NITROGENASE

DR. ALKA GUPTA

DEPARTMENT OF CHEMISTRY

BRAHMANAND COLLEGE,
KANPUR

NITROGENASE

- The third fundamental process in nature that is carried out by living cells in addition to photosynthesis and respiration is nitrogen fixation.
- Constituents of the living cell contain nitrogen and act as a reservoir of atmospheric nitrogen are amino acids, nucleic acid, purines, pyrimidines, porphyrins, alkaloids and vitamins.

COMPONENTS OF NITROGEN CYCLE

Nitrate ion	Nitrite ion	Hyponitrite ion	Nitrogen gas	Hydroxyl amine	Ammonia
NO_3^-	NO_2^-	$N_2O_2^{2-}$	N_2	NH_2OH	NH_3
+5	+3	+1	0	-1	-3

Thus, in nature nitrogen may exist in highly oxidized form (NO_3^-) or in highly reduced state (NH_3).

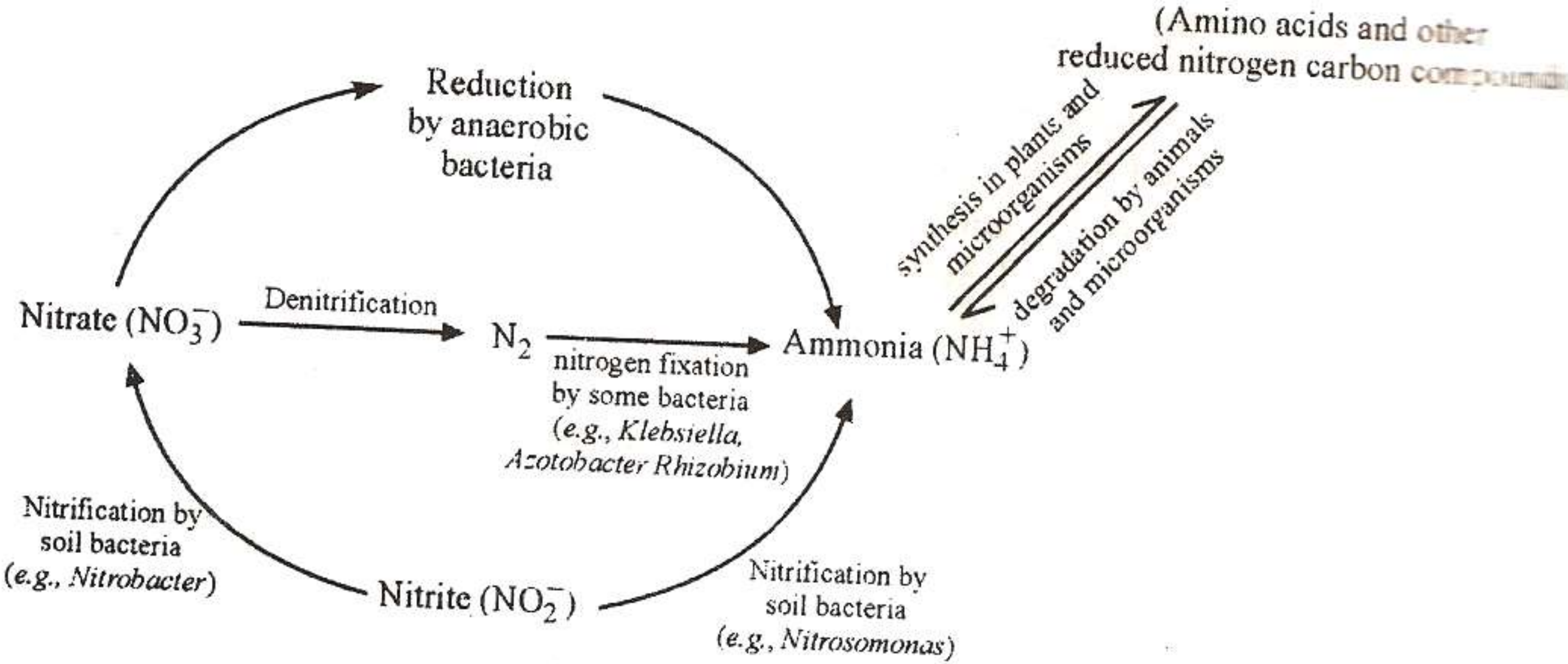


Fig.1 : Nitrogen cycle

NITROGEN FIXATION

The conversion of atmospheric nitrogen into useful nitrogenous compounds by natural or artificial methods is known as fixation of nitrogen.

Nitrogen present in various nitrogen compounds is known as combined or fixed nitrogen.

Basic requirements of nitrogen fixation

- Presence of enzyme nitrogenase and hydrogenase.
- A protective mechanism for the enzyme nitrogenase against O_2.
- A non-heme iron protein-ferredoxin as electron carrier.

- Hydrogen donating system (viz. pyruvate, hydrogen, sucrose, glucose, etc.)
- A constant supply of ATP
- Presence of thiamine pyrophosphate (TPP), Coenzyme-A, inorganic phosphates and Mg^{2+} as cofactors.
- Presence of cobalt and molybdenum
- A carbon compound for trapping released ammonia.

Methods of Nitrogen Fixation

(A) Artifical fixation

- **Fixation of nitrogen as nitric acid:** N_2 and O_2 of the air are allowed to combine to form nitric oxide at 3000ºC. The equilibrium mixture is suddenly cooled when it combines with more of O_2 to form nitrogen dioxide.

$$N_2 + O_2 \xrightleftharpoons{3000^oC} 2NO - 43.2 \text{ K. Cals}$$

$$2NO + O_2 \longrightarrow 2NO_2$$

It on dissolution in water forms nitric acid which is used for the manufacture of nitrogenous fertilizers.

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

❖Fixation of nitrogen as ammonia and ammonium salts

- **Haber's Process :** A mixture of N_2 and H_2 in the ratio 1:3 is passed at a pressure of 200-500 atmospheres into a reaction vessel containing finely divided iron as a catalyst and molybdenum as promoter heated to 450-500^oC.

$$N_2 + 3H_2 \rightleftharpoons 2\ NH_3 + 24,\ K.Cals$$

Conversion of NH_3 in to HNO_3 by ostwald's process.

The NH_3 so obtained can be oxidised to nitric oxide by passing a mixture of NH_3 and air in the ratio 1:8 over heated platinum gauze at 800^oC.

$$4\ NH_3 + 5O_2 \longrightarrow 4\ NO + 6H_2O$$

Then nitric oxide combines with the excess of O_2 of the air to form nitrogen dioxide which is absorbed in water in presence of excess of air and gives nitric acid

$$4\ NO + 2O_2 \longrightarrow 4\ NO_2$$

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

Now NH_3 and HNO_3 may be converted into ammonium salts and nitrates which are useful fertilizers.

❖Fixation of nitrogen as calcium cyanamide

When N_2 gas is passed over heated calcium carbide at 800-1000ºC, a mixture of calcium cyanamide and carbon is obtained.

$$CaC_2 + N_2 \longrightarrow [\,CaCN_2 + C\,]$$

Calcium carbide nitrolim

- It is used as a fertilizer under the name of nitrolim.
- It is added to plants before sowing below the surface of soil.
- It undergoes a series of changes producing ammonia in the soil.

$$CaCN_2 + H_2O + CO_2 \longrightarrow H_2NCN + CaCO_3$$

Calcium cyanamide   Cyanamide

$$H_2NCN + H_2O \longrightarrow NH_2CONH_2$$

Urea

$$NH_2CONH_2 + H_2O \longrightarrow 2NH_3 + CO_3$$

Ammonia

The ammonia so produced is converted into nitrates (by nitrifying bacteria) which are finally assimilated by plants.

❖ Fixation of nitrogen as nitrides

When atmospheric nitrogen is passed over red hot oxides of B, Al, Mg or Si and coke, nitrides are obtained.

$$B_2O_3 + 3C + N_2 \xrightarrow{\Delta} 2\,BN + 3CO$$

Boron nitride

$$Al_2O_3 + 3C + N_2 \xrightarrow{\Delta} 2\,Al\,N + 3CO$$

Aluminium nitrid

$$3MgO + 3C + N_2 \xrightarrow{\Delta} Mg_3\,N_2 + 3CO$$

Magnesium nitride

These nitrides are decomposed by water and NH_3 is evolved

$$Al\,N + 3\,H_2O \longrightarrow Al(OH)_3 + NH_3$$

$$Mg_3N_2 + 6H_2O \longrightarrow 3\,Mg\,(OH)_2 + 2NH_3$$

(B) NATURAL FIXATION

- **By Lightning discharges:** Under the influence of lightning discharges during thunder clouds N_2 and O_2 of the air combine together to form nitric oxide

$$N_2 + O_2 \xrightarrow{\text{Lightning}} 2NO$$

Nitric oxide

It is oxidised by excess of O_2 to give nitrogen dioxide

$$2\,NO + O_2 \longrightarrow 2\,NO_2$$

Nitrogen dioxide

It reacts with rain water in presence of excess of O_2 to produce nitric acid which is washed away by rain and form nitrates with the basic material of the soil and stored in the soil as plant food.

$$H\,NO_2 + 2H_2O + O_2 \longrightarrow 4\,HNO_3$$

Nitric acid

$$2HNO_3 + CaCO_3 \longrightarrow Ca(NO_3)_2 + H_2O + CO_2$$

❖By Symbiotic bacteria

- These bacteria grow in nodules of the plants belonging to the family leguminaced such as peas, beans, gram etc.
- These are capable of converting the atmospheric nitrogen into nitrogenous compounds. The enzymes found in bacteroids are called nitrogenase, e.g. Azotobacter, Gleocapsa and Anabaena etc.

The key enzyme- nitrogenase

Recent studies have shown that enzyme nitrogenase consists of two protein components or subunits. They are composed of two metallo proteins which are commonly referred as:

- Non heme iron protein commonly called Fe- protein or dinitrogen reductase.
- Iron molybdenum protein called MoFe protein (or dinitrogenase) or MFe (M =Mo, V and Fe) cofactor.

- The Fe protein component is common to all nitrogenases is a dimer with a single Fe_4S_4 (ferritins) cluster bound between two equivalent subunits and frequently known as “P cluster” as shown in Fig.2.

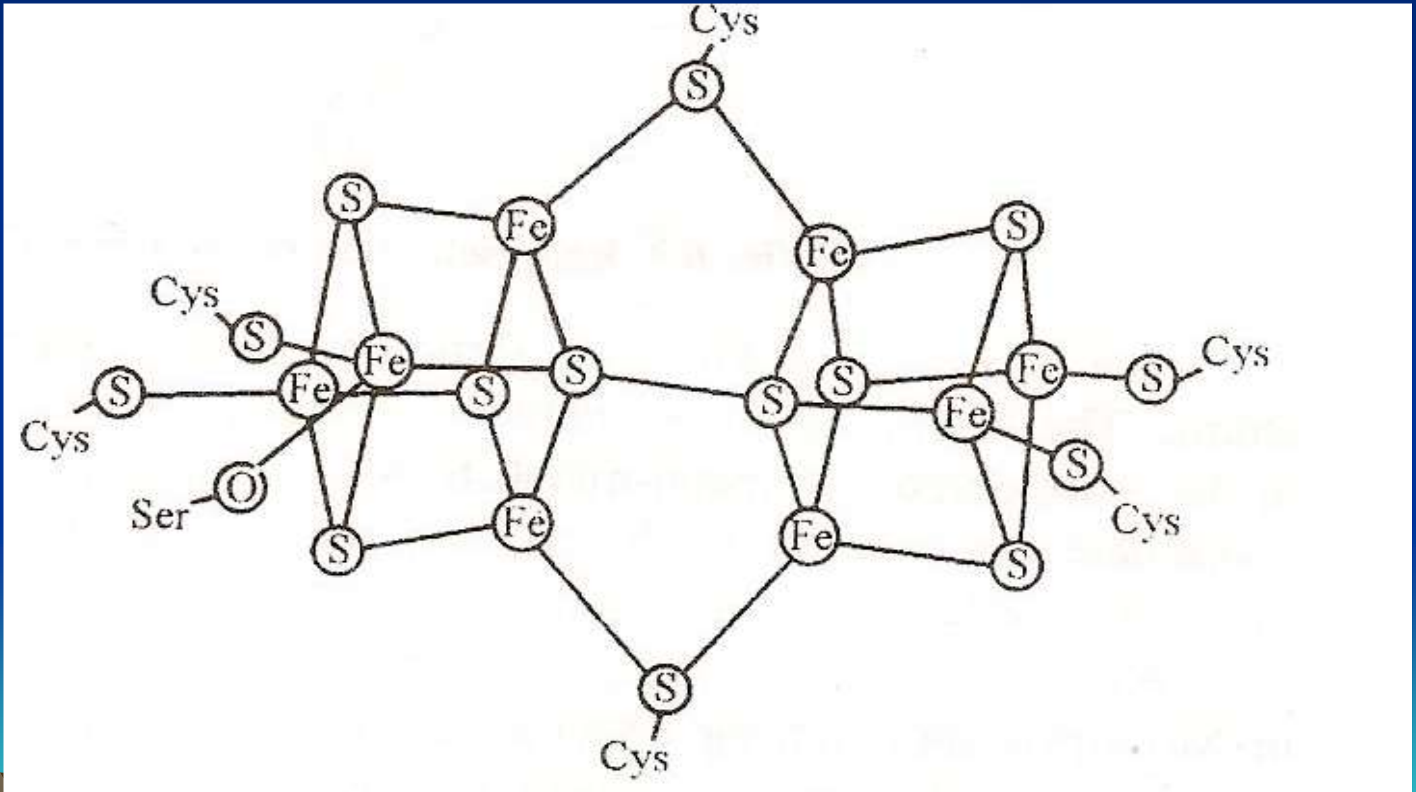


Fig.2: Model for the structure of the P cluster in nitrogenase.

The function of Fe-protein components is to react with ATP and reduce MoFe protein, which then reduces N_2 to ammonia.

- The MoFe protein contains P-cluster and the Fe-Mo co-factor. The structure of Fe-Mo cofactor cluster is shown in Fig.3.

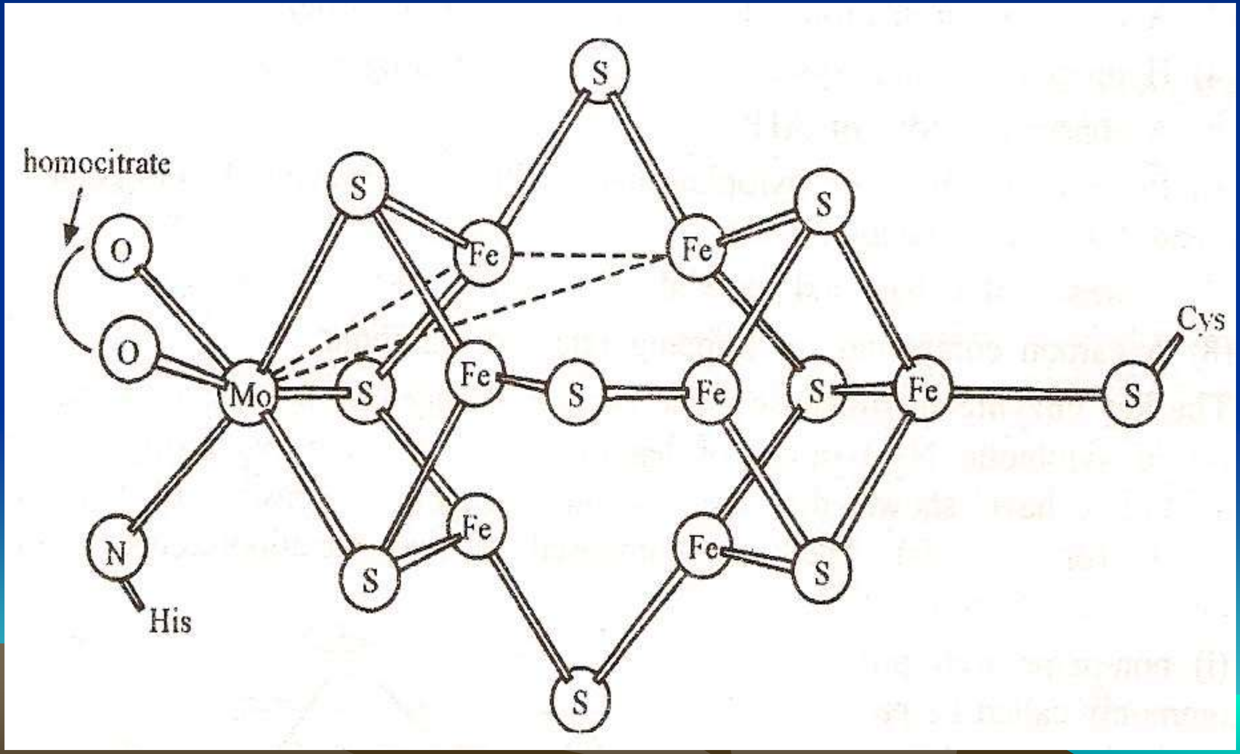


Fig.3: Representation of core of Fe-Mo-Cofactor

The two entities, $MoFe_3S_3$ (left) and Fe_4S_3 (right) are bridged by three sulphur atoms. The Fe-Fe distances between bridged ion sites average 2.5Å, close enough to be considered as metal-metal bonded. Only two protein ligands Cys and His coordinate the cofactor to the protein.

Thus nitrogenases react to natural N_2 through the metal present in these proteins.

Properties of some representative nitrogenase

Organism	Component	MW	Metal content
Azotobacter vinelandii	[MoFe] [Fe]	234000 64000	2Mo, 34-38Fe, 26-28S 3,4 Fe, 2.8S
Azotobacter chrococum	[MoFe] [Fe]	227000 65400	2Mo, 24Fe, 20S 4Fe, 3.9S
Clostridum pasterurianum	[MoFe] [Fe]	221800 55000	2Mo, 24Fe, 24S 4Fe, 4S
Klebsiella pneumoniae	[MoFe] [Fe]	299000 66800	2Mo, 32Fe, 24S 4Fe, 3.8S
Anabaena cylindrica	[MoFe] [Fe]	223000 60000	2Mo, 20Fe, 20S
Rhodospirillum rubrum	[MoFe] [Fe]	215000 60000	2Mo, 25-30Fe, 19-22S

Mode of action of nitrogenase

The overall reaction is as follows:

$$N_2 + 6H^+ + 6e^- \longrightarrow 2NH_3$$

- The reduction of nitrogen to ammonia requires at least 6 protons and 6 electrons. Besides, it requires at least 12 molecules of ATP (at least 4 ATP molecules are needed for each pair of electron transferred to N_2).
- Very recently, in view of the fact that N_2 fixation is always accompanied by evolution of some H_2, the equation is modified as follows:

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

- The enzyme hydrogenase is present in almost all the microbes involved in N_2 fixation. The enzyme catalyzes

$$H_2 \rightleftharpoons 2H^+ + 2e^-$$

BIOLOGICAL NITROGEN FIXATION

In contrast to the chemical fixation of nitrogen, biological fixation occurs at one atmosphere pressure and at temperature of living cells in the presence of appropriate enzymes.

$$N_2 + 3H_2 \xrightarrow[1\,atm]{25^{o}C} 2NH_3$$

Biological fixation of nitrogen is accomplished either by non-symbiotic micro-organisms or symbiotic system.

Non symbiotic micro-organisms: That can live independently such as aerobic organisms of the soil (e.g. Azotobacter), soil anerobes (e.g. Clostridium sp.) photosynthetic bacteria (e.g. Rhizobium rubium), and cyanobacteria (e.g. Anabaena sp.)

Symbiotic system: consists of bacteria (Rhizobia) living in symbiosis with members of the Leguminoseae such as clover, alfalfa and soyabeans. Except the legumes about 190 species of shrubs and trees, including the Sierra sweet Bay, Ceanothus and alder are nitrogen fixers.

Essential feature of symbiotic fixation

- The development of nodular tissue on the roots of legumes after infection by a strain of Rhizobia, specific for the given legume.
- Legume alone is unable to fix nitrogen, free living Rhizobia bacteria can fix N_2 only when grown with a limiting supply of organic nitrogen and oxygen.

- Biological nitrogen fixation is carried out by a highly conserved complex of proteins called the nitrogen complex.
- Two key components of this complex are dinitrogenase reductase and dinitrogenase.

Dinitrogenase reductase:

- It is a dimer of two identical subunits (Mr 60,000.
- It contains a single Fe_4-S_4 redox center which can be oxidized and reduced by one electron.
- It also has two binding sites for ATP. (Mr= relative molecular mass).

Dinitrogenase:

- It is a tetramer with two copies of two different sub units (combined Mr 240000).
- Dinitrogenase contains both iron and molybdenum, and its redox center has a total of 2Mo, 32Fe, and 30S per tetramer.
- About half of the Fe and S is present as four Fe_4-S_4 centers and remainder is present as part of a novel iron molybdenum cofactor of unknown structure.

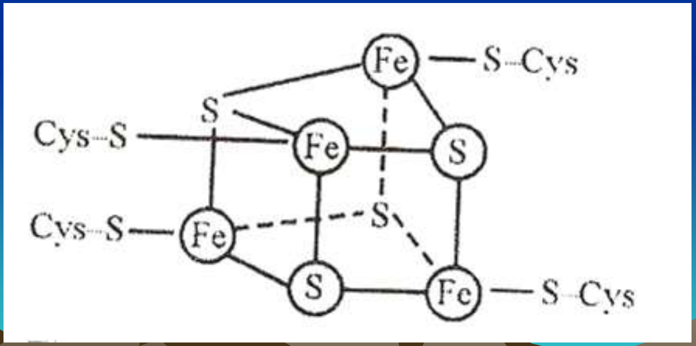


Fig.4: Fe_4-S_4 centers (ferredoxins)

Process of Nitrogen Fixation

- The laguminous plants which have nitrifying bacteria in the nodes of their roots, absorb the N_2 from atmosphere
- This N_2 is reduced to NH_3 by employing solar energy in presence of coenzymes mainly ferredoxin e.g. 8Fe-S and Mo/Fe protein known as nitrogenase.

$$N_2 + 6e^- + 2ATP \xrightarrow{\text{Nitrogenase}} 2NH_4^+ + 2ADP + 2Pi + 4H^+$$

$$\left.\begin{matrix} nATP^{-4} \\ M^{+2} \\ \text{reductant} \end{matrix}\right] \xrightarrow{H_2O} n\,[\,ADP^{-3} + H_3PO_4^- + M^{+2}]$$

oxidant

$$\text{Electron acceptor} - e^+ \longrightarrow \text{reduction product}$$

The conversion of atmospheric nitrogen into its compounds takes place as below:

			Inhibitors
	N_2 →	NH_3	H_2, CO
	N_3^- →	$N_2 + NH_3$	CO
	N_2O →	$N_2 + H_2O$	CO
Endogenous	RCN →	$RCH_3 + NH_3$	CO
	RNC →	RNH_2, CH_4, C_2H_4, C_2H_6	CO
	RCN →	$RCH_3 + NH_3$	CO

The reduction of N_2 is due to endogenous reaction of H_2O as

$$2H_2O \rightleftharpoons H_3O^+ + OH^-$$

$$H_3O^+ \longrightarrow H_2O + H^+$$

and at pH 7 $N_2 + 8H^+ + 6e^- \longrightarrow 2NH_4^+ + E^o = -280$ mV.

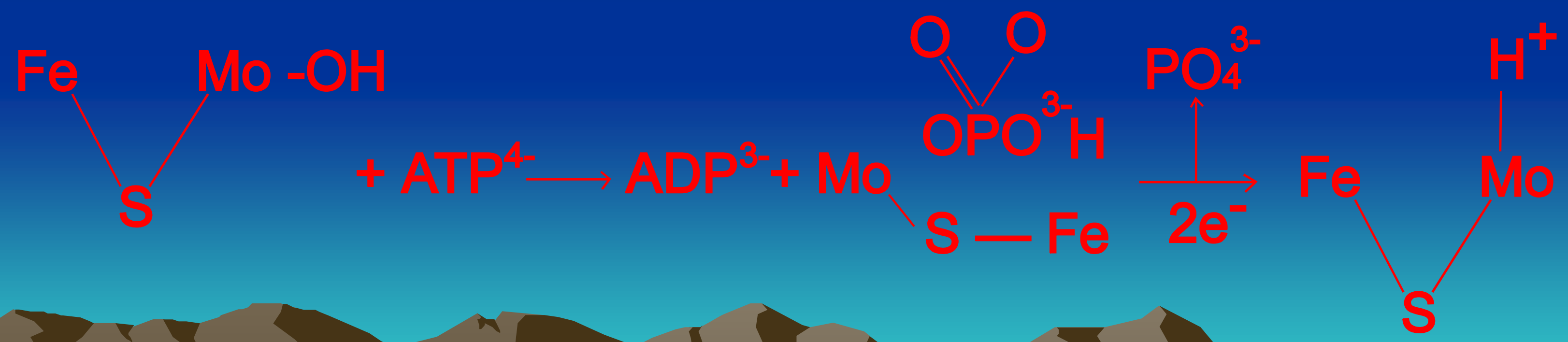
•The Mo-Fe protein of Azotobacter appears to consists two Mo-containing units. The Fe sites of Mo/Fe protein in which possesses two unique Fe sites, are specific to that protein and to nitrogen fixation.

•In oxidised state Fe^{+n} appears to have n = 3 to have high spin configuration. Reduction with HSO^{3-} converts about half the iron to high spin Fe^{+2} (or Fe^+).

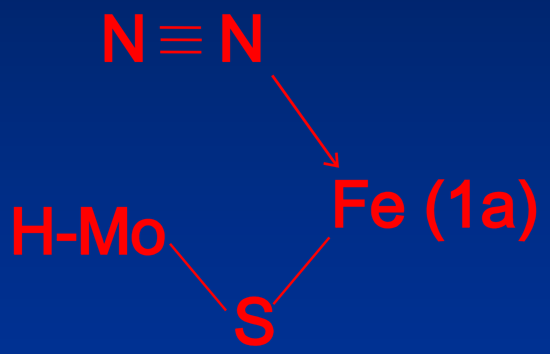
- Mo precipitate in electron transfer process, just as the Fe sites do and it seems that the processes are 2e^{-} in nature at Mo and sulphur remains present in the co-ordination sphere of Mo

$$(Mo^{+6} \rightleftharpoons Mo^{+2}, Mo^{+5} \rightleftharpoons Mo^{+3})$$

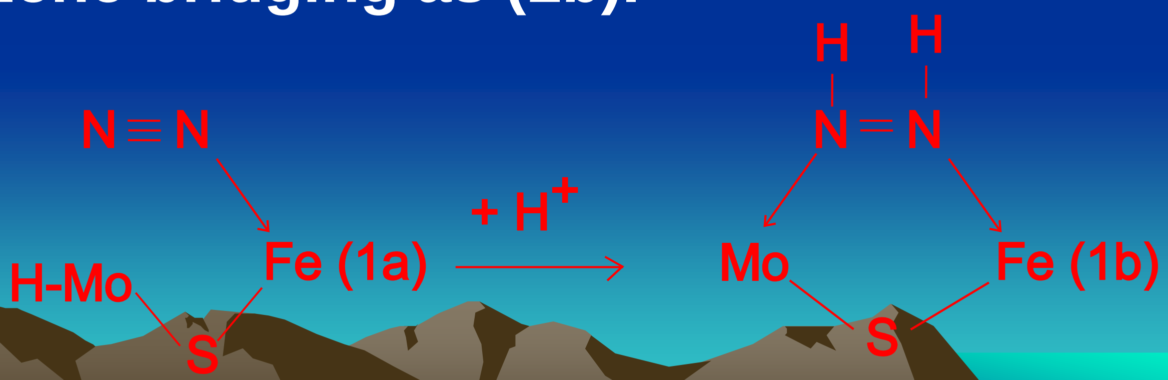
- R.W.F. Hardy et al. proposed that N_2 fixation occurs at a Mo-S-Fe site after ATP-4 induced reduction of Mo^{+n} to $Mo^{+(n-2)}$, the possibilities are



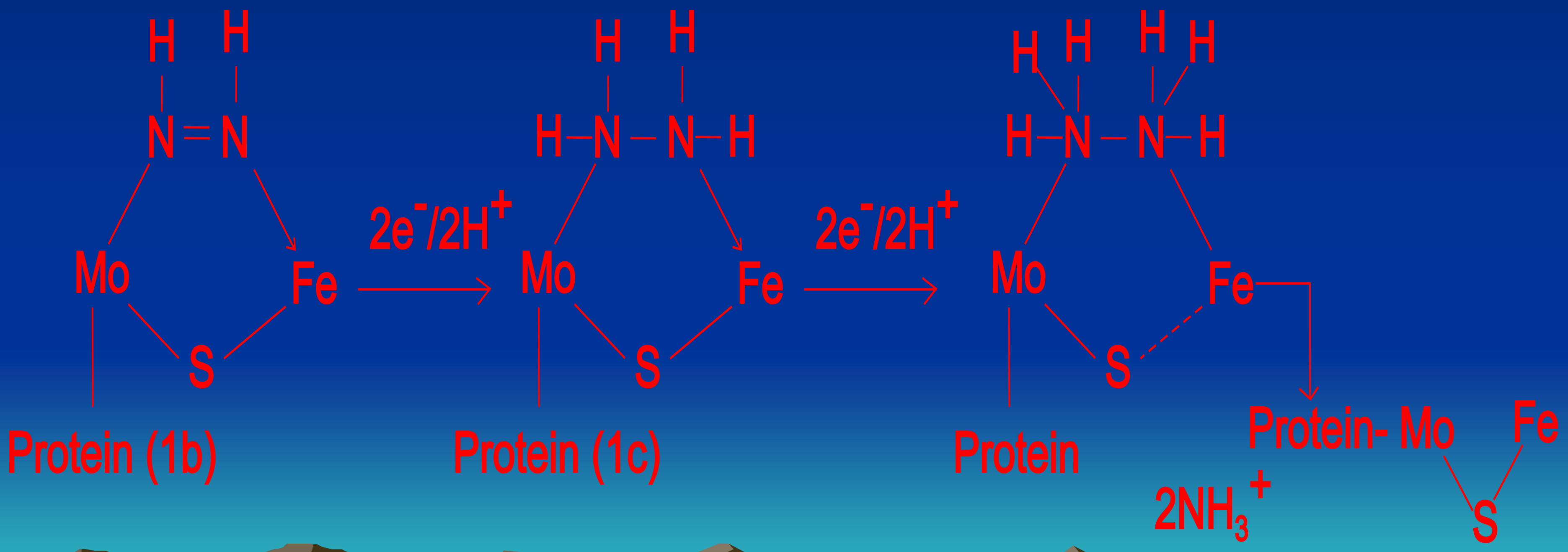
- This scheme amounts for the ATP required reductive diphosphorylation step. The Fe at this site (presumabily Fe^{+3}) is prepared for the entry of N_2 by reduction to Fe^{+}. Upon co- ordination of N_2 to Fe^{+}, the active site is suggested to be appear as (1a).



- (1a) on further reduction by H^{+} changes into a complex having diazene bridging as (1b).



- Further one or two electron reduction of this bridged diazene (1b) results in bridging hydrazine (1c) which on further reduction and protonation yields N–N bond and two NH_3 molecules as below:



- In any NO_3 or NO_2 are formed in plants, these are reduced in presence of nitrate and nitrite reductase enzymes as.

$$NO_3^- + NADH + H^+ \xrightarrow{NO_3\ Reductase} NO_2^- + NAD + H_2O$$

$$NO_2 + 6e^- + 8H^+ \xrightarrow{NO_2^-\ Reductase} NH_4^+ + 2H_2O + NH_3 + H^+$$

- The NH_3 molecules released during the electron reduction and protonation again form NH_4^+ which combines with organic acids or ketones present in the plants as.

(I) $^-OOCCH_2 - CH_2COCOO^- + NH_4^+ \rightleftharpoons {}^-OOCCH_2 - CH_2\text{-}C(=NH)\text{-}COO^- + H_3O^+$

α-Keto glutrate → α-imino glutrate

(II) $^-OOCCH_2\text{-}CH_2\text{-}CH(NH_3^+)\text{-}COO^- + TPN^+ + 2H_2O \rightleftharpoons TPNH + 2H_3O^+$

glutamate

(III) $^-OOCCH = CHCOO^- + NH_4^+ \rightleftharpoons {}^-OOCCH_2 - CH(NH_3^+)COO^-$

- **When further NH_3 reacts with glutamate or asparte or their acids in presence of ATP, Mg^{2+} and glutamine synthetase, the amines (e.g. glutamine or aspartagine), which are excellent carries of N_2 for protein synthesis are formed as.**

(I) $HOOC - CH_2 - CH_2 - CHCOO^- + ATP + NH_3$

glutamate (the CH carries NH_3^+)

$\rightleftharpoons$ glutamine synthetase Mg^{2+}

$H_2NOCCH_2 - CH_2 - CHCOO^- + HPO_4^{2-}$

glutamine (the CH carries NH_3^+)

(II) $HOOCH_2 - CHCOO^- + ATP + NH_3$

Asparte (the CH carries NH_3^+)

$\rightleftharpoons$ Aspartagine synthesis Mg^{2+}

$H_2NOCCH_2 - CHCOO^- + ADP + HPO_4^{2-}$

Aspartagine (the CH carries NH_3^+)

Molybdenum Nitrogenase

Nitrogenase is an enzyme involved in the fixation of nitrogen which occurs in bacteria. This enzyme is comprised of two protein chains.

(i)The lower molecular weight protein contains on Fe_4S_4 cluster.

(ii)The larger protein, which, itself is tetrameric, involves two molybdenum atoms and large number of iron atoms and sulphide ions.

Both proteins are required for activity. Although Fe_4S_4 clusters are thought to be the redox centers, molybdenum is vitally important.

▪It has been shown that bacteria grown in the presence of tungsten VI oxide rather than molybdenum (VI) oxide, can incorporate tungsten but show no nitrogen fixing activity. Thus nitrogen actually coordinates with molybdenum during the fixation process.

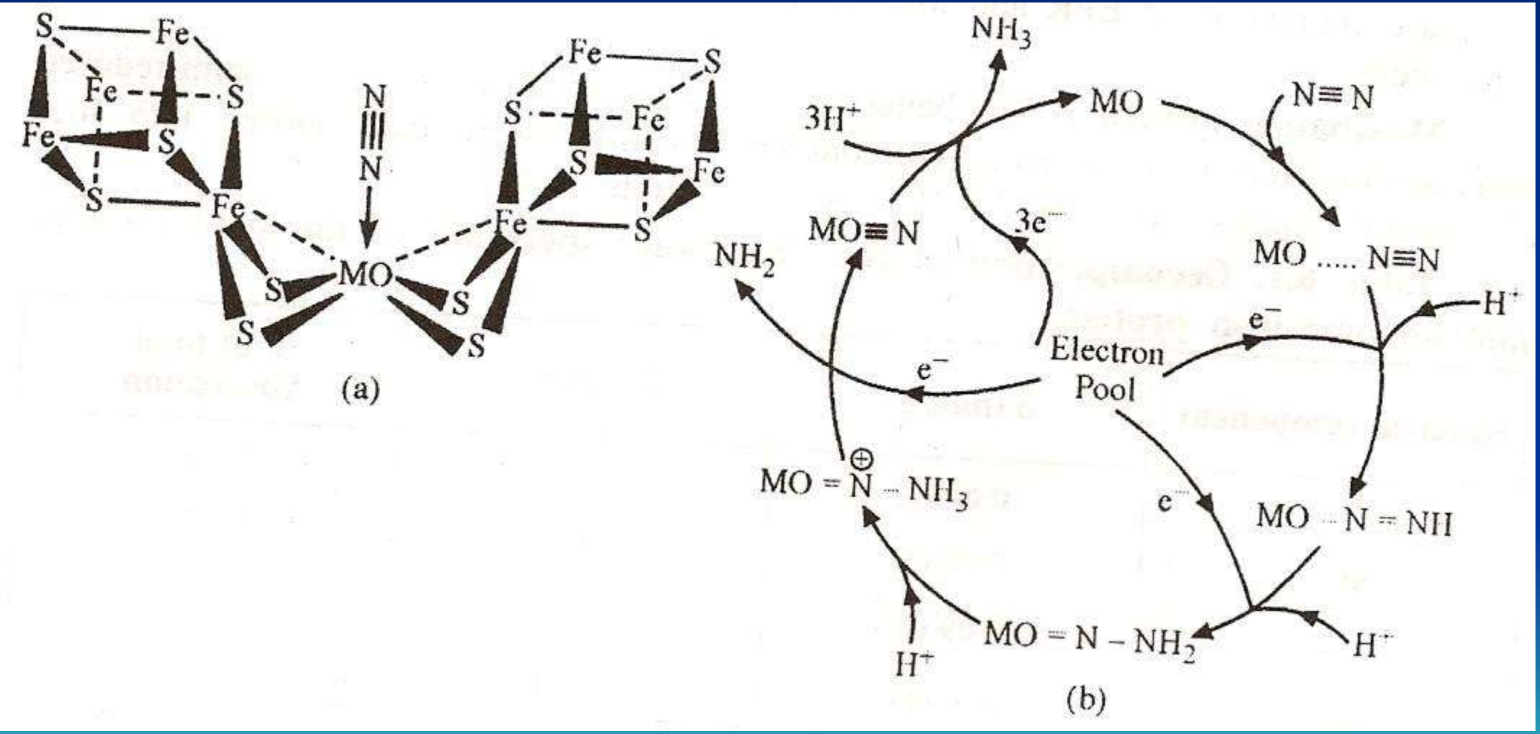


Fig.5: (a) Proposed arrangement of Mo, S and Fe in the active site of nitrogenase (b) Proposed catalytic cycle of nitrogen fixation and conversion.

- Thus the nitrogen compounds are of great importance both for constructive and destructive purposes.
- Our country is essentially an agricultural one. Hence we require nitrogenous fertilizers whose manufacture is based upon the fixation of nitrogen.
- Nitric acid is used in the manufacture of explosives viz. nitro-glycerine, picric acid, dynamite, T.N.T. etc. which can be used both for constructive and destructive purposes.

Thanks