



Uttar Pradesh Rajarshi Tandon
Open University

Bachelor of Science
UGEVS-103(N)
**Environmental Microbiology
and Biotechnology**

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COURSE INTRODUCTION

The “Environmental Microbiology and Biotechnology” course delves into the intricate and alluring world of microorganisms and their deep insight into the impact on the environment and human life. By exploring the interplay between microbes and their surroundings, this course will unravel how these tiny organisms contribute to ecological balance, environmental restoration, and biotechnological advancements.

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BLOCK INTRODUCTION

This block deals with Environmental Microbiology and Biotechnology in three units viz. Unit 1, 2 and 3. The three units are as follows:

Unit 1: Introduction to Microbes

This unit delves into fundamental microbiology, covering microorganism classification and nomenclature. It emphasizes nutritional requirements and explores different types of microorganisms based on their strategies. Additionally, it discusses media types for bacterial cultivation, highlighting preparation and storage techniques for effective microbial study.

Unit 2: Microbial Transformation

This unit explores microbial transformation, bioaccumulation, and bioleaching, emphasizing environmental and industrial importance. It discusses microorganisms' role in metal extraction, factors affecting bioleaching, and bioreactors' use in enhancing bioremediation efficiency for contaminated environments.

Unit 3: Microbial Degradation

This unit delves into biodegradation, where microorganisms break down organic materials, enhancing soil fertility and sustaining agricultural productivity. It explores stages of biodegradation, including xenobiotic breakdown, highlighting microbial strategies and challenges. Additionally, it covers microbial roles in wastewater treatment, contributing to improved water quality and environmental sustainability.

UNIT-1 : INTRODUCTION TO MICROBES

Structure

- 1.1 Introduction
 - Objectives
- 1.2 The Science of Microbiology
- 1.3 Classification of Microorganisms
 - 1.3.1 Whittaker's Five- Kingdom Concept
 - 1.3.2 Kingdom *Procaryotae* after *Bergey's Manual of Systematic Bacteriology*
- 1.4 Nomenclature of Microorganisms
- 1.5 Nutritional Requirement of Microorganisms
- 1.6 Nutritional Types of Microorganisms
 - 1.6.1 Phototrophs
 - 1.6.2 Chemotrophs
 - 1.6.3 Autotrophs and Heterotrophs
 - 1.6.4 Obligate Parasites
- 1.7 Bacterial Media
 - 1.7.1 Types of Media
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 - 1.7.1.4 Media for Bacterial Enumeration
 - 1.7.1.5 Media for Bacterial Characterization
 - 1.7.1.6 Maintenance Media
 - 1.7.1.7 Solid and Semisolid media
- 1.8 Media Preparation and Storage
- 1.9 Conditions required for Bacterial Growth
 - 1.9.1 Temperature
 - 1.9.2 Gaseous Requirement
 - 1.9.3 pH

1.9.4 Miscellaneous Requirements

1.10 Summary

1.11 Terminal Questions

1.12 Further Suggested Readings

1.1 INTRODUCTION

Microorganisms are tiny living entities, including bacteria, viruses, molds, yeasts, and algae collectively referred to as fungus, as well as bacteria and protozoa. Because viruses lack typical cell structures, therefore they are categorized differently. Fungi, algae, and protozoa are eukaryotes (cells with nuclei), whereas bacteria are prokaryotes (cells without separate nuclei). Microorganisms can be found in soil, water, the atmosphere, animals, plants, and other living things. All these environments aside from the atmosphere are favourable to their growth. All together, they greatly outnumber all other living beings on the earth. They were the first living cells to arrive on the earth more than 3 billion years ago. They have performed important roles ever since, many of which are beneficial to the biological systems.

Objectives

- To discuss the methods of classification of microorganism
- To discuss the factors involved with general classification and nomenclature used for microorganisms
- To discuss the nutritional requirements of microorganisms
- To understand the types of microorganisms on the basis of their nutrition
- To discuss the different types of media and their preparation

1.2 THE SCIENCE OF MICROBIOLOGY

Microbiology is the branch of biology that focuses on studying microorganisms, including viruses, viroids, bacteria, fungi, blue-green algae, and prions. The majority of microbes exhibit the following traits:-

1. Microscopy is usually required to analyse their structure because they are usually too small to be seen with the naked eye.
2. Compared to those of higher plants and animals, cells and other structures are comparatively less specialized and simpler.
3. They are generally treated and cultivated in a nearly identical manner in the laboratory.

Microbiology as a science has developed in a way that allows for multiple approaches. A specialized study might focus on each of the different categories that gave origin to the following disciplines:

- Mycology - the study of fungi
- Protozoology - the study of protozoa
- Bacteriology - the study of bacteria

- Virology - the study of viruses
- Phycology (algology) - the study of algae

Microbes can also be examined from an applied perspective, that is, from the perspective of how they interact with human activities, the environment, and other microbes. Therefore, this leads to several specialized research fields:

- **Medical microbiology**- includes aspects of pathology (the study of diseases), immunology (the functioning of the immune system to prevent the invasion of microorganisms), and epidemiology (the distribution and spread of diseases).
- **Agricultural microbiology**- The investigation of microorganisms in relation to plant and crop health.
- **Industrial microbiology/biotechnology**- The study of microorganisms' application in extensive industrial processes.
- **Food microbiology**- The study of the function of microorganisms in food production, food deterioration, food preservation, and food-borne illness.

These specialized areas of research are all interdependent and cannot exist alone. For example, food microbiology uses several aspects of biotechnology and industrial microbiology to produce single-cell proteins and fermented foods. The study of food-borne illness incorporates elements of agricultural and medical microbiology. Proficiency needs to be underpinned by a firm understanding of basic principles. The food microbiologist, for example, needs to understand the microbial structure, microorganism categorization and identification, how bacteria grow, factors that influence growth, and methods for controlling growth. They also need to understand nutrition, microorganism mortality, and laboratory culture procedures.

1.3 CLASSIFICATION OF MICROORGANISM

Classification in biology, like in any other discipline, refers to the systematic grouping of the units of study into larger units. The Swedish botanist Carolus Linnaeus (1707–1778) is credited with establishing modern biological categorization. All living things were categorized into one of two kingdoms plant or animal up until the eighteenth century. It was suggested that additional kingdoms be created to encompass organisms that are normally neither plants nor animals, because some organisms do not ordinarily belong in either the plant or animal kingdoms.

Haeckel's Kingdom Protista- German naturalist E. H. Haeckel offered one of these early suggestions in 1866. In order to accommodate those unicellular microbes that are usually neither plants nor animals, he proposed the formation of the third kingdom, Protista. Protists are a group of organisms that include fungi, bacteria, algae, and protozoa. (Viruses are not considered protists because they are not cellular entities.) Algae, fungi, and protozoa are referred to as higher protists, while bacteria are considered lower protists.

1.3.1 WHITTAKER'S FIVE- KINGDOM CONCEPT

R. H. Whittaker (1969) proposed the five-kingdom system, a thorough classification scheme. The three layers of cellular organization that developed to support the three main processes of nutrition photosynthesis, absorption, and ingestion are the foundation of this classification scheme. The kingdom Monera includes prokaryotes because they do not have an ingestive mechanism of nourishment. All three nutritional categories of unicellular eucaryotic microbes are included in the kingdom Protista. The

feeding pathways are continuous: the microalgae feed via photosynthetic processes; the protozoa feed through ingestive processes; and certain other protists feed through absorptive processes, which may overlap with the photosynthetic and ingestive processes. The multicellular and multinucleate eucaryotic organisms are found in the kingdoms *Plantae* (multicellular green plants and higher algae), *Animalia* (multicellular animals), and *Fungi* (multinucleate higher fungi). Microorganisms are found in three of the five kingdoms: *Monera* (bacteria and cyanobacteria), *Protista* (microalgae and protozoa), and *Fungi* (yeasts and molds).

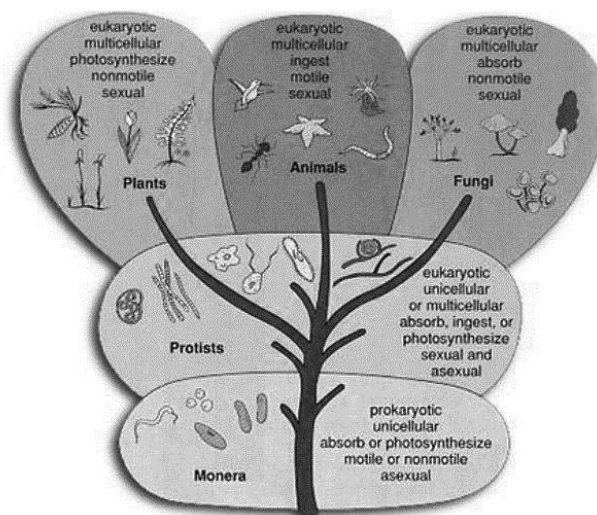


Fig. 1.1 Whittaker's 5 kingdom classification

1.3.2 KINGDOM PROCARYOTAE AFTER BERGEY'S MANUAL OF SYSTEMATIC BACTERIOLOGY

According to Bergey's Manual of Systematic Bacteriology, all bacteria belong to the kingdom *Procaryotae*, which is further divided into the following four divisions:

Division-1 Gracilicutes

Prokaryotes that resemble Gram-negative bacteria and have a complex cell wall structure

Division-2 Firmicutes

Prokaryotes with a cell-wall structure characteristic of Gram-positive bacteria

Division-3 Tenericutes

Prokaryotes that lack a cell wall

Division-4 Mendosicutes

Prokaryotes that exhibit indications of a more recent evolutionary history compared to the bacteria classified in Divisions 1 and 2 (above)

Bergey's Manual is the international standard for bacterial taxonomy.

Protists are primarily classified as algae, viruses, bacteria, protozoa, and fungi. Viruses are included despite not being protists or cellular organisms for two reasons: (1) the techniques used to study viruses are microbiological in nature; and (2) since viruses are disease-causing agents, diagnostic procedures for their identification are used in both the plant pathology and clinical microbiological

laboratories.

- **Algae** are relatively simple organisms. Unicellular forms are the most primitive. Others are collections of related cells that have little to no structural or functional distinction. Other algae, like big brown kelp, have a more intricate structure with different cell types serving different purposes. All algal cells, regardless of size or complexity, have chlorophyll and can perform photosynthesis. Algae exist most frequently in aquatic environments or damp soil.
- **Viruses** are microscopic, noncellular parasites that can infect bacteria, plants, animals, and other protists. Only an electron microscope can see them because they are so small. Only living cells can be used to cultivate viruses.
- **Bacteria** are a simple collection of similar cells or unicellular prokaryotic organisms. Binary fission is typically used in cell multiplication.
- **Protozoa** are unicellular eucaryotic organisms. Their morphological, nutritional, and physiological traits are used to distinguish them. Although they play a variety of roles in nature, the most well-known protozoa are those that infect humans and other animals with disease.
- **Fungi** are lower eucaryotic plants without chlorophyll. They lack the ability to divide into roots, stems, and leaves while being multicellular in nature. Their forms and sizes vary, ranging from enormous multicellular mushrooms and puffballs to microscopic yeasts with a single cell. The organisms known as molds, mildews, yeasts, and plant diseases termed rusts are of special interest to us. The body of a true fungus, referred to as a mycelium, is made up of filaments and masses of cells. Fungi can reproduce by budding, fission, or by producing spores on fruiting structures that are very different from one another for some species.

1.4 NOMENCLATURE OF MICROORGANISM

The set of rules and regulations that control taxonomic names is known as nomenclature. The International Code of Terminology of Bacteria (Bacteriological Code) governs the terminology used for prokaryotes. Classification is the process of organizing creatures according to phylogeny and phenotype into increasingly inclusive groupings; nomenclature is the use of formal guidelines to provide names to species. Except for epithets for species and subspecies, taxonomic names are written in italics (or underlined when handwritten) with a majuscule initial letter. The formal system of identifying species of living things known as binomial nomenclature (also known as binary nomenclature or binominal nomenclature) consists of assigning each species a name that consists of two components, both of which employ Latin grammatical forms, though they may also be based on terms from other languages. Carl Linnaeus established the binomial nomenclature system. A Latin name is another colloquial term for this type of name. It is also known as a binomial name (which can be abbreviated to simply "binomial"), a binomen, or a scientific name. Many species bear a person's name, either in honor of the discoverer or a well-known figure in the area of microbiology. For example, the species *Salmonella* is named for D.E. Salmon, who made the discovery (albeit under the name "*Bacillus typhi*"). For the generic epithet, all names derived from people must be in the female nominative case, either by changing the ending to -a or the diminutive -ella, depending on the name. For the specific epithet, the names can be converted into either adjectival form (adding -nus (m.), -na (f.), -num (n.) according to the gender of the genus name) or the genitive of the Latinised name.

Numerous species (specific epithets) have their names derived from the location in which they are found or present (e.g. *Borrelia burgdorferi*). Unless the place has a classical Latin adjective, their names are formed by connecting the locality's name with the ending -ensis (m. or f.) or ense (n.), according to the gender of the genus name. However, in the genitive case, place names should not be

used as nouns.

Although some authors refer to phyla as kingdoms, the term kingdom is not used for the Prokaryotes, which include bacteria and archaea. Rule 9 of the Bacteriological Code states that when a new or modified species is assigned to a new rank, its name is created by appending the proper suffix to the stem of the type genus's name. The recommendation form is typically followed for subclass and class, producing a neutral plural. However, some names such as the Greek plurals Spirochaetes, Gemmatimonadetes, and Chrysiogenetes, the male plurals Chloroflexi, Bacilli, and Deinococci, and the female plurals Thermotogae, Aquificae, and Chlamydiae—do not follow this recommendation and instead take into account Graeco-Latin grammar.

The Bacteriological Code does not cover phyla; instead, the scientific community primarily uses the NCBI and LPSN taxonomy, in which the phylum name is typically the plural of the type genus, except for the names of Firmicutes, Cyanobacteria, and Proteobacteria, which are not derived from genus names. The molecular phylogeny community mostly ignores the higher taxa that Cavalier-Smith postulated (see above).

1.5 NUTRITIONAL REQUIREMENT OF MICROORGANISM

For survival and proper operation, all living forms—from bacteria to humans—need some essential foods. The following observations substantiate this claim and show the diverse range of nutritional types found in bacteria.

1. It is essential for all living things to have energy. Organisms that obtain their energy from chemicals are known as chemotrophs. Others may use radiant energy or light; these are referred to as phototrophs. Both phototrophs and chemotrophs are found in bacteria.
2. Every living thing needs an electron source in order to function. The word "lithotroph" describes organisms that have the ability to use reduced inorganic compounds as electron donors; these organisms can be either photolithotrophs or chemolithotrophs. Other species, known as organotrophs (some chemoorganotrophs, others photoorganotrophs), use organic materials as providers of electrons.
3. All organisms require carbon in some form in order to use it in the creation of cell components. On the other hand, some species, referred to as autotrophs, can utilise CO₂ as their main or even exclusive source of carbon. Heterotrophs are those that obtain their carbon from organic matter.
4. All organisms require nitrogen in some form for cell components.
5. All living things require oxygen, sulphur, and phosphorus for the building blocks of their cells. Oxygen can come from a variety of sources, including water, the atoms that make up certain nutrients, molecules of oxygen, and other things. Three amino acids—cysteine, cystine, and methionine—are produced in response to sulfur. While certain bacteria can use elemental sulphide and even inorganic sulphide compounds, others require organic sulphide compounds. Phosphorus is usually provided as phosphate. Phosphorus is an essential component of nucleotides, nucleic acids, phospholipids, teichoic acids, and other compounds.
6. All microorganisms require metal ions, such as K⁺, Ca²⁺, Mg²⁺, and Fe²⁺ and other metals ions for normal growth.
7. All microorganisms contain vitamins and vitamin-like compounds. These function either as coenzymes for several enzymes or as the building blocks for coenzymes.

8. All living organisms require water, and in the case of bacteria, all nutrients must be in aqueous solution before they can enter the cells.

1.6 NUTRITIONAL TYPES OF MICROORGANISMS

Based on their dietary needs, microorganisms can be categorized into a wide range of types. The two groups—phototrophs and chemotrophs—are the main group.

1.6.1 PHOTOTROPHS

Certain kinds of bacteria that are phototrophic—referred to as photolithotrophs—get their electrons from inorganic substances. For example, *Chromatium mckenziei* uses H_2S as an electron source to oxidize it to elemental sulfur. Because they use organic materials like fatty acids and alcohols as electron donors, some phototrophic bacteria are also known as photoorganotrophs. For example, succinate can be used by *Rhodospirillum rubrum* as an electron donor.

1.6.2 CHEMOTROPHS

Chemolithotrophs, also known as chemotrophic bacteria, utilize inorganic compounds as electron sources. For instance, *Nitrosomonas* bacteria rely on ammonia for electron supply, converting it into nitrite to generate energy. Chemoorganotrophs, a subset of chemotrophic bacteria, utilize organic substances like sugars and amino acids as electron donors. Some bacteria, such as *Pseudomonas pseudoflava*, exhibit flexibility by extracting electrons from either inorganic molecule like H_2 or organic compounds like glucose. Autotrophic bacteria have relatively simple nutrient requirements, thriving on a mix of inorganic substances, showcasing their adeptness at converting these compounds into complex organic molecules essential for cell composition. In contrast, heterotrophic bacteria, a crucial nutrient group, exhibit substantial variations in their specific growth requirements, particularly organic carbon sources, nitrogen sources, and vitamins.

1.6.3 AUTOTROPHS AND HETEROTROPHS

Autotrophs are organisms capable of utilizing CO_2 as their exclusive carbon source for absorption. Some autotrophs, known as facultative autotrophs, have the flexibility to survive either as autotrophs or heterotrophs, obtaining their carbon from organic molecules or CO_2 . For example, *P. pseudoflava* can function as an autotroph when supplied with H_2 as the electron source, but it can also flourish as a heterotroph when glucose is the sole carbon source for assimilation.

1.6.4 OBLIGATE PARASITES

Certain bacteria have not been successfully cultured in an artificial medium, and their dietary and physical requirements remain unknown. Currently, these bacteria can only be proliferated by utilizing a living host as a medium. An example is *Mycobacterium leprae*, the bacterium responsible for leprosy, which can be cultivated by infecting mice or armadillos.

1.7 BACTERIAL MEDIA

Autotrophs necessitate cultivation in chemically defined media, which can also serve to delineate the nutritional requirements of heterotrophs. However, conventional practice does not typically involve

the use of chemically specified media for the routine cultivation of heterotrophs. Instead, the growth of a diverse array of heterotrophic bacteria is sustained through the utilization of complex raw materials, such as peptones, meat extract, and yeast extract. When a solid medium is preferred, agar is employed as an inert solidifying agent. Detailed information about these raw materials is provided in Table 1.1. Liquid and solid media examples that are relatively simple yet support the growth of numerous common heterotrophs include nutrient broth and nutrient agar (Table 1.2). The incorporation of yeast extract in these formulations enhances their nutritional quality due to its various B vitamins and other growth-promoting compounds. For the cultivation of some fastidious heterotrophs, additional complex supplies like bovine rumen fluid, animal blood, blood serum, or extracts of plant and animal tissues may be required.

Table 1.1 Characteristics of several Complex Materials Used as Ingredients of Media

Raw Material	Characteristics	Nutritional importance
Peptone	The final result of breaking down protein-rich substances like meat, casein, and gelatin is known as peptone. Protein digestion occurs through the action of acids or enzymes. Various peptones are accessible for bacteriological media, depending on the specific protein and digestion method employed. Peptones exhibit differing abilities to facilitate bacterial growth.	The primary supplier of organic nitrogen, it may also encompass varying amounts of vitamins and sporadically include carbohydrates, contingent upon the type of absorbed proteinaceous material.
Yeast extract	An aqueous extract of yeast cells, commercially available as a powder	A very rich source of the B vitamins; also contains organic nitrogen and carbon compounds
Agar	A complex carbohydrate obtained from certain marine algae; processed to remove extraneous substances	Agar serves as a solidifying agent in media; upon dissolving in water-based solutions, it forms a gel when the temperature falls below 45°C. However, it is not considered a nutrient source for bacteria.
Beef extract	An aqueous extract of lean beef tissue concentrated into a paste	Contains the water-soluble components of animal tissue, including salts, organic nitrogen compounds, carbohydrates, and water-soluble vitamins.

Table 1.2 Composition of Nutrient Broth, and Nutrient Agar

Nutrient broth	Quantity
Beef extract	3g
Peptone	5g
Water	1,000 ml
Nutrient agar	
Beef extract	3g
Peptone	5 g
Agar	15 g
Water	1,000 ml

1.7.1 TYPES OF MEDIA

Numerous specialized media are required to aid in the identification, counting, and separation of specific bacterial types. To meet these needs, the microbiologist has available numerous media which, on the basis of their application or function, may be classified as follows:

1.7.1.1 SELECTIVE MEDIA

These media provide nutrients that support the growth and dominance of a particular bacterium species, while inhibiting the growth (and possibly preventing) of other potential organisms. For example, if a soil sample with diverse bacteria is introduced to a medium with cellulose as the sole carbon source, the development of cellulose-utilizing organisms will be specifically favoured or enhanced.

1.7.1.2 DIFFERENTIAL MEDIA

When introduced to culture media, particular additives or reagents can facilitate the differentiation of various bacterial species. For example, some bacteria may cause hemolysis (destruction) of red blood cells when inoculated on a blood-containing agar medium (blood agar), while others do not exhibit this behavior. Therefore, on the same medium, it becomes possible to discern between hemolytic and non-hemolytic bacteria.

1.7.1.3 ASSAY MEDIA

Assay methods for vitamins, amino acids, and antibiotics utilize specified compositions of media. Additionally, there are specialized media compositions designed for evaluating disinfectants.

1.7.1.4 MEDIA FOR BACTERIAL ENUMERATION

Certain types of media are employed to assess the bacterial content in substances like milk and water. The composition of these media must conform to specified specifications.

1.7.1.5 MEDIA FOR BACTERIAL CHARACTERIZATION

Various types of media are typically employed to identify the growth characteristics of bacteria and assess their capacity to induce specific chemical alterations.

1.7.1.6 MAINTENANCE MEDIA

Sustaining the viability and physiological characteristics of a culture over time might require utilizing a medium that differs from the optimal one for growth. The rapid demise of cells towards the end of the development phase could be associated with excessive and swift growth. For example, while glucose often fosters development in a medium, it can also lead to the generation of cell-damaging acid. Hence, it is preferable to omit glucose in a maintenance medium.

1.7.1.7 SOLID AND SEMISOLID MEDIA

Bacteria are commonly studied using solid and semisolid media in addition to liquid media. Solid media is employed to isolate bacteria and analyse colony properties, with agar typically serving as the solidifying agent. Agar, used at concentrations of 1.5 to 2.0 percent, produces firm and translucent gels that resist bacterial degradation. In the case of autotrophic bacteria, silica gel may be used as an inorganic solidifying agent. Semisolid media, with a soft, custard-like consistency achieved by using agar at concentrations of 0.5 percent or less, prove useful for cultivating microaerophilic bacteria or conducting tests on bacterial motility.

1.8. MEDIA PREPARATION

To cultivate bacteria, various naturally occurring chemicals are utilized, with milk, typically in its skim form rather than whole, being one of them. The process involves straightforward dispensing into tubes or flasks, followed by sterilization, when employing such natural resources. The essential components are combined to form nutrient broth or nutrient agar media. Alternatively, water can be added to a pre-packaged dried product containing all necessary nutrients for convenience. Most media are commercially available in powdered form. The preparation of bacteriological media generally follows these steps:

1. Dissolving each ingredient or the entire dehydrated medium in an appropriate volume of distilled water.
2. Determining and adjusting the pH of the fluid medium using a pH meter.
3. Adding and dissolving bacteriological agar in the media through boiling if a solid medium is desired.
4. Pouring the medium into flasks or tubes.
5. Sterilizing the medium, typically using an autoclave, although filtration may be employed for heat-labile media or specific components.

1.9 CONDITIONS REQUIRED FOR BACTERIAL GROWTH

Understanding the optimal physical conditions for the growth of organisms is just as crucial as understanding the appropriate nutrients for cultivating bacteria. Bacteria exhibit diverse dietary requirements, and their responses vary depending on environmental factors such as pH, temperature, and gaseous conditions.

1.9.1 TEMPERATURE

The temperature conducive to the fastest growth within a brief timeframe (12 to 24 hours) is

referred to as the optimum growth temperature. Table 1.3 displays the optimal temperature for various bacteria, along with the temperature range in which they thrive.

Table 1.3 Temperature-dependent growth characteristics of various bacterial species

	Temperature of Growth, °C		
	Minimum	Optimum	Maximum
<i>Vibrio marinus</i> strain MP-1	-1	15	20
<i>Vibrio psychroerythrus</i>	0	15	19
<i>Pseudomonas fluorescens</i>	4	25–30	40
<i>Staphylococcus aureus</i>	6.5	30–37	46
<i>Corynebacterium diphtheriae</i>	15	37	40
<i>Neisseria gonorrhoeae</i>	30	35–36	38.5
<i>Streptococcus thermophilus</i>	20	40–45	50
<i>Thermoactinomyces vulgaris</i>	27–30	60	65–70
<i>Thermus aquaticus</i>	40	70–72	79

Source: Data from R. Y. Morita, *Bacterial Rev*, 39:144, 1975, and *horn Bergey's Manual of Determinative Bacteriology*, 8th ad, Williams & Wilkins. Baltimore, 1974.

On the basis of their temperature relationships, bacteria are divided into three main groups:

- i. **Psychrophiles** are capable of growth at 0°C or lower, although they prefer higher temperatures for optimal development. Microbiologists typically reserve the term "psychrophile" for organisms that can grow at 0°C but exhibit optimal growth at 15°C or lower, with a maximum temperature of around 20°C. The terms "psychrotroph" or "facultative psychrophile" are employed to describe organisms that can grow at 0°C but thrive at temperatures ranging from 20 to 30°C.
- ii. **Mesophiles** flourish in the temperature range of approximately 25 to 40 degrees Celsius. For example, all bacteria harmful to humans and other warm-blooded creatures fall under the category of mesophiles, with the majority thriving at body temperature (37°C).
- iii. **Thermophiles** thrive in environments where temperatures exceed 45°C. Numerous species of facultative thermophiles are those whose growth range extends into the mesophilic zone.

1.9.2 GASEOUS REQUIREMENT

The primary gases influencing bacterial growth are oxygen and carbon dioxide. Bacteria exhibit diverse responses to free oxygen, leading to their classification into four groups based on the following criteria:

- a) **Aerobic bacteria** depend on oxygen for growth and can thrive in an air atmosphere (e.g., *Nocardia sp.*, *Pseudomonas aeruginosa*, *E. Coli*, *Citrobacter*, *Klebsiella*, *Proteus*, *Salmonella*, *Achromobacter*, *Mycobacterium tuberculosis* etc.).
- b) **Anaerobic bacteria** are unable to flourish in an air environment due to their inability to utilize oxygen for energy production, which is toxic to them. Non-stringent or tolerant anaerobes can survive in low oxygen environments, while *strict anaerobes cannot endure such conditions and may perish after brief exposure. The common anaerobes associated are Bacteroides and Fusobacterium species.*
- c) **Facultatively anaerobic** bacteria can grow without oxygen but can use it if available for energy production. They thrive equally well in both air and oxygen-free environments, as oxygen does

not limit their growth. *Staphylococcus* spp., *Escherichia coli*, *Salmonella*, *Listeria* spp., *Shewanella oneidensis* and *Yersinia pestis*.

- d) **Microaerophilic bacteria** necessitate low levels of oxygen for growth but cannot tolerate the oxygen levels present in an air atmosphere.

1.9.3 pH

Most bacteria thrive in a pH range of 6.5 to 7.5, with limits spanning from 5 to 9. However, certain bacteria prefer more extreme pH conditions for optimal growth. For example, *Thiobacillus thiooxidans* may flourish in a pH range of 0.5 to 6.0 but shows a preference for a pH of 2.0 to 3.5. The addition of a buffer, a substance that stabilizes pH, to the medium can help prevent drastic fluctuations in pH.

1.9.4 MISCELLANEOUS REQUIREMENTS

Certain microorganisms have specific additional requirements. Phototrophic bacteria, for instance, rely on light as their energy source and therefore need exposure to a light source. Additionally, bacterial development can be influenced by hydrostatic pressure. In laboratory settings, some of these microorganisms may not thrive unless the medium is subjected to pressures resembling those found in the deepest ocean trenches, where pressure is measured in tonnes per square inch.

1.10 SUMMARY

All organisms that are very small or microscopic in size, and cannot be seen with the naked eye are referred to as microorganisms. Microorganisms are visible under the microscope. Anton van Leeuwenhoek first observed microorganisms under the microscope. Microorganisms include bacteria, archaea, algae, fungi, protozoa, etc. Although viruses are not considered living organisms, sometimes they are also included in the micro-organisms category. Classification is the process by which organisms are grouped into various categories based on morphological and physiological characteristics. There have been various attempts to classify organisms based on their morphological, physiological, cellular and molecular characteristics. Modern classification is also based on evolutionary relationships i.e. phylogenetic relationships. Based on the following characteristics, R. H. Whittaker divided living organisms into five kingdoms namely Monera (Unicellular prokaryotes), Protista (Unicellular eukaryotes), Fungi –Eukaryotic, heterotrophic (saprophytic/ parasitic) and with a cell wall (chitin), Plantae – Eukaryotic, autotrophic (photosynthetic) and with a cell wall (cellulose) and Animalia – Eukaryotic, heterotrophic (holozoic/ saprophytic etc.) and without a cell wall. Binomial nomenclature is the biological system of naming the organisms in which the name is composed of two terms, where, the first term indicates the genus and the second term indicates the species of the organism. Bacteria are prokaryotic organisms that require nutrients and energy for their growth and development like other living organisms. They require carbon, hydrogen, oxygen, nitrogen, minerals and water for their biochemical processes. Based on their energy source and the carbon, bacteria are divided into two major classes: autotrophs and heterotrophs. Organisms that obtain carbon from carbon dioxide and use light energy or inorganic chemical compounds to produce complex organic compounds are known as autotrophs. These are bacteria that can synthesize their food from inorganic compounds. Heterotrophs are organisms that cannot make their food but instead obtain nutrition from other organic sources that may be living or dead. A bacterial culture or growth media contains nutrients that are required to grow microorganisms. Different cell types are grown in various types of medium. Nutrient broths and agar

plates are the most typical growth media for microorganisms. Some microorganisms or bacteria need special media for their growth.

1.11 TERMINAL QUESTIONS

Q.1 Discuss Whittaker's Five-Kingdom Concept of microbial classification.

.....
.....

Q.2 Write an explanatory note on Bergey's Manual of Systematic Bacteriology.

.....
.....

Q.3 What nutritional requirements in terms of chemicals are needed by all forms of life for growth and cellular maintenance?

.....
.....

Q.4 Distinguish between (a) phototrophs and chemotrophs, (b) lithotrophs and organotrophs, and (c) autotrophs and heterotrophs.

.....
.....

Q.5 Discuss different kinds of media used for the cultivation of microorganisms.

.....
.....

Q.6 Discuss the importance of different components which are used in media.

.....
.....

Fill in the blanks:

- i. grow best at temperatures above 45°C.
- ii. The temperature that allows for the most rapid growth during a short period of time (12 to 24 h) is known as the.....
- iii. Organisms that can use CO₂ as their sole source of carbon for assimilation are termed
- iv. Mesophiles grow best within a temperature range of approximately
- v. A culture is a culture that contains only one species of bacteria.

1.12 SUGGESTED READINGS

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UNIT-2 : MICROBIAL TRANSFORMATION

Structure

- 2.1 Introduction
 - Objectives
- 2.2 Microbial Biotransformation
- 2.3 Bioaccumulation
- 2.4 Bioleaching
- 2.5 Copper Mining using *Acidithiobacillus*
- 2.6 Copper Bioleaching Techniques
 - 2.6.1 Heap Irrigation
 - 2.6.2 Copper Extraction from Leach Liquor
- 2.7 Microbial Extraction of Uranium from Ores
- 2.8 Factors Influencing Bioleaching
 - 2.8.1 Mineralogy of Ore
 - 2.8.2 Microorganisms
 - 2.8.3 Temperature
 - 2.8.4 pH
 - 2.8.5 Nutrients and Energy Source
- 2.9 Bioremediation
- 2.10 Use of Bioreactors in Bioremediation
- 2.11 Summary
- 2.12 Terminal Questions
- 2.13 Suggested Readings

2.1 INTRODUCTION

The sustenance of all life on Earth hinges upon the presence of metals and metalloids. Elements such as copper fall under the category of metals, exhibiting characteristics such as proficient heat and electrical conductivity. On the other hand, metalloids like selenium display metallic features only under specific crystallographic configurations. Metals can be further classified into essential and toxic elements for various organisms. Crucial metals for a wide range of organisms, spanning from simple

prokaryotes to mammals, encompass iron, copper, zinc, selenium, cobalt, manganese, molybdenum, calcium, magnesium, sodium, and potassium. Meanwhile, metals like arsenic, lead, boron, and tungsten are indispensable for some organisms while toxic to others. It is noteworthy that even essential metals can become toxic if their concentrations exceed metabolic requirements. Throughout the course of evolution, microorganisms have engaged with metals, developing an array of mechanisms to cope with metal deficiencies or surpluses. Primitive life forms, including prokaryotic and eukaryotic bacteria, archaea, and fungi (collectively termed microbes), have particularly diversified mechanisms to navigate unfavourable environmental conditions. Microorganisms leverage thirty or more essential elements, with twenty being metals, aligning their utilization with the elements' abundance in the Earth's crust and their solubility in water. The industrial revolution has significantly contributed to the large-scale transfer of metals from reserves to the exchange pool through human activities. Microorganisms have adapted by evolving strategies to maintain minimal intracellular content of toxic metals through defensive mechanisms or homeostasis. Notably, microbes can metabolize metals into organometallic compounds, such as dimethylarsinic acid or trimethylarsine, which may be soluble or volatile.

Objectives :

After reading to the unit, the Learner will able to

- To discuss the concepts of microbial transformation
- To understand the concepts of bioaccumulation and bioleaching
- To discuss the role of microorganisms involving the extraction of copper and uranium
- To understand the factors affecting the bioleaching process
- To learn the use of bioreactors in bioremediation process

2.2 MICROBIAL BIOTRANSFORMATION

Biotransformation encompasses alterations to the structure of a chemical compound facilitated by organisms or enzyme systems, resulting in the creation of molecules exhibiting increased polarity. This mechanism, evolved by microbes to adapt to environmental shifts, proves valuable in diverse biotechnological processes. A key aspect of biotransformation is its ability to preserve the original carbon skeleton while yielding products. There are two primary types of biotransformation: enzymatic and non-enzymatic. Enzymatic processes are further categorized into microsomal and non-microsomal. Enzymatic elimination occurs through various body enzymes, while microsomal biotransformation involves enzymes within the lipophilic membranes of the smooth endoplasmic reticulum. Non-microsomal biotransformation utilizes enzymes present in the mitochondria. Examples include alcohol dehydrogenase metabolizing ethanol into acetaldehyde. Microbial biotransformation finds extensive application in transforming pollutants and various compounds such as hydrocarbons, pharmaceuticals, and metals. Transformations fall into categories like oxidation, reduction, hydrolysis, isomerization, condensation, formation of new carbon bonds, and introduction of functional groups. White biotechnology utilizes microbial biotransformation to produce desired products, employing living cells like bacteria, fungi, animals, plants, algae, yeast, and actinomycetes. Wine and beer making are examples of biotransformations that have been practiced since ancient times. Vinegar has long been produced by fermentation, involving the oxidation of ethanol to acetic acid. Microbial cells are preferred for biotransformation due to their high surface-volume ratio, faster growth rates reducing biomass transformation time, efficient substrate transformation owing to heightened metabolism, and ease of maintaining sterile conditions. Since the industrial revolution, human activities have significantly

transferred metals from reserves to the exchange pool. Microbial responses to metal interactions in terrestrial and aquatic environments involve precipitation as minerals, volatilization, oxidation, and reduction of essential and toxic metal forms. Environmental interactions between metals and bacteria are categorized as beneficial and defensive.

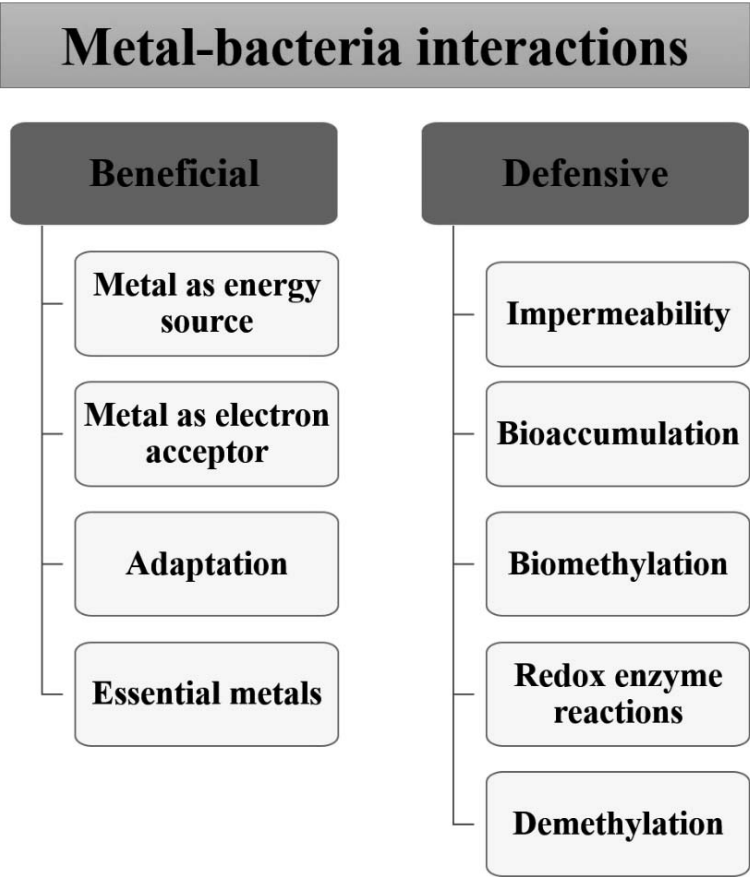


Fig 2.1 Different Types of metal-bacteria interactions

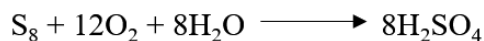
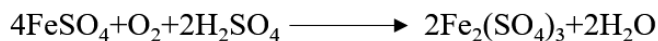
2.3 BIOACCUMULATION

Bacteria produce various types of polymers in the extra-, peri-, and endo-cellular domains, effectively sequestering metals through bioaccumulation and significantly mitigating metal toxicity. Among certain bacterial groups that generate capsules, glycocalyx, and other exudates, extracellular complexation stands out as the predominant mechanism of metal resistance. These structures play a crucial role in reducing toxicity by binding metals and preventing their adverse effects on cellular metabolism. Microbial cell surfaces typically exhibit a negative charge due to the presence of carboxyl, amino, and phosphodiester groups in the cell wall. Metals bind to both microbial surfaces and clay minerals, as the resulting surface charge is negative. Microorganisms enhance metal accumulation by producing various biological ultrastructures, including spores, capsules, S-layers, slime layers, fimbriae, flagella, sheaths, division sites (septa), and diverse extracellular and intracellular polymers. Capsules and sheaths, composed of repetitive sugar residue sequences known as glycocalyces, are highly hydrated structures. Certain bacteria, such as *Leptotrix* and *Sphaerotilus*, exhibit notable interactions with manganese and iron, respectively. S-layers, comprising proteins or glycoproteins with a molecular weight range of 24 to 150 Kilodalton, often require metal ions like Ca^{2+} and Mg^{2+} for proper assembly and adhesion, as observed in *Sporosarcinaureae*. Intracellular metal accumulation through polymers is observed in nickel-resistant blue-green algae, *Synechococcus species name*. Biological mineralization, a

unique aspect of bioaccumulation, occurs after creating a supersaturated state in the microorganism's surrounding environment. The formation of minerals in extra- or intra-cellular spaces of microbial cells is a significant phenomenon in metal cycling. Biogeomorphogenesis involves the storage of energy and metals in the reserve pool, with anaerobic bacteria primarily responsible for the authigenic formation of mineral deposits.

2.4 BIOLEACHING

Bioleaching involves extracting metals from sulphide ores or concentrates using elements naturally present in the environment, namely water, air, and microorganisms. Simply put, it is the commercial utilization of specific bacteria and archaea, naturally occurring, to facilitate the oxidation of sulphide minerals. What sets bioleaching apart from traditional acid leaching is its focus on leaching sulphide minerals, rather than oxidized ones. A related process is biooxidation, where sulphide minerals associated with, though not necessarily part of, the targeted mineral are oxidized. In contemporary commercial operations, bioleaching is employed for copper, nickel, cobalt, zinc, and uranium ores, while bio-oxidation is used in gold processing and coal desulfurization. The bioleaching process utilizes microorganisms to catalyse the oxidation of iron sulphides, producing ferric sulphate and sulfuric acid. Ferric sulphate, a potent oxidizing agent, then oxidizes copper sulphide minerals, allowing the leaching of the copper by the formed sulfuric acid. Bioleaching of metals stands in contrast to mineralization, as it involves the biological oxidation of mineral ores, releasing the metals. The environmental significance lies in the natural mobilization of metals from reserves to the exchange pool. The attention garnered by bioleaching is due to its potential application in the biorecovery of metals, particularly in extracting metals from low-grade tailings and ore deposits through a process known as "biohydrometallurgy." Bioleaching has been implemented on an industrial scale globally, involving in situ, heap, and stirred tank bioreactors for mineral ores containing copper, gold, and uranium. Natural bioleaching of metals occurs in various mining areas worldwide. The pivotal role of bacteria, specifically *Thiobacillus ferrooxidans*, in metal solubilization and acidification of coal mine waters was discovered in Montana (USA) in 1950. *T. ferrooxidans*, thriving at acidic pH levels (2-3), is omnipresent in mine drainage waters, soils contaminated by industrial wastes, and waters with high levels of reduced sulphur species and metals. This bacterium catalyses metal release in all acid aerobic environments, utilizing energy from the oxidation of ferrous iron, soluble and insoluble sulphides, sulphur, and soluble sulphur compounds such as thiosulphate, tetrathionate and possibly cuprous ions to catalyze the following reactions:



Organic acids such as gluconic acid, oxalic acid, itaconic acid, and citric acid play a crucial role in the leaching of metals from non-sulphide and non-ferrous ores, resulting from the fermentation of sugars by both prokaryotic and eukaryotic cells. Notably, a strain P6 of *Penicillium* spp., cultivated in the presence of laterite ores, demonstrated a remarkable nickel removal efficiency of 70%. The fungus's glucose fermentation produced acidity, dissolving other metals like iron and magnesium. *Penicillium* P6 exhibited superior nickel removal capabilities compared to industrially used *A. niger*, which is known for citric acid production. The nickel resistance of *Penicillium* P6 enabled its growth in nickel laterite ores, contrasting with the nickel-sensitive *A. niger*, despite the latter's proficiency in citric acid synthesis.

Concerning uranium, ferric sulfate is employed to oxidize tetravalent uranium oxide, initially insoluble in acid, into hexavalent uranium oxide. Subsequently, sulfuric acid facilitates leaching. In the biooxidation process for refractory gold ores, bacteria are utilized to oxidize the iron sulfide matrix enclosing gold particles, rendering the gold soluble and accessible for cyanide leaching. Similarly, in coal desulfurization, bacteria aid in the oxidation of pyrite contaminants within the coal, making the sulfur soluble as ferric sulfate.

Bioleach processing varies based on the resources being processed. Currently, it is employed in three distinct methods, each tailored to specific types of raw materials:

1. **Dump Leaching-** This method involves leaching waste rock, low-grade ore, or concentrator tailings (including low-grade oxides and secondary sulfides) at the location where they are disposed of. This technique is considered mature and is widely utilized.
2. **Heap Leaching-** Newly mined run-of-the-mine (ROM) material, which includes intermediate-grade oxides and secondary sulfides, is piled on an impervious natural surface or a pad for leaching. ROM can be leached as it is mined or undergo partial crushing and acid mixing before being placed on the heap. This approach is also mature and is experiencing increasing usage. Additionally, primary sulfides such as chalcopyrite can be leached using this method.
3. **Agitated Leaching-** Intermediate- to high-grade chalcopyrite concentrates are placed in a tank and subjected to leaching through mechanical agitation. This method is suitable for materials with intermediate to high grades and is particularly effective for leaching chalcopyrite.

2.5 COOPER MINING USING ACIDOTHIOBACILLUS

Generally, copper predominantly exists in ores comprising diverse copper sulfides and oxides, possessing minimal intrinsic value. Consequently, the extraction of copper involves intricate and protracted chemical procedures, employing rigorous reagents to separate the ore from rock and subsequent treatments to refine the metal, yielding pure copper. Despite their efficacy, these methods are both energetically costly and environmentally harmful, generating substantial quantities of pollutant chemicals such as sulfur dioxide and cyanide. A promising alternative to these detrimental practices is biomining, where bacterial metabolic processes are harnessed to facilitate the solubilization of metal ores. *Acidithiobacillus ferrooxidans*, a lithoautotrophic bacterium, is renowned for utilizing ferrous iron (Fe^{2+}) as an electron donor in conjunction with elemental sulphur.

2.6 COPPER BIOLEACHING TECHNIQUES

Contemporary biomining procedures encompass extensive operations, spanning the extraction of ores and processing over several months. Whether achieved through strip-mining or blasting, the primary objective is to obtain raw ore for the subsequent extraction of copper.

2.6.1 HEAP IRRIGATION

The minerals acquired through mining are crushed and stacked up to a height of 10 meters on plastic irrigation pads. Subsequently, a solution containing acid, known as the lixiviant, is irrigated through the heaps, and the resulting mixture is collected. Following the subsequent procedures to separate the dissolved copper in the leach liquor, the extracted copper is recycled and employed for irrigating the heap once more.

2.6.2 COPPER EXTRACTION FROM LEACH LIQUOR

The extraction of copper metal from leach liquor involves a two-step procedure. The leach liquor, derived from heap irrigation, contains a minimal copper concentration of approximately 0.5 - 2.0 g/L. Utilizing solvent extraction, the copper is transferred from the dilute liquor to a more concentrated solution. Subsequently, the dissolved copper ions undergo a transformation into solid copper metal through electrowinning. This process employs an electric current to reduce the dissolved metal and deposit it onto a cathode. At this stage, the resulting solid elemental copper metal can be utilized for various purposes.

The native culture of mesophilic bacteria predominantly *A. ferrooxidans* is isolated from the source mine water show good potential for the bio-leaching of copper from the low -grade ore.

2.7 MICROBIAL EXTRACTION OF URANIUM FROM ORES

Microorganisms have the potential to facilitate the recovery of uranium through their catalytic actions in the oxidation and reduction processes of uranium and associated metals, thereby influencing their movement in the environment. The industrial-scale bioleaching of uranium involves techniques such as spraying stope walls with acid mine drainage and *in-situ* irrigation of fractured underground ore deposits. This method is not only simple, effective, and relatively cost-efficient with low-energy requirements, but it is also environmentally friendly due to the uranium-solubilizing and accumulating properties of specific microorganisms. The practice of commercially applying bioleaching to extract uranium from low-grade ores has been in place since the 1960s. The leading uranium-producing countries, listed in descending order, are Canada, Australia, Niger, the Russian Federation, Kazakhstan, Namibia, and Uzbekistan. Presently, Canada and Australia alone contribute to over 50% of global uranium production. The microbial consortia responsible for extracting uranium from its minerals consist of complex mixtures of acidophilic, autotrophic, and heterotrophic bacterial strains. In most ores, uranium exists in a mix of minerals in either U(IV) or U(VI) states. While U(IV) can be oxidized to a soluble form by ferric ions, this oxidation process is significantly accelerated in the presence of *A. ferrooxidans*. A pH range of 1.0–2.5 is considered suitable for bioleaching, and a high Fe (III)/Fe(II) ratio is essential for rapid uranium extraction kinetics, influencing the redox potential (Eh) of the leach liquor. As high-grade mineral resources become depleted, there is a need to mine ores deeper underground. The leachability of uranium varies across different locations due to factors such as mineral types, their association with other ore minerals and gangue, and their responsiveness to standard leaching conditions. Minerals like betafite (samiresite), brannerite, and parsonite exhibit lower dissolution rates (16%–35%), while most others display moderate (40%–55%) to higher levels of uranium dissolution (60%–88%). The significance of microorganisms in mining, ore processing, and wastewater treatment is expected to grow in importance over time.

Microorganisms' humble nutritional needs are met through the aeration of a water-based suspension containing iron and/or sulfur-containing minerals or by irrigating a heap. To prevent limitations in nitrogen, phosphate, potassium, and trace elements, small amounts of inorganic fertilizer can be incorporated. An additional beneficial feature of bioleaching processes is their typical lack of contamination by undesired microorganisms. Table 2.1 illustrates various microorganisms with the capability to leach uranium from ores, along with the optimal leaching conditions. The correlation between the characteristics of various microbial classes and their applicability lies in their ability to utilize specific raw materials. For example, the *Acidithiobacillus* bacteria group harnesses energy from Fe (III) in an acidic environment with oxygen, achieving optimal activity at a pH of 1.5–2.5 within the mesophilic range. This enables them to leach 70%–98% of the metal content from the substrate.

Maintaining the exponential phase activity of these microbes requires careful control of nutrient levels, pH, and temperature. In the case of fungi, their primary action involves metabolite-mediated reactions. Microorganisms crucial for dissolving uranium from minerals produce Fe (III) and H₂SO₄ necessary for bioleaching reactions. These microorganisms share common features that make them well-suited for their role in mineral solubilization. They grow autotrophically by fixing atmospheric CO₂, eliminating the need for an organic carbon source. Chemolithotrophs among them derive energy from Fe (II) or reduced inorganic sulfur compounds (some use both) as an electron donor, with oxygen as the electron acceptor. From a bio-hydrometallurgy standpoint, these organisms leverage the redox potential difference between Fe (II)/Fe (III) or S⁰/SO₄²⁻ and O₂/H₂O as their energy source. In bioleaching processes operating at 40°C or lower, a consortium of Gram-negative bacteria, particularly *A. ferrooxidans*, *A. thiooxidans*, *A. caldus*, *Leptospirillum ferrooxidans*, and *L. ferriphilum*, are considered crucial microorganisms.

2.8 FACTORS INFLUENCING URANIUM BIOLEACHING

The efficiency of uranium dissolution relies on various factors such as the characteristics of microorganisms, ore/mineral species (including mineral surface area, particle size, water availability), temperature, pH, redox potential, oxygen, carbon dioxide supply, provision of other nutrients (nitrogen compounds and phosphate), and the presence of toxic substances, as well as the formation of secondary minerals. To enhance the bioleaching of uranium, it is crucial to fine-tune the bioleaching reaction rates and the growth of the involved microorganisms. Achieving optimal leaching requires a comprehensive understanding of the biotic and abiotic reactions within the system.

2.8.1 MINERALOGY OF THE ORE

Each ore is distinct, and within a single ore deposit, there is variability in both the mineral composition and uranium concentration. The bioleaching profiles of mineral samples from each mineralization are individualized due to differences in inclusion and mineral composition, acid demand characteristics, and uranium concentration.

2.8.2 MICROORGANISMS

Various bacteria and fungi have the capability to extract uranium from its ores. The efficacy of this process is influenced by factors such as bacterial population density, metal tolerance, and the organism's ability to adapt to both the mineral surface and the surrounding environment. The rate and extent of uranium dissolution are also contingent upon the specific microbial strain within a given species. Inoculum density has been identified as a factor affecting the bio-leaching rate. Additionally, ore mineralogy, particle size, medium pH, temperature, and the concentration of oxidant (Fe³⁺) play roles in influencing the rate of uranium bioleaching. Notably, *A. ferrooxidans*, *A. thiooxidans*, *Sulfolobus*, and *Leptospirillum*sp. have been utilized on an industrial scale for this purpose thus far.

Microorganism	Characteristics	Leaching Agent	Carbon Req.	Oxygen (Optimum)	pH (Optimum)	Temp range/Optimum Temp (°C)	Leachability
<i>Acidithiobacillus ferrooxidans</i>	Oxidise: Fe ²⁺ , S ⁰ ,	Fe ³⁺ , H ₂ SO ₄	OC	A	1.2–6.0 (1.5–	5–40/28–35	70%–98%

<i>ans</i>	U ⁴⁺ , Cu ⁺ , Se ²⁺ , thiosulphate, tetrathionate, S ⁺				2.5)		
<i>Consortia of Acidithiobacillus thiooxidans and Acidithiobacillus ferrooxidans</i>	Oxidise: S ⁰ , thiosulphate, tetrathionate	H ₂ SO ₄	OC	SA	0.5–6.0 (1.5–2.5)	10–40/28–30	65%–72%
<i>Acidithiobacillus acidophilus</i>	Oxidise: S ⁰ , organic compounds	H ₂ SO ₄	OC	A	1.5–6.0 (3.0)	5–35/25–30	60%–75%
<i>Leptospirillum ferrooxidans</i>	Oxidise: Fe ²⁺ , pyrite	Fe ³⁺	OC	A	1.5–4.5 (2.5–3.0)	20–40/35–45	70%–85%
<i>Pseudomonas fluorescens</i>	Accumulation by siderophores	Fe ³⁺	H	A	5.2–8.5	22–25/24	54%
<i>Aspergillus niger</i>	Metabolite-mediated leaching, with accumulation on surface	Oxalate	H	SA	3.0–6.0 (3.5–4.5)	20–40/35	85%
<i>Aspergillus terreus</i>		Oxalate	H	SA	3.0–6.3	25–30/28	75%
<i>Penicillium spinulosum</i>		Fumarate	H	SA	4.5–7.5	25–30/28	82%
<i>Cladosporium oxysporum</i>		Unknown	H	SA	3.0	30	71%
<i>Aspergillus flavus</i> and <i>Curvularia clavata</i>		Unknown	H	SA	3.0	30	59% and 50%
Note: OC=Obligate chemolithotroph; H=heterotroph; A=aerobic; SA=strict aerobic.							

Source: Abhilash, & Pandey, B. D. (2015). Microbial extraction of uranium from ores. In Abhilash, B. D. Pandey & K. A. Natarajan (Eds.), *Microbiology for minerals, metals, materials and the environment* (pp. 59–98). London, UK: CRC Press, Taylor & Francis Group. DOI: 10.1201/b18124-4.

2.8.3 TEMPERATURE

The extraction of uranium is significantly influenced by temperature, as the behavior of specific species is contingent upon it. The bioleaching processes are notably impacted by seasonal temperature variations. In summer, characterized by elevated temperatures and humidity, uranium dissolution rates are considerably higher than those observed at lower temperatures. The influence of temperature becomes evident when utilizing thermophiles, which thrive in higher temperatures. Comparisons of oxidation kinetics can be made between mesophiles and moderate thermophiles to highlight the temperature's effect.

2.8.4 pH

Maintaining an acidic environment is essential to keep Fe (III) and uranium in solution, facilitating leaching. Acidity is regulated through the oxidation of iron, sulfur, and metal sulfides, as well as the hydrolysis of ferric iron. Optimal pH values, ranging from 1.0 to 2.5, support the effective oxidation of iron and S^{2-} . Microbial growth is impeded below a pH of 1.0 due to significant cell inhibition in response to higher acidity. However, cellular adaptation is possible by adjusting the pH either upward or downward. *Acidithiobacillus* species can engage with certain sulfide minerals and soluble sulfur compounds even under neutral conditions. *A. ferrooxidans* exhibits a lower activity limit around pH 1.0 and experiences inhibition above pH 5.5.

2.8.5 NUTRIENTS AND ENERGY SOURCE

Adequate nutrients, including nitrogen, magnesium, and iron, are essential for facilitating the growth of *Acidithiobacillus* species. The energy for this process is derived from the oxidation of Fe(II), elemental sulfur, and other sulfur-containing uranium minerals. This oxidation is primarily mediated by genera such as *Acidithiobacillus*, *Leptospirillum*, *Sulfolobus*, *Sulfobacilli*, and *Acidianus* under low pH conditions, leading to the release of uranium.

2.9 BIOREMEDIATION

Bioremediation, a field within biotechnology, utilizes living organisms, including microbes and bacteria, to eliminate contaminants, pollutants, and toxins from various environments such as soil and water. This approach is employed in addressing environmental issues like oil spills or cleaning up contaminated groundwater. The process involves promoting the growth of specific microbes capable of utilizing contaminants like oil, solvents, and pesticides as sources of food and energy. These microbes then transform the contaminants into minimal amounts of water and harmless gases like carbon dioxide. Successful bioremediation hinges on maintaining optimal conditions, including the right temperature, nutrients, and suitable food sources. The absence of these elements may extend the duration of contaminants' cleanup. To enhance conditions unfavourable for bioremediation, "amendments" such as molasses, vegetable oil, or simple air can be introduced to the environment. These amendments optimize conditions for microbial flourishing, thereby expediting the bioremediation process.

2.10 USE OF BIOREACTORS IN BIOREMEDIATION

A bioreactor, in the realm of manufactured equipment or systems, is any apparatus that fosters a biologically active environment. This bioreactor approach involves an *ex-situ* biochemical processing system that employs microbes for the removal of contaminants from wastewater, pumped groundwater,

and the various stages of contaminated soil treatment, including both solid and liquid (slurry) phases. Among the array of bioremediation systems, slurry bioreactors stand out as highly sophisticated and effective solutions for treating polluted soils containing resilient contaminants within a controlled environment. Engineered with a containment system, these bioreactors utilize microorganisms to treat contaminated solid materials such as soil, silt, or sludge, as well as water. The process can take on either an aerobic or anaerobic nature. Typically cylindrical in shape, these bioreactors come in sizes ranging from liters to cubic meters and are commonly constructed using stainless steel.

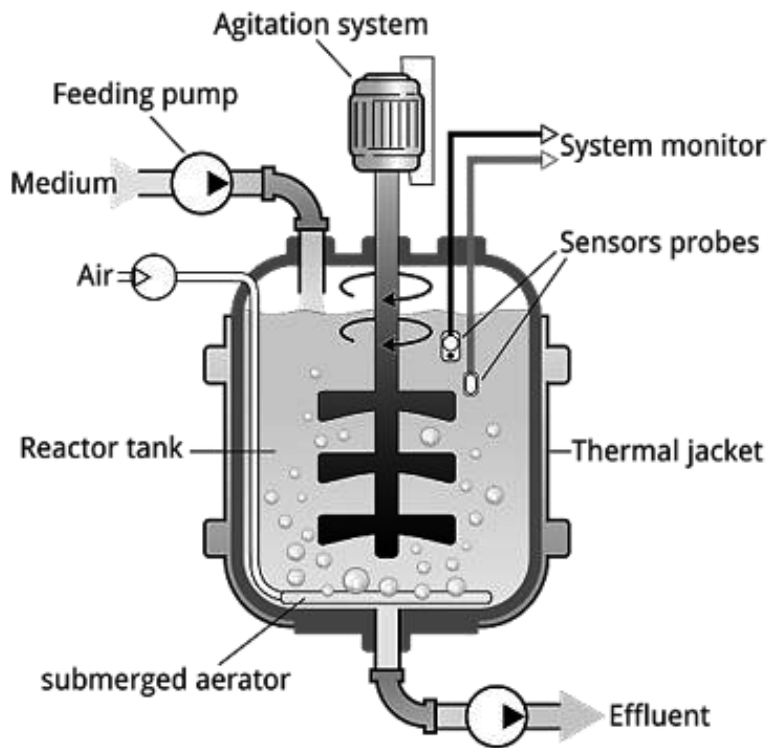


Fig. 2.3 Schematic diagram of a typical Bioreactor

Bioreactor processes involve several steps:

- **Incorporating Additives:** Contaminated soils are combined with water, wastewater, and various additives within a specially engineered bioreactor system, typically enclosed in vats, tanks, or columns. This setup ensures continuous interaction between microorganisms and pollutants within the soil, optimizing environmental conditions for biodegradation.
- **Mechanical Preparation:** Prior to initiating the biological breakdown of pollutants, the soil within the bioreactor undergoes mechanical preparation, encompassing mixing, grinding, and volumetric categorization.
- **Slurry Formation:** The soil is mixed with an appropriate amount of water, determined by the concentration of contaminants, biodegradation rate, and soil type, resulting in the creation of a slurry. Typically, slurries contain 10-30% solids by weight.
- **Nutrient Addition:** To maintain optimal conditions within the slurry, mechanical aeration provides oxygen, and nutrients are added based on the overall pollution quantity in the soil. This ensures an ideal carbon, phosphorus, and nitrogen ratio for effective biodegradation.

- **Slurry Draining:** Post-treatment, the slurry is drained using pressure filters, vacuum filters, or centrifuges.
- **Additional Treatment:** The resulting fluids undergo further treatment using conventional wastewater treatment methods.

Advantages

- **Exceptionally Effective and Efficient:** Bioreaction stands out as an exceptionally effective and efficient process when compared to other studied methods.
- **Enhanced Treatment Rate:** Closed-system slurry bioreactors accelerate and enhance the treatment rate by providing superior control, manipulation, and oversight of various environmental factors. These factors include temperature, pH, aeration, nutrients, emissions, and reactions.
- **Swift and Simple Decontamination:** Utilizing bioreactors for decontamination proves to be a swift and uncomplicated technique, yielding results of over 90%.
- **Precision in Control:** This method offers a significant advantage through precise control over the entire process. By regulating and maintaining influencing parameters, optimal yields can be achieved.

Applications

- Bioreactor technology has proven to be highly effective in treating compounds that degrade aerobically, including SVOCs, refractory insecticides, explosive substances, aromatic hydrocarbons, chlorinated organic compounds, and Polycyclic aromatic hydrocarbons (PAHs). This treatment has been successfully implemented on both laboratory and commercial scales.
- **Applications in Engineering:** These devices are being developed for applications in tissue engineering and biochemical/bioprocess engineering.
- **Full-Scale Devices and Feasibility Studies:** Slurry bioreactors have been utilized as both full-scale devices and for conducting feasibility studies across a broad spectrum of bioremediation applications.
- **Treatment of Challenging Soils:** This approach is employed for treating soils that pose challenges for conventional methods, such as those with high clay concentration (> 40%) or when a rapid treatment is required.

2.11 SUMMARY

Microbial transformation is a fascinating area of study within the field of microbiology that explores the ability of microorganisms to alter the chemical composition of various substances. In the context of environmental science and industrial processes, microbial transformation plays a crucial role in processes such as metal accumulation and leaching. This transformative ability of microorganisms has significant implications for industries involved in mining, metal extraction, and environmental remediation. One of the key aspects of microbial transformation is its role in metal accumulation and leaching. Microorganisms, particularly bacteria and fungi, possess the capability to interact with metals in their environment, leading to either the accumulation of metals within their cellular structures or the leaching of metals from solid substrates. This phenomenon has important applications in the field of biomining, where microorganisms are employed to extract valuable metals from ores. For example, bacteria such as *Acidithiobacillus ferrooxidans* are known to oxidize iron and sulfur, creating acidic

conditions that enhance the solubility of metals like copper. This process facilitates the leaching of copper from ores, providing an environmentally friendly alternative to traditional chemical methods. Moreover, microbial transformation extends its influence on the extraction of specific metals, such as copper and uranium. The use of microorganisms for metal extraction is a sustainable approach that minimizes the environmental impact associated with conventional mining and extraction processes. Microorganisms like bacteria and fungi secrete bioactive compounds, such as organic acids and chelating agents, which enhance the solubility and mobility of metals in their surroundings. This bioleaching process is particularly valuable in extracting metals from low-grade ores, contributing to the efficiency and sustainability of mining operations.

Bioremediation, the process of using living organisms to remove or neutralize pollutants from the environment, is another application of microbial transformation. Bioreactors, which are controlled environments that support the growth of microorganisms, play a crucial role in bioremediation processes. These bioreactors provide optimal conditions for the microbial transformation of pollutants, such as heavy metals (e.g., As, Ni, etc.) and organic contaminants (e.g. PAHs, etc.), into less harmful forms. The use of bioreactors in bioremediation offers a scalable and efficient approach to treating contaminated water, soil, and air. The integration of microbial transformation processes in various industrial applications represents a paradigm shift towards more sustainable and eco-friendly practices. As industries seek alternatives to traditional chemical methods with high environmental costs, the use of microorganisms in processes like metal extraction and bioremediation offers promising solutions. Harnessing the capabilities of microorganisms not only improves the efficiency of industrial processes but also reduces the ecological footprint associated with these activities. In conclusion, microbial transformation encompasses a spectrum of processes, including metal accumulation and leaching, extraction of metals like copper and uranium, and the use of bioreactors in bioremediation. These processes highlight the versatility of microorganisms in transforming and interacting with their environment. The application of microbial transformation in industrial processes signifies a transition towards sustainable and environmentally conscious practices, emphasizing the potential of microorganisms to address contemporary challenges in mining, metal extraction, and environmental remediation.

2.12 TERMINAL QUESTIONS

- Q.1 What is microbial transformation? Discuss various types of metal-bacterial interactions.
- Q.2 Discuss bioaccumulation in detail.
- Q.3 Write an explanatory note on bioleaching?
- Q.4 Discuss the microbial extraction of copper.

Q.5 Discuss the microbial extraction of uranium.

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Q.6 What is bioremediation? Discuss the use of bioreactors in bioremediation.

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Q.7 Write explanatory note on bioreactors. Discuss the various advantages and applications of bioreactors.

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2.13 SUGGESTED READINGS

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UNIT-3 : MICROBIAL DEGRADATION

Structure

- 3.1 Introduction
 - Objective
- 3.2 Biodegradation
- 3.3 Biodegradable Pollutants
- 3.4 Microorganism in Biodegradation
- 3.5 Stages of Biodegradation
- 3.6 Microbial Flora of Soil
- 3.7 Soil-Microbe Interactions
- 3.8 Role of Microbes in Soil Fertility
- 3.9 Wastewater Treatment
- 3.10 Biodegradation of Xenobiotics
- 3.11 Summary
- 3.12 Terminal Questions
- 3.13 Further Suggested Readings

3.1 INTRODUCTION

Microbial degradation, a vital ecological process, involves the enzymatic breakdown of organic compounds by microorganisms, prominently bacteria, fungi, and archaea. At its core, microbial degradation plays a pivotal role in recycling nutrients and maintaining ecosystem balance. Biodegradation, a subset of microbial degradation, specifically refers to the decomposition of synthetic compounds, pollutants, plastics, and xenobiotics by microbial action. This process is paramount for mitigating environmental pollution and managing waste streams. Microbes deploy a diverse array of enzymes to catalyse the degradation of complex molecules, transforming them into simpler, more manageable forms that can be assimilated into microbial biomass or further cycled within ecosystems. Understanding microbial degradation mechanisms holds significant implications for environmental sustainability, bioremediation strategies, and the development of innovative biotechnological solutions. Harnessing the metabolic capabilities of microorganisms offers promising avenues for addressing contemporary environmental challenges and promoting a healthier, more resilient planet.

Objectives :

After reading this unit, the Learner will be able to

- To discuss the concept of biodegradation
- To discuss the role of microbes in soil fertility

- To understand the stages of biodegradation
- To discuss biodegradation of xenobiotics
- To discuss wastewater treatment

3.2 BIODEGRADATION

Biodegradation involves the decomposition of organic matter or substances into simpler forms through a biologically catalysed reduction facilitated by living microorganisms. It is a broad term encompassing any alteration or breakdown of a substrate mediated by biological processes. Microbes can metabolically break down biodegradable matter in the presence of oxygen (aerobically) or in its absence (anaerobically). Virtually any form of matter, whether living or non-living, is susceptible to biodegradation, with the primary determinant being the duration of the breakdown process. The European Union has established a standard for biodegradability, requiring 90% of the original substance to be degraded into water, minerals, and carbon dioxide within six months through biological reactions. This degradation process is driven by the metabolic and enzymatic activities of microorganisms such as bacteria, yeast, and fungi. Microorganisms employ two primary modes for biodegradation based on the type of matter and environmental conditions: Mineralization involves the complete degradation of organic pollutants, with organisms using the matter as the sole source of carbon for energy production. In contrast, co-metabolism entails degradation initiated by the addition of a growth substrate, serving as the primary source of carbon and energy. Certain naturally occurring microbes exhibit remarkable catabolic activity, enabling them to transform and degrade a diverse range of compounds, including polychlorinated biphenyls, hydrocarbons (e.g., oils), radionuclides, metals, and more. Biodegradation is considered a highly favorable and sustainable method for removing complex organic matter, often viewed in the context of ecology, the natural environment, and waste management.

3.3 BIODEGRADABLE POLLUTANTS

Since the onset of the industrial revolution and the subsequent rise in living standards, a multitude of highly hazardous organic compounds, including fuels, polycyclic aromatic hydrocarbons (PAHs), dyes, pesticides, and synthetic chemicals such as radionuclides, have been produced and introduced into the environment either directly or indirectly. These intricate and toxic compounds pose a challenge for natural fauna and flora, as they exhibit resistance to rapid degradation upon their release. The following section outlines some specific organic pollutants.

Polycyclic Aromatic Hydrocarbons (PAHs)- Hydrocarbons are organic compounds consisting of hydrogen and carbon, serving as the primary functional units in a compound. They can be classified as either aliphatic, encompassing linear and branched structures, or aromatic, featuring compounds with a benzene ring. Aliphatic compounds comprise alkanes, alkenes, and alkynes, while aromatic compounds include benzene-ring-containing substances like phenols and toluene, etc. PAHs are identified as significant organic pollutants within the 'Hydrophobic Organic Contaminants (HOCs)' category, prevalent in sediments, soils, and air. These compounds are naturally present in gasoline, crude oil, and coal. PAHs can be generated through processes like burning coal, wood, and high-heat cooking of meat. PAHs have the propensity to bind easily, forming minute particles that accumulate in organisms. In the United States, a synthetic PAH known as 'Naphthalene' is utilized in the production of mothballs and various chemicals. Cigarette smoke is another source of releasing numerous PAHs into the environment. Accumulation of PAHs in fish and aquatic organisms can result in their transfer to humans through seafood consumption. The NHANES (National Health and Nutrition Examination Survey) conducted in 2003-2004 by CDC scientists analyzed urine samples from 2,504 individuals aged six years and above

and identified ten distinct PAH metabolites. Prolonged exposure to Naphthalene and other airborne PAHs has the potential to irritate eyes and nasal passages, in addition to causing liver problems.

Polychlorinated Biphenyls (PCBs)- PCBs, characterized as synthetic compounds possessing an oily texture, lacking taste or smell, and displaying a yellow hue, are ubiquitously present in the air, water, sediments, and soil worldwide. These chemical compounds exhibit remarkable chemical stability, high resistance to elevated temperatures, non-flammability, and excellent electrical insulation properties. Owing to these attributes, PCBs find extensive use in hydraulic and electrical equipment, serve as plasticizers in rubber products, and function as pigments in dyes. Following their release into the environment, PCBs easily disperse over long distances through air and soil, resulting in their enduring presence in the atmosphere. Human exposure to PCBs commonly occurs through the consumption of contaminated meat, fish, dairy products, fruits, and vegetables, etc. Upon absorption, PCBs accumulate in fatty tissue, posing risks such as cancer development and acting as endocrine disruptors. Monsanto Inc. commercially sold PCBs under the name Aroclor in the United States. Since their ban in 1977 due to associated health hazards, PCB levels in the food chain and environment have been gradually decreasing.

Pesticides- Pesticides are formulations of toxic compounds designed to target pathogens while remaining safe for non-pathogens such as humans and animals. This category encompasses various chemicals, including herbicides, fungicides, rodenticides, molluscicides, plant growth regulators, insecticides, and nematocides. These substances serve purposes such as weed control, pest eradication, and disease prevention in crops. The use of pesticides plays a crucial role in boosting crop productivity and preventing vector-borne diseases. The United States holds a prominent position as a major global trader, producer, and consumer of these chemicals. However, the widespread use of pesticides poses a significant health risk to non-target organisms due to their high-fat solubility and tendency to bioaccumulate. This can result in adverse effects on the populations of zooplankton and phytoplankton when pesticides are introduced into aquatic environments. Moreover, pesticides exhibit neurotoxic and carcinogenic properties, contributing to reduced fertility in various organisms such as fishes, invertebrates, insects, mammals, and amphibians. Continuous use of pesticides may lead to the development of resistance in pests, diminishing the effectiveness of these chemicals. The biodegradation of pesticides primarily occurs through the activity of soil microorganisms, which utilize them as a food source. However, persistent xenobiotics can become integrated into the environment, leading to biomagnification.

Dyes- They find utility across various industries, including cosmetics, textiles, pharmaceuticals, rubber products, and more. Predominantly employed, Azo dyes consist of diazotized amine coupled with phenol or an amine group, featuring single or multiple azo ($-N=N-$) groups. Microorganisms exhibit promising oxidoreductive enzymes and a dynamic metabolic capacity, enabling them to utilize dyes containing intricate xenobiotic compounds as substrates, thereby aiding in the decolorization of contaminated sites.

Radionuclides and Heavy metals- The nucleus of an atom possesses a notably unstable nature, capable of releasing surplus energy through the radiation process as it undergoes radioactive decay, generating subatomic alpha or beta particles or emitting gamma rays. Microbial breakdown of radionuclides leads to the creation of less hazardous and more enduring compounds. Unlike organic compounds, heavy metals such as arsenic, cadmium, chromium, and others cannot be eliminated, but they can be transformed into stable forms. The mechanism applied by microbes includes:

- **Bioleaching**– mobilization of heavy metal through methylation reactions or excretion of organic acids
- **Biosorption**– metal absorption on the cell surface.

- **Enzyme-** catalyzed redox reactions.
- Intracellular accumulation of heavy metals.
- **Extraction** of heavy metals can be achieved by recovering the precipitate of metals from microbial samples.

3.4 MICROORGANISM IN BIODEGRADATION

Biodegradation is a natural phenomenon occurring in the environment, where complex and toxic organic compounds are broken down into simpler forms by microorganisms like bacteria, fungi, and yeast. This process serves to recycle waste, making it suitable as a food source for other organisms.

Bacterial Strain- Bacteria play a crucial role in the biodegradation process and can be readily isolated from various environments.

- Bacterial strains belonging to the hydrocarbon degradation category include those from the genera *Klebsiella*, *Enterobacter*, *Bacillus*, *Staphylococcus*, *Acinetobacter*, *Brevibacillus*, and *Pseudomonas*. Species such as *Brevibacillus* and *Pseudomonas*, which anaerobically reduce nitrate-containing compounds, have been obtained from petroleum-contaminated soil.
- Gram-negative strains like *Pseudomonas* and *Aeromonas* are involved in the biodegradation of aromatic hydrocarbons.
- A diverse population of bacterial strains is more effective in degrading pollutants as they can easily exchange genetic information related to degradative enzymes and pathways.
- Polychlorinated biphenyls (PCBs) undergo aerobic and anaerobic degradation through redox and dehalogenation reactions. Gram-positive strains such as *Rhodococcus*, *Bacillus*, *Microbacterium*, and Gram-negative strains including *Pseudomonas*, *Sphingomonas*, *Ralstonia* exhibit PCB degrading activity. The degradation process involves four enzymes: biphenyl dehydrogenase, dihydro diol dehydrogenase, 2,3-dihydroxy biphenyl dehydrogenase, and hydrolase.
- Strains of *Staphylococcus* and *Stenotrophomonas* bacteria are responsible for degrading pesticides like DDT (Dichlorodiphenyltrichloroethane), while *Providencia stuartii* is known for degrading the pesticide Chlorpyrifos.
- *Shewanella decolorationis* is identified as a single bacterial species capable of efficiently removing azo dyes.
- Heavy metals undergo transformation through a dissimilatory metal reduction process, where bacterial species utilize metals as terminal electron acceptors during respiratory reactions. *Acidithiobacillus ferrooxidans*, an acidophilic iron bacterium, and sulfur-oxidizing bacteria are found to accumulate high concentrations of Arsenic, Cadmium, Zinc, Cobalt, and Copper from contaminated soils.

Plant Growth Promoting Bacteria (PGPB and PGPR)- These bacteria are commonly present in plants, either naturally occurring in the roots or in close proximity to them. They do not harm plants and play a beneficial role in their growth and development. There exists a mutualistic relationship between plants and plant growth-promoting bacteria, where plants provide shelter and food sources, and in return, bacteria aid in metabolizing toxic substances beneficial for plant use.

- *Pseudomonas* species and *Lysinibacillus* exhibit activity in degrading PAHs released by plants.

- Strains of the *Luteibacter*, *Williamsia*, and *Rhodobacter* genera display promising activity in degrading PCBs found in contaminated soil.
- *Azospirillum lipoferum*, isolated from the rhizoplane of crop plants, has been observed to degrade Malathion, an organophosphorus insecticide.

Fungal Species- They are recognized as the primary microorganisms crucial for carbon decomposition in the atmosphere. Thriving in environments with low pH and moisture, they possess specialized extracellular multienzyme complexes that facilitate the breakdown of natural polymeric compounds. Utilizing their hyphal structures, they efficiently colonize and penetrate substances, aiding in the redistribution of nutrients throughout the mycelium. The degradation mechanisms of fungi are implicated in the recycling of recalcitrant such as lignin. Fungi capable of degrading toluene include deuteromycetes species from the genera *Exophiala*, *Leptodontium*, and *Cladophialophora*, along with the ascomycete strain *Pseudeurotium zonatum*. These fungi utilize toluene as carbon and energy sources. The filamentous fungi *Cladosporium* and *Aspergillus* exhibit degrading activity towards aliphatic hydrocarbons, while *Penicillium*, *Fusarium*, and *Cunninghamella* fungi degrade aromatic hydrocarbons. *Aspergillus niger* has demonstrated biodegrading activity against PCBs. Fungi employ mechanisms such as active uptake, transformation, and extra or intracellular precipitation to detoxify metals. Examples of such fungi include *Rhizopus arrhizus* and *Aspergillus niger*.

Yeast- Various yeast species have undergone extensive examination for their ability to degrade Poly Aromatic Hydrocarbons (PAHs) and utilize them in energy-dependent uptake systems. *Trichosporon cutaneum*, a soil yeast, has demonstrated proficiency in breaking down phenols. Species such as *Candida lipolytica*, *Rhodotorula aurantiaca*, *Candida ernobii*, among others, are capable of degrading alkanes with carbon chain lengths ranging from 10 to 20. The *Candida methanosorbosa* BP-6 species have been identified as potentially effective in breaking down azo dyes like aniline. *Saccharomyces cerevisiae*, *Candida biodinii*, and various other yeast strains exhibit the ability to transform plasticizers, insecticides, fungicides, and polychlorinated biphenyls (PCBs) into simpler forms. Yeast strains like *Hansenula polymorpha*, *Cyberlindnera fabianii*, *Rhodotorula pilimanae*, etc., have been observed to accumulate heavy metals such as Cr, Co, Ni, Mg, and others. They can subsequently reduce these metals through redox reactions.

Genetically Modified Microorganisms (GMM)- Microorganisms that have undergone genetic modification exhibit changes in their genetic material, influenced by the gene transfer techniques of microorganisms using genetic engineering methods such as DNA recombinant technology. These genetically modified microorganisms (GMMs) demonstrate significant potential with enhanced capabilities for biodegrading various chemical contaminants. Key considerations for the development of GMMs in biodegradation applications include:

1. Modification of enzyme specificity and affinity.
2. Regulation and construction of metabolic pathways.
3. Development, monitoring, and control of bioprocesses.
4. Utilization of bioreporter sensory applications to reduce toxicity, sense chemicals, and conduct endpoint analysis.

3.5 STAGES OF BIODEGRADATION

The biodegradation process comprises three distinct phases: Biodeterioration, where complex structures undergo mechanical weakening; Bio-fragmentation, involving the breakdown of toxic and intricate compounds by microorganisms; and Assimilation, wherein old structures are converted into

new compounds.

Biodeterioration- The procedure includes the structural weakening of the compound through mechanical, physical, and chemical means. These changes are triggered by abiotic factors in the environment, such as light, temperature, and chemicals. The biodeterioration process is the first and it is initiated by the adherence and colonisation of microbes on the polymer surface with the sole aim of reducing the resistance and durability of the plastic materials.

Bio-fragmentation- Following the structural weakening, the cleavage of polymeric bonds induces the conversion of oligomers and monomers. This fragmentation occurs both in aerobic and anaerobic environments. Aerobic breakdown of compounds results in the generation of water, carbon dioxide, and basic molecules that serve as a nutrient source. Anaerobic digestion diminishes the mass and volume of complex materials, leading to the production of natural gases. The utilization of anaerobic reactions is prevalent in waste management facilities as a means of harnessing renewable energy.

Assimilation- The microorganisms absorb the recently created molecules through membrane carriers, utilizing them either as a source of energy in the form of ATP (Adenosine Triphosphate) or as components for cell structure.

3.6 MICROBIAL FLORA OF SOIL

Fertile soil is home to the root systems of higher plants, various animal forms such as rodents, insects, and worms, and an abundance of microorganisms. The considerable variations in soil composition, along with differences in physical characteristics and agricultural practices, lead to substantial differences in the microbial population, both in terms of total numbers and types. Few environments on earth boast such a diverse array of microorganisms as fertile soil. This microscopic community includes bacteria, fungi, algae, protozoa, and viruses, potentially reaching billions of organisms per gram. The extensive variety of microbial flora makes it challenging to accurately determine the total number present. Among these microorganisms, bacteria dominate both in quantity and diversity. Microscopic counts have reported figures as high as several billion per gram, while plate counts from the same samples yield only a fraction of this number, typically in the millions. The discrepancy arises from the vast array of nutritional and physiological types of bacteria in soil, making it impossible for a single laboratory environment (comprising medium composition and incubation conditions) to support the growth of every viable cell in the inoculum. Soil is likely to harbor various types of bacteria, including autotrophs and heterotrophs, mesophiles, thermophiles, psychrophiles, aerobes and anaerobes, cellulose digesters, sulfur oxidizers, nitrogen fixers, protein digesters, and other bacterial varieties.

The soil hosts a diverse array of fungi, with hundreds of different species thriving, particularly near the surface where favorable conditions are prevalent. These fungi exist in both mycelial and spore stages, making it challenging to accurately estimate their numbers. Reported counts range from thousands to hundreds of thousands per gram of soil. Fungi play an active role in decomposing plant tissues, breaking down cellulose, lignin, and pectin. The accumulation of molds' mycelium in the soil contributes to the improvement of its physical structure. A significant agricultural benefit lies in the binding together of fine soil particles, forming water-stable aggregates. This binding is achieved through the penetration of mycelium, creating a network that entangles small particles. In soils of vineyards, orchards, and apiaries, yeasts are more likely to be prevalent due to specific conditions, especially the presence of sugars that favor their growth.

The abundance of algae in soil is generally lower compared to bacteria or fungi. The predominant types include green algae and diatoms, primarily found on or just below the soil surface due to their photosynthetic nature. Cyanobacteria, oxygenic photosynthetic bacteria, are observed to

thrive on freshly exposed rocks, leading to the simultaneous deposition of organic matter as their cells accumulate. Most soil protozoa are flagellates or amoebas, with their numbers ranging from a few hundred to several hundred thousand per gram of soil in moist, organic-rich environments. Bacterial viruses (bacteriophages) and viruses from plants and animals may enter soils through the addition of organic waste. Additionally, soil microorganisms themselves may serve as hosts for viruses.

Rhizosphere- The area where the soil and roots come into contact is known as the rhizosphere. The microbial population in and around roots surpasses that in soil without roots, showing both quantitative and qualitative distinctions. Bacteria are predominant, and their growth is boosted by plant tissue-released nutritional substances, such as amino acids, vitamins, and other nutrients. The plant's growth is affected by microbial metabolism byproducts released into the soil. Reports suggest that amino acid-dependent bacteria are more abundant in the rhizosphere than in root-free soil. Studies have proven that the microbiota of the rhizosphere is more physiologically active than that of non-rhizosphere soil. The rhizosphere constitutes an incredibly intricate biological system, and much remains to be discovered about the interactions between the plant and the microorganisms closely linked to its root system.

3.7 SOIL-MICROBE INTERACTIONS

The soil's microbial ecosystem encompasses the entire microbial population along with the physical composition and characteristics of the soil. It constitutes the combined biotic and abiotic elements of the soil. Various microorganisms residing in the soil engage in diverse types of associations or interactions. Some associations are neutral, others are positive or beneficial, and some are negative or detrimental. Initially, the term symbiosis broadly denoted the coexistence of different organisms, but later it acquired a more specific meaning, particularly in reference to the mutually beneficial relationship between bacteria and plants known as symbiotic nitrogen fixation.

Neutral associations (Neutralism)- It is possible for two distinct microorganism species to coexist in a shared environment without mutual interference. For instance, they might employ different nutrients without generating inhibitory metabolic byproducts. This situation could be temporary, and alterations in environmental conditions, especially nutrient availability, could prompt a shift in their relationship.

Positive associations-

Mutualism exemplifies a symbiotic relationship where each organism benefits from their association, and how these benefits manifest can vary. **Syntrophism**, a type of mutualistic interaction, involves the exchange of nutrients between two species. Numerous microorganisms produce different vitamins and amino acids in excess, while others have specific nutritional needs that are met through such exchanges. Some species synthesize essential nutrients in suboptimal amounts. Consequently, certain species thrive together in low-nutrient conditions, emphasizing their interdependence.

Bees gather nectar from flowers to make honey, while inadvertently transferring pollen from one flower to another, aiding in the plants' reproduction. The bee gets food (nectar) from the flower, and the flower gets pollinated, allowing it to produce seeds and reproduce. This mutually beneficial relationship helps both the bee and the flower survive and thrive. Another example is the relationship between certain species of ants and aphids. Ants protect aphids from predators and in return, aphids secrete a sugary substance called honeydew, which ants feed on. This symbiotic relationship benefits both species involved.

Commensalism, on the other hand, describes a relationship where one species benefits while the other remains unaffected. In the context of soil, commensalism is evident in the degradation of complex molecules like cellulose and lignin. Fungi, with cellulolytic capabilities, break down cellulose into

glucose and other byproducts. Although many bacteria cannot directly utilize cellulose, they can thrive by utilizing the breakdown products produced by fungi, such as glucose and organic acids. The presence of nutritionally fastidious bacteria in soil suggests their reliance on the synthesis and excretion of essential nutrients by less fastidious species for growth and survival. The simplest example of commensalism is a bird making a nest in a tree. The tree provides shelter and protection to the bird without getting significantly harmed or affected by the bird.

Negative associations

Antagonism occurs when a species adversely impacts the environment for another species, displaying an antagonistic relationship. Such organisms hold practical significance as they often produce antibiotics or inhibitory substances, affecting the normal growth processes or survival of other organisms. Organisms producing antibiotics serve as a classic example of this phenomenon, but antibiosis can arise from various conditions within mixed populations.

Competition results in a negative association among species vying for essential nutrients. In such scenarios, the microbial species best adapted to the conditions will prevail, potentially eliminating other species dependent on the same limited nutrient substance.

Parasitism is defined as a relationship where one organism lives in or on another organism, feeding on its cells, tissues, or fluids. The host, commonly harmed in the process, becomes dependent on the parasite, establishing an intimate physical and metabolic connection. Microbial parasites can target all major groups of plants, animals, and microorganisms. Viruses that attack bacteria, fungi, and algae are strict intracellular parasites, unable to exist as free-living forms.

- i. Ticks and mammals: Ticks are ectoparasites that attach themselves to the skin of mammals, including humans and other animals, to feed on blood. They can transmit diseases such as Lyme disease and Rocky Mountain spotted fever.
- ii. Tapeworms and vertebrates: Tapeworms are internal parasites that live in the digestive tract of vertebrates, including humans and other animals. They absorb nutrients from the host's food, depriving the host of those nutrients and potentially causing health issues.
- iii. Mistletoe and trees: Mistletoe is a parasitic plant that grows on the branches of trees. It penetrates the host tree's tissue and absorbs water and nutrients from it. Although mistletoe can photosynthesize on its own to some extent, it still relies heavily on the host tree for its survival.
- iv. Dodder and plants: Dodder is a parasitic plant that lacks chlorophyll and cannot photosynthesize. It wraps itself around the stems of host plants and absorbs water, nutrients, and carbohydrates from them. Dodder weakens the host plant and can ultimately cause its death.
- v. Bedbugs and humans: Bedbugs are ectoparasites that feed on the blood of humans and other warm-blooded animals. They typically feed at night while their hosts are asleep, causing itchy bites and potential allergic reactions.

3.8 ROLE OF MICROBES IN SOIL FERTILITY

Soil microorganisms function as biogeochemical agents, facilitating the conversion of intricate organic compounds into either simple inorganic compounds or their constituent elements. This overall process is referred to as mineralization. The transformation of complex organic compounds into inorganic compounds or elements plays a vital role in sustaining elements (or their compounds) as nutrients for plants, animals, and humans. This discussion briefly addresses the role of soil microorganisms in the transformations they induce on nitrogen, carbon, sulfur, phosphorus, and their

respective compounds.

Due to the significance of nitrogen in plant nutrition, extensive research has been conducted on the biochemical events comprising the nitrogen cycle. Fig. 3.1 illustrates the sequential changes, starting from free atmospheric nitrogen to fixed inorganic nitrogen, progressing to simple organic compounds, and then complex organic compounds within the tissues of plants, animals, and microorganisms, ultimately leading to the release of nitrogen back into atmospheric nitrogen.

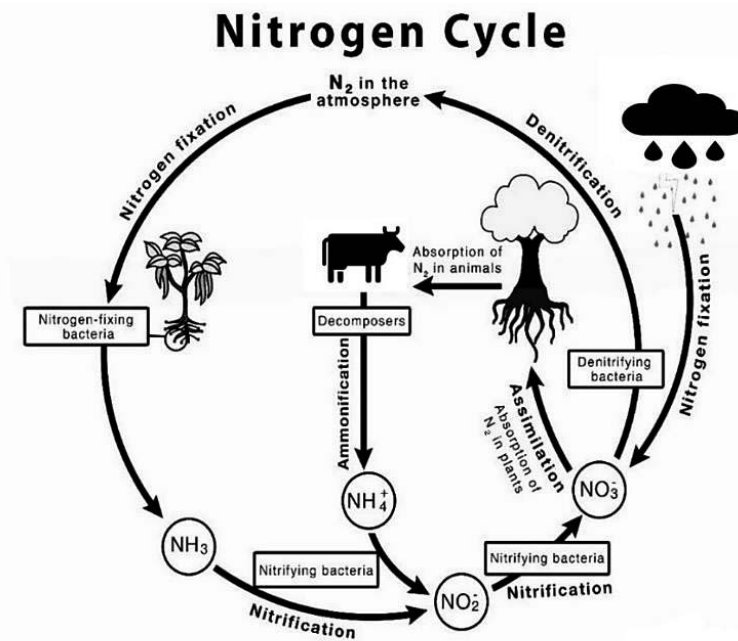


Fig. 3.1 Schematic diagram showing Nitrogen cycle in nature

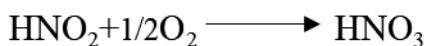
Complex organic nitrogenous substances such as proteins, nucleic acids, purine, and pyrimidine bases, as well as amino sugars (like glucosamine and galactosamine), are introduced into the soil through the deposition of animal and plant wastes or their tissues.

Nitrification is the microbial conversion of ammonia to nitrate, and it takes place in two distinct steps, with each step being carried out by a different group of bacteria.

1. Oxidation of ammonia to nitrite by ammonia-oxidizing bacteria



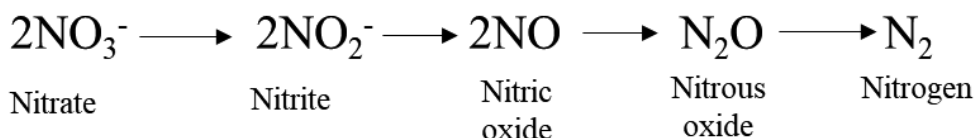
2. oxidation of nitrite to nitrate by nitrite-oxidizing bacteria



Gram-negative chemolithotrophs belong to both physiological groups of bacteria, namely ammonia oxidizers and nitrite oxidizers. Nitrifying bacteria are widely distributed in various environments such as soil, sewage, and aquatic habitats. Examples of recognized ammonia oxidizers include *Nitrosomonas europaea*, *Nitrosovibrio tenuis*, *Nitrosococcus nitrosus*, and *Nitrosococcus oceanus*. Additionally, certain heterotrophic bacteria possess the capability to convert nitrates into nitrites or ammonia, typically occurring in anaerobic conditions like waterlogged soil. In this process, the oxygen in the nitrate acts as an electron and hydrogen acceptor. The process involves several reactions, and the overall result is:



Microorganisms carry out the conversion of nitrates into gaseous nitrogen through a series of biochemical reactions, a process commonly referred to as denitrification. From an agricultural perspective, denitrification is undesirable as it leads to the depletion of nitrogen in the soil, resulting in a reduction of essential nutrients for plant growth. Various bacteria, such as *Achromobacter*, *Agrobacterium*, *Alcaligenes*, *Bacillus*, *Chromobacterium*, *Flavobacterium*, *Hyphomicrobium*, *Pseudomonas*, *Thiobacillus*, and *Vibrio*, are capable of transforming NO_3^- into N_2 . The overall biochemical reaction which expresses the process of denitrification is:



Various microorganisms can utilize atmospheric molecular nitrogen as their nitrogen source, with the conversion of molecular nitrogen into ammonia termed as nitrogen fixation. This process involves two categories of microorganisms: (1) those residing freely in the soil, and (2) symbiotic microorganisms found in the roots of plants. Symbiotic nitrogen fixation occurs through the collaboration of bacteria, specifically of the *Rhizobium* genus, with legumes such as soybeans, clover, and peas. Prior to nitrogen fixation, these bacteria need to establish themselves within the root tissue cells of the host plant. The invasion of the root system by rhizobia bacteria is closely linked to the formation of an "infection thread" in certain root hairs. Through this infection thread, nitrogen-fixing bacteria enter the host plant cells, causing infection, cell enlargement, increased cell division, and the development of abnormal growths (nodules) on the root system. The legume, the bacteria, and the nodule together form the system for this nitrogen fixation type. This mutualistic process benefits both the bacteria and the plant—the bacteria convert atmospheric nitrogen into a form accessible to the plant, while the bacteria receive nutrients from the plant tissues. It's essential to note that not all *Rhizobium* species exhibit nodulation and nitrogen fixation with every legume, as there is a specific interaction between the bacteria and legumes.

3.9 WASTEWATER TREATMENT

Treatment of wastewater is essential to prevent significant undesirable or harmful effects when disposing of it. Despite this necessity, some communities and municipalities release inadequately treated wastewater into natural water bodies, either due to indifference to consequences or the belief that the water body's size and location will dilute potential hazards. With the growing demand for domestic and industrial water, there is an increasing need to reuse water that receives wastewater. Improper disposal of untreated wastewater results in several consequences:

- Higher risk of spreading pathogenic microorganisms.
- Increased danger in using natural water sources for drinking.
- Contamination of shellfish like oysters, making them unsafe for human consumption.
- Significant losses in the waterfowl population due to pollution in their winterfeeding grounds.
- Elevated risk of swimming in the water and reduced value for recreational purposes.

- Depletion of oxygen supply in water due to unstable organic matter, leading to the death of aquatic life.
- Creation of objectionable conditions such as offensive odors and debris accumulation, reducing property values.
- Accumulation and dissemination of toxic chemicals, posing threats to ecosystems and public health.

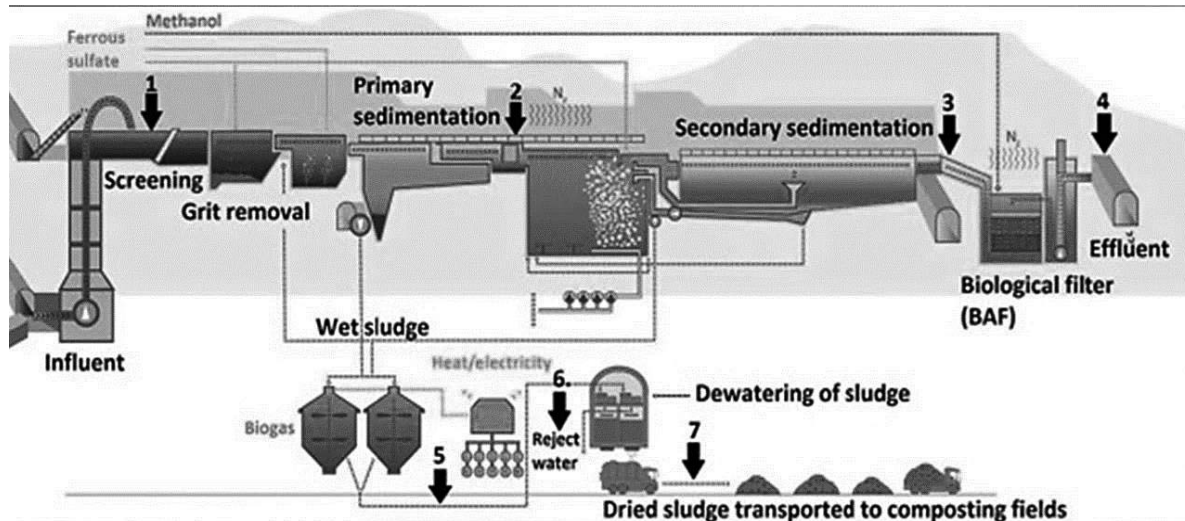
Wastewater treatment processes are diverse. In this discussion, we will explore these processes in two distinct scenarios: (1) for a single dwelling or unit structure and (2) for a community or municipality.

Single Dwelling Units- The treatment and disposal of wastewater and sewage from individual residences or other unit structures, such as certain motels or shopping centres, can be carried out through anaerobic digestion and/or aerobic metabolic processes. One commonly used system for this purpose is the septic tank, which operates as an anaerobic digestion system. The septic tank is specifically designed to retain the solids in the sewage for a sufficient duration, allowing for the effective decomposition of the sludge. In this way, the septic tank accomplishes two essential processes: sedimentation and the biological breakdown of the sludge. Upon sewage entry into the tank, sedimentation occurs in the upper section, enabling the discharge of a liquid with fewer suspended solids from the tank. The settled solids undergo degradation by anaerobic bacteria, resulting in end products that are still highly unstable, characterized by a high biological oxygen demand (BOD) and an odorous nature. The effluent from the septic tank is then distributed beneath the soil surface through a disposal field.

Municipal Treatment Processes- Municipal wastewater treatment plants undergo a sequence of treatment processes, which can be outlined as follows:

1. Primary treatment: Eliminating coarse solids and achieving the removal of settleable solids.
2. Secondary (biological) treatment: Adsorbing and ultimately oxidizing organic components in the wastewater to decrease the Biological Oxygen Demand (BOD).
3. Advanced treatment: Eliminating additional objectionable substances for further reduction in BOD; this includes the removal of nutrients like phosphorus and nitrogen.
4. Final treatment: Disinfecting and disposing of the liquid effluent.
5. Solids processing: Stabilizing solids separated from liquid processes, dewatering solids, and ultimately disposing of them through methods such as land application, landfill, or incineration.

The success of secondary waste water treatment processes relies heavily on the growth and metabolism of microorganisms. The chemical actions of these microorganisms play a significant role in stabilizing the final effluent, as well as in the processes of liquefaction, gasification, and mineralization of sludge constituents. From an ecological perspective, wastewater presents one of the most intricate microbiological environments, with total populations and the distribution of physiological types experiencing notable fluctuations. The diverse substrates are prone to dissimilation by the mixed microbial flora, and interactions among species can lead to outcomes that differ from those observed in pure cultures.



3 STAGE OF WASTEWATER TREATMENT

Fig. 3.2 Schematic representation of a modern municipal sewage treatment plant.

3.10 BIODEGRADATION OF XENOBIOTICS

Xenobiotics, derived from the Greek words "xenos" meaning foreign, encompass a wide range of unnatural, foreign, and synthetic chemicals such as pesticides, herbicides, refrigerants, solvents, and other organic compounds. The microbial degradation of xenobiotics is crucial as it offers an efficient and cost-effective method for disposing of toxic chemicals, particularly environmental pollutants. A specific microorganism strain may be capable of degrading one or more compounds, while sometimes a consortium of microbes working synergistically proves to be more efficient for the degradation of a single compound. For example, the insecticide parathion is more effectively degraded by the combined action of *Pseudomonas aeruginosa* and *Pseudomonas stutzeri*.

In general, the metabolism (breakdown) of xenobiotics does not confer any advantage to the microorganism; the pollutant chemical cannot serve as a source of carbon or energy for the organism. The term "co-metabolism" is often used to describe the non-beneficial biochemical pathways associated with the biodegradation of xenobiotics. However, co-metabolism relies on the presence of a suitable substrate for the microorganism, known as a co-substrate. Several factors influence biodegradation, including the chemical nature of the xenobiotic, the capability of the individual microorganism, nutrient and oxygen supply, temperature, pH, and redox potential. Among these factors, the chemical nature of the substrate targeted for degradation holds particular importance.

Recalcitrant Xenobiotics- Certain compounds exhibit resistance to biodegradation, leading to their prolonged persistence in the environment, often spanning years. Such substances are classified as recalcitrant. The reasons behind the resilience of xenobiotics to microbial breakdown include:

- Chemical and biological inertness, rendering them highly stable.
- Lack of suitable enzyme systems in microorganisms for biodegradation.
- Inability to enter microorganisms due to their large size or absence of transport systems.

- Potential high toxicity or the generation of highly toxic by-products that can eliminate microorganisms.

Numerous recalcitrant xenobiotic compounds exist, such as chloroform, freons, insecticides (e.g., DDT, lindane), herbicides (dalapon), and synthetic polymers (plastics like polystyrene, polyethylene, and polyvinyl chloride). The degradation of DDT in soil typically takes around 4-5 years, with a group of microorganisms (including *Aspergillus flavus*, *Mucor atenans*, *Fusarium oxysporum*, and *Trichoderma viride*) being associated with its slow biodegradation.

3.11 SUMMARY

Microbes play a pivotal role in various ecological processes, contributing significantly to waste decomposition, soil fertility, agriculture, industry, medicine, and wastewater treatment. Understanding their diverse functions sheds light on their importance in maintaining environmental balance and human well-being. Waste decomposition is a crucial aspect of microbial activity. Microorganisms, such as bacteria and fungi, are instrumental in breaking down organic matter in waste, facilitating the recycling of nutrients back into the ecosystem. In natural settings, these microbes play a vital role in decomposing dead plants, animals, and other organic materials, ensuring the efficient cycling of essential elements like carbon, nitrogen, and phosphorus. In soil fertility, microbes contribute to nutrient cycling and bioavailability. Beneficial bacteria and fungi form symbiotic relationships with plant roots, enhancing nutrient uptake and promoting plant growth. Nitrogen-fixing bacteria convert atmospheric nitrogen into a form that plants can utilize, while mycorrhizal fungi extend the plant's root system, increasing surface area and hence ability to access nutrients. The intricate web of interactions between microbes and plants is fundamental to maintaining healthy soils and sustaining agricultural productivity. Microbes also play a pivotal role in agriculture, where they are employed in practices like biofertilization and biopesticides. Certain strains of bacteria and fungi enhance nutrient availability and protect crops from pathogens, reducing the need for chemical fertilizers and pesticides. This promotes sustainable farming practices, minimizing environmental impact and ensuring long-term soil health. In the industrial sector, microbes are harnessed for various processes, including the production of enzymes, antibiotics, biofuels, etc. Biotechnological applications leverage the metabolic capabilities of microbes to manufacture a wide range of products efficiently. From pharmaceuticals to bio-based materials, the industrial use of microbes exemplifies the versatility and economic importance of these microscopic organisms. Microbes are indispensable in medicine, where they are used in the production of antibiotics, vaccines, and other pharmaceuticals. Probiotics, which are beneficial bacteria, contribute to human health by maintaining a balanced microbial community in the digestive system. Understanding the human microbiome and its role in health has led to advancements in personalized medicine and the development of innovative therapies. Waste water treatment relies heavily on microbial activity to break down pollutants and organic matter. Treatment plants utilize microbial communities to degrade contaminants, purifying water before it is released back into the environment. This sustainable approach mitigates the environmental impact of wastewater disposal and ensures the preservation of water quality. The degradation of xenobiotics, synthetic compounds foreign to the environment, is another critical role played by microbes. Certain microorganisms possess the ability to metabolize and break down pollutants, contributing to the detoxification of contaminated sites. Harnessing microbial biodegradation capabilities is a promising strategy for remediation efforts, addressing pollution challenges in diverse ecosystems. In conclusion, microbes are integral to various facets of life on Earth, influencing waste decomposition, soil fertility, agriculture, industry, medicine, and wastewater treatment. The intricate relationships between microbes and their environments highlight the interconnectedness of ecological processes and underscore the importance of sustainable practices that harness the power of these microscopic organisms for the benefit of both the environment and humanity.

3.12 TERMINAL QUESTIONS

Q.1 Discuss the role of Microorganism in Biodegradation.

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Q.2 Discuss Biodegradation in detail.

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Q.3 Write an explanatory note on Biodegradable Pollutants?

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Q.4 Discuss the Plant Growth Promoting Bacteria.

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Q.5 Discuss the microbial degradation of xenobiotics.

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Q.6 Discuss different stages of Biodegradation.

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Q.7 Write explanatory note on Nitrogen cycle.

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3.13 FURTHER SUGGESTED READINGS

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INTRODUCTION

This block deals with Environmental Microbiology and Biotechnology in three units viz. Unit 4, 5 and 6. The three units are as follows:

Unit 4: Microbes in Gaseous Production

This unit explores bioreactors, engineered systems fostering biologically active environments. It details bioreactor parts and functions, offering insights into their operation. Various bioreactor types, suited for specific biotechnological and environmental purposes, are discussed. Additionally, the unit examines biogas plants, emphasizing sustainable energy generation, and delves into microbial hydrocarbon biodegradation.

Unit 5: Ecological Restoration and Bioremediation

This unit unravels into bioremediation and phytoremediation, exploring their roles in cleaning contaminated environments. It examines different bioremediation types and focuses on pesticide degradation by microbes, revealing mechanisms and influencing factors. Insights into microbial heavy metal tolerance highlight survival strategies in polluted environments.

Unit 6: Ecologically Safe Products and Processes

This unit emphasizes into the multifaceted roles of Plant Growth Promoting Rhizobacteria (PGPR), exploring their applications across various fields such as biofertilizers, insecticides, fungicides, biocontrol agents, mining, metal biotechnology, and integrated pest management.

UNIT-4 : MICROBES IN GASEOUS PRODUCTION

Structure

- 4.1 Introduction
 - Objectives
- 4.2 Bioreactors
- 4.2 Bioreactor Design
- 4.3 Parts of the Bioreactor and Their Function
- 4.4 Types of Bioreactors
- 4.5 Biocomposting
- 4.6 Bioventing
- 4.7 Biogas
- 4.8 Biogas Plant and Its Components
- 4.9 Biodegradation of Hydrocarbons
- 4.10 Summary
- 4.11 Terminal Questions
- 4.12 Suggested Readings

4.1 INTRODUCTION

Microbes play a crucial role in gaseous production across various natural and engineered environments, significantly impacting global biogeochemical cycles. In natural settings, microbial communities contribute to the production and consumption of gases like methane, carbon dioxide, and nitrogen compounds, influencing climate patterns and ecosystem dynamics. Engineered systems such as bioreactors capitalize on microbial activities for industrial gas production, including biofuel synthesis, wastewater treatment, and biogas generation from organic waste. Bioreactors provide controlled environments where microbial populations thrive under optimized conditions, maximizing gas production efficiency. Additionally, biocomposting, a microbial-driven process, transforms organic waste into valuable compost while releasing gases like carbon dioxide and methane. Understanding microbial roles in gaseous production is essential for optimizing bioreactor performance, enhancing biocomposting processes, and mitigating greenhouse gas emissions. Harnessing microbial activities in these contexts holds promise for sustainable gas production, waste management, and environmental stewardship in a rapidly changing world.

Objectives

- To discuss the concept of bioreactors
- To discuss the parts of the bioreactor and their function
- To understand different types of bioreactors
- To explore biogas plant and its components

- To understand biogas plant and its components
- To discuss biodegradation of hydrocarbons

4.2 BIOREACTORS

A bioreactor serves as a fermentation vessel employed in the synthesis of diverse chemicals and biological reactions. This enclosed container is equipped with essential features such as aeration, agitation, temperature, and pH control, as well as a drain or overflow vent to eliminate the residual biomass of cultured microorganisms and their byproducts. Its primary functions include the generation of biomass, metabolites, and antibiotics. Key requirements for a bioreactor encompass:

1. Facilitating agitation to mix cells and the medium.
2. Providing aeration (in aerobic fermenters) for oxygen supply.
3. Regulating factors like temperature, pH, pressure, nutrient feeding, and liquid level.
4. Ensuring sterilization and the maintenance of sterility.
5. Enabling the withdrawal of cells/medium.

4.3 BIOREACTOR DESIGN

- The configuration and operational approach of a bioreactor hinge upon factors such as organism production, optimal conditions for targeted product synthesis, product value, and the scale of production.
- A well-constructed bioreactor design contributes to enhanced productivity and the provision of superior quality products at more economical prices.
- A bioreactor encompasses various elements, including an agitator system, oxygen delivery system, foam control system, and additional features such as temperature and pH control, sampling ports, as well as cleaning and sterilization systems, along with lines for charging and emptying the reactor.
- The construction material for a bioreactor must possess critical properties: it should not be corrosive, should not leach toxic substances to the fermentation media, withstand steam sterilization, tolerate high pressure, and resist pH fluctuations.
- Bioreactor sizes exhibit significant variation based on their applications. Some are tailored for small-scale fermenters, while others cater to large-scale industrial needs, ranging from microbial cells (few mm³) to shake flasks (100-1000 ml), laboratory-scale fermenters (1–50 L), pilot level (0.3–10 m³), and plant scale (2–500 m³) for substantial volumes.

Principle- The core of any biochemical process lies in the bioreactor, serving as an environment where microorganisms thrive, achieving optimal growth and generating metabolites and conversion of substrates into desired products. These reactors are adaptable and can be designed or fabricated to align with the specific growth needs of the organisms employed. Functioning as machines, reactors have the capability to convert biological materials into sought-after products, making them instrumental in the production of diverse enzymes and facilitating other bio-catalytic processes.

4.4 PARTS OF THE BIOREACTOR AND THEIR FUNCTION

These reactors are engineered to uphold specific parameters such as flow rates, aeration, temperature, pH, foam control, and agitation rate. The extent to which parameters can be monitored and controlled is determined by the inclusion of sensors and control elements in a particular bioreactor. Before designing a fermenter, several factors need consideration, as outlined below and illustrated in Figure 4.1. Various components of a standard bioreactor comprise:

1. Fermenter Vessel

- A fermenter is a sizable cylindrical structure sealed at both ends and interconnected with various pipes and valves.
- Two common types of fermenter vessels are glass and stainless steel.
- The design of the vessel is tailored to facilitate operation under controlled conditions.
- Glass vessels find application in small-scale industries due to their non-toxic and corrosion-resistant properties.
- Stainless steel vessels are employed in large-scale industries, offering resistance to both pressure and corrosion.

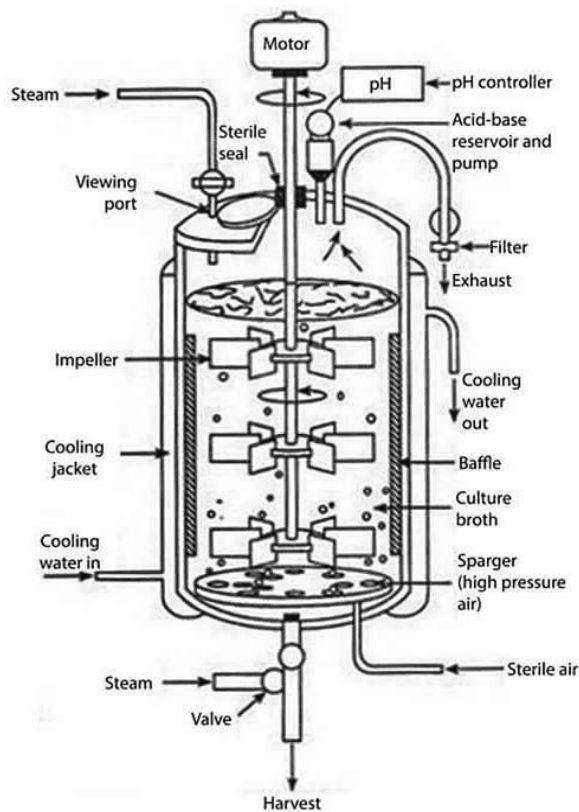


Fig 4.1: A typical Bioreactor. (Image Source: Kuila, A., & Sharma, V. (2018). Principles and applications of fermentation technology. John Wiley & Sons, Inc.)

2. Heating and Cooling Apparatus

- The outer surface of the fermenter vessel is equipped with a cooling jacket, sealing the vessel and facilitating the circulation of cooling water.
- Heat is typically supplied through thermostatically controlled baths or internal coils, while silicone jackets are employed to dissipate excess heat.

- The presence of a cooling jacket is essential for sterilizing the nutrient medium and managing the heat generated during the fermentation process.

3. Aeration System

- The aeration system stands out as a crucial component of a fermenter.
- Selecting an effective aeration system is vital to guarantee adequate aeration and consistent oxygen availability throughout the culture.
- The system comprises two distinct aeration devices, namely a sparger and an impeller, ensuring optimal aeration within the fermentor.
- Stirring serves two essential purposes:
 - Facilitating the mixing of gas bubbles throughout the liquid culture medium.
 - Ensuring the uniform dispersion of microbial cells throughout the liquid culture medium, promoting consistent access of microbial cells to nutrients.

4. Sealing Assembly

- The sealing assembly is employed to seal the stirrer shaft, ensuring effective agitation.
- Sealing assembly in the fermenter is of the following three types:
 - Packed gland seal
 - Mechanical seal
 - Magnetic drives

5. Baffles

- Baffles are integrated into fermenters to hinder vortex formation and enhance aeration.
- They typically consist of metal strips radially attached to the wall.

6. Impeller

- Impellers are employed to achieve a consistent suspension of microbial cells in various nutrient mediums.
- Comprising blades attached to a motor on the lid, impellers play a crucial role in diminishing the size of air bubbles and ensuring their uniform distribution into the fermentation media.
 - Variable impellers are employed in the fermenters and are of two types viz. disc turbines and variable pitch open turbines.

7. Sparger

- A sparger is a device designed for introducing sterile air into a fermentation vessel, ensuring adequate aeration.
- The sparger pipes are equipped with small holes, typically ranging from 5-10 mm, through which pressurized air is released.
- Three types of sparger are being used Porous sparger, Nozzle sparger, and combined sparger–agitator.

8. Feed Ports

- They are utilized for introducing nutrients and acid/alkali into the fermentor.
- Feed ports, constructed from silicone tubes, serve this purpose.
- *In-situ* sterilization is carried out before the removal or addition of products.

9. Foam-Control

- Minimizing the level of foam in the vessel is crucial to prevent contamination, representing a vital aspect of the fermenter.
- Foam control involves two units: a foam sensing unit and a control unit.
- A foam-controlling device is positioned atop the fermenter, featuring an inlet into the fermenter.

10. Valves

- Valves are employed to regulate the flow of liquid within the fermenter vessel.
- Five different types of valves in use include,
 - globe valve,
 - butterfly valve,
 - a ball valve, and
 - diaphragm valve.
- A safety valve is incorporated into the air and pipe configuration to function under pressure.

11. Controlling Devices for Environmental Factors

- Various devices are employed to manage environmental factors such as temperature, oxygen concentration, pH, cell mass, essential nutrient levels, and product concentration.

12. Use of Computer in Fermenter

- To enhance efficiency in processes, as well as for monitoring and data collection, the fermentors are commonly integrated with modern automated and semi-automated computer systems and databases.

4.5 TYPES OF BIOREACTORS

- 1. Continuous stirred tank fermenter-** A continuous stirred tank bioreactor consists of a cylindrical vessel with a centrally controlled shaft powered by a motor, supporting one or more agitators (impellers). The sparger, in conjunction with impellers, facilitates enhanced gas distribution throughout the vessel. This type of bioreactor can be operated continuously in the fermenter, providing effortless temperature control. Its construction is cost-effective, easy to operate, leading to low labour costs, and simple to clean. It stands as the most widely utilized bioreactor type in various industries.

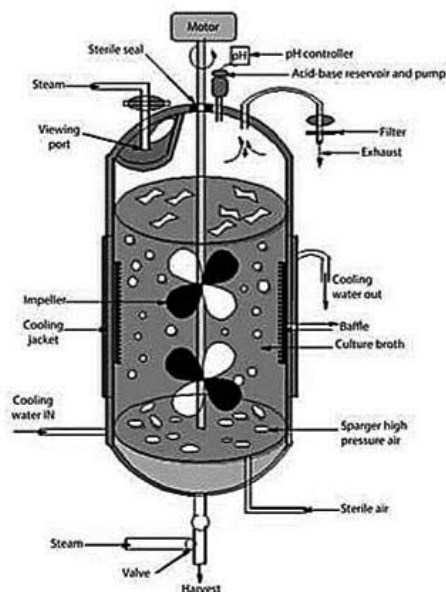


Fig 4.2. Continuous stirred tank fermenter

(Image Source: Saran, S., Malaviya, A., & Chaubey, A. (2019). *Introduction, scope and significance of fermentation technology. High Value Fermentation Products*, 1–25.

<https://doi.org/10.1002/9781119460053.ch1>)

2. **Airlift fermenter-** The airlift reactor, also referred to as a tower reactor, is commonly employed for gas-liquid or gas-liquid-solid contact applications. Utilizing an airlift system, a bioreactor employing this design divides the fluid volume into two zones to enhance circulation, oxygen transfer, and equalize forces, within the reactor. In a two-zone configuration, only one zone is subjected to gas sparging—referred to as the riser, while the non-sparged zone is the downcomer. Airlift bioreactors find application in aerobic bioprocessing technology, providing controlled liquid flow in a recycling system with the aid of pumps. Notable advantages of this equipment include its straightforward design devoid of moving parts or agitators, ease of sterilization, low energy requirements, and cost-effectiveness.

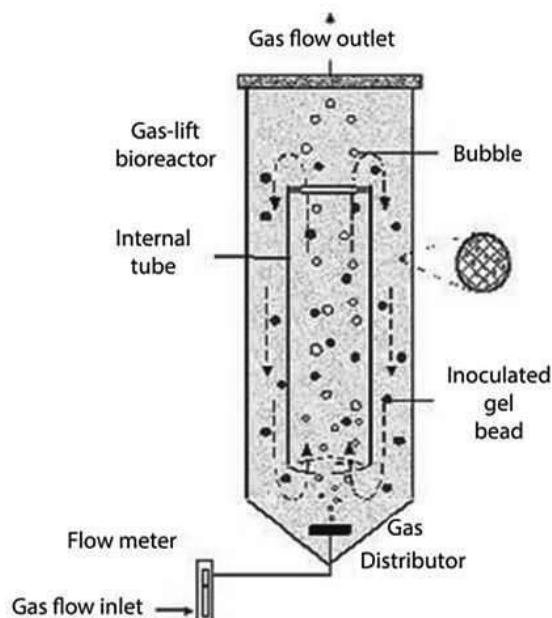


Fig 4.3. Airlift fermenter

3. **Bubble column fermenter-** A bubble column fermenter is a cylindrical vessel featuring a gas sparger that introduces gas bubbles into either a liquid phase or a liquid-solid suspension. At the column's base, air or gas is supplied via perforated pipes, plates, or a metal micro-porous sparger. The efficiency of oxygen mixing and other performance aspects are greatly affected by the fluid's rheological properties and the gas flow rate. To improve mass transfer and adapt the vessel's fundamental design, internal components such as horizontal perforated plates, vertical baffles, and corrugated sheet packings are commonly added. These reactors are appreciated for their straightforward construction, ease of maintenance, and low operational costs. Bubble column reactors are extensively employed in biochemical processes like fermentation and biological wastewater treatment, as well as in various chemical, petrochemical, and biochemical industries.

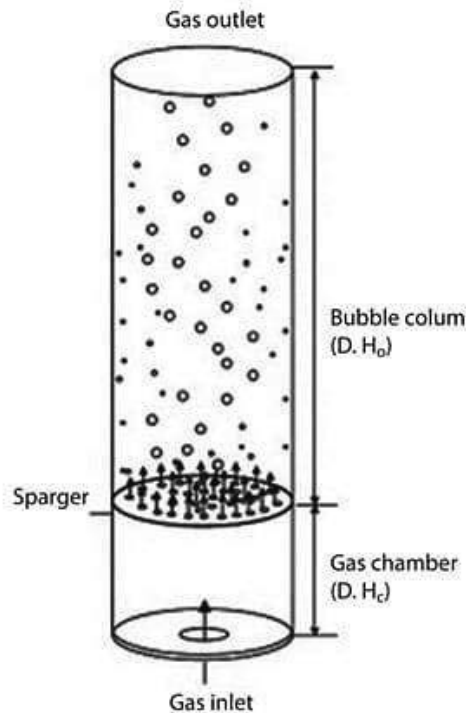


Fig 4.4 Bubble column fermenter

4. **Fluidized-bed fermenter-** Fluid bed bioreactors consist of packed beds with smaller particles, effectively addressing issues such as clogging, high liquid pressure drop, channeling, and bed compaction that are often encountered in packed bed reactors. In these bioreactors, the catalyst is placed at the bottom of the reactor, and reactants are introduced through a distributor pump to fluidize the bed. The immobilized cells, contained within small particles, move with the fluid, improving mass transfer, oxygen transfer, and nutrient supply to the cells. These bioreactors are used in reactions involving fluid-suspended biocatalysts, including immobilized enzymes, immobilized cells, and microbial flocs. Notable advantages include the ability to maintain consistent temperatures, ease of catalyst replacement and regeneration, continuous and automatic operation, and reduced contact time between gas and solid compared to other catalytic reactors.

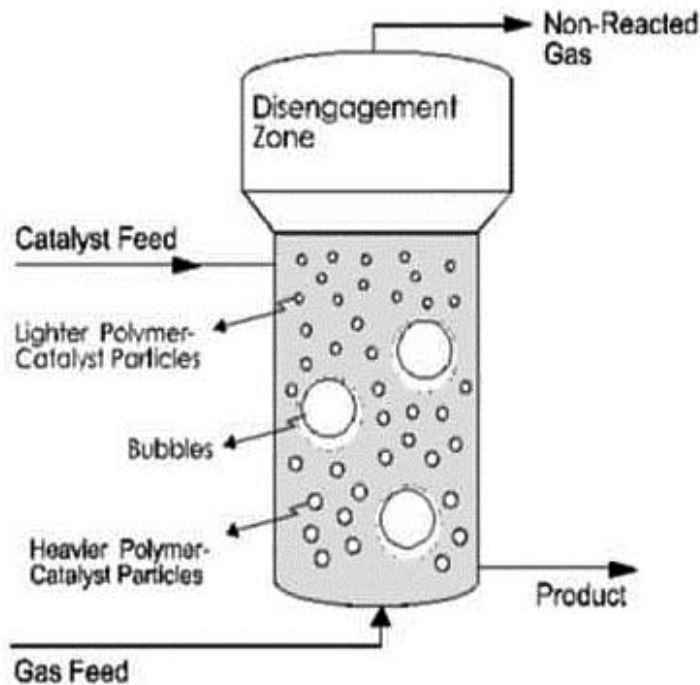
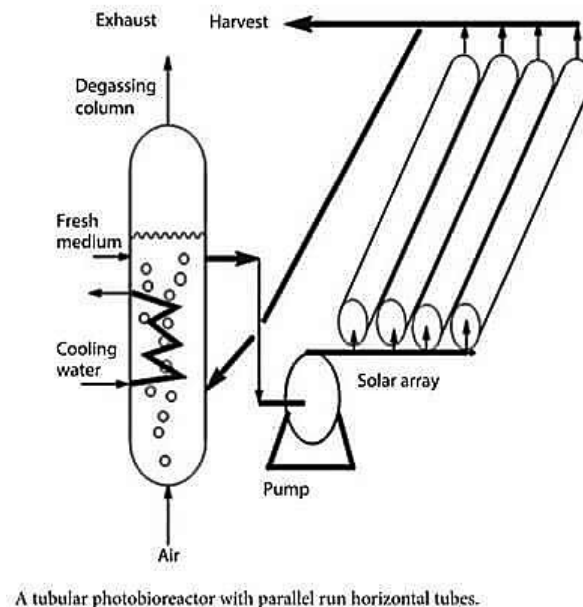


Fig 4.4. Fluidized-bed fermenter

(Image Source: Singh, J., Kaushik, N., & Biswas, S. (2014). Bioreactors – Technology & Design Analysis. April 2016.)

5. **Packed bed fermenter-** A packed bed fermenter consists of a bed of solid particles with a biocatalyst either on or embedded within the solid matrix. It can function in either submerged mode (with or without aeration) or trickle flow mode. These reactors, also known as fixed bed reactors, are commonly used in various chemical processing applications such as absorption, distillation, stripping, separation processes, and catalytic reactions. In packed-bed bioreactors, air is introduced through a sieve that supports the substrate. This type of reactor offers numerous benefits, including a high conversion rate for the catalyst, ease of operation, low construction and operating costs, enhanced contact between reactant and catalyst, and the ability to operate at high temperatures and pressures.
6. **Photobioreactor-** A photobioreactor is a specialized fermentation unit illuminated either by direct sunlight or artificial lighting. Typically constructed from transparent materials like glass or plastic, it features tubes or flat panels equipped with light-receiving systems. The bioreactor employs centrifugal pumps or airlift pumps to circulate the medium through solar receivers. Operated in a continuous mode within a temperature range of 25–40°C, photobioreactors are specifically designed for the photosynthetic cultivation of microalgae and cyanobacteria. Their applications include the production of products such as astaxanthin, β -carotene and several other secondary metabolites, etc.



A tubular photobioreactor with parallel run horizontal tubes.

Fig 4.5. Photobioreactor

(Image Source: Singh, J., Kaushik, N., & Biswas, S. (2014). Bioreactors – Technology & Design Analysis. April 2016.)

7. **Membrane bioreactor-** This system integrates conventional treatment with membrane filtration, achieving the elimination of organics and suspended solids along with the reduction of elevated nutrient levels. Membranes within this system are submerged in an aerated biological reactor, featuring pore sizes ranging from 0.035 microns to 0.4 microns. By employing pure oxygen, the advantages of this bioreactor are further enhanced, leading to even more efficient biological treatment systems that enable compact control of chemical oxygen demand (COD) and microorganisms.

4.6 BIOCOMPOSTING

Bio Composting is the controlled aerobic biodegradation of organic materials, yielding a nutrient-rich humus-like substance. In natural systems, there is no concept of "waste" as energy and matter captured through life processes are released upon the breakdown of organic substances, and subsequently reused by living organisms within the system. Natural systems maintain long-term soil fertility by reutilizing residues from biological decomposition to foster new growth. This transformation and flow of nutrient-containing chemical compounds are often termed "nutrient cycling," ensuring the stability of natural systems over time by connecting synthesis (build-up) and degradation (breakdown) processes. Composting differs from the natural decomposition of organic matter as it is a human-controlled process. This resource guide focuses on factors that can be managed to optimize composting, acknowledging that nature recycles organic materials regardless of human intervention. While compostable materials undergo decomposition in nature, human regulation can ensure a smooth process and the creation of a high-quality product. A "compostable" product refers to one that can be placed in a composition of decaying biodegradable materials, ultimately transforming into a nutrient-rich material. Though closely related to "biodegradable," it specifically pertains to solid materials and excludes liquids. In nature, composting occurs regularly as fallen leaves and tree limbs biodegrade into the forest floor. This process involves a diverse population of primarily aerobic microorganisms that decompose organic material for growth and reproduction. To encourage the activity of these microorganisms, it is

essential to manage the carbon-to-nitrogen (C:N) ratio, oxygen supply, moisture content, temperature, and pH of the compost pile. When managed correctly, composting accelerates natural decomposition, producing enough heat to eliminate weed seeds, pathogens, and fly larvae.

Advantages

1. Compost provides essential nutrients for plant growth.
2. Incorporating compost into soil builds organic reserves, gradually releasing nutrients over an extended period.
3. The application of compost in substantial amounts to soil systems poses minimal risk of excess nutrient accumulation.
4. Compost contributes varying degrees of other essential elements for plants, including phosphorus, potassium, calcium, sulfur, and other micronutrients.
5. Compost enhances soil structure and tilth by reducing bulk densities, increasing permeability and porosity, and introducing beneficial microorganisms.
6. The use of compost improves the soil's capacity to retain plant nutrients, especially those in cationic form. Elements like calcium, magnesium, and potassium can be held on exchange sites.
7. Compost aids in retaining fertilizers, pesticides, and herbicides in the soil, reducing their loss through erosion, leaching, and runoff.

4.7 BIOVENTING

Bioventing is a technique that stimulates the natural, in situ biodegradation of pollutants in soil by introducing air or oxygen to existing soil microorganisms. This method utilizes low airflow rates to provide just enough oxygen to sustain microbial activity in the vadose zone (unsaturated zone). Particularly effective for simple hydrocarbons, bioventing is applicable even when pollution is situated deep below the surface. As a form of *in-situ* bioremediation technology, bioventing promotes aerobic decomposition. Delivering oxygen into the unsaturated zone enhances the inherent ability of indigenous microorganisms to break down organic pollutants adsorbed to the soil. The process involves injecting air directly into the contaminated zone through vertical and horizontal wells, using only the necessary amount for degradation. Additionally, it reduces the volatilization of pollutants and their release into the environment. Bioventing, initially implemented on a large scale in the 1990s, has become widely utilized in commercial applications. The process can be carried out actively or passively. In passive bioventing, gas exchange from the vent wells is influenced by atmospheric pressure, while in active bioventing, the air is forcefully introduced into the ground through a blower, potentially in conjunction with vacuum extraction of the gas.

Process- The predominant method of venting involves introducing air into the vadose zone through one or more blowers, commonly known as active venting.

- **Air injection-** The blowers are capable of either injecting air or extracting soil vapours through a network of vent wells. The selection of the number, placement, and depth of these wells is determined by a range of geological and engineering considerations.
- **Vapour extraction-** During vapor extraction, a negative pressure is generated, inducing atmospheric air to be drawn into the subsurface. Conversely, extraction helps diminish (or eliminate) vapor intrusion into nearby structures. However, the introduction of air through

injection might exacerbate the situation by elevating subsurface pressure and establishing preferential pathways.

- **System operation-** Hence, in the initial months of operating a system engineered for soil gas extraction, it may be necessary to employ vapor treatment methods like thermal or catalytic oxidation or granular activated carbon (GAC) at numerous sites. Continuous monitoring of subsurface pressures, as well as concentrations of oxygen, carbon dioxide, and total petroleum hydrocarbons, is conducted using soil gas monitoring sites.
- **Respiratory tests-** During the implementation of full-scale treatment, *in-situ* respiratory tests are conducted regularly.
- **Baseline test-** A baseline test is conducted either immediately before or at the commencement of treatment. Subsequent tests can then be performed on a quarterly to annual basis to evaluate changes in degradation rates over time.
- **Shutting off systems-** The test begins by ceasing the bioventing system after the air has been introduced into the vadose zone for a minimum of 24 hours.
- **Recording-** During a period of one to five days, concentrations of oxygen, carbon dioxide, and petroleum hydrocarbons are monitored. The test is typically concluded when the oxygen concentration drops below 5%.
- **Calculation-** The rate of biodegradation can be assessed by measuring the rate of oxygen or carbon dioxide consumption. However, the use of carbon dioxide may be less reliable in alkaline soils, as elevated pH levels can convert carbon dioxide to mineral carbonate.

Applications

- **Treatment of aerobically biodegradable substances-** Conventional bioventing procedures are commonly used to address aerobically biodegradable substances, such as components of petroleum hydrocarbons, including volatile organic compounds (VOCs) and semi-volatile organic compounds.
- **Sites with mid-weight petroleum-** Bioventing is frequently applied at sites containing mid-weight petroleum compounds, such as jet fuel. This is because lighter liquids, like gasoline, tend to volatilize more rapidly and can be removed more quickly.
- **Cometabolic bioventing-** Cometabolic bioventing involves the injection of a cometabolite, like methane or propane, which is metabolized by microorganisms. This process induces the production of enzymes that incidentally react with chlorinated volatile organic compounds (VOCs). Contaminants that resist direct aerobic decomposition, such as trichloroethylene, trichloroethane, ethylene dibromide, and dichloroethylene, may be suitable for cometabolic bioventing.
- **Anaerobic bioventing-** Anaerobic bioventing is an innovative technology designed for the treatment of chlorinated substances, as well as some polychlorinated biphenyls and pesticides. In this method, air injection is replaced by delivering nitrogen gas and an electron donor to the subsurface.

Limitations

- **Proximity of water table-** Bioventing may be ineffective if the water table is relatively close to the ground surface.

- **High moisture and low temperature-** The effectiveness of bioventing can be hindered by high soil moisture or low permeability soils. Additionally, low temperatures may impede the remediation process.
- **Vapor buildup-** Within the impact radius of air-injection wells, vapor accumulation in basements may occur. To address this issue, a solution is to extract air in close proximity to the structure.
- **Low permeable soil formation-** Fluctuating water tables can create soil zones with low air permeability.
- **No effective degradation of chlorinated chemicals-** Many chlorinated chemicals may not degrade effectively in an aerobic environment unless a cometabolite is present.
- **Monitoring of vapor-** It may be required to observe vapour close to the soil surface.

4.8 BIOGAS

Biogas is a mixture of gases produced by microorganisms during the anaerobic fermentation of biodegradable materials. This biochemical process occurs in an oxygen-free environment, where specific bacteria digest biomass, resulting in the production of methane (CH₄), carbon dioxide (CO₂), hydrogen (H₂), and trace amounts of other gases, along with decomposed mass. The composition of biogas varies depending on the characteristics of the feed material, the extent of degradation, and other factors. Typically, biogas consists of 50 to 70 percent methane, 30 to 40 percent carbon dioxide, and small amounts of other gases. Methane, a combustible gas, determines the energy content of biogas, which ranges from about 50 to 70 percent methane. Biogas production involves a series of microbial reactions where complex organic molecules in the biomass are broken down into simpler compounds such as sugars, alcohols, acids, and amino acids by acid-producing bacteria. These intermediates are then converted into methane by another group of bacteria. The biogas production process generally involves three stages.

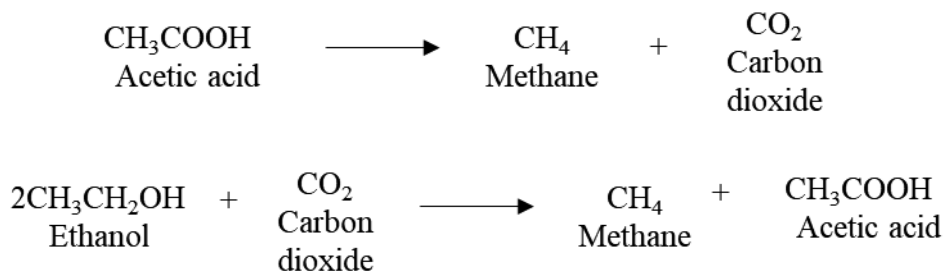
- i. Hydrolysis
- ii. Acid formation and
- iii. Methane formation

At each step of the process, different bacteria specialize in breaking down intermediate products formed during the degradation of organic material. Biogas production involves multiple stages, as shown in the figure. The efficiency of digestion is determined by the extent to which the process progresses through these stages. Improved digestion results in a shorter retention time and more efficient gas production.

Hydrolysis- In the polymer breakdown stage, complex organic molecules such as fats, starches, and proteins, which are water-insoluble and present in cellulosic biomass, are broken down into simpler compounds. This process is facilitated by enzymes secreted by bacteria. The major product of this stage is glucose, a simple compound. This stage is also referred to as the polymer-to monomer-breakdown stage.

Acid formation- The products (monomers) obtained in the hydrolysis stage serve as input for bacteria in the acid formation stage. In this stage, the products generated in the previous stage are fermented under anaerobic conditions, resulting in the formation of different acids. The major products produced at the end of this stage include acetic acid, propionic acid, butyric acid, and ethanol.

Methane formation- In the final stage, the acetic acid produced earlier is converted into methane and carbon dioxide by a group of microorganisms known as methanogens. This process, called methanogenesis, involves methanogens—obligate anaerobes that are highly sensitive to environmental changes. They utilize the intermediate products from the earlier stages and convert them into methane, carbon dioxide, and water, which make up the bulk of the biogas produced. Methanogenesis is particularly sensitive to pH levels, occurring optimally within a pH range of 6.5 to 8. The primary reactions in this stage are outlined below.



4.9 BIOGAS PLANT AND ITS COMPONENTS

- a) **Mixing tank-** Cow dung is gathered from the shed and combined with an equal proportion of water (1:1) to create a homogeneous mixture or slurry in the mixing tank.
- b) **Feed inlet pipe/tank-** The homogeneous slurry is introduced into the digester through the inlet pipe in KVIC biogas plants or the inlet tank in Janatha biogas plants.
- c) **Digester-** The fed slurry go through anaerobic fermentation with the help of microorganisms within the digester.
- d) **Gas holder-** As a result of anaerobic fermentation, the generated gas is stored in the gas holder (a drum in the case of KVIC biogas plants and a dome in the case of fixed dome biogas plants).
- e) **Slurry outlet tank/pipe-** The digested slurry is discharged from the digester through the slurry outlet pipe in KVIC biogas plants or the outlet tank in Janatha biogas plants.
- f) **Gas outlet pipe-** The stored gas is released and conveyed through the gas outlet pipe located at the top of the gas holder.

4.10 BIODEGRADATION OF HYDROCARBONS

Hydrocarbons are compounds made up entirely of carbon and hydrogen atoms. These include aromatic hydrocarbons, alkanes, alkenes, cycloalkanes, alkynes, and various combinations thereof, forming different types of hydrocarbons. They are major components of crude oil and petroleum. Naturally occurring microorganisms, such as bacteria, archaea, fungi, and algae, can biodegrade hydrocarbons in both freshwater and marine environments under aerobic and anaerobic conditions. The biodegradation of hydrocarbons forms the basis for natural and enhanced bioremediation. To encourage microbial growth and metabolism for biodegradation, amendments such as nitrogen and phosphorus fertilizers are often added. Additionally, oxygen, nitrate, or sulfates may be introduced as electron acceptors to improve biodegradation rates. Bioaugmentation, the practice of adding hydrocarbon-degrading microbial cultures to contaminated sites, is uncommon for hydrocarbons due to the natural abundance of these microbes. Most hydrocarbons act as energy sources or electron donors for microbial metabolism because of their relatively reduced nature. Their oxidation must be coupled with the reduction of a suitable electron acceptor. Aerobic degradation occurs with molecular oxygen (O₂),

which serves as both a reactant to oxidize the substrate and an electron acceptor for microbial respiration. In contrast, anaerobic degradation follows different biotransformation pathways that do not rely on oxygen, using various electron acceptors for microbial respiration. Under anaerobic conditions, biodegradation often involves the stepwise, cooperative action of multiple microbes in a process known as syntrophy.

Aerobic Degradation- Hydrocarbons are efficiently degraded under aerobic conditions by bacteria, fungi, and algae. Generally, alkenes (hydrocarbons with double bonds) and short-chain alkanes (hydrocarbons with single bonds) are the most easily degraded, followed by branched alkanes (alkanes with side chains), and finally aromatics (hydrocarbons with stable ring structures). However, degradation rates can alter depending on environmental conditions, and typically decrease as the complexity of the hydrocarbons enhances. Reported degradation rates exhibit considerable variation due to differences in hydrocarbon composition, which depend on the source of the petroleum and the spill age.

Alkanes and Alkenes- Alkanes containing 14 carbons or fewer are prone to volatilization, while alkanes with more carbons are less volatile. Nevertheless, alkanes and alkenes, excluding cyclic alkanes (alkanes in a ring structure), are the most readily degraded hydrocarbons, with reported degradation of alkanes containing up to 44 carbons. Both alkanes and alkenes are degraded with the addition of molecular oxygen. The rate-limiting factors in aerobic alkane degradation are oxygen availability and the initial step of degradation. In the initial steps of aerobic alkane degradation, enzymes known as oxygenases add molecular oxygen to the hydrocarbon molecules. This process forms alcohols, which are further oxidized to fatty acids. These fatty acids are subsequently metabolized to acetyl-CoA and ultimately to CO₂ and H₂O. Many mono- and dioxygenases, as well as other oxidases, exhibit a wide substrate range and readily act on various hydrocarbons.

Aromatic Hydrocarbons- Aromatic hydrocarbons are generally more challenging to degrade compared to shorter alkanes and alkenes, primarily due to their greater toxicity. However, they are readily degraded aerobically by many bacteria and fungi. The degradability of aromatic hydrocarbons tends to decrease with an increasing number of rings and greater molecular size, attributed to increased hydrophobicity and sorption capacity. Fungal degradation of aromatic hydrocarbons occurs through non-specific extracellular oxidizing enzymes that generate radical intermediates. Many of these reactions are like those found in bacteria, although the mechanisms may vary between the groups of microorganisms.

Anaerobic Degradation- Hydrocarbon degradation under anaerobic conditions tends to be slower than under aerobic conditions due to the less favorable energetics of reactions with alternative electron acceptors. Despite this challenge, both facultative and obligately anaerobic bacteria and archaea are capable of breaking down hydrocarbons without oxygen. These microorganisms often flourish in hydrocarbon-contaminated areas where oxygen is quickly depleted, making anaerobic processes crucial for determining the fate of hydrocarbons in such environments. The initial steps of anoxic hydrocarbon degradation generally involve the addition of an oxidized functional group to the molecule, a process that is typically rate-limiting.

Anaerobic microbes utilize terminal electron acceptors other than oxygen, such as nitrate, sulfate, carbon dioxide, oxidized metals, or certain organic compounds. At contaminated sites, microbes usually consume electron acceptors sequentially based on their reduction potential, typically starting with oxygen, followed by nitrate, ferric iron, sulfate, and hydrogen. Anaerobic degradation of hydrocarbons often involves syntrophic relationships, where the degradation of a substrate by one microbe relies on another microbe to keep intermediate product concentrations, like formate and hydrogen, low. To activate hydrocarbons without oxygen, anaerobic microbes use various strategies, including oxygen-independent hydroxylation, fumarate addition, and carboxylation.

4.11 SUMMARY

Bioreactors play a pivotal role in various environmental applications, serving as versatile tools for bioremediation, composting, bioventing, biogas production, methane production, and the biodegradation of hydrocarbons. These applications harness the power of microbial activity to address environmental challenges and promote sustainable practices. Bioremediation is a crucial process in the clean-up of contaminated environments, and bioreactors have emerged as effective tools in this domain. These systems utilize microorganisms to break down or transform pollutants into less harmful substances. Bioreactors enhance the efficiency of bioremediation by providing controlled conditions for microbial growth and activity. This ensures optimal degradation of contaminants, ranging from oil spills to industrial waste, promoting the restoration of ecosystems. Composting, another environmentally beneficial process, involves the decomposition of organic waste into nutrient-rich compost. Bioreactors facilitate and accelerate composting by maintaining optimal conditions such as temperature, moisture, and aeration. This results in faster decomposition, reduced odours, and the production of high-quality compost that can be utilized in agriculture to improve soil fertility. Bioventing is a technique that employs microorganisms to remediate soil contaminated with volatile organic compounds. Bioreactors enhance bioventing by supplying air and nutrients to the subsurface, promoting microbial activity that degrades pollutants. This approach is particularly effective in mitigating soil pollution without the need for excavation or physical removal of contaminated material. Biogas production is a sustainable energy solution that utilizes microorganisms to digest organic matter, and generating methane-rich biogas anaerobically. Bioreactors optimize this process by providing a controlled environment for the microbial consortia responsible for anaerobic digestion. The produced biogas can be used as a renewable energy source for electricity generation or as a clean fuel for various applications, contributing to a reduction in greenhouse gas emissions. Methane production, specifically, involves the cultivation of methane-producing microorganisms, such as methanogenic archaea, in bioreactors. These microorganisms play a vital role in anaerobic digestion processes, breaking down complex organic compounds into methane and carbon dioxide. Bioreactors enable precise control over conditions like temperature, pH, and substrate concentration, enhancing the efficiency of methane production for energy applications. The biodegradation of hydrocarbons is a critical process in addressing petroleum-based contamination in soil and water. Bioreactors provide a controlled environment for microbial communities capable of breaking down hydrocarbons into harmless by-products. This approach is eco-friendly and cost-effective, offering a sustainable alternative to traditional remediation methods. In conclusion, bioreactors serve as indispensable tools across various environmental applications, offering sustainable solutions to pollution and waste management challenges. Whether applied to bioremediation, composting, bioventing, biogas production, methane production, or hydrocarbon biodegradation, these systems provide a controlled environment that enhances microbial activity, thereby promoting efficient and environmentally friendly processes. As the world continues to seek innovative and sustainable solutions to environmental issues, bioreactors play a crucial role in advancing the principles of green technology and fostering a more sustainable future.

4.12 TERMINAL QUESTIONS

Q.1 What is a Bioreactor? Discuss its design and principle.

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Q.2 What are the different parts of the bioreactor and their function?

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Q.3 Discuss different types of bioreactors in detail.

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Q.4 What is Bio composting? Write its advantages.

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Q.5 What is Bioventing? Discuss its applications and limitations.

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Q.6 Discuss the Biogas production in detail.

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Q.7 Discuss the biodegradation of hydrocarbons in detail.

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4.13 SUGGESTED READINGS

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UNIT-5: ECOLOGICAL RESTORATION AND BIOREMEDIATION

Structure

- 5.1 Introduction
 - Objectives
- 5.2 Bioremediation and Phytoremediation
- 5.3 Types of Bioremediation
- 5.4 Phytoremediation
- 5.5 Biosparging
- 5.6 Degradative Plasmids
- 5.7 Pesticide Degradation by Microbes
- 5.8 Factors that Affect the Degradation of Pesticides by Microorganisms
- 5.9 The Mechanism of Microbial Degradation of Pesticides
- 5.10 Heavy Metal Tolerance in Microbes
- 5.11 Summary
- 5.12 Terminal Questions
- 5.13 Further Suggested Readings

5.1 INTRODUCTION

Ecological restoration and bioremediation stand as cornerstone methodologies in the endeavour to revive and heal ecosystems afflicted by environmental degradation and pollution. Ecological restoration encompasses a holistic approach aimed at reinstating the structure, function, and resilience of ecosystems that have been disturbed or damaged by human activities or natural disasters. It involves a range of interventions such as habitat rehabilitation, reforestation, and reintroduction of native species, all geared towards promoting biodiversity and ecosystem health. Bioremediation, a powerful subset of restoration, harnesses the metabolic capabilities of microorganisms, plants, and other biological agents to degrade, detoxify, or sequester pollutants in soil, water, or air. Phytoremediation, for instance, exploits the remedial properties of certain plant species to absorb, metabolize, or immobilize contaminants, offering a sustainable and cost-effective solution for environmental cleanup. By planting suitable vegetation in contaminated sites, phytoremediation not only removes pollutants but also enhances soil quality and biodiversity. Additionally, biosparging represents a promising bioremediation technique that involves the injection of air or oxygen into groundwater to stimulate microbial activity and accelerate the degradation of organic contaminants. This process enhances the natural attenuation of pollutants, promoting the restoration of groundwater quality. The integration of ecological restoration and bioremediation strategies not only addresses environmental degradation but also fosters ecosystem resilience, enhances ecosystem services, and promotes human well-being. By understanding the complexities of ecological systems and leveraging the inherent abilities of living organisms, we can

forge a path towards sustainable environmental management and the restoration of our planet's natural balance.

Objectives :

After reading to the unit, the Learner will be able

- To discuss the concept of Bioremediation and Phytoremediation
- To understand types of bioremediations
- To explore pesticide degradation by microbes
- To discuss factors that affect the degradation of pesticides by microorganisms
- To investigate the mechanism of microbial degradation of pesticides
- To study heavy metal tolerance in microbes

5.2 BIOREMEDIATION AND PHYTOREMEDIATION

The term 'Bioremediation' refers to the utilization of microorganisms for breaking down or eliminating hazardous components in waste, aiming to improve environmental conditions. This approach harnesses the natural capabilities of bacteria, fungi, or plants to degrade or detoxify substances harmful to both human health and the environment. Essentially, bioremediation is a treatability technology employing biological activity to reduce the concentration or toxicity of pollutants. It commonly relies on the natural processes through which microorganisms transform or break down chemicals in the environment. The use of microorganisms, primarily bacteria, for the destruction or transformation of hazardous contaminants is not a recent development. Dating back to 600 B.C., the Romans and other civilizations employed microorganisms to treat wastewater. While this technology is still utilized for wastewater treatment today, its applications have expanded to address various contaminants. Commercially, bioremediation has been in use for approximately 30 years, with the first instance recorded in 1972 when a bioremediation system was employed to clean up a Sun Oil pipeline spill in Ambler, Pennsylvania. Bioaugmentation is the term used when microorganisms are intentionally introduced to a contaminated site to enhance degradation. For bioremediation to be successful, microorganisms must enzymatically break down pollutants, converting them into less harmful or harmless byproducts. The effectiveness of bioremediation is contingent on environmental conditions supporting microbial growth and activity. Therefore, its application may involve manipulating environmental parameters to facilitate the acceleration of microbial growth and degradation. The key indicators showing bioremediation in progress:

- (a) **Biological Oxygen Demand (BOD)**- Biological Oxygen Demand (BOD) quantifies the oxygen consumption by microorganisms during the degradation of organic matter into carbon dioxide and water. An increase in BOD results in a decrease in dissolved oxygen (DO) levels, rendering the wastewater septic. Elevated BOD values signify the abundance of microorganisms, indicating a heightened degree of pollution.
- (b) **Chemical Oxygen Demand (COD)**- Chemical oxygen demand (COD) is a measure of the oxygen needed by microorganisms for the complete degradation of chemical substances. This parameter is typically expressed in milligrams per liter (mg/L), reflecting the mass of oxygen consumed per liter of solution.
- (c) **Dissolved Oxygen (DO)**- Dissolved oxygen (DO) in water refers to oxygen that has become soluble in the aquatic environment. This oxygen dissolves through processes such as diffusion from the surrounding air, aeration occurring when water cascades over falls and rapids, and as a result of aquatic photosynthesis. Unlike fish and other aquatic animals, which are unable to

extract oxygen directly from water (H₂O) or other oxygen-containing compounds, green plants and certain bacteria can perform this extraction through photosynthesis and related mechanisms. It's noteworthy that a significant portion of the oxygen we breathe is generated by green plants through these processes.

- (d) **Total Suspended Solids (TSS)**- Total Suspended Solids (TSS) encompasses solid materials, both organic and inorganic, that remain suspended in water. This category includes particles such as silt, plankton, and industrial wastes. Elevated concentrations of suspended solids can adversely impact water quality by absorbing light. Consequently, the water may experience increased temperature, reducing its capacity to retain oxygen essential for aquatic life. This decrease in dissolved oxygen levels can have detrimental effects on the well-being of aquatic ecosystems.
- (e) **Sulphides**- The occurrence of sulphide in water, particularly in the form of hydrogen sulphide, can result in unpleasant taste and odor. To address these aesthetic concerns, a recommended limit of ≤ 0.05 mg/L (≤ 50 μ g/L), expressed as hydrogen sulphide, has been established. Sulphides are produced by sulphate-reducing bacteria during the anaerobic decomposition of sewage, algae, naturally deposited organic matter, and sulfur-containing substances present in fungicides, pesticides, and fertilizers. Additionally, industrial effluents from facilities such as petroleum and petrochemical plants, gas works, paper mills, heavy water plants, and tanneries can contribute to the presence of sulphides in water.
- (f) **Coliforms**- Coliform bacteria constitute a diverse group of aerobic and facultative anaerobic, gram-negative, non-spore-forming bacilli. Among them, *Escherichia coli* and thermo-tolerant coliforms represent a subgroup capable of fermenting lactose at elevated temperatures. Traditionally associated with the genera *Escherichia*, *Citrobacter*, *Klebsiella*, and *Enterobacter*, the coliform group is more varied and encompasses a broader array of genera. This includes both faecal and non-faecal species within the total coliform group. In untreated or partially treated sewage, coliforms, particularly faecal coliforms, are inherently present, and the organic matter serves as a nutrient source, fostering their growth. This heightened microbial activity increases the septicity of the water, posing potential concerns for water quality.

5.3 TYPES OF BIOREMEDIATION

Bioremediation can be broken into two main types: a) Intrinsic, and b) Engineered.

- a) **Intrinsic bioremediation**- Intrinsic bioremediation, also referred to as natural attenuation or passive bioremediation, is favoured over engineered bioremediation primarily due to its significantly lower cost. Intrinsic bioremediation involves leveraging naturally occurring microorganisms to degrade contaminants without implementing engineered interventions to enhance the process. The success of intrinsic bioremediation relies on meeting four main requirements:
 1. Adequate populations of microorganisms capable of biodegrading the contaminant,
 2. The presence of essential nutrients,
 3. Favourable environmental conditions,
 4. Adequate time for the natural degradation process to occur.
- b) **Engineered bioremediation**- Engineered bioremediation, also termed enhanced bioremediation, involves augmenting or improving the natural degradation process. Typically employed when

any of the essential conditions for intrinsic bioremediation are lacking or when a quicker completion of the process is required, engineered bioremediation employs various techniques. One example is the installation of wells to circulate fluids and nutrients, stimulating microorganisms. Within the realm of engineered bioremediation, there are two main types:

- (i) **Biostimulation-** In biostimulation, the environmental conditions are altered to promote the proliferation of existing microbes. This may involve supplementing missing nutrients, providing oxygen, and other favorable modifications. Several essential nutrients must be accessible to microorganisms for successful bioremediation. These crucial elements include moisture, nitrogen, phosphorus, and various trace elements. Microorganisms, like all living organisms, rely on moisture for their survival and growth. Additionally, they depend on moisture for the transportation of nutrients since they lack mouths. Alongside moisture, nitrogen (in the form of ammonia) and phosphorus (as orthophosphate) stand out as significant nutrients essential for the optimal functioning of microorganisms involved in bioremediation.
- (ii) **Bioaugmentation-** Bioaugmentation is employed in situations where the desired microorganisms are present in low numbers or are undesirable. This process involves introducing specific microorganisms into the system to augment and improve the overall metabolic processes. The underlying concept is rooted in the existence of biologically degradable solids within wastewater. While biological treatment typically relies on intrinsic bacteria naturally present in wastewater, these indigenous populations are often in limited numbers. They may lack the capability to effectively biodegrade specific contaminants, and their action might not be swift enough to prevent the spread of the contaminant. In the scenarios where a rapid response is crucial, depending on the small numbers of indigenous microbial populations may not be suitable. The solution lies in bioaugmentation, where additional microbes are introduced to the site to boost their numbers, surpassing the undesirable ones. This method, commonly applied in the treatment of municipal wastewater, facilitates a more efficient and accelerated remediation process.

Advantages and Disadvantages of Bioremediation

Advantages of bioremediation include

- Bioremediation is frequently feasible on-site, minimizing significant disruptions to regular activities. This approach eliminates the necessity to transport waste or wastewater off-site, mitigating potential risks to human health and the environment that may arise during transportation.
- In place of transferring contaminants from one environmental medium to another, such as from land to water or air, bioremediation offers the advantage of complete degradation of target pollutants and transforming them from hazardous to non-hazardous. This differs from the translocation of contaminants to another site, minimizing long-term liability for the agency responsible for the contamination.
- The residues generated from bioremediation treatments are typically harmless, consisting of carbon dioxide, water, and cell biomass.
- Bioremediation stands out as a natural process, aligning with the principles of ecological systems.
- It is cost-effective and competes favourably with other treatment methods.

- As bioremediation requires no energy consumption, it is environmentally friendly and can be implemented in remote areas without access to electricity, cutting down on operational costs.
- There are no infrastructure costs or civil structures associated with bioremediation, allowing for a quick start without extensive preparation time.
- The population of microbes capable of degrading the contaminant tends to increase in response to the presence of the contaminant (substrate-dependent). As the contaminant is degraded, the microbial population subsequently declines and may eventually die off.

Disadvantages

- Bioremediation is applicable only to certain contaminated areas, limited to compounds that are biodegradable. Not all contaminants are susceptible to rapid and complete degradation through this method.
- Some sites may lack the necessary ingredients for bioremediation, making it unfeasible or economically impractical to implement the process.
- A significant challenge with bioremediation is that it involves microorganisms that are invisible to the naked eye, leading to a lack of understanding. Additionally, the association of bacteria with diseases and infections can contribute to a reluctance or resistance to accept bioremediation as a viable solution.
- Biological processes in bioremediation are often highly specific to the conditions of a particular site, posing challenges in achieving consistent and universal application.

5.4 PHYTOREMEDIATION

Phytoremediation is a process that utilizes plants to clean up the environment. The term "phytoremediation" originates from the Greek word "phyto," meaning "plant," and the Latin word "remediare," meaning "to remedy." This term broadly encompasses any system in which plants are introduced into an environment to remove contaminants. Phytoremediation is implemented through various approaches, such as introducing plants into an environment and allowing them to absorb contaminants into their leaves and roots. These plants can then be harvested to recover the contaminants or dispose of the plant parts as hazardous waste. In some cases, studies have shown that these plants can transform contaminants into harmless substances through natural processes, making them suitable for uses like mulch, or paper production. The primary goal of phytoremediation is to use plants and trees to clean up contaminated soil and water. While this technology is still in its early stages, ongoing research is being conducted to explore its potential as a widely adopted remediation technique. Current research focuses on identifying the most effective plants for specific tasks, understanding the mechanisms by which plants transform pollutants, and determining which contaminants are amenable to phytoremediation. Phytoremediation utilizes various plant types to remove, transfer, stabilize, and/or destroy contaminants in both soil and groundwater. Several mechanisms, such as:

1. **Rhizosphere biodegradation-** In this process, plants release natural substances through their roots, providing essential nutrients to microorganisms in the soil. This symbiotic relationship with microorganisms plays a crucial role in enhancing biological degradation processes.
2. **Phyto-stabilization-** In this process, the chemical compounds produced by the plant work to immobilize contaminants, preventing their movement, or spread, rather than actively degrading them.

3. **Phytoaccumulation (also called phytoextraction)**- In this process, plant roots adsorb contaminants alongside other nutrients and water. Instead of destroying the contaminant mass, the contaminants are retained in the plant's shoots and leaves. This method is particularly employed for waste containing metals. The metals are stored in the aerial shoots of the plant, which can be harvested. These harvested shoots are then either smelted for potential metal recycling and recovery or disposed of as hazardous waste. As a general guideline, readily bioavailable metals for plant uptake include Cadmium, Nickel, Zinc, Arsenic, Selenium, and Copper. Metals with moderate bioavailability include Cobalt, Manganese, and Iron. On the other hand, Lead, Chromium, and Uranium exhibit low bioavailability. However, the bioavailability of "Lead" can be increased significantly by adding chelating agents to soils. Similarly, the availability of Uranium and radio-caesium-137 can be enhanced using citric acid and ammonium nitrate, respectively.
4. **Hydroponic Systems for Treating Water Streams (Rhizofiltration)**- Rhizofiltration is akin to phytoaccumulation, but it involves cultivating plants in greenhouses with their roots immersed in water. This approach is particularly suitable for ex-situ groundwater treatment, where groundwater is brought to the surface and used to irrigate these plants. Hydroponic systems are commonly employed in rhizofiltration, utilizing an artificial soil medium like a mixture of sand with perlite or vermiculite. After the plant roots become saturated with contaminants, these are harvested and subsequently disposed of.
5. **Phytovolatilization**- Plants absorb water containing organic contaminants and subsequently release these contaminants into the air through their leaves.
6. **Phytodegradation**- In this process, plants metabolise and degrade contaminants within plant tissues.
7. **Hydraulic Control**- In this process, the trees contribute to remediation indirectly by influencing groundwater movement. When the roots of trees extend towards the water table and form a dense mass, they function as natural pumps, drawing up significant quantities of water. For instance, a poplar tree can extract 30 gallons of water from the ground per day, while a cottonwood has the capacity to absorb up to 350 gallons daily (1 gallon = 4.5 liters).

Phytoremediation operates on a fundamental principle: the plant absorbs pollutants through its roots. The pollutant can be stored in the plant tissues (phyto-extraction), released into the air by the plant (phyto-volatilization), metabolized by the plant (phyto-degradation), or a combination of these processes. However, phytoremediation is more than just planting and allowing foliage to grow; the site must be engineered to prevent erosion and flooding while maximizing pollutant uptake. A significant portion of ongoing research in the field of phytoremediation focuses on identifying the most efficient plant species for specific applications. Not all plant species handle the metabolism, volatilization, and accumulation of pollutants in the same manner. The objective is to determine which plants are most effective at remediating a given pollutant. Table 5.1 provides a partial listing of plants and the pollutants they are capable of remediating.

Plant	Chemicals
Arabidopsis	Mercury
Bladder campion	Zinc, Copper
Brassica family (Indian Mustard & Broccoli)	Selenium, Sulphur, Lead, Cadmium, Chromium, Nickel, Zinc, Copper, Cesium,

	Strontium
Buxaceae (boxwood)	Nickel
Compositae family	Cesium, Strontium
Euphorbiaceae	Nickel
Tomato plant	Lead, Zinc, Copper
Trees in the Populus genus (Poplar, Cottonwood)	Pesticides, Atrazine, Trichloroethylene (TCE), Carbon tetrachloride, Nitrogen compounds, 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5 triazine (RDX)
Pennycress	Zinc, Cadmium
Sunflower	Cesium, Strontium 90, Uranium, Chromium, Zinc
Parrot feather	Explosives wastes
Perennial ryegrass	Polychlorinatedphenyls (PCP's), Polyaromatichydrocarbons (PAH's)
Pondweed, arrowroot, coontail	TNT, RDX

Source: Green Chemistry Series Module II: Bioremediation and Phytoremediation, June, 2011

Advantages of Phytoremediation

- Aesthetically pleasing
- Solar-driven.
- Effective with metals and slightly hydrophobic compounds, including various organics.
- Can stimulate bioremediation in the soil closely associated with the plant root. Plants can enhance microbial activity by releasing nutrients and facilitating oxygen transport to their roots.
- Relatively inexpensive.
- Even if the plants become contaminated and unusable, the resulting ash is approximately 20-30 tons per 5000 tons of soil.
- Planting vegetation on a site also reduces erosion caused by wind and water, helping to maintain usable topsoil integrity.

Limitations of Phytoremediation- Phytoremediation, a site cleanup process, is influenced by seasonal and location-specific factors, often requiring multiple growing seasons and significant land area. Concerns arise due to unknown toxicity of biodegradation products, potentially affecting groundwater and bioaccumulating in animals. Further research is needed to understand their fate in plant cycles. Disposal of harvested plants, especially those high in heavy metals, poses challenges. There's a risk of contaminant introduction into the food chain by plants absorbing toxins. Compliance with regulations is crucial when returning water post-aquaculture. Phytoremediation is less effective for hydrophobic and strongly adsorbed contaminants like PCBs.

5.5 BIOSPARGING

Biosparging is an *in-situ* remediation technology designed to utilize indigenous microorganisms for the biodegradation of organic constituents within the saturated zone. In this process, air (or oxygen) and, if necessary, nutrients are injected into the saturated zone to enhance the biological activity of existing microorganisms. Biosparging proves effective in reducing concentrations of petroleum constituents dissolved in groundwater, adsorbed to the soil below the water table, and within the capillary fringe. While biosparging can treat constituents adsorbed to soils in the unsaturated zone, it is typically less effective in this scenario compared to bioventing. The biosparging process closely resembles air sparging; however, it differs in its emphasis on promoting biodegradation rather than volatilization. Biosparging generally employs lower flow rates than air sparging. In practical applications, a degree of volatilization and biodegradation occurs when either air sparging or biosparging is employed. When volatile constituents are present, biosparging is often combined with soil vapor extraction or bioventing. Additionally, it can be integrated with other remedial technologies. In cases where biosparging is combined with vapor extraction, the vapor extraction system creates a negative pressure in the vadose zone through extraction wells, effectively controlling the migration of the vapor plume.

Advantages and Disadvantages of Biosparging

Advantages

- a) Utilizes readily available equipment and is easy to install.
- b) Causes minimal disturbance to site operations.
- c) Exhibits short treatment times, ranging from 6 months to 2 years under favorable conditions.
- d) Proves cost effectiveness.
- e) Enhances the effectiveness of air sparging, particularly for treating a broader spectrum of petroleum hydrocarbons.
- f) Requires no removal, treatment, storage, or discharge of groundwater.
- g) Low air injection rates help minimize the potential need for vapor capture and treatment.

Limitations

- a) Limited applicability to environments suitable for air sparging, such as those with uniform and permeable soils, an unconfined aquifer, and the absence of free-phase hydrocarbons or nearby subsurface confined spaces.
- b) Incomplete understanding of interactions among complex chemical, physical, and biological processes.
- c) Insufficient field and laboratory data to support design considerations.
- d) Potential for inducing the migration of constituents.

5.6 DEGRADATIVE PLASMIDS

One intriguing characteristic of microorganisms, especially those recognized for degrading various aromatic and aliphatic compounds, notably within the genus *Pseudomonas*, is the presence of many genes encoding degradative pathways on plasmids. These plasmids carry the genetic information for the breakdown of diverse substances, including aliphatic compounds (such as octane and decane),

aromatic and polyaromatic hydrocarbons (like xylenes, toluene, naphthalene), oxidation products (salicylate, benzoate), terpenes (camphor), alkaloids (nicotine/nicotinate), chlorinated hydrocarbons like 2,4-D, MCPA, and 3-CBA, common environmental pollutants like polychlorinated biphenyls (pCBs), and even bacterial aphrodisiacs known as opines. This represents just a subset of what is anticipated to be a diverse group of naturally occurring plasmids that encode the degradative capabilities found in various taxonomically important soil microorganisms. A noteworthy characteristic of nearly all identified degradative plasmids is their self-transmissible nature. These plasmids can be transferred from one bacterial cell to another through direct cell-to-cell contact, a process known as conjugation.

Naturally occurring degradative plasmids

- 1. The TOL Plasmid-** Among the various plasmids known for their capability to degrade aromatic and aliphatic compounds, one of the most extensively studied is the TOL (toluate utilization) plasmid. Independently isolated from *Pseudomonas putida*, this degradative plasmid is characterized by a broad host range, conjugative nature, and a size of 117 kilobases (kb). The TOL plasmid encodes the utilization of various compounds, including toluene (TOL), m- and p-xylene (XYL), 3-ethyltoluene, and 1,2,4-trimethylbenzene. Additionally, it facilitates the degradation of their alcohol, aldehyde, and carboxylic acid derivatives through the meta cleavage pathway.
- 2. The NAH and SAL Plasmids-** The salicylate (SAL) and naphthalene (NAH) plasmids were identified in strains of *Pseudomonas putida*, and they encode similar degradative pathways. Both plasmids specify a complete meta pathway for the oxidation of catechol. Catechol is generated either directly from salicylate by a SAL plasmid-encoded hydroxylase or from naphthalene via salicylate through enzymes encoded by the NAH plasmid. Notably, these plasmids are transferable from their original host to other *Pseudomonas* strains, particularly *Pseudomonas aeruginosa*. The NAH plasmid is approximately 70 kb in size, while estimates for the size of the SAL plasmid vary, ranging from 63 to 82 kb. An intriguing aspect of the SAL plasmid is its close evolutionary relationship to the naphthalene (NAH) degradative plasmid.
- 3. The OCT Plasmid-** The presence of the OCT (octane utilization) plasmid enables its host strain, *Pseudomonas oleovorans*, to utilize n-alkenes such as octane, hexane, and decane as the sole sources of carbon and energy. The OCT plasmid encodes inducible alkane hydroxylating and primary alcohol-dehydrogenating activities, but it does not encompass the complete alkane to the fatty acid pathway. The remaining steps leading to the degradation of the plasmid-encoded end products (octanol and octanal) are presumed to be encoded in chromosomal genes. The naturally occurring OCT plasmid of *Pseudomonas oleovorans* is conjugative and appears to be a cointegrate of three distinct plasmids: OCT (octane utilization), MER (mercury resistance), and the transfer factor K.
- 4. The CAM Plasmid-** The CAM (camphor utilization) plasmid, an exceptionally large plasmid (>200 kb), carries the genetic information for the degradation of the terpene camphor. This plasmid is conjugative, readily transferring from its original host, *Pseudomonas putida*, to various species of *Pseudomonas*, including *Pseudomonas aeruginosa*. Notably, similar to the OCT plasmid but in contrast to other degradative plasmids, the CAM plasmid does not encode the entire degradative pathway.

The NIC Plasmid- In *Pseudomonas putida*, the genes responsible for the degradation of nicotine/nicotinate are located on the chromosome. In contrast, the corresponding degradative genes in *Pseudomonas convexa* are found on the NIC plasmid. The distinct pathways leading to the degradation of nicotine and nicotinic acid converge at 2,5-dihydroxy pyridine. The plasmid-encoded end products,

fumaric and maleamic acid, are easily assimilated by most strains of *Pseudomonas*.

5. **The 2,4-D Plasmids-** Phenoxyherbicides like 2,4-D and MCPA have been extensively used for over 30 years. Despite their complex nature, many pesticides, including 2,4-D, are readily degraded by microorganisms present in soil and water. Strains of soil microorganisms capable of degrading 2,4-D have been isolated from various soil types. The initial indication that plasmids played a role in the environmental degradation of the pesticide 2,4-D was reported by Pemberton and Fisher in 1977. They observed that a strain of *Alcaligenes paradoxus*, a common saprophytic soil bacterium, possessed a transmissible plasmid that conferred the ability to degrade both 2,4-D and MCPA. Subsequently, six additional 2,4-D plasmids have been isolated from strains of *Alcaligenes paradoxus* and the closely related *Alcaligenes eutrophus*.
6. **The Ti Plasmids-** *Agrobacterium tumefaciens*, a common soil bacterium, possesses the capability to instigate tumours in numerous dicotyledonous plants, and Ti plasmids play a crucial role in this tumorigenic process. The Ti plasmid harbours essential tumour-inducing (Ti) functions, with a specific section known as the T-DNA responsible for the synthesis of opines (unusual amino acids) and the initiation of tumour formation within plant cells. The genes responsible for the breakdown and utilization of opines produced by the plant are closely associated with the genes facilitating the conjugal transmission of the plasmid on these Ti plasmids, classifying them as degradative plasmids. Exploiting the T-DNA's ability to transfer from the bacterium and integrate itself into the plant cell genome has been utilized as a strategy to introduce small DNA segments into plant cells.
7. **The LAC/RAF/SCR Plasmids-** Most strains of enteric bacteria, such as *Yersinia enterocolitica* and *Salmonella* species, especially *Salmonella typhi*, typically exhibit a Lac⁻ phenotype, indicating an inability to ferment lactose. However, isolated instances have been documented where strains of these bacteria possess all the characteristic features of the species but exhibit a phenotypically Lac⁺ trait, signifying their ability to ferment lactose. In the case of *Yersinia enterocolitica*, the acquisition of a 50kb conjugative plasmid is responsible for the Lac⁺ phenotype, and this plasmid also imparts the capability to ferment raffinose. Similarly, *Escherichia coli* carries conjugative plasmids, specifically the raffinose (RAF) plasmids, which encode the utilization of raffinose and sucrose but not lactose, salicin, or dulcitol.

5.7 PESTICIDE DEGRADATION BY MICROBES

The escalated application of pesticides and herbicides in agriculture has not only become prevalent in crop cultivation but has also extended to food storage practices. Presently, more than 500 compounds are registered and globally utilized as pesticides or their metabolites. Post-World War II, the agricultural sector witnessed a gradual surge in pesticide usage, contributing to a subsequent increase in worldwide food production. Pesticides encompass insecticides, herbicides, and fungicides, with commonly employed varieties in India being organophosphates, organochlorines, and neonicotinoids, among others. Ideally, a pesticide should be effective in eradicating targeted pests while sparing non-target species, including humans. Unfortunately, the reality falls short of this ideal, sparking debates on the responsible and judicious use of pesticides.

Chemically related pesticides

1. **Organophosphate-** Most organophosphates primarily function as insecticides, exerting their effects by disrupting the neurological system. They achieve this by interfering with the activity of an enzyme that regulates a neurotransmitter.

2. **Carbamate-** Like organophosphorus pesticides, carbamate pesticides also impact the neurological system by disrupting an enzyme that regulates neurotransmitters. It is worth noting that the effects of enzymes in the case of carbamates are generally reversible.
3. **Organochlorine insecticides-** Organochlorine pesticides, which were extensively used in the past, have faced global withdrawal from the market by numerous nations. This decision stems from their documented adverse health and environmental impacts, coupled with their persistence. Examples of such organochlorine pesticides include DDT, chlordane, and toxaphene.
4. **Pyrethroid-** These pesticides are synthetic derivatives of pyrethrin, a naturally occurring pesticide found in chrysanthemum flowers. Their design aimed to maximize environmental stability.
5. **Sulfonylurea herbicides-** For weed management, sulfonylurea herbicides such as cyclosulfamuron, pyriithiobac-sodium, bispyribac-sodium,terbacil, and sulfometuron-methyl have been sold. Sulfosulfuron, rimsulfuron, imazosulfuron, nicosulfuron, primisulfuron-methyl, oxasulfuron, nicosulfuron, pyrazosulfuron-ethyl, flazasulfuron, halosulfuron-methyl, flupyrsulfuron-methyl-sodium, ethoxysulfuron, chlorimuron-ethyl,azimsulfuron, bensulfuron-methyl, and amidosulfuron.
6. **Biopesticides-** Biopesticides are pesticides derived from natural substances likeplants, animals, microbes, and certain minerals.

However, due to their unintended and indiscriminate application, only 10% of the applied pesticides effectively reach the intended target organism. The remaining substantial percentage is deposited in non-target areas, such as soil, water, and sediments, leading to severe environmental pollution. Consequently, there is a pressing need to develop strategies for the bioremediation of polluted sites and waste treatment. Utilizing native or genetically modified organisms for the degradation or removal of pesticides has emerged as a potent technology for *in-situ* remediation. Bioremediation, as an innovative technology, is frequently employed for the cleanup of polluted sites. The natural microbial degradation of contaminants is accelerated through bioremediation, achieved by supplementing these microorganisms with nutrients, carbon sources, or electron donors. The process can utilize indigenous microorganisms or introduce an enriched culture of microorganisms. Microbes leverage their inherent specific characteristics to degrade contaminants more rapidly. The intracellular or extracellular enzymes of these microbes play a crucial role in the degradation of chemical compounds. Various sources of microorganisms possessing pesticide-degrading abilities have been identified, often isolated from a diverse range of pesticide-contaminated sites.

5.8 FACTORS THAT AFFECT THE DEGRADATION OF PESTICIDES BY MICROORGANISM

- a) **The Impact of Microbes Themselves-** The breakdown of pesticides is directly influenced by various factors related to microbial species, including their metabolic activity, environmental adaptability, and other characteristics. The spatial distribution, population density, and interactions with other microorganisms also play a crucial role in determining the efficiency of pesticide degradation. Microorganisms acquire degradation capabilities through specific adaptation processes, exposure to new chemicals, or the production of relevant enzymes due to gene mutations. Their robust adaptability and domestication contribute to these capabilities.
- b) **Effects of Pesticide Structures-** The difficulty of microbial degradation is influenced by several factors related to pesticide structures. These factors include the molecular weight of pesticide compounds, spatial structure, types and quantities of substituents, and the number of substituents.

Generally, polymer compounds pose greater challenges for disintegration compared to low molecular weight compounds under normal conditions.

- c) **Impact of Environmental Factors-** Pesticide pollution and microbial degradation are subject to the influence of various environmental factors. These factors encompass environmental temperature, humidity, pH levels, the presence of carbon and nitrogen, organic matter, salinity, matrix adsorption, viscosity, and ventilation. Microorganisms often exhibit an optimal temperature for development, and the enzymes involved in degradation have specific temperature optima for their reactions. A change in temperature can affect microbial metabolism, the enzymatic activity of degrading enzymes, and even the physical state of pesticide pollutants, thereby influencing the rate of disintegration.

The following microbes are commonly involved in the breakdown of pesticides. *A. niger*, *Aspergillus fumigatus*, *Absidia*, *A. terreus*, *Pseudomonas*, *Rhodococcus* sp *Absidia*, *Rhizopus microsporus* var *corymberifera*, *microsporis*, *Fusarium*, *Botrytis cinerea* *Trichoderma viridae*, etc.

5.9 THE MECHANISM OF MICROBIAL DEGRADATION OF PESTICIDES

Microorganisms involved in pesticide breakdown can be categorized into two classes:

- i. **Direct action microorganisms:** This class consists of microorganisms that directly act on pesticides through enzymatic reactions. Pesticide microbial degradation is commonly associated with this group. These microorganisms achieve the degradation of pesticides through specific enzymatic processes.
- ii. **Indirect action microorganisms:** The second class comprises microorganisms that indirectly influence pesticides by altering the chemical and physical environment. While not directly engaged in enzymatic degradation, these microorganisms play a role in modifying the conditions that affect pesticide stability or breakdown.

Pesticides exhibit various modes of action, encompassing mineralization, co-metabolism, bioconcentration or cumulative effects, as well as microbial influences. The enzymatic reactions involved in microbial pesticide breakdown primarily include oxidation, dehydrogenation, reduction, hydrolysis, and synthesis. The intracellular enzyme-driven degradation process typically unfolds in three steps:

1. **Adsorption on Microbial Cell Surface:** Pesticides dynamically reach equilibrium by adsorbing onto the surface of microbial cells, leading to their rapid breakdown. This phase is crucial during the lag phase of degradation.
2. **Pesticide Permeation of Cell Membrane:** The permeability of the cell membrane dictates the amount of pesticide that can enter the microbial cell. Pesticide penetration through the cell membrane is often the rate-limiting step in degradation. Molecular structural factors, mainly lipophilic and steric hindrance parameters, significantly influence this penetration rate.
3. **Enzymatic Degradation Within the Cell Membrane:** Once inside the microbial cell, pesticides are rapidly degraded through enzymatic reactions involving specific degrading enzymes.

Different Pesticide Degradation Pathways:

- a) **Mineralization-** Chemical pesticides, resembling natural substances, serve as a microbial nutrition source through mineralization. This process results in the generation of inorganic

compounds, carbon dioxide, and water. Mineralization is the optimal method for degradation, ensuring the complete transformation of pesticides into non-toxic inorganic substances.

- b) **Co-metabolism-** Some synthetic chemicals resist microbial destruction, but in the presence of another carbon matrix and energy, they undergo partial decomposition through co-metabolism. Utilizing organic pesticides as a carbon or nitrogen source or breaking down complex pesticide molecules into simpler compounds like CO₂, H₂O, and NH₃, reduces pesticide residues and environmental toxicity.
- c) **Other Microbial Degradation Pathways-** Various degradation processes include hydrolysis (breaking ester and dialkylamine bonds for detoxification of pesticides like malathion and propanil), dehalogenation (DDT degradation into DDE), oxidation (carbendazim and 2,4-D degradation), nitro reduction (conversion of parathion to amino-phosphorus), and methylation and demethylation (removing two N-methyl groups during dexamethasone degradation).

Major enzymes applied for pesticide biodegradation are:

Hydrolases- Hydrolases constitute a broad category of enzymes crucial in the biodegradation of pesticides. These enzymes play a vital role in accelerating the hydrolysis of various essential biochemical groups found in pesticides, such as esters, peptide bonds, carbon-halide bonds, urea, thioesters, and more. Notably, hydrolases often function independently of redox cofactors, which makes them highly suitable candidates for various contemporary bioremediation techniques.

Phosphodiesterases- Phosphotriesterases stand out as one of the most crucial classes of enzymes extensively studied for their role in degrading pesticides. These enzymes play a key role in hydrolyzing and detoxifying organophosphate insecticides (OPs), effectively extracted from various microorganisms. Their action mitigates the toxicity of organophosphates by reducing their ability to inactivate acetylcholinesterase (AChE). The first phosphotriesterase isolated belongs to the *Pseudomonas diminuta* MG species, displaying high catalytic efficiency against organophosphate insecticides. The opd gene (organophosphate-degrading) codes for phosphotriesterases. Structurally, the phosphotriesterase enzyme is a homodimeric protein, with each monomer weighing 36 kilodaltons.

Esterase- Esterases are enzymes that catalyze hydrolysis reactions involving various substrates, including carboxylic esters, amides, and phosphate esters. In the reaction facilitated by esterases, a diverse range of ester substrates undergo hydrolysis, resulting in the production of their respective alcohol and acid components. Many insecticides, such as organophosphates, carbamates, and pyrethroids, contain a carboxylic ester, and carboxylesterases are the enzymes capable of hydrolyzing this ester linkage. Esterases form a heterogeneous group that plays a protective role at the target site, particularly acetylcholinesterase, by accelerating the hydrolysis of insecticides or by acting as substitute blanks. These enzymes exhibit a broad spectrum of substrate specificities, enabling them to bind to various compounds such as phosphate triesters, esters, thioesters, amides, and peptides, among others.

Oxidoreductases- Oxidoreductases belong to an enzyme family that facilitates the transfer of electrons from one molecule, known as the reductant or electron donor, to another, termed the oxidant or electron acceptor. In the context of xenobiotic removal, microorganisms play a pivotal role, particularly in the degradation of substances like endosulfan from contaminated sites. This is attributed to the dynamic, complex, and intricate enzyme systems present in microorganisms, which effectively break down these compounds by removing functional groups from the parent substance.

Glutathione S-Transferase (GST)- Glutathione S-transferases (GSTs), represented by the EC 2.5.1.18

enzyme classification, play a crucial role in catalyzing the conjugation of hydrophobic components with the glutathione tripeptide. In this biochemical process, the thiol group of glutathione reacts with an electrophilic site on the target compound, resulting in the formation of a conjugate. This conjugate can then undergo metabolism or be excreted from the system. The formed conjugates participate in a multitude of cellular physiological processes, including the detoxification of both endogenous and exogenous compounds, intracellular transport, biosynthesis of hormones, and protection from oxidative stress. The versatile functions of GSTs highlight their significance in maintaining cellular homeostasis and defense mechanisms.

5.10 HEAVY METAL TOLERANCE IN MICROBES

Metal-contaminated environments pose significant health and ecological risks, as metals such as aluminum, antimony, arsenic, cadmium, lead, mercury, and silver can lead to various adverse conditions. These include hypophosphatemia, heart disease, liver damage, cancer, neurological and cardiovascular diseases, central nervous system damage, encephalopathy, and sensory disturbances. Due to their toxic nature, metals are less amenable to bioremediation compared to organic pollutants. Metals can be classified into three categories: metals, metalloids, and heavy metals. Heavy metals are defined based on various criteria, including cationic-hydroxide formation, specific gravity greater than 5 g/ml, complex formation, hard-soft acids, and bases, and their association with eutrophication and environmental toxicity. Certain microorganisms are believed to have evolved metal resistance either shortly after life began, due to exposure to toxic metals, or in response to recent exposure to metal pollution over the past 50 years. Microorganisms play a crucial role in influencing the fate of metals in the environment, offering potential solutions to mitigate existing contamination. In response to metal presence, microorganisms have developed ingenious mechanisms of metal resistance and detoxification. Some resistance mechanisms are plasmid-encoded and specific to particular metals, while others are general, conferring resistance to a variety of metals. These microbial adaptations are pivotal for understanding and addressing metal pollution challenges in diverse environments.

Metal-dependent mechanisms of resistance

- a) **Metallothioneins-** Metallothioneins (MTs), discovered approximately 45 years ago, play a central role in heavy metal metabolism and the management of various stress forms. These low molecular weight proteins (6-7 kDa) are cysteine-rich and exhibit a high affinity for metals such as cadmium, zinc, copper, silver, and mercury. Metallothionein-like proteins have been identified in organisms like the Cyanobacterium *Syneococcus* spp, as well as in bacteria like *E. coli* and *Pseudomonas putida*.
- b) **Methylation of Metals-** Methylation of metals is a metal-dependent resistance mechanism involving specific metals. While methylation typically increases metal toxicity due to enhanced lipophilicity and increased permeation across cell membranes, metal volatilization allows for effective diffusion of metals away from the cell, reducing toxicity. Metal volatilization has been observed in lead, mercury, tin, selenium, and arsenic. For instance, mercury (Hg^{2+}) is oxidized to volatile and highly toxic forms like methylmercury and dimethylmercury, enabling their diffusion away from the cell. Mercury resistance may involve enzymatic reduction of Hg^{2+} to elemental mercury (Hg_0) in both gram-positive and gram-negative bacteria.
- c) **Biosorption Mechanisms-** The complex structure of microorganisms provides multiple pathways for metal uptake by the microbial cell. Biosorption mechanisms can be categorized based on their dependence on the cell's metabolism: Metabolism-dependent and Non metabolism dependent. Metabolism-dependent metal transport across the cell membrane leads to intracellular

accumulation, often associated with an active defense system reacting to toxic metals. In contrast, non-metabolism-dependent biosorption involves metal uptake through physicochemical interactions between the metal and functional groups on the microbial cell surface. This process relies on physical adsorption, ion exchange, and chemical sorption, independent of the cell's metabolism.

- d) **Extracellular Processes-** Microorganisms, including *Thiobacillus*, *Serratia*, *Pseudomonas*, *Bacillus*, *Penicillium*, and *Aspergillus*, produce organic or inorganic acids capable of extracting metals from solid substrates. Under anaerobic conditions, toxic metals can be mobilized as co-precipitates with iron oxides. Metals closely associated with iron, such as Cd and Zn, are solubilized through enzymatic reduction of ferric iron, while others, notably Pb, are solubilized through the indirect action of bacterial metabolites. The formation of insoluble metal salts results in the immobilization of toxic metals by sequestering them in sediments or adsorbing them to soil particles.
- e) **Intracellular Accumulation-** The concentration of metals within bacterial and other microbial cells can occur through interaction with surface ligands, followed by slow transport into the cell. This process may serve as a detoxification mechanism or a means of incorporating specific metals into enzymes (e.g., Cu and Zn). Bacterial assimilation of metals plays a crucial role in detoxification, enzyme function, and the physical characteristics of the cell. Extracellular or cell wall-attached ligands bind toxic metals, facilitating their transport through the cell wall in a slow transport step. Once inside the cell, metals can be released, incorporated into biochemical pathways, or trapped in an inactive form through complexation with another high-affinity ligand. The protoplasm of *E. coli*, for instance, typically contains 0.3% trace elements, including manganese, cobalt, copper, zinc, and molybdenum. Rapid accumulation of uranium has been observed in cells of *Saccharomyces cerevisiae* and *P. aeruginosa*.
- f) **Precipitation-** Precipitation processes can occur either in connection with cellular metabolism or independently from it. When linked to cellular metabolism, the removal of metals from solution often involves the active defense mechanisms of microorganisms. These microorganisms respond to the presence of toxic metals by producing compounds that facilitate the precipitation process. On the other hand, independent precipitation can arise from chemical interactions between the metal ions and the surface of the cell.

5.11 SUMMARY

Bioremediation and phytoremediation are innovative and sustainable approaches to environmental cleanup, aiming to mitigate the impact of pollutants on both water and land ecosystems. These methods leverage the natural abilities of microorganisms and plants to degrade or immobilize contaminants, offering a promising alternative to traditional remediation techniques. In water remediation, several specific bioremediation technologies have gained prominence. Biosparging is a technique that involves the injection of air or oxygen into contaminated groundwater to enhance the activity of indigenous microorganisms. By increasing the oxygen supply, biosparging stimulates the aerobic degradation of pollutants such as hydrocarbons and other organic compounds. This method has proven effective in treating groundwater contaminated with petroleum hydrocarbons. Land farming is another key technique in bioremediation, particularly for treating contaminated soils. This approach involves spreading contaminated soil in a thin layer over the land surface and promoting microbial activity through aeration and nutrient addition. As microorganisms break down pollutants, the soil undergoes remediation, resulting in a decrease in contaminant levels. Understanding the degradative pathways of plasmids is crucial in the context of bioremediation. Plasmids are extrachromosomal DNA

elements that can carry genes conferring resistance to pollutants. In bioremediation, scientists explore ways to target and eliminate these plasmids to prevent the spread of resistance genes in microbial populations. Strategies may include the use of engineered microorganisms or specific compounds that disrupt plasmid replication and transfer mechanisms. Hydrocarbons, such as those found in petroleum products, pose significant environmental challenges. Bioremediation exploits the metabolic capabilities of certain microorganisms, like bacteria and fungi, to break down hydrocarbons into less harmful substances. These microorganisms utilize hydrocarbons as a carbon source, converting them into water, carbon dioxide, and biomass. This process is cost-effective and environmentally friendly, offering a viable solution for hydrocarbon-contaminated sites. Pesticides, widely used in agriculture, can contaminate soil and water, posing threats to ecosystems and human health. Bioremediation, particularly through microbial degradation, has shown promise in breaking down pesticide residues. Certain bacteria and fungi have enzymes that can detoxify pesticides, converting them into non-toxic byproducts. Harnessing these natural mechanisms can mitigate the environmental impact of pesticide pollution. Heavy metals, such as lead, cadmium, and mercury, are persistent pollutants with detrimental effects on ecosystems. Microbes play a crucial role in heavy metal tolerance and remediation. Some bacteria and fungi possess mechanisms to sequester or transform heavy metals, rendering them less toxic. This microbial action can be harnessed in bioremediation strategies, offering a sustainable approach to mitigating heavy metal contamination. The development of heavy metal tolerance in microbes involves intricate molecular processes, including the expression of metal-binding proteins and the activation of detoxification pathways. Understanding these mechanisms is essential for optimizing bioremediation strategies and improving the efficiency of microbial metal remediation. In conclusion, bioremediation and phytoremediation are powerful tools in the environmental cleanup arsenal, offering sustainable solutions for water and land contamination. Specific technologies, such as biosparging and land farming, target different environmental media, while understanding the degradative pathways of plasmids and the tolerance mechanisms of microbes enhances the effectiveness of these approaches. By harnessing the natural capabilities of microorganisms and plants, we can address a wide range of pollutants, from hydrocarbons and pesticides to heavy metals, contributing to a cleaner and healthier environment.

5.12 TERMINAL QUESTIONS

Q.1 What is bioremediation? What are the key indicators of bioremediation?

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Q.2 What are the different types of bioremediation?

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Q.3 What is Phytoremediation? What are the different mechanisms of phytoremediation?

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Q.4 What are the advantages and limitations of phytoremediation?

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Q.5 What is Biosparging? Discuss its advantages and disadvantages.

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Q.6 Discuss different advantages and disadvantages of bioremediation.

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Q.7 Discuss the various types of degradative plasmids in detail.

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Q.8 Discuss the mechanisms of heavy metal tolerance in microorganisms.

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5.13 FURTHER SUGGESTED READING

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UNIT-6 : ECOLOGICALLY SAFE PRODUCTS AND PROCESSES

Structure

- 6.1 Introduction
 - Objectives
- 6.2 Plant Growth Promoting Rhizobacteria (PGPR): Biofertilizers
 - 6.2.1 Ways in Which PGPR Promote Plant Growth
- 6.3 PGPR as Insecticides and Pesticides
- 6.4 PGPR as Fungicides
- 6.5 PGPR as Biocontrol Agents
- 6.6 PGPR in Integrated Pest Management
- 6.7 Development of Stress Tolerant Plants
- 6.8 PGPR in Mining and Metal Biotechnology
- 6.9 Summary
- 6.10 Terminal Questions
- 6.11 Suggested Readings

6.1 INTRODUCTION

Agriculture plays a significant role in the economy of many developing nations, contributing substantially to national income and export revenue, while also ensuring food security and employment opportunities. In today's world, sustainable agriculture has become increasingly important due to its potential to meet future agricultural demands, a feat conventional agriculture may struggle to achieve. There's been a surge of interest in eco-friendly and sustainable agricultural practices recently. Plant Growth-Promoting Rhizobacteria (PGPR) have garnered attention for their ability to enhance plant growth compared to synthetic fertilizers, insecticides, and pesticides. They offer a promising avenue for maintaining both environmental safety and crop productivity. Rhizospheric soil harbors diverse communities of PGPR, which play beneficial roles in enhancing crop productivity. Numerous research studies are underway to comprehend the diversity, dynamics, and significance of soil PGPR communities, as well as their cooperative roles in bolstering agricultural productivity. Some common examples of PGPR genera exhibiting plant growth-promoting activity are: *Pseudomonas*, *Azospirillum*,

Azotobacter, *Bacillus*, *Burkholdaria*, *Enterobacter*, *Rhizobium*, *Erwinia*, *Mycobacterium*, *Mesorhizobium*, *Flavobacterium*, etc. This article presents perspectives on the role of PGPR in agriculture sustainability.

Objectives

After reading this unit, the learner will be able to :

- To discuss Plant Growth Promoting Rhizobacteria (PGPR): Biofertilizers
- To discuss Microbial (PGPR) as Insecticides and Pesticides
- To explore PGPR as Fungicides
- To understand PGPR as Biocontrol Agents
- To understand the role of PGPR in Mining and Metal Biotechnology
- To discuss the role of PGPR in Integrated Pest Management

6.2 PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) BIOFERTILIZERS

Free-living PGPR hold great potential as biofertilizers. Numerous studies and reviews have documented their ability to promote plant growth, boost yields, solubilize phosphorus (P) or potassium (K), facilitate nitrogen (N) and other element uptake when plants are inoculated with PGPR. Moreover, research has demonstrated that PGPR inoculation leads to enhanced root growth, resulting in a root system with a larger surface area and more root hairs. Extensive use of artificial fertilizers to replenish soil nitrogen (N) and phosphorus (P) comes with high costs and increased environmental pollution. Much of the phosphorus exists in insoluble compounds, rendering it unavailable to plants. Therefore, nitrogen-fixing and phosphorus-solubilizing bacteria play a crucial role in plant nutrition by enhancing the uptake of N and P by crop plants, thus contributing significantly to biofertilization. In addition to nitrogen fixation and phosphorus solubilization, PGPR contribute to agricultural ecosystems by producing antibiotics and other substances that promote plant growth. Recent research findings suggest that treating agricultural soils with PGPR inoculation leads to significantly higher agronomic yields compared to untreated soils.

6.2.1 WAYS IN WHICH PGPR PROMOTES PLANT GROWTH

Several mechanisms have been suggested by which PGPR can promote plant growth; some important ones are as follows (Table 1):

Table1- Promotion of plant growth by Plant Growth Promoting Rhizobacteria (PGPR) on diverse parameters (<https://www.ias.ac.in/public/Volumes/reso/018/03/0275-0281.pdf>).

PGPR	Crop parameters
<i>Rhizobium leguminosarum</i>	Direct growth promotion of canola and lettuce
<i>Pseudomonas putida</i>	Early developments of canola seedlings, growth stimulation of tomato plant
<i>Azospirillum brasilense</i> and <i>A. irakense</i>	Growth of wheat and maize plants
<i>P. fluorescens</i>	Growth of pearl millet, increase in growth, leaf nutrient contents and yield of banana (<i>Musa</i>)
<i>Azotobacter</i> and <i>Azospirillum</i> spp.	Growth and productivity of canola
<i>P. alcaligenes</i> , <i>Bacillus polymyxa</i> , and <i>Mycobacterium phlei</i>	Enhances uptake of N, P and K by maize crop
<i>Pseudomonas</i> , <i>Azotobacter</i> and <i>Azospirillum</i> spp.	Stimulates growth and yield of chick pea (<i>Cicer arietinum</i>)
<i>R. leguminosarum</i> and <i>Pseudomonas</i> spp.	Improves the yield and phosphorus uptake in wheat
<i>P. putida</i> , <i>P. fluorescens</i> , <i>A. brasilense</i> and <i>A. lipoferum</i>	Improves seed germination, seedling growth and yield of maize
<i>P. putida</i> , <i>P. fluorescens</i> , <i>P. fluorescens</i> , <i>P. putida</i> , <i>A. lipoferum</i> , <i>A. brasilense</i>	Improves seed germination, growth parameters of maize seedling in greenhouse and also grain yield of field grown maize

- a) **Phytohormone Production-** PGPR have been shown to increase different agronomic yields by producing growth-promoting phytohormones like indole-3-acetic acid (IAA), gibberellic acid (GA3), zeatin, ethylene, and abscisic acid (ABA).

Recent research affirms that treating seeds or cuttings with non-pathogenic bacteria like *Agrobacterium*, *Bacillus*, *Streptomyces*, *Pseudomonas*, *Alcaligenes*, etc., induces root formation in certain plants due to the natural production of plant growth-promoting substances by these bacteria. While the exact mechanisms remain incompletely understood, root induction by PGPR is attributed to phytohormones such as auxins, ethylene inhibitors, and mineralization processes. Environment friendly applications in agriculture, particularly in horticulture and nursery production, have gained increased importance. The utilization of PGPR for multiplying nursery materials holds significance for obtaining organic nursery products, as the use of all formulations of synthetic plant growth regulators like indole-3-butyric acid (IBA) is prohibited in organic agriculture worldwide.

- b) **Phosphate Solubilization-** *Rhizobium* and phosphorus (P)-solubilizing bacteria are crucial for plant nutrition and serve as important PGPR for crop biofertilization. These microbes secrete various organic acids, such as carboxylic acids, which reduce the pH in the rhizosphere, thereby releasing bound forms of phosphate like $\text{Ca}_3(\text{PO}_4)_2$ in calcareous soils. Utilizing these microorganisms as environment friendly biofertilizers helps decrease reliance on costly synthetic phosphatic fertilizers. Phosphorus biofertilizers have the potential to enhance phosphate availability through solubilization, improve biological nitrogen fixation efficiency, and facilitate the availability of elements like Fe and Zn through the production of plant growth-promoting substances.
- c) **Siderophore Production-** Siderophores, extracellular metabolites produced by certain PGPR, were first highlighted for their importance in plant growth promotion by Kloepper et al. in 1980.

These low molecular weight compounds, often termed microbial Fe-chelating agents, play a crucial role in enhancing plant growth and productivity. When siderophore-producing PGPR are present in the rhizosphere, they accelerate the supply of Fe^{3+} to plants. Additionally, after binding with Fe^{3+} , siderophores create a deficiency of Fe^{3+} in the soil, inhibiting the activity of competing soil microbes.

6.3 MICROBIAL (PGPR) AS INSECTICIDES AND PESTICIDES

Plant growth-promoting rhizobacteria (PGPR) are advantageous bacteria that inhabit the rhizosphere, the soil area influenced by plant roots, and foster plant growth through diverse mechanisms. These mechanisms include nutrient solubilization, synthesis of plant growth regulators, and biological suppression of plant pathogens. Although PGPR can indirectly support plant health and bolster resistance against pests by boosting plant vigor and immunity, they are not commonly employed as direct insecticides.

Nevertheless, emerging research indicates that particular strains of PGPR could serve as biopesticides or biocontrol agents against insect pests. This potential stems from their ability to induce systemic resistance in plants, generate insecticidal compounds, or disrupt the life cycle of insects. Although these applications are still in the investigative and developmental stages, their efficacy might vary based on factors like the bacteria's characteristics, target pests, and environmental factors. Consequently, while PGPR are not conventionally deployed as insecticides, they represent a promising component of integrated pest management approaches, aiming to foster sustainable agriculture and diminish dependence on synthetic chemical pesticides.

6.4 PGPR AS FUNGICIDES

Biocontrol agents such as PGPR (Plant Growth-Promoting Rhizobacteria) and bacterial endophytes play a crucial role in managing various fungal diseases. However, a significant challenge faced in utilizing these agents effectively lies in the lack of an appropriate delivery system. For instance, *Bacillus subtilis*, *Pseudomonas chlororaphis*, and endophytic *Pseudomonas fluorescens* have demonstrated the ability to inhibit the growth of the stem blight pathogen *Corynespora casicola*. Moreover, treatments involving seed and soil application of *P. fluorescens* have been effective in reducing root rot in black gram, which is caused by *Macrophomina phaseolina*. Similarly, both seed and foliar applications of *P. fluorescens* have shown promise in reducing sheath blight in rice crops. Furthermore, the use of *B. subtilis* in peat supplemented with chitin or chitin-containing materials has exhibited improved control against *Aspergillus niger* in groundnut and *Fusarium udum* in pigeon pea. Additionally, strains of *Burkholderia cepacia* have demonstrated biocontrol capabilities against *Fusarium* spp.

6.5 PGPR AS BIOCONTROL AGENTS

PGPR produce compounds that serve as a defense mechanism against different diseases. These bacteria can shield plants from pathogens through direct antagonistic interactions or by stimulating host resistance. Notably, the involvement of siderophore-producing PGPR in combating soil-borne plant pathogens has garnered significant attention in recent years. Microbiologists have devised methods to introduce siderophore-producing PGPR into the soil system through seeds, soil, or root systems. PGPR indirectly boost plant growth by suppressing phytopathogens through a range of mechanisms. These include:

1. The production of siderophores, as previously mentioned, which sequester iron, depriving pathogens of access to this essential nutrient.
2. Synthesizing antifungal and antibacterial compounds like fungicides, antibiotics, and enzymes that break down fungal cell walls, or hydrogen cyanide, all of which inhibit the proliferation of fungal pathogens.
3. Competing effectively with pathogens for nutrients or specific root niches, while also inducing systemic resistance in plants.

Pseudomonas fluorescens stands out as one of the extensively researched PGPRs, renowned for its ability to combat numerous plant pathogens. In regions like the Western Ghats of Tamil Nadu, India, where the banana crop (*Musa* spp.) faces severe threats from the Banana Bunchy Top Virus (BBTV), *P. fluorescens* has emerged as a promising solution. Studies have showcased the efficacy of specific strains of *P. fluorescens* in significantly reducing BBTV incidence in hill banana crops, both in controlled greenhouse environments and actual field conditions. Table 2 provides a comprehensive overview of various PGPR species utilized as biocontrol agents against diverse plant diseases.

Table 2. PGPRs as biocontrol agents against various plant diseases

(<https://www.ias.ac.in/public/Volumes/reso/018/03/0275-0281.pdf>).

PGPR	Disease resistance
<i>Bacillus pumilus</i> , <i>Kluyvera cryocrescens</i> , <i>B. amyloliquefaciens</i> and <i>B. subtilis</i>	Cucumber Mosaic Cucumovirus (CMV) of tomato (<i>Lycopersicon esculentum</i>)
<i>B. amyloliquefaciens</i> , <i>B. subtilis</i> and <i>B. pumilus</i>	Tomato mottle virus
<i>B. pumilus</i>	Bacterial wilt disease in cucumber (<i>Cucumis sativus</i>), Blue mold disease of tobacco (<i>Nicotiana</i>)
<i>Pseudomonas fluorescens</i>	Sheath blight disease and leaf folder insect in rice (<i>Oryza sativa</i>), Reduce the Banana Bunchy Top Virus (BBTV) incidence, Saline resistance in groundnut (<i>Arachis hypogaea</i>)
<i>B. subtilis</i> and <i>B. pumilus</i>	Downy mildew in pearl millet (<i>Pennisetum glaucum</i>)
<i>B. subtilis</i>	CMV in cucumber
<i>B. cereus</i>	Foliar diseases of tomato
<i>Bacillus</i> spp.	Blight of bell pepper (<i>Capsicum annuum</i>), Blight of squash
<i>Burkholderia</i>	Maize (<i>Zea mays</i>) rot
<i>B. subtilis</i>	Soil borne pathogen of cucumber and pepper (<i>Piper</i>)
<i>Bacillus</i> sp. and <i>Azospirillum</i>	Rice blast
Fluorescent <i>Pseudomonas</i> spp.	Rice sheath rot (<i>Sarocladium oryzae</i>)

6.6 PGPR IN INTEGRATED PEST MANAGEMENT

PGPR stands for Plant Growth-Promoting Rhizobacteria. They are a group of beneficial soil

bacteria that colonize the rhizosphere (root zone) of plants and promote plant growth and health through various mechanisms. While PGPR are not typically used directly as a method of pest management, they can play a significant role in integrated pest management (IPM) programs indirectly. Here's how:

1. **Enhanced Plant Health-** PGPRs promote plant growth by enhancing nutrient uptake, synthesizing growth-promoting hormones, and improving stress tolerance. Healthy plants are better equipped to withstand pest attacks and recover from damage caused by pests.
2. **Induced Systemic Resistance (ISR)-** Some PGPR strains can induce systemic resistance in plants against pathogens and pests. They trigger a plant's defense mechanisms, making it more resistant to pest infestations. This can reduce the need for chemical pesticides in pest management.
3. **Biocontrol Agents-** Certain strains of PGPRs have antagonistic effects against plant pathogens and pests. They produce antimicrobial compounds or compete with pathogens for space and nutrients, thereby suppressing pest populations indirectly.
4. **Nutrient Competition-** PGPRs compete with pathogenic organisms for nutrients and space in the rhizosphere. By outcompeting pathogens, they can reduce the incidence of soil-borne diseases that may weaken plants and make them more susceptible to pest attacks.
5. **Improved Soil Structure-** PGPRs can contribute to soil health by promoting the formation of soil aggregates and improving soil structure. Healthy soil supports diverse and balanced microbial communities, which can indirectly suppress pest populations by maintaining ecosystem resilience.

In an IPM approach, PGPRs can be integrated into pest management strategies alongside other practices such as cultural controls, biological controls, and judicious use of chemical pesticides. By promoting plant health and resilience, PGPR can help reduce reliance on synthetic pesticides, minimize environmental impacts, and promote sustainable agriculture. However, it's essential to note that the effectiveness of PGPRs in pest management may vary depending on factors such as soil conditions, plant species, and the specific PGPR strains used. Therefore, it's important to conduct site-specific evaluations and integrate PGPRs into a comprehensive IPM plan tailored to local conditions and pest pressures.

6.7 DEVELOPMENT OF STRESS TOLERANT PLANTS

The development of stress-tolerant plants using PGPRs (Plant Growth-Promoting Rhizobacteria) involves harnessing the beneficial interactions between these bacteria and plants to enhance the plant's ability to withstand various environmental stresses. Here are some examples of how PGPRs have been used to develop stress-tolerant plants:

1. **Drought Tolerance-** Some strains of *Azospirillum*, a genus of PGPR, have been found to enhance drought tolerance in plants by improving water use efficiency and root development. Researchers have demonstrated that inoculating crops such as maize and wheat with *Azospirillum* can improve their performance under drought conditions.
2. **Salinity Tolerance-** Certain PGPR strains of *Halotolerant Rhizobacteria* adapted to saline environments and have been used to improve salt tolerance in crops like rice and soybean. These bacteria help by facilitating nutrient uptake, reducing sodium uptake, and promoting the production of osmolytes, which are compounds that help plants cope with high salt concentrations.

3. **Heat Stress Tolerance-** Some species of *Bacillus*, another common genus of PGPRs, have been shown to enhance heat stress tolerance in plants by inducing the expression of heat shock proteins and antioxidants. By inoculating crops with heat-tolerant strains of *Bacillus* (e.g., *Bacillus subtilis*), researchers aim to improve crop productivity in regions prone to heat stress.
4. **Cold Stress Tolerance-** Certain strains of *Pseudomonas* (*Pseudomonas putida*, *Pseudomonas fluorescens*), a diverse genus of PGPRs, have been identified for their ability to enhance cold stress tolerance in plants. These bacteria promote plant growth at low temperatures by producing antifreeze proteins and enhancing nutrient availability in the rhizosphere.
5. **Nutrient Deficiency Tolerance-** Example: *Phosphate-Solubilizing Bacteria* (PSB): PGPR strains capable of solubilizing phosphate in the soil have been used to improve phosphate uptake by plants, especially in phosphorus-deficient soils. By increasing the availability of phosphate to plants, PSBs help alleviate nutrient stress and promote healthy plant growth.

These examples illustrate how PGPRs can be harnessed to develop stress-tolerant plants through various mechanisms, including enhanced nutrient uptake, hormone regulation, and modulation of stress-responsive genes. Integrating PGPRs into plant breeding programs and agricultural practices offers a promising approach to develop resilient crop varieties capable of thriving under challenging environmental conditions, ultimately contributing to food security and sustainable agriculture.

6.8 PGPR IN MINING AND METAL BIOTECHNOLOGY

PGPRs (Plant Growth-Promoting Rhizobacteria) play a crucial role in mining and metal biotechnology, particularly in the context of bioremediation and bioleaching processes.

1. **Bioremediation:** PGPR can assist in the remediation of metal-contaminated soils and waters by promoting the growth of plants that can accumulate metals from the environment (phytoremediation). These bacteria can enhance plant growth and metal uptake by various mechanisms, including producing plant growth regulators, facilitating nutrient uptake, and modifying the rhizosphere environment to make metals more available to plants. Here are some examples of plants commonly used in phytoremediation to accumulate metals from contaminated soils and waters, often facilitated by the presence of PGPRs.
 - a. **Sunflower** (*Helianthus annuus*): Sunflowers are known for their ability to accumulate heavy metals, including lead, arsenic, cadmium, and nickel, in their tissues. PGPR can enhance the growth and metal uptake of sunflowers by improving nutrient availability and root development.
 - b. **Indian Mustard** (*Brassica juncea*): Indian mustard is widely used in phytoremediation due to its high tolerance to heavy metals, particularly cadmium and zinc. PGPR can promote the growth of Indian mustard and enhance its metal accumulation capacity by increasing root surface area and nutrient uptake.
 - c. **Willow** (*Salix* spp.): Willows are fast-growing trees that are effective at extracting metals such as cadmium, zinc, and copper from contaminated soils and waters. PGPR can improve the establishment and growth of willow trees, leading to enhanced phytoremediation efficiency.
 - d. **Poplar** (*Populus* spp.): Poplar trees are commonly used in phytoremediation due to their deep root systems and high transpiration rates, which allow them to uptake and translocate metals from contaminated soils to their above-ground tissues. PGPR can support the

growth and metal uptake of poplar trees by promoting root development and nutrient acquisition.

- e. **Ferns** (various species): Certain fern species, such as *Pteris vittata* (commonly known as the arsenic hyperaccumulator fern), have been studied for their ability to accumulate high concentrations of arsenic from contaminated soils. PGPRs can enhance the growth and arsenic uptake of ferns by improving soil conditions and nutrient availability.

These plants, when combined with the beneficial effects of PGPR, offer promising solutions for the remediation of metal-contaminated environments through phytoremediation.

- 2. **Bioleaching:** In the mining industry, bioleaching is a process where microorganisms, including PGPRs, are used to extract metals from ores. PGPRs can enhance the efficiency of bioleaching by facilitating the oxidation of metal sulfides, which releases the target metals from the ore matrix. Additionally, PGPRs can produce organic acids and other metabolites that can aid in the solubilization of metals, making them more accessible for extraction. Some examples of microorganisms, including PGPRs, commonly used in bioleaching processes to extract metals from ores:

- a) **Acidithiobacillus ferrooxidans:** It is a Gram-negative, acidophilic bacterium that plays a key role in bioleaching processes. It oxidizes ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}), which in turn oxidizes metal sulfides present in ores, releasing target metals such as copper, gold, and zinc. This bacterium also produces sulfuric acid as a metabolic byproduct, which helps in the solubilization of metals from the ore matrix.
- b) **Acidithiobacillus thiooxidans:** It is another acidophilic bacterium involved in bioleaching. It oxidizes elemental sulfur and reduced inorganic sulfur compounds to sulfate, contributing to the generation of sulfuric acid in bioleaching environments. *A. thiooxidans* can enhance the efficiency of metal extraction by accelerating the oxidation of metal sulfides and facilitating the release of target metals.
- c) **Leptospirillum ferriphilum:** It is a Gram-negative, iron-oxidizing bacterium commonly found in bioleaching environments. It oxidizes ferrous iron to ferric iron, which contributes to the oxidation of metal sulfides in ores. *L. ferriphilum* also produces organic acids and other metabolites that aid in the solubilization of metals, thereby improving the accessibility of metals for extraction.
- d) **Sulfobacillus spp.:** These are acidophilic bacteria capable of oxidizing sulfur and iron compounds in bioleaching environments. They contribute to the generation of sulfuric acid and ferric iron, which promote the oxidative dissolution of metal sulfides. *Sulfobacillus* spp. can also produce extracellular polymeric substances (EPS) that help in biofilm formation and metal solubilization.
- e) **Fungi:** Certain fungal species, such as *Acidomyces* spp. and *Aspergillus* spp., are also used in bioleaching processes. These fungi secrete organic acids and enzymes that facilitate the decomposition of metal sulfides and the solubilization of metals from ores. Fungal bioleaching is particularly effective for refractory ores containing complex sulfide minerals.

- 3. **Environmental Sustainability:** The use of PGPRs in mining and metal biotechnology offers a more sustainable and environmentally friendly approach compared to traditional chemical methods. By harnessing the natural abilities of these bacteria, it is possible to reduce the reliance on harsh chemicals and energy-intensive processes, thereby minimizing the environmental impact of mining activities.

4. **Cost-Effectiveness:** PGPR-mediated processes can also be more cost-effective in the long run. While initial setup costs may be comparable or slightly higher than traditional methods, the reduced need for chemical inputs and lower energy consumption can lead to significant cost savings over time.
5. **Biotechnological Innovations:** Researchers are continually exploring ways to optimize the use of PGPRs in mining and metal biotechnology through biotechnological innovations. This includes the genetic engineering of bacteria to enhance their metal-mobilizing capabilities, as well as the development of consortia of microorganisms that work synergistically to improve metal extraction efficiency. Here are some examples of biotechnological innovations aimed at optimizing the use of PGPRs in mining and metal biotechnology.
 - a. **Genetic Engineering of PGPR:** Researchers are genetically engineering PGPR strains to enhance their metal-mobilizing capabilities. This includes modifying genes responsible for metal ion transport, secretion of organic acids, and production of enzymes involved in metal oxidation and solubilization. These genetically modified PGPR strains can be tailored for specific metal bioremediation or bioleaching applications.
 - b. **Biofilm Engineering:** Biofilms formed by PGPRs play a crucial role in metal biotechnology processes such as bioleaching. Biotechnological innovations focus on engineering biofilms to improve their adhesion, stability, and metal-binding capacity. This includes the development of biofilm-forming strains with enhanced metal tolerance and biofilm matrix components that can bind metals more efficiently.
 - c. **Metabolic Engineering:** Metabolic engineering techniques are used to optimize the metabolic pathways of PGPRs for enhanced metal mobilization and utilization. This includes metabolic engineering to increase the production of organic acids, siderophores, and other metabolites involved in metal solubilization and uptake. Additionally, metabolic engineering can be employed to improve the energy efficiency of PGPR-mediated processes.
 - d. **Synthetic Biology Approaches:** Synthetic biology offers innovative approaches for designing and constructing novel PGPR strains with customized functionalities for mining and metal biotechnology applications. This includes the design of synthetic gene circuits and genetic regulatory networks to control the expression of metal-related genes in response to environmental cues. Synthetic biology also enables the creation of synthetic consortia of PGPRs with complementary capabilities for synergistic metal bioprocessing.
 - e. **Omics Technologies:** Omics technologies, including genomics, transcriptomics, proteomics, and metabolomics, are used to gain a comprehensive understanding of the molecular mechanisms underlying PGPR-mediated processes in mining and metal biotechnology. These technologies enable researchers to identify key genes, proteins, and metabolic pathways involved in metal mobilization, uptake, and tolerance, providing insights for targeted biotechnological interventions.

6.9 SUMMARY

Plant Growth Promoting Rhizobacteria (PGPR) are a group of bacteria that enhances plant growth and yield via various plant growth promoting substances as well as biofertilizers. Due to the negative environmental effects and rising costs of artificial fertilizers, the global adoption of beneficial soil microorganisms like Plant Growth-Promoting Rhizobacteria (PGPR) has increased over the past two decades. PGPR biofertilizers are now widely recognized for their efficiency in promoting sustainable

agriculture and significantly improving crop yields. Overall, there has been substantial progress in the field of PGPR biofertilizer technology worldwide. Research has consistently shown that PGPR are highly effective in enhancing soil fertility and boosting agricultural productivity. They serve as excellent models for biotechnologists, offering novel genetic materials and bioactive compounds that have diverse applications in agriculture and environmental sustainability. Advancements in our understanding of PGPR diversity, colonization, mechanisms of action, formulation, and application are expected to further establish their role as dependable elements in sustainable agricultural management systems.

6.10 TERMINAL QUESTIONS

Q.1 What is a PGPR? Discuss its role as biocontrol agents.

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Q.2 How do you describe the Role of Plant Growth-Promoting Rhizobacteria (PGPR) in Biofuels?

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.....

Q.3 Discuss the role of PGPR in Mining and Metal Biotechnology in detail.

.....
.....

Q.4 What are the role of PGPRs in Integrated Pest Management? Name them.

.....
.....

Q.5 How do you develop of Stress Tolerant Plants utilizing Plant Growth-Promoting Rhizobacteria (PGPR)? Write the names of the PGPRs involved, elaborating its function.

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.....

Q.6 Multiple Choice Questions-

1. Which of the following is a common mechanism by which PGPR promote plant growth?
 - a) Nitrogen fixation
 - b) Photosynthesis
 - c) Increasing soil pH
 - d) Waterlogging prevention
2. PGPR can promote plant growth by:
 - a) Solubilizing phosphorus
 - b) Producing plant hormones like auxins
 - c) Enhancing nutrient uptake

- d) All of the above
3. How do PGPR act as insecticides?
- a) By producing toxins harmful to insects
 - b) By increasing plant photosynthesis
 - c) By fixing nitrogen
 - d) By solubilizing phosphorus
4. Which of the following is a method by which PGPR provide pest control?
- a) Competitive exclusion
 - b) Enhancing soil moisture
 - c) Providing shade to plants
 - d) Decreasing soil aeration
5. PGPR can act as fungicides by:
- a) Producing antibiotics
 - b) Enhancing leaf chlorophyll content
 - c) Increasing root length
 - d) Fixing atmospheric nitrogen
6. Which substance produced by PGPR helps in controlling fungal pathogens?
- a) Hydrogen cyanide
 - b) Water
 - c) Carbon dioxide
 - d) Oxygen
7. As biocontrol agents, PGPR can suppress plant diseases by:
- a) Producing siderophores
 - b) Increasing photosynthesis rate
 - c) Decreasing soil temperature
 - d) Enhancing soil salinity
8. Which of the following is NOT a mechanism used by PGPR to control plant pathogens?
- a) Inducing systemic resistance
 - b) Direct antagonism
 - c) Increasing soil temperature
 - d) Competition for nutrients

9. In Integrated Pest Management (IPM), PGPR contribute by:
- Reducing the need for chemical pesticides
 - Increasing the use of chemical fertilizers
 - Increasing pest populations
 - Decreasing plant root development
10. The role of PGPR in IPM includes:
- Enhancing plant tolerance to abiotic stress
 - Serving as a primary food source for pests
 - Increasing the use of synthetic pesticides
 - Reducing plant root size
11. PGPR help in developing stress-tolerant plants by:
- Producing ACC deaminase which lowers ethylene levels
 - Reducing chlorophyll content in leaves
 - Increasing plant water uptake exclusively
 - Decreasing the size of plant roots
12. Which of the following stresses can PGPR help plants to tolerate?
- Drought
 - Salinity
 - Heavy metals
 - All of the above
13. In mining and metal biotechnology, PGPR can assist by:
- Bioleaching of metals from ores
 - Increasing the pH of mining waste
 - Decreasing the concentration of metals in soils
 - Providing shade to mining sites
14. How do PGPR contribute to the phytoremediation of contaminated soils?
- By producing plant growth-promoting substances
 - By reducing the bioavailability of heavy metals

- c) By increasing soil temperature
- d) By producing large amounts of biomass

6.11 FURTHER SUGGESTED READINGS

- B R Glick, C L Patten, G Holguin and D M Penrose, Biochemical and genetic mechanisms used by plant growth promoting bacteria, Imperial College Press, London. 1999.
- A R Podile and K Kishore, Plant growth-promoting rhizobacteria. In: Plant associated bacteria. Springer Netherlands, pp. 195–230, 2007.

INTRODUCTION

This block deals with Environmental Microbiology and Biotechnology in three units viz. Unit 7, 8 and 9. The three units are as follows:

Unit 7: Structure of Nucleic Acid and Proteins

This unit covers the fundamentals of nucleotides, nucleosides, nucleic acids, including DNA and RNA, and the structure of proteins. This unit further explores their physical and chemical properties, providing a comprehensive understanding of these essential biomolecules and their roles in molecular biology and biotechnology.

Unit 8: Recombinant DNA Technology

This unit explores the origins and contemporary applications of recombinant DNA technology, covering gene cloning steps, classification of restriction enzymes, polymerases, and its role in environmental management. Delve into the fascinating world of genetic manipulation and its profound implications in biotechnology and environmental science.

Unit 9: Gene Expression

The final unit focuses into the fundamental concepts of genetics, beginning with an understanding of genes and Mendel's Laws of Inheritance. The unit explore Mendelian genetics, studying the principles of inheritance and the structure of sex chromosomes. Additionally, the unit discusses sex-linked and polygene inheritance, shedding light on the complexities of genetic transmission and variation.

UNIT-7 : STRUCTURE OF NUCLEIC ACID AND PROTEINS

Structure

- 7.1 Introduction
- 7.2 Nucleotides
- 7.3 Nucleoside
- 7.4 Nucleic acid
- 7.5 Deoxyribonucleic acid (DNA)
- 7.6 Ribonucleic acid (RNA)
- 7.7 Physical and Chemical Properties of Nucleic Acid
- 7.8 Structure of Proteins
- 7.9 Summary
- 7.10 Terminal Questions
- 7.11 Suggested Readings

7.1 INTRODUCTION

Cells contain different biomolecules including proteins, lipids, carbohydrates, and nucleic acids. Among these, nucleic acids stand out for their considerable size and weight, ranging from 30,000 to several million in molecular weight. The discovery of nucleic acids traces back to Friedrich Miescher, a Swiss physician and biologist, in 1869, when he first extracted them from pus cells and named them "nuclein." Subsequently, Oscar Hertwig, a German zoologist, and professor, proposed nuclein as the carriers of hereditary traits, eventually leading to the adoption of the term "nucleic acids" to describe them, departing from their original designation as "nucleonic acid" due to their acidic properties. Nucleic acids are ubiquitous across all domains of life, and present in plants, animals, bacteria, and viruses. These large molecules, composed of nucleotide monomers, act as repositories of genetic information and directives for cellular operations.

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) constitute the two primary forms of nucleic acids. DNA functions as the genetic blueprint found universally in living organisms, typically housed within the nucleus of eukaryotic cells. In addition to the nucleus, eukaryotic DNA also reside in extrachromosomal structures within chloroplasts and mitochondria. Unlike eukaryotes, prokaryotic cells lack a nucleus, containing their DNA within the cytoplasm. The collective genetic material of an organism encompassing DNA sequences is referred to as its genome. Inside the nucleus of eukaryotic cells, DNA associates with different proteins in a thread-like structure called as "chromosome". A single chromosome can contain tens of thousands of genes, some of them code for proteins (Fig. 1). DNA regulates cellular activities by controlling gene expression, while RNA primarily facilitates protein synthesis. Messenger RNA (mRNA) serves as the intermediary between DNA and the protein, whereas additional types of RNA such as ribosomal RNA (rRNA), transfer RNA (tRNA), and microRNA, are involved in modulating protein synthesis and its associated processes. Table 7.1 gives information about

the distinguishing features between DNA and RNA.

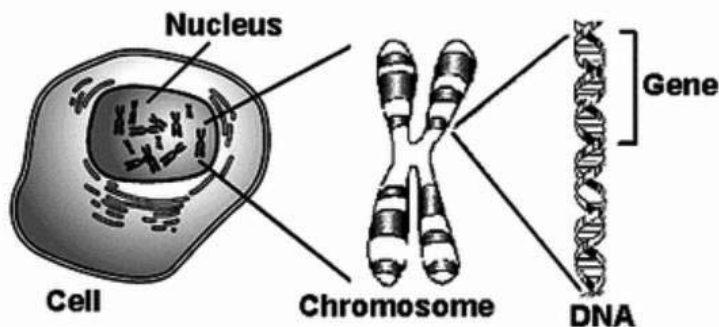


Fig. 7.1 Eukaryotic cell representing nucleus, chromosome, and DNA

Source: <https://www.microscopemaster.com/chromosomes.html>

Table 7.1 Difference between DNA and RNA

S. No.	DNA	RNA
1	DNA is a genetic material found in all living organisms (except RNA virus).	RNA is genetic material found in some viruses called retrovirus.
2	DNA is a double-stranded molecule consisting of a long chain of nucleotides.	RNA is single-stranded (mostly) except for some viruses like retrovirus which has double-stranded RNA. Relatively short chain (of nucleotides).
3	DNA contains deoxyribose sugar in which the hydroxyl group is absent at 2'-C in pentose sugar.	RNA contains oxy-ribose sugar consisting of hydroxyl group.
4	Types of DNA: Nuclear DNA, Extrachromosomal DNA (Mitochondrial and Chloroplasic)	Types of RNA found- mRNA, tRNA, and rRNA(mainly). Other types are hnRNA, snRNA, snoRNA, miRNA, siRNA
5	Nitrogenous bases found in DNA are adenine, cytosine, guanine, and thymine.	Nitrogenous bases found in RNA are adenine, cytosine, guanine, and uracil.
6	In DNA, the proportion of purines is equal to pyrimidine (Chargaff's rule).	In RNA, purine is not equal to pyrimidine.
7	In DNA, complementary pairing of Bases occurs throughout the length of the molecule.	In RNA, pairing between bases occurs only in specific regions. Most of the RNA is linear and single-stranded.
8	DNA contains a few numbers of uncommon bases.	RNA contains more uncommon bases.
9	Genetic messages are usually encoded in DNA.	The usual function of RNA is translating messages encoded in DNA into protein.
10	DNA consists of a large number of nucleotides.	RNA consists of a lesser number of Nucleotides as compared to DNA.

Objectives

- To discuss the concept of nucleotides
- To understand nucleoside and Nucleic acid
- To discuss Deoxyribonucleic acid (DNA)
- To understand ribonucleic acid (RNA)
- To discuss physical and chemical properties of nucleic acid
- To study structure of proteins

7.2 NUCLEOTIDES

Nucleotides serve as the basic building blocks of nucleic acids which get linked to one another to form a polynucleotide chain. They are composed of three essential components—nitrogen-containing bases (either purines or pyrimidines), pentose sugar (a five-carbon sugar), and phosphate groups (Fig. 7.2a). In a nucleotide, the nitrogenous base is joined covalently (at N-1 of pyrimidines and N-9 of purines) to the 1' carbon of the pentose through N- β -glycosyl bond formed by removal of the elements of water (a hydroxyl group from the pentose and hydrogen from the base). While the phosphate (PO_4^{3-}) group esterifies to the 5' carbon of the sugar molecule, see Fig. 7.2b.

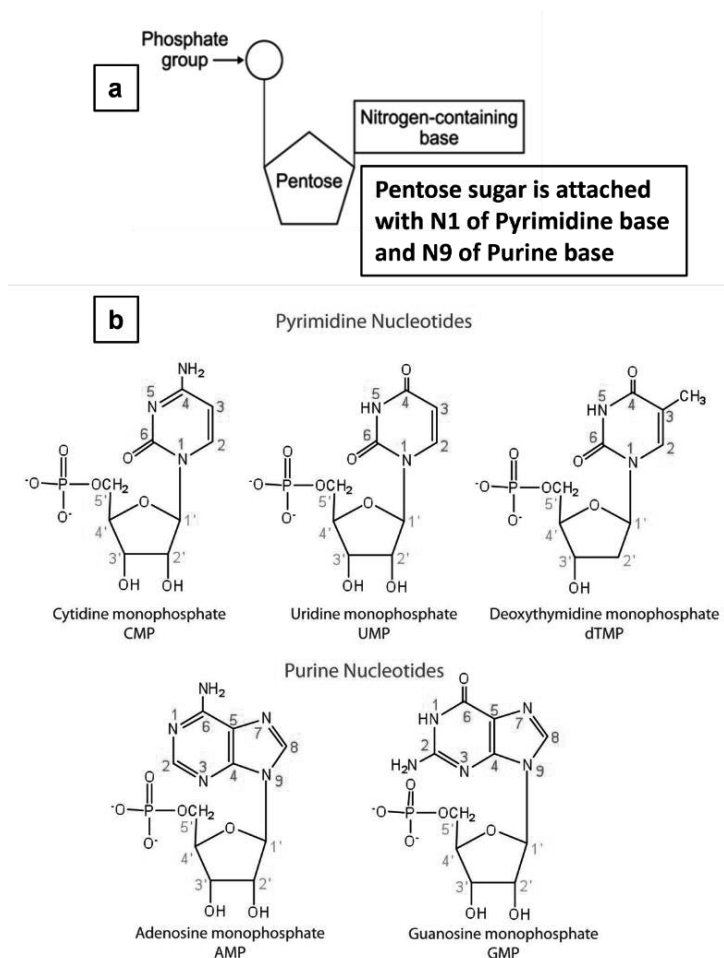


Fig. 7.2 Basic structure of a nucleotide

Source: https://saylordotorg.github.io/text_the-basics-of-general-organic-and-biological-chemistry/s22-01-nucleotides.html

- (I) **Nitrogenous base-** Nitrogenous bases present in a nucleotide are grouped into two classes called pyrimidine and purines, which play a crucial role in nucleotide structure. These nitrogenous bases are organic molecules that contain nitrogen and are bases because they contain an amino group that has the potential of binding an extra hydrogen.

Pyrimidine bases- Pyrimidines are simple aromatic compounds composed of carbon and nitrogen atoms in a six-membered, heterocyclic ring (single-ring) system. It has a chemical composition of $C_4H_4N_2$ and can be regarded as the parental structure for various types of pyrimidine bases. Refer to Fig7.3 for the chemical structure of different pyrimidine bases. The most abundant naturally occurring pyrimidine base are uracil (U) (2, 4-dihydroxypyrimidine), cytosine (C) (2-hydroxy-4-aminopyrimidine), and thymine (T) (2, 4- dihydroxy-5-methyl pyrimidine). The first two pyrimidines are primarily found in RNA, while the latter two are mainly present in DNA. In DNA, the two pyrimidines typically pair with purine residues on the complementary strand, resulting in a purine-to-pyrimidine ratio of one. However, in single-stranded RNA, this ratio can vary significantly.

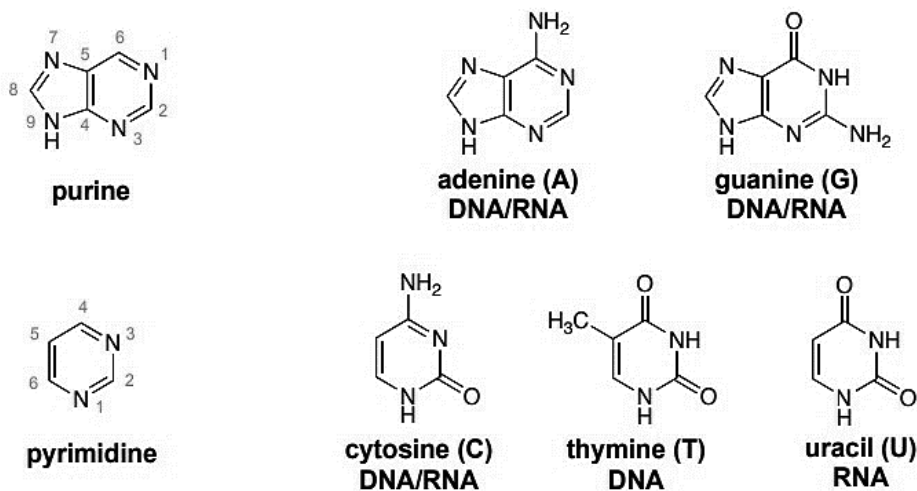


Fig. 7.3 Structure of Purine and Pyrimidine Base

Source: https://saylordotorg.github.io/text_the-basics-of-general-organic-and-biological-chemistry/s22-01-nucleotides.htmls

Purine bases- Purines (Two-ring structure) are heterocyclic aromatic organic compounds that are classified as guanine (G) (2-amino, 6-oxo purine) and adenine (A) (6-amino purine). Purines are composed of an imidazole ring fused to a pyrimidine ring. Purine is water soluble and is abundant in meat and meat products, particularly in internal organs like the kidney and liver. The two types of purines that are present in both DNA and RNA are adenine and guanine. The purine ring's carbon atoms are numbered counter-clockwise, while the imidazole ring's carbon atoms are counted clockwise. Both rings have common elements C-4 and C-5. Refer to Fig. 7.3 for the chemical structure of different pyrimidine bases.

- (II) **Pentose sugar-** A pentose sugar molecule in a nucleotide can be either ribose or deoxyribose. Each nucleotide's carbon atoms of pentose sugar molecules are numbered 1', 2', 3', 4', and 5' (1' is pronounced as "one prime"; this is done to differentiate the carbons on the bases, which have their carbons simply labelled as 1, 2, 3, etc.), from the carbons on the sugars. Fig. 7.4 displays the structure of pentose sugar present in a nucleotide. The pentose sugar in RNA is called ribose, and it has a hydroxyl group bonded to its 2' carbon. Deoxyribose, the pentose sugar found in DNA, has a hydrogen atom bonded to its 2' carbon. The absence of an oxygen atom at the 2' carbon is indicated by the term "deoxy" in the name of DNA.

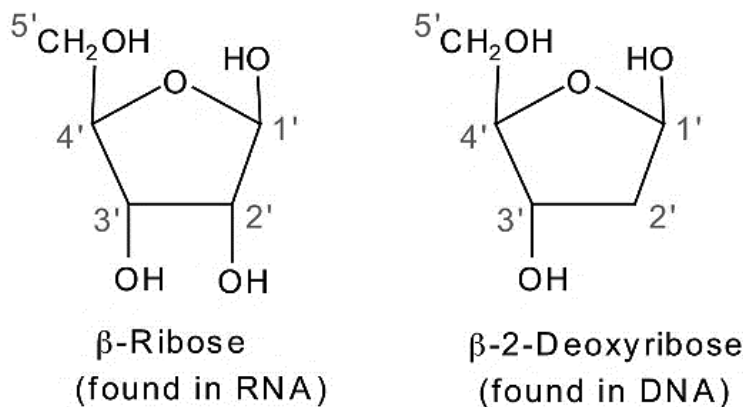


Fig. 7.4 Structure of pentose sugar present in a nucleic acid

Source: https://saylordotorg.github.io/text_the-basics-of-general-organic-and-biological-chemistry/s22-01-nucleotides.html

(III) Phosphate groups- Phosphate groups and sugar molecules together form the backbone of a polynucleotide chain. The connection between the sugar molecules of two different nucleotides, facilitated by a phosphate group, is known as a phosphodiester bond. In one strand of the DNA helix, these phosphodiester bonds are oriented in the 3' to 5' direction, while in the complementary strand, they are oriented in the 5' to 3' direction.

7.3 NUCLEOSIDE

A nucleoside is a part of a nucleotide that is made up of just a five-carbon sugar and a nitrogenous base. The name of nucleosides is based on the connection between the base (purine or pyrimidine) and the sugar (ribose or deoxyribose) at the 1' position through a beta-glycosidic bond. Cytidine, uridine, adenosine, guanosine, thymidine, and inosine are examples of nucleosides.

- uracil-containing = uridine (attached to ribose) / deoxyuridine (attached to deoxyribose)
- thymine-containing = ribothymidine (attached to ribose) / thymidine (attached to deoxyribose)
- cytosine-containing = cytidine (attached to ribose) / deoxycytidine (attached to deoxyribose)
- guanine-containing = guanosine (attached to ribose) / deoxyguanosine (attached to deoxyribose)
- adenine-containing = adenosine (attached to ribose) / deoxyadenosine (attached to deoxyribose)

A nucleotide is created when a nucleoside is combined with one or more phosphates. For this reason, nucleotides are frequently referred to as nucleoside phosphates. The relevant prefixes (mono, di, or tri) indicate how many phosphates are present in the nucleotide. Thus, cytidine, for example, refers to a nucleoside (no phosphate), but cytidine monophosphate refers to a nucleotide (with one phosphate). The addition of second and third phosphates to a nucleoside monophosphate requires energy, due to the repulsion of negatively charged phosphates and this chemical energy is the basis of the high-energy triphosphate nucleotides (such as ATP) that fuel cells.

7.4 NUCLEIC ACID

Nucleic acids are long, linear chains of nucleotides in which two adjacent nucleotides are chemically linked by phosphodiester linkages. These phosphodiester linkages are a type of covalent

bonds formed between the 3' carbon of one nucleotide and the 5' phosphate group of another by dehydration synthesis (Fig.7.5). A polynucleotide may have thousands of such phosphodiester linkages. The first nucleotide in the chain has a free phosphate group at the 5' end of the molecule, giving it a negative charge. This is how nucleic acids exhibit directionality. At the 3' end of the molecule, the final nucleotide added possesses a free 3' hydroxy group. The 3' end is always where nucleotides are introduced. Nucleic acid sequences are typically written from the 5' end to the 3' end, from left to right.

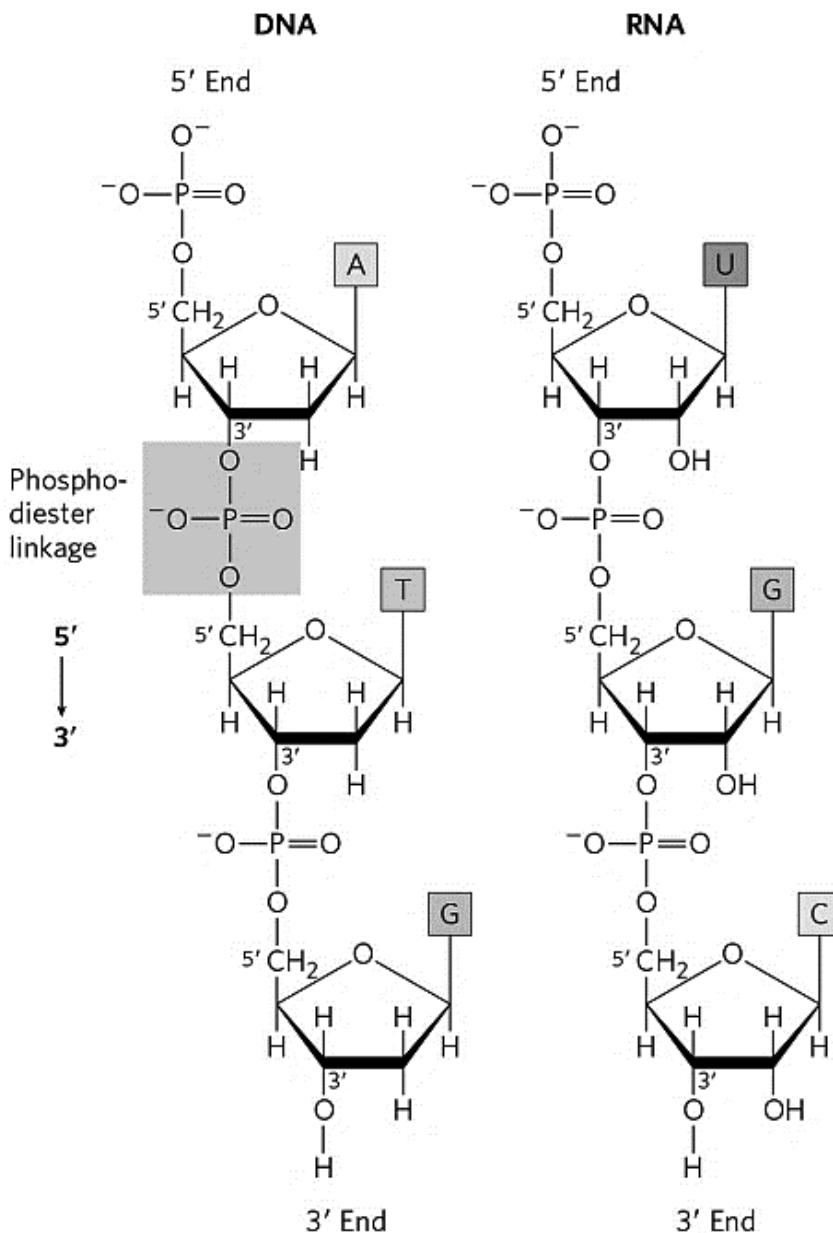


Fig. 7.5 Phosphodiester linkages in the covalent backbone of DNA and RNA which links Successive nucleotide units

Source: Lehninger, Principles of Biochemistry, 7th Edition

Nucleic acids can be broken down to create sugars, phosphoric acid, and a combination of organic bases (purines and pyrimidines). They are the main information-carrying molecules in the cell and, through controlling the production of new proteins, they define the genetic makeup of all living things. Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are the two primary forms of nucleic acids. DNA is the genetic substance present in all living things, including multicellular mammals and

single-celled bacteria. The sole known uses for DNA are in the storing and transferring biological information. The amino acid sequence of each protein and the nucleotide sequence of each RNA in a cell are determined by the nucleotide sequence found in the DNA of the cell. On the other hand, RNAs have a broader range of functions involved in protein synthesis and are classified as ribosomal RNAs (rRNAs), messenger RNAs (mRNAs), and transfer RNAs (tRNAs).

7.5 DEOXYRIBONUCLEIC ACID (DNA)

The discovery of the structure of DNA by Watson and Crick in 1953 led to the development of completely new fields of study as well as changes in many well-established ones. Deoxyribonucleic acid (DNA) is a molecule made up of two nucleotide-based chains that wind around one another to form a double helix. DNA carries the genetic information necessary for the growth and development of all living organisms and some viruses. A significant portion of DNA (over 98% in humans) is non-coding, meaning it does not encode protein sequences. The two DNA strands run in opposite directions, making them antiparallel. RNA strands are synthesized from DNA templates in a process known as transcription. According to the genetic code, these RNA strands are then translated to determine the sequence of amino acids in proteins, a process called translation.

DNA is arranged into structures called chromosomes in eukaryotic cells. These chromosomes are replicated during DNA replication before normal cell division, giving each daughter cell a full complement of chromosomes. The majority of the DNA in eukaryotic organisms is kept in the cell nucleus called chromosomal DNA, whereas a small portion is kept in organelles like mitochondria and chloroplasts termed extra-chromosomal DNA. Prokaryotes, which include bacteria and archaea, on the other hand, only store their DNA in the cytoplasm. Table 7.2 provides differential features between prokaryotic and eukaryotic DNA.

Table 7.2 Difference between prokaryotic and eukaryotic DNA

S.No.	Prokaryotic DNA	Eukaryotic DNA
1	In prokaryotes, genomic DNA is found freely in the cytoplasm and typically exists as covalently closed circular DNA.	In eukaryotes, genomic DNA is housed within a distinct nucleus and exists as linear DNA with two distinct ends.
2	The amount of DNA is relatively small.	The quantity of DNA is greater compared to that in prokaryotes.
3	It consists of a single chromosome.	Eukaryotic genomic DNA comprises multiple pairs of chromosomes, with the exact number varying from species to species.
4	Plasmids constitute the form of extra-chromosomal DNA.	Cellular organelles like mitochondria and chloroplasts contain circular extra-chromosomal DNA.
5	Only a small number of proteins interact with prokaryotic DNA, typically encoding for 300 to 500 proteins.	Eukaryotic DNA interacts with a wide array of proteins, coding for thousands of proteins. For instance, the human genome encodes approximately 40,000 proteins.

6	Most of the DNA in prokaryotes is coding, with only a small fraction consisting of non-coding regions.	The bulk of DNA comprises non-coding sequences, with the coding region being smaller in comparison to the non-coding regions.
7	Introns are not present within the coding regions of DNA. Prokaryotic DNA exclusively consists of exons, lacking introns altogether.	Introns are located within the coding region of genes, forming split genes. Eukaryotic DNA consists of both introns and exons.

(I) Structure of DNA-In 1868, Friedrich Miescher initially isolated and identified DNA, naming it "nuclein" due to its phosphorus content. However, it wasn't until the 1940s, thanks to the groundbreaking research of Oswald T. Avery, Colin MacLeod, and Maclyn McCarty, that compelling evidence emerged confirming DNA as the genetic material. Through their experiments, Avery and his team observed that an extract from a virulent strain of *Streptococcus pneumoniae*, which caused disease in mice, could transform a nonvirulent strain into a virulent one. By subjecting the extract to various chemical analyses, they conclusively demonstrated that it was the DNA from the virulent strain—not proteins, polysaccharides, or RNA—that carried the genetic instructions for virulence. Erwin Chargaff and his colleagues in the 1950s while studying DNA from different animals proposed a set of conclusions, known as Chargaff's Rule, explained as follows:

- The base composition of DNA generally varies from one species to another.
- DNA specimens isolated from different tissues of the same species have the same base composition. The base composition of DNA in a given species does not change with an organism's age, nutritional state, or changing environment.
- In all cellular DNAs, regardless of the species, the number of adenosine residues is equal to the number of thymidine residues (that is, $A = T$), and the number of guanosine residues is equal to the number of cytidine residues ($G = C$).
- The sum of the purine residues equals the sum of the pyrimidine residues; that is, $A + G = T + C$. Also, $[A+G] / [T+C] = 1$
- The $[A+T] / [G+C]$ ratio varies but remains constant within a species, serving as a marker for DNA source identification. This ratio tends to be lower in primitive organisms and higher in more advanced ones.

(II) Double Helix Structure of DNA: Watson and Crick Model- James Watson and Francis Crick in 1953 postulated a three-dimensional model of DNA called as Double Helix structure or Watson and Crick Model, that involved collective information of all the available data as well as in-depth examination of the physical and chemical properties of DNA. They proposed a three-dimensional helix model of physiological DNA (i.e. B-DNA). The model states that DNA is made up of two polynucleotide chains that wind around one another to form a right-handed double helix.

The hydrophilic backbones consisting of alternating deoxyribose and phosphate groups face the surrounding water. The purine and pyrimidine bases of both strands are stacked inside the double helix, perpendicular to the long axis due to their hydrophobic and nearly planar structure. The furanose ring of each deoxyribose sugar is in the C-2' endo conformation. The offset pairing of the two strands creates a major groove and minor groove on the surface of the duplex (Fig. 7.6). Nucleotide base in one strand is paired in the same plane with a base of the other strand via hydrogen bonding. G pairs with C by forming three hydrogen bonds ($G \equiv C$); while A pairs with T forming two hydrogen bonds $A = T$ (Fig.

7.7). Pairings of bases other than G with C and A with T tend (to varying degrees) to destabilize the double-helical structure.

The two polynucleotide chains within the double-helical DNA are antiparallel, meaning they run in opposite directions, and they are not identical in base sequence or composition. Instead, they exhibit complementary pairing to each other. Adenine in one chain always pairs with thymine in the other, and likewise, guanine in one chain always pairs with cytosine in the other.

The DNA double helix, also known as a duplex, is upheld by hydrogen bonding between complementary base pairs and by base-stacking interactions. Although hydrogen bonding between base pairs ensures complementarity between DNA strands, it does not significantly contribute to the structure's stability. Instead, the double helix's stability primarily arises from metal cations, which shield the negative charges of phosphate groups in the backbone, and from base-stacking interactions between complementary base pairs. Notably, interactions between adjacent G≡C pairs are stronger than those between adjacent A=T pairs. As a result, DNA duplexes with a higher G≡C content exhibit greater stability.

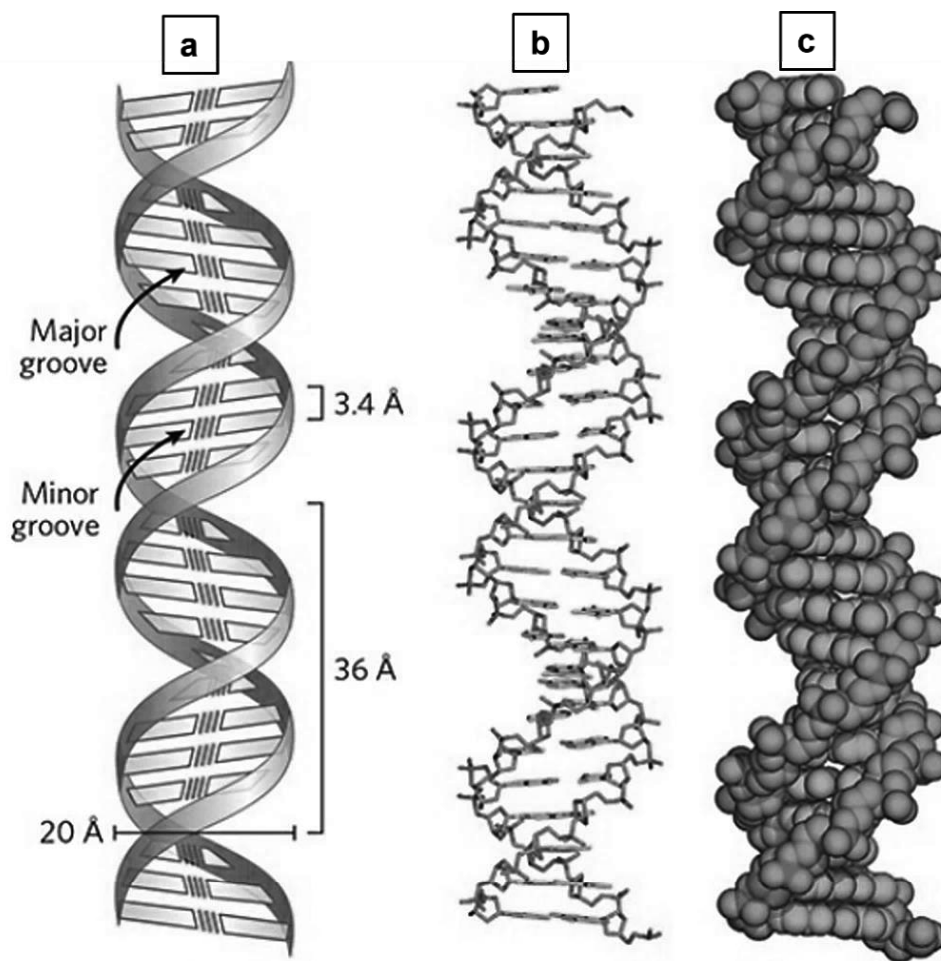


Fig 7.6 Watson-Crick model for the structure of DNA. (a) Schematic representation, showing dimensions of the helix. (b) Stick representation showing the backbone and stacking of the bases. (c) Space-filling model; Source: Lehninger, Principles of Biochemistry, 7th Edition

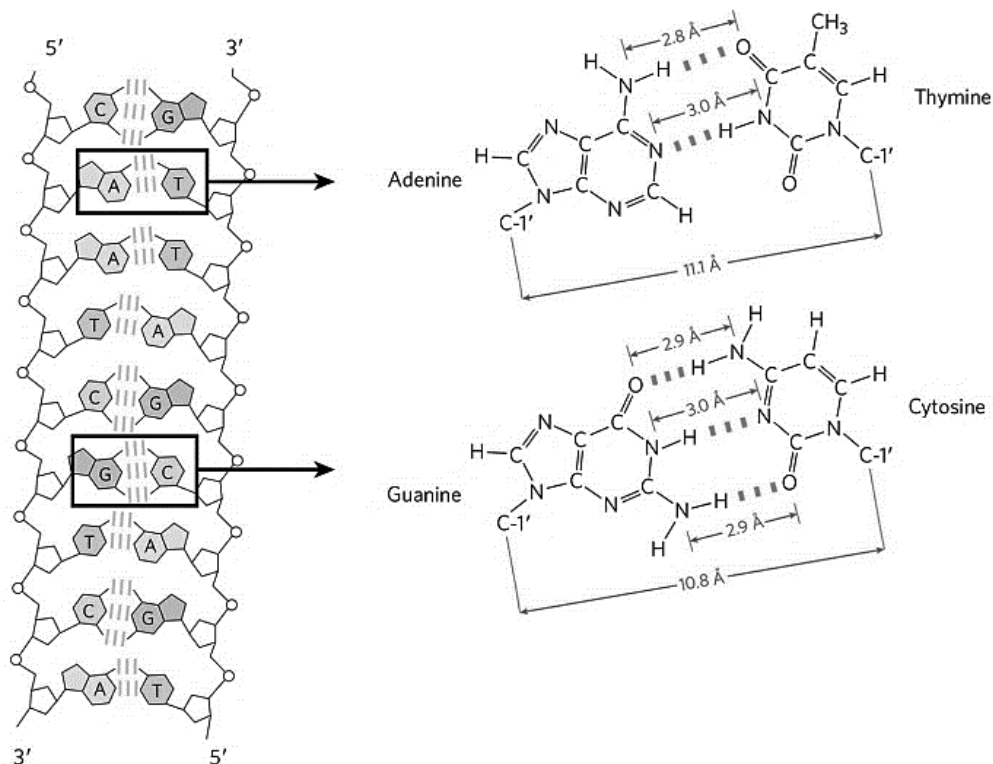


Fig. 7.7 Hydrogen-bonding patterns in the base pairs defined by Watson and Crick

Source: Lehninger, Principles of Biochemistry, 7th Edition

(III) Structural forms of DNA- DNA exhibits remarkable flexibility due to the ability for significant rotation around various bonds within its sugar-phosphate backbone. Thermal fluctuation can produce bending, stretching, and unpairing (melting) of the strands. This leads to structural variations in the DNA defined by Watson and Crick, which are influenced by several factors. Firstly, different conformations of deoxyribose contribute to structural diversity. Additionally, rotation around the bonds forming the phosphor deoxyribose backbone and free rotation around the C-1'–N-glycosyl bond further influence DNA structure. Purines within purine nucleotides are limited to two stable conformations, syn- and anti-, due to steric constraints. Pyrimidines, on the other hand, primarily adopt the anti-conformation due to steric hindrance between the sugar and the carbonyl oxygen at C-2 of the pyrimidine.

Three distinct morphological forms of the DNA double helix exist, known as A, B, and Z DNA. Among these, B-DNA and Z-DNA are observed in cellular DNA. A-DNA is typically found under rigidly controlled experimental conditions. The Watson-Crick structure is also referred to as B-form DNA or BDNA. The B form is the most stable structure for a random-sequence DNA molecule under physiological conditions and is therefore the standard point of reference in any study of the properties of DNA. Important structural features of A, B, and Z-DNA are given in Table 7.3 and represented in Fig. 7.8.

(i) A-DNA- The A form of DNA is commonly favored in solutions with lower water content i.e. dehydrating conditions. Despite still exhibiting a right-handed double helix arrangement, the A-DNA form differs from B-DNA in several ways. In this form, DNA is still twisted into a right-handed double helix, but it is wider and has 11 base pairs per helical turn instead of 10.5 like in B-DNA. The base pairs in A-DNA are not quite perpendicular to the helix axis because their plane is slanted by around 20° in comparison to the base pairs in B-DNA. Due to these structural modifications, the minor groove becomes shallower and the major groove becomes deeper. The

reagents employed to facilitate the crystallization of DNA typically induce dehydration, leading to the propensity for most short DNA molecules to crystallize in the A form.

- (ii) **Z-DNA-** Z-form DNA differs from B-form DNA primarily in its left-handed helical rotation. In Z-form DNA, each helical turn typically encompasses 12 base pairs, contributing to its slender and elongated appearance. The DNA backbone adopts a zigzag pattern with deep and narrow minor grooves and rarely visible major grooves. The purine residues flip to the syn-conformation and alternate with pyrimidines in the anti-conformation to generate the left-handed helix in Z-DNA.

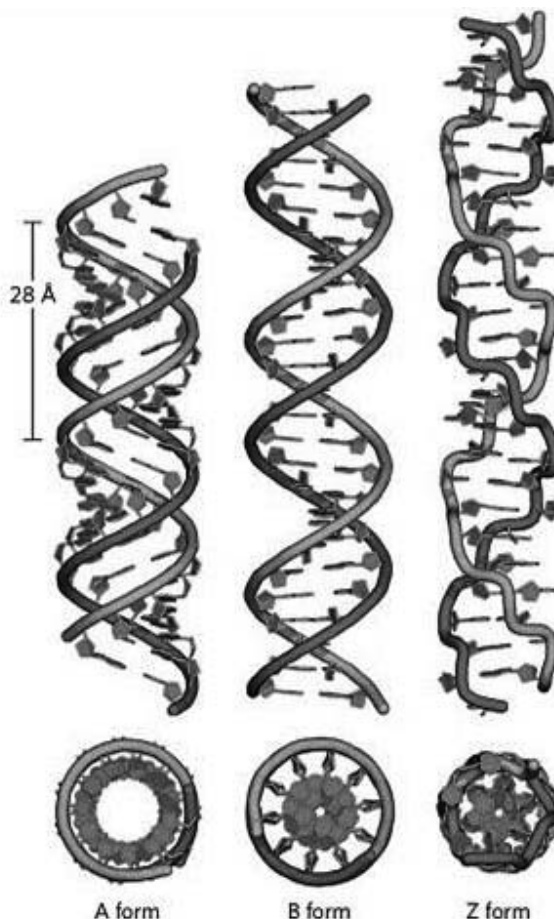


Fig. 7.8 Different forms of A, and Z- DNA

Source: Lehninger, Principles of Biochemistry, 7th Edition

Table 7.3 Difference between A, B, and Z-forms of DNA

Attribute	DNA form		
	A	B	Z
Helix sense/turn	Right-handed	Right-handed	Left-handed
Height of helical turns (helical pitch)	28.6 Å	34 Å	44 Å

Diameter	26 Å	20 Å	18 Å
Height of helical turns (helical pitch)	28.6 Å	34 Å	44 Å
Number of base pairs per helical turn	11.6	10	12
Helical twist per base pair	31°	36°	9° or 51°
Distance between each base pair (helical rise/base pair)	2.9 Å	3.4 Å	7.4 Å
Base tilt to the normal helical axis	20°	6°	7°
Major groove	Narrow and deep	Wide and deep	Flat
Minor groove	Wide and shallow	Narrow and deep	Narrow and deep
Ribose sugar conformation	C-3' endo	C-2' endo	C-2' endo for pyrimidine C-3' endo for purine
Glycosidic bond conformation	Anti-	Anti-	Anti-for pyrimidine and syn- for purine

- (iii) **Unusual DNA Structures-** DNA structures within the complex network of the genome go well beyond the traditional double helix, displaying a tapestry of sequence-dependent variants that impact genetic function and metabolism. For instance, the DNA helix bends when four or more adenosine residues appear consecutively in a single strand. A bend of roughly 18° is produced by six adenosines in succession. Certain proteins' ability to attach to DNA may be influenced by the bending seen with this and other sequences. Further palindrome and mirror sequences seen in the DNA sequences lead to the generation of unusual DNA structures. Structures such as hairpin or cruciform structures and tetraplex DNA or quadruple helices are examples of some of the unusual DNA structures.

7.6 RIBONUCLEIC ACID (RNA)

RNA serves as the second major form of nucleic acid in cells, pivotal in protein synthesis (translation) and its regulation. Serving as a mediator in gene expression, RNA transports the genetic information encoded in DNA to determine the amino acid sequence necessary for producing functional proteins. RNA is present in both the nucleus and cytoplasm of cells. Cellular RNA primarily comprises three types:

- Messenger RNA (mRNA), also referred to as template RNA, serves as a carrier of genetic information from DNA to ribosomes, where protein synthesis occurs.
- Ribosomal RNA (rRNA) forms an integral part of ribosomes, which function as the cellular machinery responsible for protein synthesis.

- Transfer RNA (tRNA), also called soluble RNA (sRNA), transports specific amino acids to the ribosome during protein synthesis.

(I) Structure of RNA- RNA is a polymer of nucleotides (nitrogenous base + pentose sugar + phosphate group) specifically containing a ribose sugar. Thus, it can specifically be regarded as a polymer of ribonucleotides. It is usually single-stranded and consists of ribonucleotides connected by 3'-5' phosphodiester bonds. However, certain viruses possess double-stranded RNA. Each ribonucleotide in the RNA chain comprises a ribose sugar, one of the four nitrogenous bases (adenine, guanine, cytosine, and uracil), and a phosphate group. Fig. 7.9 a shows the monomeric ribonucleotide unit of the RNA.

The RNA molecule typically forms a right-handed helix, primarily driven by base-stacking interactions (**Fig. 7.9b**). Despite being single-stranded, many types of RNA molecules exhibit significant intramolecular base pairing between complementary sequences within the RNA strand. This intricate base pairing gives rise to a predictable secondary three-dimensional structure that is crucial for the RNA's function.

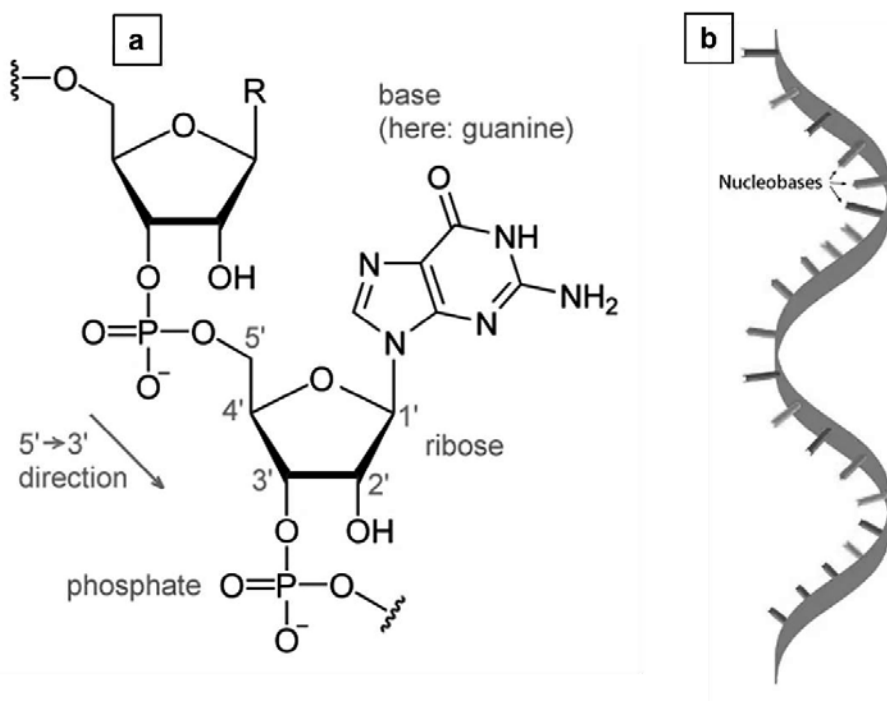


Fig 7.9. Structure of RNA. (a) Monomeric ribonucleotide unit of RNA (b) Typical right-handed stacking pattern of single-stranded RNA

Source: <https://en.wikipedia.org/wiki/RNA>

Different secondary structures assumed by RNA include A-form, Z-form, hairpin loops, hairpin double helix, and bulge (**Fig. 7.10 a & b**). When two RNA strands possess perfectly complementary sequences and pair together, they typically form a predominant double-stranded structure known as an A-form right-handed double helix. However, it's uncommon for RNA strands to be perfectly paired over long regions of sequence. Weak interactions, particularly base-stacking interactions, play a crucial role in stabilizing RNA structures, similar to their function in DNA.

Z-form helices have been artificially created in laboratory settings, typically under conditions of very high salt concentrations or elevated temperatures. In the regular A-form helix, breaks can occur due to mismatched or unmatched bases in one or both strands, leading to the formation of bulges or internal

loops. Hairpin loops emerge between adjacent self-complementary (palindromic) sequences. Many RNAs contain extensive base-paired helical segments with resulting hairpins representing the most prevalent type of secondary structure in RNA. Specific short base sequences, such as UUCG, are frequently located at the ends of RNA hairpins and are renowned for forming exceptionally tight and stable loops. These sequences serve as crucial starting points for the intricate folding of an RNA molecule into its precise three-dimensional structure, including the formation of hairpin double helices.

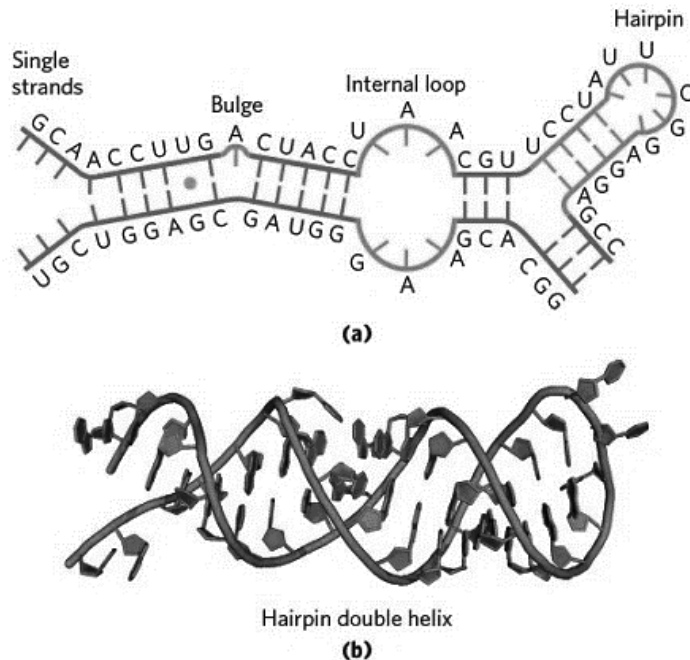


Fig. 7.10 Secondary structure of RNAs. (a) Bulge, internal loop, and hairpin loop. (b) The paired regions generally have an A-form right-handed helix, as shown for a hairpin

Source: Lehninger, Principles of Biochemistry, 7th Edition

- (II) Types of RNA-** Three distinct types of cellular RNA have been identified: Messenger RNA (mRNA), Ribosomal RNA (rRNA), and Transfer RNA (tRNA). Ribosomal and transfer RNA collectively constitute approximately 98% of all RNA within the cell. Each form of RNA is transcribed from a DNA template.

Messenger RNA and transfer RNA are synthesized based on DNA templates located within the chromosomes. In contrast, ribosomal RNA originates from DNA within the nucleolus. These three types of RNA are synthesized at different stages during early development. The majority of RNA synthesized during cleavage is mRNA. Synthesis of tRNA primarily occurs towards the end of cleavage, while rRNA synthesis initiates during gastrulation.

- (i) Ribosomal RNA (rRNA)-** Ribosomal RNA, as the name suggests, is found in the ribosomes. Ribosomes serve as the primary sites for protein synthesis within the cell. Ribosomal structure is similar in prokaryotic and eukaryotic cells which is organized into a large subunit and a small subunit along with some nucleoproteins as shown in Fig 7.11. The complete ribosome is referred to as 70S in prokaryotes and 80S in eukaryotes.

In eukaryotic animals, the large subunit of ribosome (60S) contains three different types of ribosomal RNA (rRNA): 28S rRNA (4700 bases long), 5S rRNA (12 bases long), and 5.8S rRNA (160 bases long). Conversely, the 40S subunit consists of only one type of rRNA, known as 18S rRNA, which is approximately 1900 bases long. Interestingly, the gene encoding 5S rRNA is separate from

those of other rRNAs. It is transcribed independently to produce the RNA molecule. However, all other rRNAs are synthesized as a single transcript, which is subsequently cleaved to generate individual RNA molecules.

In prokaryotic ribosomes, 30S subunit contains 16S rRNA, while the 50S subunit contains 23S and 5S rRNA. The "S" in the designation of different types of rRNA stands for the sedimentation coefficient, which is a measure of the rate at which a molecule sediment in a centrifugal field.

The precise functions of rRNAs within ribosomes are not fully understood. However, they are believed to play significant roles in the binding of messenger RNA (mRNA) to ribosomes and in facilitating the process of protein synthesis. In addition to their role in protein synthesis, ribosomal RNA (rRNA) can also exhibit enzymatic activity, leading to the classification of such RNA molecules as ribozymes. Ribozymes, primarily known for their involvement in the splicing of introns, act as catalysts in various cellular processes. In eukaryotes, the formation of rRNA originates from a specific region of DNA. These rRNA molecules are typically single-stranded but may fold into complex structures in certain regions.

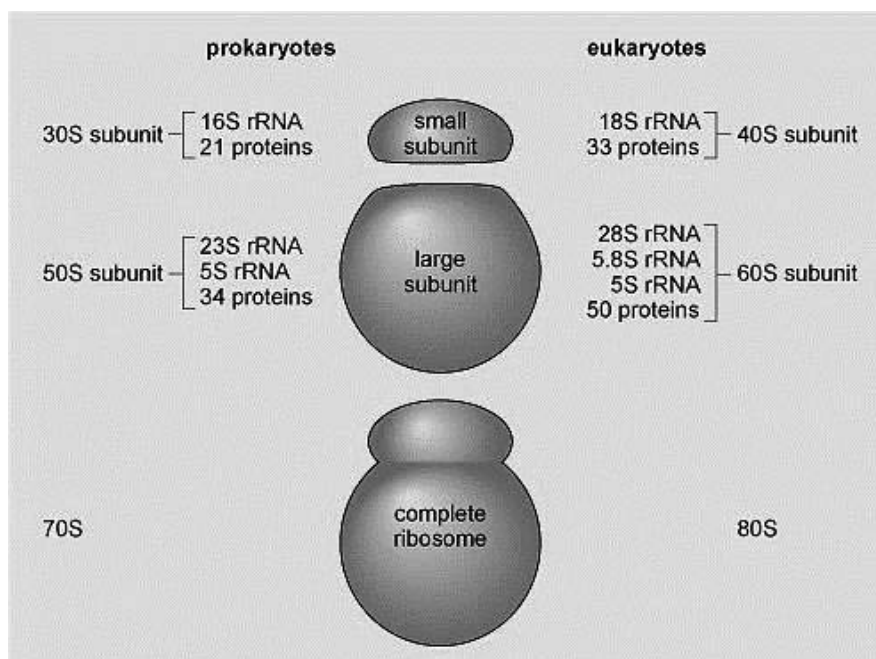


Fig. 7.11 Organization of ribosome in eukaryotic and prokaryotic cells;

Source: <https://www.open.edu/openlearn/science-maths-technology/a-tour-the-cell/content-section-4.4>

- (ii) **Messenger RNA (mRNA)**- The term "messenger RNA" was introduced by French biologist Francois Jacob and biochemist Jacques Monod. Messenger RNA (mRNA) is a linear molecule synthesized in the nucleus via transcription from DNA. Its sequence is complementary to the template DNA strand. Nitrogenous bases on mRNA form codons, each consisting of three bases. mRNA acts as a transporter of genetic information, ferrying it from the nucleus, where chromosomal DNA is located, to the cytoplasm for protein synthesis.

Once transported to the cytoplasm, messenger RNA (mRNA) associates with ribosomes, leading to the formation of polyribosomes or polysomes. Each polysome consists of several ribosomes (typically around five) forming a complex with mRNA.

In general, each gene produces its mRNA molecule, resulting in approximately as many types of

mRNA molecules as there are genes. A distinctive feature of mRNA is its heterogeneous nature, with significant differences observed in size and molecular weight among different mRNA molecules. These variations stem from differences in the size and number of cistrons present within each mRNA molecule.

The lifespan of messenger RNA (mRNA) varies between eukaryotes and prokaryotes. In eukaryotes, mRNA tends to be more stable and has a longer lifespan compared to mRNA in prokaryotes, which typically survives for a very short period, often less than a minute. Despite their short lifespan, prokaryotic mRNA molecules possess a high turnover rate.

Approximately 3%-7% of total cellular RNA comprises mRNA. Immediately after formation within the nucleus, mRNA is transported to the cytoplasm, where it becomes associated with ribosomes.

Messenger RNA can be categorized as monocistronic or polycistronic. Monocistronic mRNA codes for only one protein, containing codons for a single cistron. In contrast, polycistronic mRNA can synthesize more than one type of protein from the same mRNA molecule, as it contains codons for multiple cistrons. Monocistronic mRNA is a characteristic feature of eukaryotes, whereas prokaryotes typically contain polycistronic mRNA. Major differences between monocistronic and polycistronic-mRNA are summarized in Table 7.4 and Table 7.5 showcases differences between prokaryotic and eukaryotic mRNA.

Table 7.4 Comparison between monocistronic and polycistronic-mRNA

S.NO.	Polycistronic m-RNA	Monocistronic m-RNA
1	Contains codons for multiple cistrons.	Contains codons for a single cistron.
2	Encodes multiple proteins.	Encodes a single protein
3	Found in prokaryotes.	Found in eukaryotes.
4	Transcribed from multiple genes.	Transcribed from a single gene.

Structure of mRNA

The structure of messenger RNA (mRNA) can be outlined as follows and depicted in **Fig. 7.12**:

- **5' Cap:** The mRNA molecule begins with a cap region (N7MetG) at the 5' end. The presence of this cap has been associated with the binding of mRNA molecules to ribosomes.
- **Non-Coding Region:** Following the 5' cap, there is a non-coding region in the mRNA sequence. This region, approximately 10-100 nucleotides long, does not encode any protein. This is also called Untranslated region (5'UTR).
- **Initiation Codon (AUG):** After the non-coding region, the mRNA sequence contains an initiation codon, typically AUG. This codon marks the starting point of protein synthesis.
- **Coding Region:** After the initiation codon, there is the coding region in the mRNA strand. This region contains nucleotides that are translated into a protein.

- **Termination Codon (Stop Codon):** At the end of each coding segment, there is a termination codon. These codons (UAA, UAG, and UGA) signal the termination of protein synthesis as they do not code for any amino acids. They are also referred to as stop codons.
- **Another Non-Coding Segment:** Following the coding region, there is another non-coding segment in the mRNA sequence (3'UTR). This segment, about 50-150 nucleotides long, contains a specific sequence known as AAUAAA.
- **Poly(A) Sequence:** The final segment in the mRNA strand is the poly(A) sequence, consisting of a large number of adenine nucleotides (AAA...) and called Poly-A tail. This sequence, approximately 200-250 nucleotides long, is added to the mRNA molecule inside the nucleus before it is transported from the nucleus to the cytoplasm.

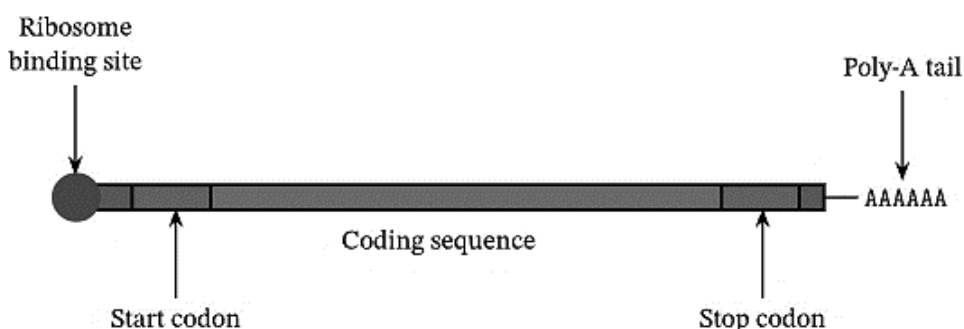


Fig. 7.12 Structure of eukaryotic mRNA

Source: <https://www.nagwa.com/en/explainers/519158434803/>

Table 7.5 Comparison between prokaryotic and eukaryotic mRNA

S.NO.	Prokaryotic mRNA	Eukaryotic mRNA
1	Translation commences while mRNA is still being transcribed from DNA.	Translation begins when the Transcription is completed.
2	Prokaryotic mRNAs have a short lifespan and are continually broken down into ribonucleotides by ribonucleases.	Eukaryotic mRNAs are long-lived. Thus, are metabolically more stable.
3	In prokaryotes mRNAs are polycistronic.	Eukaryotic mRNAs are typically monocistronic.
4	Them RNAs under go very little processing after being transcribed.	The mRNA undergoes processing after being transcribed such as polyadenylation, capping, and methylation.
5	Prokaryotic mRNA lacks a poly(A) tail.	Eukaryotic m-RNA has a poly(A)tail.

- (iii) **Transfer RNA (tRNA) or Soluble RNA (sRNA)** Transfer RNA (tRNA), also known as soluble RNA, is a molecule consisting of approximately 71-80 nucleotides, most commonly around 75, with a molecular weight of about 25,000. There are at least 20 different species of tRNA, each corresponding to one of the 20 amino acids found in protein structures. The structure of tRNA, particularly for alanine, was first elucidated by Holley.

After rRNA, tRNA is the second most abundant RNA in the cell. It is also referred to as soluble RNA due to its small size, making it incapable of precipitation by ultracentrifugation at 100,000 g. Transfer RNA comprises approximately 10-20% of the total RNA content within the cell.

Structure of tRNA- The structure of tRNA, specifically for alanine, was initially characterized by Holley. This tRNA structure is often likened to that of a cloverleaf, with the molecule comprising a single polynucleotide chain folded to form five distinct arms as explained below: Fig. 7.13a and b represents different arms of the tRNA and clover leaf model of tRNA.

- **Acceptor Arm:** This arm is marked by a sequence CCA (5' to 3') at its end, where the amino acid binds to the tRNA molecule.
- **Anticodon Arm:** The anticodon arm carries a triplet of specific nucleotide bases, known as the anticodon, which recognizes and pairs with the complementary codon on the mRNA during translation.
- **D Arm:** Named for the presence of dihydrouridine, this arm plays a structural role in the tRNA molecule.
- **TΨC Arm:** This arm contains a sequence composed of thymine (T), pseudouridine (Ψ), and cytosine (C), contributing to the structural integrity of the tRNA.
- **Variable Arm:** The variable arm exhibits the greatest variability among tRNA molecules. Based on this variability, tRNAs are classified into two categories:
 - **Class I tRNAs:** Predominant forms with variable lengths ranging from 3 to 5 base pairs.
 - **Class II tRNAs:** Containing longer arms, typically with lengths spanning 13 to 20 base pairs.

The structural integrity of transfer RNA (tRNA) is upheld by complementary base pairing within its arms. Below are the respective arms of tRNA along with the number of base pairs (bp) they form:

- Acceptor Arm: 7 base pairs (bp)
- TΨC Arm: 5 base pairs (bp)
- Anticodon Arm: 5 base pairs (bp)
- D Arm: 4 base pairs (bp)

The synthesis of tRNA takes place in the nucleus, utilizing a DNA template strand. However, only a tiny fraction of total DNA, approximately 0.025%, encodes for tRNA. Similarly, rRNA synthesis also occurs from a small portion of DNA. This contrasts with mRNA, which is synthesized from a greater portion of DNA, resulting in an expected base relationship with DNA. The primary role of tRNA is to transport amino acids to mRNA during protein synthesis. Each tRNA molecule is specific to a particular amino acid out of the total 20 amino acids.

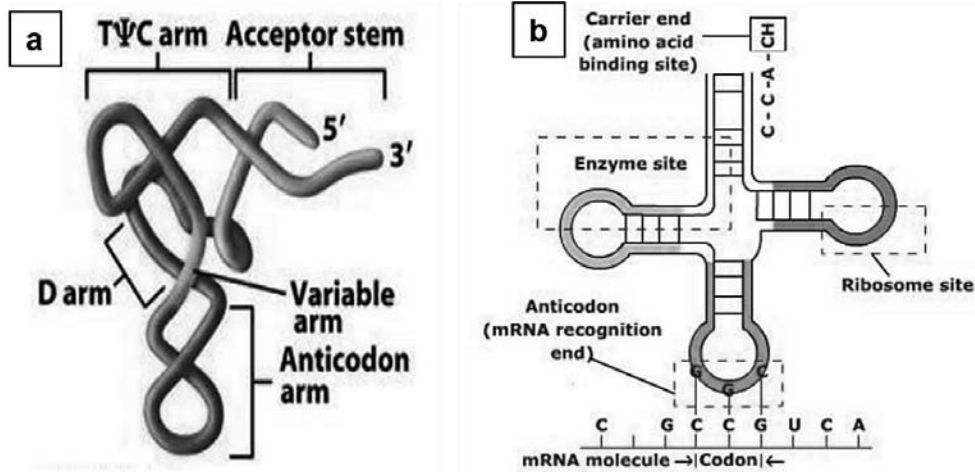


Fig. 7.13 Structure of tRNA. (a) Structure demonstrating different arms of tRNA. (b) Cloverleaf model of tRNA

Source: https://en.wikipedia.org/wiki/Transfer_RNA; <https://www.khanacademy.org/science/biology/genetic-expression-central-dogma/translation-polypeptides/a/tRNA-and-ribosomes>.

Unusual bases in tRNA-Unlike mRNA and rRNA, tRNA contains additional bases beyond the typical adenine (A), guanine (G), cytosine (C), and uracil (U). These extra bases, such as methylguanine and methyl cytosine, are formed through methylation of the standard bases. The inclusion of these unusual bases in transfer RNA (tRNA) serves an important purpose. They help safeguard the tRNA molecule from enzymatic degradation, preserving its structural integrity and functionality. This protection ensures that tRNA remains effective in its vital role of facilitating protein synthesis.

(iv) Other types of RNA- In addition to the classical types of RNA, several new types of RNA have been discovered in recent years, each with unique functions and roles in cellular processes. These other types of RNA have been summarized below:

- **Small nuclear RNA (snRNA)**- Found in multiple copies within the nucleus, snRNA, also known as U-RNA due to its richness in uridylic acid, is crucial for processes like splicing. They often form complexes with proteins, known as small nuclear ribonucleoproteins (snRNPs), and play roles in editing other RNA molecules. SnRNAs such as U1, U2, U5, U4, and U6 are involved in splicing.
- **Short interfering RNA (siRNA)**- These double-stranded RNA molecules, about 20-25 base pairs in length, participate in RNA interference. By degrading mRNA post-transcription, siRNA inhibits the expression of specific genes, thereby preventing protein translation.
- **MicroRNA (miRNA)**- A small non-coding RNA molecule approximately 22 nucleotides long, miRNA is present in plants, animals, and some viruses. It regulates gene expression post-transcriptionally by base-pairing with complementary sequences in mRNA. This interaction can lead to mRNA cleavage, poly(A) tail shortening, or improper translation, ultimately silencing gene expression.
- **Heterogeneous nuclear RNA (hnRNA)**- In contrast to prokaryotic mRNA, eukaryotic mRNAs are monocistronic. Eukaryotic cells produce a primary transcript called hnRNA, which is larger than mature mRNA and contains various RNA types and sizes within the cell nucleus. hnRNA undergoes processing to form mature mRNA, also called as pre-

mRNA. It includes both exon and intron segments, with exons encoding proteins and introns being removed by splicing. hnRNPs stabilize pre-mRNAs and facilitate splicing by binding to specific sequences along the pre-mRNA. The spliced mature mRNAs then serve as templates for protein translation.

7.7 PHYSICAL AND CHEMICAL PROPERTIES OF NUCLEIC ACID

Nucleic acids, including DNA and RNA, exhibit a variety of physical and chemical properties that are fundamental to their biological functions. The following are some properties of DNA:

- (i) **Denaturation of DNA-** The disruption of hydrogen bonds that hold the two strands of the DNA helix together can occur due to changes in pH or an increase in temperature, resulting in the separation of the polynucleotide strands. This phenomenon, involving the loss of the helical structure of DNA, is termed denaturation or melting. Notably, denaturation does not break the phosphodiester bonds in the DNA backbone. The temperature at which half of the helical structure of DNA is lost is known as the melting temperature (T_m). The stability of DNA is influenced by the base composition, with DNAs containing higher G-C content having a greater T_m due to the stronger hydrogen bonding in G-C base pairs compared to A-T base pairs.

Factors contributing to the denaturation of DNA

- **Temperature:** Heating a DNA solution to 90°C or higher can lead to the separation of the double-stranded DNA molecule into single strands. Regions rich in A-T base pairs, with only two hydrogen bonds, denature more easily, referred to as low melting areas. Conversely, regions rich in G-C base pairs, connected by three hydrogen bonds, are more stable and have higher melting temperatures (T_m).
- **Chemical agents:** Certain chemicals, such as urea and formamide, can induce denaturation of DNA by enhancing the solubility of the purine and pyrimidine groups.
- **pH:** Extreme pH values, either highly acidic (pH 2-3) or highly alkaline (pH 12), can trigger the denaturation of DNA.

When DNA undergoes denaturation, transitioning from its native double helix structure to a randomly looped, single-stranded form, several changes occur in its properties:

- **Increase in Absorption of UV-Light:** Denaturation causes a significant increase in the absorption of ultraviolet (UV) light. When measured at 260nm using a spectrophotometer, the absorbance of light increases as DNA becomes denatured. This phenomenon is known as the hyperchromatic effect, hyper chromaticity, or hyper chromism, resulting from the unstacking of base pairs.
- **Decrease in Specific Optical Rotation:** Double-stranded DNA exhibits a strong positive rotation, which decreases significantly upon denaturation.
- **Decrease in Viscosity:** DNA solutions typically have high viscosity due to the relatively rigid double helix structure. Denaturation results in a marked decrease in viscosity.

Hyperchromicity- It refers to the increased absorption of light at 260nm observed in denatured or separated DNA strands compared to intact double-stranded DNA. This effect occurs due to the unstacking of base pairs in denatured DNA, leading to a higher absorption of light energy. Hyperchromicity serves as a useful indicator to differentiate between single-stranded and double-

stranded DNA. The phenomenon is evident in plots where elevated temperatures correspond to DNA denaturation followed by increased absorbance of light at 260nm.

(ii) **Renaturation of DNA-** Renaturation, also known as annealing, is the process where separated complementary DNA strands can reform a double helix. Several factors influence the degree of renaturation:

- **C₀**- This represents the concentration of double-stranded DNA before denaturation, typically measured as nucleotides per unit volume.
- **T**- The duration of the renaturation process, measured in seconds.
- **t_{1/2}**- The time required for renaturation to reach halfway completion.
- **C₀t_{1/2} Value**- This is the product of C₀ and t_{1/2}. A higher C₀t_{1/2} value indicates greater complexity of the DNA.

By analyzing the cot curve based on renaturation kinetics, one can determine the complexity of the DNA molecule. Repetitive DNA sequences with lower complexity exhibit lower C₀t_{1/2} values compared to highly complex unique DNA sequences.

Fig. 7.14 Demonstrates the reversible thermal denaturation and annealing of DNA and **Fig. 7.15** depicts the melting curve of DNA.

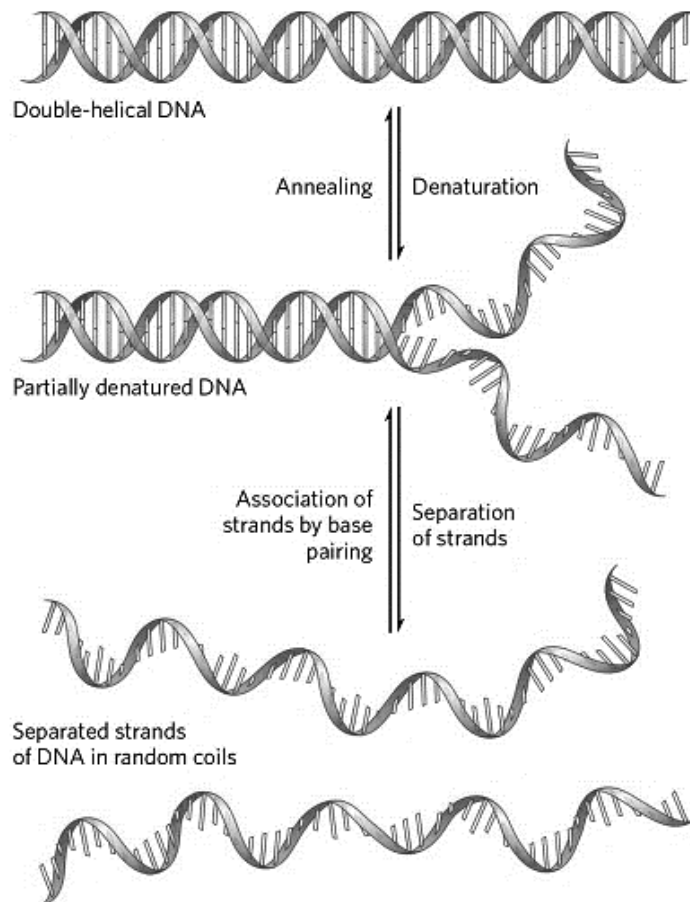


Fig. 7.14 Reversible denaturation and annealing (renaturation) DNA

Source: Lehninger, Principles of Biochemistry, 7th Edition

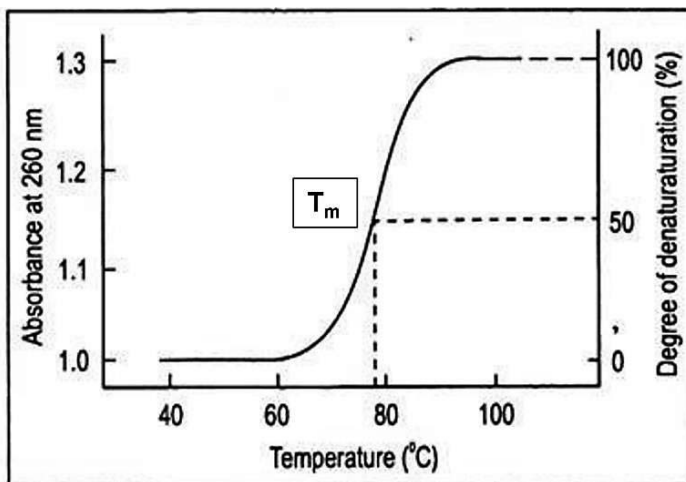


Fig. 7.15 Melting curve of DNA

7.8 STRUCTURE OF PROTEINS

Proteins are macromolecules made up of monomers called amino acids. Amino acids are the building blocks of all proteins. Proteins consist of linear sequences of amino acids connected by peptide bonds. A peptide bond forms when the α -amino group of one amino acid binds covalently to the α -carboxyl group of another amino acid (see Fig. 7.16). After two amino acids join to form a dipeptide, there remain free amino and carboxyl groups at either end, which can further link to additional amino acids. Consequently, chains of amino acids, known as oligopeptides (comprising up to 25 residues) or polypeptides (exceeding 25 residues), can form via peptide bonds. Importantly, polypeptides retain a free α -amino group and α -carboxyl group. Peptide sequences are typically depicted with the free α -amino group at the left, the free α -carboxyl group at the right, and hyphens indicating peptide bonds between amino acids. Thus, the tripeptide with the sequence H₃N-serine-leucine-phenylalanine-COO- is abbreviated as Ser-Leu-Phe or S-L-F.

The peptide bond between carbon and nitrogen exhibits a partial double-bond character because of the proximity of the carbonyl carbon-oxygen double bond, allowing for the existence of resonance structures. As a result, the bond length between carbon and nitrogen is shorter than typical single C-N bonds. Consequently, the peptide unit, comprised of CO-NH atoms, is relatively rigid and planar, although rotation can occur freely about the C α -N and C α -C bonds flanking the peptide bond. This freedom of rotation permits adjacent peptide units to adopt different angles. Notably, the hydrogen of the amino group is typically positioned on the opposite side (trans) of the double bond relative to the oxygen of the carbonyl group, rather than on the same side (cis).

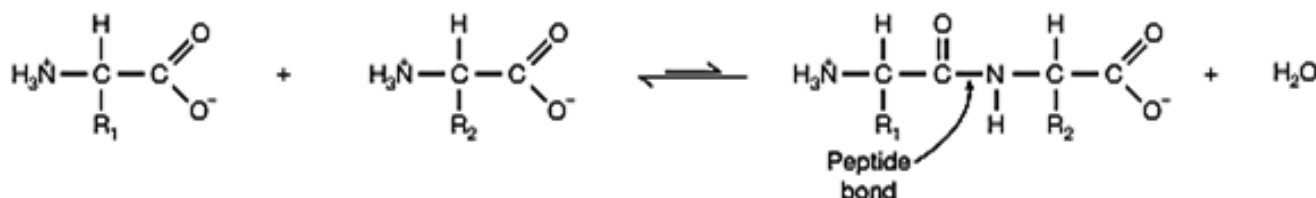


Fig. 7.16 Formation of a peptide bond

Source: BIOS, Instant Notes Biochemistry, 3rd Edition

- (I) **Ramachandran plot-** The backbone of a protein consists of a linked sequence of rigid planar peptide groups. The conformation of a polypeptide backbone is determined by the rotation angles, or torsion angles, about the C α -N bond (ϕ , φ) and the C α -C bond (ψ , ψ) of each amino

acid residue. In the fully extended (all-trans) conformation of the polypeptide chain, both ϕ and ψ angles are defined as 180° , and they increase with clockwise rotation when viewed from the C_α atom (as illustrated in Fig.7.17). The conformational range of ϕ and ψ angles is constrained by steric hindrance.

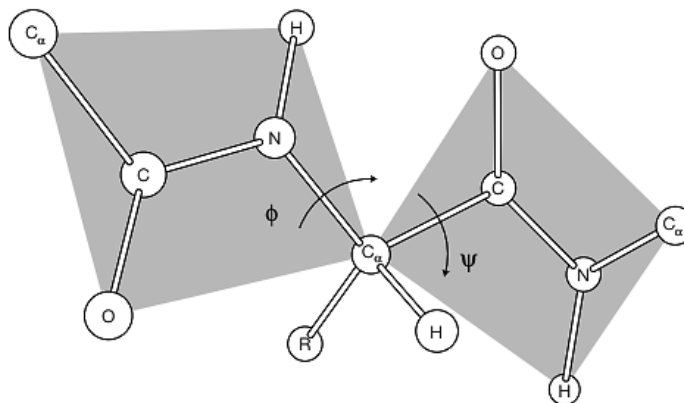


Fig. 7.17 Segment of a polypeptide chain showing the torsion angles about the C_α -N bond (ϕ) and C_α -C bond (ψ)

Source: BIOS, Instant Notes Biochemistry, 3 rd Edition

The allowed values of ϕ and ψ can be determined by calculating the distances between atoms of a tripeptide at all ϕ and ψ values for the central peptide unit. These values are represented in a steric contour diagram, also known as a conformation map or Ramachandran plot (as depicted in Fig.7.18). From the Ramachandran plot, it is evident that most areas, or combinations of ϕ and ψ angles, are inaccessible to a polypeptide chain due to steric hindrance. Only three small regions of the plot are physically accessible to a polypeptide chain, and within these regions lie the ϕ - ψ values that give rise to the right-handed α -helix, the parallel and antiparallel β -pleated sheets, and the collagen helix.

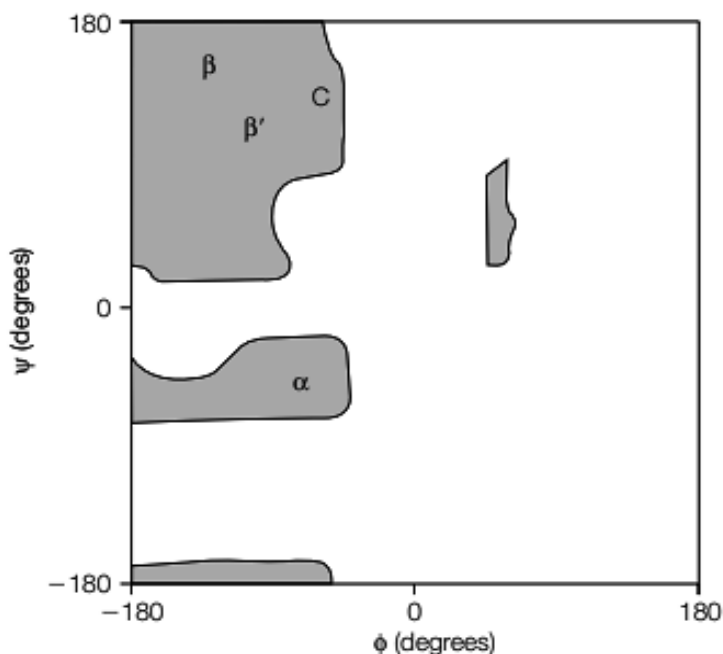


Fig. 7.18 Ramachandran plot showing the allowed angles for poly-L-alanine (grey regions). α , ϕ - ψ values that produce the right-handed α -helix; β , the antiparallel β -pleated sheet; β' , the parallel β -pleated sheet; C, the collagen helix.

- (II) **Protein conformation-** The conformation of a protein refers to how its constituent atoms are positioned in space, determining the overall structure of the molecule. This arrangement is determined by the bonding patterns within the protein's structure. In proteins, the conformation is determined by its amino acid sequence and is characterized by four levels of structure: primary, secondary, tertiary, and, in some cases, quaternary structure (Fig. 7.19).

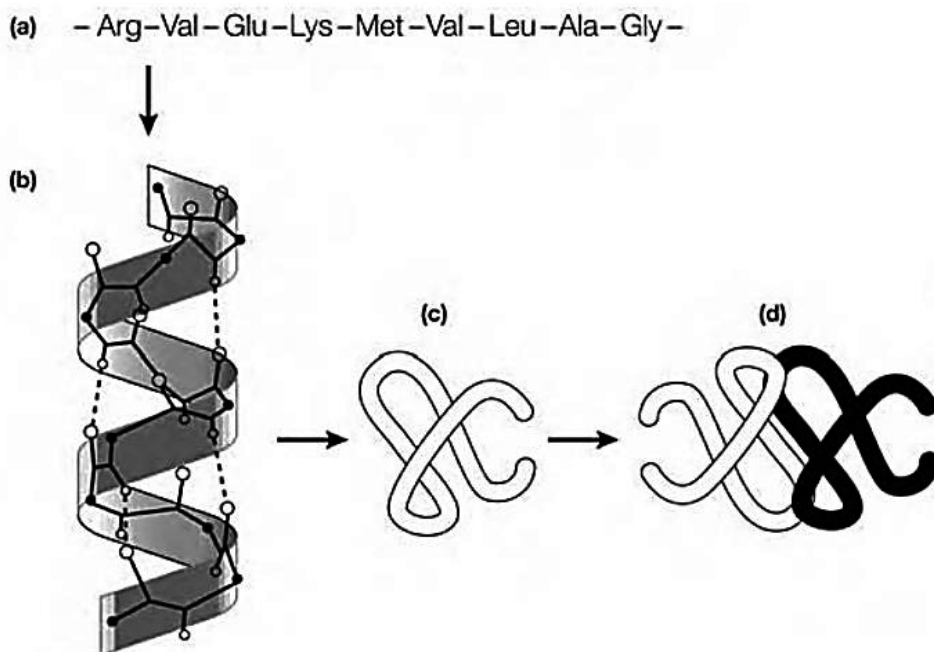


Fig. 7.19 The four levels of structure in proteins. (a) Primary structure (amino acid sequence), (b) secondary structure (α -helix), (c) tertiary structure, (d) quaternary structure

Source: BIOS, Instant Notes Biochemistry, 3 rd Edition

- (i) **Primary Structure-** The primary structure of a protein refers to the linear sequence of amino acids linked together by peptide bonds. This sequence is encoded by the gene and represents the genetic information of the protein. The primary structure represents the linear sequence of amino acids in a protein, which is determined by the sequence of nucleotide bases in the gene that encodes the protein. Each amino acid is linked to the next through peptide bonds. In addition to peptide bonds, other covalent bonds may be present in the primary structure. One common example is disulfide bonds, which form between two cysteine residues in the polypeptide chain (as depicted in Fig. 7.20). Disulfide bonds stabilize the tertiary structure of proteins by forming bridges between distant parts of the chain. These bonds are typically found in extracellular proteins, contributing to their stability. Besides disulfide bonds, some proteins may contain other types of covalent cross-links. For example, in collagen, covalent cross-links can form between lysine residues, contributing to the structural integrity of collagen fibers. Overall, the primary structure of a protein is essential for its overall folding and function. Even small alterations in the primary structure, such as changes in amino acid sequence or disruptions in covalent bonds, can have significant effects on the protein's structure and function.

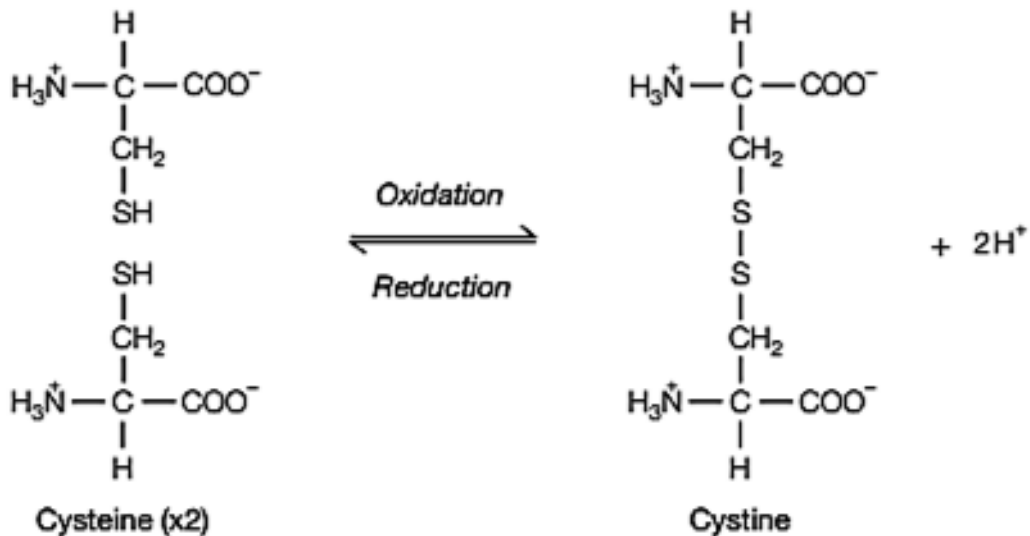


Fig. 7.20 Formation of a disulfide bond between two cysteine residues, generating acysteine residue

Source: BIOS, Instant Notes Biochemistry, 3rd Edition

- (ii) **Secondary Structure-** Secondary structure refers to the local folding patterns within a protein chain, primarily driven by hydrogen bonding between the peptide backbone atoms. The two most common types of secondary structure are the α -helix and the β -sheet. The secondary structure of a protein involves the regular folding of localized regions of the polypeptide chain into specific patterns. The two primary types of secondary structure are the α -helix and the β -pleated sheet.

α -Helix- In the α -helix structure, the polypeptide chain adopts a helical conformation, resembling a spiral staircase. The backbone of the polypeptide forms the central axis of the helix, while the side chains of the amino acids project outward from the helix. The backbone forms hydrogen bonds between the carbonyl oxygen of one amino acid residue and the amino hydrogen of an amino acid residue four positions ahead in the sequence. This regular arrangement of hydrogen bonds stabilizes the helical structure. The α -helix typically contains 3.6 amino acid residues per turn of the helix, with a rise of 0.15 nanometers per residue along the axis of the helix. The side chains of the amino acids are positioned along the exterior of the helix. Proline residues are less frequently found in α -helices due to their inability to participate in the formation of hydrogen bonds in this structure. Refer to the Fig. 7.21 for the structure of the α -Helix.

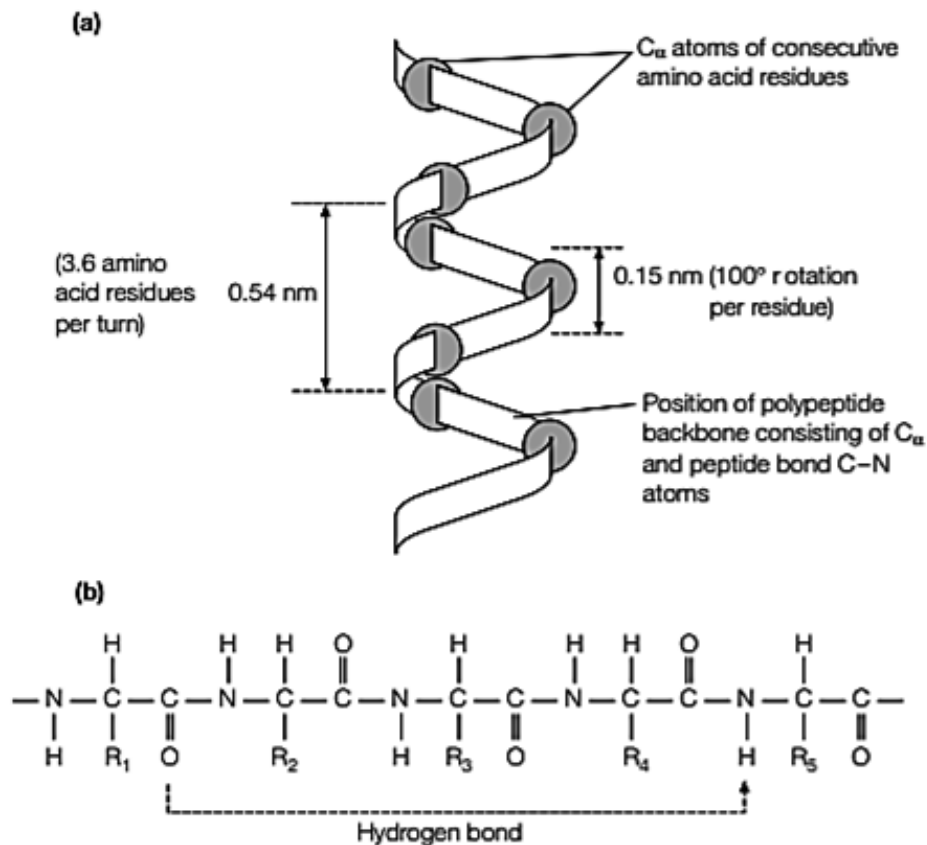


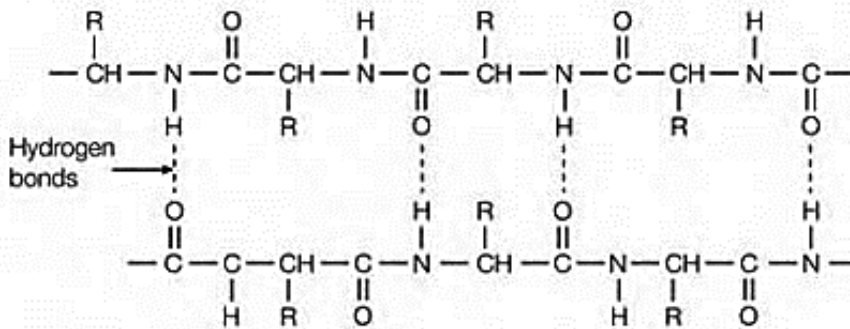
Fig.7.21 Folding of the polypeptide chain into an α -helix. (a) Model of an α -helix with only the $C\alpha$ atoms along the backbone shown; (b) in the α -helix the CO group of residue n is hydrogen bonded to the NH group on residue $(n + 4)$;

Source: BIOS, Instant Notes Biochemistry, 3 rd Edition

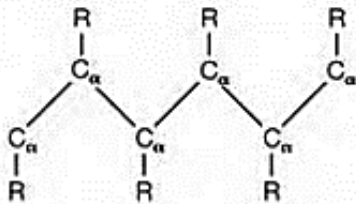
β -Pleated Sheet- In the β -pleated sheet structure, hydrogen bonds form between the peptide bonds, either connecting different sections of the same polypeptide chain or linking adjacent polypeptide chains (as illustrated in Fig. 7.22a). The flat shape of the peptide bond prompts the polypeptide to fold into a pleated pattern, with the side chains of the amino acids extending above and below the sheet (as shown in Fig. 7.22b). Depending on their alignment, adjacent polypeptide chains in β -pleated sheets can run parallel or antiparallel to each other (refer to Fig. 7.22c). The polypeptide chain within a β -pleated sheet stretches out fully, resulting in a distance of 0.35 nm between consecutive $C\alpha$ atoms. β -Pleated sheets typically exhibit a slight curvature, and when multiple polypeptides are involved, the sheet can close to form a barrel-like structure known as a β -barrel. These arrangements provide strength and stability to many structural proteins, such as silk fibroin, which primarily consists of stacks of antiparallel β -pleated sheets.

To achieve the compact shape characteristic of globular proteins, the polypeptide chain often changes direction, forming structures like hairpins or β -turns. In β -turns, the carbonyl oxygen of one amino acid forms a hydrogen bond with the hydrogen on the amino group of the fourth amino acid along the chain. β -Turns frequently serve as connectors between the ends of antiparallel β -pleated sheets. Regions of the polypeptide chain lacking a defined secondary structure are described as having a coiled or looped conformation. Approximately half of the polypeptide chain in a typical globular protein adopts such a conformation.

(a)



(b)



(c)

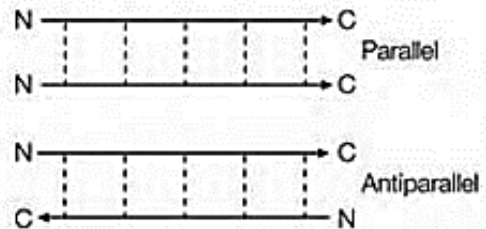


Fig. 7.22 The folding of the polypeptide chain in a β -pleated sheet. (a) Hydrogen bonding between two sections of a polypeptide chain forming a β -pleated sheet; (b) a side-view of one of the polypeptide chains in a β -pleated sheet showing the side-chains (R groups) attached to the C_α atoms protruding above and below the sheet; (c) because the polypeptide chain has a polarity, either parallel or antiparallel β -pleated sheets can form

Source: BIOS, Instant Notes Biochemistry, 3 rd Edition

- (iii) **Tertiary structure-** Tertiary structure describes the overall three-dimensional arrangement of a protein molecule, including the folding of secondary structures and additional folding and bending of the polypeptide chain. The tertiary structure is stabilized by various interactions, such as hydrogen bonds, disulfide bonds, hydrophobic interactions, and electrostatic interactions between amino acid side chains. In water-soluble globular proteins like myoglobin, the primary driving force behind the folding of the polypeptide chain is the energetic need to conceal nonpolar amino acids within the hydrophobic interior, shielded from the surrounding aqueous, hydrophilic environment. Consequently, the polypeptide chain spontaneously folds, positioning most of its hydrophobic side chains within the interior and leaving the majority of its polar, charged side chains exposed on the surface.

Once folded, the protein's biologically active (native) conformation is maintained not only through hydrophobic interactions but also via electrostatic forces, hydrogen bonding, and, if present, covalent disulfide bonds. Electrostatic forces include salt bridges formed between oppositely charged groups, along with multiple weak van der Waals interactions occurring between the closely packed aliphatic side chains within the protein's core.

- (iv) **Quaternary Structure-** Quaternary structure refers to the arrangement of multiple protein subunits (polypeptide chains) in single protein complex. Proteins with quaternary structure consist of two or more individual polypeptide chains known as subunits. The subunits may be identical (homodimers, homotrimers, etc.) or different (heterodimers, heterotrimers, etc.). The

interaction between subunits is often mediated by noncovalent bonds and sometimes by covalent bonds such as disulfide bridges.

7.9 SUMMARY

The present unit explores the fundamental components and characteristics of nucleic acids (DNA and RNA) and proteins, essential molecules in biology. Nucleic acids, made up of nucleotides, serve as carriers of genetic information and are crucial for cellular function and inheritance. The chapter delves into the detailed structure of nucleic acids, including the arrangement of nucleotides, the double helix structure of DNA, and the single-stranded nature of RNA. Nucleotides are the essential constituents of nucleic acids and are composed of three primary components: a nitrogenous base (such as adenine, cytosine, guanine, thymine, or uracil), a five-carbon sugar (ribose in RNA and deoxyribose in DNA), and one or more phosphate groups. DNA has a double helix structure of two polynucleotide strands which are wound around each other in a right-handed manner. These strands feature sugar-phosphate backbones running in opposite directions, with nitrogenous bases projecting inward. Crucially, hydrogen bonds form between complementary base pairs (adenine with thymine and guanine with cytosine), ensuring the stability of the DNA molecule and accurate replication of genetic information.

Unlike DNA, RNA generally exists as a single-stranded molecule. It can fold into complex secondary and tertiary structures through intramolecular base pairing. This structural flexibility allows RNA to undertake diverse biological roles. For instance, RNA functions as a messenger between DNA and the protein synthesis machinery, conveying genetic information for protein synthesis. Additionally, certain RNA molecules, known as ribozymes, exhibit catalytic activity, facilitating biochemical reactions within cells. RNA is classified primarily as mRNA, tRNA, and rRNA, having specific functions in gene expression and regulation. mRNA (messenger RNA) carries genetic instructions from DNA to ribosomes, where proteins are synthesized. tRNA (transfer RNA) delivers amino acids to the ribosome for protein assembly. rRNA (ribosomal RNA) forms the structural framework of ribosomes and catalyzes protein synthesis.

Various physical and chemical properties of nucleic acid have also been described in brief focusing on DNA. Denaturation of DNA is influenced by pH, temperature, and chemical agents, leading to the separation of DNA strands. Denaturation causes an increase in UV light absorption (hyperchromicity), a decrease in specific optical rotation, and a reduction in viscosity. Hyperchromicity serves as an indicator of DNA denaturation. Renaturation, or annealing, allows complementary DNA strands to reform a double helix, with factors like concentration and time affecting the process. The $C_{0t1/2}$ value indicates DNA complexity, with lower values for repetitive sequences. Moreover, the chapter discusses the structure of proteins, which are macromolecules essential for almost all biological processes. Proteins are comprised of amino acids linked together by peptide bonds to form polypeptide chains. The specific sequence of amino acids determines the protein's structure and function. Proteins exhibit a hierarchical structure, including primary, secondary, tertiary, and quaternary levels of organization, each crucial for their proper functioning.

7.10 TERMINAL QUESTIONS

Q.1 Define nucleotide and nucleoside.

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Q.2 Give a comparative account between DNA and RNA

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Q.3 Explain the Watson and Crick Model of DNA.

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Q.4 Difference between A, B and Z form of DNA.

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.....
Q.5 Write explanatory note on the structure of mRNA, rRNA and tRNA.

.....
.....
Q.6 Discuss denaturation and renaturation of DNA.

.....
.....
Q.7 Write explanatory note on structure of proteins

7.11 FURTHER SUGGESTED READINGS

- Robert F. Weaver; Molecular Biology, Mc Graw Hill, 5th Edition (2012).
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UNIT-8 : RECOMBINANT DNA TECHNOLOGY

Structure

- 8.1 Introduction
 - Objective
- 8.2 Recombinant DNA Technology: Origin and Current Status
- 8.3 Major Steps in Gene Cloning or rDNA Technology
- 8.4 Restriction Enzymes
- 8.5 Polymerases
- 8.6 r-DNA Technology in Environment Management
- 8.7 Summary
- 8.8 Terminal Questions
- 8.9 Suggested Readings

8.1 INTRODUCTION

Through the years, scientists have studied life and how it changes and adapts to the changes in the surroundings. One of their methods to cope with the changes is through genetic modification i.e., manipulation of genomic DNA. Genetic modification is the process of changing the genetic makeup of an organism to express new traits. This is a slow process and always happening to plants and animals to control or modify their characteristics. Recombinant DNA (rDNA) technology marks the beginning of a new era in biological research and application. As a part of biotechnology, it has gained significant motion in recent years, as it offers solutions to several challenges faced by conventional breeding procedures used in plants and animals that are time-consuming and labour intensive and often lead to the expression of certain undesirable genes along with the desired ones. rDNA technology also known as genetic engineering, enables scientists to manipulate DNA molecules by combining genetic material from different sources. This process also involves inserting these recombined DNA molecules into a host organism, resulting in the creation of new genetic combinations. The techniques of genetic engineering or rDNA technology include the creation of recombinant DNA by gene cloning and gene transfer. DNA found in the cells of all living organisms, acts as the genetic material carrying genetic instruction for the growth, functioning, reproduction, and development of all known living organisms. In this technology, scientists create rDNA by combining genetic material from different sources by combining genes encoding desired traits or in vitro synthesized genes into a virus, bacterial plasmid, or other vector system. Then the constructed rDNA molecule is allowed to propagate within a host organism where it does not naturally occur.

rDNA technology serves as a cornerstone in modern science, offering remarkable capabilities to manipulate genetic material for a wide range of purposes. It has become an indispensable tool in both research and applied biology, playing a central role in the field of biotechnology, which has witnessed substantial progress in recent years. This technology finds applications across diverse fields, including scientific research, medical treatments, agricultural improvements, and industrial processes. For

instance, it facilitates the production of valuable proteins for therapeutic use, the creation of genetically modified crops with enhanced traits, and the development of innovative solutions for environmental challenges. Furthermore, rDNA technology has revolutionized various sectors such as biomedical research, pharmaceutical advancements, agriculture, environmental conservation, and medical diagnosis and treatment. By employing rDNA techniques, scientists can generate essential proteins for medical treatments, improve crop productivity and resilience, devise novel strategies for environmental remediation, and create precise diagnostic methods for personalized medicine. With its transformative potential, rDNA technology continues to drive scientific progress and address significant global issues across multiple domains.

Objectives :

After reading this unit, the learner will be able to

- To discuss recombinant DNA technology: origin and current status
- To understand major steps in gene cloning or rDNA technology
- To discuss restriction enzymes and its classifications
- To study Polymerases
- To discuss r-DNA Technology in environment management

8.2 RECOMBINANT DNA TECHNOLOGY ORIGIN AND CURRENT STATUS

The following year, in 1973, the pioneers Herbert W. Boyer from the University of California San Francisco (UCSF) and Stanley N. Cohen from Stanford University engineered the first organism containing recombinant DNA. Their milestone involved introducing an antibiotic-resistance gene into *Escherichia coli* bacteria. Notably, they went a step further by incorporating genes from the toad, *Xenopus laevis*, demonstrating that DNA from different species could be spliced together. Paul Berg's ground-breaking work was recognized with the 1980 Nobel Prize in Chemistry, acknowledging his fundamental studies of the biochemistry of nucleic acids, particularly in the realm of recombinant DNA. This ability to cut, paste, and copy DNA molecules marked a crucial moment in scientific research, giving rise to an entire industry centred around genetic engineering. In 1976, Herbert Boyer, one of the early pioneers, founded Genentech, the first biotechnology company. By 1982, the U.S. Food and Drug Administration (FDA) approved Genentech's inaugural successful product: a synthetic form of human insulin. This insulin was produced by bacteria that had been ingeniously engineered to contain the insulin gene, showcasing the practical applications and success of rDNA technology in producing vital therapeutic proteins. Today, the current status of rDNA reflects ongoing innovation and its integration into diverse fields, playing a crucial role in the development of new therapies, genetically modified crops, and the exploration of fundamental biological processes.

In today's world, scientists are widely using rDNA technology in research labs around the globe to explore many aspects of genes, like how they're built, what they do, and how they're controlled. One way they use this technology is by creating special animals, usually mice, with a gene that doesn't work. These animals help scientists understand what that gene does by seeing what happens when it's missing. These specially engineered mice, often called "knock-out" mice, are super helpful, especially in developmental biology, where they've taught us a lot about the essential genes needed for an organism to grow from a single fertilized egg. These techniques are also super important in the biotechnology industry. For example; scientists can make plants that produce a toxin called Bt toxin, which comes from a bacterium called *Bacillus thuringiensis*. This toxin messes up the stomachs of certain insect larvae

(like caterpillars) that harm crops. By using rDNA technology, scientists put the Bt gene into plants, making them resistant to these crop-damaging insects. Such type of crops is known as “genetically modified (GM) crops”. Such type of application of this technology makes a big impact on our economy, reducing the need for pesticides and making crops more successful over time. This technology played a pivotal role in the production of insulin, growth hormones, and vaccines, revolutionizing the pharmaceutical industry. Moreover, rDNA Technology has facilitated the engineering of crops with enhanced nutritional content, improved resistance to pests, and increased tolerance to environmental stressors, contributing to advancements in agriculture.

8.3 MAJOR STEPS IN GENE CLONING OR rDNA TECHNOLOGY

These steps represent the general workflow of rDNA technology, which can be customized and optimized based on the specific goals and requirements of the experiment or application. The major steps in rDNA technology involve several key processes as represented in Fig. 8.1 and explained below. The process of gene cloning involves several sequential steps:

1. **Isolation of DNA Fragment-** DNA containing the gene of interest is extracted from the organism of interest, often involving cell disruption and DNA extraction methods. The gene of interest is typically a segment of DNA encoding a specific protein, enzyme, or hormone, such as the gene for insulin.
2. **Selection of Vector-** A vector, such as a plasmid or viral DNA, is chosen as a carrier molecule to transport the DNA fragment of interest into host cells. Vectors have the ability to replicate independently within host cells, allowing for the production of multiple copies of the inserted DNA fragment.
3. **Insertion of DNA into Vector-** The gene of interest is inserted into the selected vector using restriction endonucleases, which cut both the vector and the DNA fragment at specific recognition sequences called restriction sites. The cut DNA fragments are then ligated into the vector with the help of DNA ligase, resulting in recombinant DNA molecules.
4. **Transformation-** The recombinant DNA molecules are introduced into host cells through techniques such as electroporation, microinjection, or lipofection. Once inside the host cell, the recombinant DNA can replicate and express the gene of interest.
5. **Selection of Recombinant Cells-** Transformed host cells are selected from a mixture of transformed and non-transformed cells using various selection methods, such as reporter genes or colony hybridization, to isolate cells containing the recombinant DNA.
6. **Multiplication/Culturing of Recombinant Cells-** The selected transformed cells are cultured in a nutrient-rich medium and incubated under appropriate conditions to allow for cell division and replication of the recombinant DNA.
7. **Expression of Recombinant DNA-** Depending on the desired outcome, the emphasis may be on replicating the recombinant DNA or expressing the protein encoded by the gene of interest. Steps are taken to create favorable conditions for the host cells to express the desired protein, including transcription of the gene into messenger RNA (mRNA) and translation of the mRNA into protein.
8. **Harvesting and Purification-** The multiplied gene copies or the protein expressed by the recombinant DNA are isolated using various methods tailored to the specific experimental requirements. Marker genes present in the vector can aid in isolating recombinant cells from non-recombinant ones.
9. **Analysis and Characterization-** The purified protein is thoroughly analyzed and characterized to ensure it meets desired quality standards and functions as intended. This process helps in

understanding the protein's role in biological systems and guides further experimentation or modifications for improved performance.

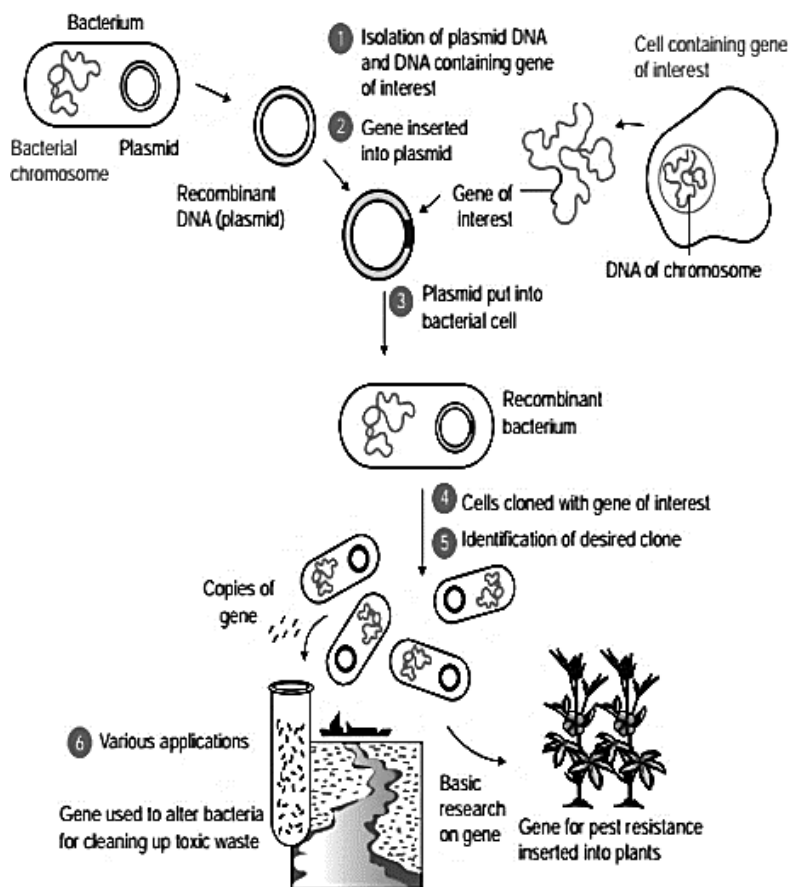


Fig. 8.1 Diagrammatic representation of steps involved in recombinant DNA technology

Source: <https://www.sarthaks.com/856174/describe-the-steps-involved-in-recombinant-dna-technology>

8.4 RESTRICTION ENZYMES

A restriction enzyme, also known as a restriction endonuclease, is a type of nuclease enzyme that functions by cutting DNA molecules at specific sites (or sequences), known as restriction sites or recognition sequences. These enzymes belong to a larger group called endonucleases. Restriction enzymes are commonly classified into five types (Type I- Type V), each with unique features in how they interact with and cut DNA. To cut the DNA, all restriction enzymes make two cuts or incisions on both strands of the DNA double helix. Type II restriction enzymes, like other types, are produced by single-celled microorganisms such as bacteria and archaea (prokaryotes). Their main function inside the prokaryotic cell is to protect themselves from invasion against viruses and other infectious DNA molecules. Inside the prokaryotic cell, a restriction enzyme selectively cleaves the foreign DNA through a process known as restriction digestion; In the meantime, the cell's DNA is protected by a modification enzyme called methyltransferase, which alters the prokaryotic cell's DNA and prevents it from being cleaved. Within a prokaryotic cell, these enzymes precisely chop up invading DNA through a process known as restriction digestion. Meanwhile, the cell's DNA is safeguarded by a modification enzyme called methyltransferase, which alters the DNA and prevents it from being cleaved. Together, these two

processes form the restriction-modification (R-M) system. Restriction enzymes have found their wide application in genetic engineering and biotechnology being extensively used for gene cloning and protein expression, DNA mapping, Restriction fragment length polymorphism (RFLP), epigenetic modification, preparation of cDNA libraries, etc.

The specific DNA sequences recognized by Type II restriction enzymes exhibit a unique structure known as a restriction site which is mostly a palindrome. Palindromes are sequences of nucleotide bases that read the same on both strands but in opposite directions. For instance, if one DNA strand reads GAATTC in the 5' to 3' direction, the complementary strand reads CTTAAG in the opposite direction. However, when both strands are read in the 5' to 3' direction, the sequence remains the same. This palindrome structure is illustrated as follows:

5' GAA↓TTC 3'
3' CTT ↓ AAG 5'

Within this palindrome, there exists a point of cutting, represented by the downward arrow (↓) where the enzyme molecule cleaves the recognized sequence. Restriction enzymes exploit this site by making cuts in the DNA molecule around this point.

Cleavage of DNA molecules by restriction enzymes leads to the formation of fragments with either sticky ends or blunt ends (see Fig. 8.2). Sticky end (or staggered end) fragments have overhanging sequences that can easily pair with complementary sequences in other DNA fragments. Blunt-end fragments lack overhangs and can be directly ligated to other blunt-ended DNA fragments using linkers or adapters. Fig. 8.2 demonstrates the sticky and blunt end generated by the cleavage restriction enzymes. The recognition and cleavage sites, as well as the cleavage patterns, of a few restriction enzymes, is shown in Fig 8.3.

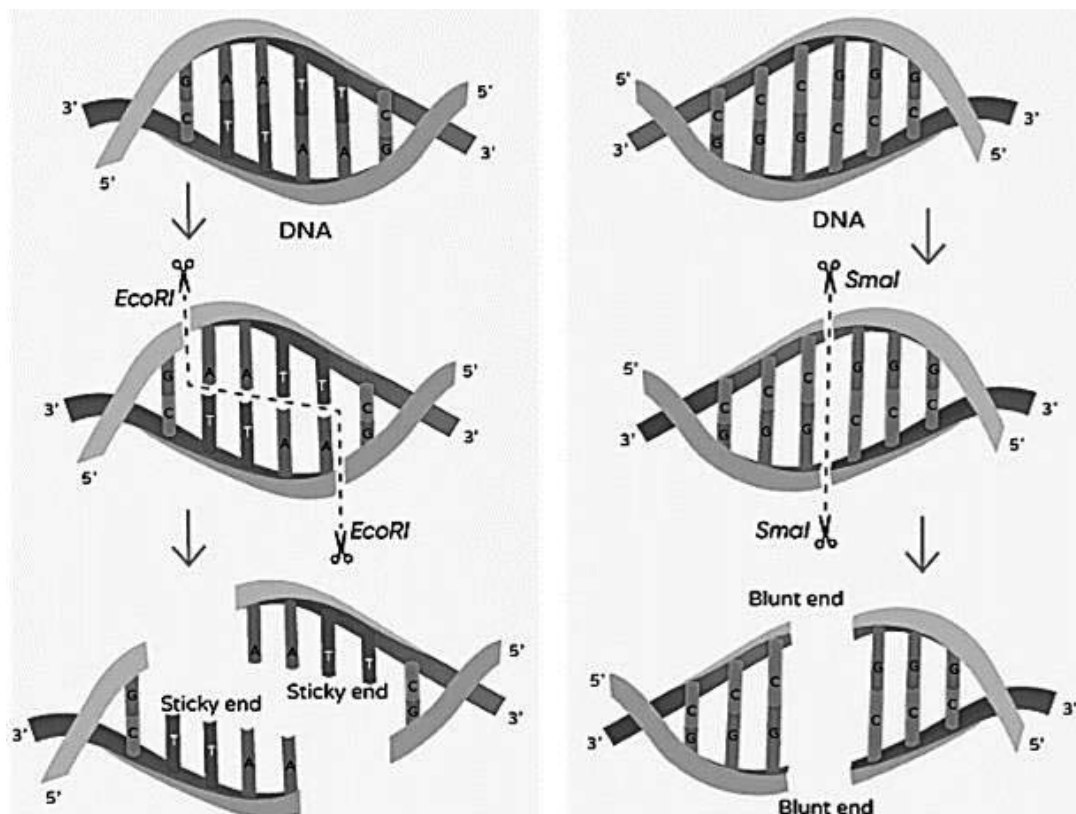


Fig. 8.2 Sticky and blunt ends generated by the cleavage of restriction enzymes

Source: <https://www.sciencefacts.net/restriction-enzymes.html>

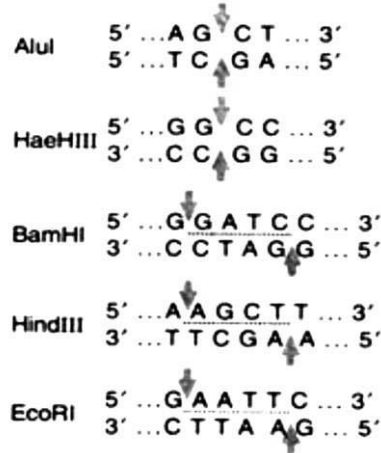


Fig 8.3 AluI and HaeIII produce blunt ends. BamHI, HindIII and EcoRI produce sticky ends

Discovery of restriction enzymes- In 1963, two enzymes crucial for limiting the growth of bacteriophage in *Escherichia coli* were identified. One of these enzymes methylated DNA by adding methyl groups, while the other enzyme was responsible for cutting DNA. This latter enzyme was termed a restriction endonuclease. Five years later in 1968, the first restriction endonuclease, Hind II, was isolated and characterized. This enzyme was capable of recognizing and cleaving specific DNA sequences of six base pairs. These specific sequences are termed recognition sequences or recognition sites. Since then, over 900 restriction enzymes have been discovered, originating from more than 230 bacterial strains, each recognizing distinct recognition sequences.

Nomenclature of restriction enzymes- Since their discovery in the 1970s, numerous restriction enzymes have been identified and characterized, particularly Type II restriction enzymes. Each enzyme is named based on the bacterium from which it was originally isolated, following a naming convention that includes the bacterial genus, species, and strain. For example, the name of the EcoRI restriction enzyme is derived as follows:

"E" represents *Escherichia*, indicating the genus of the bacterium.

"co" stands for *coli*, specifying the species of the bacterium.

"R" refers to RY13, the strain of the bacterium.

"I" denotes that it was the first enzyme identified in this particular bacterium.

Another well-known example is the *BamHI* enzyme, isolated from *Bacillus amyloliquefaciens* strain H, while *Sau3A* is derived from *Staphylococcus aureus* strain 3A. Similar naming conventions apply to hundreds of other restriction enzymes with diverse sequence specificities, all obtained from various bacterial sources and widely available for research and commercial purposes.

Classification of restriction enzymes

Restriction enzymes are categorized into two main groups: isoschizomers and neoschizomers.

- Isoschizomers are enzymes that recognize and cut the same DNA sequence. For example: *AgeI* and *BshT1* recognize and cleave 5'-A↓CCGGT.
- Neoschizomers are enzymes that recognize the same sequence but cut it at different positions. For example: *SmaI* (5'-CCC↓GGG-3') and *XmaI* (5'-C↓CCGGG-3'); both recognize 5'-CCCGGG-3' sequence but cleave them at a different position (marked with the arrow ↓)

Additionally, restriction enzymes are traditionally classified into five types: Type I, II, III, IV,

and V. This classification is based on various factors including their structure, cleavage site, specificity, and cofactors. The difference between these classes is summarised in Table 8.1 and briefly explained below:

Type I restriction enzymes cut DNA at random sites, often more than 1000 base pairs away from the recognition sequence or restriction site. These enzymes move along the DNA during the reaction and rely on co-factors such as magnesium ions (Mg^{2+}), S-adenosyl methionine (SAM), and adenosine triphosphate (ATP).

Type II restriction enzymes first isolated by Hamilton Smith, are simpler in structure. They do not require ATP for DNA degradation and cut DNA directly within the recognition sequence or restriction site. Examples: *EcoRI*, *HindIII*, *BamHI*, *NotI*, *PacI*

Type III restriction enzymes cut DNA approximately 25 base pairs away from the recognition sequence or restriction site. Similar to Type I enzymes, they move along the DNA during the reaction, but they rely on ATP as a source of energy.

Type IV restriction enzyme Type IV RE cuts modified such as typically methylated DNA. For example: McrBC and Mrr systems found in *E. coli*, have the unique ability to recognize modified DNA, typically methylated. These enzymes require GTP for DNA cleavage. They possess both methyltransferase and endonuclease activities, which are combined within a single polypeptide chain. Interestingly, the endonuclease activity of these enzymes is positively influenced by S-adenosine methionine (SAM), while ATP does not affect their activity.

Type V restriction enzyme Type V restriction enzymes, such as the cas9-gRNA complex from CRISPRs, utilize guide RNAs to target specific non-palindromic sequences present in invading organisms. Unlike traditional restriction enzymes, Type V enzymes can cut DNA of variable length, as long as they are provided with an appropriate guide RNA. The remarkable flexibility and user-friendliness of these enzymes make them highly promising for future applications in genetic engineering.

Table 8.1 Difference between Type I, II, III and IV restriction enzymes

S. No.	Feature	Type I	Type II	Type III	Type IV
1.	Subunit	Three different	Two identical	Two different	Two different
2.	Enzyme activity	Endonuclease methyltransferase, ATPase	Endonuclease or methyltransferase	Endonuclease methyltransferase, ATPase	Endonuclease GTPase
3.	Co-factor for DNA cleavage	ATP, SAM, Mg^{2+}	Mg^{2+}	ATP, Mg^{2+}	Mg^{2+} , GTP
4.	Methylation	SAM, Mg^{2+}	SAM	SAM, Mg^{2+}	-
5.	Recognition sequences	Asymmetric, bipartite	Usually symmetric	Asymmetric	Bipartite, methylated
6.	Cleavage site	Random at least 1000 bp from the recognition site	At or near the recognition site	25-27 bp from the recognition site	Between methylated bases at multiple positions
7.	DNA translocation	Yes	No	Yes	No

Mechanism of action of the restriction enzymes- Restriction enzymes display a multi-step mechanism of action. The steps involved in DNA binding and cleavage by a type II RE is depicted in Fig. 8.4 and explained below:

1. **Initial binding-** These enzymes initially make nonspecific contacts with DNA and form dimers.
2. **Locating the target site-** After initial binding, the enzyme slides down the DNA scanning for the recognition sequences through a combination of linear diffusion and hopping/jumping. During linear diffusion, the enzyme moves along the DNA over short distances, while hopping or jumping allows it to cover longer distances.
3. **Specific binding-** Once the target restriction site is located, specific binding of the enzyme with the recognition sequences occurs. This recognition process triggers significant conformational changes in both the enzyme and the DNA. These changes activate the catalytic centre of the enzyme.
4. **Catalysis-** After the specific binding, the enzyme associates with the metal cofactor (Mg^{2+}) and initiates catalysis of DNA by hydrolysis of phosphodiester bonds. This hydrolysis reaction breaks the DNA strand at specific locations using the mechanism of strain distortion and catalysis by approximation, resulting in the release of reaction products.

Overall, the mechanism involves a dynamic interplay between the enzyme and DNA, leading to the precise recognition and cleavage of specific DNA sequences.

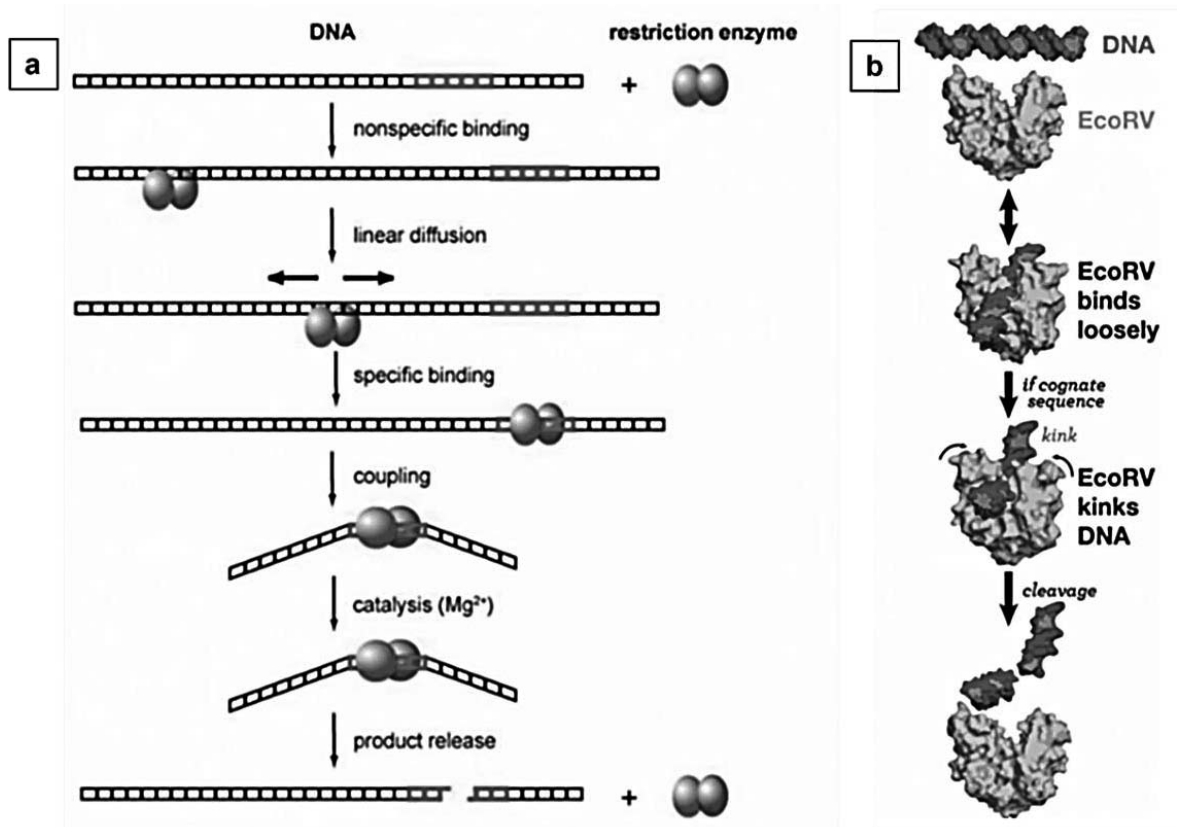


Fig. 8.4 Visual Representation of DNA recognition and cleavage by Type II restriction endonucleases. (a) Depicts an EcoRV dimer's process of scanning non-specifically along DNA until it recognizes a specific binding site, triggering coupling with the metal cofactor and strain distortion of the DNA. Subsequent hydrolysis of the phosphodiester bond leads to the release of DNA cleavage products from the enzyme. (b) Presents a space-filling model illustrating EcoRV's DNA recognition and cleavage

8.5 POLYMERASES

Polymerases are specialized enzymes that catalyse the synthesis of DNA or RNA strands with a sequence complementary to the original template sequence. They are important for numerous biological processes involving DNA and RNA, such as replication, repair, recombination, and transcription. Polymerases exhibit a diverse range of structures and functions, from single-protein units to intricate assemblies involving multiple proteins working together in a coordinated manner to synthesize nucleic acids accurately and rapidly. Due to their critical role, polymerases have been evolutionarily conserved, ensuring their importance across different life forms. Studying polymerases is crucial for unraveling the mechanisms behind growth, propagation, and evolution in living organisms.

DNA polymerases are specialized enzymes responsible for building new DNA strands using an existing DNA template. They achieve this by adding building blocks called deoxyribonucleotides to the growing DNA chain, specifically at the free 3'-OH end. The synthesis of the new DNA strand occurs in a 5'-3' direction, ensuring the correct sequence alignment. For the enzyme to function properly, three main components are required:

- **Template Strand:** The DNA polymerase needs a template strand, which serves as a guide for synthesizing a complementary DNA strand.
- **Primer:** A short piece of RNA or DNA with a free 3'-OH group is necessary to initiate the synthesis process. The primer hybridizes with the template, forming a double-stranded region where DNA polymerization begins.
- **dNTPs:** A pool containing all four types of deoxyribonucleoside triphosphates (dNTPs) is essential. These nucleotide building blocks are used by the polymerase to assemble the new DNA strand according to the template sequence.

In addition to these requirements, DNA polymerases may need cofactors such as Mg^{2+} ions, which help stabilize the enzyme and facilitate its activity. Optimal conditions, including the correct pH level, are also necessary for the polymerase to function efficiently.

Types of DNA polymerases- In rDNA technology or genetic engineering, various types of DNA polymerases are utilized for different purposes, these include, DNA polymerase I, Klenow Fragment, Thermostable DNA Polymerase, and reverse transcriptase; explained as follows:

- **DNA Polymerase I (Pol I)-** DNA polymerase I is prepared from *E. coli* and possesses both DNA polymerase and DNA nuclease activities. It binds to the 'nick' region of DNA, synthesizing a complementary strand for the nick while degrading the pre-existing strand with its exonuclease activity. These activities are exhibited by different domains of *E. coli* Polymerase I. The C-terminal is responsible for the polymerase activity whereas the N-terminal of the enzyme catalyses the 5'-3' exonuclease activity. The central region of the enzyme, distinct from both the C-terminal and N-terminal domains, houses the 3'-5' exonuclease activity. This enzymatic function serves as a proofreading mechanism by allowing the enzyme to remove misincorporated or misread nucleotides from the 3' end of the growing DNA strand during replication.
- **Klenow Fragment-** When *E. coli* Pol I holoenzyme is treated with a mild protease, it splits into two fragments. The larger fragment retains both 5'-3' polymerase and 3'-5' exonuclease activities, while the smaller fragment only has 5'-3' exonuclease activity. This larger fragment is known as

the Klenow fragment. This fragment can synthesize a new DNA strand complementary to the template but lacks the 5'-3' exonuclease activity present in the full-length DNA polymerase I enzyme. It is vastly used in rDNA technology as well as DNA sequencing.

- **Thermostable DNA Polymerase-** These enzymes are derived from thermophilic bacteria or archaea and are heat-resistant, allowing DNA amplification through techniques like polymerase chain reaction (PCR) under high-temperature conditions without denaturation. Examples include Taq Polymerase which is isolated from thermophilic bacteria *Thermus aquaticus* and remains functional at high temperatures of PCR reactions.
- **Reverse Transcriptase (RT)-** These enzymes are found in RNA viruses or retroviruses. RT is an RNA-dependent DNA polymerase involved in converting RNA genomes into DNA and integrating them into host cells. RT utilizes mRNA as a template to synthesize complementary DNA (cDNA). Additionally, RT exhibits RNaseH activity which is responsible for degrading the RNA strand from DNA-RNA hybrids. In genetic engineering, RT is utilized to generate cDNA from mRNA, which can then be cloned into expression vectors for protein expression.

8.6 rDNA TECHNOLOGY IN ENVIRONMENT MANAGEMENT

rDNA technology plays a crucial role in environmental management, particularly in the field of environmental biotechnology. By harnessing the power of genetically modified microorganisms, it offers innovative solutions for waste management, pollution monitoring, and bioremediation efforts. The main objective is to isolate the genes responsible for bioremediation in these bacteria, modify them, and incorporate them into suitable hosts, such as *Escherichia coli*, to create effective bioremediation agents. genes responsible for bioremediation in these bacteria, modify them, and incorporate them into suitable hosts, such as *Escherichia coli*, to create effective bioremediation agents. The microorganisms commonly utilized in bioremediation are *Pseudomonas putida* and *Nitrosomonas europaea*.

Here's a comprehensive overview of how rDNA technology is applied in environmental management:

- **Bioremediation enhancement-** Waste Degradation Efficiency: rDNA technology enhances the efficacy of bioremediation processes by improving the metabolic capabilities of microorganisms. Genetically engineered strains can efficiently degrade complex chemicals and process higher volumes of waste materials.
- **Bio-indicators development-** Genetically modified bacteria, known as bioluminescent biosensors, emit light in response to specific chemical pollutants. These bio-indicators are valuable tools for detecting hazardous substances in the environment, aiding in pollution monitoring efforts.
- **Genetic Sensors-** Genetic sensors are being developed to detect various chemical contaminants in groundwater. By monitoring the degradation of pollutants, these sensors provide insights into natural remediation processes.
- **Bioluminescent Biosensors-** These are used for the detection of hazardous chemicals in water and soil. By linking the Mercury Resistance Gene (mer gene) with bioluminescence genes, biosensor cells can detect low levels of inorganic mercury in contaminated waters and soils. The emitted light is measured using fiber-optic fluorometers. Likewise, Toluene Degradation Gene (tol gene) can be combined with bioluminescence genes to detect toluene contamination in the environment.

- **Bioremediation Agents-** *Pseudomonas putida* and *Nitrosomonas europaea*: These microorganisms are commonly utilized in bioremediation efforts. The goal is to isolate genes responsible for bioremediation in these bacteria, modify them, and incorporate them into suitable hosts, such as *Escherichia coli*, to create effective bioremediation agents.

In conclusion, rDNA offers promising solutions for environmental challenges by enhancing bioremediation processes, developing innovative bio-indicators, and facilitating pollution monitoring efforts. However, careful regulation and monitoring are essential to ensure the safe and responsible application of genetically modified organisms in environmental management.

8.7 SUMMARY

rDNA technology is a fundamental tool in modern biotechnology, allowing scientists to manipulate genetic material. It involves several key steps, such as isolating DNA, cutting it using specific enzymes called restriction enzymes, joining DNA fragments together using polymerases, and inserting the modified DNA into host organisms. This process facilitates the creation of genetically modified organisms with desired characteristics. In environmental management, rDNA technology is vital for bioremediation programs, where microorganisms are used to remove contaminants from different environments. Genetically modified bacteria also function as bio-indicators, helping to detect harmful pollutants. However, releasing modified organisms requires careful monitoring to prevent unintended consequences, such as disrupting natural ecosystems. Despite these challenges, rDNA technology holds great promise for addressing environmental issues while ensuring responsible usage.

8.8 TERMINAL QUESTIONS

Q.1 Define different types of DNA polymerases.

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Q.2 Differentiate between type I, II, III, and IV classes of restriction enzymes.

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Q.3 Explain the mechanistic action of restriction enzymes.

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Q.4 Define the major steps involved in gene cloning or rDNA technology.

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Q.5 Define the Klenow fragment and how it is different from *E. coli* Polymerase I holoenzyme.

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Q.6 How rDNA technology be employed for environment bioremediation?

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8.9 SUGGESTED READINGS

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UNIT-9 : GENE EXPRESSION

Structure

9.1 Introduction

Objective

9.2 Gene Concept

9.2.1 Classical Concept of Gene

9.2.2 Neoclassical Concept of Gene

9.2.3 Modern Concept of Gene

9.3 Allele Concept

9.4 Basic Concepts in Genetics

9.5 Inheritance

9.6 Mendelian Genetics

9.7 Reasons for Choosing *Pisum sativum*

9.7.1 Mendel's monohybrid cross

9.7.2 Mendel's dihybrid cross

9.8 Mendel's Law of Inheritance

9.8.1 Law of Dominance

9.8.2 Law of Segregation

9.8.3 Law of Independent Assortment

9.9 Quantitative Genetics

9.10 Multiple Factor Hypothesis

9.11 Polygene Inheritance

9.12 Sex chromosome

9.13 Structure of Sex Chromosomes

9.14 Sex-linked Inheritance

9.14.1 Sex-limited Inheritance

9.14.2 Sex-influenced Inheritance

9.14.3 Sex-linked Diseases

9.15 Summary

9.16 Terminal Questions

9.17 Further Suggested Readings

9.1 INTRODUCTION

Genetics is a fundamental branch of biological science that involves the study of the intricate mechanisms governing heredity and variation within organisms and explaining the transmission and expression of genetic material across successive generations. The origin of genetics dates back to the pioneering work of Gregor Johann Mendel during the 1860s, who is regarded as the "Father of Genetics". Mendel's ground-breaking experiments with pea plants laid the foundation for the understanding of modern genetics. He described the hereditary units as "factors" rather than employing the term "genes".

The term "gene" was later coined by Danish botanist Wilhelm Johannsen in 1905, marking a pivotal moment in genetic terminology. Before this, various hypotheses were suggested to elucidate the nature of these hereditary entities. In 1906, William Bateson and Reginald Crundall Punnett (R.C. Punnett) introduced the concept of linkage in sweet peas, proposing the presence or absence theory, which suggested that dominant characters possessed determiners while recessive characters lacked them.

Mendel's experiments on hybridization provided clear evidence for the transmission of hereditary traits from one generation to the next through germ cells. These experiments helped to establish a strong connection between genes, the units responsible for heredity, and the observable traits exhibited by organisms. Following Mendel's pioneering work, subsequent experimental efforts by researchers provided compelling evidence that genes are segments of the DNA molecule. This discovery solidified DNA's central role as the primary carrier of genetic information within cells. Independently, Sutton and Boveri proposed the idea that chromosomes act as the storage units for hereditary information. This concept gained further support from Thomas Hunt Morgan's particulate gene theory in 1926. According to Morgan's theory genes as linearly arranged entities along the length of chromosomes, drawing a parallel to "beads on a string" like structure. This analogy illustrates the orderly arrangement of genes on chromosomes. This theory illustrated that the chromosome serves as a molecular expressway housing hundreds or even thousands of genes which are positioned at specific locations known as loci. These loci act as genetic addresses, determining the precise positions of genes along the chromosome. This model provided a framework for understanding the organization and transmission of genetic information within cells, laying the groundwork for further advancements in genetics and molecular biology. As genetic understanding continued to evolve, Belling proposed an important idea in 1928, suggesting that granular structures observed within chromosomes were indicative of genes. This proposition sparked further

interest and investigation within the field of cytology, the study of cells and their structures, leading to subsequent research that provided supporting evidence for Belling's hypothesis. This ground-breaking concept contributed to our growing understanding of the complex organization and function of genes within chromosomes, shedding light on the molecular mechanisms underlying heredity and genetic inheritance.

Morgan's revolutionary contributions to genetic theory were honoured with the Nobel Prize in 1933, marking a significant milestone in the field of genetics. However, the game-changing discovery of DNA as the primary repository of genetic information covered some aspects of Morgan's earlier theory. This pivotal discovery highlighted the necessity of merging classical genetic theories with cutting-edge molecular insights. Synthesizing traditional and modern perspectives is vital for gaining a thorough understanding of genes and their complex functions in controlling hereditary characteristics and diversity across successive generations. This highlights the ever-evolving nature of scientific exploration and the ongoing enhancement of our comprehension of genetics.

Objectives

After reading this unit, the learner will be able

- To understand Gene Concept
- To discuss Mendel's Law of Inheritance
- To Study Mendelian Genetics
- To understand the structure of sex chromosomes
- To discuss Sex-linked Inheritance
- To discuss concept of Polygene Inheritance

9.2 GENE CONCEPT

The concept of the gene has evolved significantly throughout history, with distinct periods marking its progression. In the classical era, genes were seen as discrete units of inheritance responsible for observable traits in offspring. The neoclassical phase introduced the identification of DNA as the physical basis of inheritance and further divided the gene into components like mutons and recons, associated with specific nucleotide sequences. The modern concept, emerging in the 1970s, integrates DNA technologies into genetic research, recognizing diverse gene structures and functions revealed by modern methodologies. This includes phenomena such as repeated genes, alternative splicing, and complex promoters. While classical genetics predates the Central Dogma Concept of 1941, studies in genetics and molecular biology thereafter contribute to the neoclassical and modern understanding of the gene.

9.2.1 CLASSICAL CONCEPT OF GENE

The important research conducted by Gregor Mendel on *Pisum sativum* in 1865 and its subsequent rediscovery by Carl Correns, Hugo de Vries, and Erich von Tschermak in 1900 are foundational to the field of classical genetics. The formulation of the classical concept of the gene is largely attributed to the pioneering work of Thomas Hunt Morgan, together with Calvin Blackman Bridges, Hermann Joseph Muller, and Alfred Sturtevant. Their proposal of the chromosomal theory of inheritance revolutionized genetic understanding by positing that genes are arranged on chromosomes like beads on a string structure. Morgan's explanation of genetic linkage stands as a landmark achievement in the field. Additionally, the contributions of Sutton and Boveri in 1903 are notable in

classical genetics, as their research provided further support for the chromosomal theory of inheritance. They proposed that the principles outlined by Mendel's Laws find explanation and validation in the behaviour of chromosomes during the process of meiosis.

The foundational principles of classical genetics elucidate the following key concepts:

- Genes are hereditary units that can be passed from parent organisms to their offspring.
- Genes are found in pairs within organisms and are situated on chromosomes. Each species possesses a specific, fixed number of chromosome pairs.
- Chromosome pairs are referred to as linkage groups, and each chromosome carries a multitude of genes. Genes located on the same chromosome are considered linked genes.
- Genes are arranged linearly on chromosomes, similar to beads on a string. Each gene occupies a distinct position on a chromosome, known as a locus. Changes in gene position can result in alterations in observable traits.
- Genes are discrete entities inherited according to Mendelian principles, residing at specific loci on chromosomes and controlling the expression of particular phenotypic traits. Interactions between multiple gene pairs can influence trait expression.
- Gene pairs, or alleles, separate during gamete formation, with only one allele from each pair entering a gamete, as dictated by Mendel's law of segregation.
- Gene pairs situated on different chromosomes undergo independent assortment during inheritance. Since the number of genes in an organism exceeds the number of chromosomes, multiple genes are present on each chromosome.
- Genes located on the same chromosome pair may exchange positions between homologous chromosomes through processes such as crossing over or translocation.
- Genes are susceptible to sudden changes in position and composition known as mutations. They are linearly arranged on chromosomes, with their order remaining unchanged unless affected by a mutation.
- Genes possess the ability to self-duplicate, generating exact copies of themselves. They can combine with other genes or replicate during cell division processes.

9.2.2 NEOCLASSICAL CONCEPT OF GENE

The neoclassical era was a period of intensive examination of the nature of genetic material. Notably, researchers such as Oliver (1940) and Lewis (1941) observed intragenic recombination in *Drosophila melanogaster*, marking a pivotal transition towards a new understanding of the gene. Intragenic recombination refers to the exchange of genetic material within a single gene. Unlike traditional genetic recombination, which occurs between different genes or chromosomes, intragenic recombination involves the swapping of segments within the same gene locus. This phenomenon was initially observed in experiments involving *Drosophila melanogaster*, a commonly studied model organism in genetics research. The observation of intragenic recombination challenged previously held notions about the stability and immutability of genes. It demonstrated that genes are not static entities but can undergo internal rearrangements, leading to the generation of novel genetic variants. This realization had profound ideas for our understanding of genetic diversity, evolution, and the mechanisms

underlying genetic inheritance. The discovery of intragenic recombination prompted a re-evaluation of classical genetic principles and paved the way for the development of more sophisticated models of gene structure and function.

During the neoclassical era of genetics, researchers Dounce (1952) and Gamow (1954) introduced the colinearity hypothesis, a revolutionary concept that transformed our understanding of genetic information and protein synthesis. This hypothesis proposed a direct correspondence between the linear arrangement of nucleotides in DNA and the linear sequence of amino acids in proteins. In core, it suggested that the sequence of nucleotides along a DNA strand determines the sequence of amino acids in the resulting protein. The colinearity hypothesis provided deep insights into the mechanisms governing protein synthesis. It implied that the genetic code which is embedded within the linear sequence of nucleotides in DNA, serves as a blueprint for the assembly of proteins. Each triplet of nucleotides, known as a codon, corresponds to a specific amino acid, thereby determining the sequence of amino acids in the synthesized protein. Moreover, the colinearity hypothesis laid the groundwork for subsequent research into the molecular mechanisms of gene expression, including transcription and translation. It provided a framework for deciphering the genetic code and elucidating the intricate processes underlying protein synthesis. Overall, the introduction of the colinearity hypothesis represented a significant milestone in the field of molecular genetics, shaping the trajectory of genetic research for decades to come.

The revolutionary study by Avery, MacLeod, and McCarty in 1944 provided experimental evidence demonstrating that DNA is the material responsible for bacterial transformation. The phenomenon of bacterial transformation was first observed by Frederick Griffith in 1928 which involves the uptake and incorporation of foreign genetic material by bacterial cells. Griffith's experiments with *Streptococcus pneumoniae* demonstrated that non-pathogenic bacteria could be transformed into pathogenic strains when exposed to heat-killed pathogenic bacteria. Building upon Griffith's work, Avery and his colleagues (1944) sought to determine the nature of the transforming substance. Therefore, through a series of experiments, they systematically isolated various cellular components from the pathogenic strain of bacteria and tested their ability to induce transformation in non-pathogenic strains. Their results conclusively demonstrated that DNA, and not proteins or other cellular components, was responsible for the observed genetic transformation.

Furthermore, Hershey and Chase's ground-breaking experiment in 1952 provided additional confirmation of DNA's role as the carrier of genetic information. They proved this using bacteriophage, viruses that infect bacteria, and demonstrated that during viral infection, it is the viral DNA, rather than the viral proteins, that is transferred into the bacterial host cell. Their use of radioactive labelling techniques allowed them to track the movement of viral genetic material and conclusively establish DNA as the hereditary material.

Together, these landmark studies by Avery et al. and Hershey and Chase provided compelling evidence that DNA is the molecule responsible for transmitting genetic information from one generation to the next. Their findings proved DNA's central role in genetics and laid the groundwork for the molecular revolution that followed in the field of biology.

9.2.3 MODERN CONCEPT OF GENE

Modern genetics concept involves the foundational principles of the central dogma of life to contemporary molecular genetics which encompasses numerous essential discoveries. Among these, significant milestones include Beadle and Tatum's influential proposal of the "one gene, one enzyme" hypothesis, Watson and Crick's ground-breaking elucidation of the structure of DNA, and Paul Berg's pioneering advancements in genetic engineering techniques.

In addition to these achievements, the seminal contributions of Seymour Benzer stand as bases in the field of molecular biology. Benzer's research, particularly his investigations into bacteriophages, yielded invaluable insights into the nature of genetic mutations. Through meticulous experimentation, Benzer was able to identify and map hundreds of mutations within these viral genomes, providing crucial knowledge about the genetic makeup of these organisms.

In 1957, Benzer introduced a novel conceptual framework for categorizing genes and genetic material with chromosomes. He proposed three fundamental units—Cistron, Muton, and Recon—which delineate different aspects of genetic phenomena. This classification system, based on the underlying genetic processes, offered a deeper understanding of the relationship between genes and chromosomes, laying a solid foundation for further exploration in molecular genetics. Following is the generalized explanation of these units:

Cistron- Cistron is defined as the segment of DNA that codes for a single polypeptide chain. A cistron serves as a functional unit within the DNA sequence, transmitting genetic information from one generation to the next. Cistrons vary in length, ranging from approximately 100 to 30,000 nucleotide pairs. For example, in *Escherichia coli*, a cistron may contain up to 1500 base pairs, although some cistrons consist of a larger number of nucleotides.

Initiation of each cistron is marked by a specific start codon, while termination occurs with a distinct stop codon. This description ensures the accurate transcription of the cistron into functional mRNA, which subsequently guides the synthesis of the corresponding polypeptide chain. In summary, cistrons play a vital role in gene expression by encoding the information necessary for the formation of specific protein products.

Muton- The term "muton" refers to the smallest chromosomal unit capable of undergoing mutation. It represents the fundamental unit of genetic change, as alterations within the muton are adequate to induce a visible change in phenotype. Specifically, the muton corresponds to a segment of genetic material (DNA) that is susceptible to mutation, thereby giving rise to a mutant phenotype.

The muton is defined at the nucleotide level, encompassing either a single nucleotide or a set of nucleotide pairs within the DNA molecule. Even an ultra-small alteration within the muton sequence can have deep effects on the resulting phenotype, explaining the critical role of mutons in genetic variation and inheritance. Thus, the muton serves as the molecular basis for genetic mutations and their phenotypic consequences.

Recon- The term recon denotes the smallest segment of DNA capable of undergoing separation and exchange with another chromosome. It serves as the fundamental unit of recombination, representing the genetic material that can be detached from other units through genetic recombination events. Typically, a recon comprises no more than two pairs of nucleotides, making it a compact unit of genetic material composed of one to two nucleotides.

Advancements in research techniques have expanded classical and neoclassical genetics, leading to the emergence of the modern concept of the gene. This contemporary understanding of genes encompasses various forms, including those outlined below. It is essential to note that while the modern concept of the gene has expanded our understanding, the classical principles established by Mendel and Morgan remain foundational and indisputably accurate in shaping our understanding of genetics.

9.3 ALLELE CONCEPT

The concept of alleles is pivotal in genetics, representing the various forms a gene can assume at a particular locus or position on a chromosome. These alternative versions originate from mutations or

genetic variations within a population, contributing to the diversity of observable traits, known as phenotypes, within organisms. Alleles play a crucial role in shaping an organism's characteristics and are fundamental to understanding inheritance patterns and genetic diversity within populations. Additionally, alleles can exhibit different levels of dominance or recessiveness, influencing the expression of traits in individuals.

Key aspects of the allele concept include:

Genetic variation- Genetic variation demonstrated by alleles represents the variety of genetic traits found within a group of organisms. These differences come about primarily in two ways: mutations and recombination. Mutations occur when there are changes in the DNA sequence of a gene, introducing new variations into the genetic code. Recombination happens during sexual reproduction when genetic material from two parents is mixed and shuffled, creating unique combinations of alleles in offspring. Understanding genetic variation is important because it helps to explain the diversity of traits we see in different individuals and populations, and it's essential for studying how organisms evolve and adapt to their environments over time.

Dominance and recessiveness- Alleles come in various strengths, determining how traits are expressed. In complete dominance, one allele fully overshadows the other, making its traits the only ones visible in individuals with two different alleles. However, in cases of incomplete dominance or codominance, both alleles have a say. In incomplete dominance, neither allele dominates completely, resulting in a blend of their traits in the individual. Conversely, in codominance, both alleles are expressed fully, showcasing their unique traits simultaneously. Recognizing these diverse patterns of allele interaction provides valuable insights into how genetic traits are inherited and expressed across generations.

Homozygosity and heterozygosity- Individuals can have different genetic compositions, reflected in their homozygosity or heterozygosity for a specific gene. Homozygosity occurs when an individual carries two identical alleles at a particular locus, represented as either AA or aa. On the other hand, heterozygosity arises when two different alleles are present at the same locus, denoted as Aa. The combination of alleles determines an organism's genetic makeup, known as its genotype. Recognizing these genetic states sheds light on the diversity and inheritance patterns within populations.

Multiple alleles- In genetics, multiple alleles refer to the existence of more than two different versions of a gene at a specific spot on a chromosome within a population. Unlike typical genetic situations where there are only two options for a trait, multiple alleles add more diversity and intricacy. Each individual still inherits two alleles for a gene—one from each parent. However, with multiple alleles, there are more than two variations of the gene in the population. A well-known example of multiple alleles is the ABO blood group system in humans. In this system, there are three main versions of the gene—IA, IB, and i—that determine whether someone has blood type A, B, or O, respectively. Each person gets two of these alleles, resulting in various blood type combinations. Multiple alleles add to the variety of traits we see in populations and can lead to different physical characteristics. Understanding how these alleles interact and are expressed is essential for understanding inheritance patterns and the rich diversity of traits in both humans and other living organisms.

9.4 BASIC CONCEPTS IN GENETICS

- Character/Trait: The observable features of an organism.
- Pure-breeding Line: An inbred line where a specific phenotype remains constant over many generations.
- Hybridization: The mating or crossing between two individuals with differing traits.
- Hybrid: Offspring resulting from the mating of two individuals with different traits.

- **Genotype:** The genetic makeup of an organism.
- **Phenotype:** The physical appearance or characteristics of an organism.
- **Gene:** The fundamental unit of heredity passed from one generation to the next.
- **Allele:** Alternative forms of a gene located at the same position on homologous chromosomes (Fig. 9.1).
- **Locus:** The specific location of a gene on a chromosome.
- **Homozygous:** Having two identical alleles at one or more loci (Fig. 9.1).
- **Heterozygote:** Having two different alleles for one or more genes (Fig. 9.1).
- **Monohybrid Cross:** A mating between individuals differing in a single trait.
- **Dihybrid Cross:** A mating between individuals differing in two traits.
- **Reciprocal Cross:** Two crosses involving the same parents but with reversed parental roles.
- **Testcross:** Crossing an F1 hybrid with an individual having the recessive phenotype.
- **F1 Generation:** The first filial generation resulting from the crossing of two different parents.
- **Dominant:** The expressed trait in a heterozygous condition.
- **Recessive:** The trait expressed in the homozygous condition in the F2 generation.

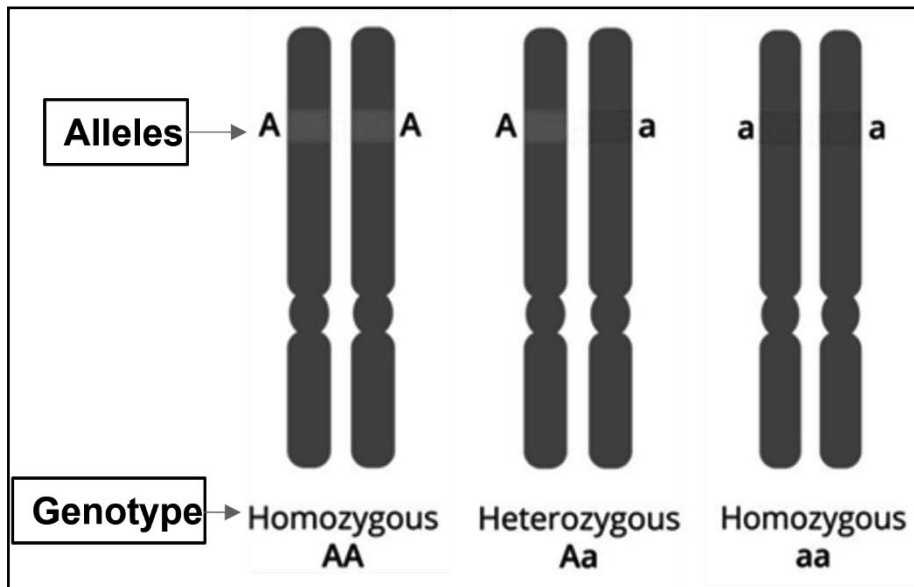


Fig.9.1 Homozygous and heterozygous allele

Source: <https://www.dreamstime.com/difference-homozygous-heterozygous-alleles-difference-homozygous-heterozygous-alleles-genotype-image248161823>

9.5 INHERITANCE

Inheritance is a fundamental biological process by which genetic material is transferred from parent organisms to their offspring or progeny. It involves the transmission of various traits, ranging from observable features like eye colour, hair texture, and height, to underlying biological functions such as metabolism and susceptibility to specific diseases. These traits are encoded within the DNA molecules found within the cells of organisms.

Inheritance follows the rules of genetics, which outline how traits are passed on and displayed in descendants. These principles were first elucidated by pioneering geneticist Gregor Mendel, who conducted experiments with pea (*Pisum sativum*) plants in the 19th century. Mendel's work paved the way for the understanding of inheritance patterns and the transmission of traits from one generation to the next.

9.6 MENDELIAN GENETICS

Gregor Johann Mendel, often referred to as the "Father of Genetics," was a pioneering scientist who laid the groundwork for our understanding of inheritance patterns long before the discovery of chromosomes. Born on July 22, 1822, in the village of Heinzendorf, now known as Hyncice in the Czech Republic, Mendel came from a family of peasant farmers.

Mendel's ground-breaking work began in 1856 when he conducted experiments with garden peas (*Pisum sativum*) over nine years. Despite facing limited resources and recognition, he meticulously bred pea plants and carefully observed their traits. In 1865, Mendel presented his findings to the Bruno Society for the Study of Natural Science, which were later published as "Experiments in Plant Hybridization" in The Annual Proceedings of the Natural History Society of Brunn in 1866. Unfortunately, his contributions went largely unnoticed during his lifetime.

It wasn't until 1900, sixteen years after Mendel's death at the age of 61 in 1884, that his work gained widespread recognition. Three botanists, Karl Correns of Germany, Hugo de Vries of the Netherlands, and Erich von Tschermak of Austria, independently rediscovered Mendel's laws and experiments. Their rediscovery marked the beginning of modern genetic research and helped establish Mendel's legacy in the field of genetics.

Mendel's original paper was later republished in 1901 in the journal 'Flora' (Vol. 89, Page 364), cementing his status as a foundational figure in the history of genetics. Today, Mendel's principles of inheritance continue to serve as the basis for understanding genetic traits and their transmission from one generation to the next.

9.7 REASONS FOR CHOOSING PISUM SATIVUM

Mendel selected garden pea (*Pisum sativum*) for his inheritance experiments because:

- Short life cycle allows for quick generation turnover.
- Self-pollination and bisexual nature simplify breeding.
- Clear, contrasting traits aid observation.
- Easy hybridization facilitates controlled experiments.

9.7.1 MENDEL'S MONOHYBRID CROSS

Mendel first performed a **monohybrid cross** which involved one pair of contrasting traits. For example, Monohybrid cross between parents with tall (TT) and dwarf (tt) height and dwarf pea plants, and carefully collected all the resulting seeds. From these seeds, he cultivated plants of the first hybrid generation, known as the F1 generation, where he noted that all plants exhibited tall height. Upon self-pollinating the F1 plants, Mendel observed a segregation of traits in the F2 generation, where some plants displayed dwarf characteristics alongside tall ones. Remarkably, he found that the ratio of dwarf plants to tall ones in the F2 generation was 1:3, with 1/4 being dwarf and 3/4 being tall. The monohybrid

ratio observed in the F₂ hybrids is 1:2:1 for genotypic and 3:1 for phenotypic traits. Fig.9.2 represents Mendel's monohybrid cross involving one pair of contrasting characters ie. height.

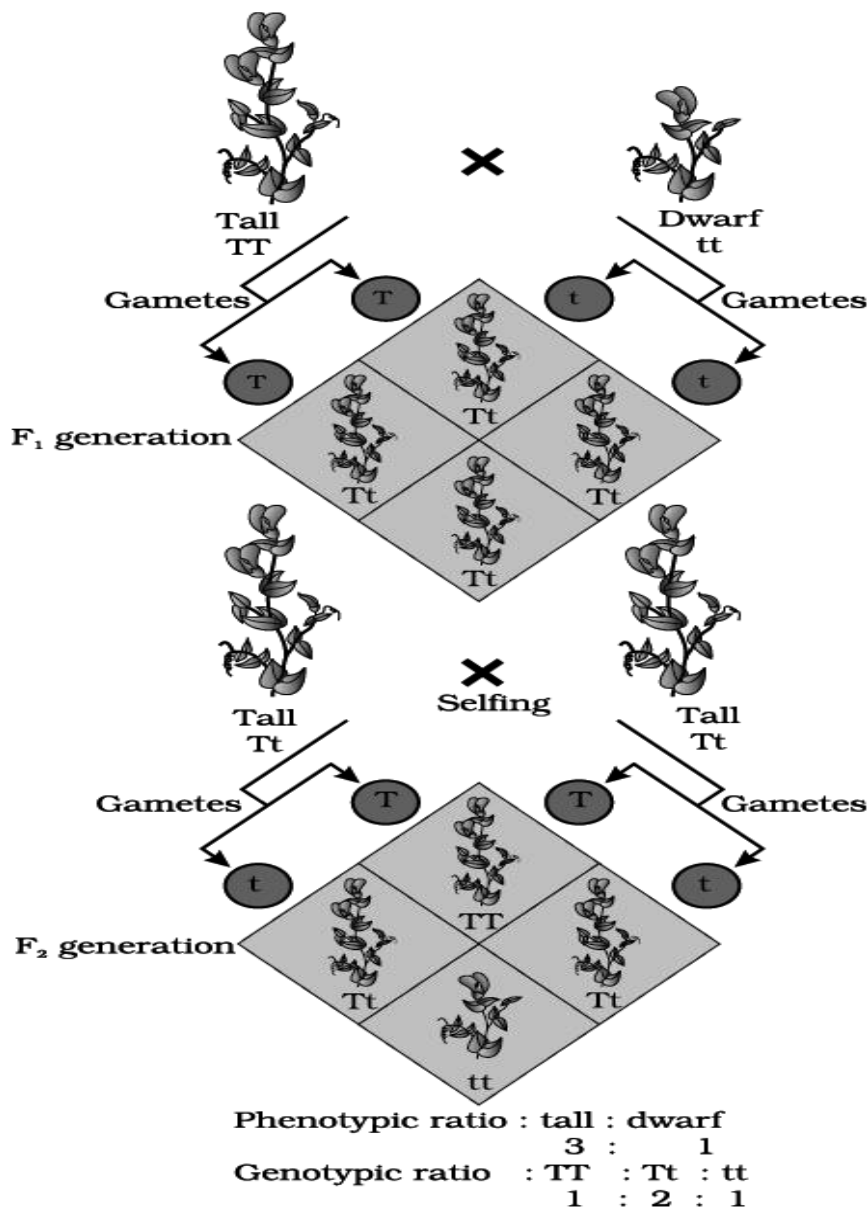


Fig.9.2Diagrammatic representation of Mendel's monohybrid cross

Source: Principles of Inheritance and Variation, Class 12 Biology, NCERT

9.7.2 MENDEL'S DIHYBRID CROSS

Mendel's **diybrid** cross involved the cross between parents that differed for two pairs of contrasting characters. For example, Mendel crossed two varieties of garden pea namely, round yellow cotyledons (RRYY) with wrinkled green cotyledons (rryy). The F₁ was the round yellow round type. When Mendel self-fertilized the F₁ plants to produce F₂ generation, four different types of plants were observed. The male and female gametes can combine in 16 possible ways as shown in Punnet's square. namely, 9/16 round yellow, 3/16 wrinkled yellow, 3/16 round green and 1/16 wrinkled green. Thus, the dihybrid phenotypic ratio is 9:3:3:1 (Fig. 9.3). While his monohybrid crosses resulted in a phenotypic

ratio of 3:1, dihybrid crosses showed a more complex 9:3:3:1 ratio. This ratio indicates that each pair of alleles segregates independently during gamete formation, resulting in various combinations of traits in offspring.

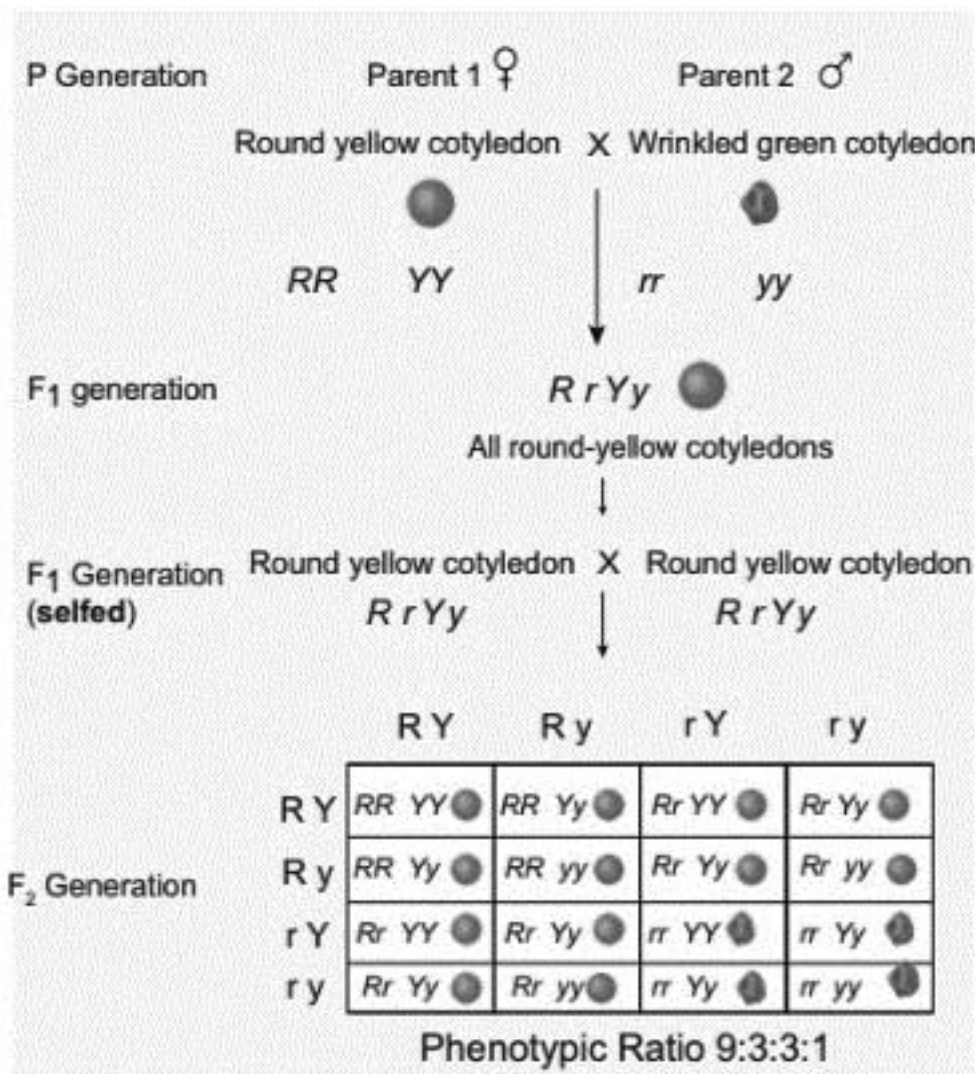


Fig. 9.3 Diagrammatic representation of Mendel's dihybrid cross involving two sets of contrasting traits seed shape and colour

Source: https://www.brainkart.com/article/Analysis-of-seed-sample-to-study-Mendelian-dihybrid-ratio_38342/

9.8 MENDEL'S LAW OF INHERITANCE

Mendel's pioneering experiments with pea plants led to the formulation of three fundamental laws that explain the inheritance patterns in diploid organisms. Mendel organized findings in the following mentioned three laws:

- Law of Dominance
- Law of Segregation
- Law of Independent Assortment

9.8.1 LAW OF DOMINANCE

The Law of Dominance states that:

- An individual's genetic makeup, or genotype is made up of the alleles it inherits.
- The observable traits, or phenotype, of an individual result from a combination of its alleles and environmental factors.
- The presence of an allele does not guarantee its expression in the individual.
- In cases where an individual inherits two different alleles for a particular trait (heterozygous condition), one allele determines the organism's appearance and is termed dominant, while the other allele has no observable effect and is termed recessive.
- Therefore, the dominant allele masks the phenotypic effects of the recessive allele.
- The Law of Dominance pertains to how alleles are expressed in the phenotype rather than how they are transmitted.
- Dominant alleles are represented by uppercase letters, whereas recessive alleles are represented by lowercase letters.

9.8.2 LAW OF SEGREGATION

The Law of Segregation states that:

- Every individual organism carries two alleles for each trait, which segregate during the formation of gametes (sex cells) through meiosis. Consequently, each gamete receives only one allele for each trait.
- Offspring inherit a pair of alleles for a trait, with one allele from each parent, through the transmission of homologous chromosomes during reproduction.
- During meiosis, the two alleles of a gene pair separate from each other, ensuring that each gamete has an equal chance of receiving either allele.

9.8.3 LAW OF INDEPENDENT ASSORTMENT

The Law of Independent Assortment states that alleles for different traits are inherited independently of each other during gamete formation and genetic recombination. In other words, the selection of an allele for one trait is not influenced by the selection of an allele for any other trait. Mendel observed evidence for this law in his dihybrid cross experiments, where he studied the inheritance of two different traits simultaneously.

9.9 QUANTITATIVE GENETICS

Quantitative genetics deals with the inheritance patterns and variability of traits that are not governed by the straightforward rules of Mendelian genetics. While Mendelian traits are usually controlled by one or a few genes with clear-cut dominant or recessive alleles, quantitative traits are far more intricate. They are governed by a combination of genetic and environmental factors, leading to a variety of phenotypic outcomes rather than discrete categories.

In contrast to Mendelian traits, which follow predictable inheritance patterns, quantitative traits exhibit continuous variation. This means that instead of distinct categories (like tall vs. short or wrinkled vs. smooth peas in Mendel's experiments), these traits can take on a range of values along a spectrum. For example, height in humans or yield in crops can vary continuously, with individuals displaying a wide range of heights or yields rather than fitting into distinct categories.

The complexity of quantitative traits arises from the interplay between multiple genetic factors and environmental influences. Unlike Mendelian traits, which are often controlled by single genes, quantitative traits are influenced by numerous genes, each exerting a small effect on the phenotype. Additionally, environmental factors such as nutrition, climate, and social conditions can further impact the expression of these traits.

Understanding quantitative genetics is crucial for decoding the genetic basis of complex traits and predicting their inheritance patterns. By studying the inheritance of quantitative traits, researchers can identify the underlying genetic factors contributing to phenotypic variation. This knowledge is invaluable for various fields, including agriculture, where breeders aim to improve crop yield or disease resistance, and medicine, where understanding the genetic basis of complex traits can lead to advancements in disease diagnosis and treatment.

9.10 MULTIPLE FACTOR HYPOTHESIS

Mendel's laws of heredity elegantly explain the distinct qualitative traits observed in plants and animals, such as flower color (red or white) and seed color (yellow or green). However, some traits are quantitative and show a range of characteristics or phenotypes, as observed in cultivated plants and domestic animals, such as seed yield, fruit yield, egg production, and milk/meat quantity. These traits are not precisely categorized into distinct classes and exhibit a continuous spectrum of variation rather than discrete categories. Instead, they exhibit a continuous spectrum of variation, ranging from the extremes of large to small, heavy to light, and so forth.

The multiple-factor hypothesis emerged to explain traits that show continuous variation, like weight, height, or intelligence, unlike Mendel's laws which account for distinct differences such as flower color or seed color. Swedish botanist H. Nilsson-Ehle and American E.N. East investigated this phenomenon and proposed that continuous traits are influenced by the combined action of multiple genes, each having a small effect on the trait. These genes, known as cumulative, additive, or polygenes, work together to shape the trait's expression. When similar or identical cumulative genes come together, they collectively impact the intensity or degree of the trait. This hypothesis suggests that quantitative traits are controlled by numerous genes, hence the term "polygenic inheritance." Examples include skin color in humans and corolla length in tobacco plants.

9.11 POLYGENE INHERITANCE

Polygenic inheritance refers to the inheritance of traits that are controlled by multiple genes, each contributing to the phenotype in an additive manner. Unlike traits governed by a single gene (monogenic traits), polygenic traits are influenced by the combined effects of several genes, along with environmental factors. This type of inheritance results in continuous variation in traits, such as height, weight, and number; that is why it is also called quantitative inheritance. These traits are heritable, meaning they can be passed down from one generation to the next.

Polygenic traits are controlled by polygenes, also known as minor genes. Each of these genes contributes a small effect to the overall expression of the trait. The more polygenes involved, the greater the intensity of the trait. Polygenic inheritance explains why these traits exhibit a wide range of

variations within a population and why they often follow a continuous distribution rather than distinct categories.

Characteristics of polygenes include:

- **Additive Effects-** Each gene involved in polygenic inheritance contributes additively to the phenotype. This means that the more alleles an individual has that promote a particular trait, the stronger the expression of that trait will be.
- **Equal Contribution-** In polygenic inheritance, each allele at a specific locus has an equal effect on the phenotype. Unlike in some other forms of inheritance where certain alleles may be dominant over others, polygenes exert their influence uniformly.
- **Absence of Dominance, Epistasis, and Linkage-** Unlike single-gene inheritance, polygenic traits do not exhibit dominance, epistasis (interaction between genes), or linkage (genes located close together on the same chromosome). Each allele contributes independently to the overall phenotype.
- **Environmental Influence-** While genetic factors predominantly determine polygenic traits, environmental factors can also play a role. However, the effect of the environment on polygenic traits is generally considered to be minor compared to the cumulative effect of multiple genes.

These features collectively contribute to the continuous variation observed in polygenic traits, such as height, skin color, and intelligence, across populations.

9.12 SEX CHROMOSOME

Chromosomes are essential structures located in the nucleus of cells, consisting of long strands of DNA, the genetic material that carries genes. DNA is vital for constructing and regulating the human body's functions. Within chromosomes, proteins help maintain DNA's structure and function properly. Typically, chromosomes occur in pairs, and a human cell typically contains 22 pairs along with X and Y chromosomes, totaling 46 chromosomes. These chromosomes are inherited from both parents, with one set originating from the mother and the other from the father. Chromosomes are classified as sex chromosomes and autosomal chromosomes. Chromosome pairs 1-22 are termed autosomal chromosomes while the X and Y are termed sex chromosomes.

Sex chromosomes, namely the X and Y chromosomes in humans, are essential in determining the biological sex of a person (Fig. 9.4a). The presence of two X chromosomes typically signifies a female (XX), while an XY configuration denotes a male. Although the X and Y chromosomes share a small region of similarity allowing them to pair during meiosis, the Y chromosome is notably shorter and contains fewer genes.

In terms of gene content, the X chromosome houses around (800-900) protein-coding genes, responsible for a wide range of functions. In contrast, the Y chromosome harbors approximately (60-90) protein-coding genes, with about half being active solely in the testes, the organs responsible for sperm production. The human Y chromosome plays a crucial role in embryo development, primarily attributed to a gene called SRY (sex-determining region of Y) (See Fig. 9.4b). SRY, located on the Y chromosome, encodes a protein that initiates the activation of other genes essential for male development. Therefore:

Embryos with two X chromosomes lack SRY and develop as females.

Embryos with an XY configuration possess SRY and develop as males.

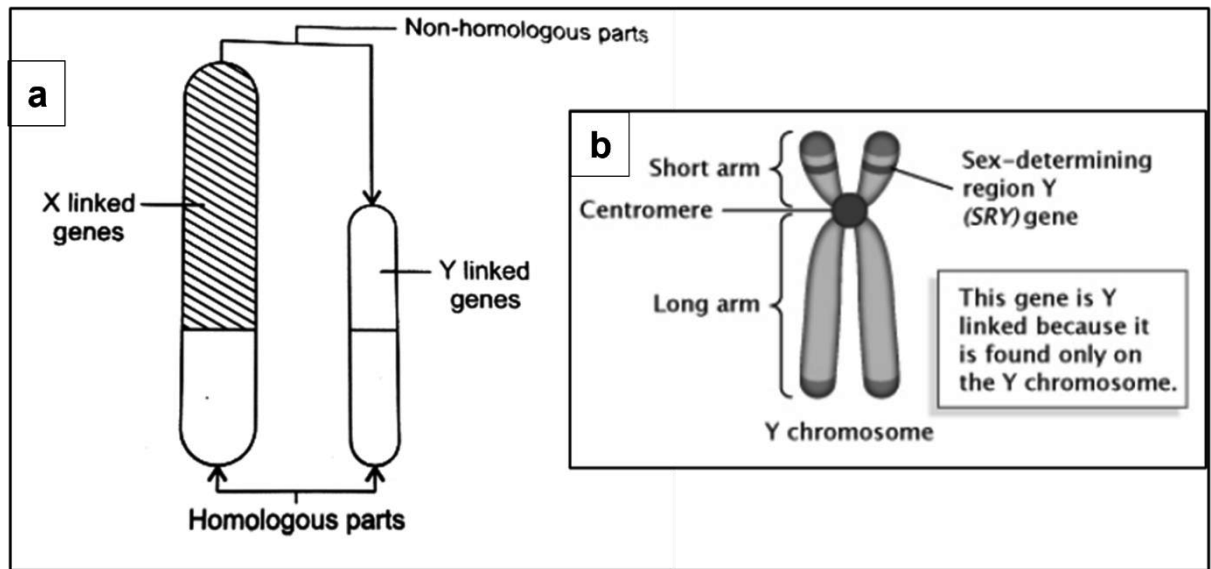


Fig.9.4 (a) Structure of X and Y chromosomes. (b) genes located on Y chromosomes

Source: <https://www.nature.com/scitable/topicpage/genetic-mechanisms-of-sex-determination-314/>;
<https://www.doubtnut.com/qna/102374704>

9.13 STRUCTURE OF SEX CHROMOSOMES

X and Y chromosomes exhibit distinct structural features:

- The X chromosome appears as a straight, rod-like structure, longer than the Y chromosome.
- While the X chromosome is metacentric, the Y chromosome is acrocentric.
- Euchromatin, which is the less condensed DNA region in a chromosome, constitutes a larger portion of the X chromosome, whereas heterochromatin, the highly condensed DNA region, dominates the Y chromosome.
- The abundance of DNA material in euchromatin renders the X chromosome genetically more active.
- Conversely, the Y chromosome, with a smaller euchromatin fraction and larger heterochromatin portion, is genetically less active or inert.
- Both chromosomes feature homologous and non-homologous regions.
- Homologous regions contain similar genes, while non-homologous regions harbor dissimilar genes.
- Crossing over-exclusively occurs between homologous regions of X and Y chromosomes.
- The non-homologous region of the X chromosome is lengthier and encompasses more genes compared to the non-homologous region of the Y chromosome.

- X-linked genes reside on the non-homologous region of the X chromosome, whereas Y-linked genes occupy the non-homologous region of the Y chromosome.

9.14 SEX-LINKED INHERITANCE

Characters for which genes are located on sex on X and Y chromosomes are known as sex-linked traits. Genes controlling these traits are called sex-linked genes. The genes that occur exclusively on the X chromosome (mammals, *Drosophila*, *Melandrium*, etc.) are called X-linked genes. The genes that exclusively occur in the Y chromosome are called holandric genes. The inheritance of X and holandric genes is called sex-linked inheritance.

Sex-linked genes can be classified into the following three types

- X-linked inheritance- The genes that are located in the non-homologous sections of the X chromosome and have no corresponding allele in the Y chromosome are called X-linked genes. The inheritance of these genes is called X-linked inheritance.

The females are homogametic with two XX chromosomes while males are heterogametic with one X and one Y chromosome. The males express the sex-linked recessive trait with a single gene but females can express such a trait only if it has two genes for it.

X-linked inheritance can be recessive or dominant. X-linked recessive inheritance is more common in males as they have only one X chromosome; examples include Haemophilia A and B. On the contrary, X-linked dominant inheritance is seen in both males and females, examples include *Incontinentia pigmenti*.

- Y-linked inheritance- The genes located in the non-homologous region of the Y-chromosome and have no homologous allele in the X-chromosome are termed Y-linked genes. The inheritance of these genes is called Y-linked inheritance, The Y-linked genes are confined to males only and are also called holandric genes. These genes are transmitted directly from father to son, generation after generation. Examples: Hypertrichosis of the ears, webbed toes, and porcupine man are examples of Y-linked inheritance in humans. Hypertrichosis of the ears (or hairy ears) is a condition wherein there is a conspicuous growth of hair on the outside rim of the ear. Webbed toe condition is characterized by having a web-like connection between the second and third toes. Porcupine man is a condition when the skin thickens and gradually becomes darker, scaly, rough, and bristle-like outgrowths.
- XY-linked inheritance- The genes located in the homologous region of both X and Y chromosomes are called XY-linked genes; and their inheritance is called XY-linked inheritance. These genes are inherited like the autosomal genes.

9.14.1 SEX-LIMITED INHERITANCE

Sex-limited genes, found on autosomes (non-sex chromosomes), express themselves based on the presence or absence of particular sex hormones, resulting in their manifestation in only one sex. These genes regulate traits such as secondary sexual characteristics, including beard growth, deepening of the male voice, and musculature development.

For example, although both males and females possess genes related to beard and mammary gland development, sex hormones dictate their expression differently. Females, due to hormonal factors, do not develop beards, whereas males do not develop fully developed breasts. Similarly, traits like milk production in cattle are sex-limited, with both sexes possessing the genetic capacity for milk production

but expressing it only in females. This phenomenon extends to other species, where certain traits like plumage or feathering patterns in birds are determined by sex-limited genes.

9.14.2 SEX-INFLUENCED INHERITANCE

Sex-influenced inheritance refers to traits governed by autosomal genes following Mendelian inheritance principles but exhibiting different expressions between males and females.

For example, consider the inheritance of baldness in humans. The gene B associated with baldness is dominant in males but recessive in females. In heterozygous individuals, where one allele is dominant and the other is recessive, the trait only manifests in the presence of male androgenic hormones, primarily found in males.

9.14.3 SEX-LINKED DISEASES

Sex-linked diseases, also known as X-linked diseases, are genetic disorders caused by mutations in genes located on the X chromosome. Since males have only one X chromosome (XY), while females have two (XX), sex-linked diseases are more commonly observed in males. This is because males inherit their X chromosome from their mothers and the Y chromosome from their fathers. Therefore, if a male inherits a mutated X chromosome carrying a disease-causing allele, he will likely develop the disorder because he lacks a second X chromosome to compensate for the mutation.

Some common examples of sex-linked diseases include:

1. **Hemophilia-** A bleeding disorder characterized by impaired blood clotting due to deficiencies in clotting factors, such as Factor VIII or Factor IX.
2. **Color blindness-** A condition where individuals have difficulty distinguishing certain colors due to mutations in genes responsible for color vision, such as the *OPN1LW* and *OPN1MW* genes.
3. **Duchenne muscular dystrophy (DMD)-** A progressive muscle-wasting disorder caused by mutations in the *DMD* gene, leading to the absence of dystrophin, a protein essential for muscle function.
4. **Fragile X syndrome-** A genetic disorder causing intellectual disability and behavioral challenges, caused by an expansion of the CGG triplet repeat in the *FMR1* gene on the X chromosome.
5. **X-linked agammaglobulinemia (XLA)-** A primary immunodeficiency disorder characterized by a lack of mature B cells and the inability to produce antibodies, caused by mutations in the *BTK* gene.

Females can also inherit and express X-linked diseases, but they are less common because they have two X chromosomes, providing a backup copy of the gene on the unaffected X chromosome. In females, the disorder typically manifests if both X chromosomes carry the mutated gene or if lyonization (random inactivation of one X chromosome) results in the inactivation of the healthy X chromosome, leaving only the mutated X chromosome active.

9.15 SUMMARY

The chapter on gene expression provides a comprehensive overview of key concepts in genetics,

beginning with an introduction to the fundamental principles. It delves into the classical, neoclassical, and modern concepts of genes, elucidating the evolution of our understanding. The allele concept is explored, followed by an examination of basic genetics principles and inheritance mechanisms. Mendelian genetics is discussed in detail, covering Mendel's experiments with *Pisum sativum* and his laws of inheritance, including dominance, segregation, and independent assortment. Quantitative genetics, multiple factor hypothesis, and polygene inheritance are also examined. The chapter further delves into sex chromosomes, their structure, and sex-linked inheritance, including sex-limited and sex-influenced inheritance. Finally, the discussion extends to sex-linked diseases, providing insights into genetic disorders associated with sex chromosomes.

9.16 TERMINAL QUESTIONS

Q.1 Write a descriptive note on gene concept.

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Q.2 Define Mendel's laws of inheritance.

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Q.3 Explain the multiple factor hypothesis.

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Q.4 What is polygene inheritance?

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Q.5 What are sex chromosomes and elucidate their structure

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Q.6 What are sex-linked diseases? Explain any two with examples.

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Q.7 What is the function of the *SRY* gene and where it is located?

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9.17 SUGGESTED READINGS

- Robert J. Brooker, Genetics: Analysis and Principles, McGraw Hill, 7th Edition (2020).
- Principles of Genetics, Wiley, 8th Edition (2006).
- Rickki Lewis, Human Genetics, McGraw Hill, 12th Edition (2020).
- Eldon John Gardner, Michael J. Simmons, D. Peter Snustad, 8th Edition (2006).
- T.A. Brown, Genetics: A Molecular Approach, Garland Sciences, 1st Edition, (1999).

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