# M. Sc. Biotechnology

[Academic Year of Implementation: 2018-2019]

# **Course Outline: Semester-VII**

Theory:

BT: 1001 Bioprocess Technology-I

BT: 1002 Enzyme Technology

BT: 1003 Advances in Instrumentation and Techniques

BT: 1004 Cell and Tissue Culture Technology-I

Practical:

BTP: 1005 Enzyme and Bioprocess Technology

BTP: 1006 Cell Culture Technology-I

# **Course Outline: Semester-VIII**

Theory:

BT: 2001 Bioprocess Technology-II

BT: 2002 Genomics and Proteomics

BT: 2003 Aquaculture Technology

BT: 2004 Agriculture Biotechnology

Practical:

BTP: 2005 Genomics and Bioprocess Technology

BTP: 2006 Agriculture Biotechnology & Aquaculture Technology

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## **BT-1001: Bioprocess Technology-I**

### **UNIT-1: Unit Operations in Bioprocess Technology:**

- 1.1 Introduction to sterilization in bioprocess industry
- 1.2 Equipment sterilization
- 1.3 Monitoring & Validation of sterilization
- 1.4 Sterility audit and automation
- 1.5 Introduction to the development of inoculums for industrial process
- 1.6 Nutritional requirements in fermentation processes
- 1.7 Introduction to Response Surface Methodology (RSM)

#### **UNIT-2: Mass Transfer and Instrument Control:**

- 2.1 Gas-liquid mass transfer in cellular systems
- 2.2 Determination of Oxygen Transfer Rates (OTR)
- 2.3 Introduction to Heat Transfer
- 2.4 Fundamentals of Process Control
- 2.5 Control loops
- 2.6 Additional forms of Control
- 2.7 Measurement Elements

#### **UNIT-3: Cell Separation Systems:**

- 3.1 Introduction to Cell Separation Systems.
- 3.2 Criteria for Decision.
- 3.3 Pre-treatment's.
- 3.4 CGMP and Regulatory Considerations.
- 3.5 Conventional Filtration & Cross flow Microfiltration.
- 3.7 Centrifugation.
- 3.7 Cell Disruption: Physical and Chemical Methods.

#### **UNIT-4: Product Recovery:**

- 4.1 Product Isolation: Extraction and Sorption
- 4.2 Precipitation
- 4.3 Chromatography and fixed bed adsorption
- 4.4 Membrane Separation: RO and UF
- 4.5 Electrophoresis
- 4.6 Product Recovery Trains (General Concepts)
- 4.7 Recovery of: Polysaccharides, Proteins & Gluconic Acid

#### **REFERENCES:**

- **1.** James, B. & Ollis David, F. (2010). *Biochemical Engineering Fundamentals*. Tata McGraw-Hill.
- **2.** Lydersen, B. K., D'Elia, N. A. & Nelson, K. L. (2010). *Bioprocess Engineering: Systems, Equipment and Facilities.* Wiley India Pvt. Ltd.
- **3.** Stanbury, P. F. & Whitaker, A. (1984). *Principles of Fermentation Technology*. Pergamon Press.
- **4.** Vogel, H. C. & Todaro, C. M. (1996). Fermentation and biochemical engineering handbook: principles, process design and equipment. William Andrew.

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## **BT-1002: Enzyme Technology**

## **UNIT – 1: Enzyme Preparation:**

- 1.1 Potential Sources of Enzymes
- 1.2 Screening for novel Enzymes
- 1.3 Media for enzyme production
- 1.4 Extraction and large scale purification of Enzymes
  - 1.4.1 Extraction of soluble and membrane-bound enzymes, Nature of the extraction medium
  - 1.4.2 Preliminary and Advanced purification procedures, Criteria of purity
  - 1.4.3 Determination of molecular weights of enzymes

## **UNIT – 2: Immobilized Enzymes and Biosensors:**

- 2.1 Preparation and properties of immobilized enzymes
- 2.2 Application of Immobilized enzymes: General principles
- 2.3 Genetic immobilization of enzymes on yeast cell surface
- 2.4 Biosensors: Calorimetric, Potentiometric and Optical

### **UNIT – 3: Large Scale/ Industrial Uses of Enzymes:**

- 3.1 Use of enzymes in detergents
- 3.2 Enzymes in the fruit juices, wine, brewing and distillation industries
- 3.3 Use of proteases in the leather and wool industry
- 3.4 Applications of glucose oxidase and catalase in the food industry
- 3.5 Use of enzymes in cellulose and starch hydrolysis
- 3.6 Use of lactases in the dairy industry
- 3.7 Medical applications of enzymes

#### **UNIT – 4: Recent advances and future prospects in Enzyme Technology:**

- 4.1 Enzymes and recombinant DNA technology.
- 4.2 Synthesis of artificial enzymes- Enzyme engineering.
- 4.3 Use of 'unnatural' substrates.
- 4.4 Coenzyme-regenerating systems.
- 4.5 Enzymes and Bioinformatics.

### **REFERENCES:**

- **1.** Enzymes: Biochemistry, Biotechnology and Clinical Chemistry Trevor Palmer, Horwood Publishing Chichester, England.
- **2.** Enzymes and Immobilized Cells in Biotechnology. Allen I. Laskin, The Benjamin/Cummings Publishing Company, INC., California.
- **3.** Fermentation Microbiology and Biotechnology. Mansi El-Mansi & Charlie Bryce, Taylor & Francis Ltd, London.
- 4. Industrial Biotechnology. S. N. Jogdand, Himalaya Publishing House, Mumbai.
- **5.** Fundamentals of Enzymology: Nicholes C. Price and Lewis Stevens, Oxford Univ. Press.

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## BT-1003: Advances in Instrumentation and Techniques

## **UNIT – 1: Advances in Spectroscopy:**

- 1.1 Principle, Instrumentation, Interpretation of results in IR spectroscopy
- 1.2 FT-IR: Principle and application
- 1.3 Working principle of NMR
- 1.4 Mass spectrometry and their applications
- 1.5 Atomic spectroscopy
  - 1.5.1 Atomic absorption spectroscopy: Principle, atomizers, sources and applications
  - 1.5.2 X-ray diffraction/crystallography: Principle and application

## **UNIT – 2: Electrophoretic Techniques:**

- 2.1 Electrophoresis of proteins
  - 2.1.1 SDS-PAGE
  - 2.1.2 Native gels and Gradient gels
  - 2.1.3 Isoelectric focusing
  - 2.1.4 Two dimensional electrophoresis
  - 2.1.5 Western blotting
- 2.2 Electrophoresis of nucleic acids
  - 2.2.1 Agarose gel of DNA and RNA
  - 2.2.2 DNA sequencing gels
  - 2.2.3 Southern Blotting
  - 2.2.4 PFGE, DGGE and TGGE

#### **UNIT - 3: GC and HPTLC:**

- 3.1 HPTLC: Methods of development and spot detection
- 3.2 GC: Principle, stationary and mobile phases
  - 3.2.1 Detectors: FID,TCD,ECD
  - 3.2.2 Quantitative analysis and qualitative analysis
  - 3.2.3 GC-MS combinations
  - 3.2.4 Limitations of GC

## **UNIT – 4: Advances in Liquid Chromatography:**

- **4.1 HPLC** 
  - 4.1.1 Components of Instrumentation
  - 4.1.2 Isocratic, binary and quaternary system
  - 4.1.3 Types of columns
  - 4.1.4 Stationary and mobile phases
  - 4.1.5 Detectors: UV absorption, PDA, RI and fluorescence
- 4.2 Ion exchange chromatography: Types of resins, principles of separation, detection and applications
- 4.3 Gel filtration chromatography: Principles and applications

#### **REFERENCE:**

1. Skoog D. Skoog and West's Fundamentals of Analytical Chemistry. Andover: Cengage Learning EMEA; 2014.

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## BT-1004: Cell and Tissue Culture Technology-I

#### **UNIT-1: Plant Genomes and Plant Tissue Culture:**

- 1.1Eukaryotic Gene structure and gene expression-regulation, Protein targeting
- 1.2 Types of plant promoters, enhancer and reporter system
- 1.3 Plant tissue culture: Culture environment and growth regulators
- 1.4 Types of culture

#### **UNIT-2: Secondary Metabolites Isolation and Identification:**

- 2.1 Classification, Biosynthetic pathway of secondary metabolites production, factors affecting production of secondary metabolites in plant tissue culture
- 2.2 Methods of extraction and isolation
- 2.3 Methods of separation
- 2.4 Methods of identification
- 2.5 Applications

#### **UNIT-3: Immortalization and Cell Separation Techniques:**

- 3.1 Control of senescence
- 3.2 Immortalization with viral genes
- 3.3 Telomerase-Induced immortalization
- 3.4 Tumorigenicity
- 3.5 Cell density and isopyknic sedimentation
- 3.6 Antibody based cell separation techniques
- 3.7 Fluorescence-Activated Cell Sorting (FACS)

#### **UNIT-4: Cell culture, Cryopreservation and Cell Viability:**

- 4.1 Primary culture
- 4.2 Criteria for subculture, subculture of cells growing in suspension and in monolayer
- 4.3 Cryopreservation, principles of cryopreservation
- 4.4 Cell viability

#### **REFERENCES:**

- 1. Plant Biotechnology: An Introduction to Genetic Engineering by Adrian Slater, Nigel W. Scott, Mark R. Fowler. Oxford University Press, 2008.
- 2. Phytochemical Methods A Guide to Modern Techniques of Plant Analysis by JB Harborne. Springer, 1998.
- 3. Introduction to Plant Biotechnology. 2<sup>nd</sup> edition. By H. S. Chawla. Oxford & IBH publishing Co. Pvt. Ltd. New Delhi.
- 4. Plant Tissue culture: Theory and Practice, a revised Edition, S. S. Bhojwani and M.K. Razdan, Elsevier.
- 5. Ian R. Freshney, Culture of animal cells: a manual of basic technique and specialized applications, 6<sup>th</sup> Ed., Willey Blackwell pub.

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## **BTP-1005: Enzyme and Bioprocess Technology**

- 1. To study time course of the reaction catalysed by alkaline phosphatase.
- 2. To determine temperature optima for alkaline phosphatase.
- **3.** To investigate the thermal stability of horseradish peroxidise.
- **4.** Preparation of double reciprocal curve.
- **5.** Study of enzyme inhibition kinetics.
- **6.** Estimation of oxygen transfer rate (OTR) by sulphite oxidation method.
- 7. Immobilization of whole cells (Yeast/Bacteria) by calcium alginate method.
- **8.** Production, estimation and purification of amylase/lipase/protease.
- **9.** Determination of thermal death point (TDP) and thermal death time (TDT) of microorganism for design of a sterilizer.
- **10.** Demonstration of fermentation process in a bioreactor.

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## BTP-1006: Cell Culture Technology-I

1.	Preparation of commonly used Plant Tissue culture media (MS and Gamborg's B5
	media) forplantlet regeneration.

- **2.** Anther culture.
- **3.** Embryo culture.
- **4.** Qualitative analysis of important phytochemicals.
- **5.** Isolation of protoplast.
- **6.** To perform suspension culture (PBLC) and prepare metaphase plate.
- 7. To perform GTG banding and learn Karyotyping of prepared metaphase plates.
- **8.** Isolation of Peripheral Blood Mononuclear Cells (PBMC).
- **9.** Assessment of cell viability by Trypan blue.
- 10. Cell Culture: Cell revival and Cell maintenance.

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## **BT-2001: Bioprocess Technology-II**

#### **UNIT-1: Fermentation of Food and Feed Products:**

- 1.1 Fermentation of Wine
- 1.2 Fermentation of Beer
- 1.3 Fermentation of Vinegar
- 1.4 Production Single Cell Proteins
- 1.5 Fermentation of Gluconic acid

#### **UNIT-2: Fermentation of Fine Chemicals:**

- 2.1 Penicillin
- 2.2 Citric acid
- 2.3 Ethanol
- 2.4 L-lysine
- 2.5 Human Recombinant Insulin

#### **UNIT-3: Industrial Production in Animal and Plant Cells:**

- 3.1 Introduction to Large Scale Animal Cell Culture
- 3.2 Animal Cells and Bioreactor Technology
- 3.3 Stirred Tank Characteristics
- 3.4 Support Systems
- 3.5 Downstream Processing
- 3.6 Non-traditional cell culture processes & Regulatory issues
- 3.7 Cell culture production runs (example) & Plant cell cultivation

#### **UNIT-4: Bioprocess Industry-Economics and Validation:**

- 4.1 General Fermentation Process Economics
- 4.2 Economics of Fine Chemicals, Bulk Oxygenates and SCP Production
- 4.3 Bioproduct Regulation
- 4.4 Introduction to Biopharmaceutical Validation
- 4.5 Need for Validation and Occurrence of Validation
- 4.6 Validation Structure and Resources for Validation
- 4.7 Validation of Systems and Processes

#### **REFERENCES:**

- **1.** Reed G. (2004). *Prescott and Dunn's Industrial microbiology*. CBS Publishers & Distributors
- **2.** Peppler, H. J., & Perlman, D. (1979). *Microbial technology. Vol. 1 and 2*. Academic Press Inc.
- **3.** Vogel, H. C., & Todaro, C. M. (1996). Fermentation and Biochemical Engineering Handbook: Principles, Process Design and Equipment. William Andrew.
- **4.** James, B., &Ollis David, F. (2010). *Biochemical engineering fundamentals*. Tata McGraw-Hill.
- **5.** Lydersen, B. K., D'Elia, N. A., & Nelson, K. L. (Eds.). (2010). *Bioprocess engineering: systems, equipment and facilities.* Wiley India Pvt. Ltd.

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#### **BT-2002: Genomics and Proteomics**

#### **UNIT-1: Methods of Studying Genomes:**

- 1.1 Genetic mapping: DNA Markers and Linkage mapping
- 1.2 Physical mapping: Restriction mapping, FISH, STS mapping
- 1.3 Chain termination sequencing: Traditional and Alternative methodology
- 1.4 NGS Techniques: Template preparation, Sequencing and Imaging, Emulsion PCR
- 1.5 NGS Platforms: Pyro-sequencing, SOLiD, Illumina, Ion Torrent, Helicose, PacBio, Nanopore
- 1.6 Assembly of a contiguous DNA sequence

#### **UNIT-2: Annotation& Functional Genomics:**

- 2.1 Locating genes in sequence
- 2.2 Determining function of individual genes
- 2.3 Global gene expression profiling: Microbial genes and Human diseases
- 2.4 Annotation case study of Sachharomyces cerevisiae
- 2.5 Comparative genomics: Bacteria, Organelles and Eukaryotes

#### **UNIT-3: Proteomics-I:**

- 3.1 2-D PAGE for proteome analysis
- 3.2 Detection of proteins in 2D gels
- 3.3 Mass Spectroscopy: Introduction, background and *de novo* sequencing using MS data
- 3.4 Use of protein microarrays
- 3.5 Structural proteomics: X-ray crystallography and NMR
- 3.6 International structural proteomics initiatives

#### **UNIT-4: Proteomics-II & International Collaborative Projects:**

- 4.1 Methods for studying protein interactions
- 4.2 Use of affinity purification
- 4.3 Bioinformatics support to study protein interaction
- 4.4 Metabolomics and global biochemical networks
- 4.5 Human Genome Project: Mapping phase, Sequencing phase and future
- 4.6 Other projects: ENCODE, HapMap, Human Microbiome Project

#### **REFERENCES:**

- 1. Brown T. Genomes 3. New York and London: Garland Science; 2007.
- **2.** Primrose S, Twyman R. Principles of genome analysis and genomics. Malden, Mass.: Blackwell Pub.; 2003.
- **3.** Pennington, S. and Dunn M. Proteomics from protein sequence to function. New Delhi: Viva Books Private Limited; 2002.



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## **BT-2003: Aquaculture Technology**

### **UNIT-1: Introduction to Aquaculture:**

- 1.1. Definition, scope & significance of aquaculture
- 1.2. General characteristics and economic importance of fishes
- 1.3. Sustainable aquaculture
- 1.4. Fish culture process
- 1.5. Components, design & types of Hatcheries
- 1.6. Different Aquaculture Systems

#### **UNIT-2: Aqua-Feed& Diseases:**

- 1.1. Importance and types of feeds
- 1.2. Feed formulations & feed developmental process
- 1.3. Prebiotics & Probiotics in Aqua-feed
- 1.4. Bacterial, Viral, Fungal diseases of fishes and their diagnosis
- 1.5. Fish spoilage & marine toxins
- 1.6. Bio-fouling

### **UNIT-3: Aquaculture Biotechnology:**

- 1.1. Transgenic fish production
- 1.2. Chromosomal manipulation Triploidy, Polyploidy, Androgenesis & Gynogenesis
- 1.3. Applications of recombinant hormone & growth factors in aquaculture
- 1.4. Fish vaccines and its development
- 1.5. Cryopreservation

#### **UNIT-4: Algal Biotechnology:**

- 1.1. Economic importance of seaweeds& algae
- 1.2. Seaweed & microalgae culture methods
- 1.3. Bioactive compounds from marine organisms
- 1.4. Molecular farming of microalgae
- 1.5. Enhancement of protein production by microalgae

#### **REFERENCES:**

- 1. General & Applied Ichthyology (Fish & Fisheries) by S. K. Gupta & P. C. Gupta, S. Chand Publication, New Delhi.
- 2. Aquaculture Technology & environment by Ujwala Jadhav, Prentice Hall of India Pvt. Ltd., New Delhi.
- 3. Biotechnology & Genetics in Fisheries & Aquaculture by Andy Beaumont, Pierre Boudry, Kathrin Hoare.
- 4. Aquaculture Farming aquatic animals & plants Edited by John S. Lucas, Paul C. Southgate, Blackwell publishing Company.
- 5. Fisheries & Aquaculture Biotechnology by Varun Mehta, Book International Pub.
- 6. A Textbook of Fish Biology & Fisheries by S. S. Khanna & H. R. Singh.
- 7. Fish Health & Diseases by B. R. Selvamani & R. K. Mahadevan, Campus books Int., New Delhi.

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## **BT-2004: Agriculture Biotechnology**

### **UNIT-1: Tissue Culture & Transgenic Technologies:**

- 1.1 Role and molecular action of growth regulators in tissue culture
- 1.2 Direct transformation of protoplasts using PEG, electroporation, particle bombardment
- 1.3 Agrobacterium biology (Ti plasmids, Ri plasmids)
- 1.4 Ti plasmid based transformation

#### **UNIT-2: Abiotic & Biotic Stress and Resistance of Crops:**

- 2.1 Abiotic stress: Physiological and molecular responses of plants to water stress, salinity stress, temperature stress
- 2.2 Stress perception and stress signaling pathways
- 2.3 Plant interaction with bacterial, viral and fungal pathogens, biochemical and molecular basis of host plant resistance
- 2.4 Herbicide (glyphosate, phosphinothricin) and Pest (Bt toxin) resistance

#### **UNIT-3: Molecular Breeding:**

- 3.1 Restriction based and PCR based markers; RFLP: methodology and applications, RAPD and AFLP: Principles, methodology and advantages, disadvantages and applications
- 3.2 Development of SCAR and SSR, ISSR markers
- 3.3 Other markers: CAPS, SNP, Comparison of different marker systems
- 3.4 QTL and MAS

#### **UNIT-4: Molecular Farming & Applications:**

- 4.1 Definition and common perception of molecular farming
- 4.2 Transgenic plants as bioreactors
- 4.3 Relevance and advantages of plant based molecular farming
- 4.4 Edible vaccine; medicinally related proteins-antibodies (plantibodies), the oleosin system –Hirudinand Insulin production

#### **REFERENCES:**

- 1. Plant Biotechnology: An Introduction to Genetic Engineering by Adrian Slater, Nigel W. Scott, Mark R. Fowler. Oxford University Press, 2008.
- 2. Introduction to Plant Biotechnology. 2nd edition. By H. S. Chawla. Oxford & IBH publishing Co. Pvt. Ltd. New Delhi.
- 3. Plant Tissue culture: Theory and Practice, A revised Edition, S.S. Bhojwani and M.K. Razdan, Elsevier.
- 4. An Introduction to Plant Tissue Culture. 2nd Edition, by M. K. Razdan. Oxford & IBH Publishing Co. Pvt. Ltd.

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## **BTP-2005: Genomics and Bioprocess Technology**

- 1. Preparation of standard curve for estimation of antibiotic Penicillin.
- 2. Preparation of standard curve for estimation of Citric acid
- **3.** Preparation of standard curve for estimation of Ethanol.
- **4.** Microbial fermentation of Penicillin
  - 4.1. Determination of optimum pH for production of Penicillin.
  - 4.2. Determination of optimum inoculum size for Penicillin production.
- 5. Microbial fermentation of Citric acid
  - 5.1. Determination of optimum pH for production of Citric acid.
  - 5.2. Determination of optimum inoculum size for Citric acid production.
- **6.** Fermentative production of Ethanol using different substrates.
- **7.** Recovery of Citric acid and Ethanol from fermented broth/medium.
- **8.** Bioassay of antibiotic Penicillin.
- **9.** Soil DNA extraction by spin column method
- **10.** Total bacterial RNA extraction and separation by electrophoresis

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## BTP-2006: Agriculture Biotechnology & Aquaculture Technology

## **Aquaculture Technology:**

- 1. To study commercially important fishes of South Gujarat region (Any 10 specimen)
- **2.** To study fresh water algae & sea weeds (Any 10 specimen)
- **3.** Proximate analysis of fish feed (Crude Proteins, Crude Lipids, Carbohydrates, Fibers, Moisture & Ash)
- **4.** Detection of White Spot Syndrome Virus in shrimps by PCR technique (Demonstration).
- **5.** Review Article on any recent/emerging areas of aquaculture (**OR**) Field Visit to any one place Processing Unit (Fish/Prawn), Culture Farm (Fish/Prawn), Fish Feed development industry, Fisheries/Aquaculture Educational/Research Institute, Seaweed/Algal Culture facility, Fish/Algal Product development industry

## **Agriculture Biotechnology:**

- **1.** Standardization and preparation of commonly used Plant Tissue culture media (MS and Gamborg's B5) for *in vitro* regeneration of agriculturally important plants media.
- **2.** Quantitative analysis of important phytochemicals.
- **3.** Estimation of antioxidants and antioxidant enzymes Ascorbate peroxidase, Superoxide dismutase, Catalase and Peroxidase.
- **4.** Rapid *in vitro* screening tests for abiotic stress tolerance (drought and salinity).
- **5.** Isolation of DNA from suitable plant source.

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