SWAMI RAMANAND TEERTH MARATHWADA UNIVERSITY NANDED

SyllabuS of M.Sc. InduSt rIa l MIcroblology. 2008-09

Sr. No.	Title of Paper	Paper No.	Marks	Semester No.
1	Introduction to Industrial Microbiology	IMB-101	50	I
2	Microbial Physiology	IMB-102	50	I
3	Metabolic pathways related to Industrial Fermentations.	IMB-103	50	I
4	Microbial Genetics and Strain Improvement Techniques	IMB-104	50	I
5	Bioanalytical Techniques	IMB-201	50	II
6	Industrial Fermentation Technologies	IMB-202	50	II
7	Bioprocessing Engg., & Down Stream processing	IMB-203	50	II
8	Enzyme Technology & Biosensors	IMB-204	50	II
9	Biostatistics & Comp., application in Industrial Fermentation	IMB-301	50	III
10	Microbial Genetics Engg., & its. Industrial application	IMB-302	50	III
11	Bioinformatics , IPR & Patents	IMB-303	50	III
12	Herbal technology	IMB-304	50	III
13	Pharmaceutical Microbiology	IMB-401	50	IV
14	Industrial Food & Dairy Fermentation	IMB-402	50	IV

15	**Environmental Microbiology Industrial Wastes Management & treatment technologies**	**IMB-403**	**50**	**IV**
16	**Project & Seminar/ Implant Training**	**IMB-404**	**50**	**IV**

Total Marks: 800

PRACTICALS

Sr. No.	Title of Practical Papers	Marks
	SEMESTER FIRST PRACTICALS	
1	**Lab course I: Practical's based on Theory paper IMB-101 & IMB-102, At least 6 practical's from each above papers should be completed**	**50**
2	**Lab course II: Practical's based on Theory paper IMB-103 & IMB-104**	**50**
	NOTE: LAB COURSE I&II ARE BASED ON ABOVE 4 THEORY PAPERS. THERE SHOULD BE ATLEAST SIX PRACTICALS BASED ON EACH THEORY PAPER.	
	SEMESTER SECOND PRACTICALS	
3	**Lab course III: Practical's based on Theory paper IMB-201& IMB-202**	**50**
4	**Lab course IV: Practical's based on Theory paper IMB-203 & IMB-204**	**50**
	NOTE: LAB COURSE III&IV ARE BASED ON ABOVE 4 THEORY PAPERS. THERE SHOULD BE ATLEAST SIX PRACTICALS BASED ON EACH THEORY PAPER.	
	SEMESTER THIRD PRACTICALS	
5	**Lab course V: Practical's based on Theory paper IMB-301 & IMB-302**	**50**
6	**Lab course VI: Practical's based on Theory paper IMB-303 & IMB-304**	**50**
	NOTE: LAB COURSE V&VI ARE BASED ON ABOVE 4 THEORY PAPERS. THERE SHOULD BE ATLEAST SIX PRACTICALS BASED ON	

	EACH THEORY PAPER.	
	SEMESTER FOURTH PRACTICALS	
7	**Lab course VII: Practical's based on Theory paper IMB-401 & IMB-402. There should be at least 15 practical's based on the above three papers{ 5 each}**	**50**
8	**Lab course VIII: Practical's based on Theory paper IMB-403 & IMB-404. Project submission (One Month duration)**	**50**
	NOTE: LAB COURSE VII&VIII ARE BASED ON ABOVE 4 THEORY PAPERS. THERE SHOULD BE ATLEAST SIX PRACTICALS BASED ON EACH THEORY PAPER.	

PAPER (IMB-101)

Introduction to Industrial Microbiology

Unit-I; History and Scope of Industrial Microbiology: Introduction; Discovery of Microbial world; The experiments of Pasteur; The discovery of Anaerobic Life; Physiological significance of Fermentation; Pasteur and Fermentation; The Era of discovery of Antibiotics; Growth of Industrial fermentations; The Chronological development of Fermentation industry.

Unit-II: General properties of Microorganisms: Fungi; Algae; Actinomycete; Mycoplasam and Viruses, With ref. to. Properties, Isolation, Classification, Cultural characteristic, Biochemical characteristics.

Unit -III:- **Bacterial Metabolism**: Definition of Metabolism, Catabolism, Anabolism. Modes of ATP generation, Structure of ATP, Respiration: EMP, HMP, and ED pathways, Kreb's cycle, Oxidative phosphorylation. Bacterial Photosynthesis: Autotrophs & heterotrophs. Brief account of photosynthetic and accessory pigments – chlorophyll – fluorescences, phosphorocenses - bacteriochlorophyll – rhodopsin – carotenoids – phycobilliproteins; Carbohydrates – anabolism – autotrophy – oxygenic – anoxygenic photosynthesis – autotrophic generation of ATP; fixation of CO_2 – Calvin cycle – C3 – C4 pathways. Chemolithotrophy – sulphur – iron – hydrogen – nitrogen oxidations. RETC in bacteria's. Bacterial fermentations: Def., Types of Fermentations: Alcohol, Mixed acid, Lactic acid etc.

Unit -IV: Microbial Metabolic Diversity: Definition, Introduction to metabolic diversity: Pathogenic & Non-Pathogenic Microorganisms Two-Component Signaling Systems (anaerobiosis, Arc & FNR systems) Synthesis of Acylated Homoserine Lactones by LuxI-Type Proteins. Introduction to Bacterial Development & to Quorum Sensing. Quorum Sensing in *Pseudomonas aeruginosa.* Introduction to Biofilms and Disease: Involvement of Host Factors in Regulating the Lux System of *V. fischeri.* Regulation of Pathogenicity in *Staphylococcus aureus*. Induction of Apoptosis by Microbial Pathogens :Genetically-Programmed Signal Transduction of Cell Death Signals. Induction of Apoptosis by Microbial Pathogens

UNIT- V: **Nanotechnology** : Definition of nano scale with reference to biosystems, Scope and future prospects. Scanning probe instrument, spectroscopy, electron microscopy, molecular resolution of bio/in-organic materials, chemical imaging by scanning force microscopy, the quantification of adhesion forces between molecules, positioning signal atoms using proximal probes and finally either destructive or constructive molecular manipulations. Manipulation of matter at the molecular level to create new products with atom by – atom precision. Molecular synthesis, Self assembly, Polymerisation, Nanoscale lithography, e-beam lithography, Heterogeneous nano structure and composites, nanoscale biostructres . Atomic force microscopy, DNA-scaffolds, polymer nano-electronics and nano-colloids.

Reference:

1.Cappuccino and James, G (1996) Microbiology a laboratory manual, Addison Wesley Publishing Company Inc. 4th edition, England, California.

2.Gerhardt, P., Murray, R.G., Wood, W.A. and Kreig, N.R. (1994) Methods of General and Molecular Bacteriology, Ed. American Society for Microbiology, Washington D.C.

3.David R. Brooke. Bergey's Manual of Systematic Bacteriology (Vol. I), Eastern Halz, Springer Publication.

4.James T. Stanley, Marvin P. Bryant. Bergey's Manual of Systematic Bacteriology (Vol. II), Nobert Pfeming Springer Publishers.

5.Wilson K. Walker (1995). Practical Biochemistry, Principles and Techniques, Cambridge University Press.

6.M.Ratner and D.Ratner, Nanotechnology –a gentle introduction to the next big idea, Pearson education 2007.

7.R.R.Birge, Protein based computers, Scientific American , 1995.

8. L.E.Foster, Nanotechnology-Science, Innovation and opportunity , Person education inc, 2007

9. Nanotechnology by Mark Ratner and Daniel Ratner, Pearson Education

PAPER (IMB-102)

MICROBIAL PHYSIOLOGY

UNIT-I: **Principles of Microbial Nutrition:** Bacterial nutrition: Basic nutritional requirements, nutritional classification, Structure and organization of membrane (Glyco-conjugants and proteins in membrane systems), fluid mosaic model of membrane. Methods to study diffusion of solutes in bacteria, passive diffusion, facilitated diffusion, different mechanisms of active diffusion (Proton Motive Force, PTS, role of permeases in transport, different permeases in E. coli.

UNIT-II: **Metabolite & Ion Transport**: Introduction, Transport of amino acids and inorganic ions in microorganisms and their mechanisms. Mitochondrial cation & metabolite transport, uncoupling protein family, bacterial transport, Transport(movement) of bacterial cell, transport of macromolecules across bacterial membranes. RETC & electron transfer systems.

UNIT-III: MICROBIAL GROWTH: Introduction, phases of growth, growth curve, kinetics of growth, measurement of growth, continuous & batch culture, synchrony, chemostat& turbidostat.

UNIT-IV : YEAST & SOLVENT TOLERANC IN MICROBES: Cytology and Physiology of wine Yeasts: types of wine yeast, the biology and cytology of yeasts, alternative nuclear phase and alternative life cycles of yeast, homothallism and heterothallism. morphology of yeast during

vegetative multiplication, sexual reproduction and spore formation, colonial morphology, formation of pseudo and true mycelia of yeast, active dry /compressed yeast. Habitat & distribution of solvent tolerant organisms, genes for solvent tolerance; Partition coefficient log P as an index of solvent tolerance in Microorganisms.

UNIT V: KINETICS OF MICROBIAL GROWTH AND PRODUCT FORMATION:

Phases of cell growth in batch cultures; Simple unstructured kinetic models for microbial growth, Monod model, Growth of filamentous organisms. Growth associated (primary) and non - growth associated (secondary) product formation Kinetics. Leudeking-Piret models, substrate and product inhibition on cell growth and product formation

PAPER (IMB-103)

METABOLIC PATHWAYS RELATED TO INDUSTRIAL FERMENTATIONS

Unit -I: **Chemolithotrophic & Phototrophic metabolism:** Hydrogen Oxidising Bacteria, Carbon mono oxidizing, Sulphur oxidizing, iron oxidizers four families of phototrophic bacteria.

Unit-II: METABOLIC STOICHIOMETRY: Microbial fermentations, Classification of fermentations: Introduction to Structured Models for growth and product formation: metabolic basis of oxygen limited growth.
Stoichiometry of Cell growth and product formation, elemental balances, degrees of reduction of substrate and biomass, available electron balances, yield coefficients of biomass and product formation, maintenance coefficients.

Energetic analysis of microbial growth and product formation, oxygen consumption and heat evolution in aerobic cultures, thermodynamic efficiency of growth.

Unit –III: Metabolic pathways: Strategies for redirecting branched and linear pathways; Auxotrophs; Protoplasts fusion; recombinant Microorganisms. Regulation mechanisms in microorganisms and their role in the production of primary metabolites: Amino acid synthesis pathways and its regulation at enzyme level and whole cell level, Alteration of feed back regulation, Limiting accumulation of end products.
Regulation mechanisms in microorganisms and their role in the production of secondary metabolites : Regulation of secondary metabolite pathways,

precursor effects, prophase, idiophase relationship, Catabolite regulation by passing control of secondary metabolism, producers of secondary metabolites, applications of secondary metabolites.

Unit –IV: **Production pathways & Bioconversions**: Role of Biotin: MSG production; Penicillin pathway; antibiotic resistance and new antibiotics, Bacteriocin production pathway, polysaccharide production pathways, Applications of Bioconversions, Factors affecting bioconversions, Specificity, Yields, Co metabolism, Product inhibition, mixed or sequential bioconversions, Conversion of insoluble substances.

Unit –V: **Applications of Metabolic Engg**.: Redirecting metabolic pathways through genetic engineering. Microarray techniques and transcriptional profiling, Yeast, bacteria, examples of Xylose to ethanol; 1,3 propanediol from glucose; acrylamide by bioactive process. Metabolic map of E. coli Metabolic engineering Signal transduction pathways Signal Transduction to Tyrosin Kinase Receptor tyrosine kinases, G-protein coupled receptors Ultrasensitivity in the MAP kinase cascade Proliferative, survival and death pathways. Applications in pharmaceuticals, chemical bioprocess, food technology, agriculture, environmental bioremediation and biomass conversion.

TEXT BOOKS:

1. Wang.D.I.C Cooney C.L., Demain A.L., Dunnil.P. Humphrey A.E. Lilly M.D. Fermentation and Enzyme Technology, John Wiley and sons 1980.

2. Stanbury P.F., and Whitaker A., Principles of Fermentation Technology, Pergamon Press, 1984.

3.Zubay G., Biochemistry, Macmillan Publishers, 1989.

PAPER (IMB-104)

Microbial Genetics and strain iMproveMent technoloGy

Unit. I: Microbial Genetics: Basics of bacterial, Phage and fungal genetics, methods in microbial genetics: mutagenesis and screening, strain improvement, DNA/ RNA Structure, replication, repair.

Unit –II :Microbial Strain improvement: Strain improvement for the selected organism: mutation and screening of improved cultures, random and strategic screening methods, strategies of strain improvement for primary, secondary metabolites with relevant examples. Use of recombinant DNA technology, protoplast fusion techniques for strain improvement of primary and secondary metabolites. Production of recombinant molecules in heterologus system, problems associated with strain improvement programme, improvement of characters other than products and its application in the industry. Preservation of cultures after strain improvement programme.

Unit-III :Genetic Engineering and Strain Improvement

Studies of auxotrophs, strain improvement improvement by UV/Chemicals method, Selection of improved Strain/Cell line, studies of phenotypic characteristic of mutantant and their comparison with wild strain.

Extraction and purification of plasmid DNA. Bacterial transformation and identification of recombinant colonies. Preparation and transformation of

competent cells, preparation of agarose gel, Restriction enzyme analysis, Identification of recombinant clones

Unit IV : GENETIC ENGG., of Microbial Systems : Microbial production of therapeutic agents: Pharmaceuticals – Isolation of interferon cDNAs; Engineering human interferon and human growth hormone; optimizing gene expression. Enzymes – DNAase I and Alginate lyase against cystic fibrosis. Monoclonal antibody as therapeutic agents – Production of antibodies in *E.coli*, HIV therapeutic agents. Vaccines: Subunit vaccines – Herpes simplex virus, Foot and mouth disease, Tuberculosis, Peptide vaccines, Genetic immunization, Attenuated vaccines – Vector vaccines.

Unit –V : **Gene Regulatory Networks (GRNs**) : The film switch in *E. coli* illustrating the dynamic nature of the genome, Transcriptional network in *S. cerevisiae*, Mathematical modeling and computer simulation of GRNs(Boolean network, Stochastic master equation), Engineering & Control of GRNs(Perturbation of GRNs: gene knock outs/knocks ins, RNAi).

PAPER (IMB-105)

BIO ANALYTICAL TECHNIQUES

Unit -I.: Basic laboratory Instruments: Principle and working of pH meter, Laminar-air flow. Centrifugation: Types of centrifuge machines, preparative and analytical centrifuges, differential centrifugation, sedimentation velocity, sedimentation equilibrium, density gradient methods and their applications; Dialysis, Ultra filtration, Seitz filter,

Unit – II: Bioreactors & Microscopy: Bioreactor: Introduction, definition, types, Design and analysis of biological reactors. Analysis of complex microbial populations. Stability of bioreactor operation with complex population. Batch and continuously reactors.

Phase contrast Microscopy, confocal microscopy Fluorescent Microscopy, Electron Microscopy, Scanning Ion Conductance Microscopy, Video Micrography, Atomic force Microscopy. Flow Cytometry.

Unit –III: Electrophoretic techniques: Basic principles of electrophoresis, theory and application of paper, starch gel, isoelectric focusing. Agarose gel electrophoresis, polyacrylamide gel electrophoresis (native and SDS-PAGE), isoelectric focusing and 2-Dimensional polyacrylamide gel electrophoresis and their uses in protein research; PCR

Unit –IV: Chromatographic techniques and Spectroscopy: Theory, principles and applications of paper, thin layer, gel filtration, ion exchange, affinity, hydrophobic, gas liquid, high pressure/ performance liquid chromatography (HPLC), FPLC, gel permeation chromatography.

Spectroscopic techniques, theory and applications of UV, Visible, IR, NMR, Fluorescence, Atomic Absorption, Mass spectroscopy, CD, ORD, Mass, Raman Spectroscopy, ESR principles - instrumentation-applications, Beer-Lambert's law, Use of NMR in elucidation biosynthesis pathways.

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

Reference:-

1. Spectrometric identification of Organic Compounds, Robert. M. Silverstein et al, 7th Edition, 1981.
2. Principles of Instrumental Analysis by Donglas A. Skoog, James, J. Leary, 4th Edition.
3. Pharmaceutical Analysis – Modern Methods – Part A, Part B, James W. Munson – 2001.
4. Vogel's Text Book of Quantitative Chemical Analysis, 6th Edition, 2004.
5. Chromatographic Analysis of Pharmaceuticals, John A. Adamovic, 2nd Edition.
6. Practical Pharmaceutical Chemistry, Part two, A. H. Beckett & J. B. Stenlake – 4^{th} Edition.
7. Instrumental Methods of Chemical Analysis – B. K. Sharma - 9th Edition.
8. Instrumental Methods of Analysis – Hobert H. Willard, 7th Edition.

9. Organic Spectroscopy – William Kemp, 3rd Edition.
10. Techniques and Practice of Chromatography – Raymond P. W. Scott, Vol. 70.
11. Identification of Drugs and Pharmaceutical Formulations by Thin Layer
12. Chromatography – P. D. Sethi, Dilip Charegaonkar, 2nd Edition.
13. HPTLC – Quantitative Analysis of Pharmaceutical Formulations – P. D. Sethi.
14. Liquid Chromatography – Mass Spectrometry, W. M. A. Niessen, J. Van Der Greef, Vol. 58.
15. Stereo Chemistry – Conformation and Mechanism by P. S. Kalsi, 2nd Edition.
16. Spectroscopy of Organic Compounds by P. S. Kalsi.
17. Instrumental Methods of Analysis. 6th Edition by H.H. Willard, L.L. Merritt Jr. and others. 1986. CBS Publishers and Distributors.
18. Instrumental Methods of Chemical Analysis. 1989 by Chatwal G and Anand, S. Himalaya Publishing House, Mumbai.
19. A Biologists Guide to Principles and Techniques of Practical Biochemistry. 1975 by Williams, B.L. and Wilson, K.
20. Spectroscopy. Volume 1. Edited by B.B. Straughan and S. Walker. Chapman and Hall Ltd.
21. Gel Electrophoresis of Proteins- A Practical Approach by Hanes.
22. Chromatography: Concepts and Contrasts- 1988 by James Miller. John Wiley and Sons. Inc., New York.
23. Analytical Biochemistry by Holme.

24. Introduction to High Performance Liquid Chromatography by R. J. Hamilton and P. A. Sewell.
25. Spectroscopy by B.P. Straughan and S. Walker.
26. Practical aspects of Gas Chromatography and Mass Spectrometry 1984 by Gordon M. Message, John Wiley and Sons, New York.
27. Gel Chromatography by Tibor Kremmery. Wiley Publications.
28. Isotopes and radiations in Biology by C.C. Thornburn, Butterworth and Co. Ltd., London.
29. The use of radioactive isotopes in the life sciences by J.M.Chapman and G.Ayrey, George Allen and Unwin Ltd., London.
30. .D.Campbell and R.T.Dwek, Biological Spectroscopy, Benjameer Cunmeib & Co., 1986
31. F. Settle. Handbook of Instrumental Techniques for Analytical Chemistry, Prence Hall, 1997
32. W.Botton, Instrumentation and Process Measurements, University Press, 1993.

PAPER (IMB-106)

INDUSTRIAL FERMENTATION TECHNOLOGY

UNIT –I :WINE TECHNOLOGY: Grape biochemistry, Varieties of wine grapes Sanitary and phytosanitary measures of wine grapes, Metabolism of organic acid and other metabolites during wine production from grapes: organic acids in grapes, organic acids from fermentation, different types of acidity (total, volatile, acidity and pH), and buffer capacity of must and wine, acidification and de-acidification of wine, tartarate crystallization and precipitation. Principles of fermentations: Design, layout and operation of fermentors, types of fermentors, inoculums, seeds, fermentation and downstream processing.

Operation of Fermentor: Media composition and preparation, media sterilization, control of fermentation parameters, periodic sampling and observing sterility, control of foaming by addition of antifoam agent or mechanical means, Enrichment shot with dilution for maximum yield, etc.

Unit -II **: METABOLITE PRODUCTION: Production of Primary Metabolites:** A brief outline of processes for the production of some commercially important Organic acids (e.g. citric acid, lactic acid, acetic acid, gluconic acid,); Amino acids (Glutamic acid, lysine, aspartic acid &Phenylalanine); and Alcohols (ethanol, 2,3- butanediol)

Secondary Metabolites : Study of production processes for various classes of low molecular weight secondary metabolites: Antibiotics-beta-lactams (Penicillin), semi synthetic Pencillins and Cephalosporins amino-glycosides

(streptomycin), macrolids (erythromycin), quinines, and aromatics. Vitamin (B12) and Steroids, dual or multiple fermentation.

Unit -III: Production of Commercially Important Enzymes & Recombinant Proteins:

Proteases, Amylases Lipases, Cellulases, Pectinases, Isomerases and other commercially important. Enzymes for the food pharmaceutical industries.

Production of recombinant proteins (Insulin, Interleukin & Interferon's) having therapeutic and diagnostic applications; production of vaccines.

UNIT IV : Microbially enhanced recovery of minerals resources, Bioleaching of metals, oil recovery, Mushroom cultivation, microbial insecticides &pesticides**,** Production of bacterial vaccines: Preparation of toxoid from a toxin.

Unit V : Bio products and other Processes: Natural Biopreservatives (Bacteriocin/Nisin), and Biopolymers (PULLULAN/Xanthan Gum and PHB); Single Cell Protein, , Steroid Bioconversions; Bioconversion of Vegetable Oils, Bioleaching. Microbial processes for the production of energy from agricultural Waste. Production of chemical solvents by microorganisms – the acetol- butanol fermentation. Production of biochemical from microalgae.

REFERENCES:

1.Microbiology: - Prescott and Dunn.

2.Microbial biotechnology: Glazer, A.N. and Nikaido, H. 1995 W.H. Freeman &Company, New York.

3. Industrial Microbiology:- A. H. Patel.. Stanier R. Y., Ingram J.L., Wheelis M.L., Painter R.R., General Microbiology McMillan Publications, 1989.

4. Foster C.F., Ware D.A., Environmental Biotechnology, E. Horwood Ltd.,1987.

5. Karrely D., Chakrabarty K., Omen G.S., Biotechnology and Biodegradation,

6. Advances in Applied Biotechnology Series, Vol.4, Gulf Publications Co. London, 1989.

7. Bioremediation engineering; design and application 1995 J. T. cookson,Jr. Mc Graw Hill, Inc.

PAPER (IMB-107)

BIOPROCESSING ENGINEERING & DOWN STREAM PROCESSING

Unit I: Introduction to Bioprocesses: Historical development of bioprocess technology, an overview of traditional and modern applications of biotechnology industry, outline of an integrated bioprocess and the various (upstream and downstream) unit operations involved in bioprocesses, generalized process flow sheets.

Unit–II Reactor Engineering: Design of a basic fermentor, bioreactor configuration, design features, individual parts, baffles, impellers, foam separators, sparger, culture vessel, cooling and heating devices, probes for on- line monitoring, computer control of fermentation process, measurement and control of process. Batch operation of mixed reactor.

Reactors for specialized applications: Tube reactors, packed bed reactors, fluidized bed reactors, cyclone reactors, trickle flow reactors, their basic construction and types for distribution of gases. Behavior of microbes in different reactors (air lift, fluidized, batch, continuous fed conditions), Role of shear in stirred fermentors

Unit – III: Mass Transfer : Definition of binary mass transfer coefficient, convective mass transfer, Liquid-Solid mass transfer, Liquid –liquid mass transfer, gas-liquid mass transfer, oxygen uptake and transfer in fermentor – bubbles, oxygen transfer from gas bubble to aerobic culture, factors affecting oxygen transport- sparging, stirring, medium properties, antifoam agents, temperature, determination of Kla, aeration/agitation, its importance

Molecular diffusion, theory of diffusion, Role of diffusion in bioprocessing, Film theory. Heat transfer: Heat transfer between fluids, design equation for heat transfer systems. Reynolds number of fluid flow & mixing, rheological properties of fermentation broths

Unit –IV: Media Design & sterilization: Medium requirements for fermentation processes, Carbon, nitrogen, minerals, vitamins and other complex nutrients, oxygen requirements, medium formulation for optimal growth and product formation, examples of simple and complex media, design and usage of various commercial media for industrial fermentations.

Thermal death kinetics of microorganisms, batch and continuous heat. Sterilization of liquid media, filter sterilization of liquid media, Air. Design of sterilization equipment.

Unit –V: **DOWNSTREAM PROCESSING :** Role and importance of downstream processing in industrial Microbiological processes. Problems and requirements of bioproduct purification. Economics of downstream processing in industrial microbiology, cost-cutting strategies, characteristics of biological mixtures, process design criteria for various classes of bioproducts (high volume, low value products and low volume, high Value products)

Physico-chemical basis of bio-separation processes. cell distrubtion methods for intracellular products removal(flocculation, sedimentation, centrifugation and filtration methods. Membrane-based separations (Micro & ultrafiltration) theory, design, and configuration of membrane. precipitation methods(with salts, organic solvents, and polymers, extractive separations, aqueous two phase extraction, integrated bioprocessing.

Electrophoresis of proteins and nucleic acids, 1D-2D gels, chromatofocusing electrophoretic separations, Distillation, Dialysis, crystallization, evaporation, HPLC, FPLC, GC,TLC, ION exchange, Gel filtration, Super liquid extraction foam based separation case study with examples for bioprocessing of Two Industrial products(Citric acid/Penicillin and low volume high values products like recombinant proteins) Whole broth processing, Detection and estimation of fermentation products, Scale up of fermentations, Basic concepts of quality assurance practices – GMP, cGMP

REFEREENCES;

1. Wankat PC. Rate controlled seperations, Elsevire, 1990.
2. Belter PA & Cussler E. Bioprocessing, Wiley 1985.
3. Product recovery in Bioprocess Technology. BIOTOL. Series VCH 1990.
4. Asenjo J.M. Seperation Processes in Biotechnology, 1993, Marcel Dekkere INC.
5. M.R. Ladisch, Bioseperation Engg.: Principls, Practice and economy, wiley Interscience 2001.
6. S.N. Mukhopadhyay Process Biotechnology fundamentals Viva Books Pvt. LTD. 2001.
7. . P.M.Doran Bioprocess Engineering Principles, Academic Press 1995

PAPER (IMB-108)

EnzymE TEchnology & Biosensors

Unit I : Introduction to Enzymology , Extraction and purification

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

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

Unit II : Production of Microbial enzymes:

Production of Glucose isomerase, Alpha-amylase, Lipase, Xylase, Pectinases, Proteases Trypsin , Beta-galactosidase and Cellulase and other commercially important. Enzymes for the food pharmaceutical industries.

Production of recombinant proteins (Insulin, Interleukin & Interferon's) having therapeutic and diagnostic applications; production of vaccines.

Unit III : Enzyme Immobilization and Design of Enzyme Reactors:

Physical and Chemical techniques for enzyme Immobilization - adsorption. Matrix entrapment, encapsulation. cross-linking. covalent binding - examples; Advantages. Mass Transfer Effects in Immobilized Enzyme Systems.

Analysis of Film and Pore Diffusion Effects on kinetics of Immobilized Enzyme. Reactions; Formulation of dimensionless groups and calculation of Effectiveness Factors, Thiele modulus and disadvantages of different Immobilization techniques. overview of applications of immobilized enzyme systems, effect of pH, temperature on immobilized reaction kinetics.

Unit – IV: Protein Engineering : Introduction : Design and construction of novel proteins and enzymes, Conformation of proteins in general and enzymes in particular, Effect of amino acids on structure of proteins, Energy status of a protein molecule, Structure function relations of enzymes, Physical

methods such as x-ray crystallography for determination of protein structure, Site directed mutagenesis for specific protein function, Basic concepts for design of a new protein/enzyme molecule, Specific examples of enzyme engineering.

Protein architecture and structure and function relationship. Functional Properties of microbial proteins. Gene shuffling and chimeric enzymes. *In vitro* directed evolution of enzymes and other proteins. Over expression and folding of proteins. Proteins post-translational modification, stability and folding, Applications of protein Engineering.

Unit – V: Biosensors: Definitions, history and market needs. Target analytes. Sensors based on: enzymes, affinity and whole-cells. Transducers: electrodes, photometric and acoustic. Immobilizations techniques: thin films, micro & nano-structures. Invasive, non- invasive, and disjointed sensors . Continuous vs. discontinuous monitoring . Pitfalls. Quality control. Signal processing. Case studies: immunosensors. Novel transducers and synthetic receptors. Clinical, environmental, industrial and military applications. Future prospects.

References

1. Methods in Enzymology. Volume 22 - Enzyme purification and related techniques. Edited by William B. Jakoby. Academic Press, New York.

2. Allosteric Enzymes - Kinetic Behaviour. 1982. by B.I. Kurganov. John Wiley and Sons. Inc., New York.

3. Biotechnology. Volume 7 A - Enzymes in Biotechnology. 1983 Edited by H. J. Rehm and G. Reed. Verlag Chemie.

4. Hand Book of Enzyme Biotechnology by Wiseman.

5. Enzymes as Drugs Edited by John S.Holcenberg and Joseph Roberts , John Wiley & Sons New York.

6. Methods of Enzymatic Analysis by Hans Ulrich, Bergmeyer, Academic Press.

7. Methods in Enzymology by W.A. Wood, Academic Press.

8. Advances in Enzymology by Alton Meister, Interscience Publishers.

9. Topics in Enzyme and Fermentation Biotechnology by L.N. Wiseman, John Wiley and Sons.

PRACTICALS

LAB COURSE- I

MARKS 50

Introduction to Industrial Microbiology & Microbial Physiology(PAPER IMB101 & IMB102)

1. ISOLATION OF FUNGI, CYANOBACTERIA, BACTERIOPHAGE**.**

2. LACTOPHENOL COTTON BLUE FUNGAL STAINING**.**

3. ISOLATION OF PHTOSYNTHETIC BACTERIA**.**

4. ISOLATION OF EXTREMOPHILS i.e. ALKALOPHILES, ACIDOPHILES, PSYCHROPHILES, THERMOPHILES, AND THEIR MAINTAINANCE**.**

5. ISOLATION OF BACTERIOCIN PRODUCERS, PHOSPHATE SOLUBILIZERS.

6. MUTANT ISOLATION BY GRADIENT PLATE TECHNIQUE

7. DEMOSTRATION OF LYSOGENY IN *E.coli***.**

8. MEASUREMENT OF AMMONIA, NITRATE& NITRITE UPTAKE BY MICROORGANISMS

9. EVOLUTION OF OXYGEN IN LIGHT AND UPTAKE OF OXYGEN IN DARK BY MICROORGANISMS.

10. PHOTOSYNTHETIC ELECTRON TRANSPORT BY 2-6-DICHLOROPHENOL INDOPHENOL REDUCTION TEST

11. DETERMINATION OF SUGAR/GLUCOSE TRANSPORT IN *S.cerevisiae*

*1***2.** Isolation and Characterization of an Aniline-Degrading Bacterium.

13. Isolation, Characterization and Biological Activities of Actinomycete.

14. Determine of growth curve and growth inhibition**.**

15. Cultivation of Anaerobes**.**

16. Estimation of Calcium ions present in sporulating bacteria by EDTA method.

17. Glucose uptake by Yeast and Other bacteria**.**

18. Ultraviolet irradiation survival curve.

19. Isolation and enumeration of bacteriophage, one-step growth curve and burst size determination.

20. Chemical analysis of bacterial cell.

21. Control of micro organism by physical and chemical factors; effects of pH, osmotic factors.

22. Isolation of Yeast and study of its characters.

23. Demonstration Chemical mutagenesis

24. Isolation of mutants: Antibiotic resistant, Azide resistant, Pigment, Respiratory deficient, Thymine requiring.

25. Induction, isolation and analysis of auxotrophic mutant.

LAB COURSE- II

Metabolic pathways related to Industrial fermentations & Microbial genetics & strain improvement.(PAPER IMB 103 & IMB 104)

Marks 50

1. Isolation of DNA from Bacteria, & Soil.
2. Determination of purity of the isolated DNA by UV spectrophotometer.
3. Quantification of DNA

a. Spectroscopic method (UV absorption method)

b. Colorimetric method (Diphenylamine reagent)

4. Thermal denaturation of DNA and demonstration of hyperchromic effect.
5. Determination of melting temperature (Tm) and estimation of GC content
6. Extraction & purification of RNA from Yeast
7. Estimation of RNA .
8. Agrobacterium culture, selection of transformants, reporter gene (GUS) assays.

9. Design of PCR primer.

10. Agarose gel electrophoresis – Separation and molecular size determination of DNA

11. Purification of DNA fragment from Agarose gels

a. Electro elution

b. Affinity purification

12. Isolation of Lac mutants

13. Isolation of Lambda phage DNA

14. Isolation of industrially important microorganisms for microbial processes (citric / lactic) and improvement of strain for increase yield by mutation.

15. DNA isolation from filamentous fungi

Lab course III

Bioanalytical techniques & Industrial fermentation technology

(PAPER IMB 201 & IMB 202) Marks 50

1. Titration of a weak acid using a pH meter, Determination of pKa value, preparation of buffers

2. Verification of Beer-Lambert's law and determination of absorption coefficients

3. Paper chromatography – Separation of amino acids and carbohydrates in a mixture

4. Thin layer chromatography of fatty acids, sugars, amino acids, toxins

5. Electrophoresis

6. Demonstration of viable cells using phase contrast microscopy.

7. Estimation of different macromolecules , SUGARS, PROTEINS, & AMINO ACIDS by visible spectrophotometer.

8. Estimation of turbidity using UV-VIS spectrophotometer.

9. Estimation of proteins & nucleic acids by U.V. method.

10. Production of Citric acid, Lactic acid, alcohol.

11. Production of Antibiotics Penicillin Streptomycin, rifamycin

12. Production of bread and yoghurt

13. Bioleaching of copper.

14. Production of Microbial Insecticide

15. Mushroom cultivation and its analysis.

16. Production of wine from grapes**:**

1. Determination of P^H, TSS (^{0}Brix), titrable acidity of wine.
2. Determination of alcohol (ethanol) percentage of wine by Ebulliometry.
3. Estimation of Total/free SO_2 in wine / juice / must by Ripper titrametric method.
4. Protein stability test / Heat stability test of wine
5. Lab scale and pilot scale production of wine from different fruits.
6. Tartarate and bitartrate stability test / Cold stability test of wine.
7. Determination of Acetaldehyde content, phenol content of wine by titrametric method.
8. Sensory analysis of hydrogen sulphide and Mercaptans in wine.
9. Methanol estimation/ alcohol estimation by specific gravity method.

10. Estimation of reducing & total sugar by copper reduction technique.
11. Determination of total tannin content by visible spectrometry.
12. Visit to nearby winery and Wine Park.

LAB COURSE- IV

Bioprocessing Engg., & Down Stream processing Enzyme Technology & Biosensors (paper IMB 203 & IMB 204) Marks 50

1. BIOPROCESS ENGINEERING LAB

1. SCREENING OF PROCESS VARIABLES: Plackett-Burman design practice

2. DEMONSTRATION OF REACTOR STUDIES

Batch, fed-batch, and continuous flow reactor analysis and residence time distribution.

1. Determination of Thermal Death Point (TDP) and Thermal Death Time (TDT) of microorganisms for design of a sterilizer.

2. Monitoring of dissolved oxygen during aerobic fermentation.
3. Preservation of industrially important bacteria by lyophilization.
4. Product concentration by vacuum concentrator
5. Cell disruption for endoenzymes by sonication.

2. **DOWNSTREAM PROCESSING LAB**

Cell disruption techniques.

Solid separation methods-filtration, sedimentation, centrifugation, product enrichment operations, precipitation, ultra filtration, two-phase aqueous extraction, high-resolution purification, preparative liquid chromatographic techniques, product crystallization and drying.

3.Enzyme Technology & Biosensor LAB

1. Isolation and purification of Amylase(Bacterial/Fungal)
2. Time course of enzymatic reaction
3. Influence of substrate concentration on the rate of enzymatic reaction
4. Effect of pH and temperature on the rate of enzyme reaction
5. Inhibition of enzyme activity. Determination of Ki values
6. Molecular weigh determination of enzyme by gel filtration
7. Isozyme detection
8. Immobilization studies:

(a) Preparation of urease/Amylase entrapped in alginate beads and determination of percent entrapment

(b) Study of the kinetics of the rate of Starch hydrolysis by Amylase entrapped alginate beads

(c) Study of reusability and storage stability of Amylase entrapped alginate beads

(d) Immobilization of urease/Amylase by covalent attachment to solid support.

FACULTY OF SCIENCE

M.Sc. induStrial MicrOBiOlOGY i SEMESTER

PRACTICAL EXAMINATION

PRACTICAL PROFORMA

TIME: 11.00 A.M TO 5.00 P.M	MARKS:50

Q.1 Major question (LAB I -101/ /LABII-103). 18 Marks

OR

a) Major question. 10 Marks.
b) Minor question. 08 Marks.

Q.2 Major question (LAB I-102 /LABII-104). 18 Marks.

OR

a) Major question. 10 Marks.
b) Minor question. 08 Marks.

Q.3 Viva Voce. 10 Marks.

Q.4 Record Book. 04 Marks.

FACULTY OF SCIENCE

M.Sc. induStrial MicrOBiOlOGY ii SEMESTER

PRACTICAL EXAMINATION

PRACTICAL PROFORMA

TIME: 11.00 A.M TO 5.00 P.M	MARKS:50
Q.1 Major question (LAB I -105/ /LABII-107).	18 Marks
OR	
c) Major question.	10 Marks.
d) Minor question.	08 Marks.
Q.2 Major question (LAB I-106 /LABII-108).	18 Marks.
OR	
c) Major question.	10 Marks.
d) Minor question.	08 Marks.
Q.3 Viva Voce.	10 Marks.
Q.4 Record Book.	04 Marks.