B. Sc. III SEMESTER V

5S MICROBIOLOGY

(Environmental Microbiology and Bioinstrumentation)

Unit II: Microbiology of Soil

a) Microorganisms in soil.

b) Rhizosphere.

c) Decomposition of plant and animal residues in soil.

d) Definition, formation, function and microbiology of humus and compost.

e) Biological Nitrogen fixation: Type of nitrogen fixing microorganisms, factors affecting and mechanism of symbiotic and non-symbiotic nitrogen fixation. Process of nodulation, nitrogenase complex, recombinant DNA and nitrogen fixation, legume inoculants.

f) Cycles of elements in nature:

i) Carbon cycle : CO_2 fixation, organic carbon degradation.

ii) Nitrogen cycle : Proteolysis, amino acid degradation, Nitrification, Denitrification, Degradation of nucleic acids.

iii) Sulphur cycle

iv) Phosphorus cycle.

v) Biofertilizers, biological pest control.

Soil Microbiology

Definition:

- It is branch of science/microbiology which deals with study of soil microorganisms and their activities in the soil.
- **Soil**:
- It is the outer, loose material of earth's surface which is distinctly different from the underlying bedrock.
- Agriculturally, soil is the region which supports the plant life by providing mechanical support and nutrients required for growth.
- From the microbiologist view point, soil is one of the most dynamic sites of biological interactions in the nature.
- It is the region where most of the physical, biological and biochemical reactions related to decomposition of organic weathering of parent rock take place.

Components of Soil:

- Soil is an admixture of five major components viz. organic matter, mineral matter, soil gases, soil water and soil microorganisms. The amount of these components varies with locality and climate.

1. **Mineral / Inorganic Matter**: It is derived from parent rocks/bed rocks through decomposition, disintegration and weathering process. Different types of inorganic compounds containing various minerals are present in soil. Amongst them the dominant minerals are Silicon, Aluminum and iron and others like Carbon, Calcium Potassium, Manganese, Sodium, Sulphur, Phosphorus etc. are in trace amount.
2. **Organic matter:** Derived from organic residues of plants and animals added in the soil. Organic matter serves not only as a source of food for microorganisms but also supplies energy for the vital processes of metabolism. Organic matter in the soil is the potential source of N, P and S for plant growth. The proportion of organic matter in the soil ranges from 3-6% of the total volume of soil.
3. **Soil Water**: The amount of water present in soil varies considerably. Soil water comes from rain, snow, dew or irrigation. Soil water serves as a solvent and carrier of nutrients for the plant growth. The microorganisms present in the soil also require water for their metabolic activities. Soil water thus, indirectly affects plant growth. Percentage of soil-water is 25% total volume of soil.
4. **Soil air (Soil gases):** Soil is lower in oxygen and higher in carbon dioxide, because CO_2 is continuous recycled by the microorganisms during the process of decomposition

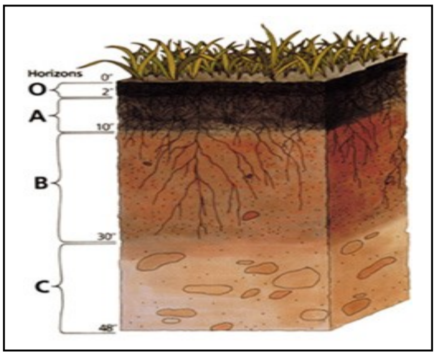
of organic matter. Soil air comes from external atmosphere and contains nitrogen, oxygen, CO_2 and water vapour. Soil aeration plays important role in plant growth, microbial population, and microbial activities in the soil.

5. **Soil microorganisms:** Soil is an excellent culture media for the growth and development of various microorganisms. Soil is not an inert static material but a medium pulsating with life. Soil is now believed to be dynamic or living system.

SOIL PROFILE

- Soil consist of a series of layers different from one another in colour, texture, composition, etc. Each layer is called a horizon.
- Soil Profile refers to the layers of soil. Soil has three main horizons (A, B, and C).
- **O Horizon** - The top, organic layer of soil, made up mostly of leaf litter and humus (decomposed organic matter).

  Horizon A - Refers to the upper layer of soil, nearest the surface. It is commonly known as topsoil. This horizon has a layer of dark, decomposed organic material known as humus, such as fallen leaves and twigs. Horizon A provides plants with nutrients they need for their life. This is the layer in which most biological activity occurs.
- **Horizon B** - Low organic matter is present in horizon B and therefore there is much less humus. The B horizon is commonly referred to as "subsoil", and consists of mineral layers which may contain concentrations of clay or minerals such as iron or aluminium oxides or organic material moved there by leaching. They may also have stronger colors than the A horizon.
- **Horizon C** - consists mostly of weatherized big rocks. The C horizon also contains parent material. It forms the framework of the soil. The A and B layers are formed by this layer.



a) **MICROORGANISMS IN SOIL** –

- It is oblivious that microorganisms are present in soil because there is food for them.
- Soils are excellent cultural media for the growth of many types of organisms.
- A spoonful of soil contains billions of microorganisms.
- In general majority of microorganisms are found in the upper 6 to 12 inches of soil and the number decreases with the depth.
- The number and kind of organisms found in soil depends upon the nature of soil, depth, season of the year, state of cultivation, organic matter, temperature, moisture, aeration, etc.
- Cultivated soil has relatively more population of microorganisms than the fallow land, and the soils rich in organic matter contain much more population than sandy and eroded soils.
- Microbes in the soil are important to us in maintaining soil fertility / productivity, cycling of nutrient elements in the biosphere and sources of industrial products such as enzymes, antibiotics, vitamins, hormones, organic acids etc. At the same time certain soil microbes are the causal agents of human and plant diseases.
- As soil contains several diverse groups of microorganisms, but the most important amongst them are:
  1. Bacteria
  2. Fungi (Molds, Yeast, Mushroom)
  3. Actinomycetes
  4. Algae (Blue Green Algae, Yellow Green Algae, Golden Brown Algae)
  5. Protozoa

- Relative percentage of various soil microorganisms are: Bacteria-aerobic (70%), anaerobic (13 %), Actinomycetes (13%), Fungi /molds (03 %) and others (Algae, Protozoa, viruses) 0.2-0.8 %.
- **Bacteria**
- Amongst the different microorganisms found in the soil, bacteria are the most abundant and predominant organisms.
- Many types of bacteria are found in the soil.
- Soil bacteria are divided into three groups viz *Cocci* (spherical shaped), bacilli (rod-shaped) and *Spirilla* (spiral shaped). *Bacilli* are most numerous followed by Cocci *and Spirilla* in soil.

- The most common soil bacteria belong to the genera *Pseudomonas, Arthrobacter, Clostridium Achromobacter, Sarcina, Enterobacter* etc. The another group of bacteria common in soils is the Myxobacteria belonging to the genera *Micrococcus, Chondrococcus, Archangium, Polyangium, Cyptophaga.*
- Among the bacteria, some are autotrophic and utilize CO2 (from atmosphere) as carbon source and derive energy either from sunlight (eg. *Chromatrum. Chlorobium. Rhadopseudomonas*) or from the oxidation of simple inorganic substances present in soil (chemoautotrophs eg. *Nitrobacter, Nitrosomonas, Thiaobacillus*).
- Majority of soil bacteria are heterotrophic and derive their carbon and energy from complex organic substances, decaying roots and plant residues.

Bacterial genera	**Process/reaction**
a) Aerobic : *Angiococcus, Cytophaga, Polyangium, Sporocytophyga, Bacillus, Achromobacter, Cellulomonas* b) Anaerobic*: Clostridium, Methanosarcina, Methanococcus*	Cellulose decomposition (celluloytic bacteria) most cellulose decomposers are mesophilic
Bacillus, Pseudomonas	Ammonification (Ammonifiers)
Nitrosomonas, Nilrobacter Nitrosococcus	Nitrification (Nitrifying bacteria)
Achromobacter, Pseudomonas, Bacillus, Micrococcus	Denitrification (Denitrifies)
a) Symbiotic- Rhizobium, *Bradyrrhizobium* b) Non-symbiotic: aerobic – Azotobacter *Beijerinckia* (acidic soils), anaerobic- *Clostridium*	Nitrogen fixing bacteria

Bacteria capable of degrading various plant residues in soil are :

Cellulose	***Hemicelluloses***	***Lignin***	***Pectin***	***Proteins***
Pseudomonas	***Bacillus***	***Pseudomonas***	***Erwinia***	***Clostridium***
Cytophaya	***Vibrio***	***Micrococcus***		***Proteus***
Spirillum	***Pseudomonas***	***Flavobacteriumm***		***Pseudomonas***
Actinomycetes	***Erwinia***	***Xanthomonas***		***Bacillus***
Cellulomonas		***Streptomyces***		

<u>Fungi</u>

- These include a group of organisms from the moulds to the large fleshy fungi such as mushrooms. Fungi in soil are present in both the mycelial and the spore stages.

- Most commonly encountered genera of fungi in soil are; *Alternaria, Aspergillus, Cladosporium, Cephalosporium Botrytis, Chaetomium, Fusarium, Mucor, Penicillium, Verticillium, Trichoderma, Rhizopus, Gliocladium, Monilia, Pythium,* etc. Most of these fungal genera belong to the subdivision Deuteromycotina / Fungi imperfecta.
- Fungi plays important role in the degradation of cellulose, hemi cellulose, starch, pectin, lignin of plant tissue.
- The Mould mycelium penetrates the soil and forms a network which entangles soil particles and forms water stable aggregates, which are of considerable agricultural importance. Thus fungi improve the physical condition of the soil.
- Some soil fungi are parasitic and causes number of plant diseases such as wilts, root rots, seedling blights eg. *Pythium, Fusarium, Verticillium* etc.
- Number of soil fungi forms mycorrhizal association with the roots of higher plants (symbiotic association of a fungus with the roots of a higher plant) and helps in mobilization of soil phosphorus and nitrogen eg. *Gigaspora, Aculospora,* and *Amanita, Boletus, Entoloma, Lactarius*.
- Yeasts are generally not found in large number.

Actinomycetes

- Actinomycetes are numerous and widely distributed in soil and are next to bacteria in abundance.
- They are heterotrophic, aerobic and mesophilic organisms.
- In the order of abundance in soils, the common genera of actinomycetes found in soil are *Streptomyces* (nearly 70%), *Nocardia* and *Micromonospora*.

Algae

- Algae are present in most of the soils where moisture and sunlight are available. Their number in soil usually ranges from 100 to 10,000 per gram of soil. They are photoautotrophic, aerobic organisms and obtain CO_2 from atmosphere and energy from sunlight and synthesize their own food.
- Soil algae are divided in to four main classes or phyla as follows:
- 1. Cyanophyta (Blue-green algae)
  2. Chlorophyta (Grass-green algae)
  3. Xanthophyta (Yellow-green algae)
  4. Bacillariophyta (diatoms or golden-brown algae)

- Out of these four classes, blue-green algae and grass-green algae are more abundant in soil.
- The most common genera of green algae found in soil are: *Chlorella, Chlamydomonas, Chlorococcum, Protosiphon* etc. and that of diatoms are *Navicula, Pinnularia. Synedra, Frangilaria.*
- The dominant genera of Blue Green Algae in soil are: *Chrococcus, Phormidium, Anabaena, Aphanocapra, Oscillatoria* etc. BGA fixes nitrogen symbiotically and non-symbiotically) in water logged paddy fields.

Protozoa

Protozoa belonging to following classes are found in soil –

1. Rhizopoda (Sarcondia)- Important genera are *Amoeba, Biomyxa, Euglypha,* etc.
2. Mastigophora - Important genera are: *Allention, Bodo, Cercobodo, Cercomonas, Entosiphon Spiromonas, Spongomions* and *Testramitus.*
3. Ciliophora (Ciliata) - The important soil inhabitants of this class are *Colpidium, Colpoda, Balantiophorus, Gastrostyla, Halteria, Uroleptus, Vortiicella, Pleurotricha* etc.
4. Sporophora (not common Inhabitants of soil)

- Protozoa are abundant in the upper layer (15 cm) of soil. . Several soil protozoa cause diseases in human beings which are carried through water and other vectors, eg. Amoebic dysentery caused by *Entomobea histolytica.*

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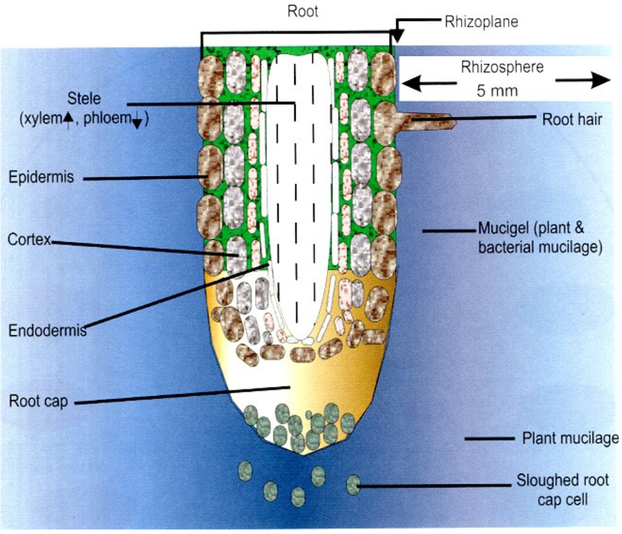
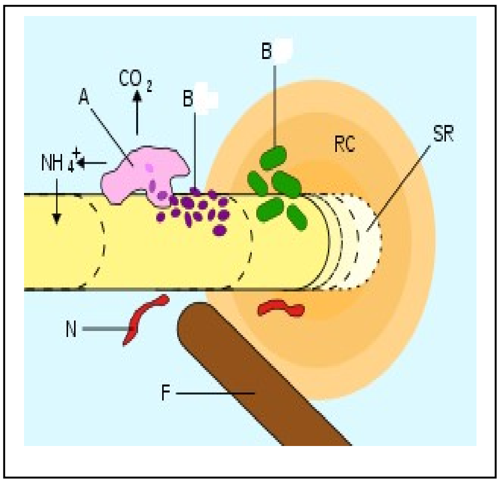
b) Rhizosphere –

- The **Rhizosphere** is the zone surrounding the roots of plants in which complex relations exist among the plant, the soil microorganisms and the soil itself.
- The **rhizosphere** is the narrow region of soil that is directly influenced by root secretions and associated soil microorganisms.
- It is the zone/region of soil immediately surrounding the plant roots together with root surfaces, or it is the region where soil and plant roots make contact, or it is the soil region subjected to influence of plant roots.
- The microbial population on & around roots is considerably higher than that of root free soil. Soil which is not part of the rhizosphere is known as bulk soil.
- Rhizosphere usually extends about 2 mm out from the root surface.
- The rhizosphere is enriched in organic material due to root exudates and sloughed off root cells. Rhizospere contains several compounds contributed by root exudates such as

amino acids, organic acids, carbohydrates (sugars) nucleic acid derivatives, growth factors (phytohormones), vitamins, enzymes and several other compounds.





B =Bacteria; A=Amoeba consuming bacteria; RC=Root derived carbon; SR=Sloughed root hair cells; F=Fungal hyphae; N=Nematode worm

Rhizosphere Effect on microorganisms

- The rhizosphere region is a highly favorable habitat for growth, activity and metabolism of numerous microorganisms.
- Microbial activity in the rhizosphere may be 2 – 10 greater than in the bulk soil.
- Rhizosphere is a tremendous complex biological system which profoundly influence the chemistry of the soil including pH and nitrogen transformations.
- Microbial floras of the rhizosphere are affected by soil type, moisture, soil PH, proximity of root with soil, plant species, and age of plant and root exudates.
- The greater rhizosphere effect is observed on bacteria.
- In rhizosphere bacteria predominate & their growth is enhanced by nutritional substances from plant tissues.
- Bacteria feed on sloughed-off plant cells, amino acids, proteins, growth factors and sugars released by roots.
- The most common genera of bacteria are: *Pseudomonas, Arthrobacter, Agrobacterium, Alcaligenes, Azotobacter, Mycobacterium, Flavobacter, Cellulomonas, Micrococcus* and others have been reported to be either abundant or sparse in the rhizosphere.
- From the agronomic point of view, the abundance of nitrogen fixing and phosphate solubilizing bacteria in the rhizosphere are of great importance.
- Protozoa and nematodes that graze on bacteria are also more abundant in the rhizosphere.

- In contrast plant roots do not enhance the fungi in the rhizosphere. However, rhizosphere effect is selective and significant on specific fungal genera *(Fusarium, Verticillium,* and *Aspergillus)* which are stimulated.
- Actinomycetes and algae are not significantly influenced by their proximity to the plant roots.
- **Activities in the Rhizosphere**
- In soil and rhizosphere region, many microorganisms live in close proximity and their interactions with each other may be associative or antagonistic.
- **Associative activities in rhizosphere**:
- In associative activity, one microorganism depends upon another for extra-cellular products (eg. amino acids & growth promoting substances). There is an increase in the exudation of amino acids, organic acids and monosaccharide by plant roots in the presence of microorganisms.
- Mycorrhiza is one of the best known associative / symbiotic interactions between the roots of higher plants and fungi. The fungi provide increased absorption area for the roots, so that greater quantities of water and nutrients can be taken up from soil. In return, the plant provides the fungi with organic nutients.
- This mycorrhizal association improves the growth through better uptake of phosphorus and zinc from soil.
- Another example is association between the bacterium *Rhizobium* and roots of legumes and *Azospirillum* with cereal crops (wheat, rye, bajara, maize etc).

- **Antagonistic activities in rhizosphere**:
- Several mutualistic, communalistic, competitive and antagonistic interactions exist in the rhizosphere.
- Antagonistic microorganisms in the rhizosphere play an important role in controlling some of the soil borne plant pathogens. It has been found that fluorescent pigments of *Pseudomonas fluorescens* helps in biological control of root pathogens. *Pseudomonas fluorescens* produce several biologically active compounds such as plant growth substances, cyanides, antibiotics and iron chelating substances called "Siderophores". Rovira and Campbell (1975) showed that bacterial strains of *P fluorescens* could lyse the hyphae of *Gaumannomyces graminis*, the causative agent of “take-all” disease of wheat.
- The successful antagonists among fungi are *Trichoderma* sp (T. viride and *T. harzianum, T. hamatum)* parasitize, lyse or kill the phytopathogenic fungi in the soil.
- Actinomycetes in the rhizosphere play an important role in controlling pathogenic fungi and bacteria, for example Micromonospora globosa is a potent antagonist of Fusarium udum.

c) Decomposition of Plant and Animal residues

- When plant and animal residues are added to the soil, their organic matters are decomposed by the activity of microorganisms and carbon is released as CO_2, and nitrogen as NH_4, NO_3 for the use by plants. Other nutrients are also converted into plant usable forms.
- This process of release of nutrients from organic matter is called mineralization. The final product of aerobic decomposition is CO_2 and that of anaerobic decomposition are Hydrogen, ethyl alcohol, various organic acids and carbon dioxide (CO_2). Soil organisms use organic matter as a source of energy and food.
- Sugars, water-soluble nitrogenous compounds, amino acids, lipids, starches and some of the hemicelluloses are decomposed first at rapid rate, while insoluble compounds such as cellulose, hemicellulose, lignin, proteins etc. which forms the major portion of organic matter are decomposed later slowly.
- Thus, the organic matter added to the soil is converted by oxidative decomposition to simpler substances which are made available for plant growth and the residue is transformed into humus.
- The decomposition of some of the major constituents (viz. Cellulose, Hemicellulose, Lignin, Proteins etc.) of soil organic matter are discussed in the following paragraphs.
- **Decomposition of Cellulose:**
- Cellulose is the most abundant carbohydrate present in plant residues. The decomposition of cellulose occurs in two stages: (i) in the first stage the long chain of cellulose is broken down into cellobiose and then into glucose by the process of hydrolysis in the presence of enzymes cellulase and cellobiase, and (ii) in second stage glucose is oxidized and converted CO_2 and water.

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                Cellulase                    Cellobiase
1. Cellulose ----------------> Cellobiose ------------------> Glucose
               Hydrolysis                   hydrolysis

             Oxidation                     Oxidation
2. Glucose ---------------> Organic Acids --------------> CO2 + H2O
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The intermediate products formed during enzymatic hydrolysis of cellulose (eg. cellobiose and glucose) are utilized by the cellulose-decomposing organisms or by other organisms as source of energy for biosynthetic processes. The cellulolytic microorganisms responsible for degradation of cellulose are fungi, bacteria and actinomycetes.

b) <u>Decomposition of Hemicelluloses</u>:

Hemicelluloses are water-soluble polysaccharides and consists of hexoses, pentoses, and uronic acids and are the major plant constituents. When subjected to microbial decomposition, hemicelluloses degrade initially at faster rate and are first hydrolyzed to their component sugars (eg. xylose, arabinose, galactose and mannose) and uronic acids. The hydrolysis is brought about by number of hemicellulolytic enzymes known as "hemicellulases" excreted by the microorganisms.

Hemicelluloses ----- Hemicellulases ------------ Sugars + Uronic Acids

Sugars are further converted to organic acids, alcohols, CO2 and H2O and uronic acids are broken down to pentoses and CO2. Various microorganisms including fungi, bacteria and actinomycetes both aerobic and anaerobic are involved in the decomposition of hemicelluloses.

c) <u>Lignin Decomposition</u>:

Lignin is the third most abundant constituent of plant tissues, and accounts about 10-30 percent of the dry matter of mature plant materials. Lignin content of young plants is low and gradually increases as the plant grows old. It is one of the most resistant organic substances for the microorganisms to degrade. However certain Basidiomycetous fungi are known to degrade lignin at slow rates. Complete oxidation of lignin result in the formation of aromatic compounds such as syringaldehydes, vanillin and ferulic acid. The final cleavages of these aromatic compounds yield organic acids, carbon dioxide, methane and water.

d) <u>Protein Decomposition</u>:

Proteins are complex organic substances. During the course of decomposition proteins are first hydrolyzed to a number of intermediate products eg. Proteases, peptides etc.

Protein ----- Proteases ------ Peptones ---- Peptides --------- Amino Acids

The intermediate products so formed are then hydrolyzed and broken down ultimately to individual amino acids, or ammonia and amides. The hydrolysis of proteins to amino acids is known as “aminization or ammonification” and is brought about by certain enzymes, collectively known as “proteases” or “proteolytic” enzymes secreted by various microorganisms. Amino acids and amines are further decomposed and converted into ammonia. During the course of ammonification, various organic acids, alcohols, aldehydes etc. are produced which are further decomposed finally to produce carbon dioxide and water. All types of microorganisms, bacteria, fungi, and actinomycetes are able to bring about decomposition of proteins.

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d) HUMUS

Definition

- A precise definition of humus is rather difficult.
- Humus is a uniform dark colored, amorphous substance obtained as a result of decompositions of organic materials of plant & animal origin, by the microbial flora comprising of actinomycetes, fungi, bacteria & worms.
- **Composition of Humus**
- Humus is a mass of resistant residual organic matter formed as a result of decomposition and synthesis of organic matter under soil conditions.
- It is generally brownish-black waxy material, known as the "fat of the land".
- Humus is very complex and varies greatly in composition. It is mainly composed of compounds of carbon and nitrogen. This combination is called ligno-protein. Besides this, it also contains number of inorganic salts such as phosphates, potash, etc.
- Its composition depends upon the following factors.
- 1) The material from which it is made- constituents of dead and living plant, and animal cells.
- 2) The interconversion of material by various types of microorganisms.
- 3) Soil conditions – temperature, moisture, aeration, soil reaction, etc.
- It must be remembered that composition of humus is not always same.

Humus formation:--

- The importance sources of humus are plant residues, stable manure, green manures and artificial composts.
- These materials when added to soil or in compost heaps are acted upon by various organisms ranging from bacteria to fungi & worms.
- The sugars & starches are easily degraded followed by some hemicelluloses, proteins and celluloses. But lignins & some of their derivatives, waxes & tannins are more resistant to decomposition.
- During decomposition there is consumption of oxygen followed by evolution of heat, liberation of considerable carbon dioxide & ammonia.
- If the material is rich in nitrogen and darkening takes place. As the process of decomposition become slower, the residual mass turns brown to black. During the process of decomposition new microbial cells are developed. This mass of decomposing

and decomposed material, together with newly synthesized microbial cells constitutes the humus.

Functions of Humus:--

Humus is an important part of the soil system; it has number of functions in agriculture.

1) It improves the physical condition of the soil, making it soft and smooth.
2) It increases the water holding capacity of soil and aerates the soil. With this soil becomes sticky and resist erosion.
3) It serves as a source of energy for development of various groups of microorganisms and as a result of its decomposition, a continuous stream of carbon dioxide and ammonia is given off.
4) Humus possesses cation exchange capacity & ability to absorb water. The cation exchange capacity of soil is directly linked to its fertility. The higher the humus content of soil more is the cation exchange capacity.
5) Humus is a great storehouse of food materials essential for higher plants, especially carbon & nitrogen, and to less extent of P, Ca, Mg, Fe, Mn etc.
6) Humus influences the chemical properties of the soil, such as the solubility of soil, the oxidation-reduction potential, and the buffering capacity.
7) It plays a very important role in soil-plant relationship by adsorbing toxic material to the plant growth.
8) In soil, humus forms clay-humus complex which is of great significance from the agriculture point of view.
9) It provides a more favourable medium for the development of root systems of plant & for the growth of microorganisms.
10) The texture and structure of soil are influenced by the presence of humus. It binds loose, porous soils into aggregate & opens up heavy solids.

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d) COMPOSTING :

- Considerable amounts of organic material is produced annually in nature, are eventually degraded by microbial action.
- **Definition :**
- Composting is the process of producing compost through aerobic decomposition of biodegradable organic matter.
- Composting is the decomposition of organic matter by a mixed population of microorganisms in a warm, moist, aerobic environment.

- Composting is the transformation of organic material (plant matter) through decomposition into a soil-like material.
- Invertebrates (insects and earthworms), and microorganisms (bacteria and fungi) help in this transformation.

Formation of Compost:

- A wide variety of organic materials are suitable for composting include livestock wastes, farm manure, crop residues, fruit and vegetable trimmings, leaves and sewage sludge etc.
- In composting, compostable material is placed in pits or in stacks on the ground & the material is turned at intervals to allow adequate aeration & mixing.
- The process of composting involves an interaction between the organic waste, microorganisms, moisture & oxygen.
- An indigenous mixed population of microorganisms under suitable moisture and aeration conditions attack organic matter of waste (sugars, proteins, fats, hemicelluloses, cellulose, lignin and minerals). Microorganisms secretes extra-cellular enzyme which hydrolyse the larger polymers into simple basic units which can be easily utilized by microorganisms.
- The natural decomposition process is a slow process. The composting process can be accelerated by gathering the material into heaps. The heat generated during decomposition raises the temperature of heaps & fastens reaction rates.
- To encourage the most active microbes, there should be a correct mixture of Carbon and Nitrogen elements in composting materials (30 parts carbon to 1 part nitrogen by weight), Oxygen (from the air) and Water.
- In attacking organic matter the microorganisms reproduce themselves and liberate carbon dioxide, water, & other organic products & energy.

Microbiology of composting:

- Compost consists of a mixed microbial population. Each is predominantly active for a limited duration. Bacteria, Actinomycetes and fungi are mainly involved involved in composting.
- Bacteria are present in vast numbers. About 2000 bacterial & 50 fungal species are present in compost. Organisms vary from psychrophiles (20^{o}C), mesophiles (20-40^{o}C) to thermophiles (40^{o}C). Actinomycetes develop far more slowly than most bacteria & fungi but become prominent at peak temperature & in the later stages of composting. The thermophilic fungi are well defined group in composting.

- In temperate climates the earthworm plays a major role in the breakdown process in the compost heap. In arid & semi- arid climates this function is usually taken up by the termites.
- **Vermi Compost** – Vermi Compost is the natural waste produced by earthworm. Vermi-compost is an excellent alternative for chemical fertilizer.

Conditions required for satisfactory composting:

1. Adequate supply of available nitrogen. 2. Optimum pH 3.Good aeration
   4. Adequate moisture 5. A warm climate.

Function : Compost produced at the end of compoting process can be used in farming and gardening to improve soil quality. It is an excellent alternative for chemical fertilizer.

Compost Boxes and Bins

e) Biological Nitrogen Fixation-

- Nitrogen is a critical limiting element for plant growth and production.
- Nitrogen fixation is essential for all forms of life because nitrogen is required to synthesize basic building blocks of plants, animals and other life forms, e.g., nucleotides of DNA and RNA, amino acids for proteins and other important biomolecules of cells such as ATP, chlorophyll etc .
- A relatively small amount of ammonia is produced by lightning. Some ammonia also is produced industrially by the Haber-Bosch process, using an iron-based catalyst, very high pressures and fairly high temperature. But the major conversion of N_2 into ammonia, and thence into proteins, is achieved by microorganisms in the process called nitrogen fixation (or dinitrogen fixation).

- There is an abundant supply of nitrogen in the earth's atmosphere - nearly 79% in the form of N_2 gas.
- However, Animals & plants are unable to utilize vast stores of nitrogen in the atmosphere because there is a triple bond between the two nitrogen atoms, making the molecule almost inert.
- In order to use nitrogen for growth it must be "fixed" (combined) in the form of ammonium (NH_4) or nitrate (NO_3) ions.
- **Definition** : The conversion of atmospheric nitrogen into compounds, such as ammonia, nitrites or nitrates by natural agencies or various industrial processes, or biological processes is called nitrogen fixation. It is process by which free atmospheric nitrogen (N2) is converted (fixed) into nitrogen compounds which are usable by plants and other forms of life.

  There are two types of nitrogen fixation:
- **1) Non-biological nitrogen fixation** - Non-biological nitrogen fixation refers to nitrogen fixation that occurs through nonbiological process either naturally or artificially. A small amount of non-biological nitrogen fixation occurs in the atmosphere through lightning where the extremely high temperatures allow nitrogen and oxygen in the air to react. There are few man made prosesses (example – haber process, cynamide process etc) where nitrogen is fixed, but they requires rather drastic conditions of temperature and pressure.
- **2) Biological Nitrogen Fixation** - Biological nitrogen fixation refers to the nitrogen fixation by biological process involving living microorganisms. Biological nitrogen fixation was discovered by the German agronomist Hermann Hellriegel and Dutch microbiologist Martinus Beijerinck.
- In biological nitrogen fixation two moles of ammonia are produced from one mole of nitrogen gas, using 16 moles of ATP and a supply of electrons and protons.
- $N_2 + 8H+ + 8e^- + 16\ ATP = 2NH_3 + H_2 + 16ADP + 16\ Pi$
- Biological nitrogen fixation is performed exclusively by prokaryotes (the bacteria and related organisms), using an enzyme complex termed nitrogenase.
- There are two main modes of Biological Nitrogen Fixation
- 1) Symbiotic Nitrogen Fixation
- 2) Non-symbiotic Nitrogen Fixation

- **Symbiotic Nitrogen Fixation**
- Symbiotic nitrogen fixation is a mutual relationship between the plant and a microbe. Since neither the plant nor the bacterium can fix atmospheric nitrogen independently, the process, therefore, is called Symbiotic nitrogen fixation.
- The symbiotic nitrogen fixing microorganisms lives in the roots of plants. They live in intimate symbiotic associations with plants.
- The microbe firstly invades the root and later forms nodules in which nitrogen fixation takes place.
- The plant or host provides the plant sugars (sucrose) to microorganism and the nitrogen fixing microbe supplies the fixed form of nitrogen to plant. Both the bacteria and the plant benefit by the association.
- The most common symbiotic relationship occurs in leguminous plants (like white clover, mung beans, soybeans, peas etc).
- The fixation of atmospheric nitrogen by legumes was first demonstrated by French chemist Boussingault in 1837. The root nodule bacteria was later isolated and identified as *Rhizobium*.
- These are gram negative, motile, aerobic, non-sporeforming bacteria.

  Bacterium *Rhizobium* shows specificity towards leguminous plants.

Bacteria	Leguminous plants
1. *Rhizobium melitoli*	Alfalfa, sweet clover
2. *Rhizobium trifoli*	Red, white and other clovers
3. *Rhizobium leguminosarum*	Peas, lentil
4. *Rhizobium phaseoli*	Beans
5. *Rhizobium lupini*	Lupines
6. *Rhizobium japonicum*	Soybeans

Non-symbiotic Nitrogen Fixation

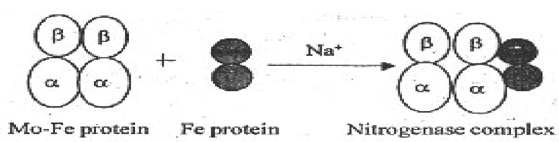
- Nitrogen fixation by microorganism living freely & independently in the soil is called Non-symbiotic nitrogen fixation.
- They live independently of other organisms. So-called free-living nitrogen-fixing bacteria.
- These microbes do not require symbiosis.
- *Clostridium pasteurianum* was the first bacterium isolated by Winogradsky that can fix atmospheric nitrogen without symbiotic aid.
- However, in recent years a large number of organisms have been isolated that can fix nitrogen non-symbiotically.

Non-symbiotic nitrogen fixing microorganisms

Aerobic		Anaerobic	
Heterotrophic	Autotrophic	Heterotrophic	Autotrophic
Azotobacter *Pseudomonas* *Achromobacter*	Blue Green Algae *Nostoc,* *Anabena,* *Calothrix,*	*Clostridium,* *Aerobacter*	Photosynthetic bacteria: *Rhodospirillum,* *Rhodopseudomonas,* *Chlorobium,* *Chromatium* etc Chemosynthetic bacteria: *Methanobacterium ,* *Desulfovibrio*

Nitrogenase Complex

- Biological nitrogen fixation is performed exclusively by prokaryotes (the bacteria and related organisms), using an enzyme complex termed nitrogenase.
- This enzyme consists of two proteins - an iron protein and a molybdenum-iron protein.
- A point of special interest is that the nitrogenase enzyme complex is highly sensitive to oxygen. The enzyme is composed of the heterotetrameric MoFe protein that is transiently associated with the homomeric Fe protein.
- MOFe proteins play a key role in nitrogen fixation (substrate binding & reduction) with Fe protein that assists in transfer of electron from flavodoxin to the bigger subunits & ATP consumption. Neither of the subunits can function independently.
- Electrons for the reduction of nitrogen are supplied to nitrogenase when it associates with the reduced, nucleotide-bound Fe protein.
- ATP supplies the energy to drive the transfer of electrons from the Fe protein to the MoFe protein.
- The reduction potential of each electron transferred to the MoFe protein is sufficient to break one of dinitrogen's chemical bonds, though it has not yet been shown that exactly three cycles are sufficient to convert one molecule of N_2 to ammonia.
- Nitrogenase ultimately bonds each atom of nitrogen to three hydrogen atoms to form ammonia (NH_3), which is in turn bonded to glutamate to form glutamine.
- The nitrogenase reaction additionally produces molecular hydrogen as a side product.



Type of nitrogen fixing microorganisms:

All the nitrogen-fixing organisms are prokaryotes (bacteria). Microorganisms that fix nitrogen are bacteria called diazotrophs. & the phenomenon of this activity is known as diazotrophy. Some higher plants, and some animals (termites) can also fix nitrogen in associations with diazotrophs.

Two groups of microorganisms are involved in nitrogen fixation–

1) **Non-symbiotic nitrogen fixing microorganisms** – Nitrogen fixation by microorganism living freely & independently in the soil is called Non-symbiotic nitrogen fixation. They live independently of other organisms. So-called free-living nitrogen-fixing bacteria

2) **Symbiotic nitrogen fixing microorganisms -** mos those living in the roots of plants. They live in intimate symbiotic associations with plants or with other organisms (e.g. protozoa).

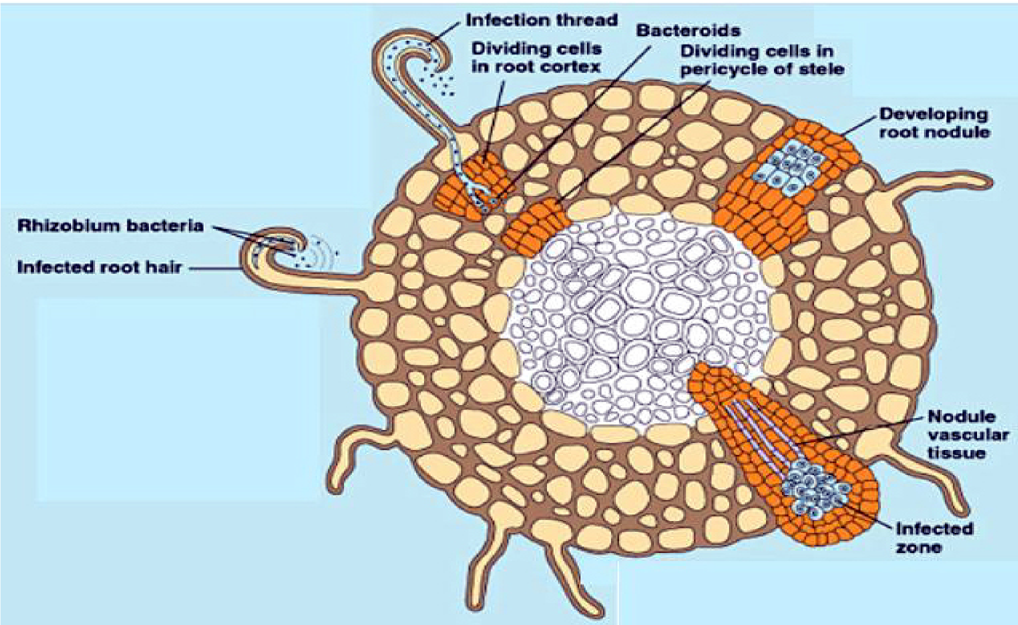
Examples are shown in the table below.

Some nitrogen fixing organisms

Free living				Symbiotic	
Aerobes		Anaerobes		Leguminous plant	Non-leguminous plant
Heterotroph	Photototroph	Heterotroph	Photototroph		
Azotobacter spp.	Various cyanobacteria	*Clostridium spp*	*Rhodospirillum,*	Soybean,	*Alnus, Myrica, Ceanthus, Casurina*
Klebsiella	*Nostoc*	*Desulfovibrio*	*Rhodopseudomonas*	Clover ,	In association
Beijerinckia	*Anabena*	*Desulfotomaculum*	*Chlorobium*	Locust, etc	With actinomycetes of the genus *Frankia*
Bacillus polymixa	*Calothrix*		*Chromatium*	In association	
Mycobacterium flavum				With a bacterium of the genus	
Azospirillum lipoferum				*Rhizobium* or	
Citrobacter freundii				*Bradyrhizobium*	

Nodulation Process

- BNF takes place in nodules located on the roots of the plants.
- Nodules are formed as a result of infection of the roots by soil bacteria.
- The bacteria most often infecting the roots are bacteria in the genus *Rhizobium*.
- Rhizobia first invade the root hairs are known as infection.
- Bacteria aggregate as threads and penetrates the plant cells.

- The presence of bacteria stimulates the multiplication of infected cells, resulting in the formation of nodules. The complex process in which nodules are formed is known as nodulation.
- The process of infection in many legumes begins when *Rhizobium* bacteria come in contact with the root hairs of the host plant.
- Roots emit chemical signals that attract Rhizobium bacteria. The bacteria emit signals that stimulate root hairs to elongate, and to form an infection thread by an invagination of the plasma membrane.
- The bacteria gradually form an infection thread which allows bacteria to enter root cells of the plant via the root hairs.
- Bacteria in the root cells gradually grow and develop into structures known as bacteroids.
- The bacteroids eventually develop the biochemical 'machinery' which is able to absorb atmospheric N_2 and convert it to N in the form of ammonia (NH_3).
- During the infection process, the bacteria stimulate cell division in the root cells.
- This results in the eventual formation of the structures known as root nodules.
- Nodules vary in size and shape.
- BNF takes place in bacteriods within these nodules.
- 

f) Cycles of Elements in Nature

- Soil microorganisms serve as biogeochemical agents for the conversion of complex organic compounds in to simple inorganic compounds or into their constituents elements. The overall process is called mineralization.

- This conversion of complex organic compounds into inorganic compounds or of elements provides for the continuity of elements or their compounds as nutrients for plants & animals including man.
- All the elements that are essential components of protoplasm undergo cyclic alternation between inorganic state, free in nature or in oxidized state & combined state or reduced state in living organisms or protoplasm.
- Important biogeochemical cycles of elements required by organisms for life are

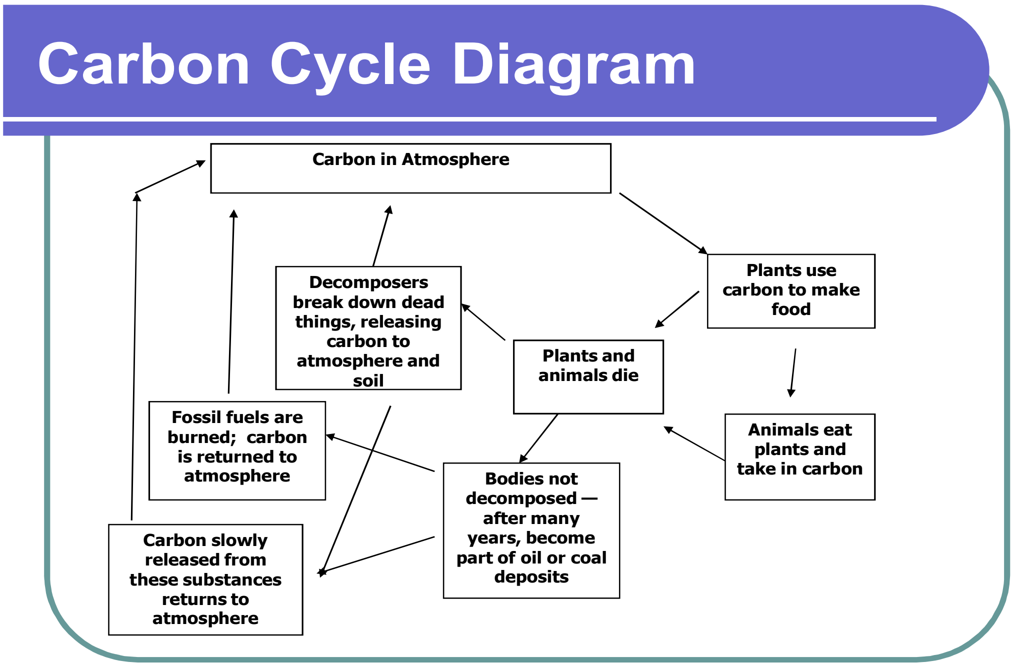
- **Carbon cycle**
- **Phosphorus cycle**
- **Sulphur cycle**
- **Nitrogen cycle**

i) Carbon Cycle

- About half of the dry weight of living organisms is composed of carbon compounds. The ultimate source of organic carbon compounds in nature is the carbon dioxide present in the atmosphere. Carbon is an element, which is the basis of life of earth, found in rocks, fossil fuels, oceans, and atmosphere.
- It is a cycle involving biological, geological and chemical processes, in which carbon atoms are exchanged between the earth and the atmosphere.
- In the biosphere, the carbon cycle is the beginning of the food chain. A primary producer establishes the food chain through the photosynthesis, upon which the remaining heterotrophs including man depends.
- **Carbon dioxide fixation** - Plants pulls carbon dioxide from the atmosphere and use it to make food by photosynthesis. The carbon becomes part of the plant (stored food). Using photosynthesis, plants converts atmospheric CO_2 to glucose, releasing oxygen (O_2) in the process. $CO_2 + H_2O \rightarrow CH_2O + O_2$
- Animals obtain their carbon by eating and digesting plants, so organic carbon is transferred form plants to the animals feeding on them.
- Carbon returns to the environment in a number of ways.
- Both plants and animals respire, so they release CO_2 during respiration. Respiration by plants and animals result in some transfer of carbon back to the atmosphere.
- Another route of CO_2 back to the physical environment occurs through the death of plants and animals. When this organism die or when wood and leaves decay or when animals

excrete, their organic carbon compounds are deposited in the soil & are degraded by microbial activity. The end product CO_2 is released into the air & soil. Bacteria & fungi are the prominent microorganisms that degrade organic carbon compounds. The most abundant organic material in plant is cellulose

- i) Cellulose ------- Cellulase -----→ Cellobiose
- ii) Cellulose ------ B Glucosidase ---→ Glucose
- iii) Glucose ----→ CO_2, Water & Other End Products
- In the process, some of the carbon returns to the environment by way of fossilization. Some of these living beings buried millions of years ago have been converted to fossil fuels made of carbon like coal and oil over millions of years. When humans burn fossil fuels, most of the carbon quickly enters the atmosphere as carbon dioxide.
- Mining and burning of fossil fuels cause this carbon to move from the lithosphere to the atmosphere.
- Some of this atmospheric carbon gets dissolved in the oceans and thus completes the cycle.
- But by far, most of the carbon returns to the physical environment through the respiration of CO_2.



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ii) NITROGEN CYCLE

- Nitrogen is the most important structural element of all living organisms.
- Air is a vast store of nitrogen, but animals and the majority of plants are unable to fix atmospheric nitrogen.
- The nitrogen cycle is chiefly concerned with the transformation of atmospheric gaseous nitrogen and organic nitrogen of dead plants and animals into the forms that are usable by higher plants.
- Higher plants generally require nitrogen in the form of nitrates, although ammonia and some organic nitrogenous compounds are also utilized to a lesser extent.
- The key processes of nitrogen cycle are – proteolysis, ammonification, nitrification, nitrate reduction, denitrification
- **1.Proteolysis**:
  Upon death, plant and animals undergo microbial decay in the soil and the nitrogen contained in their proteins is released. Thus, the process of enzymatic breakdown of proteins by the microorganisms with the help of proteolytic enzymes is known as "proteolysis".
- The breakdown of proteins is completed in two stages. In first stage proteins are converted into peptides or polypeptides by enzyme "proteinases" and in the second stage polypeptides / peptides are further broken down into amino acids by the enzyme "peptidases".
- **Proteins ------------------------> Peptides ------------------------> Amino Acids**
  Proteinases **Peptidases**
- The amino acids produced may be utilized by other microorganisms for the synthesis of cellular components, absorbed by the plants to make their proteins or may be deaminated to yield ammonia.
- The most active microorganisms responsible for proteolysis are *Pseudomonas, Bacillus, Proteus, Clostridium Histolyticum, Micrococcus, Alternaria, Penicillium* etc.

2.Ammonification(Amino-acid-degradation):

Amino acids released during proteolysis undergo deamination in which nitrogen containing amino (-NH2) group is removed. The process of deamination that leads to the production of ammonia is termed as "ammonification". Ammonification usually occurs under aerobic conditions with the liberation of ammonia (NH3) or ammonium ions (NH4) which are either released to the atmosphere or oxidized to nitrites and then to nitrates.The processes of ammonification are commonly brought about by *Clostridium* sp, *Micrococcus* sp, *Proteus sp.* etc. and it is represented as follows.

- Alanine

CH3 CHNH2 COOH + 1/2 O2 ----------------> CH3COCOOH + NH3

Alanine deaminase Pyruvic acid ammonia

- **3. Nitrification**:

Ammonical nitrogen / ammonia released during ammonification are oxidized to nitrates and the process is called "nitrification". Soil conditions such as well aerated soils rich in calcium carbonate, a temperature below 30 ° C, neutral PH and less organic matter are favorable for nitrification in soil.

- Nitrification is a two stage process and each stage is performed by a different group of bacteria as follows.
- **Stage I**: Oxidation of ammonia of nitrite is brought about by ammonia oxidizing bacteria viz. *Nitrosomnonas europaea, Nitrosococcus nitrosus, Nitrosospira briensis, Nitrosovibrio* and *Nitrocystis* and the process is known as nitrosification. The reaction is presented as follows.
- 2 NH3 + ½ O_2 ------------------> NO_2 + 2 H + H_2O

Ammonia Nitrite

- **Stage II:** In the second step nitrite is oxidized to nitrate by nitrite-oxidizing bacteria such as *Nitrobacter winogradsky, Nitrospira gracilis, Nirosococcus mobiiis* etc, and several fungi *(eg. Penicillium, Aspergillus) and* actinomycetes *(eg. Streptomyces, Nocardia).*
- NO_2^- + ½ O_2 ---------------------> NO_3

Nitrite ions Nitrate ions

- The nitrate thus, formed may be utilized by the microorganisms, assimilated by plants, reduced to nitrite and ammonia or nitrogen gas or lost through leaching depending on soil conditions. The nitrifying bacteria (ammonia oxidizer and nitrite oxidizer) are aerobic gram-negative and chemoautotrophic and are the common inhabitants of soil, sewage and aquatic environment.
-
- **4. Nitrate Reduction**:

Several heterotrophic bacteria *(E. coli, Azospirillum)* are capable of converting nitrates to nitrites and nitrites to ammonia. Thus, the process of nitrification is reversed completely which is known as nitrate reduction. Nitrate reduction normally occurs under anaerobic soil conditions (water logged soils) and the overall process is as follows:

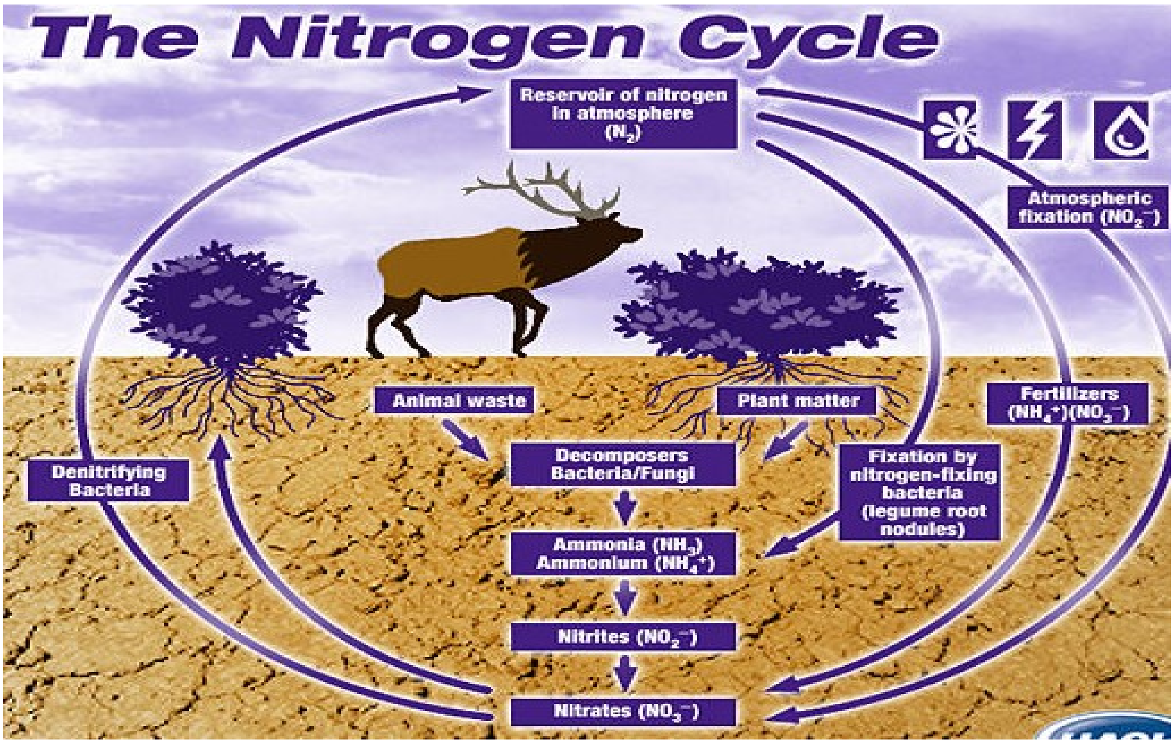
$$\underset{\text{Nitrate}}{HNO_3} + 4\ H_2 \xrightarrow{\text{Nitrate Reductase}} \underset{\text{Ammonium Ion}}{NH_4} + 3\ H_20$$

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

<u>5. Denitrification</u>:

- This is the reverse process of nitrification. During denitrification nitrates are reduced to nitrites and then to nitrogen gas and ammonia. The reduction of nitrates to gaseous nitrogen by microorganisms in a series of biochemical reactions is called “denitrification". The process is wasteful as available nitrogen in soil is lost to atmosphere. The overall process of denitrification is as follows:

  Nitrate -----> Nitrite ----> Nitric Oxide ----> Nitrous Oxide ------> Nitrogen gas
- This process also called dissimilatory nitrate reduction as nitrate nitrogen is completely lost into atmospheric air.
- The most important denitrifying bacteria are *Thiobacillus denitrificans, Micrococcus denitrificans,* and species of *Pseudomonas, Bacillus, Achromobacter, Serrtatia paracoccus* etc.
- Denitrification leads to the loss of nitrogen (nitrate nitrogen) which is essential nutrient for plant growth therefore, it is an undesirable process from the soil fertility and agricultural productivity point of view.
- Although, denitrification is an undesirable process from agricultural productivity point of view, but it is of major ecological importance since, it returns nitrogen to the atmosphere.

- 

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iii) Sulfur Cycle

- Sulphur is the most abundant and widely distributed element in the nature and found both in free as well as combined states. In the combined forms it occurs in both organic & inorganic combinations.
- Sulphur, like nitrogen is an essential element for all living systems and occurs in all living matter chiefly as a component of amino acids (methionine, serine & cystine) which are of structural & functional importance in many proteins.
- Sulfur undergoes alternations between organic & elemental state, & between oxidation & reduction.
- In soil, sulfur enters from plant residues, animal wastes, chemical fertilizers & rain water.
- In soil, sulphur is found in the organic form (sulphur containing amino acids-cystine, methionine, proteins, polypeptides, B- vitamins - biotin, thiamine etc). Of the total sulphur present is soil only 10-15% is in the inorganic form (sulphate) and about 75-90 % is in organic form.
- These organic sulphur compounds are metabolized by soil microorganisms to make it available in an inorganic form (sulphur, sulphates, sulphite, thiosulphale, etc) for plant nutrition.

- Cycling of sulphur between organic and elemental states and between oxidized and reduced states are brought about by various microorganisms, especially bacteria. The conversion of organic sulphur compounds to the inorganic state by microorganisms is termed as mineralization of sulphur".
- The sulphur / sulphate, thus released are either absorbed by the plants or escapes to the atmosphere in the form of oxides.
- Animal get sulfur by feeding on plants. The dead parts of plants, animal & also microorganisms upon incorporation into sulfate is
- Various transformations of the sulphur in soil results mainly due to microbial activity. The major types of transformations involved in the cycling of sulphur are:
  1. Mineralization 2. Immobilization 3. Oxidation 4. Reduction
- **1. Mineralization**: The breakdown / decomposition of large organic sulphur compounds to smaller units and their conversion into inorganic compounds (sulphates) by the microorganisms.
- **2. Immobilization**: Microbial conversion of inorganic sulphur compounds to organic sulphur compounds.
- **3. Oxidation**: Oxidation of elemental sulphur and inorganic sulphur compounds (such as H2S, sulphite and thiosulphale) to sulphate (SO4) is brought about by chemoautotrophic and photosynthetic bacteria.
- When plant and animal proteins are degraded, the sulphur is released from the amino acids and accumulates in the soil which is then oxidized to sulphates in the presence of oxygen and under anaerobic condition (water logged soils) organic sulphur is decomposed to produce hydrogen sulphide. H_2S can also accumulate during the reduction of sulphates under anaerobic conditions which can be further oxidized to sulphates under aerobic conditions.

Ionization

a) $2\,S + 3O_2 + 2\,H_2O \text{ -------->} 2H_2SO_4 \text{ -------------->} 2H^+ + SO_4$ (Aerobic)

light

b) $CO_2 + 2H_2S$ --------> $(CH_2O) + H_2O + 2\,S$

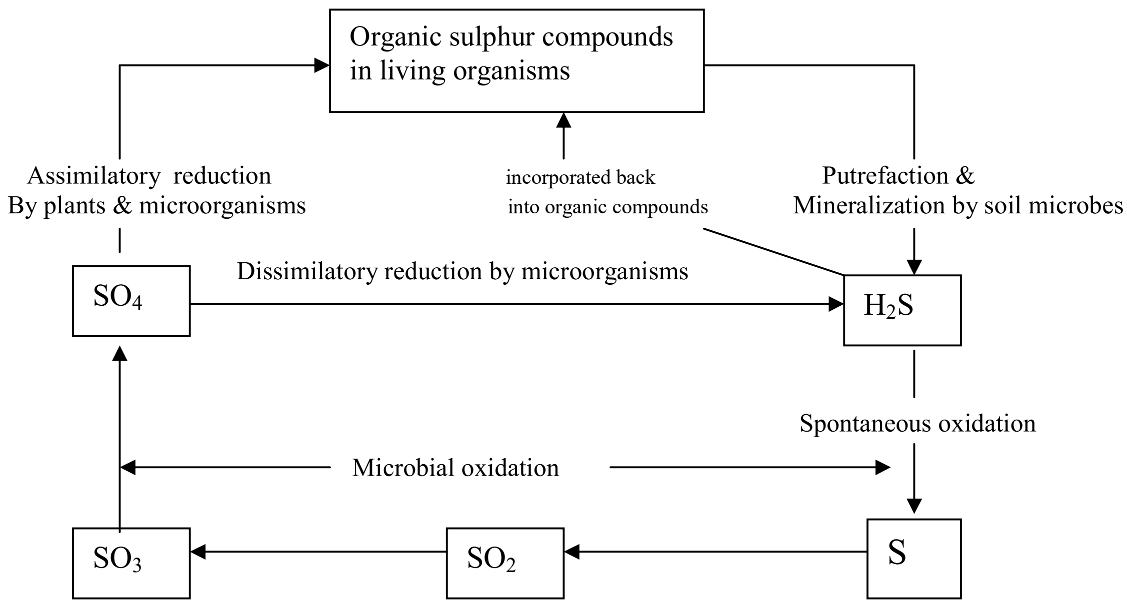
light

OR $H_2 + S + 2\,CO_2 + H_2O$ ---------> $H_2SO_4 + 2\,(CH_2O)$ (anaerobic)

- The members of genus *Thiobacillus* eg, *T. ferrooxidans* and *T. thiooxidans* are involved in the oxidation of elemental sulphur to sulphates. Other than *Thiobacillus,* heterotrophic bacteria *(Bacillus, Pseudomonas, and Arthrobacter)* and fungi *(Aspergillus, Penicillium)* and some actinomycetes are also reported to oxidize sulphur compounds. Green and purple bacteria (Photolithotrophs) of genera *Chlorbium, Chromatium. Rhodopseudomonas* are also reported to oxidize sulphur in aquatic environment.

- The formation of sulphate / Sulphuric acid is beneficial in agriculture in different ways : i) As it is the anion of strong mineral acid (H_2SO_4) can render alkali soils fit for cultivation by correcting soil PH. ii) solubilize inorganic salts containing plant nutrients and thereby increase the level of soluble phosphate, potassium, calcium, magnesium etc. for plant nutrition.
- **4. <u>Reduction of Sulphate</u>**:
- **<u>Assimilatory sulphate reduction</u>** - Sulphate in the soil is assimilated by plants and microorganisms and incorporated into proteins. This is known as "assimilatory sulphate reduction".
- **<u>Dissimilatory sulphate reduction</u>** - Sulphate can be reduced to hydrogen sulphide (H2S) by sulphate reducing bacteria (eg. *Desulfovibrio* and *Desulfatomaculum)* and may diminish the availability of sulphur for plant nutrition. This is "dissimilatory sulphate reduction" which is not at all desirable from soil fertility and agricultural productivity view point. For example, calcium sulphate is attacked under anaerobic condition by the members of the genus *Desulfovibrio* and *Desulfatomaculum* to release $H_2 S$.
- $CaSO_4 + 4H_2$ -----------> $Ca (OH)_2 + H_2 S + H_2 O$.
- Hydrogen sulphide produced by the reduction of sulphate and sulphur containing amino acids decomposition is further oxidized by some species of green and purple phototrophic bacteria *(eg. Chlorobium, Chromatium)* to release elemental sulphur.

$$CO_2 + 2H_2 + H_2S \xrightarrow[\text{Enzyme}]{\text{Light}} \underset{\text{Carbohydrate}}{(CH_2O)} + H_2O + \underset{\text{Sulphur}}{2\,S.}$$



<u>Fig. sulphur cycle</u>

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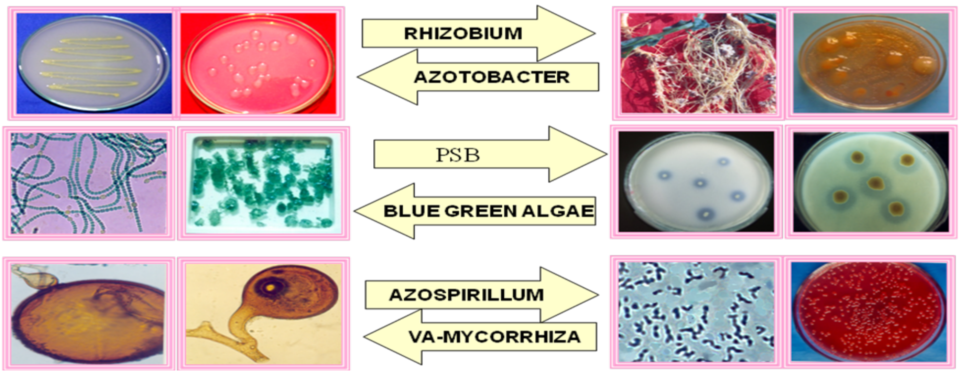
v) **BIOFERTILIZERS:-**

- Biofertilizer technology is not a new concept. Biofertilizer concept goes back as early as 300 BC when our ancestors realized the importance of legume crops bearing nodules.
- The perspective of biofertilizer came into existence through discovery of many organisms capable of nitrogen fixation, Phosphate solubilization and micronutrient transformation in the soil.
- **Definition :**
- Biofertilizers are defined as biologically active products or microbial inoculants of bacteria, algae and fungi (separately or in combination), which may help biological nitrogen fixation, solubilization and mobilization of other nutrients for the benefit of plants.
- In other words biofertilizer refers to preparation containing live microbes which helps in enhancing the soil fertility either by fixing atmospheric nitrogen, solubilization of phosphorus or decomposing organic wastes or by enhancing plant growth by producing growth hormones.
- Biofertilizers assumes special significance because 1) use of biofertilizers leads to increased crop productivity 2) Replace 25-30% chemical fertilizers that leads to damage in soil texture 3) increased cost of chemical fertilizers and their ill effects on soil health. 4) Use of biofertilizers is both economical and environment friendly.

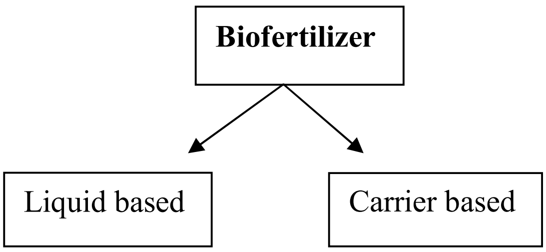
- **BIOFERTILIZER ORGANISMS**
- Biofertilizers thus include the following:
- **1) Symbiotic Nitrogen Fixers (*Rhizobium* spp.)** - Enter into symbiotic relationship with legumes. They fix atmospheric nitrogen & thus not only increase the production of inoculated crops, but also leave a fair amount of nitrogen in the soil, which benefits the subsequent crop.
- 2) **Non-symbiotic Nitrogen Fixers (*Azotobacter, Azospirillum*)** - Azobacter & Azospirillum when applied to rhizosphere fix atmospheric nitrogen & make it available to crop plants. They also synthesize growth promoting substances helpful to plant.
- **3) Blue Green Algae (blue green algae *Anabaena* in association with *Azolla*)** - Blue green algae (BGA) & Azolla constitute a system which is the main source of algal fertilizers. BGA inoculation (without Azolla) with cultures of algal genera *Anabena, Nostoc, Oscillabria* have been found to be more effective than single cultures.

- 4) **<u>Phosphate Solublizing Microorganisms (PSM)</u>** - *Bacillus, Pseudomonas, Penicillium, Aspergillus* etc. PSM convert non available inorganic phosphate into soluble organic phosphates, which can be utilized by crop plants.
- 5) **<u>Mycorrhizae</u> -** is a symbiotic association of fungi with roots of plants. Fungus absorbs nutrients from the soil which are required to the host cells & in turn the fungus takes their food from the host.
- 6) **<u>Cellulolytic microorganisms -</u>** Enhance the degradation of organic matter in soil, as well as enhance the rate of decomposition in compost pit.
- 7) **<u>Organic fertilizers</u>** - organic fertilizers (manure, compost, vermicompost) are also considered as biofertilizers
- Nobbe and hiltner (1895, USA) produced the first *rhizobium* biofertilizer under the brand name “nitragin” for 17 different legumes.



<u>Production Technology</u> –

Biofertilizer can be produced as liquid based preparation or carrier based preparations. Liquid based biofertilizer involves Culturing of microorganisms in a liquid nutrient medium, where as carrier based fertilizers involves the mixing of microbial broth culture with the carrier material (such as Peat soil, lignite, vermiculite, charcoal, press mud, farmyard manure and soil mixture) and finally packing.



- **<u>Advantages of Liquid based Biofertilizer</u> -** Longer shelf life, Easier to produce, Temperature tolerant, High cell count, Contamination free, More effective.
- **<u>Advantages of Carrier Based Biofertilizer –</u>** Cheap, Easier to produce, Less investment.

Role of Biofertilizers in soil fertility and Agriculture

- Biofertilizers are known to play vital roles in soil fertility, crop productivity and production in agriculture. Some of the important functions or roles of Biofertilizers in agriculture are:

1. Supplement chemical fertilizers for meeting nutrient demand of the crops.
2. Replace 25-30% chemical fertilizers. .
3. Add 20-200 kg Nitrogen per hector per year and thereby increases 15-25 % of total crop yield.
4. Minimize the use of chemical fertilizers.
5. Decompose plant residues, and stabilize C: N ratio of soil.
6. Use of biofertilizer results in increased mineral and water uptake, root development, vegetative growth and nitrogen fixation.
7. Some Biofertilizers *(eg, Rhizobium BGA, Azotobacter* sp) stimulate production of growth promoting substance like vitamin-B complex, Indole acetic acid (IAA) and Gibberellic acids etc and help to maintain soil fertility.
8. Phosphate mobilizing or phosphorus solubilizing Biofertilizers (bacteria, fungi, mycorrhiza etc.) converts insoluble soil phosphate into soluble forms due to which crop yield may increase by 10 to 20%.
9. They act as antagonists and suppress the incidence of soil borne plant pathogens and thus, help in the bio-control of diseases.
10. They are cheaper, pollution free, No adverse effect on plant growth.
11. Improve physical properties of soil such as texture, soil tilth, and structure.
12. Improve water holding capacity of soil and soil health in general.
13. They improve soil fertility and soil productivity.
14. Biofertilizers containing cellulolytic and lignolytic microorganisms enhance the degradation of organic matter in soil.
15. *Azotobacter* biofertilizer when applied to many non-leguminous crop plants, promote seed germination.
16. Plays important role in the recycling of plant nutrients.

Biological Pest Control (Biopestisides/Bioinsecticides)

- Nowadays the use of chemical pesticides is being discouraged due to
- i) Insect Resistance ii) Cause Environmental Pollution iii) Expensive Costs.
- iv) Poisonous & nonbiodegradable, v) non target specific vi) Some can cause health problem through bioaccumulation & biomagnifications.
- Therefore the use of bioinsecticides & an integrated approach to insect pest control have become necessary.
- **Biological control** is a method of controlling pests (including insects, mites, weeds and plant diseases) using other living organisms.
- Bioinsecticides are i) Biodegradable (Environmental Friendly), ii) Nontoxic iii) Cost Effective, iv) Safer v) Active, vi) Efficient vii) Non Hazardous.

Definition:

- Biological agents which are used in controlling insects pests are called bioinsecticides or biopesticides.

Biocontrol agents

- Biological control uses predators, parasites, or disease organisms to attack unwanted pests.
- i) Various predators of the pest insects
- ii) The insect parasites
- iii) The microorganisms pathogenic to pest insects.
- **Biological Methods Of Pest Control**
- Number of biological methods of pest control has been devised in recent years an alternative to the chemical pesticides. Some of these areas follows
- **1) Predators** - Predators are free-living species that directly consume a large number of preys during their whole lifetime. For example Ladybugs are voracious predators of aphids, and also consume mites, scale insects and small caterpillars.
- **2) Pathogens** - Pathogenic micro-organisms include bacteria, fungi, and viruses.
- Bacteria - *Bacillus thuringiensis* is the most widely applied species of bacteria used for biological control of Lepidopteran (moth, butterfly), Coleopteran (beetle) and Dipteran (true flies) insect pests. The bacteria are available in sachets of dried spores which are mixed with water and sprayed onto vulnerable plants such as brassicas and fruit trees.
- Fungi - Fungi that cause disease in insects known as entomopathogenic fungi used as biocontrol agent. *Beauveria bassiana* is used to manage a wide variety of insect pests including whiteflies, thrips, aphids and weevils. Other Examples of entomopathogenic

fungi are - *Paecilomyces fumosoroseus* (against white flies, thrips and aphids), *Metarhizium* spp. (against beetles, locusts and grasshoppers, Hemiptera, spider mites and other pests), *Lecanicillium* spp. (against white flies, thrips and aphids).

- Viruses - The most common and effective type of insect viruses are the baculoviruses, which can infect over 600 insect species worldwide. Most baculoviruses infect caterpillars, which are the immature form of moths and butterflies. Insect viruses are potent population regulators of many caterpillar pests.
- **3) Sterilization strategy**- In this method, the males of the pest species are sterilized by exposure to radiation & such sterile makes are, released in the making season to complete with the normal fertile males. This method checks insects population screwworms fly, frieutol fruit fly has been successfully controlled by sterilization strategy.
- **4) Natural insecticides**- Natural insecticides are naturally occurring chemicals extracted from plants. Some examples are nicotine, neem, rotenone, and pyrethrins.
- For eg, pyrethrins from pyrethrum, nicotine from tobacco, azadirachtin from neem (*Azadirachta indica*). These are insect repellants insecticides obtained. Insecticides obtained from microbial sources also come under this category.
- Rotenone – it is extraction from the roots and stems of plant belonging to the genera *Lonchocarpus* and *Derris*. It is applied as a spray on fruits and row crops. It is a potentially lethal toxin for aphids, cockroaches, houseflies, corn borers, Mexican bean beetles, and mosquitoes. It kills potato beetles, cucumber beetles, flea beetles, cabbage worms, raspberry beetles, and asparagus beetles, as well as most other arthropods.

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