# Soil Microbiology
# Fourth stage


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**Soil Microbiology** is the branch of science/ microbiology concerned with soil-inhabiting microorganisms, their functions, and activities.

**Soil** is one of the world's most important natural resources, and together with air and water, it is the basis for life on planet earth. Soil can be defined as a thin layer of material covering the earth's surface and is formed from the interactions between five factors called **soil-forming factors,** including the land's topography, the organisms present in the environment, the climate, the parent material, and the time.

## Soil profile

A vertical cross-section of the soil, made of layers running parallel to the surface. These layers are known as **soil horizons.** Each layer of soil has distinct features. These horizons are represented by letters O, A, E, C, B and R as shown in figure-1**.**

**1. O horizon**

The O horizon is a surface horizon that is comprised of organic material at various stages of decomposition (minimal, moderately, highly and completely decomposed organic matter). It is most prominent in forested areas where there is the accumulation of debris fallen from trees. This horizon of soil is often black brown or dark brown, mainly because of organic content.

**2. A horizon (Topsoil)**

It is commonly referred to as the topsoil, it is the top layer for many grasslands and agriculture lands and typically the A horizon is made of sand, silt and clay with high amount of organic matter, which giving them a darker color than deeper layers.

**3.B horizon (Subsoil)**

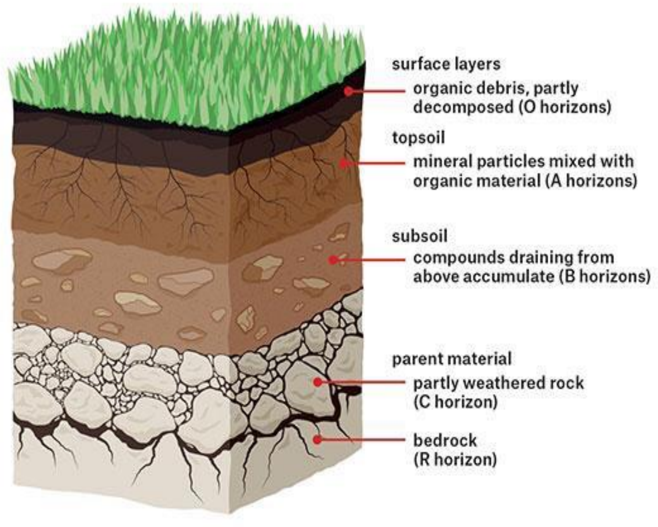
It is known as illuviation zone because of the accumulation of minerals such as iron, aluminum and carbonates. The accumulation of minerals in the lower horizons of soil from the upper horizons because of the downward movement of water is called illuviation. The B horizon has a lighter color than the A horizon.

**4.C horizon (Parent Soil)**

The C horizon is a layer of partially weathered or unweather rock. It contains a high concentration of parent material and its indicated by the absence of organic matter and microbial activities.

**3. R horizon (Rocky bed)**

It's a compacted and cemented layer, it consists of different types of rocks such as limestone, and granite.




**(Figure-1): Soil profile**

## Soil texture and soil structure

Soil texture and soil structure are both unique properties of the soil that have a profound effect on soil behaviour, such as water holding capacity, nutrient retention and supply, drainage, and nutrient leaching.

**Soil texture** is one of the most important physical properties of soil. Soil texture is the proportion of sand, silt, and clay particles that make up soil. These particles are distinguished solely by size as shown in table.1

**Table (1) Soil Texture Particles size**

| Type of Soil Particles | Size range/mm |
|---|---|
| Sand | 0.05 to 2.0 |
| Silt | 0.002 to 0.05 |
| Clay | less than 0.002 |

The texture of the soil is essential because it determines soil characteristics that affect plant growth, from these characteristics are water-holding capacity, permeability, and soil workability.

## Types of soil

### 1. Sandy Soil

It consists of small particles of weathered rock. Sandy soils are one of the poorest types of soil for growing plants because it has very low nutrients and poor water holding capacity, which makes it hard for the plant's roots to absorb water.

### 2. Clay Soil

It is mainly composed of the smallest particles of soil, which are tightly packed together with very little, or no airspace, and they effectively retain water. This soil is not suitable for growing plants as it is harder for moisture and air to penetrate the soil.

### 3.Silt Soil

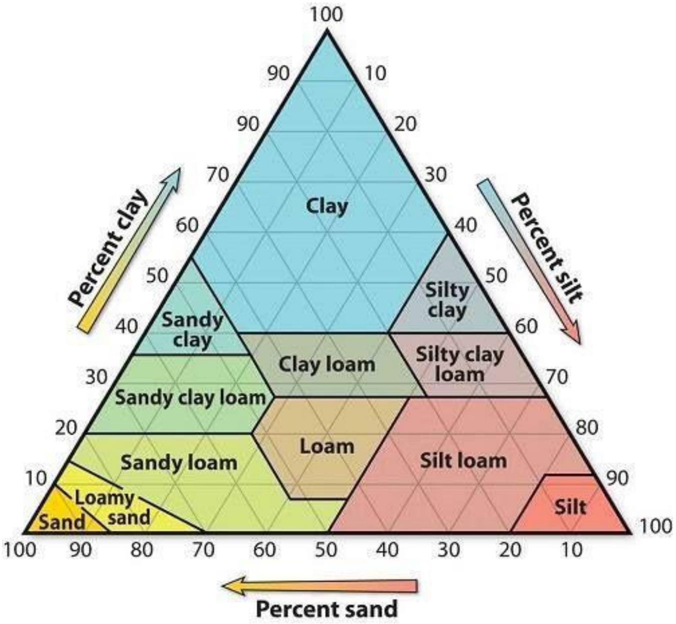
It has much smaller particles than sandy soil and larger than clay. It holds water better than sand. The silt soil is more fertile than the other two soil types. Therefore, it is also used in agricultural practices to improve soil fertility.

### 4.Loam Soil

It is a combination of sand, silt and clay such that the beneficial properties from each are included. It can retain moisture and nutrients; hence, it is more suitable for farming. This soil is also referred to as agricultural soil.

## Textural triangle

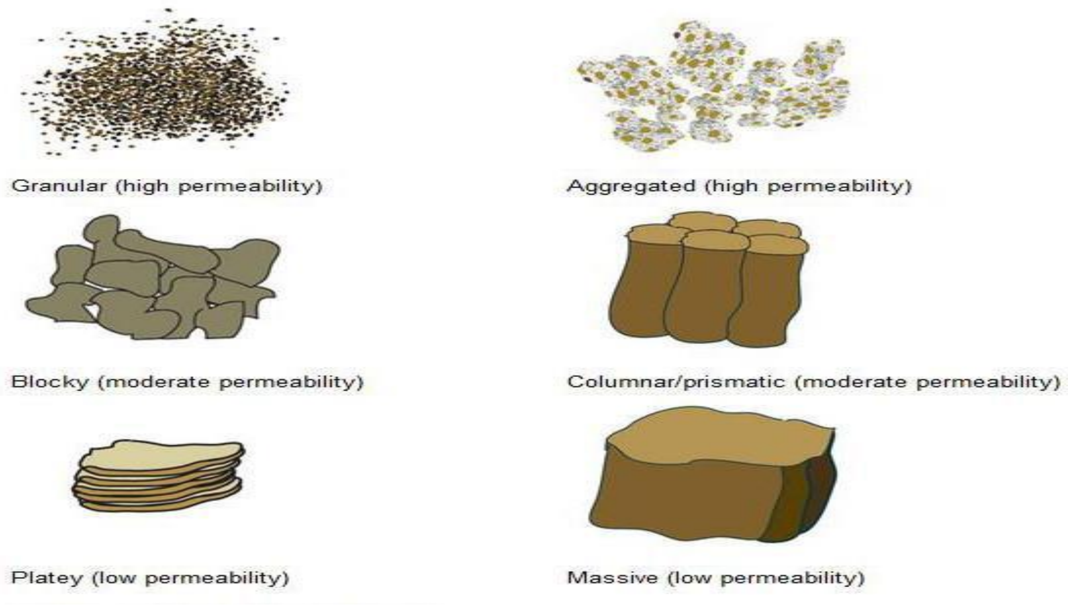
Soil textural class is a grouping of soils based upon the relative proportion of sand, clay and silt. There are different types of loams, based upon which soil separate is most abundantly present. the textural triangle **used to determine the soil type** as shown in figure-2**.**




**(Figure – 2) : Soil texture triangle**

**Soil structure**: is the arrangement of the soil particles into aggregates of various sizes and shapes. Aggregates that occur naturally in the soil are referred to as peds.

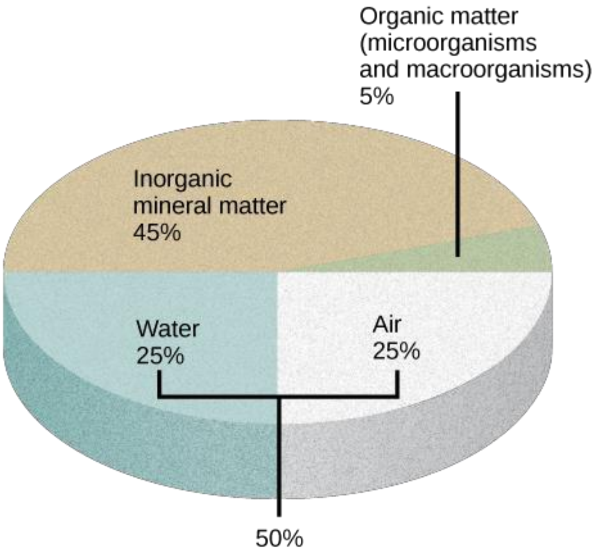
Good structure is important as it allows air movement through the soil. Soil air and water are vital for healthy plant growth and nutrient supply. The various types of soil structures are provided in figure-3




**(Figure – 3) :Types of soil structures**

## Soil composition

The basic components of soil are minerals, organic matter, water and air. The typical soil consists of approximately 45% mineral, 5% organic matter, 25% water, and 25 % air as shown in figure-4. Good healthy soil has sufficient air, water, minerals, and organic material to promote and sustain plant life.




**(Figure -4) :The four major components of soil**

### • Inorganic (minerals) materials

It is derived from parent rocks decomposed by weathering and biogeochemical factors into smaller particles that vary in size (sand, silt and clay). Inorganic compounds that are present in the soil contain various minerals , these minerals based on their chemical nature can divided into two groups:

### 1- Silicate minerals

Minerals are composed of silicate groups. They are the largest and most important class of minerals and makeup approximately 90 % of Earth's crust, and quartz is the most common silicate mineral.

### 2- Non – Silicate minerals

Minerals that are not composed of silicate groups. Makeup only 10% of Earth's crust such as copper, silver and gold.

- **Water and Air**

Soil particles pack loosely, forming a soil structure filled with pore spaces. These pores contain soil solution (water) and gas (air). Water and air in soil vary significantly with soil texture, weather, and plants uptake of water, but their percentage together in most soil types is about 50 % of total soil volume.

## Soil water

Comes from rain, snow, dew and irrigation. Soil water serves as a solvent and a carrier of nutrients for plant growth. The microorganisms inhabiting the soil also require water for their metabolic activities.

**The amount of water in the soil is dependent upon two factors**

1. Soil water is intimately related to the climate.
2. The amount of water in the soil depends upon how much water a soil may hold, the water holding capacity depends on capillary action and the size of the pores that exist between soil particles. Sandy soils have large particles and large pores. However, large pores do not have a great ability to hold water. As a result, sandy soils drain excessively. On the other hand, clay soils have small particles and small pores. Since small pores have a greater ability to hold water, clay soils tend to have high water holding capacity.

## Soil air

soil pores which not occupied with water are filled with air. Compared with atmospheric air, soil is lower in oxygen and higher in carbon dioxide because microorganisms continuously recycle CO2 during the decomposition processes of organic matter. $CO_2$ in soil air is 0.3% more than atmosphere air (0.03%). Soil aeration plays a vital role in plant growth, microbial population, and microbial activity in the soil. A good aerated soil types lead to complete oxidation of organic matter and is characterized by high redox potential capacity, which offers $e^-$ and $H^+$ donor and acceptors, resulting in thriving of aerobic and facultative microorganisms, but poor aerated soil types (saturated soils), which featured by low redox potential capacity cause continues release of $NO^{3-}$ and $SO_4^{2+}$ and accumulation of some harmfully intermediate like $CH_4$, that affected soil fertility and increase of anaerobic microorganisms population.

- **Organic matter**

Soil organic matter (SOM) is the fraction of the soil that consists of plant or animal tissue and microorganisms in different decomposition stages. Organic matter influences many of the physical, chemical and biological properties of the soil, including water holding capacity, cation exchange capacity, pH buffering capacity, chelating of micronutrients and improves soil structure by increasing aggregation and enhancing biological activities in the soil.

## Non–living organic matter can be divided into

1. **Non-humic Substances:** that are easy to decompose - like sugars, starches and proteins or compounds that take several years to decompose like cellulose and lignin.

2. **Humic Substances:** Successive decomposition of dead organic material over thousands of years results in forming of more complex and relatively stable substances, which are resistant to further decomposition commonly called humus which cannot be used by many microorganisms as an energy source and remains in the soil for a relatively long time. Humus affects soil properties, as humus is brown or black so it gives soils its dark color; increases soil aggregation and aggregate stability; increases water and nutrient retention, increases soil fertility and allows the plant's growth, about 35-55 percent of the non-living part of organic matter humus.

# • Soil Living organic matter (Soil Biota)

Soil biota consists of microorganisms (bacteria, fungi, actinomycetes and algae), soil animals (protozoa, nematodes, mites, springtails, spiders, insects, and earthworms) and plants. Microorganisms form a very small fraction of soil mass and occupy a volume of less than 1% in the upper layer of soil (topsoil up to 10 – 30 cm depth i.e., Horizon A). The microbial population is very high, which decreases with the depth of soil. Each organism or group of organisms is responsible for a specific change/ transformation in the soil. The final effect of various activities of microorganisms in the soil is to make the soil fit for the growth and development of higher plants.

**Living organisms present in the soil are grouped into two categories as follow:**

1. Soil flora (microflora) e.g. Bacteria, Fungi, Actinomycetes, and Algae.
2. Soil fauna animals include macrofauna (e.g., Earthworms), mesofauna (e.g., Mites), microfauna (e.g., protozoa, nematodes).

## Soil Microflora

### 1- Bacteria

These are unicellular organisms without organelles or nuclei, and they are one of the simplest forms of life. The size ranges from 1-5 microns, and the shape varies from cocci (round-shaped) to bacilli (rod-shaped) and spiral. They are probably the most numerous microbes whose population ranges from a few hundred to 3 billion per gram soil. They are very versatile in their metabolic activities. Some can use simple inorganic materials as an energy source, while others are heterotrophic.

**Based on their origin, soil bacteria are classified as:**

- Indigenous (true resident), or autochthonous.
- Invaders or allochthonous.

**Importance:**

1. Are very important in the general decomposition of organic matter in the soil.
2. They carry out specific functions in nutrient cycling, such as nitrification.
3. A group of bacteria is important in nitrogen fixation- converting atmospheric nitrogen to plant-available forms.
4. Biotransformation of chemicals and biogas formation.

**Examples of soil bacteria**: *Agrobacterium, Arthrobacter, Bacillus, Alcaligenes, Erwinia, Clostridium, Nitrosomonas, Nitrobacter, Rhizobium and Thiobacillus.*

## 2- Fungi

They have well-developed organelles, including nuclei and mitochondria. They are more developed than bacteria. The most important characteristic of fungi is possessing a filamentous body consisting of strands of hyphae, and they are about 5 µm in diameter. Almost all fungi are heterotrophic in nature, and all are aerobic; thus, they do not occur in a diverse environment like bacteria.

**Importance:**

- Production of fungi static products including antibiotics.
- Degrade some of the tough plant residues, like lignin.
- They play important role in soil aggregation and in the formation of humus.
- Maintain biological equilibrium in soil by their feeding on protozoa and nematodes.
- Some fungi form a symbiotic association with roots of higher plants (Mycorrhiza).
- Some soil fungi are parasitic and causes number of plant diseases such as wilts

**Example of soil fungi:** *Alternaria, Aspergillus, Cladosporium , Helimenthosporium, Humicola, Fusarium and Phytophthora*.

## 3- Actinomycetes

Structurally, these organisms lie between bacteria and fungi, they bear similarity to bacteria in terms of cell size and structure characteristics, and they are filamentous organisms like fungi. They are numerous and widely distributed in soil and are next to bacteria in abundance. They are sensitive to acidity / low PH (optimum PH range 6.5 to 8.0) and waterlogged soil conditions.

**Importance**

- They are decomposing all sorts of organic substances like cellulose, protein, and fats.
- Some actinomycetes produce antibiotics e.g., *Streptomyces sp.*
- They are responsible for subsequent decomposition of humus in soil.

**Example of actinomycetes are:** *Actinomyces, Streptomyces, Nocardia, Micromonospora, Actinoplans and Thermoactinomycetes.*

## 4- Algae

Algae are present in most of the soil where moisture and sunlight are available to perform the photosynthesis process. The prominent genera in soil are ***Anabaena, Nostoc, Calothrix*** **and** ***Oscillatoria.***

**Algae roles in the soil**

- Soil algae through photosynthesis process liberate large quantity of oxygen in the soil environment.
- Add organic matter to soil when die and thus increase the amount of organic carbon in soil.

**Rhizosphere**

The rhizosphere is the narrow soil region around the plant root that is influenced by several factors like the root exudates and the associated soil microorganisms. As plant roots grow through the soil, they release water-soluble compounds such as amino acids, sugars and organic acids that supply food for the microorganisms. The food supply means microbiological activity in the rhizosphere is much greater than in soil away from plant roots. In return, the microorganisms provide nutrients for the plants. All this activity makes the rhizosphere the most dynamic environment in the soil.

**The rhizosphere can be divided into three zones:**

1- The endorhizosphere is the internal plant tissues, including portions of the cortex and endodermis in which microbes can occupy the free space between cells

2- Rhizoplane is the medial zone directly adjacent to the root.

3- Exorhizosphere, which extends from the rhizoplane out into the soil.

**The rhizospheric M.O. have either beneficial or harmful effects on the development of plants.**

## Possible effects of rhizospheric M.O are:

- Some rhizospheric M.O. secretes plant regulators such as indole acetic acid, gibberellins and Cytokinins.

    - Rhizospheric M.O. promotes plant nutrition as bacteria like *Rhizobium* act as phosphate solubilizing bacteria increase the availability of accumulated phosphate, increasing the efficiency of biological nitrogen fixation and rendering availability of iron and zinc through the production of plant growth-promoting substances.

    - Rhizospheric M.O. protects plants against pathogens by control of pathogens (biocontrol) via synthesis of antibiotics or secondary metabolite-mediated induced systemic resistance.

    - The products of rhizospheric zone M.O. metabolism sometimes have toxic effects on plant development, and these are termed phytotoxins.

# Element cycles

Earth's biosphere can be thought of as a sealed container into which nothing new is ever added except the energy from the Sun. Since new matter can never be created, living things must reuse the existing matter again and again.

The six most common elements in organic molecules (carbon, nitrogen, hydrogen, oxygen, phosphorus, and sulfur) take a variety of chemical forms. They may be stored for long or short periods in the atmosphere, on land, in water, or beneath the Earth's surface, as well as in the bodies of living organisms. Geologic processes such as weathering of rocks, erosion and water drainage all play a role in this recycling of materials, as do interactions among organisms. The ways in which an element moves between its various living and non-living forms and locations is called **a biogeochemical cycle.**

The most important **biogeochemical cycles** are **carbon, nitrogen, oxygen, phosphorus, sulfur, and water.**

## Carbon cycle

The carbon cycle is one of several recycling processes, but it may be the most important since carbon is a basic building block of life. Carbon is the basis of carbohydrates, proteins, lipids, and nucleic acids, which form the basis of life on Earth. All the carbon we currently have on earth is the same amount we have always had. The carbon cycle is nature's way of reusing carbon atoms, which travel from the atmosphere into organisms in the earth and then back into the atmosphere over and over again. Carbon occurs in nature into two main states: complex carbonated organic compounds and inorganic carbon, most of the inorganic carbon is in form of $CO_2$. $CO_2$ makes up only about 0.04% of the atmospheric gases, on Earth most carbon is stored in rocks and sediments, while the rest is located in the ocean, atmosphere, and in living organisms. These are the reservoirs, through which carbon cycles. (Figure - 1)

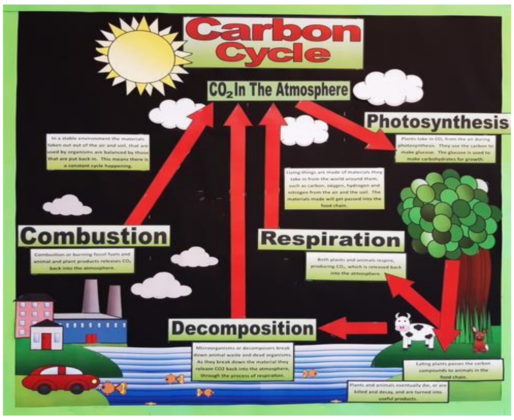



Figure -1: The carbon cycle.

## Steps of the Carbon Cycle

There are different steps by which carbon is moved from one form to another. The major of the Carbon Cycle is photosynthesis, respiration, decomposition and the combustion of fossil fuels.

1. **Photosynthesis**

Carbon exists in the atmosphere as carbon dioxide ($CO_2$). It first enters the ecological food web (the connected network of producers and consumers) when photosynthetic organisms (such as plants, algae, and a group of bacteria called cyanobacteria) absorb carbon dioxide, the plants then fix or capture the carbon dioxide and can convert it into simple sugars like glucose through the photosynthesis. Plants store and use this sugar to grow and reproduce. When plants are eaten by animals, their carbon is passed on to those animals. Since animals cannot make their food, they must get their carbon either directly by eating plants or indirectly by eating animals that have eaten plants.

2. **Respiration.**

Respiration is the next step in the cycle, it occurs in plants, animals, and even decomposers. The animal respiration is taking in oxygen (and releasing carbon dioxide) and oxidizing its food (or burning it with oxygen) to release the energy the food contains. So the carbon is returned to the atmosphere as carbon dioxide. Carbon atoms that started as components of carbon dioxide molecules have passed through the body of living organisms and been returned to the atmosphere, ready to be recycled again.

3. **Decomposition.**

Decomposition is the process by which dead organic substances are broken down into simpler organic or inorganic matter such as carbon dioxide, water, simple sugars and mineral salts. The organisms that decompose organic matter are called decomposers. Decomposers (fungi, bacteria, actinomycetes and invertebrates such as worms) are microorganisms that can feed on the rotting remains of plants and animals. It is their job to consume both waste products and dead matter. They also return carbon dioxide to the atmosphere by respiration.

The decomposition rate is affected by soil temperature, moisture, aeration and food availability.

Generally, decomposition is either aerobic or anaerobic.

- **Aerobic decomposition**

Aerobic decomposition is the process by which organic materials decompose in the presence of oxygen. Most heterotrophic microbes easily utilize aerobically soil organic compounds to get their energy and nutrients.

$$\text{Organic compound} + O_2 \xrightarrow{\text{Mineralization}} CO_2 + H_2O + \text{energy} + \text{inorganic nutrients}$$

$CO_2$ + $H_2O$ → **recycled again to atmosphere**

inorganic nutrients → **Immobilization** → **Assimilated by living organisms**

**Mineralization**: Is the process by which organic matter is decomposed to release simpler inorganic compounds (e.g., $CO_2$, $NH_4$, $SO_4$, Mg).

**Immobilization**: The nutrients that are converted into biomass become temporarily "tied up" from nutrient recycling until the organism dies. At this time, the **C** is released back into the environment via decomposition.

- **Anaerobic decomposition.**

The anaerobic decomposition ($O_2$ free) is a complex biochemical reaction carried out in a number of steps by several types of microorganisms. During the process, a gas mainly composed of methane and $CO_2$ is produced.

There are four basic steps of anaerobic decomposition. (Figure-2)

1) hydrolysis.

2) acidogenesis (Fermentation).

3) acetogenesis.

4) methanogenesis.

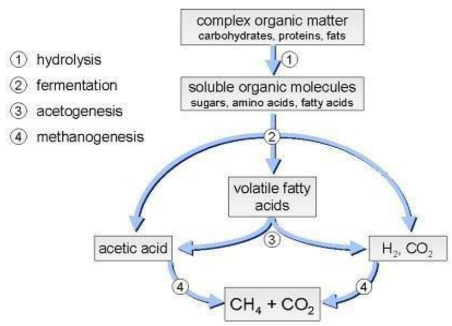



Figure-2: Anaerobic decomposition steps.

**Hydrolysis:** Complex organic matter (such as protein, carbohydrates) is decomposed into simple soluble organic molecules (such as amino acids, sugar) by water and enzymes. The enzymes are exoenzymes (protease, cellulase) from several bacteria and fungi.

**Acidogenesis (Fermentation):** During acidogenesis, soluble monomers are converted into small organic compounds, such as short chain (volatile) acids (propionic, formic, lactic, butyric, succinic acids), ketones (glycerol, acetone), and alcohols (ethanol, methanol).

**Acetogenesis:** The acidogenesis intermediates are attacked by acetogenic bacteria; the products from acetogenesis include acetic acid, $CO_2$, and $H_2$.

Several bacteria contribute to acetogenesis, including *Clostridium spp., peptococcus anaerobes, Lactobacillus,* and *Actinomyces.*

**Methanogenesis:** The last phase of anaerobic digestion is the methanogenesis phase. Several reactions take place using the intermediate products from the other phases, with the main product being methane. They were accomplished by certain soil bacteria known as **Methanogenic bacteria.** Methanogenic bacteria are a part of the carbon cycle; anaerobically, they convert the acetic acid made by acetogenic bacteria to $CH_4$, $CO_2$**,** convert alcohol (methanol or ethanol) to $CH_4$, or derive $CH_4$ from reduction $CO_2$.

$$\mathbf{2CH_3CH_2OH + CO_2 \rightarrow 2CH_3COOH + CH_4}$$

$$\mathbf{CH_3COOH \rightarrow CH_4 + CO_2}$$

$$\mathbf{CH_3OH \rightarrow CH_4 + H_2O}$$

$$\mathbf{CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O}$$

Main soil methanogenic bacteria are:

***Methanococcus, Methanobacteria and Methanosarcina.***

To complete the recycling pattern, another group of methane bacterium called **Methanotrophes** can reoxidize released **$CH_4$** again to **$CO_2$**, like ***Methylomonas*****.** This conversion also yields water and energy.

**4.Combustion.**

The process of burning fossil fuels, carbon in coal, oil and natural gas returns to the atmosphere as $CO_2$ when these fossil fuels are burned.;

Other soil autotrophic bacteria can participate in carbon cycling by oxidizing carbon monoxide CO. This gas is relatively rare under ordinary conditions, released from some activities, commonly from partial combustion. Extremely poisonous for most aerobic organisms, including man, is used as a source of energy and carbon by at least one bacterial species Carboxydomonas that oxidize CO to $CO_2$.

There is an increase of $CO_2$ in the atmosphere by about one–third, and it continues to rise. Like $CO_2$, methane concentration increases about 1% per year due to human activities (combustion of fossil fuel). The term greenhouse gases describe these gases' ability to trap heat within Earth's atmosphere, leading to an increased planet's temperature, a phenomenon known as the greenhouse effect. Soil microorganisms play a role in the generation of each of these gases.

## Nitrogen cycle

Nitrogen is a naturally occurring elements essential for growth and reproduction in both plants and animals because nitrogen is a component of proteins and nucleic acids essential to life on Earth. Although 78 % by volume of the atmosphere is nitrogen gas, this abundant reservoir exists in a form unusable by most organisms. Through a series of microbial transformations, however, nitrogen is made available to organisms, which in turn ultimately sustains all organism's life.

Nitrogen gas exists in nature in both organic and inorganic forms. The movement of **$N_2$** between the atmosphere, terrestrial and marine ecosystems in different forms is described by the **Nitrogen Cycle**, microorganisms are the key element in the cycle, providing different forms of nitrogen compounds by their metabolic activities.

In general, the nitrogen cycle has several steps: (Figure-1)

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

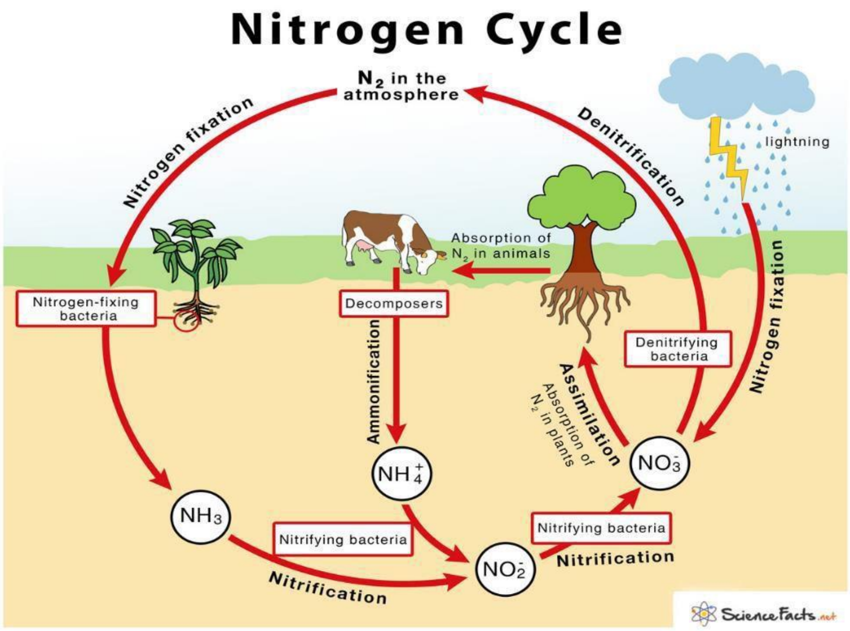



Figure-1: Nitrogen cycle steps.

**1. Nitrogen fixation**

Nitrogen fixation is a natural process. It occurs either biologically or non-biologically by which the ($N_2$) in the atmosphere is converted into ammonia or related nitrogenous compounds that plants can assimilate.

**Types of Nitrogen Fixation**

- **Atmospheric fixation:** A natural phenomenon, where the energy of lightning breaks the nitrogen molecules into nitrogen oxides and is then used by plants.

- **Industrial nitrogen fixation:** This is a man-made alternative that aids in nitrogen fixation using ammonia. Atmospheric nitrogen and hydrogen (usually derived from natural gas or petroleum) can be combined to form ammonia ($NH_3$), which is converted into various fertilizers such as urea.

- **Biological nitrogen fixation (BNF):** Most nitrogen fixation occurs naturally in the soil by microorganisms called diazotrophs that are diverse groups of prokaryotes, including free-living and symbiotic bacteria, cyanobacteria and actinomycetes. Common nitrogen fixing microorganism is given in table (1).

**Table -1: $N_2$ fixing microorganisms**

| **Nitrogen-fixing microorganisms** | | | | | |
|---|---|---|---|---|---|
| **Free-living M.O.** | | | | **Symbiotic M.O.** | |
| Aerobic | Anaerobic | Facultative Anaerobic | Photosynthetic | Legumes | *Non-legumes* |
| *Azotobacter, Azomonas* and *Beijerinckia* | *Clostridium pasteurianum* and *Desulfovibrio* | *Aerobacter* and *Klebsiella* | *Rhodomicrobium* and *Rhodopseudomonas* | *Rhizobium* | *Frankia* |

The biological conversion of atmospheric nitrogen takes place with the help of an enzyme called nitrogenase, which combines gaseous nitrogen with hydrogen to produce ammonia, which is then further converted by bacteria to make their organic compounds. Nitrogenase is a metalloprotein that consists of two proteins – an iron protein and a molybdenum-iron protein but sometimes contains vanadium instead of molybdenum.

The nitrogenase enzyme is highly sensitive to oxygen. It is inactivated when exposed to oxygen. Although this is not a problem for anaerobic bacteria, it could be a major problem for the aerobic species, but These organisms have various methods to overcome the problem. For example, *Azotobacter* species have the highest known rate of respiratory metabolism of any organism, so they might protect the enzyme by maintaining a very low oxygen level in their cells.

The fixation process requires such a large amount of energy (ATP), and it needs about 16 moles of adenosine triphosphate (ATP) to reduce each mole of nitrogen. They obtain this energy by the oxidation of organic molecules. Free-living microorganisms, which are non-photosynthetic in nature, receive these molecules from other organisms, while photosynthetic microorganisms, such as cyanobacteria, use sugars synthesized in the process of photosynthesis. Symbiotic nitrogen-fixing microorganisms obtain these compounds from their host plants.

**Symbiotic $N_2$ fixation microorganisms**

*Rhizobium* is the most well-known species of bacteria that acts as the primary symbiotic fixer of nitrogen. These bacteria can infect the roots of leguminous plants, leading to the formation of nodules where the nitrogen fixation takes place. The bacterium's enzyme system supplies a constant source of reduced nitrogen to the host plant, and the plant provides nutrients and energy for the activities of the bacterium. In the soil, the bacteria are free-living and motile, feeding on the remains of dead organisms. Free-living rhizobia cannot fix nitrogen, and they have a different shape from the bacteria found in root nodules. They are regular in structure, appearing as straight rods; in root nodules, the nitrogen-fixing form exists as irregular cells called bacteroids, often club and Y-shaped.

**Root nodule formation**

The actual process of nodulation is a very coordinated effort between legume and the *Rhizobium* in soil. One *Rhizobium* strain can infect certain species of legumes, but not others. Specificity genes determine which *Rhizobium* strain infects which legume.

The initial interaction between the host plant and free-living rhizobia is the release of a variety of chemicals by the root cells into the soil, such as **flavonoids**, rhizobia which colonize the soil in the near of the root hair will respond to the flavonoids, so the bacteria attracted to the root hair and attach themselves to the root hair surface and secrete specific nod factor, these stimulate the hair to curl. Rhizobia then invade the root through the hair tip, which induces the formation of an **infection thread**.

*Rhizobium* multiplies within the infection thread, which grows through the root hair cells and penetrates through several layers of cortical cell and then rupture, the bacteria released from the infection thread into the cytoplasm of the host cell and after the bacteria undergo alteration morphologically into larger forms called **Bacteroids**, cortex cells in turn rapidly divided to form a tumor like nodules of Bacteroids – packed cells, each nodule contains thousands of bacteroids. The nodules begin to fix nitrogen by the nitrogenase that catalyzes the conversion of atmospheric nitrogen to ammonia.

The nitrogenase complex is sensitive to oxygen, becoming inactivated when exposed. *Rhizobium* controls oxygen levels in the nodule with **leghaemoglobin**. This red, iron-containing protein has a similar function to haemoglobin, binding to oxygen. This provides sufficient oxygen for the metabolic functions of the bacteroids but prevents the accumulation of free oxygen that would destroy nitrogenase activity.

**Free-living $N_2$ fixation microorganisms**

Many heterotrophic or autotrophic microorganisms live in the soil and fix nitrogen without direct interaction with other organisms. The heterotrophic M.O. must find their own source of energy to fix $N_2$, typically by oxidizing organic molecules released by other organisms or from decomposition. Some free-living organisms have chemolithotrophic capabilities and can thereby utilize inorganic compounds as a source of energy. *Azotobacter* is the best example of free-living aerobic nitrogen fixers.

**2. Ammonification**

Before we know what ammonification is, we first must understand proteolysis. Plants use the ammonia produced by symbiotic or non-symbiotic $N_2$ fixation microorganisms to make nitrogen-containing compounds (such as proteins). The animals eat the plants, and the plant protein is converted into animals' protein. Upon death, plants and animals undergo microbial decay in the soil, and the nitrogen is released. Thus, the process of enzymatic break down of protein by the M.O. with the help of proteolysis enzyme is known as proteolysis.

$$\text{Protein} \xrightarrow{\textbf{Proteases}} \text{peptides} \xrightarrow{\textbf{peptidases}} \text{amino acids}$$

The most active microorganisms responsible for proteolysis are ***Pseudomonas, Bacillus, Proteus, Penicillium*** **and** ***Alternaria.***

Amino acids released during proteolysis undergo deamination in which amino group ($NH_2$) is removed. Thus, the process of deamination, which leads to the production of ammonia or ammonium ($NH_4^+$) is known as **ammonification.** Ammonification usually occurs under aerobic conditions (known as oxidative deamination), it is represented as follows:

$$\underset{\textbf{Amino acid}}{RCHNH_2COOH} + O_2 \xrightarrow{\textbf{deaminase}} \underset{\textbf{Keto acid}}{RCOCOOH} + \underset{\textbf{Ammonia}}{NH_3} + \text{energy}$$

Ammonification is commonly brought about by ***Proteus, Micrococcus*** **and** ***Clostridium.***

**3.Nitrification**

In soil, the liberated ammonia during ammonification is rapidly oxidized to nitrate by some soil highly specialized bacterial groups of strictly aerobic chemolithotrophs, this oxidation process is termed nitrification, which occurs in two stages, and each stage is performed by different bacteria as follows:

**Stage 1**: Oxidation of ammonia ($NH_3$) to nitrite ($NO_2^-$) and this process is known as **Nitrosofication**

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$$2\ NH_3 + 3O_2 \longrightarrow 2\ NO_2^- + 4H^+ + 2\ H_2O + \text{energy}$$

This stage is performed by soil bacteria such as ***Nitrosomonas, Nitrosococcus* and *Nitrosovibrio***.

**Stage 2:** In this stage, nitrite is oxidized to nitrate by nitrite- oxidizing - bacteria such as *Nitrobacter, Nitrococcus* and *Nitrospira* and several fungi such as *Penicillium* and *Aspergillus.*

$$2NO_2^- + O_2 \longrightarrow 2\ NO_3 + \text{energy}$$

Plants utilize the formed nitrate as their primary source of $N_2$ nutrition. Nitrate that is not used for plant nutrition is susceptible to reduce to ammonia or nitrogen gas or lost through leaching depending on soil conditions.

**Factors affecting nitrification**

- **Soil pH:** Nitrification occurs over a wide pH range in soil. The optimal pH has been estimated to be between pH 6.5 to 8.8, and Nitrification rates are slower in acid soils.
- **Soil Temperature:** The optimal temperature for nitrification ranges from 30° C – 35°C.
- **Soil aeration:** Since nitrification is an aerobic process, oxygen is necessary because it affects the M.O involved in the process.
- **Soil Moisture**: Nitrifying bacteria are sensitive to changes in soil water content since it influences the abundance and activity of bacteria.

**3. Denitrificatin**

Several heterotrophic bacteria can convert nitrate to nitrite and ammonia. This is a reverse process of nitrification known as **denitrification.** Nitrate reduction normally occurs under anaerobic soil conditions (waterlogged soil).

Nitrate reduction leading to production of ammonia is called dissimilatory nitrate reduction as some of the microorganisms assimilate ammonia for synthesis of amino acids and proteins.

Denitrification consists of four steps, and each step is carried out by a reductase enzyme. Nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase are the four main enzymes involved in denitrification, the overall process of denitrification as follows:

$$NO_3 \xrightarrow{\text{Nitrate reductase}} NO_2 \xrightarrow{\text{Nitrite reductase}} NO \xrightarrow[\text{reductase}]{\text{Nitric oxide}} N_2O \text{ Nitrous oxide}$$

Nitrate Nitrite Nitric oxide

$$N_2O \xrightarrow{\text{Nitrous oxide reductase}} N_2$$

The most important denitrifying bacteria are ***Thiobacillus denitrificans, Micrococcus denitrificans, Pseudomonas*** **and** ***Achromobacter.***

Denitrification leads to the loss of nitrogen from the soil, which results in the depletion of an essential nutrient for plant growth; therefore, it is an undesirable process for soil fertility and agricultural productivity, but it has major ecological importance since without denitrification, the supply of nitrogen, including $N_2$ of the atmosphere, would not have got depleted, and $NO_3$ (which are toxic) would have accumulated in the soil and water.

## Sulfur cycle

Sulfur, an essential element for the macromolecules of living things, is one of the components that make up proteins and vitamins. Proteins consist of amino acids that contain sulfur atoms, and sulfur is important for functioning proteins and enzymes necessary for plants and animals.

Most of the earth's sulfur is tied up in rocks and salts or buried deep in the ocean in oceanic sediments. Sulfur occurs in nature and the soil as organic forms such as proteins and inorganic forms such as sulfate ($SO_4^{2-}$); these elements undergo alteration between organic and inorganic forms and between oxidative and reductive states.

The sulfur cycle is a biogeochemical cycle in which the sulfur moves between rocks, waterways and living systems.

The weathering of rocks releases the sulfur; once sulfur is exposed to the air, it combines with oxygen and becomes sulfate ($SO_4^{2-}$). Plants and microbes assimilate sulfate and convert it into organic forms. As animals consume plants, the sulfur is moved through the food chain and released when animals and plants die and decompose.

Sulfur is also released into the atmosphere by burning fossil fuels and volcanic activities such as $SO_2$ and hydrogen sulfide ($H_2S$) gas. The $SO_2$ and $H_2S$ in the atmosphere return to the surface in rainwater/soil as sulfate or sulfuric acid along with rain.

**The Role of Microorganisms in the Sulfur Cycle**

Microorganisms play an important role in the sulfur cycle as these organisms have specialized enzyme systems and mechanisms to form different sulfur compounds.

Sulfur is microbiologically metabolized in the soil through different transformation processes:

- Mineralization (decomposition) of organic sulfur into inorganic forms, such as hydrogen sulfide ($H_2S$) and elemental sulfur.
- Oxidation of hydrogen sulfide, elemental sulfur ($S^{\circ}$) and related compounds to sulfate.
- Reduction of sulfate to hydrogen sulfide ($H_2S$).
- Microbial immobilization of the sulfur compounds and subsequent incorporation into the organic form of sulfur.

- **Mineralization (decomposition) of organic sulfur compounds**

As given below, the decomposition of large organic sulfur compounds to smaller units and finally into inorganic compounds by the microorganisms.


Cysteine

- **Oxidation of inorganic sulfur compounds**

Hydrogen sulfide (produced from sulfur-containing amino acids) oxidizes to produce elemental sulfur, which is carried out by photosynthetic bacteria from green sulfur bacteria such as ***Chlorobium*** and purple sulfur bacteria such as ***Chromatium.***

$$CO_2 + 2H_2S \xrightarrow{\text{Light}} \underset{\text{Carbohydrate}}{(CH_2O)\,n} + H_2O + \underset{\text{Elemental sulfur}}{2S^{\circ}}$$

Elemental sulfur present in the soil cannot be utilized directly by the plants. Therefore, it is converted into sulfates by chemolithotrophic bacteria such as ***Thiobacillus ferrooxidans*** and ***Thiobacillus thiooxidans*** are the main organisms involved in the oxidation elemental sulfur to sulfates.

$$2S^{\circ} + 3O_2 + 2H_2O \longrightarrow 4H^{+} + 2\,SO_4^{2-}$$

Sulfur oxidation improves soil fertility; it results in the formation of sulfate, which plants can use. The acidity produced in sulfur oxidation helps to solubilize plants nutrients and lowers the pH of alkaline soils.

- **Reduction of Sulfates**

Sulfate produced by sulfur-oxidizing bacteria may be reduced to hydrogen sulfide by sulfur–reducing bacteria. Sulfate-reducing occurs under anaerobic soil conditions (waterlogged soil). Sulfate reduction can be dissimilatory or assimilatory. In assimilatory sulfate reduction, the sulfate is reduced for the biosynthesis of amino acids and proteins. Sulfate reduction is dissimilatory sulfate reduction if the purpose of reducing the sulfate is to produce energy, in which sulfate in the absence of $O_2$ serves as terminal electron acceptors for anaerobic respiration.

The predominant sulfate-reducing bacteria in soil are ***Desulfovibrio desulfuricans*** and ***Desulfotomaculum***.

Hydrogen sulfide produced by reducing sulfate is further oxidized by some species of green and purple phototrophic bacteria (***Chlorobium*** and ***Chromatium***) to release elemental sulfur.

Sulfate reduction may decrease the availability of sulfur for plant nutrition; the dissimilatory sulfate reduction is not at all desirable from soil fertility and agricultural productivity.

- **Immobilization of inorganic sulfate compounds**

In this process, microorganisms absorb inorganic sulfate and convert it into organic form to synthesise microbial tissue.

## Phosphorus Cycle

Phosphorus is an important element for all living organisms. It forms a significant part of the structural framework of DNA and RNA, and they are also an important component of the energy storage molecule ATP. Humans contain 80% of phosphorus in teeth and bones.

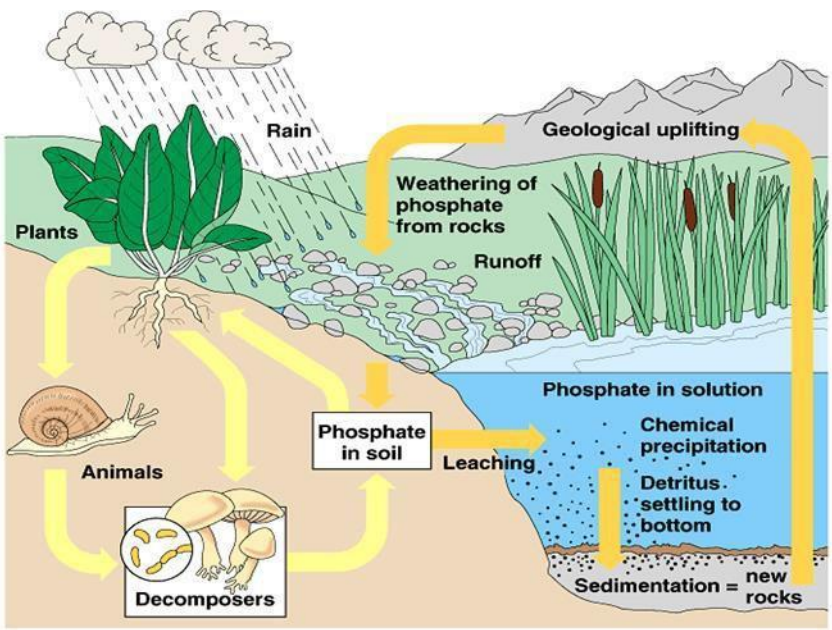
Phosphorus on the Earth is derived from rock, which is the major reservoir of phosphorus in nature. Phosphorus can be found on earth in water, soil and sediments.

The phosphorus cycle is how phosphorus moves through the lithosphere, hydrosphere, and biosphere.

Since phosphorus and phosphorus-containing compounds are present only on land, the atmosphere plays no significant role in the phosphorus cycle.

Since the main source of phosphorus is found in rocks, the first step of the phosphorus cycle involves the extraction of phosphorus from the rocks by weathering. Weather events, such as rain and other erosion sources, result in phosphorus being distributed in soils and water; once in the soil, plants take up inorganic phosphate from the soil. Animals may then consume the plants. When the plants and animals die, they are decomposed by microorganisms; during this process, the organic form of phosphorus is converted into the inorganic form, which is recycled to soil and water.

Phosphorus in the soil can end up in waterways and eventually oceans. Once there, it can be incorporated into sediments over time. As shown in figure-1




(Figure-1): Phosphorus cycle

Microorganisms are known to play a key role in transformations of phosphorus; these include:

- **Solubilization of inorganic phosphates compounds**

Phosphorus (P) is one of the essential elements necessary for plant development and growth. On average, the phosphorus content of the soil is about 0.05% (w/w), but only 0.1% of this phosphorus is available for plant use because most phosphorus complexes with calcium, iron and aluminium, thus generating insoluble phosphate salts present in the soil.

Fortunately, many microbial organisms, including bacteria, fungi, actinomycetes and algae can solubilize unavailable phosphate (P) to available forms ($H_2PO_4^-$ or $HPO^{2-}{}_4$), such transformations increase P availability and promote plant growth.

Through various solubilising mechanisms, phosphate Solubilizing Microorganisms (PSM) can transform organic and inorganic insoluble phosphorus compounds to soluble P form that plants can easily assimilate.

The main P solubilization mechanisms employed by PSM include releasing organic or inorganic acids as a side product of microorganism's activities. These acids act on converting insoluble salts to be more available to plants.

Organic and inorganic acids produced by PSM dissolve the insoluble soil phosphates by chelation of the cations bound to phosphate, thereby converting them into soluble forms and are called chelates.

$$Ca_3 (PO_4)_2 + 4HNO_3 \longrightarrow Ca (H_2PO_4)_2 + 2Ca (NO_3)_2$$

The other mechanism of solubilizing soil P is mineralization. PSM mineralize soil organic P by the production of phosphatases and phytase.

The organisms involved in phosphate solubilization include ***Pseudomonas, Bacillus***, ***Rhizobium, Streptomyces, Penicillium*** and ***Aspergillus***.

- **Mineralization of organic phosphates compounds**

Organic phosphorus compounds (e.g., nucleic acids or phospholipids) are mineralized (decomposed) to inorganic phosphate ($H_2PO_4^-$ or $HPO_4^{2-}$) forms of plant-available Phosphate known as orthophosphates by a wide range of microorganisms that include ***Bacillus, Arthrobacter, Streptomyces, Aspergillus*** and ***Penicillium***.

Phosphatases and phytases are the enzymes responsible for the degradation of phosphorus compounds.

Mineralization is highly influenced by soil moisture, temperature, pH and availability of organic phosphate.

- **Immobilization of phosphorus**

During immobilization, microorganisms convert inorganic forms to organic phosphate, which are then incorporated into their living cells.

# Biodegradation and bioremediation of environmental pollutants

Environmental pollutants are compounds that are toxic to living organisms; they are released into the ecosystem at high concentrations, usually as a consequence of human activities and that are hazardous to health. Contaminants are either compounds of industrial origin such as in chemical and pharmaceutical industries or natural compounds like hydrocarbons present in fossil fuels, Therefore the main successful strategy to fight pollution is the use of the abilities of living organisms (Biodegradation and bioremediation) as they are catabolically versatile and are capable of degrading or transforming a huge range of the toxic compounds into non-toxic ones.

## Biodegradation

**Biodegradation** is the process by which organic substances are broken down into smaller compounds by living microbial organisms. When biodegradation is complete, the process is called **mineralization**. Microbial organisms transform the substance through metabolic or enzymatic processes. It is based on two processes: **Growth** and **cometabolism**. In growth, an organic pollutant is used as a sole source of carbon and energy. This process results in a complete degradation (mineralization) of organic pollutants. Cometabolism is defined as the metabolism of an organic compound in the presence of a growth substrate that is used as the primary carbon and energy source. Several microorganisms, including fungi and bacteria are involved in the biodegradation process. microorganisms have the astonishing, naturally occurring, microbial catabolic diversity to degrade, transform or accumulate a huge range of compounds including hydrocarbons (e.g. oil), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), radionuclides and metals . Many factors influence microorganisms to use pollutants as substrates or cometabolize them, like, the genetic potential and certain environmental factors such as temperature, pH, and available nitrogen and phosphorus sources, which determine also the rate of degradation. Biodegradation processes vary greatly, but frequently the final product of the degradation is carbon dioxide. Organic material can be degraded aerobically, with oxygen, or anaerobically, without oxygen.

**There are 3 types of biodegradation**

**1.Biodeterioration:** Is the physical, mechanical, and chemical changes in the properties of the materials by the biological activities of microorganisms.

**2.Biofragmentation:** Is biologically breaking down the polymer chain into monomers and oligomers with the help of microorganisms.

**3. Assimilation:** It begins when the microorganisms are provided with nutrients, carbon, and energy sources from the resulting products from biofragmentation and are then transformed into biomass, water, and $CO_2$.

**Bioremediation**

Bioremediation is a treatment technique that uses living organisms to remove or neutralize an environmental pollutant from soil, water, and other environments. Bioremediation relies on stimulating the growth of certain microbes that utilize contaminants like oil, solvents, and pesticides for sources of food and energy. These microbes convert contaminants into water, as well as harmless gases like carbon dioxide. If eliminating or detoxifying hazardous pollutants into less or nontoxic forms is done by indigenous microorganisms without human intervention is known as **intrinsic bioremediation** , conversely, **extrinsic bioremediation** takes a more proactive stance by augmenting natural bioremediation processes, which is achieved through introducing external agents or altering environmental conditions to expedite the degradation of pollutants The effectiveness of bioremediation depends on many factors; including microbial population, types of contaminants, and physical factors such as pH, temperature, kind of soil, presence of oxygen, etc.

**Types of bioremediation based on strategies applied**

- **In-situ bioremediation** involves treating the contaminated material at the contamination site.
- **ex -situ bioremediation** involves the removal of the contaminated material from the original site to another place to be treated elsewhere.

**According to the microorganisms used, bioremediation is classified into three types**

- **Bacterioremediation** is a type of remediation, where bacteria are bioremediants and can treat various kinds of pollutants through in-situ or ex-situ techniques.
- **Phytoremediation** utilizes several plants to stabilize, remediate, reduce, or restore contaminants in sediments, soil, water, etc.
- **Mycoremediation** is a form of bioremediation in which fungi are used to degrade or isolate contaminants in soil.

**Based on the principle of degradation, bioremediation is of two types**

- **Biotransformation:** Is the chemical modification of organic compounds by microorganisms resulting in the loss or alteration of some characteristics and properties of the original compounds.
- **Biomineralization:** Is another type of bioremediation where microorganisms digest and convert organic waste nutrients into inorganic materials like water and carbon dioxide.

**Examples of Bioremediation**

- **Biostimulation**: involves the modification of the environment to stimulate indigenous microorganisms capable of bioremediation. This can be done by the addition of various forms of limiting nutrients and electron acceptors, such as phosphorus, nitrogen, oxygen, or carbon in soil.

- **Bioventing**: Is a process of aerating soils to promote bioremediation by stimulating the biological activity of the indigenous microbial population.
- **Bioaugmentation**: Is the introduction of exogenous microorganisms into a contaminated environment to enhance bioremediation of pollutants.
- **Composting**: Is a natural process of recycling decomposed solid organic wastes into a valuable fertilizer known as compost that can enrich soil and plants.

**Some biodegradable pollutants**

- **Pesticides**

Pesticides are chemical compounds that are used to kill pests, including insects, rodents, fungi and unwanted plants (weeds). Over 1000 different pesticides are used around the world. Pesticides are used in public health to kill vectors of disease, such as mosquitoes, and in agriculture to kill pests that damage crops. Pesticides are potentially toxic to other organisms, including humans, and need to be used safely and disposed of properly. They are among the leading causes of death by self-poisoning.

**Effect of pesticides**

Some of the most important effects caused by pesticides are:

1. Alterations of the ecological balance of the soil microflora.

2. Inhibition of $N_2$ fixing soil bacteria such as *Rhizobium and Azotobacter* and cellulolytic and phosphate solubilizing microorganisms and Suppression of nitrifying bacteria, *Nitrosomonas* and *Nitrobacter* (alter biogeochemical cycles).

3. Adverse effect on soil fertility and crop productivity.

Pesticides that are rapidly degraded are called nonpersistent while those which resist degradation are termed persistent. The effective persistence of pesticides in the soil varies from a week to several years depending upon the structure and properties of the constituents in the pesticide. Many of the older, cheaper pesticides, such as dichlorodiphenyltrichloroethane (DDT) and lindane, can remain for years in soil and water. These have adverse effects on larger parts of the ecosystem and can accumulate in the food chain. These chemicals have been banned by countries that signed the 2001 Stockholm Convention.

**Biodegradation of pesticides in soil**

Many soil microorganisms such as *Pseudomonas*, *Flavobacterium*, *Aspergillus* sp. and *Trichoderma* sp. can act upon pesticides and convert them into simpler non-toxic compounds. Biodegradation of pesticides is greatly influenced by soil factors like moisture, temperature, pH and organic matter content, in addition to microbial population and pesticide structure, most of the pesticides degrade within a short period under optimal conditions.

- **Hydrocarbons pollutants (oil or petroleum)**

Petroleum is a liquid mixture consisting of complex hydrocarbons. It is the main source of energy for industry and daily life. The main cause of water and soil pollution is the release of hydrocarbons into the environment whether accidentally or due to human activities.

**Effects of petroleum**

Petroleum is a serious global environmental problem that has several detrimental effects on the environment, including the health of people, plants, and animals, as well as the marine environment. The biological systems of the human body are harmed by petroleum pollution, including the hematologic, renal, liver, and hepatic as well as the respiratory and neurological. Furthermore, petroleum can profoundly influence marine animals' behaviour and development.

**Biodegradation of petroleum**

Microbial degradation is one of the most promising mechanisms for removing petroleum hydrocarbon pollutants from the environment, which consume hydrocarbons as a carbon source, carbon dioxide and water are released as by-products. Bacteria and fungi are the key agents of petroleum degradation (also known as **hydrocarbonoclastic microorganisms**), bacteria are considered primary degraders and the most active agents in petroleum pollutant degradation. Fungi are more successful degraders than bacteria for complex hydrocarbons. Bacterial hydrocarbon degraders include species of genera *Achromobacter*, *Acinetobacter*, *Arthrobacter*, *Brevibacterium*, *Cellulomonas*, *Corynebacterium*, *Flavobacterium*, *Marinobacter*, *Alcanivorax borkumensis*, *Micrococcus*, *Nocardia*, *Pseudomonas*, and *Vibrio*. Fungi degraders include those of genera *Aspergillus*, *Fusarium*, *Graphium*, *Neosartoria*, *Paecilomyces*, *Penicillium*, *Sporobolomyces*, and *Talaromyces*, and yeast of genera *Candida*, *Pichia*, *Rhodotorula*, and *Yarrowia.*

Hydrocarbonoclastic microorganisms can degrade oil to water - soluble compounds and eventually to carbon dioxide and water because they have the genetic instructions to produce oil-degrading enzymes. The tens of thousands of different compounds that make up oil can only be biodegraded by communities of microorganisms acting in cooperation, the combined action of the community can degrade almost all components of oil.

The main factors affecting the efficiency of biodegradation are the physical and chemical characteristics of the oil, the temperature, and pH of the water, the levels of nutrients and the level of oxygen present in the water.