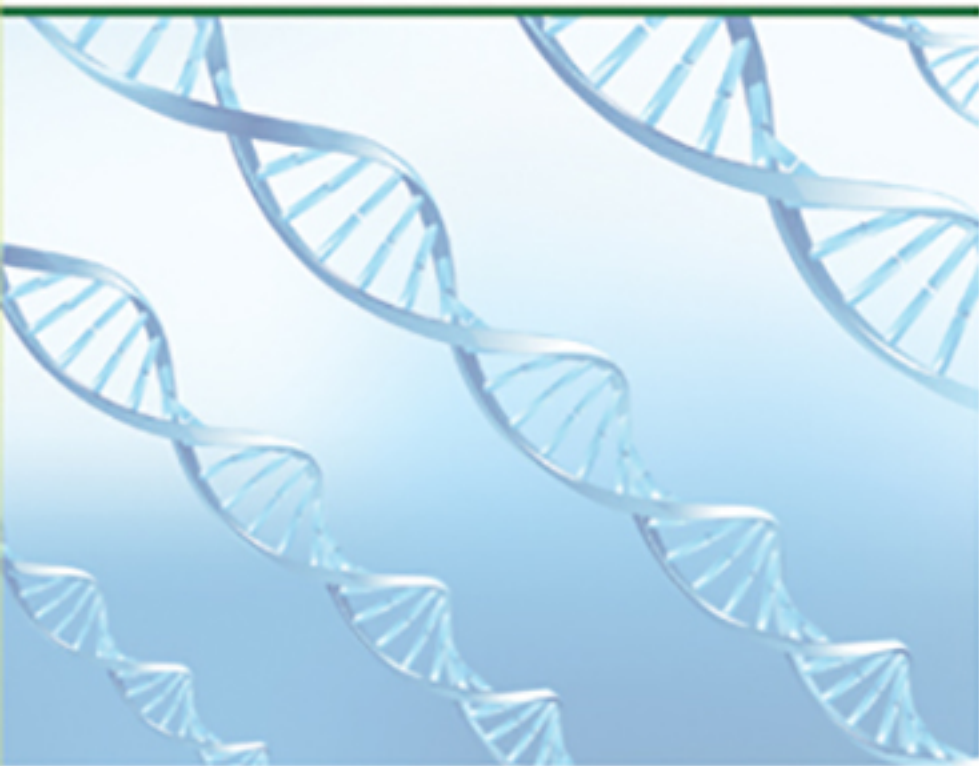


**Revised Edition** as per Latest CBSE/ISC Syllabi

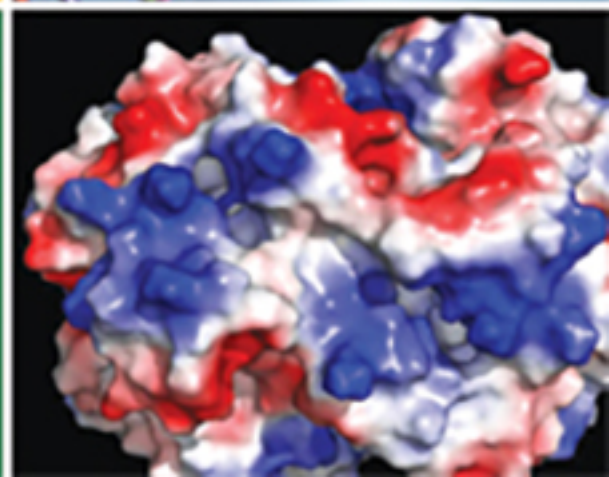
# A Textbook of Biotechnology

with Biotechnology Practicals

For Class - XII



**Dr. R.C. DUBEY**



# A TEXTBOOK OF **BIOTECHNOLOGY**

*with* Biotechnology Practicals

**For CLASS XII**

As per the CBSE Course Structure and the NCERT Guidelines (Also Useful for ISC, and Other Indian State Boards of Class XII Students)

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*To*

*My Mother, Father and Teachers*

*Matrideo bhav, Pitrideo bhav,  
Acharyadeo bhav*

*(Tattiriya Upanisad, 1.11.2)*

*(See God in Mother, Father and Teachers)*



# PREFACE TO THE THIRD EDITION

I feel pleasure in bringing out the **Third Edition** of **A Textbook of Biotechnology for Class XII**. Some additions and deletions for the improvement of the quality of the book have been done.

This revised and updated edition contains current information on different areas of biotechnology streamlining the relevance of the study of this fast-emerging discipline in various universities.

Questions asked in the annual examination of *Central Board of Secondary Education (CBSE)* as well as other boards like *ISC* have been added along with brief answers to understand the subject matter and gravity of the questions. A **Glossary** of the technical terms has been prepared and given in the book to ease the understanding of the students.

I am thankful to the management and the editorial team of *S. Chand & Company Pvt. Ltd.*, New Delhi for help and support in publication of this book.

Constructive suggestions and comments, if any, from the readers for the improvement of the book will be gratefully acknowledged and appreciated.

**Dr. R.C. DUBEY**

**Email:** profrcdubey@gmail.com

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# PREFACE TO THE FIRST EDITION

I feel pleasure in bringing out **A Textbook of Biotechnology for Class XII** students. This book is written solely as per syllabus prescribed by *the Central Board of Secondary Education (CBSE) for Class XII*. The book is also useful for Class XII, ISC students. It provides an understanding of up-to-date information on the concerned topics. This book embodies three units covered in twelve chapters. Unit III includes only practical for Classes XI and XII as per syllabus of the CBSE.

Biotechnology is a fascinating interdisciplinary subject which emerged in recent past. Being interdisciplinary area, the subject is very vast due to rapidly expanding the world of biotechnology. But in order to make the subject easy and understandable the text of the book has been illustrated with simplified well-labelled diagrams. Language of the book is simple, lucid and concise. The author has tried to arrange the matter in such a way that makes continuity of the subject and maintains curiosity of the students. This book has been brought out in multicolour edition.

India's present 95% economy is based on fossil fuel, and 1% on plants, microbes and animals. India's future is in the hands of youngsters. Hence, we have to look into the ways and means how to increase our economy based on plants, microbes and animals. Because we are the second largest country in the world rich in biodiversity. To achieve this goal, teaching of biotechnology at the level of Class XI and XII is must because the students must be enthused with the future objectives very clearly. Really, in future biotech-based economy of any country shall be viable.

The highlights of the book are: (i) important points written in Boxes, (ii) addition of six different types of questions at the end of each chapter such as very short answer type questions, short answer type questions, long answer type questions, matching type questions, filling in the blanks and multiple choice (objective type) questions with their answers (for competitive examinations).

The author expresses his sincere thanks to S. Chand & Company, Ltd. for its interest in publication of this book in multicolour. This is the first textbook of biotechnology in India that has been brought out in multicolour. Thanks are also due to Mr. R.K. Gupta, Chairman & M.D., Shri Navin Joshi (General Manager, Sales and Marketing) and Shri Shishir Bhatnagar (Editor) S. Chand & Company Ltd. for their help, suggestions and bringing out the book shortly. Special thanks to Ms Malini Kothiyal for providing colour illustrations and editorial assistance and Mr. D.K. Gupta for Designing & Layouting of the book.

Last but not and least, I extend thanks to my wife and son (master Pradeep Dubey) for their endurance, and daughters for preparation of neat and clean diagrams of the book.

Reader's perception in the form of constructive comments and suggestions for improvement of the book is highly appreciated.

**Dr. R.C. DUBEY**

# CBSE COURSE STRUCTURE

## Class XII

### **(Theory) One Paper**

*Time: 3 hours*

*Maximum Marks 70*

#### **UNIT V : Protein and Gene Manipulation**

**Marks 40**

Chapter 1.	Protein Structure and Engineering	...Marks 15
Chapter 2.	Recombinant DNA Technology	...Marks 15
Chapter 3.	Genomics and Bioinformatics	...Marks 10

#### **UNIT VI : Cell Culture Technology**

**Marks 30**

Chapter 4:	Microbial Culture and Applications	...Marks 10
Chapter 5:	Plant Cell Culture and Applications	...Marks 10
Chapter 6:	Animal Cell Culture and Applications	...Marks 10

### **Practicals**

*Time: 3 hours*

*Maximum Marks 30*

A.	Two experiments	6 + 6 (only one computer-based practical)
	Practical record	...Marks 04
	Viva on practicals	...Marks 04
B.	Project work	
	Write up	...Marks 05
	Viva on project	...Marks 05

Total	30
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# NEW CBSE SYLLABUS FOR CLASS XII

## UNIT – V

### PROTEIN AND GENE MANIPULATION

#### Chapter I. Protein Structure and Function

*Introduction to the world of proteins; 3D shape of proteins, non-covalent bonds, hydrogen bonds, Van der Waals forces, hydrophobic interaction, Structure - function relationships in proteins, Chymotrypsin- a proteolytic enzymes, Molecular diseases- sickle cell anaemia; two dimensional gel electrophoresis, Purification of proteins, Initial recovery of proteins, aqueous two phase partition, scale-up of purification, Bulk protein production, purification of proteins used for therapeutic or diagnostic purposes, Characterisation of proteins, Mass spectrometry (MALDI, Protein-based products, Blood products and vaccines, therapeutic antibodies and enzymes, therapeutic hormones and growth factors, regulatory factors, analytical applications, industrial enzymes, Functional non-catalytic proteins, Neutraceutical proteins, Designing proteins by protein engineering, improving laundry detergent subtilisin, Creation of novel proteins, Improving nutritional value of cereals and legumes- essential amino acid biological value, protein efficiency ratio, Proteomics, Genes and proteins-number of genes vs number of proteins, Types of proteomics, Expression proteomics, Structural proteomics, Functional proteomics.*

#### Chapter II. Recombinant DNA Technology

*Introduction, Tools of recombinant DNA technology, Restriction enzymes, Other enzymes used in cloning, Cloning vectors - plasmids, bacteriophages, cosmids, YAC vector, BAC vectors, animal and plant viruses, Host cells making recombinant DNA, DNA library, Introduction of recombinant DNA into host cells, Identification of recombinants, PCR, DNA Probes, Hybridisation techniques, Southern blotting technique, DNA sequencing- Sanger's method, Site-directed mutagenesis.*

#### Chapter III. Genomics and Bioinformatics

*Introduction- Structural genomics, Functional genomics, Genome sequencing projects- Direct sequencing of BAC contigs, Random shotgun sequencing, The expression sequence tag approach; Gene prediction and gene counting, Gene prediction and counting, Genome similarity, SNPs and comparative genomics, Functional genomics- microarray technology, Fluorescence in situ hybridisation, comparative cDNA hybridisation microarray, History of bioinformatics, Sequences and Nomenclature, The IUPAC symbols, DNA and protein sequences, The concept of directionality, Different types of sequences, Information sources- major databases, Data retrieval tools- BLAST family of search tools, Resources for gene level sequences, Analysis using bioinformatics tools.*

## UNIT – VI

### CELL CULTURE TECHNOLOGY

#### Chapter I. Microbial Cell Culture and Its Applications

*Introduction, Microbial cell culture techniques, Nutrients for microbial culture, Culture procedures (sterilisation, environment for microbial growth, aeration and mixing), Equipment for microbial culture (baffle flask, shakers, fermentors), Types of microbial culture (batch culture, fed-batch culture and continuous culture), Measurement of kinetics of microbial growth, Measurement of microbial growth, growth kinetics and specific growth rate, Scale-up of microbial process, Isolation of microbial products, Strain isolation and improvement, Strain isolation, Strain improvement (mutant selection, genetic engineering technique), Strain preservation, Culture collection centres, Applications of microbial culture technology, Bioethics in microbial technology.*

#### Chapter II. Plant Cell Culture and Applications

*Introduction, Cell and tissue culture techniques, Basic techniques, Nutrient media, Type of cultures- organ culture, explant culture, callus culture, cell suspension culture, mass cell culture, protoplast culture, Plant regeneration pathways (organogenesis, somatic embryogenesis), Application of cell and tissue cultures, Micropropagation, Virus-free plants, Embryo rescue, Haploids and triploids, Somatic hybrids and cybrids, Production of secondary metabolites, Somaclonal variation, In vitro plant germplasm conservation, Gene transfer methods in plants, Vector mediated gene transfer, Vector-less or direct gene transfer, Transgene analysis. Transgenic plants with beneficial trait, Stress tolerant (herbicide tolerance, insect resistance, virus resistance, fungi and bacteria, abiotic stress tolerance), Delayed fruit ripening, Male sterility, Transgenic plants as bioreactor i.e. molecular pharming (nutrient quality, seed protein quality, diagnostic and therapeutic proteins, edible vaccines), biodegradable plastics, metabolic engineering and secondary products; Diagnostics in agriculture and molecular breeding, Bioethics in plant genetic engineering.*

#### Chapter III. Animal Cell Culture and Applications

*Introduction, Animal cell culture technique, Features of animal cell growth in culture, Primary cell culture, Secondary cell culture and cell lines, Type of cell lines, Physical environment for culturing cells (temperature, pH, osmolarity, media, serum), Cryopreservation, Equipments required for animal cell culture (laminar air flow hoods, CO<sub>2</sub> incubator, centrifuge, inverted microscope), Characterisation of cell lines; Scale-up of animal culture process (roller bottles- microcarries beads, spinner cultures), Application of animal cell cultures, Tissue plasminogen activator (tPA), factor VIII, Erythropoietin, Hybridoma technology, Monoclonal antibodies, OKT3, Stem cell technology, The morphological approach, In vitro clonal assay, Long-term marrow culture, Embryonic stem cell culture, Cell and tissue engineering, Bioethics of genetic engineering in animals.*

# C O N T E N T S

## UNIT – I

### PROTEIN AND GENE MANIPULATION

#### **1. Protein Structure and Function** **3-34**

*The world of proteins- an introduction; Some examples of protein function, Mechanism of protein function, Protein deficiency-related disorders (beta-thalassaemia, mad cow disease and prions, sickle cell anaemia, SCID); Human Genome Project; Intracellular and extracellular proteins; The 3D shape of proteins, Structural organisation of protein, non-covalent interactions (hydrogen bonds, ionic interaction, hydrophobic interaction, Van der Waals interaction); Structure - function relationships in proteins, Chymotrypsin (structure of chymotrypsin, mechanism of action, regulation of chymotrypsin activity); Sickle cell anaemia- what is sickle cell anaemia, protein fingerprinting of haemoglobin, Two dimensional gel electrophoresis, Purification of proteins, Initial recovery of proteins, Aqueous two phase partition, scale up of purification, Methods of protein characterisation [electrophoresis, protein fingerprinting, two-dimensional electrophoresis, protein sequencing, Mass spectrometry (MALDI), surface enhanced laser desorption ionisation (SELDI), electrospray ionisation]; Protein-based products-Blood products, Synthetic and recombinant vaccines, Therapeutic antibodies and enzymes, Enzymes used in diagnostic assay, Therapeutic growth factors and hormones, Regulatory factors; Analytical applications; Industrial enzymes; Functional non-catalytic proteins; Neutraceutical proteins, Milk, Whey proteins, Curd; Test questions.*

#### **2. Protein Engineering, Protein Design and Proteomics** **35-51**

Protein engineering and design; *What is Protein engineering and design?* Differences between protein engineering and protein design, Steps of protein engineering and protein design; *Protein structure and function; Methods in protein engineering and design*, Properties required for protein engineering and design, Physical and computational techniques- X rays crystallography, NMR, spectroscopic technique, prediction of protein structure, computer modelling; *Some achievements in protein engineering and design*, Biochemical techniques and molecular biology- Site directed mutagenesis, Recombinant DNA techniques; *Some achievements in protein engineering and design*, Improvement in subtilisin, Recombinant vaccines, Protein engineering in enzymes, Protein design, Improvement in nutritional quality of seeds- essential amino acid profile, biological value, protein efficiency ratio, digestibility; PROTEOMICS– Protein data bank; *Relation between gene and protein, Types of proteomics*, Expression proteomics, Structural proteomics, Functional proteomics, *Test questions.*

#### **3. Recombinant DNA Technology (Genetic Engineering)** **52-90**

*Strategy of recombinant DNA technology; Basic tools of recombinant DNA technology, Isolation and purification of DNA, Restriction enzymes- types of restriction enzymes, cleavage of recognition sequences, construction of DNA molecules, Uses of other enzymes in rDNA technology, Cloning vectors- bacterial plasmids [(pBR322, pUC19, Ti-plasmid, shuttle vectors,*

expression vectors), bacteriophages (phage  $\lambda$ , phage M13), Cosmid vectors, YAC vector, BAC vectors], animal viruses, plant viruses; Host cells, Construction of recombinant DNA molecules, *DNA library*, Genomic library, cDNA library; *Transfer of recombinant vector into host cells*- Transformation, Transfection, Electroporation, Microinjection, Particle bombardment gun, *Agrobacterium*-mediated gene transfer; *Identification of recombinants*- Direct selection of recombinants, insertional selection, inactivation method, Blue-white selection method, *DNA probes*, *Colony and plaque hybridisation techniques*, Southern blotting technique, Northern blotting technique, Northern blotting technique; Polymerase chain reaction- working mechanism of PCR, application of PCR, DNA (gene) sequencing- Maxam and Gilbert method, Sanger's method, automatic DNA sequencer, Site-directed mutagenesis; *Test questions*.

## **4. Genomics**

**91-106**

*What is genomics?* Genome sequencing projects; Significance of complete genome sequencing, *Methods of gene sequencing*, Direct sequencing of bacterial artificial chromosome, Random shotgun sequencing, The expression sequence tag approach; *Gene prediction and gene counting*, Gene prediction algorithms, GenScan, Accuracy and validity of gene prediction algorithms; *Genome similarity, SNPs and comparative genomics*, Genome similarity, Single nucleotide polymorphism (SNPs), Comparative genomics; *Types of genomics*, Structural genomics, Functional genomics- microarray technology (FISH nick translation, application of FISH in detection of chromosomal defects, comparative microarray hybridisation ; *Test questions*.

## **5. Bioinformatics (In Silico Biology)**

**107-131**

*What is bioinformatics?; Historical background; Sequences and Nomenclature*, IUPAC symbols, Nomenclature of DNA sequences, Nomenclature of protein sequences, Directionality of sequences, Types of sequences used in bioinformatics- genomic DNA, cDNA, organellar DNA, ESTs, GSTs, Sequence-tagged site (STS), other biomolecules; *Information sources*, GDB, MGD, NCBI, GenBank, Data retrieval tools- Entrez, Taxonomy browsers, LocusLink, Sequence retrieval system, Similarity-based database searching- BLAST, FASTA, Resources for gene level sequences- UniGen database, HomoloGene database, RefSeq database, Use of bioinformatics tools in analysis; *Fundamentals of computers*; What is computer ?, History of computer, Classification of computer, Properties of computer; *Hardware and software*; Hardware-input devices, central processing unit (memory, RAM, control unit, ALU, microprocessor), secondary storage, output unit (monitor, printer); Software-Types of software, System software (operating system, firmware, language/compiler programme, productivity tools), programming software, application software; *Applications of computer*; Networking and internet, Alternating computing models. *Test questions*.

### **UNIT – II**

### **CELL CULTURE TECHNOLOGY**

## **6. Microbial Cell Culture and its Applications**

**135-168**

*Techniques of microbial cell culture*, Growth media, Sources of nutrition, Procedure for microbial culture (sterilisation, control of environmental conditions, aeration and mixing, vessels for microbial culture), *Types of microbial culture*- Batch culture- microbial growth kinetics and specific growth rate, Fed-batch culture, Continuous culture- the chemostat, turbidostat, *Measurement of*

*microbial growth*, Methods of measuring microbial growth- wet weight, dry weight, absorbance, total cell count, viable count, Coulter counter, *Scale-up of microbial process*, *Down stream processing*- separation of biomass, cell disruption, concentration of broth, initial purification, metabolic specific purification, *Isolation and improvement in microbial strains*, Isolation of strains, Strain improvement of microorganisms- mutation and mutant selection, recombination, *Culture preservation*, Agar slant culture, agar slant culture covered with mineral oil, storage in saline suspension, preservation by drying in vacuum, cryo-preservation, Lyophilisation or freeze-drying, *Culture collection centres*, *Applications of microbial culture technology*, Exploitation of whole cells of microorganisms, Production of primary metabolites, Production of secondary metabolites, Biotransformation, Biomining and bioremediation, Production of recombinant proteins, *Production of organic acids*; Production of citric acid (submerge culture process. chemistry of citric acid production, product recovery; *Enzyme production*, Selection of enzyme-producing microorganisms, methods of enzyme production; *Antibiotic production*, Selection of culture; Fermentation, Application of antibiotics; *Biosensor*, principles of detection; application, *Bioethics in microbial technology*, *Test questions*.

## **7. Plant Cell, Tissue and Organ Culture - I** **Tools and Techniques**

**169-192**

*History of plant cell, tissue and organ culture; Organisation of plant tissue culture laboratory; Methods of plant cell tissue and organ culture; Basic steps, Composition of nutrient media (inorganic nutrients, carbon and energy sources, organic supplements, growth regulators, solidifying agents), Maintenance of aseptic environment (sterilisation of glassware, instruments, transfer area, plant materials), Types of cultures (explant culture, callus culture, cell suspension culture, cell culture synchronization, mass cultivation of plant cells, protoplast culture, organ culture), Plant regeneration (organogenesis, somatic embryogenesis), In vitro germplasm conservation, Modes of conservation - in situ conservation, ex situ conservation, Methods of preservation (freeze preservation or cryopreservation, cold storage, low pressure and low oxygen storage), Methods of gene transfer in plants, Direct gene transfer methods (stimulation by chemical, lysosome-mediated plasmid transfer, electroporation, microinjection, particle bombardment gun), Vector-mediated gene transfer in plant cells (co-cultivation with protoplasts, leaf disc infection method), Transgene analysis, Test questions.*

## **8. Plant Cell, Tissue and Organ Culture - II** **Applications**

**193-221**

*Micropropagation*, Production of virus-free plants, Methods of virus elimination (heat treatment of meristem, meristem tip culture-virus indexing), *Artificial or somatic seeds*, *Embryo rescue*, *Haploid production*, Application of haploids, *Somatic hybrids and cybrids*, Formation of hybrids and cybrids, selection of somatic hybrids and cybrids, *Production of secondary metabolites*, *Somaclonal variation*, *Transgenic plants*, Stress tolerance-abiotic stress-tolerance herbicide resistance Production of drought-resistance- a hope from technology, the plant pathway, a promising future, biotic stress tolerance (insect resistance, virus resistance, resistance against fungi and bacteria), Delayed fruit ripening, Male sterility and fertility restoration, Transgenic plants as bioreactor *i.e.* molecular pharming (nutritional quality of seed protein, diagnostic and therapeutic proteins, edible vaccines, biodegradable plastics, metabolic engineering and secondary products); *Diagnostics in agriculture and molecular breeding*, Agricultural diagnostics, Molecular breeding, *Bioethics in plant genetic engineering*, *Test questions*.

## 9. Animal Cell Culture - Tools and Techniques

222-236

*Historical development, Techniques of animal cell culture, Some characteristics of animal cell growth in culture, Primary cell culture, Secondary cell culture and cell lines, Type of cell lines (finite cell lines, continuous cell lines), Environmental factors required to culture the animal cells (temperature, pH, osmolarity, growth media, preparation of water, serum-containing and serum-free media), Cryopreservation of animal cells, Equipments required for animal cell culture (laminar air flow, CO<sub>2</sub> incubator, centrifuge, inverted microscope), Characterisation of cell lines; Large scale cultivation of animal cells (scale-up of culture process, Methods for scale-up of cell culture process (roller bottles- microcarries beads, spinner cultures- advantages of spinner culture), Test questions.*

## 10. Applications of Animal Cell Cultures

237-261

*Choice of expression system in animals, Products of animal cell culture, Tissue plasminogen activator (tPA), Blood factor VIII, Erythropoietin, Hybridoma technology, Monoclonal antibodies- applications of monoclonal antibodies (disease diagnosis, disease treatment, therapeutic monoclonal antibodies, passive immunisation, detection and purification of biomolecules), Stem cell technology, The morphological approach, In vitro clonal assay, Long-term marrow culture, Embryonic stem (ES) cell culture (production of chimeric mouse, knockout mice, human ES cell culture), Cell and tissue engineering, Nuclear transplantation technique, Gene therapy in humans (ex vivo and in vivo gene therapy, antisense therapy), Bioethics in animal genetic engineering, Test questions.*

### UNIT – III

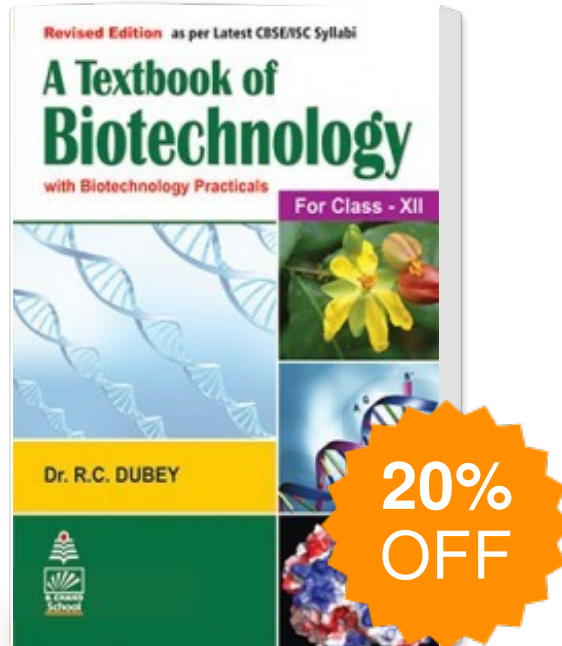
### BIOTECHNOLOGY IN HUMAN WELFARE

## 11. Biotechnology in Human Welfare

265-296

*Production of drought-and disease-resistant plants; Biofertilizers-Rhizobium biofertilizer (mass production of inoculants- methods of application, genetic modification in Rhizobium), Blue-green algal (cyanobacterial) biofertilizer (mass production of BGA, National Centre for Conservation and Utilization of BGA), Azolla spp., Mycorrhizal fungi- mass inoculum production, transfer of technology and launching of products; Biopesticides-Bacillus thuringiensis (insect pest, mode of action, bulk inoculum production, trade name of commercial products, specification of products, environmental impact and non-target toxicity, application); Biopesticide- Trichoderma spp.- culture characteristics, species of Trichoderma, why Trichoderma spp. as biopesticide ?, mode of action against plant pathogens, plant growth promotion, commercial formulation, methods of application, registered microbial pesticides; Manufacturers and distributors of biofertilizers and biopesticides; Genetically engineered vaccines- vaccine for hepatitis B virus, biotech industry in India, transmission of Hepatitis B virus; Genetically engineered hormone; Insulin-transfer and cloning of insulin gene, transfer of insulin gene into plasmid vector, cloning of insulin gene; Biotechnology of waste management; Vermicomposting; Mushroom production; Biotechnological production of renewable energy (bio-energy); Renewable energy from biomass; bio-ethanol, electricity from bagasse, Biodiesel (standards of biodiesel, composition of biodiesel, properties of biodiesel, soybean biodiesel); Biogas-construction, biogas plant, anaerobic degradation of residues, users of biogas and sludge; Test questions.*

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