Nitrogen Metabolism

CONTENTS

5. Nitrogen Metabolism

- 5.1 Introduction
- 5.2 Definition
- 5.3 Reduction of Nitrates into Ammonia in Plants
- 5.4 Biosynthesis of Amino Acids
- 5.5 Nitrogen Cycle
  - 5.5.1 Nitrogen Fixation
    - 5.5.1.1 Non-Biological Nitrogen Fixation
    - 5.5.1.2 Biological Nitrogen Fixation
  - 5.5.2 Ammonification
  - 5.5.3 Nitrification
  - 5.5.4 Denitrification
- 5.6 Protein Synthesis
  - 5.6.1 Inhibitors of Protein Synthesis
- 5.7 Human influences on the nitrogen cycle
  - 5.7.1 Wastewater treatment
  - 5.7.2 Environmental Damage
- 5.8 Points to Remember

5. NITROGEN METABOLISM

5.1 Introduction

- In addition to carbon. Hydrogen and oxygen, nitrogen is also found in sufficient quantities in plants.
- About 78 to 79 % of earth's atmosphere is composed of nitrogen.
- Protoplasm is mainly made up of proteins.
- Nitrogen is present in the organic composition of compounds which are found in plants such as proteins, amino acids, alkaloids, vitamins, enzymes and nucleic acids.
- Nitrogen is also found in the chlorophyll.
- The dry weight of Nitrogenous organic compounds is 5 to 25 % of total dry weight of a plant.
- The deficiency of nitrogen causes chlorosis in leaves of the plants and the cell division as well as cell elongation is stopped due to which plants remain dwarf and production of flowers, fruits and seeds are decreased.
- The majority of the plants cannot utilize this form of nitrogen.
- Most of the plants utilize N_2 as nitrates.
- Source of nitrogen to plants is atmospheric nitrogen; nitrites, nitrates and ammonia in the soil (Inorganic nitrogen); amino acids (Organic Nitrogen) in the soil and organic nitrogenous compounds in bodies of the insects.

5.2 Definition

Metabolism

"The chemical processes occuring within an organism, or within part of one."
Metabolism is usually divided into two parts –
"Catabolism causes complex substances are decomposed into simple ones, with the release of energy, which becomes available for the organism's activities."

"Anabolism, which comprises the building up of complex substances with the absorption or storage of energy."

- Metabolic reactions are usually under the control of enzymes, which are consequently of immense importance in the chemistry of life.
- Metabolic processes are very similar throughout the plant and animal kingdoms and there are therefore corresponding similarities between the enzymes manufactured by organisms.
- Nitrogen is one of the important elements found in living organisms.
- Supply of nitrogen from the atmosphere is maintained.
- Nitrogen is an essential constituent of amino acids and hence the proteins cannot be absorbed by the plants directly from the atmosphere though it contains 79% of nitrogen.

Nitrogen Metabolism

"It is the series of biochemical changes taking place inside or outside the plant body which results in the synthesis of complex nitrogenous substances from its simpler derivatives and destruction of complex nitrogenous substances into its components."

- This includes nitrogen fixation, protein synthesis, Ammonification, nitrification and Denitrification.

"The supply of nitrogen from atmosphere and its return back to atmosphere is termed as nitrogen cycle."

"The conversion of molecular nitrogen into compounds of nitrogen especially ammonia is called **nitrogen fixation**."

"Organic nitrogenous compounds in the soil are first decomposed to release ammonia by some saprophytic microorganisms. This process is called ammonification."

"The process of converting ammonia into nitrates is called nitrification."

"The process of converting nitrates present in the soil into atmospheric nitrogen is called denitrification."

5.3 Reduction of Nitrates into Ammonia in Plants

- Nitrogen in nitrates (NO_3^-) is present in highly oxidised state while in ammonia in reduced form.
- So, the conversion of nitrates to ammonia is a reductive process which occurs by following two steps-
  - **Reduction of Nitrate to nitrite**
    - It occurs in presence of the enzyme nitrate reductase which requires reduced coenzyme I (NADH) or coenzyme II (NADPH).
    - Nitrate reductase is a molybdoflavo protein with an operative sulfhydryl group.
    - It was isolated first time by Evans and Nason (1953) from Neurospora and soybean leaves.
    - This enzyme contains FAD as its prosthetic group with which Mo is associated.

      $$NO_3 + NADH + 4H^+ \xrightarrow[reductase]{nitrate} NO_2 + NAD + H_2O$$
    - Nitrate reductase is an inducible type of enzyme.
    - Its synthesis is induced by (NO_3^-) in many plants.

  - **Reduction of nitrite to ammonia**
    - It occurs in the presence of the enzyme nitrite reductase which requires reduced coenzyme.
    - The enzyme nitrite reductase was first isolated by Nason et.al. from *Neurospora* and Soybean leaves.
    - In green tissues, it is found in chloroplast Sirohaem (am iron porphyrin) is known to be associated with this enzyme.

      $$NO_2 + 3\,NADH + 4H^+ \xrightarrow[reductase]{nitrate} 3NAD + NH_3 + 2H_2O$$
    - Proteins are N_2 containing molecules.
    - Proteins are condensation products of amino acids.
    - Out of so many amino acid occuring in nature only 20 have been reported from the molecules of protein.
    - The sequence of amino acid for a particular protein is specific.
    - Amino acids are linked together by peptide bond.

5.4 Biosynthesis of Amino Acids

- They are synthesised by following steps-
- **Reductive Amination**
  - Ammonia by reduction of NO_3 reacts with α–Ketoglutaric acid in the presence of enzyme glutamic dehydrogenase and reduced coenzyme $NADPH_2$ to form an amino acid, the glutamic acid.

    $$\text{-Ketoglutaric acid} + NADPH_2 + NH_3 \xrightarrow[dehydrogenase]{Glutamic} \text{Glutamic acid} + NADP + H_2O$$

❖ **Transamination**
- Other amino acids are produced by Transamination reactions involving the transfer of amino group from glutamic acid to ketoposition of the corresponding keto acid.
- Transamination reactions take place in the presence of enzymes transaminases which require coenzyme pyridoxal phosphate (a derivative of vitamin B6 or pyridoxine).

Glutamic acid + Oxaloacetic acid $\rightarrow$ -Ketoglutaric acid + Aspartic acid

Glutamic acid + Pyruvic acid $\rightarrow$ -Ketoglutaric acid + -alanine

Aspartic acid + Pyruvic acid $\rightarrow$ Oxaloacetic acid + -alanine

5.5 Nitrogen Cycle
- Nitrogen is present in the air as an inert gas and constitutes about 78% of the atmosphere.
- It is a gaseous cycle.
- N_2 cycle is completed into following steps-
  - Nitrogen Fixation
  - Ammonification
  - Nitrification
  - Denitrification

5.5.1 Nitrogen Fixation
- It is the conversion of molecular nitrogen into inorganic forms.
- It is of the following types-
  - Non-biological Nitrogen Fixation
  - Biological Nitrogen Fixation

5.5.1.1 Non-Biological Nitrogen Fixation
- Due to thunder storm the atmospheric nitrogen combines with oxygen to form Nitric oxide (NO), which is finally oxidized to nitrogen oxide (NO_2).
- This reacts with water of rain in the presence of oxygen to form Nitric acid.
- The nitric acid comes to earth; here it reacts with the basic substances in the soil and form Nitrate which is the life source of the plants in the soil.
- At the time of electric discharge nitrogen combine with oxygen.

$$N_2 + O_2 \rightarrow 2NO$$

$$2NO + O_2 \rightarrow 2NO_2$$

$$4NO_2 + 2H_2O + O_2 \rightarrow 4HNO_3$$

$$HNO_3 + NH_3 \rightarrow NH_4NO_3$$

$$HNO_2 + NH_3 \rightarrow NH_4NO_2$$

$$2HNO_3 + CaO \rightarrow Ca(NO_3) + H_2O$$

or salts [Ca or K nitrates]

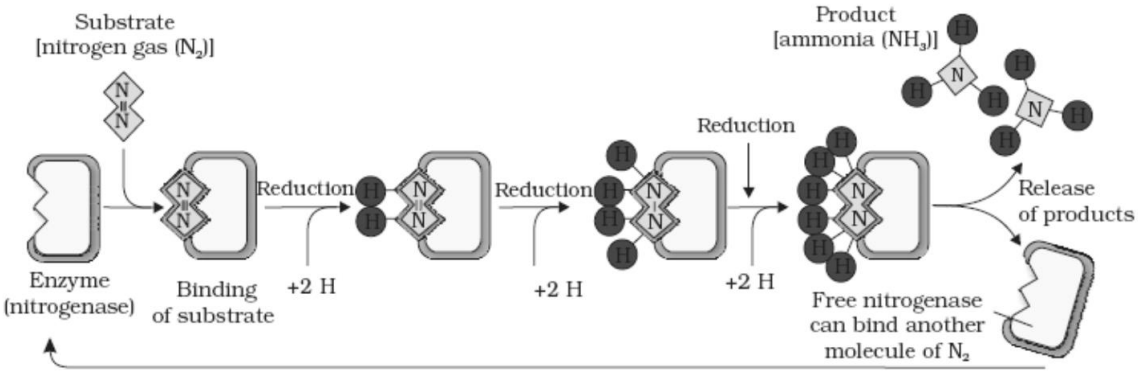
- Nitrates are soluble in water and are directly absorbed by the plants.
- This acid fall on the ground along with rain water and form nitrites and nitrates.
- By this method 16600 gm nitrogen of air comes to one acre land annually**.**

5.5.1.2 Biological Nitrogen Fixation
- Biological Nitrogen fixation is of two types-

a. Non Symbiotic N_2 Fixation
  - Free living aerobic N_2 fixing bacteria example- *Azotobactor Beijerinckia Derxia*.

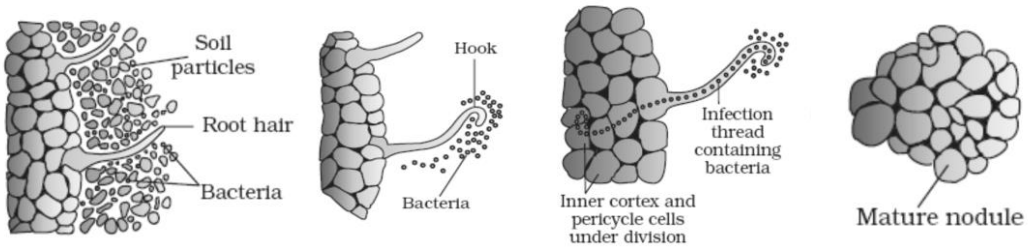
- Free living anaerobic N_2 fixing bacteria example-*Clostridium*.
- Free living photosynthetic N_2 fixing bacteria, example-*Chlorobium, Rhodospirillum, Rhodopseudomonas*.
- Free living chemosynthetic N2 fixing bacteria, example-*Disulpho vibrio*.
- Free living N_2 fixing fungus, example- *Pullularia*.
- Free living N_2 fixing blue green algae (Cyanobacteria), example-*Nostoc, Anabaena, Spirullina, Oscillatoria, Aulosiran Trichodesmium cylindrospermum.*

$$N_2 \xrightarrow{\text{oxidation}} N_2O \xrightarrow{\text{reduction}} NH_2 \xrightarrow{\text{hydrolysis}} NH_2$$



Steps of Conversion of atmospheric nitrogen to ammonia by nitrogenase enzyme complex found in nitrogen-fixing bacteria

b. Symbiotic N_2 Fixation
   - The bacterium *Rhizobium leguminosarum* lives symbiotically in the root of the leguminous plants.
   - The bacterium *Rhizobium leguminosarum infects the roots through the root hairs of pulse crops, like Soybean, groundnut, garden pea (*Pisum sativum*), hyacinth bean (Dolichos lablab), lobia (Phaseolus lunatus) etc. and reproduce in the cells of cortex of roots.*
   - Simultaneously the division of cortex cells takes place due to which the nodules are formed in the root.
   - The bacteria living in such nodules get the carbohydrates from host cells and also convert the absorbed atmospheric nitrogen into ammonia which is converted to the other nitrogenous substances.
   - Thus bacterium and its host plant are mutually beneficial (symbiosis).
   - When the pulse is harvested the roots are left in the soil and because of the presence of nitrogen the crops are beneficial for next crop.



5.5.2 Ammonification

- When the plants and animals die their protein contents are decomposed into NH3 by saprophytic decaying bacteria, example- *Bacillus mycoides*, *Bacillus ramosus*.
- Bacteria deaminates amino acids into NH3.

$$\text{Alanine} + O_2 \xrightarrow[\text{dehydrogenase}]{\text{Alanine}} NH_3 + \text{Pyruvic Acid}$$

5.5.3 Nitrification

- NH_3 is converted into nitrite by bacteria, example- *Nitrosomonas*.
- Nitrite is converted to nitrates by bacteria, example-*Nitrobacter.*
- The most limiting factor for nitrification is the pH of the soil.
- The pH of fertile soil is 6 to 7.
- Nitrification is not possible below pH 5.

5.5.4 Denitrification

- Denitrification is the reduction of nitrates back into the largely inert nitrogen gas (N_2), completing the nitrogen cycle.
- This process is performed by bacterial species such as *Pseudomonas* and *Clostridium* in anaerobic conditions.
- They use the nitrate as an electron acceptor in the place of oxygen during respiration. These facultatively anaerobic bacteria can also live in aerobic conditions.
- The denitrifying bacteria, example-*Micrococcocus*, *Pseudomonas aeruginosa*, *Bacillus denitrificans* and *Thiobacillus denitrificans* break up or dissolve nitrates into free nitrogen which passes into atmosphere.
- Anaerobic conditions help in Denitrification.
- Denitrification reduces the fertility of soil.

5.6 Protein Synthesis

- Proteins are made up of amino acids.
- Proteins are in the form of one or more chain called polypeptide chains.
- Within the polypeptide chains, amino acids and amides are linked through peptide bonds, involving the carboxyl group of one amino acid and the amino group of the next.
- The number of amino acids varies greatly among proteins and thus differs the molecular weight of proteins also.
- One way flow of information or central dogma for protein synthesis.

$$\text{DNA} \xrightarrow{\text{Transcription}} \text{mRNA} \xrightarrow{\text{Translation}} \text{Protein}$$

- Two way flow of information or central dogma for protein synthesis.

$$\text{DNA} \underset{\text{Reverse Transcription}}{\overset{\text{Transcription}}{\rightleftharpoons}} \text{mRNA} \xrightarrow{\text{Translation}} \text{Protein}$$

- Reverse transcription was discovered by H. Temin and D. Baltimore (1975).
- In tumor virus RNA provides template for DNA synthesis.

$$\text{RNA} \rightarrow \text{DNA} \rightarrow \text{mRNA} \rightarrow \text{Protein}$$

- The different steps occur during protein synthesis are-
    - Transcription in the presence of enzyme RNA polymerase.
    - mRNA transfer to cytoplasm and associated with ribosome. mRNA attached with 30s subunit of 70s ribosome with the help of rRNA.
    - T-RNA molecule picks up specific amino acid by its CCA end according to its anticodon loop.

$$\text{Amino acid} + \text{ATP} + \text{enzyme} \rightarrow \underset{\text{Enzyme complex}}{\text{Aminoacyl adenylate} + \text{Pyrophosphate}} + \text{tRNA}$$

$$\downarrow$$

$$\text{Amino acid attached to tRNA} + \text{enzyme} + \text{AMP}$$

- Protein synthesis starts with AUG and terminates with UAA, UGA, UAG.
- A peptide bond is established between two amino acids in the presence of enzyme peptide synthetase.

5.6.1 Inhibitors of Protein Synthesis

- Streptomycin- The most commonly used antibiotic for inducing mutations in many cytoplasmic genes.
- Chloramphenicol or Chloromycetin- It inhibits protein synthesis by inhibiting the transfer of amino acids to ribosome.
- Puromycin- It inhibits protein synthesis because it prevents the elongation of polypeptide chain.
- Actinomycin D- It inhibits transcription or RNA synthesis.

5.7 Human influences on the nitrogen cycle

- As a result of extensive cultivation of legumes (particularly soy, alfalfa, and clover), growing use of the Haber-Bosch process in the creation of chemical fertilizers, and pollution emitted by vehicles and industrial plants, human beings have more than doubled the annual transfer of nitrogen into biologically available forms.
- In addition, humans have significantly contributed to the transfer of nitrogen trace gases from Earth to the atmosphere, and from the land to aquatic systems.
- Human alterations to the global nitrogen cycle are most intense in developed countries and in Asia, where vehicle emissions and industrial agriculture are highest.
- N_2O (nitrous oxide) has risen in the atmosphere as a result of agricultural fertilization, biomass burning, cattle and feedlots, and other industrial sources.
- N_2O has deleterious effects in the stratosphere, where it breaks down and acts as a catalyst in the destruction of atmospheric ozone.
- N_2O in the atmosphere is a greenhouse gas, currently the third largest contributor to global warming, after carbon dioxide and methane.
- While not as abundant in the atmosphere as carbon dioxide, for an equivalent mass, nitrous oxide is nearly 300 times more potent in its ability to warm the planet.
- Ammonia (NH_3) in the atmosphere has tripled as the result of human activities.
- It is a reactant in the atmosphere, where it acts as an aerosol, decreasing air quality and clinging on to water droplets, eventually resulting in acid rain.
- Fossil fuel combustion has contributed to a 6 or 7 fold increase in NO_2 flux to the atmosphere.
- NO_2 actively alters atmospheric chemistry, and is a precursor of tropospheric (lower atmosphere) ozone production, which contributes to smog, acid rain, damages plants and increases nitrogen inputs to ecosystems.
- Ecosystem processes can increase with nitrogen fertilization, but anthropogenic input can also result in nitrogen saturation, which weakens productivity and can kill plants.
- Decreases in biodiversity can also result in higher nitrogen availability increases nitrogen-demanding grasses, causing a degradation of nitrogen-poor, species diverse heath lands.

5.7.1 Wastewater treatment

- Onsite sewage facilities such as septic tanks and holding tanks release large amounts of nitrogen into the environment by discharging through a drain-field into the ground.

- Microbial activity consumes the nitrogen and other contaminants in the wastewater.
- However, in certain areas, the soil is unsuitable to handle some or all of the wastewater, and, as a result, the wastewater with the contaminants enters the aquifers.
- These contaminants accumulate and eventually end up in drinking water.
- One of the contaminants concerned about the most is nitrogen in the form of nitrates.
- A nitrate concentration of 10 ppm or 10 milligrams per liter is the current EPA limit for drinking water and typical household wastewater can produce a range of 20-85 ppm.
- The health risk associated with drinking water (with >10 ppm nitrate) is the development of methemoglobinemia and has been found to cause blue baby syndrome.
- Several states have now started programs to introduce advanced wastewater treatment systems to the typical onsite sewage facilities.
- The result of these systems is an overall reduction of nitrogen, as well as other contaminants in the wastewater.

5.7.2 Environmental Damage

- Additional risks posed by increases in fixed nitrogen in aquatic systems include spurring the creation and growth of eutrophic lakes and oceanic dead zones through algal bloom-induced hypoxia.
- The extent and effects of the anthropogenically-induced doubling of biologically available nitrogen in the soils, waters, and air of the earth during the past century are still poorly understood.

5.8 Points to Remember

- Nitrogen is one of the important elements found in living organisms.
- Supply of nitrogen is maintained from atmosphere.
- Nitrogen, it is a highly inert gas. Most of the nitrogen exists in proteins and amino acids.
- Nitrogen is also found in large quantity in nucleic acids , cytochromes , chlorophylls , vitamins , alkaloids etc.
- It is not used directly and found in fixed forms in combination with C, H, O to constitute other compounds.
- Nitrogen combines with oxygen and hydrogen to form nitrate (NO3-), nitrite (NO2-) and ammonium salts (NH4 +).
- Bacteria, blue-green algae can algae can also fix atmospheric nitrogen.
- Soil contains nitrogen in the form of nitrate, ammonium salts and organic nitrogenous compounds.
- Ammonia is absorbed by the plants directly.
- Urea and amino acids can be absorbed directly by the plants.
- Urea is first converted into ammonia by the action of enzyme urease.

$$\underset{\text{Urea}}{NH_2 - \overset{\overset{O}{||}}{C} - NH_2} \xrightarrow{\text{urease}} 2NH_3 + CO_3$$

- Members of the family Leguminosae such as beans gram, groundnut and soybean etc., on their secondary tertiary and sometimes primary roots bears small nodule like swellings.
- *Rhizobium* penetrates to the cortex of root through infection thread.
- Simultaneous cortical cells of root are stimulated to divide more vigorously to form nodules on the root.
- Neither bacterium nor plant alone can fix nitrogen in such cases.
- Nitrogen fixation is actually the outcome of symbiotic relationship between the two.
- Root nodules have Leghaemoglobin present in it.

- Fixation of nitrogen is done with the help of enzyme nitrogenase, which functions under anaerobic conditions.
- Leghaemoglobin combines with oxygen and protects nitrogenase.
- The nitrifying bacteria are chemoautotrophs and are benefited by utilising energy released in oxidation, which is used in chemosynthesis.
- Leaf nodules develop in some members of family Rubiaceae, the bacteria being *Mycobacterium*.
- Some cyanobacteria also have symbiotic association with plants e.g., lichens, *Anthoceros* and *Azolla.*
- Ammonia by reduction of NO_3 reacts with α–Ketoglutaric acid in the presence of enzyme glutamic dehydrogenase and reduced coenzyme $NADPH_2$ to form an amino acid, the glutamic acid.
- Transamination reactions involves the transfer of amino group from glutamic acid to ketoposition of the corresponding keto acid.
- During Nitrification NH_3 is converted into nitrite by bacteria e.g., *Nitrosomonas*.
- Denitrification is the reduction of nitrates back into the largely inert nitrogen gas (N_2), completing the nitrogen cycle.