Topic: NITROGEN METABOLISM: NITROGEN CYCLE

- The nitrogen cycle refers to the movement of nitrogen through the food chain of living organisms . This complex cycle involves bacteria, plants and animals. All organisms can convert **ammonia (NH_3)** to organic nitrogen compounds, that is compounds containing C-N bonds.
- However, only a few microorganisms can synthesize ammonia from nitrogen gas (N_2).
- Although N_2 gas makes up about 80% of the earth's atmosphere, it is a chemically unreactive compound. The first stage in the nitrogen cycle is the reduction of N_2 gas to ammonia, a process called **nitrogen fixation**. Ammonia can also be obtained by reduction of nitrate ion (NO_3) that is present in the soil.
- **Nitrate reduction** can be carried out by most plants and microorganisms. The ammonia resulting from these two processes can then be assimilated by all organisms.
- Within the biosphere there is a balance between total inorganic and total organic forms of nitrogen.
- The conversion of organic to inorganic nitrogen comes about through catabolism, denitrification and decay.

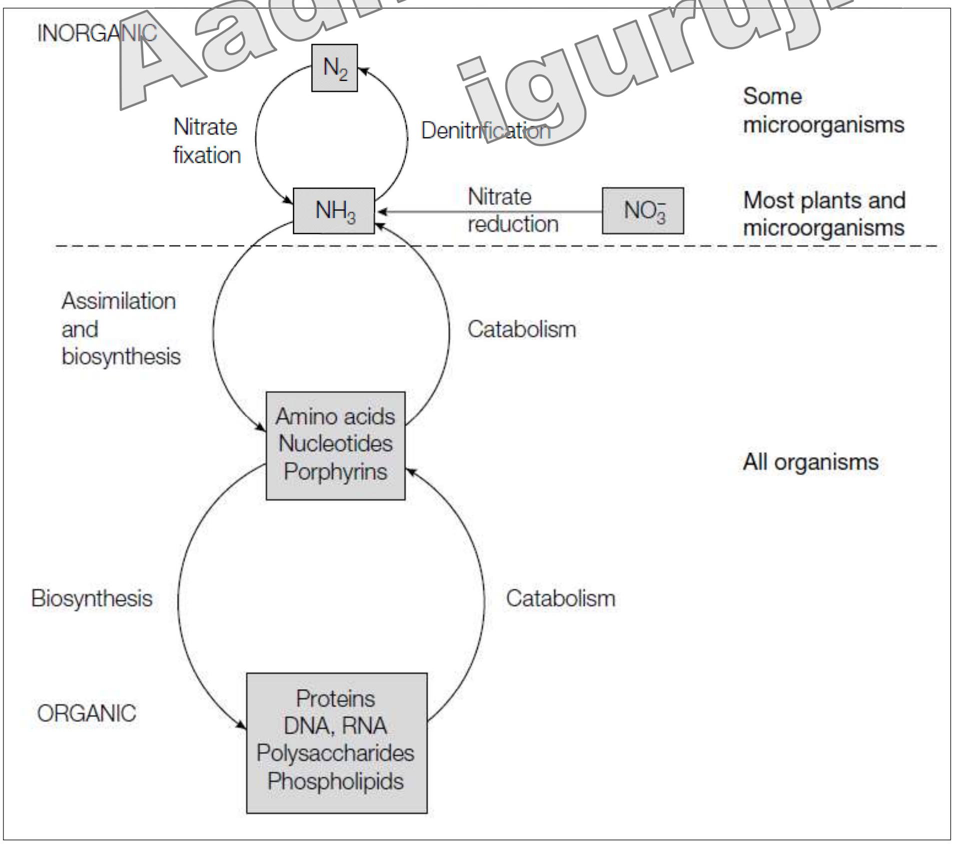

Fig. The interrelationships between inorganic and organic nitrogen metabolism.

Nitrogen fixation:

- The process of converting atmospheric N_2 gas into ammonia (nitrogen fixation) is carried out by only a few microorganisms, termed **diazatrophs**.
- These are some free-living soil bacteria such as *Klebsiella* and *Azotobacter,* cyanobac-teria (blue-green algae), and the **symbiotic bacteria** (mainly *Rhizobium).*
- The symbiotic *Rhizobium* bacteria invade the roots of leguminous green plants (plants belonging to the pea family, e.g. beans, clover, alfalfa) and form root nodules where nitrogen fixation takes place.
- The amount of N_2 fixed by these diazatrophic microorganisms has been estimated to be in the order of 10^{11} kg per year, about 60% of the earth's newly fixed nitrogen. Lightning and ultraviolet radiation fix another 15%, with the remainder coming from industrial processes.
- **The chemical unreactivity of the N=N bond is clearly seen when one considers the industrial process of nitrogen fixation.**
- **This process, devised by Fritz Haber in 1910 and still used today in fertilizer factories, involves the reduction of N_2 in the presence of H_2 gas over an iron catalyst at a temperature of 500°C and a pressure of 300 atmospheres.**

$$N_2 + 3\,H_2 \longleftrightarrow 2\,NH_3$$

Nitrogenase complex

- Biological nitrogen fixation is carried out by the **nitrogenase complex** which consists of two proteins: a **reductase**, which provides electrons with high reducing power, and a **nitrogenase**, which uses these electrons to reduce N_2 to NH_3. The reductase is a 64 kDa dimer of identical subunits that contains one iron-sulfur cluster and two ATP binding sites.
- The nitrogenase is a larger protein of 220 kDa that consists of two α and two β-subunits ($\alpha_2\beta_2$) and contains an iron-molybdenum complex. The transfer of electrons from the reductase to the nitrogenase protein is coupled to the hydrolysis of ATP by the reductase.

Although the reduction of N_2 to NH_3 is only a six-electron process:

$N_2 + 6\,e^- + 6\,H^+ \rightarrow 2\,NH_3$

the reductase is imperfect and H_2 is also formed. Thus two additional electrons are also required:

$N_2 + 8\,e^- + 8\,H^+ \rightarrow 2\,NH_3 + H_2$

The eight high-potential electrons come from reduced **ferredoxin** that is produced either in chloroplasts by the action of photosystem I or in oxidative electron transport. The overall reaction of biological nitrogen fixation:

$N_2 + 8\,e^- + 16\,ATP + 16\,H_2O \rightarrow 2\,NH_3 + H_2 + 16\,ADP + 16\,P_i + 8\,H^+$

highlights that it is energetically very costly, with at least 16 ATP molecules being hydrolyzed.

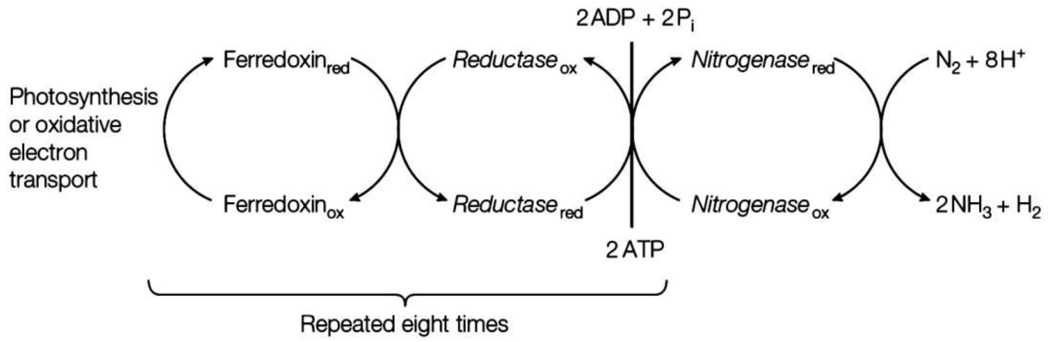

Fig. The flow of electrons in the nitrogenase-catalyzed reduction of N2

Leghemoglobin

- The nitrogenase complex is extremely sensitive to inactivation by O_2, so the enzyme must be protected from this reactive substance. In the root nodules of leguminous plants, protection is afforded by the symbiotic synthesis of leg-hemoglobin.
- The globih part of this monomeric oxygen-binding protein is synthesized by the plant, whereas the heme group is synthesized by the *Rhizobium.*
- The leghemoglobin has a very high affinity for O_2, so maintaining a low enough concentration to protect the nitrogenase.
- **Nitrogenase Components** → Nitrogenase has two components.
- Mo Fe Proteins → it is a tetramer with a molecular weight of approximately 220,000. It has two different types of subunits namely α and β. The structure of the tetramer is α2 β2. it is also called as Protein 1, Molybdo ferredoxin, Azoferno or dinitrogenase. It contains both Mo and Fe.
- **Fe Protein**:- It is a dimmer of two identical subunits. It is referred to as γ2. it contains Fe but no Mo. Fe-protein is also caused as protein 2, Azoferredoxin, Azofer or Dinitrogenase reductase. Molecular weight of the dimmer varies between 57, 000 and 72, 000.
- **Oxygen Sensitivity** → The enzyme nitrogenase is exteremely sensitive to oxygen. It is irreversibly inactivated in the presence of oxygen. It is able to reduce nitrogen to ammonia under microaerobic or anaerobic conditions. So, it can be concluded that oxygen is prevented from gaining access to the N_2 fixing enzyme sites.
- **Reason of inactivation of nitrogenase by oxygen**:-
- Both components of nitrogenase are sensitive to oxygen, though the Fe-protein, with a half decay time in air of 0.75 min is more rapidly inactivated than is the Mo-Fe protein with a half decay time in air of 10 min. Because of this extreme sensitivity shielded from oxygen.
- The mechanism of oxygen inactivation of nitrogenase is not clear. The toxicity of oxygen is usually explained in terms of the reduction of oxygen to superoxide (O_2^-).
- $O_2 + 2e^- \longrightarrow O_2^{-\cdot}$

- In aqueous environment, superoxide forms perhydroxy radical.
- $O_2^- + H^+ \longrightarrow HO_2$
- and also react with itself to form hydrogen peroxide (H_2O_2)
- $O_2^- + O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2$
- Superoxide react with hydrogen peroxide to produce hydroxyl radicals (OH).
- $H_2O_2 + O_2^- \longrightarrow O_2 + OH + OH^-$
- Hydroxyl radicals attack and degrade almost every molecule found in living organism, and ae responsible for many of thw observed toxic effects of oxygen. Many orgaism fix nitrogen only under atmosphere containing decreased concentrations of oxygen, and even those organism that can fix nitrogen aerobically are usually adversely affected by exposure to elevated concentration of oxygen.
- In some bacteria, not only does oxygen affect nitrogenase activity, but it also represses the synthesis of the enzyme. Thus, all N_2 fixing organism must protect nitrogenase from the deleterious effects of oxygen.

Nitrogen Assimilation :

- The next step in the nitrogen cycle is the assimilation of inorganic nitrogen, in the form of ammonia, into organic nitrogen-containing compounds. All organisms assimilate ammonia via two main reactions catalyzed by **glutamate dehydrogenase** and **glutamine synthetase** giving rise to the amino acids glutamate (Glu) and glutamine (Gin), respectively.

The amino nitrogen in Glu and the amide nitrogen in Gin are then used in further biosynthetic reactions to give rise to other compounds.