are converted into organic compounds in the living organisms. The energy is derived from the solar source for photosynthesis. The dead organic matter is converted into inorganic matter by a process called ***mineralization***.

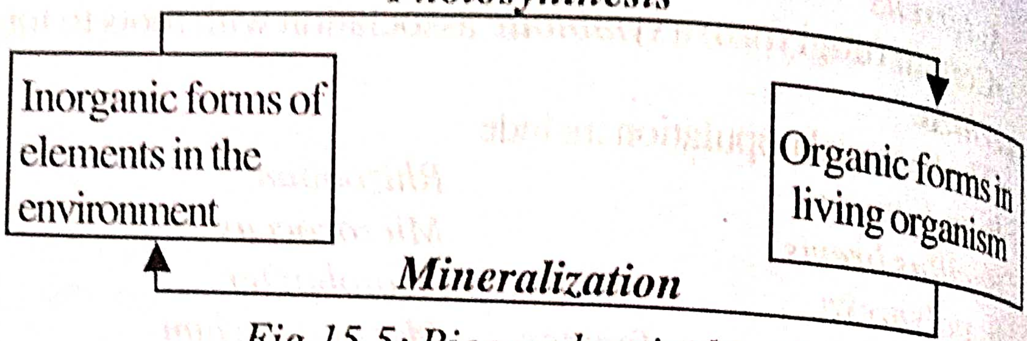

Fig.15.5: Biogeochemical cycle.

Mineralization is essential for the maintenance of life on our planet. Microorganisms play a very important role in biogeochemical cycles.

Soil microorganisms serve as biochemical agents for the mineralization of organic carbon, nitrogen, sulfur, phosphorus and other compounds. As a result of mineralization, these elements are returned to the environment, for reuse. Thus the chemical elements are shed and reused.

The following are the important biogeochemical cycles.

1. *Nitrogen cycle*
2. *Carbon cycle*
3. *Sulfur cycle*
4. *Phosphorus cycle*
5. *Iron cycle*

1. Nitrogen Cycle

The cyclic movement of nitrogen between the living organisms and the environment is referred to as ***nitrogen cycle.***

1. Nitrogen in the Living Systems

Nitrogen is a key building block of the ***protein*** molecule. It is an absolutely necessary component of the protoplasm of plants, animals and microorganisms. It is an essential constituent of the following biologically significant molecules:

1. *Amino acids* and *proteins*
2. *Nucleic acids*
3. *Enzymes*
4. *Hormones*
5. *Vitamins*
6. *Chlorophyll and other pigments*
7. *Purines*
8. *Pyrimidines*
9. *Porphyrins*
10. *Alkaloids*

2. Nitrogen in the Environment

Nitrogen makes up 79% of the volume of the atmosphere. This large reservoir (3.8 x 10^{15} metric tons) is very slowly cycled. Large reservoirs of nitrogen are present in igneous (1.5 x 10^{16} metric tons) and sedimentary rocks (4 x 10^{15} metric tons) as *bound ammonia*. These are essentially unavailable reservoirs. Several inorganic and organic nitrogen compounds can be considered as components of nitrogen cycle. The inorganic nitrogen compounds include N_2 gas, nitrate ion, nitrite ion and hydroxylamine. The nitrogen atom can possess a variety of oxidation numbers.

Nitrogen compounds	Nitrate ion	Nitrite ion	Hyponitrite ion	N_2 gas	Hydroxylamine	Ammonia
Formula	NO_3^-	NO_2^-	$N_2O_2^{2-}$	N_2	NH_2OH	NH_3
Oxidation number	+5	+3	+1	0	-1	-3

Thus, in nature, nitrogen may exist in either a highly oxidized form (NO_3^-) or a highly reduced state (NH_3). The inorganic nitrogen salts, ammonium, nitrite and nitrate are highly water soluble. They are distributed in dilute aqueous solution throughout the ecosphere. They form small, actively cycled reservoir.

Living and dead organic matter also provide relatively small, actively cycled reservoir. In temperate climates, humus forms a substantial and relatively stable nitrogen reservoir. The nitrogen of the humus becomes available through mineralization. In tropical climates, the temperature and humidity favour the rapid and direct mineralization of organic matter, limiting the accumulation of litter and humus.

Geological deposits of more readily available combined nitrogen are rare. The only natural accumulation of nitrate occur on some islands. These nitrate deposits are derived from the decomposition of guano deposited by sea birds. The dry climate of these islands has prevented the leaching of nitrate.

Nitrogen Cycling

Nitrogen cycle involves the following steps: (Fig.15.6)

1. Proteolysis

4. Nitrate reduction

2. *Ammonification* 5. *Denitrification*
3. *Nitrification* 6. *Nitrogen fixation*

1. Proteolysis

Proteolysis is *the enzymatic hydrolysis of proteins of de... organic matter into amino acids*. This is brought about by the fo... lowing microbes :

1. *Clostridium hystolyticum*
2. *Cl. sporogenes*
3. *Proteus vulgaris*
4. *Pseudomonas fluorescens*
5. *Bacillus cereus*
6. *Hay bacillus*
7. *Molds*
8. *Fungi*
9. *Actinomycetes.*

These microorganisms produce two types of ***proteolytic enzymes***. 1. ***Proteinases*** and 2. ***Peptidases***.

Proteinases convert proteins into smaller units of ***peptides***. Peptidases convert peptides to ***amino acids.*** The overall reactions may be summarized:

Proteins —*Proteinases*→ Peptides —*Peptidases*→ Aminoacids

The end products of proteolysis are ***amino acids.***

2. Ammonification

Ammonification is a *process in which organic nitrogen is converted to ammonia*. The amino acids are utilized as ***nutrients*** by microorganisms. Under aerobic conditions, the amino groups are removed from amino acids with the liberation of ***ammonia***. This is known as ***oxidative deamination.***

Similarly, ***urea*** present in the urine of man and animals is also decomposed by microorganisms such as, *Micrococcus* and *Proteus*. Ammonium is released from urea.

3. Nitrification

Nitrification is a *process in which ammonia is oxidized to nitrate*. This process consists of two steps.

In the first step, ammonia is oxidized to nitrite. This is called ***nitrosification.***

$$2NH_3 + 3O_2 \longrightarrow 2HNO_2 + 2H_2O + \text{Energy (70 Kcal)}$$

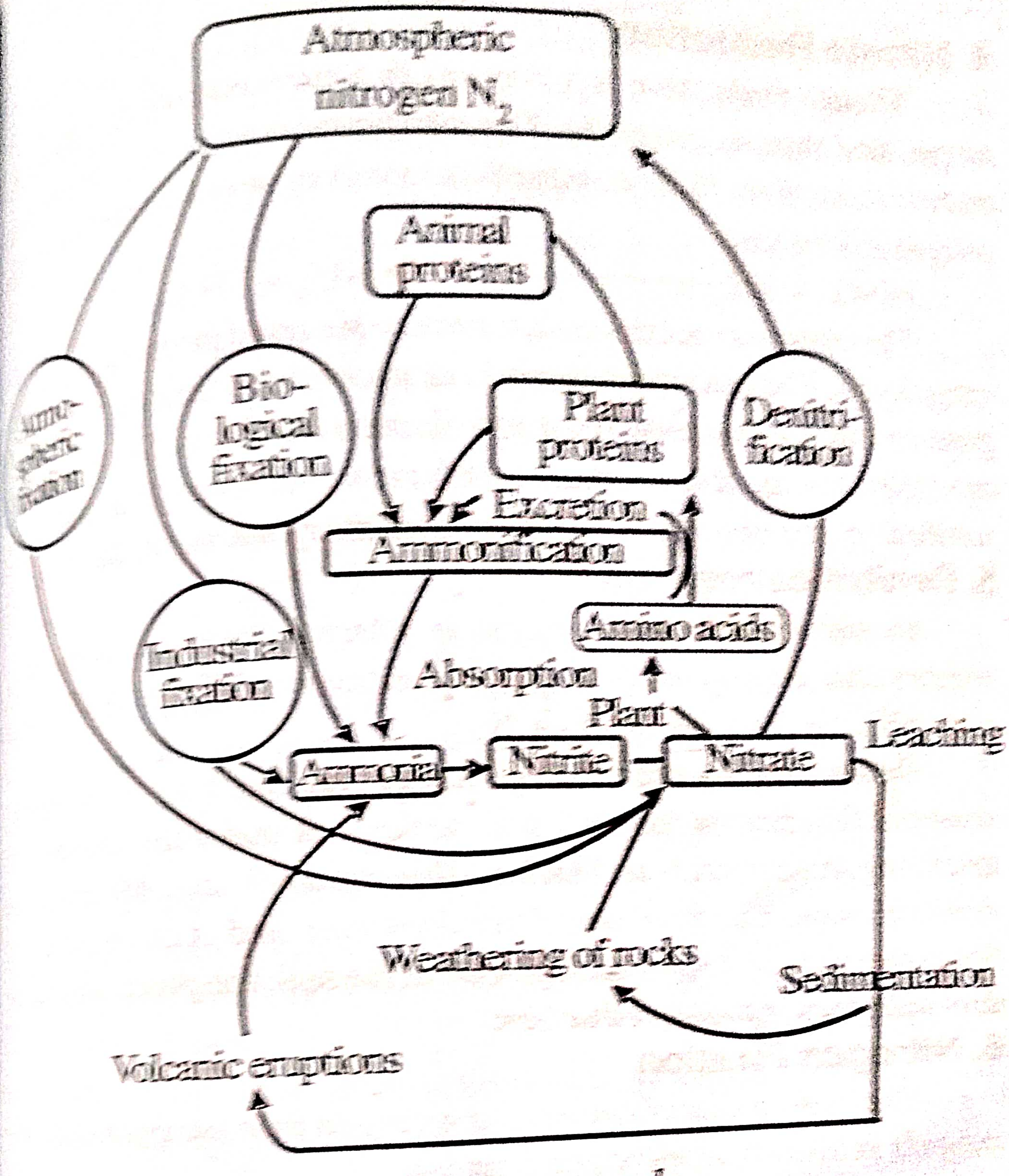

Fig.15.6: Nitrogen cycle.

The oxidation reaction provides energy and this is carried out by bacteria such as *Nitrosomonas, Nitrosococcus, Nitrosospira* and *Nitrosocystis.*

In the second step, the nitrites are oxidized to nitrates.

$$2HNO_2 + O_2 \longrightarrow 2HNO_3 + \text{Energy (20Kcal)}$$

The oxidation reaction also provides energy and this is carried out under the influence of bacteria of the genus *Nitrobacter.* Nitrates are readily used by plants and many microorganisms.

However, several species of fungi (Eg. *Penicillium, Aspergillus, Cephalosporium*) oxidize both ammonia to nitrite and nitrite to nitrate.

4. Nitrate Reduction

Nitrate reduction is a *process in which nitrate is reduced to nitrite and then to ammonia.* The nitrification process is reversed in nitrate reduction. Nitrate reduction involves several reactions. The overall reaction is:

$$HNO_3 + 4H_2 \longrightarrow NH_3 + 3H_2O.$$

This reaction occurs under ***anaerobic*** conditions, eg. in water-logged soil. The reaction is carried out under the influence of micro-organisms such as bacteria, yeast and filamentous fungi. Since these microorganisms are able to obtain cellular nitrogen through ammonia assimilation, the process is called ***assimilatory nitrate reduction.***

5. Denitrification

Denitrification is *a process in which nitrates are reduced to nitrites and subsequently to gaseous nitrogen* ($NO_2 \rightarrow N_2O \rightarrow N_2$)

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

Denitrification occurs under ***anaerobic*** conditions, eg., during seasonal flooding of the land. It is carried out under the influence of microorganisms such as *Thiobacillus denitrificans, Micrococcus denitrificans, Clostridium, Pseudomonas* and *Achromobacter*. Since there is net loss of nitrogen from the soil, the process is called ***dissimilatory nitrate reduction.***

6. Nitrogen Fixation

The conversion of molecular nitrogen into nitrogenous compounds is known as ***nitrogen fixation.***

2. Carbon Cycle

The cyclic movement of carbon between the living organisms and the environment is referred to as ***carbon cycle.***

Carbon in the Biotic Environment

All living organisms require carbon in some form. Carbon is an important ***basic element*** of organic compounds such as carbohydrates, fats, proteins, etc. It serves as the cornerstone of cell structure. Almost 50 percent of living matter is composed of carbon. Every form of plant or animal life requires carbon for survival. Most of the energy is supplied by the oxidation of carbon compounds.

1. Atmospheric Nitrogen Fixation

*The conversion of molecular nitrogen into a nitrogenous compound through lightning or meteorite trails or cosmic radiation is known as **atmospheric nitrogen fixation.***

Lightning, meteorite trails and cosmic radiation provide high energy for this physico-chemical nitrogen fixation. It takes place in several steps.

At first the atmospheric nitrogen combines with O_2 under the influence of high energy to produce ***nitric oxide***.

$$N_2 + O_2 \xrightarrow{\text{high energy}} 2NO$$

The nitric oxide is then oxidized to ***nitrogen peroxide*** (NO_2) in presence of O_2.

$$2NO + O_2 \xrightarrow{\textit{Oxidation}} 2NO_2$$

During rain the NO_2 combines with rain water to form ***nitrous acid or nitric acid.***

$$2NO_2 + H_2O \longrightarrow HNO_2 + HNO_3$$

The nitrous acid or nitric acid comes to the ground along with rain water. In the soil, nitric acid reacts with alkali radicals to produce *nitrates* and *nitrites*.

2. Industrial Nitrogen Fixation

The conversion of molecular nitrogen into a nitrogenous usable compound through a man made chemical method is known as ***industrial nitrogen fixation.***

The industrial process for example ***Haber-Bosch Process***, needs high temperature and pressure to produce ***ammonia*** from N_2.

$$N_2 + 3H_2 \xrightarrow[\text{200 atmospheric pressure}]{400^0C} 2NH_3$$

3. Biological Nitrogen Fixation

The conversion of molecular nitrogen into a nitrogenous usable compound through the agency of living organisms is known as ***biological nitrogen fixation.***

Some microorganisms fix atmospheric nitrogen very strongly at one atmospheric pressure and at a temperature of 20°C by using the enzyme *nitrogenase.*

$$N_2 + 3H_2 \xrightarrow{Nitrogenase} 2NH_3$$

Biological nitrogen fixation contributes 90% of fixed nitrogen on earth.

Biological nitrogen fixation is carried out by bacteria and blue green algae. The following are the biological nitrogen fixers:

1. *Rhizobium*	4. *Beijerinckia*	7. *Bacillus*
2. *Frankia*	5. *Klebsiella*	8. *Clostridium*
3. *Azotobacter*	6. *Enterobacter*	9. *Desulfovibrio*

There are two main types of biological nitrogen fixation.

1. Symbiotic nitrogen fixation.
2. Non-symbiotic nitrogen fixation.

1. Symbiotic Nitrogen Fixation

Symbiotic nitrogen fixation** is the fixing of molecular nitrogen by bacteria living in the roots of leguminous plants.* This was first demonstrated by the French Chemist ***Boussingault in 18[illegible]. The root nodule bacteria were later isolated by ***Beijernick*** in 18[illegible]. *Rhizobium* is the symbiotic N_2 fixing bacterium.

Since neither the plant nor the bacterium can fix the atmospheric nitrogen independently, the process is called ***symbiotic nitrogen fixation.***

Fig.15.12: Bacteroids from the nodules of pea plants.

Rhizobia are ***Gram - negative***, motile, aerobic, non-spore forming bacteria.

They are mainly ***rod-shaped.*** But a variety of morphological shapes are also observed.

The ***nodules*** are the ***sites of nitrogen fixation***. The formation of nodules in the roots of leguminous plants is known as ***nodulation process.***

In the development of nodule, initially, the plant releases some *growth factors* into the root zone. These substances are stimulatory to the nodule bacteria.

These growth factors attract the bacteria. As a result, the bacteria move and aggregate around the root hairs.

The rhizobia produce ***indole acetic acid*** (IAA) as a stimulatory substance.

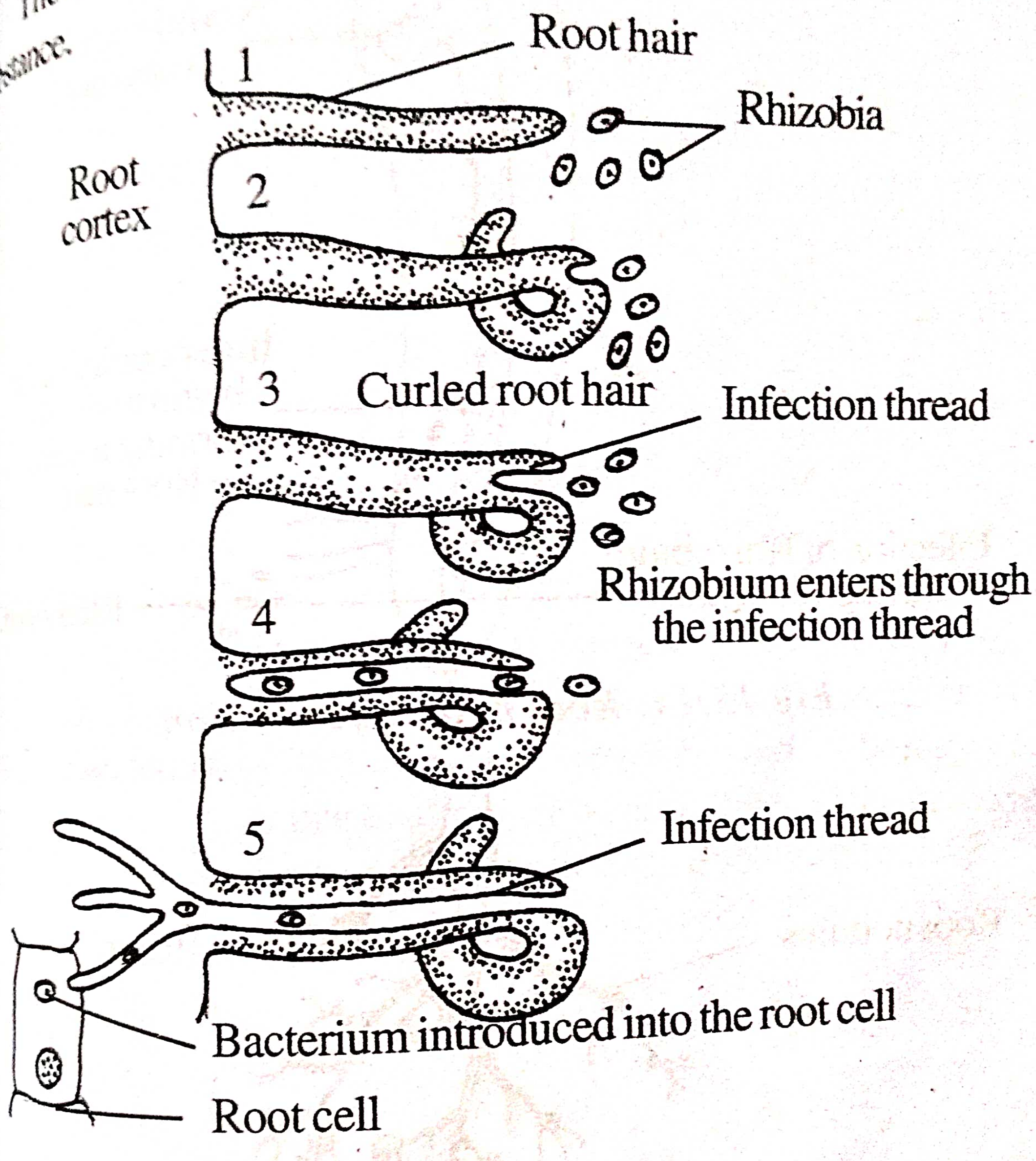

Fig.15.13: Root hair showing curling and infection thread.

The IAA brings about the ***curling*** of the tip of root hairs. The bacteria ***aggregate*** at the curled root hairs.

The *Rhizobium* penetrates the root hair. During penetration the microbe produces an enzyme called ***polygalacturonase***. The enzyme acts on the pectin of plant cell wall and makes easy penetration.

As the microbe penetrates into the root-hair, a hypha-like ***infection thread*** is formed.

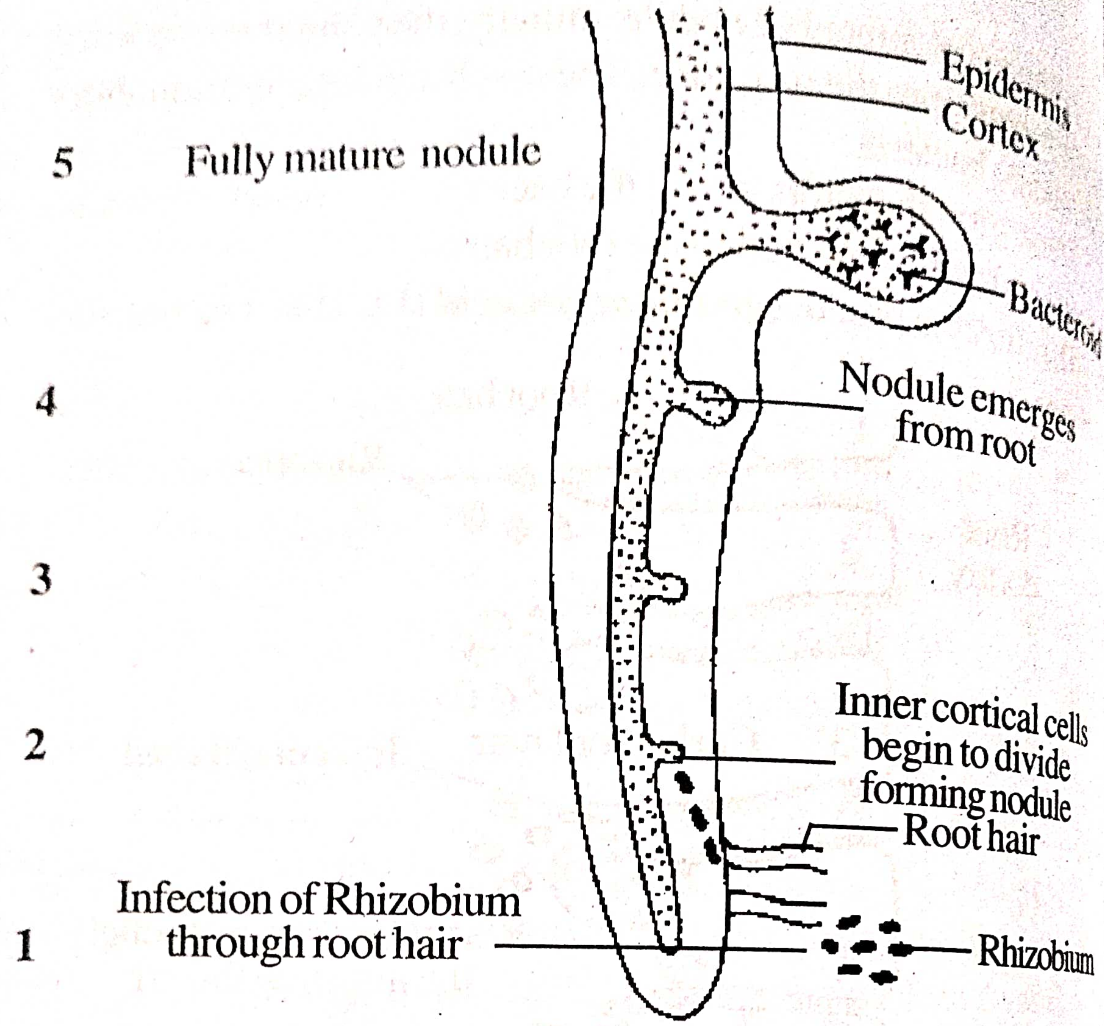

Fig.15.14: Root nodule formation.

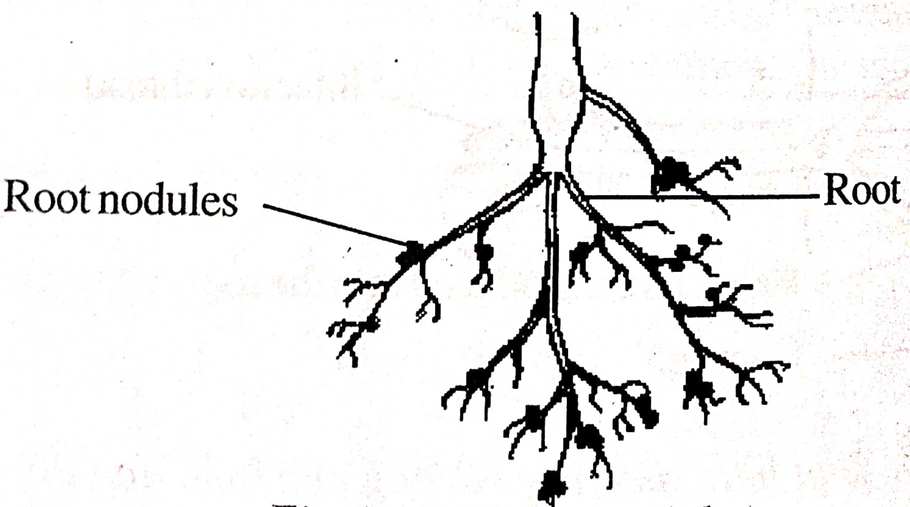

Fig.15.15: Root nodules.

The infection thread grows inwards and it becomes ***branched***. The branches reach the ***inner cortex*** of the root.

The bacteria pass through the infection thread and are released into the ***cytoplasm*** of the root cells.

As the bacteria invade the cortical cells, ***doubling*** of chromosome number takes place in the root cells. As a result, ***disomatic tissues*** or ***tetraploid cells*** are formed.

The bacterial cells multiply and colonize inside the host cells until available space is filled. They become dormant cells called *bacteroids*. The bacteroid is *irregularly shaped*. Some may be *star-shaped*, some may be *clubbed*. The bacteroids are capable of N_2 fixation.

The tetraploid cell and the surrounding diploid cells proliferate to form the *nodule*.

The bacteroids float in a reddish pigment called *leghaemoglobin* found in the cytoplasm of host cells. The pigment leghaemoglobin (similar to haemoglobin of RBC) acts as a very efficient *oxygen scavenger*. It combines with O_2 and provides a favourable *anaerobic* condition for the activity of the enzyme *nitrogenase*. At the same time, it supplies O_2 for the bacteroids.

Table.15.4: Specificity of bacteria and legumes.

No.	Bacteria	Legumes
1	*Rhizobium melitoli*	Alfalfa, sweet clover
2	*Rhizobium trifoli*	Red, white, and other clovers.
3	*Rhizobium leguminosarum*	Peas, lentil, vetch.
4	*Rhizobium phaseoli*	Beans
5	*Rhizobium lupini*	Lupines
6	*Rhizobium japonicum*	Soybeans

The nodules of legumes show significant differences as follows:

Legumes	*Morphology of nodules*
Red and clovers	- Club-shaped & lobed
Alfalfa	- Longer and branched
Cowpea, planut & lina bean	- Spherical

The leguminous plant, the bacteria and the nodule constitute the system for symbiotic nitrogen fixation. Here both the bacteria and the plant benefit by the association.

The bacteria obtain their nutrients and source of energy from the plant and in turn, fix atmospheric nitrogen and make it available to the plant. The bacterium contains *nitrogenase* for N_2 fixation.

The nitrogen is fixed in the nodules with the help of the enzyme *nitrogenase*.

2. Non-Symbiotic Nitrogen Fixation

The conversion of atmospheric nitrogen into ammonia by free living microorganisms is called ***non-symbiotic nitrogen fixation***. These organisms are called ***non-symbiotic nitrogen fixers***.

These organisms include some ***bacteria*** and a few ***cyanobacteria*** (Blue green algae).

Bacteria : *Azotobacter, Azomonas, Azospirillum.*

Cyanobacteria : *Anabaena, Nostoc.*

The non-symbiotic nitrogen fixers, fix about 20 to 50 kg/acre annually. Among non-symbiotic nitrogen fixers, the cyanobacteria are more efficient in fixing nitrogen. They fix 10 times more nitrogen than that of other free living bacteria.

Mechanism of Non-symbiotic nitrogen fixation

The mechanism of non-symbiotic nitrogen fixation has been well studied in Cyanobacteria, especially in *Anabaena.*

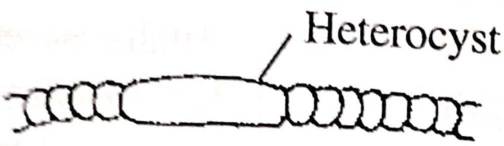

Fig.15.16: Anabaena.

In *Anabaena,* nitrogen fixation takes place in a specialized cell called ***heterocyst***. This cell provides an ***anaerobic condition*** for nitrogen fixation.

In N_2 fixation, N_2 is converted into ammonia. This conversion is a ***reduction reaction.*** Six electrons are needed to reduce 1 molecule of N_2 to produce 2 molecules of ammonia.

$$N_2 + 6H^+ + 6e \longrightarrow 2NH_3$$

This process requires the break up of three covalent bonds in nitrogen molecule $N \equiv N$.

$$N \equiv N \xrightarrow{6H} 2NH_3$$

The reduction of N_2 is catalysed by an enzyme called ***nitrogenase.*** It is sensitive to O_2 and it loses this activity in the presence of O_2. It functions in an ***anaerobic*** condition.

Mechanism of Biological N_2 Fixation

The mechanism of biological nitrogen fixation has been well studied in the non-symbiotic N_2 fixer, *Azotobacter*, an aerobic bacterium.

The biological nitrogen fixation occurs ***smoothly*** in a comfortable temperature of 20°C and a pressure of 1 atmosphere. But in industrial N_2 fixation, it takes place in a drastic environment of 400°C and in a high pressure of 200 atmosphere.

Nitrogen fixation is carried out by the irregular shaped ***bacteroid*** and not by the rod-shaped *Rhizobium.*

The process of N_2 fixation occurs in an ***anaerobic*** condition. However, the bacteroid lives in an aerobic condition. The aerobic and anaerobic conditions are provided by the protein pigment, ***leghaemoglobin.***

Biological N_2 fixation requires the following components :

Atmospheric nitrogen (N_2)	*Leghaemoglobin*
Hydrogen	*ATP*
Nitrogenase	*Ferridoxin,* etc.

In N_2 fixation, N_2 is converted into soluble ***ammonia***. This conversion is a ***reduction reaction***. Six ***electrons*** are needed to reduce 1 molecule of N_2 to produce 2 molecules of ammonia.

$$N_2 + 6H^+ + 6e^- \longrightarrow 2NH_3$$

This process requires the break up of three covalent bonds ina nitrogen molecule (N≡N).

$$N \equiv N \xrightarrow{6H} 2NH_3$$

The reduction of N_2 is catalysed by an enzyme called ***nitrogenase***. The enzyme is made up of two components. Component I is called ***iron-molybdenum protein (Fe-Mo protein)*** or ***dinitrogenase***. The component II is called ***iron-protein component (Fe- protein)*** or ***dinitrogen reductase.***

The enzyme has an ***active site***. The inert molecule of N_2 can be tightly bound to the active site. The reduction of N_2 to NH_3 takes place at the active site.

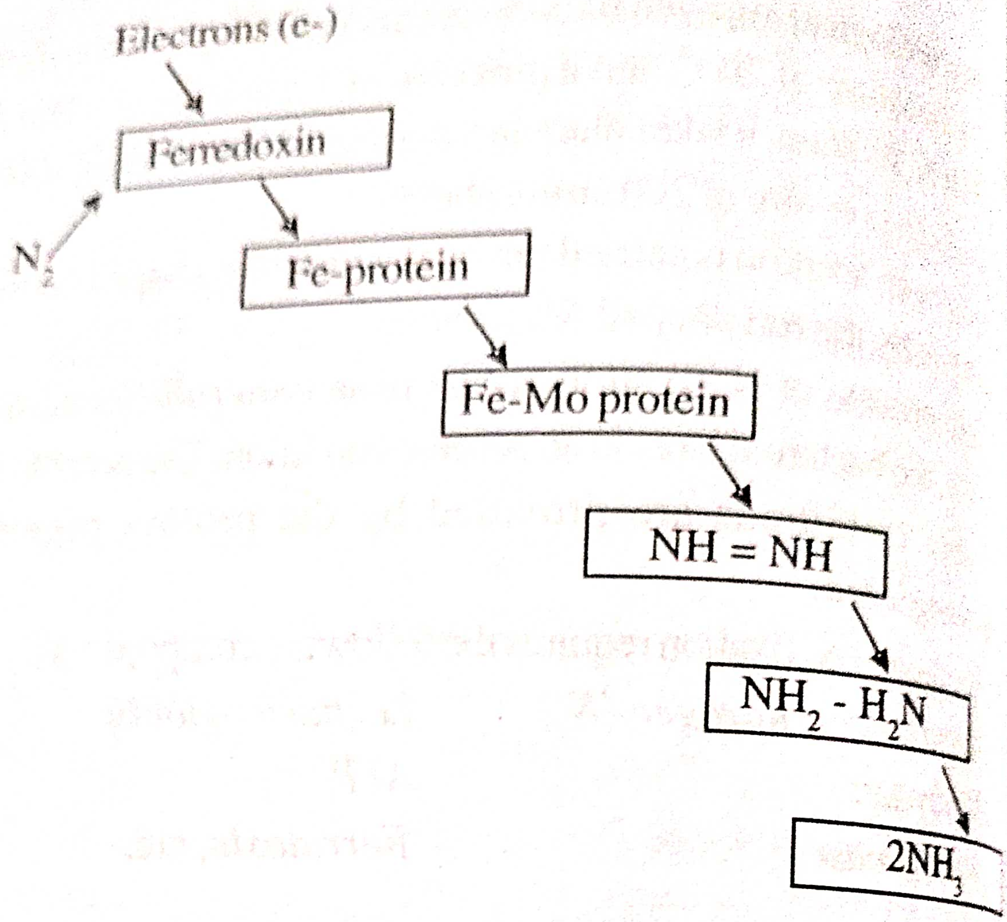

Fig.15.17: Mechanism of N_2 fixation. Arrows represent the transfer of electrons.

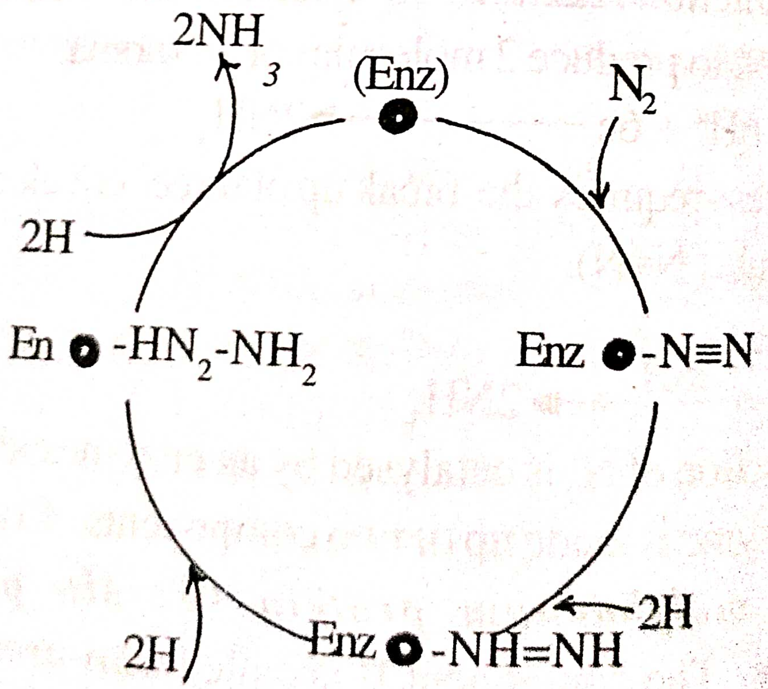

Fig.15.18: Biochemical aspects of N_2 fixation.

The 6 electrons for the reduction of N_2 into NH_3 are donated by ***Krebs cycle*** and ***electron transport system***. These electrons are accepted by ***ferredoxin*** or ***flavodoxin (fd).***

$$Fd\ (oxidised) + H \xrightarrow[\text{Nitrogenase}]{} Fd\ (Reduced)$$

The reduced ferredoxin in turn reduces the ***Fe-protein compo-*** of the nitrogenase enzyme.

$$Fd\ (reduced) + Nitrogenase + N_2 \longrightarrow Fd\ (oxidised) + Nitrogenase\text{-}N_2(reduced)$$

The reduced Fe-protein of nitrogenase is activated by ATP.

$$Nitrogenase - N_2\ (reduced) + ATP \longrightarrow Nitrogenase + ADP + Pi$$

The reduced activated Fe-protein donates its electrons to the Fe-Mo protein component of the nitrogenase enzyme. Hence the Fe-protein is oxidised and the Fe-Mo protein is reduced.

The reduced Fe-Mo protein in turn reduces the N_2 into ammonia (NH_3).

The reduction of N_2 to NH_3 occurs in three stages. First of all molecular nitrogen ($N \equiv N$) is reduced to ***diimide*** ($NH = NH$). The *diimide* is reduced to ***hydrazine*** NH_2-NH_2. The hydrazine is finally reduced to two NH_3 molecules.

Biofertilizers

Biofertilizer is a preparation of microorganisms added to the soil to improve the fertility of the soil.

The biofertilizers synthesize and release certain useful compounds which are readily utilized by the plant for their growth. Biofertilizers are used to reduce the use of chemical fertilizers.

Organisms used as Biofertilizers

Bacteria, blue green algae and a fern are used as ***biofertilizers***.

Bacteria : *Rhizobium*
Azotobacter
Azospirillum

Blue green Algae : *Nostoc*
Anabaena

Role of Biofertilizers

Some of the biofertilizers convert atmospheric N_2 into nitrogenous compounds which are used by plants. Eg. *Rhizobium, Anabaena, Nostoc*, etc.

The root nodules protect the *Rhizobium* from the destru... effects of atmospheric oxygen. In addition they give a suitable anae... condition for nitrogen fixation.

Rhizobium reduces molecular nitrogen to ammonia.

In this process, the enzyme ***nitrogenase*** plays a key role. ... ammonia may then be interconverted by host cell enzymes to ... usable forms such as ***nitrite*** and ***nitrate*** ions, amino acids ... nitrogenous bases.

Leguminous plants with *Rhizobium* containing root nodules ... able to fix ten times more nitrogen than free living diazotrophs. ... improves the crop yield and enriches the fertility of the soil.

The second way, in which plants can get usable nitro... compounds, involves nitrogen fixation by ***free living diazotroph...*** They include *Clostridium, Azotobacter, Enterobacter, Chlorobi...* and *Rhodospirillum.*

Microorganisms as Biofertilizers

The nutrients of biological origin added to the soil to enri... the soil fertility are called ***biofertilizers.***

They are often known as ***microbial fertilizers*** or ***microbi... inoculants.***

A biofertilizer may contain ***nitrogen fixing microbes*** ... ***phosphate solubilizing microbes*** or ***spores of VAM fungi.***

It is supplied to the soil either by ***seed treatment*** or by *spread...* it over the field during cultivation. Biofertilizers reduce the use ... chemical fertilizers in agriculture and cost of production.

The ***nitrogen biofertilizer*** may have nitrogen fixing bacteria ... blue-green algae.

The nitrogen fixing bacteria include *Rhizobium, Azospirillu...* *Azotobacter, Azotococcus,* etc. Blue green algae such as *Anabae...* *Aulosira, Nostoc, Plectonema* and *Tolypothrix* are used as nitro... biofertilizers.

Preparations of phosphate solubilizing bacteria such as *Bacil... megaterium, B. subtilis, Xanthomonas* and *Pseudomonas,* are us... as ***phosphate biofertilizers.***

The spores of VAM fungi like *Glomus, Gigaspora, Acaulospora, Sclerocystis* and *Endogone* are used as VAM biofertilizers.

Biofertilizers have the following advantages:

i. Biofertilizers ***reduce the use of chemical fertilizers in agriculture.***

ii. They ***never cause pollution*** in air, water and land.

iii. They ***secrete plant growth hormones*** to increase the plant growth.

iv. They ***reduce*** the attack by ***soil-borne pathogens.***

v. They ***improve*** the ***quality of soil*** for more productivity.

vi. They can be ***mass produced*** by using renewable wastes.

vii. ***No special care*** is required while using biofertilizers.

viii. The farmers themselves can grow ***BGA*** biofertilizers and *Azolla* biofertilizer in their own lands.

Rhizobium

Rhizobium is a Gram negative, aerobic, rod-shaped bacterium. It contains a ***refractive granule.***

It is a soil bacterium present in large numbers in the rhizosphere of legume roots.

Rhizobium invades the roots of legumes and forms nodules on the roots. Inside the root nodules, the bacteria exist in various *pleomorphic* forms called ***bacterioids.***

The bacterioids fix the atmospheric nitrogen into ammonia.

They provide the fixed nitrogen for plant's use and draw nourishments from the root cells.

This type of association is called ***symbiosis.***

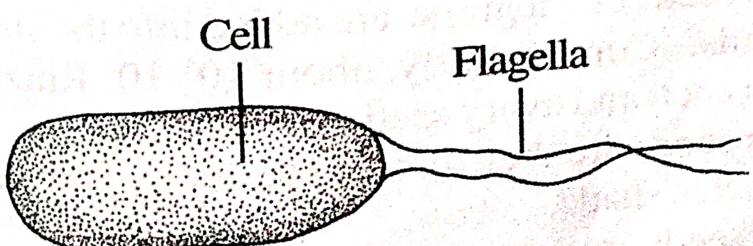

Fig.17.1: Rhizobium.

Different species of *Rhizobium* can fix 50-200 kg nitrogen/ year in leguminous crops. Therefore, they have been recommended as nitrogen biofertilizers in agriculture.

Different species of *Rhizobium* show a great degree of specificity. Hence they can be used as biofertilizers only for the specific crops-

1. *R. meliloti* (Medic-*Rhizobium*) : Lucerne and Fenugreek.
2. *R. trifolii* (Clover-*Rhizobium*) : Egyptian clover and clover.
3. *R. leguminosarum* (Pea-*Rhizobium*) : Lentil, pea, khesari (Lathyrus) and vetch.
4. *R. phaseoli* (Bean-*Rhizobium*) : Bean, kidney bean and French bean.
5. *R. lupini* (Lupin-*Rhizobium*) : Lupines and white lupines.
6. *R. japonicum* (Soybean-Rhizobium) : Soybean.
7. *Rhizobium sps.* (Chickpea-*Rhizobium*) : Chickpea and subabul.
8. *Rhizobium sps.* (Cowpea-*Rhizobium*) : Sunnhemp, cluster bean, peanut, jack bean, lablab, horsegram, moth bean, green gram, blackgram and pigeonpea.

Field Application of Rhizobium Inoculant

Rhizobium inoculants are recommended for various leguminous crops by ***seed treatment:***

1. A 50 g of cane sugar is dissolved in 500 ml of water and boiled for 15 minutes.
2. 200 g of gum arabic is added to the boiling sugar solution and stirred well to dissolve it. The sticker solution so formed is cool down.
3. Then a 200 g of *Rhizobium* inoculant is added into the sticker solution and mixed well to get a biofertilizer slurry.
4. The seeds of a legume are added into the slurry and mixed well by hands. Consequently, about 10^5-10^6 Rhizobial cells get adsorbed on each and every seed.
5. The seeds are then allowed to dry by spreading them on a polytene sheet in shade.
6. The seeds are sown in the main field.

While adopting this method, the farmers should take the following precautions-

- After seed inoculation, the seeds should not be stored for more a day.
- No fungicide should be used within one day after sowing the s.
- Use of chemical fertilizers should be avoided for more than a immediately after sowing.
- Pesticides should be used in small amounts, if required.

Crop Response

- Seed inoculation of a proper *Rhizobium* strain increases the growth and yield of many legumes. *Rhizobium* usually increases the yield of legumes upto 10-35%.
- In *Cajanus cajan*, it increases yields upto 4-40% in various states of India. In *Cicer aritinum*, it boots the yield upto 4-67%.
- In *Vigna mungo*, it increases the yield upto 4-29%.
- In *Lens culinaris*, it improves the yield upto 21%.
- The crop rotation of *Rhizobium* inoculated legumes with cereals increases the yield of the subsequent cereal crop. For example, crop rotation of *Cajanus cajan* with wheat increases the wheat yield upto 4%. Similarly, crop rotation of *Lens culinaris* with rice has increased the rice yield upto 13.2%.

Azotobacter

Azotobacter is a gram negative, non-symbiotic, nitrogen fixing bacterium. It is an aerobic bacterium present in large numbers in the rhizosphere soils. *Azotobacter* includes six species-*Azotobacter beijerinckii, A.paspali, A.chroococcum, A.haplophilus, A.vinelandii* and *A.miscellus*.

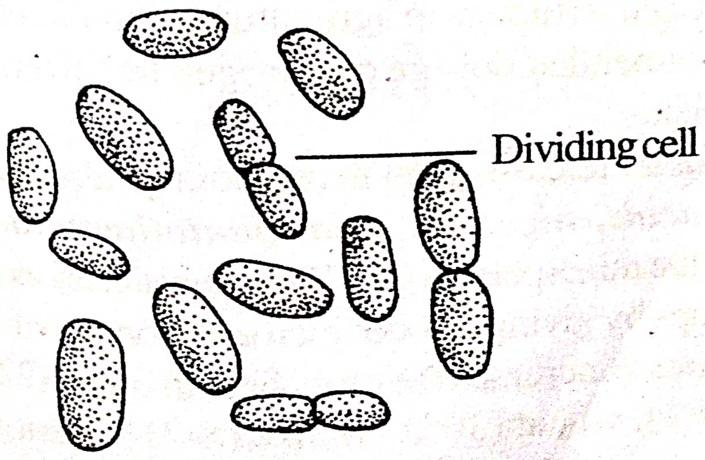

Fig.17.2: Azotobacter.

Azotobacter fixes about 20-40Kg N/ha/year. Furt... produces plant growth promoting substances like ***IAA***, gibbe... ***acid*** and ***vitamins*** to favour the plant growth. Hence it has... recommended as a ***biofertilizer*** for rice, wheat, millets, co... vegetables, mustard, sunflower, etc.

Field Applications

1. Seed Treatment

Azotobacter inoculant is mixed with a small quantity of wa... make a slurry and the seeds are kept dipped in the slurry for one... In the early morning, the seeds are taken out and sown in the... field. The remaining slurry is directly sprayed in the field.

2. Seedling Treatment

In this method, the roots of seedlings to be transplanted are... dipped in the slurry for 10-15 minutes. During this time, a suffic... number of cells gets adsorbed onto the roots. Then the ***seedlings***... ***transplanted*** in the main field.

3. Pouring of Slurry

For crops such as sugarcane, *Azotobacter* inoculant is dil... properly with water and a small volume of the slurry is poured near... root zone of the crop.

4. Top Dressing

For cereal crops like rice and wheat, *Azotobacter* inoculant (... ha) is mixed with farmyard manure (20-25 Kg/ha) and broadcas... by top dressing after transplantation.

Beneficial Roles of Azotobacter

Azotobacter fixes the atmospheric nitrogen in the soil and th... by saves nitrogen fertilizers in agriculture. *Azotobacter* together w... 75% of recommended dosage of nitrogen fertilizers boosts yiel... many crop plants.

Azotobacter releases growth promoting substances such as IA... ***gibberellic acids, nicotinic acid, pantothenic acid, pyrido...*** ***biotin***, etc. in the rhizosphere soils. These substances increase the gro... rate of the crops by giving a good microenvironment for their roots.

Azotobacter increases the crop yield in onion (22%), rice (40... cabbage (40%), wheat (30%), maize (8-71%), sorghum (9-3... barley (1-21%), oat (1-12%), potato (1-8%) and sugarbeet (1-7...

Azotobacter synthesises some antagonistic substances which suppress the growth of *Fusarium*, *Aspergillus* and *Alternaria*. Azotobacter treatment reduces crop diseases caused by these soil-borne pathogens.

Azospirillum

Azospirillum is a Gram negative, symbiotic, vibrioid soil bacterium. It occurs in large numbers in association with the roots of cereals, grasses and tuber crops. *Azospirillum* fixes about 20-40 Kg of the atmospheric nitrogen under microaerobic conditions. It increases the vegetative growth and crop yield in many plants.

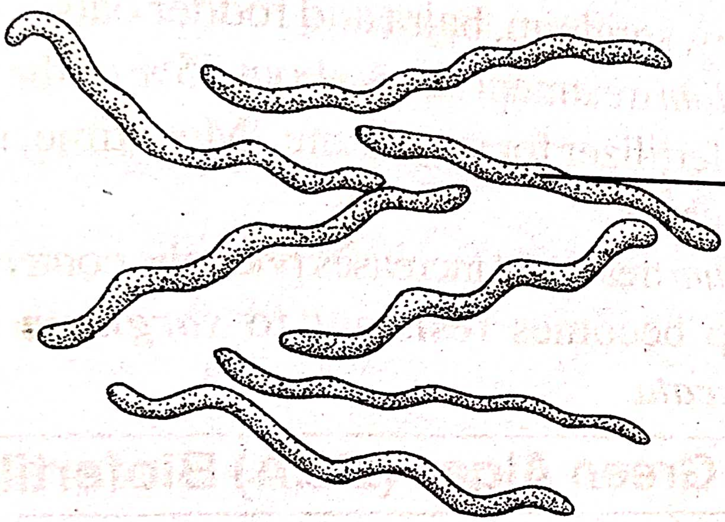

Fig.17.3: Azospirillum.

Azospirillum includes 4 species- *Azospirillum lipoferum*, *A.brasilense*, *A.amazonese* and *A.halopraeferns*. Among them, *A.lipoferum* and *A.brasilense* are in common use as biofertilizers.

Field Use of Azospirillum

Azospirillum inoculant is applied to various crops by three ways-

1. Seed Treatment

Azospirillum inoculant is mixed with water in a container to form a slurry. The seeds are soaked in the slurry overnight before sowing. About 2 kg inoculant is required for a hectare.

2. Seedling Treatment

One kilogram of *Azospirillum* inoculant is mixed with 40 litres of water to make a ***slurry.*** The roots of seedlings to be transplanted are kept dipped in the slurry for 15 minutes and then transplanted in the main field. The remaining slurry is diluted with water and sprayed in the field.

3. Top Dressing

In this method, 3 kg of *Azospirillum* inoculant is mixed well with 25 kg of FYM and 25 kg of soil. This mixture is broadcasted throughout the field by top dressing. This method is best suited for young seedling of rice in the main field.

Crop Response

Azospirillum fixes the atmospheric nitrogen and releases plant growth promoting substances. So the crops grow well and give higher yield.

1. *Azospirillum* increases the straw yield as well as grain yield in rice, wheat, barley, sorghum, bajra and fodder oats.

2. *Azospirillum* treatment saves about 25% of the recommended dose of nitrogen fertilizer for sugarcane. Meantime, it increases the sugar level in the canes.

3. *Azospirillum* treatment increases phenolic contents in sorghum. Hence, the crop becomes resistant to ***sorghum shootfly*** and *Antherigona soccata.*

Blue - Green Algae (BGA) Biofertilizer

Blue-green algae are photoautotrophic, prokaryotic algae. They are mostly ***free living***. They are otherwise called *Cyanobacteria*. Many species of BGA fix the atmospheric nitrogen in moist soils. Hence BGA inoculant has been recommended as a biofertilizer for flooded rice in many countries.

BGA include both ***unicellular*** species as well as ***filamentous*** species. *Gloeocapsa* is an example of unicellular species.

Among the filamentous forms, some have specialized thick-walled cells called ***heterocysts.*** Eg.*Nostoc, Anabaena,* etc. Almost all heterocystous species do nitrogen fixation, as the heterocyst is the site of nitrogen fixation.

Heterocyst is altogether absent in some filamentous species. Such species are known as ***non-heterocystous species***. Among them only a few species can reduce N_2 into NH_3. For example, there are 85 species of *Oscillatoria* in India, but only four species can fix the atmospheric nitrogen.

BGA inoculant being released by IARI (New Delhi) contains nitrogen fixing species of *Anabaena, Aulosira, Nostoc, Plectonema* and *Tolypothrix*. It is superior to monocultures.

Field Use of BGA Inoculants

A 10 kg of BGA inoculant is recommended for one hectare of flooded rice. The BGA flakes are dispersed and broadcasted over the field 10 days after transplantation. *The application of BGA to the crops is called **algalization.***

Crop Response

Inoculation of BGA in rice fields increases the yield upto 34 per cent (ie. 586 kh/ha). It saves about 30% of the recommended dose of nitrogen fertilizers for flooded rice.

Phosphate Biofertilizers

Microbes which solubilise the bound phosphates and rock phosphates into simple soluble phosphates are called ***phosphate solubilizers, phosphate solubilizing bacteria*** or ***phosphobacteria.*** They secrete organic acids such as formic acid, acetic acid, lactic acid, succinic acid, propionic acid and hydroxy acids to solubilize the bound phosphates in the soils. The simple phosphates are readily available for the plants.

The phosphate biofertilizers can save 30-50 kg of super phosphate per hectare and increase the yield upto 200-500 kg/ha.

The following are important phosphate biofertilizers:

Bacteria	Fungi
Bacillus megaterium var.phosphaticum	*Aspergillus awamorii*
Bacillus subtilis	*Aspergillus flavus*
Bacillus striata	*Aspergillus niger*
Bacillus pulvifaciens	*Aspergillus fumigatus*
Pseudomonas striata	*Penicillium digitatum*
Pseudomonas rathonis	
Pseudomonas liquifaciens	

Field Application

The phosphate biofertilizer is diluted with water and then treated with gum arabic and ***calcium carbonate*** to make a ***slurry.*** Seeds to be sown are soaked in the slurry for some time and dried in shade. By this process, the inoculant is made to form a pellet on the seeds. The seeds are then sown in the main field.

Uses

Phosphobacteria solubilize bound phosphates and contribute 20-30% of phosphate required for the crops. Phosphate biofertilizer is suited for all types of crops.

- In groundnut *Bacillus megaterium* together with *Rhizobium* boosts the yield upto 28%.
- *Aspergillus awamorii* increases the groundnut yield upto 25%.
- By providing 10 kg of phosphobacteria per hectare, phosphate fertilizers can be avoided for sugarcane.
- Co-inoculation of *B.megaterium* and *Pseudomonas striata* with *Azotobacter chroococcum* increases the yield in rice, sorghum and cotton.

Vesicular - Arbuscular Mycorrhizal Fungi

The vesicular arbuscular mycorrhizal fungi (VAM Fungi) are a group of symbiotic, endotrophic mycorrhizal fungi found in the roots of higher plants. They are included in the family ***Endogonaceae*** of Zygomycetes. VAM fungus infects a plant root and forms ***vesicles*** and ***arbuscles*** in the ***roots*** cortex and a permanent ***mantle of hyphae*** on the root's surface.

VAM fungi infect many crop plants like rice, maize, potato, soybean, cotton, tobacco, sugarcane, tomato, rubber, straw berry, citrus, avacado, coffee, tea, cacao, peas, apples, papaya and so on.

Genera of VAM Fungi

Gerdemann and ***Trappe*** (1973) have recognised five genera of VAM fungi based on the spore morphology. They are-

i. **Glomus:** Chlamydospores with a ***single stalk***. Eg. *Glomus microcarpus, G.fasciculatum, G.mosseae*, etc.

ii. **Gigaspora:** Chlamydospores with a ***bulbous stalk***. Eg. *Gigaspora nigra.*

iii. **Acaulospora:** Chlamydospores ***sessile***. Eg. *Acaulospora scrobiculata.*

iv. **Sclerocystis:** Chlamydospores ***thick-walled.*** Eg. *Sclerocystis clavispora.*

v. **Endogone:** ***Zygospores*** inside the ***sporocarp***. Eg. *Endogone incereseta.*

Morphology of VAM

VAM has three distinct regions-

i. External hyphae

ii. Arbuscles

iii. Vesicles

The external hypha is aseptate, dimorphic, thick-walled and closely oppressed on the root's surface. It extends upto about 1 cm from the root's surface. At the point of contact with the plant root, it bears an ***appresorium***.

An ***arbuscle*** is a dichotomously branched, brush-like haustorium produced at the tip of fungal hypha in cortical cell. It gets digested as the host cell matures.

The vesicle is a ***spherical*** or ***oval***, ***thick-walled*** structure produced at the ***tip of hypha*** in the intracellular space or inter cellular space. It is rich in fat droplets and hence serves as a ***storage organ***.

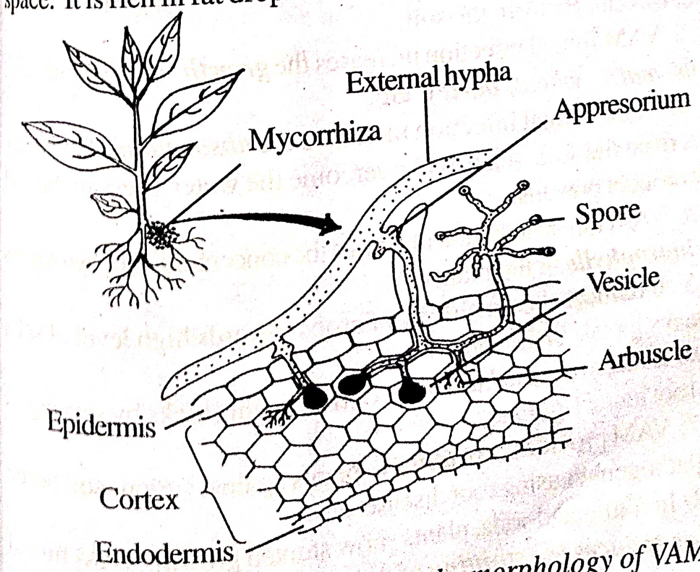

Fig.17.4: ***Illustration showing the morphology of VAM.***

The spore germinates into a hypha called ***permanent hypha*** on the root surface. This hypha is ***aseptate*** and ***thick-walled.*** Thin-walled short-lived hyphae arise from the permanent hypha and penetrate root hairs or epidermis to reach the cortex.

In the cortex, they grow through the inter cellular spaces between the cells. Tips of these hyphae enter the cells and form ***arbuscles*** and ***vesicles.***

Some of these thin-walled hyphae come out of the plant root and produce spores called ***chlamydospores.***

Field Application

VAM fungal inoculant is diluted with water and mixed with seeds to make a *pellet* of inoculant on them. The seeds are then sown in the main field as usual.

In another method, the inoculant is spread uniformly all over the field before ploughing and then crops are transplanted or seeds are sown in that field as usual.

Importance of VAM Fungi

VAM fungi play the following important roles in agriculture:

1. VAM fungi help the plants to ***intake*** more Zn, S, Cu, P, Ca, K, Fe, Mn and Br from the soil.

2. VAM fungal infection increases the ***growth rate*** in plants. Eg. *Citrus, maize, wheat, barley,* etc.

3. VAM fungal infection increases the ***absorption of water*** by plants from the soil. It helps to overcome the water stress in the soil while drought prevails.

4. VAM fungal infection increases the concentration of cytokinins and ***chlorophylls*** in the plants.

5. It reduces the sensitivity of crops towards high level of salts and heavy metals in the soil.

6. It improves the ***hardiness*** of transplant stocks by serving as extra root hairs. Eg. *Pine.*

7. VAM provides ***resistance*** to plants against various soil borne plant pathogens causing root diseases.

8. In fumigated soils, plants show stunted growth. VAM fungal infection ***reduces the stunting*** of the plants in such soils.

9. VAM fungal infection increases the ***yield*** in crops like potato, maize, barley, etc.

10. When the infected plant is starving for food, VAM gives the plant its own ***food*** and ***protect*** the plant (***Haymann***, 1974).

Azolla : A Green Manure Cum Biofertilizer

Azolla is a free-floating freshwater fern. The plant body consists of crowded mass-like leaves on a branched fragile stem and submerged roots.

The leaves are arranged in two alternate rows. The leaf is triangular or polygonal in shape. It has an upper lobe called ***aerial lobe*** and a lower lobe called ***submerged lobe.*** The ventral surface of the aerial lobe bears mucilage cavities harbouring the blue-green alga, *Anabaena azollae*.

Anabaena azollae is a filamentous, heterocystous alga lacking mucilage sheath. The cells are barrel shaped. *Azolla* synthesises sugars and provides them to *Anabaena*. On the other hand, *Anabaena* fixes the atmospheric nitrogen in the heterocysts and provides the reduced NH_3 to the *Azolla*. Thus *Azolla* and *Anabaena* are living together as ***symbionts***.

Azolla biomass gets doubled within 5-7 days by vegetative methods. It can fix 40-80 kg nitrogen / ha / year. Besides this, its forms a good green manure for flooded rice. An increase of crop yield upto 15-20% has been observed while fertilizing the rice with *Azolla*.

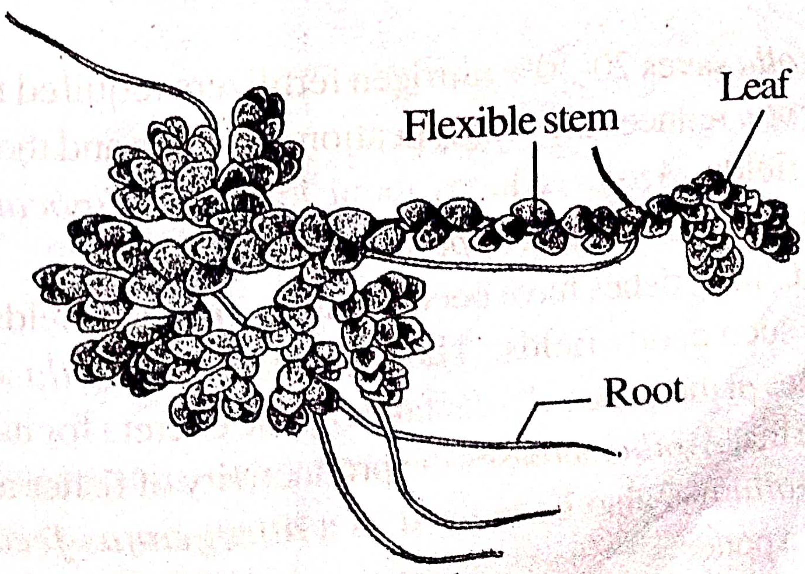

Fig.17.5: Azolla.

There are seven species of *Azolla*-*Azolla caroliniana*, *A. filiculoides*, *A. mexicana*, *A. nilptoca*, *A. microphylla*, *A. pinnata* and *A. rubra*. All these species harbour the unique blue green alga *Anabaena azollae*. *Azolla pinnata* is the common species in India.

Azolla microphylla is a heat tolerant species. It was crossed with *Azolla filiculoides*- a cold tolerant European species-to raise hybrids. The hybrids are growing faster, tolerant to heat and cold, and have 4-5% more nitrogen.

Field Application of Azolla

Azolla is added to the main fields either as a ***green manure*** or as a ***dual crop.***

Azolla As A Green Manure

The main field is ploughed and levelled about 15 days before transplanting the rice seedlings. Then *Azolla* is raised throughout the main field. After forming a dense growth, *Azolla* is mixed with the mud by gentle ploughing and levelling just before transplantation.

Azolla As A Dual Crop

Azolla is grown as an intercrop with rice 1-2 weeks after transplanting the rice. Usually it forms a dense growth within 3-4 weeks. Thereafter, the *Azolla* biomass is burried in the mud by using a weeder. *Azolla* may once again be cultivated in the same field. By this method, 0.5 -1 ton of *Azolla* biomass can be added to one hectare of rice field during a season.

Uses

- *Azolla* saves 20-30% nitrogen fertilizers required for rice.
- *Azolla* reduces the multiplication of weeds and their dispersal in paddy fields. *Azolla* reduces about 75% of *Echinocola* and 98% of *Marsilia* and eliminates *Cyperus* in paddy fields.
- In China, fishes have been cultured in paddy fields. *Azolla* is grown in such paddy fields. The fishes feed on *Azolla* and excrete their wastes in the water. *Azolla* takes up the excreta for its growth. It is noticed that *Azolla* increases the productivity of fishes and rice.
- *Azolla* has also been used as a ***nitrogenous feed*** for carps growing in ponds.

Mycorrhiza and Crop Production

Mycorrhiza is a ***fungus - root association*** (***Myco*** = fungus, *rhiza* = plant root). The term ***mycorrhiza*** is coined by ***Frank.***

Mycorrhiza is a ***beneficial symbiotic*** association between fungus and plant roots.

Mycorrhiza is common in both cultivated and wild plants. They are found in ***bryophytes, pteridophytes, gymnosperms*** and *angiosperms.* More than 90% of vascular plants form mycorrhiza. *Orchids* commonly form in mycorrhiza.

The fungi commonly occurring in mycorrhiza are the following:

1. *Laccaria laccata*
2. *Leccinum*
3. *Lepiota*
4. *Pisolithus*
5. *Gigaspora*
6. *Glomus*
7. *Scutellospora*
8. *Monotoa*
9. *Vaccinum*
10. *Rhizoctonia.*

Mycorrhiza is broadly classified into two groups, namely ***ectomycorrhiza*** and ***endomycorrhiza.***

In ***ectomycorrhiza,*** the fungus grows on the surface of the root. Outside the root surface, the fungal mycelium forms a compact and multilayered covering called ***mantle.***

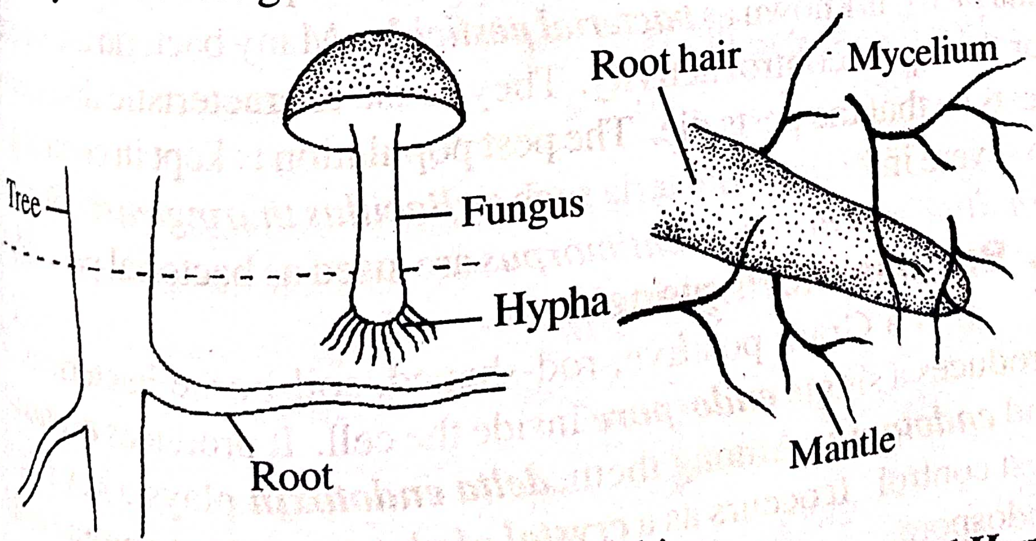

Fig.17.6: Mycorrhiza.

The fungus forms a network of mycelia in the cortex called ***Hartig net.*** The mycelia never enter the endodermis. Eg. *Laccaria laccata.*

In ***endomycorrhiza***, the fungal mycelium grows deep into the tissues of the root. Eg. *Glomus, Gigaspora.*

Vesicular - arbuscular mycorrhiza (VAM) is a typical endomycorrhiza.

Mycorrhiza is a good example for *symbiosis*. Here both partners are benefited.

The fungus accelerates the absorption of minerals by the green plants.

The fungus provides certain nutrients to the tree; the tree provides essential growth substances to the fungus.

Orchids do not have root hairs. The fungi absorb nutrients and water for orchids. Under natural conditions orchids rarely grow without fungus.

Biopesticides

Biopesticides are defined as *formulations containing the spores or vegetative cells of certain microorganisms which are pathogenic to most of the pest population.*

Over 400 species of ***fungi***, 90 species of ***bacteria***, 250 species of ***protozoa*** and more than 1000 species of ***viruses*** have been enlisted as microorganisms that can be used as biopesticides.

1. Bacterial Pesticides

The formulation containing a suitable species of bacteria that kills pests, is known as ***bacterial pesticide***. Many bacteria are known for their pest control activity. They cause characteristic diseases to pests so that the pests die. The pest population is kept in control due to severe infection. Bacteria such as *Bacillus thuringiensis*, *Bacillus popilliae* and *Bacillus lentimorpus* are used as bacterial pesticides.

a. Bacillus thuringiensis

It is a Gram positive, rod-shaped, soil-borne bacterium. It produces a single ***endospore*** inside the cell. It produces ***exotoxins*** and ***endotoxin***. Among them, ***delta endotoxin*** plays a key role in pest control. It occurs as a ***crystal of glycoprotein*** at one end of the endospore.

When the bacterial cell enters an insect, the cell wall ruptures and the endospore comes out. The endotoxin gets dissolved in ***alkaline juice*** of the digestive tract of the insect. The toxicity of the endotoxin kills the insect very soon. As a result, the pest population is reduced greatly.