JSS BIOTECHNOLOGY SKILL ENHANCEMENT PROGRAM (JSS – BiSEP)

SYLLABUS FOR

POSTGRADUATE DIPLOMA IN

PROTEIN EXPRESSION AND SCALE-UP

JSS COLLEGE OF ARTS, COMMERCE AND SCIENCE
(AUTONOMOUS; NAAC 'A' GRADE & COLLEGE WITH POTENTIAL FOR EXCELLENCE)
B. N. Road, MYSURU – 570 025

CO1-Students learn Good manufacturing practices

CO2- Students have been given training in laboratory skills and knowledge about biotech industry

Lab to Products: From DNA to Proteins

Course Code	**:**	**BiSEP101**	**Credits**	**:**	**04**
Total Lecture Hours	**:**	**52**	**Internal Marks**	**:**	**30**
Contact Hours/ Week	**:**	**04**	**External Marks**	**:**	**70**

Unit – I **6--- hours**

Introduction to biopharmaceutical industries

Major top ten Biotech industries in India and their products. State-of the art facilities available in these industries. Guidelines and basic principles of current good manufacturing practices.

Unit – II **12---- hours**

DNA

DNA amplification methods, DNA polymerases, DNase and DNA ligase - Industrial orientation/commercialization. Sequencing techniques, Gene therapy

Unit – III **10---- hours**

RNA

Types of RNA its structure and regulation. RNA extraction, mRNA isolation and cDNA conversion. RNA polymerases, Reverse Transcriptase and RNase. RNA interference technology.

Unit – IV **12---- hours**

Proteins

Chemical synthesis of peptides - Khorana's solution phase and Merrifield's solid phase synthesis-Industrial Application. Importance of peptides in research and industrial use (eg. Antibody production, biophysical studies, cyclic peptides etc.). How determination of peptides/amino acids can help in identification of proteins (Mass Spec). Introduction to proteases.

Unit – V **12 ---- hours**

Recombinant protein therapeutics

Structure, function and their applications: Insulin, Interferon alpha, Interferon gamma, Interleukin-2, Gm-CSF,G-CSF, Hepatitis B vaccine, Erythropoietin, Strptokinase, EGF, Chymotrypsin, Modification of proteins to increase their life.

Clotting, Haemophilia, Anticoagulants, Thrombolytic agents, tissue plasminogen activator, streptokinase.

Monoclonal antibodies as therapeutics: antibodies, hybridoma technology, FDA approved therapeutic antibodies, humanization. Methods for production of vaccines.

REFERENCE BOOKS:

1. Pharmaceutical biology, Concepts and applications by Gary Walsh**.**
2. Molecular Cell Biology, 4th edition - Harvey Lodish, Arnold Berk, S Lawrence Zipursky, Paul Matsudaira, David Baltimore, and James Darnell.
3. Molecular Biology of the Cell - Bruce Alberts, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, Peter Walter.
4. Molecular Biotechnology - Principles & application of r-RNA - Bernard R. Glick & Jack. J. Pasternak.
5. Principles of Gene Manipulation - Sandy Primrose, Richard Twyman & Bob Old.
6. Cells - Benjamin Lewin, Lynne Cassimeris, Vishwanath R. Lingappa , George Plopper
7. Production of Recombinant Proteins - Novel Microbial and Eukaryotic Expression
8. Systems http://www.cplbookshop.com/contents/C1627.htm - Edited by Gellissen, Gerd

9. Principles of Fermentation Technology - Peter F. Stanbury, P. F. Stanbury, Allan Whitaker, Stephen J. Hall.
10. A Text Book of Industrial Microbiology - Cruger and Cruger
11. Fermentation Biotechnology-Principles, Process and Products - Ward,O.P

Note: More importantly, students have to refer to recent research and review articles for updated information.

CO1- Students have been trained for production of recombinant proteins

CO2- Students understand the isolation and purification of proteins

Recombinant Proteins: Industrial Perspective

Course Code	**:**	**BiSEP102**	**Credits**	**:**	**04**
Total Lecture Hours	**:**	**52**	**Internal Marks**	**:**	**30**
Contact Hours/ Week	**:**	**04**	**External Marks**	**:**	**70**

Unit – I **10 ---- hours**

Cloning

General introduction to cloning and transformation techniques; Cloning tools- vectors, hosts, codon optimization, enzymes; Site directed mutagenesis methods; Engineering protein expression; Expression of various membrane proteins, cytosolic, carrier etc.

Unit – II **14 ---- hours**

Protein expression in bacteria

Applications of expression vectors, small scale isolation and regulation of protein expression, screening of recombinants, general considerations for purification of fusion proteins, detection / analysis of fusion proteins. Problems and troubleshooting of protein expression. Optimization of expression. Characteristics of small scale and large scale expression.

Protein expression in yeasts

General protein expression and regulation mechanisms in yeast *Saccharomyces cereviseae,* cloning and expression vectors in yeasts- Yip, Yep and Ycp vectors. Recombinant protein expression in yeasts- example and methodology used, advantages and disadvantages of *S. Cereviseae* as host; General protein expression and regulation mechanisms in *Pichia* species, cloning and expression in *Pichia pastoris*- example and methodology used, advantages and disadvantages of *P. pastoris* as host, other yeasts used for protein expression.

Unit – III **6 ---- hours**

Construction of expression vectors, transfection methods, transient and transduction methods. Multiplication of infection cloning strategies- advantages and disadvantages, protein production and purification methods, characterization of target protein and functional studies.

Unit – IV **14 ---- hours**

Protein expression in insect cells using baculovirus- advantages and disadvantages, methods, purification modules and protein expression analysis. Interpretation and scale-up

Protein expression in mammalian cells- Contribution to biomedical research, Requirements for mammalian expression system, Cell lines - CHO cell recombinant DNA hosts, current stratergy for CHO cell line development. Advantages and disadvantages. Interpretation and scale-up. Compare prokaryotic and eukaryotic expression system, control of expression, promoters, translation difference, codon bias selection, secondary modifications, downstream processing. Application of mammalian expression system

Unit – V **8 ---- hours**

Biosimilars

Introduction to biologics, defining biosimilars, differences between biosimilars and generics, technical challenges associated with production of biosimilar molecules, regulatory aspects of biosimilar molecules. Current status of biosimilars in different countries.

REFERENCE BOOKS:

1. Biosimilars- A new generation biologics**, Prugnaud,** Jean-Louis, **Trouvin, Jean Hugues (Eds.)**
2. Protein expression in mammalian cells, Methods and protocols, Hartley, James L
3. Biochemistry, genetics and molecular biology" New insights into cell culture technology" by SivakumarJoghi Thatha Gowder.
4. Molecular Cell Biology, 4th edition - Harvey Lodish, Arnold Berk, S Lawrence Zipursky, Paul Matsudaira, David Baltimore, and James Darnell.
5. Molecular Biology of the Cell - Bruce Alberts, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, Peter Walter.
6. Molecular Biotechnology - Principles & application of r-RNA - Bernard R. Glick & Jack. J. Pasternak
7. Principles of Gene Manipulation - Sandy Primrose, Richard Twyman & Bob Old.
8. Cells - Benjamin Lewin, Lynne Cassimeris, Vishwanath R. Lingappa , George Plopper.
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12. A Text Book of Industrial Microbiology - Cruger and Cruger
13. Fermentation Biotechnology-Principles, Process and Products - Ward,O.P

Note: More importantly, students have to refer to recent research and review articles for updated information.

CO1- Students learn the upstream and downstream processing of proteins
CO2- Students have been given training in analytical technologies

Fermentation & Downstream Processing

Course Code	**:**	**BiSEP103**	**Credits**	**:**	**04**
Total Lecture Hours	**:**	**52**	**Internal Marks**	**:**	**30**
Contact Hours/ Week	**:**	**04**	**External Marks**	**:**	**70**

Unit – I

Design of a fermenter **10--- hours**

Different types of fermentation (Solid liquid, surface etc). Detailed study of the design and construction of fermenters; Different process variables (measures to control the same). Industrial fermentations of importance (With Specific example to two products); scale-up processes-need for scale-up, factors affecting scale-up.

Microbes of industrial importance (Specific examples), strain improvement, Inoculum build up (Stages involved) and its importance.

Unit – II

Production of Fungal Proteases and other metabolites **6--- hours**

Fermentative process for the production of Proteases (Types of fermentation eg solid state, submerged, liquid surface etc) Fungal proteases: Extracellular products, Bacterial proteases: Intracellular products.

Unit – III

Culture preservation, Production of enzymes with special references to proteases
8 ---- hours

Proteases Enzyme definition with EC No, importance of EC No ,enzyme classification ,what are proteases, world production, demand and supply, production in India ,major producer of protease (global as well as national scenario), Microorganisms producing Proteases Eg (Fungal, yeasts bacteria etc).

Culture maintenance, Preservation of cultures, strain improvement techniques)

Unit – IV

Chromatographic techniques **14 ---- hours**

Principles and applications of TLC, adsorption, ion exchange, gel filtration, affinity, GLC, chromatofocusing, Liquid chromatography, HPLC.

Spectroscopic techniques

Principles- Beer-Lambert's law, limitation, extinction coefficient, Colorimetry, Turbidometry, spectrophotometer, fluorimetry. Flame photometry. Mass spec and its applications.

Unit – V

Electrophoretic techniques **14 ---- hours**

Polyacrylamide gel electrophoresis, SDS-PAGE, 2D-Electrophoresis, Isoelectric focusing, Agarose gel electrophoresis, separation of proteins, nucleic acids, visualizing separated

components - staining, fluorescence, PAS staining, zymogram and reverse zymogram, pulsed field electrophoresis, high voltage electrophoresis, capillary electrophoresis.

Ultra centrifugation

Construction of preparative and analytical ultra centrifuge, Schlieren optics for molecular weight determination, Svedberg's constant, sedimentation velocity and Sedimentation equilibrium. Step and gradient centrifugation.

REFERENCE BOOKS:

1. Principles of fermentation technology, 3rd edition, Peter stanbury,Allan Whitaker, Stephen Hal
2. Principles and techniques of Biochemistry and Molecular biology by K. Wilson and J. walker.
3. Molecular Cell Biology, 4th edition - Harvey Lodish, Arnold Berk, S Lawrence Zipursky, Paul Matsudaira, David Baltimore, and James Darnell.
4. Molecular Biology of the Cell - Bruce Alberts, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, Peter Walter.
5. Molecular Biotechnology - Principles & application of r-RNA - Bernard R. Glick & Jack. J. Pasternak.
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CO1- Students learn the techniques of GLP

Quality Control Biologist (LFS/Q2301)

Course Code	**:**	**BiSEP106**	**Credits**	**:**	**04**
Total Lecture Hours	**:**	**52**	**Internal Marks**	**:**	**30**
Contact Hours/ Week	**:**	**04**	**External Marks**	**:**	**70**

Unit 1 – Essentials of quality control **16 hours**

Preparations - buffer, solvents, solutions and microbial media for running bio-analytical quality tests, assays to carry out quality control procedures on biopharmaceutical products.
Concepts of pharmacopeia like BP, USP, EP and other applicable guidelines such as WHO, ICH and EMEA, etc., statistical tools and software like combistats, safe handling of infectious materials like cultures, strains and seed strains, procedures for handling infectious spillage control, GLP/GMP, biochemical analysis of proteins, bio analytical and microbiological methods, working of instruments/apparatus/equipment, biological assays, application of various analytical techniques such as HPLC, capillary electrophoresis including icIEF, FTIR, Circular Dichroism, UV and Fluorescence spectroscopy, ELISAs, enzyme assays and other applicable methods for the testing of biopharmaceuticals, application of microbiological techniques such as air monitoring, water testing, surface monitoring, microbial monitoring, biosafety levels and biosafety hazards

Unit 2 – Safety and Security at workplace **6 hours**

Different types of occupational health hazards, knowledge of chemical substances, characteristics & safety measures, use of safety gears, masks, gloves & accessories, evacuation procedures for workers & visitors. Health, safety & security issues – types (illness, fire accidents), company policies and procedures, When and how to report, summon medical assistance & emergency services

Unit 3 – Interpersonal Skills **6 hours**

Understand work output requirements, company rules, guidelines & policies related to the process flow, identifying and reporting issues requiring intervention, delivery of quality work on time & report any anticipated reasons for the delay, importance of team work, resolution of conflicts, multi-tasking, training the team members, knowledge of project management

Unit 4 – Clean work station **6 hours**

Cleaning the work area and equipments, materials and equipments required for cleaning, adequate ventilation for the work area, personal protective equipments, dealing with accidental damage, procuring and storing housekeeping equipment and supplies, disposal of wastes, maintain schedules and records for housekeeping

Unit 5 - Reporting and documentation in quality **8 hours**

Reporting – company procedures**,** escalation matrix for reporting identified issues - defects, problem, incidents, quality issues and test results, feedback to production manager and R&D staff. Documentation – procedures and good documentation practices, offline and online mode, accuracy, details, controlled document files and test records, regulatory and compliance requirements**,** inspection - procedures, protocols and checklists, inspection reports.

Unit 6 - Quality Assurance **10 hours**

Quality checks - quality assurance samples, master sample, internal controls, statistical analysis of test data, techniques and concepts of statistical quality control and statistical

process control, non-conformities. Operational aspects – calibration, accuracy checks of quality control equipments like stability chambers and BOD incubators, HPLC, gas chromatography, photoflourometer, etc., application softwares used in quality analysis