

Chapter - 12

Nitrogen Metabolism and Nitrogen Cycle

The nitrogen is the most abundant gas found in the atmosphere. Its quantity is approximately 78 % in the atmosphere. Nitrogen is the main component of proteins found in the body. It is present in plants in the form of nucleic acids, vitamins, proteins, amino acids, enzymes, etc as organic compounds.

Plant with air also absorbs nitrogen through stomata but it cannot be assimilated in this form. Plants get nitrogen only by soil because in soil it is present in the form of compounds containing organic and inorganic nitrogen.

Free nitrogen present in the gaseous state cannot be utilized by highly developed living organisms. But some bacteria, fungus, blue green algae, etc. are capable of fixing atmospheric nitrogen. These microbes can be found in independent or in symbiotic state.

Dry weight of nitrogen-rich compound in plants is about 5 to 30% of the entire of the plant, which demonstrates its importance in plants. It is a controlling nutrient elements. Its deficiency leads to many types of plant diseases.

Plants use the following four types of nitrogen-rich compounds :

(i) Nitrites (ii) Nitrates (iii) Ammonium compounds (iv) Nitrogen containing carbonic compounds.

After the absorbtion of nitrogen by plants, various organic compounds are produced. Some micro-organisms fix atmospheric nitrogen. Thus, fixed nitrogen is free to be used by other organisms in the entire environment by various processes. The

entire process is called nitrogen cycle. This nitrogen cycle is completed in four stages:-

- (A) Nitrogen Fixation
- (B) Ammonification
- (C) Nitrification
- (D) Denitrification

(A) Nitrogen Fixation :

Two nitrogen atoms in a molecule of nitrogen gas are inactive due to presence of triple bond. It requires large amount of energy to break the bond and form new bonds. The process of nitrogen fixation in the biosphere takes place in different steps. That could be understood with following flow chart.

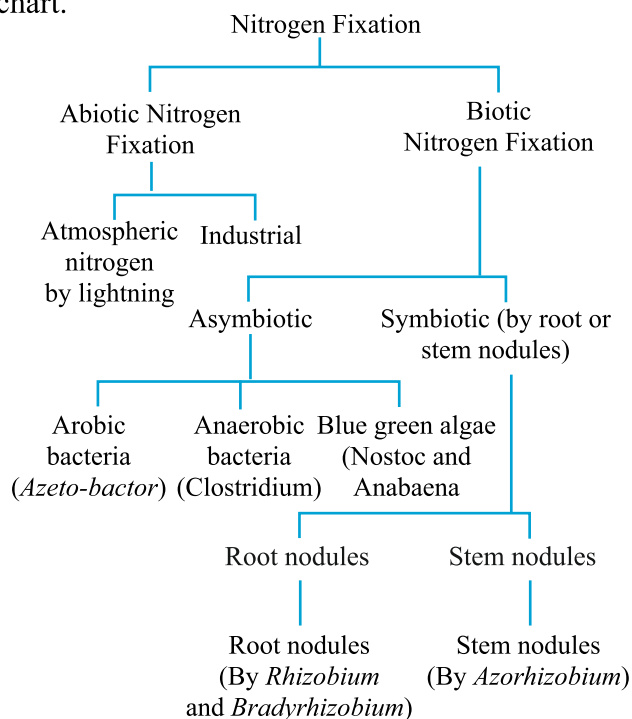
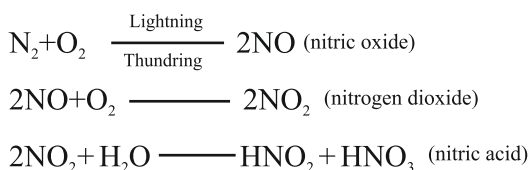


Chart : Types of Nitrogen Fixation

(1) Abiological Nitrogen (N₂) Fixation :

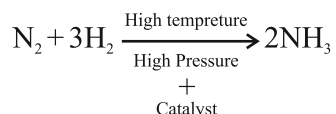
This type of nitrogen fixation is mainly done with the effect of environmental factors. It can be of two types :-

(i) Atmospheric nitrogen fixation : The effect of lightning and ultraviolet rays in nature, mainly combined with the nitrogen present in the atmosphere, and forms nitric oxide. Nitric oxide (NO) recombines with oxygen and forms nitrogen dioxide (NO₂). Nitrogen dioxide forms nitrous acid (HNO₂) and nitric acid (HNO₃) by reacting with water. Thus, the formed nitric acid goes into the soil with rain water, where it reacts with alkaline substances to form nitrate, which is absorbed by plants.



This type of nitrogen fixation is up to 10% of total nitrogen fixation. This method to form commonia is known as harbour method.

(ii) Industrial Nitrogen Fixation : In the presence of excessive heat, pressure and catalyst, atmospheric nitrogen and hydrogen combined to form ammonia which is used to make chemical fertilizers at industrial level.



(2) Biological Nitrogen Fixation:

To convert atmospheric nitrogen into organic or inorganic compounds by micro-organisms is called biological nitrogen fixation. These micro-organisms are called Dia-azotrophs, because they fixed the atmospheric dia-nitrogen (N≡N). Biological nitrogen fixation occurs by two types :-

(i) Non symbiotic Nitrogen Fixation : This type of nitrogen fixation is done by individual micro-organisms present in the soil. Following micro-organisms participate in the non- symbiotic nitrogen fixation-

(a) Aerobic bacteria :

Example : *Azotobacter*, *Azomonas*

(b) Anaerobic bacteria :

Example : *Clostridium*

(c) Photosynthetic bacteria :

Example : *Chlorobium*, *Rhodospseudomonas*

(d) Fungi : Yeast

(e) Blue Green Algae or Cyanobacteria :

Nostoc, *Anabaena* etc.

A special cell called Heterocyst is found in blue green algae which completes the work of nitrogen fixation. For activation of micro-organism an element molybdenum is required.

(ii) Symbiotic Nitrogen Fixation : In this type of nitrogen fixation, a symbiotic relationship is established between the micro-organism and the plant. In the roots of leguminous *Bradyrhizobium* plants, different species of *Rhizobium* and *Bradyrhizobium* enter and form the root nodules and convert the nitrogen of the soil into nitrate compounds. In *Sesbania* plant of leguminosae family, nitrogen fixation is done by formation of stem nodules. A micro- organism called *Frankia* found in roots of *Alnus* plant produces nodules for nitrogen fixation. A blue green algae *Anabaena* found in a pteridophyte called *Azolla* helps in nitrogen fixation.

Mechanism of Bacteria Infection and Nodulation:-

Rhizobium is a type of gram negative bacillus bacterium found in the soil. This is gathered in near areas of the roots of the plants and soon began to form the nodules. The process of formation of root nodules takes place in the following steps :-

- (i) First of all, a specific glycoprotein, lectin is released, by the legume plants. This attracts the special species of *Rhizobium* towards the root.
- (ii) By the effect of plant hormones secreted by roots of plants such as auxin, cytokinin and the nod factor secreted by bacteria, effects make the top of the roots hair coiled like hook.
- (iii) The cell wall at the end of the coiled radicles as hook breaks down from place to place and the *Rhizobium* bacteria enter the cell with a

mucous substance form a broken cell wall. *Rhizobium* bacteria is called Bacteroid after entering into the radicle.

- (iv) The root hair membrane, after curling forms an infection thread which slowly enters in cells and motivates them to increase the DNA content by multiplication. Hence, cortex cells get multiplied and these multiplied cells form nodules by continuous division. The size of nodules increases by the secretion of Indol Acetic Acid (IAA) by bacterial cells.
- (v) After the formation of the nodule, the conduction tissue develops and it establishes a connection with the conduction tissue of the root. This nodule is pink in color due to a pigment leghaemoglobin present in the peribacteroid membrane of Bacteroid present in the living nodule, while these are white or light yellow in the dead stage.

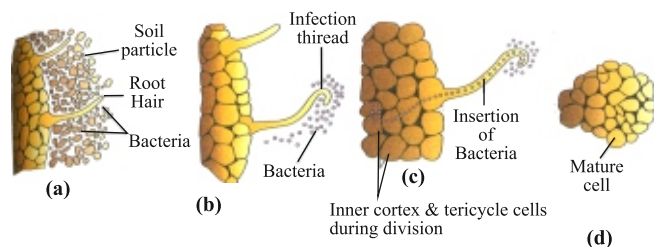


Fig. 12.1 (a) Root hair, Soil particle, Bacteria
(b) Root hair is infected by Bacteria
(c) Hook shaped root hair and infection thread in cortex
(d) Mature cells

Two types of proteins have special significance in symbiotic nitrogen fixation:

1. Leghaemoglobin : In the active and functional nodule, a red pink color pigment is found, which is called leghaemoglobin. This pigment absorbs O_2 at the time of nitrogen fixation, because the nitrogen fixation enzyme nitrogenase, is very sensitive to oxygen and it is only active in an anaerobic environment. It becomes inactive in the presence of oxygen. Hence, leghaemoglobin absorbs O_2 to prevent nitrogenase from being inactive and therefore Nitrogen fixation is not possible in the absence of this pigment.

2. Nodulin protein : There may be several types of this protein, which is related to the structure of the nodules and metabolism of nitrogen and

carbohydrate.

Nitrogen fixation by symbiosis is the result of the mutual support and expression of the genes of two symbionts.

In nitrogen fixation of nod genes of host and nod, nif and fix genes bacteria have a special contribution. The nod genes play a major role in the formation of nodule and nif genes play main role in nitrogen fixation.

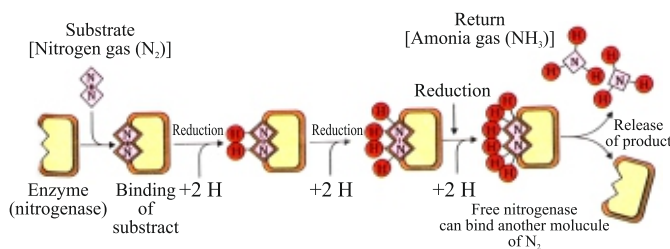
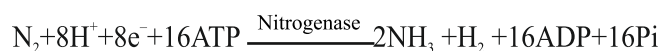


Fig. 12.2 Nitrogenase enzyme and conversion of atmosphere N_2 into NH_3

Mechanism of Symbiotic N_2 Fixation :

Symbiotic nitrogen fixation occurs in the root nodule because it contains the essential nitrogenase enzyme and leghaemoglobin pigment for fixation. Symbiotic nitrogenase is controlled by nif gene of *Rhizobium* bacteria. Nitrogenase enzyme is made up of proteins, molybdenum (Mo) and iron (Fe) elements. Nitrogenase enzyme converts free nitrogen into ammonia through following chemical reaction. For this energy is required as an ATP.



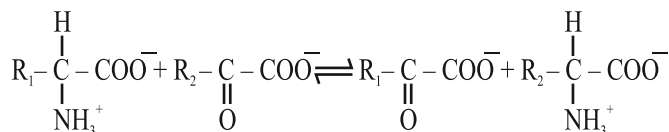
This type ammonia is used in the production of amino acids. In this case, first ammonia made α -glutamic acid. By acting with ketoglutaric and other types of amino acid are formed from this ketoglutaric acid

Ammonia assimilation : Ammonia is formed in biological nitrogen fixation but ammonia and ammonium ions are toxic for plants. Therefore, they are converted into amino acids by combining them with carbonic acids. This action is accomplished by two methods.

(i) Reducing amination : In this process ammonia acts with the α -ketoglutaric acid and form glutamic acids (amino acid).



(ii) Transaminase Reaction : In this process, the transfer of an amino acid, or a group of amino acids to a keto acid is done. As a result, new amino acids are formed.



About 17 types of amino acids can be synthesized by trans-amination.

(B) Ammonification :

The process of the formation of ammonium compounds from the disintegration of organic matter present in the soil is called Ammonification.

Inorganic nitrogen absorbed by plants is transformed into organic matter by being assimilated. After the death and decay of plants, this organic matter is re-disintegrated by micro-organisms and released as inorganic nitrogen in the atmosphere in gaseous form. This action takes place in the following steps by active purifying bacteria present in the soil.

(a) Proteolysis : In this process the protein is dissolved and converted into amino acids.

Example : *Clostridium*, *Pseudomonas*.

(b) Deamination : In this process, the amino acid is disintegrated by the bacteria, and produced ammonia is released into the atmosphere.

Example : Different varieties of *Bacillus* Bacteria.

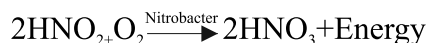
© Nitrification

Oxidation of ammonia into nitrate is called nitrification. This reaction is completed by various types of chemotropic bacteria. This reaction is completed in two stages-

(a) conversion of ammonia into nitrite : Ammonia in the presence of bacteria *Nitrosomonas* is oxidized into nitrite.



(b) Conversion of nitrite to nitrate : Nitrates are formed when nitrite is oxidized in the presence of *Nitrobacter* bacteria.



(D) Denitrification :

The conversion of nitrate into nitrogen in the soil by bacteria is called denitrification. This reduces the fertility of the land.

Example : *Bacillus denitrificans*, *Thiobacillus denitrificans*.

Thus, the nitrogen cycle in the above four stages can be explained by the following diagram.

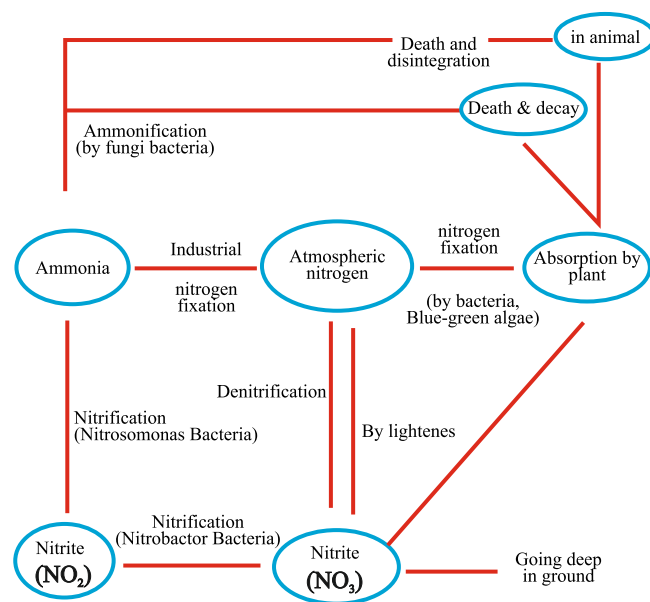


Fig. 12.3 Nitrogen cycle

Important Points

1. Nitrogen is found up to 78% in the atmosphere.
2. Nitrogen exists in all living organisms in the form of important organic compounds such as proteins, nucleic Acids.
3. Biological nitrogen fixation is done by independent and symbiotic micro-organisms.
4. Symbiotic nitrogen fixation is done by the species of *Rhizobium* bacteria in the root nodules of Leguminous plants.
5. Natural nitrification, is complete by *Nitrosomonas* and *Nitrobacter* bacteria.

6. Leghaemoglobin protects nitrogenase enzymes from oxidation.

Practice Questions

Multiple Choice Questions-

- The essential pigment for nitrogen fixation in plants of Leguminosae is:
(a) Haemoglobin (b) Leg haemoglobin
(c) Chlorophyll (d) xanthophyll.
- The essential elements for the activation of nitrogen fixation micro-organisms is :
(a) Cobalt (b) Molybdenum
(c) Iron (d) All of these
- In which form is Nitrogen absorbed by plants:
(a) Ammonia (b) Nitrogen
(c) Nitrite (d) Nitrate
- Symbiotic nitrogen fixation bacteria is:
(a) *Nitrosomonas* (b) *Nitrobacter*
(c) *Rhizobium* (d) All of these
- Which of the following is the denitrifying bacteria:
(a) *Rhizobium*
(b) *Bacillus denitrificans*
(c) *Nitrobacter*
(d) *Nitrosomonas*

Very short answer questions-

- What is the specific cell that does Nitrogen fixation in blue green algae?
- What is Leghaemoglobin?
- What is the function of glycoprotein called lectin?
- What are the two main proteins used in symbiotic nitrogen fixation ?
- What is nitrogen fixation?

Short answer questions-

- Plants cannot use nitrogen directly when there is 78% nitrogen present in Atmosphere. Why?
- What do you understand by symbiotic nitrogen fixation?
- Write a note on the following:
(a) Nitrification
(b) Denitrification
(c) Nif gene
(d) Leghaemoglobin
- Explain the method of formation of nodule in the roots of leguminous plant.

Essay Type Questions-

- What do you mean by nitrogen fixation? Explain biological nitrogen fixation in plants.
- Write an article on various events of the nitrogen cycle.

Answer Key-

1. (B) 2. (D) 3. (D) 4. (C) 5. (B)