

**INDEX**

**DEPARTMENT OF MICROBIOLOGY**

**SEMESTER-II**

<b><u>Sl.No.</u></b>	<b><u>Content</u></b>	<b><u>Page No.</u></b>
1	<b>BSc. (Hons.) Microbiology– DSC</b>  1. Bacterial Diversity and Systematics 2. Biochemistry of Nucleic Acids and Proteins 3. Food and Dairy Microbiology	2 – 11
2	<b>Pool of Generic Electives offered by Department of Microbiology</b>  1. Microbes in Environmental Management 2. Microbes in Infectious Diseases 3. Applications of Microbes in Biotechnology 4. Fundamentals of Agricultural Microbiology 5. Microbial Products in Therapeutics	12 - 26

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**DISCIPLINE SPECIFIC CORE COURSE – 4: Bacterial Diversity and Systematics**

**CREDIT DISTRIBUTION, ELIGIBILITY AND PRE-REQUISITES OF THE COURSE**

Course title & Code	Credits	Credit distribution of the course			Eligibility criteria	Pre-requisite of the course (if any)
		Lecture	Tutorial	Practical/ Practice		
<b>MICROB-DSC201:</b>  <b>Bacterial Diversity and Systematics</b>	<b>4</b>	<b>3</b>	<b>0</b>	<b>1</b>	<b>Class XII pass with Biology/ Biotechnology/ Biochemistry</b>	<b>NIL</b>

**Learning Objectives**

The Learning Objectives of this course are as follows:

- The main objective of this course is for students to acquire in-depth knowledge of bacterial cell structure and organization, cultivation methods and growth patterns, and reproduction.
- Further, the student gains insights into the vastness of bacterial diversity and its significance

**Learning outcomes**

The Learning Outcomes of this course are as follows:

- Student will be able to describe the classification of bacteria based on their modes of nutrition, and the diverse physiological types of bacteria as determined by variable environmental factors.
- Student will be able to describe the fundamental concepts and terminology of taxonomic organization and parameters used in classifying bacteria, and the molecular analytic approaches used to classify diverse bacteria. Student will be able to discuss about the use of rRNA analysis as a means of developing phylogenetic relationships.
- Student will be able to describe the major groups of archaea, their stand-out physiological and structural features, as well as their ecological niches and economic significance.
- Student will be able to discuss the major groups of eubacteria, including bacteria with special features such as mycoplasma, rickettsia, chlamydia and spirochetes.

- Student will be able to demonstrate bacteria count by serial dilution and identify different types of bacteria using various media.
- Student will be able to analyze bacteria microscopically using various staining methods.

## SYLLABUS OF DSC-4

### UNIT – I (1 Week)

**Bacterial diversity based on nutritional and physiological factors:** Classification of bacteria based on nutrition: lithotrophs, organotrophs, phototrophs, chemotrophs. Diversity based on physiological factors: solutes, pH, temperature, oxygen, pressure, radiation.

### UNIT – II (4 Weeks)

**Bacterial systematics:** Definitions: Concepts of systematics, taxonomy, taxa, species, strains. Conventional and modern approaches to classification: Phenetic, phylogenetic, genotypic classification, evolutionary chronometers, rRNA oligonucleotide sequencing (ribotyping) and signature sequences, nucleic acid hybridization, genomic fingerprinting, MLSA, RFLP to study polyphasic bacterial taxonomy, FAME analysis

### UNIT – III (4 Weeks)

**Diversity of Archaea:** General characteristics with reference to genera belonging to Crenarchaeota (*Sulfolobus*) and Euryarchaeota: Methanogens (*Methanobacterium*), thermophiles (*Pyrococcus*), acidophiles (*Picrophilus*) and halophiles (*Halobacterium*). Key features of other groups: Thaumarchaeota, Lokiarchaeota, Nanoarchaeota

### UNIT – IV (6 Weeks)

**Diversity of Eubacteria:** Key features and significance of the following genera: Deeply Branching Bacteria: *Thermotoga*, *Deinococcus*. Proteobacteria: Classes and Types. Alphaproteobacteria: *Rhizobium*, *Rickettsia*. Betaproteobacteria: *Neisseria*, *Thiobacillus*. Gammaproteobacteria: *Escherichia*, *Yersinia*. Deltaproteobacteria: *Myxococcus* and *Bdellovibrio*. Epsilonproteobacteria: *Campylobacter*, *Helicobacter*. Zetaproteobacteria: *Mariprofundus ferrooxydans*. Non-Proteobacteria: Chlamydia, Spirochaetes. Gram Positive bacteria having genomes of low GC content: Firmicutes *Clostridium*, *Bacillus*. Tenericute *Mycoplasma*. Gram Positive bacteria having genomes of high GC content: *Mycobacterium*, *Streptomyces*

## Practical component

### UNIT 1: (5 Weeks)

Use of McConkey agar medium as a differential medium to distinguish between lactose- fermenting and lactose-nonfermenting gram negative bacteria. Enumeration of viable bacterial / CFU count using serial dilution and spread plate method/pour plate method.

**Unit 2: (10 Weeks)**

**Bacterial staining methods:** Use of light microscope to observe bacteria. Simple staining, Gram staining, Negative staining and Acid-fast staining (permanent mount). Endospore staining using malachite green. Observation of bacterial capsules by negative staining. Demonstration of bacterial motility by hanging drop method/flagellar staining.

**Essential/recommended readings*****Theory:***

1. Brock Biology of Microorganisms by M.T. Madigan, J. Aiyer, D. Buckley, W. Sattley and D. Stahl. 16<sup>th</sup> edition. Pearson, USA. 2021.
2. Prescott's Microbiology by J. M. Willey, K. Sandman and D. Wood. 11<sup>th</sup> edition. McGrawHill Higher Education, USA. 2019.
3. Microbiology: Principles and Explorations by J.G. Black and L.J. Black. 10<sup>th</sup> edition. Wiley, USA. 2019.
4. Microbiology: An Introduction by G.J. Tortora, B.R. Funke, and C.L. Case. 13<sup>th</sup> edition. Pearson, USA. 2018.
5. Principles of Microbiology by R. M. Atlas. 2<sup>nd</sup> edition. W.M.T. Brown Publishers, USA. 1997.
6. Microbiology by M. J. Pelczar, E. C. S. Chan and N. R. Krieg. 5<sup>th</sup> edition. McGraw Hill, USA. 1993.

***Practicals:***

1. Microbiology: A Laboratory Manual by J. Cappuccino and C.T. Welsh. 12<sup>th</sup> edition. Pearson Education, USA. 2020.
2. Basic Lab Manual of Microbiology, Biochemistry and Molecular Biology by A. Ray and R. Mukherjee. Taurean Publisher, India. 2019.
3. Benson's Microbiological applications: Laboratory manual in general microbiology by A.E. Brown and H. Smith H. 15<sup>th</sup> edition. McGraw-Hill Education, USA. 2022.
4. Manual of Microbiology: Tools & Techniques by A.K. Sharma. 1<sup>st</sup> edition. Ane Books, India. 2007.

**Suggestive readings**

**Note:** Examination scheme and mode shall be as prescribed by the Examination Branch, University of Delhi, from time to time.

**DISCIPLINE SPECIFIC CORE COURSE – 5: Biochemistry of Nucleic Acids and Proteins**

**CREDIT DISTRIBUTION, ELIGIBILITY AND PRE-REQUISITES OF THE COURSE**

Course title & Code	Credits	Credit distribution of the course			Eligibility criteria	Pre-requisite of the course (if any)
		Lecture	Tutorial	Practical/ Practice		
<b>MICROB-DSC202:</b>  <b>Biochemistry of Nucleic Acids and Proteins</b>	<b>4</b>	<b>3</b>	<b>0</b>	<b>1</b>	<b>Class XII pass with Biology/ Biotechnology/ Biochemistry</b>	<b>NIL</b>

**Learning Objectives**

The Learning Objectives of this course are as follows:

- The main objective of this course is to enable the students to develop a clear understanding of the structures and properties of biomolecules: proteins, lipids, carbohydrates and nucleic acids, and lays the foundation for a basic understanding of cellular processes.
- The students will gain an understanding of the principles of thermodynamics and bioenergetics, and will be introduced to the basic concepts of enzymes and enzyme kinetics.
- This course will empower the students with essential knowledge to support learning in subsequent courses offered in the program.

**Learning outcomes**

The Learning Outcomes of this course are as follows:

- Student will be able to describe the chemical structures of the building blocks of nucleic acids and the structures of the different types of DNA.
- Student will be able to describe the composition of proteins, and the structure and chemical properties of the different amino acids.
- Student will be able to describe the structural attributes of some classical proteins.
- Student will be able to analyze the constituents of an active enzyme, the interactions at enzyme active sites, and steady- state kinetics, allosteric regulation, and will be able to describe many different forms of enzymes found in living cells.

- Student will be able to analyze the structures of biomolecules using different types of models.
- Student will be able to analyze proteins qualitatively and quantitatively using different biochemical tests.

## SYLLABUS OF DSC-5

### UNIT – I (3 Weeks)

**Nucleic acids:** Introduction to importance of nucleic acids. Structures of purines and pyrimidines, nucleosides and nucleotides. Formation of DNA chains by phosphodiester bonds. Structure of DNA: the double helix. Types of DNA: A, B and Z. Properties of DNA. Types of RNA: rRNA, mRNA, tRNA

### UNIT – II (3 Weeks)

**Composition of Proteins:** Introduction to the importance of proteins. Amino acids as building blocks: structures and properties of standard amino acids. Zwitterion, titration curves of amino acids, and determination of pKa and pI of monocarboxylic amino acid. Ninhydrin reaction. Essential amino acids, non-protein amino acids: beta-alanine, D-alanine and rare amino acids: selenocysteine, hydroxyproline. Oligopeptides: structure and functions of glutathione and aspartame

### UNIT – III (2 Weeks)

**Protein structure:** primary, secondary ( $\alpha$  helix,  $\beta$  sheets), super secondary (collagen), tertiary (myoglobin) and quaternary (haemoglobin). Structure of insulin

### UNIT – IV (7 Weeks)

**Enzymes:** Concept of holoenzyme, coenzyme and apoenzyme. Cofactors: prosthetic group, Coenzyme: NAD, metal cofactors. Enzyme nomenclature and classification. Active site and activation energy. Lock and key hypothesis, induced fit hypothesis. Concept of steady state kinetics,  $V_{max}$  and  $K_m$ , significance of hyperbolic and double reciprocal plots. Enzyme unit, specific activity and turnover number. Temperature and pH effects on enzyme activity. Michaelis-Menten kinetics versus kinetics of allosteric enzymes. Competitive, non-competitive and uncompetitive enzyme inhibition. Allosteric enzymes: Phosphofructokinase. Multienzyme complex: pyruvate dehydrogenase. Isozyme: lactate dehydrogenase. RNA as enzymes: Hammerhead ribozyme

## Practical component

### UNIT 1: (5 Weeks)

Study of biomolecules with the help of models: The use of different types of models for visualizing molecular structures of biomolecules: Space filling models, Ball and stick models, Ribbon Models. Study of protein secondary and tertiary structures with the help of photographs/ models: collagen, myoglobin, hemoglobin.

### Unit 2: (10 Weeks)

**Qualitative and quantitative analysis of proteins:** Qualitative analysis of proteins using Xanthoproteic Test, Millon's Test, Biuret Test, Ninhydrin Test. Quantitative estimation of proteins by Lowry's method using bovine serum albumin as the standard. Demonstration of enzyme activity (amylase / urease / catalase) and effect of temperature, pH and heavy metal salt on activity.

### Essential/recommended readings

#### *Theory:*

1. Lehninger Principles of Biochemistry by D.L. Nelson and M.M. Cox. 8th edition. W.H. Freeman and Company, UK. 2021.
2. Biochemistry by J.M. Berg, J.L. Tymoczko, G.J. Gatto, and L. Stryer. 9th edition. W.H. Freeman and Company, UK. 2019.
3. Biochemistry by T.A. Brown and S.N. Mukhopadhyay. 1st edition. Viva Books, India. 2018.
4. Fundamentals of Biochemistry by D. Voet, J.G. Voet and C.W. Pratt. 5th edition. John Wiley and Sons, UK. 2016.

#### *Practicals:*

1. Practical Biochemistry by R.C. Gupta and S. Bhargava. 5th edition. CBS Publishers and Distributors, India. 2018.
2. An Introduction to Practical Biochemistry by D. Plummer. 3rd edition. McGraw Hill Education, India. 2017.
3. Introduction to Practical Biochemistry (ebook) by G. Hegyi, J. Kardos, M. Kovacs, A. Malnasi-Csizmadia, L. Nyitray, G. Pal, L. Radnai, A. Remenyi and I. Venekei. Eotvos Lorand University. 2013.
4. Modern Experimental Biochemistry by Rodney Boyer. 3rd edition. Pearson, India. 2002.

### Suggestive readings

**Note:** Examination scheme and mode shall be as prescribed by the Examination Branch, University of Delhi, from time to time. **CREDIT DISTRIBUTION, ELIGIBILITY AND PRE-REQUISITES OF THE COURSE**

**DISCIPLINE SPECIFIC CORE COURSE – 6: Food and Dairy Microbiology**

Course title & Code	Credits	Credit distribution of the course			Eligibility criteria	Pre-requisite of the course (if any)
		Lecture	Tutorial	Practical/ Practice		
<b>MICROB-DSC203:</b>  <b>Food and Dairy Microbiology</b>	<b>4</b>	<b>3</b>	<b>0</b>	<b>1</b>	<b>Class XII pass with Biology/ Biotechnology/ Biochemistry</b>	<b>NIL</b>

### Learning Objectives

The Learning Objectives of this course are as follows:

- The main objective of this course is to familiarise students with the importance of microorganisms in food spoilage as well as in preparation of certain foods, and to acquaint the students with quality control and safety indices used in the food industry

### Learning outcomes

The Learning Outcomes of this course are as follows:

- Student will be able to evaluate the factors governing microbial growth in foods and sources of food contamination.
- Student will be able to discuss the factors that govern spoilage of some common foods due to microbial activity.
- Student will be able to describe various physical and chemical methods used for food preservation.
- Student will be able to analyse the role of microorganisms in the production of fermented dairy and non-dairy food products. Will understand the health benefits of prebiotics, probiotics and synbiotics.
- Student will be able to discuss on the common food-borne diseases and preventive measures to be used, as well as methods for detection of food-borne pathogens.
- Student will be able to determine the importance of quality control in the food industry and describe various indices being used to measure quality and safety in the food industry.

### SYLLABUS OF DSC-6

#### UNIT – I (3 Weeks)



**Foods as a substrate for microorganisms:** Natural microflora and contamination sources of foods. Factors impacting growth and survival of microbes in foods. Intrinsic : pH, moisture content, nutrient availability, Eh values, antimicrobial substances and biological structures. Extrinsic: temperature, relative humidity and gaseous storage. Spoilage of foods by microorganisms: Factors responsible for food spoilage. Non- perishable, -semi perishable and - highly perishable foods. Spoilage of vegetables, fruits, meat, eggs, milk, butter, bread, and canned foods

### **UNIT – II (3 Weeks)**

**Food preservation methods:** Physical methods of food preservation: Temperature control (low: refrigeration, freezing; high: boiling, blanching, pasteurization, UHT, aseptic packaging). Canning: home and commercial. Dehydration: natural drying, artificial drying, freeze drying, smoking and tying of water molecules, reduced water activity products. Irradiation: radication, radurization, radappertization. Hydrostatic pressure, high voltage pulse, microwave processing. Chemicals used in food preservation: salt, sugar, organic acids, SO<sub>2</sub>, nitrites and nitrates, ethylene oxide, antibiotics and bacteriocins

### **UNIT – III (3 Weeks)**

**Fermented dairy and non-dairy foods:** Starter cultures. Fermented foods: yogurt, acidophilus milk, kumiss, kefir, dahi, cheese, bread, dosa, kanji, sauerkraut, soy sauce, tempeh, and fermented meat (sausages). Concept, health benefits and limitations of prebiotics, probiotics and synbiotics. Selection criteria for probiotic. Probiotic foods available in the market.

### **UNIT – IV (4 Weeks)**

**Food intoxications, food infections and detection of food borne pathogens.** Causative agents, foods involved, symptoms and preventive measures in food-borne diseases caused by Clostridium botulinum, Shigella (bacillary dysentery), Vibrio cholerae, Escherichia coli, Yersinia enterocolitica, Salmonella (food infection), Entamoeba histolytica. Mycotoxins: aflatoxins (Aspergillus). Detection of food-borne pathogens: culture-based as well as rapid detection methods

### **UNIT – V (2 Weeks)**

**Quality control in the Food Industry:** Total Quality Management (TQM): concepts and approaches. Hazard Analysis of Critical Control Point (HACCP) for food safety: principles and limitations. Indices of food quality (IFQ): FSSAI standard, ISO certification.

### **Practical component**

### **UNIT 1: (7.5 Weeks)**

#### **Microbial spoilage of food and fermented foods:**

Isolation and identification of spoilage fungi from various spoiled vegetables/ fruits: collection of spoilt food samples, point inoculation on suitable media, preparation of temporary mounts, and microscopic observations. Isolation and identification of spoilage fungi from spoiled breads using similar methods. Comparison of the fungi identified in the two categories of foods. Fermented

foods: Production of fermented foods using starter cultures and normal microflora of food. Preparation of yogurt / dahi. Preparation of sauerkraut / kanji. Preparation of buttermilk and butter. Preparation of kefir using kefir grains. Student research study project: unusual fermented foods from India and around the world.

## Unit 2: (7.5 Weeks)

### Food Quality Control :

Methylene Blue Dye Reduction Test (MBRT) to assess the microbiological quality of raw versus pasteurized milk: principle of the method, performance of the test with various samples of milk, evaluation and grading of milk quality based on the results obtained. Evaluation of milk quality by assessing its bacterial load using the standard plate count with serial dilutions of the milk. Clot on boiling (COB) test of milk samples: principle, performance of the test with milk samples, and evaluation of milk quality based on results obtained. Alkaline phosphatase test to check efficiency of pasteurization of milk: principle, performance of the test with various pasteurized milk samples, evaluation of milk quality based on results obtained.

### Essential/recommended readings

#### *Theory:*

1. Antimicrobials in Foods edited by P.M. Davidson, T.M. Taylor, and J.R.D. David. 4<sup>th</sup> edition. CRC Press, UK. 2020.
2. Food Microbiology by W.M. Foster. CBS Publishers & Distributors Pvt. Ltd. 2020
3. Food Microbiology by W.C. Frazier, D.C. Westhoff, and N.M. Vanitha. 5<sup>th</sup> edition. TataMcGraw-Hill Publishing Company Ltd, India. 2017.
4. Food Microbiology by M.R. Adams, M.O. Moss and P. McClure. 4<sup>th</sup> edition. Royal Society of Chemistry, UK. 2015.
5. Fundamental Food Microbiology by B. Ray and A. Bhunia. 5<sup>th</sup> edition. CRC Press. 2013.
6. Basic Food Microbiology by G.J Banwart. 2<sup>nd</sup> edition. CBS Publishers and Distributors, India. 2004.
7. Modern Food Microbiology by J.M. Jay, M.J. Loessner and D.A. Golden. 7<sup>th</sup> edition. Springer, Switzerland. 2005.
8. The Microbiological Safety and Quality of Foods. Vol. 1-2 by B.M. Lund, T.C. Baird-Parker, and G.W. Gould. ASPEN Publication, USA. 2000.

#### *Practicals:*

1. Analytical Food Microbiology: A Laboratory Manual by A.E. Yousef, J.G. Waite-Cusic and J.J. Perry. 2<sup>nd</sup> Edition. Wiley Publishers, UK. 2022.
2. Laboratory Manual of Food Microbiology by N. Garg, K.L. Garg and K.G. Mukerji. Dreamtech Press, India. 2021.
3. Microbiology: A Laboratory Manual by J. Cappuccino and C.T. Welsh. 12th edition. Pearson Education, USA. 2020.
4. Laboratory manual of Microbiology and Biotechnology by K.R. Aneja. 2<sup>nd</sup> edition. Scientific International Pvt. Ltd., Delhi. 2018.

### Suggestive readings

**Note:** Examination scheme and mode shall be as prescribed by the Examination Branch, University of Delhi, from time to time.

**COMMON POOL OF GENERIC ELECTIVES (GE) COURSES OFFERED BY THE DEPARTMENTS**

**GENERIC ELECTIVES (GE-6: MICROBES IN ENVIRONMENTAL MANAGEMENT)**

**Credit distribution, Eligibility and Pre-requisites of the Course**

Course title & Code	Credits	Credit distribution of the course			Eligibility criteria	Pre-requisite of the course	Department offering the course
		Lecture	Tutorial	Practical/ Practice			
<b>MICROB-GE6:  MICROBES IN ENVIRONMENTAL MANAGEMENT</b>	<b>4</b>	<b>2</b>	<b>0</b>	<b>2</b>	<b>None</b>	<b>NIL</b>	<b>Microbiology</b>

**Learning Objectives**

The Learning Objectives of this course are as follows:

- The main objective of the course is for students to appreciate how various microorganisms are bestowed with the capacity to modulate the environment.
- Students will get acquainted with the role of microbes in biodegradation, biogeochemical cycling, and production of biofuels.
- They will become aware of environmental problems and how microorganisms are used to manage these problems.
- This course will motivate them to think of novel ways to solve various environmental issues, including newer challenges such as e-waste management and plastic degradation using suitable microbes

**Learning outcomes**

The Learning Outcomes of this course are as follows:

- The student will be able to recall the importance of microbes in any ecosystem with reference to nutrient cycling/ biogeochemical cycling, and biofuels and the role of microbes in mineral recovery.
- The student will be able to describe BOD, COD and various methods of waste treatment (solid and liquid) utilizing diverse microorganisms.

- The student will be able to describe microbial bioremediation, including petroleum products, microbial degradation of pesticides, plastics and e-waste management for a cleaner environment.
- The student will be able to describe the concept of potability of water and demonstrate various tests to check the potability of given water samples.
- The student will be able to demonstrate isolation of microorganisms with special and unique properties from natural reservoirs of soil and landfills etc. and analyse how they keep reclaiming and rejuvenating our environment.
- The student will be able to demonstrate the use of conventional methods with innovative solutions to preserve and enhance environmental sustainability.

## **SYLLABUS OF MICROB-GE6**

### **UNIT – I (5 Weeks)**

**Role of microbes in biodegradation, biofuels and bioleaching:** Role of microbes in biodegradation and maintaining a continuous supply of nutrients like carbon, nitrogen (nitrogen fixation, ammonification and denitrification) and phosphorus in the ecosystem. Microbes as sources of Biofuels: bioethanol, algal biofuels, biogas, microbes in mineral recovery (iron, gold).

### **UNIT – II (6 Weeks)**

**Microbes in waste management:** Sources and types of solid waste, sanitary landfill, composting. Liquid waste management: composition and strength of sewage (BOD and COD). Primary, secondary (aerobic: Oxidation pond, Trickling filter, Activated sludge process; anaerobic: Septic tank, Imhoff tank, anaerobic sludge digester); and tertiary sewage treatment

### **UNIT – III (4 Weeks)**

**Microbial bioremediation:** Bioremediation of contaminated soils (heavy metals and petroleum) and marine pollutants. Microbial degradation of pesticides (2,4-D and 2,4,5-T). Role of microbes in e-waste management and plastic degradation

### **Practical component –**

#### **UNIT – 1 (5 Weeks)**

**Determination of water potability:** Water potability, Safety standards of drinking (potable) water. Methods to determine potability of water samples, standard qualitative procedure - presumptive test/MPN test, confirmed and completed tests for faecal coliforms; membrane filtration technique and Presence/Absence tests for coliforms using rapid detection kit

#### **UNIT – 2 (6 Weeks)**

**Isolation of microbes important in environment management:** Detection of starch/ cellulose-degrading and dye (malachite green/ crystal violet/ methylene blue) decolorising microorganisms from the soil. Isolation of heavy metal-accumulating

(copper/ nickel/ zinc/ cobalt/ aluminium) microorganisms from soil, and plastic-degrading microbes from landfills

**UNIT – 3 (4 Weeks)**

Preparation of compost using composting pits on college premises or elsewhere. Student Idea Presentation on environment protection. Visit to a wastewater treatment plant/solid waste treatment site. Understanding eutrophication and algal blooms with the help of pictures

**Essential/recommended readings**

1. Brock Biology of Microorganisms by M.T. Madigan, J. Aiyer, D. Buckley, W. Sattley and D. Stahl. 16th edition. Pearson, USA. 2021.
2. Prescott's Microbiology by J. M. Willey, K. Sandman and D. Wood. 11th edition. McGrawHill Higher Education, USA. 2019.
3. Soil Microbiology by N.S. Subba Rao. 5th edition. Medtech, India. 2017.
4. Environmental Microbiology edited by I.L. Pepper, C.P. Gerba, T.J. Gentry. 3rd edition. Academic Press, USA. 2014.
5. Advances in Applied Bioremediation edited by A. Singh, R.C. Kuhad and O. P. Ward. Springer-Verlag, Germany. 2009.
6. Microbial Ecology: Fundamentals and Applications by R.M. Atlas, R. Bartha. 4th edition. Benjamin Cummings, USA. 2000.
7. An Introduction to Soil Microbiology by A. Martin. 2nd edition. John Wiley and Sons Co, UK. 1991.

**Suggestive readings (if any)**

**Note:** Examination scheme and mode shall be as prescribed by the Examination Branch, University of Delhi, from time to time.

**GENERIC ELECTIVES (GE-7: MICROBES IN INFECTIOUS DISEASES)**

**Credit distribution, Eligibility and Pre-requisites of the Course**

Course title & Code	Credits	Credit distribution of the course			Eligibility criteria	Pre-requisite of the course	Department offering the course
		Lecture	Tutorial	Practical/ Practice			
<b>MICROB-GE7:  MICROBES IN INFECTIOUS DISEASES</b>	<b>4</b>	<b>2</b>	<b>0</b>	<b>2</b>	<b>None</b>	<b>NIL</b>	<b>Microbiology</b>

**Learning Objectives**

The Learning Objectives of this course are as follows:

- The major objective of this course is to give students of other disciplines an overview of the fundamentals of principles of immunology, infection and disease.
- The students will become aware of the whole spectrum of infectious diseases caused by different classes of microbes.
- They will become familiar with methods of disease diagnosis, the identification of the causative microbe and the latest immunological techniques.

**Learning outcomes**

The Learning Outcomes of this course are as follows:

- The student will be able to describe the basic concepts associated with infectious diseases and the principles and types of infection.
- The student will be able to describe different immune organs, immune cells, and their functions, and discuss the role of antigens and antibodies in fighting infection.
- The student will be able to describe different types of microbial diseases, their symptoms, and mode of transmission.
- The student will be able to demonstrate the complete blood count (TLC and DLC), and able to identify the human blood groups and different immune cells.
- The student will be able to describe about the different selective and differential media for culturing bacteria, and the principle and working of PCR-based tests for disease diagnosis.
- The student will be able to identify pathogenic bacteria by performing biochemical tests.

## **SYLLABUS OF MICROB-GE7**

### **UNIT – I (3 Weeks)**

**Introduction to basic concepts of infection and disease:** Infection, colonization, pathogenicity, virulence and its determinants (adhesion, enzymes, toxins - exotoxins and endotoxins), transmission (direct and indirect) of infectious diseases. Types of infections (acute, latent, chronic), opportunistic and nosocomial infections. Reservoir and source of infection.

### **UNIT – II (6 Weeks)**

**Basic principles of immunology:** Basic concepts of innate and adaptive immunity. Cells and organs of the immune system. Characteristics of antigen (foreignness, molecular size and heterogeneity), haptens, adjuvant. Structure, types and functions of antibodies. Cell mediated immunity. Primary and secondary immune response. Principles of immunization and types of vaccines

### **UNIT – III (6 Weeks)**

**Infectious diseases and their transmission:** Symptoms and mode of transmission of diseases. Bacterial : tuberculosis, tetanus, anthrax. Viral: chicken pox, measles, mumps, polio, COVID-19, AIDS, dengue. Fungal: athlete's foot, histoplasmosis, candidiasis. Protozoan: malaria, amoebiasis

### **Practical component –**

#### **UNIT – 1 (5 Weeks)**

**Immunological techniques:** Use of the haemocytometer. Analyzing total leucocyte count and differential leukocyte count in blood sample: determining percent count neutrophils, lymphocytes, eosinophils, basophils and monocytes in a blood smear. Identification of human blood groups and different immune cells

#### **UNIT – 2 (5 Weeks)**

**Culturing of microorganisms and diagnosis:** Use of various selective and differential media for culturing and identification of bacteria: mannitol salt agar, deoxycholate citrate agar / Salmonella Shigella (SS) agar, MacConkey / EMB Agar. Use of PCR based techniques to identify the infectious agent. Student group project: Different methods used to diagnose the following diseases: COVID19, tuberculosis

#### **UNIT – 3 (5 Weeks)**

**Biochemical tests for identifying bacteria:** Bacterial identification based on morphological features: Gram staining, capsule, endospore and motility characteristics. Bacterial identification based on biochemical characteristics: IMViC (Indole test, Methyl Red test, Voges-Proskauer test, Citrate test), Triple Sugar Iron (TSI) test, and catalase test. Kit based identification of a microbial pathogen.

### **Essential/recommended readings**

1. Textbook of Microbiology by R. Ananthanarayan and C.K.J. Paniker. 11<sup>th</sup> edition. Universities Press, India. 2020.



2. Prescott's Microbiology by J. M. Willey, K. Sandman, K. and D. Wood. 11th edition. McGraw Hill Higher Education, USA. 2019
3. Basic Immunology: Functions and Disorders of the Immune System by A. K. Abbas, A. H. Lichtman, S. Pillai. 6<sup>th</sup> edition. Elsevier, India. 2019.
4. Kuby Immunology by J. Punt, S. Stranford, P. Jones, and J. Owen. 8<sup>th</sup> edition. W.H. Freeman and Company, USA. 2018.
5. Jawetz, Melnick and Adelberg's Medical Microbiology by K.C. Carroll, S. A. Morse, T.A. Mietzner, and S. Miller. 28<sup>th</sup> edition. McGraw Hill Education, USA. 2016. Immunology by C. Richard and S. Geoffrey. 6<sup>th</sup> edition. Wiley- Blackwell Scientific Publication, UK. 2009.

**Suggestive readings (if any)**

**Note:** Examination scheme and mode shall be as prescribed by the Examination Branch, University of Delhi, from time to time.

**GENERIC ELECTIVES (GE-8: APPLICATIONS OF MICROBES IN BIOTECHNOLOGY)**

**Credit distribution, Eligibility and Pre-requisites of the Course**

Course title & Code	Credits	Credit distribution of the course			Eligibility criteria	Pre-requisite of the course	Department offering the course
		Lecture	Tutorial	Practical/ Practice			
<b>MICROB-GE8:  APPLICATIONS OF MICROBES IN BIOTECHNOLOGY</b>	<b>4</b>	<b>2</b>	<b>0</b>	<b>2</b>	<b>None</b>	<b>NIL</b>	<b>Microbiology</b>

**Learning Objectives**

The Learning Objectives of this course are as follows:

- The main objective of this course is to provide the students a clear understanding on the biotechnological potential of microorganisms in production of important industrial products like amino acids, antibiotics, vitamins, biopolysaccharides, bioplastics, pharmaceutical products, high fructose corn syrup, biofertilizers, biopesticides, transgenic plants, biofuels and biogas.
- They will also learn about the use of microorganisms for detoxification of industrial effluents, biogas production and extraction of metals from even low-grade ores.

**Learning outcomes**

The Learning Outcomes of this course are as follows:

- The student will be able to describe the concept of genetic manipulation of microbes by metabolic engineering and the production of important microbial products of immense industrial and medical/therapeutic value.
- The student will be able to describe the use of microbes in agricultural biotechnology for the formulation of biopesticides, biofertilizers, transgenic plants with desirable traits like disease resistance etc; and the importance of microorganisms in environmental management and biofuels production.
- The student will be able to demonstrate whole cell and enzyme immobilization techniques with strategies of dye decolorization using microorganisms.
- The student will be able to demonstrate the isolation and screening of enzyme producers from soil and symbiotic & asymbiotic nitrogen fixers.

- The student will be able to collect, analyse and interpret data on commercially available microbial products; and describe the cultivation and importance of edible mushrooms as well as single cell proteins.

## SYLLABUS OF MICROB-GE8

### UNIT – I (2 Weeks)

**General Microbial Biotechnology:** Scope of microbial biotechnology in agriculture, healthcare, environmental management, genomics, and proteomics, with suitable examples. Microbes commonly used in microbial biotechnology: viruses, bacteria, fungi. Relevance of natural, laboratory-selected mutant and genetically engineered microbes (GEMs), primary and secondary metabolites, metabolic engineering.

### UNIT – II (6 Weeks)

**Biotechnological potential of microbes in industry and medicine:** Production and applications of microbial products: amino acids (glutamic acid), antibiotics (streptomycin), vitamins (vitamin B12), polysaccharide (xanthan gum), bioplastic (PHB), high fructose corn syrup using immobilized microbial enzyme glucose isomerase. Production and applications of important medicinal products: Insulin, recombinant vaccine (Covishield) and Microbial biosensor (glucose oxidase), gene therapy for SCID in humans using virus

### UNIT – III (7 Weeks)

**Agricultural and Environmental Biotechnology:** Biofertilizers and biopesticides in agriculture: definition, classification with examples, advantages and disadvantages. Fertilizers from agricultural waste. Development of transgenic crops with important traits such as resistance to insects and viruses, herbicide resistance and environmental stress (drought and frost). Brief description of Bt cotton and Golden rice. Biofuel production from lignocellulosic waste and algal biomass, biogas (methane and hydrogen) production using microbes. Role of microbes in bioremediation (superbug, oilzapper, concentration of uranium from waste using bacteria). Biodegradation of xenobiotics (types of xenobiotics, hazards from xenobiotics, origin of microbial capacity to degrade xenobiotics and suitable examples) and microbial mining (mineral recovery of metals by bioleaching)

### Practical component –

#### UNIT – 1 (4 Weeks)

**Microbial enzyme immobilization and dye degradation:** Performing yeast cell immobilization and enzyme immobilization in suitable polymers by calcium alginate method, studying the activity and reuse of the immobilized enzyme for recycling purpose, observing dye decolorization/degradation using bacteria or fungi.

#### UNIT – 2 (6 Weeks)

**Enzymes and microbes from soil:** Screening of soil samples for isolation of hydrolytic enzymes: protease, lipase, cellulase, xylanase (any two) producing microorganisms using plate assay, isolation of symbiotic nitrogen fixer: Rhizobium from root nodules, isolation of asymbiotic nitrogen fixers from soil: Azotobacter and Azospirillum

### **UNIT – 3 (5 Weeks)**

**Microbial products:** Student group project: Conducting a market survey to identify any five popular microbial products and working to identify the microbe(s) involved in its production and the method of its preparation. Study of mushroom cultivation: importance, types of edible mushrooms and their cultivation, introduction to medicinal mushrooms. Single cell protein from algae Spirulina & Chlorella: medicinal importance, advantages, disadvantages and production strategies.

#### **Essential/recommended readings**

1. Prescott's Microbiology by J. M. Willey, K. Sandman and D. Wood. 11<sup>th</sup> edition. McGrawHill Higher Education, USA. 2019.
2. Biotechnology: A Textbook of Industrial Microbiology by W. Crueger, A. Crueger and K.R.Aneja. 3<sup>rd</sup> edition. Medtech Publisher, India. 2017.
3. Principles of Fermentation Technology by P.F. Stanbury, A. Whitaker and S.J. Hall. 3<sup>rd</sup> edition. Elsevier Science Ltd, Netherlands. 2016.
4. A Textbook of Biotechnology by R.C. Dubey. 5<sup>th</sup> edition. S. Chand and Co, India. 2014.
5. Molecular Biotechnology by B.R. Glick, J.J. Pasternak and C.L. Patten. 4<sup>th</sup> edition, ASM Press, USA. 2009.
6. Microbial Biotechnology by A.N. Glazer and H. Nikaido. 2<sup>nd</sup> edition. Cambridge University Press, UK. 2007.
7. Elements of Biotechnology by P.K. Gupta. 2<sup>nd</sup> edition. Rastogi Publications, India. 2009.
8. Basic Biotechnology by C. Ratledge and B. Kristiansen. 3<sup>rd</sup> edition. Cambridge University Press, UK. 2006.
9. Modern Industrial Microbiology and Biotechnology by Naduka Okafor. Science Publishers, USA. 2007.
10. Manual of Industrial Microbiology and Biotechnology by A.L. Demain, J.E. Davies and R.M. Atlas. 2<sup>nd</sup> edition. ASM Press, USA. 1999.

#### **Suggestive readings (if any)**

**Note:** Examination scheme and mode shall be as prescribed by the Examination Branch, University of Delhi, from time to time.

**GENERIC ELECTIVES (GE-9: FUNDAMENTALS OF AGRICULTURAL MICROBIOLOGY)**

**Credit distribution, Eligibility and Pre-requisites of the Course**

Course title & Code	Credits	Credit distribution of the course			Eligibility criteria	Pre-requisite of the course	Department offering the course
		Lecture	Tutorial	Practical/ Practice			
<b>MICROB-GE9:  FUNDAMENTALS OF AGRICULTURAL MICROBIOLOGY</b>	<b>4</b>	<b>2</b>	<b>0</b>	<b>2</b>	<b>None</b>	<b>NIL</b>	<b>Microbiology</b>

**Learning Objectives**

The Learning Objectives of this course are as follows:

- The major objective of this paper is to develop clear understanding of the role of soil and soil microbes in agriculture.
- The student will get an overview of plant microbe interaction and the role of microbes in nutrient cycles and their importance in agriculture.
- The students will have an in- depth knowledge of biofertilizers, composting and their importance for improving crop productivity.
- They will get familiarized with the significance of biocontrol agents and organic farming.

**Learning outcomes**

The Learning Outcomes of this course are as follows:

- The student will be able to describe the overview of soil and its characteristics and, the important microorganisms involved in mineralization of essential nutrients present in the soil and their significance in agriculture. Students will be able to describe various plant-microbe interactions including symbiotic and non-symbiotic associations.
- The student will be able to describe various microorganisms acting as biofertilizers including bacterial, fungal and algal biofertilizers. Students will be able to recognize the benefits of biofertilizers as compared to chemical fertilizers in terms of increased crop productivity and mass culturing of biofertilizers. Students will be able to describe various aspects of composting.
- The student will be able to discuss types and applications of bacterial and fungal biocontrol agents in agriculture and importance of organic farming.

- The student will be able to determine soil type, texture and its characteristics. Students will be able to describe the microbial interactions with plants, the different stages of nodules in leguminous plant roots and nodule forming bacteria under microscope. Student will be able to identify the stages of Mycorrhizal colonization through pictures.
- The student will be able to describe soil microbiology and microbial ecology, including the types of organisms living in soil. Students will be able to demonstrate the presence of microorganisms in soil by CO<sub>2</sub> evolution and enzyme activity.
- The student will be able to describe the recycling of organic matter for an easy and cheap way to make compost to enhance soil quality. They will also know about the antagonistic potential of *Trichoderma* spp. as biological control agent against other fungi.

## SYLLABUS OF MICROB-GE9

### UNIT – I (6 Weeks)

**Microbes and soil fertility:** Study of soil properties and microbial interactions: Study of soil types and its texture with the help of Sieve method/Mason jar method. Hands-on analysis of different soil characteristics - pH, moisture content, water holding capacity, percolation, capillary action of a soil sample. Isolation of phylloplane microflora on nutrient agar and potato dextrose agar by leaf impression technique. Demonstration of stages of nodule formation in leguminous plant with the help of photographs. Slide preparation of crushed nodule to observe nodule forming bacteria. Study of mycorrhizal association through pictures

### UNIT – II (6 Weeks)

**Biofertilizers and composting:** Introduction and scope of biofertilizers. Types, characteristics, mass production and methods of applications of the following: Bacterial biofertilizers: *Rhizobium*, *Azotobacter*, *Azospirillum*. Algal fertilizer: blue green algae, *Azolla*- *Anabaena*. Fungal biofertilizers: mycorrhiza. Quality testing of biofertilizers (ISI standards). Role of microbes in organic matter decomposition and different methods of composting.

### UNIT – III (3 Weeks)

**Biocontrol agents and organic farming:** Importance, potential and types of biocontrol agents. Application of *Trichoderma* spp. and *Bacillus thuringiensis* as biocontrol agents in agriculture. Concept of organic farming, types, methods and advantages.

## Practical component –

### UNIT – 1 (7 Weeks)

**Study of soil properties and microbial interactions:** Study of soil types and its texture with the help of Sieve method/Mason jar method. Hands-on analysis of different soil characteristics - pH, moisture content, water holding capacity, percolation, capillary action of a soil sample. Isolation of phylloplane microflora on nutrient agar and potato dextrose agar by leaf impression technique. Demonstration of stages of

nodule formation in leguminous plant with the help of photographs. Slide preparation of crushed nodule to observe nodule forming bacteria. Study of mycorrhizal association through pictures.

### UNIT – 2 (5 Weeks)

**Evaluation of microbial activity in soil:** Study of microbial activity in soil by CO<sub>2</sub> evolution: determination of CO<sub>2</sub> by trapping it in alkali solution and its estimation by titration. Detection of microbes in soil by Dehydrogenase/Urease/Amylase activity: reduction of triphenyl tetrazolium chloride (TTC) by dehydrogenases/ detection of ammonia by phenol red or Nessler's reagent/ detection of amylase using iodine solution

### UNIT – 3 (3 Weeks)

**Biodegradation of organic matter and Trichoderma as biocontrol agent:** Demonstration of steps of organic matter decomposition: composting of plant and food wastes containing organic compounds-lignin, cellulose, hemicellulose, polysaccharides, proteins, lipids, etc. into simple inorganic compounds/elements to be used as soil conditioner. Demonstration of antagonistic activity of *Trichoderma sp.* against different fungi (any 2) using dual culture plate technique.

### Essential/recommended readings

1. Benson's Microbiological Applications, Laboratory Manual in General Microbiology by A. Brown and H. Smith. 15th edition. McGraw-Hill Education, USA. 2022.
2. Biopesticides and Bioagents: Novel tools for pest management by M. A. Anwer. 1st edition. Apple Academic Press, USA. 2021.
3. Brock Biology of Microorganisms by M.T. Madigan, J. Aiyer, D. Buckley, W. Sattley and D. Stahl. 16th edition. Pearson, USA. 2021.
4. Microbiology: A Laboratory Manual by J. Cappuccino and C.T. Welsh. 12th edition. Pearson Education, USA. 2020.
5. Soil Microorganisms and plant growth by N.S., Subba Rao. 4th edition. Oxford & IBH Publishing Co. Pvt. Ltd. India. 2020.
6. Prescott's Microbiology by J. M. Willey, K. Sandman and D. Wood. 11th edition. McGraw Hill Higher Education, USA. 2019.
7. Biofertilizers in Agriculture and Forestry by N.S., Subba Rao. 4th edition. Biogreen Publisher, India. 2009.
8. Agricultural Microbiology by G. Rangaswami. and D. J., Bagyarai. 2nd edition, Prentice-Hall of India Private Limited, New Delhi. 2005.
9. Principles and Applications of Soil Microbiology by D.M., Sylvia. J.J., Fuhrmann. P.J. Hartel and D.A., Zuberer. 2nd edition Pearson, Prentice Hall, USA. 2005.
10. Agricultural Biotechnology by S.S., Purohit. 2nd edition. Agrobios Publisher, Jodhpur, India. 2003.

### Suggestive readings (if any)

**Note:** Examination scheme and mode shall be as prescribed by the Examination Branch, University of Delhi, from time to time.

**GENERIC ELECTIVES (GE-10: MICROBIAL PRODUCTS IN THERAPEUTICS)**

**Credit distribution, Eligibility and Pre-requisites of the Course**

Course title & Code	Credits	Credit distribution of the course			Eligibility criteria	Pre-requisite of the course	Department offering the course
		Lecture	Tutorial	Practical/ Practice			
<b>MICROB-GE10:</b>  <b>MICROBIAL PRODUCTS IN THERAPEUTICS</b>	<b>4</b>	<b>2</b>	<b>0</b>	<b>2</b>	<b>None</b>	<b>NIL</b>	<b>Microbiology</b>

**Learning Objectives**

The Learning Objectives of this course are as follows:

- The major objective of this course is to give students an in-depth knowledge of the commercially available microbial products used in the treatment of human diseases and their management.
- Students will be acquainted with the large-scale culturing of microorganisms to produce various metabolites used for therapeutic purposes.
- Students will get an hands-on experience in the production of enzymes by microorganisms and production of fermented foods.
- They will learn to use bioassay for detecting an antibiotic in a sample and they will get familiar with the technique to determine antibiotic sensitivity of any bacterial culture..

**Learning outcomes**

The Learning Outcomes of this course are as follows:

- The student be able to describe the techniques involved in isolation, screening and mass culturing of microorganisms to produce microbial metabolites at the industrial scale.
- The student would be able to describe the microbial therapeutics used in the management of infectious and non-infectious diseases in humans.
- The student be able to demonstrate and/or describe the extracellular enzyme production by microorganisms and its detection in the broth, and the production of fermented food products involving microorganisms.
- The student would be able to demonstrate the concept of bioassay for the detection of an antibiotic in the sample, and differentiate between antibiotic sensitive and antibiotic resistant bacteria.



- The student be able to collect and analyse data of commercially available therapeutic products and on locally available fermented foods.

## SYLLABUS OF MICROB-GE10

### UNIT – I (5 Weeks)

**Isolation, screening and mass culturing of microorganisms to produce useful metabolites:** Sources of industrially important microbes, their isolation and screening (primary and secondary). Fermentation techniques for large scale culturing: batch, fed-batch, continuously stirred tank reactor, solid-state fermentation. Different methods for recovery of microbial products

### UNIT – II (5 Weeks)

**Microbial therapeutics in the treatment of infectious diseases:** Antibiotics: mode of action, uses, and producer organisms of penicillin, streptomycin, tetracycline, cephalosporin, neomycin, erythromycin, augmentin, vancomycin and griseofulvin. Antimicrobial Resistance (AMR) phenomenon. Enzybiotics: Mode of action, uses and producer microorganisms of bacteriocins and lysozyme. Probiotics: Features of effective probiotics, benefits, commonly used probiotic microorganisms (Lactobacillus sp., Bifidobacterium sp., Saccharomyces boulardii). Bacto therapy by microbiota transplant.

### UNIT – III (5 Weeks)

**Microbial therapeutics in the treatment of non -infectious diseases:** Mode of action, uses and producer microorganisms of the following biopharmaceuticals: anti-inflammatory agents (serrapeptidase and collagenase), thrombolytic agents (streptokinase, nattokinase, tissue plasminogen activator ), digestive aids (fungal amylase and lipase), anticancer agents (asparaginase, methioninase), vitamins (cyanocobalamin, riboflavin), hormones (insulin and somatostatin). Production of steroid- based pharmaceuticals by microbial transformation: dehydrogenation (cortisol to prednisolone), hydroxylation (progesterone to 11 $\alpha$  hydroxyprogesterone).

### Practical component –

#### UNIT – 1 (6 Weeks)

**Production of enzymes and fermented foods:** Production of amylase from fungi and its detection in the culture broth: medium preparation, sterilization by autoclaving, inoculation, fermentation under specified condition of temperature and product harvesting from the broth by filtration. Production of any fermented product having probiotic bacteria or yeast (sauerkraut / curd / kanji ). Estimation of lactic acid produced during curd formation by titration

#### UNIT – 2 (6 Weeks)

**Detection of antibiotics and determination of antibiotic susceptibility:** Bioassay to detect the presence of an antibiotic in the broth/ provided samples: spreading an antibiotic sensitive bacterial culture on nutrient agar plates, making wells in the plates and dispensing antibiotic dilutions in the wells. Measuring zone of inhibition following incubation. Determination of the sensitivity of a bacterial culture to antibiotics using Kirby -Bauer disc diffusion method: spreading a bacterial culture

using sterile swab on Mueller -Hinton agar and determination of susceptibility of the bacterial culture to different antibiotic discs

**UNIT – 3 (3 Weeks)**

**Data collection and report preparation: Student research study project:** Market survey of commercially available pharmaceutical products of microbial origin. Report preparation of locally fermented food and dairy products. Presentation of main findings.

**Essential/recommended readings**

1. Industrial Microbiology by A.H. Patel. 2nd edition. Laxmi publication Pvt Ltd/Trinity Press. 2022.
2. Microbiology: A Laboratory Manual by J. Cappuccino and C.T. Welsh. 12th edition. Pearson Education, USA. 2020.
3. Industrial Microbiology by L.E. Casida. 2nd edition. New Age International publisher. 2019.
4. Prescott's Microbiology by J. M. Willey, K. Sandman and D. Wood. 11th edition. McGrawHill Higher Education, USA. 2019.
5. Crueger's Biotechnology: A Textbook of Industrial Microbiology by W. Crueger,
6. A. Crueger and K.R.Aneja. 3rd edition. Medtech Publisher, India. 2017.
7. Principles of Fermentation Technology by P.F. Stanbury, A.Whitaker and S.J. Hall. 3rd edition. Elsevier Science Ltd, Netherlands. 2016.
8. Benson's Microbiological applications: Laboratory manual in general microbiology by A.E. Brown and H. Smith H. 15th edition. McGraw-Hill Education, USA. 2022.
9. Pharmaceutical Biotechnology: Fundamentals and Applications edited by J. Crommelin, R. Sindelar and B Meibohm B. 4th edition. Springer, UK. 2013.
10. Manual of Industrial Microbiology and Biotechnology edited by R.H. Baltz, A.L. Demain, and J.E. Davies. 3rd edition. American Society for Microbiology. 2010.
11. Pharmaceutical Biotechnology: Concepts and Applications by G. Walsh. John Wiley and Sons. 2007.

**Suggestive readings (if any)**

**Note:** Examination scheme and mode shall be as prescribed by the Examination Branch, University of Delhi, from time to time.