Nitrogen fixation is the reduction of molecular nitrogen to ammonia and assimilation into amino-acids. It is carried out by the prokaryotes. The nitrifying bacteria belong to the α-, β- and γ proteobacteria. Very few microorganisms can reduce nitrogen gas and use it as a nitrogen source, they must incorporate either ammonia or nitrate. As nitrogen is a major component of proteins, nucleic acids, coenzymes and many cell constituents, the cell should be able to assimilate inorganic nitrogen.

Nitrogen fixation provides the earth’s ecosystem with 200 million tons of nitrogen per year. These nitrogen fixing prokaryotes exist as free living or in symbiotic relationship. Symbiotic relationship exist between rhizobia and roots of leguminous plants. Symbiosis exists between aerobic diazotrophic actinomycete, *Frankia* with roots of actinorrhizal plants. Association between oxygenic photosynthetic cyanobacteria with higher plants can also fix nitrogen. They are found in flooded rice fields and marshes. *Anabaena* lives in symbiotic association with *Azolla*, a water fern producing as much as 50 kg per hectare annually enough to fertilize a crop of rice.

**Nitrogen fixation**

Reduction of nitrogen to ammonia is called nitrogen fixation. It is catalyzed by the enzyme nitrogenase. Nitrogen is reduced by two electron additions and is an exergonic reaction having high activation energy because molecular nitrogen is an unreactive gas with a triple bond between two nitrogen atoms. It is an expensive reaction requiring 8 electrons and 16 ATP molecules, 4 ATPs per pair of electrons.

\[
\text{N}_2 + 8\text{H}^+ + 8\text{e}^- + 16\text{ATP} \rightarrow 2\text{NH}_3^+ + \text{H}_2 + 16\text{ATP} + 16\text{Pi}
\]

The electrons are from ferredoxin that has been reduced by photosynthesis in bacteria, respiratory processes in aerobic nitrogen fixers or fermentation in anaerobic bacteria. Eg. *Clostridium pasteurianum*, an anaerobic bacterium reduces ferredoxin during pyruvate whereas the aerobic *Azotobacter* uses electrons from NADPH to reduce ferredoxin.
Fig. 1. Nitrogenase structure

Structure of the nitrogenase enzyme complex (PDB code 1n2c) showing the MoFe and Fe protein components, with the three metalloclusters revealed on the left-half of the complex (abbreviated as F, [4Fe4S]; P, P cluster and M, FeMo-co, respectively). ATP hydrolysis by the Fe protein, the route of electron transfer to the catalytic site and the enzyme reaction are also illustrated on the left half of the structure.

Nitrogenase consists of two major protein components, a MoFe protein (MW 220,000) joined with one or two Fe proteins (MW 64,000). The MoFe protein contains 2 atoms of molybdenum and 28 to 32 atoms of iron; the Fe protein has 4 iron atoms. Fe protein is first reduced by ferredoxin, then it binds ATP. ATP binding changes the conformation of the Fe protein and lowers its reduction potential, enabling it to reduce the MoFe protein, ATP is hydrolysed when this electron transfer occurs.

MoFe protein donates electrons to atomic nitrogen. In cyanobacteria, nitrogenase is protected from oxygen inactivation by the heterocysts.

Reduction of N\textsubscript{2} to NH\textsubscript{3} takes place in 3 steps each requiring an electron pair, a total of 8 electrons and 16 ATPs. 6 electron transfer takes place which requires 12 ATPs per N\textsubscript{2} reduced.

H\textsubscript{2} reacts with diimine (HN=NH) to form N\textsubscript{2} and H\textsubscript{2}. Symbiotic nitrogen fixing bacteria can consume almost 20% of the ATP produced by the host plant.

Nitrogenase can reduce a variety of molecules containing triple bonds such as acetylene, cyanide and azide.

HC≡CH + 2H\textsuperscript{+} + 2e\textsuperscript{-}\rightarrow H\textsubscript{2}C=CH\textsubscript{2}

Ammonia can then be incorporated into organic compounds when molecular nitrogen is reduced to ammonia. In \textit{Rhizobium}, the ammonia diffuses out of the bacterial cell and is assimilated in the surrounding legume cell.

**Assimilatory Nitrate reduction**

The reduction of nitrate to ammonia is known as assimilatory nitrate reduction where nitrate is incorporated into organic material and does not participate in energy generation. This is different from that occurring during anaerobic respiration and dissimilatory nitrate reduction. Assimilatory nitrate reduction takes place in the cytoplasm of bacteria.

Source: https://www.researchgate.net/figure/Nitrogenase-structure-and-genes-required-for-its-biosynthesis-a-The-nitrogen-fixation_fig1_261218148
Reduction of nitrate to nitrite takes place with the help of nitrate reductase, an enzyme that contains both FAD and Molybdenum. NADPH is the electron source.

\[ \text{NO}_3^- + \text{NADPH} + \text{H}^+ \rightarrow \text{NO}_2^- + \text{NADP} + \text{H}_2\text{O} \]

Nitrite is reduced to ammonia with a series of two electron additions catalyzed by nitrite reductase and other enzymes. Hydroxylamine may be intermediate, ammonia is then incorporated into amino acids.

**Ammonia incorporation:**

The amino acid alanine is formed in a reductive amination reaction catalyzed by alanine dehydrogenase.

\[ \text{Pyruvate} + \text{NH}_4^+ + \text{NADH} (\text{NADPH}) + \text{H}^+ \rightarrow \text{L-alanine} + \text{NAD}^+ (\text{NADP}) + \text{H}_2\text{O} \]

Two possible routes have been recognized for ammonia assimilation:

i) Formation of glutamate from α-ketoglutarate is the major route for ammonia incorporation which involves one enzyme, glutamate dehydrogenase. When the ammonia concentration is high, many bacteria and fungi employ the glutamate dehydrogenase.

\[ \text{α-ketoglutarate} + \text{NH}_4^+ + \text{NADPH} (\text{NADH}) + \text{H}^+ \rightarrow \text{glutamate} + \text{NADP}^+ (\text{NAD}^+) + \text{H}_2\text{O} \]

Once alanine or glutamate is synthesized, the α-amino groups can be transferred to other carbon skeletons by transamination reactions to form amino acids. Transaminases possess the coenzyme pyridoxal phosphate, which is responsible for the amino group. Each transaminase catalyze the formation of several amino-acid using the same amino-acid as an amino group donor. When glutamate dehydrogenase works in cooperation with transaminases, ammonia can be incorporated into amino acids.

ii) The other route involves two enzymes-glutamine synthetase and glutamate synthase.

\[ \text{Glutamate} + \text{NH}_3 + \text{ATP} \rightarrow \text{Glutamine} + \text{ADP} + \text{Pi} \]

Glutamine synthetase and glutamate synthase are two enzymes which act in sequence for incorporation of ammonia. Ammonia is used to synthesize glutamine from glutamate, the amide nitrogen of glutamine is transferred to α-ketoglutarate to generate a new glutamate molecule.

Glutamate acts as an amino donor in transaminase reactions and hence, ammonia may be used to synthesize all common amino acids when suitable transaminases are present. ATP and a source of electron such as NADPH or reduced ferredoxin are required. This route is present in *Escherichia coli*, *Bacillus megaterium* and other bacteria and is effective at low ammonia concentration.