KRISHNA UNIVERSITY :: MACHILIPATNAM
DEPARTMENT OF BIOTECHNOLOGY
Part I: MPhil/PhD Syllabus

Paper-I: Research Methodology (Common to all scholars registered in the department of Biotechnology) :100M
Paper-II: Broad Area of Research Subject: 100M
Paper –III: Research Specialization Subject: 100M
(Paper II & Paper III be different among scholars depending on their area of research)

Paper I: RESEARACH METHODOLOGY (Common Paper)

UNIT – I

Basic principles and applications of the following biophysical and biochemical techniques: Buffers – Acids, bases, ionization. Preparations of buffers. Measurement of pH. Standard hydrogen electrode, reference electrode, calomel electrode and silver-silver chloride electrode, COD and BOD. Centrifugation techniques – RPM, RCF, Svedberg coefficient, Differential, rate-zonal, and equilibrium density-gradient centrifugation. Isolation of cells, subcellular organelles and macromolecules. Microscopy – Phase contrast, fluorescent and electron microscopy (SEM, TEM). Preparation of biological samples of microscopy.

UNIT – II

Chromatography – Adsorption, partition, ion-exchange, gel filtration, affinity chromatography-based techniques: paper, TLC, GLC, HPLC, FPLC. Microscopy – Phase contrast, fluorescent and electron microscopy (SEM, TEM). Electrophoresis – Paper, gel (starch, agarose, acryl amide). Disc, slab, 2-dIrectional, gradient electrophoretic techniques. Pulse-field and capillary electrophoresis. Blotting techniques – Western blotting, Southern blotting, and Northern blotting. Electro-elution of biomolecules from gel/paper.

UNIT – III

Immuno-chemical techniques – Production of monoclonal and polyclonal antibodies. Functions of adjuvants and haptens. Immunoprecipitin reactions, immunoelectrophoresis and serum electrophoresis. Labelled antibody-based techniques – RIA, ELISA and fluorescent antibody technique. Flow cytometry and complement fixation test. Recombinant DNA Technology – Construction of genomic and cDNA libraries, Screening for recombinant clones, PCR based diagnosis, Sanger's and Maxam-Gilbert's methods for sequencing. Method for recombinant vaccine production.

.UNIT –IV

Spectroscopic techniques – Visible, UV, IR, NMR and atomic absorption spectro-photometry. Turbidometry, nephelometry, fluorimetry, flame photometry. X-Ray diffraction – X-Ray diffraction and X-Ray crystallography of biomolecules. Isotope techniques – Stable and radioactive isotopes, nature and types of radio activity, decay reaction, decay units. Detection and measurement of radioactivity - GM, scintillation, Gamma-ray counter, Cerenkov radiation and autoradiography. Biological uses and effects of radiation, mass spectrometry.

UNIT – V

Elements of biostatistics – Experimental design. Collection and presentation of data, tests of significance, analysis of variance, Duncan's multiple range test. Biological databases. Sequence alignment and sequence analysis. Construction of phylogenetic tree. Construction of protein 3D structures. Primer designing. Significance and application of proteomics.

Paper II: METABOLIC ENGINEERING (Broad area)

UNIT – I

Glycolysis and its regulation. TCA cycle – function and regulation. Electron transport chain-Energy yield during aerobic and anaerobic conditions, Glyoxylate cycle, Gluconeogenesis and its regulation, HMP shunt and its significance, Uronic acid pathway, Glycogen metabolism and its regulation with special reference to phosphorylase and glycogen synthase.

Biosynthesis and regulation of branched chain amino acids, aromatic amino acids, histidine and methionine. Metabolic breakdown of individual amino acids. Ketogenic and glycogenic amino acids. Formation of creatinine, ammonia and urea. Regulation of urea cycle. Proteins turn over – Role of ubiquitin.

UNIT – I I

Biosynthesis of purines and pyrimidines; Degradation of purines and pyrimidines and their regulation.

Oxidation of fatty acids, Formation and utilization of ketone bodies. Biosynthesis of fatty acids and regulation. Metabolism of arachidonic acid – formation of prostaglandins, thromboxanes, leucotrienes. Biosynthesis of triglycerides. Metabolism of phospholipids, sphingolipids. Biosynthesis of cholesterol and its regulation, Formation of bile acids. Role of liver and adipose tissue in lipid metabolism.

UNIT – III

Components, properties of lipid bilayer, Membrane models-Singer and Nicolson - fluid mosaic model. Biosynthesis and transport of phospholipids to plasma membrane and other organelle membranes. Biosynthesis of membrane proteins.

Active, passive transport and ion channels. Symport and antiport system. Organization, mechanism and significance of Na+ - K+ ATPase, Na+ - H+ ATPase, and Ca++-ATPase pumps. Special bacterial transport systems. Permeases, Phosphotransferase system, transport through binding proteins. Transport of macromolecules.

UNIT – IV

Structure and functions of membrane receptors. Excitable membrane ion gates and action potential generation, neurotransmitter types and action, G Protein – coupled receptors, Activation & inhibition of adenyl cyclase, Cell adhesion – Cadherins and Integrins. Extracellular matrix of cells. Membrane biogenesis - Liposomes and drug targetting. Types of receptors used for cell signaling, pathway of intracellular signal transduction using secondary messengers, Apoptosis

UNIT – V

Nomenclature and classification of enzymes. Factors affecting enzymes. Enzyme specificity. Enzymes assay. Kinetics of single substrate enzyme catalysed reaction, equilibrium steady state

assumption (Michaelis-Menten), transformation of Michaelis Menten equation, Lineweaver Burk, Eadie-Hofstee, Hanes plots. Determination of $V_{max,}$ $K_{m,}$ K_{cat} and their significance. Enzyme Inhibition: Reversible inhibition – Competitive, uncompetitive and non competitive inhibition, allosteric and irreversible inhibitions.

BOOKS RECOMMENDED:

1. Biochemistry and Molecular Biology, Third Edition by William H. Elliott and Daphne C. Elliott, Oxford University press.
2. Biochemistry L.Stryer Third Edition
3. Biochemistry White, Handler and R.B.Smith 7th Ed. 1983
4. Principles of Biochemistry A.Lehninger 1987.
5. Fundamentals of Biochemistry by J.L. Jain, Sunjay Jain AND Nitin Jain, S. Chand and Company LTD

PAPER II: MICROBIAL BIOTECHNOLOGY (Broad area)

UNIT – I

Heterologous Expression: Expression vectors and hosts Generally Regarded as Safe (GRAS) organisms. Production of active recombinant proteins of mammalian/Eukaryotic origin in prokaryotes. Large-scale production of proteins from recombinant microorganisms. Principles of microbial growth. Batch fermentation, feed-batch fermentation, continuous fermentation. High density cell cultures. Bioreactors – Large scale fermentation system – tandem Airlift reactors – Single stirred tank reactors.

UNIT – II

Downstream processing: Harvesting microbial cells – Membrane filtration system, high-speed semi continuous centrifugation. Disrupting microbial cells. Gram-scale purification of recombinant proteins. Chromatography systems and analytical methods for large-scale purification. Stabilization of proteins.

UNIT – III

Processing technology: Microbial metabolites – Organic solvents (Alcohol), Organic acids (Citric acid), Wines and beers, Antibiotics (Penicillin), Vitamins (Vitamin B12), Amino acids (Aspartic acid). Production of single cell proteins.

UNIT – IV

Environmental pollution – Types of soil and air pollutants, methods for the measurement of air pollution. Water pollution and its control – Need for water management, sources of water pollution. Measurement of water pollution. Microbiology of wastewater treatment: Aerobic process – Activated sludge, oxidation ditches, trickling filter, towers, rotating discs, rotating drums, oxidation ponds. Anaerobic treatment. Treatment schemes for wastewater of sugar industry. Microbiology of degradation of substituted hydrocarbons (*p*-nitrophenol), pesticides (2,4-D). Oil pollution, surfactants. Bioremediation of contaminated sites. Biopesticides in integrated pest management.

UNIT – V

Enzyme technology: Sources of production, isolation and purification of enzymes for the industrial use. Application of enzymes in pharmaceutical, food processing and other industries. Different techniques of immobilization of enzymes, applications and kinetics of immobilized enzymes. Design and operation of immobilized enzyme systems and bioreactors. Whole cell immobilization. Biosensors – Principle and types.

BOOKS RECOMMENDED:

1. Microbial Biotechnology. Glazer and Nikaido. 1995.
2 Biotechnology – A Text Book of Industrial Microbiology, Crueger and Crueger. 2000
3. Principles of Fermentation Technology, Stanbury. Whitaker & Hall. 1997
4. Microbial Technology Vol. I & II. Peppler & Perllman (EDS)
5. Industrial Microbiology. Prescott &Dunn's. Reed (Ed)
6. Concepts in Biotechnology. Balasubramanian, Bryce, Dharmalignam, Green & Jayaraman
7. Microbial Ecology – Fundamentals and applications. Atlas and Bartha

8. Environmental Microbiology. Mitchall
9. Environmental Microbiology. Grant & Long
10. Microbial aspects of Pollution. Skyes and Skinner. 01. Biotechnology – Volumes 1 to 5 by Rehem.

Paper-II: ANIMAL BIOTECHNOLOGY (Broad Area)

UNIT 1

Animal Cell culture Laboratory design and Equipments – Planning, construction and services; Layout; Sterile handling area; Incubation; Hot room; Air circulation; Service bench; Laminar flow; Sterilizer; Incubator; CO2 incubator; Refrigerators and freezers; Centrifuge; Inverted stage microscope; Magnetic stirrer; Liquid nitrogen freezers; Slow cooling system for cell freezing; Water bath; Autoclaves and hot air oven; Pipette washers; Water purification system; Fluid handling systems and other equipments; Washing, packing and sterilization of different materials used in animal cell culture; Aseptic concepts; Maintenance of sterility; Cell culture vessels. Principles of sterile techniques. Sources of tissues, types of tissues – Epithelial, muscle, connective, nerve and blood. Introduction to balanced salt solutions.

UNIT 2

Culture Media – Types of cell culture media; Ingredients of media; Physiochemical properties; CO2 and bicarbonates; Buffering; Oxygen; Osmolarity; Temperature; Surface tension and foaming; Balance salt solutions; Antibiotics and growth supplements; Foetal bovine serum; Serum-free media; Trypsin solution; Selection of medium and serum; Conditioned media; Other cell culture reagents; Preparation and sterilization of cell culture media, serum and other reagents. Role of antibiotics in media. Measurement of cell number – Hemocytometer, Coulter counter. Measurement of cell viability and cytotoxicity. Dye exclusion and inclusion tests, colonigenic assay, macromolecular estimation, MTT-based assay. Measurement of growth – Growth curves, PDT, Plating efficiency and factors influencing growth.

UNIT 3

Types of Cell culture – History of animal cell culture; Different tissue culture techniques; Types of primary culture; Chicken embryo fibroblast culture; Chicken liver and kidney culture; Secondary culture; Trypsinization; Cell separation; Continuous cell lines; Suspension culture; Organ culture etc.; Behavior of cells in culture conditions: Division, growth pattern, metabolism of estimation of cell number; Development of cell lines; Characterization and maintenance of cell lines, stem cells; Cryopreservation; Common cell culture contaminants.

UNIT 4

In vitro fertilization – Concept of superovulation, collection, maintenance, and maturation of oocytes, fertilization of oocytes, Cloning – concept of nuclear transfer, nuclear reprogramming and creation of Dolly. Stem cells – Isolation and culture, embryonic and adult stem cells, plasticity and concept of regenerative medicine. Transgenic animals – Retroviral, microinjection, and engineered embryonic stem cell method of transgenesis. Application of transgenic animals – Biopharming, disease models, functional knockouts. Genetherapy – ex vivo and in vivo gene therapy methods, applications. Implications of Biotechnology on health.

UNIT 5

Scale-up and application of animal cell culture – Vaccine production, specialized cell types. Concepts of tissue engineering – Skin, liver, kidney, balder and heart. Principles and species suitable for aquaculture (Indian major carps and prawns). Genetic status of culture stocks. Cell culture reactors; Scale-up in suspension; Scale and complexity; Mixing and aeration; Rotating chambers; Perfused suspension cultures; Fluidized bed reactors for suspension culture; Scale-up

in monolayers; Multisurface propagators; Multiarray disks, spirals and tubes; Roller culture; Microcarriers; Perfused monolayer cultures; Membrane perfusion; Hollow fiber perfusion; Matrix perfusion; Microencapsulation; Growth monitoring. Chromosome manipulations – Production of all male and sterile populations. Hypophysation in fishes and prawns. Molecular tools of identification of diseases in aquatic species.

Paper III: CANCER BIOLOGY (Research Specialization)

UNIT – I

Introduction, growth characteristics of cancers cells; Morphological and ultrastructural properties of cancer cells. Types of growth: hyperplasia, dysplasia, anaplasia and neoplasia. Nomenclature of neoplasms. Differences between benign and malignant tumors. Epidemiology of cancer.

UNIT – II

Cancer biology and biochemistry – Aberrant metabolism during cancer development; Paraneoplastic syndromes; Tumor markers; cellular protooncogenes- oncogene activation. Growth factors – EGF, TNF and TGF and growth factor receptors. Signal transduction in cancer. Role of transcription factors.

UNIT – III

Carcinogenesis – Radiation and chemical carcinogenesis. Stages in chemical carcinogenesis – Initiation, promotion and progression. Free radicals, antioxidants in cancer. Viral carcinogenesis – DNA and RNA viruses. Hormone-mediated carcinogenesis in humans.

UNIT – IV

Cell cycle regulation – Tumor suppressor genes p53, p21, Rb, BRACA1 and BRACA2. Telomeres, Telomerase, and Immortality. Cell–cell interactions, cell adhesion, invasion and metastasis. VEGF signaling, angiogenesis. Epigenetics – Role of DNA methylation in gene silencing, epigenetic silencing of tumor-suppressor genes. Apoptosis in cancer – Cell death by apoptosis, role of caspases. Death signaling pathways – Mitochondrial and death receptor pathways.

UNIT – V

Detection of cancers, Prediction of aggressiveness of cancer. Different forms of therapy – Chemotherapy, Radiation therapy, and Immuno therapy. Advantages and limitations.
Resistance to chemotherapy and radiotherapy and the signaling mechanisms involved in this process

PAPER III: ENVIRONMENTAL BIOTECHNOLOGY (Research Specialization)

UNIT -1

Structure of model ecosystem: terrestrial, aquatic ecosystems - Energy flow - Degradation of ecosystem. Consequences - Ecosystem managements - Energy conservation - Alternative energy sources - Biofuels: Production of bioethanol, biobutanol from agriculture waste - Problems and perspectives - Biodiesels: mass cultivation and use of Jatropa, marine algae for production of biodiesel.

UNIT-II

Types and sources of pollution - Inorganic, organic and biotic, Clinical examples of air, water and land pollutions. Environmental impact of pollution and measurement methods, Composting of organic wastes, microbial bioremediation of oil spills, Waste water treatment - sewage treatment and common industrial effluent treatment. Microbial leaching of ores – direct and indirect mechanisms

UNIT- III

Biofertilizers and their importance in crop productivity, Algal and fungal (mycorrhizae) biofertilizers, Bacterial biofertilizers (rhizobial, free living N2 fixers and phosphate solublizing bacteria), their significance and practice, Biopesticides : Bacterial (Bt pesticides), Production of biofertilizers and biopesticides for large scale application.

UNIT- IV

Genetically engineered microorganisms in environmental health, Genetically engineered plants and microorganisms in agriculture and productivity, Genetically engineered bacteria in bioremediation of organic pesticides, insecticides oil spills, Hazards of genetically engineered microorganisms, plants and animals.

UNIT-V

Hazardous Waste Management: Introduction - Xenobiotic compounds, recalcitrance. hazardous wastes - biodegradation of Xenobiotics. Biological detoxification - market for hazardous waste management. Biotechnology application to hazardous waste management - examples – cyanide detoxification - detoxification of oxalate.

Paper III: AQUATIC BIOTECHNOLOGY (Research Specialization)

UNIT: 1

Types of Aquatic systems- Fresh water, marine, brackish. Importence of aquatic animals and their culture. Culture practice and types. Fish culture. Water quality management, importance of plankton and their role in aqua culture.

UNIT: 2

Microbial isolation and identification techniques- Bacteria, fungi, and viruses. Culture media. Cultivation of bacteria, viruses and fungi. Identification protocols of micro organisms- cultural, staining techniques, biochemical techniques, molecular methods, ELISA and PCR.

UNIT: 3

Microbial diseases of fishes- bacterial, fungal, and viral diseases and their control. Mode of transmission of microbial diseases in fishes.

Unit: 4

Disease diagnosis in aquatic animals- principles applied in fish disease diagnosis. Diagnosis of bacterial, fungal, and viral diseases using molecular methods, epizootology, symptomology, aetiology, histopathology, hematological and immunological diagnosis of fish diseases.

UNIT: 5

Therapy and control of fish diseases- chemical treatment, drug therapy, vaccination, adjuvants, sanitizers, probiotics treatment and bio control of fish pathogens- phage therapy.

Model Question Paper

Paper-I, Paper-II & Paper III

Time: Three Hours Maximum Marks: 100

Answer each question (not less than 500 words each) (5 x 20=100 Marks)

1. One out of two from Unit I
2. One out of two from Unit II
3. One out of two from Unit III
4. One out of two from Unit IV
5. One out of two from Unit V

Minimum Pass marks: 50%

2012-13 BOS recommended subject areas

PAPER I	PAPER II	PAPER III
Research Methodology	Metabolic Engineering	Cancer Biology
	Microbial Biotechnology	Environmental Biotechnology
	Animal Biotechnology	Aquatic Biotechnology

Krishna University - Machilipatnam
Ph.D Program 2010-11
Department of Biotechnology
Part – 1: Paper – 1: Research Methodology
Model Question paper – 1

Time: 3 hours Total marks: 100

Answer all the following questions. Each question carries equal marks. 5x20 = 100

1. Write an account on preparation of biological samples of microscopy and explain in detail about scanning electron microscopy.

(Or)

2. Write an account on the selection of biochemical buffers and their applications.

3. Describe the principle, methodology and applications of gas liquid chromatography.

(Or)

4. Describe agarose gel electrophoresis and its applications.

5. Describe PCR concept, technology and applications

(Or)

6. Give brief account on i) RIA ii) Flow cytometry

7. Describe the principle, instrumentation, methodology and applications of NMR

(Or)

8. Write an account on detection and measurement of radioactivity.

9. Write an account on construction of genomic libraries.

(Or)

10. Write in detail about tests of significance.

Krishna University - Machilipatnam
Ph.D Program 2010-11
Department of Biotechnology
Part – 1: Paper – 2: Metabolic Engineering

Model Question paper

Time : 3 hours Total marks :100

Answer all the following questions. Each question carries equal marks. 5x20 = 100

1. Discuss in detail about various reactions involved in TCA cycle and describe its regulation.

(Or)

2. Write an account on Metabolic breakdown of amino acids.

3. Describe the Biosynthesis of Purines with regulation.

(Or)

4. Write an account on Oxidation of Fatty acids.

5. Describe the mechanism of Transport of bio molecules through plasmamembranes.

(Or)

6. Write an account on Membrane models.

7. Describe cell-cell interactions.

(Or)

8. Write an account on Apoptosis.

9. Give an account on classification of enzymes.

(Or)

10. Write a note on Michaelis – Menten equation and Reversible enzyme inhibition.

Krishna University - Machilipatnam
Ph.D Program 2012-13
Department of Biotechnology
Part – 1: Paper – 2: Microbial Biotechnology
Model Question paper – 1

Time: 3 hours **Total marks: 100**

Answer all the following questions. Each question carries equal marks. 5x20 = 100

1. Describe the production of active recombinant proteins of prokaryotes.

(Or)

2. Describe the principles of microbial growth.

3. Describe high speed semi continuous centrifugation and its applications.

(Or)

4. Write an account on stabilization of proteins.

5. Describe the production of single cell proteins.

(Or)

6. Explain the processing technology and production of citric acid.

7. Describe the different sources of water pollution and their control.

(Or)

8. Describe various types of treatment methods of sewage.

9. Describe the methods of purification of enzymes for industrial use.

(Or)

10. Describe various types of Biosensors and their principles.

MODEL QUESTION PAPER
KRISHNA UNIVERSITY:: MACHILIPATNAM
DEPT OF BIOTECHNOLOGY
Part – 1: Paper – 2: Animal Biotechnology
Model Question paper

Time : 3 hours Total marks :100

Answer all the following questions. Each question carries equal marks. 5x20 = 100

1. Write a detailed note on design of animal cell culture laboratory
(Or)
2. Explain which selective tissues can be grown on medias in detail

3. Write a detailed description about cell culture medium
(Or)
4. Write a note on cell viability and cytotoxicity tests

5. Write a note on cell culture types
(Or)
6. Write a note on behavior of cells in cell culture conditions

7. Write a detailed on invitro fertilization and it's importance
(Or)
8. Write a clerical note on regenerative therapy

9. Write a detailed note on molecular tools in identification of disease
(Or)
10. What is scale up and how it is applicable to chalk out the cells from media discuss in detail.

Krishna University - Machilipatnam
Ph.D Program 2012-13
Department of Biotechnology
Part – 1: Paper – 3: Cancer Biology

Model Question paper – 1

Time : 3 hours **Total marks :100**

Answer all the following questions. Each question carries equal marks. 5x20 = 100

1. **Describe the Ultrastructural properties of cancer cells.**

(Or)

2. **Write an account on the differences between benign and malignant tumors**

3. **Describe the signal transduction in cancer cells.**

(Or)

4. **Write an account on the aberrant metabolism during cancer development.**

5. **Describe various stages in chemical carcinogenesis**

(Or)

6. **Write an account on the hormone mediated carcinogenesis in humans.**

7. **Describe cell-cell interactions.**

(Or)

8. **Write an account on Apoptosis.**

9. **Describe various forms of therapy to prevent the aggressiveness of cancer.**

(Or)

10. **Describe various methods to detect cancer.**

Krishna University - Machilipatnam
Ph.D Program 2012-13
Department of Biotechnology
Part – 1: Paper – 3: Environmental Biotechnology
Model Question paper – 1

Time: 3 hours **Total marks: 100**

Answer all the following questions. Each question carries equal marks. 5x20 = 100

1. Describe the structure and management of aquatic ecosystem.

(Or)

2. Explain the process of production of biofuels from agricultural waste.

3. Describe the direct and indirect mechanisms of microbial leaching of ores.

(Or)

4. Describe various types of treatment methods of sewage.

5. Describe various types of mycorrhizae and its importance in crop production.

(Or)

6. Write an account on various types of biopesticides, their production and large scale applications.

7. Describe how the genetically engineered plants are developed and their role in agriculture and productivity.

(Or)

8. Explain the role of genetically engineered bacteria in bioremediation of organic pesticides, insecticides and oil spills.

9. Describe how you manage the hazardous waste from biotechnological applications.

(Or)

10. Describe the processes of biodegradation of xenobiotic compounds.

MODEL QUESTION PAPER
Krishna university:: Machilipatnam

Department of Biotechnology

Part – 1: Paper – 3: **Aquatic Biotechnology**
Model Question paper

Time : 3 hours Total marks :100

Answer all the following questions. Each question carries equal marks. 5x20 = 100

1. Write a note on culture practices and it's types? 1*20: 20M

(Or)

2. Write a note on how water quality play role in health management of fish?
3. Write a note on isolation protocols of microorganisms from fishes? 1*20: 20M

(Or)

4. How PCR helpful in disease diagnosis of fishes?
5. Write assay on mode transmission of pathogens in fishes? 1*20:20M

(Or)

6. Write general note on bacterial infections on fishes?
7. Write a clerical note on disease diagnosis in fishes? 1*20: 20M

(Or)

8. Write a note on symptomology with special reference to different diseases?
9. Write a note on role of probiotics in Aqua culture and their importance? 1*20:20M

(Or)

10. Write a note on different therapy and control measures of fish diseases?