Table 7.3: Difference between Capsule and Slime layer

Capsule	Slime layer
Capsule is a glycocalyx layer, consisting of firmly associated polysaccharide molecules with the cell wall.	Slime layer is a glycocalyx layer that consists of loosely associated glycoprotein molecules.
It is a well-organized layer, difficult to be washed off.	It is an unorganized layer and can be easily washed off.
It is tightly bound to the cell wall.	It is loosely bound to the cell wall.
It is thicker than slime layer.	It is a thin glycocalyx layer.
It acts as a virulence factor that helps to escape phagocytosis.	It mainly helps in adherence. It protects the cell from dehydration and nutrient loss.

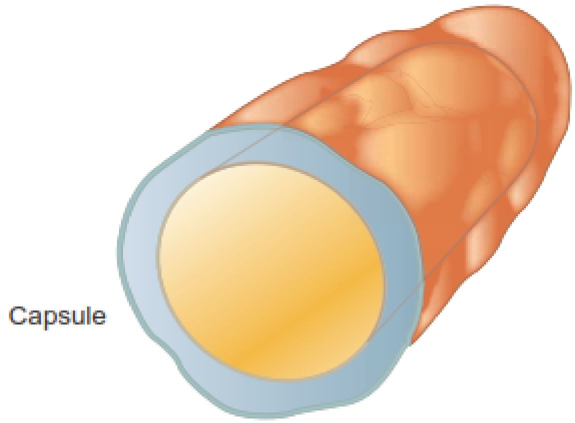


Figure 7.6: Structure of capsule

The role of the capsule varies depending on the bacterium.

- A thick capsule protects cells from dehydration.
- Capsules protect the pathogenic bacteria from being engulfed and destroyed by white blood cells (phagocytes).
- Capsules are virulence factors of many pathogenic bacteria, such as *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Bacillus anthracis*. Encapsulated bacterial cells generally have greater virulence.

Slime layer

Some bacteria are covered with a surface layer that is loosely distributed around the cell and diffuses into the medium, this surface layer is referred to as slime layer. (Figure 7.7) The slime layer is a structure that is easily washed off. Slime layer protects bacteria from loss of water and nutrients. Slime has little affinity for basic dyes and is ivisible in Gram stained smears.

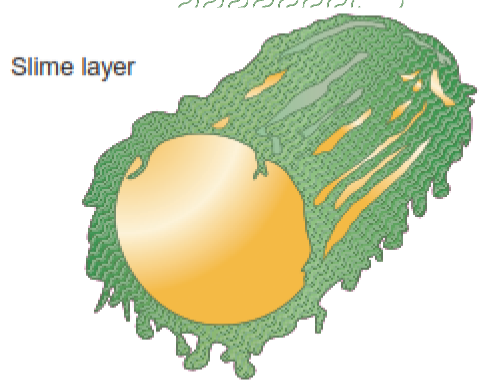


Figure 7.7: Structure of slime layer

7.2.3 Other Appendages

Sheath

Sheathed bacteria are bacteria that grow as long filaments in the form of chain or trichome. These bacteria are enclosed by a hollow tube like structure known as sheath

(Figure 7.8). Within the shealth, the bacteria are capable of growth and division. Aquatic bacteria mostly form sheath. Examples of sheathed bacteria include *Leptothrix discophora* (also known as iron bacteria), *Sphaerotilus* and *Clonothrix*.

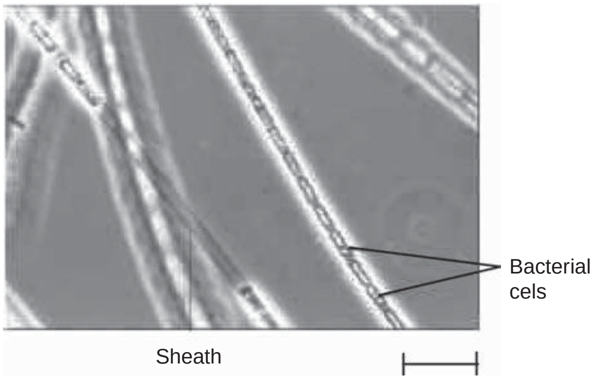


Figure 7.8: Sheathed bacterium

Function:

- It provides mechanical support.
- In a few bacteria, shealth is strengthened by the deposition of ferric and manganese hydroxides.

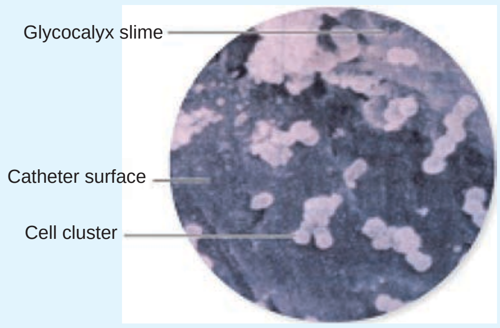
Prosthecae

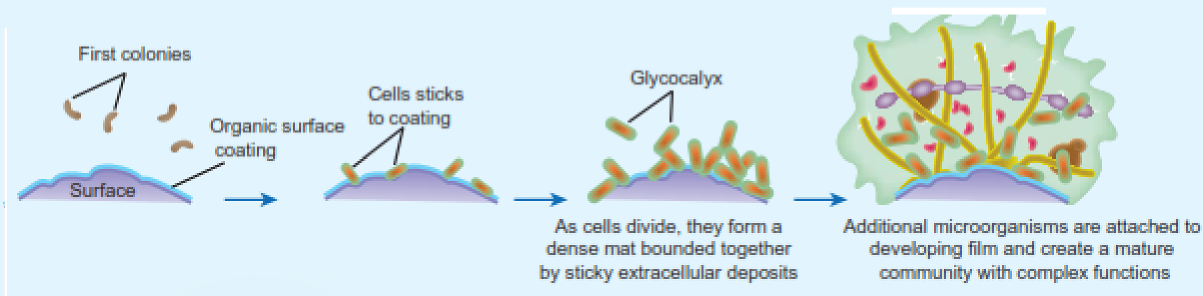
They are semi rigid extensions of cell wall and cell membrane. Some bacteria may contain more than one prosthecae (Figure 7.9). Aerobic bacteria in fresh water and marine environment possess prosthecae. Some of the prosthecate bacteria are *Caulobacter, Stellar, Prosthecobacter and Hyphomicrobium.*

Infobits

Biofilms:

Microbial adhesion to animate or inanimate surfaces can be mediated by polysaccharides capsules or slime. These adherence polymers are collectively called as adhesions. Microorganism tend to adhere to any surface and the layer they produce is called Biofilm. Biofilm can be harmful or beneficial to humans. Biofilm formation is a critical issue for almost all surfaces in health care and food preparation settings. Biofilms may form on a wide variety of surfaces, including living tissues, medical devices, industrial or portable water piping system, etc., Biofilm formation is a multi-step process starting with attachment to a surface, then formation of three dimensional structure and finally ending with maturation and detachment. During biofilm formation many species of bacteria are able to communicate with one another through specific mechanism called quorum sensing.





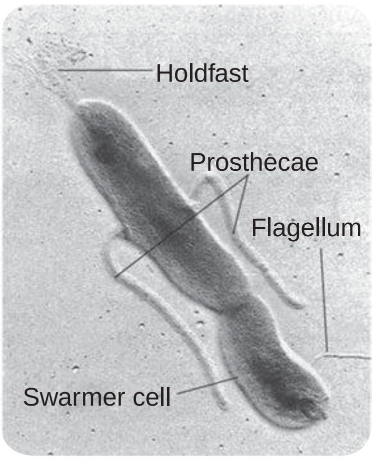


Figure 7.9: Prosthecate bacteria

Function:

- Prosthecae increase surface area for absorption of nutrients from the dilute aquatic environment.
- Helps in adhesion.
- Some prosthecae develop bud at the tip and helps in asexual reproduction.

Stalk

It is a nonliving ribbon like tubular structure. It is formed by excretory product of bacteria. Some of the stalked bacteria are *Gallionella, Planctomyces* (Figure 7.10).

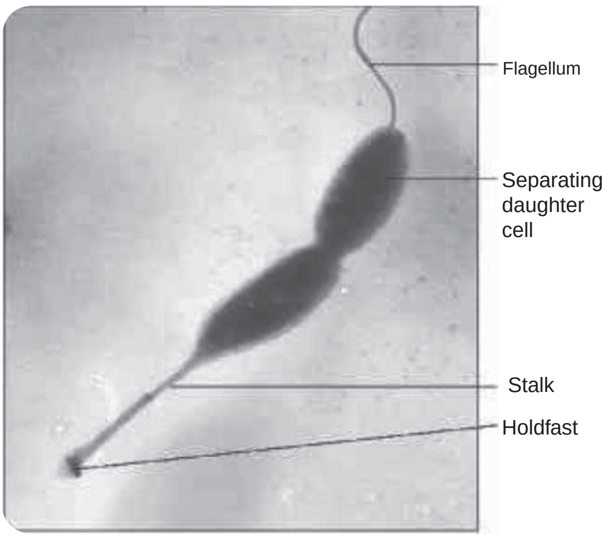


Figure 7.10: Stalked bacteria

Function:

- Stalk helps in attachment of cells to solid surface.

7.3 Cell Envelope of Bacteria

Cell envelope is an external covering that lies outside the cytoplasm. It is composed of two or three basic layers: the cell wall, the cell membrane and in some bacteria the outer membrane.

7.3.1 Structure of Prokaryotic Cell Wall

Prokaryotic cells almost always are bounded by a chemically complex cell wall. Cell wall lies beneath the external structures (capsules, sheaths and flagella). Cell wall lies external to the plasma membrane (cell membrane). Cell wall of eubacteria is made up of **peptidoglycan** or **murein**, whereas that of Archaeobacteria is composed of proteins, glycoproteins or polysaccharides. A few genera such as *Methanobacterium*, have cell walls composed of **pseudomurein**, a polymer whose structure superficially resemble eubacteria peptidoglycan of eubacteria but differs markedly in chemical composition.(Note: Ordinary or typical bacteria are sometimes called eubacteria to distinguish them from the phylogenetically distinct group known as archaeobacteria). Peptidoglycan is a cross linked polymer of enormous strength and rigidity. It is a polymer composed of many identical subunits (Figure 7.11). Peptidoglycan differs somewhat in composition and structure from one species to another, but it is basically a polymer of N-acetylglucosamine(NAG), N-acetylmuramic acid(NAM), L-alanine, D-alanine, D-glutamate, and a diamino acid (LL- or meso-diaminopimelic acid, L-lysine, L-ornithine, or L- diaminobutyric acid).

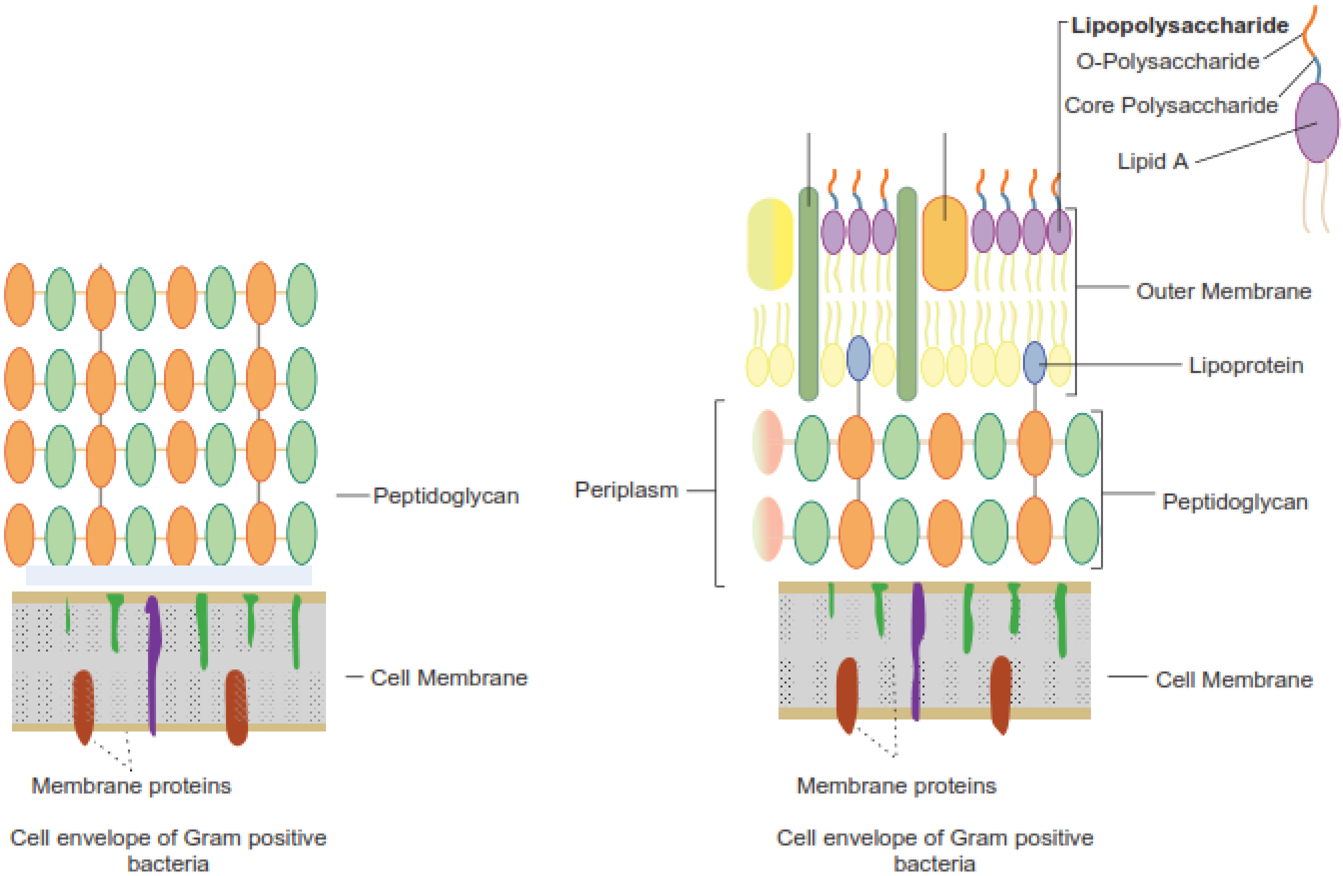


Figure 7.11: Cell envelope of Gram positive and Gram negative bacteria

Cell wall may contain other substances in addition to peptidoglycan. For instance, *Staphylococcus aureus* and *Streptococcus fecalis* contain **teichoic acids** (polymer of acidic polysaccharides) covalently linked to peptidoglycan. Cell wall of Gram positive bacteria contain very little lipid but *Mycobacterium* and *Corynebacterium* cell walls are rich in **mycolic acid** (or Cord factor) which make them acid fast. When stained, the cells cannot be decolorized easily despite treatment with dilute acids. *Mycoplasma* lack cell wall.

Protoplast is a bacterial cell consisting of cell material bound by a cytoplasmic membrane.

Spheroplast is a bacterial cell with two membranes namely the cytoplasmic membrane and the outer membrane but no cell wall.

Functions of cell wall

- It gives shape to bacteria like a bicycle tyre that maintains the necessary shape and prevents the more delicate inner tube (the cytoplasmic membrane) from bursting when it is expanded.
- It protects bacteria from osmotic lysis in dilute solutions (hypotonic environment).
- It protects cell from toxic substances.

HOTS

How do bacteria maintain their shape?

7.3.2 Structure of Outer Membrane

Eubacteria and Archaeobacteria (Gram positive and Gram negative) differ with respect to their cell walls. Gram negative cell walls are more complex. An outer membrane surrounds a thin underlying layer of peptidoglycan (Table 7.4). Outer membrane is bilayered, consisting mainly of phospholipids, proteins and lipopolysaccharide (LPS).

LPS is composed of three parts which are covalently linked to each other. They are

1. **Lipid A** which is firmly embedded in the membrane,
2. **Core polysaccharide** that is located at the membrane surface and
3. **Polysaccharide O antigens** that extend like whiskers from the membrane surface into the surrounding medium

Special protein channels called porins span the membrane. The points of contact between outermembrane and cytoplasmic membrane are known as adhesions. Outer membrane is anchored to peptidoglycan layer by means of **Braun's** lipoprotein. Periplasmic space between the cell membrane and the outer membrane.

Functions of outer membrane

- It serves as an impermeable barrier to prevent the escape of important enzymes (such as those involved in cell wall growth) from the periplasmic space.
- It serves as a barrier to various external chemicals and enzymes that could damage the cell. For example, the walls of many Gram positive bacteria can be easily destroyed by treatment with an enzyme called lysozyme, which selectively

Table 7.4: Difference between Gram positive and Gram negative bacteria

	Gram positive bacteria	**Gram negative bacteria**
Gram reaction	The bacteria that retain the colour of the primary stain (crystal violet) are Gram positive	The bacteria that cannot retain the primary stain but takes on the colour of the counterstain safranin are called Gram negative
Cell wall	The cell wall is thick (20-30nm thick)	The cell wall is thin (8-12nm thick)
Peptidoglycan layer	Thick (multilayered)	Thin (single layered)
LPS content Lipopolysaccharide	None	High
Periplasmic space	Absent	Present
Outer membrane	Absent	Present
Lipid and lipoprotein content	Low (acid fast bacteria have lipids linked to peptidoglycan)	High due to the presence of outer membrane
Teichoic acids	Present in many	Absent
Example:	*Streptococcus, Staphylococcus, Corynebacterium, Bacillus, Clostridium*	*Escherichia coli, Pseudomonas, Haemophilus, Salmonella, Shigella.*

dissolves peptidoglycan. However, Gram negative bacteria are refractory to this enzyme because large protein molecules of enzyme cannot penetrate the outer membrane. Only when outer membrane is damaged the enzyme can penetrate.

- Porins allow the smaller molecules, such as amino acids, monosaccharides to pass across.
- Adhesions are export sites for newly synthesised LPS and porins, and are sites at which pili and flagella are made.

7.3.3 Structure of Cytoplasmic Membrane

Immediately beneath the cell wall is the cytoplasmic membrane also known as plasma membrane or cell membrane. It is composed of phospholipids and proteins. The phospholipids form a bilayer. Integral proteins are embedded within this bilayer. Surface proteins or peripheral proteins are loosely attached to the bilayer. The lipid matrix of the membrane has fluidity, allowing the components to move around laterally. In eubacteria, the phospholipids are phosphoglycerides, in which straight chain fatty acids are ester linked to glycerol. In archaeobacteria, the lipids are polyisoprenoid branched-chain lipids, in which long-chain branched alcohols (phytanols) are ether linked to glycerol.

Functions of the cell membrane

- Prokaryotes do not have intracellular membrane bound organelles as present in eukaryotic organelles. Thus cell membrane provides a site for functions such as energy reactions, nutrient processing and synthesis.
- It regulates transport, the passage of nutrients into the cell and the discharge of wastes. It is a selectively permeable membrane.
- It is also involved in secretion or discharge of a metabolic product into extracellular environment.
- Cell membrane is an important site for a number of metabolic activities. Most enzymes of respiration and ATP synthesis reside in the cell membrane since prokaryotes lack mitochondria.

Significance of cell envelope

- It has toxic properties (Example: LPS)
- It stimulates antibody production by immune system
- The cell walls of many pathogens have components that contribute to their pathogenicity. Example mycolic acids of *Mycobacterium tuberculosis*
- Cell wall is a site of action of several antibiotics.
- Many of the serological properties of Gram negative bacteria are attributable to O antigens; they can also serve as receptors for bacteriophage attachment.

7.4 Structures Internal to Cell Membrane of Bacteria

Cytoplasm is called as the internal matrix of the cell inside the cell membrane. Its major component is water (70-80%). It also contains proteins carbohydrates, lipids, inorganic ions, and certain low molecular weight compounds. Inorganic ions are present in much higher concentrations in cytoplasm than in most media.

Cytoplasm is thick, aqueous, semi-transparent and elastic. The major structures in the cytoplasm of prokaryotes are nucleoid (containing DNA), ribosomes and reserve deposits called inclusions. Prokaryotic cytoplasm lacks certain features of eukaryotic cytoplasm such as a cytoskeleton and cytoplasmic streaming.

Ribosomes

All living cells contain ribosomes. They are the sites of protein synthesis. High number of ribosomes represents the high rate of protein synthesis. Prokaryotic ribosomes are freely found in the cytoplasm, whereas eukaryotic ribosomes are attached to the cell membrane. Prokaryotic ribosomes consists of protein and a type of RNA called ribosomal RNA. They are smaller and less dense than the eukaryotic ribosomes. The ribosomes of prokaryotes are 70S where as that of eukaryote are 80S (Figure 7.12).

Nucleus

The nuclear area has the hereditary material of most bacteria. It contains a single, circular, long, continuous, thread like double stranded DNA called the bacterial chromosome. Some bacteria with linear chromosome also exist. It carries the information required for the cells structure and function. They are not surrounded by a nuclear envelope and are devoid of highly conserved histone proteins. The nuclear area can be spherical, elongated or dumbbell shaped. In actively growing bacteria, as much as 20% of the cell volume is occupied by DNA, because such cells presynthesize nuclear material for future cells. The chromosome is attached to the cell membrane. Proteins in the plasma membrane are believed to be responsible for the replication of the DNA and segregation of the new chromosomes to daughter cells in cell division.

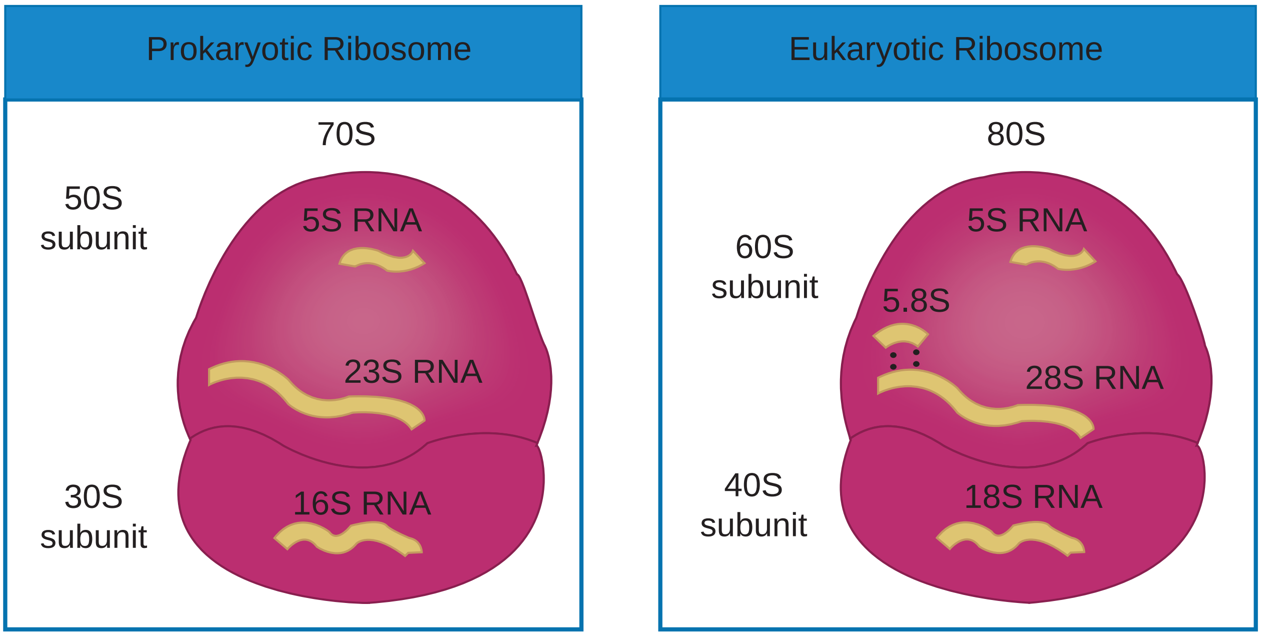


Figure 7.12: Prokaryotic and Eukaryotic Ribosomes

Plasmids

Apart from the bacterial chromosome, bacteria also contain small circular, double stranded DNA molecules called plasmids (Figure 7.13). Plasmids are self replicating extra chromosomal genetic elements. Plasmids may carry genes for activities such as antibiotic resistance and tolerance to toxic metals. Examples: Fertility plasmid (F plasmid), Resistance plasmid (R plasmid) and colicin plasmid (Col plasmid).

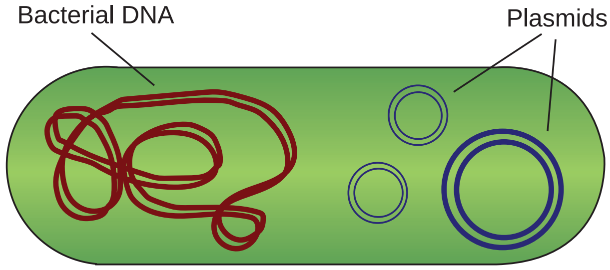


Figure 7.13: Plasmids in Prokaryotes

Molecular Chaperones

They are the helper proteins which recognize the newly formed polypeptides and fold them into their proper shape of secondary and tertiary structure. Many chaperones are involved in proper folding of bacteria. They were first identified in *Escherichia coli* mutant. Example: Heat shock proteins are produced in *Escherichia coli* cells subjected to live at high temperatures, or in any other stressful unfavorable conditions.

Inclusions

The cytoplasm of prokaryotic cells has several kinds of reserve deposits known as inclusions. Cells may accumulate certain nutrients when they are plentiful and use them when they are deficient. Some inclusions are common to a wide variety of bacteria whereas others are limited to certain species (Table 7.5).

Endospores:

Some species of bacteria produce metabolically dormant structures called spores. They are highly durable and dehydrated resting bodies produced inside the cells. They are formed by bacteria only when there is lack of water or depletion of essential nutrients in the environment. Endospores are coated with a specific chemical compound diaminopimelic acid. It binds with the Calcium and forms Calcium dipicolinate which removes the water from it and makes the spore resistant to extreme conditions Example: *Bacillus anthracis* and *Clostridium tetani* possess endospores.

Mesosomes

Generally prokaryotes do not have cytoplasmic organelles like mitochondria and chloroplast. It contains mesosome as their organelle. They are the invaginations of the cell membrane and they are in the form of tubules, vesicles or lamellae. They are seen in both Gram positive and Gram negative bacteria, generally more in Gram positive bacteria. They are located next to the septa or cross walls in dividing bacteria (Figure 7.14). They may be involved in cell wall formation during division or play a role in chromosome replication and distribution to daughter cells. If they are located near to the surface they are called peripheral mesosomes and if they are located deep into the cytoplasm they are called central mesosomes.

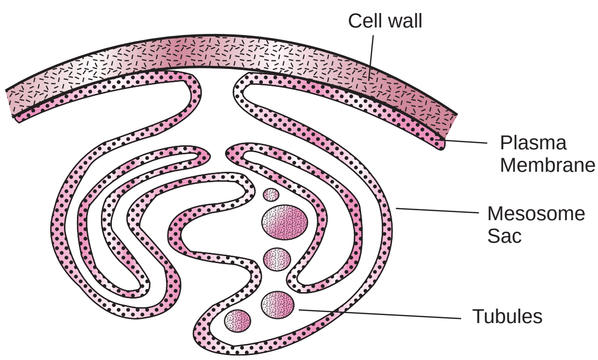


Figure 7.14: Bacterial Mesosome

Table 7.5: Different types of inclusion bodies in bacteria

Type of inclusion bodies	Example of organisms possessing	Significance
Polyhydroxybutyrate (PHB)	*Bacillus megaterium*	Reserve of Carbon and energy sources. Sudan dye is used to observe lipid inclusions
Polyphosphate (volutin granules) or metachromatic granules	*Corynebacterium diphtheriae*	Reserve of phosphate
Sulphur globules	Phototrophic bacteria Like purple and green Sulphur bacteria Example: *Thiobacillus*	Elemental sulphur, reserve of electrons in phototrophs. Reserve of energy source in lithotrophs
Gas vesicles	Aquatic bacteria, *Cyanobacterium*	They are protein shells filled with gases. They provide buoyancy and keep the cells floating in vertical water column
Parasporal crystals	Genus *Bacillus*	It is a proteinaceous compound, It is toxic to certain insects
Magnetosomes	*Aquaspirillum magnetotacticum*	They are like intracellular chains of magnetite particles. They help the bacteria to swim to nutrient rich sediments. It protects the cell against H_2O_2 accumulation
Carboxysomes	Photosynthetic Bacteria, cyanobacteria Autotrophic bacteria	They contain the enzyme Ribulose 1-5 bisphosphate carboxylase which is involved in Carbon dioxide fixation during photosynthesis
Phycobilisomes or cyanophycin Granules	Cyanobacteria	They have a long polypeptide with equal proportion of Arginine and Aspartic acid. They store Nitrogen
Chlorosomes	Green bacteria	They contain bacteriocholorophyll pigments which are involved in bacterial photosynthesis

HOTS

Why are endospores so difficult to destory?

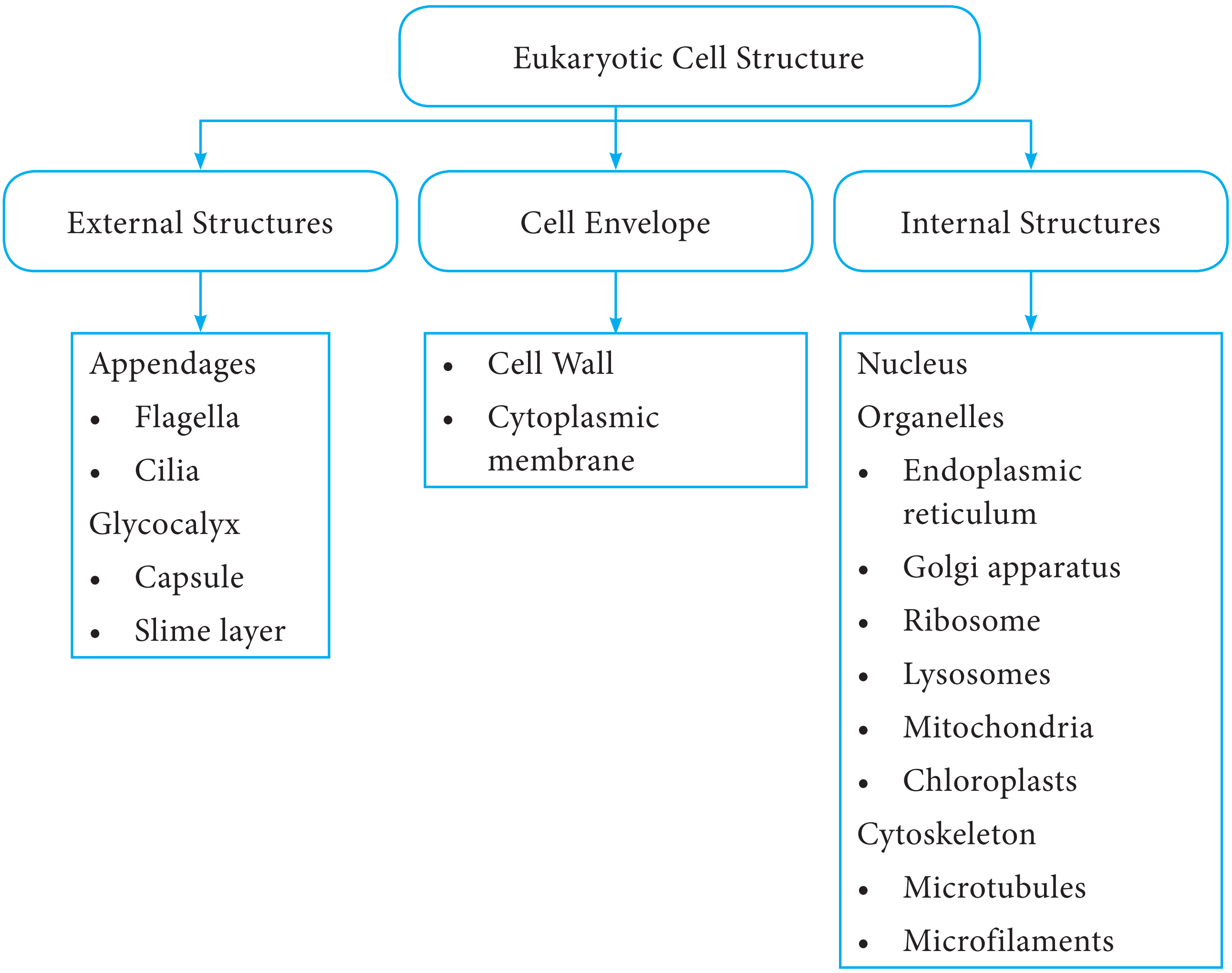
EQCG4P

7.5 Eukaryotic Cell Structure

As mentioned earlier, eukaryotic organisms include algae, protozoa, fungi, higher plants and animals. The eukaryotic cell is typically larger and structurally more complex than the prokaryotic cell (Flowchart 7.1).

Prokaryotes and Eukaryotes are chemically similar, in the sense that they both contain nucleic acids, proteins, lipids, and carbohydrates (Figure 7.15). They use the same kinds of chemical reactions to metabolize food, build proteins, and store energy. It is primarily the structure of cell walls and membranes, and the absence of organelles (specialized cellular structures that have specific functions), that distinguish prokaryotes from eukaryotes (Table 7.6).

The general, eukaryotic microbial cells have a cytoplasmic membrane, nucleus, mitochondria, endoplasmic reticulum, Golgi apparatus, vacuoles, cytoskeleton, and glycocalyx. A cell wall, locomotor appendages and chloroplasts are found only in some groups. The structure and functions of the eukaryotic cells are discussed in (Table 7.7).



Flowchart 7.1: Eukaryotic Cell Structure

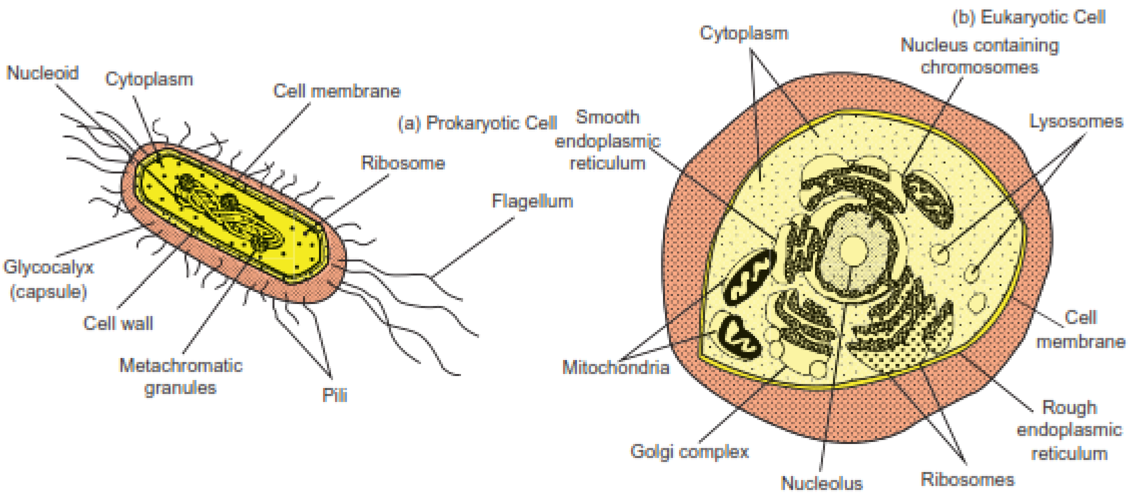


Figure 7.15: Structure of prokaryotic and eukaryotic cell

Table 7.6: Differences between prokaryotic and eukaryotic cell

S. No	Characteristic	Prokaryotic	Eukaryotic
1	Size of cell	Typically 0.2-2.0nm in diameter	Typically 10-100 nm diameters
2	Nucleus	No nuclear membrane or nucleoli	True nucleus, consisting of nuclear membrane and nucleoli
3	Membrane enclosed organelles	Absent	Present. Example: lysosomes, Golgi complex, endoplasmic reticulum, mitochondria and chloroplasts
4	Flagella	Consist of two protein building blocks	Complex, consist of multiple micro tubules
5	Glycocalyx	Present as a capsule or slime layer	Present in some cells that lack cell wall
6	Cell wall	Usually present and is chemically complex (typical bacterial cell wall includes peptidoglycan)	When present, chemically simple
7	Plasma membrane	No carbohydrates and generally lacks sterols	Sterols and carbohydrates that serve as receptors are present
8	Cytoplasm	No cytoskeleton or cytoplasmic streaming	Has cytoskeleton and shows cytoplasmic streaming
9	Ribosomes	70S	80S (70S in organelles)

(Continued)

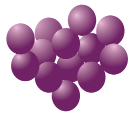
Table 7.6: Differences between prokaryotic and eukaryotic cell *(Continued)*

10	Chromosome (DNA)	Single circular chromosome, lacks histone	Multiple linear chromosomes with histone arrangement
11	Cell division	Binary fission	Mitosis
12	Sexual recombination	No meiosis (transfer of DNA fragments only)	Involves meiosis

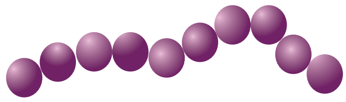
Table 7.7: Functions of Eukaryotic organelles

Eukaryotic organelles	Functions
Plasma membrane	Mechanical cell boundary, selectively permeable barrier with transport systems, mediates cell to cell interactions and adhesion to surfaces, secretion
Cytoplasmic matrix	Environment for other organelles, location of many metabolic processes
Microfilaments, intermediate filaments, and Microtubules.	Cell structure and movements from the cytoskeleton
Endoplasmic reticulum	Transport of materials, protein and lipid synthesis
Ribosome	Proteins synthesis
Golgi apparatus	Packaging and secretion of materials for various purposes, lysosome formation
Lysosomes	Intracelluar digestion
Mitochondria	Energy production through use of the tricarboxylic acid cycle, electron transport, oxidative hosphorylation, and other path ways
Chloroplasts	Photosynthesis, trapping light energy and formation of carbohydrate from CO_2 and water
Nucleus	Repository for genetic information, control center for cell
Cell wall and pellicle	Strengthen and give shape to the cell
Cilia and flagella	Cell attachment and Cell movement
Vacuole	Temporary storage and transport, digestion (food vacuoles), water balance(contractile vacuole)

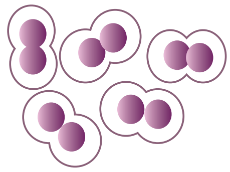
Different Shapes of Bacteria



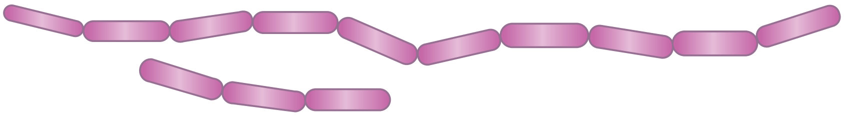
Staphylococcus aureus

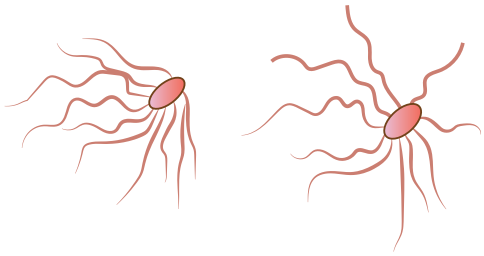


Streptococcus pyogenes

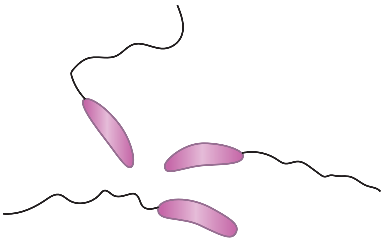


Streptococcus pneumoniae

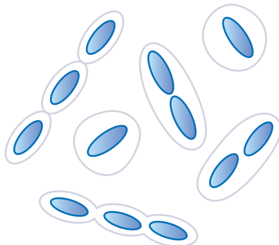




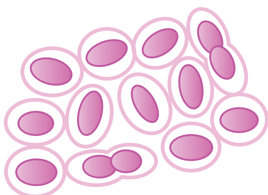
Escherichia coli; Salmonella



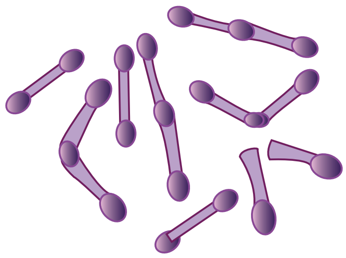
Vibrio cholerae



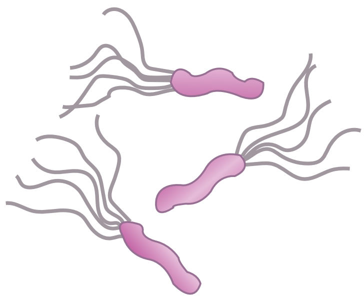
Klebsiella pneumoniae



Bordetella pertussis



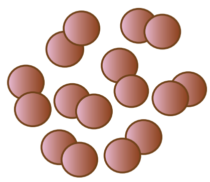
Corynebacterium diphtheriae



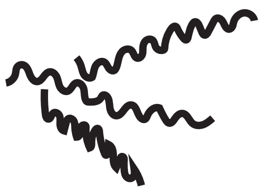
Helicobacter pylori

Clostridium botulinum

Clostridium tetani



Neisseria gonorrhoeae

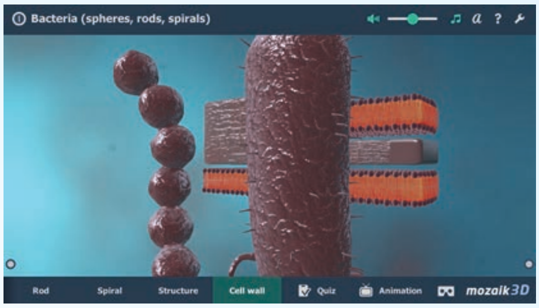


Treponema pallidum

ICT CORNER

Bacteria

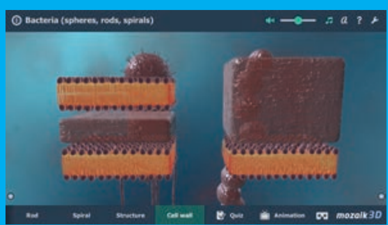
Know the various shapes of Bacteria



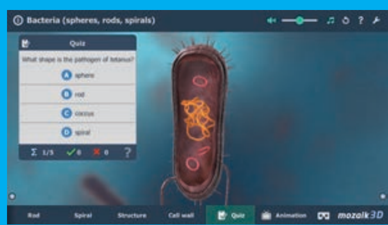
STEPS:

- Use the URL or scan the QR code to download the Bacteria interactive educational VR 3D app.
- Select sphere, rod and spiral to observe the structure of bacteria shapes.
- Select 'structure' tab and note the internal structure of bacteria.
- Click cell wall and note the difference between different shapes.

Step1



Step2



Step3

URL:

https://play.google.com/store/apps/details?id=com.rendernet.bacteria&hl=en

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Summary

Most prokaryotes have one of three general shapes coccus (round), bacillus (rod), or spiral, based on the configuration of the cell wall. Two types of spiral cells are spirochetes and spirlla. Shape and arrangement of cells are key factors for describing prokaryotes. Arrangements of cells are based on the number of planes in which a given species divides.

Cocci can divide in many planes to form pairs, chains, packets, or clusters. Bacilli divide only in the transverse plane. If they remain attached, they form chains or palisades.

Some bacterial cell walls are covered by capsules or slime which protect the cell from phagocytosis, drying and nutrient loss. Fimbriae and Pili are involved in attachment and in transfer of genetic information between bacterial cells. Flagella are involved in cell motility.

The cell envelope is the complex boundary structure surrounding a bacterial cell. In Gram negative bacteria, the envelope consists of outer membrane, the cell wall, and the cell membrane. Gram positive bacteria have only cell wall and cell membrane. Gram positive bacteria have thick murein and teichoic acid. The cell walls of Gram negative bacteria are thinner and have wide periplasmic space. The outer membrane of Gram negative cells contains LPS toxic to mammalian hosts. The cell membrane is typically composed of phospholipids and proteins, and it performs many metabolic functions as well as transport activities.

The cytoplasm of bacterial cell serve as a solvent for material used in all functions. The genetic material of bacteria is DNA and the genes are arranged on larger, circular chromosomes. Additional genes are carried on plasmid. Bacterial ribosomes are dispersed is the cytoplasm in chains and are also embedded in the cell membrane.

Bacteria may store nutrients in their cytoplasm, in form of inclusions. Inclusion vary in structure and the materials that are stored. A few bacteria produce dormant bodies called endospores, which are the hardiest of all life forms, survival for hundreds or thousands of years. The genus *Bacillus* and *Clostridium* are spore forming deadly pathogens.

Eukaryotes are cells with nucleus and membrane bound organelles. Cell structures common to most eukaryotes are the cell membrane, nucleus, vacuoles, mitochondria, endoplasmic reticulum, golgi apparatus and a cytoskeleton. Cell wall, chloroplast and locomotory organelles are present in some eukaryote groups.

Evaluation

Multiple choice questions

1. The arrangement of flagella on cell surface can sometimes help in the identification of an organism for example, *Escherichia coli* to has flagella throughout the cell surface that is referred to as.
   a. Lophotrichous
   b. Monotrichous
   c. Peritrichous
   d. None of the above

2. The movement of an organism toward or away from a chemical substance in its environmentis called.
   a. Tracking
   b. Chemotaxis
   c. Tumbling
   d. Tumbling of none of the above.
3. Bacterial cell wall is composed of
   a. Lipid
   b. Murein
   c. Cellulose
   d. Chitin
4. Cell wall shows
   a. Semipermeability
   b. Complete permeability
   c. Differential permeability
   d. Impermeability
5. Gram negative differ from Gram positive in having
   a. Thick wall
   b. Absence of wall lipids
   c. Complete wall
   d. Simple wall
6. Lipopolysaccharide is found in cell wall of
   a. Gram positive bacteria
   b. Gram negative bacteria
   c. Both Gram positive and Gram negative
   d. Algae
7. Cell wall of archaeobateria contain
   a. Peptidoglycan
   b. Murein
   c. Pseudomurein
   d. All the above
8. The components of cell wall which the outer membrane of enteric Gram negative bacteria to murein layer
   a. Lignic acid
   b. Pseudomurein
   c. Murein
   d. Teichoicacid
9. Endotoxin present in
   a. Outer membrane
   b. Plasma membrane
   c. Murein
   d. All the above.
10. ____________________ has fluidity
   a. Cell wall
   b. Cell membrane
   c. Outer membrane
   d. All the above
11. The organelle of prokaryote involved in active cell division.
   a. Mesosomes
   b. Mitochondria
   c. Ribosomes
   d. Endoplasmic reticulum
12. The metachromaticgranules are seen in the bacteria
   a. *Escherichia coli*
   b. *Corynebacterium diptheriae*
   c. *Pseudomonas aeroginosa*
   d. *Bacillus antharcis*
13. The extra chromosomal DNA is called
   a. Plasmid
   b. Episome
   c. Nucleus
   d. Nucleoid
14. The siderophores has high affinity towards.
   a. Iron
   b. Magnesium
   c. Chloride
   d. Copper

15. The molecular chaperones are involved in
   a. Folding of proteins
   b. Folding of carbohydrates
   c. Folding of lipids
   d. Folding of fatty aids

Answer the following

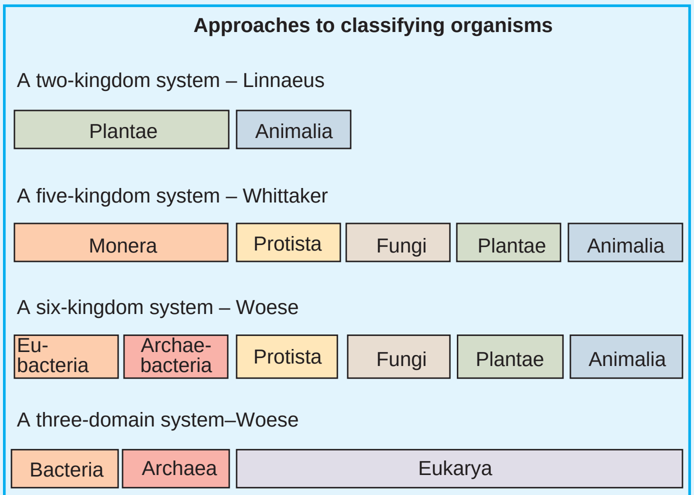
1. What is Glycocalyx?
2. What is a capsule? What are its functions?
3. What is a pilus, what is its function?
4. What is chemotaxis?
5. Explain the arrangement of flagella in bacteria with example.
6. Define carboxysomes
7. State volutin granules
8. What is called magnetosomes?
9. What is the role of ribosomes involved in protein synthesis?
10. Define periplasmic space.
11. What is LPS composed of?
12. List functions of cell wall
    a. Cell membrane
    b. Outer membrane
13. Discuss why a cell lyses without cell wall
14. Give the significance of cell envelope.
15. What is the role of siderophores?
16. Differentiate between capsule and slime/pili and fimbriae.
17. Diagrammatically explain structure of cell wall.
18. Differentiate Gram positive and Gram negative bacteria.
19. Explain any five cytoplasmic inclusions
20. Differentiate between Prokaryotes and Eukaryotes.

Student Activity

1. Students will prepare clay model of bacteria.
2. Students will collect the pictures of different Eukaryotic microorganisms.

Chapter 8

Microbial Taxonomy



The correct identification of micro organisms is of fundamental importance to microbial systematists as well as to scientists involved in many other areas of applied research and industry (Example: agriculture, clinical microbiology and food production).

Chapter Outline

Learning Objectives

After studying this chapter the student will be able,

- *To understand the concept of taxonomy, taxon and phylogeny.*
- *To appreciate the contribution of Linnaeus and Whittaker.*
- *To learn the characteristics of Kingdom Monera, Protista, Fungi, Plantae and Animalia.*
- *To know some special methods used in classification of microorganisms.*

8.1 Diversity of Living Organisms is Fascinating

The branch of science which deals with the classification, nomenclature and identification of all living organisms is called Taxonomy. (Greek taxis means arrangements and nomos means law or to distribute). Because of large number and great diversity of organisms, biologists use the characteristics of different organisms to identify and group them. To understand life, it is essential to understand taxonomy. The method of grouping related organisms is the basis of classification (Figure 8.1). The objectives of taxonomy are:

- To establish the criteria for identifying organisms
- To arrange related organisms into groups
- To provide evolutionary information of the organism

The system of naming living organism is called Nomenclature.

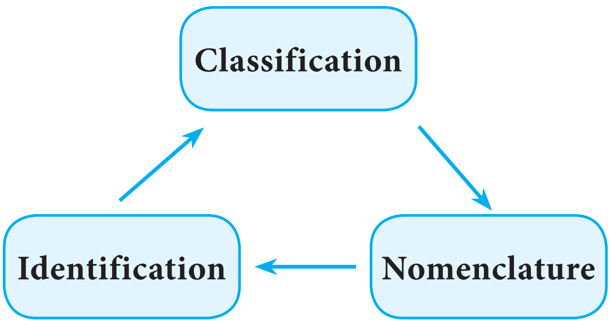


Figure 8.1: The three facets of taxonomy

8.2 Binomial Nomenclature

Swedish botanist Carolus Linnaeus in 1735 introduced a formal system of classification which divided all living organism into two kingdoms-Animalia and Plantae. He introduced "two name" system, the first name, genus and second name species. The name often gives information on something special about it. Taxa (the basic taxonomic group) are constructed from strains which are successions of cultures derived from an initial colony. The basic taxonomic group is called the species (a collection of strains having similar characteristics). The special bacterial strain which is the permanent reference specimen for the species is called the "**type strain**" (Figure 8.2).

HOTS

Why is type strain refered as the most important strain in a bacterial species?

A variant strain that differ physiologically and biologically from other strains in a particular species is called as "Biovar". Variations in a species is biological in nature. One biovar in a species may grow on sucrose, while another cannot. If the biovars are very similar except for one property, they belong to the same genus and species, though vary in biological growth properties.

The strain that differ morphologically are called as Morphovar or Morphotypes. Serovars or Serotypes are those strains that differ in their antigenic properties. It refers to immunological variations in a species. An example of differing serovars is *Salmonella*. Cell surface of *Salmonella* varies slightly from one serovar to another. Because of this cell surface change, a person who has been infected by or become resistant or immune to one serovar will not be immune to a second type, because the immune system cannot recognize a similar bacterium with a new surface cover.

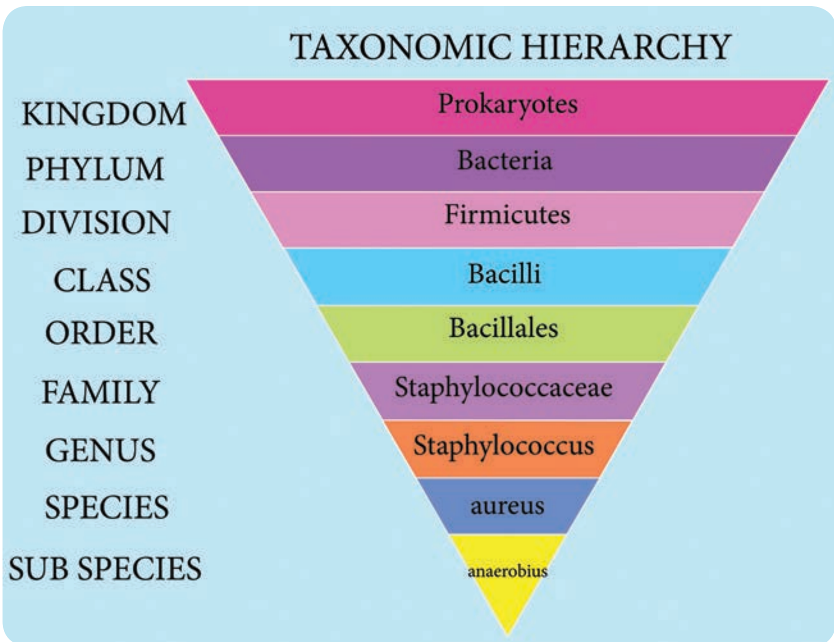


Figure 8.2: Taxonomic hierarchy-an example of hierarchy in microbial taxonomy

Infobits

The Microbial Type Culture Collection and Gene Bank (MTCC), a national facility established in 1986 is funded jointly by the Department of Biotechnology (DBT) and the Council of Scientific and Industrial Research (CSIR), Government of India. The MTCC, housed at the Institute of Microbial Technology (IMTECH), Chandigarh, has established itself as a distinguished culture collection centre for microbial resources in India. It is an affiliate member of the World Federation for Culture Collections (WFCC) and is registered with the World Data Centre for Microorganisms (WDCM). The main objectives of this national facility are to act as a depository, to supply authentic microbial cultures and to provide related services to the scientists working in research institutions, universities and industries.

8.3 Whittaker's System of Classification

It is the five kingdom classification. In the 20th century, advances in cell biology and interest in evolutionary biology led scientists to question the two or three-kingdom classification schemes. In 1969, Robert H. Whittaker proposed a system which recognizes five kingdoms of living things: Monera (Bacteria), Protista, Fungi, Plantae and Animalia (Table 8.1).

Whittaker's system of classification is based on 1) complexity of cell structure 2) mode of nutrition 3) body organization 4) phylogenetic or evolutionary relationship.

Monera: This kingdom includes all prokaryotic organisms. Unicellular microorganism such as Mycoplasma, Bacteria, Actinomycetes and Cyanobacteria are grouped under kingdom Monera.

DO YOU KNOW?

Phylogeny is the evolutionary history of organisms that refer to the relationship between life forms.

Table 8.1 Properties of the five kingdoms

Kingdom	**Monera**	**Protista**	**Fungi**	**Plantae**	**Animalia**
Cell type	Prokaryotic	Eukaryotic	Eukaryotic	Eukaryotic	Eukaryotic
Cell organization	unicellular	unicellular	Multicellular and unicellular	Multicellular	Multicellular
Cell Wall	Present in most	Present in some absent in others	Present	Present	Absent
Nutritional Class	Phototrophic, heterotrophicor chemoautotrophic	Heterotrophic and phototrophic	Heterotrophic	Phototrophic	Heterotrophic
Mode of nutrition	Absorptive	Absorptive or ingestive	Absorptive	Mostly Absorptive	Mostly ingestive

> **Infobits**
>
> **Hints of life:**
>
> The Precambrian was the age of microorganisms. They were macroscopically expressed in a colonial structure called **stromatolite**. It is a layer produced by live or fossilized mats of photosynthetic prokaryotes (cyanobacteria) associated with warm lagoons or hot springs. The ancient **stromalite** belongs to anoxygenic phototrophic filamentous bacteria and modern **stromalite** belongs to oxygenic photo trophic cyanobacteria.

Protista: This kingdom includes eukaryotic unicellular Protozoans, slime molds and algae. The kingdom is made up of more than 250000 species. These organisms have typical eukaryotic cell organization.

Fungi: This kingdom includes non green, non photosynthetic eukaryotic fungi. molds, mushroom, toad stools, puffballs and bracket fungi are grouped under this kingdom. They are multicellular and consist of specialized eukaryotic cells arranged in a filamentous form.

Plantae: It includes all multicellular plants of land and water. They use photosynthesis to synthesize their organic molecules.

Animalia: This kingdom includes all multicellular eukaryotic animals. They are also referred to as Metazoans. Animals ingest their food through one of any ingestion portal and then use digestive enzymes to break food particles into absorbable fragments (Figure 8.3).

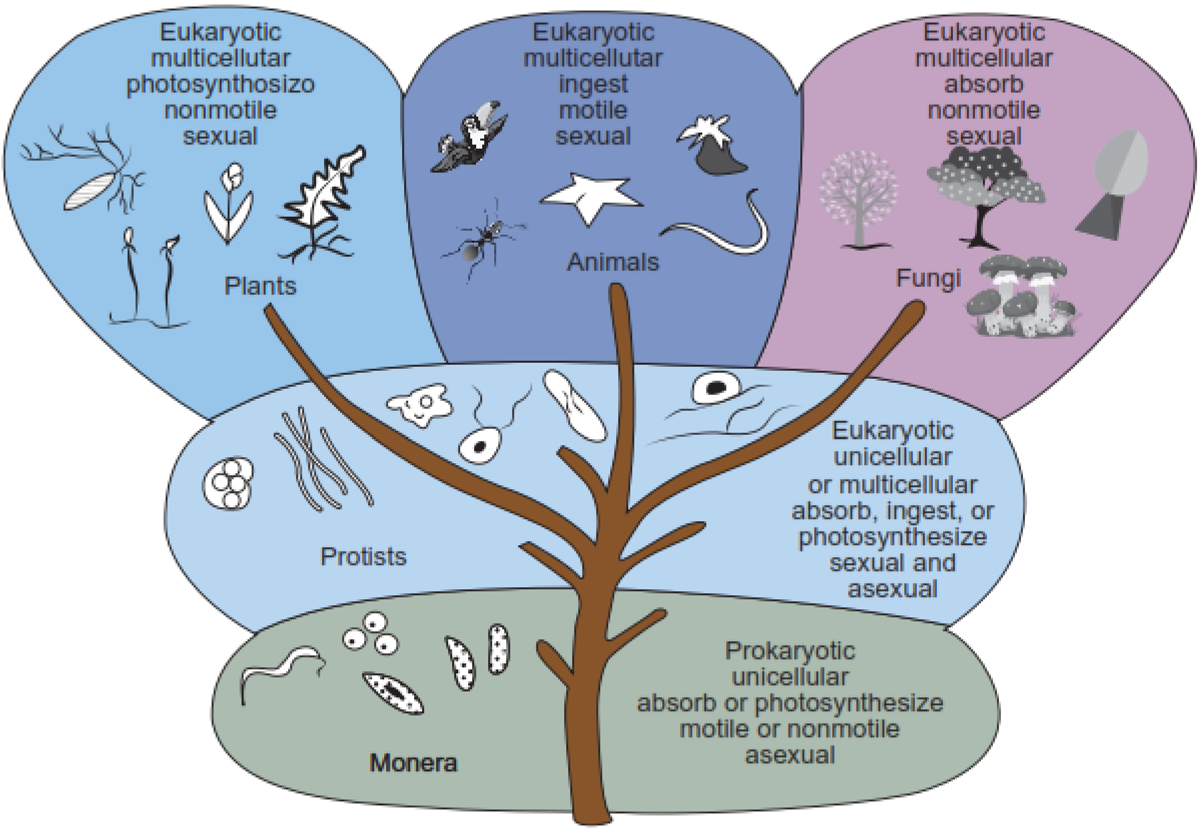


Figure 8.3: Whittaker's Five Kingdom classification

8.4 Taxonomic Systems

Classical Taxonomy

Classical taxonomy is a method of classification based on morphology, physiology, biochemical and ecological characteristics of the microorganisms.

- **Morphological Characteristics:** The structural characteristics are the usual tools which help in classification. Cell morphology gives little information about phylogenetic relationship. The first step in identification of bacteria is differential staining.
- **Physiological and metabolic characteristics:** These characteristics are useful because they are directly related to nature and activity of microbial enzymes and transport protein. Since proteins are gene products, analysis of these characteristics provides an indirect comparison of microbial genomes.
- **Biochemical characteristics:** Enzymatic activities are widely used to differentiate bacteria. Bacteria can be separated into separate species by various biochemical tests. Example: Carbohydrate fermentation ability of bacteria.
- **Ecological characteristics:** Many properties are ecological in nature since they alter the relation of microorganism to their environment. Microorganisms living in various parts of the human body markedly differ from one another and from those growing in freshwater, terrestrial and marine environments.

Prokaryotes have only a few structural characteristics and these characteristics are subject to rapid change due to change in environment. In classifying prokaryotes, metabolic reactions, genetic relatedness and other specialized properties are used (Figure 8.4).

HOTS

If two microorganisms have an identical mol% G+C value for their DNA, are they necessarily related? Explain

If two micro organisms have very different mol% G+C values for their DNA, are they necessarily unrelated? Explain

Numerical Taxonomy

The objective classification system deals with the grouping by numerical methods of taxonomic units based on their character and does not use subjective evaluation of their properties. To be more objective about grouping bacteria, the scientists determine many characteristics (usually 100 to 200) for each strain studied, giving equal weightage for each character. Then by using computer **%similarity** is calculated **(%S** of each strain to every other strain). For any two strains, this is

$$\%S = \frac{NS}{NS + ND}$$

where, NS is the number of characteristics that are the same (positive or negative) for the two strains, and ND is the number of characteristics that are different. Those strains having a high %S to each other are placed into groups; and those groups having a high %S to each other are in turn placed into larger

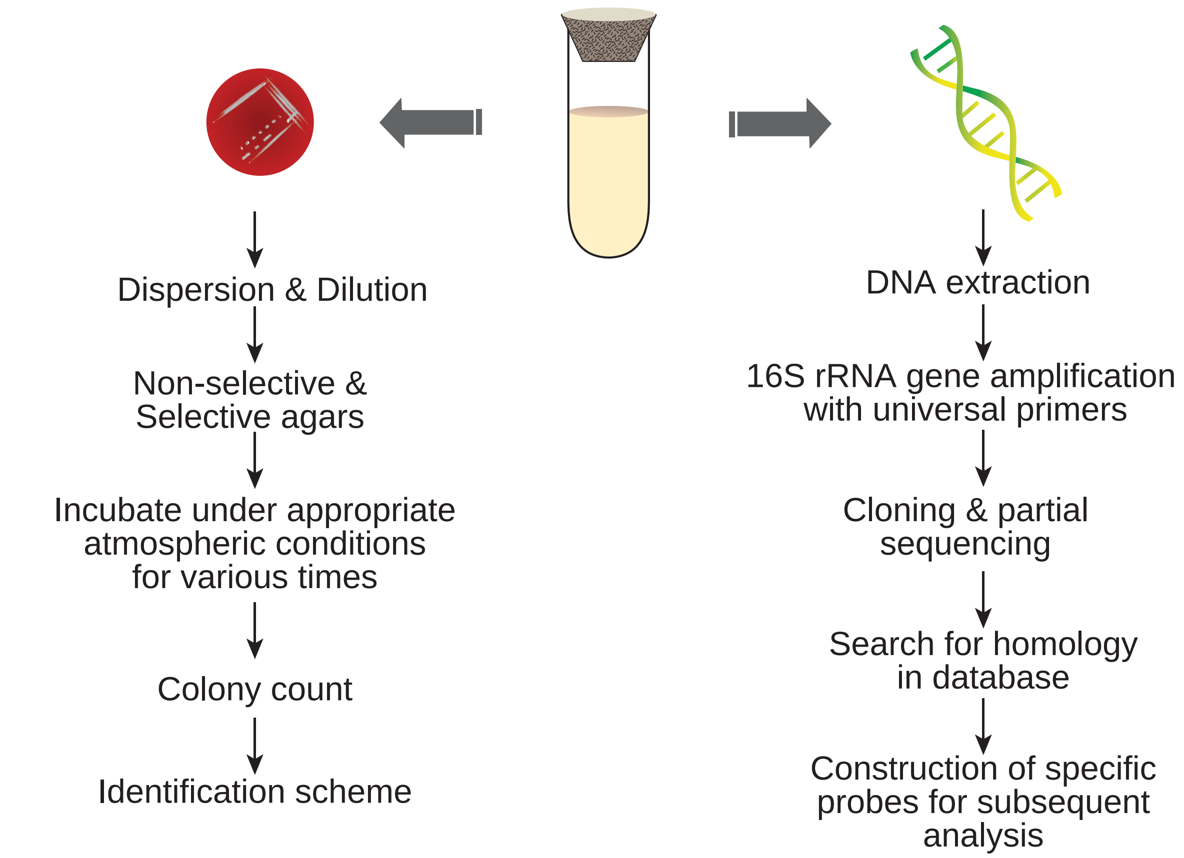


Figure 8.4: General scheme for classification and identification in microbial taxonomy

groups. Numerical taxonomy also yields classification that has a high degree of stability and predictability.

DO YOU KNOW?

In Numerical Taxonomy, which was defined by Peter H.A.Sneath and Robert Sokal, each characteristic is given equal weightage and it is converted into numerical form and compared by means of a computer.

Atleast 50 and preferably several hundred charactericties are compared.

The presence and absence of selected characters in the group of organism is calculated by simple matching coefficient (SSM), called Jaccard coefficient.

Molecular Taxonomy

ER549H

Molecular techniques in the field of biology has helped to understand genetic relationship between the numbers of different taxonomic categories.

DNA and protein sequencing, immunological methods, DNA-DNA or DNA-RNA hybridization methods are very helpful in studying different species.

The data or information from such studies are used to construct phylogenetic tree (a branching diagram showing the evolutionary relationship among various biological species based on similarities and difference in their physical or genetic characteristics).

A classification technique that is widely used is DNA base composition which is expressed as the percentage of Guanine plus Cytosine (G+C). It is a fixed property that reveals the degree of species relatedness. Ribosomal RNA sequencing is used to determine the diversity of organisms and the phylogenetic relationship. Basically ribosomes consists of two subunits, each of which is composed of protein and a type of RNA. Specific base sequences called as signature sequences are found in all groups of organisms. These unique DNA sequences are 5-10 bases long and found in 16s rRNA location and unique to major groups of prokaryotic organisms.

Nucleic acid based detection methods help in the detection of genomic materials. The 16s rRNA gene sequencing has been established as the "gold standard" for identification & taxonomic classification of microbial species.

8.5 The Three Domain System

This system of classification was introduced by C.Woese, O. Kandler and M.L.Wheelis, is an evolutionary model of phylogeny based on cells rRNA sequences(differences in the sequences of nucleotides) studies. They grouped all living organisms into three domains: Bacteria, Archaea and Eukaryota (Figure 8.5).

Bacteria and Archaea are two different groups of prokaryotes. The domain Bacteria comprise the vast majority of prokaryotes. The domain Archaea contains prokaryotes that live mostly in extreme environments. The domain Eukaryota contains living organisms that includes Kingdom Protista, Kingdom Fungi, Kingdom Plantae and Kingdom

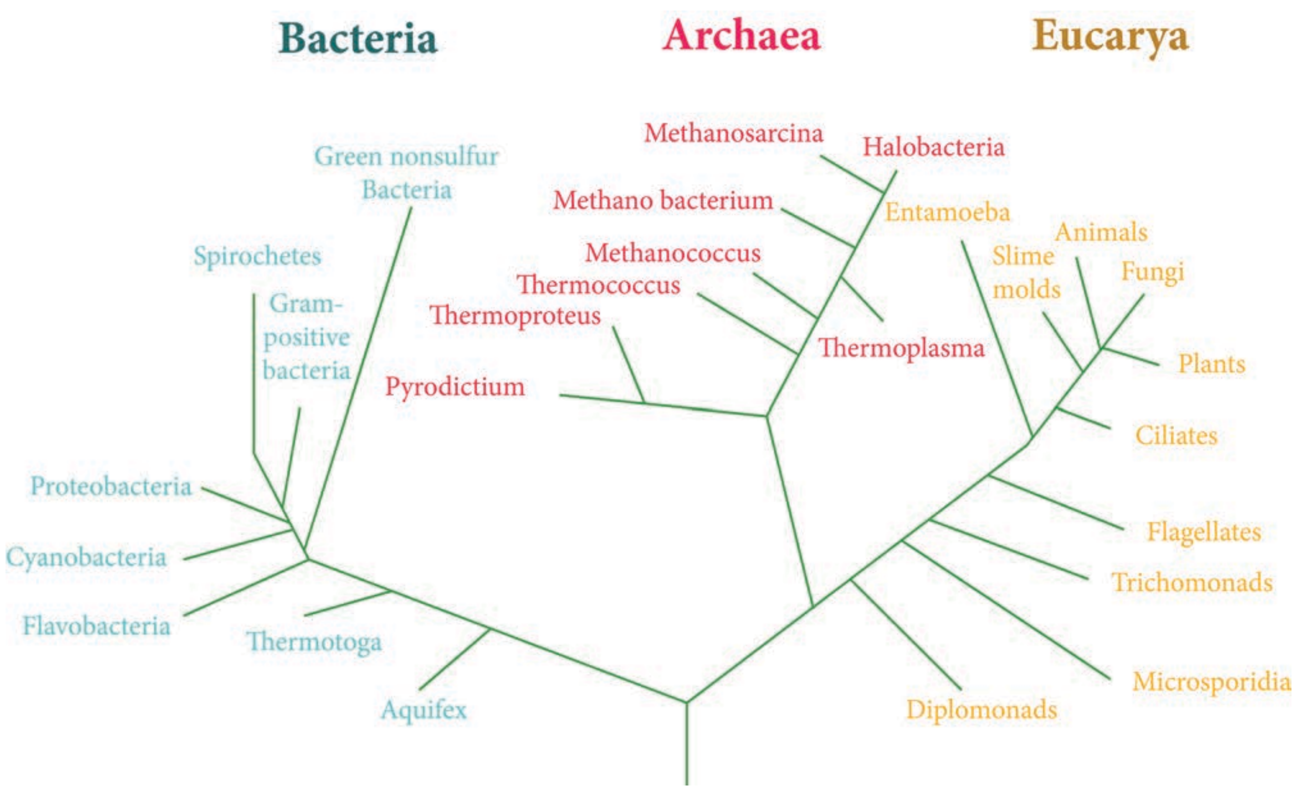


Figure 8.5: A phylogenetic tree based on rRNA analysis. Organisms are classified into three domains: Bacteria, Archaea and Eukaryotes as proposed by Carl Woese et al.

Animalia. This system of classification is currently accepted by most biologist.

The three domain system is based on the current state of knowledge. As knowledge of organisms increase in the future, classification will undoubtedly continue to change.

Infobits

The ***Bergey's Manual of Systematic Bacteriology's*** first edition was published initially in four volumes. Volume 1 included Gram negative bacteria of general, medical or industrial importance, Volume 2 included Gram positive bacteria other than actinomycetes, Volume 3 included Cyanobacteria, Archaebacteria and remaining Gram negative bacteria and Volume 4 included Actinomycetes.

The current grouping edition 2 (2012) has five volumes based on 16S rRNA sequencing:

Volume 1 (2001) includes Archaea and the deeply branching and phototrophic Bacteria.

Volume 2 (2005) includes Proteobacteria.

Volume 3 (2009) includes Firmicutes.

Volume 4 (2011) includes Bacteroidetes, Spirochaetes, Tenericutes (Mollicutes), Acidobacteria, Fibrobacteres, Fusobacteria, Dictyoglomi, Gemmatimonadetes, Lentisphaerae, Verrucomicrobia, Chlamydiae, and Planctomycetes.

Volume 5 (in two parts) (2012) includes Actinobacteria.

8.6 The Past and Present State of Bacterial Taxonomy

The first classification scheme for bacteria was published in 1773 based on morphological characteristics. One of the unique, broadscope and widely accepted classification scheme was published in 1927 by David Bergey & colleagues is Bergey's Manual of Determinative Bacteriology.

It provides identification schemes for identifying Bacteria and Archae based on their morphology, differential staining and biochemical tests. Whereas in 1984, a more detailed work was entitled. Bergey's manual of Systematic Bacteriology provides information on Bacteria and Archaea based on rRNA sequencing. The classification in Bergey's Manual is accepted by the most microbiologists as the best consensus for prokaryotic taxonomy.

The present classification scheme based on genetic relatedness has more practical value. This is expected to provide greater stability and predictability. It would lead to improved identification schemes, and to aid our understanding of the origin of present day genera and species.

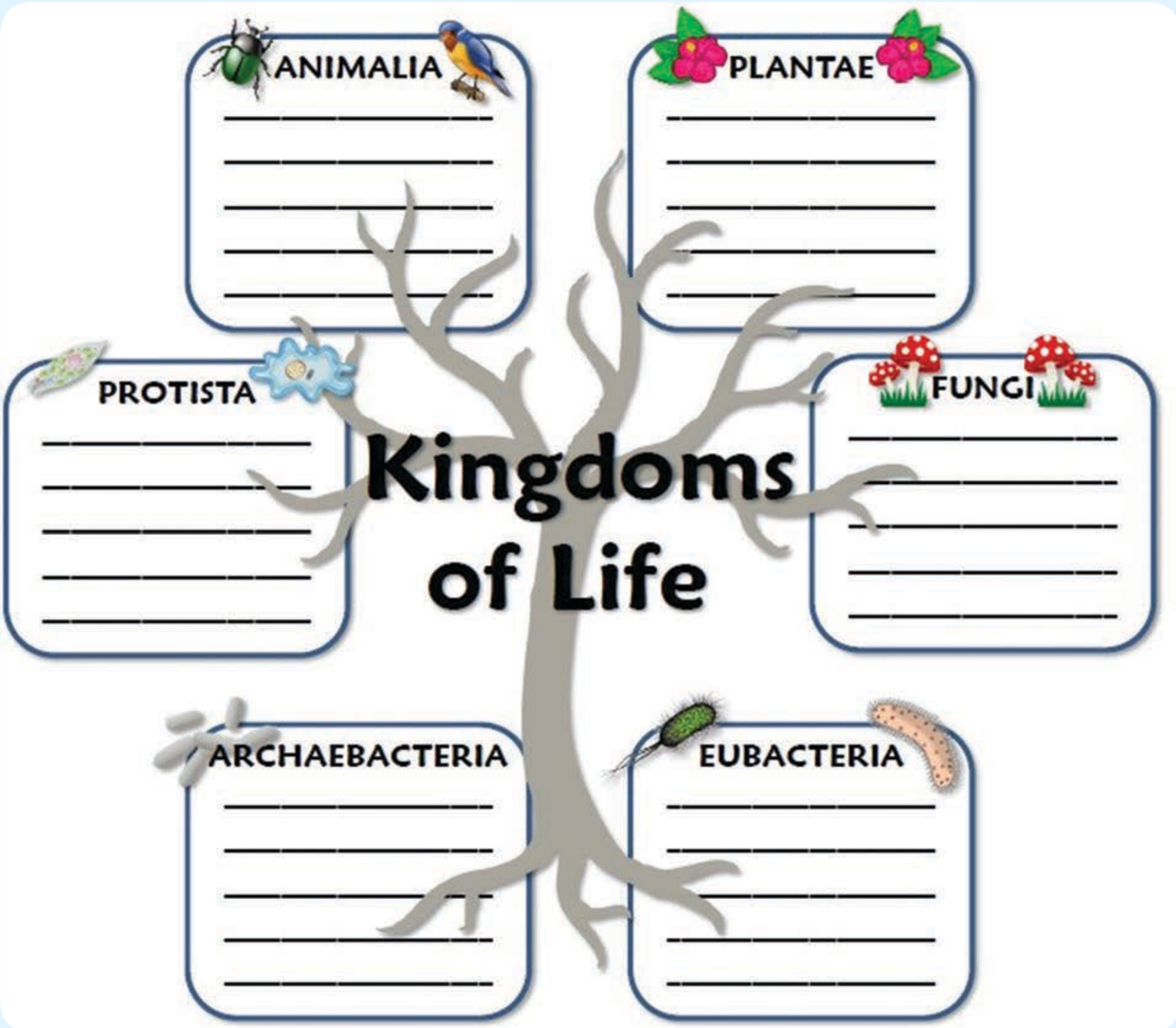
Summary

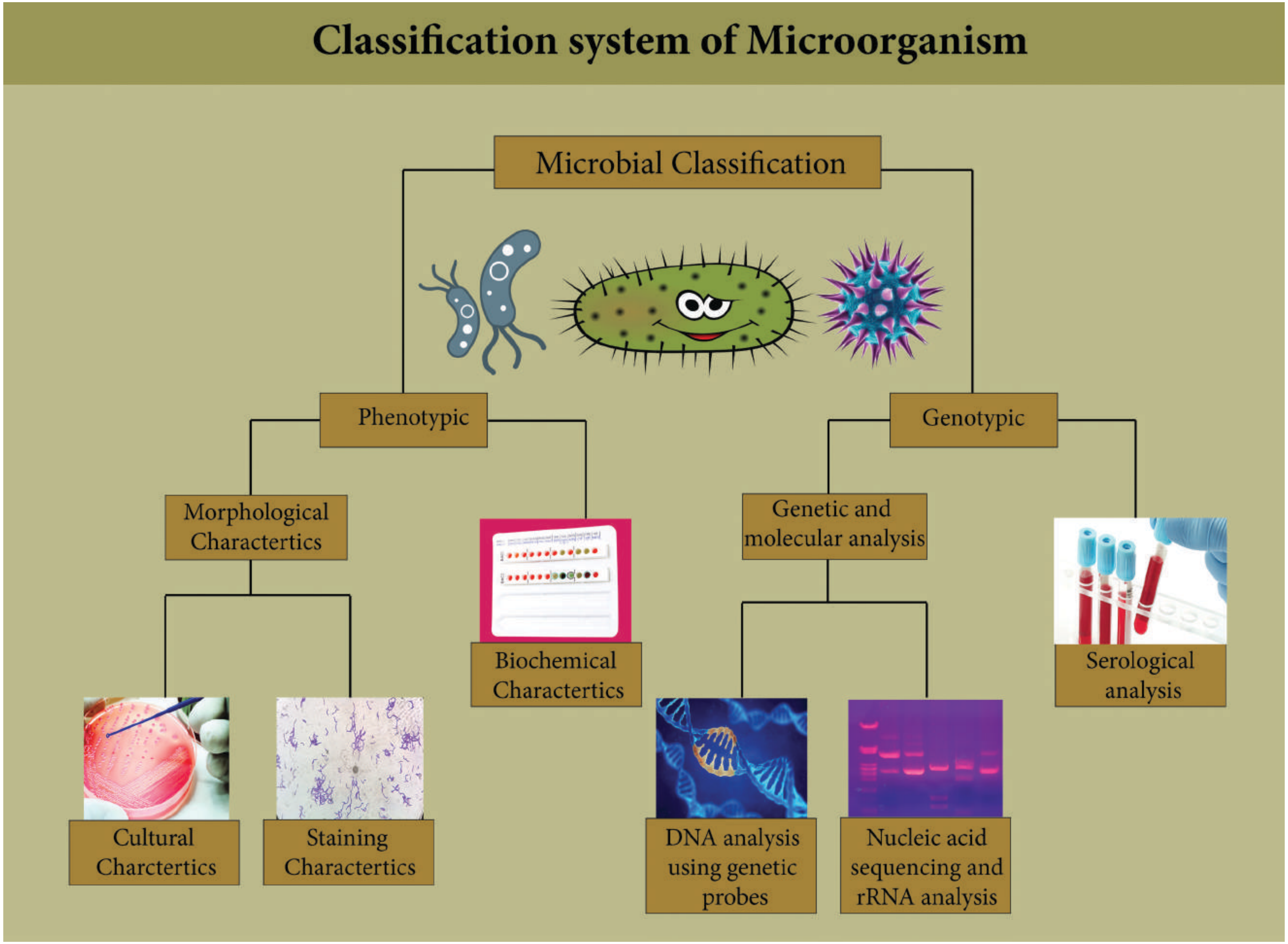
The branch of science which deals with the classification, nomenclature and identification of all living organisms is called taxonomy. The system of naming living organisms is called as nomenclature. Carolus Linnaeus divided all living organisms into two kingdoms- Animalia and Plantae. He introduced Binomial Nomenclature for naming living organisms. Whittaker proposed five kingdom classification based on various properties of living organisms. Currently accepted classification proposed by Woese, Kandler and Wheelis is the three domain classification. Modern developments of sequencing technologies and recognition of rDNA sequences are of now cornerstone for identification purposes.

Overall, it is important to recognize that microbial diversity is very much linked to its environment and the correlation has to be established by description of environmental parameters whenever sampling is carried out. It is also important to study the phenotypic characteristics and link them to the observations obtained from genotyping techniques. The link between habitat and diversity then becomes easier to understand for future studies.

Student Activity

The student must understand the characteristics of each domain under the five kingdom classification and fill in the chart below.




Classification system of Microorganism
Microbial Classification
Phenotypic
Genotypic
Morphological Charactertics
Biochemical Charactertics
Genetic and molecular analysis
Serological analysis
Cultural Charctertics
Staining Charactertics
DNA analysis using genetic probes
Nucleic acid sequencing and rRNA analysis

Evaluation

Multiple choice questions

1. Which of the following is a reasonable representation of phylogenetic diversity?
   a. The Chain of Being
   b. The Ladder of Life
   c. The 5-Kingdom Tree
   d. The 3-Domain Tree
2. Microorganisms belonging to the same are expected to have the most characteristics in common with eachother.
   a. Order
   b. Species
   c. Family
   d. Kingdom
3. What was the first and most useful microscopic tests for classifying bacteria?
   a. Gramstain
   b. Flagellar stain
   c. Simple stain
   d. Capsular stain
4. Which of the following is the arrangement of organisum into groups or taxa?
   a. Nomenclature
   b. Identification
   c. Systematics
   d. Classification
5. Binomial nomenclature means writing the name of microorganism in two words is
   a. Order and family
   b. Family and genus
   c. Species and variety
   d. Genus and species

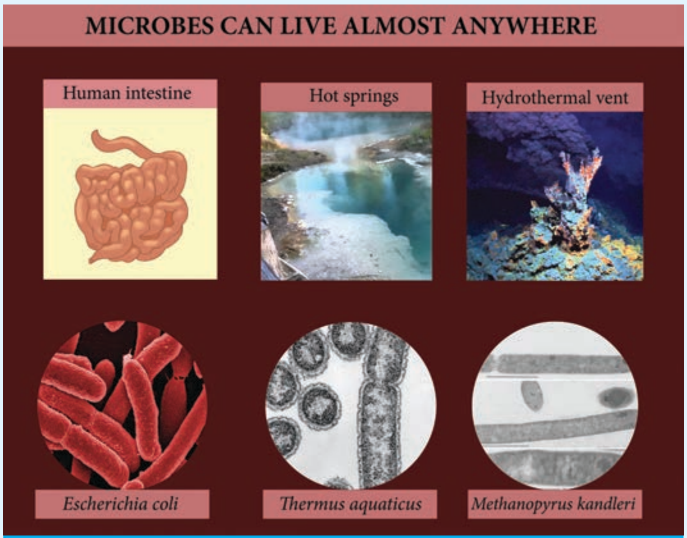
Answer the following

1. Define: Taxonomy and what is the here inter related parts of taxonomy?
2. Define: Classification, Nomenclature and Identification.
3. What is meant by binomial system?
4. Who developed the Bionomial system in the year?
5. What is taxonomic rank and why are we using this?
6. What is the difference between biovars, serovars and morphovars?
7. What is type strain and why it is called as type strain?
8. Write down the techniques which are used to identify the taxonomic characters of an organism?
9. Explain in detail about the molecular characteristics which are used to identify the taxonomic orders?
10. What are the five kingdom classifications?

Chapter 9

Environmental Microbiology

Chapter Outline



Microorganisms are found in every corner of the earth from miles below the surface to boiling hot springs to Antartic ice. Every ecosystem on earth contains microorganisms thay occupy unique niches based on their specific metabolic properties.

Learning Objectives

After studying this chapter the student will be able,

- *To gain knowledge of various layers of atmosphere and micro fauna of air.*
- *To understand air pollution and air borne diseases.*
- *To learn water borne diseases and water treatment procedures.*
- *To know Eutrophication.*
- *To know composting techniques.*
- *To gain knowledge of biogas production.*

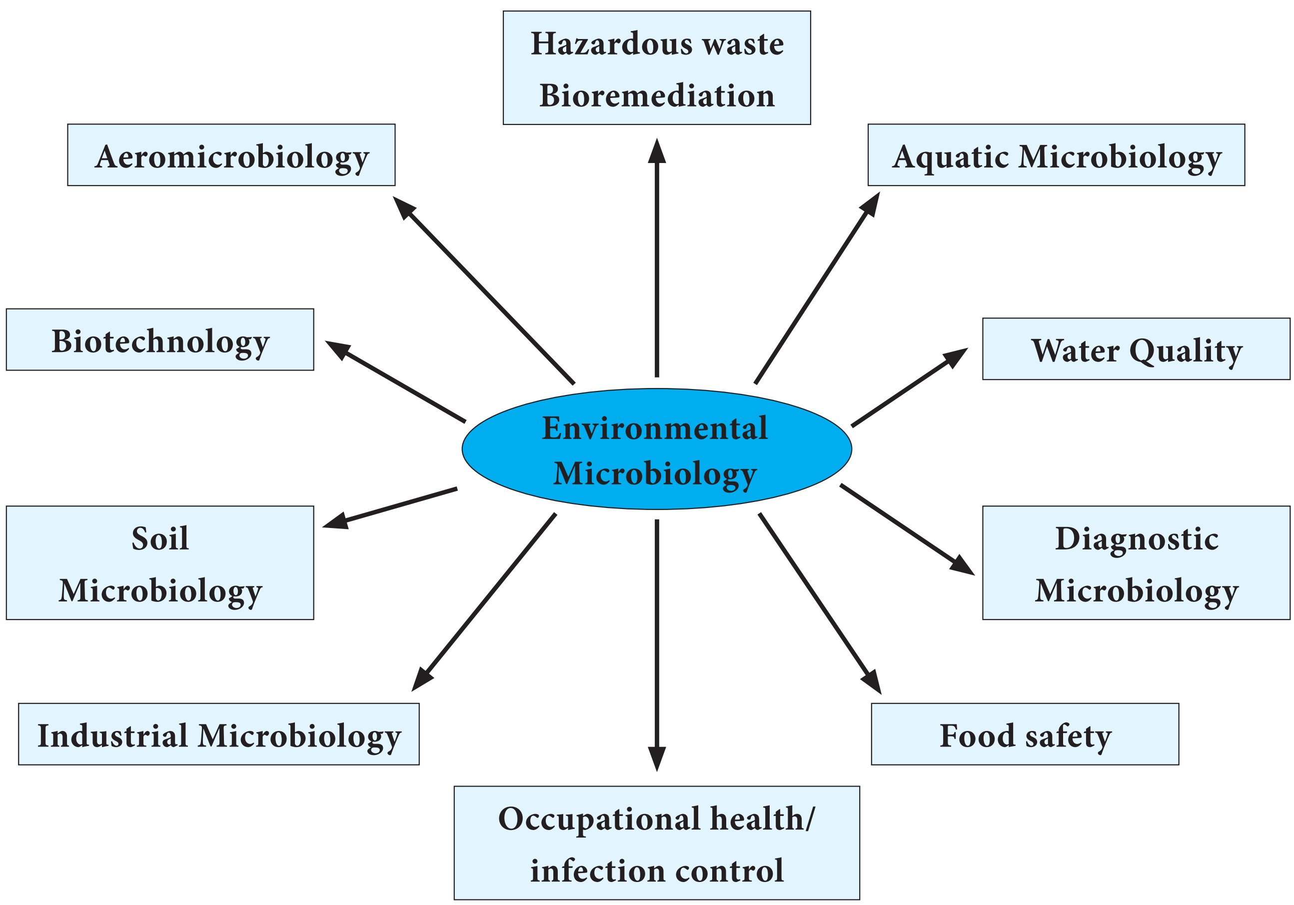
Environmental Microbiology is the field of science that examines the relationship between microorganisms and their biotic and abiotic environments. Microorganisms in the environment are diverse in origin and ubiquitous. Environmental Microbiology involves the study of the applied effects of microorganisms on the environment and on human activity, health and welfare.

It was in the 1970s that a new area of Microbiology emerged and developed into the field of Environmental Microbiology. The initial focus was on water quality pathogens in the environment in the context of public health safety. The developing field of environmental microbiology expanded to several other areas of applied research. These include microbial interactions with chemical pollutants in the environment and the use of microorganisms for resource production and resource recovery.

Chemical pollutants in soil and ground water have pronounced effects on human population. The cost of cleanup or remediation of the contaminated sites is too high. The overall objective of this chapter is to define the important microbes involved in environmental microbiology, the nature of the different possible environments in which the microbes are situated, the methodologies used to monitor the microbes and their activities and finally the possible effects of the microbes on human activities.

9.1 Interrelationship with other Fields

Since environmental microorganisms affect so many aspects of life and are easily transported between environments, the field of Environmental Microbiology interfaces with a number of subspecialities. It includes soil, aquatic, aeromicrobiology, as well as bioremediation, Water quality, Occupational health, Infection control, Food safety and Industrial Microbiology.



Modern environmental microbiology has a much wider scope. There are many different fields which recognises the problems in various fileds of Environmental Microbiology. It includes discovery and identification of new microbes and their products that may have practical application for protection of environment and human health and commercial applications.

9.2 Air Microbiology

Earth's environment is endowed with atmosphere, hydrosphere, lithosphere and biosphere. In the 1930s, **F.C. Meier** coined the term "Aerobiology". Air is a natural resource and is fundamental to human life as it makes breathing possible. Its universal presence and requirement for the survival of human beings make air an important environmental factor. Air contains significant number of microorganisms. It also acts as a medium for the transmission of microorganisms including bacteria, viruses, fungi, yeast and protozoa. Airborne transmission is one of the important modes for the transmission of infectious diseases. Aeromicrobiology is the study of air borne microorganisms.

Infobits

Miquel (1883) and Carnally & colleagues (1887) carried out the most systematic studies in airborne microorganisms.

9.2.1 Layers of Atmosphere

Earth's atmosphere is divided into five major layers (Figure 9.1) they are:

- Exosphere : 700 to 10,000 km
- Thermosphere : 80 to 700 km
- Mesosphere : 50 to 80 km
- Stratosphere : 12 to 50 km
- Troposphere : 0 to 12 km

The air in the troposphere, the layer close to the earth is constantly in motion and the temperature decreases with increasing altitude, reaching a low of -57°C at the apex of this region. Microorganisms are frequently found in the lower portion of the troposphere, where they are dispersed by the air currents. Most of the microbes present in the troposphere are either spore formers or microbes that are easily dispersed in air. The stratosphere has a temperature range of -80°C to -10°C. The temperature in the stratosphere can reach a maximum of several thousand degrees. Microorganisms are not found in the upper regions of the atmosphere because of the temperature extremes, scarcity of available oxygen, absence of nutrients and moisture, and low atmospheric pressure. The relatively low humidity in the atmosphere (especially during daylight hours) and UV rays from the sun, limit the types and number of microorganisms that are able to survive in this part of the biosphere.

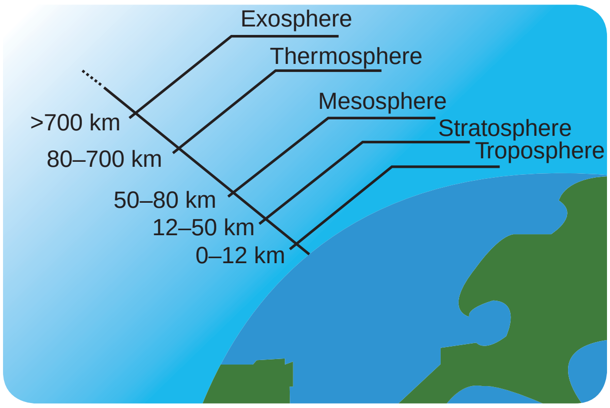


Figure 9.1: Diagram showing layers of atmosphere

9.2.2 Composition of Air

The air in our atmosphere is composed of different gaseous molecules. The most common gases are nitrogen (78%), CO_2 (0.034%) oxygen (21%) argon (1%) and other molecules in trace level are present in the atmosphere.

Table 9.1: Composition of Air

Gas	Symbol	Content
Nitrogen	N_2	78.084% ⎤
Oxygen	O_2	20.947% ⎥ 99.99%
Argon	Ar	0.934% ⎦
Carbon dioxide	CO_2	0.033%
Neon	Ne	18.20 ppm
Helium	He	5.20 ppm
Krypton	Kr	1.10 ppm
Sulphur dioxide	SO_2	1.00 ppm
Methane	CH_4	2.00 ppm
Hydrogen	H_2	0.50 ppm
Nitrogen oxide	N_2O	0.50 ppm
Xenon	Xe	0.09 ppm
Ozone	O_3	0.07 ppm
Nitrogen dioxide	NO_2	0.02 ppm
Iodine	I_2	0.01 ppm

9.2.3 Microflora of Air

Human beings and animals are continuously inhaling the microbes present in the air that cause various infectious diseases. Most of the respiratory tract infections are acquired by inhaling the air pathogen. The microflora of air is studied under two categories such as indoor and outdoor microflora.

Indoor microflora

The air found inside the closed environment (building/ room) is referred as indoor air and the microbes present in this region is called indoor microflora.

Example: *Staphylococcus, Bacillus, Penicillium*.

Outdoor Microflora

The air in the exterior environment is called outdoor air and the microbes that reside there are called outdoor microflora.

Example: *Bacillus, Aspergillus*.

9.2.4 Sources of Microorganisms in Air

Air is not a natural environment for microorganisms as it doesn't contain enough moisture and nutrients to support their growth and reproduction. Soil microorganisms when disturbed by the blow of wind, gets liberated into air and remain suspended there for a long period.

Man-made actions like digging, ploughing the soil may also release soil borne microbes into the air. Microorganisms found in water may also enter air by wind action or tidal action in the form of droplets or aerosols. Air currents may bring the microorganism from plant or animal surfaces into air.

Commensal as well as pathogenic flora of the human beings may enter the air by activities like coughing, sneezing, talking and laughing. The microorganisms are discharged out in different forms of particles which are grouped on the basis of their relative size and moisture content. These are aerosols, droplets, droplet nuclei and infectious dust.

Aerosols

An aerosols are mixture of small liquid or solid particles dispresed in air. Aerosols can be natural or anthropogenic. Example: Dust and smoke.

Droplets

Droplets are formed by sneezing, coughing and talking which consists of saliva and mucus (Figure 9.2). Droplets are relatively large, about 10µm or more in size, and they tend to settle rapidly in still air. When inhaled these droplets are trapped on the moist surfaces of the respiratory tract. Thus, the droplets containing pathogenic microorganisms may be a source of infectious disease.

Figure 9.2: Droplets released during sneezing

Droplet nuclei

Small droplets in a warm, dry atmosphere tend to evaporate rapidly and become droplet nuclei. These are 1-4µm in size. They can remain in air for longer period and transmit various infectious airborne diseases.

Infectious Dust

Large aerosol droplets settle out rapidly from air on to various surfaces and get dried. Nasal and throat discharges from patient can also contaminate surfaces and become dry. Microorganisms can survive for longer period in dust. This creates a significant hazard, especially in hospital areas.

9.2.5 Air Borne Diseases

Many microbial diseases are transmitted through the air (Table 9.2). The incidence of diseases caused by airborne transmission can be reduced by covering one's nose and mouth during coughing or sneezing and by the use of face masks.

Table 9.2: Important airborne human diseases and causative agents (pathogens)

Human Diseases	Pathogens
Bacterial diseases	
Pulmonary tuberculosis	*Mycobacterium tuberculosis*
Pneumonia	*Klebsiella pneumoniae*
Streptococcal respiratory infections	*Streptococcus pyogenes*
Fungal diseases	
Aspergillosis	*Aspergillus fumigatus*
Cryptococcosis	*Cryptococcus neoformans*
Viral diseases	
Influenza	*Influenza Virus*
Common cold	*Picorna Virus*
Protozoal diseases	
Pneumocystosis	*Pneumocystis carinii*

Nosocomial infection

Hospital acquired infection are also known as a nosocomial infection. It is acquired in a hospital or other health care facility. Infection is spread to the susceptible patient in the clinical setting by various means, one of them being air droplets. The infection can originate from another infected patient, staff, or in some cases, the source of the infection cannot be determined. The most common pathogens that cause nosocomial infections are *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli.* One of the common nosocomial infections is respiratory pneumonia.

9.2.6 Enumeration of Microorganisms in Air

There are several methods adopted to enumerate microorganisms in air. The most important methods are solid impingement and liquid impingement, filtration, sedimentation, centrifugation electrostatic precipitation. Many tools have been designed for the collection of air samples. Choosing an appropriate sampling device is based on many factors, such as availability, cost, volume of air to be sampled, sampling efficiency and the environmental conditions under which sampling will be conducted. One of the methods is Settle plate technique, where the microorganism carrying particles are allowed to settle onto the medium (solid impingement) for a given period of time and incubated at the optimum temperature (Figure 9.3). It works under the principle of gravitational force.

Figure 9.3: Settle plate technique showing bacterial and fungal growth

Choice of the medium depends upon the kind of microorganisms to be enumerated. For bacterial isolation Nutrient agar and for fungal isolation Sabourauds dextrose agar (SDA) can be used.

During sampling it is better to keep the plates about one meter above the ground level. Then the plates are uncovered in the selected position for the required period of time. Immediately after exposure for the given period of time the plates are closed with the lids. Then the plates are incubated for 24 hrs at 37°C for aerobic bacteria and for 2 days at room temperature (27°C) for fungi. Enumeration results are expressed as numbers of viable organisms per unit area of air colony forming unit (CFU/mm^3).

HOTS

Can microorganisms grow in clouds?

9.3 Microbiology of Water

Aquatic microbiology is the study of microorganisms and microbial communities in water environments. Aquatic environments occupy more than 70% of the earth's surface. Water is essential for life and one of the most important natural resource. Thus, protection and preservation of aquatic environments are vital for the continuation of life. There are two kinds of water found on earth:

1. Salt water (97%)
2. Fresh water (3%)

9.3.1 Salt Water Microflora

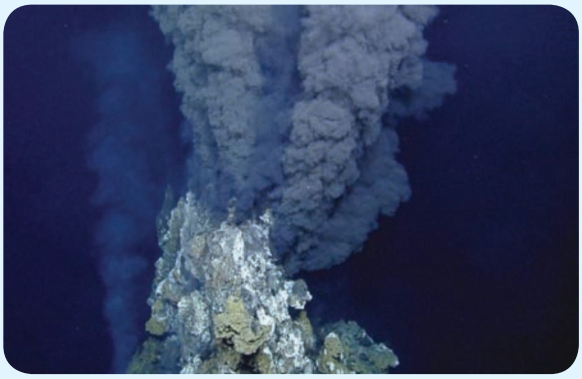
Salt water contains a significant level of dissolved salts. The major bodies of salt water are oceans, seas, estuaries, and certain salt water lakes. Average salinity of ocean is 3.5 grams per 100 grams of water. The pH of salt water remains relatively constant at a range of 8.3 to 8.5. The temperature of seawater fluctuates depending on location, season and depth.

9.3.2 Estuaries

A partly enclosed coastal body of water in which fresh water is mixed with seawater is called an estuary. As a consequence of this geographical location, estuaries are of salinity levels that range from less than 0.5 to 2.5 grams of dissolved salts per 100 grams of water at their mouths, where the water flows into the marine environment. Estuaries are ecologically sensitive environments and serve as habitats for fish and wide variety of marine life. There is an increasing concern about the quality of life in estuaries seriously damaged by human impact, including over development and pollution from industrial and waste water discharges. The bacterial population in estuaries consists of *Pseudomonas, Flavobacterium,* and *Vibrio*, as well as enteric organisms. The quantities and types of organisms vary, and depend on the tide, rainfall, salinity, depth and temperature. Most of the bacteria found in water runoff come from animal and

DO YOU KNOW?

The hot, sulphur-rich, acidic pool of yellow stone national park (U.S.) is home to many Archaebacteria. The colour differences in the pool result from the different communities of microbes that are able to thrive at extreme water temperatures. *Pyrolobus fumarii* is a unique Archaebacteria, which is hyperthermophilic that can grow at the temperature of 113°C. Some Archaebacteria live in thousands of miles deep in ocean near superheated volcanic vents.



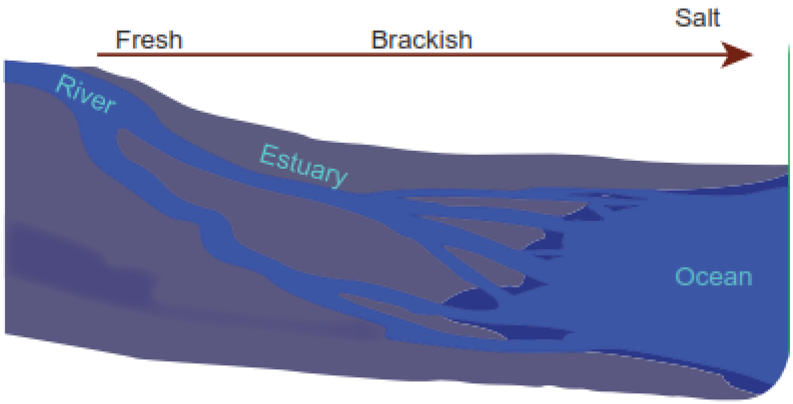


Figure 9.4: Estuary

fowl fecal matter deposited on the ground. Sometimes overflow from sewage systems contributes to these higher number of bacteria in the water. Microbiota are the primary producers in the aquatic ecosystem since they play a major role in food chain in water. Drifting microbial life of aquatic environment is called plankton. It is composed of phytoplankton and zooplankton. The bottom region of the water harbours largest number of microorganisms called as benthic microorganisms. Microorganisms have been found at all depths and at all latitudes within the ocean. Microorganisms are abundant near shore regions, where organic pollution as well as microbial pollution from the surrounding environment occurs.

Algae are common in these environments. Because of their dependence on light as a source of energy, algae occur primarily in the upper strata of the oceans and seas. Although they constitute a vital part of the food chain in the marine environments, they also can be a nuisance and threat to other forms of life. Nutrient levels in such environments can significantly increase as a result of sewage plant discharges. Under such conditions algal blooms are common.

DO YOU KNOW?

Barophiles are bacteria that thrive deep in the ocean and live at pressures of 400-700 atmosphere but die at 1 atmosphere. A strain MT-41, a bacteria is isolated from the marine sediments in the Mariana Trench near the Phillippines where the depth exceeds 10 kilometres. This strain has optimum growth at a pressure of 709 bar.

9.3.3 Freshwater Microflora

The study of fresh water ecosystem is referred to as Limnology. The major bodies of fresh water are rivers, streams, swamps, marshes and lakes. The fresh water ecosystem is divided into lentic (still water) and lotic (flowing water) ecosystems. Lentic ecosystem is divided into zones based on light penetration and temperature. They are littoral, limnetic and profundal zones (Figure 9.5).

Most lakes are surrounded by rooted vegetation in a large littoral zone along the shore. Light penetrates the shallow littoral and open-water limnetic zone but is unable to reach the profundal zone in the deep portions of many lakes.

As lake vegetation and animal life decompose, their organic matter provides a source of nutrients. Lakes that have very high concentrations of nutrients (particularly nitrogen and phosphorus) are termed eutrophic and have low oxygen concentrations because of extensive microbial decomposition of organic matter. In comparison, the lakes that receive small amounts of nutrients and are nutrient sparse are oligotrophic. Caulobacter grows well in oligotrophic lakes.

The common microorganisms found in fresh water are *Pseudomonas, Flavobacterium, Serratia, Chromobacterium, Micrococcus, Aeromonas* and *Alcaligenes*.

As these waters reach the surface or inland bodies of water, they become contaminated with different types of microorganisms. Rivers flowing water (lotic) in close contact with the soil may contain large numbers of soil bacteria (Bacillus, *Actinomyces* and *Streptomyces*), Fungi (*Polyphagus, Penicillium* and *Aspergillus*), and algae (*Microcystis* and *Nostoc*). These microorganisms frequently impart an earthy odour and taste to river water. Rivers also receive high concentration of bacteria and agricultural chemicals through surface runoff water from adjoining soil during heavy rains and irrigation. In many countries, rivers are heavily polluted with sewage bacteria, especially *Escherichia coli, Enterobacter fecalis, Proteus vulgaris* and other intestinal bacteria.

Infobits

Red Tides

Red tide is a common name for a phenomenon known as an algal bloom which is caused by a species of Dinoflagellates (*Gymnodinium*) and the bloom takes on a red colour due to the presence of photosynthetic pigments with the production of toxins. The most conspicuous effects of red tides are associated with mortalities of marine species

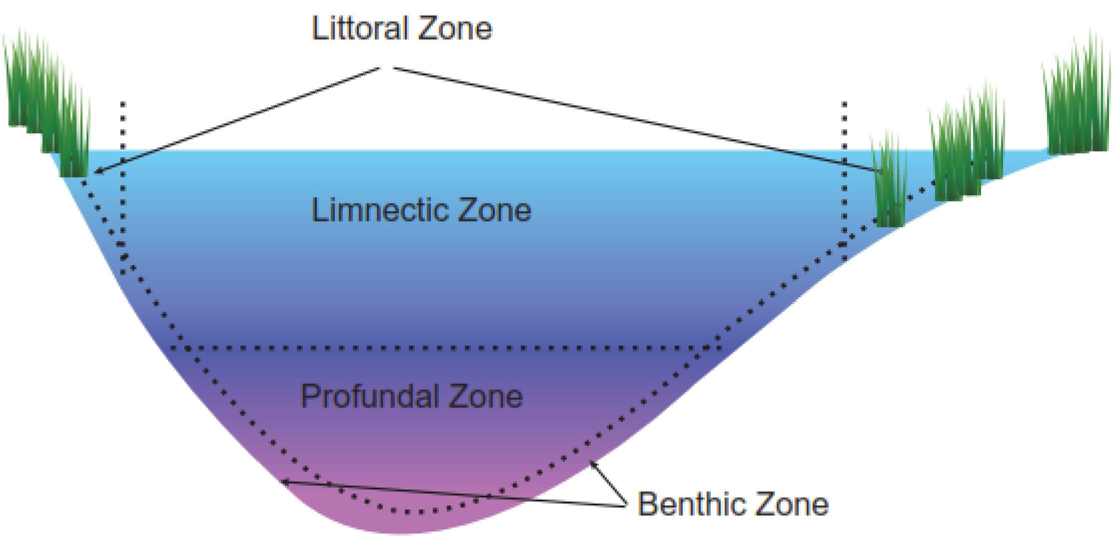


Figure 9.5: Light penetration zones of a freshwater lake

9.3.4 Eutrophication

The addition of excess quantities of nutrients to aquatic ecosystems termed eutrophication often cause damage on the communities involved. The sudden influx of abundant nutrients along with warm temperatures encourages a heavy surface growth of cyanobacteria and algae called a bloom (Figure 9.6). This heavy matt of biomass effectively shuts off the oxygen supply to the lakes below. The oxygen content below the surface is further depleted by aerobic heterotrophs that actively decompose the organic matter. The lack of oxygen greatly disturbs the ecological balance of the community. It causes massive death of strict aerobes. Fish invertebrates and anaerobic or facultative microbes will survive. Eutrophication is an enrichment of water by nutrients, especially nitrogen and phosphorus which are maintained at high levels that causes structural changes to the eco system.

Effects of eutrophication

- Eutrophication can have serious, long term effect. The most notable effect of eutrophication is **algal blooms**.
- When a bloom occurs, the river, lake or ocean becomes covered with algae, which is usually bright green.
- Blue green algae like *Microcystis, Anabaena, Gonyaulax* and other Dinoflagellates produce toxins which are poisonous to aquatic lives such as fish.
- Algal toxin may also contaminate the drinking water supply.
- Plankton blooms of green alga create problems of O_2 supply in the water.
- Musty tastes and odors in drinking water are the other effects of Eutrophication.
- Excessive growth of aquatic weeds which impair fishing, swimming, boating, shell fish production.
- Irrigation canals may become clogged.

Control measures

Five different methods have been suggested to control eutrophication they are,

1. Ecological management (control the flow of nutrients into natural wastes)

Figure 9.6: Algal blooms in a polluted eutrophic lake

2. Advanced treatments Example: Phosphate removal is effected by precipitation with lime.
3. Chemical algicides Example: copper sulphate
4. Biological algicides Example: Bacteria
5. Destratification Example: physical/ mixing / forced aeration.

9.4 Water Pollution and Microbial Contamination

Water is polluted by both natural as well as man-made activities. Polluted water is one which consists of undesirable substances rendering it unfit for drinking and domestic use.

Sources of water pollutants

1. Industrial waste
2. Sewage waste
3. Mining activities
4. Marine dumping
5. Accidental oil leakage
6. Burning of fossil fuels
7. Chemical fertilizers and pesticides
8. Radio-active waste

The most prevalent biological contaminants in water are microbes, particularly bacteria and viruses. Most of the bacteria carried by storm runoff originate from animal fecal matter. Studies have shown that during storms, the water that drains off the land and into sewage systems also carries large quantity of bacteria and chemicals. The chemicals include pesticides applied to lawns, chemical wastes near industrial plants, and organic matter deposited o the ground by different sources. In addition to the chemical and biological contaminants, physical properties also affect the quality of biological life in water. Among these are pH, temperature, dissolved oxygen concentration and salinity.

Potable Water

Clean water free from odour, disagreeable taste, harmful chemicals, turbidity and microorganisms is called potable water, which is safe to drink and can use for food preparation without risk of health problems.

Biological oxygen demand (BOD)

Biological oxygen demand is one of the common parameter to monitor water quality and purity. BOD is the amount of dissolved oxygen needed by aerobic organisms to break down organic material present in a given water sample at certain temperature over a specific period of time. The amount of decomposable organic material in sewage is measured by the biochemical oxygen demand, or BOD. The BOD of the water sample is determined by aerating it, measuring the amount of oxygen in sample before incubation, placing the sample in a sealed container (BOD bottle) and incubating the container for five days at 20°C. During this five day period, microorganisms in the water grow and oxidize any organic materials in it. After incubation period, the BOD of the water can be determined by measuring the quantity of residual oxygen in the container. BOD of drinking water should be below 3ppm or 3mg/litre.

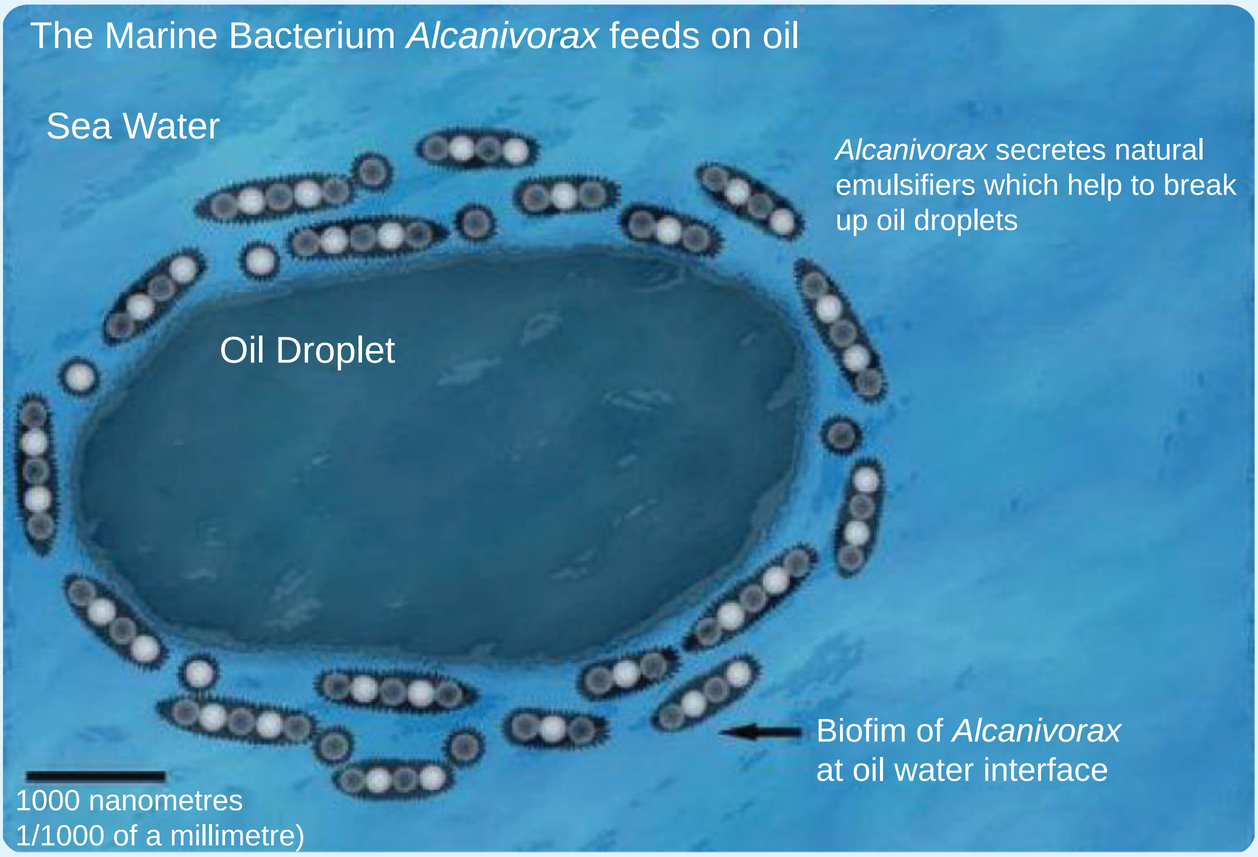
Indicator Microorganisms

Indicator organism are frequently used to monitor bacterial contamination of

Infobits

Microbes at work:

One of the major environmental problems today is hydrocarbon contamination resulting from the activities related to the Petrochemical Iindustry. Accidental releases of petroleum products are of particular concern in the environment. Hydrocarbon components have been known to belong to the family of carcinogens and neurotoxic organic pollutants. Petroleum-based products are the major source of energy for industry and daily life. Leaks and accidental spills occur regularly during the exploration, production, refining, transport, and storage of petroleum and petroleum products. There are the two main approaches to oil spill bioremediation: (a) Bioaugmentation, in which known oil-degrading bacteria are added to supplement the existing microbial population, and (b) Biostimulation, in which the growth of indigenous oil degraders is stimulated by the addition of nutrients or other growth-limiting cosubstrates. Bacteria like *Pseudomonas putida* and *Alcanivorax borkumens* are the most active agents in petroleum degradation, and they work as primary degraders of spilled oil in environment. Bioremediation is a potential source for clean and green environment.



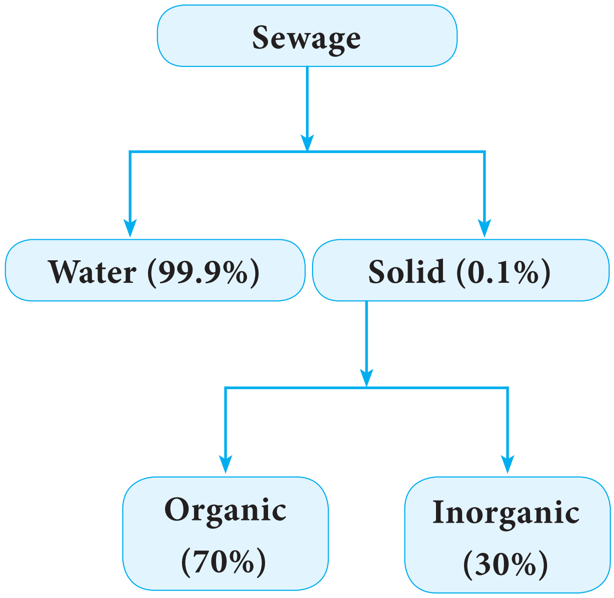
water. These indicator organisms provide a representative index of the water contamination by pathogenic microbes. The indicator organisms generally used in water quality monitoring are those that are associated with the gastrointestinal tract and fecal matter. The most common group of indicator organisms used in water quality monitoring are the coliforms, bacteria that are Gram negative, aerobic or facultative anaerobic, non-spore forming rods that ferment lactose with gas production within 48 hours at 35°C. Examples of coliforms are *Escherichia coli, Enterobacter aerogenes* and *Klebsiella pneumonia*. Two analytical procedures were followed to check the presence of coliforms in water. They are Most Probable Number (MPN) and Membrane Filteration (MF) technique. The number of coliforms per 100ml of water sample is estimated to find the quality of water and its suitability for drinking purposes. In addition to coliforms, coli phages, *Clostridia* and human enteric viruses are also monitored in drinking water.

Waterborne diseases

Waterborne diseases are posing a serious threat to health (Table 9.3).

9.5 Sewage Treatment

Wastewater treatments also called **sewage treatment** which removes the impurities from wastewater, or sewage, before disposal into natural bodies of water. In broad terms, water is said to be polluted when it contains enough impurities to make it unfit for a particular use, such as drinking, swimming, or fishing. Water pollution is caused primarily by the drainage of contaminated wastewater into surface water or groundwater. The wastewater treatment is a major element of water pollution control.



Goal of Sewage Treatment

- To convert waste water into a reusable resources.
- To reduce the spread of pathogenic microorganisms
- To avoid health hazards while swimming and boating in the water.
- To prevent the development of objectionable colours and tastes

The predominant method of wastewater disposal in large cities and towns is discharge into a body of surface water. Suburban and rural areas rely more on subsurface disposal. In either case, wastewater must be purified or treated to some degree in order to protect both public health and water quality. Suspended particulates and biodegradable organics must be removed to varying extents. Pathogenic bacteria must be destroyed. It may also be necessary to remove nitrates, phosphates (plant

HOTS

List the major oil spills that occurred recently in India and other countries. Is it practically possible to clean up these oil spills using bacteria?

Table 9.3: Waterborne diseases

Waterborne diseases	Causative agent	Symptoms
	Bacterial diseases	
Enteric fever	*Salmonella typhi*	Fever & enlargement of spleen
Cholera	*Vibrio cholerae*	Vomiting & watery diarrhea
Leptospirosis (Weils Disease)	*Leptospira interrogans*	High fever, red eyes, muscle pain and vomiting
	Viral diseases	
Infectious Hepatitis	*Hepatitis A*	Jaundice, vomiting & abdominal pain
Gastroenteritis	*Rotavirus*	Diarrhoea, vomiting
Poliomyelitis	*Coxsackie Virus*	Head ache, neck stiffness, flaccid paralysis.
	Protozoan diseases	
Giardiasis	*Giardia lamblia*	Chronic diarrhoea, abdominal cramp, fatigue & weight loss
Amoebiasis	*Entamoeba histolytica*	Stomach pain, bloody stools, fever
Meningoencephalitis	*Naegleria fowleri*	Ulceration, watery, bloody diarrhoea
	Treamatode disease	
Schistosomiasis	*Schistosoma*	Drowsy, confusion, head ache, stiff neck, Diarrhoea

nutrients) to neutralize or remove industrial wastes and toxic chemicals. The degree to which wastewater must be treated varies, depending on local environmental conditions and governmental standards.

Sewage Treatment Methods

Sewage treatment defined as an artificial process in which sewage is subjected to remove / alter its constituents to render it less offensive. There are three levels of wastewater treatment (Figure 9.7): primary, secondary, and tertiary (or advanced).

1. Primary treatment
2. Secondary treatment
3. Tertiary / Final treatment

Primary treatment

Primary treatment removes about 60 percent of total suspended solids and about 35 percent of BOD; dissolved impurities are not removed. It is the physical method which remove large floating and suspended solids from sewage water. Example: papers, leaves, bottles, rocks, pieces of metal or wood. These objects are removed by passing the sewage through screens (Figure 9.8) and grit chambers. The screened water is then sent to settling tanks or basin, where the suspended solids are allowed to settle as primary sludge. Materials such as oil or grease, which float on the surface, are removed with a skimmer. The liquid

Figure 9.8: Bar Screening in Sewage Treatment

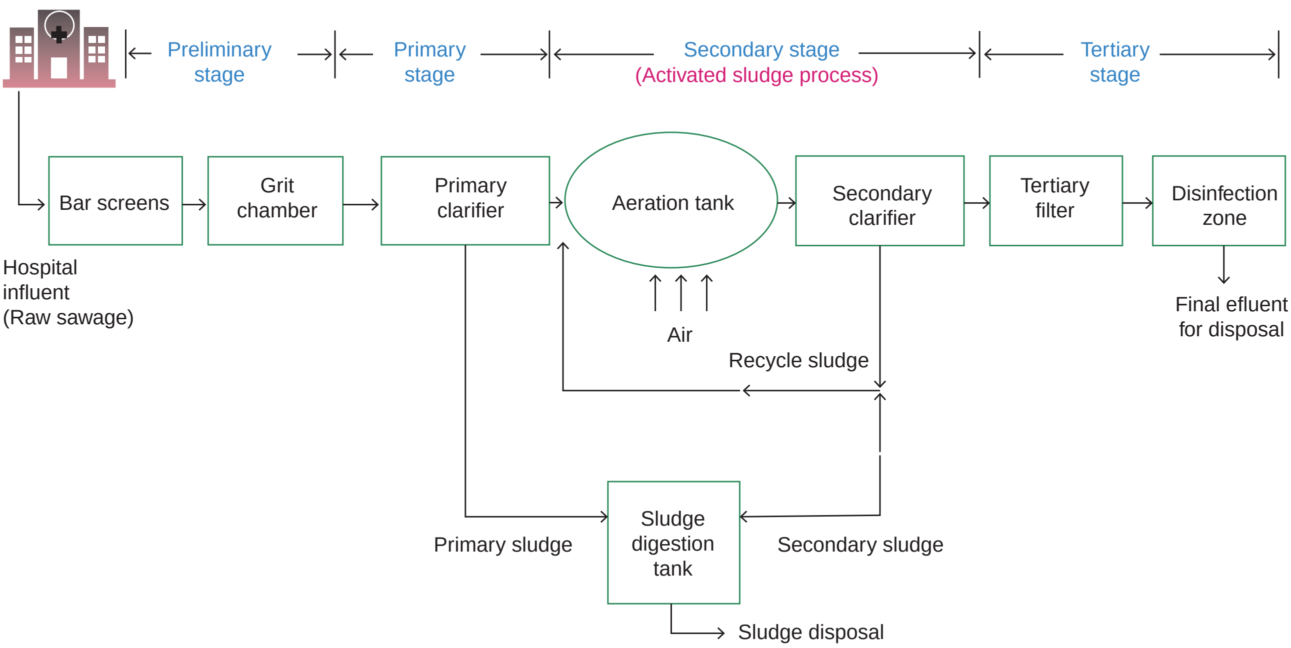


Figure 9.7: Schematic diagram of waste water treatment

wastewater remaining in the settling tank or basin is then ready for secondary treatment. The fluid from primary treatment is called primary effluent.

Secondary treatment

Secondary treatment removes more than 85 percent of both suspended solids and BOD. Secondary treatment involves the oxidation of the primary effluent by microorganisms. The common types processes used are:

1. Trickling filter process
2. Activated Sludge process
3. Oxidation ponds

A trickling filter consists of a large tank or basin filled with a bed of crushed stone, gravel, slag or other porous material. Sewage is sprayed in a fine mist over the rocks. As the sewage trickles through the bed, organic matter clings to the rocks, where it is digested by heterotrophic microorganisms (Figure 9.9). The microorganisms are contained within a biofilm which are produced by slime forming bacteria such as Zooglea and the organic matter, is oxidized to gases and inorganic products.

In the activated sludge process sewage is mixed with a slime forming bacteria (Zooglea) in a large aeration tank. As the mixture is aerated, large flocs, or clumps, form. These clumps contain not only the original slime forming bacteria but also large population of heterotrophs, which oxidize organic matter within these clumps. In this system wastewater is continuously pumped into the tank and the treated water is removed into a holding tank and the flocs are allowed to settle. The settled floc material is recycled into the tank as an inoculums to continue the process. The remaining floc is further treated or removed for burial or incineration.

Oxidation ponds (Figure 9.10) are used in some communities to handle small loads of sewage. Small communities and isolated areas frequently use oxidation ponds for treatment of waste water. Sewage is channeled into an initial pond where the sludge settles out. The liquid portion of the sewage is then pumped into an adjacent series of ponds where aeration allows bacterial growth and degradation of organic matter. These secondary ponds often are seeded with algae which provide oxygen for the growth of aerobic, heterotrophic bacteria.

Tertiary treatment

Tertiary processes can remove 99 percent of all the impurities from sewage. Although effluents from secondary treatment have a low BOD, they may contain eutrophication inducing salts (Phosphorus and Nitrogen compounds), organic and inorganic suspended solids, and poorly biodegradable organic materials. Advanced or tertiary treatment process are designed to reduce or eliminate these materials depends more on physical and chemical processes than biological processes. For phosphorus elimination the phosphates are converted to poorly soluble aluminum, calcium or iron compounds and removed by precipitation. Nitrogen in sewage effluent is removed primarily through nitrification by microorganisms. The extent of nitrification during tertiary treatment depends on adequate treatment plant designed and the proper

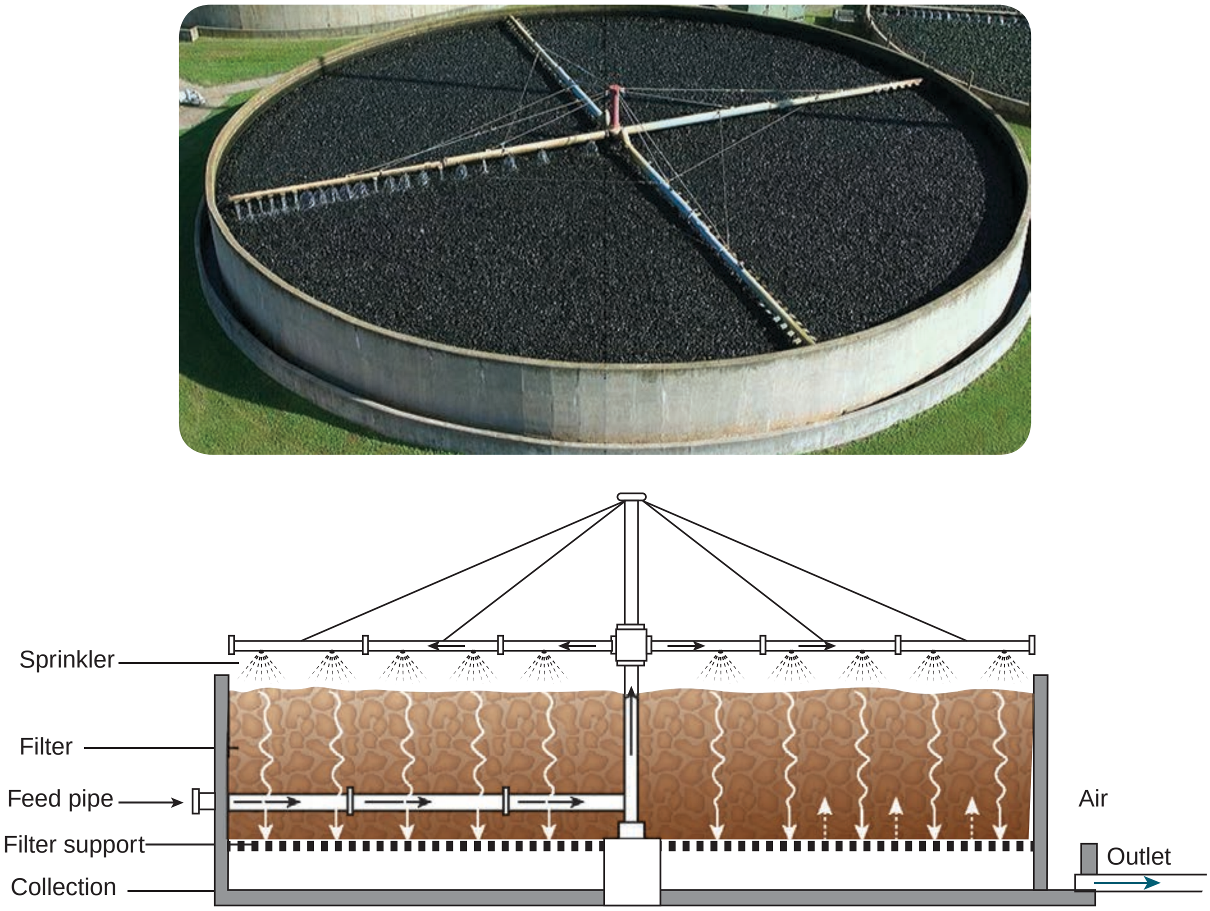


Figure 9.9: Trickling Filter in sewage treatment

removal of sludge so that these bacteria are grown under optimal conditions. Otherwise, large amounts of nitrogenous compounds may escape tertiary treatment and released in the effluent. Suspended solids are eliminated in sewage through filtration or sedimentation. Poorly biodegradable substances can be removed by the use of specialized microorganisms capable of using them as substrates. Chlorine is frequently added to tertiary treated effluent to kill any remaining microorganism.

9.6 Recycling of Treated Sewage

Water recycling is reusing treated wastewater for beneficial purposes such as agricultural and landscape irrigation, industrial processes, toilet flushing, and replenishing a ground water basin. Water recycling offers resource and financial savings. Recycled water for landscape irrigation requires less treatment than recycled water for drinking purpose. Recycled water can satisfy most water demands, as long as it is adequately treated to ensure water quality appropriate for the use.

The residue that accumulates in sewage treatment plants is called sludge. Treatment and disposal of sewage sludge are major factors in the design and operation of all wastewater treatment plants. Two basic goals of treating sludge before final disposal are to reduce its volume and to stabilize the organic materials. Stabilized sludge does not have an offensive odour and can be handled without causing a nuisance or health hazard.

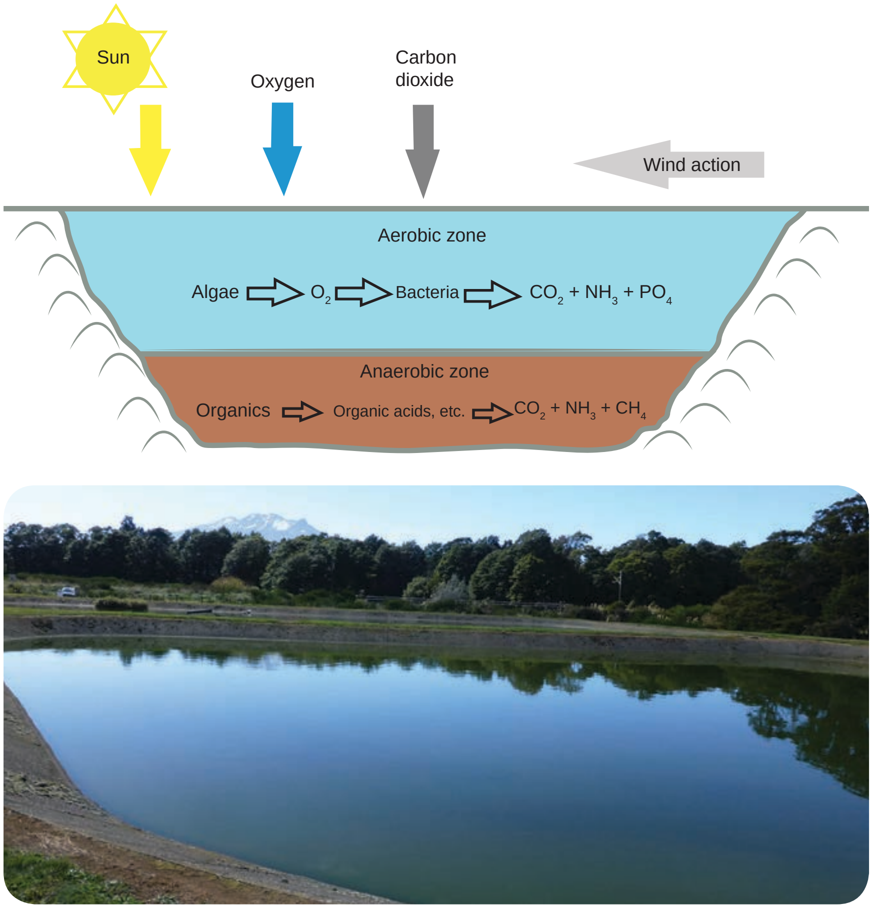


Figure 9.10: Oxidation Ponds

Sludge Digestion

Treatment of sewage sludge may include a combination of thickening, digestion, and dewatering processes. Among these digestion is mediated by microbes. Sludge digestion is a biological process in which organic solids are decomposed into stable substances. Digestion reduces the total mass of solids, destroys pathogens, and makes it

DO YOU KNOW?

Septic tank is a small scale anaerobic treatment process. It is commonly used in rural areas. It is simple, inexpensive and satisfactory if properly operated. The septic tank consists of an underground sedimentation container into which sewage from a home enter and is retained for a short time. The organic matter in the sewage settles to the bottom of the tank where it is covered by a thin organic film that excludes oxygen. Anaerobic bacteria in the sediment digests the organic matter into simpler chemical compounds and gases. The gases are then discharged through a vent in the tank. Liquids in the tank rise and overflow through an outlet pipe and are distribute in the surrounding soil. As the water trickles through the soil any remaining organic matter is decomposed by aerobic prokaryotes. Septic tank should not be located near water supplies because not all bacterial pathogens are removed by this treatment. Undigested solids in the bottom of the septic tanks must be periodically removed.

easier to dewater or dry the sludge. Digested sludge is inoffensive, having the appearance and characteristics of a rich potting soil.

Most large sewage treatment plants use a two-stage digestion system in which organics are metabolized by bacteria anaerobically (in the absence of oxygen). In the first stage, the sludge, thickened to a dry solids (DS) content of about 5%, is heated and mixed in a closed tank for several days. Acid forming bacteria hydrolyze large molecules such as proteins and lipids, breaking them into smaller water-soluble molecules, and then ferment those smaller molecules into various fatty acids. The sludge then flows into a second tank, where the dissolved matter is converted by other bacteria into biogas, a mixture of carbon dioxide and methane. Methane is combustible and is used as a fuel to heat the first digestion tank as well as to generate electricity for the plant.

Sludge digestion may also take place aerobically The sludge is vigorously aerated in an open tank for about 20 days. Methane gas is not formed in this process.

Digested sewage sludge is usually dewatered before disposal. Sludge-drying beds provide the simplest method of dewatering. A digested sludge slurry is spread on an open bed of sand and allowed to remain until dry. Drying takes place by a combination of evaporation and gravity drainage through the sand.

Sludge Disposal

The final destination of treated sewage sludge usually is the land. Dewatered sludge can be buried underground in a sanitary landfill. It also may be spread on agricultural land in order to make use of its value as a soil conditioner and fertilizer. Since sludge may contain toxic industrial chemicals, it is not spread on land where crops are grown for human consumption.

When a suitable site for land disposal is not available, as in urban areas, sludge may be incinerated. Incineration completely evaporates the moisture and converts the organic solids into inert ash. The ash must be disposed of, but the reduced volume makes disposal more economical. Air pollution control is a very important consideration when sewage sludge is incinerated. Appropriate air-cleaning devices such as scrubbers and filters must be used.

Benefits of Sewage Treatment

- Water recycling has proven to be effective and successful in creating a new and reliable water supply, while not compromising on public health.
- Water recycling can help us find ways to decrease the diversion of water from sensitive ecosystems
- Water users can supplement their demands by using recycled water.
- Decreases wastewater discharges
- Reduces and prevents water pollution
- Recycled water can be further used in Thermal power plant (for cooling), Municipal use, Irrigation and Agricultural use.

Infobits

There is a huge business opportunity in finding ways to use these waste dumps for productive purposes - energy, organic fertilizer. This requires methods of dealing with **old waste** that has been accumulating over the years as opposed to new/fresh waste.

9.7 Composting

Compost is organic matter that has been decomposed and recycled as a fertilizer and soil amendment. It is a mass of rotted organic matter made from waste. Example: garbage, paper, sugarcane trash, paddy straw, aquatic weeds, other agricultural waste.

Composting is a natural process in which aerobic and anaerobic microorganisms decomposes organic matter into valuable manure called as compost. The primary objective of composting is to convert an unstable material into stable end product (Figure 9.11).

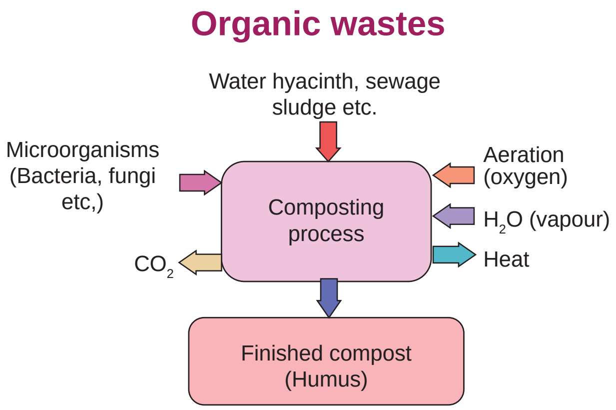


Figure 9.11: Composting process

The humification of organic material occurs in three stages

1. **Mesophilic stage** - Mesophilic is the initial stage of decomposition, lasting for about a week, during which sugars and other simple carbohydrates are rapidly metabolized. This is an exothermic process and may cause an increase in temperature by 40°C. Example: *Bacillus subtilis*
2. **Thermophilic stage** - Thermophilic is the second stage, lasting for about two weeks, during which the temperature may rise to about 50 to 75°C. Such a drastic increase in temperature is accompanied by the decomposition of cellulose and other resistant materials. It is important that the material be thoroughly mixed and kept aerated during this stage. Example: *Bacillus stearothermophilus*
3. **Curing stage** - The temperature decreases during this final stage and the material being composted is recolonized by mesophillic organisms, which often produce plant-growth stimulating compounds.

The humification of organic material is characterized by an increase in concentration of humic acids approximately from 4 to 12 percent, and decreases during the composting process.

What should you compost?

When selecting materials for your compost pile avoid the following:

- Wastes that attract pests
- Diseased / insect ridden plants
- Non-biodegradable things

Compost bed types

1. Pit method
2. Heap method

Pit method

The compost pits dug in soil with dimension of 3.5m × 2.5m × 1.5m

Figure 9.12: Pit method

(L×B×H). The pits are filled layer by layer using green plants and animal excreta. The layering is repeated until the pit is filled. Finally a layer of mud is plastered on the top of the pit (Figure 9.12).

Heap method

In regions with heavy rainfall, the compost may be prepared in heaps above the ground level and protected by a shed. The pile is made with dimension of 2m × 2m × 1.5m (L×B×H) (Figure 9.13).

Methods of compost preparation

1. Indore method
2. Bangalore method

Indore method

This method was developed at Indore, India. In this method organic wastes are spread in the cattle shed to serve as bedding. Trenches are dug with dimension of 10ft × 6ft × 2ft.

Figure 9.13: Heap method

Dry wastes with cattle dung and soil are added in ratio of 4: 2: 1 up to 2 inches layer in composting pit. A moisture level of about 40-50% is ideal for good composting. Odour and insect problems can be controlled by covering the piles with a layer of soil or wood chips.

The heap is left undisturbed for about 8 to 9 months. Turning the pile for every 15 days is important for coplete composting because pile needs a periodic influx of O_2. Plant residues, weeds, sugarcane leaves, grass, wood ashes, animal dung, and water urine soaked mud can also be used as raw materials for this type of composting.

Two types of microbes which help in composting process are

Aerobes

which decompose organic matter matter in the presence of oxygen

Example: *Bacillus subtilis*

Anaerobes

which decompose organic matter in the absence of oxygen

Example: *Clostridium thermocellum*

Bangalore method

- This method was developed at Bangalore, India. It is recommended as a satisfactory method for disposal of town wastes and night soil.
- The compost pits dug in soil with dimension of 4.5m × 2.5m × 90cm (L×B×H)
- In the Bangalore method of composting, dry waste material of 25cm thick is spread in a pit and a thick suspension of cow dung in water is sprinkled over for moistening.
- A thin layer of dry waste is laid over the moistened layer
- The pit is filled alternatively with dry layer of material and cow dung suspension till it rises 0.5m above the ground level and plastered with wet mud and left undisturbed for about 4-6 months or till required.
- This method saves labour cost because there is no need of turning & regular sprinkling of water.

Benefits of compost

- Compost improves the quality of soil hence called as a soil conditioner.
- Compost contains a variety of the basic nutrients required for healthy growth of the plant.
- Nitrogen, phosphorous, potassium and certain micronutrients *viz*, manganese, copper, iron and zinc are found in compost.
- The composted product is safe and easy to handle, and does not induce nitrogen deficiency in recipient plants by nitrogen stabilization in the compost.
- It suppresses disease infestation by partial sterilization and detoxifies pollutants.
- Compost material is principally used for the reclamation of drastically disturbed. Example: mined soil, landscaping and agriculture.
- Compost finds unrestricted application in parks and gardens for ornamental plants, in land reclamation and highway beautification projects.

9.8 Biogas Production

Worldwide energy consumption and demand are growing up since past 50 years. With the growth of population, demand for energy is also increasing leading to an uneven supply and distribution of resources. Therefore, the requirement of sustainable and eco friendly energy in India to satisfy the energy demand is inevitable. Along with the source of sustainable green energy, biogas production is an alternative way to produce clean energy through solid waste management.

Biogas is a type of renewable energy that can be produced from decomposition of animal and plant waste. It is composed of 50–75% methane, 25–50% carbon dioxide, 0–10% nitrogen, 0–3% hydrogen sulphide, 0–1% hydrogen and traces of other gases. The term "anaerobic" suggests that the process occurs in the absence of free oxygen and produces CH_4 through decomposition of waste in nature and reduces environmental pollution.

Biogas generating technology is a favorable dual purpose technology at present since used as fuel and fertiliser.

Leftover foods fruit & vegetable wastes and cow dung can be subjected to anaerobic digestion for energy production in a variety of ways.

Figure 9.14(a): Biogas production schematic diagram

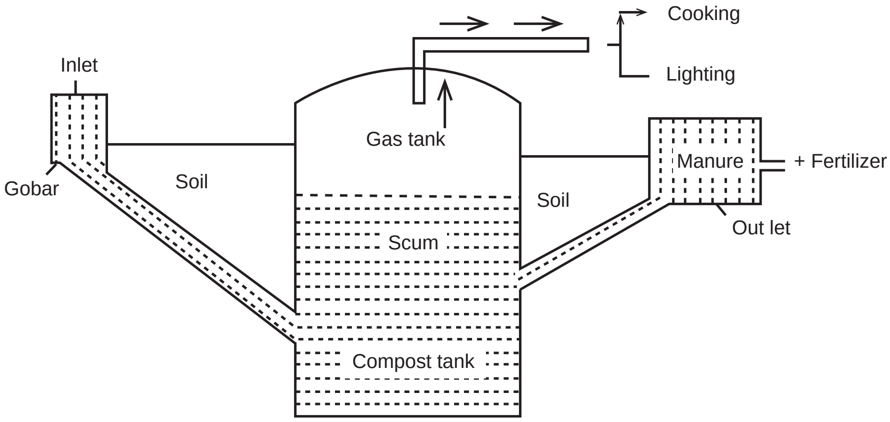


Figure 9.14(b): Biogas Digester

Production of Biogas

Biogas production is carried out in an airtight cylindrical tank called biogas digester Cow dung is mixed with equal volume of water and made into slurry and fed through the inlet of the biogas unit. The digestion proceeds at 37°C with sufficent amount of nitrogen and phosphorus. The production of biogas sets around 40-50 days, under anaerobic conditions. Production of biogas accomplished in 3 stages namely Hydrolysis, Acetogenesis and Methanogenesis

Steps

Hydrolytic fermentative stage

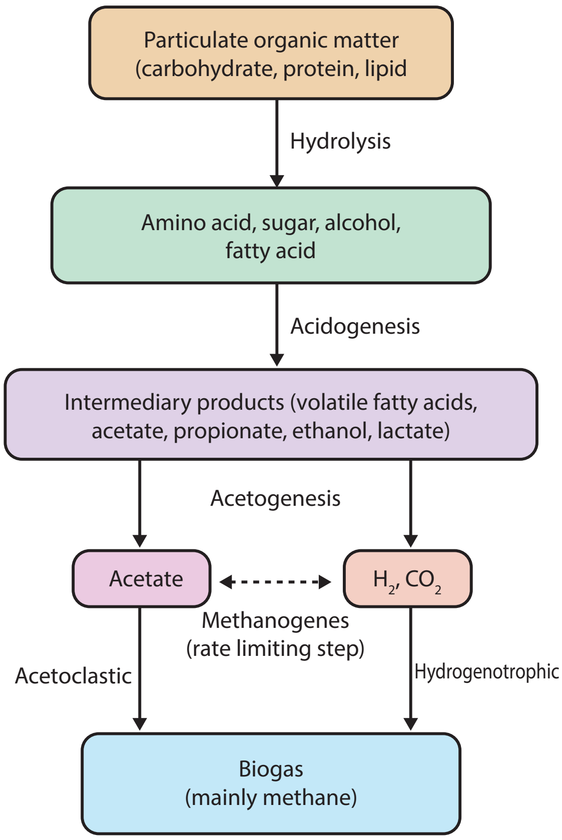
In this step, several microbes secrete different enzymes, which cleave the complex macromolecules into simpler forms. Organisms that are active in a biogas process during the hydrolysis of polysaccharides include various bacterial groups such as *Bacillus*, *Clostridium*, *Cellulomonas*.

Acetogenic stage

Through various fermentation reactions, the products from hydrolysis are converted mainly into various organic acids (acetic acid, propionic acid, butyric acid, succinic acid, lactic acid), alcohols, ammonia (from amino acids), carbon dioxide and hydrogen. Facultative anaerobes and hydrogen producing bacteria Example: *Acetovibrio cellulosolvens, Bacteroid cellulosolvens* are involved.

Methanogenic stage

In this step, obligate anaerobic methane

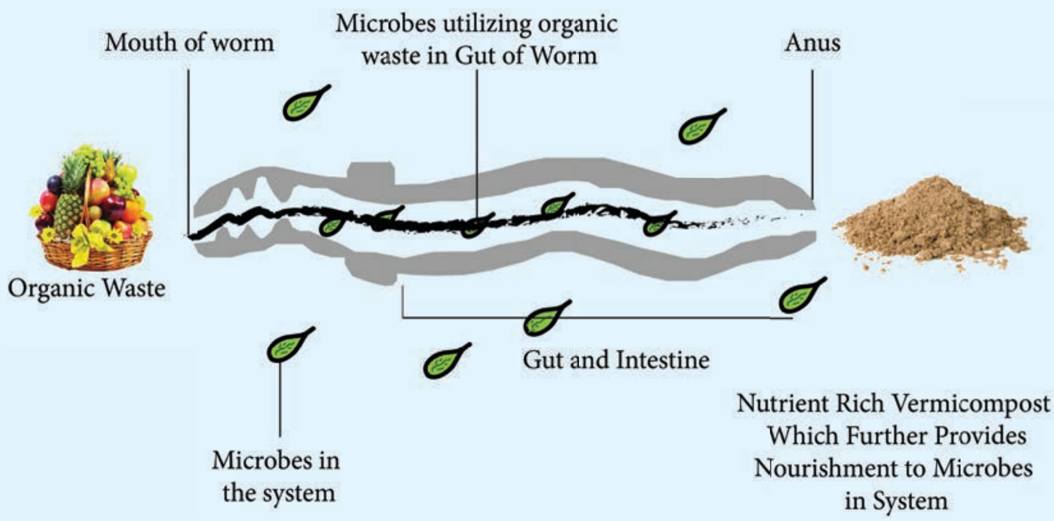


Infobits

Role of microbes in vermicomposting:

Recycling organic wastes through Vermiculture Biotechnology (VBT) is being considered an economically viable solution. Earthworms are regarded as natural bioreactors which proliferate along with other microorganisms and provide required conditions for the biodegradation of wastes. Vermicomposting involves bio oxidation and stabilization of organic material through the interactions between earthworms and microorganisms.

Worms like to feed on slowly decomposing organic materials like fruit and vegetable scraps. Worms produce castings that contain beneficial microbes and nutrients, which makes a great soil amendment. Worms are very efficient at breaking down food scraps and can eat over half their body weight in organic matter every day. Vermicasting, also called vermicomposting, is the processing of organic wastes through earthworms . It is a natural, odourless, aerobic process, much different from traditional composting. Earthworms ingest waste, then excrete casts – dark, odourless, nutrient- and organically rich, soil mud granules that make an excellent soil conditioner.



producing bacteria produce Methane gas as the major end product along with Carbon dioxide, Hydrogen and traces of other gases. Methanogenesis has six major pathways, each converting a different substrate into Methane gas. The six major substrates used are Carbon dioxide, Formic acid, Acetic acid, Methanol, Methylamine, and Dimethyl sulphate. The methanogenic bacteria include *Methanococcus voltae* and *Methanobacterium formicum* (Figure 9.14 a, b).

Small scale biogas unit

The biogas production is carried out in an air tight cylindrical tank called biogas digester (Figure 9.15).

DO YOU KNOW?

Deenabandhu model

It is a biogas production model popular in India which means "Friend of the helpless"

Applications

1. Biogas used as fuel
2. Used to generate electricity
3. Biogas is used to run any type of heat engine in order to generate electrical and mechanical power.
4. Producing high quality fertilizer.
5. Reducing water and air pollution.

Summary

Environmental microbiology is the field of science that examines the relationship between microorganism and their biotic and abiotic environments.

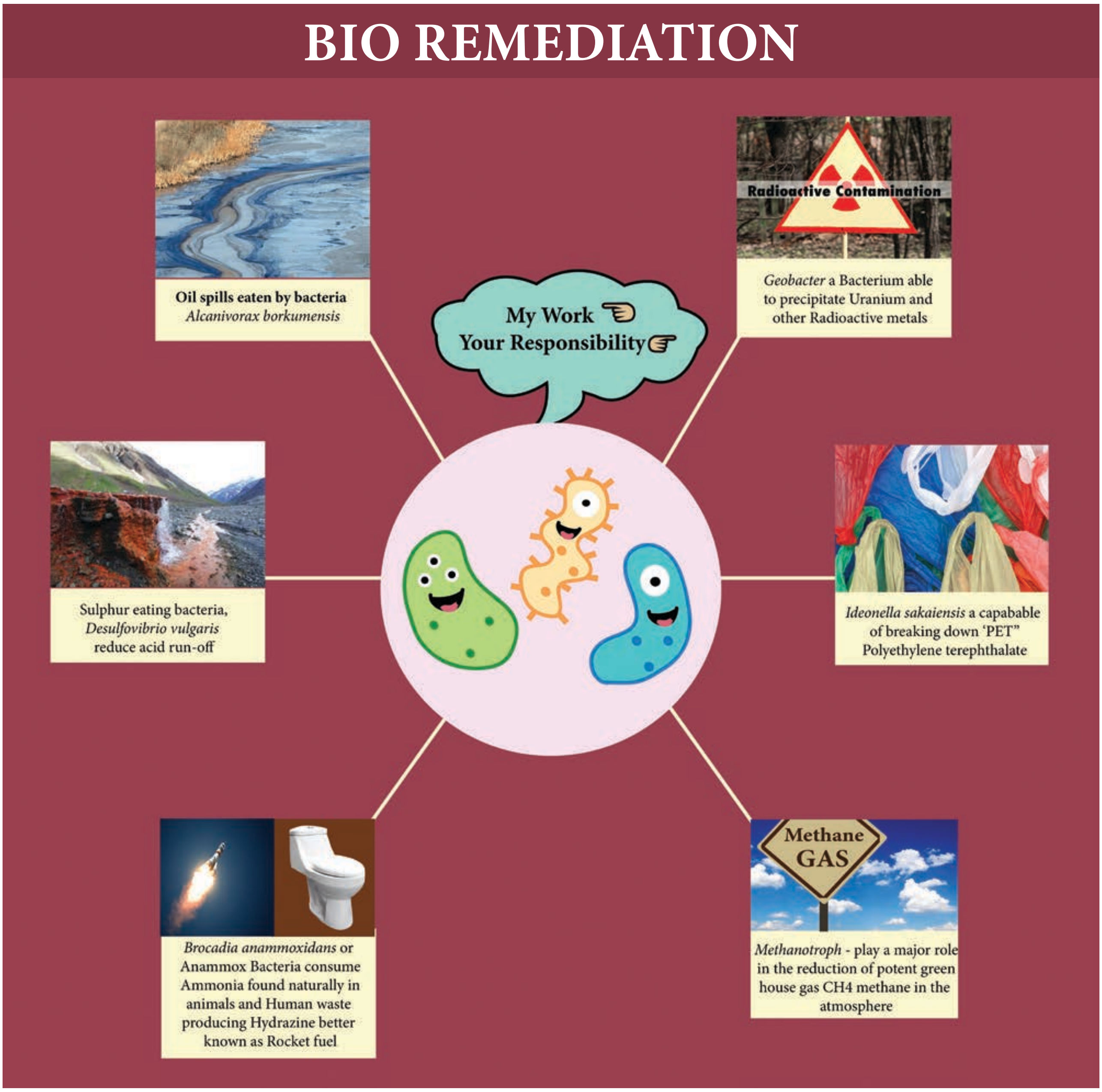
Identification of new microbes and their products have practical application on protecting the environment as well as human health. Areomicrobiology is the study of airborne microorganisms and is one of the important modes for the transmission of infectious diseases. The air in our atmosphere is composed of different gaseous molecules. The air present both interior and exterior of the environment is called indoor air and outdoor air. The microorganisms are discharged out in different forms which are grouped on the basis of their relative size and moisture content. They are aerosols, droplet, droplet nuclei and infectious dust. Hospital – acquired infection are also known as nosocomial infection. Solid and liquid impingement, filtration, sedimentation, centrifugation, electrostatic, precipitation are used to enumerate microorganisms in air.

The Aquatic Microbiology is the study of microorgansims and microbial communities in the water environment. The partly enclosed coastal body of water in which river water is mixed with sea water is called an estuary. Lentic zone can be divided into different zones based light penetration and temperature. Eutrophication is an enrichment of water by nutrients, especially nitrogen and phosphorus, which makes the overgrowth of the "algal bloom". Apart from microbes and chemicals, pH, temperature, dissolved oxygen concentration and salinity are the physical properties that affect the quality of biological life.

BOD is the amount of dissolved oxygen needed by aerobic organisms to breakdown organic material present in a given water sample at certain temperature over a specific period of time. Indicator organisms are frequently used to monitor bacterial contamination of water. The most common group of indicator organisms used in water quality monitoring are the coliforms.

Waste water treatment are called sewage treatment which removes the impurities from waste water and sewage. Primary, secondary, tertiary treatment are employed in the sewage treatment process. Trickling filter, activated sludge, oxidation ponds are generally used. The dewatered sludge used as a fertilizer. Compost is a natural process in which aerobic and anaerobic microorganisms decomposes organic matter in to valuable manure called as compost.

Indore method and Bangalore method are the different methods used in composting. Biogas is a type of renewable energy that can be produced from decomposition of animal and plant waste. Hydrolytic fermentative stage, Acetogenic stage, Methanogenic stage are the three stages involved in the Biogas production. Biogas has many applications.



Evalution

Multiple choice questions

1. In 1930, the term aerobiology was coined by ______________________.
   a. F.C.Meier b. Miquel
   c. Carnelly and colleagues
   d. None of the above
2. The gas molecules which are more in atmosphere.
   a. Nitrogen b. Oxygen
   c. Carbon dioxide d. Neon
3. _____________ are water droplets containing several types of microorganisms released in to the air from the water sources.
   a. Droplet nuclei
   b. Infectious dust
   c. Droplet d. Aerosols
4. Hospital acquired infections are otherwise called as
   a. Nosocomial infection
   b. Gastro intestinal infections

c. Ocular infection

d. All the above

5. During summer the upper layer of the lake in called

a. Hypolimnion b. Epilimnion

c. Lentic

d. None of the above

6. ________________ Is the amount of dissolved oxygen needed by aerobic organisms to breakdown organic materials?

a. BOD b. COD

c. DOB d. DOC

7. _____________________ is called as Indicator organisms

a. *Escherichia coli*

b. *Staphylococcus aureus*

c. *Pseudomonas aeruginosa*

d. None of the above

8. In which process of treating sewage,99 percent of all the impurities from the sewage are removed.

a. Primary treatment process

b. Secondary treatment process

c. Tertiary treatment process

d. None of the above

9. Primary treatment is a ____ method

a. Physical b. Chemical

c. Biological d. All of the above

10. Activated sludge process is an example for_____________________treatment

a. Physical b. Chemical

c. Biological d. Composting

11. Chlorination is an example for _____________________ treatment

a. Physical b. Chemical

c. Biological

d. None of the above

12. _________________ is an open tank where algal forms are allowed to grow

a. Trickling filter

b. Oxidation pond

c. Sludge digester

d. None of the above

13. Trickling filter is an example for _____________________ treatment

a. Physical b. Chemical

c. Biological d. None of the above

14. Which one of the following is a good source for making compost?

a. Plastic b. Aluminum foil

c. Vegetable peel d. Polythene

15. Algal boom in pond water is called

a. Eutrophication

b. Acclimatization

c. Algalization

d. Green manuring

16. The most common toxic algal bloom among the following

a. Euglena b. Microcystis

c. Paramaecium d. Hydra

17. Organism inhabiting the bottom sediment of aquatic environments constitute _____________ community

a. Pelagic b. Benthic

c. Abyssopelagic d. Episammon

18. Study of flora and fauna of fresh water

a. Ornithology b. Zoology

c. Paleontology d. Limnology

19. A partly enclosed coastal body of water in which river water is mixed with sea water

a. Lake b. Estuary

c. Creek d. Bay

20. Chemical agent used for disinfecting water

a. Glycols b. Chlorine
c. Hydrogen peroxide
d. None of the above

21. The main component of natural gas is_____________
a. CO_2 b. Carbon monoxide
c. O_2 d. Methane

22. Biogas is a mixture of
a. Methane, Nitrogen, Hydrogen
b. Methane, Nitrogen, Oxygen
c. O_2, CO_2, N_2
d. None of these

23. Which compost method is employed in the regions with heavy rainfall?
a. Heap method
b. Pit method
c. Indore method
d. Banglore method

Answer the following

1. What are properties favour survival of microorganisms in the atmosphere?
2. Define Nosocomial infections
3. What is droplet nuclei?
4. What is the purpose of bacteriological analysis of water?
5. What is potable water?
6. What is sewage?
7. Define sludge.
8. Explain the sewage treatment processes.
9. Is compost a fertilizer?
10. Describe the process of composting?
11. What is Biogas?
12. What role can biogas play in supplying our energy needs?
13. Draw the light penetration zones of a fresh water lake.
14. What is Eutrophication?
15. Write a note on trickling filter.
16. Discuss the air borne diseases.
17. Write in detail about settle plate technique.
18. Write in detail about biogas production processes.
19. Discuss the Indore method of composting in detail.
20. Discuss the sludge digestion methods.
21. Explain the benefits of waste water treatment?

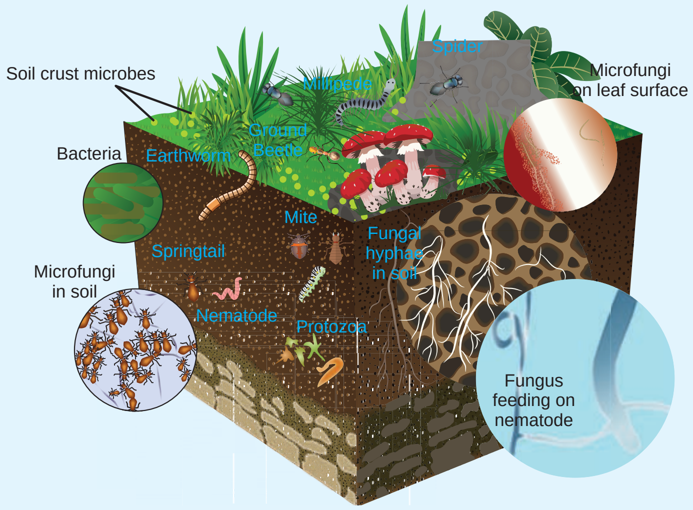
Student Activity

1. Set up a small scale anaerobic digester for anaerobic digestion using cow dung / fruits & vegetable waste.
2. Instruct the students to bring algal bloom sample from their residential area.
3. Visit nearby sewage treatment plant
4. Set up a mini composter using plastic bin/bottles (Ask students to collect rubbish such as fruit and vegetables wastes from their houses and separate degradable and biodegradable waste and build mini composter). Requirements: soda bottle, soil and organic wastes.

Chapter 10

Soil Microbiology

Chapter Outline



Soil is inhabited by a living microscopic population which is responsible for the numerous reactions taking place in the soil. The soil microorganisms affects the life economy of man in many ways.

Learning Objectives

After studying this chapter the student will be able,

- *To know the composition of soil.*
- *To understand the importance of soil microorganisms in soil fertility.*
- *To learn about the beneficial and harmful interaction between soil microorganisms.*

Knowledge of Soil Microbiology is essential to understand the agricultural and environmental science. Without soil microorganisms, life as we know could not exist on earth. Organic matter would accumulate in the form of undecomposed substances. Why should we study soil Microbiology? If we understand what is happening in soil, we get a better idea of how other biological systems work on earth.

10.1 Soil in General

Soil is the outer covering of the earth. It consists of loosely arranged layer of materials composed of inorganic and organic constituents. Soil provides the physical support needed for the anchorage of root system and serves as the reservoir of air, water and nutrients that are essential for plant growth.

10.1.1 Formation of Soil

The processes involved in the formation of soil are slow, gradual and continuous. The sum total of environmental effects on rocks collectively known as the weathering of rocks. Weathering of rocks is a continuous phenomenon and add more and more soil to the surface of the earth. There are different types of parent materials of rocks available for the formation of soil.

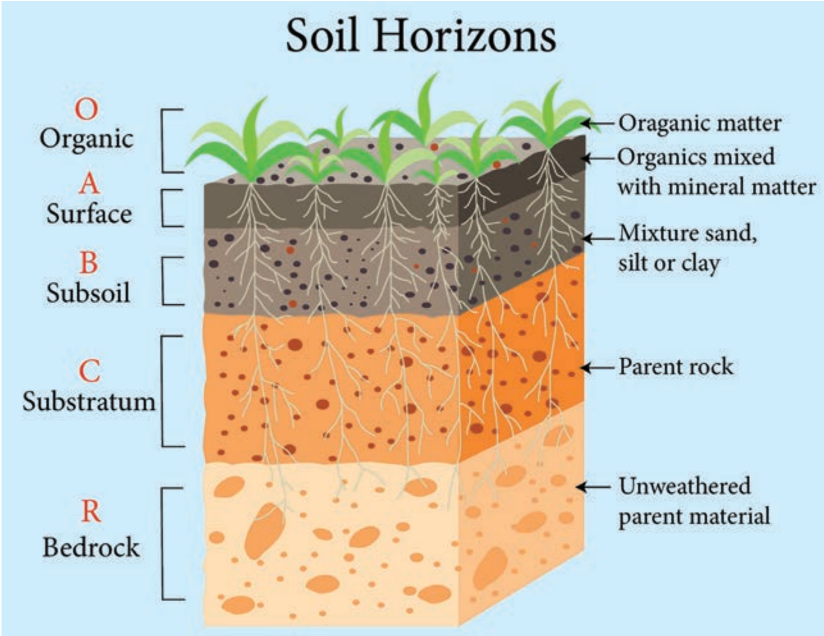


Figure 10.1: Soil Horizons

10.1.2 Soil Horizons

Each type of soil is characterized by the presence of different horizons which can be seen in a soil profile (Figure 10.1). The formation of soil horizons depends on climate, living organisms, parent rock material, topography and time; all of which control the weathering of rocks.

10.1.3 Physical and Chemical Properties of Soil

Physical properties of a soil type depends on the size of particles, soil texture, soil temperature and soil pH.

Chemical properties of soil includes three main components which provides nutrients for plant growth. The three components are the organic matter, the derivatives of parent rock materials and the clay fraction.

The fertility of soil depends not only on its chemical composition, but also on the qualitative and quantitative nature of microorganisms inhabiting it. The branch of science dealing with study of soil microorganisms and their activities in soil is known as '**Soil Microbiology**'.

10.2 Pioneers of Soil Microbiology

Scientists studied the microorganisms from water, air and soil. They recognized the role of microorganisms in natural processes. They realized the importance of soil microorganisms in growth and development of plants. Soil Microbiology emerged as a distinct branch of soil science during first half of the 19^{th} century.

Sergei N. Winogradsky discovered the autotrophic mode of life among bacteria and established the microbiological transformation of Nitrogen and Sulphur. He isolated nitrifying bacteria for the first time and demonstrated the role of these bacteria in nitrification (1890). Further he demonstrated that free-living *Clostridium pasteurianum* could fix atmospheric Nitrogen (1893). He developed the Winogradsky column (Figure 10.2), a self contained ecosystem for studying the

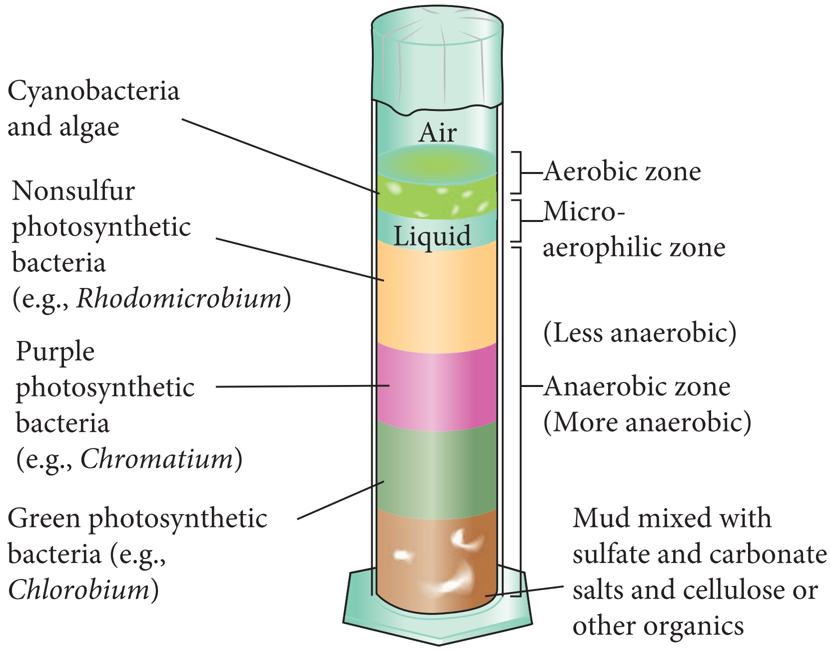


Figure 10.2: Winogradsky column

Sulphur cycle. Therefore, he is considered as the '**Father of Soil Microbiology**'.

M. W. Beijerinck (1888) isolated root nodule bacteria in pure culture from nodules in legumes and named them as *Bacillus radicola*. Thus, he is considered as the '**Father of Microbial ecology**'.

Beijerinck and Winogradsky (1890) developed the enrichment culture technique for isolation of soil organisms, proved independently that transformation of nitrogen in nature is largely due to the activities of various groups of soil microorganisms (1891). Therefore, they are considered as '**Pioneers in Soil Bacteriology**'.

10.3 Components of Soil

The soil is composed of five major components

- Mineral matter
- Water
- Air
- Organic matter
- Living organisms

One gram of soil contains approximately one million microorganisms. The soil has organic matter, soil solution and soil air. All these components are affected by the activities of microorganisms. Soil is a constantly changing medium. The soil solution in agricultural soil has ions like K^+, Na^{++}, Mg^{++}, Ca^{++}, Fe^+, S^-, NO_3, SO_4, PO_4 and others.

These ions are very essential in culture media. In a fertile soil, these elements in mineral form are supplemented by organic compound, derived from the decomposition of animal and plant residues. Thus the soil is an excellent natural medium for growth of microorganisms.

10.4 Soil Microorganisms

Soil contain five major groups of microorganisms. They are Bacteria, Actinomycetes, Fungi, Algae and Protozoa (Table 10.1).

Table 10.1: Soil Microorganisms with example

Soil Microorganisms	Examples
Bacteria	*Agrobacterium* *Bacillus* *Clostridium* *Pseudomonas*
Actinomycetes	*Actinomyces* *Nocardia* *Streptomyces*
Fungi	*Aspergillus* *Fusarium* *Alternaria* *Cladosporum*
Soil algae	*Anabaena* *Oscillatoria* *Nostoc*
Protozoa	*Colpoda* *Nematodes* *Pleurotricha* *Heteromita*
Bacteriophages	T4 Bacteriophages

DO YOU KNOW?

One teaspoon of productive soil can contain between 100 million and 1 billion bacteria. These living microorganisms recycle organic material, promoting soil fertility and supporting plant growth. By practicing conservation tillage, farmers can maintain biodiversity in their soil.

Soil Bacteria

Among the soil microorganisms, bacteria are most dominant group of organisms. All kinds of bacteria are found in soil. This is because all kinds of organic refuse are disposed on the soil

Many of the soil bacteria perform useful functions like decomposition of organic matter, conversion of soil constituents into useful materials, production of antibiotics in the soil and biogeochemical cycling of elements like Carbon, Nitrogen, Phosphorus, Iron, Sulphur and Manganese. The bacterial population of the soil exceed the population of all other groups of microorganisms in both number and variety.

Soil Actinomycetes

The actinomycetes population is present as many as millions per gram of soil. The most predominant genera present in the soil are *Nocardia, Streptomyces* and *Micromonospora*. Actinomycetes are capable of degrading many complex organic substances and therefore play an important role in building soil fertility. One of the most notable characteristics of the actinomycetes is their ability to produce antibiotics. Examples: Streptomycin, neomycin, erythromycin and tetracycline.

Soil Fungi

Next to bacterial population in soil, fungi dominates in all kinds of soil. It possess filamentous mycelium composed of individual hyphae. All environmental factors which influence the distribution of bacteria and actinomycetes also influence the fungal flora of soil. The quality and quantity of organic matter present in the soil have a direct influence on the fungal numbers in soil. Fungi are dominant in acidic soils because acidic environment is not supportive for the existence of either bacteria or actinomycetes.

Soil microbes create humus: Humus is the dark organic matter in soil. The humus is formed when dead plant and animal matter are decayed by soil microorganisms. Humus has many nutrients that improve the fertility of soil, Nitrogen being the most important.

Humus helps soil to retain moisture, and encourages the formation of soil structure. Soil organisms promote plant growth.

Soil Algae

Soil algae are ubiquitous in nature wherever moisture and sunlight are available. They are visible to the unaided eye in the form of green scum on the surface of soils. Numerically, they are not as many as Fungi, Bacteria or Actinomycetes. Some of the common algae in Indian soil are *Chlorella, Chalmydomonas, Chlorochytrium, Chlorococcum* and *Oedogonium*.

Blue green algae, or *Cyanophyceae,* are responsible for Nitrogen fixation. The amount of Nitrogen they fix depends more on physiological and environmental factors rather than the organism's abilities. These factors include intensity of sunlight, concentration of inorganic and organic Nitrogen sources and ambient temperature and stability.

Soil Protozoa

Soil protozoa are unicellular. They are characterized by a cyst in their life cycle which can help the species to withstand adverse soil conditions. The protozoans prefer certain species of bacteria for their nutrition. Protozoa are abundant in the upper layer of the soil and their numbers are directly dependent on bacterial population.

HOTS

"Without fungi even death will be incomplete" - Pasteur

Justify the statement.

10.4.1 Factors Influencing Microbial Population in Soil

The major factors that influence the microbial community in soil are

ESPEJI

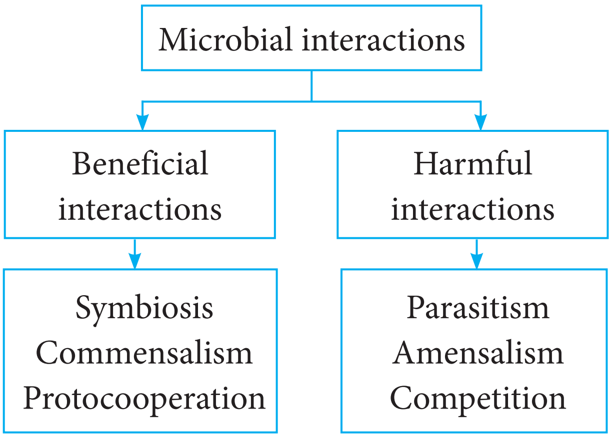
- Moisture
- pH
- Temperature
- Gases
- Organic and inorganic fertilizer
- Organic matter of soil
- Types of vegetation and growth stages
- Ploughing
- Season
- Depth of soil

Infobits

Some soil microbes produce a variety of substances that promote plant growth, including Auxins, Gibberellins and antibiotics.

10.5 Microbial Interactions

Microorganisms in soil interact with themselves and lead to beneficial and harmful relationships (Flowchart 10.1). Some of the interaction and interrelationship have been discussed in this connection in Table 10.2.



Flowchart 10.1: Microbial interactions

10.5.1 Beneficial Interactions

The beneficial interactions such as symbiosis (mutualism) and commensalism are found to operate among the soil inhabitants.

Symbiosis (mutualism)

Mutualism is an example of symbiotic relationship in which each organisms benefits from the association. One type of mutualistic association is involving the exchange of nutrients, between two species, a phenomenon called syntrophism. Many microorganisms synthesise vitamins and amino acids in excess of their nutritional requirements. Other have a requirement for one or more of these nutrients. Symbiosis is an obligatory relationship between two populations that benefit both the population. Both populations live together for mutual benefit.

Table 10.2: Types of microbial interaction in soil

Interaction	Microorganisms A	Microorganisms B
Neutralism	No effect	No effect
Commensalism	+	No effect
Amensalism	No effect	-
Mutualism Synergism Protoco-operation Symbiosis	+	+
Competition	-	-
Parasitism	+	-
Predation	+	-
+ = positive effect – = negative effect		

The relationship between algae and fungi that result in the formation of lichen is a classical example of mutualistic intermicrobial relationship (Figure 10.3).

Lichens are composed of primary producer, the phycosymbiont (algae) and a consumer the mycosymbiont (fungus)

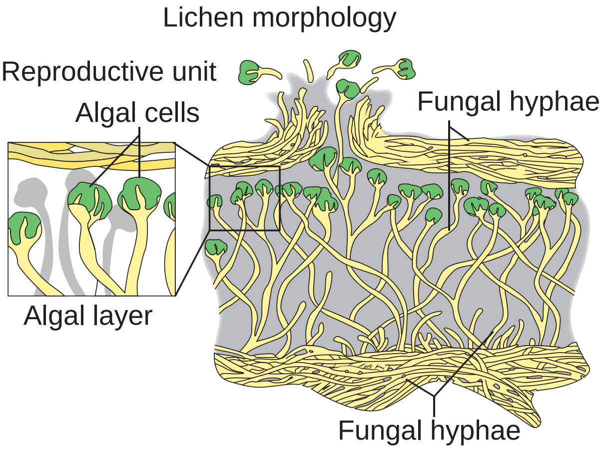
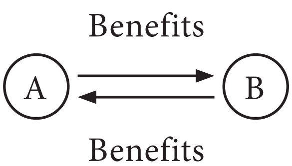


Figure 10.3: Lichen Morphology

Commensalism

In a commercial relationship between two microbial population, one population is benefited and other population remains unaffected. Commensalism is an unidirectional relationship between two population. The unaffected population does not benefit by the action of second population. For the receiving population, the benefit provided may be essential. In commensalism, the unaffected population modifies the habitat in such a way that another population is benefited.



For example: A population of facultative anaerobes utilizes oxygen and creates a habitat suitable for the growth of anaerobes. In soil, vitamin and growth factor producing organisms benefit vitamin and growth factors requiring organisms.

10.5.2 Harmful Microbial Interactions

Harmful microbial interaction is otherwise described as negative interaction or antagonistic interaction. Any inhibitory effect of an organism created by any means to the other organism is known as harmful interaction or antagonistic interaction and the phenomenon of this activity is called antagonism

Ammensalism

Ammensalism is the phenonmenon where one microbial species is affected by other species, where as other species is unaffected by first one. Ammensalism is accomplished by secretions of inhibitory substances such as antibiotics. Certain organisms may be of great practical importance, since they often produce antibiotics or other inhibitory substances, which affect the normal growth of other organisms. Antagonistic relationships are quite common in nature. For example: *Pseudomonas aeruginosa* is antagonistic towards *Aspergillus terreus* (Figure 10.4).

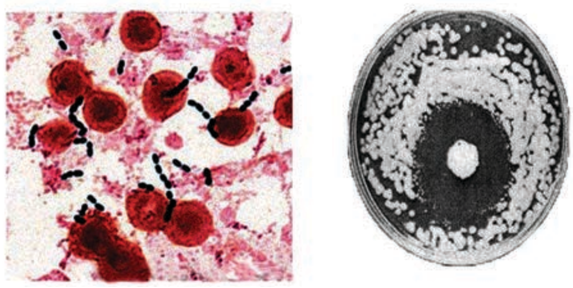
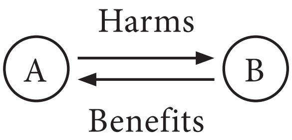


Figure 10.4: Microbial antagonism

Parasitism

This is a relationship in which one of the population benefits from the other and the host is usually harmed. Parasitism is one of the most complex microbial interactions. The line between parasitism and predation is difficult to define. The parasites feed on the cells, tissues or fluids of another organisms the host, which is harmed in this process.



The parasites depends on the host and lives in intimate physical and metabolic contact with the host. All types of plants and animals are susceptible to attack by microbial parasites.

10.6 Rhizosphere

In 1904, L.Hiltner for the first time coined the term "rhizosphere" to denote the area of intense microbiological activity that extends several millimeters from the root system of the growing plants.

The region which is adjacent to the root system is called rhizophere. The microbial population on and around root system is considerably higher than the root free soil or non rhizophere soil. This may be due the availability of nutrients from plants root in the form of root nodules, secretion, lysates, mucigel and sloughed off cells (Figure 10.5).

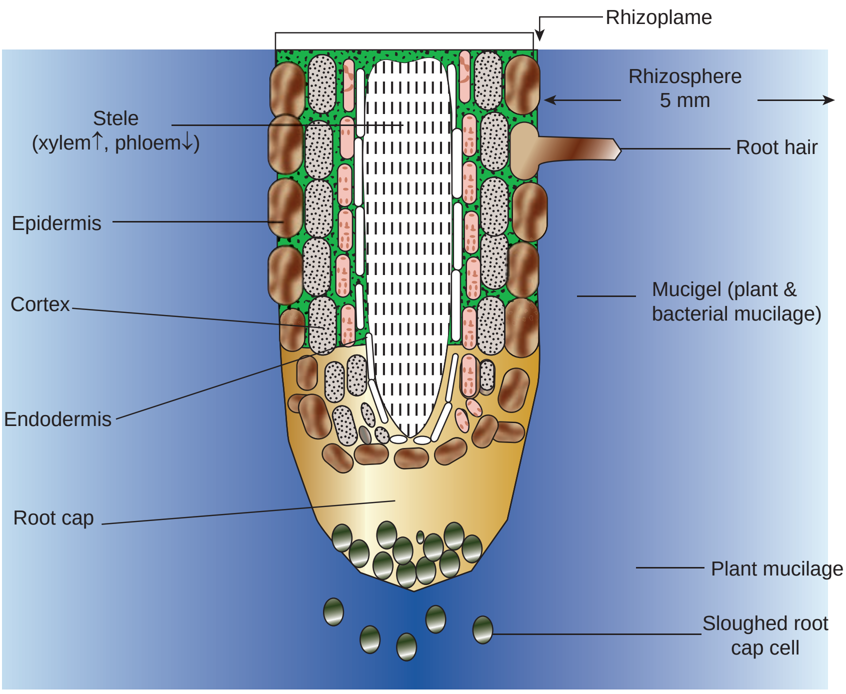


Figure 10.5: Root hair and Rhizosphere

The rhizospere region can be divided into two zones.

- Exorhizosphere
- Endorhizophere

However the root surface is termed as "rhizoplane".

Rhizophere Effect

The rhizophere is a zone of increased microbial community as well as microbial activities influenced by the root itself.

Greater rhizosphere effect is seen with bacteria (R: S values ranging from

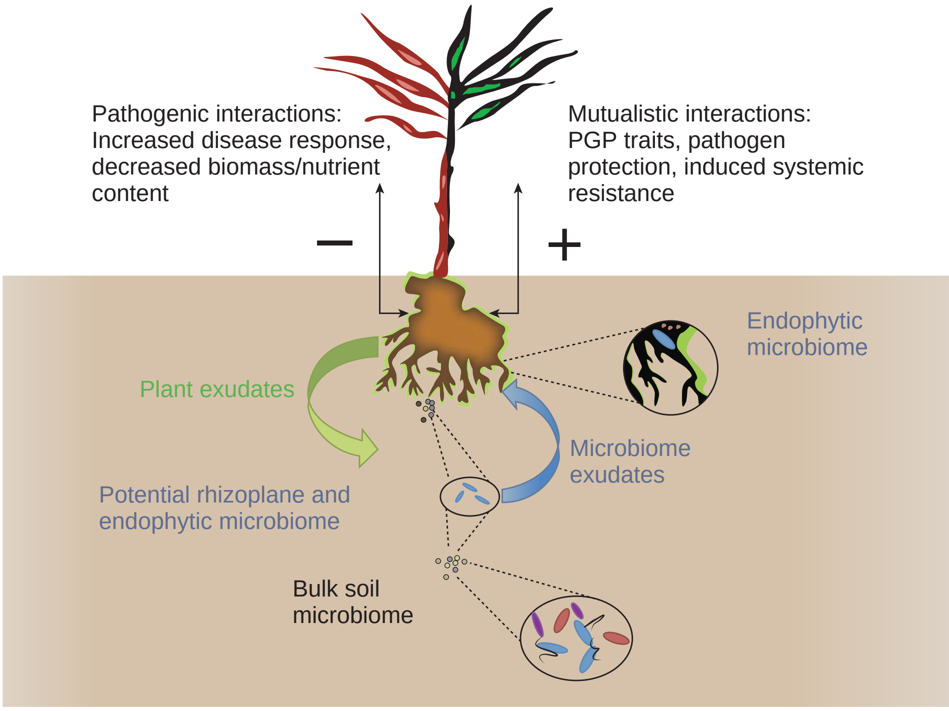


Figure 10.6: Effect of Rhizosphere in plant growth

> ### Infobits
>
> **Bioleaching:**
>
> Soil microorganisms are very closely involved as catalytic agents in many geological processes. These include mineral formation, mineral degradation, sedimentation and geochemical cycling. In recent years, a new discipline of mineral science namely bio-hydrometallurgy or microbial mining (mining with microbes) is rapidly growing. Broadly speaking, bio-hydrometallurgy deals with the application of biotechnology in mining industry. In fact, microorganisms can be successfully used for the extraction of metals (Example: copper, zinc, cobalt, lead, uranium) from low grade ores. Mining with microbes is both economical and environmental friendly.

10 to 20 or sometimes more) than with actinomycetes or fungi (Figure 10.6). From the agronomic point of view, the abundance of Nitrogen fixing and Phosphate solublising bacteria in the rhizosphere of crop plants assumes a natural significance.

It has been reported that amino acid requiring bacteria exist in the rhizosphere in large numbers than in the root free soil. The rhizosphere effect improves the physiological conditions of the plant and ultimately result in higher yield.

10.7 Phyllosphere

The term "Phyllosphere" was coined by the Dutch Microbiologist Ruinen. The leaf surface has been termed as Phylloplane and the zone on leaves inhabited by the microorganisms as Phyllosphere (Figure 10.7). In forest vegetation, thick microbial epiphytic

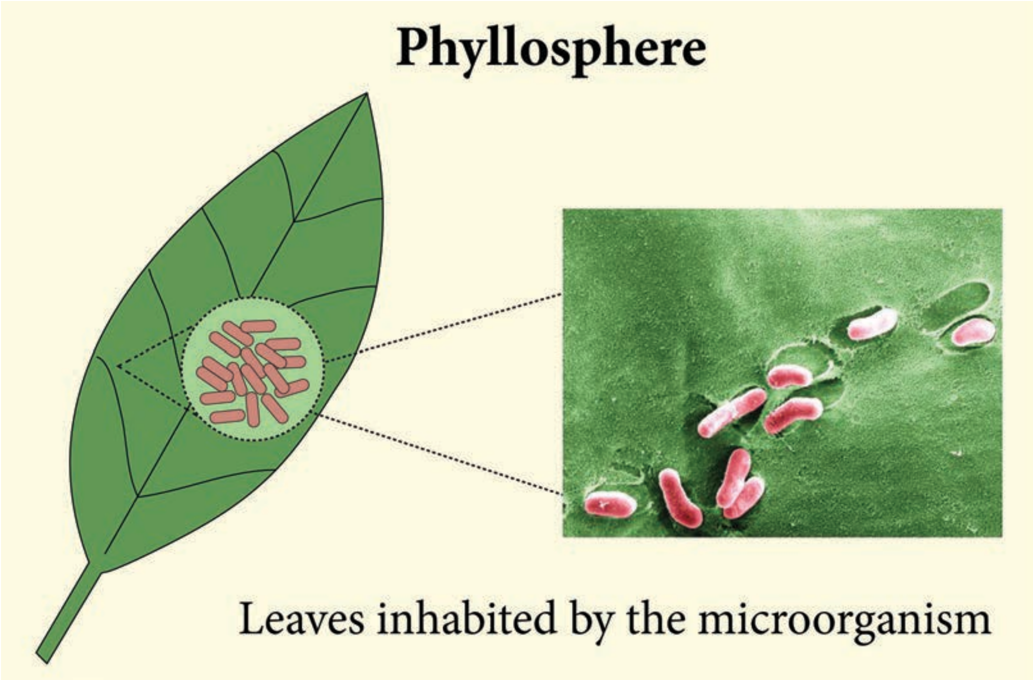


Figure 10.7: Microscopic appearance of Phyllosphere Bacteria

Infobits

PGPB can promote plant growth. The bacteria include those that are free-living, those that form specific symbiotic relationships with plants (Example: *Rhizobia* and *Frankia*), bacterial endophytes that can colonize some or a portion of a plant's interior tissues, and cyanobacteria (blue-green algae). PGPB may promote plant growth directly usually by either facilitating resource acquisition or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogenic agents on plant growth and development, that is, by acting as biocontrol bacteria. It is envisioned that in the not too distant future, plant growth-promoting bacteria (PGPB) will begin to replace the use of chemicals in Agriculture, Horticulture, Silviculture, and environmental cleanup strategies.

associations exist on leaves. The dominant and useful microorganisms on the leaf surfaces in the forest, vegetation happened to be Nitrogen fixing bacteria like *Beijerinckia* and *Azotobacter*.

Apart from Nitrogen fixing bacteria, other genera such as *Pseudomonas, Pseudobacterium, Phytomonas* are also encountered on the leaf surface. The age of plant, its leaf spread, morphology and maturity level and the atmospheric factors greatly influence the phyllosphere microflora.

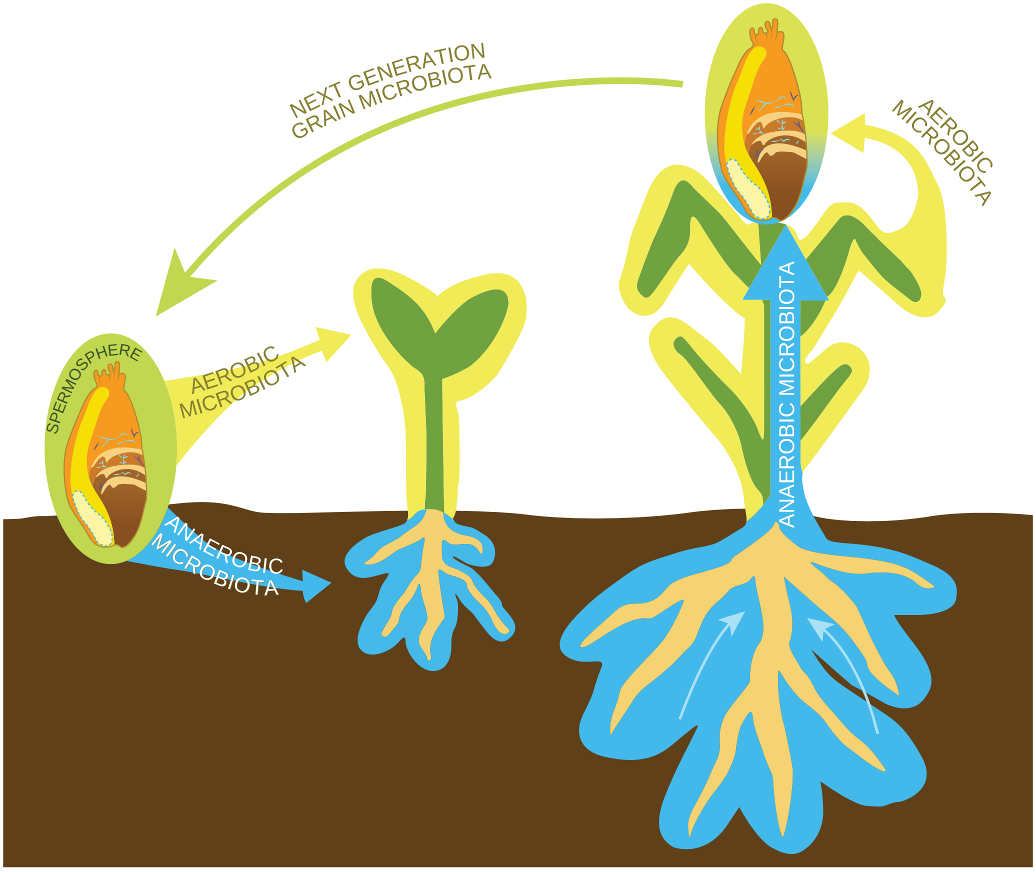


Figure 10.8: Spermosphere

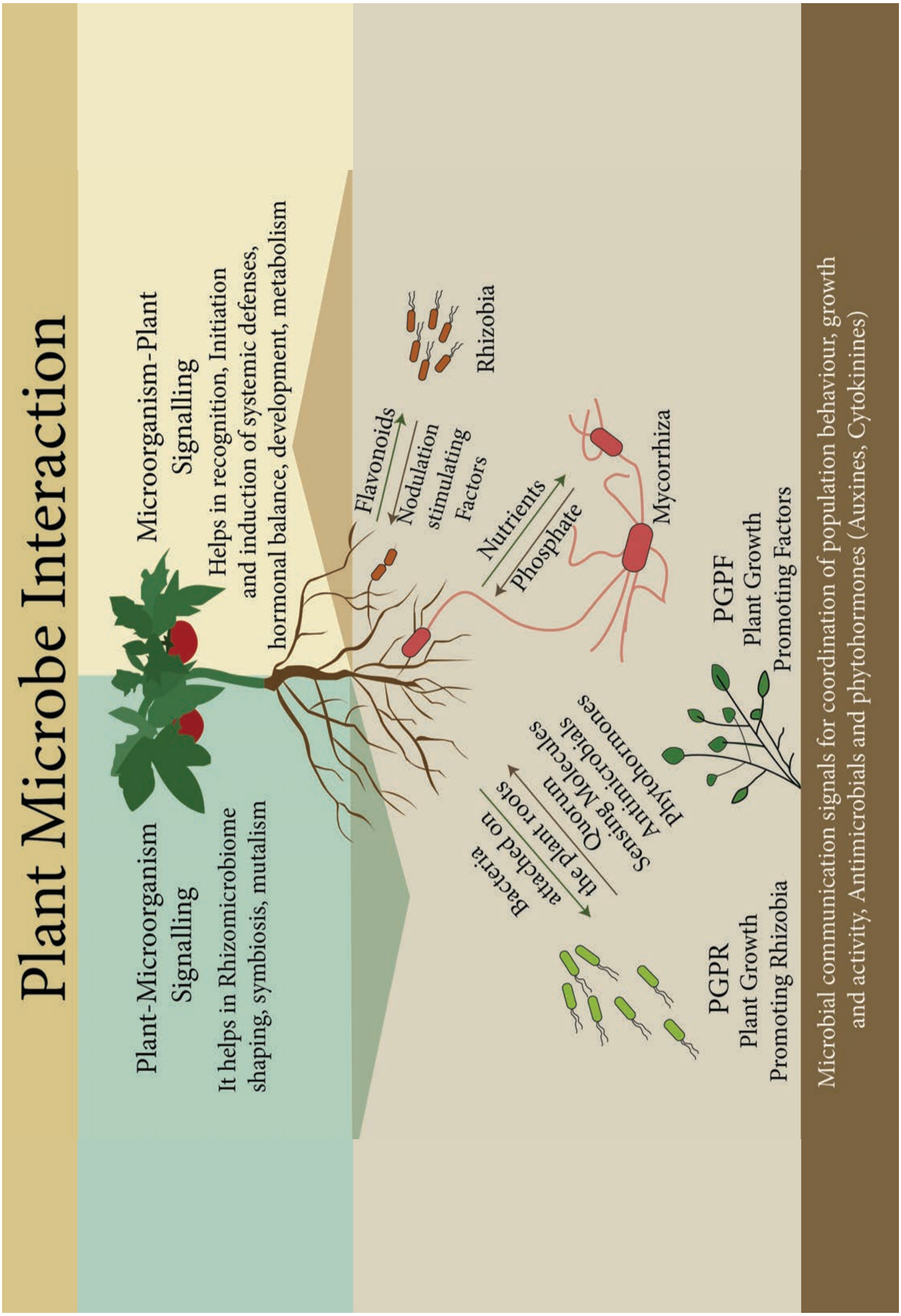
10.8 Spermosphere

The region which is adjacent to the seed surface is termed as spermosphere (Figure 10.8). Healthy seeds carry specific bacterial flora in respect to number and species. There are several reports in the literature on the quantity and quality of microorganisms carried by the seeds of different plants species both externally and internally. When the seed is sown in soil, certain interactions between the seed borne microflora and the soil microorganisms take place under the influence of chemicals excluded by the germinating seed.

Summary

Soil is the outer most covering of the earth. Soil consists of living and non living components that contribute its fertility. There are five major components in the soil, that includes mineral matter, water, air organic matter and living organisms. The soil environment is unique in several ways. It consists of bacteria, fungi, actinomycetes, algae and protozoa. Several factors influences the moisture, pH, temperature, organic and inorganic matter of the soil.

Microorganisms in soil interact themselves and lead to both beneficial and harmful interactions. Beneficial interaction includes symbiosis and commensalism. Harmful microbial interaction includes parasitism. The region adjacent to the root system is called rhizosphere. Bacteria predominate in rhizophere. Soil and their growth is influenced by nutritional substances released from plant tissues.


Plant Microbe Interaction
Plant-Microorganism Signalling
It helps in Rhizomicrobiome shaping, symbiosis, mutalism
Microorganism-Plant Signalling
Helps in recognition, Initiation and induction of systemic defenses, hormonal balance, development, metabolism
Flavonoids
Nodulation stimulating Factors
Rhizobia
Nutrients
Phosphate
Mycorrhiza
Bacteria attached on the plant roots
Quorum Sensing Molecules
Antimicrobials
phytohormones
PGPF
Plant Growth Promoting Factors
PGPR
Plant Growth Promoting Rhizobia
Microbial communication signals for coordination of population behaviour, growth and activity, Antimicrobials and phytohormones (Auxines, Cytokinines)

The leaf surface has been termed as phylloplane and the zone on leaves inhabited by the microorganisms is phyllosphere. The region, which is adjacent to the seed surface is termed as spermosphere.

Evaluation

Multiple choice questions

1. Example for soil algae ______________
   a. *Anabaena*
   b. *Oscillatoria*
   c. *Nostoc*
   d. All the above

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2. Lichens are example for
   a. Symbiosis
   b. Parasitism
   c. Commensalism
   d. All the above
3. The relationship between _______ and that result ______________ in the formation of lichen
   a. Bacteria and virus
   b. Algae and bacteria
   c. Algae and fungi
   d. Virus and fungi
4. Harmful interaction is otherwise called as ______________________
   a. Mutualism
   b. Antagonism
   c. Commensalism
   d. Symbiosis
5. _______________ first coined the term rhizophere
   a. L. Hiltner
   b. Ruinen
   c. Pasteur
   d. Koch
6. Leaf surface has been termed as __________________________ .
   a. Rhizosplane b. Spermoplane
   c. Phylloplane d. All the above
7. Spermosphere is __________________
   a. Leaf and microorganisms
   b. Root and microorganisms
   c. Seed and microorganisms
   d. All the above

Answer the following

1. What is soil?
2. Give examples for the soil bacteria?
3. What are the types of microbial interaction?
4. What is harmful microbial interaction?
5. Define rhizophere.
6. Define rhizoplane.
7. Define phyllosphere.
8. Define spermospere.
9. Explain parasitism.
10. Explain commensalism.
11. Give examples for the soil Fungi & Actinomycetes.
12. What are the components of soil?
13. Mention the different types of soil microorganism with help of chart.
14. Explain – factors influencing microbial population in soil.
15. Explain – microbial interaction.
16. Write about symbiosis or mutualism.
17. Describe rhizosphere.
18. Explain rhizophere effect.

Chapter 11

Agricultural Microbiology

Chapter Outline

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The *Anabaena azollae-Azolla* association is a symbiotic relationship between a blue green alga and a rice plant. *Azolla* is a small, fast growing floating water fern. In the cavities of leaves of azolla, *Anabaena azollae* a blue green algae (a cyanobacterium) fixes nitrogen from the air.

Learning Objectives

After studying this chapter the student will be able,

- *To understand the various biogeo chemical cycle*
- *To know the nitrogen fixation process*
- *To infer about the biofertilizer*
- *To learn the role of biopesticides in agriculture*
- *To discuss the plant diseases*

Agricultural microbiology is a branch of microbiology which deals with the study of microorganisms that are involved in various agricultural processes taking place in soil, plants and agro industries.

Agriculturally important processes like the Biological nitrogen fixation, nutrient cycling of Carbon, Sulphur, Phosphorus and Nitrogen are microbe mediated and play a very significant role in replenishing the nutrient supply in soil. Certain microorganisms like bacteria, fungi and viruses are economically important since they cause plant diseases and are responsible for severe crop losses. The study that deals with plant diseases is called plant pathology.

There are microorganisms capable of producing plant hormones thereby enhancing the plant growth. Some micro organisms can be used as biopesticides as they have ability to kill insects that damage plants.

11.1 Biogeochemical Cycles

Biogeochemical cycling is defined as the movement and conversions of materials by biochemical activities throughout air, water and soil. All living organisms participate in biogeochemical cycling but microorganisms play a major role. This is because of their high enzymatic activity and their ubiquity.

Most macro elemental components of living organisms like Carbon, Nitrogen, Sulphur, Oxygen, Phosphorus and Hydrogen are cycled most intensely and other elements like Copper, Chromium, Iron, Zinc, Nickel are cycled less intensely.

11.1.1 Carbon Cycle

Carbon is a macro element present in all living cells. In microorganisms, they are present in all macromolecules like cell wall, cytoplasmic membrane, proteins and nucleic acids.

Reservoirs of Carbon:

Reservoirs are the storage places of nutrients that are present in nature. They store nutrients in large amounts for longer periods of time.

Atmospheric CO_2, dissolved carbon in oceans and freshwater, organic matter are actively cycled carbon reservoirs. Sediments and fossil fuels like coal, petroleum and natural gas are slowly cycled carbon reservoirs. Carbon is cycled between these reservoirs by the biochemical activities of micro organisms and other living things (Figure 11.1).

The different stages or processes involved in carbon cycle are

1. Photosynthesis
2. Decomposition
3. Methanogenesis

1. Photosynthesis

It is a process where atmospheric CO_2 is converted to organic carbon $(CH_2O)n$. This is carried out by higher plants, photosynthetic bacteria, cyanobacteria and algae using radiant energy from

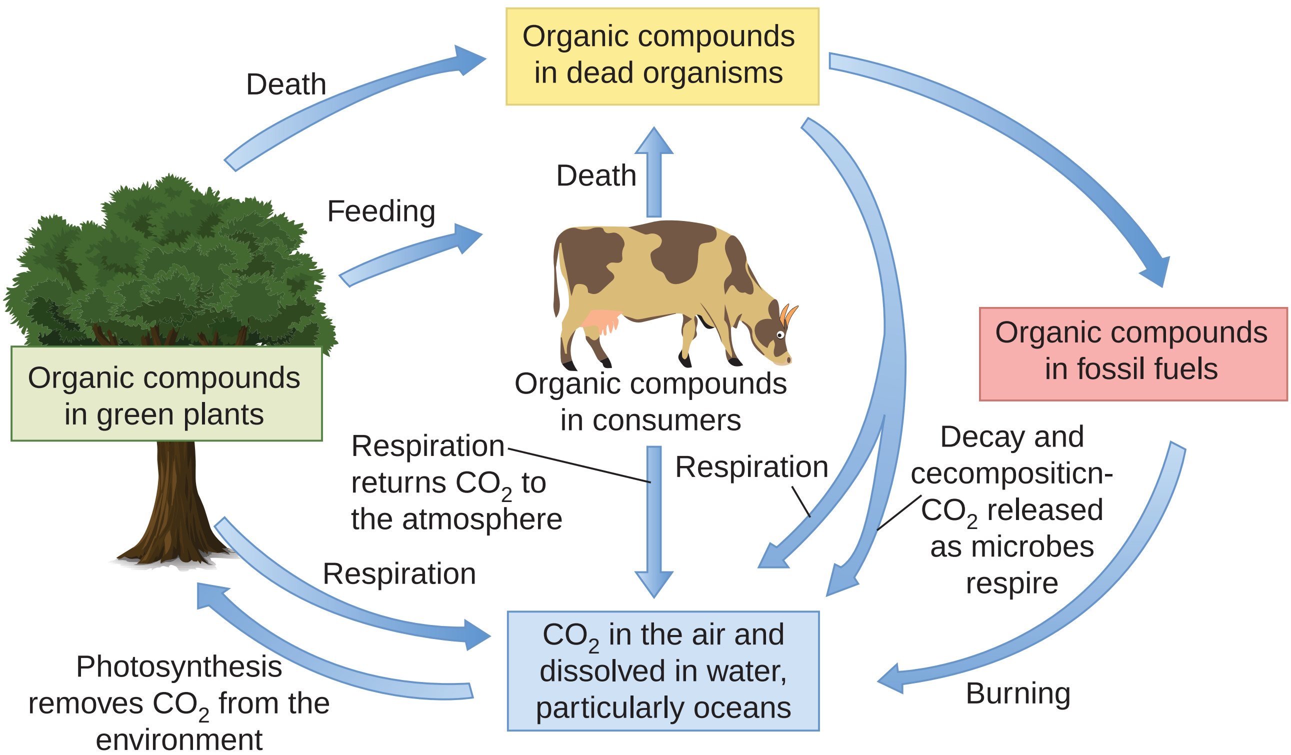


Figure 11.1: A simplified diagram of Carbon cycle is as follows

the sun. This can be explained by the equation,

$$CO_2 + H_2O \rightarrow (CH_2O)_n + O_2$$

where $(CH_2O)_n$ represents the organic form of carbon (Example: Carbohydrates) which gets incorporated into the photosynthetic organisms. This organic carbon serves as food for herbivores and in turn for carnivores.

2. Decomposition

The organic matter fixed as a result of photosynthesis is eventually degraded by microorganisms to CO_2 during processes like respiration and decomposition. When aerobic and anaerobic organisms respire, CO_2 is released into the atmosphere. Much of the CO_2 is released when dead organisms decompose in the soil predominantly by the activities of soil microorganisms. Burning of fossil fuels also release CO_2 into the atmosphere.

3. Methanogenesis

It is an anaerobic process where CO_2 gets converted to CH_4 (methane) by strict anaerobes like methanogens (Example: *Methanobacterium*). Methanogens are a group of Archaebacteria found in anaerobic environments like swamps, marshes, rumen of ruminants, paddy fields and gut of termites.

$$H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$$

Methane is converted back to carbondioxide by a process called **Methylotrophy**.

11.1.2 Nitrogen Cycle

The element Nitrogen (N) is a key constituent in microbial cell. Nitrogen is needed for the formation of proteins, amino acids, nucleotides and is present in a number of oxidation states inside the cell. Nitrogen is cycled between atmosphere, organic compounds in living things, soil and sediments.

The processes that are involved in Nitrogen cycle are

1. Nitrogen fixation
2. Nitrification
3. Ammonification
4. Denitrification

Nitrogen fixation

Nitrogen is present as N_2 ($N \equiv N$) in air (78% N_2). The triple bonded state of nitrogen makes it very stable and nitrogen in its gaseous state cannot be assimilated by plants or animals for their metabolism. Only few groups of prokaryotes are

Infobits

Fistulated cow:

Fistulated cows are very useful in studying rumen microorganisms and ruminant nutrition and are used to treat indigestion in cows. Fistula is a sampling port that allows access to the rumen. Rumen is the first and the largest chamber of the stomach of cows and other ruminant animals.

capable of breaking the triple bond and use it for building up their proteins and aminoacids. The process of reduction of gaseous nitrogen (N_2) to ammonia (NH_4) is called Nitrogen fixation. This process is carried out by a group of prokaryotes called diazotrophs.

$$N_2 + 8H \rightarrow 2\ NH_3 + H_2$$

Cyanobacteria, *Rhizobium* and *Frankia* are some of the examples of **diazotrophs** that can fix atmospheric nitrogen. The fixed ammonia gets incorporated into proteins and amino acids, thus building up organic nitrogen.

$$NH_4 \xrightarrow{\text{assimilation}} \text{Proteins, amino acids}$$

Ammonification

The production of ammonia during the decomposition of organic nitrogen compounds, by micro organisms after the death of plants and animals is called **ammonification** (Figure 11.2). Much of the ammonia released by aerobic decomposition in soil is taken up rapidly by plants and micro organisms and is converted to amino acids.

Bacteria like *Bacillus, Clostridium, Pseudomonas* and fungi like *Aspergillus, Mucor* and *Penicillium* are few examples of micro organisms that can ammonify.

Nitrification

The oxidation of ammonia (NH_3) to nitrate (NO_3) is called **nitrification**. It is carried

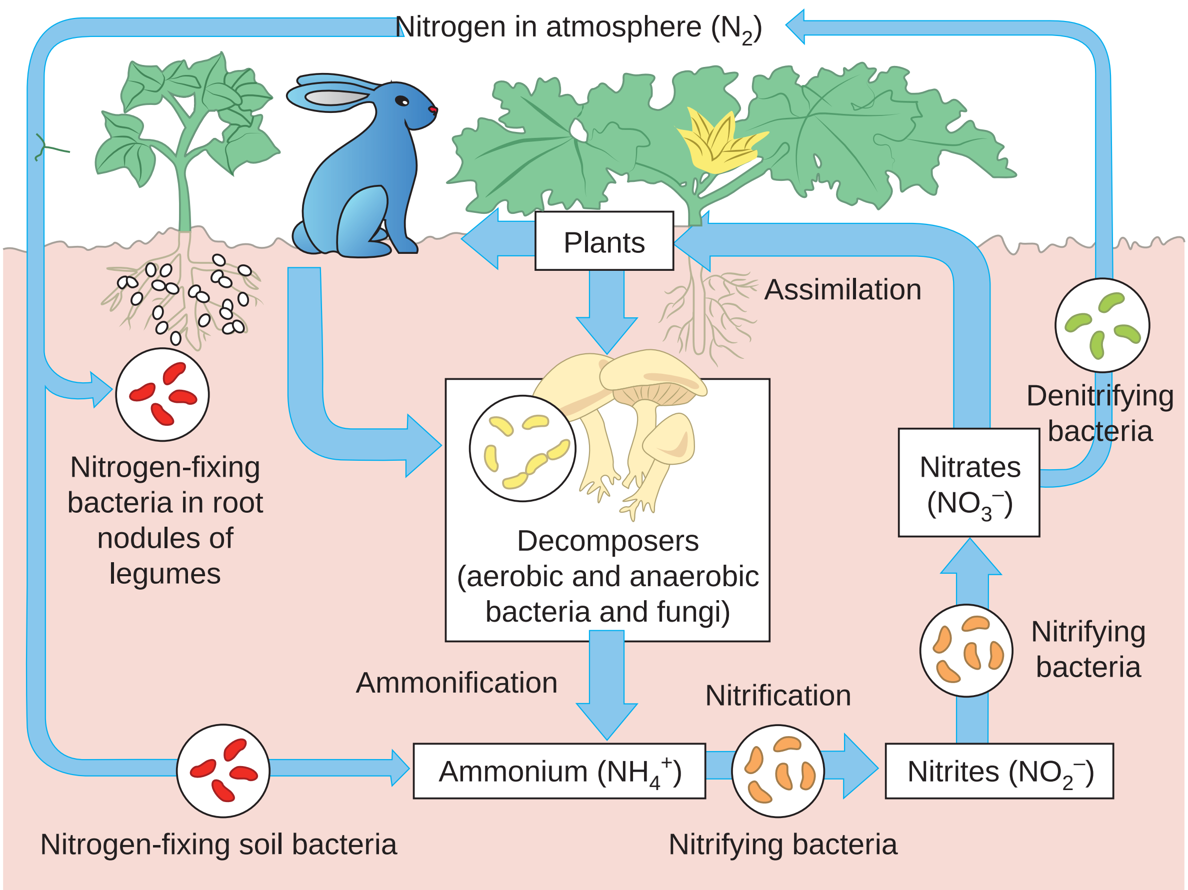


Figure 11.2: Simplified diagram of nitrogen cycle

out by nitrifying bacteria. It is a two step process where ammonia is first converted to nitrite (NO_2) and then to nitrate (NO_3).

$$2NH_3 + 3O_2 \rightarrow 2HNO_2 + 2H_2O$$

The above given oxidation reaction is the first step that produces nitrite. This reaction provides energy and is carried out by *Nitrosomonas* and *Nitrosococcus*.

In the second step, the nitrite is oxidized to nitrate

$$2HNO_2 + O_2 \rightarrow HNO_3$$

This reaction is carried out by *Nitrobacter*.

Nitrates are readily assimilated by plants but are very water soluble and rapidly leached from soil.

Denitrification

The reduction of NO_3^- from soils by denitrifying bacteria to gaseous nitrogen is called **denitrification**. In this process, carried out by bacteria like *Pseudomonas, Thiobacillus denitrificans,* organic compounds serve as hydrogen donors and nitrates serve as electron acceptor.

$$NO_3 \rightarrow NO_2 \rightarrow N_2O \rightarrow N_2$$

Biological Nitrogen Fixation

One of the most significant biological process taking places on the Earth is biological nitrogen fixation (BNF). This fixation of atmospheric nitrogen carried out by few prokaryotes is cost efficient because industrial production of ammonia by Haber's Bosch process is very expensive.

Organisms carry out BNF in a free living state in soil or they can establish symbiotic association with other plants or micro organisms and fix N_2.

Organisms capable of BNF are

Free living	
Aerobic	*Azotobacter* Cyanobacteria
Anaerobic	*Clostridium*
Symbiotic	*Rhizobium* *Frankia.*

BNF by *Rhizobium* in leguminous plants

Leguminous plants belong to the family Leguminaceae and bear seeds in pods. Example: Black gram, Green peas, Soyabean, Subabul. The bacteria belonging to the genus *Rhizobium* which can exist in free living state in soil but can enter into symbiosis with legume plants and carry out nitrogen fixation.

Process of BNF

It consists of the following steps

1. Infection of legume roots by *Rhizobium*
2. Formation of root nodules
3. Reduction of $N\equiv N$ to NH_4 in root nodules

1. Infection of legume roots by *Rhizobium*

Rhizobium recognises and attaches to the root hairs of legume plant. It invades the root hairs and secretion of certain *nod* factors result in root hair curling typically called **Shepherd's crook symptom** which leads to the formation of infection thread. Infection thread is a cellulosic tube like structure through which *Rhizobium* moves into the cortex from root hairs.

2. Formation of root nodules

The invaded plant cells are stimulated to divide repeatedly thus forming a mass of tissue on the roots which are called root nodules (Figure 11.3). Root nodules are fleshy light pink colored globose structures seen on the roots. The bacteria inside the root nodules transform into swollen, mishappened forms. These are called **bacteroids**. The bacteroids are capable of nitrogen fixation (Figure 11.4)

3. Reduction of N≡N to ammonia in root nodules

This biochemical process is catalysed by an enzyme called **Nitrogenase** present in bacteroids and happens under diminished O_2 levels. The O_2 levels in the nodules are controlled by an oxygen binding protein called **leghemoglobin**. This is a red, iron containing protein which can keep the nodule environment free of oxygen.

Nitrogenase

The enzyme nitrogenase is a complex enzyme consisting of 2 enzymes, dinitrogenase reductase and dinitrogenase. Electrons from organic compounds like pyruvate are passed on to dinitrogenase reductase first and then to dinitrogenase which in turn passes them to N≡N thus reducing it to NH_4. This reduction needs 16 ATP, ferredoxin and cytochromes.

$$N_2 + 8_e^- + 8_H^+ \rightarrow 2NH_3 + H_2$$

$$N{\equiv}N + 4H \longrightarrow HN{=}NH + 2H \longrightarrow H_2N\text{-}NH_2 + 2H \longrightarrow 2NH_3$$

HOTS

Will nitrogen fixation occur in the presence of air? What will be the fate of nitrogenase enzyme in aerobic condition?

Figure 11.3: Root nodules in the leguminous plant root

11.1.3 Phosphorus Cycle

The element phosphorus (P) is an essential macro element in all living organisms. They are found in nucleic acids and phosphate esters. It is an essential component of ATP and other high energy phosphates and phospholipids.

Reservoirs of Phosphorus

1. Phosphate rock like apatite, a large inert reservoir
2. Marine and aquatic sediments
3. Dissolved phosphates in soils and waters
4. Organic phosphates in dead and living organisms.

Phosphorous transformations mostly happen as inter conversion of inorganic to organic phosphate and insoluble form to soluble phosphates.

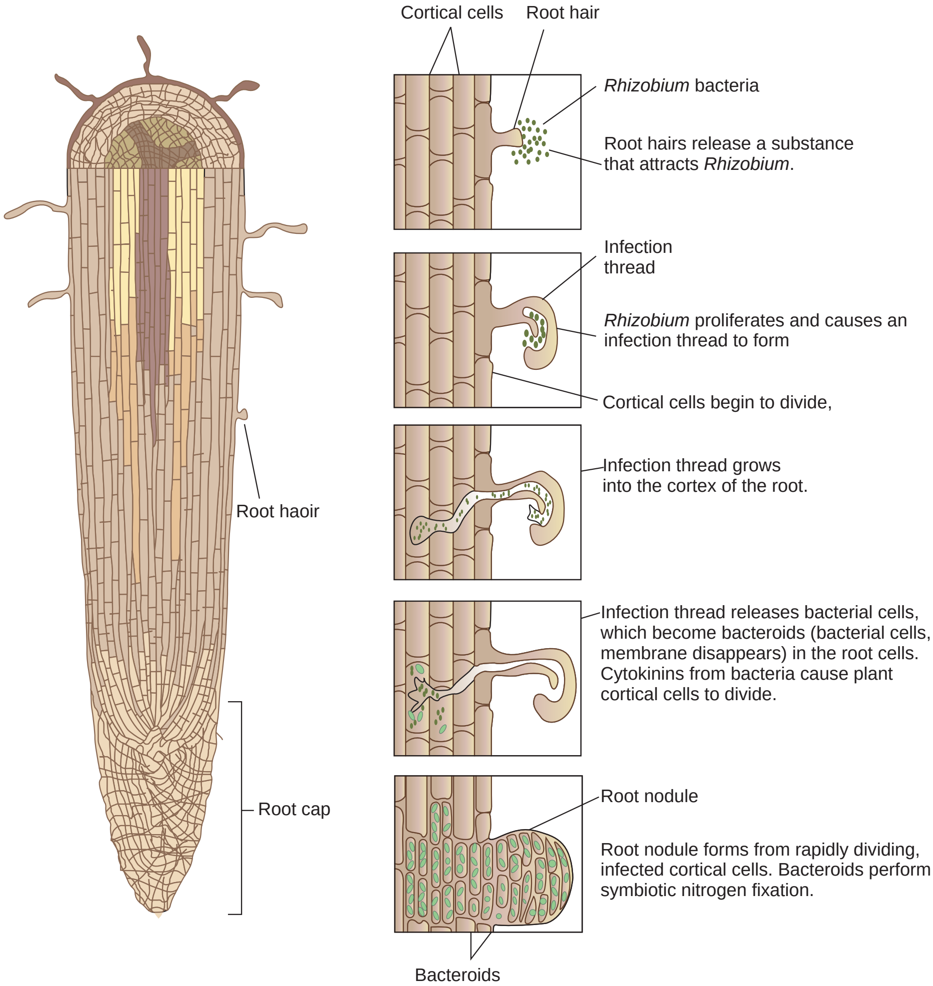


Figure 11.4: Showing stages of formation of root nodules on legume plant

Phosphate solubilizations

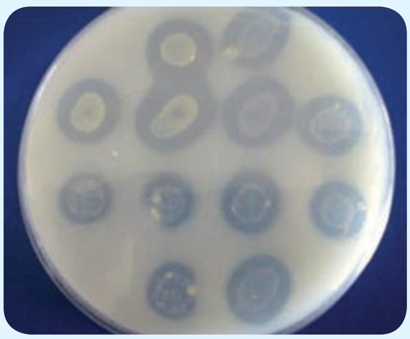
Most of phosphates occur in combination with Calcium, Iron, Magnesium and Aluminium (inorganic P) and thus are insoluble and unavailable to plants and micro organisms. Some micro organisms solubilize those insoluble phosphates by producing organic acids. Example: *Thiobacillus, Bacillus* thus enabling the plants to utilize it.

Phosphate assimilation

Plants and micro organisms can readily assimilate soluble forms of inorganic phosphates like $H_2PO_4^-$, HPO_4^{-2} and HPO_4^{-3} and incorporate them as organic forms of phosphates like ATP, nucleic acids.

Infobits

The ability of bacteria to solubilise phosphates can be tested in the laboratory by streaking the bacterial culture in Pikovaskaya agar which contains tricalcium phosphate. Positive cultures show clear halo around growth.



Phosphate mineralisation

Breakdown of organic phosphates to form soluble inorganic phosphates is called mineralisation. Organisms produce phosphatase enzymes and catalyse mineralisation. The mineralised phosphates can be utilized by plants (Figure 11.5).

$$\text{Phytic acid} \xrightarrow[\text{Phytase}]{} \text{Inositol}$$

11.1.4 Sulphur Cycle

Sulphur is present in sulphur containing aminoacids. The sulphur cycle involves oxidation – reduction reaction between Sulphate (SO_4), Elemental S and H_2S and hence there is change in the valence states of sulphur from -2 to +6.

The basic steps involved in sulphur cycle are

1. Sulphide/ sulphur oxidation
2. Sulphate reduction
3. Sulphur reduction
4. Organic sulphur compound oxidation or reduction
5. Desulfurylation

Sulphide/Sulphur oxidation ($H_2S \rightarrow S^0 \rightarrow SO_4^{2-}$)

It is carried out by prokaryotes under aerobic and anaerobic conditions. Under aerobic conditions, H_2S is spontaneously oxidized at neutral pH to elemental sulphur. Elemental sulphur is oxidized to suphates by chemolithotrophic bacteria like *Thiobacillus, Beggiatoa.*

If light is available, H_2S can be used as electron donor to carry out photosynthesis

HOTS

If there is no biogeochemical cycle in the ecosystem, What will happen to the earth?

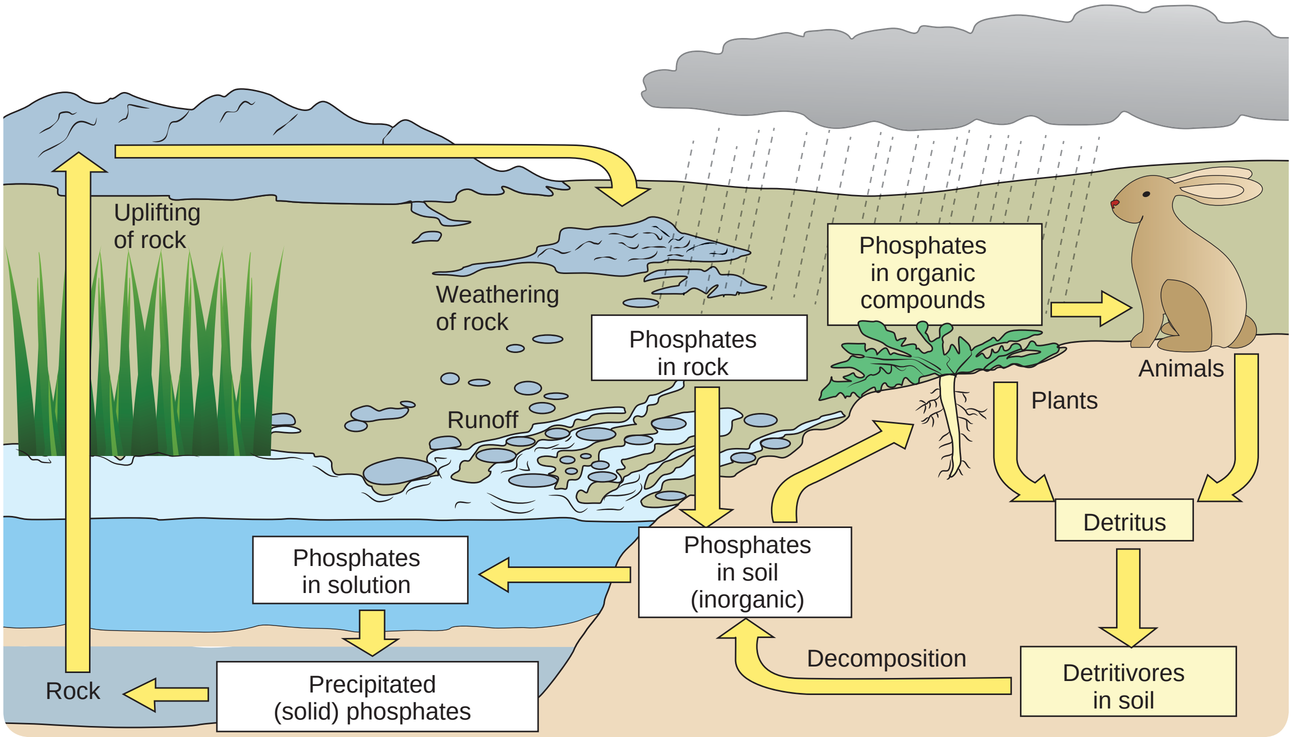


Figure 11.5: Phosphorus cycle

under anoxic conditions by phototrophic sulphur bacteria like *Chromatium* and *Chlorobium*.

Sulphate reduction

When sulphate is present in habitats, different groups of microorganisms are capable of carrying out sulphate reduction.

Beijerinck described the use of sulphate (SO_4^{2-}) as a terminal electron acceptor during anaerobic respiration to form sulphide (H_2S). This process is called **Dissimilatory Sulphate Reduction(DSR)**. The anerobic bacteria capable of carrying out DSR are *Desulfovibrio, Desulfococcus, Desulfotomaculum* (Figure 11.6) This reaction by sulphate reducers requires organic carbon sources like pyruvate or lactate. H_2S accumulated in such habitats by the action of sulphate reducers is toxic to aerobic organisms.

The reduction of sulphate to H_2S, for building up aminoacids and proteins is called as **assimilatory sulphate reduction**. The H_2S thus produced is immediately incorporated into organic compounds.

Sulphur reduction ($S^0 \rightarrow H_2S$):

The dissimilative sulphur reducing bacteria can reduce elemental sulphur to hydrogen sulphide. Example: *Desulfuromonas*, an obligate anaerobe. Under aerobic conditions, organisms like *Pseudomonas, Proteus* and *Salmonella* are also capable of performing this reaction.

Organic sulphur compounds reduction/oxidation

Organic sulphur compounds like dimethyl sulphide can be used as carbon and energy source for many microorganisms.

Desulfurylation

It is a process where organic sulphur compounds are used up by microorganisms for energy to produce H_2S.

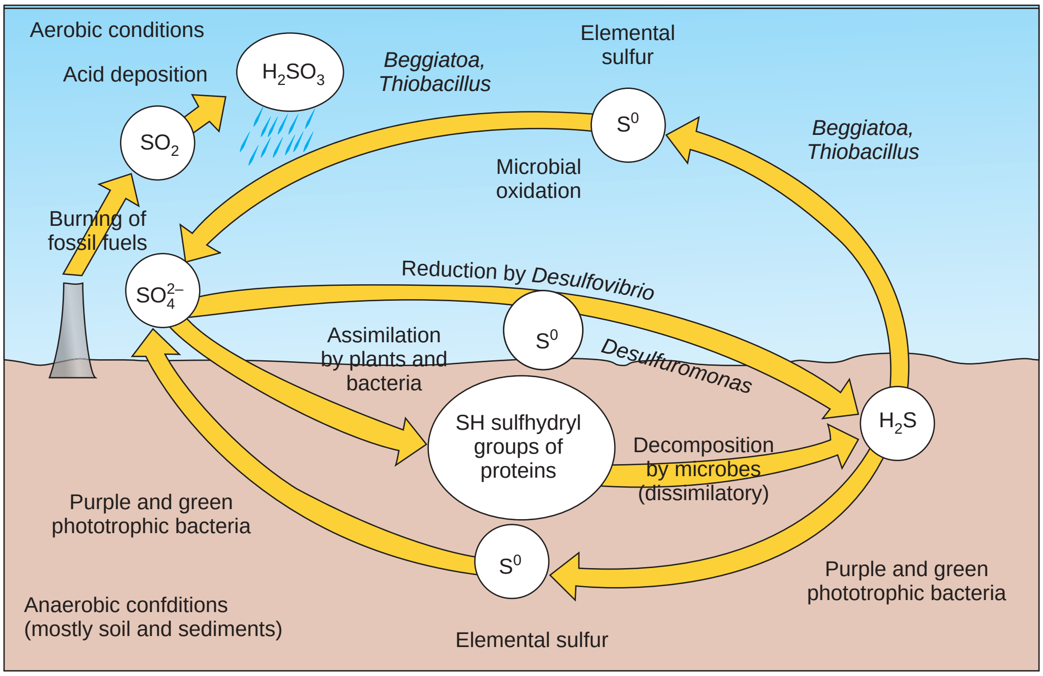


Figure 11.6: Sulphur cycle

11.2 Biofertilizers

In India, the availability and affordability of fossil fuel based chemical fertilizers at the farm level have been ensured only through imports and subsidies. Indiscriminate and imbalanced use of chemical fertilizers, especially urea, along with chemical pesticides and unavailability of organic manures has led to considerable reduction in soil health. Biofertilizers can act as a renewable supplement to chemical fertilizers and organic manures. They have the capacity to produce natural resistance in plants against pests and soil borne diseases, adding fertility to soil.

Nitrogen fixation by leguminous and other crops is reported to be 44 million metric tons per annum. The appropriate strain of *Rhizobium* can increase the crop yield up to 10-35%. Also, residual N is beneficial for the next crops grown in the same field.

It has been estimated that 40-250 kg N / hectare(ha) / year is fixed by different legume crops by the microbial activities of *Rhizobium*.

Definition

Biofertilizers are preparations containing beneficial micro organisms like N_2 fixers, PO_4 solubilizers in a viable static state intended for seed or soil application and designed to improve soil fertility.

Advantages

1. They reduce the need for chemical fertilizers.
2. They provide the plant with certain vitamins, plant growth promoting substances and increase the vigour of the plant.
3. It is cheap and cost effective.

Based on the nutrients that they provide, biofertilizers are of the following types

Nitrogenous biofertilizers-

- *Rhizobium,*
- *Azotobacter,*
- *Azospirillum*
- *Frankia*

Phosphate solubilisers

- *Bacillus*
- VAM

11.2.1 Rhizobium

Rhizobium – legume symbiosis is a well studied plant microbe interaction and *Rhizobium* is the most extensively used nitrogenous biofertilizer in India.

Rhizobium is a gram negative, non-spore forming aerobic bacillus inhabiting the soil in a free living state. The colonies of *Rhizobium* on YEMA (Yeast Extract Mannitol Agar) plate are gummy, pale white in colour (Figure 11.7). They can establish symbiotic relationship with leguminous plants and fix atmospheric nitrogen thereby greatly improving soil fertility.

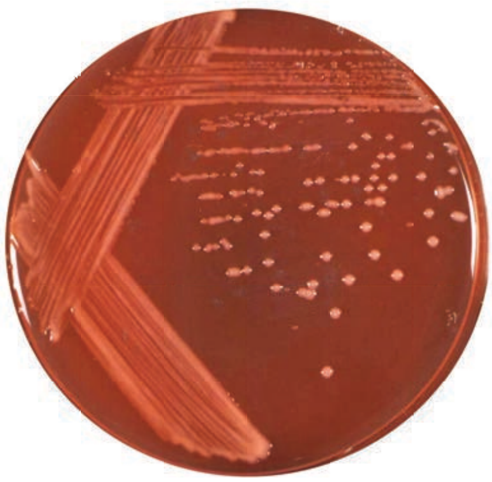


Figure 11.7: Pale pink mucoid colonies of *Rhizobium* on Yeast Extract Mannitol Agar plate

Mass production of *Rhizobium*

The flowchart explaining the mass production of *Rhizobium* biofertilizer is given below

Efficient N_2 fixing strain of *Rhizobium* are selected and initially grown in YEM broth for 24 hours.

↓

Inoculum level is increased from 250 ml to 1000 ml in large flasks

↓

Cultures are then grown in large fermentor for large scale production

↓

Broth is blended with powdered carrier material (peat or lignite)

↓

Packing in polythene bags 200g per bag

↓

Curing at 25°C

↓

Dispatch to farmers

Method of application of *Rhizobium* to plants

Carrier based *Rhizobium* inoculants are mixed with water to form slurry to which the seeds of plants are added (Figure 11.8). The coated seeds are dried in shade and used for sowing.

11.2.2 Phosphate Solubilizers

Several soil bacteria like *Pseudomonas* and *Bacillus* possess the ability to convert insoluble mineral phosphates into soluble form by secreting organic acids thereby making it available to plants.

For mass cultivation and inoculant preparation, the cultures are grown in Pikovaskaya broth for 7-18 days and mixed in suitable carrier like peat or lignite. After curing for a week, the inoculants are packed and made ready for use in a similar manner as *Rhizobium* inoculants.

11.2. 3 VAM

Mycorrhiza means fungus root. It describes the symbiotic association between plant and fungus. Vesicular Arbuscular Mycorrhiza (VAM) is an endomycorrhiza which is used as a fungal biofertilizer. They mobilize the soluble

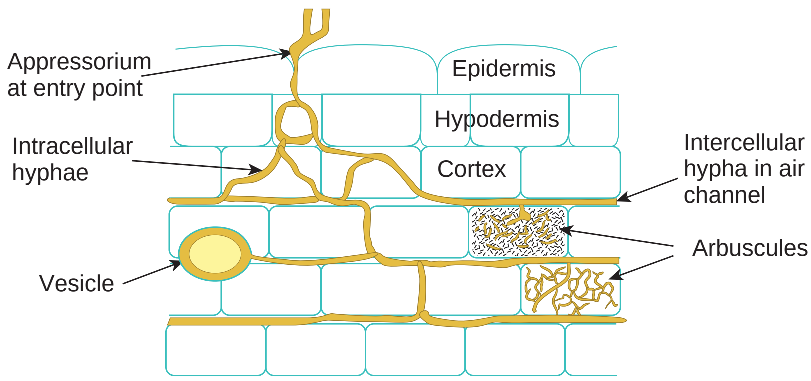


Figure 11.8: Showing the colonization of VAM fungi in root cells of plants

phosphates in the root zone of plants and satisfy the phosphorus nutrition of plants.

Morphology

VAM is an example of endomycorrhiza meaning, the storage organelles of phosphates like vesicles and arbuscles are seen intracellularly. Vesicle is a globose structure and arbuscle is a tree like branching structure present in the root cortical cells (Figure 11.9). VAM fungi are naturally most prevalent in angiosperms. gymnosperms, pteridophytes and bryophytes.

Figure 11.9: The fresh water fern *Azolla.*

Mass production

Root based inoculum is used for preparing VAM biofertilizer (Figure 11.10). The selected spores of VAM fungi are allowed to infect plants like onion, sorghum and other grasses. After 3-4 months, the roots along with the soil are macerated or pelleted with an inert material and packed in polythene pouches which can be used as biofertilizer.

11.2.4 Cyanobacteria / Blue green Algae

Blue green algae are single celled or filamentous prokaryote capable of nitrogen

Figure 11.10: Microscopic view of *Anabaena azollae*

fixation and photosynthesis. Most of the filamentous forms have specialized large, thick walled cells called heterocysts which are sites of nitrogen fixation.

Example: *Nostoc*, *Anabaena* is examples for filamentous BGA. *Gleocapsa* is an example of unicellular BGA. Some of the filamentous forms do not possess heterocysts but still fix atmospheric nitrogen. Since they need standing water for their growth, BGA can effectively colonize paddy fields and enrich the soil with nitrogen.

Mass cultivation of BGA

Applying BGA to paddy fields can reduce the amount of chemical nitrogenous fertilizer applied for the growth of paddy crop. Therefore cultivation of BGA in large quantities is necessary. Mass cultivation of BGA has the following steps.

1. Isolation of BGA
2. Mass cultivation of BGA

Isolation of BGA

BGA can be isolated from soil or paddy fields. Appropriate dilutions from serially diluted algal sample are inoculated in the liquid flasks containing algal media like BG-11 or Pringsheim's media. After several weeks of incubation at 28°C, the individual colonies are picked up, identified and stored. This can be used as starter culture for mass cultivation. Mass culture can be done in 2 ways.

Mass cultivation of BGA

1. Open air shallow culture:

Pits are prepared (6′ × 3′ × 9′)

↓

Filled with soil and 200g of superphosphate and water is added

↓

Starter culture is sprinkled over water

↓

1 week

↓

Algal scum is formed on the surface

↓

Water is allowed to dry and the dried up algal flakes are collected and stored in poly bags

The dried algal flakes around 10kg/ha can be applied in paddy fields after transplantation.

11.2.5 Azolla

Azolla is a floating freshwater fern. The plant has a branched stem, deeply bilobed leaves which are arranged alternately on the stem and each leaf has a dorsal and ventral lobe (Figure 11.9). The dorsal lobe houses the cyanobacterial symbiont *Anabaena azollae* (Figure 11.10). The fern and the cyanobacteria exhibit symbiotic relationship in which *Anabaena* provides the fern with fixed nitrogen and fern provides niche for the cyanobacteria free from competition from other microorganisms.

Azolla can be used as a nitrogenous biofertilizer for paddy crop. When applied into the paddy fields, *Azolla* provides nitrogen nutrition to standing rice crop and can reduce the need for synthetic fertilizers.

Infobits

Mycorrhiza and orchid germination

In the early stages of their life cycle, all terrestrial orchids are non photosynthetic, totally lacking chlorophyll and relying on carbon(C) acquired from a fungal symbiont (Mycorrhiza) for growth until the production of the first green leaves above the ground, a nutritional strategy termed mycoheterotrophy. Around 200 species of orchids remain achlorophyllous throughout their lifetimes. Species such as *Galeola*, *Gastrodia*, *Corallorhiza*, *Rhizanthella* and many others continue to gain carbon from mycorrhizal fungi.

Mass multiplication of *Azolla*

Small plots (50-100 sq.m) or concrete tanks with standing water are inoculated with *Azolla*

↓

pH is adjusted to 8.0 with lime.

↓

Superphosphate is applied as a nutrient and carbofuran is applied to deter insects

↓

After 20 days, *Azolla* biomass can be harvested and used as biofertilizer for rice plantings

Method of application of *Azolla* in rice fields

Azolla is grown on the flooded rice fields prior to planting for 2-3weeks. Then water is drained and *Azolla* is incorporated into the soil followed by rice transplantation within a week's time.

HOTS

Why bio- fertilizer are preferred to chemical fertilizer?

11.3 Biopesticides

Pests are insects that damage crop plants and stored products. They feed on leaves and roots or suck the sap of the plants causing severe crop losses. Chemical pesticides sprayed on plants can be detrimental to ecosystem if the usage is prolonged and pests may develop resistance to the pesticides.

The term **biopesticides** refers to compounds that are used to manage agricultural pests by means of specific biological effects. It refers to products containing biocontrol agents like natural substances such as plants, certain minerals, animals, micro organisms including their genes or metabolites.

They are an important part of Integrated Pest Management (IPM) strategy in controlling the pest.

Advantages

They are less toxic to humans and environment and they do not leave harmful residues.

They affect only the target pest.

They cause long term suppression of pest populations since they persist in the environment.

Microbial biopesticides are of three kinds

1. Bacterial biopesticide
2. Fungal biopesticide
3. Viral biopesticide

11.3.1 Bacterial Biopesticide

Bacteria like *Bacillus thuringiensis, Bacillus papillae* and *Bacillus lentimorbus* have the potential to kill certain insect pests and are entomopathogenic.

Bacillus thuringiensis

It is a gram positive, spore forming, rod shaped soil bacterium. During sporulation, the bacterium produces insecticidal proteins as parasporal crystals. These are called ***delta endotoxin*** also called as ***Cry proteins***. ***Cry proteins*** are specifically toxic to insects belonging to Lepidoptera, Coleoptera and other few insect orders.

Mode of action of Bt

The Bt cells sprayed on the leaves have to be ingested by the larval forms of the insects in order to exert its action. This is because the Bt toxin gets activated in the insect gut at a specific pH.

Process

Insect ingests the parasporal crystals containing Cry protein

↓

The crystals dissolve in the alkaline environment of the insect gut

↓

The solubilised inactive delta endotoxin are cleaved rendering them active

↓

The activated toxin binds to specific receptor of the midgut epithelium

↓

This leads to the insertion of toxin thereby developing pores in the epithelium

↓

Cell lysis and disruption of epithelium releases the cell contents leading to insect death.

Symptoms

- Larvae stops feeding
- Larvae becomes sluggish and static
- Water oozes out from the body
- Larvae dies and falls off the leaf

Various species of Bt are able to work against cotton boll worm, cabbage worm and gypsy moths.

Infobits

Photograph of a cotton plant showing opened and unopened bolls. BT cotton is a genetically modified cotton plant (GM crop) which has the gene for the crystal toxin integrated in its genome. Crystal toxin is expressed in plant parts which reduce the need for spraying pesticides. BT cotton is the only GM crop approved for commercial cultivation in India.

11.3.2 Fungal Biopesticides

These entomopathogenic fungi attack insects and cause diseases in insect body which lead to insect death. Two prominent fungi used as mycopesticide are

- *Beauveria bassiana* which causes white muscardine disease
- *Metarhizium anisopliae* which causes green muscardine disease

Mode of action of *Beauveria bassiana*

Beauveria bassiana, a filamentous fungus belongs to class Deuteromycetes also called imperfect fungi. It can be successfully used against Colarado potato beetle, (Figure 11.11) Codling moth and American boll worm.

This fungus invades the haemocoel of insects through spores. Once the spores attach to the cuticle, it germinates and the hyphae penetrates the insect cuticle (cuticle is the outer membrane of insects) Penetration is aided by formation of appresorium and penetration peg. The fungi secrete chitinases, lipases and proteases which can dissolve the cuticle. The hyphae enter the haemolymph and proliferate and colonise the entire insect and release blastospores. Insect death occurs due to nutrient depletion of the haemolymph or by toxaemia by secretion of toxic metabolites.

Figure 11.11: Picture of insect infected with *Beauveria bassiana*

> DO YOU KNOW?
>
> **Biopesticides registered in India:**
>
> 1. *Bacillus thuringiensis var. israelensis*
> 2. *Bacillus thuringiensis var. kurstaki*
> 3. *Bacillus thuringiensis var. galleriae*
> 4. *Bacillus sphaericus*
> 5. *Trichoderma viridae*
> 6. *Trichoderma harzianum*
> 7. *Pseudomonas fluorescens*

11.3.3 Viral Biopesticides

Viral insecticides are pathogens that attack insects and other arthropods. Viral pesticides are used to control Lepidopteran larvae like *Helicoverpa, Spodoptera* sp on Cotton, Corn, Sorghum, tomatoes. Baculoviruses are the commonly used viral biopesticide. They are extremely small and are composed of double stranded DNA. The genus Baculoviruses contains 3 subgroups.

- Nuclear Polyhedrosis viruses (NPVs)
- Granulosis viruses (GVs)
- Non occluded viruses

Mode of action of NPV

ETH2PG

The virus enters the insect body via ingestion by insects and infects the midgut cells by membrane fusion. The NPV uncoat within the nucleus of cells and pass through the intestinal epithelium (Figure 11.12) and establish a systemic infection of the haemocoel.

Symptoms

Discoloration (larvae turns brown or yellow)

- Decomposition or softening of larvae
- Lethargy
- Infected larvae hang upside down twigs
- Larvae become swollen with fluid containing virus and eventually die turning black in color.

Mass production of NPV

NPV are mass produced in laboratory using suitable larval hosts. The fifth stage larvae are fed with food infected with

Figure 11.12: Picture of larvae infected with NPV

NPV. After 4-5 days, the dead larvae are collected and macerated. The liquid is centrifuged and the pellet containing the viruses is suspended in sterile distilled water. This viral suspension can be used for spraying in the fields.

11.4 Plant Diseases

The study of the nature, causative agent, development and control of plant diseases is called plant pathology. The study of plant diseases is important because man is directly and indirectly dependent upon plants for his survival and plants are the source of food, fibre and drugs.

Impact of plant diseases on human welfare

History proves us that plant diseases had caused severe famine in the early centuries as a result of which millions of people died and was forced to migrate out of their country. Plantations were wiped out and agro industries failed and shortage of food resulted due to microbial attack on plants.

Example: In 1840, late blight disease of potato has caused severe famine in Ireland

In 1942, Bengal famine in India was due to Helminthosporium disease of rice caused by *Helminthosporium oryzae*.

The various diseases caused on plants by micro organisms can be broadly grouped into the following kinds.

11.4.1 Fungal Diseases

Most of the diseases of plants are caused by fungi. They exhaust all the nutrients from the plants and exhibit a wide variety of symptoms on leaves, stem and inflorescence. The common symptoms are given below.

Rusts

It is a plant disease caused by the rust fungus of the class Uredinomycetes. Example: *Puccinia* sp. The leaves have characteristic spots or pustules which are rust colored, yellow or brown that bear spores of the infecting fungi.

Smuts

This plant disease is caused by a fungus of the order Ustilaginales. The plant parts contain black, powdery masses of spores that appear as sooty smudges.

Wilts

It is due to the deficiency of water in the foliage, which results in the death of shoots and large branches. Several fungi like *Fusarium, Verticillium* cause wilt by plugging up the xylem and phloem vessels of the plant.

Downy mildew

This disease is characterised by the formation of downy growth (covered with soft, fine hairs) on the undersurface of the leaves with a corresponding yellowish patch on the top of the leaves. The causative fungus belongs to the order Perenosporales

The other fungal diseases of plants include Powdery mildews, Rots, Damping off, Leaf spots (Figure 11.13) and Blights.

11.4.2 Bacterial Diseases

The common bacterial diseases of plants are bacterial leaf spot, crown gall and fire blight.

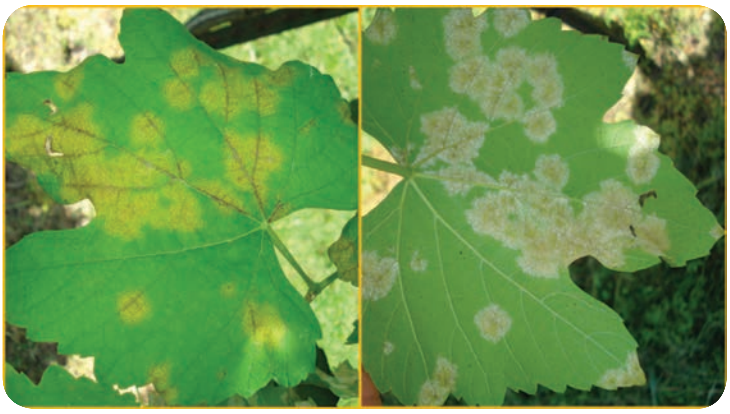
Bacterial leaf spot

Plants that are infected with bacterial leaf spot will develop dark-colored, water-soaked spots that are accompanied by

Rust

Smut of corn

Verticillium wilt



Downy mildew on upper and lower surface of grape leaf

Figure 11.13: Various fungal diseases in plants

encasing yellowing halos. Continuous rain and moisture will cause the coalescence of the spots. Severely infected leaves will defoliate prematurely. Bacterial leaf spot is a common nuisance of citrus (Figure 11.14) and stone fruit trees and vegetables, as well as other indoor and outdoor foliage plants.

Crown gall

Crown gall is a root and stem disease that is most commonly found on woody plants. Infected plants will develop smooth, light-colored galls on its roots and stems. These formations inhibit the plant's ability to transport nutrients and water throughout

Figure 11.14: Showing citrus canker lesions caused by the bacteria *Xanthomonas citri*

Figure 11.15: Photograph of a tree showing galls caused by *Agrobacterium*

Figure 11.16: Fire blight of apple caused by *Erwinia amylovora*

the plant. This results in loss of vigor of plant which is also accompanied by growth stunt and dieback of branch and twigs (Figure 11.15) Crown gall disease is a soil-borne bacterial disease caused by *Agrobacterium* sp.

Fire blight

Fire blight is a destructive bacterial disease that is especially threatening to rosebushes and pome fruit trees like apple and pear. Trees and plants that are infected with fire blight will display tan-colored, bacterial ooze near the points of infection (Figure 11.16) The infected areas become necrotic, turn black, wilt and become deformed.

11.4.3 Viral Diseases

Viruses are obligate parasites and require a wound to gain entrance to a plant cell. In nature, they depend primarily on biological agents such as nematodes, insects and man for their dissemination. Once duplication starts, the virus is translocated from cell to cell through the plasmodesmata and to distant plant parts by the phloem. Some viral diseases are very host specific, and others are general, over many hosts. Symptoms include stunting, mosaic, ring spots, color breaks, and distortion.

Common plant viruses are tobacco mosaic virus (Figure 11.17) Cauliflower mosaic virus, Impatiens Necrotic Spot Virus (INSV), tomato spotted wilt virus, ajuga viruses, and cucumber mosaic virus

Figure 11.17: Photograph showing tobacco leaves affected with TMV

Late blight of potato

Causative organism: *Phytophthora infestans*

Late blight is the disease that triggered the Irish potato famine of the 1840s which resulted in the death and starvation of 1 million people. It also was the first plant disease for which a microorganism was proved to be the causal agent, leading to the birth of plant pathology as a science (Figure 11.18)

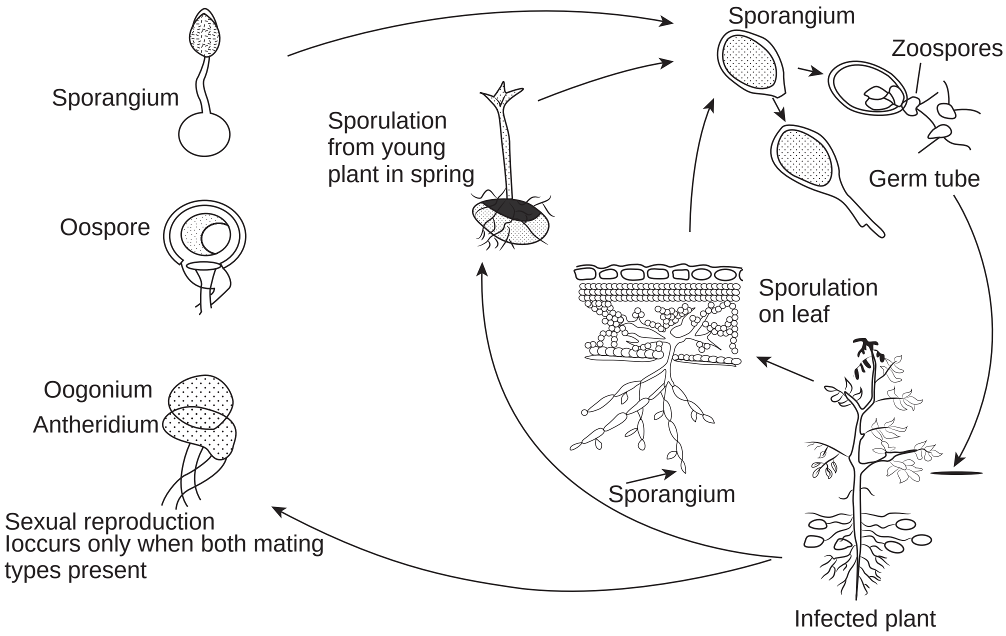
Phytophthora infestans is a member of the oomycetes, a group of organisms sometimes referred to as the "**water molds**". During asexual reproduction, *Phytophthora infestans* produces sporangia on sporangiophores. In cool, wet conditions, zoospores will form and emerge from the sporangia after about two hours. In warmer conditions, sporangia may function as a single spore and germinate directly. Zoospores are biflagellate (have two flagella) with one tinsel flagellum directed anteriorly and one whiplash flagellum directed posteriorly. After swimming on the surface of the host plant surface, zoospores encyst and infect the plant. During sexual reproduction, mating between opposite mating types happen. A nucleus from the antheridium (Male) enters the oogonium (female). Following karyogamy (the fusion of two nuclei), a thick-walled, diploid oospore is formed. From oospore, sporangium develops and the life cycle (Figure 11.19) continues.

Lesions on stems and leaves

Late blight of potato is identified by black/brown lesions on leaves and stems that may be small at first and appear water soaked or have chlorotic borders, but soon expand rapidly and become necrotic. In humid conditions, *Phytophthora infestans* produces sporangia and

Symptoms

Figure 11.18: Photograph showing blight lesions on potato leaves, stem and tuber.



This is a simplified disease cycle for late blight of potato.

Figure 11.19: Disease cycle for late blight of potato

sporangiophores on the surface of infected tissue. This sporulation results in a visible white growth at the leading edge of lesions on abaxial (lower) surfaces of leaves. As many lesions accumulate, the entire plant can be destroyed in only a few days after the first lesions are observed

Lesions on tubers

Tuber infection symptoms are a darker brown sometimes purplish area on the tuber surface. The internal rot is a reddish brown granular rot which can remain close to the surface or progress to the centre of the tuber. Rot development is irregular and sometimes threadlike through the tuber flesh. Late blight causes skin damage which allows opportunistic bacteria to invade and cause soft rotting.

Epidemiology

Temperature and moisture are the most important environmental factors affecting late blight development. Sporangia are formed on the lower leaf surfaces and infected stems when relative humidity is < 90%. Sporulation can occur from 3-26°C, but the optimum range is 18-22°C. Sporangia germinate directly via a germ tube at 21-26°C.

Control

Fungicide applications are an important means of late blight management, particularly in humid areas. They must be applied before plants are exposed to spores. Copper hydroxide (2g/lit), chlorothalonil (2g/lit) and mancozeb (2.5g/lit) fungicides are the standard protectants used for control. they are usually applied every seven to ten days for best protection.

Summary

Carbondioxide fixation and Biological Nitrogen Fixation are the most significant biological processes taking place on planet Earth. Methanogenesis is an anaerobic process converting CO_2 to CH_4. It is carried out by methanogens like *Methanobacterium* sp. Phosphorous transformations mostly happen as inter conversion of inorganic to organic phosphate and insoluble form to

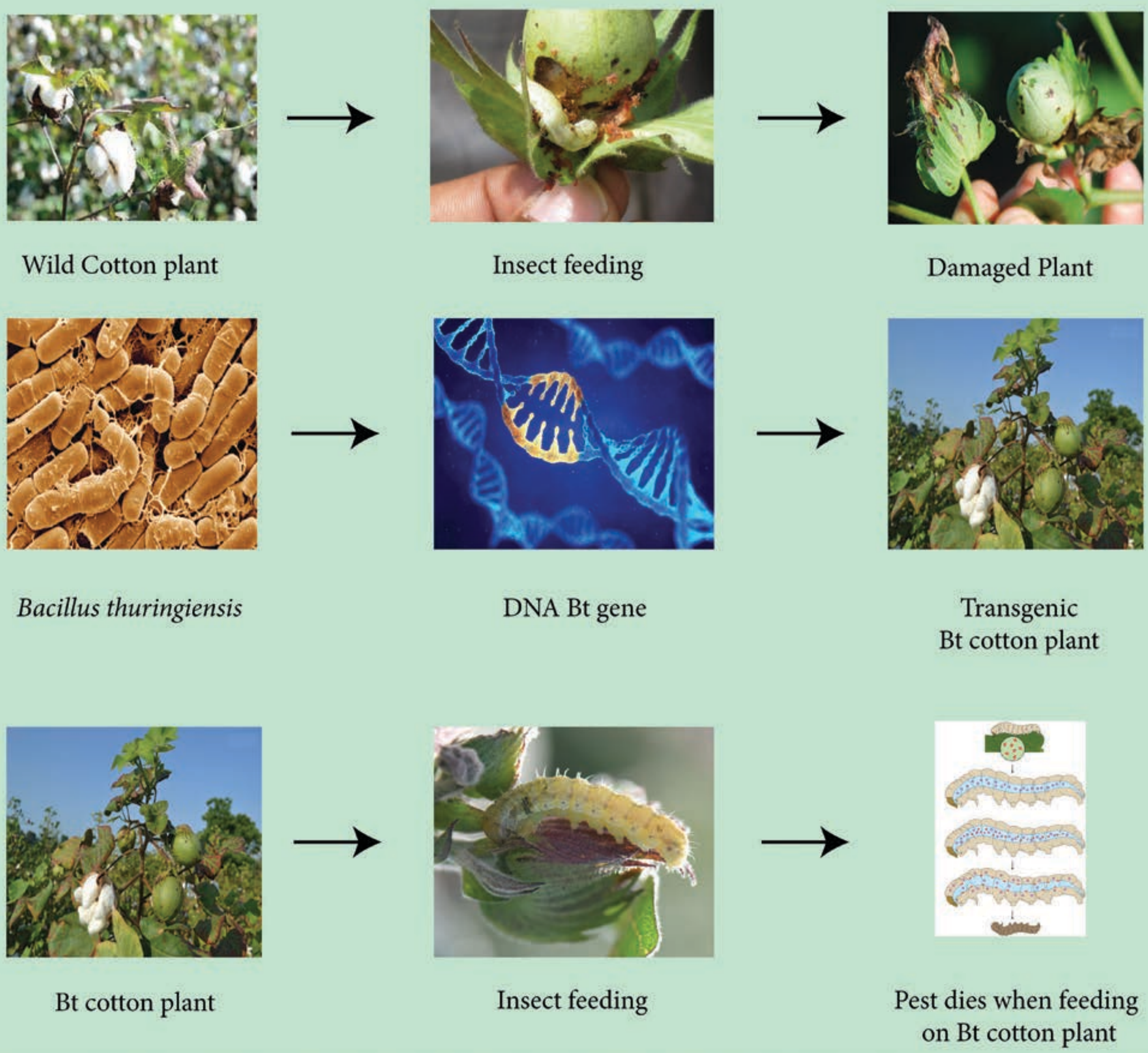
soluble phosphates.Purple and green sulphur bacteria store sulphur as granules as a result of which they appear yellow in colour.

Biofertilizers are preparations containing beneficial micro organisms like N_2 fixers, PO_4 solubilizers in a viable static state intended for seed or soil application and designed to improve soil fertility. *Azolla* and *Anabaena* share symbiotic relationship in which *Anabaena* provides fixed atmospheric nitrogen to *Azolla*.

Vesicles and arbuscles in VAM fungi are the storage organelles of polyphosphates.Blue Green algae are prokaryotes that can perform both photosynthesis and nitrogen fixation.

Biopesticides refer to compounds that are used to manage agricultural pests by means of specific biological effects. *Bacillus thuringiensis* produces crystal toxin which is detrimental to insects. Fungal and viral biopesticides establish systemic infection in the body of insects to kill them. Smuts, rusts, downy and powdery mildews are some of the important plant diseases caused by fungi.

Bacterial Biopesticide - *Bacillus thuringiensis*



Evaluation

ETQXR4

Multiple choice questions

1. The conversion of atmospheric nitrogen to ammonia by prokaryotes is called
   a. Biological Nitrogen Fixation
   b. Nitrification
   c. Ammonification
   d. Denitrification
2. The oxidation of sulphide is carried out by
   a. *Thiobacillus*
   b. Purple bacteria
   c. *Beggiatoa*
   d. Both a and b
3. Phosphate solubilisation by bacteria is mediated by the production of
   a. Organic acids
   b. Phosphatases
   c. Phosphoric acid
   d. Phytases
4. The process of production of CH_4 from CO_2 is called
   a. CO_2 fixation
   b. Methylotrophy
   c. Methanogenesis
   d. Photosynthesis
5. The reduction of sulphate for building up aminoacids and proteins is called
   a. Desulfurylation
   b. Assimilatory sulphate reduction
   c. Dissimilatory sulphate reduction
   d. Sulphur reduction
6. An example of nitrogenous biofertilizer
   a. *Bacillus*
   b. *Pseudomonas*
   c. *Rhizobium*
   d. VAM
7. The other name for Blue Green Algae is
   a. Green algae
   b. Brown algae
   c. Cyanobacteria
   d. Blue algae
8. The selective media for Rhizobium YEMA contains the sugar
   a. Maltose
   b. Mannitol
   c. Glucose
   d. Lactose
9. Solubilisation of inorganic phosphates by *Bacillus* is brought about by the
   a. Production of enzymes
   b. Production of organic acids
   c. Production of alkali
   d. Mineralisation
10. The mass cultivation of VAM is preferably done in
    a. Sorghum roots
    b. Rice roots
    c. Potato roots
    d. Cotton roots
11. ________________ is an example of entomopathogenic fungi
    a. *Verticillium*
    b. *Beauveria bassiana*
    c. *Metarhizium anisopliae*
    d. All of the above
12. The toxic effect of *Bacillus thuringiensis* is due to
    a. Cry protein
    b. Delta endotoxin
    c. Parasporal crystals
    d. All of the above

13. The action by mycopesticide depends on the
    a. Ingestion of fungi by insects
    b. Penetration of cuticle by fungi
    c. Ingestion of leaves infected with fungi
    d. Ingestion of spores by fungi.
14. Crown gall is caused by *Agrobacterium* on
    a. Monocots
    b. Dicots only
    c. Monocots and dicots
    d. Ferns
15. Smut is a disease caused by
    a. *Puccinia sp*
    b. *Ustilago sp*
    c. *Verticillium*
    d. None of the above

Answer the following

1. What is the role of purple and green bacteria in sulphur cycle?
2. What is nitrogenase? Give its function.
3. Define biofertilizers.
4. What is VAM?
5. Define biopesticides.
6. What is a smut?
7. What is NPV?
8. List out Bacterial diseases of plants.
9. What is the end result of decomposition of organic matter in carbon cycle? Give the role of microorganisms with examples.
10. What is the function of leghemo-globin?
11. Give the method of application of *Azolla* in paddy fields.
12. Give the salient features of *Rhizobium*.
13. Explain the mass production of phosphate solubilisers.
14. What is *Azolla-Anabaena* symbiosis?
15. What is crystal toxin? Write short notes on BT cotton.
16. Explain the process of root nodule formation with appropriate diagram.
17. Give in detail the reactions involved in phosphorus/carbon/sulphur/nitrogen cycle.
18. Explain the mass production of VAM.
19. Give detailed account on *Bacillus thuringiensis*.

Student Activity

- Sow two groundnut seeds in a plastic cup/earthern pot. After one month, pull out the plant and observe the root system for nodules.
- Collect pictures of all organisms involved in sulphur cycle and prepare a collage showing its role.
- Prepare a chart work showing the biogeochemical cycles of carbon, nitrogen and phosphorus
- Collect pictures of various diseases of plants and prepare a chart.
- Prepare a model on the mode of action of BT.
- Collect diseased parts of plants and identify the symptoms of the disease.

Late blight of potato

https://youtu.be/2Y77KEYuw_g

https://www.youtube.com/watch?v=p9Koqk

Chapter 12

Medical Microbiology

Chapter Outline

ETZTSR

Medical Microbiology or Clinical Microbiology plays an important role by providing the necessary diagnostic testing, means of epidemiological detection, and future innovation required in an era of emerging and reemerging infectious diseases.

Learning Objectives

After studying this chapter the student will be able,

- *To describe the importance of medical microbiology.*
- *To understand the types and sources of infections.*
- *To know the types of infectious diseases and virulence factors of the pathogen.*
- *To tell the etiological agents of skin wound respiratory, gastro intestinal, ocular, urinary, reproductive, nervous system and systemic infections.*
- *To know the causative agents of varoius human diseases and their portal of entry.*

12.1 Microbial Infections of the Human Body

Medical microbiology is the branch of microbiology which deals with prevention, diagnosis and treatment of infectious diseases. There are four kinds of microorganisms that cause infectious diseases. They are bacteria, fungi, parasites and viruses. Any disease that spreads from one host to another, either directly or indirectly is said to be a communicable disease. Chicken pox, measles, genital herpes, typhoid fever and tuberculosis are examples of such diseases, that are easily spread from one person to another.

A non communicable disease does not spread from one host to another. For example, *Clostridium tetani,* a soil inhabitant, produces Tetanus when it is introduced into a wound or an abrasion. Tetanus is thus an infectious disease, but not communicable.

Infectious disease occurs when the infecting microorganism causes damage to the host. The term infection refers to the establishment of the microorganisms in the tissues resulting in injury or harmful effect to the host. Infection is a pathological condition due to the growth of microorganisms in a host. To initiate an infection, a pathogenic microbe enters the tissues of the body by a characterization route, the portal of entry.

12.1.1 Routes of Infections

There are various ways in which microorganisms enters into the host are explained below.

a. Contact

Infection may be acquired by contact which may be direct or indirect. Sexually transmitted diseases such as syphilis and gonorrhea spread by direct contact. Indirect contact may be through the agency of inanimate objects such as clothing, pencils or toys which may be contaminated by a pathogen from one person to another. Pencils shared by school children may act as fomites in the transmission of diphtheria, and face towels in trachoma.

b. Inhalation

Respiratory infections such as influenza and tuberculosis are transmitted by inhalation of the pathogen in droplet and droplet nuclei that are shed by the patients during sneezing, speaking or coughing. Common cold virus, Adenovirus is some of the virus producing respiratory infections.

c. Ingestion

Intestinal infections are generally acquired by the ingestion of food or drinks contaminated by pathogens. Infection transmitted by ingestion may be waterborne (cholera), food borne (typhoid) or fecal-oral route (dysentery).

d. Inoculation

Pathogens, in some instances, may be inoculated directly into the tissues of the host. Tetanus spores implanted in the depth of wounds, rabies virus deposited subcutaneously by dog bites, inoculation through unsterile syringes and surgical equipments are examples that enter through direct inoculation.

e. Congenital

Some pathogens are able to cross the placental barrier and infect the fetus in uterus. Bacteria like *Treponema pallidum*, viruses like *Rubella, Cytomegalovirus* parasite like *Toxoplasma gondi* are some of the organisms that enter through placenta and cause disease in the newborn.

12.1.2 Types of Infections

Infections may be classified in various ways. Initial infection with a parasite in a host is called a primary infection. Subsequent infections by the same parasite in the host are termed reinfections. When a new parasite sets up an infection in a host whose resistance is lowered by a preexisting infectious disease, this is termed secondary infection.

When in a patient already suffering from a disease, a new infection is setup from another host or other external sources it is termed cross infection. Cross infections occurring in hospitals are called nosocomial infections. Iatrogenic infection refers to physician induced infections resulting from investigative, therapeutic or other procedures.

Depending on whether the source of infection is from the host's own body or from external sources, infections are classified as **endogenous** or **exogenous**, respectively.

Endogenous infection

Endogenous infections are acquired from the host himself from the normal flora of the body.

Microorganisms are present in certain areas of the body in all human beings. They are called normal flora. The common areas are Nose, Mouth, Teeth, Throat, Intestine, Urethra, Vagina and Skin (Figure 12.1).

1. When the skin is breached normal flora enters the tissues.
2. When the urethral organisms ascend, they cause urinary tract infection
3. When a patient is treated with antibiotics, normal flora is eliminated and replaced by potential pathogens
4. When the intestine is perforated, normal flora enter the previously sterile body parts
5. Similarly when the pH of the vagina increases potential pathogens occupy the space.

However normal flora helps host against pathogen and benefits the host in many ways

- Normal flora of skin produces fatty acids which inhibit other species
- Intestinal bacteria secrete antibacterial substances (bacteriocins, colicins) and many metabolic products that prevent other species to survive.

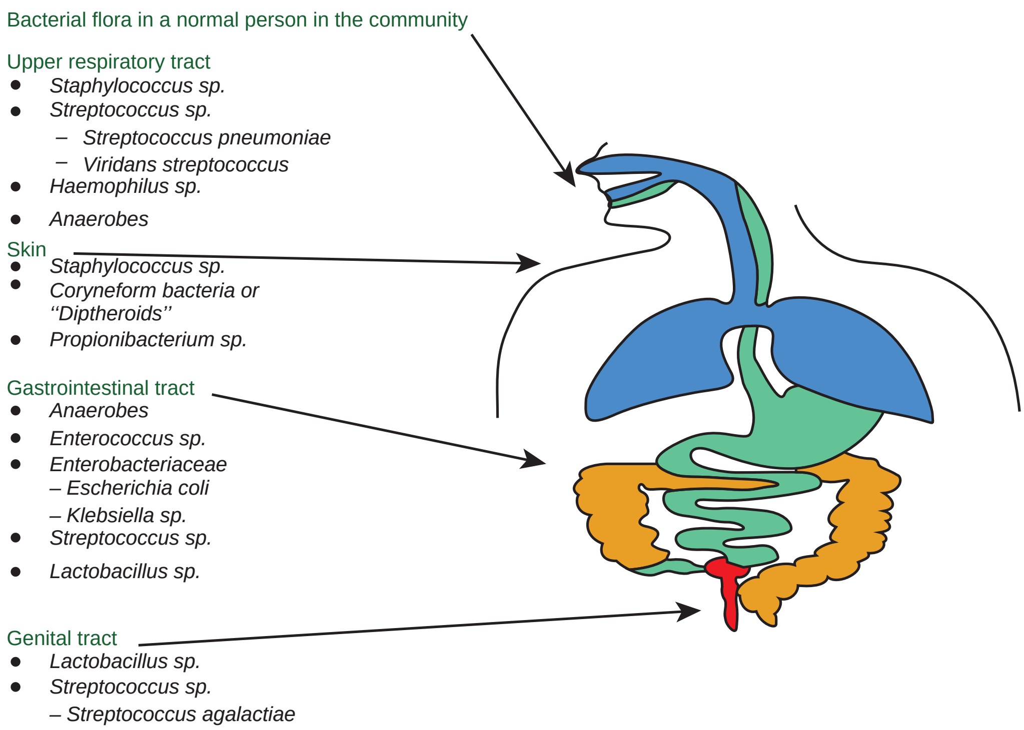


Figure 12.1: Microorganisms present as normal flora (There are many organisms in a site. Only few are listed)

- Because of their large numbers other species do not have space in the intestine
- Acidic environment created by vaginal Lactobacilli suppresses growth of other bacteria.

HOTS

How do normal flora help host against pathogenic microorganisms?

Exogenous sources of infections

Human beings: The commonest source of infections in human are from other human beings. The parasite may originate from a patient or a carrier. A patient is a person who harbours the pathogenic microorganism and suffers from ill effect because of it. A **healthy carrier** is the one who harbours the pathogens but has never suffered from the disease caused by the pathogen. A **convalescent carrier** is one who has recovered from the disease and continues to harbor the pathogen in his body (Figure 12.2).

Animals: Many pathogens are able to infect both human beings and animals. Infectious disease transmitted from animals to human beings are called zoonoses. Zoonotic diseases may be bacterial (Example: plague from rats) or viral (Example: rabies from dogs).

Insects: Blood sucking insects may transmit pathogens to human beings. The diseases so caused are called arthopod borne diseases. Insects such as mosquitoes, ticks, mites, flies, fleas and lice that transmit infections are called vectors.

Transmission may be mechanical or biological. Mechanical transmission is the passive transport of the pathogens on the insects feet or other body parts. Example: Houseflies can transfer the pathogens of Typhoid fever and Bacillary dysentery

Infobits

The story of typhoid Mary

The classic example of role of carriers in disease transmission is the story of Mary Mallon.

Mary Mallon was an Irish immigrant who worked as a cook in New York in the early twentieth century. Over seven years, from 1900 to 1907, Mallon worked for number of different households. Unknowingly spreading illness to the people who lived in each one. Later George Soper, tracked Mallon linked 22 cases of typhoid fever through her. He discovered that Mallon was a carrier for typhoid but was immune to it herself. Although active carriers had been recognized before, this was the first time that an asymptomatic carrier of infected had been identified. Epidemiologists were able to trace 51 cases of typhoid fever and three deaths directly to Mallon, who is remembered as "Typhoid Mary". She was forced to prison and then released under the conditions that she could no longer be a cook. She assumed a false name and began cooking again and of course, infecting numerous people. She was again prisoned where she died 26 years later of pneumonia.

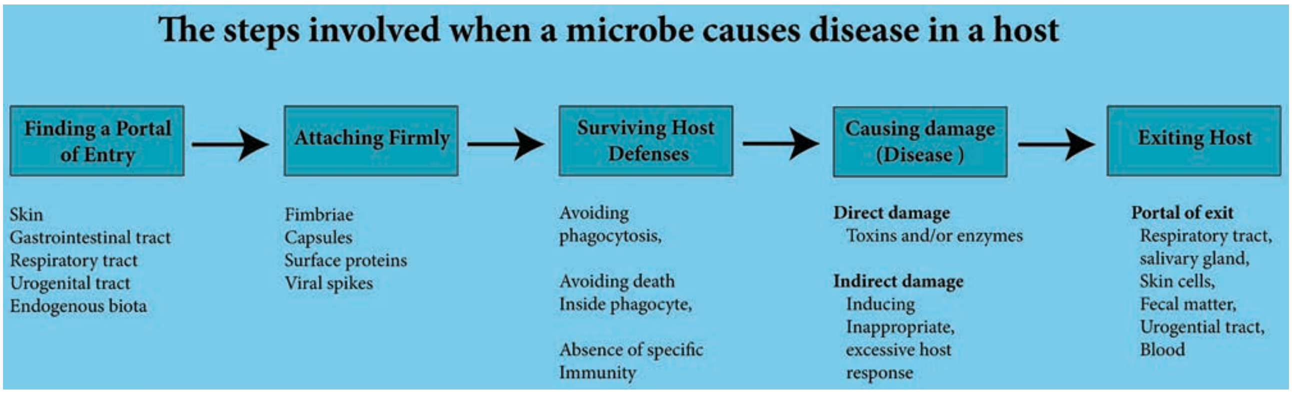


Figure 12.2: The steps involved when a microbe causes disease in a host

(shigellosis) from feces of infected people to food. Such vectors are called mechanical vectors.

Biological transmission is an active process and is more complex. The pathogens multiply in the body of the vectors often undergoing part of a developmental cycle in it. Such vectors are termed biological vectors. Example: *Aedes aegypti* mosquito transmitting dengue, *Anopheles* mosquito transmitting malaria.

Soil: Some pathogens are able to survive in the soil for very long periods. Spores of tetatus bacilli may remain viable in the soil for several decades and serve as the source of infection.

Water: Water may act as the source of infection due to contamination with pathogenic microorganisms. Example: Cholera causing *Vibrio cholerae*.

Food: Contaminated food materials may act as source of infection. The presence of pathogens in food may be due to external contamination. Example: Food contaminated by *Staphylococcus*.

12.1.3 Types of Infectious Diseases

Infectious diseases may be localised or generalised.

Localised infections: An infection that is restricted to a specific location or region within the body of the host is called localised infection.

Generalised infections: An infection that has spread to several regions or areas in the body of the host. This involves the spread of the infecting agent from the site of entry through tissue spaces or channels, along the lymphatics or through the bloodstream.

Circulation of bacteria in the blood is known as **Bacteremia**. **Septicemia** is the condition where bacteria circulate and multiply in the blood, form toxic products and cause high fever. **Pyemia** is a condition where pyogenic bacteria produce septicemia with multiple abscesses in the internal organs such as the spleen, liver and kidney.

Occurrence of a disease

To understand the full scope of a disease, we should know about its occurrence. Epidemiology involves in the study of the frequency and distribution of disease and other health related factors in defined populations. The incidence of a disease is the number of people in a population who develop a disease during a particular time period. The prevalence of a disease is the total number of existing cases with respect to the entire population.

Depending on the spread of infectious disease in the community, they may be classified into different types.

- Endemic diseases are those which are constantly present in a particular area. Typhoid fever is endemic in most parts of India.
- Epidemic disease is one that spreads rapidly, involving many persons in an area at the same time. Example: Epidemic of Dengue in 2017.
- A pandemic is an epidemic that spreads through many areas of the world involving very large numbers of persons within a short period. Example: H1N1 Influenza outbreak in 2009. Ebola outbreak in 2014-2016 in West Africa was the largest in history and first ever epidemic, affecting multiple countries.
- If a particular disease occurs only occasionally, it is called a sporadic disease. The most commonly occurring sporadic diseases in India are Diphtheria and Hepatitis A and E.

Severity or duration of a disease

Another useful way of defining the scope of a disease is in terms of its severity or duration.

- An **acute** disease is one that develops rapidly but lasts for a short time.
- A **chronic** disease develops more slowly, and the body's reactions may be less severe, but the disease is likely to be continual or recurrent for long periods.
- A disease that is intermediate between acute and chronic is described as a subacute disease.

Facts about Fever:

DO YOU KNOW?

Fever is as more healthful than harmful. An experiment with vertebrates shows that fever increases the rate of antibody synthesis. Increased temperatures stimulate the activities of T cells and increase the effectiveness of interferon. Fever appears to enhance phagocytosis. Fever almost never occurs as a single response; it is usually accompanied by chills. The explanation lies in the natural physiological interaction between the thermostat in the hypothalamus and the temperature of the blood. For example: If the thermostat has been set (by pyrogen) at 102°F but the blood temperature is 99°F, the muscles are stimulated to contract involuntary (shivering) as a means of producing heat. In addition, the vessels in the skin constrict, creating a sensation of cold and the piloerector muscles in the skin develops 'goose bumps'.

- A latent disease is one in which the causative agent remains inactive for a time but then becomes active to produce symptoms of the disease.

12.1.4 Interaction between Microbes and Host

Pathogen is a microorganism which causes disease.

Pathogenecity is the ability of a pathogen to produce disease.

Virulence is the degree of pathogenecity of a microorganism. Virulence is not generally attributable to a single property but depends on several parameters related to the organism, the host and their interaction.

Microorganisms first enter the body, survive, multiply and elaborate many factors and produce the disease.

Adhesion: The initial event in the pathogenesis of many infections is the attachment of the bacteria to body surfaces. Adhesions may occur as organized structures, such as fimbriae and pili. Adhesions serve as virulence factors.

Capsule: It is an envelope or slime layer surrounding the cell wall of certain microorganisms. Capsule plays important roles in immune evasion as it inhibit's phagocytosis, as well as protecting the bacteria while outside the host.

Toxins: Toxins are specific chemical products of microbes, plants and some animals that are poisonous to other organisms. Toxigenicty is the power to produce toxins.

A toxin is named according to specific target of action: Neurotoxin acts on the nervous system. Enterotoxin acts on the intestine, Haemotoxin lyses red blood cells, and Nephrotoxins damages the kidneys.

A toxin molecule secreted by a living bacterial cell into the infected tissue is an **exotoxin**. A toxin that is not actively secreted but is shed from the outer membrane is an **endotoxin**. the difference between exotoxin and endotoxin were given in Table 12.1.

Production of enzymes

Some enzymes like proteases, DNAases and phospholipases are produced and they help in destruction of the cell structure and to hydrolyse host tissues.

Antigenic variation

Microorganisms evade the host immune responses by changing their surface antigens. Antigenic drift and antigenic shift are common in influenza viruses. The distinction between the commensal and the organism associated with disease.

12.1.5 Diagnostic Cycle

Specific diagnosis is important for better patient care, use of appropriate antibiotics

Table 12.1: Differences between endotoxin and exotoxin

Exotoxins	Endotoxins
Heat labile proteins, secreted by certain species of bacteria and diffuse readily into the surrounding medium	Heat stable polysaccharide proteins, lipid complex which form an integral part of the cellwall of Gram negative bacteria
Proteins with a strong specificity to a target cell and extremely powerful sometimes deadly	A Lipopolysaccharide (LPS), which is part of the outermembrane of gram negative cell walls
Highly immunogenic	Less immunogenic
Toxoids can be made by treating toxins with formalin	Toxoids cannot be made
Produced mainly by Gram positive bacteria but also by some Gram negative bacteria	Produced by Gram negative bacteria