

PROTEIN EXPRESSION AND SCALE UP

Syllabus of the theory papers

BiSEP1: Lab to products: From DNA to Proteins

Total Hours: 52

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.

BiSEP2 : Recombinant proteins: Industrial perspective

Total Hours: 52

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 cerevisiae*, 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. Cerevisiae* 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 strategy 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
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BiSEP3: Fermentation process & Downstream processing

Total Hours: 52

Unit – I

(10 hours)

Design of a fermenter : 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

(6 hours)

Production of Fungal Proteases and other metabolites: 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

(8 hours)

Culture preservation, Production of enzymes with special references to proteases:
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

(14 hours)

Chromatographic techniques :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

(14 hours)

Electrophoretic techniques: 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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BiSEP 104 : Syllabus of the Elective theory papers

(Choose any one from the following)

BiSEP 104a : Production/ Manufacturing Biologist (LFS/Q2201)

Unit - I

10hours

Supervision of bio pharmaceutical production process

Supervise bio pharmaceutical production activities: Bio pharmaceutical production schedule and guidelines to production operators to handle production activities, Directions for junior biologists/ production operators - proper ingredients, temperatures, pressure and mixing times, etc.

Unit - II

10 hours

Documentation and Reporting

Documentation - Documentation of activities in the production process, Record of production output for each shift operation in the Batch Process.

Reporting – Following of approved guidelines of respective Drug Administration Body (MHRA, USFDA, CDSCO, etc.), Standard Operating Procedures and other statutory requirements, Reporting of breakdowns, Maintenance of GMP standards at shop floor and conditions suitable for production of quality products.

Unit - III

8 hours

Manage staff and inventory

Staff - Procedures to be followed in Managing staff details at production site. Role of production staff during audit

Inventory - Stock of raw materials and chemicals for production activities, Requirement and source materials as per daily production schedule, labeling, raw material conditions, batch no., shelf life and quantities, etc.

Unit - IV

8 hours

Maintain a healthy, safe and secure working environment in the life sciences facility

Self monitor and safety principles and standards, behavioural safety of workmen to current Good Manufacturing Practices (cGMP). Shop floor standards. Reporting of health issues, safety and security policies and procedures.

Managing emergency procedures: illness, accidents, fires, evacuation of worker/s during emergency.

Unit - V**8 hours****Coordination with Shift Supervisor**

Work instructions from reporting supervisor, Reporting to supervisor - process-flow improvements and production defects received from previous process, potential hazards or expected process disruptions, maintenance and repair schedule proactively, handover of completed work.

Unit – VI**Coordination within the team and with cross functional teams****8 hours**

Team player: Working with colleagues and sharing of work, work flow related difficulties. Interact with colleagues from cross functional teams: Feedback from Quality Control and Quality Assurance, Completion of work on time, support to Quality Assurance team during audits, coordination during breakdowns and for preventive and corrective maintenance, Coordination with Stores.

BiSEP104 b :Quality Control Biologist (LFS/Q2301)

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

Syllabus of the practical papers

BiSEP5:Lab1

1. Isolation of DNA from bacteria
2. *Isolation of total RNA*
3. *DNA ligation*
4. DNA fingerprinting by RAPD method
5. Restriction digestion of DNA
6. *In vitro* transcription
7. Designing of primers using software
8. Designing of SiRNA
9. Applications of BLAST, FASTA, Multiple sequence alignment in understanding genomics and proteomics
10. Isolation of mRNA by GTC method
11. Preparation of cDNA and agarose gel electrophoresis
12. Isolation of protein by gel chromatography
13. Purification of protein by HPLC
14. Mol. Wt. determination of proteins by SDS-PAGE
15. Isolation of splenocytes
16. Fusion of Splenocytes.

BiSEP6:Lab2

1. Bacmid vectors, pET vectors in bacteria, pcDNA mammalian vector – characteristic, yeast vectors, antibody markers using kit method.
2. Preparation of media for growing SF 21 cells
3. Expression of Fusion proteins (GST, HIS, FC –tag) in DH5 α cells.
4. Growing of mammalian cells – NIH 3T3, HEK 293, CHO.
5. Transformation in Prokaryotes
6. Transformation in mammalian cells
7. Identification of expressed proteins by Western blot.
8. Kinetics of cell growth, Sterilization of air and media, scale-up of microbial process.
9. Growth curve of microorganisms
10. Antibiotic sensitivity assay of microbes,
11. Production of protease in lab fermenter.
12. Estimation of protein by spectrophotometric method
13. Determination of the activity of enzyme protease
14. Estimation of protein by Lowry,s method
15. Estimation of protein by Bradford,s method