high

NITROGEN IN SOIL

Normally plants absorb nitrogen from soil in the inorganic forms either as nitrate (NO_3^-) or as ammonium (NH_4^+). Nitrate is first reduced to ammonium level in a series of steps in order to be absorbed by the plants. The average Indian soil contains about 0.05 per cent nitrogen and is the main source of nitrogen to plants. Nitrogen occurs in soil as inorganic and organic compounds—inorganic compounds commonly being nitrate and nitrites of calcium, potassium, etc. and organic forms chiefly proteins. Various processes rendering soil nitrogen absorbable to plants are described here.

(1) Denitrification

The process involved in the conversion of nitrates and nitrites, into ammonia, nitrous cxide and nitrogen are defined as denitrification. These processes are necessary to supply ammonia for the metabolic process and to maintain the balance of nitrogen in the atmosphere.

In denitrification through a series of reactions the nitrates are reduced to ammonia and free nitrogen is released back to the atmosphere. The reduction of nitrate is brought about by the activity of certain micro-organisms (bacteria) such as *Pseudomonas denitrificans, P. stutzeri, Bacillus subtilis, Thiobacillus denitrificans, Micrococcus, Hansenula, Azotobacter, Clostridium* etc.

In presence of sulphur and water *Thiobacillus* converts nitrates into sulphates and nitrogen, releasing energy in the process.

$$\underset{\text{(Pot. nitrate)}}{6KNO_3} + 5S + 2H_2O \xrightarrow{\textit{Thiobacillus}} \underset{\text{(Pot. sulphate)}}{K_2SO_4} + \underset{\text{(Pot. hydrogen sulphate)}}{4KHSO_4} + 3N_2 + \text{energy}$$

Clostridium species converts hydroxylamine into ammonia and water.

$$\underset{\text{(Hydroxylamine)}}{NH_2OH} + H_2 \xrightarrow{\textit{Clostridium}} NH_3 + 2H_2O$$

Nitrous acid is converted into ammonia and water.

$$\underset{\text{(Nitrous acid)}}{HNO_2} + 3H_2 \xrightarrow{\textit{Clostridium}} NH_3 + 2H_2O$$

Clostridium also converts nitric acid into ammonia and water

$$HNO_3 + 4H_2 \xrightarrow{\textit{Clostridium}} NH_3 + 3H_2O$$

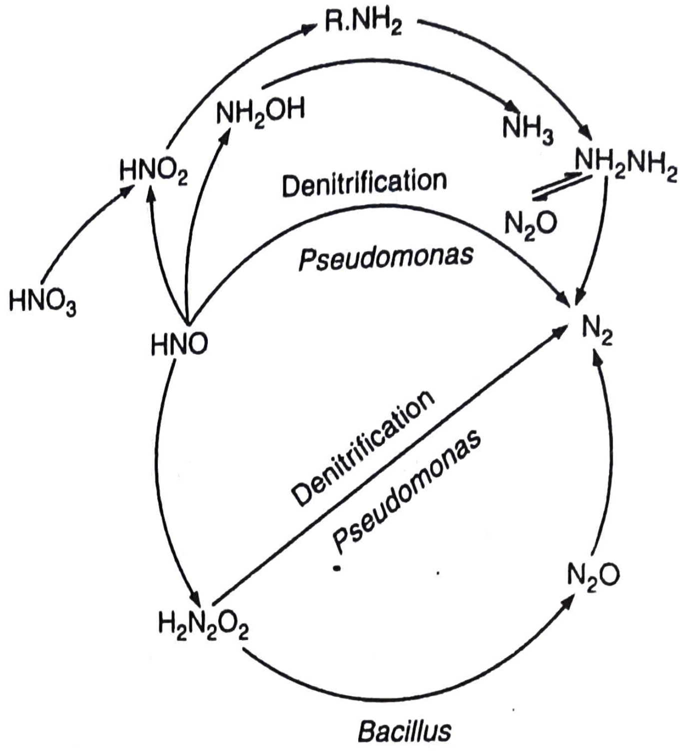


Fig. 17.5. A summarised account of denitrification.

In nature, nitric acid is converted to nitrous acid which converts into amine (R.NH_2). Amine gets converted into $H_2N_2O_2$ which may release free nitrogen or may get converted into nitrous oxide (N_2O). Nitrous oxide may be converted back to $H_2N_2O_2$. Nitrous acid produced in the process may also get converted into hyponitrous acid *via* HNO and hyponitrous acid under the influence of *Bacillus* may produce nitrous oxide which may be converted to free nitrogen by the activity of *Pseudomonas*. HNO may get converted to free nitrogen under the influence of *Pseudomonas* via hydroxylamine. HNO may also get converted into ammonia.

(2) Ammonification and Nitrification

The soil contains a large number of organic compounds in the form of animal excretion and plant remains. These compounds are first decomposed to release ammonia and this ammonia is then oxidised to produce nitrates. Both these processes occur under the influence of certain micro-organisms. The process of release of ammonia is known as ammonification and the process of further oxidation of ammonia to nitrates as nitrification. The organisms responsible for the release of ammonia are saprophytic bacteria like *Bacillus mycoides*, *B. ramosus* and *B. vulgaris*. These bacteria are called ammonifying bacteria. Many fungi and actinomycetes also help in this process.

Once ammonia has been produced it is converted into nitrates by nitrifying activities and the process is called nitrification.

For nitrification ammonia is first oxidised to nitrites by *Nitrosomonas* and *Nitrosococcus* (bacteria).

$$2NH_3 + 3O_2 \xrightarrow[\text{Nitrosococcus}]{\text{Nitrosomonas and}} 2HNO_2 + 2H_2O$$

Nitrites are then oxidised to nitrates by *Nitrobacter*.

$$2HNO_2 + O_2 \xrightarrow{\text{Nitrobacter}} 2HNO_3$$

In the process the bacteria are also benefited as they utilise the energy released in oxidation which is used in chemosynthesis.

At soil temperature between 30 to 35°C, in alkaline soils, in soils with lesser carbohydrate contents and in soils with sufficient moisture and aeration, the activity of ammonifying bacteria is found to be maximum.

(3) Nitrogen Assimilation

The atmospheric nitrogen is fixed in the soil in the form of nitrates which are absorbed by the plants and converted into ammonia through a series of steps catalysed by various enzymes.

$$\text{Nitrate} \xrightarrow{} \underset{\text{aerobic}}{\text{Nitrite}} \longrightarrow \text{Hyponitrite} \longrightarrow \underset{\text{anaerobic}}{\text{Hydroxylamine}} \longrightarrow \text{Ammonia}$$

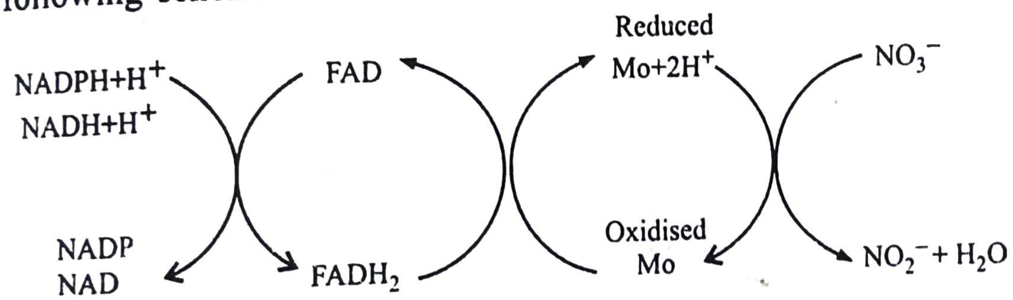
$$N_2 \longrightarrow N \longrightarrow N^+ \qquad\qquad N_2$$

$$\downarrow \qquad\qquad\qquad \downarrow$$

$$N_2O \longrightarrow (NOH)_2 \longrightarrow NH_2OH \longrightarrow NH_3$$

(The Scheme as proposed by Doby, 1965)

Step 1: *Nitrate reduction.* Evans and Nason (1954) demonstrated the presence of *reductase* from *Glycine max* (soya bean) leaves and *Neurospora.* The *reductase* enzyme contains FAD (Flavine adenine dinucleotide) as prosthetic group which receives hydrogen from reduced NADP or NAD. Both reduced NADP or NAD serve as hydrogen donor and *molybdenum serves as an electron carrier* as shown in the following scheme:



It is one reason that in Mo deficient soils, plants accumulate nitrogen.

Nitrate reduction takes place either in the root itself or in the leaves in the presence of the enzyme *nitrate reductase.*

Nitrate reductase enzyme is a homodimer with monomer size ranging from 100 to 120 K Dalton. Molybdenum is an essential constituent of the enzyme and FAD is present as a tightly bound prosthetic group of the enzyme protein. *Nitrate reductase* can reduce cytochrome c also. It is therefore believed that the enzyme consists of two components: *a component* which transfers electrons (reductant) for NAD(P)H to FAD and *a component* transferring electrons from FAD to nitrate by the way of cytochrome and molybdenum. The direction of electron transfer may be as follows:

$$\text{NAD(P)H} \longrightarrow \text{FAD} \longrightarrow \text{Cytochrome b-557} \longrightarrow \text{Mo} \longrightarrow NO_3^-$$

In the enzyme, NAD(P)H binds with its active centre involving a sulfhydryl group where it is oxidised to NAD(P) and the electron is transferred to FAD, cytochrome, Mo and finally to nitrate which is reduced to nitrite.

Step 2: *Nitrite reduction.* Vaneco and Varner (1955) demonstrated that nitrite is reduced to aminonitrogen by the leaves in presence of light more rapidly and in dark with lesser speed. It may be due to the available reducing power for the reaction from photochemical splitting of water.

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

A *nitrite reductase* is yet to be found out in pure form. There are supporting views and NADH and NADPH act as hydrogen donors and iron and copper play an important role in this reaction. Medina and Nicholas (1957) isolated an enzyme from *Neurospora crassa* which converts hyponitrite to hydroxylamine in presence of NADH requiring iron and copper for its activity.

Step 3: *Reduction of hydroxylamine.* Tucker and Nason (1955) isolated an enzyme from *Neurospora* and higher plants called *hydroxylamine reductase* which requires manganese for its activity.

$$NH_2OH + NADH + H^+ \xrightarrow{Mn} NH_3 + NAD + H_2O$$

The ammonia combines with organic acid to produce amino acids.