

## 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

<b>PAPER NO.</b>	<b>TITLE OF THE THEORY PAPER</b>	<b>MARKS</b>	<b>PRACTICAL</b>	<b>MARKS</b>
<b>Semester I</b>				
Th-I	Biostatistics, Computer Applications and research methodology	50	P-I	25
Th-II	Bioenergetics and Enzymology	50	P-II	25
Th-III	Bioinstrumentation techniques and applications	50	P-III	25
Th-IV	Industrial Food and Dairy Microbiology	50	P-IV	25
<b>Semester II</b>				
Th-V	Recent trends in Virology	50	P-V	25
Th-VI	Molecular Immunology	50	P-VI	25
Th-VII	Microbial Physiology	50	P-VII	25
Th-VIII	Microbial diversity and Extremophiles	50	P-VIII	25

<b>Semester III</b>				
Th-IX	Enzyme Technology	50	P-IX	25
Th-X	Bioprocess Engineering and Technology	50	P-X	25
Th-XI	Molecular microbial Genetics	50	P-XI	25
Th-XII	Environmental Microbial Technology	50	P-XII	25
<b>Semester IV</b>				
Th-XIII	Recombinant DNA Technology	50	P-XIII	25
Th-XIV	Fermentation Technology	50	P-XIV	25
Th-XV	Bioinformatics, Microbial Genomics and Proteomics	50	P-XV	25
Th-XVI	Pharmaceutical Microbiology	50	P-XVI	25

\*Semester IV practical (P-XV and P -XVI) or a research project of 50 marks.

**M. Sc. Microbiology [Semester I]**  
**PAPER TH-I**  
**BIostatistics, COMPUTER**  
**APPLICATIONS AND RESEARCH METHODOLOGY**

**Marks 50**

**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, poisson and normal (only definitions and problems)

Computer oriented statistical techniques. Frequency table of single discrete variable, bubble plot, 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.
6. Creating files, folders and directories.
7. Applications of computers in biology using MS-Office.
  - A] MS-Word
  - B] Excel
  - C] Power Point
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**

1. Statistics in biology, Vol. 1 by Bliss, C.I.K. (1967) Mc Graw Hill, NewYork.
2. Practical Statistics for experimental biologist by Wardlaw, A.C. (1985).
3. Programming in C by E. Ballaguruswamy
4. How Computers work - 2000. by Ron White. Tech. Media
5. How the Internet Work 2000 by Preston Gralla Tech. Media.
6. Statistical Methods in Biology - 2000 by Bailey, N.T. J. English Univ. Press.
7. Biostatistics - 7th Edition by Daniel
8. Fundamental of Biostatistics by Khan
9. Biostatistical Methods by Lachin.
10. Statisticsfor Biologist by CampbellR.C.(1974)Cambridge University Press , UK.
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, Mannitol, 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 alanine, serine, 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, non-terminal, 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 ENZYMOLOGY**

**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.
3. Quantitative estimation of sugars by Summner's method.

4. Demonstration of endogenous metabolism in *B megaterium* or *E. coli* and their survival under starvation conditions
5. Quantitative estimation of proteins by Folin-Lowry / Biuret method.
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,  $K_m$ ,  $V_{max}$  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
2. Enzyme Kinetics by Paul Engel. 1977. John Wiley and Sons. Inc., New York.
3. Enzymes by Dixon and Webb, 3rd Edition 1979. Academic Press, New York
4. Biochemistry by Stryer 5th Edition WH Freeman 2001
5. Laboratory techniques in Biochemistry and Molecular Biology by Work and Work.
6. Principles of Enzyme Kinetics. 1976. by Athel Cornish - Bowden.
7. Butterworth and Co.
8. Fundamentals of Enzymology. 3rd Edition by Price
9. Biochemistry by Chatwal
10. Methods in Enzymology by Drolittle
11. Biochemistry by Garrett
12. Principles of Biochemistry. 2nd Edition by Horton
13. Biochemistry by Voet.
14. Methods of Biochemical Analysis by David Glick, John Wiley and Sons, New





## **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.
3. Separation of serum protein by horizontal submerged gel electrophoresis.
4. Study of UV absorption spectra of macromolecules (protein, nucleic acid, bacterial pigments).
5. Quantitative estimation of hydrocarbons/pesticides/organic Solvents /methane by Gas chromatography.
6. Demonstration of PCR, DNA sequencer.
7. Separation of haemoglobin or blue dextran by gel filtration.
8. Paper electrophoresis.
9. Fricke dosimetry.
10. Density gradient centrifugation.

#### **References**

1. Instrumental Methods of Analysis. 6th Edition by H.H. Willard, L.L. Merritt Jr. and others. 1986. CBS Publishers and Distributors.
2. Instrumental Methods of Chemical Analysis. 1989 by Chatwal G and Anand, S. Himalaya Publishing House, Mumbai.
3. A Biologists Guide to Principles and Techniques of Practical Biochemistry. 1975 by Williams, B.L. and Wilson, K.

4. Spectroscopy. Volume 1. Edited by B.B. Straughan and S. Walker. Chapman and Hall Ltd.
5. Gel Electrophoresis of Proteins- A Practical Approach by Hanes.
6. Chromatography: Concepts and Contrasts- 1988 by James Miller. John Wiley and Sons. Inc., New York.
7. Analytical Biochemistry by Holme.
8. Introduction to High Performance Liquid Chromatography by R. J. Hamilton and P. A. Sewell.
9. Spectroscopy by B.P. Straughan and S. Walker.
10. Practical aspects of Gas Chromatography and Mass Spectrometry 1984 by Gordon M. Message, John Wiley and Sons, New York.
11. Gel Chromatography by Tibor Kremmery. Wiley Publications.
12. Isotopes and radiations in Biology by C.C. Thornburn, Butterworth and Co. Ltd., London.
13. The use of radioactive isotopes in the life sciences by J.M.Chapman and G.Ayrey, George Allen and Unwin Ltd., London.
14. Analytical biotechnology edited by Thomas G M Schalkhammer.
15. Principles and techniques of biochemistry and molecular biology by Keith Wilson and Walker.

## **PAPER TH-IV INDUSTRIAL FOOD AND DAIRY MICROBIOLOGY**

**Marks 50**

### **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 cassava flour, fermented pea nut milk, and grape based fermented products- wine (pre fermenting, 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 thickeners, 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 aflatoxin 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.
9. Production of Yoghurt.

## References

1. Food Microbiology. 2nd Edition By Adams
2. Basic Food Microbiology by Banwart George J.
3. Food Microbiology: Fundamentals and Frontiers by Dolle
4. Biotechnology: Food Fermentation Microbiology, Biochemistry and Technology. Volume 2 by Joshi.
5. Fundamentals of Dairy Microbiology by Prajapati.
6. Essentials of Food Microbiology. Edited by John Garbult. Arnold International Students Edition.
7. Microbiology of Fermented Foods. Volume I and II. By Brian J. Wood. Elsevier Applied Science Publication.
8. Microbiology of Foods by John C. Ayres. J. Orwin Mundt. William E. Sandinee. W. H. Freeman and Co.
9. Dairy Microbiology by Robinson. Volume II and I.
10. Food Microbiology: Fundamentals and Frontiers. 2nd Edition by Michael P. Doyle, Larry R. Beuchat and Thomas I. Montville (Eds.), ASM Publications.
11. Bacterial Pathogenesis A Molecular Approach. 2nd Edition. 2001 by Abigail A. Salyers and Dixie D. Whitt. ASM Publications.
12. Advances in Applied Microbiology by D. Pearlman, Academic Press.
13. Microbial biotechnology- principles and applications- by Lee Yuan Kun
14. Biotechnology Vol. III and V edited by H J Rehman and G Reed
15. Applied dairy microbiology edited by Elmer Marth and James Steele.

## SEMESTER II

PAPER TH-V

RECENT TRENDS IN VIROLOGY

Marks 50

### **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**

1. Medical Virology 10 Th Edition by Morag C and Tim bury M C 1994.. Churchill Livingstone, London.
2. Introduction to Modern Virology 4th Edition by Dimmock N J, Primrose S. B. 1994. Blackwell Scientific Publications. Oxford.
3. Virology 3 rd Edition by Conrat H.F., Kimball P.C. and Levy J.A. 1994. Prentice Hall, Englewood Cliff, New Jersey.
4. Text Book on Principles of Bacteriology, Virology and Immunology Topley and Wilsons 1995.
5. Molecular Biology, Pathogenesis and Control by S.J. Flint and others. ASM Press, Washington, D.C.



6. Applied Virology. 1984. Edited by Edonard Kurstak. Academic Press Inc.
7. Introduction to Modern Virology by Dimmock.
8. Prion diseases by Gaschup, M.H.
9. Clinical virology Manual by Steven, S., Adinka, R.L., Young, S.A.
10. Principles of Virology. 2000 by Edward Arnold .

**PAPER TH-VI**

**MOLECULAR IMMUNOLOGY**

**Marks 50**

**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 immunogenicity, superantigen.

Immunoglobulins: Structure and properties of immunoglobulin classes. Theories of antibody formation, hybridoma technology for monoclonal antibodies and designer monoclonal antibodies. Multiple myelomas and structural basis of antibody diversity. Freund's adjuvants and its significance.

**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
    - Immunoelectrophoresis
  - 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.
6. Estimation of Alkaline phosphatase from patient's serum.
7. Demonstration of Western blotting.
8. Detection of isozymes of Lactate dehydrogenase by PAGE
9. Clinical diagnosis of viral diseases by PCR, ELISA.

### **References**

1. Essentials of Immunology by Riott I .M. 1998. ELBS, Blackwell Scientific Publishers, London.
2. Immunology 2 nd Edition by Kuby J. 1994. W.H. Freeman and Co. New York.
3. . Immunology - Understanding of Immune System by Claus D. Elgert. 1996. Wiley - Liss, New York.
4. Fundamentals of Immunology by William Paul.
5. Cellular and Molecular Immunology. 3rd Edition by Abbas.
6. Immunobiology: The Immune System in Health and Disease. 3rd Edition by Travers.
7. Immunology- A short Course. 2 nd Edition by Benjamin.
8. Manual of Clinical Laboratory and Immunology 6th Edition. 2002 by Noel R. Rose, Chief Editor: Robert G. Hamilton and Barbara Detrick (Eds.) , ASM Publications.
9. Pocket Guide to Clinical Microbiology. 2 nd Edition. 1998 by Patrick R. Murray, ASM Publications.

**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.

Bacterial anaerobic respiration: Introduction. Nitrate, carbonate and sulfate as electron acceptors. Electron transport chains in some anaerobic bacteria. Catalase, super oxide dismutase, mechanism of oxygen toxicity.

**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**

Sporulating bacteria, molecular architecture of spores, induction and stages of sporulation, Influence of different factors on sporulation. Cytological and macromolecular changes during sporulation. Heat resistance and sporulation.

**Unit -5 Bacterial Chemolithotrophy**

Physiological groups of chemolithotrophs, ammonia oxidation by members of Genus

Nitroso group, nitrite oxidation by Nitro group of genera. Oxidation of molecular hydrogen by *Hydrogenomonas* species. Ferrous and sulfur/sulfide oxidation by *Thiobacillus* species.

**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**

1. Microbial Physiology and Metabolism by Caldwell D.R. 1995 Brown Publishers.
2. Microbial Physiology by Moat A.G. and Foster J. W. 1999.. Wiley.
3. . Prokaryotic Development by Brun. Y.V. and Shimkets L.J. 2000. ASM Press.
4. Advances in Microbial Physiology. Volumes. Edited by By A.H. Rose. Academic Press, New York.
5. Applied Microbial Physiology by Rhodes.
6. Biosynthesis by Smith.
7. The Bacteria. Volumes by I.C. Gunsalus and Rogery Stanier, Academic Press.
8. Microbial Physiology by Benjamin

**PAPER TH-VIII : MICROBIAL DIVERSITY AND  
EXTREMOPHILES**

**Marks 50**

**Unit - 1 Biodiversity**

Introduction to microbial biodiversity – distribution, abundance, ecological niche.  
Types- Bacterial, Archaeal and Eucaryal.

**Unit - 2 Characteristics and classification of Archaeobacteria.**

Thermophiles: Classification, hyperthermophilic habitats and ecological aspects.  
Extremely Thermophilic Archaeobacteria, Thermophily, commercial aspects of  
thermophiles. Applications of thermostzymes.

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**

Aims and objectives of Space research. Life detection methods a] Evidence of  
metabolism (Gulliver) b] Evidence of photosynthesis (autotrophic and  
heterotrophic) c] ATP production d] Phosphate uptake e] Sulphur uptake . Martian  
environment (atmosphere, climate and other details).

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**

1. Extremophiles by Johri B.N. 2000. Springer Verlag, New York
2. Microbial Diversity by Colwd, D. 1999, Academic Press.
3. Microbial Life in Extreme Environments. Edited by D. J. Kushner. Academic Press.
4. Microbiology of Extreme Environments. Edited by Clive Edward. Open University Press. Milton Keynes.
5. Microbiology of Extreme Environments and its potential for Biotechnology. Edited by M.S. Da Costa, J.C. Duarate, R.A. D. Williams. Elsevier Applied Science, London.
6. Extreme Environment. Mechanism of Microbial Adaptation. Edited by Milton R. Heinrich. Academic Press.
7. Thermophiles. General, Molecular and Applied Microbiology. Edited by Thomas D. Brock. Wiley Interscience Publication.
8. Microbiology: Dynamics and Diversity by Perry.
9. Microbial Ecology. Fundamentals and Applications by. Ronald M. Atlas and

- Richard Bartha. 2nd and 4th Edition. The Benjamin Cummins Publication Co. Inc.
10. Microbial Ecology. 2nd Edition. by R. Campbell. Blackwell Scientific Publication.
11. Brocks Biology of Microorganisms. 8th Edition. (International Edition - 1997) by Michael T. Madigan, John M. Martinko. Jack Parker. Prentice Hall International Inc.
12. Advances in Applied Microbiology. Vol. 10. Edited by Wayne W. Umbreit and D. Pearlman. Academic Press.

### **Semester III**

**PAPER TH-IX          ENZYME TECHNOLOGY          Marks 50**

#### **Unit - 1          Extraction and purification of microbial enzymes**

Importance of enzyme purification, different sources of enzymes. Extracellular and intracellular enzymes. Physical and Chemical methods used for cell disintegration. Enzyme fractionation by precipitation (using Temperature, salt, solvent, pH, etc.), liquid-liquid extraction, ionic exchange, gel chromatography, affinity chromatography and other special purification methods. Enzyme crystallization techniques. Criteria of purity of enzymes. Pitfalls in working with pure 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**

Methods viz. adsorption, covalent bonding, entrapment & membrane confinement and their analytical, therapeutic & industrial applications. Properties of immobilized 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  $V_{max}$  and  $K_m$  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**

1. Methods in Enzymology. Volume 22 - Enzyme purification and related techniques. Edited by William B. Jakoby. Academic Press, New York.
2. Allosteric Enzymes - Kinetic Behaviour. 1982. by B.I. Kurganov. John Wiley and Sons. Inc., New York.
3. Biotechnology. Volume 7 A - Enzymes in Biotechnology. 1983 Edited by H. J. Rehm and G. Reed. Verlag Chemie.
4. Hand Book of Enzyme Biotechnology by Wiseman.
5. Enzymes as Drugs Edited by John S.Holcenberg and Joseph Roberts , John Wiley & Sons New York.
6. Methods of Enzymatic Analysis by Hans Ulrich, Bergmeyer, Academic Press.
7. Methods in Enzymology by W.A. Wood, Academic Press.
8. Advances in Enzymology by Alton Meister, Interscience Publishers.
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  $K_L a$ , 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 ( $\mu$ ), 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
- 8 Cell disruption for endoenzymes by sonication.

### **References**

1. Principles of Fermentation Technology by Stanbury, P.F., Whitekar A. and Hall. 1995., Pergaman. McNeul and Harvey.
2. Fermentations - A practical approach. IRL.
3. Bioprocess Technology: Fundamentals and Applications. Stockholm KTH.
4. Biochemical Reactors by Atkinson B., Pion, Ltd. London.
5. Biotechnology - A Text Book of Industrial Microbiology by Cruger.
6. Fermentation Biotechnology: Industrial Perspectives by Chand.
7. Biochemical Engineering Fundamentals by Bailey and Ollis, Tata McGraw Hill, N.Y.
8. Biotechnology. Volume 3. Edited by H. J. Rehm and G. Reed. Verlag Chemie. 1983.
9. Advances in Biochemical Engineering by T.K. Bhosh, A.Fiechter and N. Blakebrough. Springer Verlag Publications, New York.
10. Biotechnology- A textbook of Industrial Microbiology by Creuger and Creuger, Sinaeur Associates.
11. Bioprocess Engineering Kinetics, Mass Transport, Reactors, and Gene expressions by Veith, W.F., John Wiley and Sons.
12. Applied Microbiology Series.
13. Industrial Microbiology by L.E. Casida, Wiley Eastern
14. Bioseparation: Down stream processing for Biotechnology by Belter, P.A. Cussler, E.L. and Hu, W.S., John Wiley and Sons, N.Y.
15. Separation process in Biotechnolgy by Asenjo, J.A. Eds. Marcel Dekkar, N.Y.
16. Bioprocess Engineering Principles by Doran, Acad. Press, London.
17. Bioreaction Engineering Principles by Nielsen, J. and Villadsen, plenum Press, N.Y.
18. Fermentation, Biocatalysis and bioseparation, Encyclopedia of Bioprocess Technology by Chisti, Y., Vol. 5, John Wiley and Sons, 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 glycosylase 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 foot printing], 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.

Conjugation: Discovery, F<sup>+</sup>, F<sup>-</sup> and Hfr cells, types of Hfr; F<sup>+</sup> and F<sup>-</sup> and Hfr and F<sup>-</sup> genetic crosses. Mechanism of conjugation. Sexduction, conjugational transfer of colicinogenic and resistance transfer factors. Genetic mapping. Plasmid Replication and Incompatibility, Control of copy number.

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**

T4 virulent phage: structure, life cycle, genetic map and DNA replication. Lambda temperate phage: Structure, genetic map, lytic and lysogenic cycle, lysogenic repression and phage immunity. [Lambda regulon] applications of phages in microbial genetics.

## PRACTICAL

PAPER - P-XI

MICROBIAL GENETICS

Marks 25

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  $T_m$  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.
4. Ampicillin selection method for isolation of auxotrophic mutant.
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.
9. Generalized transduction in E. coli using P1 phage.

### References

1. Microbial Genetics by Maloy ET. Al. 1994. Jones and Bartlett Publishers.
2. Molecular Genetics of Bacteria by J. W. Dale. 1994. John Wiley and Sons.
3. Modern Microbial Genetics. 1991 by Streips and Yasbin. Niley Ltd.
4. Molecular Biology of the Gene 4th Edition by J.D. Watson, N.H. Hopppkins, J.W. Roberts, J.A. Steitz and A.M. Weiner. 1987, The Benjamin / Cummings Publications Co. Inc. California.
5. Gene VII by Lewin Oxford University Press. 2000.
6. Bacterial and Bacteriophage Genetics. 4 th Editions by Birge.
7. Microbial Genetics by Frefielder. 4th Edition.
8. Organization of Prokaryotic Genome. 1999 by Robert L.Charlebois, ASM Publications.
9. DNA repair and mutagenesis. 1995 by Errol C. Friedberg, Graham C. Walker and Wolfram, Siede, ASM Publications.



10. Molecular Genetics of Bacteria, 1997 by Larry, Snyder and Wendy, Champness, ASM Publications.
11. Methods of General and Molecular Bacteriology, 1993. Edited by Philip. Gerhardt, ASM Publications.
12. Recombinant DNA by Watson, J.D.
13. Essentials of Molecular Biology by Malacimski.
14. Mobile DNA II by Nancy Craig, Martin Gellert Allan Lambowitz.

## **PAPER TH-XII : ENVIRONMENTAL MICROBIAL TECHNOLOGY**

**Marks 50**

### **Unit – 1 Environment and Ecosystems**

Definitions, biotic and abiotic environment. Environmental segments. Composition and structure of environment. Concept of biosphere, communities and ecosystems. Ecosystem characteristics, structure and function. Food chains, food webs and trophic structures. Ecological pyramids.

### **Unit – 2 Eutrophication**

Water pollution and its control: Need for water management. Sources of water pollution. Measurement of water pollution, Eutrophication: Definition, causes of eutrophication, and microbial changes in eutrophic bodies of water induced by various inorganic pollutants. Effects of eutrophication on the quality of water environment, factors influencing eutrophication. Qualitative characteristics and properties of eutrophic lakes. Measurement of degree of eutrophication. Algae in eutrophication, algal blooms, their effects and toxicity, coloured waters, red tides, and cultural eutrophication. Physico-chemical and biological measures to control eutrophication

### **Unit –3 Effluent treatment techniques**

Microbiology of wastewater and solid waste treatment: - Waste-types-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

[Municipal 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**

1. Bioremediation by Baker K.H. And Herson D.S. 1994.. MacGraw Hill Inc. N.Y.
2. Waste Water Engineering - Treatment, Disposal and Re-use by Metcalf and Eddy, Inc., Tata MacGraw Hill, New Delhi.
3. Pollution: Ecology and Biotreatment by Ec Eldowney, S. Hardman D.J. and Waite S. 1993. - Longman Scientific Technical.
4. Environmental Microbiology edited by Ralph Mitchell. A John Wiley and Sons. Inc.
5. Waste Water Microbiology 2nd Edition by Bitton.
6. Chemistry and Ecotoxicology of pollution. Edited by Des. W. Connell, G.J. Miller. Wiley Interscience Publications.
7. Environmental Biotechnology. Edited by C. F. Forster and D.A., John Wase. Ellis Horwood Ltd. Publication.
8. Advances in Waste Water Treatment Technologies. 1998. Volumes II and I by R. K. Trivedy. Global Science Publication.
9. Biocatalysis and Biodegradation: Microbial transformation of organic compounds. 2000 by Lawrence P. Wacekett, C. Douglas Hershberger. ASM Publications.
10. A Manual of Environmental Microbiology. 2nd Edition. 2001 by Christon J. Hurst (Chief Editor), ASM Publications.
11. Biodegradation and Bioremediation, Academic Press, San Diego.
12. Biotechnology in the sustainable environment, Plenum Press, N.Y.
13. Basic Principles of Geomicrobiology by A. D. Agate, Pune.

## **Semester IV**

### **PAPER TH - XIII RECOMBINANT DNA TECHNOLOGY**

**Marks 50**

#### **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 Plasmids**

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 ( $\lambda$ ) as a vector: Essential features, organization of  $\lambda$  genome, general structure, rationale for vector construction, improved  $\lambda$  vectors,  $\lambda$  gt series,  $\lambda$  EMBL vectors, invitro packaging, cosmids, phasmids, filamentous phage vectors,  $\lambda$  zap,  $\lambda$  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**

1. Principles of Gene Manipulations 1994 by Old and Primrose Blackwell Scientific Publications.
2. DNA Cloning: A Practical Approach by D.M. Glover and B.D. Hames, IRL Press, Oxford. 1995.
3. Molecular Biotechnology 2nd Edition by S.B. Primrose. Blackwell Scientific Publishers, Oxford. 1994.

4. Genetic Engineering and Introduction to Gene Analysis and Exploitation in Eukaryotes by S.M. Kingsman and A.J. Kingsman, Blackwell Scientific Publications, Oxford 1998.
5. PCR Technology - Principles and Applications for DNA Amplification by Henry A. Erlich (Ed.) Stockton Press. 1989.
6. Biotechnology: A Guide to Genetic Engineering by Peters.
7. Genetic Engineering – 2000 by Nicholl.
8. Recombinant DNA and Biotechnology: Guide for Teachers. 2nd Edition by Helen Kreuz. 2001.ASM Publications.
9. Molecular Biotechnology: Principles and Applications of Recombinant DNA. 2nd Edition. 1998 by Bernard R. Glick and Jack J. Pastemak, ASM Publications.
10. From genes to clones by Winnaker.
11. Manipulations and expression of recombinant DNA by Robertson.
12. Gene targeting – A practical approach by Joyner.

**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 ( $\beta$  lactam, aminoglycosides, Ansamycins (Rifamycin), peptide antibiotics Quinolinsones), 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

1. Biotechnological Innovations in Chemical Synthesis. BIOTOL. Publishers / Butterworth - Heinemann.
2. Industrial Microbiology by G. Reed (Ed), CBS Publishers (AVI Publishing Co.)
3. Biology of Industrial Microorganisms by A.L. Demain.
4. Genetics and Biotechnology of Industrial Microorganisms by C.I. Hershenberg, S.W. Queener and Q. Hegeman. Publisher. ASM. Ewens ET. Al. 1998. Bioremediation Principles. Mac Graw Hill.
5. Annual Reports in Fermentation Processes by D. Pearlman, Academic Press.
6. Fundamentals of Biochemical Engineering by Bailey and Ollis.
7. Annual Review of Microbiology by Charles E. Clifton (Volumes)
8. Biotechnology, A textbook of industrial Microbiology by Creuger and Creuger, Sinauer associates.
9. Manual of industrial Microbiology and Biotechnology 2<sup>nd</sup> edition by Davis J.E. and Demain A.L. ASM publications.

## **PAPER TH-XV BIOINFORMATICS, MICROBIAL GENOMICS AND PROTEOMICS.**

**Marks 50**

### **Unit - 1 Bioinformatics and its applications**

Databases, types, pairwise and multiple alignments. Structure-function relationship. Sequence assembling using computers. Computer applications in molecular biology, Protein domains and human genome analysis program (BLAST, FASTA, GCC etc.) Search and retrieval of biological information and databases sequence, databank. (PDB and gene bank), accessing information (Network expasy, EMB Net, ICGEB Net).

### **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.**

**Marks 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.
3. Nucleic acid Research 2001. Jan. Genome database issue.
4. The Internet and the new Biology: Tools for Genomics and Molecular Research by Peruski, Jr. and Peruske (ASM) 1997.
5. Functional Genomics. A Practical Approach Edited by Stephen P Hunt and Rick Liveey (OUP) 2000.
6. DNA microarrays: A practical approach edited by Mark Schena (OUP)
7. Bioinformatics - A Practical Guide to the Analysis of Genes and Proteins. 2nd Edition by Baxevanis.
8. Bioinformatics: Sequence, structure and Data Bank: A Practical Approach by Higgis.
9. Bioinformatics - from Genomes to drug. 2 volumes by Lenganer.
10. Bioinformatic Methods and Protocols - Misener.
11. Bioinformatics: Sequence and Genome analysis.
12. Introduction to Bioinformatics by Altwood.
13. Proteome Research: New Frontiers in Functional Genomics: Principles and Practices.
14. Genomics: The Science and Technology behind the human project.
15. Protein Biotechnology. Edited by Felix Franks. Humana Press, Totowa, New Jarsey.
16. Protein Engineering: Principles and Practice by Cleland.
17. Computer analysis of sequence data by Colte.
18. Web sites for Proteomics and Genomics  
Www.geneprot.com,      www.hybrigenis.com,      www.mdsproteomics.com,  
www.stromix.com, www.syrrx.com.

## PAPER TH -XVI PHARMACEUTICAL MICROBIOLOGY

Marks 50

### Unit - 1 Antibiotics and synthetic antimicrobial agents

Antibiotics and synthetic antimicrobial agents

(Aminoglycosides,  $\beta$  lactams, tetracyclines, ansamycins, macrolid antibiotics)

Antifungal antibiotics, antitumor substances.

Peptide antibiotics, Chloramphenicol, Sulphonamides and Quinolone 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 quionolinones.

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.

Quality assurance and quality management in pharmaceuticals ISO, WHO and US certification.

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.

## PRACTICAL

**PAPER P- XVI**

**PHARMACEUTICAL MICROBIOLOGY**

**Marks 25**

1. Spectrophotometric / Microbiological methods for the determination of Griesofulvin.
2. Bioassay of chloremphenicol by plate assay method or turbidimetric 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, LD<sub>50</sub> 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.

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