

Biotechnology: Application in Environment and Forestry



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Unit 1: Introduction to Biotechnology

Unit Structure

1.0 Learning Objectives

1.1 Introduction

1.2 Definition

1.3 History of Biotechnology

1.4 Types of Biotechnology

1.5 Importance and uses of Biotechnology

1.6 Scope of Biotechnology in Forestry

1.7 Pros and Cons of Biotechnology in Forestry

1.0 Learning Objectives

After going through this unit, you will be able to:

- Understand the term Biotechnology
- Understand the definition of Biotechnology
- Understand the importance of Biotechnology
- Discuss the scope of Biotechnology in Forestry

1.1 Introduction

At its simplest, biotechnology is technology based on biology. The term Biotechnology was coined by Hungarian Engineer, **Karl Ereky** in 1917 to describe a process for large scale production of pigs. According to him all types of work are biotechnology by which products are produced from raw materials using living organisms. During the end of 20th century biotechnology emerged as a new discipline of biology integrating with technology; but the route of biotechnology lies in biology. There was no sudden sprout of this discipline, but some of the methods for production of products were developed centuries back. Therefore, biotechnology is concerned with exploitation of biological components for production of useful products.

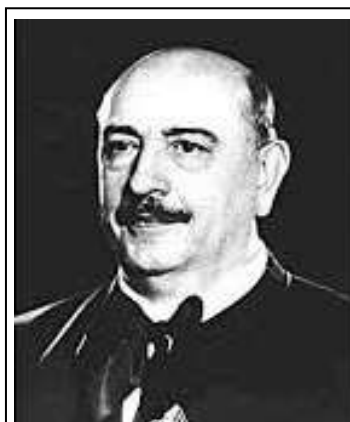


Figure 1. Karl Ereky

1.2 Definition

Biotechnology is defined by different organizations in different ways. It has been broadly defined as, “the development and utilization of biological processes, forms and systems for obtaining maximum benefits to man and other forms of life”. Biotechnology is “the science of applied biological process” (Biotechnology: A Dutch Perspective, 1981). Following are some of the definitions given by other organizations:

- Biotechnology is the application of scientific and engineering principles to the processing of materials by biological agents to provide goods and service [The Organization for Economic Cooperation and Development (OECD), 1981].
- The integrated use of biochemistry, microbiology and engineering sciences in order to achieve technological application of the capabilities of microorganisms, cultured tissue, cells, and parts they're of [The European Federation of Biotechnology (EFB), 1981; O'Sullivan, 1981].
- The application of biochemistry, biology, microbiology and chemical engineering to industrial process and products and on environment [International Union of Pure and Applied Chemistry (IUPAC), 1981.]
- Biotechnology is the “controlled use of biological agents such as microorganisms or cellular components for beneficial use” (U.S. National Science Federation).
- In the definition given by OECD, “scientific and engineering principles” refer to microbiology, genetics, biochemistry, etc. and “biological agents” means microorganisms, enzymes, plant and animal cells. The meaning of these three definitions and others given by many organisations are more or less similar.

A unified definition of genetic engineering has been given by Smith (1996) as “the formation of new combinations of heritable material by the insertion of nucleic acid molecules produced by whatever means outside the cell, into any virus, bacterial plasmid or other vector system so as to allow their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation”.

1.3 History of Biotechnology

Since ancient times, man has been using living things to improve his life. In the early times (about 8000-4000 B.C.) man has been using plants and animals for various

services. As the time passed, various developments took place in the area of biotechnology. Various stages in the development of Biotechnology. The development of biotechnology can be categorized as

Ancient biotechnology (8000–4000 BC): Early history of biotechnology relates to domestication of animals and cultivation of plants. In the period before the year 1800, events such as domestication of animals to get milk or meat, cultivation of plants such as rice, barley and wheat as major source of food were categorized as biotechnological developments. Breeding of plants and animals has been done to improve them by introducing desirable characteristics.

Classical biotechnology (2000 BC; 1800–1900 AD): This era of biotechnology was based on use of microbes in fermentation, production of food and medicine. In this period Genetics was developed (1900–1953) and DNA research was initiated (1953–1976).

The production of cheese, yogurt and bread from micro-organisms, alcoholic drinks such as beer and wine via fermentation has been done since ancient times. The role of microorganisms (such as bacteria, yeast or molds) in hydrolysis sugars and hence fermentation was discovered. In 1796, Edward Jenner demonstrated role of vaccination in providing immunity against smallpox, hence is considered the founder of vaccinology. Scientific evidence for fermentation was first described by Louis Pasteur in the late 1800s. He proposed a theory known as germ theory stating microorganisms play a major role in the process of fermentation. Honey, soybean curds has been used as natural antibiotic and found effective in healing wounds since ancient times. Ukrainian farmers used moldy cheese to treat infected wounds. Later it was found that antibiotics present in molds killed bacteria and prevented the spread of infection. Enzymes and proteins have been discovered in 1830.

In 1919, Hungarian engineer Karl Ereky coined the term "Biotechnology". According to him, biotechnology is the use of living organisms to produce materials or products of importance. In other words, it is the technology that uses biological processes, organisms or systems to produce products that improve human lives.

In 1920, Chaim Weizman tried to produce useful chemical compounds through biological techniques. He used *Clostridium acetobutylicum* that converted starch to butanol. Later, biotechnology such as fermentation was refined to develop paint solvents for the automobile industry and acetone from starch. These processes were promoted during

World War I. During World War II, penicillin was discovered. In 1929, Alexander Fleming produced antibiotic penicillin from cultures of *Penicillium notatum*. The penicillin proved useful for the treatment of wounded soldiers during World War II (Fig. 10.1). The focus of biotechnology shifted to pharmaceuticals in 1930s. Also in 1930s use of biotechnology shifted to agriculture sector.

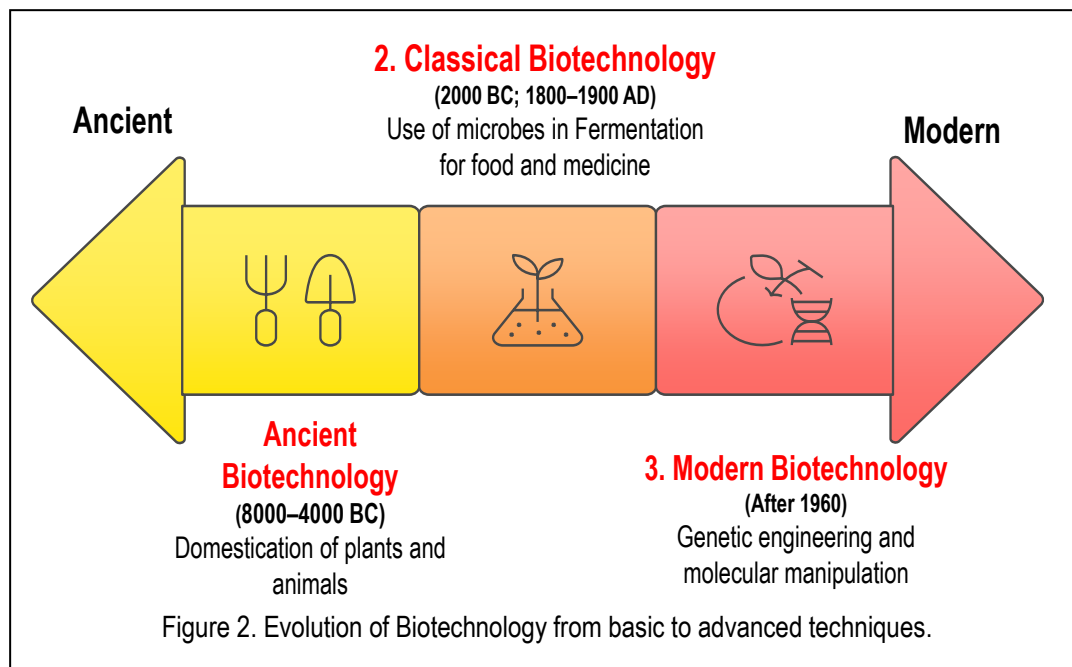
Selective breeding of plants and animals has been done in the past. In this procedure organisms with desirable traits were allowed to mate to further enhance these traits in their offspring. It was found that selective breeding could improve yield as well as productivity. Mule is one of the most primitive examples of crossbreeding for the benefit of humans. Man started using mules for transportation, carrying loads and farming. Later on, focus of biotechnology moved to pharmaceuticals. The microorganisms were used in preparation of antibiotics.

At the end of the eighteenth century and the commencement of nineteenth century, focus of biotechnology was shifted to crop rotation (including leguminous crops), development of vaccination and animal-based studies. The complexity of biotechnology increased owing to the evolution of new technologies and better understanding of various principles of life science.

By the end of the nineteenth century, many microorganisms were discovered, Mendel's work on genetics was accomplished and microbial processes such as fermentation were explored. At the beginning of the twentieth century, industry and agriculture started using biotechnology methods.

Modern biotechnology (1977): In this period, genetic information in organisms was manipulated via genetic engineering. Later in 1960s and 70s molecular, cellular technologies began to emerge. In the period between 1953 to 1976, DNA research was initiated. Cells and biological molecules (DNA, RNA and proteins) were used to make useful products. The real biotechnology was initiated with the production of Insulin in 1970. The first restriction enzyme was also discovered in 1970. The discovery enabled the researchers to insert foreign genes in bacteria. This formed the basis of recombinant DNA technology and is considered the birth of modern-day biotechnology. The DNA of living organisms was manipulated and the products obtained from genetically modified organisms were used in the manipulation of molecules. In 1977, researchers in Scotland cloned a sheep named Dolly, later on, sheep named Polly was cloned using nuclear transfer technology as it had human gene encoding Factor IX, a protein involved in

preventing haemophilia. Companies such as Genentech, Amgen, Biogen, Cetus, and Genex started were established in the mid-to late 1970s. They manufactured genetically engineered substances primarily for medical and environmental uses. In 1998, the importance of cloning Dolly was realized. Molecular biologists became interested in studying stem cells. In 1999, antibody analysis was found as another tool of use.



Modern biotechnology, thus has developed as a science with enormous potential for human welfare in areas ranging from food processing to human health and environmental protection. Biotechnology is now being used in numerous disciplines including bioremediation, energy production, agriculture and food processing. Biotechnology has vast scope in agriculture for the production of plants that are resistant to insects, weeds and plant diseases. Production of medicines (biopharmaceuticals) through cloning of vectors with genes of interest (GOIs) has been made possible via biotechnology. Modern biotechnology is applied in medicine and healthcare in therapeutics, mainly for the discovery, development and production of novel drugs and in diagnostics, for protein and nucleic acids-based tests. In the area of environment, biotechnology has been primarily used to treat waste and prevent pollution. Thus, modern biotechnology makes significant contribution in improving the lives of humans.

1.4 Types of Biotechnology

In the recent years, the scope of biotechnology has expanded. It has been categorized into different types depending on the field in which it is used.

1. Medical Biotechnology

It refers to use of living cells and other materials to improve health of humans. It is used for finding cure for diseases or prevention of diseases, as a research tool for studying human health, understanding pathogens and human cell biology. The technique is used for producing pharmaceutical drugs as well as other chemicals to combat diseases. The field is used for development of new drugs and treatments. Examples- vaccines, an anti-lymphoma vaccine has been developed using genetically engineered tobacco plants that exhibit RNA (a similar chemical to DNA) from malignant (actively cancerous) B-cells.

2. Agricultural Biotechnology

Biotechnology has been used in developing genetically modified plants to increase crop yields or introduce characteristics that impart tolerance against biotic, abiotic stress, and provide resistance against pest and pathogen attack. Examples- development of crops that express anti-pest characteristics. The genes of *Bacillus thuringiensis* have been transferred to crops. A protein (Bt) produced by *Bacillus thuringiensis* found to be very effective against pests such as the European corn borer.

3. Industrial Biotechnology

Biotechnology also finds its applications in industrial sector. It includes use of microorganisms, or components of cells like enzymes, to generate products such as food and feed, chemicals, detergents, paper and pulp,

textiles, biofuels, and biogas that are industrially useful. Examples Biocatalysts have been developed via industrial biotechnology to synthesize chemicals.

4. Environmental Biotechnology

Biotechnology is used in treatment of waste and prevention of pollution. These methods efficiently clean up wastes and significantly reduce our dependence on methods for land-based disposal. Examples- in Bioremediation, potential of living organisms are exploited for removal/treatment of wastes. Enzyme bioreactors that pretreat industrial and food waste components and allow their efficient removal via sewage system has been developed.

Recently a new system of classifying Biotechnology has been adopted. In this type of classification, various colours have been given to various branches of biotechnology (Table 1)

Table 1. Table. Categorization of Various Branches of Biotechnology on the Basis of Colour

Color Designation	Description
Gold Biotechnology / Bioinformatics / Computational Biology	Use of computational techniques for analysis of biological data.
Red Biotechnology (Biopharma)	Relates to medicine and veterinary products. Includes development of new drugs, vaccines and antibiotics, regenerative therapies, molecular diagnostics, and genetic engineering techniques to cure diseases by applying genetic manipulation.
White Biotechnology	Involves designing energy-efficient, less polluting, and low resource-consuming processes and products.
Yellow Biotechnology	Relates to the use of biotechnology in food production such as making wine, cheese, and beer by fermentation.
Grey Biotechnology	Includes environmental applications of biotechnology such as removal of pollutants or contaminants using microorganisms and plants.
Green Biotechnology	Use of biotechnology in agriculture for creating new plant varieties of agricultural interest and biopesticides.
Blue Biotechnology	Use of marine resources to create products and their application in various sectors.
Violet Biotechnology	Deals with the legal, ethical, and philosophical issues related to biotechnology.
Dark Biotechnology	Relates to bioterrorism, biological weapons, biowarfare using microorganisms, and toxins to cause diseases and death in humans, domestic animals, and crops.

1.5 Importance and uses of Biotechnology

Biotechnology has been successfully applied in various areas such as the environment, medicine, agriculture, industries and many more.

1. Environment

Biotechnological techniques have been used in the diagnosis of environmental problems, their monitoring and abatement. This technology has been used in areas of waste management, bioremediation (use of living organisms to treat environmental pollutants), phytoremediation (use of plants to remove pollutants from the environment). Apart from these renewable resources, biodegradable products and alternative energy sources have also been developed using biotechnology. Biotechnology is used to engineer microbes that show immense potential for environmental clean-up by removing a wide range of pollutants from various sectors of the environment.

2. Medicine

Biotechnological techniques have been used in the diagnostics, therapeutics, vaccines, human Genome Research. The development of insulin and vaccines such as hepatitis B are some of the major applications of biotechnology in medicine.

Recombinant DNA technology has made an immense impact in the area of healthcare by enabling the mass production of effective therapeutics. Transgenic animals are been used to understand how genes contribute to the development of human diseases such as cancer, cystic fibrosis, rheumatoid arthritis and Alzheimer's. Gene therapy had made the treatment of hereditary diseases possible via the insertion of genes into individual's cells and tissues. Agriculture

Biotechnological techniques have proved very useful in the area of developing crops with better nutrition content, high yield, and resistance to various abiotic and biotic stresses. Food, horticultural crops and tree species have been modified successfully using genetic engineering. For e.g. Oil seed can be modified to produce fatty acids for petrochemicals, fuels. Efforts have been made to develop vaccines from plants. Crops that show tolerance against a broad range of pests/insects have been developed.

4. Industries

Biotechnology has been applied for the production of cellular structures or production of biological elements for numerous uses such as development of new materials in the

construction industry, and the manufacture of beer and wine, washing detergents, and personal care products.

Biotechnology is used in the textile industry for the finishing of fabrics and garments. It helps in production of warmer, stronger, wrinkle and shrink resistant clothes along with other properties such as improved dye uptake and retention, enhanced absorbency. Biotechnology has been successfully applied in the food processing industry.

5. Biofuels

Biotechnology has been used in sector of energy production. Bioremediation waste can be converted to biofuel to run generators. Bacteria that degrade sulfur liquor, which is a waste product of the paper manufacturing industry, can help in production of methane.

6. Evolutionary studies

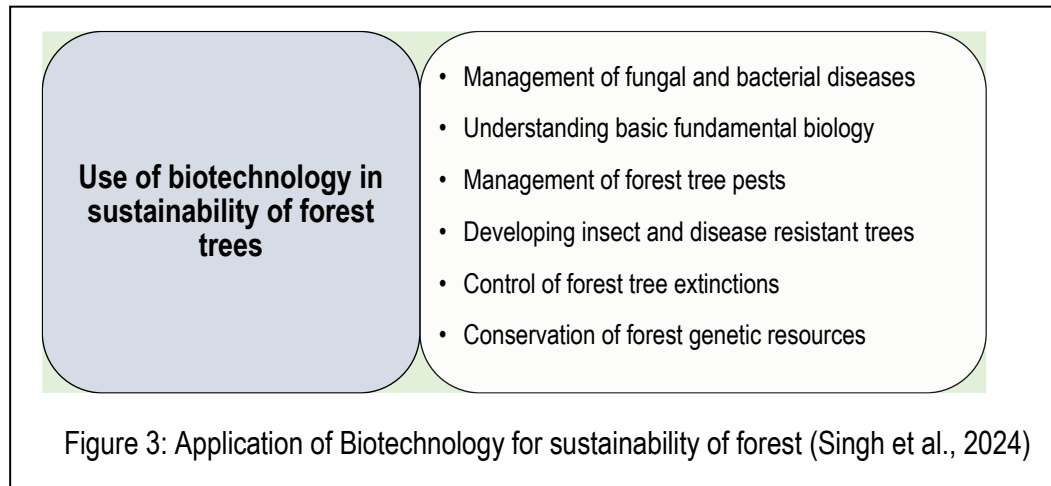
Genes associated with ecological traits and evolutionary diversification can easily be traced using biotechnological tools.

Other Uses

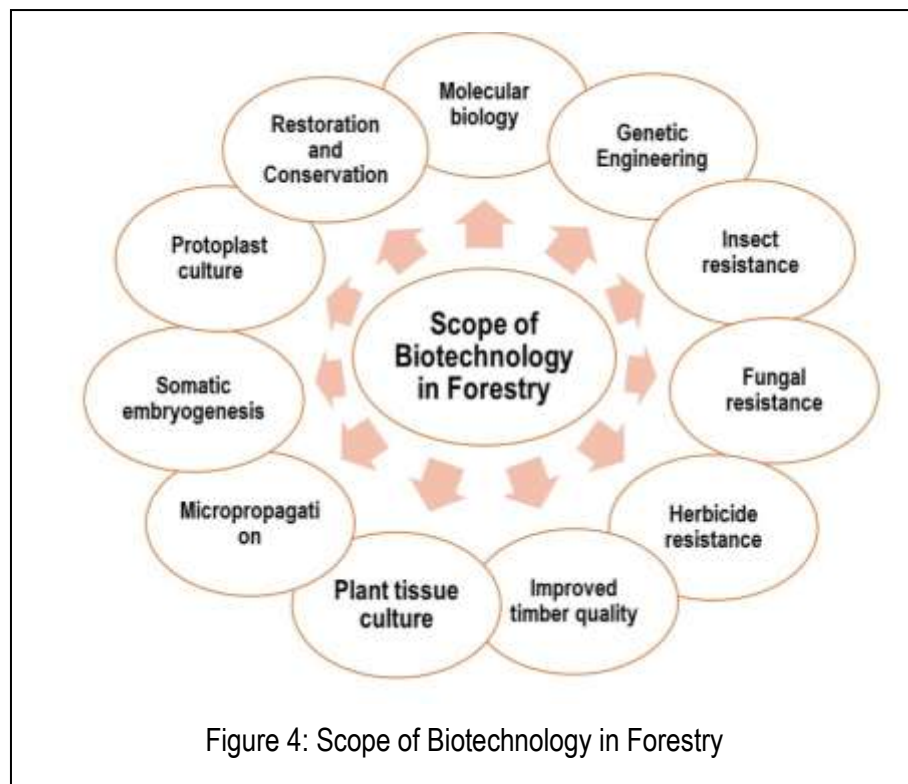
Microbes can be induced to produce enzymes required to convert plant and vegetable materials into building blocks for biodegradable plastics.

1.6 Scope of Biotechnology in Forestry

Biotechnological innovation in forestry falls into three main areas: the use of vegetative reproduction methods; the use of genetic markers; and the production of genetically modified organisms (GMOs), or transgenic trees. Most of the biotechnologies used in forestry today involve vegetative reproduction through tissue culture and molecular marker applications. However, GMOs are also likely to play a major role in forestry. Using techniques adapted from agriculture, selected foreign genes are introduced into the plant genome. In one such approach, specific genes are identified and modified to affect biochemical pathways and the resulting phenotypes. For example, the promise of controlling the lignin—cells that impart strength to the tree's structure but that must be removed in papermaking—depends on the ability to identify and modify lignin genes, thereby altering the amount, type, and form of lignin that is produced in the tree. The ease of gene transformation varies with different species, generally being more difficult in conifers than in hardwoods (Sedjo, 2001).



Forest biotechnology allows scientists to better understand tree genetics, create new tree varieties, and produce materials that can adapt to changing environments. Since forest trees have high genetic diversity but have not been widely improved through breeding, there is great potential for research. Using biotechnology, we can quickly multiply seedlings, restore damaged forests, conserve genetic diversity, and detect diseases (Singh et al., 2021). The overview of scope of Biotechnology in Forestry is represented in figure 4.



1.7 Pros and Cons of Biotechnology in Forestry

Biotechnology in forestry offers pros like faster growth, pest/drought resistance, improved wood quality, and reduced logging pressure on natural forests, aiding conservation and economic gains, but faces cons such as biodiversity loss, potential "superweed" spread, high R&D costs, and ecological risks from gene flow to wild relatives, necessitating careful regulation and long-term study.

Pros (Benefits)

Increased Productivity: Faster-growing trees mean quicker yields for wood and fiber.

Enhanced Traits: Developing trees resistant to pests, diseases, and harsh conditions (drought, saline soils).

Reduced Logging Pressure: High-yield plantations can meet demand, reducing logging in natural forests.

Environmental Benefits: Can adapt trees to degraded lands, create carbon sinks, and use biopesticides.

Economic Advantages: Lower production costs, higher returns, and potential for novel products (e.g., medicinal compounds).

Customization: Tailoring wood properties (strength, density) for specific uses.

Cons (Risks & Challenges)

Reduced Biodiversity: Monocultures and genetically uniform trees decrease ecosystem resilience.

Gene Flow: Transgenes spreading to wild relatives, creating "superweeds" or altering natural populations.

Unintended Consequences: Negative impacts on wildlife, soil microbes, or broader ecosystems.

High Costs & Risks: Expensive R&D, long development times, and regulatory hurdles.

Socio-Economic Issues: Unequal access to technology, potential displacement of traditional forestry practices.

Ecological Disruption: Introduction of novel traits can disrupt delicate forest food webs and balances.

In essence, biotechnology offers powerful tools for sustainable forestry by boosting efficiency and resilience, but requires careful management to avoid irreversible ecological damage and ensure equitable benefits.

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Unit 2: Biochemistry and Molecular Biology

Unit Structure

2.0 Learning Objectives

2.1 Introduction

2.1.1 Chemical foundations of life

2.2 Biomolecules

2.2.1 Carbohydrates

2.2.2 Structure and functions of proteins

2.2.3 Lipids

2.2.4 Nucleic acids

2.3 Molecular biology

2.3.1 Organization of genome

2.3.2 Chemistry of DNA synthesis

2.3.3 Mechanism of DNA replication in eukaryotes

2.0 Learning Objectives

After going through this unit, you will be able to:

- Explain the introduction and function of carbohydrates
- Understand classification and characteristics of carbohydrates
- Understand the properties of mono-, di- and polysaccharides
- Determine the structure of proteins and peptide bond
- Understand the general importance of lipids
- Describe nucleic acids and their components
- Explain gene organization and the structure of nucleosome
- Understand the initiation, elongation and termination of DNA replication
- Describe the structure and roles of enzymes involved in DNA replication

2.1 Introduction

The study of biological processes at the molecular level is known as biochemistry or in simple words “the study of the chemistry or chemical processes related to living organisms”. Biochemistry is the language of life; to say it simply, communication is easy if we are familiar with the vocabulary and grammar of any language. In a comparable manner, anyone who works with life and how it relates to health has to understand the biochemistry of living systems. Understanding the molecular processes

occurring within living cells makes this process of grasping life easier. Among the related biological sciences, biochemistry is one of the most recognized fields. This is because it is an interdisciplinary subject with a wide range of applications. You'll be surprised to learn that understanding biochemistry will enable you to fully grasp the molecular processes and metabolic reactions occurring in biological systems. In a sense, nonliving inorganic materials are the source of parts of living cells. Especially, in forestry, biochemistry analyses the cellular foundations of tree life, from photosynthesis and nutrient uptake to stress responses (drought, pests) and bioremediation. It uses biochemical markers (enzymes, proteins) to better understand forest health, enhance tree breeding, and manage sustainable forestry by analysing chemical processes, creating bio-pesticides, and evaluating environmental effects like pollution. It is essential for improving wood quality, understanding carbon cycles, and insuring the resilience of ecosystems.

Forests are an essential resource and habitat; they protect the soil, regulate water and absorb carbon, along with nurturing and sheltering a lot of the world's diversity. Forests are used for wood and non-wood production in combination with other functions such as soil and water protection, biodiversity conservation and recreation. They need to be managed using scientific methods. The aim of this book is to present a core of biochemical knowledge that is desirable for students. Every mother who makes dosa (food) batter or curdles milk is a biochemist! It may sound a bit silly, but it is accurate. Applications of biochemistry, such as pickling, tanning, and fermentation, have been used by humans since time immemorial. The historic project was completed in the middle of the 1800s. According to Pasteur, yeast cells promote the fermentation process, which produces alcohol. The term "biochemistry" was later created by Carl Neuberg in 1903. Some of the fundamental events that take place inside biological systems were explained in the first half of the 20th century. Among the important metabolic cycles occurring within a living cell, Hans Adolf was awarded the Nobel Prize in Physiology for his description of the krebs cycle / citric acid cycle. In a similar vein, Gustav Embden and Otto Meyerhof described the processes of glycolysis (the breaking of sugar) in 1940. These are a few of the important findings that have had a fantastic influence on the subject of biochemistry. Proteins were thought to be inherited until the middle of the 20th century. However, Hershey and Chase's 1952 research demonstrated that DNA, not proteins, is the genetic material. Later on, in 1953, James

Watson and Francis Crick made the most significant discovery of the 20th century by describing the double helical structure of DNA. This finding assisted those working in science in understanding how DNA stores genetic information. Indian American biochemist Har Gobind Khorana made the discovery of genetic code in 1968 and was awarded the Nobel Prize in physiology.

2.1.1 Chemical foundations of life

A living system's organizational hierarchy is similar to that of cells to tissues, tissues to organs, organs to systems, and systems to organisms. The intricacy of structure and organization varies slightly between invertebrates and vertebrates, or, to put it another way, from smaller to higher organisms. At the same time, different organisms have different cell compositions. We are all aware that life occurs on Earth in a variety of forms, including microbes, plants, and animals. Certain characteristics are shared by all of these living organisms, including growth, reproduction, and movement (apart from plants). All these species are composed of identical organic and inorganic chemical entities, such as proteins, carbohydrates, lipids, nucleic acids and essential minerals.

2.2 Biomolecules

In biochemistry, the four primary kinds of molecules known as biomolecules are proteins, lipids, carbohydrates, and nucleic acids. Many biological molecules are polymers i.e., made up of monomeric units. Monomers go through a process known as dehydration synthesis when they are joined to form a biological polymer. Larger complexes made up of several macromolecules are frequently required for biological function.

This section's key point is that "these biomolecules are common in their basic composition (precursor elements like carbon, nitrogen, oxygen, phosphorous, etc.) but differ in their arrangement from one organism to another." major biomolecules are synthesized from non-living inorganic chemical elements. However, these non-living components come together to produce biomolecules such as lipids, proteins, carbohydrates, and nucleic acids. You may have learnt in your lower grades that DNA functions as a genetic substance that transmits information from one generation to the next. DNA is physically comparable in both plant and animal cells (double helix), made up of nitrogenous bases such as adenine, guanine, cytosine, and thymidine.

2.2.1 Carbohydrates

Carbohydrates are one of the three major macronutrients in our diet representing a wide group of substances which include the sugars, starches, glycogen, gums and celluloses. Carbohydrates are known as the important energy source for living things. They come from the process of photosynthesis, an endothermic reaction in which condensation of carbon dioxide takes place and which requires light energy and the pigment chlorophyll. The chemical component of carbohydrates is carbon, hydrogen, and oxygen. Generally, but not always the hydrogen and oxygen in carbohydrates are present in the proportion of two hydrogen atoms to one oxygen atom as in H_2O , from which fact the term “carbohydrates” was derived. The carbohydrates are actually or potentially hydroxy aldehydes or ketones, and in most cases, they are polyhydroxy aldehyde or ketones compounds that can be hydrolyzed to them. It also means “hydrate of carbon” Plant biomass is made up of three fourths of carbohydrates but in animal body it is present in small quantities. In animal it is stored in the form of glycogen, sugar and their derivatives. All living organism contains derived monosaccharides such as the sugar acids, amino and the deoxy sugar. Derivatives of the carbohydrates also contain nitrogen, phosphates and sulfur compounds. Carbohydrates can combine with lipid and protein to form glycolipids and glycoproteins. Carbohydrates and their derivatives are present in all animal tissues and tissue fluids, blood and milk. The glucosides are a class of carbohydrates are derivatives which are frequent constituents of plants. The major components of living matter are formed from carbohydrates, proteins and fats. This complex form of carbohydrates is required for the maintenance of cell structure and functions. Presence of functional group like alcohol on almost every carbon makes the chemistry of carbohydrates a complex structure Carbohydrate may be exist in either a straight chain or a closed ring structure. The general reaction between the aldehyde and ketone part of the carbohydrates and alcohol groups forms the derivative called hemiacetals and hemiketals leads to the formation of ring structure. The most common basic structure of carbohydrates is closed rings Glucose is an example of a single closed ring carbohydrate.

Classification

The carbohydrates are sometimes referred to as the saccharides. The word saccharides come from the Greek word sakcharon meaning sugar. Those

carbohydrates such as glucose and fructose that cannot be hydrolyzed into simpler compounds are called simple sugars or monosaccharides. Certain carbohydrates are called disaccharide, made of two molecules of monosaccharides such as sucrose, lactose and maltose. Similarly, trisaccharides are composed of three and tetrasaccharides of four monosaccharides molecules as shown in figure 1. All these compound carbohydrates may be readily hydrolyzed into their constituent monosaccharides by heating with dilute acids or by the action of specific enzymes. According to the number of C atoms and functional groups in its simplest form of carbohydrates are further classified. The suffix –ose indicates the types of sugar. Nomenclature of carbohydrates based on number of carbon atoms as given below-

1. Triose= 3 carbon atom (Glyceraldehyde, Dihydroxyacetone)
2. Tetrose= 4 carbon atom (Erythrose)
3. Pentose= 5 carbon atom (Ribose, Ribulose, Xylulose)
4. Hexose=6 carbon atom (Glucose, Galactose, Mannose, Fructose)
5. Heptose=7 carbon atom (Sedoheptulose) and so on.

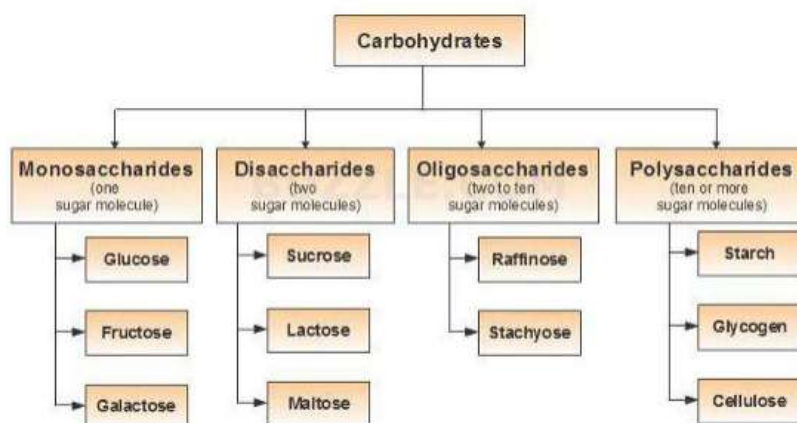


Figure 1: Classification of carbohydrates

Functions of carbohydrates

The simplest carbohydrates are initially synthesized in plants by the process of photosynthesis which is a series of reactions. Carbohydrate functions as... bio-Fuel, primary Source of Energy, storage food, framework in body, anticoagulant, antigen, hormone, provide raw material for industry, Essential for Fat Oxidation, plays role in

gastro-intestinal function, add flavour to the diet and play an important role in molecular recognition.

I. Properties of Mono saccharides

Glucose

Glucose is an aldohexose. It is a poly hydroxyaldehyde and contains 6-carbon. Carbohydrates are transformed into simplest glucose which is the primary carbohydrates utilized by the body tissues. Glucose is the sugar of blood and other body fluids. Disaccharide like sucrose, maltose and lactose and polysaccharides such as starch, cellulose and glycogen contains glucose as one of the monomers. Glucose is 25% less sweet than sucrose.

Galactose

Galactose is an aldohexose. It is a poly hydroxyaldehyde and a 6-carbon containing compound. It is found in dairy products, sugar beets, pectin and gum. Galactose is one of the monomers of the disaccharide lactose. Galactose is 80% less sweet than sucrose. It is epimers of glucose.

Fructose

Fructose is a ketohexose. It is a poly hydroxyketone and contains 6-carbon. It is found in fruit, flowers, root vegetables and honey. Disaccharide sucrose contains fructose as one monomer. It is the sweetest of all naturally occurring carbohydrates and 175% sweeter than sucrose. Fructose has higher solubility than other sugars.

II. Properties of oligosaccharides: The oligosaccharides are composed of the disaccharides, trisaccharides and tetrasaccharides and are so designated to indicate the number of monosaccharides units involved in the structures. Disaccharides is a sugar formed when a bond between the first carbon of one sugar and a hydroxyl group at any position on the other sugar is formed and a molecule of water is removed. Sucrose, maltose, and lactose are the most important disaccharides. Each one of these on hydrolysis with either an acid or an enzyme gives two molecules of the monosaccharides.

III. Properties of polysaccharides

Carbohydrates composed of ten or more monosaccharides units are generally classified as polysaccharides and their molecules are colloidal in size.

Polysaccharides may be considered as condensation polymers in which the monosaccharides are joined together by glycosidic linkages. Polysaccharides are also termed as glycans. Polysaccharides when composed of a single monosaccharide building block are called homopolysaccharides (homoglycans). Polysaccharides when composed of more than one type of monosaccharide are called heteropolysaccharides (heteroglycans). Glucose polysaccharides are “glucans”, fructose polysaccharides are fructans and polysaccharides made of mannose and xylose are called mannans and xylans, respectively. Some polysaccharides contain units that are derivatives of the monosaccharides for example chitin is made up of the amino sugar, glucosamine and hyaluronic acid is composed of glucuronic acid and glucosamine. Monosaccharide units of polysaccharides are joined by glycosidic linkage; the polysaccharides are readily hydrolyzed by mineral acids but are resistant to alkaline hydrolysis.

2.2.2 Structure and functions of proteins

Proteins are large complex molecules and play several vital roles in the body. These roles include regulatory, extracellular as well as intracellular and many others. How will you understand the function of proteins? The answer lies in the structure of proteins. In the previous unit you learnt that the amino acids are building blocks of proteins. A linear chain of amino acid residues is known as polypeptide. The amino acids are joined together by the peptide bond. Proteins contain at least one big polypeptide. The smaller polypeptides with amino acid sequences generally less than 20-30 amino acid residues are simply called as peptides or sometimes oligopeptides. The synthesis of proteins in vivo occurs in a systematic manner in the order of the sequence of nucleotides in a gene. Since there are twenty different amino acids, for a protein within amino acid residues; there can be 20^n possible sequences. Each amino acid has characteristic structure and function. Its place in the structure of proteins influences the function of protein as well as its properties. The characteristics of an individual protein are therefore determined by its amino acid composition. Several combinations of amino acids lead to wide variety of proteins and diversity of their functions. Proteins fold in three dimensions. Protein structures are organized hierarchically from primary, secondary, tertiary to quaternary structures.

I. Peptides

Amino acids with their functional groups such as carboxyl, amino and R groups can undertake several chemical reactions. Reactions due to amino group include transamination, amide bond formation (amidation) and oxidative deamination; for carboxylic acid groups, reactions include formation of esters (esterification), amides and acid anhydrides; for R groups such as –OH and –SH reactions include oxidation and esterification. The most important reaction of amino acids is the condensation reaction that leads to the formation of a peptide bond. The peptide bond is a covalent bond between α -amino group of one amino acid and α -carboxyl group of another forming an amide linkage (CO-NH). Peptide bond formation is a condensation reaction which involves removal of water (dehydration).

II. Functions of proteins

Proteins perform wide range of functions in living organism. Some of the fundamental roles played by enzymes are as follows:

- 1. Proteins as Enzymes:** Almost all of the biochemical reactions are catalyzed by enzymes. All enzymes are specialized proteins along with catalytic activity involved in several biological processes such as photosynthesis (where RuBisCO is a key enzyme), metabolism and cell signaling. Some of the examples include amylase, catalase etc.
- 2. Structural Proteins:** Proteins provide structural support at the cellular level, forming parts of the cell's structure and membranes.
- 3. Transport or carrier proteins:** Proteins on the plasma membrane regulate the transport of ions and other substances into and out of the cell.
- 4. Storage proteins:** Seeds and tubers store proteins to provide essential nutrition for growth when the plant starts to develop. Plant seeds store nutrient proteins required for the growth of the germinating seedlings. Ferritin protein found in bacteria and in plant and animal tissues stores iron.
- 5. Defense:** Plants produce a variety of defense proteins to protect themselves from pathogens like bacteria, viruses, and insects. Examples include lectins, which have anti-insect, anti-fungal, anti-viral, and anti-bacterial properties.

2.2.3 Lipids

All the classes of biological molecules discussed so far had a structural basis. Carbohydrates are sugars or their dimers/oligomers or polymers. Proteins and nucleic acids are made up of amino acids and nucleotides as monomers respectively. Lipids differ in their structures widely. As we will see, structure of a fatty acid has little resemblance to a cholesterol molecule. Both nevertheless are classified as lipids. To start with, both show higher solubility in organic solvents than in water. So, it is a physical property which was responsible for their being classified together as lipids. Interesting enough, later work showed that fortuitously, this turned out to be a judicious choice. Cholesterol and lipoproteins have a close relationship in metabolism. Look at the clinical reports on the blood of the people. You would find triglyceride content, cholesterol, LDL and HDL all mentioned together. So, it turns out that biologically, it is not a bad idea to classify these different kinds of molecules as lipids.

The simplest sub-classes of lipids are fatty acids and lipids which are derivatives of glycerol. Glycerol is a trihydric alcohol. It is used as moisturizer/solvent and commonly called glycerin. Glycerol is freely soluble in water, esterifying its alcoholic groups with long chain fatty acids turns this into fats/oils. During soap making, a process which has been called saponification for a long time, glycerol is produced as a byproduct. Currently, most of the industries which were major suppliers of glycerol in the world are shut down, it is mostly available as a byproduct during biodiesel production!

(A) Neutral Fats

1. Mono-, Di-, Triglycerides
2. Glyceryl Ethers
3. Glycosyl Glycerides

Neutral fats are actually what we generally call fats or oils. Structurally, both fats and oils are identical. If triglycerides are present as a free-flowing liquid, these are called oils. If present as solid, we call these fats. While something like lard will be solid at wide range of temperatures which will be prevalent as room temperature, in a country like India where temperature varies widely in different seasons, this may be confusing. In Delhi winter, "coconut oil" is generally solid! In other seasons, it may be semi solid. While trimesters of glycerol, fats and oils are well known compounds, glycerol also forms ethers. Here are examples of one of its terminal C-atom as a part of ether

linkage. As the other two –OH groups are free in these compounds, these compounds are called alcohols. The long chain R of the other component of the ether glycerol –OR ensures poor solubility of these compounds in water and solubility in organic solvents. Hence glycerol ethers are lipids.

(B) Phospholipids

1. Phosphatides or glycerophospholipids

The nitrogenous compounds choline, ethanolamine, L-serine etc. containing the alcohol group forms ester bonds with the free acidic group of the phosphoric acid of the L- α -phosphatidic acid. These are now generally called glycerophospholipids. The isomer is called L-form because of its stereochemical relationship with L-glycerol phosphate. One way to look at phosphatide structure is as monophosphate ester of 1,2 diglycerides. When the phosphate groups form another ester bond with these nitrogenous bases containing alcohol, many important phospholipids are formed. As we will see in a later module, glycerophospholipids are the phospholipids which are generally present in biological membranes.

2. Phosphoglycerides

These are derivatives of glycerol phosphate and do not contain nitrogen. All these are, as we just learnt, also called phosphatidic acids. The common phosphoglycerides present in animals and plants are diphosphatidyl glycerols and mono phosphatidyl glycerols respectively.

So, it is necessary to realise that phosphatidic acid is not a single component but a class of phosphoglycerides, different members have different fatty acids esterifying the –OH on the glycerol chain.

2.2.4 Nucleic acids

Nucleic acids constitute the most important biomolecules of the cell and are critical entities for all known forms of life.

Discovery: Nucleic acids were discovered by Friedrich Miescher in 1869. He reported that he had found a substance within the nuclei of human white blood cells, which was weakly acidic in nature and whose function was unknown. He had named this material as "nuclein". A few years later, Miescher was successfully able to separate nuclein into protein and nucleic acid components. Nuclein was later named as nucleic acid in 1889

by Richard Altmann. They were so named because of their initial discovery from within the nucleus (~nucle), and due to the presence of phosphate groups in their molecules (phosphoric acid ~ ic acid).

Function: Nucleic acids are present in all living beings as well as in bacteria, archaea, mitochondria, chloroplasts, viruses and viroids. Nucleic acids are involved in the storage and transfer of genetic information in living organisms.

Types: There are two types of nucleic acids in cells, Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA). Both DNA and RNA are the molecular repositories of genetic data.

Components of nucleic acid The basic components of a nucleic acid include three different entities, namely a nitrogenous base, a sugar moiety and a phosphate group. These combine to give one unit of a nucleotide (discussed later), which are stacked in a nucleic acid molecule (Fig. 2).

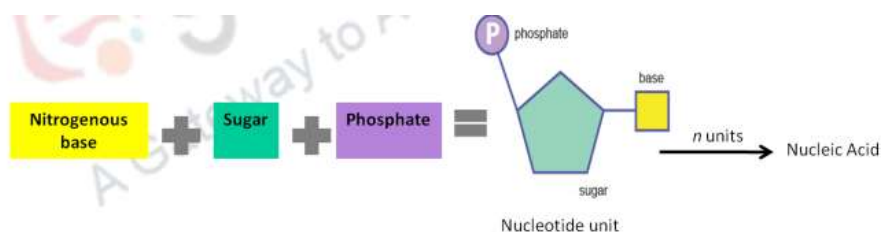


Figure 2: Component of nucleic acid

I. Nitrogenous Bases

The nitrogenous bases are nitrogen-containing bases, which are derivatives of two heterocyclic compounds: purine and pyrimidine. Pyrimidines are monocyclic, whereas purines are bicyclic. These bases are all polyfunctional in nature. Purine bases are composed of a 9-membered double-ring structure with four nitrogens and five carbons while pyrimidine bases are composed of a 6-membered ring with two nitrogens and four carbons. The carbon and nitrogen atoms in purines and pyrimidines are numbered based on convention. The basic structures of purines and pyrimidines with appropriate numbering are shown in Fig. 3 below.

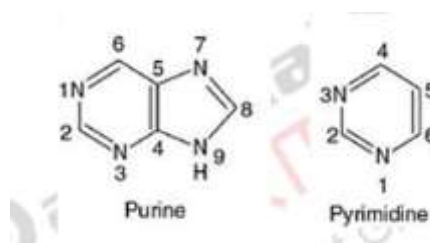


Figure 3: Structure of purine and pyrimidine

Inside the cells, five major nucleobases or nitrogenous bases are found. The derivatives of purine are called adenine (A) and guanine (G) bases, while the derivatives of pyrimidine are called thymine (T), cytosine (C) and uracil (U) bases. The DNA contains A, G, C and T, whereas RNA contains A, G, C and U bases. The chemical structures of the principal bases in nucleic acids are shown in Fig. 4.

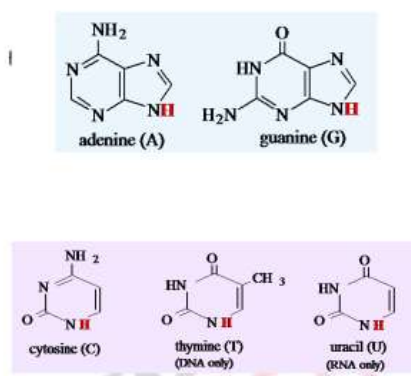


Figure 4: Chemical structures of the principal bases in nucleic acids.

Base pairing of Purines and Pyrimidines: Purines and pyrimidines, being complementary bases, can participate in base pairing, based on the specific shapes and hydrogen bond properties. Guanine, being a complement of cytosine, pairs with cytosine through three hydrogen bonds. Adenine (A) is the complement of thymine (T) in DNA and uracil (U) in RNA. Adenine base pairs with thymine and uracil through two hydrogen bonds. Chargaff's Rule Erwin Chargaff (1905-2002), an Austrian-American biochemist gave the Chargaff's rule, according to which DNA always contains equal amounts of certain base pairs. He observed that the amount of adenine (A) always equalled with the amount of thymine (T), and the amount of guanine (G) always equalled the amount of cytosine (C), regardless of the DNA source.

$$\%A = \%T \text{ and } \%C = \%G$$

Sugars: Two types of pentose sugars are found in nucleic acids, namely ribose and 2-deoxy ribose. The carbons in the ribose sugar are numbered according to convention. Ribose differs from deoxyribose in the presence of a hydroxyl group at the 2'C. The D-ribose and D-deoxyribose are found in RNA and DNA respectively, in their furanose (closed five-membered ring) forms.

Phosphates: Phosphate is another important component of the nucleic acid molecule. It gets attached to C-5' OH group of the sugar and gets incorporated into nucleic acid (both DNA and RNA).

Nucleosides: A nucleoside consists of a combination of a nitrogenous base and a sugar (ribose or deoxyribose).

Nucleosides = nitrogenous base + sugar

The bond between them is called the beta-glycosidic linkage. The position of attachment is shown below. Examples of nucleosides include cytidine, uridine, adenosine, guanosine, thymidine

Nucleotides: Nucleotides comprise of a nitrogenous base linked to a 5-carbon sugar and one or more phosphate group. The phosphate is attached to 5' CH₂OH group of sugar part of nucleoside. They function as the building blocks of nucleic acids.

Nucleotides = nitrogenous base + sugar + phosphate
Nucleotides = Nucleosides + phosphate

Examples of nucleotides include deoxyadenosine monophosphate, deoxycytidine monophosphate, deoxyguanoside monophosphate, deoxythymidine monophosphate

Nucleotide di- and tri-phosphates: The term "nucleotide" generally refers to a nucleoside monophosphate, but in case additional phosphoric acid groups are present.

Table 1. Summary of nucleobase derivatives

Base	Symbol	Nucleoside		Nucleotide	
		(Base+ deoxyribose sugar)	(Base+ ribose sugar)	(Base+ deoxyribose sugar + phosphate)	(Base+ ribose sugar+ phosphate)
Adenine	A	2'-Deoxyadenosine	Adenosine	2'-Deoxyadenosine-5'-monophosphate (dAMP)	Adenosine monophosphate (AMP)
Cytosine	C	2'-Deoxycytidine	Cytidine	2'-Deoxycytidine-5'-monophosphate (dCMP)	Cytidine monophosphate (CMP)
Guanine	T	2'-Deoxyguanosine	Guanosine	2'-Deoxyguanosine-5'-monophosphate (dGMP)	Guanosine monophosphate (GMP)
Thymine	G	2'-Deoxythymidine	Thymidine	2'-Deoxythymidine-5'-monophosphate (dTMP)	Thymidine monophosphate (TMP)
Uracil	U	2'-Deoxyuridine	Uridine	2'-Deoxyuridine-5'-monophosphate (dUMP)	Uridine monophosphate (UMP)

II. Polynucleotides

A polynucleotide formation is initiated when many nucleotides continue to join together by phosphodiester linkages. The formation of polynucleotide is catalyzed by polymerase enzymes (DNA polymerase in case of DNA or RNA polymerase in case of RNA). The -OH group on the 3' -carbon of sugar in one nucleotide reacts with the phosphate attached to the 5' -carbon of another to form phosphate ester bonds and a dinucleotide. Repeated formation of such bonds leads to further elongation of the polynucleotide chain. DNA and RNA are examples of polynucleotides (Fig. 5), where the nucleotides are arranged in linear way and proceeds in the 5' ----> 3' direction. A common representation of polynucleotide is given below: 5'pApTpGpC OH3' While RNA is single stranded, DNA is double stranded and contains two such polynucleotide chains spiralling round each other to form a double helical structure. The two chains in the double helix are held together by hydrogen bonds by complementary bases on different chains.

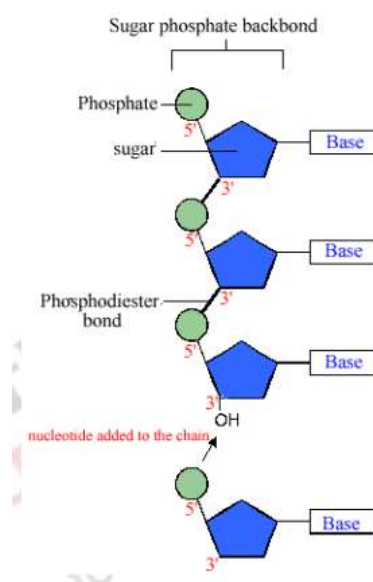


Figure 5: Formation of polynucleotides

2.3 Molecular biology

Molecular Biology is the broad term to decode molecular mechanism of life at gene level. The study of this unit will make you aware of the molecular Biology of the cell, information about gene, genome, nucleosomes and chromatin and DNA replication, transcription and translation. You know that DNA is the biomolecule which carries

information for the complete structural and functional organization of an organism. This information is contained in the sequence of its building block comprising of nucleotides, which form a polymer of a single DNA molecule and can be up to 109 bases long. Genes are the fundamental units of heredity, composed of DNA, that carry instructions for the development, functioning, and maintenance of living organisms. These instructions are encoded in the sequence of nucleotide bases—adenine (A), thymine (T), cytosine (C), and guanine (G)—that make up the DNA molecule. Genes serve as templates for the synthesis of proteins, which are essential for the structure and function of cells. A genome is the complete set of genetic material present in an organism. It includes all the genes and non-coding regions of DNA. Genomes vary in size and complexity across different species. For example, the flowering plant with the smallest genome is *Genlisea tuberosa*, a tiny carnivorous plant found in Brazil, have 61 million base pairs genome size. Genome size, or the amount of genetic material contained in an organism's genome, varies widely among different species. The genome size has a wide range and is made of both unique as well as repetitive sequences.

2.3.1 Organization of genome

The specific sequence of DNA that is transcribed into a sequence of polypeptides is known as a gene. It can be housekeeping / constitutive gene, structural gene, regulatory gene or pseudogene. The gene therefore provide the DNA template using which RNA is transcribed in a complementary sequence using monomers of ribonucleotide triphosphate except that uracil replaces thymine, The polymerization is facilitated by RNA polymerase in the direction 5' to 3'. The base pair from which RNA polymerase starts transcription is designated as +1 and it moves downstream on the DNA template in the 3' to 5' direction. All base pairs are denoted by a (+) sign in this direction and with a (-) sign in the upstream or opposite direction to +1. Here in this sequence, we shall focus on the organisation of genome only at two levels, prokaryotic and eukaryotic genome. Let us see how at the level of gene this genome is organised.

I. Eukaryotic Gene

In eukaryotes the genes are discrete units and unlike in prokaryotes. These genes which encode functionally similar proteins are not found together and are found on different chromosomes. Each gene has a separate promoter and there are three

different types of promoters associated with the three different polymerases and some can be located upstream and some are downstream from the start site.

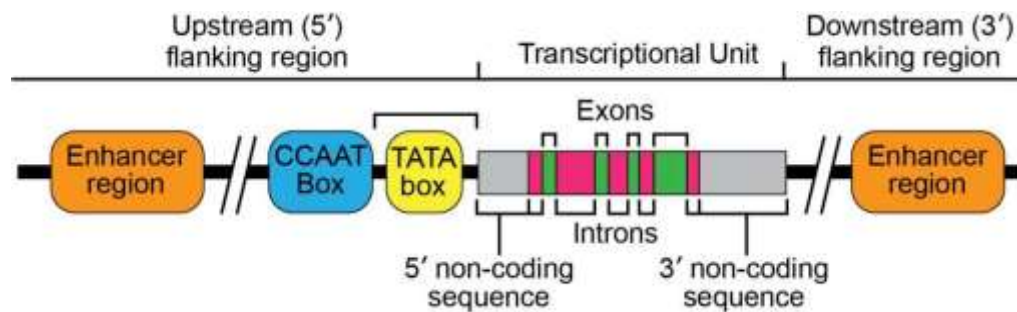


Figure 6: Organization of a eukaryotic gene and its promoter region

The RNA polymerase II promoter is also known as a core promoter and is made up of two elements:

- TATA box: Similar to the prokaryotic Pribnow box at -30 with the conserved sequence of 5'...TATAAA...3'. The pre-initiation complex is assembled here.
- The initiator sequence has the consensus 5' – Py2CAPy5 – 3' and is located around +1.
- Some genes have a downstream promoter element with a variable sequence and located at +28.
- Regulatory sequences: Found upstream and are cis- acting and bind factors that control expression of individual genes. These are enhancers, upstream activators and silencers among others and are located hundreds of kilobase pairs away. Two such regulatory elements commonly found within the gene are the CAAT box and the GC box (GGGCGG).
- Some genes have only one of the two core promoter elements. Some don't have any and are known as null genes. Moreover, a gene can have a TATA box or a downstream promoter element box but not both.
- The RNA polymerase I promoter has a GC rich core promoter from -45 to +20.
- It also has an upstream control element (UCE) which is 100 base pairs upstream. The promoter for RNA polymerase III is found downstream of the start site and falls within the gene. These are of three types:

i) Internal promoter I: found in the 5SrRNA genes.

ii) Internal promoter II: found in the tRNA genes.

iii) Upstream promoter: Found in the U6snRNA genes.

II. Chromatin

The name 'Chromatin' has originated from a description of a complex of nucleic acids and proteins in the cell nucleus that stained readily with basic dyes and condensed to form chromosomes during cell division. The chromatin is a complex of macromolecules consisting of DNA, RNA and proteins. The major proteins associated with DNA are histones and these are responsible for compacting approximately 3 meter long DNA, composed of 3 billion base pairs (bp) into a structure that can fit inside a typical eukaryotic nucleus of 10-100 micrometer in diameter. The role of histone proteins in making this task possible is immense. Apart from histone proteins, a number of non-histone proteins are also present in the chromatin. The basic composition of chromatin remains the same throughout the genome, however, there are variations in the extent of packaging. In areas where the genome is open for undergoing transcription it is known as euchromatin (stains lighter with dyes). While in non-coding areas of high compaction and tighter association between structural proteins, it is known as heterochromatin (stains dark with dyes) about which you study in later sections.

III. Euchromatin and Heterochromatin

Euchromatin and heterochromatin are two distinct forms of chromatin, the complex of DNA and proteins that make up chromosomes within the nucleus of a eukaryotic cell. They differ in their structural organization, level of condensation, and transcriptional activity. Euchromatin is a less condensed and more loosely packed form of chromatin. It appears as a diffuse and extended network during interphase. It is transcriptionally active, meaning that genes within euchromatic regions are more accessible and can be readily transcribed into RNA. This is because the DNA is less tightly wound around histone proteins, allowing regulatory proteins and transcription factors to access the DNA sequence. Euchromatin often contains genes that are actively involved in cellular processes. It is associated with the synthesis of RNA and the expression of functional proteins. It stains less intensely with DNA-specific dyes because of its less condensed structure. Heterochromatin is a highly condensed and tightly packed form of chromatin. It appears as distinct, densely staining regions in the nucleus during interphase. It is generally transcriptionally inactive or less active compared to euchromatin. The tight packing of DNA makes it less accessible to the transcriptional machinery. Heterochromatin often contains repetitive DNA sequences, such as satellite DNA and transposons. It may also include genes that are temporarily silenced during certain

stages of development or under specific cellular conditions. It stains more intensely with DNA-specific dyes due to its higher degree of condensation.

It's important to note that euchromatin and heterochromatin are not fixed states but rather dynamic and can undergo changes in response to various cellular processes and signals. The transitions between euchromatin and heterochromatin are influenced by processes such as DNA methylation, histone modifications, and the binding of specific proteins. During cell division, euchromatin condenses further into heterochromatin to facilitate the segregation of chromosomes. In interphase, euchromatin becomes more accessible for gene expression. The regulation of these chromatin states is crucial for cellular functions, including gene expression, DNA replication, and repair.

A nucleosome is the basic structural unit of DNA packaging in eukaryotic cells. It consists of a segment of DNA wound around a core of histone proteins.

- Histones are a family of proteins which are involved in forming the chromatin. These are highly alkaline or basic proteins and are perfect for interacting with the acidic DNA.
- There are five major families of histone proteins; H1/H5, H2A, H2B, H3 and H4. Of these, H2A, H2B, H3 and H4 are known as the core histones.
- The major function of histones is to compact the DNA. They also carry out the function of chromatin regulation.
- Non-histone proteins form an integral part of gene regulation. The presence and absence of these proteins drive transcription and provides a mechanism of regulation of gene expression.
- The chromatin is a complex of macromolecules consisting of DNA, RNA and proteins. The major proteins associated with DNA are histones and these are responsible for compacting approximately 3 meter long DNA, composed of 3 billion base pairs (bp) into a structure that can fit inside a typical eukaryotic nucleus of 10-100 micrometer in diameter.
- The highly compacted regions of chromatin are called heterochromatin while those involved in active transcription are called euchromatin. While it may appear that the DNA is a static structure, which is not quite true.
- The histone proteins are essential in packaging the DNA into a compact structure. The DNA takes up various structures categorized as secondary,

tertiary and quaternary depending on the degree of compaction. The DNA is highly dynamic, in which stretches of DNA can change their degree of compaction based on modifications on the histone protein and their interaction with non-histone proteins.

- SMC (Structural Maintenance of Chromosomes) complexes are involved in various processes such as chromosome condensation, sister chromatid cohesion, DNA repair, and regulation of gene expression. The SMC complexes are named after their function. Metaphase chromosomes are symmetrical i.e. the two sister chromatids are mirror images of one another. The electron microscopic studies have revealed that, each chromatid consists of a central non-histone core called scaffold or nuclear matrix, from which loop of 30 nm chromatin fiber fan out radially.
- Numbering and ordering of chromosomes is done by standard terminology used by Cytogeneticists by referring to the bands of a chromosome, and describing any chromosomal abnormalities.
- The p arm is the short arm at the top and the long arm is the q arm. Numbering of the bands begins at the centromere and progresses to the terminal of an arm.

2.3.2 Chemistry of DNA synthesis

DNA synthesis always proceeds in the 5'- 3' direction. Synthesis of DNA requires the presence of four deoxynucleoside triphosphates, namely dATP, dTTP, dGTP, dCTP, a single stranded portion of template DNA associated with a primer, forming a primer - template junction in which the primer has a terminal 3'-OH group to which the new nucleotide can be added via the catalytic action of a DNA polymerase enzyme which requires Mg^{2+} as a cofactor. Both the strands of the double helix can separately act as a template, directing the synthesis of the corresponding complementary strands. The exposed 3'-OH group of the primer initiates nucleophilic attack on the α phosphate of an incoming dNTP. This results in an extension of the primer at the 3' end by one nucleotide and concomitant release of pyrophosphate which then gets rapidly hydrolysed into two molecules of phosphate. This rapid hydrolysis of pyrophosphate releasing energy, makes the DNA synthesis reaction thermodynamically feasible. The polymerization reaction requires energy. The release of energy during the breaking of

the bond between the phosphates is in turn utilized for formation of the phosphodiester bonds in the new DNA strand being synthesized.

I. Semi conservative model of DNA replication

DNA replication is said to occur in a semi conservative mode. In this model the strands of the double helix separate and serve as templates for synthesis of new DNA strands with a complimentary sequence. At the end of the process the double stranded DNA products include one parental or old strand along with the new strand which is also referred to as the daughter strand.

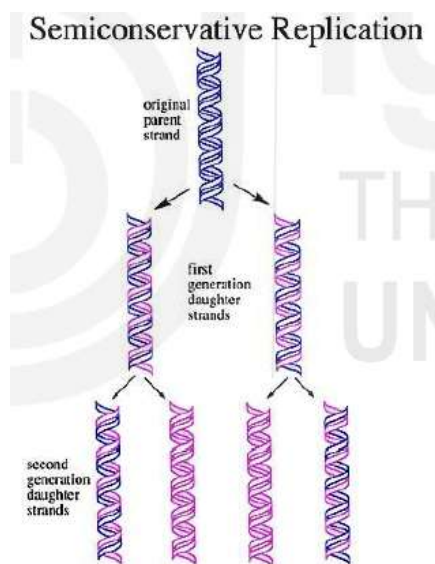


Figure 7: Semiconservative DNA replication.

2.3.3 Mechanism of DNA replication in eukaryotes

Recognition of replication origin and the mechanism of origin firing in eukaryotes is very different from that of prokaryotes. Since eukaryotic genomes are usually much larger and complex, they require multiple origins of replication (several thousands in humans) from which DNA replication starts simultaneously, so that the entire genome is replicated within a reasonable time frame. Multicellular eukaryotes do not have any consensus sequences representing the origin of replication. It is the DNA, topology Chromatin modification and allied structural signatures that distinguish the replication origin from other parts of the genome. Further in eukaryotes the timing of DNA replication has to be linked with the S phase of the cell cycle. The protein complex that recognizes the origin is fairly conserved. It recruits helicases to the origin during the M and G1 phases of the cells cycle. This mechanism is called origin licensing. During S

phase these helicases initiate the unwinding of DNA at about 20% of the licensed origin for starting replication. This process is called origin firing. The rest of the licensed origins remain as backup and they are activated only when an adjacent replication fork stalls. This backup mechanism ensures the completion of replication even in conditions that may adversely affect the process or slow it down.

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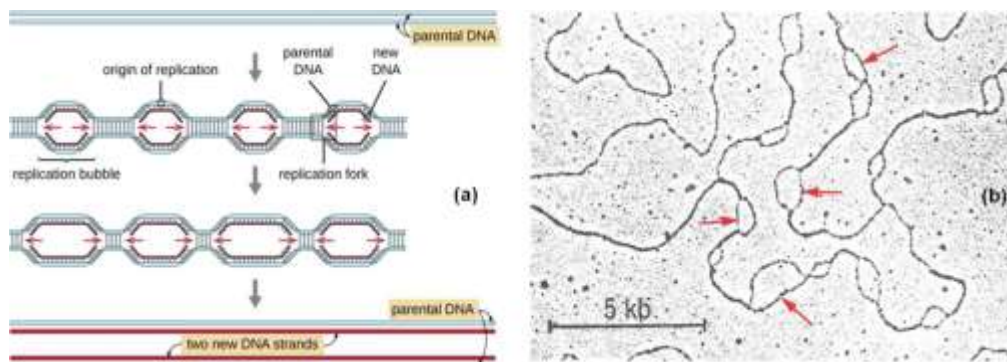


Figure 8: (a) Schematic diagram of Eukayotic origins of replication, b) The Cryoelectron micrograph of Eukayotic origins of replication

Eukaryotic DNA Polymerase Enzymes

DNA replication in eukaryotes is also bidirectional and semi conservative. The DNA polymerase ϵ takes care of the continuous leading strand replication while DNA polymerase δ is responsible for the discontinuous synthesis on the lagging strand. The stabilization of unwound single stranded DNA is done via binding of the replication protein A (RPA) which is a heterotrimeric complex. Ligation of the Okazaki fragments

ends of the chromosomes from damage is one of the functions of the telomeres. Every time a round of DNA replication is completed some telomeric sequences are lost (50-200 bp) although since telomeres contain non coding sequences this loss does not affect cellular functions and the cells survive. However, after many many rounds of DNA replication significant amount of telomere shortening will take place and beyond a threshold the shortening will obviously hamper cellular functions. The maximum number of rounds of DNA replication that can occur before telomeres get too short is believed to be about 100 and this limit is called the Hayflick limit. Shortening of telomeres also occurs due to DNA damage caused by intracellular Reactive Oxygen Species (ROS). In order to replicate telomeric DNA and maintain telomere length, rapidly dividing cells like germ cells, stem cells, hematopoietic cells, activated lymphocytes, and cancer cells express an enzyme called Telomerase which consists of a short RNA molecule called TERC and reverse transcriptase called TERT. Using the TERC RNA as a template, TERT can synthesize telomeric DNA repeat sequences. Somatic cells however have very low Telomerase activity. Telomerase activity also reduces with age and so telomere shortening is also considered as one of the hallmarks of ageing in mammals.

Reference

- Adopted from EPG pathshala, Paper: 04 (Metabolism of Carbohydrates) Module: 01, Paper: 05 (Metabolism of Lipids) Module: 01, and paper 15 Module 3 (Molecular Biology, Genetic Engineering and Biotechnology).

Unit 3: Molecular Biological Techniques

Unit Structure

3.0 Learning Objectives

3.1 Introduction

3.2 Gene regulation

3.3 Genetic engineering

3.4 DNA purification

3.5 RNA purification

3.6 Plasmid

3.7 Polymerase Chain Reaction

3.8 ELISA

3.9 UV- visible Spectroscopy

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3.11 Electron microscopy

3.11.1 Transmission electron microscopy (TEM)

3.11.2 Scanning Electron Microscopy (SEM)

3.12 High Performance Liquid Chromatography (HPLC)

3.13 Laminar air flow

3.14 Hot air oven

3.15 Incubator

3.16 Gel electrophoresis

3.17 pH meter

3.18 Fourier Transform Infrared Spectroscopy (FTIR)

3.19 X-ray Diffraction (XRD)

Summary

References

3.0 Learning Objectives

After completion of this unit, you will be able to:

- learn the concept and regulation of gene;
- discuss different genetic engineering techniques;
- understand the concept of isolation of plasmid;
- know different biological techniques like ELISA, PCR, TEM, SEM and HPLC;

3.1 Introduction

Gene regulation is the process through which cells control the expression of genes, ensuring that the right genes are turned on or off at the correct time. This regulation allows organisms

to respond to environmental changes, maintain cellular function, and support development and differentiation. Gene regulation occurs at multiple levels, including transcription, translation, and post-translational modifications, and involves various factors such as promoters, enhancers, transcription factors, and epigenetic mechanisms. In this chapter you will study the principles and concept of gene regulation and plasmid. Molecular biological techniques are essential tools used to study and manipulate gene regulation. These techniques include DNA extraction, PCR amplification, gel electrophoresis and cloning. You will also understand about different instruments such as UV- visible and fluorescence spectroscopy, SEM, TEM, HPLC, gel electrophoresis and X- ray diffraction.

3.2 Gene regulation

Gene regulation refers to the mechanisms and processes that control the timing, location, and amount of gene expression within a cell or organism. It ensures that specific genes are activated or suppressed as needed, allowing cells to function efficiently, respond to environmental changes, and differentiate during development. Gene regulation occurs at several levels, including transcriptional control (regulation of mRNA synthesis), post-transcriptional modifications (splicing, mRNA stability), translational control (protein synthesis), and post-translational regulation (protein modification and degradation). Regulatory elements such as promoters, enhancers, silencers, operons (in prokaryotes), transcription factors, and epigenetic modifications like DNA methylation and histone acetylation play key roles in controlling gene expression.

The set of gene that express in a cell determine its properties and functions. Expression of genes involves transcription and translation. Transcription is the first step in the process of gene expression, and initiation of transcription is an important control point for gene expression. In prokaryotes, translation of a transcript begins well before the completion of transcription, due to the proximity of ribosomes to the new mRNA molecules. In eukaryotes, however, transcripts are modified in the nucleus before they are exported to the cytoplasm for translation. Therefore, transcript processing provides an additional level where regulation of gene expression could be exercised in eukaryotes.

Positive regulation is achieved in the presence of a specific protein (activator) which leads to increase in transcription. The activator may provide assistance either to the binding of

RNA polymerase to promoter, or to initiation of transcription by a bound RNA polymerase. Negative regulation can be termed as the binding of a specific protein (repressor) inhibits transcription from occurring. DNA bound repressors often act to prevent RNA polymerase from binding to the promoter, or by blocking preventing initiation of transcription.

3.3 Genetic engineering

Genetic engineering is the process of transferring specific genes from the chromosome of one organism and transplanting them into the chromosome of another organism in such a way that they become a reproductive part of the new organism. Genetic engineering is a molecular genetic technique that is used for the direct manipulation, alteration or modification of genes or genome of an organisms in order to manipulate the phenotypes. This technique is also known as genetic manipulation, genetic modification or genetic alterations. Humans are manipulating the genetic material of many organisms since long. Using selective breeding and cross-hybridization, economically important plant species were created by humans.

The purpose of developing the genetic engineering or genetic manipulating technique is to produce organisms or phenotypes which are useful to us. By inserting a gene of interest or by deleting the unwanted DNA sequences from the genome, altered gene or DNA is constructed called as recombinant DNA which is transferred into the host genome using the vectors. The first recombinant DNA was constructed by Paul Berg in 1972. It is employed for the production of improved plant species, therapeutic drugs or proteins, prevention of inherited genetic disorders and construction of a genetically modified organism.

The process of genetic engineering involves two different cell's DNA that are combined and inserted into the host genome with the help of a vector. The process that produces the resulting recombinant DNA involves four steps:

- i. The target DNA sequence is cleaved from the donor chromosome using restriction endonucleases, that identify and cleave specific recognition sites and generate cohesive (sticky) ends at both termini. The same restriction enzymes are then used to cut the recipient chromosome at a corresponding site, producing complementary sticky ends that facilitate the insertion and ligation of the donor DNA fragment.

- ii. The isolated DNA fragment is incorporated into a vector commonly a plasmid for delivery into the host genome. Plasmids serve as efficient vectors due to their autonomous replication within bacterial cells and their capacity to acquire and express foreign genetic material. These circular extrachromosomal DNA molecules reside in the bacterial cytoplasm and are covalently joined to the inserted DNA fragment through the catalytic activity of DNA ligase, resulting in the formation of recombinant plasmid DNA.
- iii. The host cell undergoes replication and the plasmids gets duplicated, generating multiple copies of their inserted DNA sequences. Since the plasmid population includes both target and non-target DNA inserts, the collective set of recombinant plasmids constitutes a gene library.
- iv. To isolate the target DNA sequence, unwanted recombinant cells are first eliminated through selective screening. The remaining DNA fragments are then separated using gel electrophoresis, a technique that exploits the migration of negatively charged DNA molecules toward a positively charged electrode, with fragment separation. Subsequently, radioactive or fluorescent-labeled probes complementary to the target sequence are applied to hybridize specifically with the desired DNA, producing detectable bands.

The important components required for gene manipulation experiments includes gene of interest, vector and target cells. The different types of genetic engineering techniques include recombinant DNA technology, gene delivering and editing technique.

3.4 DNA purification

Genomic DNA resides within the nucleus of eukaryotic cells and retains its characteristic as double-stranded helical configuration. The methodology for genomic DNA extraction varies between plant and animal tissues due to structural and biochemical differences. In plants, the presence of a rigid cell wall and secondary metabolites make DNA isolation more challenging compared to animal cells. The yield and purity of isolated genomic DNA are largely influenced by the cellular composition and physiological state of the source material.

DNA cell also contains some quantity of RNA and protein that is purified or removed by the process of organic extraction and enzymatic digestion for the removal of contaminants.

The procedure includes the addition of a phenol–chloroform mixture (1:1) to the cell lysate to facilitate phase separation and protein removal. During centrifugation, denatured proteins form an interphase layer between the upper aqueous phase, which contains nucleic acids, and the lower organic phase. Supplementing the extraction with proteolytic enzymes such as pronase or protease further enhances the removal of protein contaminants. Residual RNA can be selectively degraded by RNase, which enzymatically hydrolyzes RNA into ribonucleotide monomers. Excessive or repeated phenol treatment is avoided, as it may cause shearing or chemical degradation of the DNA.

Another method includes separation by the ion-exchange chromatography method. This technique separates charged ions and polar biomolecules including proteins, nucleotides, and amino acids on the basis of their net electrical charge. Negatively charged DNA molecules interact electrostatically with a positively charged resin or matrix within the column. Elution is achieved by applying a salt gradient, whereby the increasing ionic strength progressively disrupts the DNA–resin interaction, resulting in the sequential release of bound molecules.

3.5 RNA purification

RNA (ribonucleic acid) is a single-stranded polynucleotide composed of repeating ribose phosphate units with several nitrogenous base like adenine, guanine, cytosine, and uracil attached to the ribose moieties. It is present in all living cells and many viral systems. The standard workflow for RNA isolation typically includes tissue homogenization, phase separation of cellular components, selective precipitation of RNA, subsequent washing to remove contaminants, and final solubilization of the purified RNA. The method for isolation and purification of RNA includes organic extraction method, direct lysis methods, filter-based, spin basket formats and magnetic particle methods.

Organic extraction method: This technique employs phase separation by adding a phenol–chloroform mixture combined with a chaotropic agent, such as guanidinium thiocyanate, to the aqueous biological sample followed by centrifugation. Guanidinium

thiocyanate disrupts protein structure and inactivates RNases, thereby releasing ribosomal RNA from ribosomes. Upon chloroform addition, centrifugation yields a clear stratification: an upper aqueous phase enriched with RNA, an intermediate phase containing genomic DNA, and a lower organic phase harboring proteins and other hydrophobic components. RNA is then selectively recovered from the aqueous layer through precipitation with alcohol (typically 2-propanol or ethanol) and subsequently rehydrated to obtain purified RNA.

Direct lysis methods: This approach utilizes a lysis buffer formulated under defined conditions to facilitate sample disruption while simultaneously preserving nucleic acid integrity. When required, nucleic acids can be further purified from the stabilized lysate. Since this strategy does not rely on solid-phase binding and subsequent elution, it minimizes variability associated with matrix-dependent recovery efficiency and reduces methodological bias.

3.6 Plasmid

Plasmids are autonomously replicating circular DNA molecules found in bacteria. They have their own origin of replication, and they replicate independently of the origins on the "host" chromosome. Replication is usually dependent on host functions, such as DNA polymerases, but regulation of plasmid replication is distinct from that of the host chromosome. The size of a plasmid is 4361 bp, and the cloning limit is 0.1-10 kb. The term plasmid was introduced in 1952 by the American molecular biologist Joshua Lederberg to refer to "any extrachromosomal hereditary determinant". On the basis of possibility to do horizontal gene transfer, plasmid is of two types; Conjugative plasmids (F plasmids) and non-conjugative plasmids.

Conjugative plasmids contain a set of transfer or tra genes which promote sexual conjugation between different cells. In the complex process of conjugation, plasmids may be transferred from one bacterium to another via sex pili encoded by some of the tra genes.

Non-conjugative plasmids are incapable of initiating conjugation; hence they can be transferred only with the assistance of conjugative plasmids. Non-conjugative plasmids include: Resistance (R) plasmids, Col plasmids, Degradative plasmids and Virulence plasmids.

F plasmid is a circular DNA molecule having a molecular weight of 62.5×10^6 (94.5kbp). It contains *tra* genes that allow the plasmids DNA to be transferred between cells. It is found in the bacterium *E. coli*; *E. coli* containing this F factor are known as F^+ and those without are known as F^- . The F stands for fertility and the F factor is around 100000 bases in length.

R plasmid is plasmid which contain genes that provide resistance against antibiotics or poisons. *r* plasmid consists of two contagious segments of DNA. one of this segment is the resistance transfer factor (RTF) required for transfer of the plasmid between bacteria, and the *r*-determinants (genes conferring antibiotic resistance). R plasmids were first isolated from strains of *Shigella dysenteriae* that showed resistance to multiple antibiotics.

Col plasmids contain genes that make bacteriocins (also known as colicins), which are proteins that kill other bacteria and thus defend the host bacterium. Bacteriocins are found in many types of bacteria including *E. coli*, which gets them from the plasmid ColE1.

Degradative plasmids help the host bacterium to digest compounds that are not commonly found in nature, such as camphor, xylene, toluene, and salicylic acid. These plasmids contain genes for special enzymes that break down specific compounds. Degradative plasmids are conjugative.

Virulence plasmids are usually large (>40 kb) low copy elements and encode genes that promote host–pathogen interactions. Although virulence plasmids provide advantages to bacteria in specific conditions, they often impose fitness costs on their host. it turn the bacterium into a pathogen. e.g. Ti plasmid in *Agrobacterium tumefaciens*.

Check your progress

1. What do you understand by gene regulation?
2. Briefly explain genetic engineering and its importance.
3. Explain the steps involved in the process of DNA and RNA purification.
4. What is plasmid and explain different types of plasmids.

3.7 Polymerase Chain Reaction

Polymerase chain reaction (PCR) is a strong tool that amplifies even the tiniest amount of DNA and gives millions of copies of that DNA to analyze the property of a gene. Sometime

we also use PCR amplicon (PCR product is called as amplicon) for gene cloning purpose where desired gene can be cloned in a suitable vector to check the expression and function of a particular gene. Usually, PCR comprises of many cycles i.e. 20-40 rounds of denaturation (90-94°C), primer annealing (50-65°C) and elongation (72°C) at different temperatures and different times. The reaction stops at ~4°C for brief time period. Kary Mullis along with Michael Smith received Nobel Prize in Chemistry in 1993 for the discovery of PCR.

PCR is divided into three stages where first stage is **denaturation** of double-stranded DNA into single stand, and the timing of denaturation is important. If DNA template originated from plasmid, then 3-5 minutes of denaturation is adequate. However, if DNA template is of genomic in origin, the 7-10 minutes of first denaturation is recommended. This is called as first cycle of PCR where double-stranded DNA is completely denatured and separated in the form of ssDNA. In order to avoid renaturation of two ssDNA, a short denaturation period of 30 seconds to 2 minutes is advisable from second cycle to penultimate cycle where a short denaturation is followed by **annealing** (primer is bound) and elongation. In the final stage, last **elongation** is required in order to complete any elongated product. Once the final cycle is completed, the entire reaction is at risk at 4°C till the use of product.

In the PCR reaction one of the most crucial steps is primer annealing because if the primer anneals properly, it will provide a full-length amplicon. Therefore, while designing primers primer length, annealing or melting temperature, primer dimer formation and G-C rich content should be especially considered for effective PCR process. There are different types of PCR such as hot start PCR, nested specific PCR, intergenic PCR, multiplex PCR, colony PCR and Reverse Transcription PCR.

3.8 ELISA

Enzyme-linked immunosorbent assay (ELISA) is a biochemical method, wherein target antigen (or antibody) in a sample is detected using specific enzyme-labeled antibody (or antigen), by measuring enzymatic reaction with its substrate calorimetrically. This technique was introduced in 1970s and has since then several modifications has been introduced in the conventional ELISA to improve its specificity and sensitivity. Currently Many variations of ELISA are available. Among them “sandwich” assay is popular format and is used in many

commercially available immunological kits. Here the antigen is bound (sandwiched) between capture antibody and the detection antibody, thereby making the assay more sensitive and robust. ELISA-array has also been developed to detect multiple antigens simultaneously. This has the potential to further revolutionize the food testing sector with a rapid, user friendly and inexpensive method of pathogen detection.

Principle:

ELISAs are typically performed in 96-well polystyrene plates. The serum is incubated in a well, and each well contains a different serum. A positive control serum and a negative control serum would be included among the 96 samples being tested. Antibodies or antigens present in serum are captured by corresponding antigen or antibody coated on to the solid surface. After some time, the plate is washed to remove serum and unbound antibodies or antigens with a series of wash buffer. To detect the bound antibodies or antigens, a secondary antibody that are attached to an enzyme such as peroxidase or alkaline phosphatase are added to each well. After an incubation period, the unbound secondary antibodies are washed off. When a suitable substrate is added, the enzyme reacts with it to produce a color. This color produced is measurable as a function or quantity of antigens or antibodies present in the given sample. The intensity of color/ optical density is measured at 450 nm. The intensity of the color gives an indication of the amount of antigen or antibody. There are different types of ELISA like Direct ELISA, Indirect ELISA, Sandwich ELISA and Competitive ELISA.

Application of ELISA:

1. Presence of antigen or the presence of antibody in a sample can be evaluated.
2. Determination of serum antibody concentrations in a virus test.
3. Used in food industry when detecting potential food allergens.
4. Applied in disease outbreaks- tracking the spread of disease e.g. HIV, bird flu, common, colds, cholera, STD etc.

3.9 UV- visible Spectroscopy

Spectrophotometers are used for measuring the absorbance of visible light and UV-visible light respectively. Spectrophotometers operate on both the ultraviolet (UV) and the visible

range as they are fitted with deuterium or hydrogen lamp for ultraviolet light range (200-400 nm) and tungsten lamp as light source for visible range. Since both the lamps continuously emit all wavelengths within their range, the emitted light consists of many different wavelengths (polychromatic). Spectrophotometers can measure absorbance of both color and colorless solutions. A monochromator is used to convert the polychromatic radiation to an individual wavelength of very narrow width before it reaches the sample. Monochromator consists of either a prism or rotating metal grid of high precision called grating placed between the light source and the sample. The selection of a particular wavelength depends on the colored filters as monochromators because they absorb all but a certain limited range of wavelengths. The limited range is known as bandwidth of the filter. Filter based wavelength selection is used in colorimeters. On the other hand, wavelength selection by prisms or grating is used in spectrophotometers.

Principle of UV-VIS spectrometry

In a typical absorption spectral measurement, a monochromatic radiation is made to fall on a sample taken in suitable container called cuvette. In such a situation a part of the radiation is reflected, a part is absorbed, and a part is transmitted. The intensity of original radiation, P_o is equal to the sum of the intensities of reflected (P_r), absorbed (P_a) and transmitted (P_t) radiation.

$$P_o = P_r + P_a + P_t$$

The effect of reflection can be compensated by passing equal intensities of beams through the solution and through the solvent contained in the same or similar container and comparing the transmitted radiations. We can then write the above equation as follows:

$$P_o = P_a + P_t$$

The intensity of the transmitted light is measured and is found to depend on the thickness of the absorbing medium and the concentration, besides the intensity of the incident radiation. This dependence forms the basis of spectrometric determinations and is given in terms of two fundamental laws: **Lambert's Law** (Lambert (1760) and Bouguer independently studied the decrease in the intensity of radiation when it passes through a substance). **Beer's law** (In 1852, Beer and Bernard independently studied the dependence of intensity of transmitted radiation on the concentration of the solution).

3.10 Fluorescence spectroscopy

It is also known as fluorometry or spectrofluorometry which is used to measure fluorescence emitted from a sample after it has absorbed photons. It is a complementary technique to absorption spectrophotometry. Fluorescence phenomenon is observed when light is emitted by certain molecules in the form of a band spectrum and not as a discrete line spectrum. This type of spectroscopy is associated with the electronic and vibrational states. On the absorption of radiation energy (photons), electrons of a molecule get elevated from their ground state to one of the various vibrational states in the excited electronic state. Collisions with other molecules cause the excited molecule to lose its vibrational energy until it reaches the lowest vibrational state of the excited electronic state. The excited molecule then drops back to one of the various vibrational levels in the ground electronic state by emitting light (fluorescence). The emitted photons will carry different number of energies and frequencies. The emitted light has less energy of longer wavelength than the absorbed light. The shift in the wavelength is known as Stokes shift. The compounds which show significant measurable Stokes shift can be measured fluorometrically using a spectrofluorometer. All excited molecules do not fluoresce.

Check your progress

1. Briefly explain PCR, its type and processes.
2. Explain the principle and application of ELISA?
3. Briefly explain Fluorescence spectroscopy.
4. Explain UV- visible Spectroscopy and its principle.

3.11 Electron microscopy

Electron microscopy is a powerful imaging technique used to observe structures at a very high resolution, far beyond what can be achieved with conventional light microscopy. The theoretical resolution of an optical light microscope is 200 nm. This barrier to higher resolution had to be overcome for detailed ultra-structural investigations to be possible. Instead of using visible light, electron microscopes use a beam of electrons to create an image of the specimen under study. The first prototype of the transmission electron microscope Knoll and Ernst Ruska in 1931. Today there are two major types of electron

microscopes used in clinical and biomedical research namely, Transmission electron microscope (TEM) and Scanning electron microscope (SEM).

3.11.1 Transmission electron microscopy (TEM)

In TEM, electrons pass through the specimen to form an image of internal structure of the specimen. It requires ultrathin sections for electrons to penetrate with ease. The specimen is placed along the direction of the electron beam. The source of illumination in an electron microscope is the electron gun that is fitted with a tungsten filament cathode as the electron source. The electron beam is accelerated by the anode at very high voltage. The effect of the gun is to produce a narrow beam of electrons passing at high speed in a given direction. They are focused on the specimen by the electromagnetic lenses. The electrons that are transmitted through the specimen are either unscattered or scattered electrons depending on their interaction. The emerging beam carries information about the structure of the specimen and it is further magnified by other lenses to generate a high-resolution image at atomic level. The image is recorded by projecting it on a fluorescent screen or photographed using charged coupled device (CCD) camera. TEM is widely used in Cell and Molecular biology research for the histological analysis of cells and tissue, drug investigation, diagnosis of diseases and the study of sub cellular organelles such as nucleus, mitochondria, endoplasmic reticulum etc. and biomolecules.

3.11.2 Scanning Electron Microscopy (SEM)

The scanning electron microscope (SEM) is another variation of an electron microscope. The basic parts of SEM are similar to the TEM except it has a secondary electrons detector. SEM produces an image by detecting low energy secondary electrons that are reflected back from the surface of the specimen due to excitation by the primary electron beam or backscattered electron signal. These secondary electrons strike a detector that is located near the surface of the specimen. SEM uses a fine beam of accelerated electrons that scans line by line on the specimen surface to deliver an image with information of surface topology. The detectors build up the image by mapping the detected signals with beam position. Therefore, the Image formation in the SEM is indirect. The resolution limit of SEM is about 3- 15 nm as compared to resolution of 0.2 nm by TEM but SEM has a greater depth of view

and the images produced closely represent the 3D structure of the sample. The SEM is primarily used to study of topography, composition and morphology of surface of biological specimens. Standard SEM also provides three-dimensional image of cell surface.

3.12 High Performance Liquid Chromatography (HPLC)

This is a very fine and high precision technique but the successful use of the techniques for a given problem requires the right combination of a variety of operating conditions such as the type of column, packing and mobile phase, column length and diameter, flow rate, column temperature and sample size. In fact, newer columns and packing materials offer high performance at moderate pressure (although still high relative to gravity flow). HPLC methods have been developed in a variety of areas including organics, biological, small molecules, macromolecules, pollutants, polymers, and many others. Chromatography can be done in a number of modes. The best mode for a particular separation depends on the structural characteristics of the solutes to be separated and the analysis requirements. HPLC methods can be selected based on the molecular weight and the solubility of the substance. In many samples separation can be achieved by reverse phase chromatography using a bonded silica, stationary phase. Reverse phase HPLC is the first choice because it is often faster, cheaper and easier than other alternatives.

3.13 Laminar air flow

Laminar air flow is one of the essential equipment in microbiology. It is utilized for inoculation of microbes in culture flask. Hence laminar airflow is also known as inoculation chamber. The laminar airflow provides the sterile environment for bacterial inoculation and it also protects the cell culture from pathogenic contaminants. However, sterile working environment is one of the essential requirements in microbial research laboratories. The laminar airflow (LAF) system works by generating a one directional airflow to produce a contaminants free air drawn through the highly efficient filter known as HEPA filters which effectively eliminate airborne contaminants. The basic components of laminar airflow are cabinet, working station, filter pad, fan, UV lamp, lighting lamp and HEPA Filter. Generally, there are two types of laminar airflow: Vertical laminar airflow cabinet and Horizontal airflow cabinet. In a horizontal airflow cabinet, the position of the airflow is horizontal. Air enters the

system from the backside of the HEPA filter, and the pure air (free from contaminants) is forced in a back to-front direction across the workspace. Conversely, in a vertical airflow cabinet, the airflow is vertical. Air is directed downward from the top of the cabinet onto the working area. This configuration ensures that contaminants are filtered out before the air reaches the work surface, providing a sterile environment for handling sensitive biological materials.

3.14 Hot air oven

Oven is used for drying glassware and heating the samples, particularly in the determination of moisture and fat percentage in milk and milk products. The temperature range may be from 10°C to 150°C or higher. It should be thermos-statistically controlled and equipped with air circulating fan. Hot air oven are electrical devices used in sterilization. The oven uses dry heat to sterilize articles over several hours to destroy microorganisms and bacterial spores. Generally, they can be operated from 50 to 200 °C. It is found in hospitals and laboratories where medical professionals and laboratory technicians use it. A dry heat cabinet is easy to install and has relatively low operating costs, it penetrates materials, it is nontoxic and does not harm the environment and it is noncorrosive for metal and sharp instruments.

Check your progress

1. What do you understand by the term electron microscopy.
2. Differentiate between SEM and TEM.
3. Write a short note on High Performance Liquid Chromatography.
4. Briefly explain laminar air flow and hot air oven.

3.15 Incubator

An incubator is a tool used to maintain and preserve cell or microbiological cultures by offering ideal temperature conditions in a controlled condition. The idea behind an incubator is to maintain the natural environments needed for the growth and development of microorganisms or cells by keeping the temperature, humidity, and gas levels constant. It is essential device for experimental work of in vitro laboratory work such as animal cell culture and bacterial cultures. The CO₂ incubators are extensively utilized for cultivating cells and

microorganisms that require CO₂. Basic incubators are capable of maintaining temperatures between 60 to 65 °C (140 to 149 °F), with a few standard models can rise slightly higher temperatures (often not exceeding 100 °C). The most ideal temperature is about 37 °C employed for cultivating bacteria (i.e. E. Coli) as well as mammalian cells. These organisms can grow under these artificial conditions. The lab incubator comprises of cabinet, perforated shelves, thermostat, thermometer, heating unit and control knob.

3.16 Gel electrophoresis

This technique is based on hydrated gel networks which have many desirable properties. They are beneficial in wide variety of experimental formats like vertical or horizontal electrophoresis in slab gels and in tubes or capillaries. The mechanical stability present in these gels also facilitates manipulation after electrophoresis and further experimentation becomes easier, for example: finger printing and blotting of intact proteins/digested proteins in the gel slices. In gel electrophoresis, charged molecules move in an electric field is through a matrix of a polymerized substance like agarose or polyacrylamide etc. It depends on the rate of moving of the individual molecules in the gel which is further based on the properties of molecules and the separation system. Gel matrices are penetrated with pore networks through which the molecules are moving. Moreover, substantially gels are chemically unreactive and are generally employed in biochemistry. Also, the gels have recorded minimum interaction with other biomolecules during electrophoresis, therefore, allows for the separation easily based on physical variances between the components of the sample (Adamson and Reynolds,1997). The agarose and acrylamide are the most commonly selected gels, used for the separation of nucleic acids and proteins.

3.17 pH meter

It is used for the determination of pH and oxidation-reduction potential of samples or other substances like buffers and other reagent solutions. It must have the ability for automatic temperature compensation, recorder connection and calibrated data memory. This instrument measure the potential difference between a glass electrode and a standard calomel electrode and is calibrated with buffers of known pH. Temperature compensation devices are incorporated to correct for temperature deviations. Liquid and semisolid foods

can be examined for their pH but solid food is used only after making slurry with water. The pH value ranges from 0 to 14 and the primary working mechanism of the pH meter is based on the exchange of ions from the sample solution to the inner solution (pH seven buffer) of the glass electrode through the glass membrane that generates electric voltage. Therefore, the result of the pH meter is based on the combined relation between electric voltage, ion concentration, and pH reading. There are basically three types of pH meter: Pen tester pH meter, Handheld/Portable pH meter and Benchtop/Desktop pH meter.

3.18 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared (FTIR) spectroscopy is a precise and highly effective analytical technique frequently used in biomolecular research due to its ability to provide detailed structural and functional insights into biomolecules such as proteins, carbohydrates, nucleic acids, and lipids. FTIR spectroscopy can also be utilized as an efficient tool in biofuel production processes for monitoring the lipid accumulation in algal cells. FTIR spectroscopy has proven to be a powerful analytical technique for assessing and quantifying the extent of lipid oxidation in oils by detecting characteristic changes in functional groups and monitoring the formation of oxidation products.

3.19 X-ray Diffraction (XRD)

The atomic planes of a crystal cause an incident beam of X-rays to interfere with one another as they leave the crystal. The phenomenon is called X-ray diffraction. It is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. Max von Laue, in 1912, discovered that crystalline substances act as three-dimensional diffraction gratings for X-ray wavelengths similar to the spacing of planes in a crystal lattice. X-ray diffraction is now a common technique for the study of crystal structures and atomic spacing. X-ray diffraction is based on constructive interference of monochromatic X-rays and a crystalline sample. These X-rays are generated by a cathode ray tube, filtered to produce monochromatic radiation, collimated to concentrate, and directed toward the sample.

The interaction of the incident rays with the sample produces constructive interference (and a diffracted ray) when conditions satisfy Bragg's Law ($n\lambda = 2d \sin\theta$). This law relates the

wavelength of electromagnetic radiation to the diffraction angle and the lattice spacing in a crystalline sample. These diffracted X-rays are then detected, processed and counted. By scanning the sample through a range of 2θ angles, all possible diffraction directions of the lattice should be attained due to the random orientation of the powdered material. Conversion of the diffraction peaks to d-spacings allows identification of the mineral because each mineral has a set of unique d-spacings. Typically, this is achieved by comparison of d-spacings with standard reference patterns. All diffraction methods are based on generation of X-rays in an X-ray tube. These X-rays are directed at the sample, and the diffracted rays are collected. A key component of all diffraction is the angle between the incident and diffracted rays.

Check your progress

1. Explain the use of incubator in molecular lab.
2. Briefly describe the process of gel electrophoresis.
3. Write short note on:
 - a. pH meter
 - b. Fourier Transform Infrared (FTIR) spectroscopy
 - c. X-ray diffraction (XRD)

Summary

This unit describes the process of gene regulation and about different genetic engineering techniques useful for the study of molecular biology. Gene regulation is the process through which cells control the expression of genes, ensuring that the right genes are turned on or off at the correct time. Genetic engineering is the process of transferring specific genes from the chromosome of one organism and transplanting them into the chromosome of another organism in such a way that they become a reproductive part of the new organism. This unit also discusses about the different processes useful for replication of DNA like PCR and ELISA. Polymerase chain reaction (PCR) is a strong tool that amplifies even the tiniest amount of DNA and gives millions of copies of that DNA to analyze the property of a gene. Enzyme-linked immunosorbent assay (ELISA) is a biochemical method, wherein target antigen (or

antibody) in a sample is detected using specific enzyme-labeled antibody (or antigen), by measuring enzymatic reaction with its substrate calorimetrically.

Spectrophotometers are used for measuring the absorbance of visible light and UV-visible light respectively. In a typical absorption spectral measurement, a monochromatic radiation is made to fall on a sample taken in suitable container called cuvette. In such a situation a part of the radiation is reflected, a part is absorbed, and a part is transmitted. In this unit you will also understand the advantage and principle of different instruments used in molecular biology like electron microscopy, TEM, SEM, incubator, pH meter, Fourier Transform Infrared (FTIR) spectroscopy and X-ray diffraction (XRD).

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Unit 4. Propagation Techniques

Unit Structure

4.0. Learning Objectives

4.1 Introduction

4.2 Propagation in Biotechnology

4.3 Propagation by Seed

4.4 Propagation by Cutting

4.5 Advantages and Disadvantages of Propagation by Cuttings

4.5.1 Advantages of Propagation by Cuttings

4.5.2 Disadvantages of Propagation by Cuttings

4.6 Propagation by Layering

4.7 Advantages and Disadvantages of Propagation by Layering

4.7.1 Advantages of Propagation by Layering

4.7.2 Disadvantages of Propagation by Layering

4.8 Propagation by Budding

4.8.1 Methods of Propagation by Budding

4.8.2 Advantages of Budding

4.9. Propagation by Grafting

4.9.1 Methods of Grafting

4.9.2 Cleft Grafting

4.9.3 Approach Grafting

4.9.4 Side Grafting

4.9.5 Veneer Grafting

References

4.0. Learning Objectives

After you have studied this unit, you should be able:

4.1 Introduction

Biological **propagation** is the process by which new organisms, or new individuals of a species, are created. It is fundamental to the continuation of life and is divided into two primary modes:

- **Sexual Reproduction:** Involves the fusion of male and female gametes, leading to genetic recombination and variation in the offspring. This is the basis of traditional breeding programs.

- **Asexual Reproduction (Cloning):** Involves a single organism producing offspring that are genetically identical to itself. This mode is the foundation for most biotechnological propagation techniques.

The primary role of controlled propagation is to produce desired organisms **in scale** for agriculture, horticulture, forestry, and various industrial applications.

4.2 Propagation in Biotechnology

Biotechnology utilizes living systems and organisms to develop or make products. In the context of propagation, this involves utilizing controlled laboratory and industrial environments to manipulate and rapidly multiply organisms or their components.

- **Rationale for Biotechnological Methods:**
 - **Speed and Scale:** Enables the mass production of thousands of identical plants (clones) or billions of microbial cells in a short timeframe.
 - **Disease-Free Stock:** Techniques like meristem culture allow for the elimination of systemic pathogens (e.g., viruses) from mother stock.
 - **Genetic Stability:** Asexual methods ensure the maintenance of desirable genetic traits (e.g., high yield, pest resistance) without genetic drift.

4.3 Propagation by Seed

When using an asexual technique, seed production is mostly taken into account when true-to-type plants are anticipated as the end output. Only in the case of polyembryonic seedlings is it true. As the name suggests, polyembryony describes seeds that contain many embryos. The term "gametic and sexual embryo" refers to an embryo that is created when male and female gametes unite, while the other embryos are created by simple mitotic division of nucellar embryo cells without the assistance of male gametes. Nucellar embryos are a frequent occurrence in citrus and mango.

4.4 Propagation by Cutting

Using a portion of a stem, root, or another plant part for plant multiplication is known as cutting, which is a type of vegetative propagation. Cutting-derived plants are true to their

mother plants. This technique is frequently applied to plants since they root easily and readily, making plant multiplications and inexpensive. Commercial cuttings are used to produce ornamental plants like carnations, chrysanthemums, and numerous foliage plants, as well as fruit plants like phalsa, Baramasi lemons, and grapes, among others.

The two most popular ways of propagation are hardwood and softwood cuttings, which are made from completely developed and immature tissues, respectively. Immature angular cuttings are not favored over round cuttings. Cuttings of hardwood are easily prepared from shoots that are at least a year old. Cuttings are taken from deciduous fruit plants, such as figs, pomegranates, grapes, and phalsa. Cuttings from decorative foliage plants and evergreen fruit trees, such as Baramasi lemon, can be prepared in the spring (February to March) and rainy season (August to September). Typically, 3-5 bud cuttings, around 15-20 cm in length, are taken. To improve nutrition absorption, the bottom cut is made slantingly directly below the bud or node. The upper cut is made as far away from the upper bud as feasible to prevent drying and at right angles to minimize the size of the wound. The cuttings should be let to dry after they are processed. Fruit plant cuttings are typically bundled into small bundles of 20 to 25 cuttings, buried in wet soil for a certain amount of time to promote wound healing (a process known as "callusing"), and then planted in the field to take root. Ornamental plant cuttings are either directly planted into media for rooting, or they are dipped in water to ensure proper absorption of water, followed by the application of rooting hormones to the lower cut below the node.

4.5 Advantages and Disadvantages of Propagation by Cuttings

4.5.1 Advantages of Propagation by Cuttings

1. It's an easy, affordable, and practical solution.
2. The costs are kept to a minimum.
3. A great deal of technical expertise is not needed.
4. There are no intricate issues regarding stock–scion connections.
5. Quick multiplication is feasible in a brief amount of time.

6. Traveling is simple.

4.5.2 Disadvantages of Propagation by Cuttings

1. Not every plant can root readily.
2. It is not possible to fully benefit from rootstock.

4.6 Propagation by Layering

Using layering, roots are induced on shoots while they are still attached to their mother plants. For fruit and ornamental plants that are difficult to root after being separated from their mother plants, this is an alternative propagation technique. Mound, ground, and air layering are the three most popular layering techniques. Different methods of layering are:

i) Air Layering

This technique involves forming roots while the shoot remains attached to the mother plant. A straight, healthy one-year-old shoot is selected, and a 2.5-cm-tall ring of bark is removed just below a bud. The exposed area is wrapped with moist sphagnum moss, which is then covered with a polythene strip. Sphagnum moss has a very high water-holding capacity and is lightweight. If unavailable, any material that can hold moisture for a long time may be used. The polythene wrapping retains moisture while still allowing gaseous exchange, so no further watering of the layer is required—saving labor. This method is also known as the **goottee approach**.

Air layering is practiced on ornamental plants such as guava, litchi, sapota, and loquat during February–March and July–August. Within a few weeks, roots appear and are visible through the polythene. About 15 days before separation from the mother plant, the rooted layer should be half-cut at the base. A few leaves or a small portion of the stem are retained at detachment. It is preferable to plant these rooted layers in a nursery for better care rather than directly in the field. They can be transplanted to the field in February or in September–October of the following year.

ii) Ground Layering

In this method, a low-lying branch of the plant is selected. A 2.5-cm-wide ring of bark is removed just below a bud. The branch is then bent down and buried in soil while still attached to the mother plant. The soil is kept moist through regular watering. After a few weeks, when roots have formed, the young plant is separated from the mother plant, ensuring that the new roots remain with the detached plant. These young plants should then be raised in nursery rows or pots before being transplanted to the field so that they develop stronger root and shoot systems. This technique is commonly used for ornamentals such as jasmine and Baramasi lemon.

iii) Mound Layering (Stooling)

In this technique, the plant is cut back in July or February. New shoots emerge from the base in April and September. Rings of bark are removed from these shoots, and soil is heaped around them. During the rainy season, stools rooted from April stooling are separated, and those from August stooling are removed in spring. These separated stools are planted in a nursery for further growth. This method, also known as stool layering, is commonly used in guava propagation. Early rooting can be improved by applying rooting hormones around the ring using a paintbrush.

4.7 Advantages and Disadvantages of Propagation by Layering

4.7.1 Advantages of Propagation by Layering

1. It is simple to do and does not require a lot of equipment.
2. A dependable technique for plant species that are difficult to root.
3. Does not need exact environmental control parameters.
4. It is feasible to grow big plants quickly.

4.7.2 Disadvantages of Propagation by Layering

1. Comparatively more scion wood is needed than with other techniques.
2. It is not feasible to generate a large number of plants quickly.
3. The resulting plants have fragile, small roots.

4. The advantageous benefits of rootstocks are not usable.
5. Layer mortality is typically high.

4.8 Propagation by Budding

Budding

One bud is placed into the rootstock using the budding technique. This process is quick and simple. Compared with cutting and grafting procedures, this propagation technique conserves budwood. The ideal time for budding is when the bark begins to “slip” on both the scion and the stock—an indication of active cambium, the tissue responsible for union formation. This method is usually practiced in spring and during the rainy season. The three most commonly used budding techniques are **T-budding**, **patch budding**, and **chip budding**.

4.8.1 Methods of Propagation by Budding

There are several budding techniques, which are explained in detail below:

i) T-Budding (Shield Budding)

A horizontal cut is made on the stock (stem) 15–20 cm above the ground, extending about one-third of the way around the stem. A second cut, 2–3 cm long, is made vertically downward from the center of the horizontal cut. Using the ivory end of the budding knife, the bark flaps are lifted to expose the underlying tissue.

A bud is then removed from the bud stick. A slicing cut is made beginning about 1.25 cm below the bud and extending upward to about 2.5 cm above it, removing the bark that encloses the bud. Another horizontal cut, 1.25–2 cm above the bud, releases the shield piece, which retains a thin sliver of wood.

The shield is inserted beneath the bark flaps of the T-cut so that its upper edge aligns with the horizontal cut on the stock. The bud remains exposed while the bark flaps cover the rest of the shield. The union is secured by wrapping polythene strips around the budding area. T-budding can be performed year-round as long as the sap is flowing freely. It is used on most fruit trees, roses, and several woody ornamentals during spring (March–April) and the

rainy season (July–September). This is a particularly popular method for propagating roses and citrus trees.

ii) Patch Budding

This method is highly effective for guava propagation, achieving a 60–70% success rate when performed in May and June. Freshly harvested angular budwood from the current growing season is used as scion material.

A rectangular or square patch of bark, about 1.5 cm wide and 2.5 cm long, is removed from the rootstock at a height of 15–20 cm above the ground. A similar patch containing a bud is carefully removed from the bud stick without damaging the bark beneath the bud. The patch is then fitted onto the rootstock and immediately secured with polythene tape.

For higher success, a patch containing **two buds** can be used as the scion. This modified technique is known as **improved patch budding**.

iii) Chip Budding

This technique is typically performed just before new growth begins, while both stock and scion remain dormant.

A slanting cut about 2.5 cm long is made in the stock, followed by a second cut at the base of the first to remove a chip of bark. A matching chip containing a bud is removed from the scion wood in the same manner. This bud-bearing chip is inserted into the cut on the rootstock, ensuring that the cambium layers of stock and scion match on at least one side. The bud is then wrapped with a polythene strip to prevent drying.

4.8.2 Advantages of Budding

- Compared with grafting, this method of propagation is faster and more effective.
- Less scion material is required.
- It benefits plants (especially stone fruits) that exude excessive gum from wounds made during grafting, which may obstruct the xylem.

- Because the union is stronger than in grafting, budded plants are less prone to storm damage and strong winds.
- This method is somewhat simpler than grafting.

4.9. Propagation by Grafting

Another technique for vegetative propagation is grafting, which involves joining two plant sections so that they merge and grow into a single plant. The scion twig in this manner has more than two buds upon it. Fruits like pears, peaches, plums, almonds, mangos, etc. are frequently grafted. Grafting is done during dormancy in seasonal fruits like peaches, plums, and almonds, but during active development in mango trees.

4.9.1 Methods of Grafting

There are several grafting techniques, including veneer, approach, side, cleft, and tongue grafting.

Tongue Grafting:

When the diameters of the stock and the scion are equal, this technique is frequently used. First, a 4–5 cm long, smooth, sloping cut is made on the rootstock. A second downward cut, about one-third of the way from the top and 3–4 cm long, is then made. The scion wood is sliced in the same manner, matching the cuts made on the rootstock exactly. The scion, containing two to three buds, is then firmly inserted into the rootstock, ensuring that the cambium layers of the scion and stock meet on at least one side. Finally, the union is wrapped securely with a polythene strip.

4.9.2 Cleft Grafting

Another name for this technique is **wedge grafting**. When tongue grafting is not effective and the rootstock is thicker than the scion, this method is useful in the nursery. With this technique, a rootstock up to **8 cm thick** can be grafted.

The rootstock is cut smoothly with a secateur or blade. It is then split down the middle to a depth of about **4 cm**. The scion, which contains three or four buds, is trimmed at its base into a **wedge shape**, with the outer side slightly wider than the inner side.

To ensure proper alignment, the lower bud of the scion should fit just inside the split of the rootstock so that the **cambium layers of both stock and scion match accurately**.

4.9.3 Approach Grafting

This technique is called **approach grafting** because the scion is approached by the rootstock while it **remains attached to its mother plant**. Alternatively, the rootstock may be planted beneath the canopy of mother plants trained to have low heads.

The best time to perform approach grafting is **late July to early August**. The diameters of the stock and scion should be nearly equal. Matching sections of stock and scion are cut, and a thin slice of wood (about **4 cm long**) along with bark is removed from each.

The two exposed surfaces are then brought together so that at least **one side of their cambium layers makes contact**. The union is tightly secured using polythene strips or other tying material. Regular irrigation is provided to speed up union formation.

The graft union is completed within **2–3 months**. At that time, the scion branch is cut halfway through. After about a week, if the shoot shows no wilting, it can be fully separated from the parent plant. If wilting occurs, union is incomplete and separation must be delayed.

This method is widely used for **mango propagation**. It is also known as **inarching**.

4.9.4 Side Grafting

At a height of **15–20 cm** above the ground, a three-sided rectangular cut (measuring **4 × 1.25 cm**) is made on the rootstock. The bark of this section is gently lifted. A corresponding cut is also made at the base of the scion to expose its cambium.

The scion should be prepared **in advance**. For this, healthy scion shoots from the most recent mature flush are selected. The terminal buds must be plump. The leaf blades are removed carefully, leaving the petioles intact. After **7–10 days**, the petioles fall off and the terminal buds swell—at which point the scion is ready.

The prepared scion is then placed beneath the bark flap of the rootstock so that the exposed **cambium layers match closely**. The bark flap is returned to its original position and the graft is tied securely with a strong polythene strip.

Once grafting is completed, a portion of the rootstock above the graft union is removed to encourage scion growth. After the scion begins to grow and its leaves turn green, the entire rootstock above the union is removed.

Side grafting is practiced from **March to October**, though May and October are less favorable. It is especially common in **mango propagation**.

4.9.5 Veneer Grafting

In this technique, a shallow downward cut about **4 cm long** is made on the rootstock, 15–20 cm above the ground. A second short downward cut at the base of the first removes a slice of wood and bark.

The preparation of the scion is the same as in side grafting. The cuts on both scion and stock must be of the **same width and length** for proper cambial alignment.

The prepared scion is inserted into the cut on the stock and firmly tied with a polythene strip. After the union completes, the rootstock is reduced above the graft to allow the scion to grow—thus completing the veneer grafting process.

Summery

You have learned about many asexual plant propagation techniques and nursery management tactics in this course. Plants can be mass-propagated using asexual techniques. The technique used will vary depending on the plant being propagated. Certain fruit plants, such as papayas, can only be commercially propagated through seeds, which is asexual propagation; in contrast, bananas can only be propagated through vegetative (asexual) techniques, such as suckers. With both asexual and sexual methods, which have benefits and drawbacks, the majority of horticultural plants are propagated. While homogeneous plants cannot be produced via sexual propagation, true-to-type plants can be produced using an asexual technique. Understanding the many contemporary nurseries and the techniques—such as root media—that are employed to generate commercially.

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Unit 5: Microbiology and Industrial Applications

Unit Structure

5.0 Learning Objectives

5.1 Introduction

5.2 Classification of microorganisms

5.3 Pathogenic and useful microorganisms

5.4 Microbial enzymes in industrial applications

5.5 Involvement of microorganisms in fermentation

5.6 Production of biofertilizers

5.7 Biogas

5.8 Bioethanol and biopolymers

5.9 Food Industry

5.0 Learning Objectives

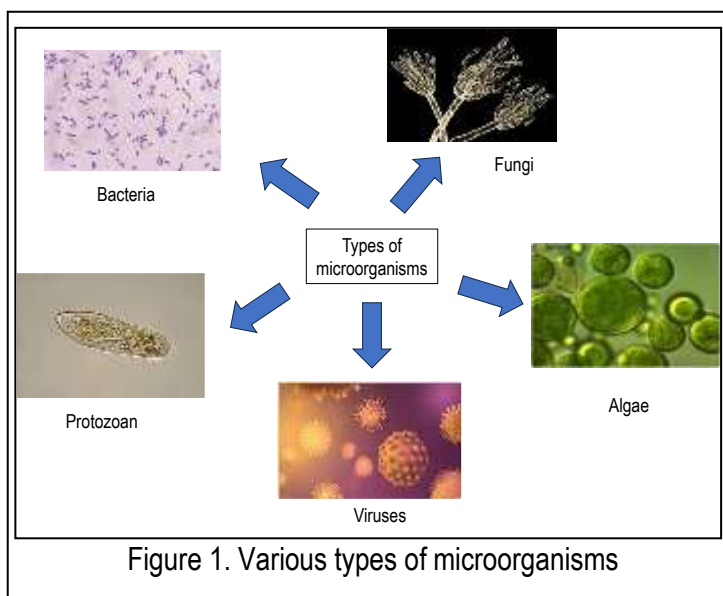
After completing this unit, learners shall be able to:

- Discuss the microorganisms and its classification?
- Understand the microbes in different habitat.
- Understand the pathogenic and useful microorganism
- Discuss the industrially important microorganisms
- Understand the microbial fermentation
- Discuss the application of microbes in production of biofertilizer, biogas, bioethanol and food industry.

5.1 Introduction

The present unit deals with the Microorganisms and their contribution in economy and ecology and their significant contribution for human wellbeing. Microorganisms are very small organisms that cannot be seen with the unaided eye. They can be seen only with a magnifying glass or microscope. They may be unicellular or multicellular. Microorganisms may be single-celled like bacteria, some algae and protozoa, or multicellular, such as many algae and fungi (Figure 1) Microorganisms may exist alone or in colonies and in association with other organisms.

Microorganisms live in all types of environments, ranging from ice cold climate to hot springs; and deserts to marshy lands. They are also found inside the bodies of animals including humans. Some microorganisms grow on other organisms while others exist freely.



Significance of microorganisms in Environment

1. Fixation of atmospheric nitrogen
2. Decomposition of organic wastes and residues
3. Suppression of soil-borne pathogens
4. Recycling and increased availability of plant nutrients
5. Degradation of toxicants including pesticides
6. Production of antibiotics and other bioactive
7. Production of simple organic molecules for plant uptake
8. Complexation of heavy metals to limit plant uptake
9. Solubilization of insoluble nutrient sources
10. Production of polysaccharides to improve soil aggregation

Significance of microorganisms in Food

1. Spoilage, Fermentation, Food production
2. Microorganisms are used in brewing, wine-making, baking, and other food-making processes.
3. They are also used to control the fermentation process in the production of cultured dairy products such as yogurt and cheese.
4. The cultures also provide flavor and aroma, and inhibit undesirable organisms

Significance of microorganisms in Industry

In large-scale industrial processes, microbes are widely used to synthesize a number of products valuable to human beings such as:

1. Food additives.
2. Alcoholic and non-alcoholic beverages.
3. Biofuels, metabolites, and biofertilizers.
4. Few Chemicals, Enzymes and other Bioactive Molecules.
5. Vaccines and other Antibiotics to kill or retard the growth of disease-causing microbes.

5.2 Classification of microorganisms

Micro-organisms may be classified in the following large biological groups: 1. Algae 2. Protozoa 3. Slime moulds 4. Fungi 5. Bacteria 6. Archaea 7. Viruses.

The algae (excluding the blue–green algae), the protozoa, slime moulds and fungi include the larger eukaryotic micro-organisms; their cells have the same general type of structure and organization as that found in plants and animals.

The bacteria, including organisms of the mycoplasma, rickettsia and chlamydia groups, together with the related blue– green algae, comprise the smaller micro-organisms, with the form of cellular organization described as prokaryotic.

The archaea are a distinct phylogenetic group of prokaryotes that bear only a remote ancestral relationship to other organisms. As the algae, slime moulds and archaea are not currently thought to contain species of medical or veterinary importance, they will not be considered further. Blue– green algae do not cause infection, but certain species produce potent peptide toxins that may affect persons or animals ingesting polluted water.

The viruses are the smallest of the infective agents; they have a relatively simple structure that is not comparable with that of a cell, and their mode of reproduction is fundamentally different from that of cellular organisms. Even simpler are viroids, protein-free fragments of single-stranded circular RNA that cause disease in plants. Another class of infectious particles are prions, the causative agents of fatal neurodegenerative disorders in animals and man. These are postulated to be naturally occurring host cell membrane glycoproteins that undergo confirmative changes to an infectious isoform.

5.3 Pathogenic and useful microorganisms

Microorganisms are considered useful because they help in agriculture, industry, medicine, environmental cleanup, and digestion.

1. Useful Microbes in Agriculture

Nitrogen Fixation: These bacteria convert atmospheric nitrogen into nitrogenous compounds, increasing soil fertility. Examples: *Azotobacter*: Free-living aerobic nitrogen-fixing bacterium, *Clostridium*: Free-living anaerobic nitrogen-fixing bacterium. *Rhizobium*: Symbiotic nitrogen fixer present in root nodules of leguminous plants.

All nitrogen-fixing bacteria possess 'nif genes' responsible for nitrogenase enzyme production.

Nitrification: These bacteria convert ammonia into usable nitrate and helps maintain soil fertility.

Nitrosomonas – Converts ammonia → nitrites

Nitrobacter – Converts nitrites → nitrates

2. Useful Microbes in Dairy Industry

Lactic Acid Bacteria: These bacteria convert lactose in milk into lactic acid, which leads to curd formation and souring of milk. Examples: *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Lactococcus lactis*, *Streptococcus thermophilus*

3. Useful Microbes in Vinegar Industry

Converts alcoholic sugar solutions into acetic acid, used to make vinegar. Examples: *Acetobacter aceti*

4. Useful Microbes in Industrial Production

Clostridium acetobutylicum → Produces acetone and butyl alcohol

Clostridium butyricum → Used in retting of fibres like jute, flax

Bacillus megatherium → Flavours tobacco leaves

Mycococcus candidans → Cures tobacco leaves

5. Useful Microbes in Medicine (Antibiotics) Many antibiotics are produced from bacteria (Table 1).

Table 1. Microbes used in antibiotic preparations

Microbes in Medicine	Medicine (Antibiotics)
<i>Bacillus subtilis</i>	Subtilin
<i>Bacillus polymyxa</i>	Polymyxin

<i>Streptomyces griseus</i>	Streptomycin
<i>Streptomyces venezuelae</i>	Chloramycetin (Chloramphenicol)
<i>S. rimosus</i>	Terramycin (Oxytetracycline)
<i>S. aureofaciens</i>	Aureomycin (Chlortetracycline)
<i>S. erythraeus</i>	Erythromycin
<i>S. fradiae</i>	Neomycin

6. Sewage Disposal

Bacteria help break down organic waste in sewage into simpler substances.

Algae + bacteria play a major role in purification of wastewater.

7. Petroleum Spill Cleanup

Pseudomonas putida (Superbug) Genetically engineered strain that digests petroleum, used for oil-spill cleanup.

8. Useful Microbes in Human Body

Escherichia coli (*E. coli*) Lives in the large intestine, helps in digestion and vitamin synthesis.

HARMFUL MICROBES

Bacteria are also responsible for many diseases in humans, animals, and plants, along with food spoilage and poisoning.

Table 2. Diseases and causative microorganism

Disease	Causative Bacterium	Disease	Causative Bacterium
Pneumonia	<i>Diplococcus pneumoniae</i>	Gonorrhoea	<i>Neisseria gonorrhoeae</i>
Typhoid	<i>Salmonella typhosa</i>	Syphilis	<i>Treponema pallidum</i>
Cholera	<i>Vibrio cholerae</i>	Diphtheria	<i>Corynebacterium diphtheriae</i>
Plague	<i>Pasteurella pestis</i>	Tuberculosis	<i>Mycobacterium tuberculosis</i>
Meningitis	<i>Neisseria meningitidis</i>	Gangrene	<i>Clostridium perfringens</i>
Jaundice (Weil's disease)	<i>Leptospira</i>	Bacterial dysentery	<i>Shigella dysenteriae</i>
Whooping cough	<i>Bordetella pertussis</i>	Leprosy	<i>Mycobacterium leprae</i>
Tetanus	<i>Clostridium tetani</i>		

2. Harmful Microbes Causing Diseases in Animals

Disease like anthrax caused by *Bacillus anthracis* responsible for kills cattle, sheep, goats, and can infect humans.

3. Harmful Microbes Causing Plant Diseases

These bacteria cause economic losses by destroying crops. Som of the plant diseases are discussed as below (Table 3).

Table 3. Harmful Microbes Causing Plant Diseases

Plant Disease	Causative Bacterium
Black leg disease	<i>Clostridium chauvei</i>
Soft rot of potato	<i>Pseudomonas solanacearum</i>
Citrus canker	<i>Xanthomonas citri</i>
Bacterial blight of paddy	<i>Xanthomonas oryzae</i>
Tundu disease of wheat	<i>Corynebacterium tritici</i>
Potato wilt	<i>Pseudomonas solanacearum</i>
Fire blight of apple & pear	<i>Erwinia amylovora</i>
Crown gall in sugar beet	<i>Agrobacterium tumefaciens</i>
Black rot of cabbage	<i>Xanthomonas campestris</i>

4. Other Harmful Microbial Activities

(A) Cotton Spoilage by *Spirochaete cytophaga*

(B) Food Poisoning: *Clostridium botulinum* (Produces botulin toxin, one of the deadliest toxins known)

(C) Reduction of Soil Fertility as denitrifying bacteria (*Bacillus denitrificans*, *Micrococcus denitrificans*, *Thiobacillus denitrificans*) convert nitrates into nitrogen gas and reduce soil nitrogen level and harm crops.

5.4 Microbial enzymes in industrial applications

Microbial enzymes produced from industries are selected from different groups of microorganisms and they include bacteria, fungi and yeasts. Many enzymes are produced in industries but most predominant enzymes that are produced on large scale in industries include protease, α -amylase, glucose isomerase and glucamylase [8]. Enzymes produced in industries with the help of microorganisms were found to exhibit good biological activity. Microbial source is preferred over plants and animals for production of enzymes mainly

because of the following reasons [9]. 1) Enzymes can be produced on large scale and are economical [10, 11]. 2) The process of extraction and purification of enzymes from microbial sources is easier in comparison with plant and animal sources [12]. 3) Microbial sources are capable of producing variety of enzymes in different environmental conditions in limited space and time period [13]. 4) Genetic manipulation is carried out to yield higher quantity of enzymes produced from microbial sources [14]. Some of the industrially produced enzymes produced in large scale using microorganisms as source are mentioned below in (Table 4).

Table 4. Industrial enzymes and their source of microorganisms (Vittaladevaram, 2017)

Source	Enzyme	Microorganism
Bacterial	Proteases	<i>Bacillus subtilis</i>
	Amylases	<i>Bacillus subtilis</i>
	Penicillinase	<i>Bacillus subtilis</i>
Yeast	Lactase	<i>Saccharomyces fragilis</i>
	Invertase	<i>Saccharomyces cerevisiae</i>
Fungal	Proteases	<i>Aspergillus niger</i>
	Amylases	<i>Aspergillus oryzae</i>
	Pectinases	<i>Aspergillus niger</i>
	Catalase	<i>Aspergillus niger</i>
	Glucose oxidase	<i>Penicillium notatum</i>
	Glucosidases	<i>Aspergillus flavus</i>

Initially, enzymes were extracted from the stomach of calves, lambs, and baby goats, but now are produced by microorganisms like bacteria, fungi, yeast and actinomycetes. Enzymes obtained from microorganisms are better than those of animal and plant origin. Microorganisms can be genetically manipulated to improve the production of commercial scale [21-23]. Enzymes can hydrolyze complex molecules into simple monomer units, like carbohydrates into simple sugars, which are natural substances involved in all types biochemical processes. Every enzyme is substrate, pH, and temperature-specific for catalyzing the reaction to convert a reactant into a product [23, 24]. The food-processing industry uses more than 55 different microbial enzymes (Table 5).

Markets for traditional industrial enzymes remain to grow while the continued prominence on biotechnological accomplishments has generated demand for an ever increasing number of additional biocatalysts [25]. The emergences of genetic manipulations have now enabled the large-scale production of enzymes and other proteins which are produced naturally only in minute quantities. The level of downstream processing to which any enzyme is subjected is depending on its proposed application. Industrial enzymes produced in bulk usually need little downstream processing, and hence are relatively crude preparations [25, 26]. The production of commercial enzymes from fungi are 60% followed by 24% bacteria, 4% yeast, 2% Streptomyces, 6% higher animals, and 4% plants. In earlier technologies animal and plant origin enzymes were largely used and till now for specific enzymes they are the main sources. The animal tissues and organs are very fine sources for enzymes such as proteases, lipases, and esterases for example, lysozyme is mostly get from hen eggs. Similarly, some enzymes are originated only from plants as brilliant sources such as papain (papaya) and bromelain (pineapple). There are several down sides related with the enzymes making from plant and animal sources. Due to limited quantities there is a wide disparity in distribution. Apart from all the difficulties, the most important is the isolation, purification of the enzymes, and the cost factor as regard to industrial enzymes extracting from bovine source which contains heavy risk of contamination due to bovine spongiform encephalopathy (BSE is a prion disease occurred due to the ingestion of abnormal protein) therefore, microbial production of enzymes is used. There exists a likelihood of producing commercial enzymes straight by mammalian cell cultures. Other than the most important limit is the cost factor which is extremely high. Although some therapeutic enzymes are prepared through cell culture technique like tissue plasminogen, microorganisms are the most noteworthy and suitable source of commercial enzymes. They can be made to prepare large amounts of enzymes under optimal growth conditions. Cultivation of microorganism by using low cost media as well as the growth of microorganism takes in short span of time. In addition, by using genetic engineering techniques on microorganism, desired product is produced. Isolation, purification, and recovery processes are easy with microbial enzymes as compared to plant and animal sources. In fact, most of the enzymes used in industrial processes are produced from microorganisms. Variety of fungi, bacteria, and yeast are produced for this purpose (Kaur and Gill, 2019). The

microbial origin and the enzymes application in the food and beverages operations are listed in (Table 5) and (Figure 2) [25]

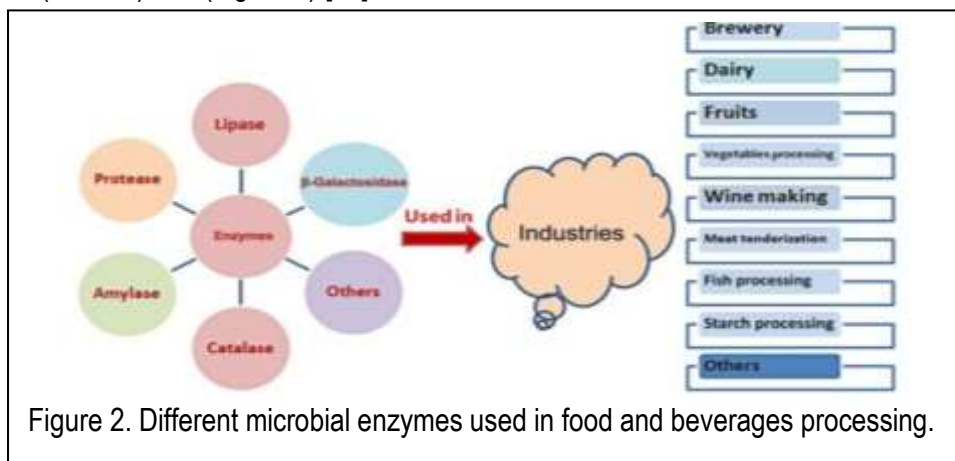


Figure 2. Different microbial enzymes used in food and beverages processing.

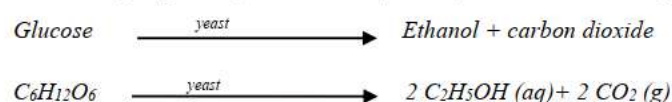
Table 5. Industrial Applications of Enzymes Produced by Solid-State Fermentation Processes (Kaur and Gill, 2019).

Process	Enzyme
Enzyme-assisted ensiling	Fungal cellulases and hemicellulases
Bioprocessing of crops and crop residues	Fungal cellulases and hemicellulases
Fiber processing (retting)	Fungal pectinases, cellulases, and hemicellulases
Feed supplement	Amylases, proteases, lipases, cellulases, and hemicellulases
Biopulping	Xylanases
Directed composting	Hydrolytic enzymes
Soil bioremediation	Laccases, ligninases
Postharvest residue decomposition	<i>Trichoderma harizanum</i> cellulases
Biopesticide	<i>T. harzianum</i> cellulase for helper function

5.5 Involvement of microorganisms in fermentation

Fermentation is a biological process in which microorganisms convert carbohydrates (usually sugars) into other compounds, such as alcohols, acids, or gases, in the absence of oxygen (anaerobic conditions) (Katz, 2012). This ancient process has been used for centuries in food preservation, beverage production, and various industrial applications. Microorganisms, such as yeasts, bacteria, and molds, are the primary agents in fermentation, each contributing to different types of fermentation processes. These microbes not only drive fermentation but also enhance the nutritional value, flavor, and safety of fermented products. This article explores the role of

microorganisms in fermentation and their significance across various industries (Mahajan, et al., 2020).



Microorganisms Involved in Fermentation

Yeasts: The most common yeast used in fermentation is *Saccharomyces cerevisiae*, which plays a central role in the production of alcoholic beverages like beer, wine, and spirits, as well as in bread making. Yeasts convert glucose into ethanol and carbon dioxide through alcoholic fermentation, a process that is crucial in baking (for leavening) and in brewing (for alcohol production).

Bacteria: Lactic acid bacteria (LAB), such as *Lactobacillus*, *Bifidobacterium*, and *Streptococcus*, are key players in lactic acid fermentation, where sugars are converted into lactic acid. This process is integral to the production of fermented dairy products like yogurt, kefir, and cheese, as well as vegetables like sauerkraut, kimchi, and pickles. Lactic acid fermentation preserves the food while imparting a tangy flavor and health benefits.

Molds: Fungi, especially molds like *Aspergillus oryzae*, are used in the fermentation of foods such as tempeh, soy sauce, and certain types of cheeses (like blue cheese). Molds secrete enzymes (e.g., proteases, amylases) that break down complex carbohydrates and proteins into simpler compounds, often enhancing the flavor, texture, and nutritional value of the food.

Microbial Fermentation Processes

Fermentation can be classified based on the type of product formed and the microorganisms involved. The main types of microbial fermentation include: (Nishida and Okada 2017)

Alcoholic Fermentation: In the absence of oxygen, yeasts like *Saccharomyces cerevisiae* convert glucose into ethanol and carbon dioxide. This process is the basis of alcoholic beverage production (beer, wine, spirits) and bread baking. The carbon dioxide produced causes the dough to rise, while the ethanol evaporates during baking.

Lactic Acid Fermentation: This type of fermentation is carried out by lactic acid bacteria, which convert glucose into lactic acid. This process is used in the production of yogurt, kefir, pickles, and fermented meats. The production of lactic acid lowers the pH, helping to preserve the food and contributing to its sour taste.

Acetic Acid Fermentation: Bacteria like *Acetobacter* convert ethanol into acetic acid (vinegar). This fermentation is aerobic, requiring oxygen, and is used in the production of vinegar, a staple in culinary and preservation processes.

Butyric Acid Fermentation: Some bacteria, such as *Clostridium butyricum*, are involved in the production of butyric acid, which has applications in both the food industry and in the production of certain biofuels.

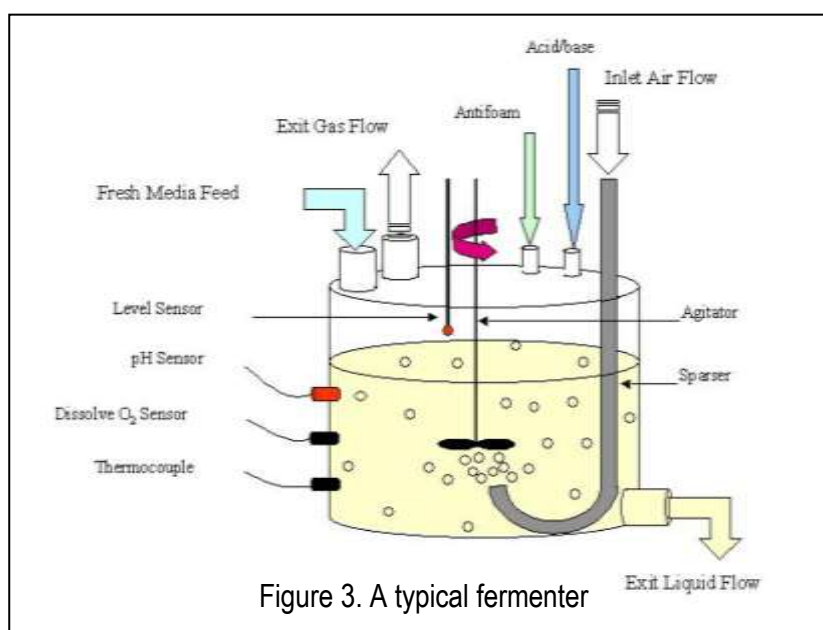
Methods of Fermentation

Submerged Fermentation

Submerged fermentation is the cultivation of microorganisms in liquid nutrient broth. Industrial enzymes can be produced using this process. This involves growing carefully selected microorganisms (bacteria and fungi) in closed vessels (Figure 3) containing a rich broth of nutrients (the fermentation medium) and a high concentration of oxygen. As the microorganisms break down the nutrients, they release the desired enzymes into solution (Renge et al., 2012).

Due to the development of large-scale fermentation technologies, the production of microbial enzymes accounts for a significant proportion of the biotechnology industries total output. Fermentation takes place in large vessels (fermenter) with volumes of up to 1,000 cubic metres. The fermentation media sterilizes nutrients based on renewable raw materials like maize, sugars and soya. Most industrial enzymes are secreted by microorganisms into the fermentation medium in order to break down the carbon and nitrogen sources. Batchfed and continuous fermentation processes are common. In the batch-fed process, sterilized nutrients are added to the fermenter during the growth of the biomass. In the continuous process, sterilized liquid nutrients are fed into the fermenter at the same flow rate as the fermentation broth leaving the system. This will achieve a steadystate production. Parameters like temperature, pH, oxygen consumption and carbon dioxide formation are measured and controlled to optimize the fermentation process. Firstly, in harvesting enzymes from the fermentation medium one must remove insoluble products, e.g. microbial cells. This is normally done by centrifugation. As most industrial enzymes are extracellular (secreted by cells into the external environment), they remain in the fermented broth after the biomass has been removed. The biomass can be recycled as a fertilizer, but first it must be treated with

lime to inactivate the microorganisms and stabilize it during storage. The enzymes in the remaining broth are then concentrated by evaporation, membrane filtration or crystallization depending on their intended application. If pure enzyme preparations are required, they are usually isolated by gel or ion exchange chromatography. Certain applications require solid enzyme products, so the crude powder enzymes are made into granules to make them more convenient to use. Sometimes liquid formulations are preferred because they are easier to handle and dose along with other liquid ingredients. Enzymes used in starch conversion to convert glucose into fructose are immobilized, typically on the surfaces of inert granules held in reaction columns or towers. This is carried out to prolong their working life as these enzymes normally go on working for over a year (Renge et al., 2012).



Solid State Fermentation

Solid-state fermentation (SSF) is another method used for the production of enzymes. Solid-state fermentation involves the cultivation of microorganisms on a solid substrate, such as grains, rice and wheat bran, bagasse, and paper pulp (Renge et al., 2012; Subramaniyam and Vimala, 2012). This method is an alternative to the production of enzymes in liquid by submerged fermentation. SSF has many advantages over submerged fermentation. These include, high volumetric productivity, relatively high concentration of product, less effluent generated and simple fermentation equipment. There are many substrates that can be utilized for the production of enzymes by SSF.

These include wheat bran, rice bran, sugar beet pulp and wheat and corn flour. The selection of substrate depends on many factors, which is mainly related to the cost and the availability of the substrate. Other factors include particle size and the level of moisture. Smaller substrate particles have a larger surface area for the proliferation of the microorganisms, but if too small the efficiency of respiration will be impeded and poor growth and hence poor production of enzymes will result. Larger particles provide more efficient aeration and respiration, but there is a reduction in the surface area (Renge et al., 2012). A compromise must be reached, regarding the particle size of the substrate for a particular process. SSF requires moisture to be present on the substrate, for the microorganisms to produce enzymes. As a consequence, the water content of the substrate must also be optimized, as a higher or lower presence of water may adversely affect the microbial activity. Water also has implications for the physicochemical properties of the solid substrate. Enzymes of industrial importance have been produced by SSF. Some examples are proteases, pectinases, glucoamylases and cellulases (Renge et al., 2012; Suganthi et al., 2011).

Types of Fermentation Process

Fermentation in liquid media is of two types depending upon the mode of operation:

Batch Fermentation

Batch reactors are simplest type of mode of reactor operation. In this mode, the reactor is filled with medium and the fermentation is allowed to proceed. When the fermentation has finished the contents are emptied for downstream processing. The reactor is then cleaned, re-filled, re-inoculated and the fermentation process starts again (Renge et al., 2012).

Continuous Fermentation

Continuous reactors: Fresh media is continuously added and bioreactor fluid is continuously removed. As a result, cells continuously receive fresh medium and products and waste products and cells are continuously removed for processing. The reactor can thus be operated for long periods of time without having to be shut down. Continuous reactors can be many times more productive than batch reactors. This is partly due to the fact that the reactor does not have to be shut down as regularly and also due to the fact that the growth rate of the bacteria in the reactor can be more

easily controlled and optimized. In addition, cells can also be immobilized in continuous reactors, to prevent their removal and thus further increase the productivity of these reactors. Continuous reactors are as yet not widely used in industry but do find major application in wastewater treatment. Fed batch reactor is the most common type of reactor used in industry. In this reactor, fresh media is continuous or sometimes periodically added to the bioreactor but unlike a continuous reactor, there is no continuous removal. The fermenter is emptied or partially emptied when reactor is full or fermentation is finished. As with the continuous reactor, it is possible to achieve high productivities due to the fact that the growth rate of the cells can be optimized by controlling the flow rate of the feed entering the reactor (Renge et al., 2012).

5.6 Production of biofertilizers

Biofertilizers are biologically active organic products containing specific living micro-organism (bacteria, algae or fungi) in cultured form which are isolated either from roots of certain plant species or from root zone soil of some specific plants. These biofertilizers improve the nutrient level and quality of soils that facilitate the plants to absorb the essential nutrients. Hence, the soil becomes more healthy and helps the budding seeds and roots to grow to their full potential. Biofertilizers work by activating the soil microorganisms, thus restore the soils' natural nutrients and protect it against diseases and other harsh conditions, and promotes the growth of plants. When Biofertilisers are applied to plant, it colonizes the rhizosphere in root and help promotes growth of plant by augmenting the supply of nutrients to the plant (Figure 4). Hence, biofertilizers enhances nutrients uptake through the natural processes of N_2 fixation, phosphorus solubilizing, and stimulating the plant growth (Raja N, 2013).

Production of Biogas

Biofertilisers work by fixing atmospheric N_2 in root nodules of legume crop or in the soil and make it available to the plants and it solubilize the insoluble forms of phosphates into readily available forms. It also produce hormones and antimetabolites which enhances root development, decompose the organic matter and that help in soil mineralization. They supply the plants with high amount of nitrogen and phosphorus in the readily available forms. In the contrary the chemical fertilizers deteriorate the environment and leads to harmful impacts on living beings. The microorganisms that

are present in these bio-fertilizers also reduce the stress in plants by competing with the plant pathogens and suppressing them (Araujo., 2008). However, the quality and productivity is a major factor in their limited acceptance by the local farmers (Figure 5). This limitations are primarily linked to strains selection and efficient biofertilizers development and production technology.

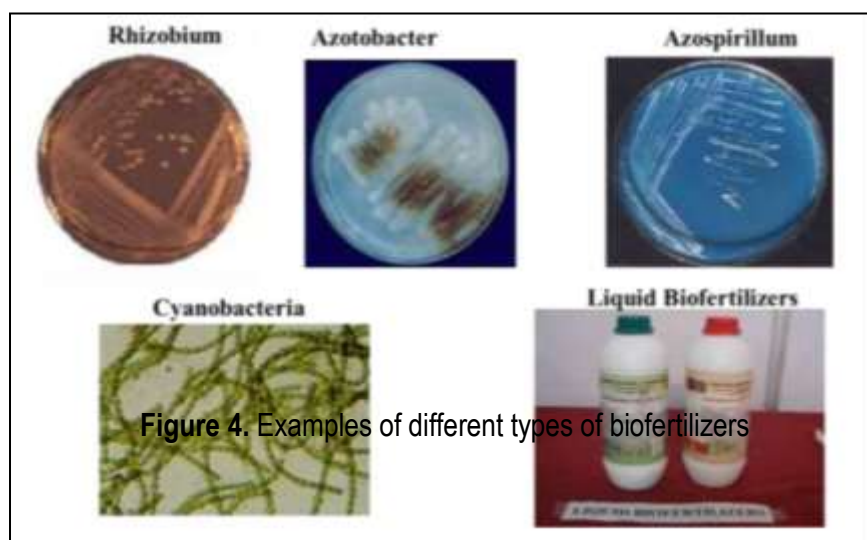


Figure 4. Examples of different types of biofertilizers

(Source: http://agritech.tnau.ac.in/org_farm/orgfarm_biofertilizers.html)

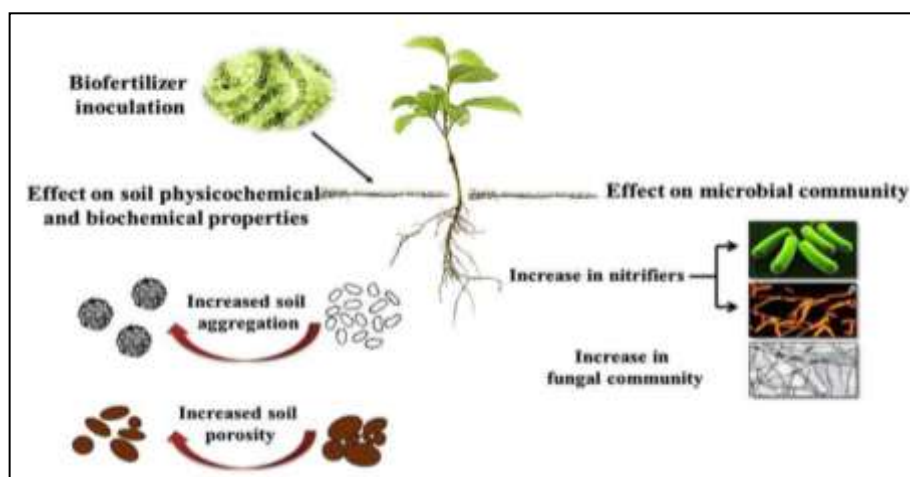


Figure 5: Effects of biofertilizers on physiological and biochemical properties of soil.

(Source: https://www.researchgate.net/profile/A_Chatterjee/publication/316700544/figure/fig1/AS:49)

5.7 Biogas

Biogas is a renewable energy source produced by the breakdown of organic matter by certain bacteria under anaerobic conditions. Biogas is the mixture of gases (about 60%

methane) produced by anaerobic bacterial digestion of organic matter. Biogas consists mainly of methane (CH_4 ; ca. 60%) and CO_2 (ca. 40%) plus traces of hydrogen (H_2) and several other gases. It is a mixture of methane, hydrogen, and carbon dioxide. It can be produced by agricultural waste, food waste, animal dung, manure, and sewage. It is produced by anaerobic degradation of a variety of organic materials, ranging from simple sugars to polymers like cellulose and nucleic acids by a community of organisms. Biogas recycles the waste products naturally and converts them into useful energy, thereby, preventing any pollution caused by the waste in the landfills, and cutting down the effect of the toxic chemicals released from the sewage treatment plants. Biogas converts the harmful methane gas produced during decomposition, into less harmful carbon dioxide gas. The organic material decomposes only in a wet environment. The organic matter or the waste dissolves in water and forms a sludge which is rich in nutrients and used as a fertilizer. It is produced by anaerobic degradation of a variety of organic materials, ranging from simple sugars to polymers like cellulose and nucleic acids by a community of organisms. Anaerobic digestion occurs in nature in the sediments of lakes and ponds, and in rumen of cattle; it is carried out by man in a variety of reactors called digesters and even in land-fill sites under non-septic conditions. A land-fill site is a location at which a pit was filled with, usually, domestic refuse, covered with soil and, generally, landscaped.

Uses of Biogas:

- (i) Direct combustion,
- (ii) Generation of electricity and
- (iii) Purification and export as compressed gas or in liquid form.

Use of gas for direct combustion close to the site of production is the most economic. It may be used to heat green-houses, boilers, furnaces, fire brick or cement kilns, for household work like cooking etc.

Alternatively, electricity may be generated by using modified internal combustion engines or gas turbines. The main problems arise due to the contaminating gases present in traces which are corrosive to the metal parts of engines; this problem can be partially overcome by cleaning of the biogas and by a suitable modification of the engines and engine oils. Biogas is used for electricity generation in USA and UK by

utilizing the gas generated at landfill sites. Lastly, the gas may be purified (pure CH₄) and exported via a pipeline as compressed gas or liquid.

5.8 Bioethanol and biopolymers

Biofuels made from biomass are attracting increasing interest worldwide. Biofuels have the potential to fulfill the energy needs and contribution in energy security, diversifying energy sources, and mitigation of GHG emissions from the transport sector and also in delivering rural economic development benefits (Winrock, 2009). The first generation (1G) has specific limitation and feedstocks used for biofuels especially food grains, sugars, edible oil cannot be diverted entirely for biofuels production. However, second (2G) and third-generation (3G) biofuel production technologies have enormous potential, because they are produced from biomass feedstock (residues and waste materials) and can be converted into varieties of biofuels such as bioethanol, Bio-DME, Fischer-tropsch diesel, biodiesel, mixed alcohols, bio-oil and biohydrogen, syngas and biogas, etc.

Bioethanol is considered a prospective substitute for fossil energy because of its clean-burning characteristics and high octane value (Prasad et al., 2007). Currently, ethanol has been promoted as an alternative automotive fuel, because of its anti-knocking properties which help to increase octane ratings and improve fuel efficiency (Prasad et al., 2014). Also, the use of biomassbased transportation biofuels can help in reducing CO₂ build up by recycling CO₂ that is liberated when ethanol is combusted as fuel (Hasunuma and Kondo, 2012). The ethanol yields per acre from various feedstocks at several locations is presented in Table 6.

Table 6. Ethanol yields by feedstocks gallons per acre

Feedstocks	Ethanol Yield (gallons per acre)
Maize (US)	401
Maize (China)	213
Sugar Cane (Brazil)	585
Sugar Cane (India)	483
Cassava (Nigeria)	158
Cellulosic Switchgrass	1150

Source: CSS, 2018

Global production peaked in 2017 after a dip in 2011 and 2012. The United States (US) is the world's largest producer of ethanol, has produced nearly 16 billion gallons in 2017 alone. Together, the U.S. and Brazil produce 85% of the world's ethanol in 2016 (CSS, 2018). The reason for the large volume of US production is, among others, the Renewable Fuel Standard (RFS). In force since 2005 and enhanced and extended in 2007, the standard requires transport fuels that are sold in the US to contain a specific, annually increasing percentage of renewable transport fuels. The second largest bioethanol-producing country is Brazil with an output of 7.06 billion gallons (Table 7). Brazil adopted its National Alcohol Programme in response to the oil crisis in the seventies to decrease the country's dependence on fossil-energy imports. In the EU-28, the directive on the promotion of the use of biofuels or other renewable fuels for transport (28/30/EG) and the Energy Tax Directive (30/96/EG) created the framework conditions for European biodiesel and bioethanol production in 2003 (AFDC, 2019).

Table 7. Countries share in Global Ethanol Production

Country/Region	Billion Gallons	Country Share (%)
United States	15.80	58%
Brazil	7.06	26%
Europe	1.42	5%
China	0.88	3%
Rest of World	1.90	8%

Source: CSS, 2018

5.9 Food Industry

Biotechnology has made a significant impact on the food sector by improving the quality, safety, and sustainability of food production. One of the key advancements is the development of functional foods that provide health benefits beyond basic nutrition, such as probiotic-rich products that support digestive health and foods fortified with vitamins and minerals to reduce nutritional deficiencies. Genetically modified crops (GM) are another important contribution, offering resistance to pests and diseases, tolerance to harsh environmental conditions, and reduced dependence on chemical pesticides, which helps increase agricultural productivity and sustainability.

Biotechnology has also introduced innovative methods of food preservation using enzymes and microorganisms to naturally extend shelf life without artificial preservatives. Additionally, modern biotechnology supports alternative food production, including lab-grown meat and plant-based substitutes, which aim to address environmental concerns and meet the growing demand for sustainable protein sources. Overall, biotechnology plays a vital role in ensuring food security and promoting healthier, more sustainable diets.

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Unit 6. Biotechnological applications in Forestry and Environment

Unit Structure

6.0. Learning Objectives

6.1 Introduction

6.2. Applications in Agriculture

6.2.1. Development of New Hybrids

6.2.2. Synthetic or Encapsulated Seeds

6.2.3. Production of Disease-Free and Disease-Resistant Plants

6.2.4. Producing Stress-Resistant Plants (Herbicide/Stress Tolerance)

6.2.5. Transfer of Nitrogen-Fixing Genes (nif Genes)

6.3. Applications in Forestry

6.3.1 Micropropagation (Tissue Culture Propagation)

6.3.2. In Vitro Mycorrhiza Establishment

6.3.3. Somatic Embryogenesis and Artificial Seeds

6.3.4. Genetic Transformation of Forest Trees

6.3.5. Conservation of Forest Genetic Resources

6.4. Industrial Applications: Production of Secondary Metabolites

6.5. Biotechnology for Environmental Management

6.5.1 Bioremediation

6.5.2. Biofertilizers and Mycorrhizae for Sustainable Forestry

6.6. Waste Management Using Biotechnology

6.7. Biotechnology for Climate Change Mitigation

6.8. Applications in Forest Health and Disease Management

6.9. Environmental Biotechnology in Biodiversity Conservation

6.10. Industrial and Commercial Applications Related to Forestry

6.0. Learning Objectives

After studied this unit, you will be able:

- To have basic understanding of about biotechnological applications in forestry and environment
- To understand how biotechnology applied for agriculture, waste management and biodiversity conservation.

6.1 Introduction

Forests are indispensable for ecological balance, biodiversity conservation, climate regulation, and sustainable development. However, rapid deforestation, climate change,

pests, and industrial pressures threaten forest ecosystems worldwide. Modern biotechnology offers powerful tools to conserve, regenerate, and sustainably manage forests while mitigating environmental challenges. This chapter highlights how plant biotechnology, molecular biology, microbial biotechnology, and environmental engineering are applied to forestry and environmental management.

In the last few decades, plant cell, tissue, and protoplast cultures have emerged as powerful tools for **crop improvement, forestry, natural product production**, and other fields of biotechnology. As early as 1887, Swaminathan highlighted how cultured plant cells could transform agriculture and forestry by:

Harnessing **totipotency**—the ability of a single plant cell to regenerate into a whole plant.

Using **cell and protoplast cultures** with DNA vectors to overcome barriers to sexual hybridization.

- Producing valuable **phytochemicals** in cell culture.
- Enhancing and extending **biological nitrogen fixation**.
- Transferring **nitrogen-fixing (nif) genes** to non-fixing plants.

Because these techniques let scientists “play” with plant tissues under controlled conditions, some researchers even call plant tissue culture a “**botanical laser**”—precise, powerful, and still full of unexplored potential.

6.2. Applications in Agriculture

6.2.1. Development of New Hybrids

Cell fusion and hybridization have made it possible to bypass sexual incompatibility between species. For example:

- **Kuchko (1985)** produced a somatic hybrid between wild and cultivated potato species (*Solanum tuberosum* × *S. chacoense*).
- The hybrid combined desirable traits such as better yield, prolonged flowering, virus resistance, and improved tuber quality.

Anther Culture in Plant Breeding

China has successfully developed **50 rice** and **20 wheat varieties** through anther culture (Hu, 1985).

Advantages include:

- Rapid production of **homozygous lines**
- Generation of **gametoclonal variation**
- Early expression of **recessive traits**

Protoplast and cell culture systems are now also used to introduce foreign genes through fusion, transformation, and organelle transfer.

6.2.2. Synthetic or Encapsulated Seeds

Using *somatic embryogenesis* and *organogenesis*, embryos can be coated to form artificial seeds. Kitto and Janick (1985) pioneered this by coating citrus and carrot embryos with a polyethylene oxide polymer.

Hardening treatments—like higher sucrose, chilling, high inoculum density, and ABA—improved the survival of synthetic seeds. Similar success has been reported in Eucalyptus. Artificial seeds promise low-cost propagation and easier storage and handling.

6.2.3. Production of Disease-Free and Disease-Resistant Plants

Many vegetatively propagated crops accumulate viruses, bacteria, fungi, and nematodes over generations. Tissue culture solves this by allowing:

- Elimination of pathogens
- Rapid indexing of disease resistance
- Controlled pathogen–host studies

Virus-Free Plants

Morel and Martin (1952) first obtained virus-free plants using shoot meristem culture. Since then, grapes, peas, clover, citrus, and many grasses have been cleaned of systemic infections.

Selection for Disease Resistance

By exposing cells or protoplasts to toxins or pathogens, resistant lines can be selected and regenerated. Examples:

- Potato protoplast-derived clones resistant to *Phytophthora infestans* (late blight)
- Tobacco cells resistant to chlorosis-inducing toxins
- Callus cultures showing resistance to fungal filtrates

These in vitro techniques allow screening millions of cells—equivalent to thousands of field acres—in a single flask.

6.2.4. Producing Stress-Resistant Plants (Herbicide/Stress Tolerance)

Cells can be selected for herbicide tolerance by growing them in gradually increasing concentrations of the chemical.

- Zenk (1974) selected 2,4-D-tolerant cells.
- Chaleff and Parsons (1978) regenerated tobacco plants resistant to picloram.
- Protoplasts also allow potential transfer of cytoplasmic factors and chloroplasts involved in herbicide resistance.

6.2.5. Transfer of Nitrogen-Fixing Genes (nif Genes)

Nitrogen fixation naturally occurs only in prokaryotes. A major research goal has been transferring this ability into crops.

Breakthrough studies:

- Holsten et al. (1971) showed *Rhizobium japonicum* could infect soybean root cell cultures without nodules.
- Child and LaRue (1974) observed functional nitrogenase activity in soybean callus infected with rhizobia.
- Long-term goals include enabling non-legumes like wheat or sugarcane to fix their own nitrogen.

A major future goal is modifying Rubisco to reduce photorespiration, which wastes up to 50% of biomass in C3 plants. This could convert C3 plants into C4-like high-efficiency plants, potentially boosting global food production.

6.3. Applications in Forestry

6.3.1 Micropropagation (Tissue Culture Propagation)

Micropropagation—clonal propagation of plants through tissue culture—has become one of the most important applications in forestry. Micropropagation is preferred to conventional methods because:

- A tiny piece of tissue can yield **millions of clones annually**
- Pathogen-free plants can be produced
- Germplasm can be conserved
- Propagation is **season-independent**
- Safe international exchange of plant material becomes possible

Examples of Trees Successfully Micro propagated

- *Eucalyptus* spp.
- *Tectonagrandis* (teak)
- *Dalbergiasissoo* (rosewood)
- *Pinus* spp.
- *Bamboo* and *Dendrocalamus* spp.
- *Azadirachta indica* (neem)

Micropropagation has become foundational for clonal forestry programs in India, Brazil, Japan, and several European countries. Plants must be gradually transitioned from in vitro conditions to greenhouse and field environments.

Advantages

1. Rapid multiplication of elite, high-yielding, or rare tree genotypes

2. Production of disease-free planting material
3. Year-round propagation irrespective of seasons
4. Conservation of valuable and endangered germplasm

6.3.2. In Vitro Mycorrhiza Establishment

- Mycorrhizae rarely grow on artificial media, so researchers attempt to culture them using host root tissues.
- Kiernan et al. (1984) successfully produced strawberry plants infected with *Glomus* species via tissue culture.
- Some systems require a **three-member symbiosis** (plant + fungus + bacterium), as reported by Mosse (1962).
- These systems can help mass-produce mycorrhizal inoculum for forestry and horticulture.

6.3.3. Somatic Embryogenesis and Artificial Seeds

Somatic embryogenesis allows development of embryos from somatic (non-sexual) cells. These embryos can be encapsulated to produce **synthetic/artificial seeds**.

Benefits to Forestry

- Easy transport of elite genotypes as synthetic seeds
- Long-term storage and exchange
- Large-scale plantation programmes
- Possibility of mechanized sowing
- Examples: *Eucalyptus*, *Picea*, *Pinus*, *Coffea*, *Citrus*.

6.3.4. Genetic Transformation of Forest Trees

Modern genetic engineering enables the introduction of desirable traits into forest species:

Important Traits Introduced

- **Insect resistance** (e.g., Bt genes in poplars)

- **Disease resistance** (against fungal pathogens)
- **Herbicide tolerance**
- **Improved lignin content** for better pulping
- **Salt and drought tolerance** for climate-stressed areas
- **Enhanced growth rate and biomass production**

Significance

1. Writable, genetically improved trees can:
2. Withstand environmental stresses
3. Reduce dependency on chemical pesticides
4. Enhance carbon sequestration
5. Improve wood quality for furniture and paper industries

6.3.5. Conservation of Forest Genetic Resources

Biotechnology provides advanced tools for conservation of rare, endangered, and economically important species.

Techniques Used

1. **Cryopreservation** of seeds, embryos, and shoot tips
2. **In vitro germplasm banks**
3. **DNA fingerprinting** to identify and preserve genetic diversity
4. **Marker-assisted selection (MAS)** in forest breeding

These methods are essential for long-term sustainability and biodiversity protection.

6.4. Industrial Applications: Production of Secondary Metabolites

Plant cell cultures are increasingly used to produce pharmaceuticals, flavors, pigments, fragrances, and nutraceuticals. Although secondary metabolites often occur in low amounts,

techniques like clonal selection, optimized media, and two-stage culture systems have dramatically improved yields.

Examples of compounds produced in vitro:

Plant Species	Compound
<i>Catharanthus roseus</i>	Serpentine
<i>Daucus carota</i>	Anthocyanins
<i>Lithospermum erythrorhizon</i>	Shikonin
<i>Mentha</i> spp.	Menthol and terpenes
<i>Morinda citrifolia</i>	Anthraquinones
<i>Nicotiana</i> spp.	Nicotine and related alkaloids
<i>Panax ginseng</i>	Saponins

Commercial examples include:

- Anthocyanin production by Canadian and Israeli biotech firms
- Shikonin production by Mitsui Petrochemical, Japan

Secondary metabolites such as alkaloids, glycosides, tannins, terpenoids, and phenolics have major uses in:

- Medicine
- Perfumery
- Food flavoring
- Cosmetics

6.5. Biotechnology for Environmental Management

6.5.1 Bioremediation

Bioremediation uses microorganisms or plants to detoxify polluted environments.

Types

- Microbial bioremediation: Bacteria degrade pollutants like oil spills, industrial effluents, pesticides, and plastics.

- Phyto-remediation: Plants absorb and detoxify heavy metals, radioactive elements, or organic pollutants.
- Myco-remediation: Fungi break down toxic compounds, petroleum products, and dyes.

Common Plants Used in Phytoremediation

- *Brassica juncea* (Indian mustard) – heavy metals
- *Populus* spp. (poplar) – groundwater pollutants
- *Vetiveria zizanioides* – soil remediation
- *Pteris vittata* – arsenic hyper-accumulator

6.5.2. Biofertilizers and Mycorrhizae for Sustainable Forestry

Beneficial microbes improve soil fertility and plant growth, reducing the need for chemical fertilizers.

Important Microbial Groups

- **Rhizobium** – nitrogen fixation
- **Azospirillum, Azotobacter** – free-living nitrogen fixers
- **Phosphate-solubilizing bacteria**
- **Mycorrhizal fungi** – enhance water and nutrient uptake

Applications in Forestry

- Establishment of seedlings in degraded lands
- Improvement of survival rates in plantations
- Restoration of nutrient-poor or eroded soils

6.6. Waste Management Using Biotechnology

Biofilters

Microbial biofilters degrade industrial gases and organic vapors.

Wastewater Treatment

Activated sludge systems using microbes clean municipal and industrial wastewater.

Biodegradation of Plastics

Bioplastic-producing microbes and enzymes like PETase help reduce plastic accumulation in the environment.

6.7. Biotechnology for Climate Change Mitigation

Forests play a key role in reducing atmospheric carbon dioxide. Biotechnology enhances this role through:

a) Genetically Improved Carbon-Capturing Trees

Trees with:

- Higher photosynthetic efficiency
- Improved growth rate
- Superior biomass accumulation

b) Monitoring and Assessment Using Molecular Tools

- DNA barcoding for species identification
- Molecular markers for stress-resistant genotypes
- Remote sensing integrated with bioinformatics to monitor forest health

6.8. Applications in Forest Health and Disease Management

Biotechnology enables early detection, containment, and control of forest diseases:

- **PCR-based diagnostics** detect fungal or bacterial pathogens rapidly.
- **Tissue culture** produces disease-free planting material.
- **Genetic engineering** develops resistant lines (e.g., chestnut blight resistance).
- **Protoplast fusion** creates hybrids with wide resistance traits.

These tools strengthen forest productivity and stability.

6.9. Environmental Biotechnology in Biodiversity Conservation

Biotechnology directly supports biodiversity through:

DNA Fingerprinting & Genomic Databases

Used to:

- Identify threatened species
- Prevent illegal logging and wildlife trafficking
- Maintain genetic purity in breeding programs

In Vitro Conservation

- Tissue culture preserves:
- Endangered orchids
- Medicinal plants
- Rare forest species with recalcitrant seeds
- Reintroduction Programs

Plants regenerated in vitro can be reintroduced into natural habitats to restore degraded forests.

6.10. Industrial and Commercial Applications Related to Forestry

Production of Valuable Metabolites

Plant cell cultures produce:

- Essential oils
- Flavors and fragrances
- Pigments like anthocyanins
- Medicinal compounds like taxol

Improved Pulp and Paper Processing

Genetically engineered trees with modified lignin reduce:

- Chemical use
- Energy consumption
- Environmental pollution

Conclusion

Biotechnological applications in forestry and environmental management have significantly transformed how forests are conserved, restored, and utilized. Modern biotechnology serves as a "botanical laser," utilizing the principle of cellular totipotency to transform forestry, agriculture, and environmental conservation. Through advanced propagation techniques like micropropagation and somatic embryogenesis, scientists can rapidly produce millions of disease-free, high-yielding clones and synthetic seeds, bypassing traditional seasonal limitations and sexual incompatibility. Beyond mere multiplication, these tools allow for the development of stress-resistant hybrids, the transfer of nitrogen-fixing genes to non-legumes, and the genetic modification of forest trees to improve biomass and wood quality. Furthermore, biotechnology addresses critical ecological challenges through bioremediation—using microbes and plants to detoxify pollutants—and climate change mitigation by engineering trees with superior carbon-capturing capabilities. By integrating molecular diagnostics and in vitro conservation, these technologies ensure the protection of biodiversity and the sustainable industrial production of valuable secondary metabolites like pharmaceuticals and fragrances.

References:

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- **Child and LaRue (1974):** Observed nitrogenase activity in soybean callus infected with rhizobia.
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- **Hu (1985):** Reported on China's success in developing rice and wheat varieties through anther culture.
- **Kiernan et al. (1984):** Produced strawberry plants infected with *Glomus* species via tissue culture.

- **Kitto and Janick (1985):** Pioneered the coating of citrus and carrot embryos with polyethylene oxide to create synthetic seeds.
- **Kuchko (1985):** Produced a somatic hybrid between wild and cultivated potato species.
- **Morel and Martin (1952):** First obtained virus-free plants using shoot meristem culture.
- **Mosse (1962):** Reported on three-member symbioses involving plants, fungi, and bacteria.
- **Swaminathan (1887):** Early highlight of how cultured plant cells could transform agriculture and forestry.
- **Zenk (1974):** Selected cells tolerant to the herbicide 2,4-D.

Unit 7: Biofertilizers

Unit Structure

7.0 Learning Objectives

7.1 Introduction

7.2 Biofertilizer

7.3 Types of biofertilizer

7.3.1 Nitrogen Fixing Biofertilizers

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7.3.1.2 Blue Green Algae (BGA)

7.3.1.3 Azospirillum

7.3.1.4 Azotobacter

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7.3.1.6 Frankia

7.3.2 Phosphate-Solubilizing Biofertilizers (PSB)

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7.4 Mechanisms of Biofertilizer

7.5 Biofertilizer Inoculation

7.6 Advantages of Biofertilizers

7.7 Biopesticides

7.8 Types of biopesticides

7.9 Advantages of biopesticide

Summary

References

7.0 Learning Objectives

After completion of this unit, you will be able to:

- understand the concept and importance of biofertilizer;
- discuss different types of biofertilizer;
- understand the concept and importance of mycorrhiza;
- understand bio-pesticide and its types;

7.1 Introduction

Biofertilizer plays an important role in improving the nutrient supplies and their availability in crop husbandry. These beneficial microbes, such as nitrogen-fixing bacteria, phosphate-solubilizing bacteria, and mycorrhizal fungi, enhance the availability of essential nutrients to plants through biological processes. Unlike chemical fertilizers, biofertilizers are eco-friendly,

cost-effective, and help maintain long-term soil health. They play a vital role in sustainable agriculture by increasing crop productivity while reducing harmful environmental impacts.

7.2 Biofertilizer

Biofertilizers are biologically active organic products containing specific living micro-organism (bacteria, algae or fungi) in cultured form which are isolated either from roots of certain plant species or from root zone soil of some specific plants. These biofertilizers improve the nutrient level and quality of soils that facilitate the plants to absorb the essential nutrients. Hence, the soil becomes healthier and helps the budding seeds and roots to grow to their full potential. Biofertilizers work by activating the soil microorganisms, thus restore the soils' natural nutrients and protect it against diseases and other harsh conditions, and promotes the growth of plants. When biofertilizers are applied to plant, it colonizes the rhizosphere in root and help promotes growth of plant by augmenting the supply of nutrients to the plant. Hence, biofertilizers enhances nutrients uptake through the natural processes of N_2 fixation, phosphorus solubilizing, and stimulating the plant growth.

Additionally, biopesticides, or also called as biological pesticides, are natural pest controlling agents that are isolated from natural sources. Using biopesticides has advantages over using conventional pesticides, since biopesticides are less harmful to the environment (Raja, 2013). Among all the biofertilizer, Rhizobium is the maximum researched bio-fertilizer. The nutrients fixed by the soil microbes are more effective than outside application. It has been estimated that nearly 80,000 tonnes of nitrogen is available over an area of one hectare land (as we know that atmospheric air contains 78% nitrogen). The biofertilizers trap some amount of this nitrogen and fix in the soil which benefits the plant. Since biofertilizer consist of many beneficial microbes, the nutrients availability in soil is improved after their application due to many dimensions.

7.3 Types of biofertilizer

There are different types of biofertilizer such as Nitrogen Fixing Biofertilizers, Phosphate-Solubilizing Biofertilizers (PSB), Potassium-Solubilizing Biofertilizers (KSB), Sulphur-Oxidizing Biofertilizers, Zinc-Solubilizing Biofertilizers, Plant Growth Promoting

Rhizobacteria and Compost and Organic Matter Bio-inoculants. All these biofertilizer are discussed below:

7.3.1 Nitrogen Fixing Biofertilizers

The nitrogen fixing bacteria work under two conditions, symbiotically and as free living bacteria (non-symbiotic). The symbiotic bacteria make an association with crop plants through forming nodules in their roots. The free-living bacteria do not form any association but live freely and fix atmospheric nitrogen. These contain microorganisms that fix atmospheric nitrogen and make it available to plants.

7.3.1.1 Rhizobium

This is the most common biofertilizer as stated earlier. Rhizobium lives in the root hairs of the legumes by forming nodules. First time, Beijerinck from Holland isolated this bacterium from nodules of a legume in 1888. Later on, the bacterium was reported in Bergey's Manual of Determinative Bacteriology under the genus Rhizobium. The name Rhizobium was established by Frank in 1889. This genus has seven distinct species based on "Cross Inoculation Group Concept". A new classification has been established for Rhizobium. That is 'slow growing rhizobia' known as Bradyrhizobium and the other group is 'fast growing rhizobia' called Rhizobium. This is called "the principle of cross inoculation" which relies on the assumption that legumes within a particular infection group may be nodulated by another species of nodule forming bacteria.

7.3.1.2 Blue Green Algae (BGA)

This is another important class of biofertilizer. The Blue-green algae are small organisms and can be seen under the microscope as a single cell or large accumulation of cells (colonies) or strings of cells (trichomes). Some accumulations may be so large that they are easily seen with the naked eye. Blue- green algae are also known by different nomenclature such as, cyanophytes, cyanobacteria and most recently cyanoprokaryotes. They have a similar external appearance to that of algae and their requirements for light, nutrients and carbon dioxide are also similar. Certain types of blue-green algae have tiny gas vesicles in their cells, which regulate them to float to the water surface or sink to the bottom in response to the changing of light and nutrient availability.

The Blue-Green Alga (*Anabaena azollae*) forms a symbiotic relationship with Azolla (aquatic fern) and fixes atmospheric nitrogen. BGA is associated with the Azolla occurring in a ventral pore in the dorsal lobe of each vegetative leaf. The endophyte fixes atmospheric nitrogen and resides inside the tissue of the water fern. Individually, BGA and Azolla can also be used in paddy fields. BGA are capable of performing photosynthetic activity as well as fix the atmospheric nitrogen in flooded rice ecosystem. Azolla is a fast-growing water fern and can double its weight within a week. Azolla is rich organic manure also. It mineralizes the soil nitrogen rapidly which is made available to the crop in a very short period.

7.3.1.3 Azospirillum

This is a free living or non-symbiotic bacteria (does not form nodules but makes association by living in the rhizosphere). Azospirillum species establish an association with many plants particularly with C₃ plants such as maize, sorghum, sugarcane, etc. It is the most common organism and can form associative symbiosis on a large variety of plants. Beijerinck in 1925 reported a nitrogen fixing bacterium under the name of *Spirillum lipoferum*. Tarrand et al., (1978) later on renamed this organism as *Azospirillum* (Nitrogen fixing *Spirillum*). *Azospirillum* is recognized as a dominant soil microbe. *Azospirillum* also forms a close associative symbiosis with the higher plants. The bacteria live on root surface, sometimes also penetrates into the root tissues but do not produce any visible nodule or out growth on the root tissue. They fix nitrogen from 10 to 40 kg/ha. The *Azospirillum* inoculation helps better vegetative growth of the plants, saving nitrogenous fertilizers by 25-30%. So far only four species of *Azospirillum* have been identified. They are *A. lipoferum*, *A. brasilense*, *A. amazonense*, *A. iraquense*. In Indian soils *A. brasilense* and *A. lipoferum* are very common.

7.3.1.4 Azotobacter

Azotobacter is a heterotrophic free living nitrogen fixing bacteria present in alkaline and neutral soils. *Azotobacter chroococcum* is the most commonly occurring species in arable soils of India. Apart from its ability to fix atmospheric nitrogen in soils, it can also synthesize growth promoting substances viz., auxins, and gibberellins and also to some extent the vitamins. Many strains of *Azotobacter* also exhibit fungicidal properties against certain species of fungus. Response of *Azotobacter* has been seen in rice, maize, cotton, sugarcane, pearl millet, vegetable and some plantation crops. Its population is very low in

uncultivated lands. Presence of organic matter in the soil promotes its multiplication and nitrogen fixing capacity.

7.3.1.5 Acetobactor

Acetobactor diazotrophicus is a newly discovered nitrogen fixing bacteria associated with sugarcane crop. This bacterium belongs to the alpha group of proteobacteria. It was isolated from leaf, root, bud and stem samples of sugarcane. *Acetobactor* is located in apoplastic fluid of sugarcane stem and to some extent in xylem vessels. It is an acid and high salt tolerant and sucrose loving bacteria which can fix up to 200 kg nitrogen per hectare. Under field condition, the yield of sugarcane increased after its inoculation. Productions of auxins and antibiotic type substance have also been noticed after its application.

7.3.1.6 Frankia

Frankia is actinomycetes which also fixes atmospheric nitrogen. It forms a symbiotic association by forming root nodules in some non-leguminous trees such as *Casuarina* and *Alnus*. The wasteland soil fertility can be improved by growing *Casuarina*. *Frankia* makes *casuarina* tree suitable for agro-forestry system in nitrogen deficient soils. In the beginning of nodulation, *Frankia* occurs as small lateral swelling on roots and then develops into new lobes at their apices and form cluster coralloid structure. Its inoculation enhances growth, nodulation, nitrogenase activity of nodule and nodule dry weight of *Casuarina* and *Alnus* plants.

7.3.2 Phosphate-Solubilizing Biofertilizers (PSB)

These microbes convert insoluble phosphates into forms available to plants. Only 15 to 20 per cent of applied phosphorus is recovered by the crops and remaining get fixed in the soil. The fixed form does not contribute to the available phosphorous content in the soil. A group of heterotrophic microorganisms solubilize this fixed phosphorous by producing organic acids and enzymes and make them available to the crops. This group of microorganisms is called Phosphorous Solubilising Microorganisms (PSM). The group includes various species of *Bacillus*, *Aspergillus*, *Penicillium* and *Trichoderma*. When applied at rock phosphate these organisms solubilize the fixed soil phosphorus and release the citrate and water-soluble phosphorus. The microorganisms also help mineralizing organic phosphate compounds present in the organic wastes. While composting, they can be used to hasten composting

process when thermophilic phase is over. The use of bacteria in neutral to alkaline and fungus in acid soils improve the efficacy of applied soil phosphorus. Also, the fixation of phosphorous is prevented. As we know, vesicular arbuscular mycorrhiza is a fungus which help in diverse ways to benefit the crop plants under natural conditions.

7.3.2.1 Vesicular Arbuscular Mycorrhiza (VAM)

The term mycorrhiza was taken from Greek language meaning 'fungus root'. This term was coined by Frank in 1885. Mycorrhiza is a mutualistic association between fungal mycelia and plant roots. VAM is an endotrophic (live inside) mycorrhiza formed by aseptated phycomycetous fungi. They are associated as an obligate symbiont with majority of crops growing under broad ecological range. Many leguminous and Graminae family plants are highly susceptible to VAM colonization. VAM help in nutrient transfer mainly of phosphorus, zinc and sulfur. They also mobilize different nutrients like Cu(copper), K(potassium), Al(aluminum), Mn(manganese), Fe (iron) and Mg (magnesium) from the soil to the plant roots. They penetrate into root cortex and form intracellular obligate fungal endo-symbiont. They possess vesicles (sac like structure) for storage of nutrients and arbuscular for funneling them into root system. Hyphae of VAM fungi do not solubilize the insoluble unavailable phosphorus but assimilate phosphorus and other nutrients from soil for their own requirement. In addition, help transfer them in different forms to the host roots. It also improves water absorption by the roots.

Functions of mycorrhiza: It enhances the feeding areas of the plant root as the hyphae spreads around the roots and mobilizes the nutrients from distance to root. It stores nutrients particularly phosphorus and removes the toxic chemicals like phenolics which otherwise hinder nutrient availability. Mycorrhizae provide protection against other fungi and nematodes.

Check your progress

1. What do you understand by the term Biofertilizers?
2. Briefly explain different types of biofertilizers.
3. Describe any 2 Nitrogen Fixing Biofertilizers?
4. Explain Phosphate-Solubilizing Biofertilizers.

5. Write a short note on Vesicular Arbuscular Mycorrhiza.
6. What are the different functions of a mycorrhizae.

7.4 Mechanisms of Biofertilizer

Biofertilizers function through a series of microbiological processes that enhance nutrient availability and promote plant growth. These microbial inoculants fix atmospheric nitrogen either within the root nodules of leguminous plants or freely in the soil, thereby converting inert N_2 into assimilable forms for plant uptake. They also solubilize insoluble phosphatic compounds into bioavailable phosphate ions through the production of organic acids and enzymatic activity. Additionally, many biofertilizers synthesize phytohormones and antimicrobial substances that stimulate root growth, accelerate organic matter decomposition, and contribute to soil nutrient mineralization. As a result, plants receive increased supplies of nitrogen and phosphorus in readily utilizable forms.

7.5 Biofertilizer Inoculation

Biofertilizer inoculation refers to the process of applying beneficial microorganisms to seeds, seedlings, or soil so that they can colonize the plant root zone and enhance nutrient availability. It ensures that the microbial cultures effectively reach and establish in the rhizosphere for maximum plant growth benefits. The biofertilizers can be inoculated on seeds as well as in the roots of different crop plants under ideal conditions. They can also be applied directly to the soil. The inoculation of biofertilizer can be done by different methods, these are discussed below:

Seed Inoculation (Seed Treatment): This is the most common practice of applying biofertilizers. In this method, the biofertilizers are mixed with 10 per cent solution of jaggary. The slurry is then poured over the seeds spread on a cemented floor and mixed properly in a way that a thin layer is formed around the seeds. The treated seeds should be dried in the shade overnight and then they should be used. Generally, 750 grams of biofertilizer is required to treat the legume seeds for one hectare area.

Root and Seedling treatment (Root Inoculation): The seedling roots of transplanted crops are treated for half an hour in the solution of biofertilizers before transplanting in the field. In this method, seedlings required for one acre are inoculated using 2-2.5 kg biofertilizers. For

this, in a bucket having adequate quantity of water is taken and biofertilizer is mixed properly. Roots of the seedlings are then dipped in this mixture so as to enable roots to get inoculum. These seedlings are then transplanted. This method has been found very much suitable for crops like Tomato, Rice, Onion, Cole Crops and flowers.

Soil Application: This method is mostly used for fruit crops, sugarcane, and other crops where localized application is needed. At the time of planting of fruit trees, 20 g of biofertilizer mixed with compost is to be added in the ring of one sapling. We may add same quantity of biofertilizer in the ring soil of the seedling after it has attained maturity. Sometime, the biofertilizers are also broadcasted in the soil but we may require four to ten times more bio fertilizers. Before broadcasting, the inoculants should be incubated with the desired amount of well decomposed granulated FYM for 24 hours. The FYM acts as food and adjuvant (carrier) for biofertilizers.

Self-Inoculation or Tubez Inoculation: This method is exclusively suitable for application of Azotobacter. In this method, 50 liters of water is taken in a drum and 4-5 kg of Azotobacter biofertilizer is added and mixed properly. Planting materials required for one acre of land are dipped in this mixture. Similarly, if we are treating the potato, then the tubers are dipped in the mixture and planting is done after drying the materials in the shade.

Importance of Biofertilizer Inoculation:

- A. Enhances nutrient fixation (N), solubilization (P, K, Zn).
- B. Improves root development and plant growth.
- C. Reduces the dependency on chemical fertilizers.
- D. Eco-friendly and cost-effective.
- E. Improves soil health and microbial diversity.

7.6 Advantages of Biofertilizers

The major advantages are it has longer shelf life and use to improve the soil profile and fertility, and to promote plant growth without any adverse effects on ecosystem. It increases the crop yields by 20-25 % and improves the soil structure by enhancing the aggregation of the soil particles, help in better water retention. It also improves the leaf water and turgor potential that maintain the stomatal functioning as well as transpiration, increasing root

length and development. Biofertilizers are formulated mainly from commonly available organic materials such as agricultural residues e.g. rice husks hence, it has low the input expenses and lower production costs in compare to the chemical fertilizers or pesticides. It helps in improvement and sustainability of agriculture by secreting growth factors, e.g. aquatic cyanobacteria provide natural growth hormone, protein, vitamins and minerals to the soil which help in growth of crop and yield. They have excellent buffering capabilities because of their organic matters contents that help in balancing of the pH of the soil. The organic materials and the acids that are found in the bio-fertilizers include humic and fulvic acids, fungi, and organic fertilizer nutrients. Humic acids are plant bio stimulants that can increase soil fertility, enhance microbial activity and reduce water evaporation. Ultimately, bio-fertilizers are environmentally friendly that not only help protect against the drought and diseases, but also prevent from damaging the natural sources, as well as cleansing the plant from precipitated chemical fertilizers. Often effective in very low concentration and decompose in shorter time in nature.

7.7 Biopesticides

Biopesticides are natural pest-control agents derived from microorganisms, plants, animals, or certain minerals. They are used to manage agricultural pests such as insects, fungi, weeds, and pathogens in an eco-friendly and sustainable manner. Unlike chemical pesticides, biopesticides are target-specific, biodegradable, and safer for humans, animals, and the environment. Biopesticides are derivatives obtained from natural materials such as plants, animals, and bacteria (Gupta *et al.*, 2010). These are widely used for controlling insects and disease-causing pathogens.

The biopesticides are categorized under microbial pesticides, plant-incorporated-protectants and biochemical pesticides, and are produced through various naturally occurring substances that control pests by non-toxic mechanisms. Biopesticides are generally inherently less toxic; affect only the target pest, it has highly effective in very small quantity and are easily biodegradable, thereby resulting in lower exposures and pollution problems. When used as a component of pest management strategies, it can greatly control major pest problems while keeping the crop yields high. Increasing demand for organic farming are expected to be the driving force for high global demand of biopesticides in future pest

controlling agents. Generally, bio-pesticides are considered to be less harmful than the synthetic pesticides because, as mentioned earlier, they are obtained from natural sources. These are effective in very small concentrations and often get decomposed very quickly in comparison to synthetic pesticides so as to avoid the associated pollution problems.

7.8 Types of biopesticides

There are different types of biopesticide available nowadays and each type can be used to utilize or target a specific group of pests. Such as specific types of fungi can kill certain types of unwanted weeds, while various groups of bacteria can kill different types of insect larvae, and mosquitoes. The different types of biopesticides are discussed below:

Biochemical pesticides: These are naturally occurring compounds that check the pest populations by non-toxic mechanisms. Since, it interferes with mating procedures or insect sex pheromones, as well as various plant extracts that attract insect pests to traps such as plant extracts, pheromones, and fatty acids, natural plant growth regulators (PGR). e.g. Avermectin, Pyrethrins, Spinosad from natural products.

Microbial pesticides: It consists of specific microorganisms e.g., bacteria, fungi, viruses as an active ingredient. Microbial pesticide is able to control many different kinds of pests, although each ingredient is specific for its target pests. e.g., specific fungi can control certain weeds and different fungi kill specific insects. *Bacillus thuringiensis* strains or Bt. strains produce a mix of proteins and that specifically kills certain species of insect larvae. Certain strains of *Bacillus* e.g. *Bacillus subtilis*, *B. pumilus*, *Pseudomonas* spp. and *Streptomyces* spp. increase crop yield and prevent plant diseases by competing with other plant pathogens. Certain viruses and fungi used as biopesticides are: Cydia pomonella granulosis virus (CpGV), cytoplasmic polyhedrosis virus and fungi include- *Aschersonia aleyrodis*, *Metarhizium anisopliae*.

Plant-Incorporated-Protectants (PIPs): These are the pesticidal molecules produced by plant from its genetic material that has been deliberately added to the plant. e.g. plant producing Bt toxin. In this case the Bt toxin or pesticidal protein coding gene can be introduced into the plant's genome. Then the plant, starts manufacturing the substance that destroys the pest.

7.9 Advantages of biopesticide

1. Biopesticides are biodegradable and do not leave harmful residues in soil, water, or crops. They reduce environmental pollution compared to chemical pesticides.
2. They are highly selective, acting only on specific pests or pathogens without harming beneficial insects, pollinators, natural predators, or humans.
3. They help in maintaining ecological balance and soil biodiversity, supporting long-term soil health and productivity.
4. Biopesticides are generally non-toxic, reducing risks of poisoning, contamination, and health hazards associated with chemical pesticides.
5. Many biopesticides (like *Trichoderma* or PGPR) produce hormones and enzymes that stimulate plant growth and improve nutrient uptake.

Check your progress

1. Explain the mechanism and inoculation process of biofertilizers.
2. Write in brief the advantages of biofertilizers.
3. What do you understand by the term biopesticides.
4. Explain different types and advantages of biopesticides.

Summary

This unit describes the importance of biofertilizer and biopesticides. Biofertilizers and biopesticides are one of the essential inputs in organic farming they not only stimulate plant growth and product yield but also enhances the soil fertility and soil health. The biological pest management is an important area that require intensive research to enhance its effectiveness mainly to achieve food security of increasing population and restore soil health. The potential uses of biopesticides and biofertilizers are in sustainable agriculture. A number of government agencies are already involved in production and application of these biopesticides. Biofertilizers function through a series of microbiological processes that enhance nutrient availability and promote plant growth. These microbial inoculants fix atmospheric nitrogen either within the root nodules of leguminous plants or freely in the soil, thereby converting inert N_2 into assimilable forms for plant uptake. There are different types

of biofertilizer such as Nitrogen Fixing Biofertilizers like Rhizobium, BGA, Azospirillum, Azotobacter, Acetobacter and Frankia. They all increases the crop yields by 20-25 % and improves the soil structure. Biopesticides are biodegradable and do not leave harmful residues in soil, water, or crops. They reduce environmental pollution compared to chemical pesticides. Biopesticides are generally non-toxic, reducing risks of poisoning, contamination, and health hazards associated with chemical pesticides.

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Unit 8: Biodegradation and Biotechnology

Unit Structure

8.0 Learning Objectives

8.1 Introduction

8.2 Genetically modified crops and ethical issues

8.3 Modification of plant species to practically desired products

8.3.1 Safety assessment

8.3.2 Regulatory issues

8.3.3 Advantages of Genetically modified crops have been developed with various purposes in mind, including

8.3.4 Current status of GM Crops in India

8.4 Biodegradation of forestry wastes through genetically engineered microbes

8.0 Learning Objectives

After completing this unit, learners shall be able to:

- Understand the biodegradation process in nature
- Discuss role of biotechnology in biodegradation.
- Understand the genetically modified crops (GMOs) and related ethical issues
- Understand the process of modification in plant species to get the desired products
- Understand the role of GEMs in biodegradation

8.1 Introduction

This particular unit will make you understand the production process for the development of Genetically Modified (GM) crops like Bt Cotton, Bt brinjal etc. and what are the issues and challenges in GM crops. The unit also covers the regulatory issues and approval protocols followed by Government of India for the consideration and approval of GM crops. This unit will how biotechnology can be useful in fighting against food security and has a potential to provide sustainable solution to fulfill Sustainable Development Goal (SDG) of “Zero Hunger”. Major public concerns will also be been discussed in this unit. The adoption of GM crops in India has been a subject of controversy and polarized opinions. While proponents argue that GM crops can increase agricultural productivity, reduce pesticide use, and improve farmers livelihoods, opponents raise concerns about potential environmental impacts, health risks, and corporate control over seeds.

8.2 Genetically modified crops and ethical issues

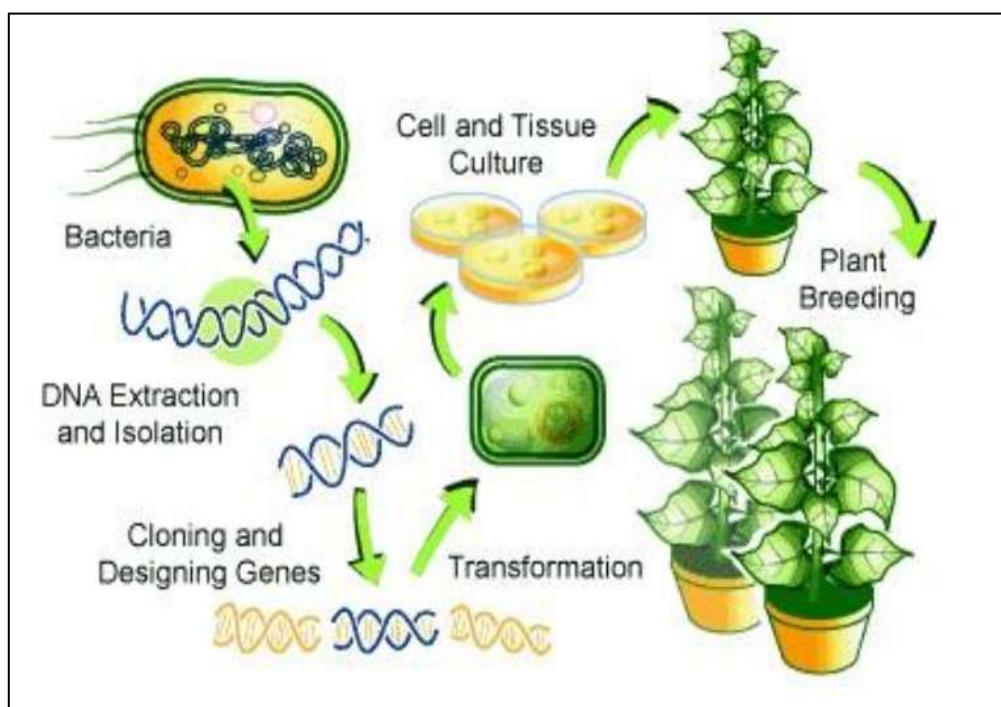
Genetically modified crops, often referred to as genetically modified organisms (GMOs), are plants whose genetic material has been altered using genetic engineering techniques. This involves the introduction of specific genes from other organisms into the target plant's DNA to confer desirable traits or characteristics.

In the process of seed domestication farmers have been selecting and growing crops producing plants with desired characters like easier to grow, climate resilient and high yield without knowing the exact mechanism of how this occurs. Recognizing valuable traits and incorporating them into future generations is very important in plant breeding. Advances in plant biotechnology have made it possible to identify and modify genes controlling specific characteristics. In last three decades scientists have developed genetic engineering tools through which they can transfer genes from one organism to another unrelated organism, producing what is now known as “Genetically Modified Organism” (GMO) or “Genetic Modified crop” (GM Crop). Any food produced this way is called genetically Modified food (GM food). The principal transgenic crops grown commercially in field are herbicide and insecticide resistant like soybeans, corn, cotton and canola (Table 1).

Table. 1 GM foods application and its history

What is GM Food? ✓ Food with added genes ✓ Food with deleted/modified genes ✓ Genetically edited crops ✓ Products from GM-fed animals ✓ Processed foods from GM crops	Why GM Food is Needed? ✓ Higher productivity ✓ Disease resistance ✓ Herbicide tolerance ✓ Biofortification (better nutrition) ✓ Longer shelf life ✓ Climate-resilient crops
Key Developments 1946 – Gene transfer discovered 1954 – DNA identified 1883 – First GM plant 1990 – China commercializes GM crops 1994 – First GM food (Flavr Savr Tomato) 2002 – Bt Cotton in India	Commercial GM Crops • Corn • Soybean • Canola • Sugar beet • Alfalfa • Cotton • Potatoes • Papaya • Pink pineapple • Summer squash

Genetically modified crops are being used in agriculture extensively; currently more than 10% of the world's croplands are planted with GM crops. Genetic engineering and plant transformation have played a pivotal role in crop improvement via introducing beneficial foreign gene(s) or silencing/editing the expression of endogenous gene(s) in crop plants. Genetically modified crops possess one or more useful traits, such as, herbicide tolerance, insect resistance, abiotic stress tolerance, disease resistance, and nutritional improvement. Basic steps involved in GM crop development are illustrated as follow:



Basic steps involved in GM crop development. (Source: <https://www.iasexpress.net/gm-crops-in-india-pros-cons-regulations-upsc/>).

ETHICAL ISSUES RELATED TO GM CROPS

Five sets of ethical concerns have been raised about GM crops:

- [1] – Potential harm to human health
- [2] – Potential damage to the environment
- [3] – Negative impact on traditional farming practice
- [4] – Excessive corporate dominance
- [5] – Unnaturalness of the technology

GM crops can have serious implications on the environment. Proper research must be carried out to study all aspects of GM crops. The reason behind the elaborate approval process is to ensure that the GM crop does not affect the health of human beings, animals, and the environment. Since the species have been artificially created, if it breeds with wild relatives, the natural ecosystem can result in genetic contamination. An example is the pollination and fertilization of Bt. Corn crop with a non-Bt. Corn. This will result in problems for the certified organic producers of corn. Another argument from ecologists is that the Bt. crops can harm non-target insects. In the case of Bt. Corn, Monarch butterflies feeding on wild milkweed that grows near cornfields may be harmed. Studies are still ongoing about the environmental effects of GM crops. Sustainability of the technology, consumer choice and labelling for monitoring are some other concerns with respect to GM crops in India. That is reason even after two decades of Bt cotton launching our policy makers hesitate to commercialize it in food chain crops.

So far, GEAC approved the cultivation of only Bt. Cotton in 2002. This implies that the cultivation of GM versions of other crops is banned in the country. In spite of the ban, incidences of suspected open cultivation of Bt. Brinjal were reported in the states of Andhra Pradesh, Haryana, Maharashtra, and Punjab which is illegal. The Environment Protection Act, 1989 makes use of the unapproved GM crops a punishable offence attracting a penalty up to Rs. 1 Lakh and imprisonment for a term of five years. States have been asked to strengthen Biotechnology Coordination Committees and District Level Committees to monitor the illegal cultivation as well as take necessary action.

As per those who support the introduction of GM crops, depending on the variety of crop, farmers will benefit using GM seeds. Loss due to pest attacks can be averted. Further, use of chemical pesticides and herbicides can also be reduced. Production costs can therefore be reduced and increase production stability. The technology is also being used now to develop drought tolerant, nutrient and water efficient varieties which will help farmers more.

8.2.1 International Scenario:

More than 30 countries from all over the world growing 14 GM crops, led by the top five countries USA, Brazil, Argentina, Canada, and India. Among these countries USA is the largest producer of GM crops in the world, planting 37.6% of the total global biotech crop area (The International Service for the Acquisition of Agri-biotech Applications- ISAAA Inc). To date, nearly 525 different transgenic events in 32 crops have been approved for cultivation in different parts of the world. The adoption of transgenic technology has been shown to increase

crop yields, reduce pesticide and insecticide use, reduce CO₂ emissions, and decrease the cost of crop production (Kumar K, et. al., 2020).

8.2.2 National Scenario:

India was an early adopter of GM crops when the regulatory body, Genetic Engineering Appraisal Committee (GEAC), cleared the cultivation of Bt cotton in 2002. For developing resistance to Lepidoptera order of insects such as butterflies and moths, a gene from *Bacillus thuringiensis* var. *kurstaki* bacteria is introduced, which produces a protein insecticidal (Cry1Ac) protein to Lepidoptera larvae, thereby aiding cotton production without excessive use of chemical insecticides. Bt cotton remains the only GM crop approved for cultivation in India till date. This implies that the cultivation of GM versions of other crops is banned in the country. In spite of the ban, incidences of suspected open cultivation of Bt. Brinjal have been reported in the states of Andhra Pradesh, Haryana, Maharashtra, and Punjab. Also, the area under Bt Cotton cultivation has increased from less than 1% in 2002-03 to almost 94% in 2019-20. Currently India stands at fifth position in producing GM crops in the world.

8.3 Modification of plant species to practically desired products

A. Isolation of the gene(s) of interest: Existing knowledge about the structure, function or location on chromosomes is used to identify the gene(s) that is responsible for the desired trait in an organism, for example, drought tolerance or insect resistance. A molecular biologist provides regulators detailed information about the characteristics of the gene of interest and other functional sequences such as promoters and terminators. These elements are required for functional gene and its products in the donor organism and intended function in the recipient organism. A detailed in silico study is required prior to begin wet lab work e.g., primer designing, gene sequencing analysis through different bioinformatics tools like Basic Local Alignment Search Tool - BLAST search (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), translational codon analysis and protein folding etc.

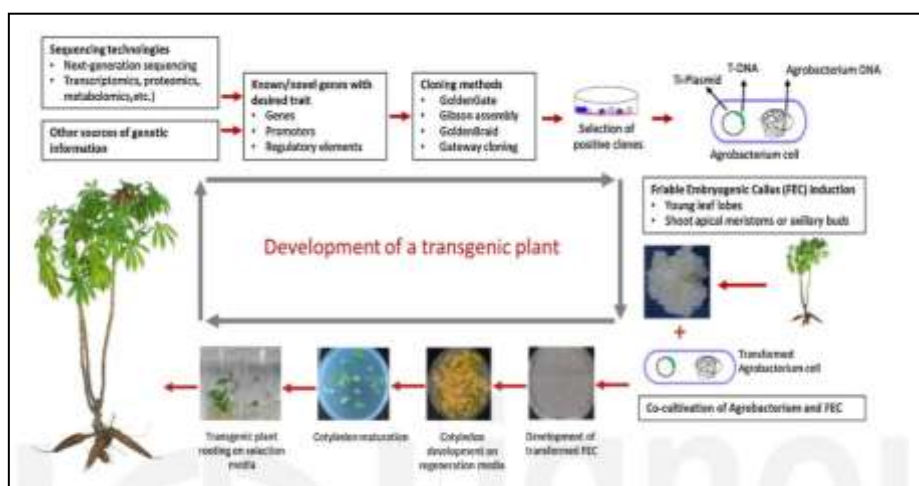
B. Gene cloning and bacterial transformation

After gene amplification through Polymerase Chain Reaction (PCR) amplified gene, we clone into plasmid vector which replicates inside *E. coli* bacteria so that it can easily amplify in vivo (inside bacteria) along with bacterial multiplication. Moreover, these plasmid vectors provide suitable restriction sites which is required for gene construct preparation into binary vector. There are different ways of gene cloning now like

gateway cloning, Gibson assembly etc. Transformed bacterial colonies are white in colour which shows that foreign genes have been inserted otherwise it will be blue in colour on selection media.

C. Binary vector and plant transformation:

Agrobacterium tumefaciens is the most commonly used gene transfer tool for plants transformation, which is a naturally occurring soil bacterium. The gene(s) of interest is inserted into the plasmid using recombinant DNA (rDNA) techniques. The modified *A. tumefaciens* cells containing the plasmid with the new gene are mixed with plant cells or cut pieces of plants such as leaves or stems (explants). Some of the cells take up a piece of the plasmid known as the T-DNA (transferred-DNA). The *A. tumefaciens* inserts the desired genes into one of the plant's chromosomes to form GM (or transgenic) cells. The other most commonly used method to transfer DNA is particle bombardment (gene gun) where small particles coated with DNA molecules are bombarded into the cell.



Production process of genetically modified (GM) crops

D. Transformed plant cells into whole plants

After transformation, only a small fraction of the plant cells takes up the gene of interest and most often, selectable marker genes that confer antibiotic or herbicide resistance are used. This will help us to screen transformed cells from the non-transformed cells. For this method, genes responsible for resistance are generally present in binary vector and transferred along with the gene(s) conferring desired traits to the plant cells. When the cells are exposed to the antibiotic or herbicide, only the

transformed cells containing and expressing the selectable marker gene will survive. The transformed cells are then regenerated into whole plants using plant tissue culture techniques and by using different rooting and shooting media. Information about the marker genes and whether they will be present or absent in the developed GM plant has to be provide to the regulators at the time of biosafety or field trial

E. Molecular characterization of the transgenic lines:

Molecular characterization involves presence of gene of interest in transgenic plants and its stable insertion is inherited normally or not. Tests are done to determine the number of copies inserted, whether the copies are intact, and whether the insertion does not interfere with other genes to cause unintended effects. Testing of gene expression (i.e., production of messenger NA and/or protein, evaluation of the trait of interest) is done to make sure that the gene is functional (Table 2).

Table 2. Different techniques for molecular characterization of transgenic plants.

S. No	Molecular Techniques	Molecular Characterization
1	Southern blotting	Gene copy number, presence of gene, etc.
2	Northern blotting	Presence of RNA in transgenic lines
3	Western blotting	Presence of protein in transgenic lines
4	Transcriptomics (qRT-PCR)	Level of foreign gene expression and associated genes
5	Metabolomics (High Performance Liquid Chromatography), (Gas Chromatography), (Nuclear Magnetic Resonance)	Presence of metabolites in transgenic lines

8.3.1 Safety assessment

Food and environmental safety assessment are must and carried out in conjunction with testing of transgenic plant performance in the field under controlled condition. Several biosafety issues like risk to human health, risk to environment, ecological concern has been raised after the rapid commercialization of GM crops every year across the world. As per Convention on biodiversity (CBD), Biosafety is a term used to describe efforts to reduce and eliminate the potential risk resulting from biotechnology and its product. Though many concerns being raised time to time, strict biosafety

guideline must be followed before introducing a GM crop in public domain especially in resource poor developing countries. In India Genetic Engineering Appraisal Committee (GEAC) functions under the Ministry of Environment, Forest and Climate Change (MoEF&CC) as per Rules, 1989, which is responsible for all the biosafety issues related to GM crops in India.

8.3.2 Regulatory issues

Regulation of all activities related to GMOs and products derived from GMOs is governed by “Rules for the Manufacture/Use/Import/Export and Storage of Hazardous Microorganisms, Genetically Engineered Organisms or Cells, 1989” (commonly referred to as Rules, 1989) under the provisions of the Environment (Protection) Act, 1986 through the Ministry of Environment and Forests (MoEF). The Rules, 1989 are primarily implemented by MoEF and the Department of Biotechnology (DBT), Ministry of Science and Technology through six competent authorities:

- i- Recombinant DNA Advisory Committee (RDAC)
- ii- Review Committee on Genetic Manipulation (RCGM)
- iii- Genetic Engineering Approval Committee (GEAC)
- iv- Institutional Biosafety Committees (IBSC)
- v- State Biosafety Coordination Committees (SBCC)
- vi- District Level Committees (DLC)

The Rules, 1989 are very broad in scope and essentially capture all activities, products and processes related to or derived from biotechnology including foods derived from biotechnology, thereby making GEAC as the competent authority to approve or disapprove the release of GM foods in the marketplace. Following the promulgation of the Food Safety and Standards Act, 2006, processed foods containing one or more ingredients derived from a genetically modified organism would be regulated under the FSSA, 2006. In order to manage the administration of the regulatory program for GM foods, the FSSAI has established a new secretariat within the FSSAI, namely the Office of GM Foods and the GM Food Safety Assessment Unit (GMFSAU) which is situated at the National Institute of Nutrition, Hyderabad (Table 3).

Table 3. Responsibilities of governmental authorities as regards the regulation of GMOs in India (excluding pharmaceuticals applications)

Activity	Responsible Authority
Contained research (laboratory and greenhouse)	RCGM (DBT)
Event selection trials/BRL 1 trials	RCGM and GEAC (MoEF)
Food safety assessment of GM foods (viable and processed)	FSSAI
Environmental risk assessment of GM organisms	GEAC
Approval for commercial release of GM foods (processed)	FSSAI
Approval for commercial release of GM foods (viable i.e., LMOs)	GEAC
Approval for environmental (commercial) release of GM organisms	GEAC

The food safety assessment and the environmental risk assessment of GM organisms are separate and distinct evaluations that share some common elements of information provided through the molecular characterization of the GM organism and characterization of the expressed, transgenic proteins.

8.3.3 Advantages of Genetically modified crops have been developed with various purposes in mind, including

Pest resistance: Adding genes that produce toxins harmful to specific pests, reducing the need for chemical pesticides.

Herbicide tolerance: Introducing genes that allow the crop to withstand certain herbicides, making weed control more efficient.

Improved nutritional content: Enhancing the nutritional value of crops by increasing the levels of specific vitamins, minerals, or other nutrients.

Disease resistance: Incorporating genes that confer resistance to viral, bacterial, or fungal diseases, reducing crop losses.

Drought and stress tolerance: Introducing genes that help crops withstand environmental stresses like drought, heat, or cold.

It's worth noting that the advantages of resistance traits in GM crops should be considered within the context of responsible and sustainable agricultural practices. Proper stewardship, adherence to regulations, and monitoring for any potential

environmental and health impacts are essential to ensure the safe and effective use of GM crops.

8.3.4 Current status of GM Crops in India

Bt Cotton: For the time being, the only genetically modified crop that is under cultivation in India is Bt cotton which is grown over 10.8 million hectares.

Bt Brinjal: The GEAC in 2007, recommended the commercial release of Bt Brinjal, which was developed by Mahyco (Maharashtra Hybrid Seeds Company) in collaboration with the Dharward University of Agricultural sciences and the Tamil Nadu Agricultural University. But the initiative was blocked in 2010.

GM-mustard: Dhara Mustard Hybrid-11 or DMH-11 is a genetically modified variety of mustard developed by the Delhi University's Centre for Genetic Manipulation of Crop Plants. The researchers at Delhi University have created hybridised mustard DMH-11 using "barnase / barstar" technology for genetic modification. It is Herbicide Tolerant (HT) crop. If approved by the Centre, this will be the second GM crop, after Bt Cotton, and the first transgenic food crop to be allowed for cultivation in the country.

8.4 Biodegradation of forestry wastes through genetically engineered microbes

Forestry wastes—such as bark, sawdust, branches, and logging residues—are primarily composed of lignocellulosic biomass, which includes cellulose, hemicellulose, and the highly recalcitrant polymer lignin. Lignin's complex aromatic network makes biological degradation difficult, leading researchers to investigate genetically engineered microbes (GEMs) capable of accelerating breakdown processes (Janusz et al., 2017). Natural microbial degradation relies on extracellular enzymes such as cellulases, xylanases, laccases, and peroxidases; however, native microbes often produce these enzymes in insufficient quantities for rapid industrial-scale biomass conversion (Kumar et al., 2020). To address this limitation, scientists have engineered bacteria and fungi to overexpress ligninolytic enzymes or to secrete enzyme cocktails more efficiently. For example, *Pseudomonas putida* has been engineered to metabolize lignin-derived aromatics into central metabolic pathways, improving both lignin depolymerization and the production of value-added chemicals (Brown & Chang, 2014). Another notable example is the development of engineered *Saccharomyces*

cerevisiae strains that secrete cellulases, enabling consolidated bioprocessing in which the same organism hydrolyzes cellulose and ferments resulting sugars—streamlining biofuel production from forestry residues (Rafeeq et al., 2023).

Beyond individual strains, synthetic microbial consortia have also been created to divide labor between lignin breakdown and sugar fermentation. Linger et al. (2014), for instance, demonstrated a system in which engineered microbes funnel lignin oligomers into specific metabolic intermediates before subsequent chemical catalysis converts them into industrially relevant compounds. Such innovations significantly reduce the need for harsh chemical pretreatments traditionally used to process woody biomass. Despite these advances, large-scale field deployment of GEMs remains limited due to ecological risks, potential horizontal gene transfer, and regulatory hurdles for environmental release (Janssen, 2020). Nonetheless, research continues to show that genetically engineered microbes hold substantial promise for transforming forestry waste management by enhancing biodegradation efficiencies and enabling sustainable bioproduct generation (Grgić et al., 2023; Singh et al., 2021).

Genetic engineering aims to (a) raise enzyme titers, (b) assemble multi-enzyme pathways in a single framework for consolidated bioprocessing (CBP), (c) broaden substrate range (e.g., introduce ligninolytic activity into robust industrial strains), and (d) route degradation products into value-added chemicals (aromatic monomers, biofuels, platform chemicals). Engineered microbes can in principle speed breakdown, work under tailored conditions (thermophilic or acidic), and convert waste into higher-value outputs (Rafeeq et al., 2023).

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Unit 9: Environmental remediation

Unit Structure

9.0 Learning Objectives

9.1 Introduction

9.2 Environmental remediation or Bioremediation

9.3 Significance of microorganisms in Bioremediation

9.4 Technologies for Bioremediation

9.4.1 In situ Bioremediation

9.4.2 Ex situ Bioremediation

9.5 Factors affecting Bioremediation

I. Hydrogen ion concentration (pH)

II. Temperature

III. Water content geological characters

IV. Nutrient availability

V. External electron availability

VI. Bioavailability of pollutants

VII. Co-metabolism

VIII. Gene expression

9.6 Bioaugmentation

9.0 Learning Objectives

After going through this unit you will be able to:

- Understand the benefits of Bioremediation to the ecosystem
- Describe the in-situ and ex-situ Bioremediation methods
- Explain detailed account on Factors affecting Bioremediation
- Describe Bioaugmentation

9.1 Introduction

The global environment is under great stress due to urbanization and industrialization as well as population pressure on the limited natural resources. The problems are compounded by drastic changes that have been taking place in the life style and habits of people. The environmental problems are diverse and sometimes specific with reference to time and space. The nature and the magnitude of the problems are ever changing, bringing new challenges and creating a constant need for evolving newer and more appropriate technologies. In this context, biotechnology has tremendous potential to cater to the need and holds hope for environmental protection and management. While some application such as for bioremediation are direct applications of biotechnology there are many which are indirectly beneficial for the environment. Environmental remediation in biotechnology, or bioremediation, means using living

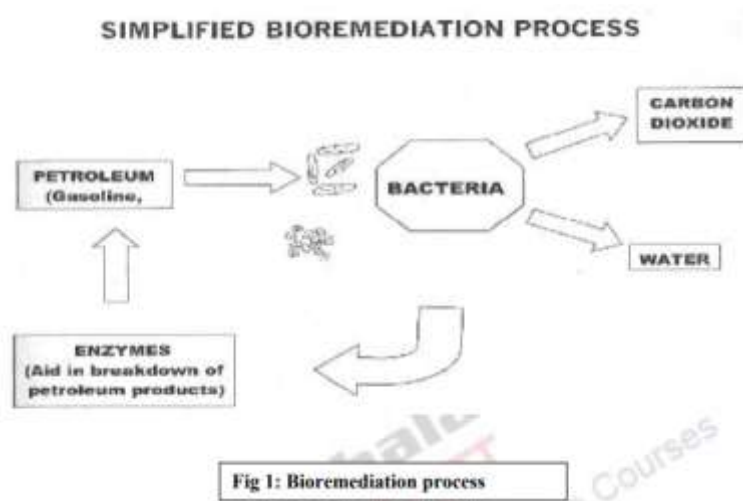
organisms like microbes, fungi, and plants, or their enzymes, to break down, neutralize, or remove harmful pollutants (contaminants) from polluted soil, water, and air, turning toxic substances into less harmful or harmless ones, offering a natural, sustainable cleanup for environmental restoration.

9.2 Environmental remediation or Bioremediation

Bioremediation is a greek word “Bio meaning Life” and latin word “Remedian meaning Restoring balance”. “Bioremediation is the process of using biological organisms to solve an environmental problem such as contaminated soil or groundwater”. It is clean up technology that uses naturally occurring organisms to degrade or transform various hazardous chemicals to less harmful or nontoxic compounds”.

Bioremediation of a contaminated site typically works in one of two ways:

- (1) In one case / microbes eat and digest organic substances as their food for nutrients and energy. When microbes completely digest chemicals they change them into water and harmless gases with an end product of clean soil, CO₂, and H₂O, and cell material.
- (2) In another case microbes degrade hazardous organic substances into inert products.



The problems of environment can be classified into the following subheads as most of the problems can be traced to one or more of the following either directly or indirectly. 1.

Waste generation

- a. Domestic waste (sewage, waste water, kitchen waste)
- b. Industrial waste (by-products, effluents)

- c. Agricultural waste (materials other than produce or materials left after separation/extraction of produce)
- d. Food waste (food processing industries)
- e. Wood waste

2. Use of chemicals for various purposes

- a. Insecticides
- b. Pesticides
- c. Chemical fertilizers
- d. Toxic products and by-products from chemical industries

3. Over exploitation of natural resources and loss

- a. By conversion to non-recoverable forms, interference with biogeochemical cycles.

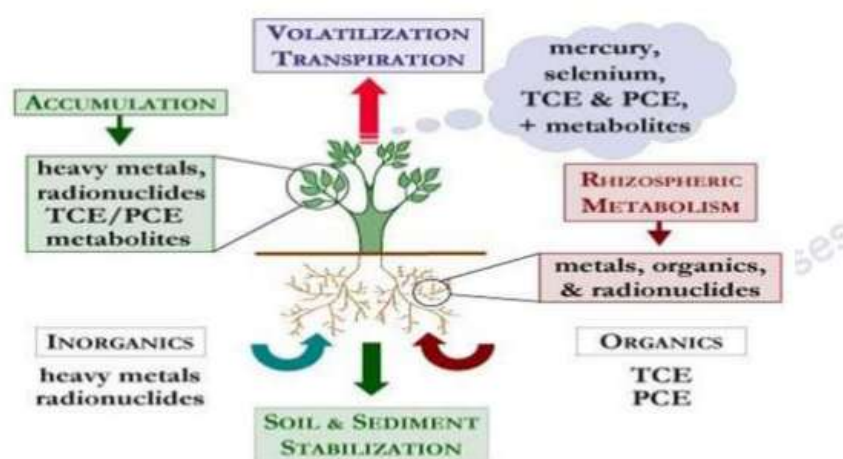
Waste generation

Waste generation is a side effect of consumption and production activities and tends to increase with economic advance. What is of concern is the increased presence of toxic chemicals such as halogen aliphatics, aromatics, polychlorinated biphenyls, polycyclic hydrocarbons etc. in the natural environment. These pollutants may reach air, water or soil and affect environment in several ways threatening the self regulating capacity of the biosphere. They may be present in high levels at the point of discharge or may remain low but can be highly toxic. The underground water sources are increasingly becoming contaminated. Some substances may reach environment in small concentrations but may lead subjected to biomagnifications wherein their concentrations increase as they pass through food chain e.g. DDT. A number of reasons including our dependence on industries for economy, the extent of damage involved, as well as the extent of the area involved makes it difficult to totally prevent such pollution or adopt classical methods to remove the pollutants effectively and economically. There are three main approaches in dealing with contaminated sites:

- A. Identification of the problem of the problem
- B. Assessment of the nature and degree of the hazard
- C. Choice of remedial action

9.3 Significance of microorganisms in Bioremediation

- Microorganisms play a pivotal role in biotechnology, especially in dealing with environment though we cannot exclude the use of higher organisms, especially plant and animal cell cultures in biotechnology.
- Microorganisms are primary fixers of photosynthetic energy and they act as systems for bringing about chemical changes in almost all types of natural and most synthetic organic molecules.
- Exploitation of the degradative capacities of microorganisms is the fundamental basis of organic pollutant bioremediation.
- The development and application of cost effective, efficient and environmentally sound bioremediation technologies depends on the integration of many different disciplines as well as on advances in our understanding of the physicochemical, biological, and ecological factors that govern microbial degradation of organic contaminants both in situ and ex situ.



Concept of bioremediation

Fig 2: Concept of Bioremediation

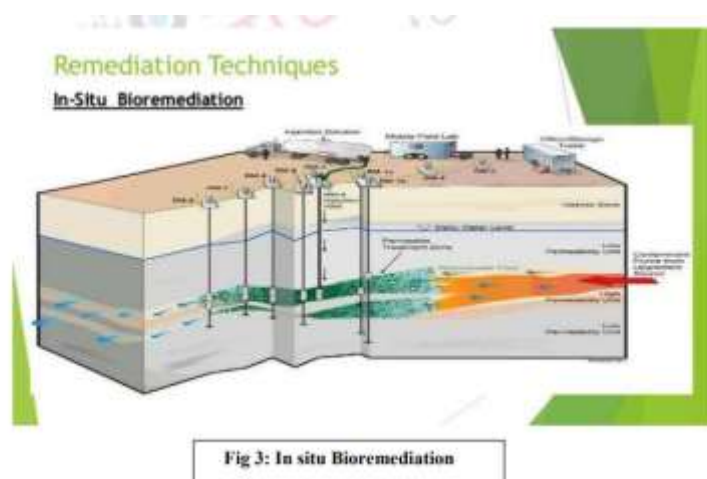
9.4 Technologies for Bioremediation

Bioremediation treatment technologies have been broadly divided into two categories based on whether biodegradation is stimulated in situ i.e. within the affected environment (soil, sediment, surface water, ground water etc.) or ex situ i.e. under contained conditions (compost pits, bioreactors etc.). The latter usually involves removal of the

contaminated material to the place of treatment with increased chances of exposure of the persons involved.

9.4.1 In situ Bioremediation

In any natural setting there is an existing microbial population which is ultimately responsible for the stabilization and treatment of waste products containing organic matter. This microbial population consists of a variety of microorganisms which invariably have minimum oxygen and nutrient requirements. Many of the problems encountered during handling of waste materials can be directly related to insufficient quantities of the two limiting factors namely oxygen and available nutrients. When the system is oxygen deficient, oxygen requiring bacteria do not thrive well and they give way to anaerobic bacteria. The natural respiration process of anaerobic bacteria can lead to generation of volatile organic compounds that are particularly noxious. The natural respiration process of these anaerobes involves the degradation of organic material into carbon dioxide and water. Many treatment systems attempt to encourage the proliferation of these anaerobes in order to breakdown waste material without generating odors.



Availability of nutrients to the microbial populations within the waste material is another limiting factor determining the rate of breakdown of waste. They may be insufficient or may not be readily available. The nutrients which may act as limiting factors for the growth of microorganisms are amino acids, purines, pyrimidines and vitamins. These substances are often referred to as growth factors. Thus, in situ bioremediation involves stimulation of the degradative activities of endogenous microbial populations by the provision of nutrients and or external electron receptors. Another way of enhancing

microbial action is by the introduction of competent, exogenous microbes with or without nutritional enrichment. This procedure which is used to enhance microbial activity is called **Bioaugmentation**.

The addition of nutrients has also been employed for treating contaminated soils. For soil bioremediation, apart from the addition of nutrients, oxygen is provided through oxygen saturated water, **air sparging** or **Bioventing**. Water containing water oxygen and nutrients is added to the surface of the contaminated area and allowed to percolate through the soil and mix with the groundwater. Hydrogen peroxide has also been widely used as an alternate oxygen source in the percolation technology. In case of air sparging air is injected into the water saturated zone of soils and sediments. Bioventing involves supply of air to soil to be treated using a combination of pumps and blowers. Using these devices, vacuum is applied to the target area, while continuously injecting low volumes of air. This procedure of bioventing is aimed at stimulating in situ aerobic degradation of contaminants, for its successful functioning, nutrients are often required to be added and adequate water levels must be present in the unsaturated zone to support microbial degradative activity. Bioventing has been extensively used for in situ remediation of unsaturated soils contaminated by petroleum hydrocarbons. Often bioventing is combined with another technique called soil vapour extraction for faster remediation of highly contaminated sites.

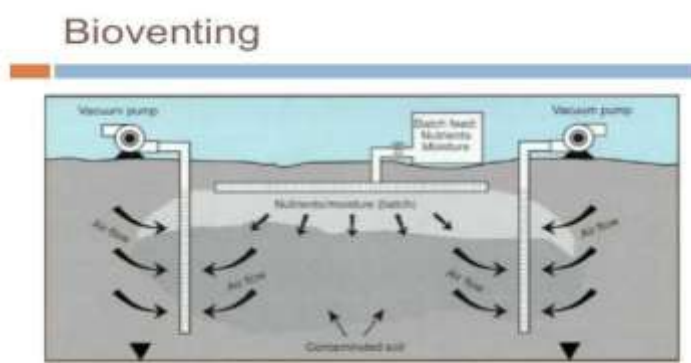


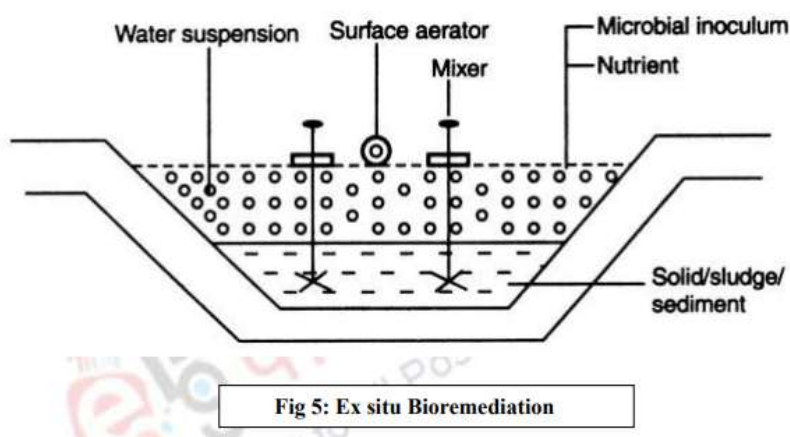
Fig 4: Bioventing

9.4.2 Ex situ Bioremediation

These techniques are usually aerobic and involve treatment of contaminated soils or slurry phase systems. Solid phase systems include land farming (soil treatment units), compost heaps and engineered biopiles. Land farming involves plowing, tilling, and raking of the contaminated soil on site together with application of water, nutrients and

microbial inocula if required. Care should be taken to prevent contamination of groundwater by leachate from the tilled zone.

Above ground composting techniques involve physical removal of the contaminated soil or sediment to specially constructed platforms, lined pads, or compost sheds. Compost heaps consist of contaminated soil or sediment supplemented with composting material (straw, wood chips, etc.) in order to enhance water and air holding capacity and improve physical handling properties. Periodic mixing or turning is applied in order to ensure to adequate aeration. In engineered biopiles, aeration is constantly supplied via a network of pipes. Nutrients and microbial inocula are added, if required to compost heaps and biopiles. Composting techniques are used for remediation of highly contaminated sites and have proved successful for military sites contaminated with explosives such as TNT, RDX and tetryl. Slurry phase bioremediation is a batch treatment technique in which excavated soils or sediments are mixed with water and treated in reactor vessels or in contained ponds or lagoons.



9.5 Factors affecting Bioremediation

A number of environmental factors influence in situ bioremediation rates. Some of them can be controlled or modified while some are difficult to control or modify.

I. Hydrogen ion concentration (pH)

Majority of bacteria exhibit growth optima at or near neutral pH. Many soils are acidic throughout the world. Treatment commonly known as liming involves addition of finely ground agricultural limestone, calcium carbonate, calcium hydroxide, magnesium carbonate etc. Treatment may affect solubility, bioavailability and chemical form of the organic pollutant and of soil macro and micro nutrients. Decrease in soil pH decreases the availability of nitrate and chloride.

II. Temperature

Temperature affects (a) bacterial metabolism (b) growth rates (c) soil matrix and (d) physicochemical state of the contaminants. Generally, in situ bioremediation is carried under mesophilic conditions (20° to 40° C).

III. Water content geological characters

Water content especially water availability influences bioremediation rates. • Water in soils or sediments may not be available to microorganisms because it is absorbed to solid substances or tied up as water of hydration to dissolved solutes. This can be solved up by irrigating the contaminated soil, compost, heaps and biopiles.

IV. Nutrient availability

Nutrients are generally supplemented in both in situ and ex situ bioremediation of soils, ground and surface waters. Nutrient requirement depends on the nature of contaminants and the extent to which polluted site has been subjected to agricultural use.

V. External electron availability

Oxygen is used as an electron acceptor to increase bioremediation activity. A number of aerobic bacteria can break down a variety of aliphatic and aromatic organic compounds of both natural and anthropogenic origin wholly or partially by denitrifying bacteria by sulphate, iron, and molybdenum reducers and by methanogenic consortia.

VI. Bioavailability of pollutants

Anthropogenic organic polymers such as polystyrene PVC etc. are highly recalcitrant because of their insolubility and the lack of extracellular microbial enzymes capable of catalyzing depolymerisation. However, non-polymer degrading bacteria and actinomycetes are able to degrade oligomeric polystyrene fragments and low molecular weight fragments of lignin resulting from fungal attacks on the lignin polymer.

VII. Co-metabolism

Co-metabolism is a process whereby microorganisms involved in the metabolism of a growth promoting substrate also transform other organic contaminants which can be called as co-substrates which not growths are supporting if these co substrates are provided as the only sources of carbon and energy.

VIII. Gene expression

The ability of indigenous microorganism to degrade organic pollutants is dependent on expression of the genes encoding the required enzymes. These genes may not express

if these substances are available in very low concentrations. This can be overcome by adding substances that are structurally related to the organic pollutants which will act as inducers.

9.6 Bioaugmentation

Where degradative microbes do not exist or where the process is too slow, microbial inoculants may be added to enhance bioremediation rates. This technique is known as bioaugmentation and may involve

- (a) addition of natural isolates of bacteria or
- (b) genetically engineered organisms.

Biological degradation or biodegradation is generally considered as a phenomenon of biological transformation of organic compounds by living organisms, particularly microbes.

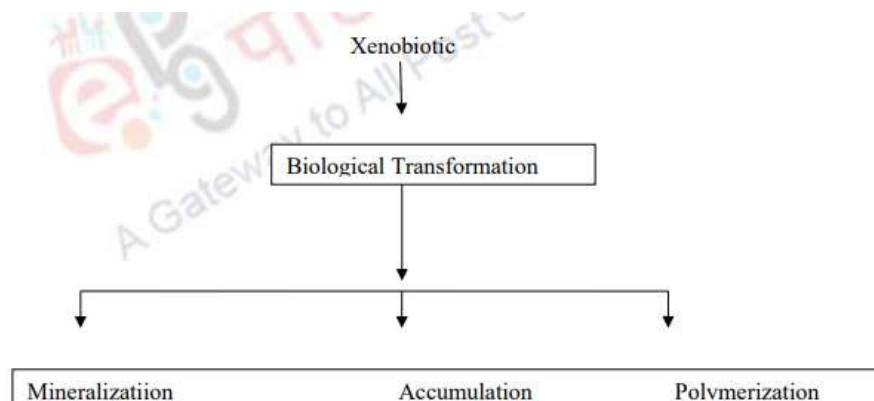


Fig 6: Biological fate of Xenobiotic compounds

The role of microorganisms in the decomposition of sewage and other organic wastes is long known. It has been considered as a natural process in the microbial world as carbon and energy source for their growth and takes a pivotal role in the recycling of materials in the natural ecosystem. It brings about changes in the molecular structure of a compound ultimately yielding simpler (mineralization) and comparatively harmless (non toxic) products like CO_2 , H_2O , NH_3 , CH_4 , H_2S , or PO_3 . When the compound is not fully broken, it is termed as biotransformation. Many of the recalcitrant substances produced by biotransformation may sometimes be more toxic than the original compound. Such changes are brought about by the catabolic activities of bacteria or fungi by their intracellular or extracellular enzymes secreted in the medium. Biological fate of xenobiotic compounds in the environment can be indicated as shown in figure 6.

Perhaps biosynthetic abilities of the living world and the possibility of the degradation by catabolic enzymes evolved parallel but slowly in nature. This has apparently ensured that under suitable conditions all natural organics get decomposed and are not deposited in the environment with the exception of natural polymers like lignin and soil humus getting degraded very slowly. This has helped microbes to act as scavengers and reduce the pollution load in natural ecosystem. Bioremediation of polluted environment capitalizes on the activities of aerobic or anaerobic heterotrophic microbes.

Reference

Adopted from epg-pathshala, Paper number 05 (Environmental Biotechnology) and Module number: 09 (Bioremediation and Biodegradation).

Unit 10: Advanced Environmental biotechnology applications

Unit Structure

10.0 Learning Objectives

10.1 Introduction

10.2 Biofilms

10.3 Biosensors and genetically engineered microorganisms in environmental applications

10.4 Biocorrosion

10.4.1 Significance of Biocorrosion

10.4.2 Importance of Studying and Controlling Biocorrosion

10.5 Bioleaching

10.5.1 Significance & Importance of Bioleaching

10.0 Learning Objectives

After completing this unit, learners shall be able to:

- Understand the biofilm
- Discuss biosensors and its application in environmental applications.
- Understand the biocorrosion and its important
- Understand the process of bioleaching

10.1 Introduction

Environmental biotechnology is an advanced and rapidly evolving field that applies biological systems, microorganisms, and biochemical processes to address environmental challenges and promote sustainable development. It plays a crucial role in solving issues such as pollution control, waste management, resource recovery, and ecosystem restoration. This discipline integrates principles of microbiology, molecular biology, ecology, and engineering to develop eco-friendly technologies that minimize environmental damage while enhancing productivity.

One of the key aspects of environmental biotechnology is its ability to harness natural microbial processes for the degradation and transformation of pollutants. Microorganisms possess diverse metabolic capabilities that enable them to break down complex organic and inorganic contaminants into less harmful substances. These processes are widely applied in bioremediation, wastewater treatment, and air purification systems. Compared to conventional chemical and physical methods,

biological approaches are often more cost-effective, sustainable, and environmentally safe.

Advanced applications of environmental biotechnology include the use of biofilms, biosensors, genetically engineered microorganisms, biocorrosion control, and bioleaching. Biofilms, which are structured communities of microorganisms enclosed in a self-produced matrix, exhibit enhanced resistance and metabolic efficiency, making them highly effective in pollutant degradation and nutrient cycling. Similarly, biosensors provide rapid and sensitive detection of environmental contaminants, enabling real-time monitoring and improved environmental management.

In addition, environmental biotechnology contributes significantly to sustainable industrial practices. Techniques such as bioleaching allow the recovery of valuable metals from low-grade ores using microorganisms, reducing the need for energy-intensive and polluting extraction methods. At the same time, understanding processes like biocorrosion helps in preventing material degradation and minimizing economic losses.

Overall, advanced environmental biotechnology offers innovative and sustainable solutions to modern environmental problems. It not only helps in pollution mitigation but also supports resource conservation and ecological balance, making it an essential tool for achieving long-term environmental sustainability.

10.2 Biofilms

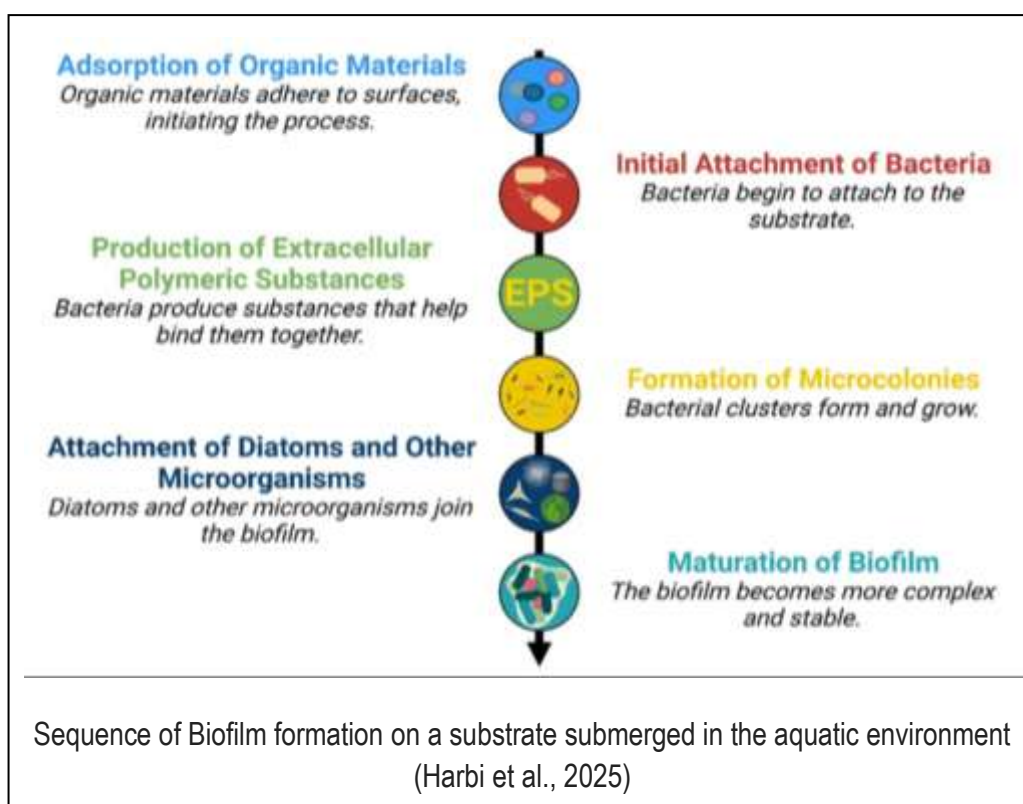
Biofilms, microbial communities in protective slime, are central to environmental biotechnology, offering powerful tools for bioremediation (cleaning pollution), wastewater treatment, and nutrient cycling, while also posing challenges in biofouling and corrosion, all through enhanced pollutant tolerance and metabolic activity within their self-made matrices. Environmental biotech uses these natural systems for sustainable solutions like cleaning contaminated soil/water, improving air quality, and even enhancing agriculture, leveraging their superior resilience compared to free-floating microbes (Krishnan et al., 2023).

Working mechanism of Biofilm

Formation: Microbes stick to surfaces (rocks, pipes, roots) and secrete an extracellular polymeric substance (EPS) matrix, forming a robust, protective community.

Enhanced Resilience: This matrix protects microbes from harsh conditions, increasing their tolerance to pollutants, antibiotics, and stress.

Metabolic Power: Biofilm communities efficiently break down pollutants (hydrocarbons, pesticides, heavy metals) and cycle nutrients, making them ideal bioremediation agents.



Application of Biofilm

Bioremediation & Pollution Control: Degradation of Toxins: Biofilms effectively break down persistent pollutants (PAHs, pesticides, heavy metals) in soil, water, and air, protecting microbes from toxicity.

Phytoremediation: Biofilms on plant roots enhance nutrient uptake and pollutant breakdown, aiding in soil restoration.

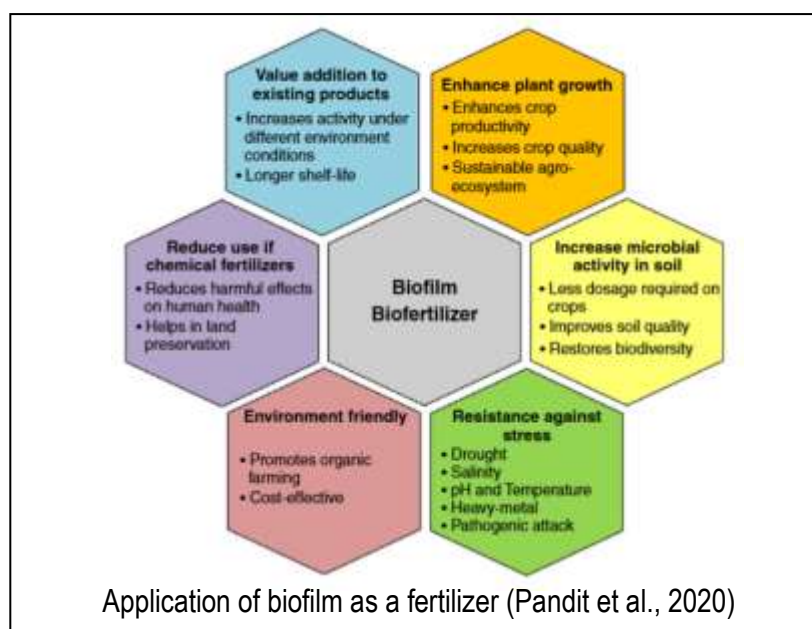
Air Filtration: Biofilms in biofilters clean harmful air pollutants, improving air quality.

- **Wastewater Treatment**

Recalcitrant Compounds: High microbial biomass in biofilms excels at treating tough industrial wastewater.

Nitrogen Removal: Anammox bacteria in biofilm granules remove nitrogen efficiently.

Engineered Systems: used in moving bed biofilm reactors (MBBRs), trickling filters and activated sludge for carbon and nutrient removal.



- **Agriculture & Soil Health**

Nutrient Cycling: Help cycle carbon, nitrogen, and other elements in soil and aquatic environments.

Biocontrol: Biofilms of beneficial microbes act as biofertilizers and biopesticides, reducing chemical use.

Soil Stabilization: Cyanobacterial biofilms (biocrusts) improve semi-arid soil quality and fertility.

- **Energy & Resources**

Bioelectricity: Bio-electrochemical systems use biofilms to generate electricity while treating wastewater.

Resource Recovery: Biofilm systems can help recover valuable resources from wastewater.

- **Environmental Monitoring**

Biomarkers: Biofilms act as natural sensors for monitoring water quality, especially from mine drainage

10.3 Biosensors and genetically engineered microorganisms in environmental applications

A biosensor is an analytical device consisting of an immobilised layer of biological material (e.g. enzyme, antibody, organelle, hormones, nucleic acids or whole cells) in the intimate contact with a transducer, i.e. sensor (a physical component) which analyses the biological signals and converts into an electrical signal. A sensor can be anything, a single carbon electrode, an ion-sensitive electrode, oxygen-electrode, a photocell or a thermistor.

The principle of the biosensor is quite simple. The biological material is immobilized as described earlier on the immobilization support, the permeable membrane, in the direct vicinity of a sensor. The substances to be measured pass through the membrane and interact with the immobilized material and yield the product. A product (i.e. monitored substrate) may be heat, gas (oxygen), electrons, hydrogen ions or the product of ammonium ions. The product passes through another membrane to the transducer. The transducer converts product into an electric signal which is amplified. The signal processing equipment converts the amplified signals into a display most commonly the electric signal which can be read out and recorded.

A glucose-electrode can be built up by immobilizing glucose oxidase in polyacrylamide gel around a platinum oxygen-electrode separated by a teflon membrane. KCl solution is placed around the platinum-oxygen electrode. From upper surface glucose oxidase is intimately covered by a cellulose acetate membrane.

When glucose solution is brought into the contact of membrane, glucose and oxygen pass through membrane into the enzyme layer and, as a result of oxidation-reduction reactions, converted into gluconic acid and hydrogen peroxide in the presence of water, oxygen and glucose oxidase. Consequently, oxygen concentration in the vicinity

of oxygen electrode is lowered down. Hydrogen peroxide brings about a change in oxygen concentration proportional to glucose concentration. The rate of diminution of current i.e. measurable signal. Electrode records the rate of the sample. It responds linearly to glucose concentrations over a range of $10^{-1} - 10^{-5} \text{ mol dm}^{-3}$ with a response time of 1 minute.

In 1987, for the first time Yellow Springs Instruments Co., USA developed a biosensor for diagnostic purposes for measuring glucose in blood plasma. It is a hand-held machine which measures six components of blood plasma for example, glucose, urea, nitrogen, sodium, potassium and chloride.

An indigenous glucose sensor has been developed by the scientists at Central Electro-chemical Research Institute (CECRI), Karaikudi. It gives electrical signal for a glucose concentration as low as 0–15 millimoles.

1. Types of Biosensor

Biosensor are of different types based on the use of different biological material and sensor devices; a few of them are discussed below:

(a) Electro-chemical Biosensor

This type of biosensor has been developed by using electronic devices such as field effect transistors or light emitting diode; the former measures charge accumulation on their surface and the later photoresponse generated in a silica based chip as an alternating current. Hence, the field effect transistor measures a biochemical reaction at the surface and induce into current. Moreover, the field effect transistors can be modified to ion sensitive, enzyme sensitive or antibody sensitive ones by using selective ions, enzymes or antibodies respectively.

(b) Amperometric Biosensor

Amperometric biosensors are those which measure the reaction of analyte with enzyme and generate electrons directly or through a mediator. The amperometric biosensors contain either enzyme-electrode or without a mediator, or chemically modified electrodes. The oxygen and peroxide-based biosensor and others discussed earlier are enzyme electrode biosensor. Some advancement has been brought into this type of biosensor by using a mediator. In addition, more advanced types are the direct electron transfer systems.

In this biosensor, a redox reaction catalysed by an enzymes is directly coupled to an electrode where enzyme is presented with the oxidizable substrate. The electrons are transferred from the substrate to the electrode via enzyme and redox mediator. In this biosensor the oxidase replaces the oxygen requirement of the enzymes.

(c) Enzyme Electrodes

Enzyme electrodes are a new type of biosensors which have been designed for the amperometric assay of potentiometric assay of substrates such as urea, amino acid, glucose, alcohol, and lactic acid. The electrode consists of a given electrochemical sensor in close contact with a thin permeable enzyme membrane capable of reacting with the given substrates. The enzyme is embedded in the membrane and produce O_2 , H^+ , NH_4^+ , CO_2 or other small molecules depending on enzymatic reactions. This is detected by the specific sensor. The magnitude of the response determines the concentration of substrates.

(d) Thermistor Containing Biosensor

Thermistor is used to record even a small temperature changes (between $0.1-0.001^\circ C$) during biochemical reactions. By immobilizing enzymes like cholesterol oxidase, glucose oxidase, invertase, tyrosinase, etc. thermistors have been developed (Gronow et al. 1988). Moreover, thermistors are also employed for the study of antigen-antibody with very high sensitivity ($10^{-13} \text{ mol dm}^{-3}$) in case of thermometric Enzyme Linked Immunosorbent Assay (ELISA).

(e) Bio affinity Sensor

Bio affinity sensors are developed recently. It measures the concentration of the determinants, i.e. substrates based on equilibrium binding. This shows a high degree of selectivity. These are of diverse nature because of the use of radiolabeled, enzyme labelled or fluorescence-labelled substance.

In this biosensor, a receptor is radiolabeled and allowed to bind with determinant analogue immobilized onto the surface of a transducer. When concentrations of a determinant are increased, the labelled receptor forms an intimately bound complex with determinant.

2. Applications of Biosensor

In the beginning biosensor was applied in the field of medicine and industry. But in recent years, biosensors are becoming popular in many areas due to the small size,

rapid and easy handling, low cost, and greater sensitivity and selectivity. Application of biosensor in some of the areas is described as below:

(a) Uses in Medicine and Health

Biosensors have tremendous potential for its application in the field of medical science. In 1979, the first glucose analyser using biomolecule for the detection of blood glucose was commercialized by Yellow Springs Instruments Co., USA. A device, a minipump filled with insulin, has been constructed to deliver insulin to diabetics based on glucose levels of blood. When biosensor provides information, the device delivers accurate amount of insulin required by the diabetics. Mitomycin, an aflatoxin, causes cancer in borne infants. Therefore, mutagenicity of such chemicals can be detected by using the biosensor. Similarly, any other abnormal toxic substance produced in body due to infectious disease can also be detected.

(b) Uses in Pollution Control

Biosensors are very helpful in environmental monitoring and pollution control, since they can be miniaturized and automated. As far as quality control of drinking water is concerned, the monitoring biosensors are successful in monitoring of pesticides in water. In Japan, a biosensor coupled with oxygen electrode and immobilized *Trichosporon cutaneum* is used for measuring biological oxygen demand (BOD) (Gronow et al., 1988).

The whole cell biosensor developed by immobilising *Salmonella typhimurium* and *Bacillus subtilis* in conjugation with oxygen electrode can be used to measure mutagenicity and carcinogenicity of several chemical compounds.

(c) Uses in Industry

Generally, spectrophotometer and autoanalyser are used to estimate the substrates utilized and the products formed in the fermented broth. In addition, there are a lot of problems associated with these. So the biosensors can be designed to measure the fermentation products to improve the feedback control, to carry out rapid sampling and rejection of below standard raw materials to improve the efficiency of workers. Isaokabure and coworkers of Tokyo University Research Centre of Advance Science & Technology have recently developed an ion sensitive field effect transistor (ISFET). This device is highly sensitive to change the ion concentration. Using this biosensor, it is possible to measure the odour, freshness and taste of foods. In determining fish

freshness either ATPase, aminooxidase or putrescine oxidase is used. ATPase detects the presence of ATP in fish muscle. As ATP is not present in staled food, therefore, signals do not occur. Recently, a biosensor has been developed at Cransfield Institute of Technology, UK which measures cholesterol levels in butter. The enzyme cholesterol oxidase, when immobilized on the electrodes, reacts with cholesterol of food.

(d) Biosensor for Military

The darker side of biosensor application is to provide support to military with such a biosensor that can detect toxic gases including chemical warfare agents. Such biosensors have advantages over the traditional methods of sensing of chemicals.

10.4 Biocorrosion

Biocorrosion, also known as microbiologically influenced corrosion (MIC), refers to the deterioration of metals and other materials caused or accelerated by the activities of microorganisms. These microbes such as sulfate-reducing bacteria (SRB), iron-oxidizing bacteria, manganese-oxidizing bacteria, and certain fungi or algae, interact with material surfaces, altering local chemical conditions and increasing corrosion rates beyond what would occur chemically alone (Beech and Sunner, 2004). Microorganisms contribute to corrosion through several mechanisms, including (Videla, and Herrera, 2005):

- Production of corrosive metabolites (e.g., H_2S , organic acids)
- Biofilm formation that creates differential aeration cells
- Direct electron transfer between microbes and metal surfaces (EMIC – extracellular microbial electron transfer)
- Modification of pH or redox potential at the material interface

10.4.1 Significance of Biocorrosion

Widespread Industrial Occurrence

Biocorrosion affects a broad range of industrial systems, such as pipelines, storage tanks, cooling towers, power plants, wastewater facilities, offshore structures, and marine vessels. The presence of biofilms in these environments leads to accelerated

material degradation, reduced system efficiency, and heightened risk of structural failure.

High Economic Impact

MIC contributes significantly to global corrosion-related losses. Studies estimate that 20–40% of all corrosion damage in certain industries may involve microbial activity, translating into billions of dollars annually when considering repairs, replacements, shutdowns, and preventive treatments.

Environmental and Safety Concerns

Microbially induced corrosion can cause leakage of hazardous materials (e.g., oil, gas, chemicals), posing risks to human health and the environment. Failures in critical infrastructure such as pipelines or storage tanks can lead to large-scale contamination or accidents.

Complex Interdisciplinary Challenge

Understanding biocorrosion requires knowledge of microbiology, electrochemistry, materials science, and environmental engineering. Microbial communities are dynamic and often difficult to detect, making diagnosis and prediction challenging.

10.4.2 Importance of Studying and Controlling Biocorrosion

Infrastructure Protection

Monitoring and controlling MIC is essential to prolonging equipment life and preventing catastrophic failures in industries such as petroleum, maritime, and water treatment.

Development of Efficient Mitigation Strategies

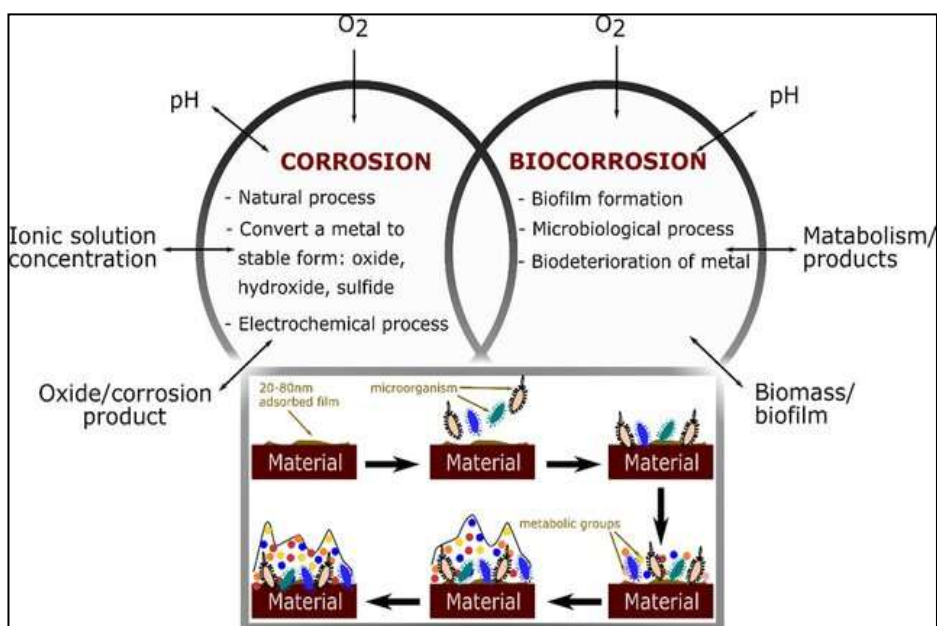
Research into biocorrosion enables the improvement of biocides, protective coatings, cathodic protection systems, and anti-biofilm technologies.

Optimization of Industrial Processes

Reducing MIC leads to increased operational efficiency by minimizing downtime, preventing blockages from biofilms, and ensuring smoother fluid transport.

Environmental Sustainability

Controlling MIC reduces unnecessary material waste and prevents environmentally damaging leaks, supporting more sustainable industrial practices.



Various factor influence corrosion versus biocorrosion—here, we show the formation of the microbial biofilm involved in the biocorrosion process (Dang et al., 2022)

10.5 Bioleaching

Microbial leaching is a technique in which microorganisms are used to extract metals from ore-bearing rocks. For many centuries, microbes have played a role in recovering metals such as copper from drainage waters. In recent years, this approach has developed into an important area of biotechnology known as biomining. Microbial methods are particularly useful for extracting metals from low-grade ores that cannot be processed economically using conventional chemical techniques. During the extraction of high-grade ores, large amounts of low-grade material are generated and often discarded as waste. These waste heaps may contain valuable metals like nickel, lead, and zinc, which can be effectively recovered through microbial leaching. Bioleaching is already widely used on a commercial scale for metals such as uranium and copper. However, if not properly managed, large-scale leaching operations can pose environmental risks. Leakage of acidic leach solutions containing dissolved metals can contaminate nearby water bodies and groundwater.

Despite these challenges, biomining is considered an economically viable hydrometallurgical process with relatively lower environmental impact compared to traditional methods. It is an interdisciplinary field that combines metallurgy, chemical engineering, microbiology, and molecular biology, and holds significant practical importance. In countries like India, where vast mineral resources remain underutilized, biomining has considerable national importance.

1. Microorganisms used for Leaching

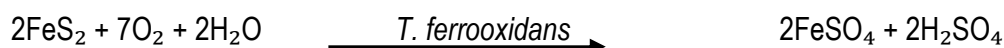
The microorganisms most frequently employed in bioleaching include *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans*. In addition to these, several other microbes are also involved in the bioleaching process, such as *Bacillus licheniformis*, *Bacillus luteus*, *Bacillus megaterium*, *Bacillus polymyxa*, *Leptospirillum ferrooxidans*, *Pseudomonas fluorescens*, *Sulfolobus acidocaldarius*, *Thermothrix thioparus*, *Thiobacillus thioparus*, and fungi like *Penicillium*.

(a) Chemistry of Microbial Leaching

Thiobacillus thiooxidans and *Thiobacillus ferrooxidans* are commonly found together in leaching dumps. Among these, *Thiobacillus* is a well-studied Gram-negative, rod-shaped bacterium that obtains its energy by oxidizing ferrous iron (Fe^{2+}) or insoluble sulfur compounds. In the process of bioleaching, two main reaction mechanisms are involved:

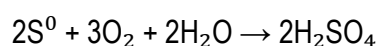
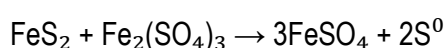
(i) Direct bacterial leaching

In this method, bacteria come into direct contact with the ore surface, allowing mineral oxidation to occur through a series of enzyme-mediated reactions. For instance, pyrite is converted into ferric sulphate during this process, as illustrated below:

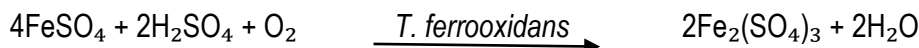


(ii) Indirect bacterial leaching

In indirect bacterial leaching, microbes are not in direct contact with minerals but leaching agents are produced by microorganisms which oxidise them.



Oxidation of ferrous (Fe^{2+}) to ferric (Fe^{3+}) by *T. ferrooxidans* at low pH is given below:



2. Leaching Process

Three commercial methods are involved in leaching process as given below:

Types of Bioleaching Methods

(a) Slope Leaching

In this method, approximately 10,000 tonnes of ore are first crushed into smaller fragments. The crushed material is then arranged in large heaps along a sloping surface, such as a mountainside. A solution containing *Thiobacillus* is continuously sprayed over the pile. As the solution percolates downward, it collects at the base, where it is processed for metal recovery and further bacterial growth in an oxidation pond.

(b) Heap Leaching

In heap leaching, the ore is piled into a large heap known as a leach dump. The treatment process that follows is essentially the same as in slope leaching, where bacterial solution is applied and the leachate is collected for metal extraction.

(c) In situ Leaching

In this technique, the ore remains in its natural underground location. The rock surface is lightly blasted to improve water permeability. A solution containing *Thiobacillus* is then pumped through drilled channels into the ore body. The acidic solution moves through the rock, dissolving the minerals, and accumulates at the bottom. This solution is then pumped out, metals are extracted, and the water is recycled after reintroducing bacteria.

10.5.1 Significance & Importance of Bioleaching

1. Environmentally Friendly Metal Extraction

Application of such as *Acidithiobacillus ferrooxidans* instead of toxic chemicals like cyanide or mercury produces significantly less air and water pollution than traditional smelting (Johnson, 2014).

2. Economically Viable for Low-Grade Ores

Bioleaching reduces the need for high-energy, high-cost roasting or smelting procedures (Rawlings, 2002).

3. Lower Energy Consumption

Bioleaching occurs at low temperatures ($\approx 30\text{--}50^\circ\text{C}$), unlike smelting ($>1000^\circ\text{C}$). It reduces overall energy demands and lowers operational costs (Olson et al., 2003).

4. Useful for Extracting Critical & Strategic Metals

It is efficient for copper, gold, nickel, cobalt, zinc, and rare earth elements. It is also important for sustainable supply of metals used in electronics, EV batteries, and renewable energy technologies (Brierley, 2008).

5. Cleaner Processing of Mining Waste

Microorganisms can treat tailings and waste piles by recovering residual metals and reduce long-term environmental hazards such as acid mine drainage (Johnson and Hallberg, 2005).

6. Supports Circular Economy & E-Waste Recycling

Bioleaching helps in the recovery of precious metals (e.g. gold, palladium) as microorganisms can dissolve metals from electronic waste or bioleaching of e-waste.

7. Scalable, Safe, and Adaptable Technology

Biomining can be applied in situ (directly in the ore body) or in bioreactors. Therefore, it is safer for workers due to reduced exposure to toxic fumes and chemicals.

8. Promotes Sustainable Mining Practices

Aligns with global initiatives for greener, low-carbon metal production. Contributed in reducing the ecological footprint of mining operations (Dutta et al., 2025).

3. Examples of Bioleaching

Bioleaching has been discussed with copper, uranium, gold, silver and silica.

Copper Bioleaching

In copper bioleaching, low-grade ores like chalcocite or chalcopyrite are piled up and sprinkled with dilute sulphuric acid. Bacteria such as *T. ferrooxidans* oxidize the copper sulfide minerals and release soluble Cu^{2+} ions. The copper-rich solution collected at the bottom is processed to extract pure copper. This method is cost-effective and environmentally friendly compared to traditional smelting.

Uranium Bioleaching

Uranium ores contain insoluble U^{4+} , which is converted into soluble U^{6+} using ferric ions in acidic conditions. *T. ferrooxidans* helps by regenerating Fe^{3+} from Fe^{2+} , maintaining continuous leaching. The dissolved uranium sulfate is then recovered through chemical processing. This technique allows uranium extraction from very low-grade ores.

Gold/Silver Bioleaching

Microbes are used to break down sulfide minerals like arsenopyrite that trap gold and silver. Once the mineral structure is degraded, gold and silver become accessible for extraction, usually by cyanidation. Silver dissolves more easily than gold during this process. This method improves recovery from refractory ores.

Silica Bioleaching

Certain bacteria such as *Bacillus licheniformis* can dissolve silica-containing minerals like magnesite. The bacteria interact with the mineral surface, breaking it down and releasing silicon. This bioleaching method is used in pilot plants to process large deposits of silica-rich ores in a sustainable way.

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Unit 11: Biotechnological methods of Pollution Detection

Unit Structure

11.0 Learning Objectives

11.1 Introduction

11.2 Types of pollutants

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11.0 Learning Objectives

After completing this unit, learners shall be able to:

- Understand the pollution and different types of pollutants
- Discuss different methods of detoxification.
- Understand the various types of degradation techniques
- Understand role of microbial enzymes in degradation of pollutant

11.1 Introduction

We know that the limited availability of fresh water is a global crisis. The growing consumption of fresh water by anthropogenic activities has taken its toll on available water resources. Unfortunately, water bodies are still used as sinks for wastewater from domestic and industrial sources. However, in recent times, the need to replenish our water resources has been receiving increasing attention. This has led to the development of strategies to return water to its source in the least toxic by detoxification of pollutants. The strategies and processes may be collectively termed as 'wastewater treatment'.

This unit attempts to explore and evaluate the types of pollutants and mechanism of detoxification. In this unit we also study the degradation of highly toxic pollutants along

with degradation techniques Types of microbial enzymes and technologies used for enzymatic treatment of wastewater will also be discussed.

11.2 Types of pollutants

Many different chemicals are regarded as pollutants, ranging from simple inorganic ions to complex organic molecules. The pollutants are all divided up into various types. Every class of pollutants has its own specific ways of entering the environment and its own specific dangers. All classes have major pollutants in it that are known to many people, because of the various health effects. The pollutants are of following types as:

A) Organic pollutants

Organic compounds are compounds that consist of long bonds, usually made up of carbon. Many organic compounds are basic fabrics of living organisms. Molecules built of carbon and of carbon and hydrogen are non-polar and have little to no water solubility. They have little to no electrical charge.

The behaviour of organic compounds is dependent upon their molecular structure, size and shape and the presence of functional groups that are important determinants of toxicity.

It is important to know the structure of organic compounds, in order to predict their fate in living organisms and the environment. The organic compounds that are dangerous to the environment are all man-made and have only existed during the last century.

11.2.1 Types of Organic pollutants

There are many different types of organic pollutants, examples are:

Hydrocarbons: These are carbon-hydrogen bonds. They can be divided up into two classes, the first being single-bonded alkanes, double bonded alkenes and triple bonded alkynes (gasses or liquids) and the second being aromatic hydrocarbons, which contain ring structures (liquids or solids). Aromatic hydrocarbons such as PAH's are much more reactive than any of the first class kinds of hydrocarbons.

B) PCB's: These are stable and unreactive fluids that are used as hydraulic fluids, coolant/ insulation fluids in transformers and plasticizers in paints. There are many different PCB's. None of them are water-soluble. In many countries PCB's are restricted.

C) Insecticides: As DDT's are very dangerous because they accumulate in fat tissues of lower animals and then enter the food chain. They have been restricted for decades.

D) Detergents: These can be both polar and non-polar.

E) Inorganic fertilizers: Some inorganic pollutants are not particularly toxic, but are still a danger to the environment because they are used so extensively. These include fertilizers, such as nitrates and phosphates. Nitrates and phosphates cause algal blooms in surface water, which causes the oxygen level of the water to decline. This causes oxygen starvation because of the uptake of oxygen by microorganisms that break down algae. This is called **eutrophication**.

F) Metals

The first class we will refer to here is metals. Metals are good conductors of electricity and generally enter chemical reactions as positive ions, known as cations. Metals are natural substances that have consisted through weathering of ore bodies, where they were deposited during volcanic action. They can be relocated into situations where they can cause serious environmental damage. Examples of metals are: lead, zinc, manganese, calcium and potassium.

They can be found in surface waters in their stable ionic forms. Unnatural metals can be very dangerous, because they often come from man-made nuclear reactions and can be strongly radioactive. Metals can react to dangerous products with other ions. They are often involved in electron transfer reactions involving oxygen. This can lead to the formation of toxic oxyradicals. Metals can form metalloids and then bond to organic compounds to form lipophilic substances that are often highly toxic and can be stored in the fat-supply of animals and humans. Metals can also bond to cellular macromolecules in the human body. They have a density greater than 5 and are therefore called heavy metals, cannot be broken down into less harmful components, as they are non-biodegradable. Organisms need metals, as they are essential for their health and are usually essential components of enzymes.

G) Radioactive isotopes

The half-lives and the ways of decay of radioactive isotopes determine how dangerous they are to humans. Humans create all radioactive isotopes in the nuclear industry. There are still debates going on about whether the benefits of nuclear power exceed

the dangers of radioactive radiation. When an atom of a radioactive substance decays, it can produce four kinds of particles: alpha, beta, gamma and neutrons.

Alpha particles can only travel a short distance through air and human tissues, but they can be very damaging if they collide with cells because of their large mass. They are positively charged.

Beta particles are more penetrating, but they do much less damage than alpha particles. They are negatively charged.

Gamma rays are highly penetrating. Their damage is similar to that of beta rays. Neutrons are liberated through radiation and react with other elements through collision. They are the basis for nuclear fission in a reactor. The radioactivity of a substance is measured in becquerel. Different kinds of radiation can do different kinds of damage, because the energy is imparted into tissues in different ways. This is expressed in sieverts. An amount of alpha radiation can do twenty times the damage of the same amount of beta radiation. Radioactive matter has to be held in storage for different periods of time, in order to erase the danger. How long it has to be stored depends upon the half-life of the isotopes; the time taken for half of the atoms of a radioactive isotope to decay.

11.3 Mechanism of detoxification

In microbial degradation the wastes can usually be broken down or even consumed as food by some organisms. When degraded, the waste may be made less toxic and its harmful effects reduced. Remineralisation is the complete breakdown of an organic chemical (for example, into its basic components such as carbon dioxide, nitrogen, phosphorus, and water), such as may occur by microbial digestion. Remineralisation completely destroys toxicity inherent in such waste. Most remineralisation is conducted by microbes (primarily bacteria and fungi). The ability of microbes to metabolize and remineralize different wastes is highly variable.

Remineralisation depends on the type and number of microbes in the total community that are capable of degrading a particular waste. The number of waste degrading microbes in turn depends on prior exposure to the waste (or similar types of waste) through chronic or single-event exposures. Degradation of the wastes may increase after exposure as a result of an increase in number of waste-degrading microbes or the

induction of appropriate enzyme systems. The rates at which microbes can degrade wastes are influenced by temperature and thus may change seasonally. The presence of oxygen often exerts a strong influence on degradation rates, and wastes may persist for very long periods of time in sediments that have little or no oxygen, even though microbes in oxygen-rich environments may readily degrade the same waste.

Marine and aquatic sediments and water-saturated soils in wetlands are often low in oxygen. Hence, the water availability in a specific location, acting through changes in the amount of saturated sediments and soils or even through changes in soil moisture, may have significant effects on contaminant degradation rates. Some wastes break down in anaerobic conditions. Specifically, the removal of chlorine atoms from PCB molecules, a necessary first step in their remineralisation, seems to occur (though slowly) in anaerobic conditions. High concentrations of waste contaminants may also be toxic to the bacteria and fungi that could otherwise degrade the contaminant where it presents in lower concentrations. This restricts the breakdown of a contaminant to the less contaminated areas of a site, slowing the overall degradation (Table 1).

Table 1. Showing Processes Involved in Waste Processing and Detoxification

Processes	Factors Affecting Processes
Contaminant movements in water bodies	
Mixing or dilution	volume of receiving waters, stratification
Advection and dispersion	velocity of water, turbulence regime
Water residence time	water input rate, tidal height, salinity distribution
Particle and sediment interactions and scavenging to sediments	solubility of contaminant (K_{ow}), particle deposition rate
Sediment-water exchange	diffusion rate, bioturbation rate, water level fluctuations
Sequestering of chemicals (acid volatile sulfides in sediments)	oxidation state of sediments, availability of sulfides
Scavenging to (accumulation at) sea–air interface	solubility of contaminant
Volatilization	vapor pressure of contaminant, wind speed and water surface roughness
Aerosol formation	breaking waves

Precipitation from solution	solubility limits
Biological transports	uptake by organisms, settling of organic materials, food chain transfers

Chemicals that can be used as food by microbes tend to break down more quickly than poor food-quality chemicals. Some organic chemicals are especially slow to break down in the environment. For many of these, their resistance to microbial degradation results from the presence of many attached chlorine or bromine atoms. While there are many naturally occurring organic compounds incorporating chlorine or bromine which degrades, albeit slowly, naturally many of those synthesized by the chemical industry are quite resistant to microbial degradation.

The chlorine carbon bonds in these synthetic chemicals are not naturally abundant, and organisms do not have enzymes effective at degrading these chemicals. One group of such resistant chemicals with known toxic effects are the persistent organic pollutants, some of which have been banned from further production by international treaties. The time required for the concentration of POPs in a contaminated area to decrease measurably is typically measured in decades. The decrease is partly due to slow degradation of the PCBs but also to their dilution and spreading.

Where large amounts of waste are present, such as in an oil spill, the degradation rates of the wastes may be limited by factors needed for microbial growth such as nitrogen and phosphorus. If bacteria cannot increase in number, the degradation rates of compounds will remain slow. The physical nature of spills may also inhibit microbial breakdown. If the spilled waste remains in large clumps, or patches, the microbes cannot reach or degrade the interior of the clump, and only the surface of the clump is subject to microbial decay, greatly slowing the digestion of the waste.

For many organic wastes, and particularly large (high molecular weight) compounds, it is important to distinguish between an initial alteration of the chemicals in the waste (a relatively small change in the form of the waste) and complete remineralisation. A small alteration of a parent compound may result in persistent and/or toxic daughter products that may be of as much concern as the parent waste chemical. With such a complex array of factors that can influence biodegradation, it is not usually a simple matter to predict the persistence of a degradable waste in an environment. However,

experience with wastes has been gained and models have been developed that allow such predictions to be made with good confidence.

11.3.1 Degradation of highly toxic pollutants

What is toxic pollution?

Toxic pollution is contaminated water, soil, and air that is harmful or poisonous. It includes industrial wastes like toxic heavy metals from mining or chemicals from factories, and also sewage and particulates from power plants. The term “toxic” is used to differentiate it from pollution that comes from increased levels of carbon dioxide, which causes climate change but does not have direct health impacts.

Toxic pollutants can poison drinking water, the fish in rivers and ponds, food grown on contaminated farmland, as well as playgrounds, homes, and the very air we breathe.

In fact, toxic pollution is the largest cause of death in the world. Yet it is one of the most underreported and underfunded global problems.

How does toxic pollution affect public health?

Pollution comes in many forms, and it affects people differently, sometimes in ways that may not be immediately noticeable. That is why some people call toxic pollution “the invisible killer.”

Pollution can cause birth defects and irreversible developmental and neurological disabilities, and immune system damage. Pollution causes various cancers, heart and lung diseases, to name just a few. Comparatively, death by pollution is larger than any other major cause. Often whole communities are affected, and economic growth impaired as well through degradation of human and natural resources.

11.3.2 Degradation Techniques

We know that continuous increase of pollutants in water bodies has necessitated the need to develop cost-effective methods for their removal. Destroying the pollutants to benign chemicals and/or removing these pollutants from contaminated water is imperative for a green environment. There are various treatment processes that have been applied for pollutant removal from wastewater, such as electrochemical oxidation,

biodegradation membrane process, coagulation, adsorption, precipitation, sonochemical degradation, micellar enhanced ultrafiltration, and AOP.

Though these methods are considered as efficient methods for pollutant removal, each method has its own benefits and drawbacks. In this section, we will explain some of the most common methods that are frequently used for pollutant removal and their basic principles.

a) Electrochemical Oxidation

It is an efficient and economic method, suitable when the wastewater contains non-biodegradable organic pollutants. This method poses several advantages since it does not require auxiliary chemicals, high pressures, or high temperatures. By its versatility and cost-effectiveness, electrochemical techniques have gained great attention for the removal of pollutants. The process of electrochemical oxidation mechanism is mainly based on the generation of the hydroxyl radicals at the electrode surface.

Two different types of mechanisms have been elaborated for electrochemical oxidation, such as **direct** and **indirect** oxidation methods.

* **In direct electrochemical oxidation**, the degradation of organic compound occurs directly over the anode material, where the hydroxyl radical or the reactive oxygen species react with the organic compound. The pollutants are first adsorbed at the surface of the anode and are then degraded by an anodic electron transfer reaction.

* **In the indirect electrochemical oxidation**, the organics are treated in the bulk solution by oxidants, such as Cl_2 , hypochlorite, peroxodisulfate and ozone (O_3), which are electrochemically generated at the electrode surface. Even though high removal efficiencies are achieved by both the direct and indirect electrochemical oxidation processes, their effectiveness strongly depends on the treatment conditions including pH, current density, types and concentration of pollutants, supporting electrolyte, flow rate, electrode preparation method and nature of the electrode materials.

Several electrode materials that include Pt, PbO_2 , Ti-SnO₂, Ti/Pt, Ti/Pt-Ir, Ti/PbO₂, Ti/PdO-Co₃O₄, Ti-TiO₂, Ti coated oxides of Ru/Ir-Ta, IrO₂, Ti/RuO₂, SnO₂, PbO₂, and so forth, and boron doped diamond (BDD) have been listed as efficient electrodes for the degradation of organics by electrochemical oxidation. Apart from these anode materials, graphite anodes are also considered as efficient materials for anodic oxidation of several organic pollutants. Particularly, the high oxygen over potential, high electro catalytic activity, chemical stability, long lifetime, and cost-effectiveness

has been credited for the high efficiency of these graphite electrodes, shown in figure 1.

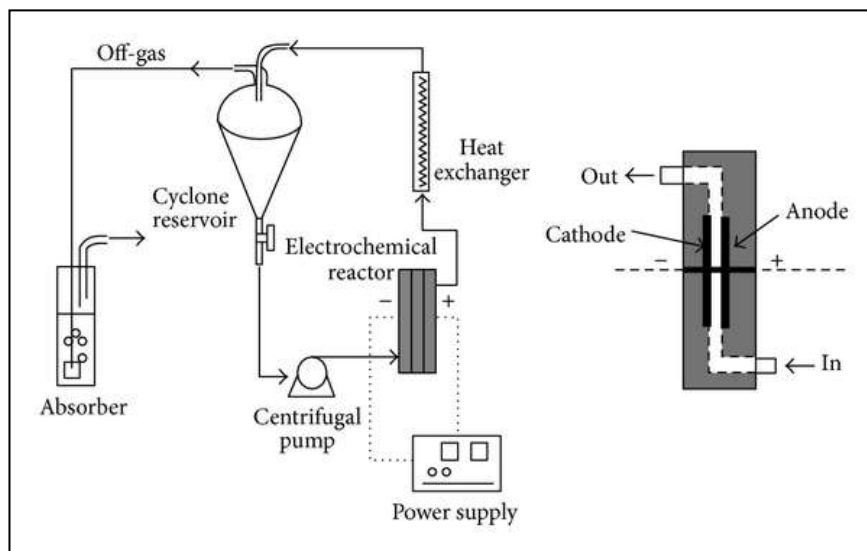


Figure 1. Showing the pilot plant arrangement and illustration of electrochemical oxidation.

The electrochemical performance was examined by measuring COD and the concentrations of all phenolic compounds formed during the electrolysis process. However, the addition of chloride ion to the electrolyte solution caused a major change in the reaction kinetics. The difference in the reaction kinetics was explained by the oxidation mediated by the chloride ions, which was formed at low pH values. Though the concentration of chloride ions helped to rapidly increase the reaction rate, increment in the supporting electrolyte concentration also assisted to improve the reaction kinetics.

b) Biological Process

Biological degradation technology with high removal efficiency has been successfully applied in industries for effluents and waste gas treatments. Even though biological processes have been used for several applications that include heavy metal ion removal and indoor air purification, its application has not been intensely investigated for numerous reasons. Thus, biological process combined with other techniques such as chemical processes, photocatalysis and AOP.

Combined biological and chemical degradation methods were carried out to evaluate the effectiveness of mature municipal landfill leachate. The biological treatment was

followed by chemical oxidation for further removal of COD. Higher removal efficiency was obtained due to the use of chemical treatment, a combined AOP and biological process was carried out to remove pesticides in aqueous solution. The experimental setup for their study is illustrated in Figure 2. The chemical oxidation process was carried out in the tank labelled as 1. The UV emitting device (labelled as 5) consists of a stainless-steel tube with a coaxial mercury vapour lamp. Ozone was produced from air by an ozone generator and was continuously fed into the oxidation tank. Finally, the pH of the system was adjusted to 7, and then the pollutant was fed to the biological treatment tank labelled as 9.

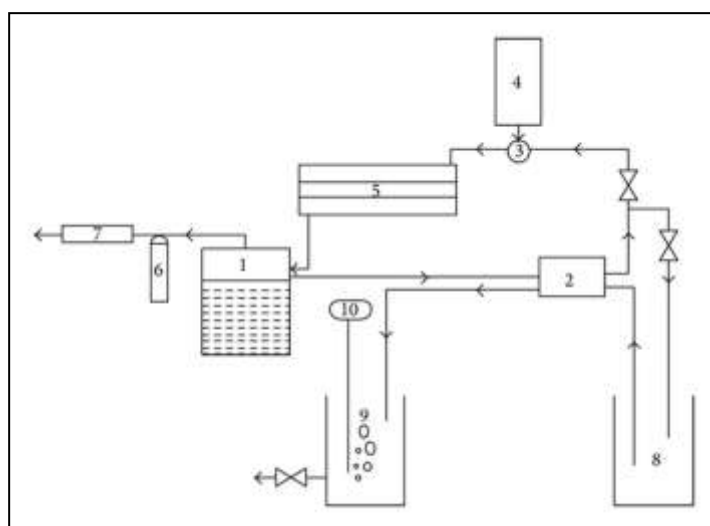


Figure 2. Showing experimental setup for combined chemical and biological degradation: 1. chemical oxidation tank, 2) peristaltic pump, 3) diffuser valve, 4) ozone generator, 5) UV emitting device, 7) excess KI trap, 8) ozone destruction device, neutralizer, 9) biological treatment tank, and 10) air sparger.

c) O₃ and O₃/UV oxidation treatment

This O₃ and O₃/UV oxidation treatment was able to achieve 90 and 100% removal of the pesticide deltamethrin, in a period of 210 min. Utilization of ozone with UV irradiation was found to enhance the degradation of pesticides. It has been well documented elsewhere that the rate of pesticide removal mainly depends on both the chemical nature of the pesticides being treated and the treatment conditions. Under their reaction conditions, pentachlorophenol degraded faster compared with other phenolic compounds and 4-chlorophenol degraded the slowest.

A combined biological and chemical procedure was also used as an ecologically and economically favourable remediation technique for 2,4,6-trinitrotoluene (TNT) reduction in contaminated ground and surface water. It was observed that the anaerobic transformation process resulted in a faster reduction of TNT due to significant change in the redox potential of the solutions under both aerobic and anaerobic conditions. Biodegradation of organic pollutants by halophilic bacteria.

Wastewater from a pharmaceutical formulation facility was treated with a biological activated sludge system followed by ozonation. The by-products identified from the incomplete oxidation of ozonation were likely to be more biodegradable than the parent compounds; thus, a postozonation biological treatment was endorsed for the efficient removal of these toxic pharmaceuticals from the wastewater.

d) Adsorption

This method is an effective and well-known process and has been widely explored as an alternate technique compared with the other waste removal methods due to the lower cost, flexibility and simplicity of design, and ease of operation. Moreover, adsorption does not result in production of any harmful substances. Discharge of several types of pollutants that include household wastes, phenolic wastes, dyes, pesticides, herbicides, and metal ions into the water body causes health issues not only for humans but also for aquatic life.

Dyes have been identified as a major contaminant in wastewater. Many industries, such as textile, leather, paper, plastics, food, and cosmetics, use several dyes as coloring materials. Most of these dyes are very toxic, and when released into the environment, they can be transported over long distances in water sources resulting in their widespread dispersal. More than a million tonnes of dyes and coloring materials are produced annually, and the drinking water quality is greatly affected by the unsafe release of these dyes into the water body. In addition, the presence of even very small amounts (<1 ppm) of dyes, in particular the synthetic dyes, in water is undesirable. Therefore, the presence of dyes in wastewater is a major concern for toxicological and esthetical reasons.

Adsorption is a method that is capable of removing nondegradable waste pollutants. There are several adsorbents that include clay minerals, activated carbon, coal, wood, fly ash, and biomaterials that have been listed for the removal of industrial wastes. However, due to the lack of effective adsorbate-adsorbent interactions, some of the

above-mentioned materials are found to be non effective for the adsorption of pollutants. Consequently, oxide materials, which are considered as an important class of adsorbents, have been explored for adsorption.

e) Advanced Oxidation Processes (AOP)

In this process oxidation takes place, which typically uses ambient conditions (room temperature and atmospheric pressure). Several AOP techniques such as ozonation, H_2O_2 photolysis process and heterogeneous photocatalysis have been explored for the elimination of pollutants, particularly from water sources. These AOP techniques destroy the pollutants by chemical oxidation or reduction.

In particular, AOP relies on the production of hydroxyl radicals, which are short-lived, extremely highly reactive species and attack most organic molecules with rate constants of . Moreover, the versatility of AOP is enhanced by different possible ways for the production of hydroxyl radicals

f) Ozonation

We know that Ozone is unstable in water and the molecular ozone can react as a dipole, electrophile, or nucleophile due to the two different resonance structures. In addition, depending on the pH, temperature, and concentration of organic and inorganic compounds in water, the half-life of ozone varies from a few seconds up to a few minutes. Ozone is a powerful oxidant and it can oxidize a large number of organic and inorganic materials. Ozone reacts either directly or indirectly with aqueous compounds. In the direct reaction, the molecular ozone directly reacts with the compounds, whereas the radicals resulting from the decomposition of ozone.

In this process the direct reactions are very slow and solute selective, whereas the indirect radical reactions are fast and nonselective. Additionally, the direct reactions are dominant in acidic solutions, while the indirect reactions occur mostly at basic pH values. Ozonation of phenol gave catechol, hydroquinone, benzoquinone, maleic acid, and oxalic acid as the ring cleavage intermediate products and it was noticed that some of the intermediates such as catechol, hydroquinone, and benzoquinone can be destroyed completely using ozone to CO_2 and H_2O . However, destruction requires long ozonation time and high dosage of ozone. The hydroxyl radicals are the active species in the decomposition of organics.

g) UV/ H_2O_2 Treatment

This treatment in AOP also involves the formation of radicals generated by the photolysis of H_2O_2 in the presence of UV irradiation and is very effective for the degradation of most of the organic pollutants. Owing to the higher molar absorption coefficient of the peroxide anion, the photolysis rate has been found to be pH dependent and increases at high pH values. Owing to the commercial availability, thermal stability, infinite solubility in water, storage, and ease of formation of hydroxyl radical, the use of hydrogen peroxide as an oxidant has received significant attention for water purification.

However, the rate of formation of hydroxyl radicals influences the oxidation of organic contaminants and this has been noted as the main disadvantage of the UV/ H_2O_2 treatment process. According to this study, an acidic medium is more effective for the degradation of DMSO.

There are a large number of organic pollutants, such as phenols and phenolic compounds, benzene and substituted benzene, salicylic acid, proline, pyridine, and dyes, that have been degraded or mineralized using UV/ H_2O_2 treatment. However, this treatment method is not the focus of this study. In addition to the UV/ H_2O_2 treatment for oxidation of pollutants, another process involves the use of O_3/UV . Even though H_2O_2 increases the generation of radicals in the ozone process. The $\text{H}_2\text{O}_2/\text{UV}$ method removed 99% of total organic carbon (TOC) from the effluent.

11.4 Microbial cell/enzyme technology

Enzymes are biocatalysts produced by living cells to cause specific biochemical reactions. These are highly specific in their action on substrates and often many different enzymes are required to bring about sequence of metabolic reactions performed by living cells.

Each strain of a microorganism produces a large number of enzymes which can be hydrolysing, oxidizing or reducing and metabolic in nature. Microbial enzymes are known to play a crucial role as metabolic catalysts, resulting in their use in various industrial applications. The end use market for industrial enzymes is extremely widespread with numerous industrial commercial applications. Microbes have served and continue to serve as one of the largest and useful sources of many enzymes.

Many industrial processes have several disadvantages like low catalytic efficiency, lack of enantiomeric specificity for chiral synthesis, requirement of high temperature, low pH

and high pressure. Also, the use of organic solvents leads to organic waste and pollutants. Enzymes are more useful for these applications as they work under mild reaction conditions (e.g., temperature, pH, atmospheric conditions).

Enzymes can be selected genetically and chemically-modified in order to improve their key properties: stability, substrate specificity and specific activity. In the case of ocean and river quality, pollution is primarily caused by the discharge of inadequately treated industrial and municipal wastewater.

On initial discharge, these wastewaters can contain high levels of inorganic pollutants which can be easily biodegraded, but whose impact load on the ecosystems, either in Total Suspended Solids (TSS), Biochemical Oxygen Demand (BOD), or Chemical Oxygen Demand (COD), may be in the tens of thousands mg/L. To combat this increasing burden on our aquatic environment, increasingly strict regulation on pollution discharge is being implemented by various governmental bodies, with focus primarily on waste reduction. Compliance to environmental legislations should not necessarily lead to the creation of additional costs, but can instead provide a secondary source of income. One possible source of increased revenue available to industries is through taking advantage of the incentives awarded by the Clean Development Mechanism (CDM) under the Kyoto Protocol 1997.

The characteristics of wastewater can differ considerably both within and among industries. In the treatment of wastewater, biological treatment appears to be a promising technology to attain revenue from Certified Emission Reduction (CER) credits, more commonly known as carbon credits, as methane gas is generated from anaerobic digestion and can be utilized as renewable energy. With appropriate analysis and environmental control, almost all wastewaters containing biodegradable constituents with a BOD/COD ratio of 0.5 or greater can be treated easily by biological means.

In comparison to other methods of wastewater treatment, it also has the advantages of lower treatment costs with no secondary pollution. Both aerobic and anaerobic processes can be used; the former involves microorganisms (aerobes) which utilise free or dissolved oxygen and convert organic wastes to biomass and CO₂ while in the latter complex organic wastes are degraded into methane, CO₂ and H₂O through three

basic steps (hydrolysis, acidogenesis including acetogenesis and methanogenesis) in the absence of oxygen. Aerobic biological processes are commonly used in the treatment of organic wastewaters for achieving high degree of treatment efficiency, while in anaerobic treatment, considerable progress has been achieved in anaerobic biotechnology for waste treatment based on the concept of resource recovery and utilization while still achieving the objective of pollution control.

Most of the waste treatment processes can be categorised as either physico-chemical or biological processes. Enzymatic treatment falls between these two conventional categories since it involves chemical processes based on the action of biological catalysts. The potential advantages of enzymatic treatment as compared with conventional treatment include: application to bio refractory compounds; operation at high and low contaminant concentrations; operation over a wide range of pH, temperature and salinity; absence of shock loading effects; absence of delays associated with the acclimatization of biomass; reduction in sludge volume and the ease and simplicity of controlling the process.

11.4.1 Types of microbial enzymes:

a) Microbial oxidoreductases: The detoxification of toxic organic compounds by various bacteria and fungi and higher plants through oxidative coupling is mediated with oxidoreductases.

b) Microbial oxygenases: Oxygenases belong to the oxidoreductase group of enzymes and participate in oxidation of reduced substrates by transferring oxygen from molecular oxygen (O_2) utilizing FAD/NADH/NADPH as a co-substrate. These enzymes have an important role in the metabolism of organic compounds as they increase their reactivity or solubility in water or bring about cleavage of the aromatic rings. Oxygenases also mediate dehalogenation reactions of halogenated methanes, ethanes, and ethylenes in association with multifunctional enzymes.

c) Monooxygenases: These enzymes catalyze oxidative reactions of substrates from alkanes to complex molecules as steroids and fatty acids and require only molecular oxygen for their activity. These enzymes require only molecular oxygen for their activities and utilize the substrate as reducing agent. The desulfurization, dehalogenation, denitrification, ammonification, hydroxylation, biotransformation, and

biodegradation of various aromatic and aliphatic compounds are catalyzed by monooxygenases.

d) Microbial dioxygenases:

These dioxygenases catalyze enantio specifically the oxygenation of wide range of substrates. Aromatic compounds are primarily oxidized by dioxygenases, reflecting the applications of dioxygenases in environmental remediation. The catechol dioxygenases are found in the soil bacteria and involved in the transformation of aromatic precursors into aliphatic products.

e) Microbial laccases:

Laccases (p-diphenol: dioxygen oxidoreductase) constitute a family of multicopper oxidases produced by certain plants, fungi, insects, and bacteria, that catalyze the oxidation of a wide range of reduced phenolic and aromatic substrates with concomitant reduction of molecular oxygen to water. Many microorganisms produce intra and extracellular laccases capable of catalyzing the oxidation of ortho and paradiphenols, aminophenols, polyphenols, polyamines, lignins, and aryl diamines as well as some inorganic ions.

f) Microbial peroxidises:

These enzymes catalyze the oxidation of lignin and other phenolic compounds at the expense of hydrogen peroxide (H_2O_2) in the presence of a mediator. Due to their high potential to degrade toxic substances in nature, lignin peroxidase (LiP) and manganese-dependant peroxidase (MnP) have been studied the most.

g) Microbial lipases:

These enzymes can catalyze various reactions such as hydrolysis, interesterification, esterification, alcoholysis and aminolysis. Along with its diagnostic usage in bioremediation, lipase has many potential applications in food, chemical, detergent manufacturing, cosmetic, and paper making industries, but its production cost has restricted its industrial use.

h) Microbial cellulases:

During the enzymatic hydrolysis, cellulose is degraded by the cellulases to reducing sugars that can be fermented by yeasts or bacteria to ethanol. Cellulases cause removal of cellulose microfibrils, which are formed during washing and the use of cotton-based cloths. In paper and pulp industry, cellulases are used for the removal of ink during recycling of paper.

i) Microbial proteases:

Proteases belong to group of enzymes that hydrolyze peptide bonds in aqueous environment and synthesize them in nonaqueous environment. Proteases have wide range of applications in food, leather, detergent, and pharmaceutical industry (Table 2).

Industries and pollutants Aromatic compounds, including phenols and aromatic amines, comprise one of the major classes of pollutants and are stringently regulated in many countries. They are found in the wastewaters of a wide variety of industries including coal conversion, petroleum refining, resins and plastics, wood preservation, metal coating, dyes and other chemicals, textiles, mining and dressing, and pulp and paper. The Kraft process which is widely used in wood pulping leaves 5-8% (w/w) of residual modified lignin in the pulp.

This residual is responsible for the characteristic brown colour of the pulp and is commercially removed by the use of bleaching agents such as chlorine and chlorine oxides. Bleaching operations produce dark brown coloured effluents which contain toxic and mutagenic chlorinated products that constitute an environmental hazard.

There have been a number of studies including the use of peroxidases and laccases for the treatment of bleaching effluents. Pesticides, which include herbicides, insecticides and fungicides, are widely used throughout the world today for crop protection and it is expected that this use will continue to grow. The potential adverse effects that the pesticide industry can have on the environment arise from the disposal of wastes formed during production and formulation of pesticides, detoxification of pesticide containers and spray tanks, and the pollution of surface and groundwater by pesticide runoff. It is estimated that 3 million tons of cyanide are used yearly throughout the world in different industrial processes including the production of chemical intermediates, synthetic fibers, rubber and pharmaceuticals, as well as in ore leaching, coal processing and metal plating.

Enzymes could be used to decrease food wastes via enzymatic processing to yield higher-value by-products and to aid in the clean-up of food waste streams. For the past decade, there has been an increasing interest in the enzymatic hydrolysis of cellulose. This interest stems from the advantages that such a process would offer, namely, the conversion of lignocellulosic and cellulosic wastes to a useful energy source through the production of sugars, ethanol, biogas or other energetic end products.

Heavy metals such as arsenic, copper, cadmium, lead and chromium among others, are dangerous contaminants found in a number of industrial and mining waste streams as well as in solid wastes, municipal sewage sludges and landfill leachate. Surfactants or surface active agents are organic substances that have rather large polar molecules and are basic ingredients of detergents. Surfactants may cause significant pollution problems when high concentrations from shampoo formulation factories, for instance, enter municipal sewerage systems and generate undesirable conditions such as foaming. Wastewaters from dairies and slaughterhouses are often rich in biodegradable organic molecules and nutrients and contain high levels of fats and proteins that have a low biodegradability coefficient.

Some dyestuffs are highly structured polymers and are very difficult to decompose. Many of the dyes are carcinogenic, mutagenic and detrimental to the environment. Leather processing industries are also a huge source of waste which include wastes from untanned hides/skins (trimmings, fleshing wastes), wastes from tanned leather (shaving wastes, buffing dust), wastes from dyed and finished leather (trimmings from leather) toluene and benzene, shown in table 2.

Table 2. Showing microbial enzymes and applications

ENZYMES	APPLICATIONS
Alkyl sulfatase	Surfactant degradation
Amylase: α -amylase, Glucoamylase	Starch hydrolysis and production of glucose
Cellulolytic enzymes: Cellulase, Cellobio-hydrolase, Cellobiase, Exo-1,4-b-D-glucosidase	Hydrolysis of cellulosic sludges from pulp and paper to produce sugars and alcohol, hydrolysis of cellulose in municipal solid waste to sugars and other energy sources

Chitinase	Bioconversion of shellfish waste to N-acetyl glucosamine
Chloro-peroxidase	Oxidation of phenolic compounds
Cyanidase	Cyanide decomposition
Cyanide hydratase	Cyanide hydrolysis
L-Galactono-lactone oxidase	Conversion of galactose from whey hydrolysis to L-ascorbic acid
Laccase	Removal of phenols, decolourization of Kraft bleaching effluents, binding of phenols and aromatic amines with humus
Lactases	Dairy waste processing and production of value-added products
Lignin peroxidase	Removal of phenols and aromatic compounds, decolourization of Kraft bleaching effluents
Lipase	Improved sludge dewatering
Lysozyme	Improved sludge dewatering
Mn-peroxidase	Oxidation of monoaromatic phenols and aromatic dyes
Parathion hydrolase	Hydrolyzation of organophosphate pesticides
Pectin Lyase	Pectin degradation
Peroxidase	Removal of phenols and aromatic amines, decolourization of Kraft bleaching effluents, sludge dewatering
Phosphatase	Removal of heavy metals
Proteases	Solubilisation of fish and meat remains Improving sludge dewatering
Tyrosinase	Removal of phenols

11.4.2. Technologies used for enzymatic treatment of wastewater:

The conformation of enzymes mainly decides their functionality. The conformation might change under harsh physical and chemical conditions of temperature, pH and ionic strength thus altering the function of enzymes. Such drastic conditions are often encountered in effluent streams.

a) Immobilization method

It reduces the loss of enzymes increasing their reusability and also minimizes the chances of loss of enzyme activity under harsh conditions. Use of immobilized enzymes in effluent treatment, as compared to free enzymes, results in multiple advantages like increased stability, reusability, ease of handling, reduction in running cost.

b) Horseradish peroxidase

Covalently immobilized onto magnetic beads retained a high activity and stability and performed higher phenol conversions.

c) Fungal laccase

Introduction of cells or tissues producing an enzyme into the effluent directly is one of the simplest method of enzyme administration to target effluent. This method is employed when suitably adapted strains of microorganisms are used to co metabolize target pollutants.

In cases where the effluent to be treated contains pollutants which cannot support growth, cell free or isolated enzymes are preferred for use over intact microorganism. Nanotechnology is another area which is gaining importance in wastewater treatment. The use of nanoparticles in Reactive Remediation Technology is of great interest to wastewater treatment, since it involves the complete degradation of contaminants to harmless products such as carbon dioxide and water.

The remediation of contaminated wastewater can be achieved by using a combination of enzyme technology and nanotechnology known as the SEN, i.e., Single Enzyme Nanoparticle. A SEN may be described as an enzyme covered by a protective few nanometers thick cage. Cell free crude extracts or purified forms of enzymes like peroxidases, polyphenol oxidases, dehalogenases, hydrolases can be used for SEN synthesis. A variety of recalcitrant compounds such as phenols, polyaromatic dyes, pesticides can be degraded by these enzymes.

Similarly, recalcitrant pollutants in wastewater can also be treated by using nanotubes carrying oxidative enzymes as laccases and peroxidases. Membrane bioreactors

constitute an interesting possibility to be applied in wastewater treatment. The combination of membrane technology with enzymatic reactors for wastewater treatment has led to the development of three generic systems: Immobilised enzyme membrane reactor (IEMR), Extractive membrane bioreactor (EMB) and Direct contact membrane reactor (DCMR).

11.4.3. Advantages of enzymatic treatment over other techniques

The continuously expanding application of enzymes is creating a growing demand for biocatalysts that exhibit improved or new properties. By specifically acting on certain recalcitrant pollutants, enzymes can remove them by precipitation or transformation to other products. Biological (enzymatic) processes have an added advantage over traditional chemical/physical methods as they are regarded as clean and green.

The various physicochemical treatments like chemical precipitation, coagulation, flocculation, floatation, membrane filtration offer advantages like ease of operation and control, flexibility to change in temperature and are rapid but their benefits, however, are outweighed by a number of drawbacks such as their high operational costs due to the chemicals used, high energy consumption and handling costs for sludge disposal. Stringent government policies regarding permitted levels of pollutants, high costs of specialized chemical treatments for pollutant removal and the fact that some of these treatments create additional solid waste has led to the development of many effective, yet simple biological methods.

Enzymes are highly specific and extremely efficient catalysts. They can selectively degrade a target pollutant without affecting the other components in the effluent. More importantly, they can operate under mild reaction conditions, especially temperature and pH. In this respect, enzymes outperform the regular catalysts (transition elements like Cu, Ni etc.). From the environmental perspective, enzymes are more acceptable due to their biodegradability. In the case of reactions wherein the target pollutant is oxidized, the enzyme receives one or more electrons from the substrate and donates these electrons to an electron acceptor.

Hence, at the end of the reaction the enzyme is regenerated and is available for the next catalytic cycle. The biological origin of enzymes reduces their adverse impact on

the environment thereby making enzymatic wastewater treatment an ecologically sustainable technique.

11.5 Advantages of Biotechnological Methods

- Highly sensitive
- Cost-effective
- Real-time & in-situ monitoring
- Environmentally friendly
- Detect bioavailable pollutant fractions
- Portable devices available

11.6. Limitations

- Environmental interference (temperature, pH)
- Short operational lifespan of biological components
- Requirement for calibration
- Genetic stability issues for GMOs

References

Adopted from unit 6 EVS 507, Environmental Microbiology and Biotechnology

Unit 12: Implication of IPRS on commercialization of biotechnology

Unit Structure

12.0 Learning Objectives

12.1 Introduction

12.2 Commercialization Process

12.3 The Innovation cycle

12.4 IP Commercialisation structures

I. Direct Exploitation

II. Licensing

A. Exclusive license:

B. Non-exclusive license:

C. Sole license:

III. Assignment

IV. Spin-Off

V. Joint ventures

12.0 Learning Objectives

After going through this unit you will be able to:

- Understand the benefits of biotechnology to the human society as well to the organisms in the ecosystem
- Describe the role of Innovation cycle
- Explain detailed account on commercialization process and structure
- Understand the need of commercialization of the product

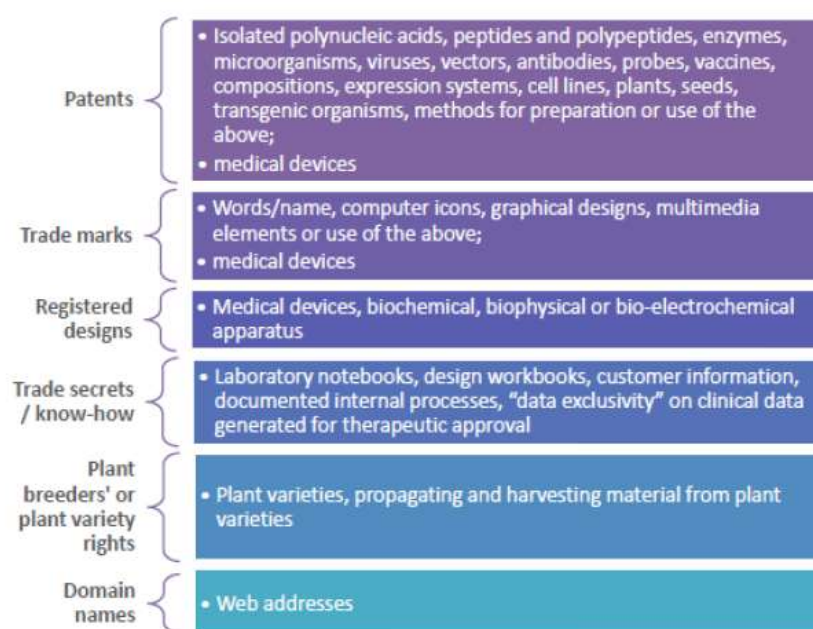
12.1 Introduction

The World Intellectual Property Organisation refers Intellectual Property Rights (IPR) to innovations, inventions, literary and artistic works, symbols, names and images or in general the creations of the mind for ultimate use in commerce. IPR grant statutory rights to the creator to commercialize his/her invention and also prevents others from exploiting it commercially. The role of Intellectual property Right in biotechnology sector is increasing at a rapid pace. The intellectual property commercialization is an important factor in transferring the technology from lab to field or to the business sector.

12.2 Commercialization Process

Commercialization is the process of bringing Intellectual Property to the market so that the patented or IPR product to be exploited in return of business profits and growth. Biotechnology mainly focuses its research on three segments. They include the

Industrial sector, Healthcare sector and Agricultural sector. Biotechnology companies produce many products starting from life saving drugs for human to different varieties of plants, animals, medical instruments, softwares, domain names etc. These different resources may take up any one form of intellectual property. The most commonly used one is the patent. The other forms of IP such as trademarks, tradeseecrets, copyright, domains, plant breeders right, design registry are also used and applied.



Source: iprhelpdesk.eu

The number of biotechnology products invented or innovated is at intensification but patenting living forms and hereditary materials is of prime concern with regard to ethical issues. Commercialization of biotechnology and its products have been acknowledged by public with mixed concern. The laws regarding patenting biotechnology products for commercialization bestow significance to ethical considerations. There are three international intellectual property treaties which are of particular importance for the protection of biotechnology: the Paris Convention for the Protection of Industrial Property (the Paris Convention); the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure (the Deposit Treaty) and the Patent Cooperation Treaty (PCT). With these treaties there are also some restrictions in patenting some biotechnological products.

Life forms such as microorganisms, plants and animals, are not patentable in India under the provisions Indian patent Act (1970). However, patent can be obtained for various biotechnological processes and product applications within the scope of International

conventions. Microorganisms isolated from nature or are obtained by simple manipulations are not patentable. But microorganisms obtained by novel techniques like genetic engineering are patentable. The first patent of GMO (Genetically Modified Organisms) was allowed by US Supreme Court in 1980 as described in utility patent. A maize plant over producing tryptophan amino acid was patented in USA in 1985. This was beginning of patenting of high organisms for patenting. For animals, a patent was granted in 1988 for 'oncomouse', genetically modified mouse in USA. But the patenting of the entire human body in all its developmental phases is not allowed and also processes for modifying the germ line genetic identity of human beings and the use of human embryos for industrial or commercial purposes is not acceptable.

Table 1: List of Patentable and not patentable products

Patentable	Not Patentable
Genes and nucleic acid molecules	Sequences without a known function
Proteins	Genetically modified organism suffering but has no medical benefit
Enzymes	Plant varieties as such
Antibodies	Animal Varieties
Virus and virus sequences	Human embryos
Cells	Process that involve destruction of human embryo
Microorganism	Human germ cells
Transgenic plants and animals	Human animal chimera

12.3 The Innovation cycle

It is a cyclic process which undergoes the steps discovery, validation, development, registration and commercialization. Research for new innovations arises mainly due to the social needs of the society. For example increasing number of cancer patients all over the world has made us to realize to concentrate towards a drug against cancer. Once a social need is there, the researchers develop interest towards the proposal and develop partnerships with public or private institutions. Next the innovation cycle enters into the developmental stage. The development of an innovation requires monetary fund that may be provided by government or public or private parties through partnership.

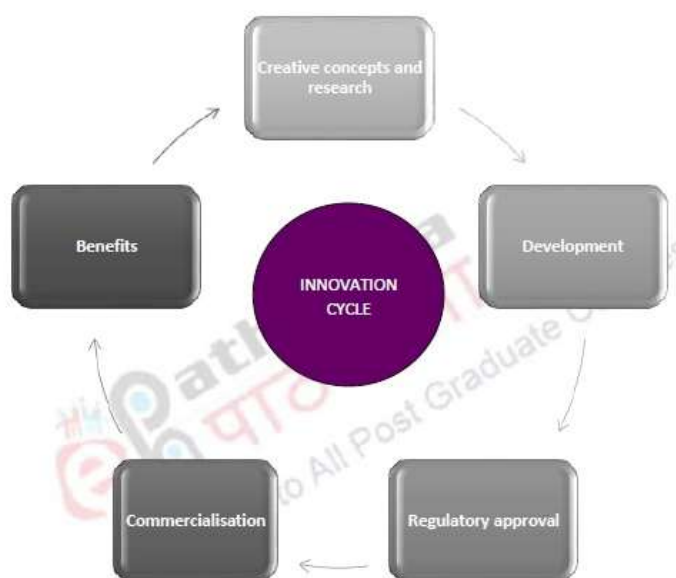


Fig 1: Steps in the development of a commercialization of a product

Once a product is fully developed it is taken for regulatory approval. Approval from the regulating body provides the innovator with a license for commercialization which may be a direct or indirect process. Newly commercialized products provide benefits to the researchers, entrepreneurs and society. The companies which enter into commercialization may be a Biotechnology R&D firm, Small and Medium sized Enterprises (SME) or larger companies. Mostly educational institutes and Universities, collaborate with industries in developing a product. Universities use technology transfer mode to acquire investment from R&D companies and technology can be granted to companies where the company pays royalty to acquire it.

The success of any IP commercialization depends on the choice of the most appropriate commercial tool that is based on

1. The organization's business objectives
2. The form of intellectual property
3. The economic resources at its disposal

12.4 IP Commercialization structures

- Direct Exploitation by Owner
- Licence
- Assignment
- Spin-off Company

- Joint Venture

I. Direct Exploitation

Direct exploitation involves the commercialization by the company on its own after developing the product. The organization choose to commercialize its IP by developing and supplying goods or services based on the IP on its own, instead of have the provision of providing its asset to some external party. Development of a biotechnology product and its commercialization requires research and development, product development, clinical trials and as well techniques for high level production to suit industry and market needs. Generally direct exploitation is less exploited because of the limiting resources of most organization to commercialize the IP.

II. Licensing

Licensing an IP and allowing the third party to utilize the IP is the most common way adopted in many organizations. It is a powerful way of allowing a third party to utilize IP without them taking ownership. License grants the right to access the product for a period of time as agreed by the parties or otherwise would infringe the owners right of IP. It allows for a temporary transfer of ownership and the rights provided to it. License allows both parties to meet their demands with a mutual agreement and it is the most used form of commercialization process. The person granting the license is called the licensor and the person receiving the license is called the licensee. There are different types of licenses:

A. Exclusive license: This involves license to a single company which may extend its commercialization in different fields and territories. This type of license will be mostly provided with high royalty rate. But this will have less competition as well more incentive to develop product for commercialization as only one company is involved.

B. Non-exclusive license: This type of license permits more than one company to use the technology. All the companies that have procured license will have equal benefit and applicable rules as declared by the IP owner. For example like a research tool or one having many fields of use that everyone uses. A non-exclusive license is the most common type of license used within Academia.

C. Sole license: It is a single licensee and the IP owner has the right to use the IP.

III. Assignment

This is a type of commercializing IP. Assignment involves the transfer of the entire rights of the IP property to another individual or company or entity permanently. This form of practice is usually allocated for financial consideration which means the IP property is sold by the IP owner for a lump sum amount. By selling the IP property the owner of IP loses all his ownership rights and cannot claim anything in the future. For example the particular IP product, commercialization may generate a revenue for the buyer three or four times more than the amount he had spent for buying it or the product may an everlasting demand etc...but the IP owner once sells it he/she cannot claim it in future. If the IP owner continues to use it after selling it becomes an infringement on the new owner's rights.

IV. Spin-Off

A spin-off company is a separate company established by an organization for the purposes of undertaking a particular activity, such as the commercialization of a specific IP asset. Spin-Off companies are created using IP. This can be any form of IP ranging from know-how to patents.

V. Joint ventures

A joint venture is a collaboration of two or more parties to undertake a common project or to pursue a specific objective, such as commercializing IP. All parties to the joint venture will contribute their efforts, personnel, financial resources and/or existing IP towards the joint venture project. It can be a unincorporated joint venture or incorporated joint venture.

Summary

Commercialization of biotechnology products with IP enhances the chances for innovating new products, process and also for a revenue generation with these products which will ultimately uplift the society.

Reference

Adopted from epg-pathshala, Paper No. : 05 (Environmental Biotechnology)

Module : 37 (Implication of IPRS on commercialization of biotechnology products).

Unit 13: Forms of Intellectual Property Rights

Unit Structure

13.0 Learning Objectives

13.1 Introduction

13.2 Patents

13.2.1 Types of patent

I. Product patent

II. Process patent

13.2.2 Filing of applications in India

13.3 Trademark

13.3.1 Symbols of trademark

13.4 Trade secret

13.5 Industrial Design

13.6 Geographical Indications

13.6.1 Geographical Indications in India

13.7 Copyrights

13.7.1 Copyright in India

13.8 Plant Variety Protection

13.0 Learning Objectives

After going through this unit you will be able to:

- Describe the concept of Intellectual Property Rights (IPR)
- Explain different types of IPR that includes patent, trademarks, industrial design, Geographical Indications, copyrights and plant variety protection

13.1 Introduction

Human intelligence, creativity and idea has changed the lifestyle of human race in various aspects. A human mind is behind every invention. Inventions mostly arise out of creativity of the inventor. Protecting the invention of the inventor is indispensable. Hence, the basic concept behind Intellectual Property Rights is to protect the invention and the idea. According to World Intellectual Property Organization (WIPO), Intellectual property refers to creations of the mind; literary and artistic works and symbols, names and images for ultimate use in commerce. Intellectual property rights, gives the creator the statutory rights to explore the benefits from the work and also for commercial exploitation. It excludes or prohibits others to use the same commercially or for any other purpose.

Intellectual property is divided in to two categories

1. Industrial property

2. Literary and Artistic works

- Industrial property comprises of ideas or concepts for commercial purpose. This includes patents for rights, trademarks, industrial designs, geographical indications, and trade secrets.
- Literary and Artistic works covers literary works. This includes books, paintings, performances, movies and artistic works

Intellectual property rights in India are governed in accordance with the provisions of the corresponding legislations and can be protected under the following different acts.

1. The Patent Act 1970, Amended 1999, 2002 and 2005
2. The Designs Act, 2000
3. The Copyright Act 1957, Amended in 1984, 1992, 1994 & 1999
4. The Trade Marks Act 1999, Amended in 1994, 1996 and 2000
5. The Geographical Indication Act, 1999
6. Plant Varieties Protection and Farmers Rights Act, 2001

13.2 Patents

Patent is an exclusive monopoly rights granted for an invention by the government under the provision of patent laws for disclosure of the invention. It is a legal document which provides the inventor the rights to commercialize the product or process. Patent prohibits others from using the invention commercially i.e. from manufacturing and selling.

The person patenting a product or process gets benefitted economically and professionally. Patent provides the inventor the monetary benefit for his/her invention. It provides the patentee the recognition for his creativity and also protection of the invention. The exclusive monopoly rights and economical benefit of patent motivates research and also enhances quality of human life.

A patent can be filed by the inventor who invented the invention or his assignee or by legal representative of any deceased inventor. Patent application can be filed by the inventor himself or by patent agent. A legal representative or attorney on behalf of the inventor can also file the application. Patent can be obtained only when it's a new product or process or it should involve a inventive step and which has industrial application. The invention should be novel and useful.

A patent is provided for an invention. An invention means a new product or process which does not exist already. The invention should have novelty, inventiveness and

usefulness. If the invention is a process, it should be compiled of inventive steps. The invention should have industrial application otherwise it may not be patented. Everything cannot be patented and patenting has restrictions too. Patent cannot be obtained if it is aimed in bringing destruction to human beings, public health and if contrary to universal laws. Mere discovery of a how to use principle cannot be patented. Scientific principles, theories, traditional knowledge, methods in agriculture cannot be patented. Rearrangement of machine parts, formation of a new product with already existing chemicals, computer programs cannot be patented.

13.2.1 Types of patent

Patent can be obtained for product or process. Based upon this patents may be divided into two types

I. Product patent:

II. Process patent

I. Product patent

It provides the owner the exclusive rights to manufacture and sell the product while restricting others from the same. It gives the patentee the monopoly rights to produce, manufacture and sell the product.

II. Process patent

This type of patent gives the inventor or the patentee the monopoly rights to produce a product by a process while restricting others from doing so. This usually involves processes associated with food, drugs or medicines.

13.2.2 Filing of applications in India

Application for patent can be filed in any one of the Indian Patent office located at Chennai, Delhi, Mumbai or Kolkata.

Documents required for filing a patent application

The following documents are required to file a application of patent for a product or process.

1. The application form - FORM 1

2. The Patent specification – Provisional or Complete – FORM 2

- A patent specification contains the details of the invention. For filing patent, a patent specification is a must. The specifications are of two types.

- Provisional specification is a document that contains description of the nature of invention but does not contain the claims or details regarding the invention. It is filed to claim the priority date of an invention.
- Complete specification is a document that contains a detailed description of the claims and drawings of the invention. It is filed within 12 months from the date of Provisional specification. This specification also includes details regarding prior art.

3. Drawings if any

4. Undertaking under Section 8 – FORM 3

5. Power of Authority – This is needed only if the patent is filed through a patent attorney

Term of Patent

The duration of a patent is for 20 years. The date of patent is calculated from the date of filing of the application for patent. For renewal and maintenance of the patent, the patentee has to pay annual fee every year without which the patent term may expire.

Patents can also be classified as:

- Utility patent – for a new machine, process, article or any new improvement
- Design Patent – for a design
- Plant Patent – for a new plant variety

13.3 Trademark

Trademark is a design, sign or expression that is associated with a product. Trademark helps to protect the possessor his product and ideas and avert others from misusing it. A trademark used to identify a service is known as the service mark. Trademarks help an individual to identify the products or services of a particular company and to distinguish it from other goods. Trademark includes word, letter, figure, number, name, shape, color, design or the combination of these. Examples:

- The apple logo of laptop
- The logo of adidas
- The f in facebook
- The M in Mc donalds



Source: <https://featuredcontent.psychonomic.org/when-south-butt-makes-you-think-of-buying-north-face/>

13.3.1 Symbols of trademark

- TM – an unregistered trademark which is used to promote goods
- SM – an unregistered service mark which is used to promote services
- R – registered trademark

A registered trademark helps to have legal security for the mark throughout the country. In India, registration of trademarks is governed by the Trademarks Act 1999. The main objective of the act is to register the trademarks and protect trademarks for goods and services. It also intends to prevent counterfeit of the trademark. An amendment of this act was done in 2010 and called the Trademarks (Amendment) Act, 2010. For registration of trademarks in India applications are to be submitted to the Controller general of Patents, Designs and Trademarks, Ministry of Commerce and Industry, Government of India. Trademarks can also be registered worldwide using Madrid – the International Trademark system. Registering trademarks using Madrid system gives protection for that trademark in nearly 116 countries. India is a member of the Madrid protocol and so citizen of is eligible to register the trademark in the Madrid system. Nationals of nonmember countries cannot register their trademark under Madrid system.

Other trademarks

I. Collective trademark

It is a trademark used by a particular association or organization that indicates membership in the group. The members of the group use the mark for their goods and services and distinguish goods and services of members of an association from those that of nonmembers. Collective marks are usually set with a high level of quality or

standards or geographical location or any other characteristic that differentiates them clearly. Example:

CA - Institute of Chartered Accountants

II. Certificate mark

Certificate marks are marks that denote that the product comply the defined standard as set by the regulatory body or by the owner of the certification mark Examples:

BIS Hallmark – Certificate for purity of gold

ISO 14000 – Certificate of standards for an effective Environmental management System

Agmark – Certificate for agricultural products

FPO mark – Certificate mark for all processed fruit products

Silk Mark – Certificate for silk textiles

Trademark Protection

All countries protect trademarks through their national or regional office. However the World Intellectual Property Organization has brought an international registration system for trademarks through Madrid Protocol. Registering trademarks using Madrid system gives protection for that trademark in nearly 116 countries. Trade-Related Aspects of Intellectual Property Rights (TRIPS) also protects trademarks of member countries which have been emphasized in Articles 15 to 20 of TRIPS agreement.

In India, trademarks are protected legally by Trademarks Act, 1999. This act registers trademarks, provides protection of trademarks for goods and also prevents fraudulent use of the mark. New elements are added in Trademark Act, 1999 and came into force since 15 September, 2003 replacing Trade and Merchandise Marks Act, 1958. The changes include the addition of shape of goods, packaging and combination of colors as trademarks, service mark, collective mark and certificate mark. Also the renewal period year changed from 7 years to 10 years. The Trademarks Act was amended in the year 2010.

13.4 Trade secret

A trade secret is valuable information that deserves secrecy because of its commercial value. It is kept confidential by the holder or the authorized organization or the company. Trade secret includes formula, instrument, process, pattern, program, technique or

information. Generally trade secrets are not accessible to public and anyone who unlawfully uses it would be considered as an unfair practice and breach of confidence. North American Free Trade Agreement (NAFTA) defines a trade secret as “information having commercial value, which is not in the public domain and for which reasonable steps have been taken to maintain its secrecy.” According to Trade-Related Aspects of Intellectual Property Rights (TRIPS Agreement), trade secret should be kept secret, have commercial value and have been subject to reasonable steps such as confidential agreements, non disclosure agreements, work for hire to keep the information in secret.

International treaties for trade secret

Trade Secrets are protected in different countries by a large number of agreements. Some of them include

- North American Free Trade Agreement (NAFTA)
- General Agreement on Tariffs and Trade (GATT)
- Related Aspects of Intellectual Property Rights (TRIPS)

According to the Article 39 of TRIPS agreement trade secrets are protected lawfully and any approach contrary to honest commercial practice would be considered as breach of confidence or contract.

India and trade secret

In India, there is no specific legislation for trade secret but they are protected by legislations that have connection with trade secrets. Some of them are,

1. Copyright Act, 1957
2. The Designs Act, 2000
3. The Information Technology Act, 2000
4. Indian Penal Code
5. The Indian Contract Act
6. The Competition Act, 2002
7. Civil Procedure Code
8. Criminal Procedure Code

13.5 Industrial Design

Industrial design right is a form of an intellectual property right. The design pattern may be three dimensional or two dimensional features. The design includes the creation of the shape, configuration, pattern, color, lines containing aesthetic value. Industrial

designs are employed in industrial products, electrical appliances, jewellery, medical instruments, architectural designs and handicrafts.

In India, Industrial designs are protected by The Designs Act, 2000. This act confers full rights to the proprietor for the created new design. The owner of the design can license or sell his design for royalty. The application for the design registration is processed to, The Controller of Designs, Indian Patent office. The Design Wing of the patent office may be approached to know whether a design is registered previously or not. The Patent office, Kolkatta maintains a document of registered designs called – ‘The Register of Designs’ which contains all the details regarding registered designs. Once a design is registered a Certificate of Registration can be obtained by paying the required fee for legal proceeding.

The registration of a design confers copyright to the design to apply in a product. The Registration of the design is for 10 years from the date of registration and renewable for another five years. The Design Rules, 2001 came into force on May 11, 2001 and was amended by ‘The Design (Amendment) Rules’ on 2008 and The Design (Amendment) Rules’ 2014. International registration of Designs is administered by the WIPO treaty – The Hague Agreement Concerning the International Registration.

13.6 Geographical Indications

Geographical Indication is a sign used on goods that have a specific geographical origin which may be a country or a place and possess specific quality or status or uniqueness because of its origin. Geographical Indications are used for agricultural products, wine and spirit drinks, handicrafts, food items and also for industrial products.

The WTO Agreement on Trade-Related Aspects of Intellectual Property Rights ("TRIPS") defines "geographical indications" as indications that identify a good as "originating in the territory of a Member, or a region or locality in that territory, where a given quality, reputation or other characteristic of the good is essentially attributable to its geographic origin.

TRIPS

TRIPS agreement gives protection against misleading of the public, act of unfair competition and turn down to register an existing trademark. TRIPS agreement through Articles 22 to 24 protects Geographical Indications of member countries and makes it mandatory for every nation to protect its own GI. India being a member of TRIPS is required to protect GI and hence enacted “The Geographical Indications of Goods

(Registration and Protection) Act, 1999. The act aims to protect geographical indications against unauthorized users, provide legal protection in India and other WTO countries.

13.6.1 Geographical Indications in India

In India, Darjeeling tea was awarded the first GI tag in the year 2004 -05. List of a few GI products of India is given below:

S.No	Geographical Indication	Type	State
1	Darjeeling Tea (word & logo)	Agricultural	West Bengal
2	Mysore silk	Handicraft	Karnataka
3	Kancheepuram Silk	Handicraft	Tamil Nadu
4	Nashik valley wine	Manufactured	Maharashtra
5	Banaganapalli Mangoes	Agricultural	Andhra Pradesh
6	Mysore Sandal Soap	Manufactured	Karnataka
7	Madhubani paintings	Handicraft	Bihar
8	Malabar Pepper	Agricultural	Kerala
9	Bhalia Wheat	Agricultural	Gujarat
10	Kashmir Walnut Wood Carving	Handicraft	Jammu & Kashmir

GI list of Indian products is on rise every year and it has reached 301 till now. The Hyderabad Haleem is the only Indian dish to have a GI status. The list of products that have received GI tag from April 2017, includes the following

- Banaganapalle Mangoes of Andhra Pradesh
- Pochampally Ikat (Logo) of Telangana
- Gobindobhog Rice of West Bengal
- Durgi Stone Carvings of Andhra Pradesh
- Etikoppaka Toys of Andhra Pradesh
- Tulapanji Rice of West Bengal
- Chakshesang Shawl of Nagaland
- The first GI product of Uttarakhand state is the Uttarakhand Tejpatta

13.7 Copyrights

Copyright is a form of intellectual property that grants the owner the exclusive rights to use and distribute it to the public. Copyright provides the owner the rights to reproduce, to sell and also to modify it to other form of artistic work, for example the novels - Harry Potter fantasy series is authored by J K Rowling and the novels are created as films by Warner Bros who have purchased the film rights of the novel from Rowling. Copyrights protects the original work of the author which includes literary works such as plays, poems, novels and artistic works like music, songs, movies, drama. Copyright is also granted for architectural works, software, drawings and photos.

13.7.1 Copyright in India

In India, original works are protected legally with the aid of The Copyright Act 1957. This Act came in to effect from January 1958. This act protects the literary works, artistic works, films, recordings from unauthorized users. Copyright protects the expressions and not the ideas. The Copyright Act has been amended six times since 1957. The amendment years include 1983, 1984, 1991, 1994, 1999 and 2012. Apart from this Copyright rules was framed in 2013 and the same has been amended on 2016. The copyright protection term depends on the type of work. Literary or artistic or musical works have copyright protection throughout the life of the author and extends till 60 years from the year of death of the author. Performers and broadcast channels have 25 years. For software copyright is for 60 years.

Copyright of Indian Copyright Act is valid only in India and not applicable in other countries. Indian works can be protected in foreign countries by the following conventions.

- Berne Convention for the Protection of Literary and Artistic works.
- Universal Copyright Convention.
- Convention for the Protection of Producers of Phonograms against Unauthorized duplication of their Phonograms.
- Multilateral Convention for the Avoidance of Double Taxation of Copyright Royalties.
- Trade Related Aspects of Intellectual Property Rights (TRIPS) Agreement.

India is a signatory in all the above conventions.

Copyright in cyberspace is an imminent issue and so the World Intellectual Property organization (WIPO) has come up with two treaties to protect creative works on the internet. The treaties include WIPO Copyright Treaty and the WIPO Performances and Phonograms Treaty (WPPT), known as the “Internet Treaties”. These treaties prevent unauthorized use of creative works on the internet.

13.8 Plant Variety Protection

Plant Variety Protection and Farmers Rights Act provide the breeders to protect new variety of plant or crop. The rights under this act include the right to produce, sell, market, directly or export a variety. Farmers can use the registered variety of seed in an unrestricted manner. They can sell, save, sow or re-sow seeds. Farmers varieties can be registered without any fee and the period of protection for field crops cover 15 years and for trees and vines registration covers for 18 years. Annual fee has to be paid to uphold registration and also renewal fee for extended period of registration. Internationally, plant varieties are protected by the International Union for the protection of new varieties of Plants established by The International Convention for the protection of new varieties of Plants. The objective of the convention is to protect new plant varieties by IPR.

Summary

Intellectual property rights provide the inventor or the creator their credit and also the invented products entry in to the commercial sector for a commercial benefit and also protection of the inventors creativity. The government also through its statutory laws protects the inventions and creations of the inventors. Different types of IPR and the statutory laws governing them ought to be known to protect the inventor’s creativity or knowledge.

Reference

Adopted from epg pathshala, Paper No. : 05 (Environmental Biotechnology) and Module number and name: 36 (Intellectual Properties)

Unit 14: Release of GEOs

Unit Structure

14.0 Learning Objectives

14.1 Introduction

14.2 Genetic Engineering

14.3 Genetical Engineered Organisms

14.4 Environmental release of GEOs and its consequences

14.5 Application of GEOs

14.6 Ethical issues of safety of GEOs.

14.0 Learning Objectives

After completing this unit, learners shall be able to:

- Understand the genetic engineering organisms (GEOs)
- Discuss the application of genetic engineering organisms (GEOs).
- Understand the environmental issues related to GEOs
- Understand the ethical issues related to GEOs

14.1 Introduction

As you know that many sciences are useful to human kind and genetic engineering is one of them. Genetic engineering is also called recombinant DNA technology. It involves the group of techniques used to cut up and join together genetic material, especially DNA from different biological species. Genetic engineering is literally technology of biological sciences. Many animal and plant have been developed due to genetic engineering. Genetic engineering also produces different types of microbes which can degrade the environmental pollutants. The term 'genetic engineering' stands for human alteration of the genetic code of an organism, so that its biosynthetic properties are changed. An organism that is generated through genetic engineering is considered to be genetically modified (GM) and the resulting entity is a genetically modified organism (GMO). The first GMO was a bacterium generated by **Herbert Boyer** and **Stanley Cohen** in 1973. Rudolf Jaenisch created the first GM animal when he inserted foreign DNA into a mouse in 1974. Genetically engineered human insulin was produced in 1978 and insulin-producing bacteria were commercialized in 1982.

Genetically modified food has been commercialized since 1994, with the release of the Flavr Savr tomato. The Flavr Savr was engineered to have a longer shelf life, but most current GM crops are modified to increase resistance to insects and herbicides. Therefore, genetic engineering is boon to human being. In this unit you will learn about the genetic engineering, selection of cloning and genes, genetic modifies organisms and their applications.

14.2 Genetic Engineering

Genetic engineering, also called genetic modification or genetic manipulation, is the direct manipulation of an organism's genes using biotechnology. It is a set of technologies used to change the genetic makeup of cells, including the transfer of genes within and across species boundaries to produce improved or novel organisms. New DNA is obtained by either isolating or copying the genetic material of interest using recombinant DNA methods or by artificially synthesizing the DNA. A construct is usually created and used to insert this DNA into the host organism. Paul Berg is regarded as father of genetic engineering. The first recombinant DNA molecule was made by Paul Berg in 1972 by combining DNA from the monkey virus SV40 with the lambda virus. As well as inserting genes, the process can be used to remove, or "knock out", genes. The new DNA can be inserted randomly, or targeted to a specific part of the genome.

Genetic engineering has been applied in numerous fields including research, medicine, industrial biotechnology and agriculture. In research GMOs are used to study gene function and expression through loss of function, gain of function, tracking and expression experiments. The same techniques that are used to produce drugs can also have industrial applications such as producing enzymes for laundry detergent, cheeses and other products.

The rise of commercialized GMOs has provided economic benefit to farmers in many countries. Genetic engineering is a process that alters the genetic structure of an organism by either removing or introducing DNA. Unlike traditional animal and plant breeding, which involves doing multiple crosses and then selecting for the organism with the desired phenotype, genetic engineering takes the gene directly from one organism and inserts it in the other. This is much faster, can be used to insert any

genes from any organism and prevents other undesirable genes from also being inserted.

The DNA can be introduced directly into the host organism or into a cell that is then fused or hybridised with the host. This relies on recombinant nucleic acid techniques to form new combinations of heritable genetic material followed by the incorporation of that material either indirectly through a vector system or directly through micro-injection, macro-injection or micro-encapsulation.

Plants, animals or micro organisms that have been changed through genetic engineering are termed genetically modified organisms or GMOs. If genetic material from another species is added to the host, the resulting organism is called transgenic. If genetic material from the same species or a species that can naturally breed with the host is used the resulting organism is called cisgenic. If genetic engineering is used to remove genetic material from the target organism the resulting organism is termed a knockout organism. The deliberate modification in genetic material of an organism by changing the nucleic acid directly is called genetic engineering. Genetic engineering holds the potential to extend the range and power of every parameter of biotechnology.

The techniques will be used widely to improve the existing microbial processes through improving the existing cultures and discarding the unwanted by-products. Of course, during this decade the rDNA technology will establish fully the basis of new microorganisms with new metabolic properties.

14.3 Genetical Engineered Organisms

Genetically Modified Organisms (GMOs) include plants, animals and microbes which have been engineered in the laboratory. In conventional livestock production, crop farming, and even pet breeding, it has long been the practice to breed select individuals of a species in order to produce offspring that have desirable traits. In genetic modification, however, recombinant genetic technologies are employed to produce organisms whose genomes have been precisely altered at the molecular level, usually by the inclusion of genes from unrelated species of organisms that code for traits that would not be obtained easily through conventional selective breeding. It must isolate the gene they want to insert into the host organism and combine it with other genetic elements, including a promoter and terminator region and often a

selectable marker. A number of techniques are available for inserting the isolated gene into the host genome. Recent advancements using genome editing techniques, notably CRISPR, have made the production of GMO's much simpler. **Herbert Boyer** and **Stanley Cohen** made the first genetically modified organism in 1973, bacteria resistant to the antibiotic kanamycin. The first genetically modified animal, a mouse, which was developed by **Rudolf Jaenisch** in the year 1974. In 1994 the **Flavr Savr tomato** the first commercialized genetically modified food.

Bacteria are the simplest organisms to engineer and have been used for research, food production, industrial protein purification, agriculture, bioremediation etc. There is potential to use them for environmental purification or as medicine. Fungi have been engineered with much the same goals. Viruses play an important role as vectors for inserting gene into host. This use is especially relevant to human gene therapy. There are proposals to remove the virulent genes from viruses to create vaccines. Plants have been engineered for scientific research, to create new colors in plants, deliver vaccines and improved crops. The majority of GEOs are engineered for herbicide tolerance or insect resistance. Golden rice has been engineered with three genes that increase its nutritional value. GM crops are as bioreactors for the production of biopharmaceuticals, biofuels or medicines. Application of GEOs are given table 1.

Table 1. Showing important GEOs (GMOs) and their applications

Name of GMO	Application
Microorganisms	
<i>Aspergillus</i>	Food fermentations
<i>Escherichia coli</i>	Therapeutic protein production
<i>Streptomyces</i>	Antibiotics, antitumor
<i>Bacillus</i>	Industrial enzymes, Fine chemicals, Antibiotics, Insecticides
Plants	
Cotton	Herbicide tolerant, Insect tolerant
Ornamental Flowers	Altered colour Longer Shelf life
Maize	Herbicide tolerant, Insect tolerant
Oil Seed rape	Herbicide tolerant

Potato	Increase Starch Content
Soybean	Herbicide tolerant
Sugarbeet	Herbicide tolerant
Golden Rice	Enhance Vitamin A
Papaya	Virus Resistance
Animals	
Genetically Modified Salmon Fish	High Production of fish
Genetically Modified Pig (ENVIROPIG)	Less phosphorus consumed by the pig.
Genetically Modified Mosquitoes	Control Disease in Human

Fauna are generally much harder to transform as compared to plants. The massive majority of animals or fauna are still at the research stage. Mammals are the best model organisms for humans, making ones genetically engineered to resemble serious human diseases important to the discovery and development of treatments. Human proteins expressed in mammals are more likely to be similar to their natural counterparts than those expressed in plants or microorganisms. Livestock are modified with the intention of improving economically important traits such as growth-rate, quality of meat, milk composition, disease resistance and survival. Genetically modified fish are used for scientific research, as pets and as a food source. Genetic engineering has been proposed as a method to control population of insects specially mosquitoes, a vector for many deadly diseases.

As only a single cell is transformed with genetic material, the organism must be regenerated from that single cell. In plants this is accomplished through tissue culture. In animals it is necessary to ensure that the inserted DNA is present in the embryonic stem cells. Further testing using PCR, Southern hybridization, and DNA sequencing is conducted to confirm that an organism contains the new desirable genes.

Gene targeting techniques, which create double-stranded breaks and takes advantage on the cells natural homologous recombination repair systems, have been developed to target insertion to exact locations. Genome editing uses artificially engineered

nucleases that create breaks at specific points. There are four families of engineered nucleases: meganucleases, zinc finger nucleases, transcription activator-like effector nucleases. etc. Transcription activator-like effector (TALENs) has greater target specificity, while CRISPR is easier to design and more efficient.

14.4 Environmental release of GEOs and its consequences

Survival of GEOs is one of the major issues for biotechnologists. As you know that Genetically Engineered Organisms or Genetically Modified Organisms are those plants and animals which are produced by genetic engineer. These organisms can survive in extreme environmental conditions. These can survive in drought, low or high temperature, xeric condition. The survivorship of these organisms totally depends on the genes. Genes make them adapted to different environmental conditions. The development of gene transfer system together with combined “omics” platforms has facilitated the understanding of physiology and biochemistry of plants adaptive responses to unfavourable conditions. Gene transfer responses include profound morphological, physiological and metabolic changes which depend on the induction and repression of numerous genes and allow plants to survive and reproduce under unfavourable condition.

Bacteria were the first organisms to be genetically modified in the laboratory, due to the relative ease of modifying their chromosomes. This ease made them important tools for the creation of other GMOs. Genes and other genetic information from a wide range of organisms can be added to a plasmid and inserted into bacteria for storage and modification. Bacteria are cheap, easy to grow, clonal, multiply quickly and can be stored at -80°C . Once a gene is isolated it can be stored inside the bacteria. A large number of custom plasmids make manipulating DNA extracted from bacteria relatively easy.

The simplest model organisms come from bacteria. Scientists can easily manipulate and combine genes within the bacteria to create novel or disrupted proteins and observe the effect this has on various molecular systems. Researchers have combined the genes from bacteria and archaea, leading to insights on how these two diverged in the past.

For over a century bacteria have been used in agriculture. Crops have been inoculated with Rhizobia to increase their production or to allow them to be grown outside their original habitat. Survival of bacteria in this environment is easy. Application of *Bacillus thuringiensis* (Bt) and other bacteria can help protect crops from insect infestation and plant diseases. Markers have also been added to aid in tracing the spread of the bacteria. The bacteria that naturally colonize certain crops have also been modified, in some cases to express the Bt genes responsible for pest resistance. *Pseudomonas* strains of bacteria cause frost damage by nucleating water into ice crystals around themselves.

Most vaccines consist of viruses that have been attenuated, disabled, weakened or killed in some way so that their virulent properties are no longer effective. Genetic engineering could theoretically be used to create viruses with the virulent genes removed. This does not affect the viruses infectivity, invokes a natural immune response and there is no chance that they will regain their virulence function, which can occur with some other vaccines. As such they are generally considered safer and more efficient than conventional vaccines, although concerns remain over non-target infection, potential side effects and horizontal gene transfer to other viruses. Another potential approach is to use vectors to create novel vaccines for diseases that have no vaccines available or the vaccines that do not work effectively, such as AIDS, malaria, and tuberculosis. The most effective vaccine against Tuberculosis, the *Bacillus Calmette–Guérin* (BCG) vaccine, only provides partial protection.

Plants have been engineered for scientific research, to display new flower colors, deliver vaccines and to create enhanced crops. Many plants are pluripotent, meaning that a single cell from a mature plant can be harvested and under the right conditions can develop into a new plant. This ability can be taken advantage of by genetic engineers; by selecting for cells that have been successfully transformed in an adult plant a new plant can then be grown that contains the transgene in every cell through a process known as tissue culture.

Much of the advances in the field of genetic engineering has come from experimentation with tobacco. Major advances in tissue culture and plant cellular mechanisms for a wide range of plants has originated from systems developed in tobacco. It was the first plant to be altered using genetic engineering and is considered

a model organism for not only genetic engineering, but a range of other fields. As such the transgenic tools and procedures are well established making tobacco one of the easiest plants to transform. Another major model organism relevant to genetic engineering is *Arabidopsis thaliana*. Its small genome and short life cycle makes it easy to manipulate and it contains many homologues to important crop species. It was the first plant sequenced, has a host of online resources available and can be transformed by simply dipping a flower in a transformed *Agrobacterium* solution.

There are three main aims to agricultural advancement; increased production, improved conditions for agricultural workers and sustainability. GM crops contribute by improving harvests through reducing insect pressure, increasing nutrient value and tolerating different abiotic stresses. Despite this potential, as of 2018, the commercialized crops are limited mostly to cash crops like cotton, soybean, maize and canola and the vast majority of the introduced traits provide either herbicide tolerance or insect resistance. Geographically though the spread has been uneven, with strong growth in the Americas and parts of Asia and little in Europe and Africa. Its socioeconomic spread has been more even, with approximately 54% of worldwide GM crops grown in developing countries in 2013.

The majority of GM crops have been modified to be resistant to selected herbicides; usually a glyphosate or glufosinate based one. Genetically modified crops engineered to resist herbicides are now more available than conventionally bred resistant varieties; in the USA 93% of soybeans and most of the GM maize grown is glyphosate tolerant. Most currently available genes used to engineer insect resistance come from the *Bacillus thuringiensis* bacterium and code for delta endotoxins. A few use the genes that encode for vegetative insecticidal proteins. The only gene commercially used to provide insect protection that does not originate from *B. thuringiensis* is the Cowpea trypsin inhibitor (CpTI). CpTI was first approved for use cotton in 1999 and is currently undergoing trials in rice. Less than one percent of GM crops contained other traits, which include providing virus resistance, delaying senescence and altering the plants composition. Golden rice is the most well known GM crop that is aimed at increasing nutrient value. It has been engineered with three genes that biosynthesise beta-carotene, a precursor of vitamin A, in the edible parts of rice.

Plants and plant cells have been genetically engineered for production of biopharmaceuticals in bioreactors, a process known as pharming. Work has been done with duckweed *Lemna minor*, the algae *Chlamydomonas reinhardtii* and the moss *Physcomitrella patens*. Biopharmaceuticals produced include cytokines, hormones, antibodies, enzymes and vaccines, most of which are accumulated in the plant seeds. Many drugs also contain natural plant ingredients and the pathways that lead to their production have been genetically altered or transferred to other plant species to produce greater volume. Other options for bioreactors are biopolymers and biofuels. Unlike bacteria, plants can modify the proteins post-translationally, allowing them to make more complex molecules. They also pose less risk of being contaminated. Therapeutics have been cultured in transgenic carrot and tobacco cells, including a drug treatment for Gaucher's disease.

The vast majority of genetically modified animals are at the research stage with the number close to entering the market remaining small. As of 2018 only three genetically modified animals have been approved, all in the USA. A goat and a chicken have been engineered to produce medicines and a salmon that has increased growth. Despite the differences and difficulties in modifying them, the end aims are much the same as for plants. GM animals are created for research purposes, production of industrial or therapeutic products, agricultural uses or improving their health. There is also a market for creating genetically modified pets. The process of genetically engineering mammals is slow, tedious, and expensive. However, new technologies are making genetic modifications easier and more precise. The first transgenic mammals were produced by injecting viral DNA into embryos and then implanting the embryos in females. The embryo would develop and it would be hoped that some of the genetic material would be incorporated into the reproductive cells. Then researchers would have to wait until the animal reached breeding age and then offspring would be screened for presence of the gene in every cell. The development of the CRISPR-Cas9 gene editing system as a cheap and fast way of directly modifying germ cells, effectively halving the amount of time needed to develop genetically modified mammals.

Significance: Significance of GEOs includes bioremediation, where the bacteria are used to convert pollutants into a less toxic form. Genetic engineering can increase the levels of the enzymes used to degrade a toxin or to make the bacteria more stable

under environmental conditions. Bio art has also been created using genetically modified bacteria.

Domestic animals are modified with the intention of improving economically important traits such as growth-rate, quality of meat, milk composition, disease resistance and survival. Animals have been engineered to grow faster, be healthier and resist diseases. Modifications have also improved the wool production of sheep and udder health of cows. Goats have been genetically engineered to produce milk with strong spiderweb-like silk proteins in their milk. They could reduce water pollution since they excrete 30 to 70% less phosphorus in manure. Dairy cows have been genetically engineered to produce milk that would be the same as human breast milk.

Gene therapy uses genetically modified viruses to deliver genes which can cure disease in humans. Although gene therapy is still relatively new, it has had some successes. It has been used to treat genetic disorders such as severe combined immunodeficiency, and Leber's congenital amaurosis. Treatments are also being developed for a range of other currently incurable diseases, such as cystic fibrosis, sickle cell anemia, Parkinson's disease, cancer, diabetes, heart disease and muscular dystrophy. Germline gene therapy results in any change being inheritable, which has raised concerns within the scientific community. Genetically modified fish are used for scientific research, as pets and as a food source. Aquaculture is a growing industry, currently providing over half the consumed fish worldwide. Two species of fish, zebrafish and medaka, are most commonly modified because they have optically clear chorion (membranes in the egg), rapidly develop, and the one-cell embryo is easy to see and microinject with transgenic DNA. Zebrafish are model organisms for developmental processes, regeneration, genetics, behaviour, disease mechanisms and toxicity testing.

14.5 Application of GEOs

There are various applications of GEOs which are summarized in Figure 1 and also discussed below:

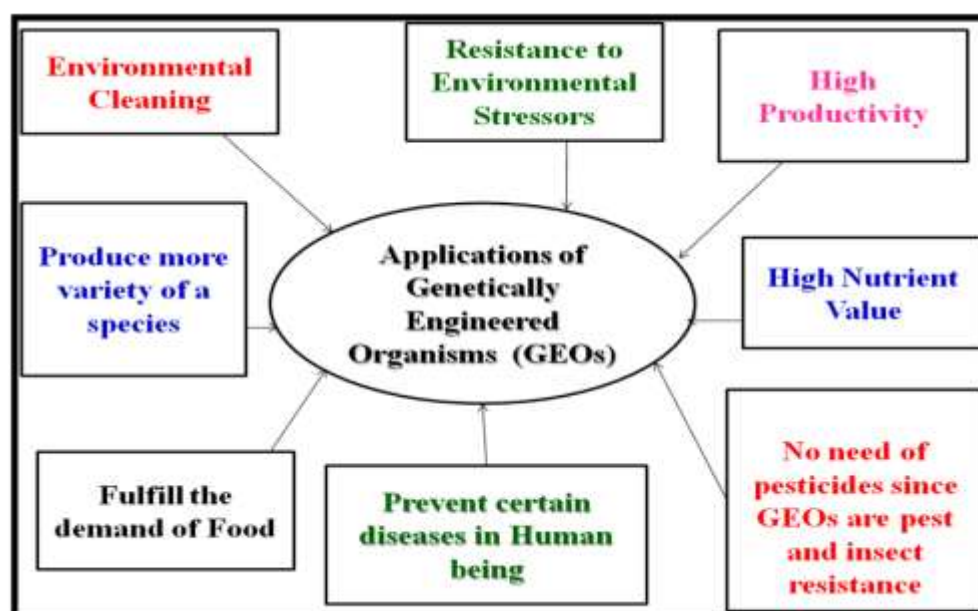


Figure 1. Showing applications of Genetically Engineered Organisms (GEOs)

As you know genetically modified organism is an organism whose genetic material or sequence has been altered. They are the sources of genetically modified foods. Genetic alteration can occur naturally or artificially. Naturally, genetically modified organisms occur as an impact of other factors apart from human will. For instance, the penetration of a gene into a foreign cell could result to development of a naturally genetically modified organism. Artificially, GEOs have been developed depending on desired characteristic. Numerous genetic engineering techniques have been developed and used in the development of these organisms. These techniques which are based on circumstances which could occur naturally include; deletion, insertion and mutation of specific genes. Some organisms whose genetic makeup has been successfully modified include bacteria, yeasts, plants, fish and mammals. Other than food, GEOs have been used in the production of other commercially products like proteins.

Genetically modified bacteria have numerous benefits. For example there has been production of large amounts of commercially viable human proteins that is used in production of medicine. Insulin, a protein used to treat diabetes has been easily produced by these bacteria. Human growth hormone, used to treat dwarfism has also been produced. Other genetically modified organisms have been used in production of

enzymes and clotting factors. For example, a drug Atryn, an anticoagulant has been obtained from the milk of a genetically modified goat.

Organisms that are of medicinal value have been cultivated in bioreactors rather than in the fields. Proper examples include; *Chlamydomonas reinhardtii* which is an alga and *Physcomitrella patens* which is a moss. Cultivating of organisms in bioreactors allows close monitoring so as to provide the required results. Other genetically modified organisms have been used as models in researches that are important in the development and establishment of treatments for different diseases.

In agriculture, crops with better traits like yield, vigor and resistance to pests and diseases have been developed through genetic engineering. A good example is the genetically modified papaya grown in Hawaii. The modified papaya is resistant to the Ring spot virus and it also produces the *Bacillus thuringiensis* toxin which is considered not harmful to human but plays a great role as an insecticide. Crops have been engineered to enhance production with longer shelf life. For example, corn and the poplars have been genetically modified and used to produce biofuel which have been used as an efficient substitute of petroleum products. There is development of animals with traits that are desirable compared with their natural counterparts. For example, Yorkshire pigs have been genetically modified to produce a type of pig that digests plant phosphorus. They are capable of producing enzyme phytase in their saliva, which digests phosphorous unlike their counterpart pigs. According to Centre for ecogenetics and Environmental Health "USA is the largest producer of GMOs, followed by, Brazil, Argentina, India, Canada, China, Paraguay, South Africa, Uruguay and Bolivia".

Transgenics have been produced in a variety of animal species, e.g., mice (including rats), rabbits, swine, sheep, goat, cattle, poultry, fish, amphibians, insects and nematodes. However, these activities are so far limited to experimental stages, and production technologies based on transgenic animals have not yet reached commercial applications:

Advantages of GMOs: There are various advantages and disadvantages of GMOs which are summarized in Figure 2 and also pointed below:

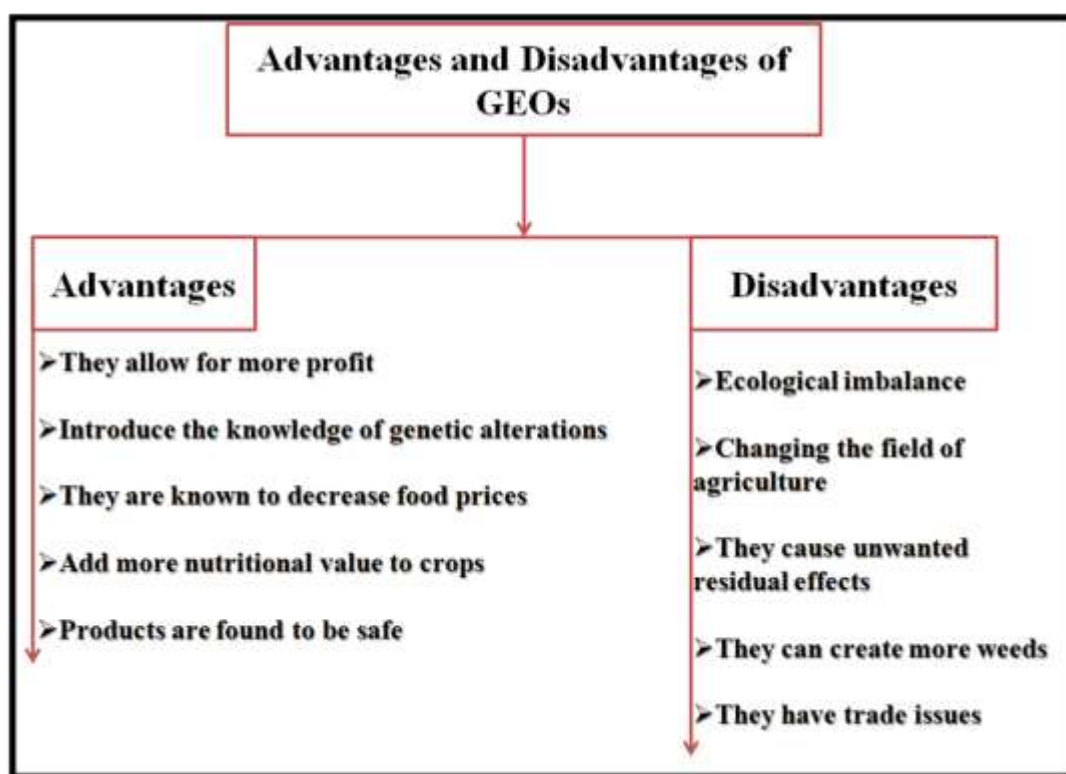


Figure 2 Showing advantages and disadvantages of Genetically Engineered Organisms (GEOs)

Advantages

They allow more profit: GMOs are an effective way to provide farmers a larger profit, while making them spend less time on resources. GMOs provide better economic value as compared to other non-GMOs. Since GMOs are designed to resist pests, there will be no need for pesticides to be used, which means more savings.

Introduce the knowledge of genetic alterations: It is another important advantage of GMOs. This is done through mapping genetic material for GM crops. This way, we would get the ability to enhance crop genes and make them more beneficial for human production and consumption. Plants can be engineered to resist temperature or produce higher yields, which is good for regions where climate limits productivity.

They are known to decrease food prices: Advanced crops and lower costs can lead to cheaper food. This will certainly help families who cannot afford to buy their needed supply for everyday consumption. Therefore, food supply and availability of food is assured.

Add more nutritional value to crops: The GMO method can put in added nutritional value to crops that lack necessary vitamins and minerals. Considering that there are places in the world relying on rice or corn as their daily staple, plant genes may be added to these crops to increase their nutritional value. Therefore, malnourished populations receive more nutrients from diet. For example Golden rice which has more vitamin A.

Products are found to be safe: The precise evaluation and testing of GMOs crops and other products means they are safe for human consumption. In fact, research shows that they are safer compared with traditional crops.

Disadvantages:

Ecological imbalance: GMOs are believed to be dangerous to some insects because new crop genes can be deadly to them. This is worth noting when it comes to certain insects, such as butterflies, that are not actually dangerous to agricultural ecology.

Changing the field of agriculture: The process of making GMOs includes adding new genetic material into an organism. In agricultural ecology, this means introducing new genes in the genome of crops like corn. Research on the effects of cultivation of GM crops in a large scale has sparked various concerns, specifically those ideas on ecosystems with GMO strains. As proven by certain studies, GMO strains have the potential to change agriculture.

They can damage the environment: Genetically modified organisms specially crops can cause a threat to the environment due to the fact that they are not a natural way to plant and cultivate plants.

They cause unwanted residual effects: A genetically modified organism can leave unwanted residual substances that can remain in the soil for extended periods of time. Agricultural regulators were alerted by research that strains from GM crops would remain in the soil for years after the crops were removed. Scientists even reported that despite the absence of these plants, the strain persisted for up to more than 5 years.

They can create more weeds: Take note that engineered crops can act as mediators in transferring genes to wild plants, which can create more weeds. To keep these new weeds under control, scientists then invented new herbicides that were not necessary

for non-GMO weeds. These chemicals are also toxic to various mammals and amphibians, who are feeding on GMO crops. Researcher also observed that the uptake of these herbicides also has toxic consequences on aquatic ecosystems.

May be threatened to crop diversity: There is opposition to introducing GM genes on genetic diversity because these genes can spread to other organic farm crops and threaten crop diversity in agriculture. And if crop diversity decreases, it will have a direct impact on our entire ecosystem and would affect the population dynamics of other plants and animals. However, when a large scale plantation releases a GM strain during pollination, this risk increases, where the cross pollination to non-GM plants could create a hybrid strain. This means there is a greater possibility of ecological novelty or new artificial strains that are being introduced into the environment that could potentially reduce biodiversity through competition.

They have trade issues: In many countries, there may be problems regarding trade matters, such as tariff and quota.

14.6 Ethical issues of safety of GEOs.

Five sets of ethical concerns have been raised about GM crops:

1. Potential harm to human health
2. Potential damage to the environment
3. Negative impact on traditional farming practice
4. Excessive corporate dominance
5. Unnaturalness of the technology

GM crops can have serious implications on the environment. Proper research must be carried out to study all aspects of GM crops. The reason behind the elaborate approval process is to ensure that the GM crop does not affect the health of human beings, animals, and the environment. Since the species have been artificially created, if it breeds with wild relatives, the natural ecosystem can result in genetic contamination. An example is the pollination and fertilization of Bt. Corn crop with a non-Bt. Corn. This will result in problems for the certified organic producers of corn. Another argument from ecologists is that the Bt. crops can harm non-target insects. In the case of Bt. Corn, Monarch butterflies feeding on wild milkweed that grows near cornfields may be harmed. Studies are still ongoing about the environmental effects of GM crops.

Sustainability of the technology, consumer choice and labelling for monitoring are some other concerns with respect to GM crops in India. That is reason even after two decades of Bt cotton launching our policy makers hesitate to commercialize it in food chain crops.

So far, GEAC approved the cultivation of only Bt. Cotton in 2002. This implies that the cultivation of GM versions of other crops is banned in the country. In spite of the ban, incidences of suspected open cultivation of Bt. Brinjal were reported in the states of Andhra Pradesh, Haryana, Maharashtra, and Punjab which is illegal. The Environment Protection Act, 1989 makes use of the unapproved GM crops a punishable offence attracting a penalty up to Rs. 1 Lakh and imprisonment for a term of five years. States have been asked to strengthen Biotechnology Coordination Committees and District Level Committees to monitor the illegal cultivation as well as take necessary action.

As per those who support the introduction of GM crops, depending on the variety of crop, farmers will benefit using GM seeds. Loss due to pest attacks can be averted. Further, use of chemical pesticides and herbicides can also be reduced. Production costs can therefore be reduced and increase production stability. The technology is also being used now to develop drought tolerant, nutrient and water efficient varieties which will help farmers more.

References

Adopted from Unit 11 Genetic Engineering: Basic Concepts, EVS 507

Adopted from Unit 13 GMOs AND GM FOODS.

Unit 15: Regulatory Measures

Unit Structure

15.0 Learning Objectives

15.1 Introduction

15.1 Acts, Rules, and Regulations: The Indian Biosafety and Biopharma Framework

15.1.1 Environmental and Biosafety Laws (India)

15.1.2 Public Health and Drug Regulation (India)

15.1.3 Biodiversity, IPR, and International Frameworks

15.2 Policies: Shaping Biotechnology Innovation and Regulation in India

15.3 Guidelines and Protocols for Biosafety and Biopharma Regulation in India (DBT)

Summary

15.0 Learning Objectives

After going through this unit, you will be able to understand:

- the fundamental national and international Acts and Rules that govern biosafety.
- the DBT's policy strategy for streamlining regulation through decentralization (empowering IBSCs) and digitization (Indian Biosafety Knowledge Portal - IBKP)
- the guidelines and protocols for the execution of the biosafety framework.

15.1 Introduction

Biotechnology is very important to the way our society works today. It has become an important tool for national growth since it helps crops grow better, makes medications that save lives, improves industrial processes, and protects the environment. However, the successful use of biotechnology depends not just on scientific innovation but also on the capacity to transfer technology efficiently. Many underdeveloped countries often rich in genetic resources but lacking advanced technologies still deal with low technical skills and legal preparation. For them, establishing trained people and forming collaborative, mutually beneficial initiatives with other nations becomes crucial. As biotechnology evolves, its relationship with the legal system becomes increasingly crucial. This interface functions on various levels:

First, current biotechnological breakthroughs require substantial legal protection through intellectual property rights (IPR) (as discussed in unit 13). Since developing a

biotechnological product takes significant expenditure, innovators need assurance that their work will not be stolen or exploited without authorization. Biotechnology research and industrial expansion may substantially slowdown in the absence of such protection.

Second, genetic resources are the foundation of biotechnology research. Access to these resources (plants, animals, microorganisms, and traditional knowledge) must be carefully controlled. Legal frameworks guarantee that access is equitable, transparent, and respects the rights of local communities and nations.

Third, biotechnology goods can have a wide-ranging impact on human health, biodiversity, and the environment. Concerns about the loss of agricultural biodiversity caused by hybrid seeds, as well as the possible hazards of genetically modified organisms (GMOs), underscore the importance of adequate regulation. Laws are thus necessary not to limit innovation, but to assure safety, ethical standards, and environmental sustainability.

In this framework, international agreements and global norms are quite important. They assist developing nations in establishing strong national biotechnology laws that are consistent with international standards. Nations that embrace these frameworks can harmonize their policies, enable safe innovation, and work toward a unified international biotechnology governance structure. India's biotechnology regulatory system is an excellent example of this technique. Many of its domestic rules and standards are designed to complement or implement international treaties, assuring both global alignment and national protection.

15.1 Acts, Rules, and Regulations: The Indian Biosafety and Biopharma Framework

"India has developed a comprehensive set of laws and regulations to govern biotechnology. Because of the close connection of biotechnology with science and innovation". This system assures that all biotech work, from new research to product launch in fields such as health, agriculture, and medicine, is done safely, ethically, and in accordance with international best practices. As biotechnology grows, it becomes increasingly important to have clear supervision. India's regulatory system has evolved into:

- Protect the environment and public health.

- Control access to India's valuable genetic resources.
- Ensure research is conducted ethically.
- Ensure safe usage of genetically altered organisms (GMOs).
- Promote new ideas while preventing harm or overuse.

Essentially, these frameworks serve as the strong backbone for controlling India's biotech sector, assuring its worldwide competitiveness and compliance with international accords.

15.1.1 Environmental and Biosafety Laws (India)

This category includes the fundamental laws that safeguard the environment and regulate biological and agricultural safety.

1. **The Environment (Protection) Act, 1986 (EPA 1986):** The Environment (Protection) Act of 1986 is a strong, comprehensive Indian law enacted following the 1972 Stockholm Conference. It gives the Central Government the authority to set environmental standards, control industrial pollutants and dangerous substances, enforce compliance by inspections and legal action, and create testing facilities. The Act requires industries to fulfill discharge limitations and report spills, and it imposes severe penalties, including 5-7 years in prison and large fines, establishing an effective legal foundation for pollution prevention.
2. **The Rules for the Manufacture, Use, Import, Export and Storage of Hazardous Microorganisms/Genetically Engineered Organisms or Cells, 1989 (Rules, 1989):** The Rules for Hazardous Microorganisms/Genetically Engineered Organisms (GMOs), 1989, were established under the Environment (Protection) Act of 1986 to strictly regulate all operations involving hazardous microorganisms and GMOs, with the goal of preserving human health and the environment. These regulations apply to manufacturing, development, and usage of GMO-based food items. The Genetic Engineering Appraisal Committee (GEAC) leads the regulatory system, which requires needed permission for large-scale usage, import, and environmental discharge of GMOs. Other organizations, such as the RCGM, IBSC, and local committees (SBCC/DLC), oversee safety and monitoring. The guidelines provide strict

liability standards, mandating rapid accident reporting and punishing penalties for breaking the rules.

3. **Plant Quarantine Order, 2003:** The Plant Quarantine Order of 2003 is India's legal defense under the Destructive Insects and Pests Act of 1914, which prohibits foreign pests and diseases from entering the nation through imported plants, seeds, or associated things. To import, two documents are required:

- a) **Import Permit (IP):** Issued in advance by Indian authorities (e.g., PPA).
- b) A phytosanitary certificate (PSC) issued by the country of origin confirms that the item has been inspected and complies with Indian health regulations.

Consignments must arrive at designated points of entry for inspection by a PQ (Plant Quarantine) officer. If necessary, the importer must pay for and schedule mandated treatment (fumigation). Special products, such as seeds or GMOs, require additional scrutiny. If restrictions are broken or pests have been found, the PPA may impose deportation or destruction at the importer's expense.

15.1.2 Public Health and Drug Regulation (India)

These regulations concern the safety and quality control of food and pharmaceuticals.

1. **Food Safety and Standards Act (FSSA), 2006:** The Food Safety and Standards Act (FSSA) of 2006 is India's primary, consolidated legislation aimed at assuring the availability of safe and nutritious food. It established the Food Safety and Standards Authority of India (FSSAI), an independent central agency in charge of developing science-based standards and regulating the licensing of all Food Business Operators (FBOs). FBOs are primarily responsible for food compliance and must initiate immediate recalls of dangerous items. State Commissioners and local Food Safety Officers (FSOs) are in charge of enforcement. The Act provides severe penalties, including fines and imprisonment, for violations such as selling dangerous or misbranded food.
2. **New Drug Approval (1988/Schedule Y):** The medications and Cosmetics (Eighth Amendment) Rules, 1988, and the detailed Schedule Y (which was later replaced by a 2005 amendment), established a strong regulatory framework for new medications in India. This framework requires prior written authorization from the central licensing body (the Drugs Controller of India) before importing or manufacturing any new medicine. A "new drug" is widely characterized as

newly discovered compounds, established medications with new claims, or novel fixed-dose combinations.

To obtain approval, applicants must provide extensive data, including detailed animal toxicology results from Clinical Trials (Phases I, II, and III), chemical and pharmacological information, and evidence of safety and efficacy in accordance with Schedule Y. Schedule Y sets strict guidelines for clinical trials, requiring prior permission from the Licensing Authority and approval from an Ethics Committee, as well as freely given informed written consent from all study subjects. It also defines responsibilities for the Sponsor (SAE reporting) and Investigator (protocol adherence). The framework also mandates post-marketing surveillance using Periodic Safety Update Reports (PSURs) to continuously assess clinical safety immediately after approval.

15.1.3 Biodiversity, IPR, and International Frameworks

This topic includes genetic resources, rights of traditional knowledge holders, and international treaties.

1. **Protection of Plant Varieties and Farmers' Rights Act, 2001:** The Protection of Plant Varieties and Farmers' Rights Act, 2001 (PPV&FR Act) establishes an intellectual property framework in India to safeguard plant varieties and the rights of both breeders and farmers. The Act establishes an Authority and a Registry to oversee the registration of new, existing, and farmer-developed varieties.

Key Rights

- Breeders get exclusive rights to registered varieties. The Authority also ensures benefit sharing with populations that provided genetic material.
- Farmers who create new varieties have equal protection as professional breeders. Farmers have the right to save, use, sow, resow, exchange, share, or sell their agricultural produce, including protected variety seed, as long as they do not sell branded seed.

The Act comprises two enforcement processes: compulsory licensing (to assure public availability) and the Plant Varieties Protection Appellate Tribunal.

2. Biological Diversity Act, 2002: The Biological Diversity Act of 2002 implements the worldwide CBD in India. Its three primary objectives are conservation, sustainable use, and Fair and Equitable Sharing of Benefits (FEBS) from biological resources and traditional knowledge.

A three-tier structure governs access:

- a. The National Biodiversity Authority (NBA) regulates international access and authorizes IPR applications.
- b. Indian enterprises must follow State Biodiversity Boards (SBBs) regulations for commercial use.
- c. FEBS (Fair and Equitable Sharing) is a mandatory condition of approval that compensates local communities and knowledge holders.

Violations can result in imprisonment up to five years and significant penalties.

3. Convention on Biological Diversity (CBD), 1992: The Convention on Biological Diversity (CBD), signed in 1992, is an important turning point in international law that recognizes biodiversity as a vital asset within the authority of sovereign governments. It has three primary and co-equal aims ("pillars"): conservation of biological diversity, sustainable use of biological components, and fair and equitable sharing of benefits (FEBS) produced from genetic resources.

Global commitments create an international framework for the responsible management of biological resources and traditional knowledge, principally through the Convention on Biological Diversity (CBD) and its associated protocols. The framework requires that access to genetic resources be allowed only with the providing country's prior informed consent (PIC) and on mutually agreed-upon terms (MAT). Furthermore, governments must respect and conserve indigenous and local populations' traditional knowledge and traditions, as well as ensuring equitable benefit sharing from this knowledge. In terms of biosafety, countries must regulate and control the possible risks connected with the use of Living Modified Organisms (LMOs) created by biotechnology. Finally, the agreement promotes international cooperation, particularly the transfer of appropriate technology to underdeveloped nations, with financial support provided by the Global Environment Facility (GEF).

4. Cartagena Protocol on Biosafety (2000): The Cartagena Protocol on Biosafety (2000) is an international agreement under the CBD that serves as a global guideline for the safe transboundary movement of Living Modified Organisms (LMOs/GMOs). Its key provision is the Advanced Informed Agreement (AIA) protocol, which requires an exporting country to provide specific information to the importing country prior to shipping LMOs intended for environmental release. The importing country may restrict the import based on a scientific risk assessment, even if complete assurance is not available, under the Precautionary Principle. The Protocol demands the use of the Biosafety Clearing House (BCH) for information sharing, as well as unambiguous labeling and documentation for all LMOs that cross borders.

5. Nagoya-Kuala Lumpur Supplementary Protocol: The Nagoya-Kuala Lumpur Supplementary Protocol is the global "cleanup clause" for biosafety. It addresses the culpability and restitution for measured and significant biodiversity loss caused by Living Modified Organisms (LMOs), including accidental or unlawful cross-border movement. The Operator bears primary accountability for the LMO. The Operator must quickly take necessary action to confine the LMO, reduce damage, and restore the environment. If the Operator fails, the national authorities can carry out the cleanup and collect the costs from them. The Protocol compels countries to include certain liability provisions in their national legislation.

15.2 Policies: Shaping Biotechnology Innovation and Regulation in India

1. The fundamental policy mandate: The DBT's principal policy purpose is a twofold commitment: to support biotechnology research and development (R&D) while also assuring the safe and secure implementation of the technology through a strong regulatory framework.

This requirement is based on the Rules for the Manufacture, Use, Import, Export, and Storage of Hazardous Microorganisms/Genetically Engineered Organisms or Cells, 1989 (Rules, 1989), which are governed by the Environment (Protection) Act, 1986.

DBT's policy approach is dynamic and flexible, incorporating worldwide best practices and adapting to changing science and technology.

2. Policy Changes in Regulatory Governance: DBT's latest policy focuses on streamlining the regulating process, especially through decentralization and digitization, in order to improve efficiency while maintaining safety.

(i) **The decentralization of regulatory powers:** The policy authorizes the Institutional Biosafety Committees (IBSCs) to speed up low-risk research approvals at the local level:

- IBSC Empowerment: IBSCs can approve low-risk trials (Category I and II), as well as import, export, and exchange regulated materials under particular quantity criteria (the "Simplified Procedure").
- The Risk-Proportionate Approach policy promotes lower-risk activities, allowing the central Review Committee on Genetic Manipulation (RCGM) to prioritize monitoring high-risk/large-scale initiatives.

(ii) **Policy on Digitization (Indian Biosafety Knowledge Portal - IBKP):** Implementing the Indian Biosafety Knowledge Portal (IBKP) is a key reform promoted by the "Digital India" project.

- The portal allows for online submission of forms for research, import/export, and safety studies, eliminating the need for paper in the regulatory process.
- The site facilitates IBSC registration, renewal, and timely submission of compliance papers, including Annual Reports and Medical Surveillance Reports.

3. Policy for Key Biotechnological Sectors: The DBT has implemented sector-specific strategies to meet particular scientific and public confidence challenges.

(i) **Policy for Genome Edited Plants (SDN Exemption):** The most major recent policy move concerns Genome Edited (GE) Plants, namely those developed with Site-Directed Nuclease (SDN) technology.

- The policy governs GE facilities based on the end-product, not the manufacturing process.
- GE plants developed using SDN-1 and SDN-2 are excluded from Rules 7-11 of the Rules, 1989, since they do not include foreign DNA in the stable plant. These products are regarded identical to those obtained through traditional

mutagenesis or natural variation, keeping with the notion of equal treatment for products with similar risk profiles.

- GE plant development is overseen by IBSC during the initial enclosed facility phase until foreign DNA is scientifically determined to be absent.

(ii) Policy on Biopharma and Vaccines: This sector's policies prioritize speed and rigor, especially in reaction to public health disasters (such as COVID-19).

- Fast-track regulatory framework: Special Rapid Response Regulatory Frameworks have been established to expedite the processing of vaccination, diagnostic, prophylactic, and therapeutic application submissions.
- Streamlining PCT: The policy allows applicants to proceed with PCT research protocols if the RCGM does not react within 15 days and the application is complete.
- Quality assurance policy requires data from at least three consistent manufacturing batches for PCT investigations to ensure product purity and stability.

4. Policy for Biosafety and Compliance Adherence: Policy decisions strengthen institutional accountability, ongoing monitoring, and training to ensure compliance with safety standards.

- The Head of the Organization and Principal Investigator (PI) are legally responsible for ensuring safe conduct and compliance with all regulations and guidelines (institutional accountability).
- IBSCs must provide ongoing biosafety training to all researchers, covering correct handling, decontamination, and emergency protocols.
- The policy requires mandatory Medical Surveillance Reports for personnel handling Risk Group 2 and higher pathogens, demonstrating a commitment to occupational health.

15.3 Guidelines and Protocols for Biosafety and Biopharma Regulation in India (DBT)

Guidelines and protocols are aimed at ensuring biosafety, biosecurity, and streamlined regulation across biotechnology research, development, and application in India.

1. The Indian Biosafety Regulatory Ecosystem

The "Rules for the Manufacture, Use, Import, Export, and Storage of Hazardous Microorganisms/Genetically Engineered Organisms or Cells, 1989" (Rules, 1989), adopted under the Environment (Protection) Act, 1986 (EPA 1986), serve as the foundation for biosafety in India. This framework is executed via a multi-tiered structure consisting of six competent authorities:

Competent Authority	Primary Function
Recombinant DNA Advisory Committee (RDAC)	Advisory; evaluates national and international biotech breakthroughs and suggests suitable safety standards.
Institutional Biosafety Committee (IBSC)	Nodal Point and Institutional Approval; reviews applications, enforces policies, and develops on-site emergency plans for the company.
Review Committee on Genetic Manipulation (RCGM)	Monitoring and Central Approval; evaluates ongoing research, creates guidelines, and grants approval for high-risk, large-scale undertakings.
Genetic Engineering Appraisal Committee (GEAC)	Environmental Release Approval: Approves large-scale usage and release of genetically modified organisms (GEOs) into the environment.
State Biotechnology Coordination Committee (SBCC)	State-Level Monitoring; conducts periodic reviews of safety and control procedures in various facilities across the state.
District Level Committee (DLC)	Monitoring at the district level includes monitoring local safety laws and developing off-site emergency plans for operations such as field trials.

2. The Institutional Biosafety Committee (IBSC)

The IBSC is the primary regulatory authority for any organization that works with GE organisms, recombinant DNA, or hazardous microorganisms (HMO).

(i) Composition and Core Functions

- The committee must have a Chairperson, Member Secretary, Biosafety Officer, and a DBT Nominee.

- To ensure effective execution, the Biosafety Officer must be a registered Medical Practitioner with the Medical Council of India (MCI)/National Medical Commission (NMC).
- Decentralized approval powers allow IBSCs to directly approve low-risk operations (Category I and II experiments).
- IBSCs must submit Annual Compliance Reports and Medical Surveillance Reports (for facilities handling Risk Group 2/3/4 organisms) to the RCGM by February 15th annually (or as per updated schedules).

(ii) Biosafety Levels (BSL) and Risk Groups (RG)

The type of laboratory confinement (Biosafety Level, BSL) is decided by the related risk posed by the microorganism (risk Group, RG) and the nature of the experiment.

- Microorganisms are classified into Risk Groups (RGs) based on their pathogenicity, mode of transmission, host range, treatment options, and potential for epidemics.
- RG 1 (Low Risk): *Bacillus subtilis* and *E. coli* B series are unlikely to cause disease in humans, animals, or plants. This requires BSL-1.
- RG 2 (Moderate danger): Can cause disease, but effective treatment and prevention methods are available, and the danger of transmission is low. This requires BSL-2.
- RG 3 (High Individual Risk): Causes serious or fatal disease but does not transmit between individuals; appropriate therapy is typically available. This requires BSL-3.
- RG 4 (High Individual and Community Risk): Causes significant disease and is easily transferred; appropriate therapy is not always available. Requires BSL 4.
- To ensure safety, BSL-3 facilities must comply with national criteria for establishment, testing, commissioning, and certification. The certification is valid for three years, with important components requiring annual revalidation.

3. Protocols for Biopharma and Vaccine Development

The regulatory process for producing biological pharmaceuticals and vaccines with genetically modified (GE) organisms is continually being optimized.

(i) General Streamlined Measures

- Applicants developing biopharma products can now submit their Pre-clinical Toxicity (PCT) procedures to RCGM for review prior to the main scientific presentation. If the RCGM does not provide a decision within 15 days, the protocol may be declared accepted.
- DBT offers generic PCT techniques to evaluate the toxicity of recombinant DNA-derived products, including monoclonal antibodies (Mabs), hormones, and vaccinations, to standardize data collection.
- Three consistency batches of drug substance (DS) must be described to determine consistency, purity, and impurity profile before starting PCT investigations. The batch size should be large enough to provide material for all CMC (Chemistry, Manufacturing, and Controls) and preclinical toxicity investigations.
- Simplified Regulatory Pathway: The application process includes IBSC evaluation, RCGM approval of the PCT protocol (Form C3), submission of the final PCT report (Form C5), and issuance of a No-Objection Certificate (NOC) to the Drug Controller General of India (DCGI) for clinical trials.

(ii) Guidelines for PCT Studies (Key Considerations)

The generalized protocol for determining toxicity focuses on comparative assessment with a Reference Biologic (RB):

Parameter	Key Requirements
Dose Levels	At least one Human Equivalent Dose (HED) must be evaluated; up to three levels (1X, 2X, and 5X HED) may be employed. The dosage calculation and total volume limits must be justified.
Species & Strain	The safety profile must be determined in a sensitive species (preferably pharmacologically relevant) that produces an immunological response to the vaccination antigen. Typically, beginning clinical trials require only one relevant species.
Dosing Frequency	Vaccine dosing should be based on the suggested clinical schedule (e.g., set time intervals/weeks), not daily doses.

End-points	Standard observations include daily clinical symptoms, weekly body weights, haematology, and serum chemistry. Histopathological examinations must encompass key organs and the site of administration. A recovery period (e.g., 14 days) is required to assess reversibility and delayed effects.
Compliance	All toxicity studies must adhere to Good Laboratory Practices (GLP).

4. Protocols for Genome Edited (GE) Plants

CRISPR/Cas9 and other genome editing technologies allow for precise changes to a plant's DNA. The regulatory attitude (Rules, 1989) is based on the end product rather than the procedure, categorizing GE plants into three types of Site-Directed Nucleases (SDN) based on whether foreign DNA remains in the finished plant.

Category	Description	Final Product Status	Regulation Status
SDN-1	Targeted break, healed without a template. This produces modest, naturally similar mutations.	Free of exogenous DNA (DNA/RNA editing reagents separated).	EXEMPT from Rules 7–11 of 1989 (no additional GE regulation).
SDN-2	A targeted break is repaired with a tiny DNA template. This results in changed expression, which is regarded akin to spontaneous allelic variation.	Free of exogenous DNA (DNA/RNA editing reagents separated).	EXEMPT from Rules 7–11 of 1989 (no additional GE regulation).
SDN-3	A targeted break is repaired using a large DNA template (the full gene/genetic element).	Contains foreign gene(s)/novel DNA inserted at a specific location.	FULLY REGULATED under the Rules of 1989, just like conventionally

			modified GE plants.
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- The early research phase for all SDN categories, prior to effective removal of editing reagents and exogenous DNA (e.g., genetic segregation), is fully regulated under Rules, 1989 and overseen by the IBSC.
- Exemption Logic: SDN-1 and SDN-2 plants' end products are considered equivalent to those obtained through classical breeding or chemical/radiation mutagenesis, and hence do not require ongoing biosafety regulation after foreign DNA is eliminated.

5. Protocols for Import, Export, and Exchange of Materials

The DBT regulates the transfer of GE organisms, HMOs, and their products to ensure biosecurity, using streamlined processes for low-risk commodities.

(i) Simplified Procedure for R&D (2020)

- The IBSC can approve the import, export, and exchange of regulated biological materials up to a certain quantity for R&D (e.g., 100 μ g of pure nucleic acids, 20g of protein, 1kg of medicinal product/substance).
- Prior permission from RCGM is required for high-risk/large quantity items that exceed the prescribed amount or fall under Risk Groups 3 (RG 3) and 4.

(ii) Biorepository and Sample Exchange

- When transferring COVID-19 samples, biorepositories must ensure the destination organization has a registered IBSC and a containment facility (BSL-2 or above) suitable for the sample's Risk Group.
- Documentation for transfers includes a signed Requisition Form from the applicant, a Declaration Form from the applicant's IBSC, and RCGM permission for RG 3/above samples or R&D involving BSL-3 facilities.
- All personnel must follow transport, storage, decontamination, disposal, and emergency protocols.

(iii) Special Trade Considerations

The export of biological materials from the Special Chemicals, Organisms, Materials, Equipment and Technologies (SCOMET) list (Category 2: Micro-organisms, Toxins),

applicants must obtain a separate export permission from the Directorate General of Foreign Trade (DGFT), Ministry of Commerce, in addition to IBSC/RCGM approval.

Summary

The regulatory measures for biotechnology in India establish a robust framework to promote innovation while ensuring public health, environmental sustainability, and equitable use of genetic resources. This system is anchored by key domestic laws, including the Environment (Protection) Act, 1986 and the Rules, 1989, which strictly govern Genetically Modified Organisms (GMOs), alongside the Food Safety and Standards Act (FSSA), 2006, for food safety, and the Biological Diversity Act, 2002, which enforces the global principles of conservation and Fair and Equitable Sharing of Benefits (FEBS). The operational control is managed through a multi-tiered structure, with the Institutional Biosafety Committee (IBSC) acting as the primary institutional approval body for low-risk work, monitored by central bodies like the RCGM and GEAC for high-risk and environmental release decisions.

Recent policy evolution has focused on streamlining this process through digitization (IBKP) and a risk-proportionate approach, most notably by exempting Genome Edited plants (SDN-1 and SDN-2) from full regulation if they contain no foreign DNA in the final product. This regulatory philosophy ensuring safety and ethical conduct without unnecessarily impeding progress is further enforced by strict protocols for biopharma development and mandatory adherence to corresponding Biosafety Levels (BSL) based on the Risk Group (RG) classification of the pathogen, guaranteeing institutional accountability and compliance.

References

1. National Biotechnology Development Strategy (2015-2020), promoting bioscience research, education and entrepreneurship, Department of Biotechnology, Minister of Science & Technology, Government of India.

Check Your Progress

1. The Environment (Protection) Act, 1986 (EPA 1986) is the foundational law for biosafety. Under this Act, which specific set of Rules was established in 1989 to strictly regulate all operations involving hazardous microorganisms and Genetically Engineered Organisms (GMOs)?

2. What are the three primary objectives (the 'pillars') of the Convention on Biological Diversity (CBD), 1992, which the Indian Biological Diversity Act, 2002, implements?
3. Identify the six competent authorities that constitute India's multi-tiered biosafety regulatory ecosystem, starting with the advisory body (RDAC) and ending with the local monitoring body (DLC).
4. What is the core policy principle behind the decentralization of regulatory powers, and which committee is empowered to speed up approvals for low-risk research (Category I and II) at the institutional level?
5. Microorganisms are classified into four Risk Groups (RG) based on factors like pathogenicity and transmission risk. If a pathogen is classified as RG 4, what are the characteristics of the risk it poses, and what Biosafety Level (BSL) is required?