

# BIOTECHNOLOGY (878)

## Aims:

1. To enable candidates to acquire the knowledge and develop an understanding of how materials are provided by biological agents to provide goods and services.
2. To appreciate the role played by biotechnology in improving health care for human beings.
3. To understand the interdisciplinary nature of this subject.
4. To create awareness about the appreciation of biological processes to industries.
5. To develop the ability to appreciate biological phenomenon in nature and the contribution of biotechnology to human welfare.
6. To develop scientific attitude towards biological phenomenon.

## CLASS XI

There will be two papers in the subject:

Paper I: Theory..... 3 hours ... 70 marks

Paper II: Practical..... 3 hours ... 15 marks

Project Work..... ... 10 marks

Practical File..... ... 5 marks

### PAPER I –THEORY- 70 Marks

There will be **one** paper of **three** hours duration divided into **two** parts.

**Part 1 (20 marks)** will consist of compulsory short answer questions, testing knowledge, application and skills relating to elementary/fundamental aspects of the entire syllabus.

**Part 2 (50 marks)** will consist of **eight** questions out of which the candidates will be required to answer **five** questions. Each question in this part shall carry 10 marks.

#### 1. Introduction to Biotechnology

- (i) Historical background; definition; a brief introduction of the traditional and modern techniques of Biotechnology and their applications.

*Definition of biotechnology by OECD and EFB; contributions of Karl Ereky and Louis Pasteur; use of various fermented products in ancient civilisations;*

*Kitchen (traditional), the first biotechnological laboratory -reasoning behind the technology involved in simple biological products like curd and beer; names*

*of microorganisms involved in their production.*

*Application of these technologies for large-scale production, with special reference to fermentation (Beer production only). Quality control management of the products, good laboratory practices.*

- (ii) Scope and importance of biotechnology; different branches of biotechnology and different regulatory guidelines; ethical, legal and social issues (ELSI) that a biotechnologist comes across while doing the work. Various organisations in the field of biotechnology.

*Names, definitions and importance of various fields that can be covered under biotechnology such as - agricultural/ plant biotechnology, animal biotechnology/medical biotechnology, nanobiotechnology, industrial biotechnology, immunology and health care, energy and environment.*

*Intellectual Property Rights (IPRs) in biotechnology- concept of intellectual property, types of IPR and its need; intellectual property rights and the choice of intellectual property rights protection. Discovery and invention; Concept of patenting, trademark, trade secrets, copyright, geographical indications and PBRs and their need.*

*Concept of ethical, legal and social issues with one common example IVF.*

*Biosafety issues: release of genetically modified organisms into the environment and their impact; GEAC and its objectives.*

*Biotechnology - global and Indian scenario. Various institutes, centres and funding agencies - NBTB, CCMB, ICGEB, ICMR, ICAR, DBT, DST which deal with biotechnology and bioinformatics in India: names only.*

- (iii) Basic concepts of Biochemical technology and biostatistics: What does the biochemical technology mean? An understanding of various statistical methods involved in biotechnology.

*Concept of buffer, type and preparation of buffers, pH, physical variables; fermentation; An understanding of bio-reactors, idea of sampling – quadrat and transect; measures of central tendency – mean, median, mode; standard deviation and standard error; concept of probability – theoretical and experimental.*

## 2. Cell Biology

- (i) Cell: Justification of cell as a basic unit of life. Prokaryotic cell and eukaryotic cell; A brief note on the cell components with special reference to nucleus. Various cytological techniques used in identifying the cell and chromosomes.

*Differentiation prokaryotic and eukaryotic cellular systems.*

*Structure of bacteria (in brief, with reference to plasmid). Gram+ and Gram- bacteria.*

*An understanding of cell components, their basic structure and functions - cell wall, cell membrane, cytoplasmic reticulum, Golgi apparatus, mitochondria, ribosomes, vacuoles, plastids, lysosomes, nucleus and other important inclusions of the cell.*

*Chromosomal structure and composition – organisation of chromatids, concept of homologous and non-homologous chromosomes, sister and non-sister chromatids, classification of chromosomes on the basis of position of the centromere on the chromosome, basic idea about telomere,*

*chromatin and nucleosome. An idea about banding patterns (Q, R, C and G) and their application.*

*Concept of chromosomal number in different species, e.g. man, mouse, Drosophila and pea.*

*Techniques in cytology – microscopy (light and electron microscope), karyotyping and centrifugation (principle and applications only).*

- (ii) Cell Division and cell cycle: types of cell divisions and various other activities of cell such as biochemical transformations.

*Types and significance of cell division and a brief note about the different stages of cell division – mitosis and meiosis.*

*Basic concept of cell cycle and cell cycle regulation – CdK method only, definition of Mitotic Index.*

*Biochemical Transformations:*

*An understanding of biochemical transformations, different biochemical pathways involved in respiration - aerobic and anaerobic.*

*Aerobic respiration - Glycolysis, Krebs' cycle, electron transport chain and oxidative phosphorylation.*

*Anaerobic respiration - lactic acid, fermentation and alcohol fermentation – definition only.*

- (iii) Errors in cell division: what happens if the cell does not divide normally? An understanding of different numerical and structural abnormalities.

*Concept of mutation: causes; types –somatic, germinal, spontaneous, induced, gene, chromosomal and genomic mutations, euploidy, aneuploidy, monosomy, nullisomy, trisomy and tetrasomy; various factors causing mutations.*

*Concept of non-disjunction: meiotic non-disjunction and mitotic non-disjunction. Non-disjunction in sex chromosomes – Turner's syndrome and Klinefelter's syndrome - chromosomal composition and symptoms only.*

*Numerical chromosomal aberrations with respect to autosomes, i.e. Down's syndrome – chromosomal composition and symptoms only.*

*Structural chromosomal abnormalities – deletions, duplications, translocations, inversions.*

*Polyploidy and its significance in plants.*

*Inborn errors of metabolism - basic concept and examples like albinism, sickle cell anaemia, phenylketonuria and alkaptonuria.*

### **3. Biomolecules and related techniques**

- (i) Introduction to biomolecules- definition and types. Carbohydrates, proteins, lipids, vitamins and enzymes – their structure and properties.

*Biomolecules – definition and types*

*Structure and functions of carbohydrates.*

*Sugars and derivatives; classification of some important mono, di and polysaccharides - glucose, fructose, glycogen, cellulose, chitin and peptidoglycan. Physical and chemical properties of sugars.*

*Structure, functions and classification of proteins i.e. simple, complex and derived; building blocks of proteins - the amino acids: chemical structure, types (acidic, basic and neutral); physical and chemical properties of amino acids. 3D - structure of proteins. Different types of protein structures - primary, secondary (alpha helix, beta pleated sheet and random structures), tertiary, quaternary; protein sequencing by MALDI-MS.*

*Structure and functions of lipids – fatty acids and alcohol; types (simple, conjugated and derived lipids with one example of each); chemical and physical properties of lipids.*

*Vitamins: Definition, types (fat soluble and water soluble vitamins); co-enzyme forms of water soluble vitamins; deficiency diseases of vitamins.*

*Enzymes: Structure and functions of enzymes: chemical nature of enzymes; characteristics and properties of enzymes. An understanding of enzyme activity on the basis of activation*

*energy; mechanism of enzyme action - lock and key model; induced fit hypothesis; factors affecting enzyme activity (temperature, pH, substrate concentration, enzyme concentration, inhibitors (competitive, non-competitive).*

*Optical activity of biomolecules (dextrorotatory and laevorotatory).*

*Concept of supramolecular assembly.*

- (ii) Techniques used for separation of biomolecules

*Ion exchange chromatography and paper chromatography.*

### **4. Developmental Biology and Immunology**

- (i) Animal and plant development: development of an organism from zygotic cell in both plants and animals.

*Animal development – fertilisation, zygote to blastocyst formation.*

*Plant development. Double fertilisation including formation of primary endosperm nucleus.*

- (ii) An understanding of defence strategies in living organisms.

*Immune system in higher animals, concept of immunity, immunisation, antigen and antibody. Various cells involved in immune response in humans. An introduction to human leukocyte antigens with reference to organ transplantation; Types of immunity - innate and acquired. ELISA Technique (Enzyme Linked Immuno Sorbent Assay).*

*Secondary metabolites in plants and their significance*

*Defence strategies in bacteria – endospores and R plasmids.*

### **5. Genetics**

- (i) Laws of Inheritance: An account of Mendel's experiments. Different types of genetic inheritance.

*Mendel's experiment on pea plant and his laws of inheritance.*

*Concept of trait, gene, allele, phenotype, genotype, homozygosity, heterozygosity and hemizyosity. Types of inheritance: autosomal inheritance - dominant, co-dominant, recessive, polygenic, pleiotropic and cytoplasmic inheritance (plastidial inheritance).*

*Pedigree construction using different standard symbols.*

*Sex chromosome inheritance - with special reference to X chromosomal inheritance with suitable examples (colour blindness and haemophilia).*

- (ii) Gene Mapping: mapping of genes on chromosomes using linkage analysis. Cancer and its genetics.

*Mapping of genes on chromosomes with respect to COV (Crossing Over Value).*

*Basic concept of linkage (types not required) and crossing over. Genetic recombination.*

*Cancer: Causes (physical, chemical, biological – TSG and oncogenes); diagnosis and treatment.*

- (iii) Genes in populations: how do genes behave in populations from generation to generation? Various ways of studying population genetics.

*Concept of gene pool and allele frequency, definition of Hardy Weinberg law, its applications.*

*Possibility of disease resistant and susceptible genes in population. Definition and application of pharmacogenetics and pharmacogenomics.*

## PAPER II

### PRACTICAL WORK – 15 Marks

Candidates are required to complete the following experiments.

1. Determination of blood group by using antisera.

*The students can perform this experiment on their own blood groups. Proper instructions however are to be given for 'prick' – e.g. (a) Sterilize finger with alcohol/disinfectant. (b) Use only disposable sterile needle. (c) Use the needle only*

*once and destroy it. (d) Do not prick or use blood drop in an indiscriminatory way.*

2. Identification of different types of blood cells by preparing blood smear using Leishmann's stain.

*Requirements: Blood sample, disposable needles, slides, Leishmann's stain. Make a blood smear on a slide, use the stain to colour the smear, wash and observe under microscope.*

3. Instruments – their names, use and principles (if applicable).

*Water bath, pH meter, weighing balance, desiccators, microfiltration unit, magnetic stirrer, LAF, haemocytometer, micropipette, vortex mixer, colorimeter/spectrophotometer, hot air oven, autoclave, incubator, electrophoresis chamber, colony counter, autoclave, hot plate.*

4. Finding out the pH of water by using pH meter or pH paper on tap water and water containing acid, base.

*Take tap water in three test tubes, add two drops of dil. HCl in one, two drops of NaOH in the second while leaving the third test tube with tap water. Use pH meter or pH paper to find their specific pH.*

5. Observation of steps of mitosis by using the root tip of onion.

*The students should be given practice in preparing slides for study of mitosis by crush smear method. They should be able to identify different stages (at least four stages). The requirement for this set of experiments is Acetocarmine stain slides, coverslips, microscopes and spirit-lamp.*

6. Measurement of mitotic index.

*Mitotic index is the ratio of number of cells undergoing mitosis to the number of cells in the field.*

*No. of cells showing mitosis*

$$MI = \frac{\text{No. of cells showing mitosis}}{\text{Total no. of cells in the field}}$$

7. Observation of various stages of meiosis under microscope.

*For the study of meiosis, the students should be shown permanent slides of meiosis and they should be able to identify at least six stages of meiosis from the slides.*

8. Effect of temperature on curdling of milk by using *Lactobacillus* bacteria at 37°C, 60°C and 10°C.

*Optimum temperature for curdling of milk is 37°C due to active form of bacteria at this temperature; it is inactive at low temperature and dies at high temperature.*

9. Food tests:

(i) *Carbohydrates – starch by iodine solution turning blue - black in colour.*

*Reducing and non-reducing sugars by using Fehling's solution / Benedict's solution – reducing the cupric ion (blue) to cuprous ion (red).*

(ii) *Protein test – Biuret test, Xanthoproteic and Millon's test*

(a) **For Biuret test** – *The protein produces deep blue – violet colour due to the involvement of cupric ion in the product formed.*

(b) **For Millon's Reagent** – *A pinkish red colour is observed with mercuric chloride.*

(c) **For Xanthoproteic Test:** *When concentrated nitric acid is boiled with protein a yellow colour is observed. On addition of ammonium hydroxide or liquor ammonia orange yellow precipitate is obtained.*

(iii) *Lipids – Sudan III, Acrolein test, paper test*

(a) *Sudan III is a red fat-soluble dye used for identification of the presence of lipids, triglycerides and lipoproteins. It reacts with the lipids or triglycerides and gives red colour.*

(b) *Acrolein test is used to detect fat. When fat is heated strongly in the presence of potassium bisulphate/ sodium bisulphate (KHSO<sub>4</sub>/NaHSO<sub>4</sub>) that acts as a dehydrating agent, the glycerol is dehydrated to form an unsaturated aldehyde called acrolein that gives a pungent and irritating odour.*

10. Finding out the purity of milk by using lactometer.

*Put the instrument in milk. If it sinks down and reaches the mark 'M' mentioned on lactometer, it means that the milk is pure or if not, it means that the milk is impure. If the*

*milk is mixed with water, it would sink higher than mark 'M'. If it stands at the mark 3 it means that the milk is 75% pure and respectively 2 for 50% purity and 1 for 25% purity.*

11. Construction of pedigree showing different types of inheritance.

*The students are to observe the traits like, rolling of the tongue/ attached earlobe/ widow's peak.*

12. Preparation of karyotypes.

*Demonstration of any metaphasic plate of mitosis.*

13. Sampling methods – quadrat and transect by using different techniques.

*To be done in groups. Use yellow and green pea seeds. Make a quadrat (30 cm X 30 cm) with blocks of 6 cm X 6 cm. Spread the seeds randomly on the table top. Put the quadrat and count the number of yellow and green peas per block; find the frequency of each type of pea seed.*

14. Data collection – primary and secondary.

*Collect any type of primary data and secondary data, tabulate the data and draw conclusion.*

## PROJECT WORK AND PRACTICAL FILE

– 15 marks

### Project Work – 10 Marks

Candidates are to creatively execute **one** project/assignment on any aspect of Biotechnology. Teachers may assign or students may choose any one project of their choice. The report should be kept simple, but neat and elegant. No extra credit shall be given for type-written material/decorative cover, etc.

### Practical File – 5 Marks

Teachers are required to assess students on the basis of the practical file maintained by them during the academic year.

## LIST OF ABBREVIATIONS

1. CCMB: Centre for Cellular and Molecular Biology
2. CdK: Cyclin dependent Kinase
3. COV: Cross Over Value
4. CSIR: Council of Scientific and Industrial Research
5. DBT: Department of Biotechnology
6. DST: Department of Science and Technology
7. EFB: European Federation of Biotechnology
8. ELISA: Enzyme Linked Immuno Sorbent Assay
9. ELSI: Ethical, Legal and Social Issues
10. ETS/ETC: Electron Transport System / Electron Transport Cycle
11. FMN/FAD: Flavin Mono Nucleotide / Flavin Adenine Dinucleotide
12. GEAC: Genetic Engineering Approval Committee
13. HLA: Human Leucocyte – associated Antigen
14. ICAR: Indian Council for Agricultural Research
15. ICGEB: International Centre for Genetic Engineering and Biotechnology
16. ICMR: Indian Council for Medical Research
17. IEF: Iso Electro Focussing
18. IPP: Intellectual Property Right Protection Act
19. IPR: Intellectual Property Right
20. IVF: In–Vitro Fertilization
21. MALDI-MS: Matrix Assisted Laser Desorption Ionization – Mass Spectrometry
22. MI: Mitotic Index
23. NADPH/NADP: Nicotinamide Adenine Dinucleotide Phosphate (reduced) / Nicotinamide Adenine Dinucleotide Phosphate
24. NBTB: National Biotechnology Board
25. OECD: Organization for Economic Cooperation and Development
26. PBR: Plant Breeder’s Right
27. TPP: Thiamine Pyrophosphate
28. TSG: Tumour Suppressor Gene

## CLASS XII

There will be two papers in the subject:

**Paper I:** Theory..... 3 hours ... 70 marks

**Paper II:** Practical..... 3 hours ... 15 marks

Project Work..... ... 10 marks

Practical File..... ... 5 marks

### PAPER I: THEORY- 70 Marks

There will be **one** paper of **three** hours duration divided into **two** parts.

**Part 1 (20 marks)** will consist of compulsory short answer questions, testing knowledge, application and skills relating to elementary/fundamental aspects of the entire syllabus.

**Part 2 (50 marks)** will consist of **eight** questions out of which the candidates will be required to answer **five** questions. Each question in this part shall carry 10 marks.

#### 1. Molecular Biology

- (i) Nucleic acids and their estimation: an understanding of nucleic acids, their biochemical structure.

*DNA as the genetic material (Hershey and Chase experiment).*

*DNA (B-DNA)– physical and chemical structure; definition, double helical model of DNA, (Watson and Crick's); Nucleotide and nucleoside; Chargaff's Law, method of replication of DNA, various replicative enzymes in both prokaryotic and eukaryotic organisms, example topoisomerases, helicase, SSBs polymerases, primases, ligases. Concept of semi conservative (with respect to Messelson and Stahl experiment and Taylor et.al experiment on Vicia faba using radiolabelled thymidine) and semi-discontinuous replication, (leading and lagging strands), okazaki fragments.*

*RNA – definition, various types of RNAs such as mRNA, tRNA (Clover leaf model with diagram; brief introduction to L-shaped model), rRNA their structure and functions.*

*Techniques of nucleic acid estimation – colorimetry and UV-visible spectrophotometry.*

- (ii) Protein Synthesis: synthesis of different RNAs, and the complete mechanism of polypeptide chain formation.

*Concept of central dogma.*

*From genes to proteins:*

- (a) *Concept of transcriptional unit, promoter, structural and terminator region; concept of split gene - intron and exon; monocistronic and polycistronic RNA, hnRNA;*
- (b) *Transcription – explanation of the complete process including enzymes involved in the process; Post-transcriptional changes and their significance in eukaryotes – polyadenylation, capping and RNA splicing;*
- (c) *Concept of reverse transcription;*
- (d) *Genetic code – properties of genetic code, start and stop codons, anticodons.*
- (e) *The translation of RNA to protein – complete mechanism of chain initiation, elongation and termination, the role of tRNA, mRNA and rRNA in protein synthesis. (Post translational changes not included).*

- (iii) Gene regulation in prokaryotes

*Operon concept – lac operon and trp operon.*

#### 2. Genetic Engineering

- (i) Introduction to gene cloning and genetic engineering: concept of cloning and vectors.

*Tools of recombinant DNA technology, types of restriction endonucleases and other enzymes used in gene cloning; techniques involved in extraction and purification of DNA from bacterial, plant and animal cells.*

*Selection of host cells: eukaryotic and prokaryotic.*

*Vectors: Characteristics and types such as plasmids -pBR322, pUC (in pBR322- presence of two antibiotic resistant genes and in pUC presence of lac Z gene to be taught), cosmids, phages (M13 and λ), YACs, BACs (to be taught with reference to stability and their carrying capacity), animal and plant viruses (CaMV, retrovirus, SV40 – only names of viruses, no details).*

*Transfer of recombinants into host cells –*

- (a) *Vectorless methods - basic concept of transformation, transfection, electroporation, liposome mediated gene transfer, microinjection, biolistic*
- (b) *Vector-mediated method - Agrobacterium tumefaciens induced gene transfer.*

*Methods of identification of recombinants- Direct selection (green fluorescent selection) and Insertional inactivation (Blue-white selection, antibiotic resistance).*

*A basic understanding of DNA libraries – construction of genomic and cDNA libraries.*

*Construction of a recombinant DNA molecule.*

- (ii) *Innovations in Biotechnology: produced by using modern biotechnological tools, (select examples of products already available)*
  - (a) *Plants: Production of Flavr Savr tomatoes, Bt-crops and Golden rice.*
  - (b) *Healthcare: Production of recombinant hepatitis-B vaccine, Humulin, interferon and edible vaccines.*
  - (c) *Animal: Dolly the cloned sheep, Sources and characteristics of stem cells and their applications.*
  - (d) *Environmental biotechnology: bioremediation using oil-eating bacteria as an example.*
  - (e) *Industrial biotechnology: applications of industrial enzymes – rennet, subtilisin, amylase, papain.*

- (iii) *Gene analysis techniques: various techniques involved in recombinant DNA technology.*

*DNA probes – definition and use.*

*Low resolution mapping techniques: gel electrophoresis, southern blotting (details of the technique to be taught), western and northern blotting (a brief idea and their uses).*

*High resolution techniques: DNA sequencing- sequencing by chain termination, automated DNA sequencing. Site directed mutagenesis.*

*DNA amplification by Polymerase chain reaction (PCR)– applications of PCR, steps and application of DNA profiling or DNA finger printing.*

### 3. Cell culture technology

A brief idea of tools and techniques involved in cell culture technology and their applications in microbial, plant tissue and animal cell cultures respectively.

- (i) *General tools and techniques used in cell culture technology*
  - (a) *Instruments - centrifuge, LAF hood and biosafety cabinets, pH meter, autoclave, vortex mixer, hot air oven, magnetic stirrer, weighing balance, micro filtration unit, incubator, CO<sub>2</sub> incubator, inverted microscope, bioreactor (diagram, its components and their function)-stirred tank and sparged type (brief idea only), use of T flasks to propagate animal cells.*
  - Only uses of the above instruments to be studied.***
    - (b) *Sterilization techniques for culture room, apparatus, transfer area, media, vitamins, and living material;*
    - (c) *Cryopreservation (need and steps).*
    - (d) *Cell counting (direct counting by haemocytometer), cell viability by Evan's blue stain and cell sorting (FACS only)*
    - (e) *Types of media (synthetic /defined, semi-synthetic/differential, complex/natural)*



*Preparation of media: microbial media-LB agar and LB broth; Plant media-MS and White's media; Animal media-RPMI, DMEM and FBS - brief idea only. (includes inorganic and organic macronutrients and micronutrients, antibiotics, growth regulators for plants: auxins and cytokinins).*

*Importance of pH and solidifying agents.*

(ii) Microbial culture and its application.

*Fermentation process and growth kinetics-batch culture, fed batch culture, continuous culture (with the help of graphs only): Definition of turbidostat and chemostat: Products and application-SCP (definition and use), industrial enzyme-subtilisin (source and its use).*

(iii) Plant tissue culture and its application.

*Isolation of single cell by mechanical and enzymatic methods, synchronisation of cell culture by chemical methods like starvation, inhibition and mitotic arrest.*

*Cellular totipotency-definition of cellular differentiation, de-differentiation, re-differentiation. Application of plant cell culture technology (**methodology not required, only brief idea needed**):*

- (a) *Haploid production-androgenesis and gynogenesis and their significance.*
- (b) *Triploid production-understanding and need for triploid production and its application (seedless crops).*
- (c) *In-vitro pollination- concept and its application.*
- (d) *Zygotic embryo culture- concept and its application, Embryo rescue (brief idea only).*
- (e) *Somatic hybridisation-protoplast fusion (Pomato).*
- (f) *Micropropagation and its significance.*
- (g) *Developing virus free plants and synthetic seeds.*
- (h) *Biodegradable plastics (concept of PHB).*

(iv) Animal cell culture and its application.

*Primary cell culture with mechanical and enzymatic disaggregation and its drawbacks; Types of cell-lines: finite, continuous, adherent and suspension; scale up-mono layer by Roller bottle, application of animal cell culture-tissue, hybridoma technology, tissue engineering (definition only).*

#### 4. Bioinformatics

(i) Introduction to bioinformatics; global bioinformatics databases and data retrieval tools; genomics, different types of sequences, types of sequence analysis.

*Introduction to bioinformatics: definition and need.*

*An introduction to global bioinformatics databases (nucleotide and protein databases). Information sources such as EMBL, NCBI, DDBJ, SWISSPROT, GenBank, GENSCAN.*

*Data retrieval tools- ENTREZ, Taxonomy Browser.*

(ii) Genomics: Definition, introduction, tools used in Genomics and its applications.

*Definition of genomics. Types of genomics-structural and functional. Basic criteria in selecting the organism for its genome sequencing. Different types of sequences – cDNA, genomic DNA, ESTs (Expressed Sequence Tags) and STSs (Sequence Tagged Sites) and the different softwares (example gene scan).*

*Types of sequence analysis by using BLAST and FASTA –global, local, pair wise and multiple.*

*Human Genome Project - its objectives, the countries involved, its achievements and significance.*

*DNA microarray technology – definition and application only.*

*Concept of Single Nucleotide Polymorphisms (SNPs).*

(iii) Proteomics: definition, introduction and databases.

*Types of Proteomics – structural, functional and expression; Important protein databases available for the public on the internet like PDB (Protein Data Bank), PIR (Protein Identification Resources).*

## PAPER II

### PRACTICAL WORK – 15 marks

Candidates are required to complete the following experiments.

1. Paper Chromatography – separation of photosynthetic pigments  
*Take any leaf. Extract chlorophyll in 80% acetone. Take a strip of paper or prepare a thin layer of silica gel on a slide. Load chlorophyll extract at one end of the paper/gel. Keep paper or gel in the rising medium in test tube or jar for about 30 minutes. The rising medium should have methanol/ acetic acid, n-butanol or benzene. The rising fluid should always be at the bottom below the point of loading of chlorophylls. After 30 minutes, three spots: yellow, bluish green and light green will be observed corresponding to carotenes, chlorophyll A & chlorophyll B.*
2. Preparation of buffers – phosphate, acetate and borate buffers  
*This experiment should be done to make the basics clear to the students. Basic calculation for buffer preparation should be known. The approach should be to utilize easily available chemicals at reasonable costs. Phosphate, borate and acetate buffers can give the range of pH 4 - pH 9.2*
3. Preparation of culture media
  - (i) *Bacterial culture Media - Luria Bertani (L.B.) media - Peptone/ Tryptone, yeast extract and NaCl. (Nutrient broth / Nutrient Agar).*
  - (ii) *Plant Tissue culture medium (Sugars + Coconut milk + Agar Agar).*
4. Sterilization of culture medium and other materials.
  - (i) *Dry Physical method – heat or radiation.*
  - (ii) *Wet Physical methods – steam sterilization.*
  - (iii) *Chemical Sterilization/ Surface sterilization Disinfection with 70% alcohol and Sodium hypochlorite solution carbolic acid*
5. Preparation of various forms of culture media – Petri plate, slant and suspension.  
*Luria Bertani (L.B) media to be prepared, autoclaved and cooled to 60 degrees C. To prepare nutrient plates the media is poured into presterilized petri-dishes under a LAF. To prepare slants the media is poured into several test tubes, plugged and kept in a tilted position (at an angle of 45°) until it sets.*
6. Inoculation and incubation of *Lactobacillus* on the culture medium in the Petri plate.  
*Use of inoculation loop or inoculation needle for the purpose.*
7. Identification of bacteria by Gram +ve and Gram –ve (from curd /saliva and/or soil solution)
  - (i) *Prepare a bacterial smear on a slide*
  - (ii) *Stain with crystal violet stain.*
  - (iii) *Rinse with water.*
  - (iv) *Add a few drops of iodine solution.*
  - (v) *Add few drops of 90 % ethanol*
  - (vi) *Counterstain with safranin solution*
  - (vii) *Observe the red and blue colonies under the microscope*
8. Action of enzymes on starch under: (a) variable temperature (b) variable substrate concentration – plotting of  $K_m$  value by graph
  - (i) *Soluble starch solution (0.5% - 1%) to be prepared. Test with iodine. Collect saliva, dilute 1: 5, add 1 ml of saliva to 10 ml of starch solution. Incubate for 15 minutes. Again test for presence of starch with iodine. Also test for the presence of reducing sugars in solution. Repeat the same process at the variable volumes of starch*
  - (ii) *To study the effect of variable temperature on the activity of the enzyme salivary amylase.*
9. Isolation of DNA from plants  
*Take half a ripe and peeled banana into a beaker and add 50 ml of extraction fluid (1.5gm table salt +10 ml liquid detergent +90 ml distilled water). Place the beaker in a water bath set at 60 degrees C for 15 minutes. Stir gently with a glass rod. Filter 5ml of cooled content into a clean test tube and add 5ml of cold 90% ethanol. DNA molecules separate out and appear as white fibres. [DNA can also be extracted from pea seeds and soaked wheat grains]*
10. DNA estimation by colorimeter by DPA method.
11. Protein estimation by colour reaction – Bradford test.  
*Bradford's Assay is a Dye binding assay based on the differential change of colour of a dye in response to various concentrations of proteins. Bradford's assay can be performed for qualitative*

as well as quantitative assessment of proteins in a sample.

*Dilute 1 volume of Bradford's dye with 4 volumes of distilled water. Filter the dye through Whatman filter paper and store at room temperature in a brown glass bottle. Take different aliquots of standard Bovine Serum Albumin (BSA solution), for example (0.2, 0.4, 0.6, 0.8 and 1.0 ml) in different test tubes. Make up the volume to 1ml with distilled water. To each tube add 2ml of Bradford's dye. Extent of colour development can be made by rough estimate using + signs to show the concentration of protein in the sample. Alternatively, OD can be read using colorimeter or spectrophotometer. Take the unknown sample to be estimated and perform the experiment. Similarly read the OD and note the corresponding concentration of protein in it using the graph.*

12. Cell viability test by Evan's blue dye.
13. Isolation of milk protein – wet weight and dry weight.  
*Milk proteins are isolated by adding 0.4 N HCl into the milk sample. Casein start coagulating at its isoelectric point (i.e. at pH 4.6). The precipitate is filtered and weighed to quantify the protein present.*
14. Chromatography to find adulteration in spices by using mixer of turmeric and metanil yellow.
15. Demonstration of cell counting by haemocytometer by using diluted blood.
16. Experiment to show the process of saponification.

## **PROJECT WORK AND PRACTICAL FILE**

**– 15 Marks**

### **Project Work – 10 Marks**

The Project Work is to be assessed by a Visiting Examiner appointed locally and approved by the Council.

Candidates are to creatively execute **one** project / assignment on an aspect of Biotechnology.

Teachers may assign or students may choose any one project of their choice. The report should be kept simple, but neat and elegant. No extra credit shall be given for type-written material/decorative cover, etc.

A list of suggested projects is as follows:

1. Effluent analysis.
2. A study of the technological details of malt preparation.
3. A study of the technological details of the brewing industry.
4. A study of the organisation of a fermenter.
5. Technological analysis of the process of drug development, drug designing and drug targeting.
6. A study of the technological details of vaccine development.
7. Diagnosis of diseases by modern techniques like ELISA, RIA and Antibody targeting.
8. DNA finger-printing.
9. DNA foot-printing.
10. Microbiological contaminants in food and food products.
11. Isolation of microbes from air, water and soil.
12. Methods of identifying microbes (various staining techniques and biochemical reactions).
13. Tissue Culture and its applications.
14. Stem Cell Technology
15. Nanotechnology
16. Bioinformatics
17. Genetic Engineering
18. Cloning
19. Instrumentation in biotechnology
20. Forensic Biotechnology
21. Ethical, Legal and Social Issues (ELSI) related to Biotechnology/ GMOs
22. Biopiracy- Case Studies

### **Practical File – 5 Marks**

The Visiting Examiner is required to assess students on the basis of the practical file maintained by them during the academic year.

### **Suggested Evaluation Criteria for Project Work:**

#### Format of the Project:

- Content
- Introduction
- Presentation (graphs, tables, charts, newspaper cuttings, handmade diagrams, photographs, statistical analysis if relevant)
- Conclusion/ Summary
- Bibliography

Projects should be handwritten by the candidate. Written pages should not exceed 15-20 pages.

## LIST OF EQUIPMENT FOR BIOTECHNOLOGY PRACTICALS FOR CLASSES XI & XII

1. Table-top Centrifuge
2. Vortex - Mixer
3. Thermostatic water-bath
4. Spectrophotometer (UV visible range)/ Colorimeter
5. Refrigerator
6. Lactometer
7. pH meter
8. Hot air oven
9. Autoclave
10. Desiccators
11. Micro-filtration unit
12. Incubator
13. Magnetic stirrer with hot plate
14. Laminar flow cabinet
15. Weighing Balance (Electrical)
16. Hot plate
17. Binocular Microscope
18. Haemocytometer
19. Colony counter
20. Antiserum
21. Electrophoresis chamber
22. Micropipettes

## LIST OF ABBREVIATIONS TO BE STUDIED

1. BAC: Bacterial Artificial Chromosomes
2. BLAST: Basic Local Alignment Search Tool
3. CTAB: Cetyl Trimethyl Ammonium Bromide
4. DBM: Diazo-benzyl oxy-methyl paper
5. DDBJ: DNA Database/ Data Bank of Japan
6. ddNTP: Dideoxy Nucleoside triphosphate
7. DMEM: Dulbecco Modified Eagle Medium
8. EBI: European Bioinformatics Institute
9. EMBL: European Molecular Biology Laboratory
10. EST: Expressed Sequence Tag
11. FACS: Fluorescence Activated Cell Sorting
12. FASTA: Fast All
13. FBS: Foetal Bovine Serum
14. HEPA: High Energy Particulate Air
15. HGP: Human Genome Project
16. IBPGR: International Board of Plant Genetic Resources
17. ICGEB: International Centre for Genetic Engineering and Biotechnology
18. IFN: Interferon
19. LB medium: Luria and Bertani Medium
20. MS medium: Murashige and Skoog medium
21. NCBI: National Centre for Biotechnology Information
22. NHGRI: National Human Genome Research Institute
23. PAGE: Polyacrylamide Gel Electrophoresis
24. PCR: Polymerization Chain Reaction
25. PDB: Protein Database/ Data Bank
26. PHB: Poly 3-Hydroxyl Butyrate
27. PIR: Protein Information Resource
28. RFLP: Restriction Fragment Length Polymorphism
29. RNA: Ribonucleic acid
30. RPMI medium: Roswell Park Memorial Institute medium
31. SCP: Single Cell Protein
32. SDS – PAGE: Sodium Dodecyl Sulphate– Polyacrylamide Gel Electrophoresis
33. SNP: Single Nucleotide Polymorphism
34. SSBs: Single Stranded Binding Proteins
35. STS: Sequence Tagged Site
36. VNTR: Variable Number of Tandem Repeats
37. YAC: Yeast Artificial Chromosome