

**BHARATHIAR UNIVERSITY  
COIMBATORE- 641 046**

**M.PHIL./ PH.D- Biotechnology  
(W.e.f. 2008-2009 and onwards)**

**PART-1 SYLLABUS**

PAPER I –Research Methodology

PAPER II- Recent Trends in Biotechnology

PAPER III- 1. Animal Biotechnology

2. Enzyme Biotechnology

3. Microbes in Biotechnology

4. Plant Biotechnology

5. Food Biotechnology

**BHARATHIAR UNIVERSITY-COIMBATORE- 641 046**  
**M.Phil./ Ph.D,-BIOTECHNOLOGY**  
**PART-I SYLLABUS**  
**PAPER-I : Research Methodology**

**Unit I Introduction to research methodology:**

What is research? Basic and applied research, Essential steps in research, Defining the research problem, Research/Experimental design, Literature collection, Literature citation, Research report: components, Format of thesis and dissertation, Manuscript/research article, Review monographs, Bibliography and Reference, Significance of research.

**Unit II**

**Biophysical methods:** Analysis of biomolecules using UV/Visible, fluorescence, circular dichroism, NMR and ESR spectroscopy, structure determination using x-ray diffraction and NMR; Analysis using light scattering, different type of mass spectrometry and surface plasma resonance methods.

**Radiolabeling techniques:** Properties of different types of radioisotopes normally used in biology, their detection and measurement; incorporation of radioisotopes in biological tissues and cells, molecular imaging of radioactive material safety guidelines.

Unit III

**Microscopic techniques:** Visualization cells and subcellular components by light microscopy, resolving powers of different microscopes, microscopy of living cells, scanning and transmission microscopes, different fixation and staining techniques for EM, freeze-tech and freeze- fracture methods for EM, image processing methods in microscope.

**Histochemical and Immunotechniques:** Antibody generation, detection of molecules using ELISA, RIA, western blot, immunoprecipitation, flowcytometry and immunofluorescence microscopy, detection of molecules in living cells, *in situ* localization such as FISH and GISH.

Unit IV

**Electrophysiological methods:** Single neuron recording, patch-clamp recording, ECG, Brain activity recording, lesion and stimulation of brain, pharmacological testing, PET, MRI, fMRI, CAT.

**Computational methods:** Nucleic acid and protein sequence database; data mining methods for sequence analysis, web-based tools for sequence searches motif analysis and presentation.

Unit V.

**Statistical Methods:** Measures of central tendency and dispersal; probability distributions (Binomial, Poisson and normal); sampling distribution; difference between parametric and non-parametric statistics; confidence interval; errors; levels of significance; regression and correlation; t-test; analysis of variance;  $X^2$  test; basic introduction to Multivariate statistics, etc.,

## Reference

1. C.R. Kothari, IInd edition (2004) Research methodology, Methods and techniques, New Age Internation (P) Ltd, Publishers, New Delhi.
2. Jerrod H. Zar (1999) Biostatistical analysis by, Prentice Hall International, Inc. Press, London.
3. Attwood. T.K and Parry-Smith D.J. (2002) Introduction to Bioinformatics, Pearson education Singapore.
4. Sharma.BK.Instrumental methods of chemical analysis.
5. Upadhyay, Upadhyay and Nath, Biophysical chemistry.
6. KhandpurR.S. Handbook of biomedical instrumentation ,Tata Mc GrawHill.
7. Brigal.L.Williams,A biologist guide to principle and techniques of practical biochemistry.

**Paper-II Recent trends in biotechnology**

**Unit 1: Molecular Cloning**

Vectors in Molecular Biology- Modifying Enzymes- Polymerase chain reaction- DNA/Protein sequencing – Mutagenesis- Transposable Elements- rRNA/ Genomic/ c DNA Library construction and screening – Map based cloning

**Unit-II: Cloning in Microorganisms**

Cloning Techniques: Cloning in E-coli- Cloning in *Bacillus subtilis*- Cloning in Yeast  
Specialized vectors: Artificial chromosomes- Operons- Expression of cloned genes- site directed mutagenesis- fusion proteins- Degradative plasmids.

**Unit III: Cloning in higher Organisms:**

DNA mediated transformation – Gene transfer by viral transduction – Genetic manipulation of mammals- DNA transfer to other vertebrates- Gene transfer in plants- Direct and indirect gene delivery systems- plant viruses as vectors.

**Unit IV: Applications of Genetic Engineering**

Nucleic acid sequences as diagnostic tools- New drugs/ Therapies for genetic diseases- combating infectious diseases- Protein engineering- Metabolic Engineering- Molecular Breeding of plants- Production of interferons- DNA vaccines

**Unit V Recent advances in Biotechnology**

DNA/ Protein micro arrays- DNA/ Protein Markers- DNA finger printing- Gene knock out – RNAi and Gene silencing – Metagenomics- Bioethics and IPR

**Reference:**

1. Bowtell, D and Sambrook, J. DNA Microarrays: A Molecular cloning manual. CSHL press
2. Glick, BR., Pasternak, JJ (1998) Molecular Biotechnology: Principles and Applications of recombinant DNA, ASM Press.
3. Grandi, G (2004) Genomics, Proteomics and Vaccines. Wiley press.
4. Hannon, GJ, RNAi: A guide to gene silencing. CSHL Press
5. Kirby, LT (1990) DNA finger printing: An introduction,. Stockton press.
6. Lewin, B (2004). Genes VIII. Pearson- Prentice Hall Press
7. Primrose, S.B., Twyman, R.M., Old. R.W. (2001) Principles of Gene Manipulation Blackwell Science Limited.

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M.Phil./ Ph.D., Biotechnology  
Paper-III- Animal Biotechnology

Unit I BASICS OF ANIMAL CELL CULTURE

Introduction to animal tissue culture: culture media; Serum and animal protein free defined media and their applications. Balanced salt solutions and simple growth medium, Physical, Chemical and metabolic functions of different constituents of culture medium; Role of carbon dioxide, serum, growth factors, glutamine in cell culture; Types of cell culture; primary and established culture; organ culture; three dimensional culture and tissue engineering; feeder layers; cell synchronization; cryopreservation.

Unit.II MOLECULAR TECHNIQUES IN ANIMAL CELL CULTURE

Biology and characterization of cultured cells: tissue typing; cell-cell interaction; measuring parameters of growth; measurement of cell death; Apoptosis and its determination; cytotoxicity assays. Molecular techniques in cell culture; cell transformation; physical, chemical and biological methods; manipulation of genes; cell cloning and microorganisms;

Unit III IMMUNITY IN HEALTH CARE

Tumor immunology, immuno diagnosis and therapy with monoclonal antibodies. Cell fusion methods Hybridoma technology and its applications;; Stem cell research; Hemopoietic stem cells, embryonic stem cells, stem cells in health care. Cell culture products. Production of interferons, interleukins and vaccines through mammalian cell cultures

Unit IV: GENTIC DISORDERS AND GENE THERAPHY; HUMAN GENOME PROJECT

Human genetics; tracing the inheritance of disease, tracking the genes, molecular biological techniques for rapid diagnosis of genetic disease, gene mapping; gene therapy, targeting, silencing and knockout. Human genome project, gene-based therapies. Biochemical and immune disorders; infectious and parasitic disease. Cancer multistep carcinogenesis, oncogenes, anti-ancogenes

Unit V: TRANSGENIC ANIMALS AND THEIR APPLICATIONS

Transgenics; Transgenic animal; production and application; transgenic animals as models for human disease; transgenic animals in live- stock improvement; expression of the bovine growth hormone; transgenics in industry; Ethical issues in animal biotechnology.

References:

1. Animal cell culture; A practical approach, 4<sup>th</sup> Edition by Freshney. R.I. John, Wiley Publications
2. Methods in cell biology; Volume 57, Animal cell culture methods, d. Jennie P, Mather, David Barnes, Academic press

3. Mammalian cell biotechnology; A practical approach, Ed. M. Butler, Oxford University Press.
4. Exploring genetic mechanism; Ed. Maxine Singer and Paul Berg.
5. Principles of genetic manipulation; Ed. Old and Primrose, 6<sup>th</sup> Edition. Blackwell science publication
6. Animal cell culture, a practical approach; Ed. John R.W. Masters, Third edition Oxford University Press.
7. In Vitro cultivation of animal cells; Published on behalf of Open University Alkenburgerrweg 167, 6401 DL Heerlen Nederland and University of Greenwich ( Formerly Thames Polytechnic) Averu Jo; Rpad. E;tja., pmdpm SE9 2HB, United Kingdom
8. Immunology by Janus Kuby, 4<sup>th</sup> edition (Freeman).
9. Fundamentals of Immunology, adited by William Paul. 4<sup>th</sup> edition (Lippincott Rave

**BHARATHIAR UNIVERSITY- COIMBATORE- 641 046**  
**M.PHIL/ Ph.D.- BIOTECHNOLOGY**  
**PAPER III- ENZYME BIOTECHNOLOGY**

**Unit I STRUCTURE AND FUNCTION OF ENZYMES**

What are enzymes- A brief history of enzymes- The naming and classification of enzymes- Introduction- Amino acids, the building blocks of proteins- The basis of protein structure- Types of specificity- The active site- The Fischer 'lock-and-key ' hypothesis- The Koshland 'induced-fit hypothesis- Hypotheses involving strain or transition- state stabilization- Monomeric enzymes- Oligomeric enzymes.

**Unit II KINETIC AND CHEMICAL MECHANISMS OF ENZYME-CATALYSED REACTIONS**

An introduction to Bioenergetic, catalysis, kinetics and inhibition:

Some concepts of bioenergetics- Factors affecting the rates of chemical reactions- Kinetics of enzymes-catalysed reactions: an historical introduction- Reversible inhibition- Irreversible inhibition

Kinetics of single-substrate and multi substrate enzyme-catalysed reactions.

The relationship between initial velocity and substrate concentration- Rapid- reactions- The kind and Altman procedure- Examples of possible mechanisms- Steady state kinetics-

**Unit III THE INVESTIGATION OF ACTIVE SITE STRUCTURE**

The identification of binding sites and catalytic sites- The investigation of the three dimensional structures of active sites- Site directed mutagenesis: chemical modification of enzyme- Mechanisms of catalysis- Mechanism of reactions catalysed by enzymes without cofactors- Metal-activated enzymes and metalloenzymes- The involvement of coenzymes in enzyme – catalysed reactions.

The binding of ligands to protein:

Determination of protein ligand interaction- Use of DNA microcircles in protein- DNA binding studies; Electrophoretic mobility shift assay.

Sigmoidal kinetics and allosteric enzymes:

Introduction - Differentiation between models for cooperative binding in proteins- Sigmoidal kinetics in the absence of cooperative binding – The physiological importance of cooperative oxygen- binding by haemoglobin- Allosteric enzymes and metabolic regulation

**Unit IV: INVESTIGATION OF ENZYMES IN BIOLOGICAL PREPARATIONS**

Choice of preparation for the investigation of enzyme characteristics- Extraction of enzymes- Purification of enzymes- Enzyme assay- Determination of molecular weights of enzymes- Investigation of sub-cellular compartmentation of enzymes

Principle of the available detection techniques- Automation in enzymatic analysis – Principles of enzymatic analysis- handling enzymes and coenzymes

## Unit V: APPLICATION OF ENZYMATIC ANALYSIS IN MEDICINE AND INDUSTRY

Biotechnological application of enzymes:

Large- scale production of enzymes- Immobilized enzymes- Enzyme utilization in industry- Enzymes and recombinant DNA technology- Applications in medicine – Applications in industry

### Reference:

1. Trevor Palmer, Enzymes: Biochemistry, Biotechnology, Clinical chemistry, East-West Press, New Delhi.
2. A . Travers & M. Buckle (2004), DNA- Protein interaction, Oxford University Press, India edition.
3. Alan Fersht (1999) 2<sup>nd</sup> edition, structure and mechanism in protein science, W.H.Freeman and company, New York.



**BHARATHIAR UNIVERSITY: COIMBATORE- 641 046**  
**M.PHIL/PH.D- BIOTECHNOLOGY**  
**PAPER-III MICROBES IN BIOTECHNOLOGY**  
**(For the year 2006-07 and onwards)**

**Unit-I MICROBIAL DIVERSITY:**

Classification-Bacteria, Fungi, actinomycetes, Bergey's system: Molecular techniques for classification- Biochemical, microbiological, 16s rRNA sequencing, DNA-DNA hybridization, construction of phylogenetic tree, G+C analysis  
Preservation and maintenance of microbes.

**Unit II FERMENTATION ENGINEERING:**

Fermenter: Types- stirred tank, deep-jet, air-lift and sparged tank fermenters; monitoring and control parameters  
Bioreactors: types- batch, fed batch, continuous, CSTR, fluidized, immobilized cell reactors; mode of operation  
Optimisation of conditions: screening of factors- Plackett Burman design. Fractional factorial design, Pareto chart; Optimisation of factors- Response Surface methodology; Model Confirmation- experimental, ANOVA, normal plot.

**Unit III DOWN STREAM PROCESSING:**

Separation of cells- flocculation, filtration, plate filters, rotary vacuum filters;  
Disintegration- mechanical and non-mechanical; Filtration- membrane filtration, ultra filtration, reverse osmosis: Extraction- two phase, organic solvents, salts;  
Chromatography- adsorption, adhesive; drying- spray driers, drum driers, freeze driers.

**Unit IV MICROBES IN PHARMACEUTICAL AND FOOD INDUSTRIES:**

Production, harvest, recovery, uses and mode of action- enzymes, antibiotics, vitamins (B12, B2) organic acids (acetic acid, lactic acid, citric acid), alcohol (ethanol), organic solvents (acetone- butanol), amino acids, beverages (beer, wine, brandy), microbial supplements (Lactic acid bacteria) as medicine, biopolymer, biofertilizers, biocides, Steroid biotransformation  
Improvement in production – improved strains by protoplast fusion, recombination, alteration in metabolic pathway; immobilization of cells.

**Unit V BIOREMEDIATION:**

Xenobiotics-microbial mechanism; Microbial mining, ore leaching, oil recovery; solid waste treatment- composting, vermicomposting, biofuel, animal feed, mushroom cultivation, oil spill remediation, biomedical waste treatment; Wastewater treatment- primary, secondary and tertiary (Biological), heavy metal removal, industrial waste treatment

## REFERENCES

1. Microbial Biotechnology- Fundamentals of applied Microbiology by A.N. Glazer and H. Nikaido. W.H. Freeman and company.
2. Principles of Fermentation Technology, P.F. Stanbury & A. Whitaker, Pergamon Press.
3. Microbial Process Development by H.W. Woelle, World Scientific
4. Biotechnology Text book of Industrial Microbiology by W. Creuger and A. Creuger
5. Industrial Microbiology by Casida
6. Industrial Microbiology by Prescott
7. Biochemical Engineering Fundamentals (2<sup>nd</sup> Ed) by J.E. Bailey and D. Ollis. McGraw Hill Book Company.
8. Biochemical Engineering Fundamentals, Bailey, J.E. and Ollis, D.F., McGraw-Hill Book Co. New York.
9. Bioprocess Technology: Fundamentals and Applications, KTH, Stockholm.
10. Bioprocess Engineering: Basic Concepts, Shuler, M.L. and Kargi, P., Prentice Hall Engelwood Cliffs.
11. Principles of Fermentation Technology, Stanbury, P.F. and Whitaker, A. Pergamon Press, Oxford.  
Bioreaction Engineering Principles. Neilson, J. and Villadsen, J., Plenum Press.

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**PAPER III- PLANT BIOTECHNOLOGY**

**Unit I GENOME ORGANIZATION AND ENGINEERING**

Plant genome organization, structure of plant gene, Gene family, Chloroplast genome, Mitochondrial genome. Importance of RFLP and RAPD in plant breeding, Construction of cDNA library. Protein targeting to chloroplast and mitochondria, heat shock proteins.

**Unit II TISSUE CULTURE TECHNIQUES**

Brief historical account: Laboratory organization: Preparation of media: Aseptic manipulation: Sterilization of media, Culture vessels and explants: Single cell culture, Suspension culture. Cellular totipotency, Somatic embryogenesis: Synthetic seeds: Somaclonal and gametic Clonal variation. Shoot tip culture, Haploid production: Anther and Pollen culture: Triploid production: In vitro pollination and Fertilization, Embryo culture.

**Unit III APPLICATION OF TISSUE CULTURE**

Protoplast isolation and culture: Somatic hybridization, Cybrid technology, Micropropagation: Gemplasm conservation: Production of secondary metabolites: Genetic engineering of metabolic pathways, Production of secondary metabolites in Bioreactors and downstream procession.

**Unit IV MOLECULAR BIOLOGY OF PLANT MICROBIAL INTERACTION AND TRANSFORMATION**

Biofertilizers: Symbiotic and Non-symbiotic nitrogen fixation, Biochemistry and Molecular biology of biological nitrogen fixation, Genetic engineering of nif genes and nod genes. Mycorrhizae: Ecto and Endo Mycorrhizae, Agrobacterium and Crown gall tumours, Mechanism of T- DNA transfer. Ti and Ri plasmid vectors Agro infection. Direct transfer of plants by physical methods. Selectable marker and reporter genes, Chloroplast transformation.

**Unit V TRANSGENIC PLANTS AND THEIR APPLICATIONS**

Transgenic plants: Genetic engineering of plants for herbicide resistance, Pest resistance, Virus resistance, Disease resistance, Stress tolerance, Cytoplasmic male sterility, Delayed fruit ripening. Genetic engineering in floral industries, Genetic engineering of seed storage proteins. Vaccine production in plants, Edible vaccine, Transgenic plants as bioreactors.

**References**

1. Chrispeels M.J. and Sadava D.E. (2002). Plants, genes and agriculture. The American Scientific publishers.

2. Chawla H.S. (2004) Biotechnology in crop improvement. International book Distribution Company.
3. Donal Grierson and Convey S.V. (1984). Plant Molecular Biology by Blackie & Son Limited, Newyork.
4. Hammond J. Mc Garvey P. and Usibov V. Y (Eds) (2000). Plant Biotechnology Springer Verlag.
5. Moncia, A. Hughes. (1999). Plant Molecular genetics by Pearson education limited, England.
6. Razdan M.K. (2003) . Introduction to plant tissue culture Oxford- IBH publishing Co. Pvt.Ltd.
7. Slater, A. Scott, N and Fowler, M. (2003). Plant Biotechnology: The genetic manipulation of plants. Oxford press.

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**M. Phil./Ph. D-BIOTECHNOLOGY**  
**PART-I SYLLABUS**  
**PAPER-III-FOOD BIOTECHNOLOGY**

**UNIT- I**

**STRUCTURE, FUNCTION AND CLASSIFICATION OF FOOD BIOMOLECULES:** Food ingredient challenges, carbohydrates, proteins, lipids, energy, minerals, vitamins-sources, biofunctional properties, physiological role, bioavailability and nutritional significances. Antinutritional factors: enzyme inhibitors, phytohemagglutinin/lectins, glucosinolates, cyanogenic glucosides/cyanogens, tannins, gossypol, saponins, phytates, toxic non-protein amino acids, allergens, oxalic acid, oligosaccharides, nitrate and nitrite and antivitamins-nature, occurrence and health effects, toxic factors induced by processing. Nutrient evaluation of proteins and carbohydrates of legumes and grains.

**UNIT-II**

**FOOD PREPARATION, PROCESSING AND PRESERVATION:** Biotechnology in relation to the food industry. Cooking of Foods: Objectives of cooking foods, methods of cooking, cooking media, microwave cooking, nutritional changes by cooking. Food processing: Milling, soaking, cooking, germination, microwave heating, thermal inactivation of microorganisms, thermal process, freezing and thawing of foods, biotechnological approaches, nutritive value of processed foods, packing, canning, labeling. Food preservation: preservation by heat, cold, chill storage, deep freezing, drying, concentration, fermentation, radiation and dehydration. Food quality: sensory evaluation, objective methods, nutritional constituents and food safety, food adulteration, control of food quality.

**UNIT-III**

**FOOD AND FOOD PRODUCTS:** Origin, scope and development. Fruits: tropical and subtropical fruits, dry fruits, vegetables, cereals, pulses, nuts, oils and fats in foods. Beverages: Coffee, tea, cocoa, soft drinks, fruit juices. Fermented products: Dairy products. Fermented vegetable products- miso, sufu, natto, tempeh, idli, dosa, sour kraut, pickles, soy sauce, fermented fish products, organic acids, amino acids, vitamins. Regulatory and social aspects of biotechnology of foods, safety assessment of nutritionally improved foods and feeds developed through the application of modern biotechnology.

**UNIT-IV**

**FOOD SPOILAGE AND FOOD ADDITIVES:** Food borne illness, quality control, case studies on biotechnology in the evaluation of food quality, contamination and spoilage of fruits, vegetables, cereals, legumes, milk products, egg, meat, poultry, sea foods. Food additives: Introduction, chelating agents, coloring agents, curing agents, emulsions, flavours and flavouring enhancers. Flour ingredients, humectants, anticaking agents, leavening agents, nutritional supplements and non nutritive sweeteners, pH

control agents, preservatives, stabilizers, and thickeners. Additives and food safety – Evaluation of safety, safety versus hazards, unintentional additives.

#### UNIT-V

**FUNCTIONAL FOODS:** Dietary fiber-physicochemical properties, functional ingredients, significance and role in health and disease, Resistant starches-Fermentation, large bowl health and lipid metabolism; natural food antioxidants-nature and occurrence in vegetables, grains, fruits, non-alcoholic beverages, biofunctional and health effects, probiotics and prebiotics-introduction, probiotic food ingredients, source, human health; Nutraceuticals-historical and technological aspects, sources, properties, nutraceutical potential. Nutraceutical proteins and peptides in health and disease. Microbes as direct food-single cell proteins, baker's yeast, Antimicrobial in foods-introduction, role of natural and synthetic bioactive molecules.

#### REFERENCES

- Food Biotechnology, 2005. K. Shetty, G. Paliyath, A. Pometto and R. E. Levin (Eds.), CRC Press, Florida, USA.
- Foods-Facts and Principles, 2005. Shakuntala Manay and M. Shdaksharaswamy, IInd edition, New Age International Pvt Ltd, New Delhi, India.
- Handbook of Nutraceuticals and Functional Foods, 2006. R. E. C. Wildman (Ed.), CRC Press, Florida, USA.
- Hand book of Dietary Fiber, 2001. S. S. Cho and M. L. Dreher (Eds.), Marcel Dekker, Inc. New York, USA.
- Probiotics in Food Safety and Human Health, 2005. I. Goktepe, V. K. Juneia and Mohamed Ahmedna (Eds.), CRC Press, Florida, USA.
- Food Science, 2007. B. Srilakshimi, IVth Edition, New age International Pvt Ltd, New Delhi, India.
- Functional Foods and Biotechnology, 2006. K. Shetty, G. Paliyath, A. Pometto and R. E. Levin (Eds.), CRC Press, Florida, USA.
- Post Harvest Biotechnology of Food Legumes, 1985. D. K. Salunkhe, S. S. Kadam and J. K. Chavan, CRC Press, Florida, USA.
- Food and Feed from Legumes and Oilseeds, 1996. E. Nwokolo and J. Smartt (Eds.), Chapman & Hall, London, UK.
- Toxic Constituents of Plant Foodstuffs, 1980. I. E. Liener, IInd Edition, Academic Press, New York, USA.
- Antimicrobial in Food, 2005. M. Davidson, J.N. Sofos and A. L. Branen (Eds.), CRC Press, Florida, USA.
- Toxic Substances in Crop Plants, 1991. J. P. F. DMello, C. M. Duffs and J. H. Duffs (Eds.), Royal Society of Chemistry, UK.
- Plant Nonprotein Amino and Imino Acids-Biological, Biochemical and Toxicological properties, 1982. G.A. Rosenthal, Academic Press, New York, USA.
- Food Antioxidants-Technological, Toxicological, and Health Perspectives, 1996. D. L. Madhavi, S. S. Deshpande and D. K. Salunkhe (Eds.), Marcel Dekker, Inc, New York, USA.