresis in gels: direct double-antibody sandwich method: Dot ELISA: Immunosorbent electron microscopy (ISEM): Decoration technique: Gene silencing: PTGS & TGS: viral suppressors of gene silencing.

## Texts/References:

- 1. Voet D; Voet JG & Pratt CW; Fundamentals of Biochemistry; 2nd Edition. Wiley 2006
- 2. Brown TA; Genomes; 3rd Edition. Garland Science 2006
- 3. Campbell AM & Heyer LJ; Discovering Genomics; Proteomics and Bioinformatics; 2nd Edition. Benjamin Cummings 2007
- 4. Primrose S<sup>\*</sup> & Twyman R; Principles of Gene Manipulation and Genomics; 7th Edition; Blackwell; 2006.
- 5. Glick BR & Pasternak JJ; Molecular Biotechnology; 3rd Edition; ASM Press; 1998.

## Genomics & Proteomics

## 3 Credits

#### Unit I

Introduction Structural organization of genome in Prokaryotes and Eukaryotes; Organelle DNA-mitochondrial; chloroplast; DNA sequencing-principles and translation to large scale projects; Recognition of coding and non-coding sequences and gene annotation; Tools for genome analysis-RFLP; DNA fingerprinting; RAPD; PCR; Linkage and Pedigree analysis-physical and genetic mapping.

#### Unit II

Genome sequencing projects Microbes; plants and animals; Accessing and retrieving genome project information from web; Comparative genomics; Identification and classification using molecular markers-16S rRNA typing/sequencing; EST's and SNP's.

#### Unit III

Proteomics Protein analysis (includes measurement of concentration; amino-acid 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 and Differential display proteomics; Protein-protein interactions; Yeast two hybrid system.

## Unit IV

Pharmacogenetics High throughput screening in genome for drug discoveryidentification of gene targets; Pharmacogenetics and drug development

#### Unit V

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

#### Texts/References:

- 1. Voet D; Voet JG & Pratt CW; Fundamentals of Biochemistry; 2nd Edition. Wiley 2006
- 2. Brown TA; Genomes; 3rd Edition. Garland Science 2006

- 3. Campbell AM & Heyer LJ; Discovering Genomics; Proteomics and Bioinformatics; 2nd 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.

## Plant Biotechnology

## Credit: 3

#### Unit I

Conventional plant breeding. Introduction to cell and tissue culture; tissue culture as a technique to produce novel plant and hybrids. Tissue culture media (composition and preparation). Initiation and maintenance of callus and suspension culture; single cell clones. Organogenesis; somatic embryogenesis; transfer and establishment of whole plants in soil.

## Unit II

Shoot-tip culture: Rapid clonal propagation and production of virus-free plants. Embryo culture and embryo rescue. Protoplast isolation; culture and fusion; selection of hybrids cells and regeneration of hybrid plants; symmetric and asymmetric hybrids; hybrid biotransformation. Anther; pollen; and ovary culture for production of haploid plants and homozygous lines. Cryopreservation; slow growth and DNA banking for germ plasm conservation.

#### Unit III

Plant transformation technology: Basis of tumor formation; hairy root; features of T1 and R1 plasmids; mechanisms of DNA transfer; role of virulence genes; use of T1 and R1 as vectors; binary vectors; use of 35S and other promoters; genetic markers; use of reporter genes; reporter genes with introns; use of scaffold attachment regions; methods of nuclear transformation; viral vectors and their applications; multiple gene transfers; vectorless or direct DNA transfer; particle bombardment; electroporation; microinjection transformation of monocots. Transgene stabilityand gene silencing/ plant transformation. Application of plant transformation for productivity and performance promoter trapping; activation tagging. Herbicide resistance; phosphoinothricin;glyphosate; sulfonyl urea; atrazine; insect resistance; Bt. Genes; non-Bt like protease inhibitors; alpha amylase inhibitor; virus resistance; coat protein mediated; nucleocaspid gene; disease resistance; chitinase; 1 – 3 beta glucanase; RIP antifungal proteins; thionines; PR proteins; nematode resistance abiotic stress post-harvest losses; long shelf life of fruits and flowers; use of ACC synthase; poly- galactrunase; ACC oxidase; male sterile lines; bar and barnase systems; carbohydrate composition and storage; ADP glucose pyrophosphatase; terminator gene technology. Chloroplast transformation: Advantages; vectors; success with tobacco and potato plants.

## Unit IV

An introduction to plant conservation biotechnology, Molecular approaches to assessing plant diversity; molecular marker system; molecular markers in germ plasm characterization; population genetics; biodiversity characterization, plant germplasm acquisition; plant genetic resource conservation; acquisition procedures; planning methods involved, Phytosanitary aspects of plant germplasm conservation; safe movement of germplasm; quarantine; virus detection; production of pathogen free plants, Cryopreservation; principle; preparation and pretreatment, cryoprotection

procedure, Stability assessment; natural variation; techniques; morphological variation; cytological, biochemical and molecular analysis, biotechnological advances in conservation of root and tuber crops; economical important plants; endangered plants; rain forest conservation.

#### Unit V

Metabolic engineering and industrial products: Plant secondary metabolites; control mechanisms and manipulation of phenylpropanoid pathway; alkaloids; industrial enzymes; biodegradable plastics; polyhydroxybutyrate; therapeutic proteins; lysosomal enzymes; antibodies; edible vaccines; purification strategies; oleosin partitioning technology. Molecular marker-aided breeding: 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. Arid and semi-arid plant biotechnology; Green house and Green-home technology.

#### References:

- 1) Plant cell & tissue culture Ed: Pollard JW & Walker JM. (1990) Humana Press
- 2) Principles of Plant Biotechnology Ed: Mantell, Mckee RA, Matthews JA. (1987) Blackwell Scientific Publications
- 3) Plant Biotechnology & Transgenic Plants, Ed: Oksman, CKM & Barz WH. (1999) Kluwer Academic/Plenum
- 4) Plant Conservation Biotechnology by Benson EE (1999) CRC
- 5) Fundamentals of Plant Biotechnology by Amla Batra. (2006) Capital Publishing House

## IPR & Biosafety

## **Non-Credit**

#### Unit I

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

#### Unit II

Agreements and Treaties History of GATT & TRIPS Agreement; Madrid Agreement; Hague Agreement; WIPO Treaties; Budapest Treaty; PCT; Indian Patent Act 1970 & recent amendments

#### Unit III

Basics of Patents and Concept of Prior Art Introduction to Patents; Types of patent applications: Ordinary; PCT; Conventional; Divisional and Patent of Addition; Specifications: Provisional and complete; Forms and fees Invention in context of "prior art"; Patent databases; Searching International Databases; Country-wise patent searches (USPTO; esp@cenet(EPO); PATENTScope(WIPO); IPO; etc.)

Unit IV

Patent filing procedures National & PCT filing procedure; Time frame and cost; Status of the patent applications filed; Precautions while patenting – disclosure/non-disclosure; Financial assistance for patenting introduction to existing schemes Patent licensing and agreement Patent infringement- meaning; scope; litigation; case studies

#### Unit V

Biosafety Introduction; Historical Backround; 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; Environmental release of GMOs; Risk Analysis; Risk Assessment; Risk management and communication; Overview of National Regulations and relevant International Agreements including Cartegana Protocol.

#### Texts/References:

- 1. BAREACT; Indian Patent Act 1970 Acts & Rules; Universal Law Publishing Co. Pvt. Ltd.; 2007
- 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
  - www.patentoffice.nic.in
  - www.iprlawindia.org/ 31k Cached Similar page
  - http://www.cbd.int/biosafety/background.shtml
  - <u>http://www.cdc.gov/OD/ohs/symp5/jyrtext.htm</u>
  - http://web.princeton.edu/sites/ehs/biosafety/biosafetypage/section 3.html

# **SEMESTER IV**

#### **Bio-entrepreneurship**

#### 3 Credits

#### Unit I:

#### Accounting and Finance

Forms of Business Organizations; Statutory and legal requirements for starting specific Organisation; Special provisions for Venture Capital Financing; Basics of accounting conventions; Difference between receipts and income; payment and expenses; Concept Profit and Loss Account; Understanding Balance Sheet related concepts; Preparation of Project for Bank Finance

#### Unit II

#### Marketing

Assessment of market demand for potential product(s) of interest; Identifying needs of customers including gaps in the market; Market Segments; Prediction of market changes; packaging the product; Market linkages; branding issues; Developing distribution channels; Pricing / Policies / Competition; Promotion / Advertising; Services Marketing

#### Unit III

#### **Negotiations Strategies**

With financiers, bankers, government, law enforcement authorities and companies & Institutions; Technology transfer and dispute resolution skills; Changes in External environment; Crisis Management; Use IT for business administration and financial management; E-business setup and management

#### Unit IV

#### Human Resource Development (HRD):

Different types of Organizational structures; Leadership and Managerial skills;Team building, teamwork, performance appraisal, rewards system; Fundamentals of Entrepreneurship: Support mechanism for entrepreneurship in India

#### Unit V

## Role of knowledge centre and R&D:

Knowledge centers like universities and research institutions; Role of technology and upgradation; Assessment of scale of development of Technology; Managing Technology Transfer; Regulations for transfer of foreign technologies; Technology transfer agencies.

#### Case Study:

- 1. Candidates should be made to start a 'mock paper company'; systematically following all the procedures.
  - The market analysis developed by them will be used to choose the product or services.
  - A product or service is created in paper and positioned in the market. As a product or services available only in paper to be sold in the market through the existing links. At this juncture; the pricing of the product or the service needs to be finalized; linking the distribution system until the product or services reaches the end consumer.
  - Candidates who have developed such product or service could present the same as a project work to the Panel of Experts; including representatives from industry sector. If the presented product or service is found to have real potential; the candidates would be exposed to the next level of actual implementation of the project.
- 2. Go to any venture capital website (like sequoiacap.com) and prepare a proposal for funding from venture capital.
- Note: Names of specific cases to be discussed in the class shall be specified from time to time

LABS

Semester I

#### Lab I: Biochemistry and Analytical Techniques

- 4 Credits
- 1. Preparation of Acetic-NaAcetate Buffer system and validation of the Henderson-Hasselbach equation.
- 2. Determination of protein concentration in unknown solution/biological sample plotting a standard graph of BSA using UV-Vis Spectrophotometer and validating the Beer-Lambert's Law.
- 3. pH meter titration of Amino Acids and separation of aliphatic, aromatic and polar amino acids by TLC.
- 4. An enzyme purification theme (such as E.coli alkaline phosphatase or any other enzyme).
  - (a) Preparation of cell-free lysates
  - (b) Ammonium sulfate precipitation
  - (c) Ion-exchange chromatagraphy
  - (d) Gel filtration
  - (e) Affinity chromatography
  - (f) Generating a purification table
  - (g) Assessing purity by SDS-PAGE gel electrophoresis
  - (h) Assessing purity by gel electrophoresis
  - (i) Enzyme Kinetic Parameters: Km, Vmax and Kcat.
- 5. Biophysical methods (Circular dichroism spectroscopy, fluorescence spectroscopy).

#### Lab II: Molecular Biology

- 1. Plasmid DNA isolation and DNA quantitation: Plasmid minipreps
- 2. Restriction digestion
- 3. Preparation of competent cells.
- 4. Agarose gel electrophoresis
- 5. Transformation of E.coli with standard plasmids, calculation of transformation efficiency
- 6. Polymerase chain reaction (PCR), using standard 16srRNA eubacterial primers
- 7. RFLP analysis of the PCR product
- 8. Transformation of the yeast Saccharomyces cerevisiae

# Semester II

#### Lab I: Immunology

- 1. Selection of animals, preparation of antigens, immunization and methods of bleeding, serum separation, storage.
- 2. Antibody titre by ELISA.
- 3. Double diffusion, immuno-electrophoresis and radial Immuno diffusion.
- 4. Complement fixation test.
- 5. Isolation and purification of IgG from serum or IgY from chicken egg.
- 6. SDS-PAGE, Immunoblotting, Dot blot assays
- 7. Blood smear identification of leucocytes by Giemsa stain
- 8. Separation of leucocytes by Dextran density gradient method
- 9. Demonstration of Phagocytosis of latex beads
- 10. Separation of mononuclear cells by Ficoll-Hypaque

#### Lab II: Microbiology and Industrial Applications

- 4 Credits
- 1. Sterilization, disinfection, safety in microbiological laboratory.
- 2. Preparation of media for growth of various microorganisms.

# 4 Credits

4 Credits

- 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. Assay of antibiotics production and demonstration of antibiotic resistance.
- 7. Isolation and screening of industrially important microorganisms.
- 8. Determination of thermal death point and thermal death time of microorganisms.

# Semester III

5.

10.

## Lab I: Genetic Engineering and Immunology

#### 4 Credits

4 Credits

- 1. Isolation of genomic DNA from Bacillus subtilis\* genome.
- 2. PCR amplification of scoC gene and analysis by agarose gel electrophoresis
- 3. Preparation of plasmid, pET-28a from E.coli DH5α and gel analysis.
- 4. Restriction digestion of vector (gel analysis) and insert with Nco I and Xho I
  - (a) Vector and Insert ligation
    - (b) Transformation in E.coli DH5a.
- 6. Plasmid isolation and confirming recombinant by PCR and RE digestion.
- 7. Transformation of recombinant plasmid in BL21 (DE3).
- 8. Induction of ScoC protein with IPTG and analysis on SDS-PAGE
- 9. Purification of protein on Ni-NTA column and analysis of purification by SDS-PAGE
  - (a) Random Primer labeling of scoC with Dig-11-dUTP
  - (b) Southern hybridization of B. subtilis genome with probe and non-r radioactive detection.
- 11. Antibody titre against model against model antigen by Sandwich Elisa method
- 12. Lymph node Immunohistochemistry (direct and indirect peroxidase assay)
- 13. Antibody isotype determination
- 14. SDS-PAGE profile of IgG, IgM and IgA class of antibodies
- 15. Western blotting using anti-sera from *Candida albicans* infected animals.

#### Lab II: Bioprocess Engineering and Technology

- 1. Determination of oxygen transfer rate and volumetric oxygen mass transfer coefficient (KLa) under variety of operating conditions in shake flask and bioreactor.
- 2. Determination of mixing time and fluid flow behaviour in bioreactor under variety of operating conditions.
- 3. Rheology of microbial cultures and biopolymers and determination of various rheological constants.
- 4. Production of microbial products in bioreactors.
- 5. Study of 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.