M. Sc. Microbiology Syllabus

M. Sc. Microbiology course of Two (2) years is divided into 4 semesters. Each semester is of 300 marks. Each semester (I II & III) will have 4 theory papers

### SYLLABUS AT A GLANCE

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*Semester IV practical (P-XV and P-XVI) or a research project of 50 marks.*
Unit – 1   Introduction to Biostatistics

Basic definitions and applications. Sampling: Representative sample, sample size, sampling bias and sampling techniques. Data collection and presentation: Types of data, methods of collection of Primary and Secondary data, methods of data presentation, Graphical representation by histogram, polygon, Ogive curves and pie diagram.

Unit – 2   Measures of central tendency

*Measures of central tendency: Mean, Median, Mode.*

Measures of variability: Standard deviation, standard error, range, mean deviation and coefficient of variation. Correlation and regression: Positive and negative correlation and calculation of Karl-Pearsons co-efficient of correlation. Linear regression and regression equation and multiple linear regression, ANOVA, one and two way classification.

Calculation of an unknown variable using regression equation.

Unit – 3   Tests of significance

Tests of significance: Small sample test (Chi-square t test, F test), large sample test (Z test) and standard error.

Introduction to probability theory and distributions, (concept without deviation) binomial, poison and normal (only definitions and problems)

Computer oriented statistical techniques. Frequency table of single discrete variable, bubble spot, computation of mean, variance and standard Deviations, t test, correlation coefficient
**Unit- 4 Introduction to computers and computer applications**

Introduction to computers: Computer application, basics, organization, PC, mainframes and Super-computers, concept of hardware and software, concept of file, folders and directories, commonly used commands, flow charts and programming techniques. Introduction to Q basic and C. Introduction in MS Office software concerning Word processing, spreadsheets and presentation software. www, introduction to internet, Medline and Pubmed for accessing biological information.

**Unit - 5 Research Methodology**

Research institutes, research schemes (minor and major), preparation of research scheme proposals, formats, funding agencies, scientific writing: research article, dissertation, review, abstract, synopsis, technical report.

Literature search, analysis of scientific report, compilation of data, presentation of experimental data, tabulation, graph, diagrams, histograms, interpretation of tables, graphs, photographs, and diagrams.

**PRACTICAL PAPER P-I**

**BIOSTATISTICS, COMPUTER APPLICATIONS AND RESEARCH METHODOLOGY**

Marks: 25

1. Representation of Statistical data by
   a) Histograms    b) Ogive Curves   c) Pie diagrams
2. Determination of Statistical averages/ central tendencies.
   a) Arithmetic mean   b) Median   c) Mode
3. Determination of measures of Dispersion
   a) Mean deviation
   b) Standard deviation and coefficient of variation
   c) Quartile deviation
4. Tests of Significance-Application of following
   a) Chi-Square test   b) t-test   c) Standard error
5. Computer operations-getting acquainted with different parts of Computers. [DOS] and basics of operating a computer.
7. Applications of computers in biology using MS-Office.
8. Creating an e-mail account, sending and receiving mails.
9. An introduction to INTERNET, search engines, websites, browsing and Downloading.
10. Searching research articles in Medline and Pubmed.
11. writing of abstracts, synopsis, research paper.
12. Presentation and analysis of experimental data.
13. oral presentation of research article.

References

3. Programming in C by E. Ballaguruswamy
7. Biostatistics - 7th Edition by Daniel
8. Fundamental of Biostatistics by Khan
11. INTERNET – CDC publication, India.
12. UGC, DST and DBT web-sites.
Unit – 1       Carbohydrate catabolic pathways and microbial growth on C1 Compounds

EMP, HMP, ED, Phosphoketolase pathway, TCA cycle, Glyoxylate bypass. Anaplerotic sequences, catabolism of different carbohydrates (Fructose, Lactose, Manose, Allose, Gluconate, Manitol, Sorbitol, Arabinose, Xylose), Polyol, glycol and 2,3 butanediol metabolism, regulation of aerobic and anaerobic carbohydrate metabolism,

Microbial growth on C1 Compounds (Cyanide, Methane, Methanol, methylated amines and carbon monoxide ) with reference to microorganisms and biochemical reactions with enzymes involved.

Unit - 2       Bacterial fermentations and Biosynthesis

Principal classes of carbohydrate fermentations. Carbon energy and balance. Alcohol, lactate, mixed acid, butyric acid, acetone-butanol, propionic acid, succinate, methane, and acetate, butanediol, acetoin fermentations. Fermentation of single nitrogenous compounds [amino acids] - alanine, glutamate and glycine with reference to microorganisms and biochemical reactions with enzymes involved.

Biosynthesis of amino acids (formation of glutamic acids, conversion of glutamic acid to glutamine, proline and arginine, formation of alanin, serinine, glycine and cysteine), Biosynthesis of nicotinic acid and pantothenic acid, biosynthesis of Purines and Pyrimidines.

Unit – 3       Endogenous metabolism and degradation of aliphatic and aromatic compounds.

Functions of endogenous metabolism, types of reserve materials, enzymatic synthesis, degradation and regulation of reserve materials - glycogen, polyphosphates and polyhydroxybutyrate (PHB), PHB production and its futuristic applications.
Microbial degradation of aliphatic hydrocarbons (microorganisms involved, mon-terrestrial, bi-terminal oxidation of propane, decane, etc.) and aromatic hydrocarbons and aromatic compounds (via catechol, protocatechuate, meta-cleavage of catechol and protocatechuate, dissimilation of catechol and protocatechuate, homogentisate and other related pathways).

**Unit – 4 Properties of Enzymes**

Classification of enzymes into six major groups with suitable examples. Numerical classification of enzymes. Different structural conformations of enzyme proteins (Primary, secondary, tertiary and quaternary structures). Forces that maintain protein structures. Sources of enzyme. Enzymes as biocatalysts, catalytic power, activation energy, substrate specificity, active site, theories of mechanisms of enzyme action (Induced fit and lock and key). Mechanism of action of lysozyme, chymotrypsin and ribonuclease.

Monomeric, Oligomeric and multienzyme complex, isozymes and allosteric enzymes. Extremozymes - thermostable, solventogenic and non-aqueous enzymes. Synthetic enzymes, Ribozymes and abzymes

**Unit – 5 Enzyme kinetics**

Importance of enzyme kinetics, factors affecting rates of enzyme mediated reactions (pH, temperature, substrate concentration, enzyme concentration and reaction time). Derivation of Michaelis - Menton equation and its significance in enzyme kinetic studies. Lineweaver-Burke plot, Haldane-Briggs relationship, sigmoidal kinetics steady state kinetics and transient phases of enzyme reaction.

**Practical Paper P-II : BIOENERGETICS AND ENZYMEOLOGY**

**Marks 25**

1. Isolation and Identification of Reserve food material (Glycogen / polyphosphates, PHB) of B. megaterium and Azotobacter SP.
2. Quantitative estimation of amino acids by Rosen’s method.
4. Demonstration of endogenous metabolism in B megaterium or E. coli and their survival under starvation conditions


6. Production of fungal alpha amylase using solid-state fermentation / production of protease by bacterial species and confirmation by determining the achromic point.

7. Purification of fungal alpha-amylase or bacterial protease by fractionation, chromatographic techniques and electrophoretic separation.

8. Studies on enzyme kinetics of alpha amylase / Protease [Optimization of parameters viz. Substrate, enzyme concentration, reaction temperature, reaction pH, Km, Vmax and metal ions as activators and inhibitors].

9. Bacterial fermentation (Detection of Acetic acid, lactic acid 2,3 butanediol and acetoin)

References

1. Understanding Enzymes by Trevor Palmer
5. Laboratory techniques in Biochemistry and Molecular Biology by Work and Work.
9. Biochemistry by Chatwal
10. Methods in Enzymology by Drolittle
11. Biochemistry by Garrett
14. Methods of Biochemical Analysis by David Glick, John Wiley and Sons, New
York.

16. Bacterial metabolism 2nd edition by H. W. Doelle

**PAPER TH-III  BIOINSTRUMENTATION TECHNIQUES AND APPLICATIONS**

**Marks 50**

**Unit –1  Basic laboratory Instruments**
Principle and working of pH meter, Laminar-air flow. Biosafety cabinets
Centrifugation: Types of centrifuge machines, preparative and analytical centrifuges, differential centrifugation, sedimentation velocity, sedimentation equilibrium, density gradient methods and their applications. Introduction to PCR, Gel documentation and water purification systems.

**Unit – 2  Chromatographic techniques**
Theory, principles and applications of paper, thin layer, gel filtration, ion-exchange, affinity, hydrophobic, gas liquid, high pressure/ performance liquid chromatography (HPLC)

**Unit – 3  Electrophoretic techniques**
Basic principles of electrophoresis, theory and application of paper, starch gel, agarose, native and denaturing PAGE, isoelectric focusing, capillary , microchip and 2 D electrophoresis.

**Unit – 4  Spectroscopy**
Unit - 5  Radioisotopic techniques
Use of radioisotopes in life sciences, radioactive labeling, principle and application of tracer techniques, detection and measurement of radioactivity using ionization chamber, proportional chamber, Geiger- Muller and Scintillation counters, autoradiography and its applications. Dosimetry.

Practical PAPER P-III BIOINSTRUMENTATION TECHNIQUES AND APPLICATIONS Marks 25

1. Studies on pH titration curves of amino acids/ acetic acid and determination of pKa values and Handerson-Hasselbach equation.
2. Separation of bacterial lipids/amino acids/sugars/organic acids by TLC or Paper Chromatography.
4. Study of UV absorption spectra of macromolecules (protein, nucleic acid, bacterial pigments).
6. Demonstration of PCR, DNA sequencer.
7. Separation of haemoglobin or blue dextran by gel filtration.
10. Density gradient centrifugation.

References
3. A Biologists Guide to Principles and Techniques of Practical Biochemistry. 1975 by Williams, B.L. and Wilson, K.

5. Gel Electrophoresis of Proteins- A Practical Approach by Hanes.


7. Analytical Biochemistry by Holme.


15. Principles and techniques of biochemistry and molecular biology by Keith Wilson and Walker.
Unit - 1  Industrial Food fermentations
Introduction, food fermentation, the science and technology.
Oriental fermented foods (Soya sauce, Natto, Miso), Cerel products, mixed preparations (Idle, Dhokala, Khamang, Papadam and Jilebies), Fermented cassaea flour, fermented pea nut milk, and grape based fermented products- wine (pre fermentating, fermentative and post fermentative practices, general methods of wine preparations), Fermented vegetables – Saurkraut, Fermented Meat – Sausages.

UNIT - 2  Industrial Dairy fermentations.
Taxonomy of lactic acid bacteria present in fermented products, Acid fermented milks (acidophilus milk, yoghurt). Slightly acid fermented milks (Cultured butter milk), Acid-alcoholic fermented milk (Kefir). Fermented milk production with extended self life (labneh).
Starter cultures for fermented dairy products (Strptococcus thermophilus, Lactobacillus bulgaricus,). Metabolism of starter cultures, biochemical changes in fermented milk (Fermentation of lactose to lactic acid, production of aromatic compounds, hydrolysis of proteins and lipids and Vit. B content).
Cheese- biological entities in cheese systems (Mlk, microorganisms, enzymes and other additives). Cheese production (Milk quality and composition, steps involed in mfg of cheese, preservation, classification and nutritional aspects)

Unit - 3  Advanced Food and dairy Microbiology
Genetically modified foods. Probiotic role of lactic acid bacteria and fermented milk products. Biosensors in food, Applications of microbial enzymes in food and dairy industry [Protease, Lipases], microbial anti oxidants, biosurfactants as emulsifiers, microbial polysaccharides as stabilizers and thinkers, flavors (esters, diaacetyl, pyrazines, lactones and terpenes, monosodium glutamate and microbial colors from molds). Production and application of Bakers Yeast, Tea, coffee and vinegar
fermentation

**Unit –4   Food preservation methods and utilization of dairy waste**

Food preservation by Radiations (UV, Gamma and microwave), Food preservation by low and high Temperature, chemicals and naturally occurring antimicrobials. 

Biosensors in food industry.

Utilization and disposal of dairy by-product - whey.

**Unit – 5   Food spoilage and Quality assurance**

Food borne infections and intoxications; bacterial with examples of infective and toxic types –, Clostridium, Salmonella, Shigella, Staphylococcus, Campylobacter, Listeria.

Mycotoxins in food (Types, structures, producer organism and its toxicity).

Quality assurance: Microbiological quality standards of food. Government regulatory practices and policies. FDA, EPA, HACCP, ISI.

**PRACTICAL**

**PAPER - P-IV   INDUSTRIAL FOOD AND DAIRY MICROBIOLOGY**

**Marks 25**

1. Production and estimation of lactic acid by Lactobacillus Sp. or Streptococcus Sp.
2. Extraction and estimation of diacetyl.
3. Sauerkraut fermentation
4. Isolation of food poisoning bacteria/ fungi from contaminated foods,
   a. Dairy products
5. Extraction and detection of afla toxin for infected foods.
6. Preservation of potato/onion by UV radiation
7. Production of fermented milk by Lactobacillus acidophilus.
8. Rapid analytical techniques in food quality control using microbial
   a. Biosensors.
References

1. Food Microbiology. 2nd Edition By Adams
2. Basic Food Microbiology by Banwart George J.
3. Food Microbiology: Fundamentals and Frontiers by Dolle
13. Microbial biotechnology- principles and applications- by Lee Yuan Kun
15. Applied dairy microbiology edited by Elmer Marth and James Steele.
Unit -1  Classification and Morphology of Viruses
Cataloging the virus through virus classification schemes of ICTV / ICNV. Morphology and ultra-structure of viruses. Virus related agents, viroids and prions.

Unit – 2  Cultivation and assay of viruses
Cultivation of viruses using embryonated eggs, experimental animals and cell cultures (Cell-lines, cell strains and transgenic systems). Purification of viruses by adsorption, precipitation, enzymes, serological methods – haeme agglutination and ELISA.
Assay of viruses – Physical and Chemical methods (Electron Microscopy and Protein and Nucleic acids studies.)
Infectivity Assays (Plaque and end-point)
Genetic analysis of viruses by classical genetic methods.

Unit – 3  Viral Multiplication
Mechanism of virus adsorption and entry into the host cell including genome replication and mRNA production by animal viruses, mechanism of RNA synthesis, mechanism of DNA synthesis, transcription mechanism and post transcriptional processing, translation of viral proteins, assembly, exit and maturation of progeny virions, multiplication of bacteriophages.

Unit – 4  Pathogenesis of Viruses
Host and virus factors involved in pathogenesis, patterns of infection, pathogenesis of animal viruses Adenovirus, Herpes virus, Hepatitis virus, Picorna virus, Poxvirus and Orthomyxovirus, pathogenesis of plant [TMV] and insect viruses [NPV]. Host cell transformation by viruses and oncogenesis of DNA and RNA viruses.
Unit – 5  Control of Viruses and Emerging Viruses

Control of viral infections through vaccines, interferons and chemotherapeutic agents.

Structure, genomic organization, pathogenesis and control of Human immunodeficiency virus. Emerging viruses

PRACTICAL

PAPER - P-V RECENT TRENDS IN VIROLOGY Marks 25

1. One step growth curve for determination of virus titre.
2. Phage typing of E.coli bacteriophages.
3. Induction of lambda lysogen by UV radiations.
4. Studies on Specialized transduction
5. Isolation of lambda DNA and their characterization.
6. Amplification of lambda DNA by PCR
7. Cultivation and assay of viruses using embryonated eggs and Tissue culture Technique.

References

5. Molecular Biology, Pathogenesis and Control by S.J. Flint and others. ASM Press, Washington, D.C.
Unit – 1 Immune System
Organs and cells involved in immune system and immune response. Lymphocytes, their subpopulation, their properties and functions, membrane bound receptors of lymph cells, helper T cells, T cells suppression, lymphocyte trafficking.

Unit – 2 Antigens and Immunoglobulins
Concept of haptens, determinants, conditions of antigenicity, antigens and immunogenecity, superantigen.

Unit – 3 Antigen – Antibody reactions
Antigen-Antibody reaction by precipitation, agglutination and complement fixation. Non-specific immune mechanism: - Surface defenses, tissue defenses, opsonization, inflammatory reaction, and hormone balance. Tissue metabolites with bactericidal properties (lysozyme, nuclein, histone, protamine, basic peptides of tissues – leukins, phagocytins, lecterins, haemocompounds)
Unit – 4  Expression and Regulation of Immune Response

Regulation of immune response: antigen processing and presentation, generation of humoral and cell mediated immune response, activation of B and T lymphocytes, cytokines and their role in immune regulation, T cell regulation, MHC restriction, immunological tolerance. Cell mediated cytotoxicity: Mechanism of T cells and NK mediated lysis, antibody dependent cell mediated cytotoxicity, and macrophage mediated cytotoxicity

Complement system: Classical, alternate, lectin pathway of complement activation.

Regulation of complement activation.

Transplantation immunology: MHC, types of grafts, grafts rejection, GVH reactions, mechanism of graft rejection, and prevention of graft rejection.

Unit - 5: Immunity and Immunoassays

Defense against bacteria, viruses, fungi and parasites. Immunodiagnostics and immunotherapy in virology – Serological methods for detection and quantitation of viruses including Hepatitis, Influenza, HIV and others.

Immuno-assays: SRID, ELISA, ELISA-PCR, RIA, Western Blotting, Immunofluorescens and their application. Immune deficiencies and autoimmunity.

PRACTICAL PAPER P-VI  MOLECULAR IMMUNOLOGY  Marks 25

1. Diagnostic immunologic principles and methods
   Precipitation method - Immunodiffusion
   - Immunelectrophoresis
   Agglutination method - Widal test
   - Haemagglutination
   - ELISA method

2. Separation of serum protein by submerged agarose gel electrophoresis.

3. Purification of human immunoglobulins from serum and confirmation of its antigenicity.

4. Identification of S.typhi by serotyping. [Purification of H and O antigens from
S. typhi

5. Clinical diagnosis of Rheumatoid arthritis by purifying immunoglobulins and albumins and confirmation by lattice agglutination test.


7. Demonstration of Western blotting.

8. Detection of isozymes of Lactate dehydrogenase by PAGE

9. Clinical diagnosis of viral diseases by PCR, ELISA.

References


5. Cellular and Molecular Immunology. 3rd Edition by Abbas.


Unit – 1  Bacterial photosynthesis
Photosynthetic microorganisms, photosynthetic pigments, and generation of reducing power by cyclic and non-cyclic photophosphorylation, electron transport chain in photosynthetic bacteria. Carbon dioxide fixation pathways.

Unit – 2  Bacterial Respiration
Bacterial aerobic respiration, components of electron transport chain, free energy changes and electron transport, oxidative phosphorylation and theories of ATP formation, inhibition of electron transport chain. Electron transport chain in some heterotrophic and chemolithotrophic bacteria.

Unit – 3  Bacterial Permeation
Structure and organization of membrane (Glyco-conjugants and proteins in membrane systems), fluid mosaic model of membrane. Methods to study diffusion of solutes in bacteria, passive diffusion, facilitated diffusion, different mechanisms of active diffusion (Proton Motive Force, PTS, role of permeases in transport, different permeases in E. coli. Transport of aminoacids and inorganic ions in microorganisms and their mechanisms.

Unit – 4  Bacterial Sporulation

Unit – 5  Bacterial Chemolithotrophy
Physiological groups of chemolithotrophs, ammonia oxidation by members of Genus

**Practical PAPER P-VII MICROBIAL PHYSIOLOGY Marks 25**

1. Isolation of Photosynthetic bacteria
2. Glucose uptake by *E. coli / Saccharomyces cerevisiae* [Active and Passive diffusion]
3. Effect of UV, gamma radiations, pH, disinfectants, chemicals and heavy metal ions on spore germination of Bacillus SP.
4. Determination of Iron Oxidation Rate of *Thiobacillus ferrooxidans*.
5. Determination of Sulfur Oxidation Rate of *Thiobacillus thiooxidans*.
6. Microbial degradation, decolorization and adsorption of organic dyes (by free and immobilized cells).
7. Estimation of calcium ions present in sporulating bacteria by EDTA method.
8. Demonstration of utilization of sugars by oxidation and fermentation techniques.

**References**

5. Applied Microbial Physiology by Rhodes.
8. Microbial Physiology by Benjamin
Unit - 1  **Biodiversity**
Introduction to microbial biodiversity – distribution, abundance, ecological niche. Types- Bacterial, Archael and Eucaryal.

**Unit – 2  Characteristics and classification of Archaebacteria.**
Thermophiles: Classification, hyperthermophilic habitats and ecological aspects. Extremely Thermophilic Archaebacteria, Thermophily, commercial aspects of thermophiles. Applications of thermozymes.
Methanogens: Classification, Habitats, applications.

**Unit – 3  Alkalophiles and Acidophiles**
Classification, alkaline environment, soda lakes and deserts, calcium alkalophily. Applications.
Acidophiles: Classification, life at low pH, acidotolerance, applications.

**Unit – 4  Halophiles and Barophiles**
Classification, Dead Sea, discovery basin, cell walls and membranes – Purple membrane, compatible solutes. Osmoadaptation / halotolerance. Applications of halophiles and their extremozymes.
Barophiles: Classification, high-pressure habitats, life under pressure, barophily, death under pressure.

**Unit – 5  Space Microbiology**
Antartica as a model for Mars. Search for life on Mars, Viking mission, Viking
landers, and Biology box experiment. Gas exchange, Label release and pyrolytic release experiments. Monitoring of astronauts' microbial flora: Alterations in the load of medically important microorganisms, changes in mycological autoflora, and changes in bacterial autoflora.

**Practical PAPER- P-VIII**  
**MICROBIAL DIVERSITY AND EXTREMOPHILES**  

**Marks 25**

1. Isolation of thermophiles from hot water spring [Study at least one enzyme].
2. Studies on halophiles isolated from seawater. [Pigmentation and Salt tolerance]
3. Studies on alkalophiles isolated from lonar water/sea water. [Study at least one enzyme]
4. Biogenic methane production using different wastes.
5. Isolation of *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* cultures from metal sulfides, rock coal and acid mine waters.

**Reference**

8. Microbiology: Dynamics and Diversity by Perry.


Semester III

PAPER TH-IX  ENZYME TECHNOLOGY   Marks 50

Unit – 1  Extraction and purification of microbial enzymes

Unit - 2  Enzyme inhibition and Co-factors
Irreversible, reversible, competitive, non-competitive and un-competitive inhibition with suitable examples and their kinetic studies.
Allosteric inhibition, types of allosteric inhibition and their significance in metabolic regulation & their kinetic study Vitamins and their co-enzymes: structure and functions with suitable examples Metalloenzymes and Metal ions as co-factors and enzyme activators.
Unit - 3  **Immobilization of microbial enzymes**

Unit – 4  **Enzyme Engineering**
Chemical modification and site-directed mutagenesis to study the structure-function relationship of industrially important enzymes.

Unit – 5  **Applications of microbial enzymes**
Microbial enzymes in textile, leather, wood industries and detergents. Enzymes in clinical diagnostics.
Enzyme sensors for clinical processes and environmental analyses. Enzymes as therapeutic agents.

**PRACTICAL**
**PAPER -P-IX ENZYME TECHNOLOGY Marks 25**

1. Microbial production, Extraction, purification and Confirmation of alpha amylase/ Lipase
2. Determination of efficiency of enzyme purification by measuring specific activity at various stages viz. Salt precipitation, dialysis, electrophoresis etc.
3. Studies on enzyme Activation and Inhibition of extracted alpha amylase/Lipase. Effect of Heavy metal ions, Chelating agents activators and inhibitors
4. Immobilization of cells and enzyme using Sodium alginate and egg albumin and measurement of enzyme activity [amylase/ Lipase]
5. Studies on impact of immobilization on enzyme activity in terms of Temperature tolerance and Vmax and Km using various forms Of alpha amylase/Lipase
6. Determination of molecular weight of enzymes using PAGE technique.

7. Preparation of biosensors of urease and determination of its activity.

References


9. Topics in Enzyme and Fermentation Biotechnology by L.N. Wiseman, John Wiley and Sons.
Unit–1  Bioreactors
Design of a basic fermenter, bioreactor configuration, design features, individual parts, baffles, impellers, foam separators, sparger, culture vessel, cooling and heating devices, probes for on-line monitoring, computer control of fermentation process, measurement and control of process.
Reactors for specialized applications: Tube reactors, packed bed reactors, fluidized bed reactors, cyclone reactors, trickle flow reactors, their basic construction and types for distribution of gases.

Unit – 2  Mass transfer in reactors
Transport phenomena in fermentation: Gas- liquid exchange and mass transfer, oxygen transfer, critical oxygen concentration, determination of Kla, heat transfer, aeration/agitation, its importance.
Sterilization of Bioreactors, nutrients, air supply, products and effluents, process variables and control, scale-up of bioreactors.

Unit – 3  Fermentation process
Growth of cultures in the fermenter Importance of media in fermentation, media formulation and modification.
Kinetics of growth in batch culture, continuous culture with respect to substrate utilization, specific growth rate, steady state in a chemostat, fed-batch fermentation, yield of biomass, product, calculation for productivity, substrate utilization kinetics.
Fermentation process: Inoculum development. Storage of cultures for repeated fermentations, scaling up of process form shake flask to industrial fermentation.

Unit – 4  Down stream processing
Biomass separation by centrifugation, filtration, flocculation and other recent developments.
Cell disintegration: Physical, chemical and enzymatic methods.
Extraction: Solvent, two phase, liquid extraction, whole broth, aqueous multiphase extraction. Purification by different methods.
Concentration by precipitation, ultra-filtration, reverse osmosis.
Drying and crystallization.

Unit - 5  Microbial strain improvement
Isolation, selection and improvement of microbial cultures: Screening and isolation of microorganisms, primary and secondary metabolites, enrichment, specific screening for the desired product.
Strain improvement for the selected organism: mutation and screening of improved cultures, random and strategic screening methods, strategies of strain improvement for primary, secondary metabolites with relevant examples. Use of recombinant DNA technology, protoplast fusion techniques for strain improvement of primary and secondary metabolites.
Production of recombinant molecules in heterologous system, problems associated with strain improvement programme, improvement of characters other than products and its application in the industry.
Preservation of cultures after strain improvement programme.

Practical Paper - P-X : Bioprocess Engineering and Technology

Marks 25

1. Isolation of industrially important microorganisms for microbial processes (citric / lactic/ alpha amylase) and improvement of strain for increase yield by mutation.
2. Determination of Thermal Death Point (TDP) and Thermal Death Time (TDT) of microorganisms for design of a sterilizer.
3. [a] Determination of growth curve of a supplied microorganism and also determines substrate degradation profile.
   [b] Compute specific growth rate (m), growth yield (Y x/s) from the above.
4. Extraction of Citric acid/Lactic acid by salt precipitation.
5. Monitoring of dissolved oxygen during aerobic fermentation.
6. Preservation of industrially important bacteria by lyophilization.
7. Product concentration by vacuum concentrator

References
2. Fermentations - A practical approach. IRL.
5. Biotechnology - A Text Book of Industrial Microbiology by Cruger.
6. Fermentation Biotechnology: Industrial Perspectives by Chand.
12. Applied Microbiology Series.
13. Industrial Microbiology by L.E. Casida, Wiley Eastern
17. Bioreaction Engineering Principles by Nielsen, J. and Villadsen, plenum Press, N.Y.
Unit – 1    DNA Structure and Mutagenesis

Historical developments in genetics, discovery of DNA and experimental evidence, Structure of Circular DNA molecule, Primary, Secondary, Tertiary and Quaternary structure of DNA, Watson and Crick model of double stranded DNA the law of DNA constancy and C value paradox and topological manipulations.

DNA replication: DNA replication mechanism, enzymes involved in DNA replication and models of DNA replication.

Molecular basis of spontaneous and induced mutations [physical and chemical mutagenic agents], types of mutation: point, frameshift, lethal, conditional lethal, inversion and deletion, null mutation, reversion of mutations, intra and intergenic suppression mutations. Environmental mutagenesis, toxicity testing and population genetics.

Systems that safeguard DNA. DNA methylation and DNA repair mechanisms - excision, mis-match, SOS, photoreactivation, recombination repair and glycycylase system.

Unit – 2    Prokaryotic Transcription and Translation

Organization of transcriptional units and regulation of gene expression Mechanism of transcription of prokaryotes-Structure and function of RNA polymerase, [DNA footprinting], termination and antitermination – N proteins and nut sites in DNA binding proteins, enhancer sequences and control of transcription, RNA processing (Capping, polyadenylation, splicing, introns and exons) Ribonucleoprotein, structure of mRNA, rRNA, tRNA. Direction of protein synthesis, RNA template, direction with experimental proof, tRNA as adaptor, ribosomes and their organization in prokaryotes, polycistronic mRNA in bacteria, initiation of translation in bacteria, small sub-units, its accessory factors, SD sequence in bacteria, initiator tRNA, elongation of translation, translocation and termination mechanisms. Post-translational modification. Salient features of genetic code.
Unit – 3  Regulation of gene expression in prokaryotes
Operon concept, co-ordinated control of structural genes, stringent response, catabolite repression, instability of bacterial RNA, positive regulation in E.coli [Arabinose operon] and negative regulation in E.coli [lac operon], inducers and repressors, regulation by attenuation by trp operon.

Unit - 4  Genetic recombination
Genetic recombination processes: Role of rec proteins in homologous recombination.
Transposons – Insertion sequences and composite transposons, phages as transposons, replicative, non-replicative and conservative transposition. Mutations i.e. deletions, inversions and frame-shift due to transposition. Mechanism of transposition, controlling elements of maize – autonomous and non-autonomous elements. Types of transposons and their properties.

Unit – 5  Phage Genetics
1. Purification of chromosomal / plasmid DNA and study of DNA profile:
   * Confirmation of nucleic acid by spectral study.
   * Quantitative estimation by diphenylamine test.
   * DNA denaturation and determination of Tm and G+C content.
   * Agarose gel electrophoresis of DNA.

2. Effect of UV radiations to study the survival pattern of E. coli/yeast. Repair mechanisms in E. coli/yeast (Dark and photoreactivation)

3. Isolation of antibiotic resistant mutants by chemical mutagenesis.


5. Extraction and Purification of RNA from S. cerevisiae.

6. Studies on gene expression in E.coli with reference to lac operon.

7. Study of conjugation in E. coli.

8. Restriction digestion and agarose gel electrophoresis of DNA.


References

12. Recombinant DNA by Watson, J.D.

PAPER TH-XII: ENVIRONMENTAL MICROBIAL TECHNOLOGY

Marks 50

Unit – 1 Environment and Ecosystems

Unit – 2 Eutrophication

Unit – 3 Effluent treatment techniques
Microbiology of wastewater and solid waste treatment: Types of solid and liquid waste characterization, physical, chemical, biological, aerobic, anaerobic, primary, secondary and tertiary treatments.
Anaerobic processes: Anaerobic digestion, anaerobic filters, and upflow anaerobic sludge. Treatment schemes for effluents of dairy, distillery, tannery, sugar and antibiotic industries (Types, microbes used, types of Effluent Treatment Plants). Bioconversion of Solid Waste and utilization as fertilizer. Bioaccumulation of heavy metal ions from industrial effluents.

Unit – 4  Bioremediation of Xenobiotics
Microbiology of degradation of xenobiotics in the environment, ecological considerations, decay behaviour, biomagnification and degradative plasmids, hydrocarbons, substituted hydrocarbons, oil pollution, surfactants and pesticides. Genetically Modified Organisms released and its environmental impact assessment and ethical issues.

Unit – 5  Global environmental problems
Ozone depletion, UV-B, green house effect and acid rain, their impact and biotechnological approaches for management. Containment of acid mine drainage applying biomining [with reference to copper extraction from low grade ores].

PRACTICAL
PAPER - P-XII  ENVIRONMENTAL MICROBIAL TECHNOLOGY

Marks 25

1. Physical analysis of sewage/industrial effluent by measuring total solids, total dissolved solids and total suspended solids.
2. Determination of indices of pollution by measuring BOD/COD of different effluents.
3. Bacterial reduction of nitrate from ground waters
4. Isolation and purification of degradative plasmid of microbes growing in polluted environments.
5. Recovery of toxic metal ions of an industrial effluent by immobilized cells.
6. Utilization of microbial consortium for the treatment of solid waste
7. Biotransformation of toxic chromium (+ 6) into non-toxic (+ 3) by Pseudomonas species.
8. Tests for the microbial degradation products of aromatic hydrocarbons /aromatic compounds
9. Reduction of distillery spent wash (or any other industrial effluent) BOD by bacterial cultures.
10. Microbial dye decolourization/adsorption.

References
Unit - 1  Techniques and enzymes in genetic recombination
Core techniques and essential enzymes used in recombination: restriction endonucleases, type I, II, III, recognition sequences, properties, nomenclature, classification of type II endonucleases, their activity. DNA ligase: Properties and specificity, S1 nuclease, BAL 31 nuclease, DNA polymerase, polynucleotide kinase, phosphatase, reverse transcriptase its activity and mode of action. Chemical synthesis of DNA. Restriction digestion, ligation and transformation.

Unit - 2  Plasmons
Properties, incompatibility, isolation and purification techniques, plasmid vectors and their properties, PBR 322 – its construction and derivatives, single stranded plasmids, promoter probe vectors, runaway plasmid vectors.
Bacteriophage lambda (λ) as a vector: Essential features, organization of λ genome, general structure, rationale for vector construction, improved λ vectors, λ gt series, λ EMBL vectors, invitro packaging, cosmids, phasmids, filamentous phage vectors, λ zap, λ blue print vectors.

Unit- 3  Specialized cloning strategies
Expression vectors, promoter probe vectors, vectors for library construction, genomic DNA libraries, chromosome walking and jumping, cDNA libraries, short gun cloning, directed cloning, phage display. Recombinant DNA technology with reference to cloning and production of interferon and insulin. Miscellaneous applications of Genetically engineered micro organisms (GEMS) / genetically modified organisms (GMO’s).

Unit - 4  PCR methods and Applications
PCR methods and Applications DNA sequencing methods, Dideoxy and Chemical method. Sequence assembly. Automated sequencing.

**Unit - 5  Molecular mapping of genome**

Genetic and physical maps, physical mapping and map -based cloning, choice of mapping population, simple sequence repeat loci, southern and fluorescence in situ hybridization for genome analysis, Chromosome microdissection and microcloning, molecular markers in genome analysis: RFLP, RAPD and AFLP analysis, molecular markers linked to disease resistance genes, Application of RFLP in forensic, disease prognosis, genetic counseling, pedigree, varietal etc. animal trafficking and poaching: Germplasm maintenance, taxonomy and Biodiversity.

**PRACTICAL**

**Paper - P-XIII  RECOMBINANT DNA TECHNOLOGY  Marks 25**

1. Isolation of genomic DNA and its confirmation by southern blotting.
2. Isolation of plasmid DNA and its restriction digestion.
3. DNA sequencing by Sangers method / or other method.
4. DNA cloning using plasmid vectors and expression vectors.
5. RFLP analysis.
6. Isolation of poly-A + RNA
7. Amplification of DNA by PCR.

**References**

10. From genes to clones by Winnaker.
11. Manipulations and expression of recombinant DNA by Robertson.

PAPER TH-XIV: FERMENTATION TECHNOLOGY Marks 50

Unit – 1 Microbial Fermentations
Metabolic pathways and metabolic control mechanisms, industrial production of citric acid, lactic acid, enzymes (alpha-amylase, lipase, xylase, pectinases, proteases), acetone-butanol, lysine and glutamic acid.

Unit – 2 Microbial production of therapeutic compounds
Microbial production of therapeutic compounds (β lactam, aminoglycosides, Ansamycins (Rifamycin), peptide antibiotics Quinolinones), biotransformation of steroids, vitamin B12 and riboflavin fermentation.

Unit – 3 Modern trends in microbial production
Modern trends in microbial production of bioplastics (PHB, PHA), bioinsecticides (thuricide), biopolymer (dextran, alginate, xanthan, pullulan), Biofertilizers
(nitrogen fixer Azotobacter, Phosphate solubilizing microorganisms), Single Cell Protein and production of biological weapons with reference to anthrax.

**Unit – 4  Biofuels**

Useful features of bio-fuels. The substrate digester and the microorganisms in the process of biogas production (biomethanation). Production of bioethanol from sugar, molasses, starch and cellulosic materials. Ethanol recovery. Microbial production of hydrogen gas, biodiesel from hydrocarbons.

**Unit – 5  Immobilization techniques, IPR and Patents**

Some industrial techniques for whole cell and enzyme immobilization. Application and advantages of cell and enzyme immobilization in pharmaceutical, food and fine chemical industries.

Intellectual Property Rights (IPR), Patents, Trademarks, Copyrights, Secrets, Patenting of biological materials, international co operation, obligations with patent applications, implication of patenting, current issues, hybridoma technology etc. Patenting of higher plants and animals, transgenic organisms and isolated genes, patenting of genes and DNA sequences, plant breeders right and farmers rights.

**PRACTICAL PAPER P-XIV  FERMENTATION TECHNOLOGY  Marks 25**

1. Production and characterization of citric acid using A. Niger.
2. Microbial production of glutamic acid.
3. Production of rifamycin using Nocardia strain.
4. Comparison of ethanol production using various Organic wastes /raw Material [Free cells/ immobilized cells].
5. Production and extraction of thuricide.
6. Laboratory scale production of biofertilizers [Nitrogen fixer/Phosphate Solubilizers/siderophore producers].
7. Microbial production of dextran by Leuconostoc mesenteroides
8. Microbial production of hydrogen gas by algae/bacteria
References

2. Industrial Microbiology by G. Reed (Ed), CBS Publishers (AVI Publishing Co.)
7. Annual Review of Microbiology by Charles E. Cliffton (Volumes)

PAPER TH-XV  BIOINFORMATICS, MICROBIAL GENOMICS AND PROTEOMICS.

Marks 50

Unit – 1  Bioinformatics and its applications

Unit – 2  Whole genome analysis
Preparation of ordered cosmid libraries, bacterial artificial chromosomal libraries, shotgun libraries and sequencing, conventional sequencing (Sanger, Maxam and Gilbert Methods), automated sequencing.
UNIT - III  **Sequence analysis**
Computational methods, homology algorithms (BLAST) for proteins and nucleic acids, open reading frames, annotations of genes, conserved protein motifs related structure / function (PROSITE, PFAM, Profile Scan). DNA analyses for repeats (Direct and inverted), palindromes, folding programmes. Use of Internet, public domain databases for nucleic acid and protein sequences (EMBL, GeneBank), database for protein structure (PDB).

UNIT - IV  **DNA Microarray**
Printing or oligonucleotides and PCR products on glass slides, nitrocellulose paper. Whole genome analysis for Global patterns of gene expression using fluorescent-labelled cDNA or end labelled RNA probes. Analyses of single nucleotide polymorphism using DNA chips.

UNIT - V  **Proteome analysis**
Two dimensional separation of total cellular proteins, isolation and sequence analysis of individual protein spots by Mass Spectroscopy. Protein microarray advantages and disadvantages of DNA and protein microarrays

PRACTICAL PAPER-P-XV BIOINFORMATICS, MICROBIAL GENOMICS & PROTEOMICS.  
M**arks  25**
Use of Internet/software for sequence analysis of nucleotides and proteins.
1. Studies of public domain databases for nucleic acid and protein sequences.
2. Determination of protein structure (PDB)
3. Genome sequence analysis
References
1. Bioinformatics. 1998 by Baxevanis
2. Bioinformatics 2000 by Higgins and Taylor OUP.
6. DNA microarrays: A practical approach edited by Mark Schena (OUP)
9. Bioinformatics - from Genomes to drug. 2 volumes by Lenganer.
12. Introduction to Bioinformatics by Altwood.
16. Protein Engineering: Principles and Practice by Cleland.
18. Web sites for Proteomics and Genomics
Unit – 1  
**Antibiotics and synthetic antimicrobial agents**

Antibiotics and synthetic antimicrobial agents
(Aminoglycosides, β-lactams, tetracyclines, ansamycins, macrolid antibiotics)
Antifungal antibiotics, antitumor substances.
Peptide antibiotics, Chloramphenicol, Sulphonamides and Quinolinone antimicrobial agents.
Chemical disinfectants, antiseptics and preservatives.

Unit – 2  
**Mechanism of action of antibiotics**

Mechanism of action of antibiotics (inhibitors of cell wall synthesis, nucleic acid and protein synthesis).
Molecular principles of drug targeting.
Drug delivery system in gene therapy
Bacterial resistance to antibiotics.
Mode of action of bacterial killing by quinolinones.
Bacterial resistance to quinolinones.
Mode of action of non-antibiotic antimicrobial agents.
Penetrating defenses – How the antimicrobial agents reach the targets (cellular permeability barrier, cellular transport system and drug diffusion).

Unit – 3  
**Microbial production and Spoilage of pharmaceutical Products**

Microbial contamination and spoilage of pharmaceutical products (sterile injectibles, non injectibles, ophthalmic preparations and implants) and their sterilization.
Manufacturing procedures and in process control of pharmaceuticals.
Other pharmaceuticals produced by microbial fermentations (streptokinase, streptodornase).
New vaccine technology, DNA vaccines, synthetic peptide vaccines, multivalent subunit vaccines. Vaccine clinical trials.

Unit – 4  Regulatory practices, biosensors and applications in Pharmaceuticals

Financing R&D capital and market outlook. IP, BP, USP.
Government regulatory practices and policies, FDA perspective.
Reimbursement of drugs and biologicals, legislative perspective.
Rational drug design.
Immobilization procedures for pharmaceutical applications (liposomes).
Macromolecular, cellular and synthetic drug carriers.
Biosensors in pharmaceuticals.
Application of microbial enzymes in pharmaceuticals.

Unit – 5: Quality Assurance and Validation

Good Manufacturing Practices (GMP) and Good Laboratory Practices (GLP) in pharmaceutical industry.
Regulatory aspects of quality control.
Sterilization control and sterility testing (heat sterilization, D value, z value, survival curve, Radiation, gaseous and filter sterilization)
Chemical and biological indicators.
Design and layout of sterile product manufacturing unit.
(Designing of Microbiology laboratory)
Safety in microbiology laboratory.
1. Spectrophotometric / Microbiological methods for the determination of Griesofulvin.

2. Bioassay of chloremphenicol by plate assay method or turbidiometric Assay method.

3. Treatment of bacterial cells with cetrimide, phenol and detection of Leaky substances such as potassium ions, aminoacids, purines, Pyrimidines and pentoses due to cytoplasmic membrane damage.

4. To determine MIC, LD₅₀ of Beta-lactum/aminoglycoside/ tetracycline/ansamycins.

5. Sterility testing by Bacillus stearothermophilus

6. Sampling of pharmaceuticals for microbial contamination and load (syrups, suspensions, creams and ointments, ophthalmic preparations).

7. Determination of D value, Z value for heat sterilization in pharmaceuticals.

8. Determination of antimicrobial activity of a chemical compound (Phenol, resorcinol, thymol, formaldehyde) to that of phenol under Standardized experimental conditions.

References


10. Quality Assurance in Microbiology by Rajesh Bhatia, Rattan Ial


1. Bacterial viruses:
   a) One-step growth experiment, single burst and premature lysis experiments.
   b) Productive cycle of lambda and phi X174 virus.
   c) Lysogeny: brief details on P2, P22, P1, and Mu1 phages.
   d) RNA phages.
   e) Isolation and cultivation of bacteriophages.

Shivaji University, Kolhapur Syllabus for M.Sc. T. & D (Microbiology) - Part II. MIC T/D - 09: Biostatistics, Bioinformatics and Scientific Writing.