BHARTI UNIVERSITY

DURG (C.G.)

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SCHEME OF EXAMINATION & SYLLABUS Of Master of Science

UNDER

FACULTIES OF MICROBIOLOGY

(Approved by Board of Studies)
Effective from Nov. 2021

EXAMINATION SCHEME

M. Sc. Microbiology

M. Sc. examination will be conducted in FOUR SEMESTERS.

SEMESTER – I

THEORY

PAPER	COURSE	DURATION	THEORY MARKS	TEACHER ASSESSMENT	TOTAL MARKS
MBT- 101	CELL BIOLOGY	3 Hrs	70	30	100
MBT- 102	BIOMOLECULES	3 Hrs	70	30	100
MBT- 103	MICROBIOLOGY	3 Hrs	70	30	100
MBT- 104	BIOLOGY OF IMMUNE SYSTEM	3 Hrs	70	30	100

PAPER	COURSE	DURATION	PRACTICAL MARKS	TEACHER ASSESSMENT	TOTAL MARKS
MBL- 101	Lab Course I (Based on paper I & II)	4 Hrs	70	30	100
MBL- 102	Lab Course II (Based on paper III & IV)	4 Hrs	70	30	100

M. Sc. Microbiology SEMESTER – II

THEORY

PAPER	COURSE	DURATION	THEORY MARKS	TEACHER ASSESSMENT	TOTAL MARKS
MBT- 201	GENETICS AND MOLECULAR BIOLOGY	3 Hrs	70	30	100
MBT- 202	BIOENERGETICS AND METABOLISM	3 Hrs	70	30	100
MBT- 203	INSTRUMENTAT ON AND MOLECULAR TECHNIQUES	3 Hrs	70	30	100
MBT- 204	BIOMETRY, COMPUTER AND SCIENTOMETRY	3 Hrs	70	30	100

PAPER	COURSE	DURATION	PRACTICAL MARKS	TEACHER ASSESSMENT	TOTAL MARKS
MBL- 201	Lab Course I (Based on paper I & II)	4 Hrs	70	30	100
MBL- 202	Lab Course II (Based on paper III & IV)	4 Hrs	70	30	100

M. Sc. Microbiology SEMESTER – III

THEORY

PAPER	COURSE	DURATION	THEORY MARKS	TEACHER ASSESSMENT	TOTAL MARKS
MBT- 301	MICROBIAL PHYSIOLOGY	3 Hrs	70	30	100
MBT- 302	FERMENTATION TECHNOLOGY	3 Hrs	70	30	100
MBT- 303	ENVIRONMENTAL MICROBIOLOGY	3 Hrs	70	30	100
MBT- 304	MEDICAL MICROBIOLOGY	3 Hrs	70	30	100

PAPER	COURSE	DURATION	PRACTICAL MARKS	TEACHER ASSESSMENT	TOTAL MARKS
MBL- 301	Lab Course I (Based on paper I & II)	4 Hrs	70	30	100
MBL-					
302	Lab Course II (Based on paper III & IV)	4 Hrs	70	30	100

M. Sc. Microbiology SEMESTER – IV

THEORY

PAPER	COURSE	DURATION	THEORY MARKS	TEACHER ASSESSMENT	TOTAL MARKS
MBT- 401	MICROBIAL BIOTECHNOLOGY	3 Hrs	70	30	100
MBT- 402	ADVANCED IMMUNOLOGY, DIAGNOSTICS AND PROPHYLAXIS	3 Hrs	70	30	100
MBT- 403	A) FOOD MICROBIOLOGY B) MICROBIAL ECOLOGY	3 Hrs	70	30	100
MBT- 404	A) AGRICULTURE MICROBIOLOGY B) INDUSTRIAL MICROBIOLOGY	3 Hrs	70	30	100

PAPER	COURSE	DURATION	PRACTICAL MARKS	TEACHER ASSESSMENT	TOTAL MARKS
MBL- 401	Lab Course I (Based on paper I & II)	4 Hrs	70	30	100
401	on paper 1 & m				
MBL-	Lab Course II	4.11	70	20	100
402	(Based on paper III & IV)	4 Hrs	70	30	100
MBP-					
101	Project Work		70	30	100

SCHEME FOR PRACTICAL EXAMINATION

EXPERIMENT	MARKS
Experiment	25
Viva-voce	10
Teacher Assessment	15
TOTAL MARKS	50

FIRST SEMESTER

MBT-101: CELL BIOLOGY

UNIT I

Molecular organization of membranes- asymmetrical organization of lipids, proteins and

carbohydrates. Osmosis, ion channels, membrane pumps and electrical properties of

membranes. Active transport by ATP- powered pumps: types, properties and

mechanisms.

UNIT II

Transport of proteins into mitochondria, chloroplast and endoplasmic reticulum.

Transport of proteins into and out of nucleus. Transport by vesicle formation:

exocytosis, endocytosis and its molecular

UNIT III

Cell signaling: Signaling via G-protein linked and enzyme linked cell surface

receptors, MAP kinase pathways. Eukaryotic cell division cycle: different phases and

molecular events, regulation and control of cell cycle. Apoptosis. Oncogenes and

tumor suppressor genes: viral and cellular Oncogenes,

UNIT IV

Organization of chromosomes: Structure of chromosomes, centromere and telomere.

States of chromosomes during cell cycle. Mitotic chromosome. Organization of genes in

chromosomes Banding. Pattern of chromosomes. Lampbrush and Polytene

chromosomes. Chromatin, nucleosomes, DNA packaging, heterochromatin and

euchromatin.

Lab Course:

1. Study of chromosome behaviour during Mitosis and meiosis (Onion / Garlic root

tips, Onion buds, human lymphocytes, rat or bird testis /grass hopper testis or any

other materials).

2. Calculation of mitotic index in growing Onion / Garlic root tips

- 3. Squash preparation: Polytene chromosome (in chironomus / Drosophila or other insect salivary gland) and Barr body (in buccal epithelial cells).
- 4. Demonstration of secretory granules in the salivary gland cells of insect.
- 5. Demonstration of mitochondria by vital staining.
- 6. Study of permanent slides.
- 7. Estimation of DNA
- 8. Estimation of RNA
- 9. Sub-cellular fractionation and marker enzymes
- 10. Identification of biomolecules in different tissues by histochemical techniques
- 11. Preparation of mitotic plate by carmine squashing method and phase identification.
- 12. Demonstration of the nuclear matrix networks in onion cells.
- 13. Study of the effect of chemical agents on chromosomes plant cells.
- 14. Isolation of protoplast, measurement of cell density plating efficiency.
- 15. Preparation of Karyotype of metaphase plate.

- H Lodish et al Molecular Cell Biology B Alberts et al. Essential Cell Biology H Lodish et al. Molecular Cell Biology
- 2. B Alberts et al. Molecular Biology of the Cell
- 3. G Karp Cell and Molecular Biology: Concepts and Experiments

MBT-102: BIOMOLECULES

UNIT I

Carbohydrates: structure, classification, properties and function; derivatives of monosaccharides, homo and hetero-polysaccharides, Peptidoglycan, glycoproteins and liposaccharide. Lipids: Classification, structure and function. Nucleic Acid: Structure of purine and pyrimidine bases, nucleoside and nucleotide; DNA- structure and conformation; RNA - Structure, types and functions.

UNIT II

Amino acids: structure, classification and functions; Synthesis of peptides and protein sequencing. Proteins- properties, covalent structure; secondary, tertiary and quaternary structure of proteins,

UNIT III

Enzyme classification, coenzymes, active site of enzyme, factors contributing to the catalytic efficiency of enzyme; enzyme kinetics- Michaelis-Menten equation, determination of Km, enzyme inhibition, allosteric enzymes, isoenzymes, ribozyme, multienzyme complexes

UNIT IV

Chemistry of porphyrins: Importance of porphyrins in biology; structure of hemoglobin and chlorophyll porphyrins, structure and biological role of animal hormones, structure and biological role of water soluble and fat soluble vitamins.

Lab Course:

- 1. Specific tests for sugars, amino acids and lipids
- 2. Formal titration of amino acids
- 3. Estimation of proteins using ninhydrin and biuret method
- 4. Estimation of sugar by anthrone and Folin-Wu method.
- 5. Saponification value and iodine number of fat.
- 6. Estimation of ascorbic acid.

- 7. Achromic point determination using salivary amylase
- 8. Effect of ions on salivary amylase activity.
- 9. Enzyme assay and kinetics (ex. Amylase, Protease)

- 1. Principles of Biochemistry by Nelson, Cox and Lehninger
- 2. Biochemistry by G. Zubay
- 3. Biochemistry by Stryer
- 4. Biochemistry by Garrett and Grosham
- 5. Text book of Biochemistry by West, Tood, Mason & Bruglen
- 6. Biochemistry by White, Handler & Smith Biochemistry by D. Voet and J C Voet

MBT-103: MICROBIOLOGY

UNIT I

General characteristics of fungi, classification of fungi, life cycle of selected fungal genus (*Aspergillus*, *Pencillium*, *Fusarium* and *Mucor*). Economic importance of fungi. Fungi and bioremediation, parasitism, mutualism and symbiosis with plants and animals. Heterothallism, sex hormone in fungi, Mycorrhiza, VAM. Algae: Distribution, classification, reproduction, ecology and importance.

UNIT II

Morphology and ultra-structure of bacteria, morphological types, cell wall of archaebacteria, gram negative, gram positive eubacteria, eukaryotes.

Cell membranes – structure, composition and properties. Structure and function of flagella, cilia, pili, gas vesicles. Cyanobacteria, protozoa, mycoplasma and Rickettsia.

Gene transfer mechanisms, transformation, transduction, conjugation and transfection.

Plasmids F: factors colicins and col factors, plasmids as a vector for gene cloning.

UNIT III

Nutritional types (autotrophs, heterotrophs, phototrophs, chemotrophs), growth curves, measurement of growth, factors affecting growth, generation time, growth kinetics. Batch and continuous culture, asynchronous and synchronous culture.

Basis of microbial classification, classification and salient feature of bacteria according to Bergey's manual of determinative bacteriology, cyanobacteria, prochlorons and cyanelles.

UNIT IV

Viruses: Structure and classification of viruses; morphology and ultra-structure; capsids and their arrangements, types of envelopes, viral genome, their types and structure, virus related agents (viroids, prions). General feature of virus reproductions, early events in virus multiplication, virus restriction and modification of host, virus mRNA. General overview of bacterial viruses, RNA and DNA bacteriophages (MS2, □ X174, M13, T3,

T4). Lysogeny and Lytic phase. General account of plant and animal viruses

Lab Course:

- 1. Glassware preparation and sterilization techniques- wet heat- dry heat- filter typeslaminar flow chamber types- CDC- safety levels.
- 2. Preparation of liquid & solid media, plating, pouring, inoculation and incubation for growth of microorganism
- 3. Methods of obtaining pure culture of microorganisms (a) streak plate (b) Pour plate, and (c) spread plate methods
- 4. Microscopic examination of the microorganisms, identification and staining methods
- 5. Micrometry and camera lucida drawings
- 6. Study of bacterial growth by turbiditimetry/ spectrophotometry
- 7. Biomass measurement for fungi
- 8. Isolation and enumeration of microorganisms from soil by serial dilution agar plating method.
- 9. Enumeration of viruses by plaque assay technique.
- 10. Motility of bacteria by hanging drop technique.

- Microbiology: L.M. Prescott, J.P. Harley and D.A. Klein, McGraw Hill Publication.
- General Microbiology: Stanier, Ingrahamana, Wheelis and Painter, Mac Millian Press
- Principles of Microbiology: R.M. Atlas
- Microbiology: Peleczar, Chan & Krieg
- General Virology: Luria, Darnell, Baltimore and Campell
- Introduction to Mycology: CJ Alexopoulos and CW Mims, Wiley Eastern Ltd, New Delhi

MBT-104: BIOLOGY OF IMMUNE SYSTEM

UNIT I

Innate immune mechanism and characteristics of adaptive immune response. Cells of immune system: Hematopoisis and differentiation, mononuclear cells and granulocytes. Antigen presenting cells. Primary and Secondary lymphoid organs and tissues. Ontogeny and phylogeny of lymphogytes. Lymphogytes treffic

and phylogeny of lymphocytes. Lymphocyte traffic.

UNIT II

Antigen receptor molecules: B-cell receptor complex, Immunoglobulin- structure, types and function. T- cell receptor complex. Major Histocompatibility Complex- types, structural organization, function and distribution. Transplantation and Rejection.

Complements in immune function.

UNIT III

Antigens: nature of antigens, factor affecting immunogenicity, Haptens and super antigens. Antigenic determinants. Recognition of antigens by T and B cell. Antigen processing. Role of MHC molecules in antigen presentation and co-stimulatory signals. Antigen and antibody interaction.

UNIT IV

Cell mediated immune response. Cytokines and interleukins- structure and function. Immunity to infections. Hypersensitive reactions and their types. Immunodeficiency disorders. Autoimmunity

Lab Course:

1. Identification of cells of immune system

2. Separation of mononuclear cells by Ficoll-Hypaque

3. Identification of Lymphocytes and their subsets

4. Lymphoid organs and their microscopic organization

5. Isolation and purification of Antigens

6. Purification of IgG from serum

- 7. Estimation of Levels of gamma globulins and A/G ratio in blood
- 8. Antigen antibody interaction

- Kuby's Immunology: R.A. Goldsby, Thomas J Kindt and Barbara A. Osborne
- Immunology- A short Course: E. Benjamini, R. Coico and G. Sunshine
- Immunology: Roitt, Brostoff and Male
- Fundamentals of Immunology: William Paul
- Immunology: Tizard
- Immunology: Abbas et al

SECOND SEMESTER

MBT-201: GENETICS AND MOLECULAR BIOLOGY

UNIT - I:

Mendelian principles: Dominance, segregation, independent assortment. Concept of gene: Allele, multiple alleles, pseudoallele, complementation tests

Extensions of Mendelian principles: Codominance, incomplete dominance, gene interactions.

Mutation: Types, causes and detection, mutant types – lethal, conditional, biochemical, loss of function, gain of function, germinal verses somatic mutants, insertional mutagenesis.

UNIT – II:

DNA replication, repair and recombination: Mechanism of replication, enzymes involved, replication origin and replication fork, fidelity of replication, extrachromosomal replicons, DNA damage and repair mechanisms; Repair of Base-excision, Nucleotide excisions, Mismatch and Double Strand. Guardian of DNA; *p53* and *p21*. Homologous and site-specific recombination.

UNIT – III:

RNA synthesis and processing: transcription factors and machinery, formation of initiation complex, transcription activator and repressor, RNA polymerases, elongation, and termination, RNA processing, capping, RNA editing, splicing, and polyadenylation, structure and function of different types of RNA, RNA transport.

UNIT – IV:

Protein synthesis and processing: Ribosome, formation of initiation complex, initiation factors and their regulation, elongation and elongation factors, termination, genetic code, aminoacylation of tRNA, tRNA- identity, aminoacyl t-RNA synthesise, and translational proof-reading, translational inhibitors, Post Translational modification of proteins.

Protein targeting.

Lab Course:

- 1. Isolation, purification and estimation of RNA
- 2. Isolation, purification and estimation of DNA
- 3. Determination of Tm of nucleic acid
- 4. Fraction of poly (A) RNA
- 5. Restriction Digestion
- 6. Ligation
- 7. DNA molecular size determination

- 1. H Lodish et al.: Molecular Cell Biology B
- 2. Alberts *et al.* : Essential Cell Biology
- 3. B Alberts et al.: Molecular Biology of the Cell
- 4. G Karp: Cell and Molecular Biology: Concepts and experiments JD Watson *et al.*: Molecular Biology of the Gene
- 5. J Wilson and T Hunt: Molecular Biology of the Cell: The Problems
- 6. B Lewin: Genes VIII
- 7. JE Krebs *et al.* (Ed.) : Genes X (Lewin's), Jones and Bartlett Publishers, Sudbury, Massachusetts, (2011)

MBT-202: BIOENERGETICS AND METABOLISM

UNIT I

First and second laws of thermodynamics. Concept of free energy, High – energy compounds, ATP cycle, structural basis of free energy change during hydrolysis of ATP. Other high – energy biological compounds

UNIT II

Basic concepts of intermediary metabolism. Carbohydrate metabolism: Glycolysis, Kreb's cycle, glycogenolysis, glycogenesis, pentose phosphate pathway, gluconeogenesis, and glyoxylate pathway, inborn errors of carbohydrate metabolism. Regulation of carbohydrate metabolism.

UNIT III

Electron transport and oxidation phosphorylation: electron carriers, Complexes I to IV, substrate level phosphorylation, mechanism of oxidative phosphorylation, Shuttle system for entry of electron, Biosynthesis and degradation of Lipids, Regulation of lipid metabolism.

UNIT IV

Nitrogen Assimilation. Biosynthesis of amino acids. Degradation of amino acids. Regulation of amino acid metabolism. Biosynthesis and degradation of purine and pyrimidine nucleotides.

Lab Course:

- 1. Protein estimation by Lowry, Bradford and Spectrophotometric method
- 2. Estimation blood cholesterol
- 3. Estimation of sugar by Nelson-Somogyi and Benedict's reagent
- 4. Isolation and estimation of lipid from seeds and egg.
- 5. Estimation of inorganic and total phosphorus by Fiske-Subba Rao method

- 6. Assay of phosphatases in blood and seeds
- 7. Urease estimation in plant tissues

- Principles of Biochemistry by Nelson, Cox and Lehninger
- Biochemistry by G. Zubay
- Biochemistry by Stryer
- Biochemistry by Garrett and Grosham
- Text book of Biochemistry by West, Tood, Mason & Bbruglen
- Biochemistry by White, Handler & Smith
- Biochemistry by with clinical application
- Biochemistry by D Voet and J C Voet
- Enzymes by Dixon and Webb
- Fundamentals of Enzymology by Price and Steven
- Practical Biochemistry by Plummer
- Enzyme Biotechnology by G. Tripathi
- Enzyme Reaction Mechanism by Walsh.
- Enzyme Catalysis and Regulation by Hammes

MBT-203: INSTRUMENTATION AND MOLECULAR TECHNIQUES

UNIT I

Centrifugation: Principle, techniques. Preparative, analytical and ultracentrifuges, sedimentation coefficient and factors affecting sedimentation coefficient. Application of centrifugation.

Photometry: Basic principles of colorimetry, UV- visible spectrophotometry & IR-spectrophotometry. Spectro flurometry Atomic absorption spectroscopy: Principle, Instrumentation and applications Electrophoresis: Paper electrophoresis, Starch gel, agarose, PAGE-type, 2D-E.

UNIT II

Microscopic techniques: light microscopy, microscopy of living cells, scanning and transmission microscopes, different fixation and staining techniques for EM, freeze-etch and freeze-fracture methods for EM, image processing methods in microscopy

Microtomy: types, principle and applications

Lyophilization: Principle, instrumentation and applications

UNIT III

Chromatography: Paper and Thin Layer Chromatography. Gel filtration, Ion exchange chromatography and Affinity chromatography. Gas-liquid chromatography and HPLC. Histochemical and immuno techniques: Antibody generation, detection of molecules using ELISA, RIA, western blot, immunoprecipitation, flow cytometry and immunofluorescence microscopy, detection of molecules in living cells, *In situ* localization; FISH and GISH.

UNIT IV

Molecular techniques: Isolation and purification of RNA, DNA (genomic and plasmid) and proteins, separation methods; RNA, DNA and proteins; 1-D and 2-D, isoelectric focusing gels; Molecular cloning of DNA and RNA fragments in bacterial systems; Expression of recombinant DNA; DNA sequencing. Gene expression; mRNA, cDNA

using PCR and qRT-PCR. Micro array based techniques.Molecular Markers for diversity analysis: RFLP, RAPD, AFLP, VNTR, SSR, ISSR, SNP, DArT.

Lab Course:

- Verification of Beers Law
- Determination of absorption maxima
- Quantitative determination, Enzyme kinetics
- Amino acid and carbohydrate separation by paper and TLC
- Ion exchange and gel filtration chromatography
- SDS Polyacralamide Gel Electrophoresis
- DNA electrophoresis
- Separation of sub-cellular organelles by differential centrifugation.
- Isolation of DNA and Agarose gel Electrophoresis
- Amplification of RAPD and AFLP markers.
- Isolation of RNA and Electrophoresis of RNA on denaturing gels.
- cDNA synthesis and cloning
- Isolation of Protein and SDS-PAGE
- In vitro DNA ligation, transformation of E. coli
- Characterization of transformants: DNA gel electrophoresis, Restriction map analysis.

- 1. K Wilson and John Walker Practical Biochemistry: Principles & Techniques
- 2. RF Boyer Biochemistry Laboratory: Modern Theory & Techniques
- 3. S Carson, H Miller and D Scott Molecular Biology Techniques: A Classroom Laboratory Manual
- 4. TC Ford and J. M. Graham An Introduction to Centrifugation
- 5. R Baserga and D Malamud Autoradiography: techniques and application
- 6. T Chard An Introduction to Radioimmunoassay and Related Techniques, Volume 6
- 7. MD Bruch NMR Spectroscopy Techniques
- 8. BA Wallace and R William Modern Techniques for Circular Dichroism and

- Synchrotron Radiation, Volume 1
- 9. J Sambrook, EF Rritsch and I Maniatis Molecular cloning: A Laboratory Manual
- 10. PD Dabre Introduction to Practical Molecular Biology
- 11. JD Watson, NH Hopkins, JW Roberts, JA Steitz and AM Weiner
- 12. Molecular Biology of Gene (4th Edition)
- 13. J Darnell, H Lodish and D Baltimore Molecular Cell Biology (2nd Edition)
- 14. B Alberts, D Bray, J Lewis, M Raff, K Roberts and JD Watson Molecular Biology of the Cell (2nd Edition)
- 15. Benjamin Lewin Gene VII
- 16. JM Walker and R Rapley Molecular Biology and Biotechnology
- 17. SB Primrose Molecular Biotechnology

MBT-204: BIOMETRY, COMPUTER AND SCIENTOMETRY

UNIT I

Introduction to biostatistics. Types of biological data: data on different scales. Frequency distributions. Cumulative frequency distributions. Random sampling. Parameters and statistics. Measures of central tendency and dispersion: Mean, Median, Mode, Range, Variance and Standard deviation. Coefficient of variation. The effects of coding data. Data transformations: Log-transformation, Square-root transformation and Arcsine transformation. Distribution: normal and binomial.

UNIT II

Statistical errors in hypothesis testing. Testing goodness of fit: Chi-square goodness of fit. Heterogeneity Chi-square. The 2 x 2 contingency table. One sample hypothesis. Two- sample hypothesis. Testing for difference between two means (*t*-test). Testing for difference between two variances (*F*-test). The paired sample *t*-test. Multiple-sample hypothesis (ANOVA): Single factor and two factors ANOVA. Simple linear regression. Regression *vs.* Correlation. Regression equation. Interpretations of regression functions. Simple linear correlation. The correlation coefficient.

UNIT III

Introduction to MS-Office software: Word processing; Creating new document, Editing documents, Adding graphics to documents, Word tables. Management of Workbook & Worksheets; Applications, Features, Using formulas and functions, Features for Statistical data analysis, Generating charts/ graph. Presentation software; Working in PowerPoint, Creating new presentation, Working with slides.

UNIT IV

Introduction to Internet and Applications. Basics of internet, e-mailing, Search engine – Google and Yahoo; PubMed, Scopus, Web of Science, Google Scholar, Indian Citation Index, Science Citation Index (SCI), h- index, i-10-index. Journal Impact Factor (JIF).

Introduction to Plagiarism and Cyber laws.

Lab Course:

- 1. Exercises for data distribution
- 2. Exercises for computation of measures of central tendency
- 3. Exercises for computation of measures of variability
- 4. Computation of correlation coefficient, r, and regression constants
- 5. Data analysis by ANOVA and multiple-range tests
- 6. Hypothesis testing by *t*-test, F-test, and Chi-square test
- 7. Graphical presentation of data using a suitable package
- 8. Statistical analysis of a data using a suitable package
- 9. Preparation of document using a suitable package
- 10. Preparation of slides using a suitable package

- i. Campbell RC Statistics for biologistsii. Zar JH Biostatistical Analysis
- iii. Wardlaw AC Practical Statistics for Experimental Biologists Snedecor GW& Cochran WG Statistical Methods
- iv. Sokal RR & Rohlf FJ Introduction to Biostatistics Sumner M
- v. Computers: Concepts & Uses
- vi. White R How Computers Work
- vii. Cassel P *et al.* Inside Microsoft Office Professional Coleman P and Dyson P

 Mastering Internets
- viii. Gralla P How the Internet Works
 - ix. Shelly GB, Vermaat ME, Microsoft 2007: Introductory Concepts and Techniques Cashman TJ
 - x. Habraken J Microsoft Office 2003 All in One Microsoft Office 2010 InDepth
 - xi. Gilmore B Plagiarism: Why it happens, How to prevent it?

- xii. Buranen L and Roy AM Perspectives on Plagiarism and Intellectual Property in a Post-Modern World
- xiii. Kumar Anupa P Cyber Law
- xiv. Sood V Cyber Law Simplified

THIRD SEMESTER

MBT-301: MICROBIAL PHYSIOLOGY

UNIT - I

Aerobic metabolism of methane and methanol: Methane and methanol users, Oxidation of methane, Formaldehyde and formic acid, assimilation of C-1 compounds.

Anaerobic respiration: Sulphur compounds and nitrate as electron acceptors, electron transport in SO_4 and NO_3 reducers. Anaerobic metabolism of glucose, Fermentation process, modes of glucose fermentation (lactic acid, ethanol, acetic acid, butyric acid, acetone and butanol, format and propionate). Transport of nutrients across membrane.

UNIT - II

Biosynthesis of peptidoglycan, teichoic acid, lipopolysaccharide, biosynthesis and degradation of essential amino acids, microbial degradation of aromatic, polycyclic and halogenated aromatic compounds. Microbial metabolism of hydrogen.

UNIT - III

Microbial photosynthesis: Historical account, structure of photosynthetic pigments i.e., chlorophylls and bacterio-chlorophylls, carotenoids, phycobilins, primary photochemistry and electron transport (light harvesting, charge-separation and electron transport in anoxygenic photosynthesis), ATP synthesis.

Eubacterial photosynthetic microbes, development of photosynthetic apparatus, carbon metabolism. Cynobacterial organization of photosynthetic apparatus. Halobacterial photo-phosphorylation.

UNIT-IV

Nitrogen metabolism: Biological nitrogen fixation, Mechanism of nitrogen fixation, ammonia assimilation, properties and regulation of glutamine synthetase, glutamate synthetase, glutamate dehydrogenase.

Biochemistry of methanogenesis; bio-transformation of steroid and non-steroid

compounds.

Lab Course:

- 1. Qualitative of assay of different extra-cellular enzymes
- 2. Quantitative assay of alkaline and acid phosphatases from microorganisms.
- 3. Determination of Km value of beta- fructofuranosidase from yeast
- 4. Antibiotic sensitivity test
- 5. Measurement of CM-cellulase by viscometric and reducing sugar method.
- 6. Experiment on production of enzymes and optimizing parameters for enzyme production in shake flask culture using *Aspergillus niger*, *Saccharomyces cerevisiae* for production of amylase, invertase respectively.
- 7. Experiment on production of citric acid and optimizing parameters for citric acid production in shake flask culture using *Aspergillus niger*.

- 1. Brown TA (1999) Genome. John Wiley & Sons (Asia) PTE. LTD.
- 2. Goeddel DV (1990) Methods in Enzymology, vol 185, Gene Expression Technology. Academic Press, San Diago.
- 3. Kaufman PB, Wu W, Kim D and Cseke LJ (1995) Molecular and Cellular Methods in Biology and Medicine. C. Press, Florida.
- 4. EL-Mansi E.M.T. and Bryce C.F.A. Fermentation Microbiology and Biotechnology. Taylor & Francis.

MBT-302: FERMENTATION TECHNOLOGY

UNIT – I

General Considerations: Fermentation biotechnology – An historical perspective, metabolic pathways and metabolic control mechanisms, primary and secondary metabolites, genetic regulation and catabolite repression, Fermentation kinetics,

kinetics of substrate utilization, product formation.

UNIT - II

Types of bio-reactors, their design and instrumentation: Fed batch bio-reactors, continuous stirred tank bio- reactors, plug flow tubular reactors; multiphase reactors, packed bed, bubble column, fluidized bed and trickle bed bio-reactors, animal and plant cell bio-reactors, non-ideal mixing, batch and continuous sterilization, immobilized bio-catalysts, sensors for medium and gases.

UNIT - III

Industrial production of microbial biomass (SCP, and mushrooms), alcohol, organic acid (citric acid, gluconic acid, itaconic acid), amino acids (L- glutamic acid, L- lysine and L- aspartic acid), enzymes and antibiotics (Penicillin), microbial polysaccharides and polyesters.

UNIT - IV

Scale up, instrumentation control, Bio-sensors in bio-process monitoring and control.

Downstream processing: Removal of microbial cells and solid matter, precipitation,

filtration, centrifugation, disintegration of cells, extraction methods, concentration

methods, purification and resolution of mixtures, drying and crystallization.

Lab Course:

1. Experiment on production of alcohol and optimizing parameters for alcohol production in shakeflask culture using *Saccharomyces cerevisiae*.

- 2. Experiment on production and optimizing parameters for SCP in shake flask culture.
- 3. Experiment on production of enzymes and optimizing parameters for enzyme production in solid-state fermentation using wheat bran and other agricultural solid waste.
- 4. Protein purification methods: affinity chromatography, ion exchange and gel filtration.
- 5. Recovery of products from solid state cultures -Recovery of intracellular products: Celldisruption procedures by sonication,
- 6. Carbohydrate catabolism by microorganisms (oxidation and fermentation of glucose)
- 7. Fermentation of carbohydrates.

- EL-Mansi E.M.T. and Bryce C.F.A. Fermentation Microbiology and Biotechnology. Taylor & Francis.
- Alberghina Lilia. Protein Engineering in Industrial Biotechnology. Harwood Academic Publishers.
- Jogdand S. N. Gene Biotechnology. Himalaya Publishing House.
- Olguin J. Eugenia, Sanchez Gloria & Hernandez Elizabeth. Environmental Biotechnology and Cleaner Bioprocesses. Taylor & Francis.
- Prescott & Dunn's. Industrial Microbiology. 4th ed, CBS publishers & Distributors.
- Bullock John and Kristiansen Bjorn. Basic Biotechnology. Academic Press.
- A.H. Patel. Industrial Microbiology

MBT-303: ENVIRONMENTAL MICROBIOLOGY

UNIT - I

Distribution and ecology of microorganism: airspora- concepts and components, indoor and outdoor air spora, aeroallergens, Ecosystem- concept, components, food chains, food webs, and trophic levels. Energy transfer efficiencies between trophic levels. Environmental factors influencing the growth and survival of microorganism. Physical factors- temperature, light, osmotic pressure and hydrostatic pressure. Chemical factors-pH, O2 and CO2. Microorganisms of extreme environments: Psychrophiles, Mesophiles, thermophiles, acidophiles, alkalophiles, halophiles and specific habitats.

UNIT - II

Microbiology of water: aquatic ecosystems-types- fresh water (ponds, lakes, streams) - marine (estuaries, mangroves, deep sea, hydrothermal vent, saltpans, coral reefs). Zonation of water ecosystems— upwelling- eutrophication- food chain. Drinking and potable water, ecology of polluted water, microbiological treatment processes. Waste water disposal and reclamation. Brief account of major water borne diseases and their control measures.

UNIT - III

Soil microbiology: Micro flora of various soil types (bacteria and nematodes): rhizosphere- phyllosphere- brief account of microbial interactions symbiosis, mutualism, commensalism, competition, amensalism, synergism, parasitism, predation, biological N2 fixing organisms, symbiotic fungi, Phosphate solubilizing organisms, Ecology of litter decomposition; extracellular enzymes (hydrolases), heterotrophic potential

UNIT-IV

Biodegradation of Cellulose, Lignins and hydrocarbons (superbug). Composting, treatment of solid wastes. Bioaccumulation of metals. Biodeterioration: classification of Biodeterioration of materials (monuments, paints, rubbers, plastics, fuels,

Lab Course:

- 1. BOD & COD estimation in water sample
- 2. Study of microbial contaminants from water and wastewater.
- 3. Study of air borne microorganisms using various methods.
- 4. Assay of anti-fungal and antibacterial properties of agro-chemicals and fungicides.
- 5. Assessment of quality of oils using saponification value, iodine number, and free fattyacid composition.
- 6. Study of thermophilic microorganisms.
- 7. Bacteriological examination of water by multiple-tube fermentation test.
- 8. Determination of coliforms to determine water purity using membrane filter method.
- 9. Lipase production test.
- 10. Isolation of Rhizobium from root nodule.
- 11. Measurement of spore size using micrometry
- 12. Isolation of microorganisms from rhizosphere and phylloplane.

- Michael, T. Madican; John. M. Mmmartinko and Jack Parker. Brock. Biology of Microorganisms.
- Microbiology of Extreme Environments edited by Clive Edwards
- Olguin J. Eugenia, Sanchez Gloria & Hernandez Elizabeth. Environmental Biotechnology and Cleaner Bioprocesses. Taylor & Francis.
- Michel. R. Introduction to Environmental Microbiology. 1999

MBT-304: MEDICAL MICROBIOLOGY

UNIT - I

Normal microbial flora of human body, role of resident flora, host microbe interactions. Classification of medically important microorganisms. Infection and infectious process routes of transmission of microbes in the body. Source of infection for man; vehicles or reservoirs of infection. Mode of spread of infection. Pathogenesis: Infectivity and virulence.

UNIT -II

Classification of pathogenic bacteria. Staphylococcus, Streptococcus, Pneumococcus, Neisseria, Corynebacterium, Bacillus, Clostridium, Non sporing Anaerobes, Organism belonging to Enterobacteriacea, vibrios, Non fermenting gram negative bacilli Yersinia; haemophilus; Bordetelia; Brucella; Mycobacteria, Spirochaetes, Actinomycetes; Rikettsiae, Chlamdiae.

UNIT-III

General properties of Viruses; Viruses Host Interactions, Pox viruses, Herpes viruses, Adeno viruses; Picarno viruses; Orthomyxo viruses; Paramyxo viruses; Arboviruses, Rhabdo viruses, Hepatitis viruses; Oncogenic viruses; Human Immuno deficiency viruses.

UNIT-IV

Mycology - Human mycotic infections caused by Dermatophytes, Histoplasma,

Cryptococcus, Candida, opportunistic mycoses. Mycotoxins. Description and classification of pathogenic fungi and their laboratory diagnosis.

Parasitology - Medical importance of Entamoeba, Giardia, Plasmodium, Taenia, Ascaris, Wucherhiria. Laboratory techniques in parasitology.

Lab Course:

- 1. Identification of micro flora of mouth, skin and wounds
- 2. Identification of enteric pathogens by TSIA medium
- 3. Identification of dermatophytic fungi
- 4. Identification of important human parasites
- 5. IMVIC test/other specific tests

- Prescott & Dunn's. Microbiology. CBS Publishers & Distributors.
- Anantnarayan R and Panikar CKJ: Text book of Microbiology, Orient Blackswan Pvt. Ltd.
- Broude AI: Medical Microbiology and Infectious Diseases, WB Saunders Co.
- Chapel and Haeney: Essentials of Clinical Immunology, Blackwell Scientific Publications
- Forbes BA, Sahm DF and Weissfeld AS: Bailey & Scott's Diagnostic Microbiology, Mosby

FOURTH SEMESTER

MBT-401: MICROBIAL BIOTECHNOLOGY

UNIT - I

Techniques of Microbial technology: Scope of genetic engineering, restriction and modification enzymes, ligation and transformation, agarose and polyacrylamide gel electrophoresis, Southern, northern, western blotting, polymerase chain reaction, DNA sequencing, cloning vectors- plasmids, bacteriophages, phagemids, cosmids. YAC, BAC.

UNIT - II

Basics of Genomics, RNA interference, Cloning strategies, cDNA synthesis and cloning, mRNA enrichment, DNA primers, linkers, adaptors and their synthesis, library construction and screening; Cloning interacting genes, two and three hybrid systems, cloning differentially expressed genes, nucleic acid microarrays; Site directed mutagenesis and protein engineering, immobilization techniques.

UNIT - III

Microbial screening, selection and strain improvement, bacterial enterotoxins, peptide hormone, interferons.

Biofertilizers, Biopesticides, Enzyme in pulp and paper industry, Bioremediation.

UNIT-IV

Role of national and international organization in biotechnology, cooperative efforts, government programs for biotechnology development and applications, patenting biotechnological process and products in different fields, regulation for bio-hazardous products.

Lab Course:

1. Bacterial culture and antibiotic selection media. Preparation of competent cells.

- 2. Isolation of plasmid DNA.
- 3. Isolation of Lambda phage DNA.
- 4. Estimation of nucleic acids.
- 5. Agarose gel electrophoresis and restriction mapping of DNA.
- 6. Construction of restriction map of plasmid DNA.
- 7. Cloning in plasmid/phagemid vectors.
- 8. Preparation of single stranded DNA template.
- 9. Gene expression in *E. coli* and analysis of gene product
- 10. PCR

- 1. Bruce A White (1997) PCR Cloning Protocols. Hanuman Press Totowa, New Jersey.
- Bruce Birren, Eric D Green, Sue Klapholz, Trichard M Myers, Horald Riethman, & Jane Roskenus (1999) Genome Analysis: A Lab Manual vol.1,vol.2,vol.3, Cold Spring Harbor Lab. Press.
- 3. Daniel L Hartl, Elizabeth & Jones W (1998) Genetics: Principles and Analysis. Jones & Bartlett Publishers.
- 4. Davies JA & Rez WS (1992) Milestones in Biotechnology Classic papers on Genetic Engineering. Butterworth-Heinemann, Boston.
- 5. Glick Molecular Biotechnology.
- Glover DM and Hames BD (1995) DNA Cloning: A practical approach, IRL Press, Oxford.
- Kaufman PB, Wu W, Kim D and Cseke LJ (1995) Molecular and Cellular Methods in Biology and

MBT-402: ADVANCED IMMUNOLOGY, DIAGNOSTICS AND PROPHYLAXIS

UNIT - I

Generation of diversity in BCR and TCR. Light and heavy chain gene recombination. Recombination Signal sequences. Heavy chain constant region genes. Class switching. Membrane and secreted immunoglobulins. Organization and arrangement of T-cell receptor genes.

UNIT - II

Synthesis and production of immunoglobulins. Monoclonal antibody. Designer antibody. Regulation of immune response by antigen, antibody, immune complex, MHC and cytokines. Autoimmunity and autoimmune disorders. Immunological tolerance. Immunity to microbial and parasitic infections. Immunodeficiency diseases.

UNIT – III

Principles of antimicrobial action and resistance of antibiotics. Antimicrobial susceptibility testing Anti- fungal and anti-cancer compounds. Nosocomial infection, common type of hospital infections and their diagnosis and control.

Immunoprophylaxis: Principles of Vaccination. Immunization practices. Vaccines against important bacterial and parasitic diseases. DNA vaccines; passive prophylactic measures. Viral vaccines and antiviral

UNIT - IV

Diagnosis of microbial diseases - Collection, transport and preliminary processing of Clinical pathogens. Clinical, microbiological, immunological and molecular diagnosis of microbial diseases. Modern methods of microbial diagnosis.

Principles of immunodiagnostics. Antigen-antibody based immunodiagnosis and the techniques involved – Enzyme, Radio and Fluorescence Immunoassays, Immunoblotting, Flow cytometry. Effector cell assays, Cytotoxic assays. Isolation of pure antibody. Application of monoclonal antibodies in immunodiagnostics.

Lab Course:

- Preparation of Parasite/ microbe Antigen and analysis by PAGE
- Immunizations and Production of Antibody
- Antigen antibody reaction by Double Diffusion, Counter Current and Immuno electrophoresis, RID and ELISA
- Western Blot Analysis
- Immunodiagnosis using commercial kits
- VDRL and RPR Test.

- Prescott and Dunn's. Microbiology. CBS Publishers & Distributors
- Anantnarayan R and Panikar CKJ: Text book of Microbiology, Orient Blackswan Pvt. Ltd.
- Broude AI: Medical Microbiology and Infectious Diseases, WB Saunders Co.
- Chapel and Haeney: Essentials of Clinical Immunology, Blackwell Scientific Publications
- Kuby's Immunology: R.A. Goldsby, Thomas J Kindt and Barbara A. Osborne
- Immunology- A short Course: E. Benjamini, R. Coico and G. Sunshine
- Immunology: Roitt, Brostoff and Male
- Forbes BA, Sahm DF and Weissfeld AS: Bailey & Scott's Diagnostic Microbiology, Mosby

Special Paper - MBT-403 (A): FOOD MICROBIOLOGY

UNIT-I

Microbial flora of fresh food, grains, fruits, vegetables, milk, meat, eggs and fish. Microbiological examination of foods for their infestation by bacteria, fungi & viruses. Chemical preservatives and food additives. Factors influencing microbial growth in food-Extrinsic and intrinsic factors. Food as a substrate for micro-organism.

UNIT-II

Canning, processing for heat treatment - D, Z and F values and working out treatment parameters; microbial spoilage of canned foods, detection of spoilage and characterization. Mold and mycotoxin contamination of food, aflatoxins, ochratoxins, trichothenes, zearalenone, ergot mycotoxins. Role of microorganisms in beverages—beer, wine and vinegar fermentation.

UNIT-III

The roles of microorganisms in the food industry, positive and negative perspectives. Food-borne infections and intoxications: Bacteria and nonbacterial-with examples of infective and toxic types- *Brucella, Bacillus, Clostridium, Escherichia, Salmonella, Shigella, Staphylococcus, Vibrio, Yersinia*; nematodes, protozoa, algae, fungi and viruses. Food borne outbreak- laboratory testing procedures; Sources and transmission of bacteria in foods: human, animal, and environmental reservoirs; cross- contamination.

UNIT- IV

Prevention Measures-Food sanitation in manufacture and retail trade; Plant sanitation-Employee's Health standards-waste treatment-disposal- quality control. Government Agency and Food Safety Policy: Government Branches (FDA, CDC, USDA and how they work to control food safety), HACCP, Risk Assessment.

Lab Course:

- 1. Isolation and identification of microorganisms from fermented food, fruits, cereal grains and oil seeds.
- 2. Determination of quality of milk sample by methylene blue reductase test.

- M.R. Adams and M.O. Moss: Food Microbiology, Royal Society, Cambridge
- William, C. Frazier and Dennis C. Westhoff: Food Microbiology, Tata McGraw Hill
- Banwart GJ: Food Microbiology CBS Publishers & Distributors, New Delhi.
- Hobbs BC and Roberts D: Food Poisoning and Food Hygiene, Edward Arnold, London

Special Paper - MBT-403 (B): MICROBIAL ECOLOGY

UNIT-I

History, significance and developments in the field of microbial ecology Contributions of Beijerinck, Winogradsky, Kluyver, Van Niel, Martin Alexander, Selman A. Waksman, Environmental chemistry, Atmosphereic pollutants, Types of wastes, The Atmosphere, Organization of life, Ecosystems.

UNIT-II

Microorganisms & their natural habitats

- A. Terrestrial Environment: Soil characteristics, Soil profile, Soil formation, Soil as a natural habitat of microbes, Soil micro flora
- B. Aquatic Environment: Stratification & Micro flora of Freshwater & Marine habitats. Atmosphere: Stratification of the Atmosphere, Aeromicroflora, Dispersal of Microbes
- D. Animal Environment: Microbes in/on human body (Microbiomics) & animal (ruminants) body.
- E. Extreme Habitats: Extremophiles: Microbes thriving at high & low temperatures, pH, high hydrostatic & osmotic pressures, salinity, & low nutrient levels.

UNIT-III

Succession of microbial communities in the decomposition of plant organic matter Biological Interactions

- A. Microbe–Microbe Interactions: Mutualism, Synergism, Commensalism, Competition, Amensalism, Parasitism, Predation, Biocontrol agents
- B. Microbe–Plant Interactions: Roots, Aerial Plant surfaces, Biological Nitrogen fixation (symbiotic/ nonsymbiotic biofertilizers)
- C. Microbe–Animal Interactions: Role of Microbes in Ruminants, Nematophagus fungi, Luminescent bacteria as symbiont

UNIT-IV

Biogeochemical cycles an introduction

Carbon cycle: Microbial degradation of polysaccharide (cellulose, hemicellulose, lignin, chitin) Nitrogen cycle: Ammonification, nitrification, denitrification & nitrate reduction. Nitrate pollution. Phosphorous cycle: Phosphate immobilization and phosphate solubilization

Lab Course:

Analysis of soil - pH, moisture content, water holding capacity, percolation, capillary action Isolation of microbes (bacteria & fungi) from soil (28°C & 45°C) Isolation of microbes (bacteria & fungi) from rhizosphere and rhizoplane.

Detection (qualitative) of the presence of enzymes (dehydrogenase, amylase, urease) in soil. Isolation of Rhizobium from root nodules of legumes

Isolation of Azotobacter/Azospirillum from soil Isolation of phosphate solubilizers from soil

- Atlas RM and Bartha R. (2000). Microbial Ecology: Fundamentals & Applications. 4th edition. Benjamin/Cummings Science Publishing, USA.
- Atlas RM. (1989). Microbiology: Fundamentals and Applications. 2nd Edition,
 MacMillan Publishing Company, New York.
- Madigan MT, Martinko JM and Parker J. (2009). Brock Biology of Microorganisms.
 12th edition.

Special Paper - MBT-404 (A): AGRICULTURE MICROBIOLOGY

UNIT-I

Structure and characteristic features of the following Biofertilizer organisms: Bacteria: Azospirillum, Azotobacter, Bacillus, Pseudomonas, Rhizobium and Frankia. Cyanobacteria: Anabaena, Nostoc, Fungi: Glomus, Gigaspora, Sclerocystis, Amanita, Laccaria.

Biofertilization processes - Decomposition of organic matter and soil fertility and vermicomposting. Mechanism of Phosphate Solubilization and phosphate Mobilization.

UNIT-II

Biofertilizers – biological nitrogen fixation – Nitrogenase enzyme – symbiotic nitrogen fixation- (Rhizobium, Frankia) – non symbiotic nitrogen fixation (Azotobacter - Azospirillum), VAM- ecto- endo- ectendo mycorrhizae and their importance in agriculture.

UNIT-III

Major Biogeochemical Cycles and the organisms: carbon – nitrogen - phosphorous and sulphur. Biopesticides: toxin from *Bacillus thuringiensis*, *Psuedomonas syringae*. Biological control - use of Baculovirus, protozoa and fungi.

UNIT-IV

Microbial diseases of crop plants: symptoms, causal organisms and control.

Fungal diseases (Late blight of potato, Tikka disease of groundnut, red rot of sugarcane). Bacterial diseases (bacterial blight of rice, citrus canker, Tundu disease of wheat) and Viral diseases (Tobacco mosaic, leaf curl of papaya, yellow vein mosaic of bhindi).

Lab Course:

- 1. Isolation and enumeration of bacteria from different soil type.
- 2. Isolation and enumeration of fungi from different soil type
- 3. Preparation of Winogradsky Column to study the various soil microflora.

- 4. Isolation of Rhizobium from root nodules.
- 5. Isolation of Azotobacter from soil.
- 6. Isolation of Cyanobacteria from paddy field.
- 7. Measurement of pH of soil sample.

Bagyraj and Rangasamy: Agricultural Microbiology

Special Paper - MBT-404 (B): INDUSTRIAL MICROBIOLOGY

UNIT-I

Introduction to industrial microbiology

Brief history and developments in industrial microbiology Fermentation processes Solid-state and liquid-state (stationary and submerged) fermentations; Batch, fedbatch and continuous fermentations

UNIT- II

Bioreactors/fermenters

Components of a typical bioreactor, types of bioreactors-Laboratory, pilot- scale and production fermenters; constantly stirred tank fermenter, tower fermenter, fixed bed and fluidized bed bioreactors and air-lift fermenter.

UNIT-III

Control parameters, industrially important strains, media ingredients Measurement and control of fermentation parameters

Control and monitoring of different parameters in a bioreactor; pH, temperature, dissolved oxygen, foaming and aeration

Isolation of industrially important microbial strains

Primary and secondary screening, strain development, preservation and maintenance of industrial strains Media and ingredients for industrial fermentations

Crude and synthetic media; molasses, corn-steep liquor, sulphite waste liquor, whey and yeast extract.

UNIT-IV

Down-stream Processing

Filtration, centrifugation, cell disruption, solvent extraction, precipitation and ultrafiltration, lyophilization, spray drying

Lab Course:

1. Microbial fermentations for the production and estimation (qualitative and quantitative) of:

(a) Enzyme: Amylase

(b) Amino acid: Glutamic acid(c) Organic acid: Citric acid

(d) Alcohol: Ethanol

(e) Antibiotic: Penicillin

2. A visit to any educational institute/ industry to see an industrial fermenter, and other downstream processing operations.

- Casida LE. (1991). Industrial Microbiology. 1st edition. Wiley Eastern Limited.
- Crueger W and Crueger A. (2000). Biotechnology: A textbook of Industrial Microbiology. 2nd edition. Panima Publishing Co. New Delhi.
- Patel AH. (1996). Industrial Microbiology. 1st edition, Macmillan India Limited.
- Stanbury PF, Whitaker A and Hall SJ. (2006). Principles of Fermentation Technology. 2nd edition, Elsevier Science Ltd.