DEPARTMENT OF MICROBIOLOGY

M.Sc. DEGREE (SEMESTER) COURSE UNDER CBCS SCHEME

SCHEME OF TEACHING AND EXAMINATION

(Effective from the academic year 2023-24 and onwards)

PAPER	Teaching Hours/week	Examination Hours	Marks	IA	Credits
III SEMESTER:					
3.1 HC Recombinant DNA Technology	4	3	75	25	4
3.2 HC Immunology and Immunotechnology	4	3	75	25	4
3.3 SCTFood and Dairy Microbiology (A)	4	3	75	25	4
3.3 SCT Microbial Enzymology (B)	4	3	75	25	4
3.4 OE Microbes and Environment	2	2	35	15	2
3.5 Practical Based on paper 3.1	4	3	35	15	2
3.6 Practical Based on paper 3.2	4	3	35	15	2
3.7 Practical Based on paper 3.3 (A)	4	3	35	15	2
3.8 Practical Based on paper 3.3 (B)	4	3	35	15	2

HC – Hard core, SC – Soft core, OE – Open Elective

Department: **Microbiology** Course: **M.Sc Microbiology**

Paper Code	Paper and Title	Credits	No of Hours/ week Theory/ Practical	Duration of Exam (SEE)	Marks		Total
					Internal 25	SEE 75	
MICHCT31	Recombinant DNA Technology	4	4	3	25	75	100
MICHCT32	Immunology and Immuno-technology	4	4	3	25	75	100
MICSCT33	Food and Dairy Microbiology (A)	4	4	3	25	75	100
	Microbial Enzymology (B)	4	4	3	25	75	100
MICOET31	Microbes and Environment	2	2	2	15	35	50
	Practical Based on paper 3.1	2	4	3	15	35	50
	Practical Based on paper 3.2	2	4	3	15	35	50
	Practical Based on paper 3.3	2	4	3	15	35	50

Paper-3.1 HC: Recombinant DNA Technology

1.	Methods of studying DNA – Density gradient sedimentation, zonal centrifugation, isopycnic separation, electrophoretic separation, agarose, polyacrylamide, pulse field electrophoreses, southern blotting, northern blotting, labeling – radioactive and non-radioactive labeling. DNA sequencing - direct sequencing, indirect sequencing, Maxam and Gilbert method, Sangers method, RNA sequencing,	**12 h**
2.	Nucleic acid hybridization – Design and construction of probes, nick translation, hybridization, liquid hybridization, solid hybridization, determination of stringency conditions. Applications of nucleic acid hybridization. Enzymes used in recombinant DNA technology, Restriction endonucleases – Type, I, II &III, Nucleotide kinase, reverse transcriptase, T4 DNA ligase, Klenow polymerase and others, restriction mapping, RFLP and RAPD.	**12 h**
3.	Plasmid vectors - Use of natural plasmids as vectors, artificial plasmid vectors, pSC 101, pBR 322, pUC 18, Ti and Ri plasmid vectors. Bacteriophage vectors – Insertion vectors, replacement vectors, cosmid vectors, phagemid vectors, shuttle vectors and M13 based vectors. BACs, YACs and HAC	**12 h**
4.	Hosts for recombinant DNA technology; Prokaryotes –Bacteriophages, *E. coli, B. subtilis, Streptomyces,* Eukaryotic – Yeasts and Fungi Construction of recombinant DNA, selection of DNA fragments for cloning, chemical synthesis, gene synthesizers, ligation with RES, homopolymer tailing, blunt end ligation, linkers, monitoring restriction and ligation. Genome libraries – construction and screening of genome libraries, chromosome walking, cDNA libraries.	**12 h**
5.	Insertion of recombinant DNA – Host selection, transformation, transfection, electroporation, lipofection, Screening of recombinant, Applications of rDNA technology PCR – principles, types and applications, primer design and applications. DNA micro array – principle, types, construction and applications, *in vitro* approach for studding DNA- Protein interactions.	**12 h**

After successful completion of this course students are expected to be able to:

CO-1: Demonstrate a comprehensive understanding of the multidisciplinary as well as interdisciplinary fundamental concepts in Genetic Engineering and Biotechnology.

CO-2:Describe the applications of nucleic acid hybridization.

Identify the enzymes used in recombinant DNA technology, including restriction endonucleases (Type I, II & III), nucleotide kinase, reverse transcriptase, T4 DNA ligase, Klenow polymerase, and others.

Explain restriction mapping, RFLP and RAPD.

CO-3:Describe the use of natural plasmids as vectors and artificial plasmid vectors such as pSC 101, pBR 322, pUC 18, Ti and Ri plasmid vectors.

CO-4:Describe the construction of recombinant DNA including selection of DNA fragments for cloning, chemical synthesis, gene synthesizers, ligation with RES, homopolymer tailing, blunt end ligation, linkers, monitoring restriction and ligation.

CO-5:Describe genome libraries including construction and screening of genome libraries, chromosome walking and cDNA libraries.

Reference Books:

1. Brown, T. A. (2017). Molecular biology: Labfax (2nd ed.). Academic Press.
2. Karp, G. (2019). Cell and molecular biology (8th ed.). John Wiley & Sons.
3. Miller, G., & Levine, J. (2019). An introduction to genetic analysis (12th ed.). W.H. Freeman.
4. Watson, J. D., &Caudy, A. A. (2017). Recombinant DNA (2nd ed.). Scientific American Books.
5. Nicholl, D. S. T., & Errington, M. L. (2016). An introduction to genetic engineering (3rd ed.). Cambridge University Press.
6. Trapp, B. E., &Freifelder, D. (2018). Molecular biology: Genes to proteins (5th ed.). Jones & Bartlett Learning.
7. Clark, D. P., Pazdernik, N., &Tillier, E. R. M. (2021). Molecular biology: Academic cell update edition (3rd ed.). Academic Press.
8. Lodish, H., Berk, A., Zipursky, S. L., Matsudaira, P., Baltimore, D., & Darnell, J. E. (2016). Molecular cell biology (8th ed.). W.H. Freeman.
9. Mechardt, C., & McElroy, K. E. (2018). Molecular biology and genomics: The experimenter series (2nd ed.). Academic Press.

Practical Based on paper 3.1 Recombinant DNA Technology

1. Isolation of Genomic DNA From Bacterial Cell.
2. Quantitative Estimation of DNA By DPA Method.
3. Quantitative Estimation of RNA By Orcinol Method
4. Estimation of Purity and Concentration of DNA By Spectrophotometric Method
5. Restriction Digestion of Lambda DNA
6. Ligation of Lambda DNA Hind-III
7. Amplification of DNA Fragment by Polymerase Chain Reaction.
8. Southern Blotting and Northern Blotting
9. Restriction Fragment Length Polymorphism (RFLP)
10. Random Amplification Of Polymorphic DNA (RAPD)
11. Complementary DNA (cDNA)
12. DNA Microarray

Paper-3.2 HC: Immunology and Immunotechnology

1.	Introduction: Origin, concept and historical development of immunology. Immunity: Definition, Types of immunity-Innate and Acquired immunity. Cells and organs of immune system: Circulatory and lymphatic systems. Hematopoiesis. Cells of immune system. Types, structure and functions of lymphoid organs. Biology of immune cells: B cells-Origin, development, maturation and surface molecules. T cells- Origin, development, maturation and surface molecules; Subsets of T cells. Structure and function of T Cell receptors	**12 h**
2.	MHC molecules-Types, structure, genetics and functions. Complement system-Components and pathways of component activation**.** Antigens and Antibodies: Antigens - Physical and chemical properties of antigens, Epitopes, Antigenicity and Immunogenicity; Types of antigens. Antibodies- Physical and chemical structures of antibodies, Types and biological functions of immunoglobulins. Monoclonal and Polyclonal antibodies- Production and applications.	**12 h**
3.	Antigen-Antibody reactions: Mechanism and principles of antigen antibody reactions. Types and determination of antigen antibody reactions – Radio immune assay, Ouchterlony double diffusion technique, Complement fixation test, Enzyme linked immunosorbent assay and Immuno blotting Immune response: Antigen processing and presentation; Activation of T and B cells; Differentiation and formation of functional T cells; Differentiation of B cells and formation of plasma and memory cells. Immune response-Primary and secondary. Effector mechanism of HMI and CMI. Cell mediated cytotoxicity, ADCC and Inflammation. Cytokines- Types, functions and applications	**12 h**

4.	Hypersensitivity- Mechanism and types of hypersensitivity. Autoimmunity and Immuno deficiency syndrome: Autoimmunity and autoimmune disorders. Immuno deficiency syndrome: IDS due to deficient T and B cells, phagocytes, complement. Severe combined immunodeficiency syndrome.	**12 h**
5.	Tumor and Transplantation immunology: Tumor antigens and immunology to tumor cells. Transplantation immunology-Blood transfusion, Tissue transplantation and HLA typing. Immunotolerance andImmuno modulators Vaccines- Types, production and immunization schedules. Recent advances in vaccines and their developments: Recombinant vaccine development (Covishield) attenuated vaccine (Covaxin)	**12 h**

After successful completion of this course students are expected to be able to:

CO-1 Compare and contrast innate and adaptive immunity

CO-2 Design a model of Immunoglobulins

CO-3 Describe which cell types and organs present in the immune response.

CO-4 To make them understand the salient features of antigen antibody reaction &its uses in diagnostics and various other studies. Illustrate various mechanisms that regulate immune responses and maintain tolerance

Reference Books:

1. Bradley, J. and Mecharty, M. (2022). Clinical Immunology. Oxford University Press,New York.
2. Abbas, A.K., Lichtman, A.H., and Pillai, S. (2022). Cellular and Molecular Immunology. Elsevier.
3. Murphy, K. (2022). Janeway's Immunobiology. Garland Science.
4. Catty, D. (2018). Antibody Production and Maintenance of Laboratory Animals. Wiley-Blackwell.
5. Kubey, J.K., Goldsby, R.A., Kindt, T.J., and Osborne, B.A. (2018). Kuby Immunology. W.H. Freeman.
6. Male, D., Brostoff, J., Roth, D.B., and Roitt, I.M. (2019). Immunology. Elsevier.
7. Stites, D.P., Terr, A.I., and Parslow, T.G. (2012). Medical Immunology. McGraw-Hill Education.
8. Coico, R., Sunshine, G., and Benjamin, E. (2014). Immunology: A Short Course. Wiley-Blackwell.
9. Topley, W.W.C., Wilson, G.S., and Collier, L.H. (1990). Topley & Wilson's Principles of Bacteriology and Immunity: Virology and Immunology (8th ed.). Edward Arnold.
10. Roitt, I.M., Brostoff, J., and Male, D.K. (1998). Immunology (3rd ed.). Mosby-Year Book

Digital References/ Study material:

https://onlinecourses.nptel.ac.in/noc22_bt40/preview

Practical Based on paper 3.2 Immunology and Immunotechnology

1. Estimation of Haemoglobin content in blood.
2. Total RBC count.
3. Total WBC count.
4. Differential WBC count.
5. Determination of Erythrocyte sedimentation rate (ESR).
6. Radial Immunodiffusion test.
7. Ochterlony double diffusion test.
8. Study of organs of immune system
9. Determination of hypersensitivity reaction by tuberculin test.
10. Preparation of antigen for polyclonal antibody of production
11. Methods of antigen injection
12. Separation of plasma and serum
13. Determination of antibody titer of the serum.

14 Separation of serum proteins by SDS-PAGE
15 Isolation of IgG from serum
16 Isolation of IgY from egg yolk
17 Agglutination Tests.
a. VDRL test.
b. Haemeagglutination test.
18 Detection of *Salmonella* by WIDAL test.
19 Immunochromatography- HCG/HIV/HBSAg/ detection
20 ELISA/ Western blot

Paper-3.3 SCT (A): Food and Dairy Microbiology

1.	Introduction: Origin, Concept, Scope and historical developments Food as substrate for microorganisms: Hydrogen ion concentration (pH), Moisture requirement, Water activity, Oxidation-Reduction potential, Nutrient content, Inhibitory substances and Biological structure. Food contamination: Contamination of foods from green plants, animals, sewage, soil, water, air and handling.	**12 h**
2.	Food spoilage: General principles of food spoilage, Causes of food spoilage, Factors affecting kind and number of microorganism. Chemical changes caused by microorganisms. Spoilage of Meat and Meat products, Egg and Egg products, Fish and Marine products, Cereal and Cereal products, Fruits and Vegetables. Food borne diseases and their control: Food Infection and Intoxication. Detection of food borne pathogens and their toxins by various methods.	**12 h**
3.	Food Preservation: General principles, Physical methods of food preservation (High temperature, Low temperature and Drying), Chemical methods of food preservation (Food additives) and Biological methods of food preservation.	**12 h**
4.	Fermented foods (Bread, Sauerkraut and Tempeh), Probiotics and Prebiotics. Concept and importance of Nutraceuticals and Nutraceutical products. Milk: Definition, Composition, Nutritive value and Properties. Microbiology of milk. Testing of milk quality. Contamination, spoilage and preservation of milk and milk products.	**12 h**
5.	Fermented milk products: Production, Quality control and Significance of Cheese, Yogurt, Shrikhand and Acidophilus milk. Food sanitation and food safety: Concept, Importance and Safety laws, GMP and LP. Quality control and food standards: Bureau of Indian Standard (BIS). PFAA, FPO, MPO, CSO, Agmark Standards, International standards – HACCP, ISO 9000 Series. Food testing laboratories.	**12 h**

After successful completion of this course students are expected to be able to:
CO-1 Know the details of food borne pathogens, fermented food products and role of microorganisms in dairy industry
CO-2 Understand concept and use of probiotics and illustrate the role of microorganisms in food safety
CO-3 Cultivate and enumerate microorganisms from various food samples
CO-4 Compare various physical and chemical methods used in the control of microorganisms

Reference Books:

1. Doyte, M. P., Loory, R. B., & Thomas, J. M. (2019). *Food microbiology* (6th ed.). ASM Press Washington DC.

2. Jay, J. M. (2018). *Modern food microbiology* (9th ed.). Chapman & Hall.
3. Joshi, V. K., & Pandey, A. (2019). *Biotechnology of food fermentation* (2nd ed.). Asia Tech Publications.
4. Frazier, W. C., &Westhof, D. C. (2019). *Food microbiology* (4th ed.). Tata McGraw Hill Education.
5. Doyle, M. P., Beuchat, L. R., & Montville, T. J. (2013). *Food microbiology: Fundamentals and frontiers* (4th ed.). ASM Press.
6. Danwart, G. J. (2018). *Basic food microbiology* (2nd ed.). CBS Publishers & Distributors.
7. Pitt, J., & Hocking, A. D. (2009). *Fungi and food spoilage* (3rd ed.). Springer.
8. Sareen, S., &Soni, S. (2018). *Food preservation* (2nd ed.). Sarup& Sons.
9. Ananthakrishnan, C. P., & Gunasekaran, P. (2017). *Dairy microbiology* (2nd ed.). Sreelakshmi Publications.
10. Robinson, R.K., Tamime, A.Y., & Robinson, R.K.(Eds.) (1990). *Dairy microbiology: a practical approach* (1st ed.). Elsevier Applied Science.

Digital References/ Study material:

https://onlinecourses.swayam2.ac.in/cec23_ag03/preview
https://archive.nptel.ac.in/course.html
https://archive.nptel.ac.in/courses/102/103/102103015/
https://onlinecourses.swayam2.ac.in/cec19_bt11/preview

Practical Based on paper 3.3 Food and Dairy Microbiology (A)

1. Microbiological Examination of Utensils.
2. Methylene blue reduction test
3. Enumeration of microorganisms from healthy and spoiled fruits and vegetables
4. Enumeration of microorganisms from cereals, spices and dry products
5. Enumeration study of spoilage of stored meat and fish
6. Study of microbiology of milk and milk products
7. Production of yoghurt, acidophilus milk, tempeh and cheese
8. Estimation of lactic acid in milk and curd
9. Estimation Fat in milk and milk products
10. Estimation of ascorbic acid from tomato, chilly and lemon
11. Mushroom cultivation (Oyster) and Spirulina, Agar-agar and single cell proteins
12. Mandatory visit to food research institutes/Industries

SCT 3.3 (B) MICROBIAL ENZYMOLOGY	
Course Credits: 4	**Total No. of Hours: 60** **No. of Teaching Hours per Week: 4 Hrs**

Unit 1. Introduction to enzymes. 12Hrs

Historical developments. Classification of enzymes into six major groups with suitable examples. Numerical classification of enzymes. Methods & structural conformations of enzymes. Properties of Enzymes, laws of thermodynamics , factors affecting the rate of chemical reactions. Arrehenius theory, collision theory . free energy. Enzymes as biocatalysts, catalytic power, activation energy, substrate specificity, active site.

Unit 2. Enzyme kinetics: 12Hrs

Importance of enzyme kinetics, Variations of velocity with [E], [S], pH and temperature, time of incubation , Derivation of Michaelis - Menton equation and its significance in enzyme kinetic studies. Lineweaver-Burke plot, Haldane-Briggs relationship. Concept and significance of K_m and V_{max}. Concept of enzyme inhibition; types of enzyme inhibitors-reversible, competitive, non-competitive, uncompetitive and irreversible; significance and applications of enzyme inhibitors. Basics of enzyme turnover- Kinetics, measurement and rates of enzyme turn over..

Unit 3. Mechanism of enzyme action.12 Hrs

Theories of mechanisms of enzyme action. Mechanism of action of lysozyme, chymotrypsin and

ribonuclease.Monomeric, Oligomeric and multi-enzyme complex (PDH and fatty acid synthase), isozymes (Lactate dehydrogenase, creatine phosphokinase, alcohol dehydrogenase, alkaline phosphatase and isocitrate dehydragenase) and Allosteric enzymes (Threonine dehydratase and aspartate transcarbomylase); covalently modulated enzymes (Glycogen phosphorylase) and Membrane bound enzymes (ATPase).

Unit 4. Enzymes from microbial sources.12 Hrs

Screening by plate assay methods, large scale production of enzymes, recovery of enzymes enzyme purification methods - enzyme precipitation, separation by chromatography, enzyme reactors. Immobilized enzymes: Physical and chemical methods of immobilization, immobilization supports, kinetics of immobilized enzymes. Enzyme electrodes, Enzyme catalysis in a pola medium, reverse micellar entrapment of enzymes and its applications.

Unit 5. Application of enzymes:12 Hrs

Synthesis of chemicals using enzymes, food technology and medicine. Enzymes in diagnostic assays. Immune-enzyme techniques. Commercial products of microbes: Antibiotics, biopolymers, biosensors, biopesticides Production of biofuels. Microbial toxins: Types, biochemical and molecular basis of toxin production, implications. Genetically engineered microbes, anti-HIV, anticancer, antifungal, anti-plasmodial, anti-inflammatory compounds.

Course Outcome for M.Sc. Microbiology:

After successful completion of this course students are expected to be able to:

CO-1 Acquire the knowledge of enzymes their properties and classification, Mechanism of action, Michaelis-Menten initial rate equation, methods for the determination of Km and Vmax.

CO-2 Analyses the mathematical derivations in understanding enzyme kinetics and different transformation and its application.

CO-3 Learn about enzyme kinetics, effect of enzymes concentration, pH and temperature on kinetics of enzyme reactions, enzyme inhibition and activation, and Multi substrate enzyme kinetics.

CO-4 Learn different immobilization techniques and Industrial and clinical scope of enzymes and preparation of various culture media, Purification techniques

References:

1. Palmer, T., & Bonner, P. L. (2007). *Enzymes: Biochemistry, Biotechnology, Clinical Chemistry* (2nd ed.). Elsevier
2. West, T. S., & Told, L. (2019). *Textbook of Enzymology* (4th ed.). Wiley-Blackwell
3. Bhatt, S. M. (2018). *Enzymology and Enzyme Technology* (2nd ed.). New Age International Publishers
4. Punekar, N. S. (2020). *Enzymes* (1st ed.). Springer Nature Switzerland AG.
5. Arora, N. K., Mishra, J., & Mishra, V. (Eds.). (2020). *Microbial enzymes: Roles and applications in industries*. Springer Singapore
6. Brahmachari, G., Demain, A. L., &Adrio, J. L. (Eds.). (2017). *Biotechnology of microbial enzymes: Production, biocatalysis and industrial applications*. Elsevier
7. Singh, S., & Singh, R. (Eds.). (2020). *Microbial enzymes and biotechniques*. Springer Singapore

Practical Based on paper 3.3 Microbial Enzymology (B)

1. Population growth of bacteria (*E.coli*) and yeast (*S. cerevisiae)*
2. Sugar fermentation tests, Catalase activity, Hydrolytic rancidity, Casein hydrolysis
3. Study of Temperature (Heat stress) and acid and pH stress tolerance by microbes
4. Study of oxidative stress
5. Isolation of Thermophiles, acidophiles, alkalophiles and halophiles
6. Isolation of aerobic, facultative aerobic, anaerobic and microaerophilic microbes.
7. Screening of microorganism for invertases, amylase, proteases, lipases
8. Determination of optimum pH, temperature, enzyme and specific activity of microbial enzyme (invertase, amylase)
9. Effect of inhibitor on microbial amylase activity
10. Determination of Km and Vmax of microbial amylase
11. Isolation of streptomycin resistant strain of *E .coli*by gradient plate method.
12. Ames test

Paper-3.4 OE: Microbes and Environment

1.	Concept of environment: Atmosphere, lithosphere, hydrosphere and biosphere; Ecological niche - ecosystems, organization of ecosystems, food and energy triangles, position of microorganisms in the ecological niche. Origin and evolution of microorganisms: Origin and early evolution of microorganisms, relationship with the early stages of life on the earth, microbes as models for understanding how evolution works and the origin of all life on earth. Biodiversity of microorganisms: Richness and expanding microbial world, distribution of microorganisms in various environments, tools used for studying *in situ* and *ex situ* microbial diversity, culturable and non-culturable bacteria.	**10 h**
2.	Cosmopolitan nature of microorganisms: Exobiology – does life exists elsewhere in the universe? X- files, news stories of 1996 – evidence for microbial life on Mars, debate, exploration for extraterrestrial life based on microbial life. Microbial Ecology: Use of microorganisms as clues to study complex ecosystems; Natural resources – renewable and non-renewable, microorganisms as renewable resources; Microbial community within a human being - humans are microbes' invention to move around.	**10 h**
3.	Microbial interactions: Mutualism, synergism, commensalism, competition, amensalism, parasitism, predation. Microbe-Plant interaction: Symbiotic and non-symbiotic interactions. Biodegradation and bioremediation: Principles and degradation of common pesticides, organic (hydrocarbon, oil spills), industrial wastes, biomagnifications and bio-augmentation.	**10 h**

After successful completion of this course students are expected to be able to:

CO-1: Learn about the four major components of the environment - atmosphere, lithosphere, hydrosphere, and biosphere. They be able to identify the position of microorganisms in the ecological niche.

CO-2: Explore the origin and evolution of microorganisms.

CO-3: Understand biodiversity of microorganisms.

CO-3: Explore cosmopolitan nature of microorganisms.

CO-4: Understand what greenhouse gases are and their sources. Explain how microorganisms can be used as indicators of water quality. Describe how greenhouse gases contribute to the greenhouse effect.Understand the role of microorganisms in El Nino effect and Global warming and Gaia.

Reference Books:

1. Steinhaus, M. (2022). Insect pathology (Vol. I & II). Academic Press, New York.
2. Burges, H. D. (1981). *Microbial control of pest and plant diseases*. Academic Press.
3. Agrios, G. N. (2005). Plant pathology. Academic Press.
4. Atlas, R., & Bartha, R. (2018). Microbial ecology: Fundamentals and applications. Benjamin/Cummings Science Publis.
5. Agrios, G. N. (2005). Plant pathology. Academic Press.
6. Hurst, C. J., Crawford, R. L., Garland, J. L., Lipson, D. A., Mills, A. L., &Stetzenbach, L. D. (2019). Manual of environmental microbiology (4th ed.). ASM Press.
7. Fletcher, M., & Grey, T.R.G. (1987). Ecology of microbial communities. Cambridge University Press.
8. Rose, R.D. (1998). Air pollution & industry. Reinhold Co.
9. Metcalf, E., & Eddy, H.P.Wastewater engineering: Treatment and reuse (4th ed.). McGraw-Hill Education.
10. American Public Health Association (APHA), American Water Works Association (AWWA), & Water Environment Federation (WEF). (2017). Standard methods for the examination of water and wastewater (23rd ed.). APHA.