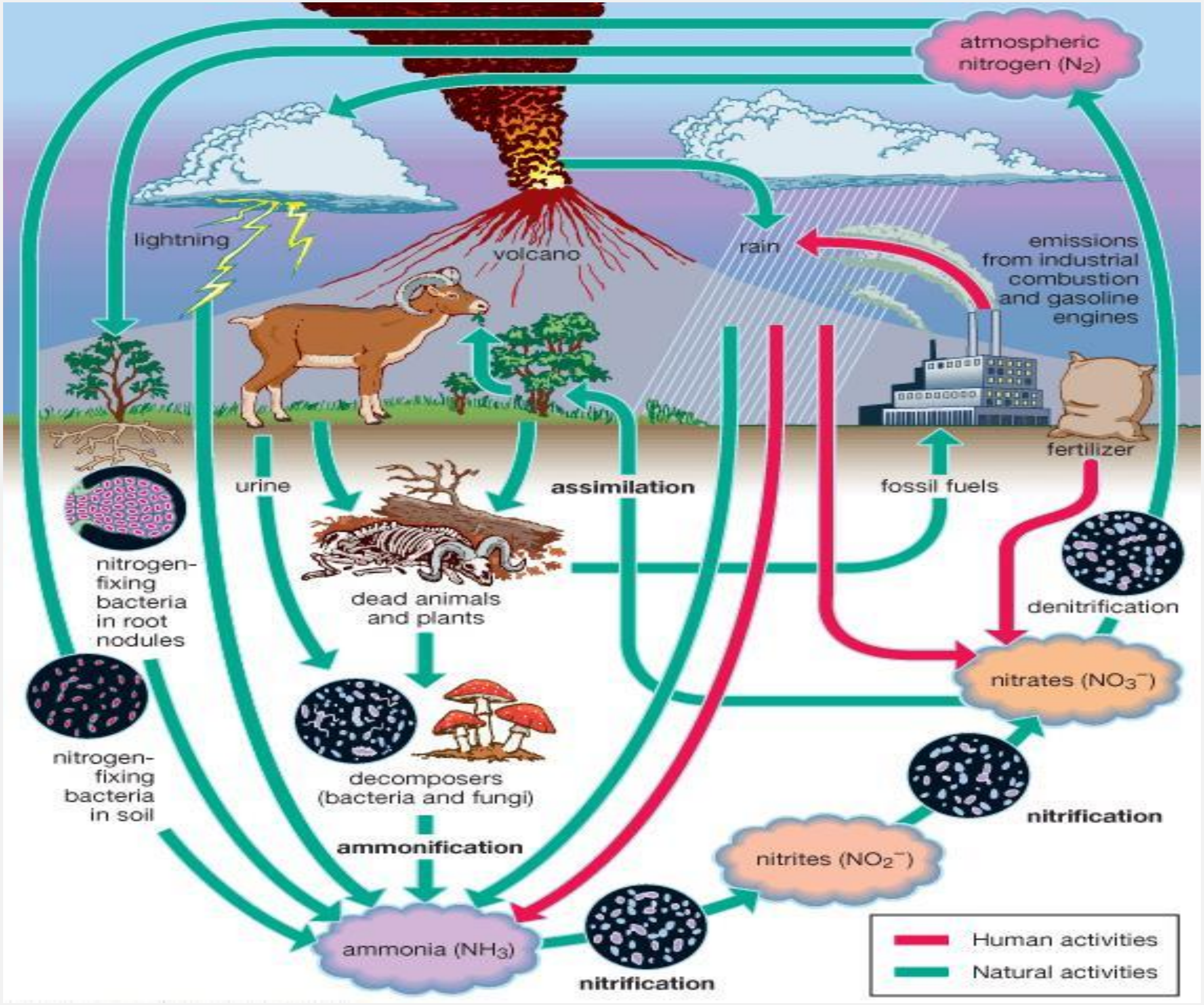
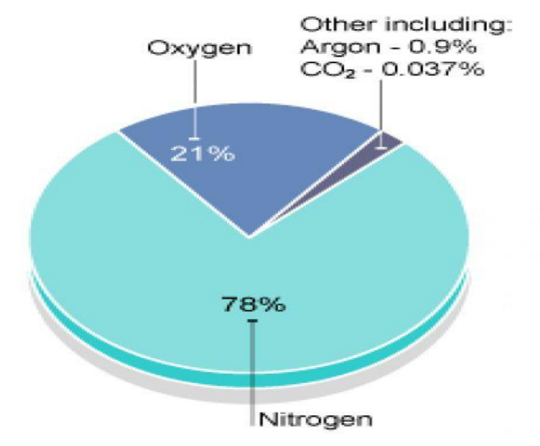
Nitrogen Fixation

Prof. Ramesh Chandra
Professor
Department of chemistry
University of Delhi


atmospheric nitrogen (N_2)
lightning
volcano
rain
emissions from industrial combustion and gasoline engines
urine
assimilation
fossil fuels
fertilizer
nitrogen-fixing bacteria in root nodules
dead animals and plants
denitrification
nitrates (NO_3^-)
nitrogen-fixing bacteria in soil
decomposers (bacteria and fungi)
nitrification
ammonification
nitrites (NO_2^-)
ammonia (NH_3)
nitrification
Human activities
Natural activities

NITROGEN

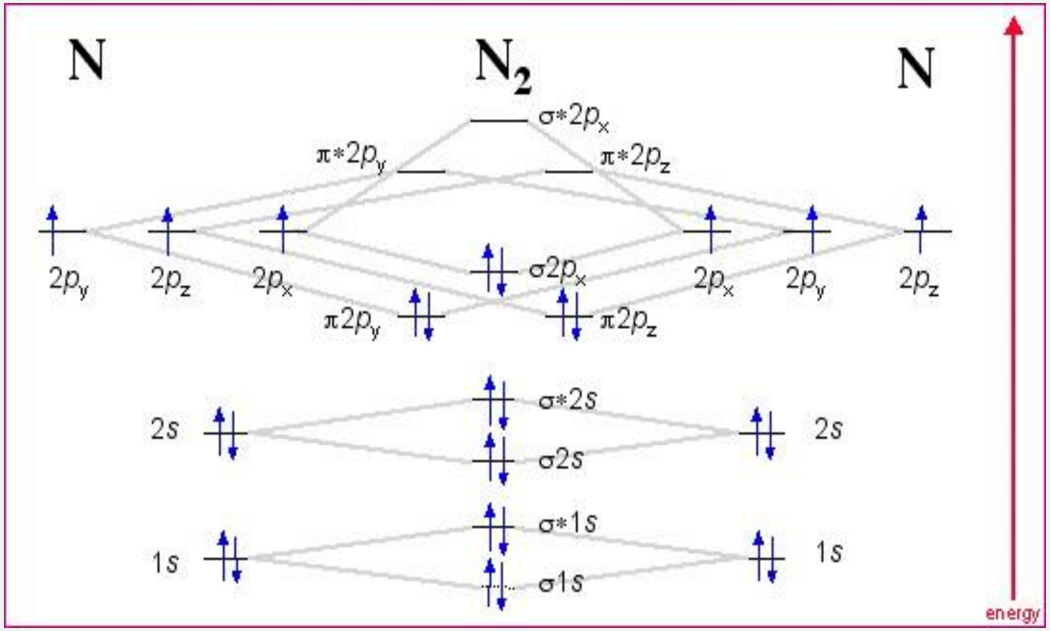
- Molecular nitrogen (N_2) is the major component (approximately 80%) of the earth's atmosphere.



- The element nitrogen is an essential part of many of the chemical compounds, such as proteins and nucleic acids, which are the basis of all life forms.
- However, N_2 cannot be used directly by biological systems to build the chemicals required for growth and reproduction. Before its incorporation into a living system, N_2 must first be combined with the element hydrogen. This process of reduction of N_2, commonly referred to as "nitrogen fixation"(N-fixation) may be accomplished chemically or biologically.

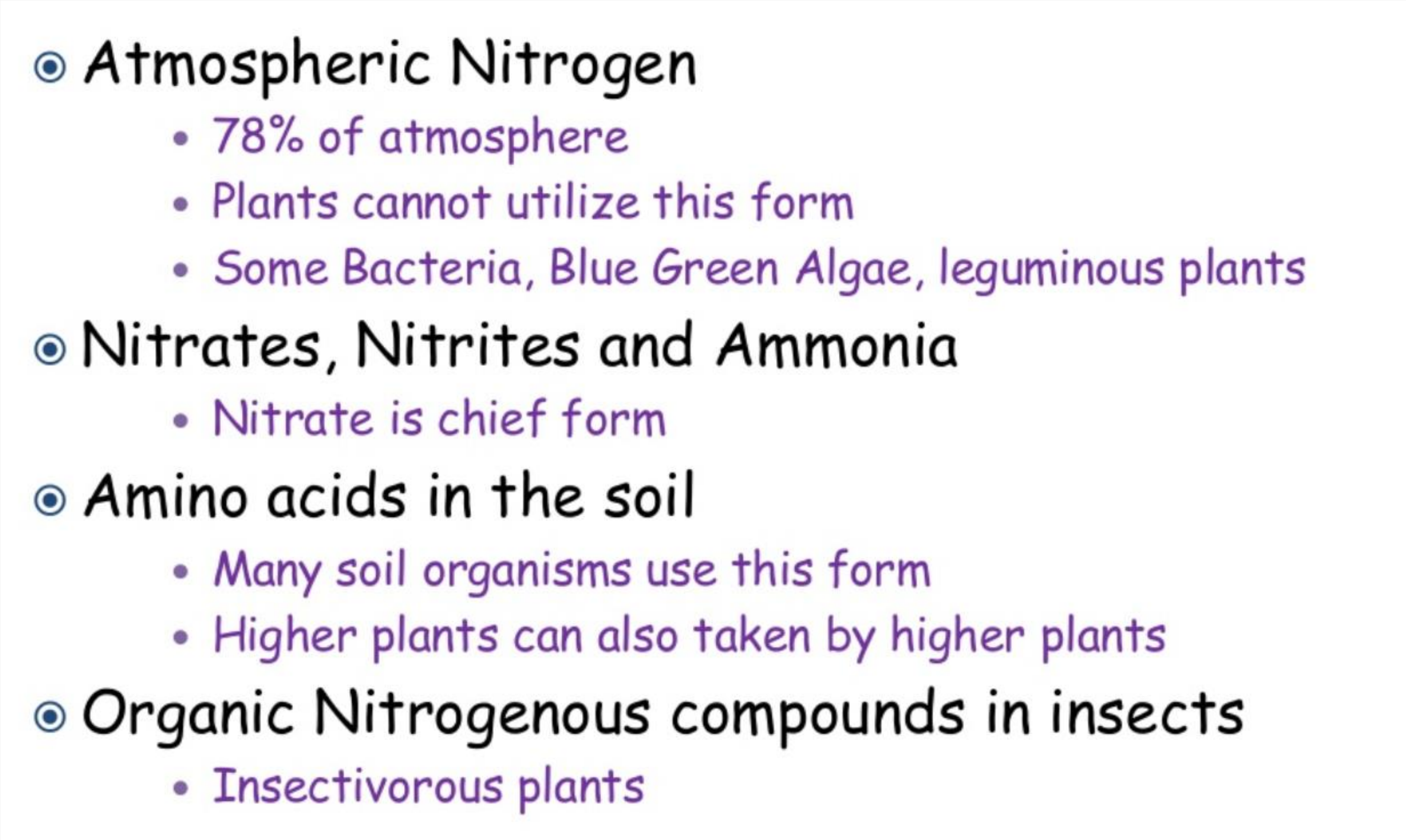
Properties of Dinitrogen which makes it inert

- Dinitrogen- Two N atoms connected by triple bond.
- Breaking the N≡N bond is difficult- high dissociation energy of 942 kJ mol^{-1}
- High ionization potential (15.058 eV).
- Inert.
- Nonpolar.
- Weak σ donor.
- Poor π acceptor.



Difference between its HOMO and LUMO is very large (22.9 eV).
As a result it is very difficult to add electrons to dinitrogen molecule or to remove them from it in the ground state.

Sources of Nitrogen



- Atmospheric Nitrogen
    - 78% of atmosphere
    - Plants cannot utilize this form
    - Some Bacteria, Blue Green Algae, leguminous plants
- Nitrates, Nitrites and Ammonia
    - Nitrate is chief form
- Amino acids in the soil
    - Many soil organisms use this form
    - Higher plants can also taken by higher plants
- Organic Nitrogenous compounds in insects
    - Insectivorous plants

Nitrogen Fixation: Need

It is one of nature's great ironies…

Nitrogen is an essential component of DNA, RNA, and proteins—the building blocks of life.

Although the majority of the air we breathe is *nitrogen*, most living organisms are *unable to use nitrogen* as it exists in the *atmosphere*!

What is Nitrogen Fixation?

"*Nitrogen Fixation*" is the process that converts the atmospheric nitrogen to useful/simpler compounds of nitrogen.

The conversion of N_2 to reactive forms of N is nitrogen fixation

- ***Nitrogen fixation done naturally by microbes***
- ***Humans do N fixation chemically = fertilizer production***

Nitrogen gets "*fixed*" when it is combined with oxygen or hydrogen.

The very first step of all fertilizer production is based on
Reactions discovered in the early 20th century by two scientists:
Haber & Bosch

What is Nitrogen Fixation?

Nitrogen fixation, any natural or industrial process that causes free nitrogen (N_2), which is a relatively inert gas plentiful in air, to combine chemically with other elements to form more-reactive nitrogen compounds such as ammonia, nitrates, or nitrites.

Or

Nitrogen fixation is the process by which atmospheric nitrogen is converted either by a natural or …

- Nitrogen is fixed, or combined, in nature as nitric oxide by lightning and ultraviolet rays, but more significant amounts of nitrogen are fixed as ammonia, nitrites, and nitrates by soil microorganisms
- More than 90 percent of all nitrogen fixation is effected by them. Two kinds of nitrogen-fixing microorganisms are recognized: free-living (non-symbiotic) bacteria, including the cyanobacteria (or blue-green algae) *Anabaena* and *Nostoc* and genera such as *Azotobacter, Beijerinckia*, and *Clostridium;* and mutualistic (symbiotic) bacteria such as *Rhizobium*, associated with leguminous plants, and various *Azospirillum* species, associated with cereal grasses.

What is Nitrogen Fixation?

- The symbiotic nitrogen-fixing bacteria invade the root hairs of host plants, where they multiply and stimulate the formation of root nodules, enlargements of plant cells and bacteria in intimate association. Within the nodules, the bacteria convert free nitrogen to ammonia, which the host plant utilizes for its development.

- Within the nodules, the bacteria convert free nitrogen to ammonia, which the host plant utilizes for its development. To ensure sufficient nodule formation and optimum growth of legumes (e.g., alfalfa, beans, clovers, peas, soybeans), seeds are usually inoculated with commercial cultures of appropriate *Rhizobium* species, especially in soils poor or lacking in the required bacterium.

- Nitrogenous materials have long been used in agriculture as fertilizers, and in the course of the 19th century the importance of fixed nitrogen to growing plants was increasingly understood. Accordingly, ammonia released in making coke from coal was recovered and utilized as a fertilizer, as were deposits of sodium nitrate (saltpetre) from Chile.
- Wherever intensive agriculture was practiced, there arose a demand for nitrogen compounds to supplement the natural supply in the soil. At the same time, the increasing quantity of Chile saltpetre used to make gunpowder led to a worldwide search for natural deposits of this nitrogen compound.

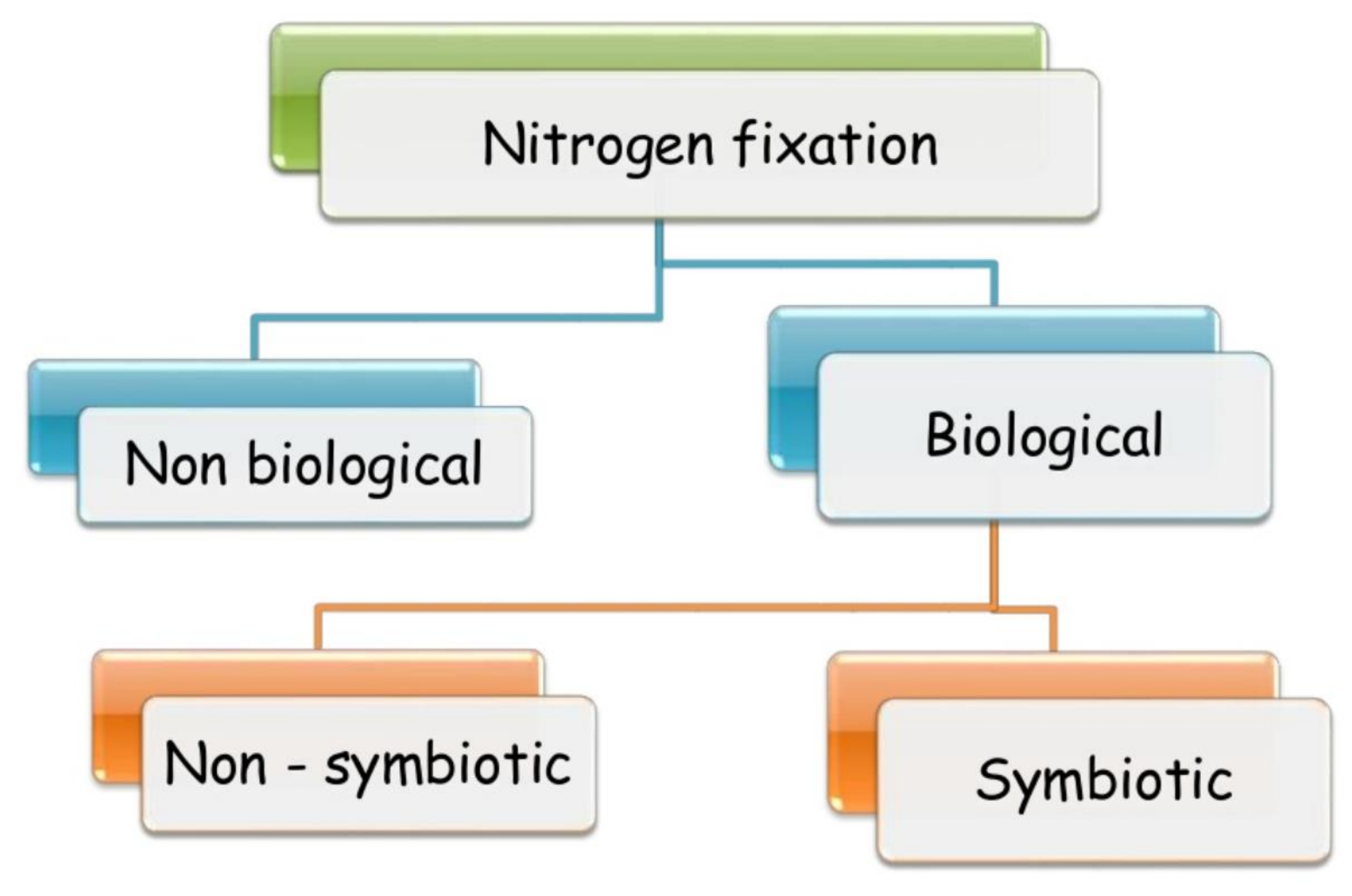
The Mechanism of Nitrogen Fixation

- The general chemical reaction for the fixation of nitrogen is identical for both the chemical and the biological processes.

$$N_2+3H_2+Energy = 2NH_3$$

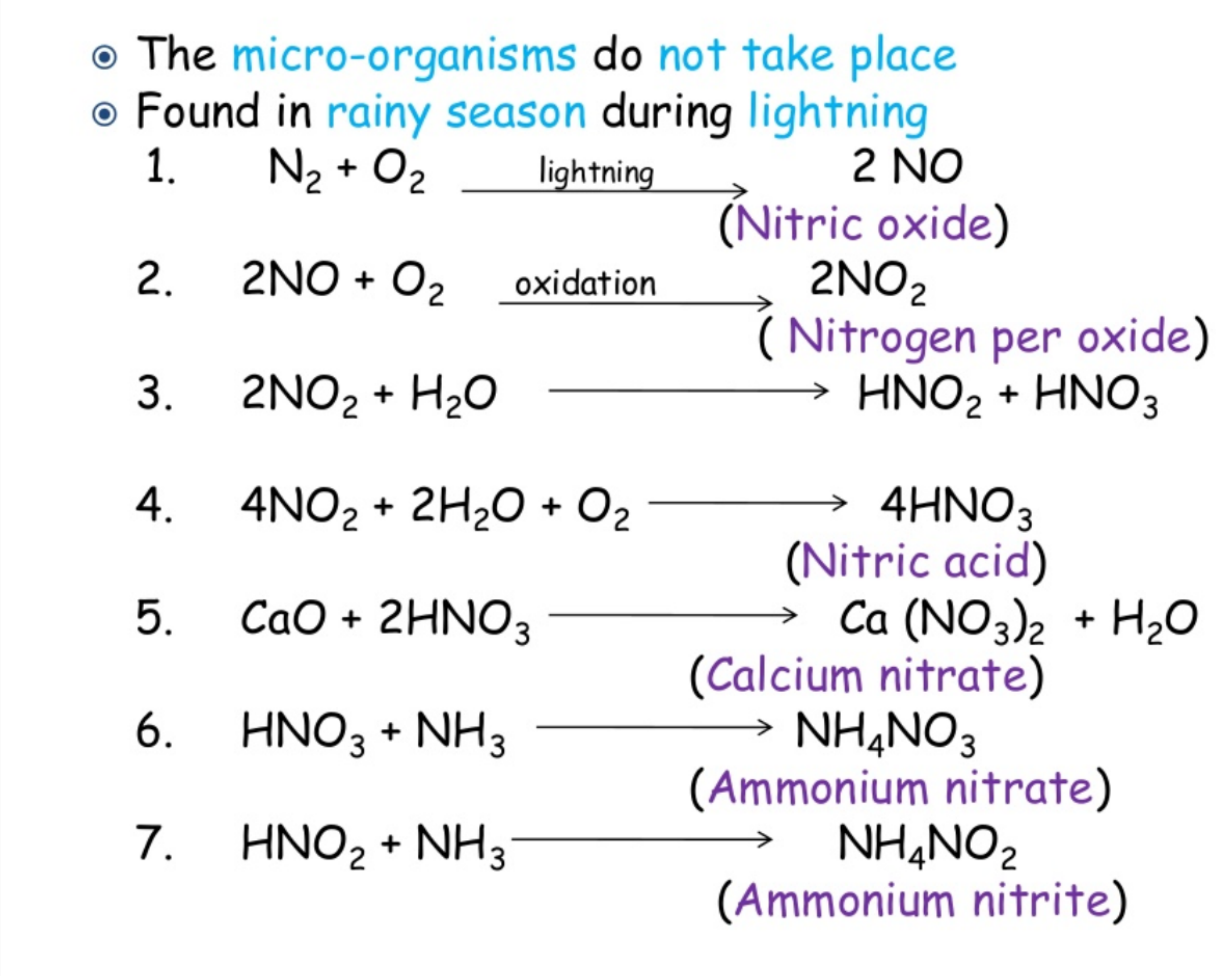
- The triple bond of N must be broken and three atoms of hydrogen must be added to each of the nitrogen atoms. Living organisms use energy derived from the oxidation ("burning") of carbohydrates to reduce molecular nitrogen (N_2) to ammonia (NH_3). The chemical process of nitrogen fixation involves "burning" of fossil fuels to obtain the electrons, hydrogen atoms and energy needed to reduce molecular nitrogen.

Types of Nitrogen Fixation



- Nitrogen fixation
  - Non biological
  - Biological
    - Non - symbiotic
    - Symbiotic

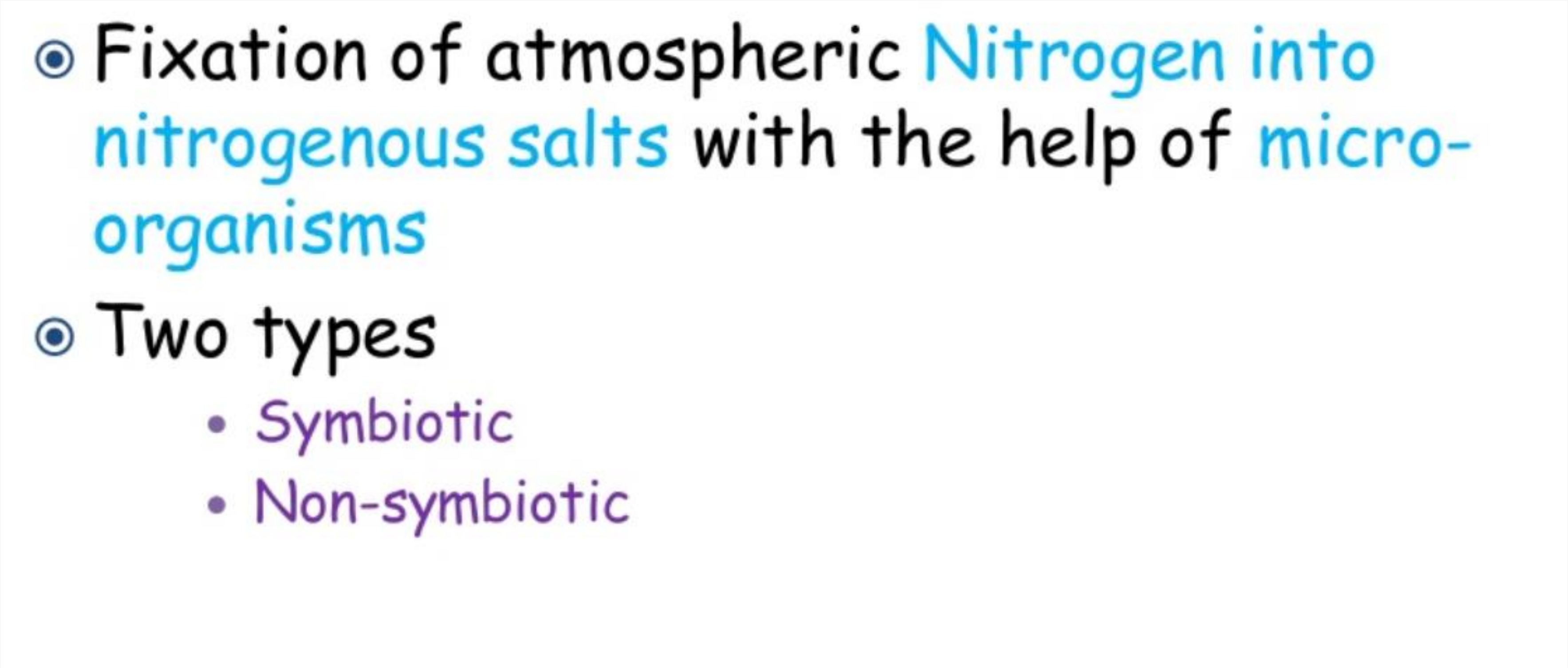
Non-Biological Nitrogen Fixation



- The micro-organisms do not take place
- Found in rainy season during lightning

1. $N_2 + O_2 \xrightarrow{\text{lightning}} 2\ NO$ (Nitric oxide)
2. $2NO + O_2 \xrightarrow{\text{oxidation}} 2NO_2$ (Nitrogen per oxide)
3. $2NO_2 + H_2O \longrightarrow HNO_2 + HNO_3$
4. $4NO_2 + 2H_2O + O_2 \longrightarrow 4HNO_3$ (Nitric acid)
5. $CaO + 2HNO_3 \longrightarrow Ca\,(NO_3)_2 + H_2O$ (Calcium nitrate)
6. $HNO_3 + NH_3 \longrightarrow NH_4NO_3$ (Ammonium nitrate)
7. $HNO_2 + NH_3 \longrightarrow NH_4NO_2$ (Ammonium nitrite)

Biological Nitrogen Fixation



- Fixation of atmospheric Nitrogen into nitrogenous salts with the help of micro-organisms
- Two types
    - Symbiotic
    - Non-symbiotic

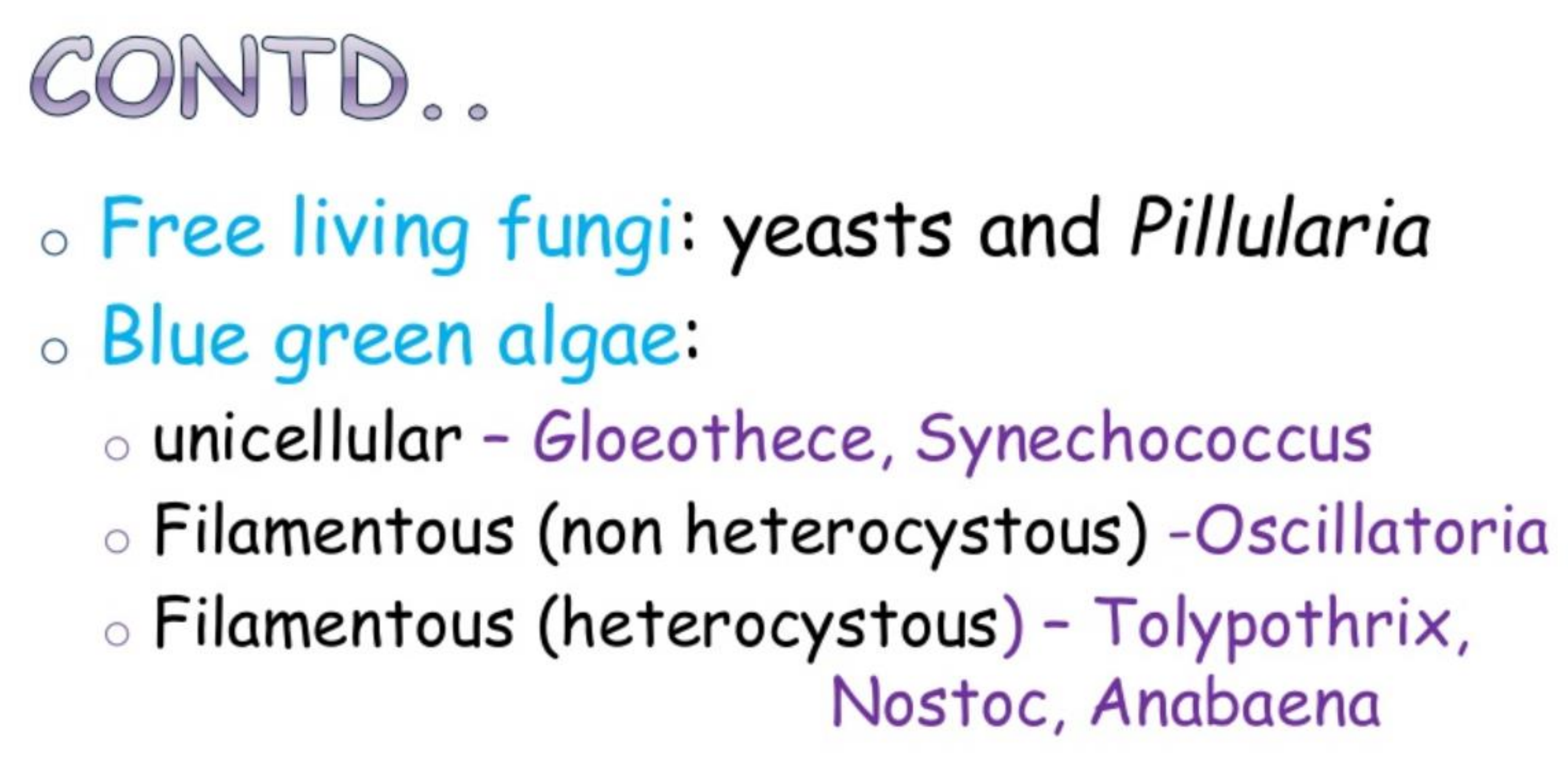
Non-Symbiotic

- Fixation carried out by free living micro-organisms
- Aerobic, anaerobic and blue green algae
- Bacteria: special type (nitrogen fixing bacteria) types -
    - Free living aerobic : *Azotobacter, Beijerenckia*
    - Free living anaerobic : *Clostridium*
    - Free living photosynthetic : *Chlorobium, Rhodopseudomonas*
    - Free living chemosynthetic :*Desulfovibro,Thiobacillus*

Non-Symbiotic

- Fixation carried out by free living micro-organisms
- Aerobic, anaerobic and blue green algae
- Bacteria: special type (nitrogen fixing bacteria) types -
  - Free living aerobic : *Azotobacter, Beijerenckia*
  - Free living anaerobic : *Clostridium*
  - Free living photosynthetic : *Chlorobium, Rhodopseudomonas*
  - Free living chemosynthetic :*Desulfovibro,Thiobacillus*

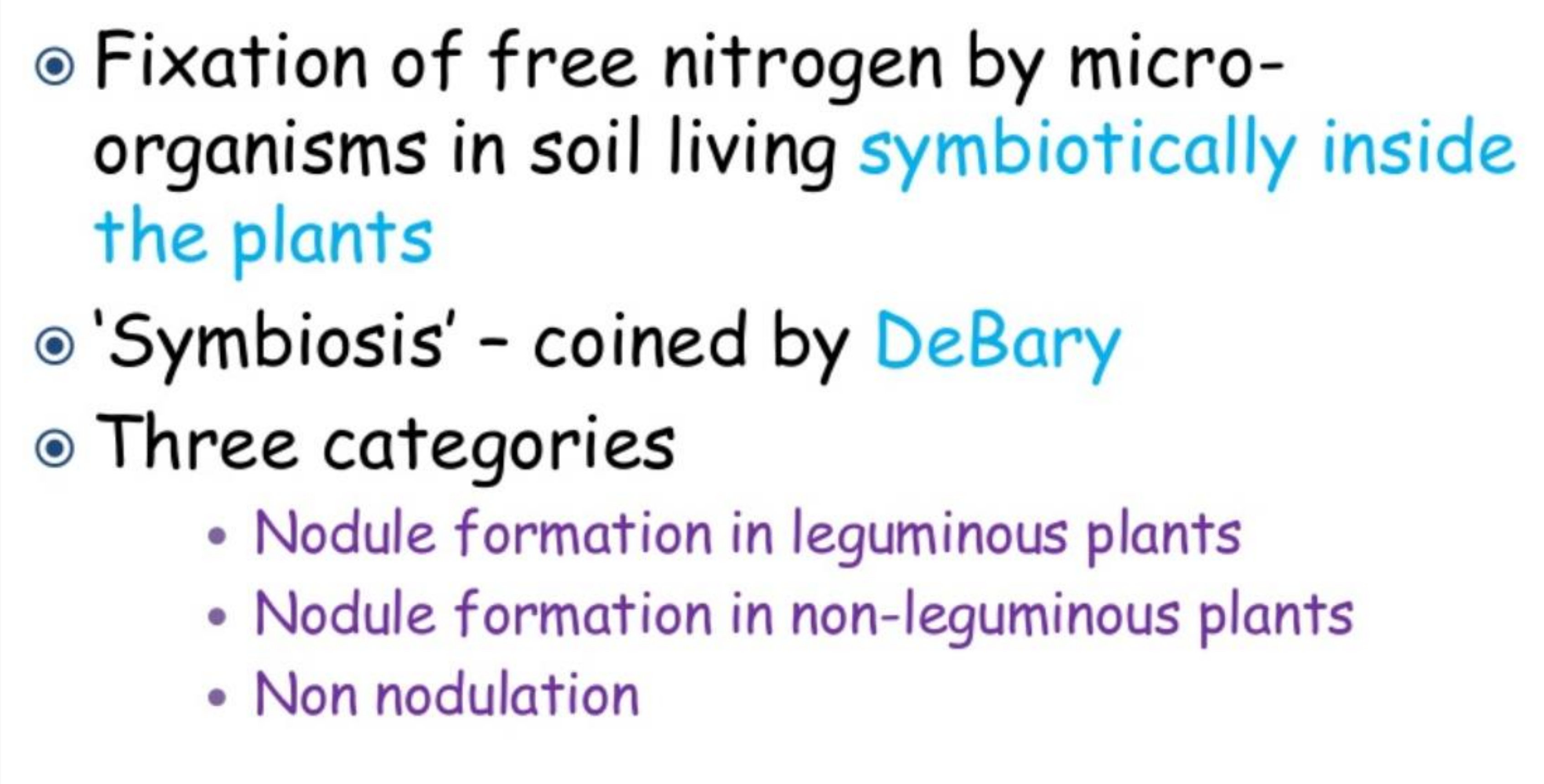
Non-Symbiotic



CONTD..

- Free living fungi: yeasts and *Pillularia*
- Blue green algae:
  - unicellular – Gloeothece, Synechococcus
  - Filamentous (non heterocystous) -Oscillatoria
  - Filamentous (heterocystous) – Tolypothrix, Nostoc, Anabaena

Symbiotic



- Fixation of free nitrogen by micro-organisms in soil living symbiotically inside the plants
- 'Symbiosis' – coined by DeBary
- Three categories
    - Nodule formation in leguminous plants
    - Nodule formation in non-leguminous plants
    - Non nodulation

Nitrogen Fixation: Processes

Processes

In-vivo

Within a living organism

Biological Fixation

In-vitro

In an artificial environment outside a living organism

Atmospheric Fixation

Industrial Fixation

Biological Fixation(BNF)

(where MOST nitrogen fixing is completed)

There are two types of "Nitrogen Fixing Bacteria"

Free Living Bacteria
("fixes" 30% of N_2)

Symbiotic Relationship Bacteria
("fixes" 70% of N_2)

In Vitro Nitrogen Fixation

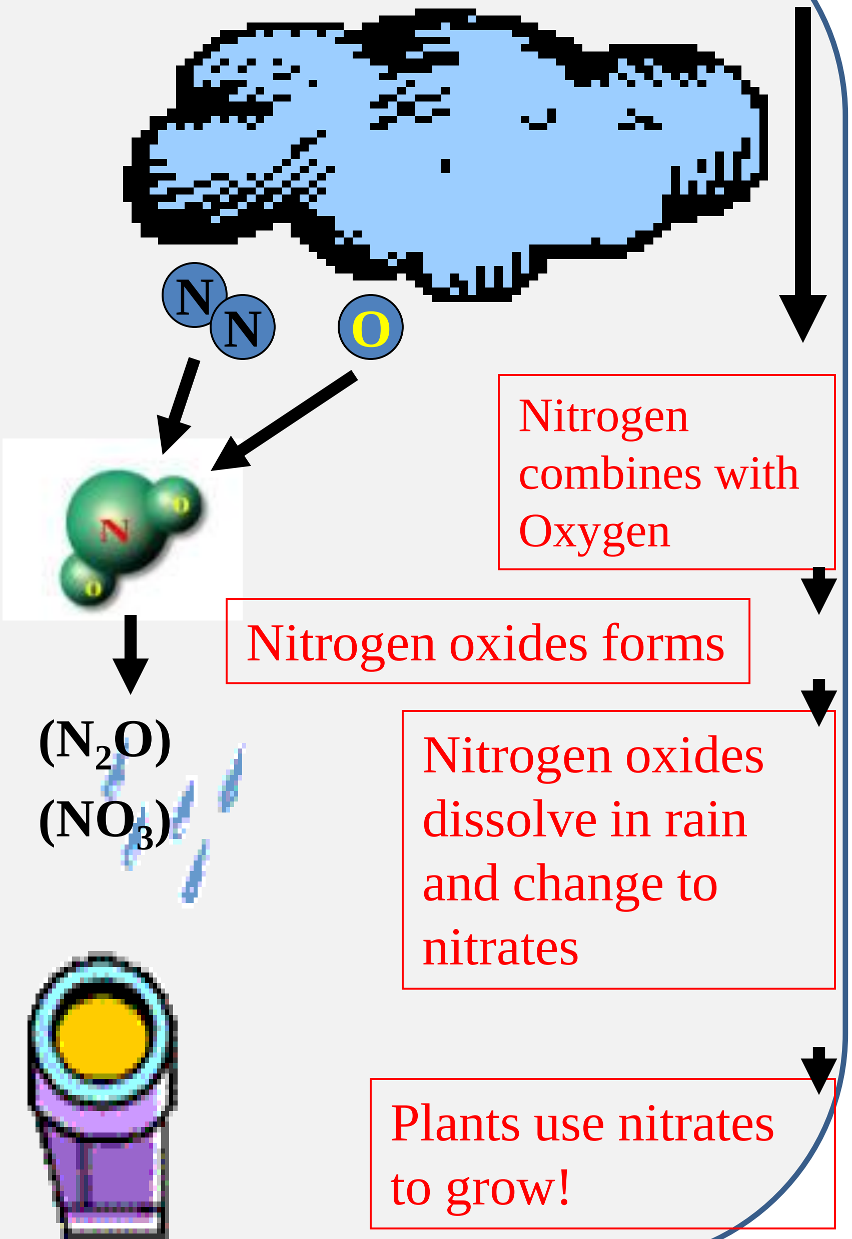
- “*in vitro*” is a Latin word which means “within the glass”.
- A procedure performed ***in vitro*** is performed not in a living organism but in a controlled environment, such as in a test tube or Petri dish.
- Because the test conditions may not correspond to the conditions inside of the organism, this may lead to results that do not correspond to the situation that arises in a living organism. Consequently, such experimental results performed *in vitro*, are in contradiction with *in vivo*.
- *in vitro* experiments are performed to model *in vivo* experiments.

Atmospheric Fixation

(Only 5 to 8% of the Fixation Process)

The enormous energy of *lightning breaks nitrogen molecules apart* and enables the nitrogen atoms to combine with oxygen forming *nitrogen oxides (N_2O)*. Nitrogen oxides dissolve in rain, forming nitrates. *Nitrates (NO_3)* are carried to the ground with the rain.

Lightning "fixes" Nitrogen!



(N_2O)

(NO_3)

Nitrogen combines with Oxygen

Nitrogen oxides forms

Nitrogen oxides dissolve in rain and change to nitrates

Plants use nitrates to grow!

INDUSTRIAL FIXATION

During the first decade of the 20th century, intensive research efforts culminated in the development of several commercial nitrogen-fixation processes.

- **The arc process-** Air is passed through an electric arc & about 1% nitric oxide is formed which can be chemically converted to nitrates.By 1902 electric generators were in use at Niagara Falls, N.Y. This venture failed commercially because it was costly and inherently inefficient in its use of energy
- **The cyanamide process-** reaction of nitrogen with calcium carbide at high temperatures to form calcium cynamide, which hydrolyzes to ammonia and urea. This was utilized on a large scale by several countries before and during World War I, but it too was energy-intensive.
- **The Haber-Bosch process** -directly synthesizes ammonia from nitrogen and hydrogen and was the Most economical nitrogen-fixation process

HABER-BOSCH PROCESS HISTORY

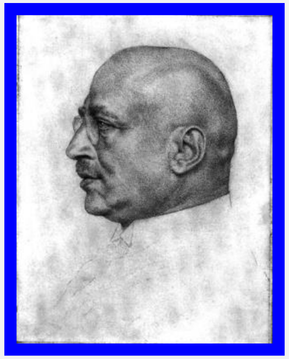
- During nineteenth century, due to the increased demand for nitrates and ammonia for use as fertilizers and industrial feedstocks , there was need for the potential source of ammonia because the main source which was the mining of niter deposits was being predicted to be unable to satisfy future demands.

- The most obvious source was atmospheric nitrogen (N_2), which makes up nearly 80% of the air, however N_2 is exceptionally stable and will not readily react with other chemicals. Converting N_2 into ammonia therefore posed a chemical challenge which occupied the efforts of chemists across the world.

- Haber together with his assistant Robert Le Rossignol developed the high-pressure devices and catalysts used to demonstrate the Haber process at laboratory scale. They demonstrated their process in 1909 by producing ammonia from air drop by drop, at the rate of about 125 ml (4 US fl oz) per hour. The process was purchased by the German chemical company BASF, which assigned Carl Bosch the task of scaling up Haber's tabletop machine to industrial-level production.

- Haber and Bosch were later awarded Nobel prizes, in 1918 and 1931 respectively, for their work in overcoming the chemical and engineering problems posed by the use of large-scale, continuous-flow, high-pressure technology.

Haber-Bosch process 1918



Fritz Haber
(1868-1934)

Carl Bosch
(1874-1940)

$$N_2 + 3H_2 \rightleftharpoons 2NH_3 \quad \Delta H = -92\ \text{kJ mol}^{-1}$$

- In 1909 **Fritz Haber** established the conditions under which N_2 and H_2 would combine to yield ammonia. He got Nobel Prize in Chemistry in1918 for the synthesis of ammonia from its elements.

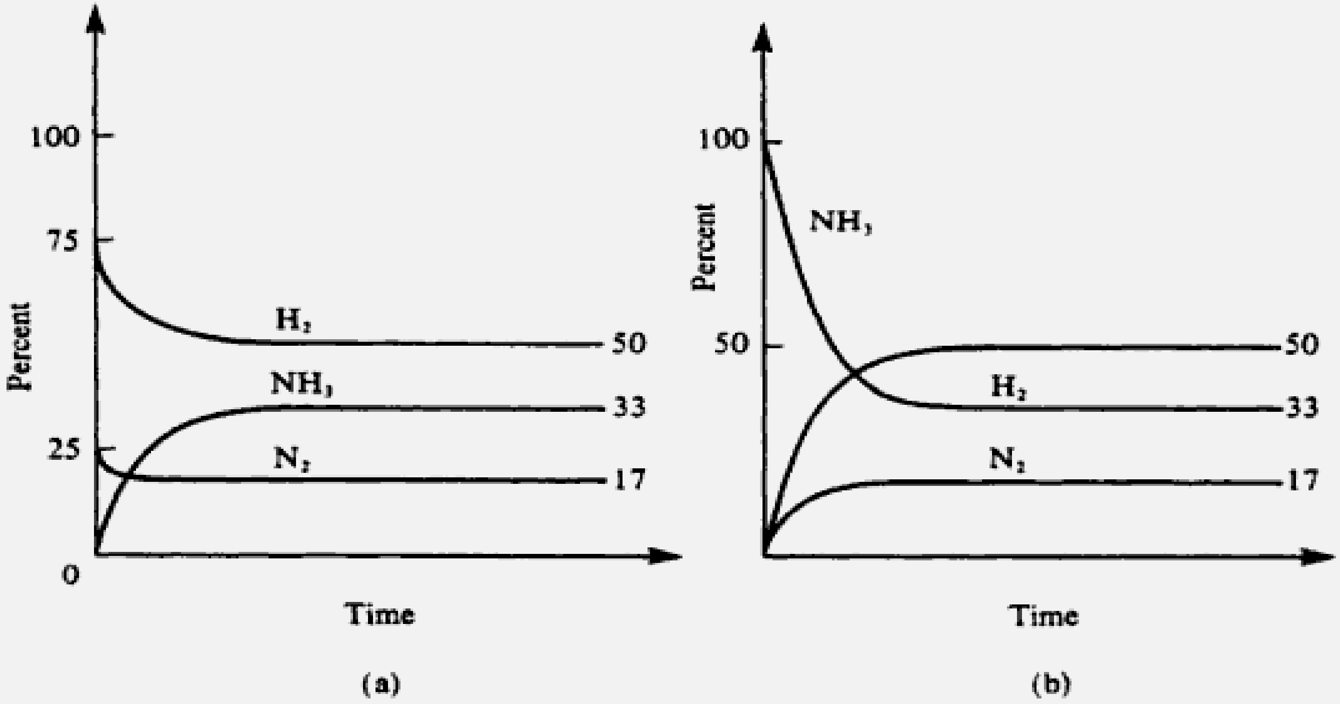
This process, made commercially feasible by **Carl Bosch**, came to be called the **Haber-Bosch process**, or the synthetic ammonia process..

The Haber-Bosch process is used to more than 500 million tons (453 billion kilograms) of artificial fertilizer per year produce;

- Roughly 1% of the world's energy is used for it, and it sustains about 40% of World's population.
- Haber-Bosch was the first industrial chemical process to use high pressure for a chemical reaction.

The Haber-Bosch Process

Reversible Reactions and Dynamic Equilibrium



(a) (b)

Attainment of equilibrium for the reaction

$$N_2(g) + 3\,H_2(g) \rightleftharpoons 2\,NH_3(g)$$

at 520°C, 500 atm total pressure. In case a, the N_2 and H_2 are mixed in a 1:3 ratio (1 mol of N_2 + 3 mol of H_2, or 25 mol % N_2 + 75 mol % H_2) and allowed to reach equilibrium. The equilibrium mole percentages are 17% N_2, 50% H_2, and 33% NH_3. In case b, 2 mol of NH_3 (100 mol % NH_3) is allowed to reach equilibrium. The equilibrium mole percentages of N_2, H_2, and NH_3 are exactly the same as in case a.

Reversible Reactions

- The Haber process is a **REVERSIBLE** reaction

$N_2(g) + 3H_2(g) \rightleftharpoons 2NH_3(g) \quad \Delta_{rxn}H^0 = -92\ \text{kJ/mol}$

nitrogen + hydrogen $\rightleftharpoons$ ammonia

A reversible reaction is one where the products of the reaction can themselves react to produce the original reactants.

Thermodynamic data(at 298 K)

$$N_2(g) + 3H_2(g) \rightleftharpoons 2NH_3(g)$$

	$N_2(g)$	$3H_2(g)$	$2NH_3(g)$	
$\Delta_f H^0$(kJ mol^{-1})	0	0	2× -46.11	-92.11
S^0(JK^{-1}mol^{-1})	191.61	3× 130.684	2×192.45	-198.76
$\Delta_f G^0$(kJ mol^{-1})	0	0	2× -16.45	-32.90
C_p(JK^{-1}mol^{-1})	29.125	3× 28.824	2× 35.06	

$$\Delta_r H^0 = -92.11\ \text{kJ mol}^{-1}$$

$$\Delta_r S^0 = -198.8\ \text{J K}^{-1}\text{mol}^{-1}$$

$$\Delta_r G^0 = -32.90\ \text{kJ mol}^{-1}$$

$$K = \exp(-\frac{\Delta_r G^0}{RT})$$

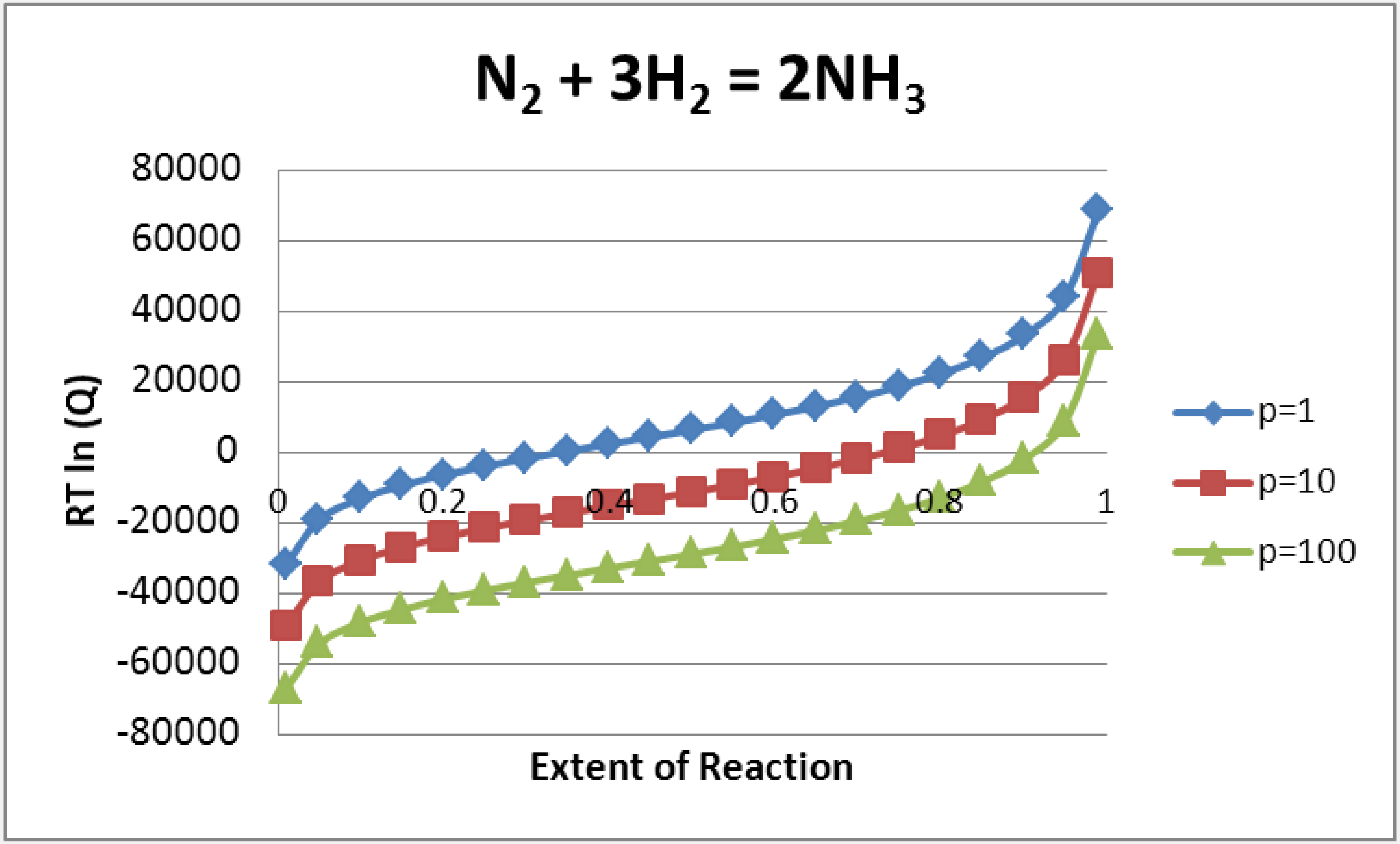
$$= \exp(-\frac{32.90 \times 1000}{8.314 \times 298})$$

$$= 5.85 \times 10^5$$

Reversible Reactions

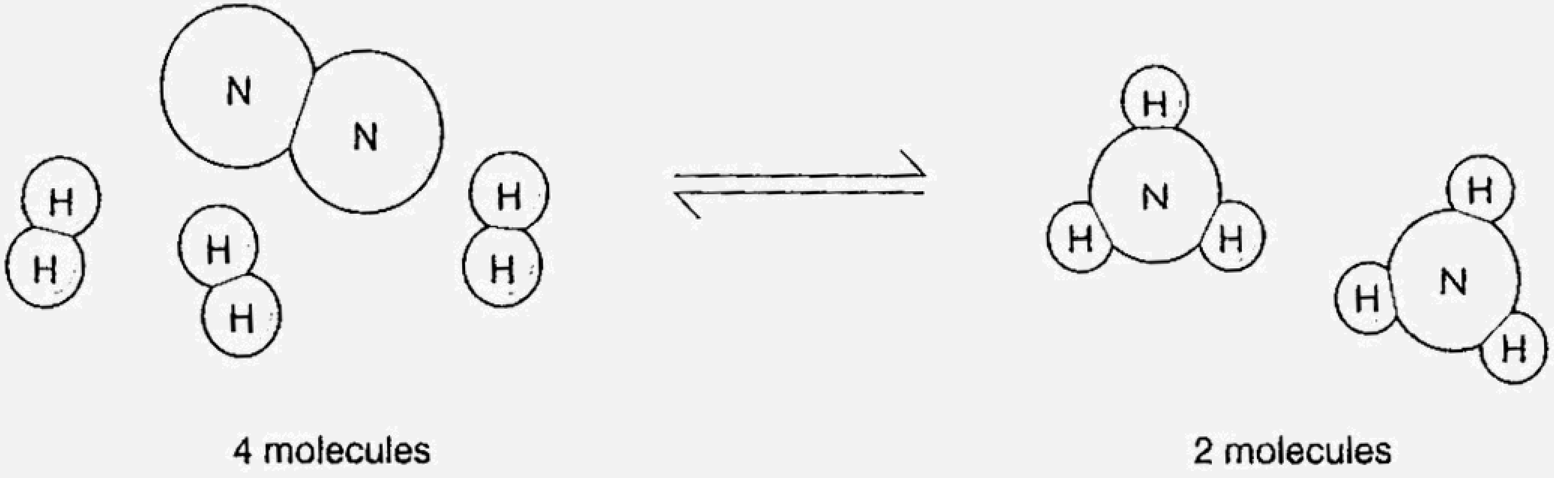
- Factors that can affect a reversible reaction include:
  - Changing pressure(or volume, concentration)
  - Changing temperature

Effect of Pressure



Here,
R = Gas constant,
T= Temparature,
Q = reaction quotient.

Effect of Pressure



Effect of pressure

- On the left hand side there are 4 moles of gas, whilst on the right hand side there are 2 moles
- Any increase in pressure will favour the forward reaction to produce more ammonia.
- This is because the forward reaction will tend to **decrease** the pressure in the system.

For High Yield

- In the Haber process the pressure is set as high as possible to give the best % yield.
- High pressure containers are VERY expensive.
- It could be possible to carry out the reaction at 1000 atmospheres – but this would not be economical (it would cost more than the product is worth).
- The typical pressure used is 200 to 350 atmospheres.

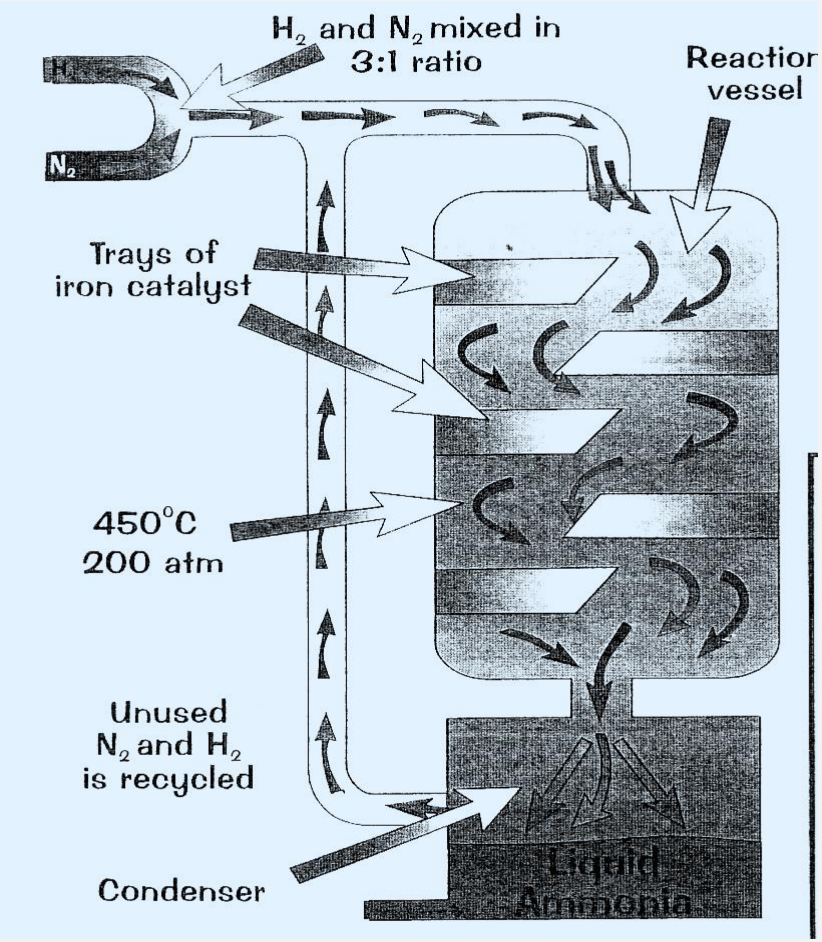
Improving the yield of ammonia in the Haber process

- $\Delta_{rxn}H^0 = -92$ kJ/mol. LOW temperature!

BUT….

- Reactions is slower at lower temperatures!
- In operating the Haber process you have to decide what is more important, the higher YIELD you can get at lower temperatures or the higher RATE at higher temperatures.
- In order to get ammonia produced at a quicker RATE the reaction is carried out at a high temperature (450°C).
- It is better to get just a 10% yield in 20 seconds (at a high temperature) than a 20% in 60 seconds (at a lower temperature)

Catalyst



- An IRON catalyst makes the reaction occur more quickly, (it does not affect the % yield i.e. the position of the dynamic equilibrium).

- Without the catalyst the temperature would have to be much higher (this would lower the yield).

Why bother with an alternative to Haber-Bosch?

- Haber-Bosch process requires high pressure and temperature, in addition to a catalyst to induce combination of H_2 and N_2.

- That is, what nature does apparently quite readily under mild conditions with no extremes of pressure and temperature, we need to apply the extreme conditions.

- So, a desirable improvement would be a process that operates at much lower temperature and pressure.

N_2 activation by coordination to transition metals:

Coordination of dinitrogen to metal offers a means of increasing its reactivity.

N_2 is a poor donor, but this does not exclude the possibility of complex formation.

CO has poor σ donor properties, but stable carbonyl complexes are formed as a result of synergic effect in which essentially weak σ and π bonding effects reinforce each other to form strong metal carbonyl bonds at the expense of C-O bond.

Same is the case with dinitrogen.

Bonding:

N_2 binds end on as well as side on to transition metals with N-N bond length 1.123-1.24 Å, which is longer than N-N bond length of 1.097 Å in free N_2.

$$M - N^{+} \equiv N \longleftrightarrow M^{+} = N^{+} = N^{-}$$

The lengthening of N-N bond arises due to the back donation of d-electron density of metal into the π^* orbital of N_2 i.e metal-Nitrogen bond is formed at the expense of N-N bond.

A brief history:

- The discovery that N_2 forms stable complexes with transition metals led to extensive investigation of the possibility of nitrogen fixation via such complexes. The key events in the history of dinitrogen coordination chemistry are listed here:
- In 1965, Allen and Senoff prepared and isolated $[Ru(NH_3)_5(N_2)]^{2+}$ from reduction of $RuCl_3$ by hydrazine, and discovered that $[Ru(NH_3)_5]_2^+$ could reversibly coordinate dinitrogen.

$$RuCl_3.3H_2O \xrightarrow{\qquad} [Ru(NH_3)_5N_2]Cl_2$$

- Later other methods were also developed for its preparation

$$[Ru(NH_3)_5Cl]^{+2} \xrightarrow[H_2O]{Zn\ (Hg)} [Ru(NH_3)_5H_2O]^{+2}$$

$$[Ru(NH_3)_5H_2O]^{+2} + N_2 \xrightarrow{\qquad} [Ru(NH_3)_5N_2]^{+2} + H_2O$$

In 1968 Room temperature and pressure complexation of dinitrogen was done

$$2[Ru(NH_3)_5(H_2O)]^{2+} \xrightarrow{\qquad} [(NH_3)_5Ru\text{-}N{=}N\text{-}Ru(NH_3)_5]^{4+}.$$

Since then a large number of transition metal compounds that contain dinitrogen as a ligand have been discovered.

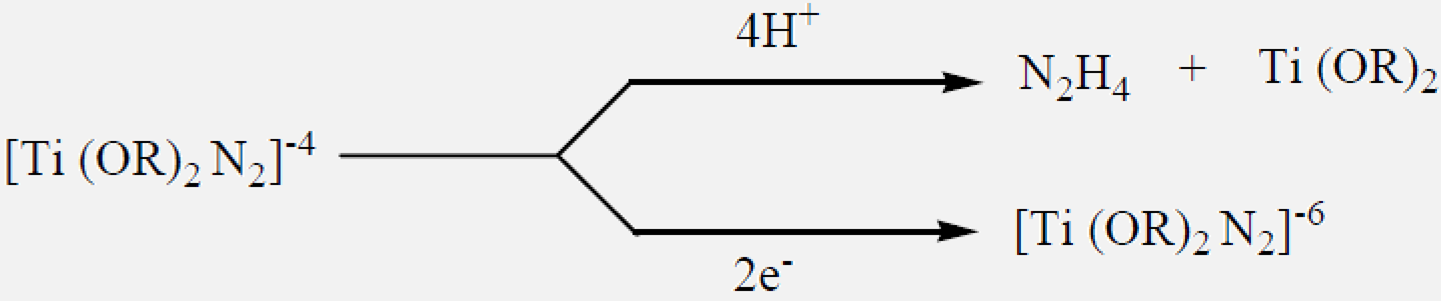
This discovery that dinitrogen was capable of forming stable complexes with transition metals led to extensive investigation of the possibility of fixation via such complexes. The first complex that was successful to fix N_2 was Ti complex.

Ti (+2) alkoxides form dinitrogen complexes which may then be reduced with subsequent release of hydrazine or NH_3. (An alkoxide is the conjugate base of an alcohol and therefore consists of an organic group bonded to a negatively charged oxygen atom. They can be written as RO−, where R is the organic substituent. Alkoxides are strong bases and, when R is not bulky, good nucleophiles and good ligands. Metal alkoxide involve a metal-oxygen-carbon bond system rather than a metal-carbon system they are not organometallic compounds but constitute an important allied field.)

$$\mathrm{Ti(OR)_4} + 2e^- \rightarrow \mathrm{Ti(OR)_2} + 2\mathrm{RO}^-$$

$$\mathrm{Ti(OR)_2} + \mathrm{N_2} \rightarrow [\mathrm{Ti(OR)_2 N_2}]$$

$$[\mathrm{Ti(OR)_2 N_2}] + 4e^- \rightarrow [\mathrm{Ti(OR)_2 N_2}]^{-4}$$



$$[Ti\ (OR)_2\ N_2]^{-4} \xrightarrow{4H^+} N_2H_4 + Ti\ (OR)_2$$

$$[Ti\ (OR)_2\ N_2]^{-4} \xrightarrow[2e^-]{} [Ti\ (OR)_2\ N_2]^{-6}$$

$$[Ti\ (OR)_2\ N_2\ ]^{-6} + 6H^+ \longrightarrow 2NH_3 + Ti\ (OR)_2$$

Such a process is not commercially competitive with the Haber process for the synthesis of ammonia but promises to be useful in the synthesis of other nitrogen compounds such as hydrazine and other organic nitrogen compounds.

- All methods for converting dinitrogen complexes into ammonia required very powerful reducing agents, the dinotrogen in the complex was almost as unreactive as atmospheric nitrogen.

- An important development was the discovery that certain phosphine complexes of molybdenum and tungsten containing dinitrogen readily yield ammonia in acidic media.

- Reaction occurs when compounds of the type $[M\ (N_2)_2\ (PR_3)_4]$ (M = Mo or W; R = alkyl or aryl) are treated at room temperature with H_2SO_4 in methanol solution.

$$[M\ (N_2)_2\ (PR_3)_4] + H_2SO_4 \xrightarrow{\text{MeOH}} 2NH_3 + N_2 + \text{other products}$$

Another example taking Mo complex

$[MoCl_3(THF)_3] + 3e^- + 2N_2 + \text{excess dppe} \rightarrow [Mo(N_2)_2(dppe)_2] + 3Cl^-$

$[Mo(N_2)_2(dppe)_2] + 6H^+ \rightarrow 2NH_3 + N_2 + \text{Mo products}$

Where THF = Tetrahydrofuran

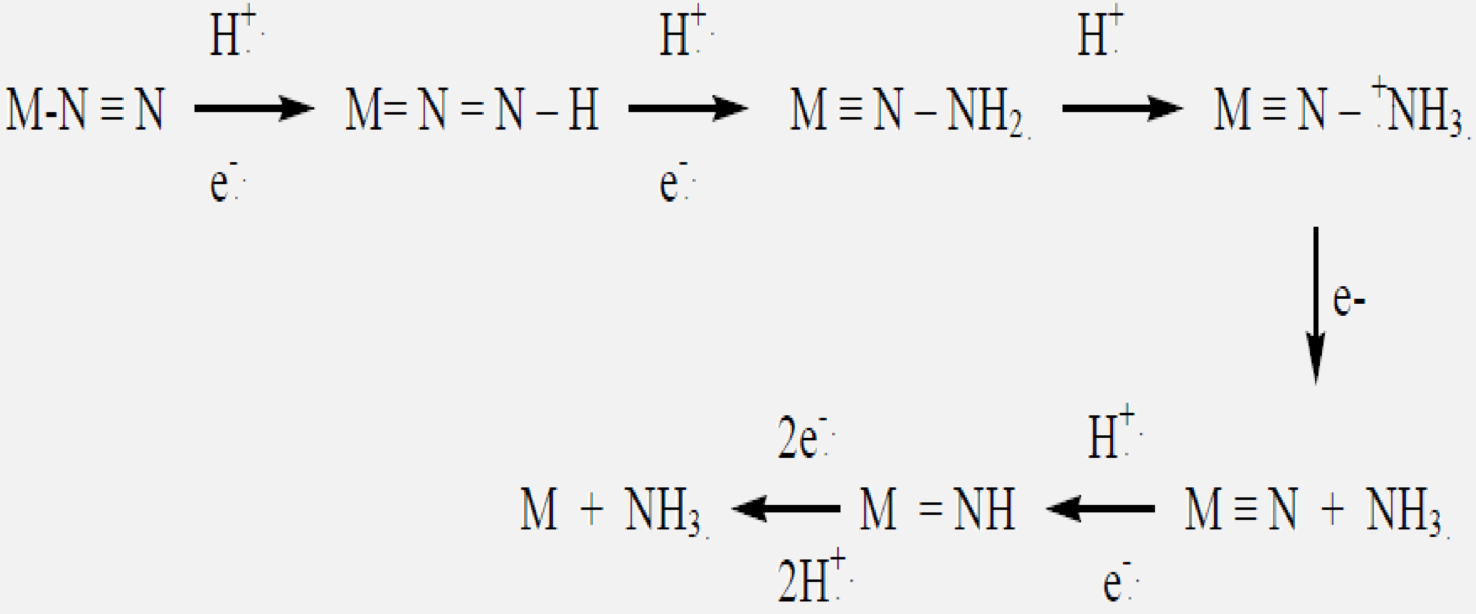
And dppe=1,2-bis(diphenylphosphino)ethane, $Ph_2PCH_2CH_2PPh_2$.

Both reactions takes place at room temperature and atmospheric pressure. The reducing agent is a Grignard reagent.

This reaction sequence is important because it models in vivo nitrogen fixation.

Two equivalents of a molybdenum complex reacted with one equivalent of dinitrogen, creating a triple bonded MoN complex.

At molybdenum and tungsten centers of this type, the bound N_2 can be reduced by protons at the terminal nitrogen, with electrons supplied by the metal to give the cycle of reduction.



$$\begin{tikzcd}
\mathrm{M{-}N\equiv N} \arrow[r, "H^+", "e^-"'] & \mathrm{M{=}N{=}N{-}H} \arrow[r, "H^+", "e^-"'] & \mathrm{M\equiv N{-}NH_2} \arrow[r, "H^+"] & \mathrm{M\equiv N{-}{}^+NH_3} \arrow[d, "e-"] \\
 & \mathrm{M + NH_3} & \mathrm{M{=}NH} \arrow[l, "2e^-", "2H^+"'] & \mathrm{M\equiv N + NH_3} \arrow[l, "H^+", "e^-"']
\end{tikzcd}$$

- If the system is quenched early in its reduction cycle, the intermediate

  $$M\equiv N-NH_2$$

  produces hydrazine N_2H_2. It seems reasonable to propose this type of reduction cycle for nitrogenase.

- The sequence might closely resemble that of biological nitrogenase but here the cycle stops after one turn, giving two NH_3 molecules per metal complex.

- This is due to the fact that the source of electrons being the metal, which is then completely oxidized after the conversion to NH_3.

- To restart, more electrons must be supplied from the electron transfer system as in biological nitrogenase.

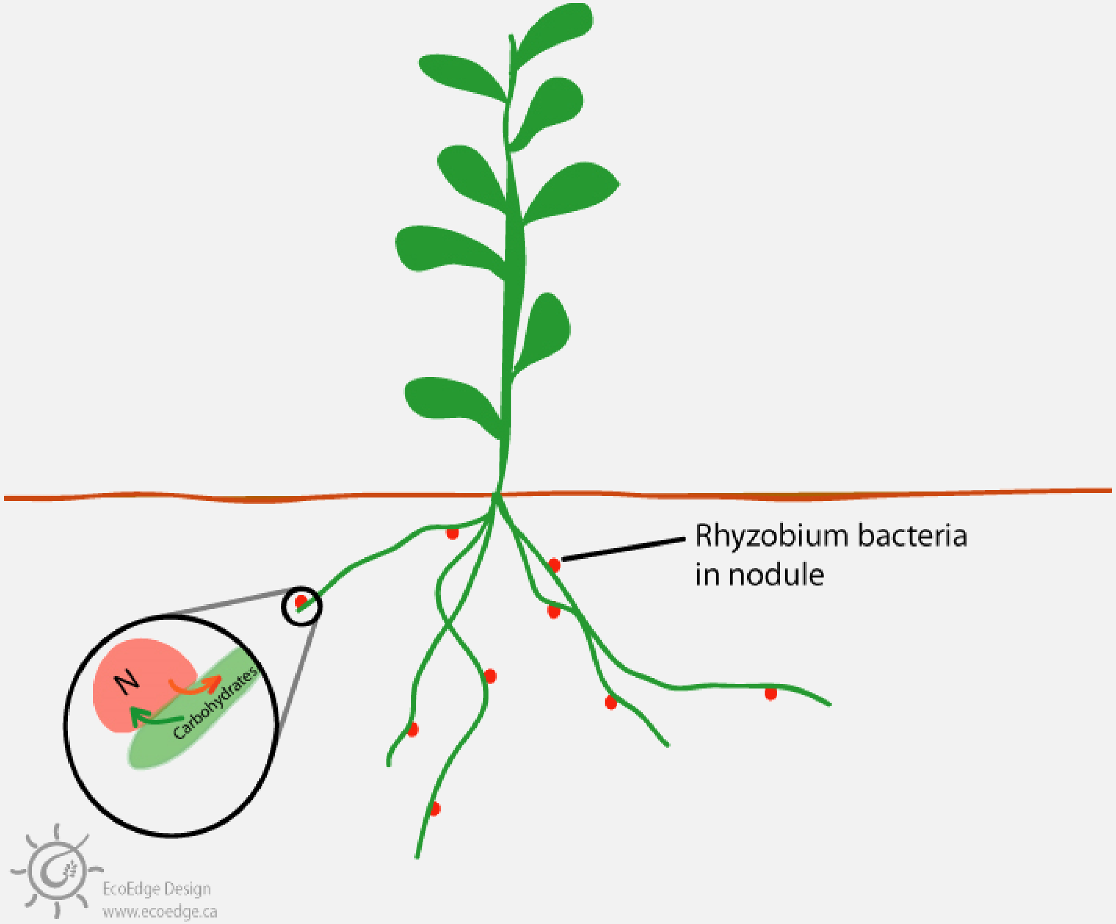
Importance of in vitro nitrogen fixation

- It provides a model for in vivo nitrogenase systems and to employ molybdenum.
- It provides in sight into the development of useful catalyst for the industrial fixation of nitrogen.

Limitations of in vitro nitrogen fixation

- It is not a continuous process, i.e., after fixing one nitrogen molecule to two molecules of NH_3 , reaction stops.
- Reaction stops when the metal is completely oxidized as there is no more electron supply but in *in vivo*, the reaction goes on because there is an enzyme nitrogenase which assists the continuous supply of oxygen and hence the electrons.

In vivo Nitrogen fixation



In vivo Nitrogen fixation

- In vivo processes are the processes which occur or made to occur within a living organism.

- Certain symbiotic or free living bacteria are able to fix nitrogen into useful compounds.

- Symbiotic bacteria fixes dinitrogen in association with plants e.g. the bacterium Rhizobium which is associated with the nodules on the roots of leguminous plants.

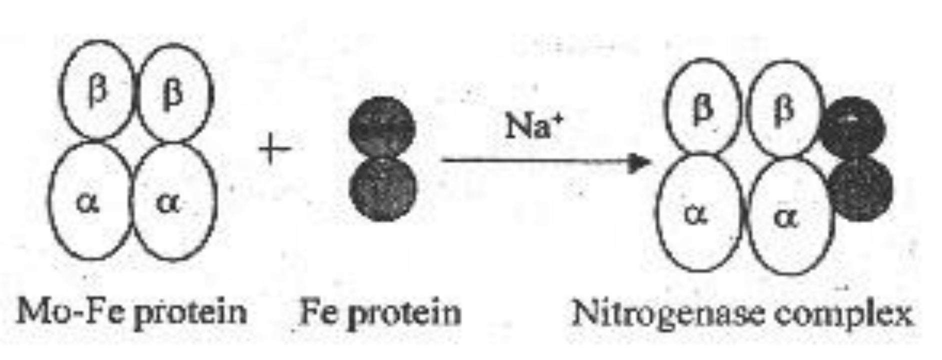
- Asymbiotic bacteria are free living bacteria which fixes atmospheric nitrogen e.g. Azotobacter.

- In biological nitrogen fixation two moles of ammonia are produced from one mole of nitrogen gas, using 16 moles of ATP and a supply of electrons and protons (hydrogen ions):

$$N_2 + 8H+ + 8e^- + 16\ ATP = 2NH_3 + H_2 + 16ADP + 16\ Pi$$

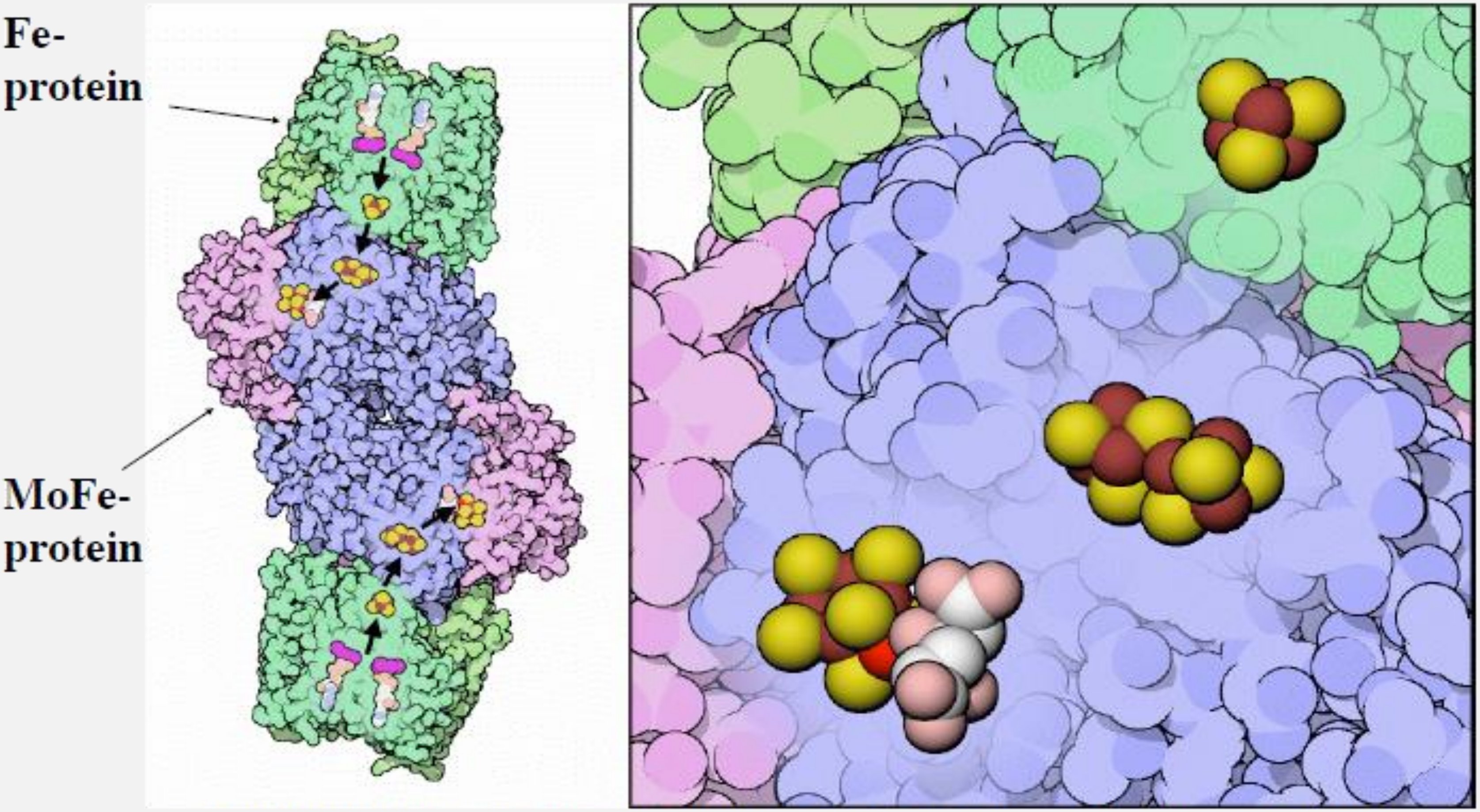
- These bacteria contain the enzyme called nitrogenase which is responsible for nitrogen fixation

Composition of Nitrogenase



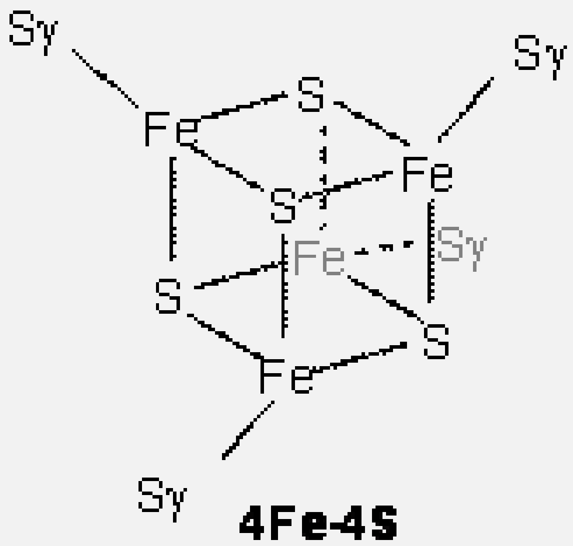
- composed of two proteins
- Fe protein also called reductas
- Fe-Mo protein
- Fe-Mo protein contains Mo-Fe as cofactor and protein P cluster.
- Mo-Fe cofactor is the active site of nitrogen fixation where dinitrogen is supposed to bind.
- These components are present in the ratio of one Mo-Fe protein to two Fe proteins .It is anaerobic in nature.
- Nitrogenase also catalyses reduction of acetylene to ethylene apart from nitrogen to ammonia.

Nitrogenase is a hetero-tetramer. 2 Fe-proteins, 2 MoFe-proteins. With 3 Iron-containing clusters.



The Fe-protein cluster passes e- to the P-cluster, which passes them to the FeMo-cluster. e- accumulate at the FeMo-cluster, where the reduction of N takes place.

Fe protein



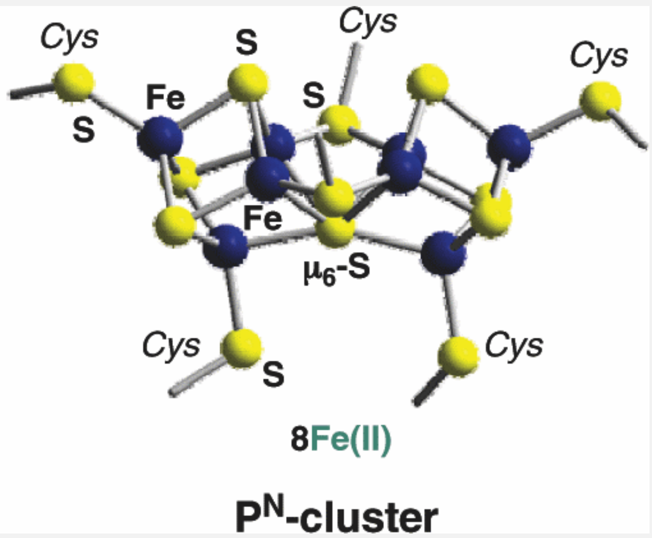
- ➢It is protein with molecular weight near 66 kD and contains a single 4Fe-4S cluster.

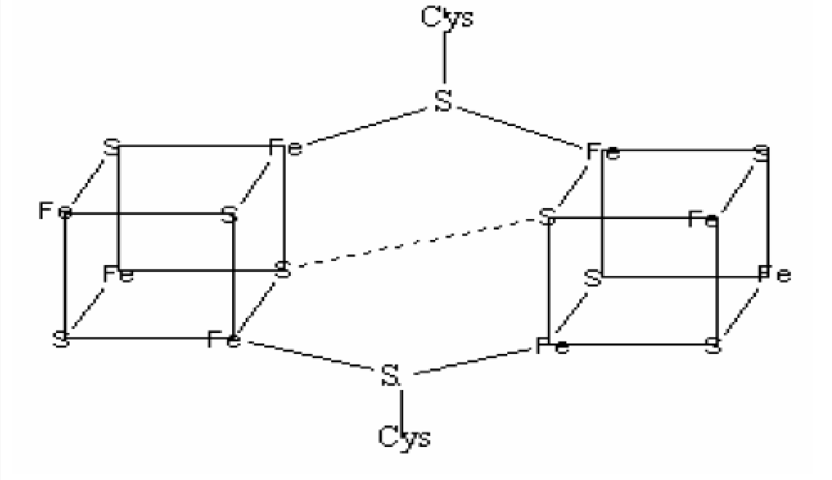
- ➢Also known as Nitrogenase reductase

- ➢Binds MgATP

- ➢It passes electrons from electrons source like ferredoxin and passes them to P-cluster

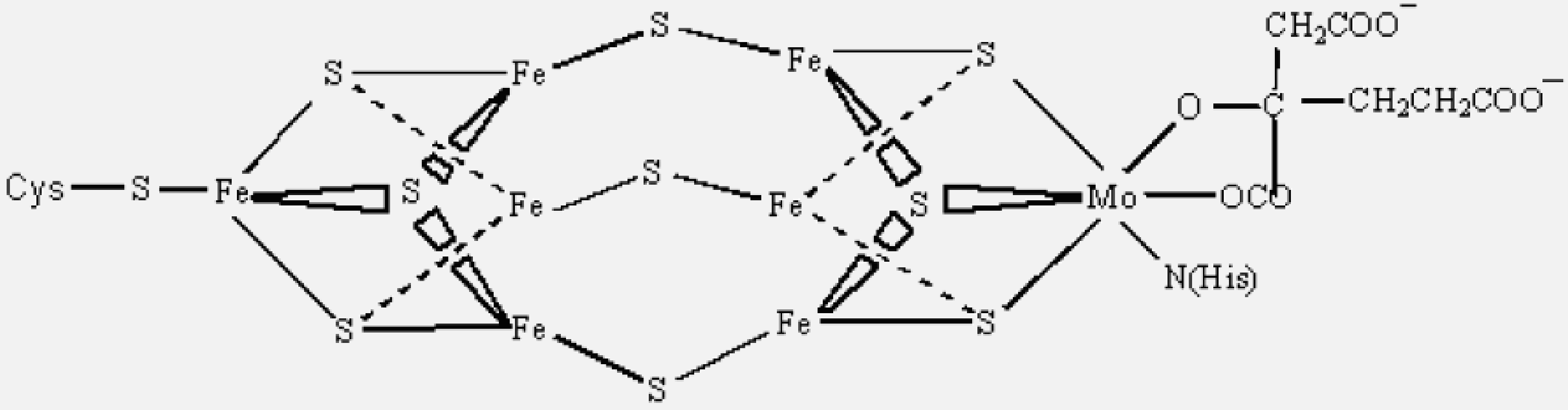
P-cluster





- ➢ it mediate electron transfer to the active cofactor site during catalysis
- ➢It has chemical composition of 8Fe & 7S.

Mo-Fe cofactor



- It consist of two cuboidal fragments with molecular weight of 220kD
- One contains four iron atoms and the other 3 Fe atoms & 1Mo
- Mo is octahedrally coordinated at the corner of heterocuane structure and is coordinated to histidine & a tetraanionic chelating homocitrate ligand.
- The vital feature of the Fe_7MoS_9cluster is FeMoco active site situated in the center.

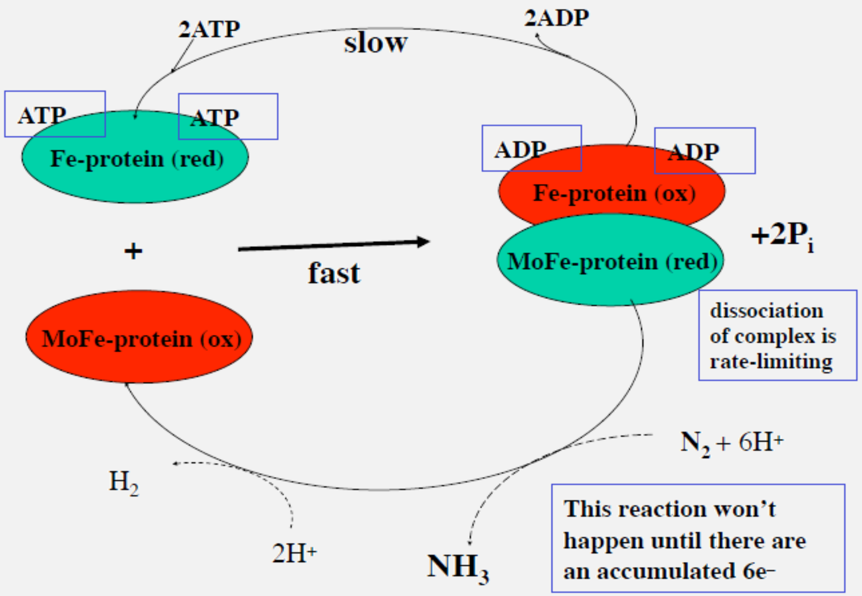
Mechanism of action

- The Fe protein is first reduced by electrons donated by ferredoxin which is generated by photosynthesis, respiration or fermentation.

- For each cycle of e^- transfer, Fe protein binds two ATP, which is then able to interact with Fe-Mo protein and transfer electrons to it. which donates electrons to N_2, producing HN=NH.

- In two further cycles of this process (each requiring electrons donated by ferredoxin) HN=NH is reduced to $H_2N\text{-}NH_2$, and this in turn is reduced to $2NH_3$.

Mechanism: simplified



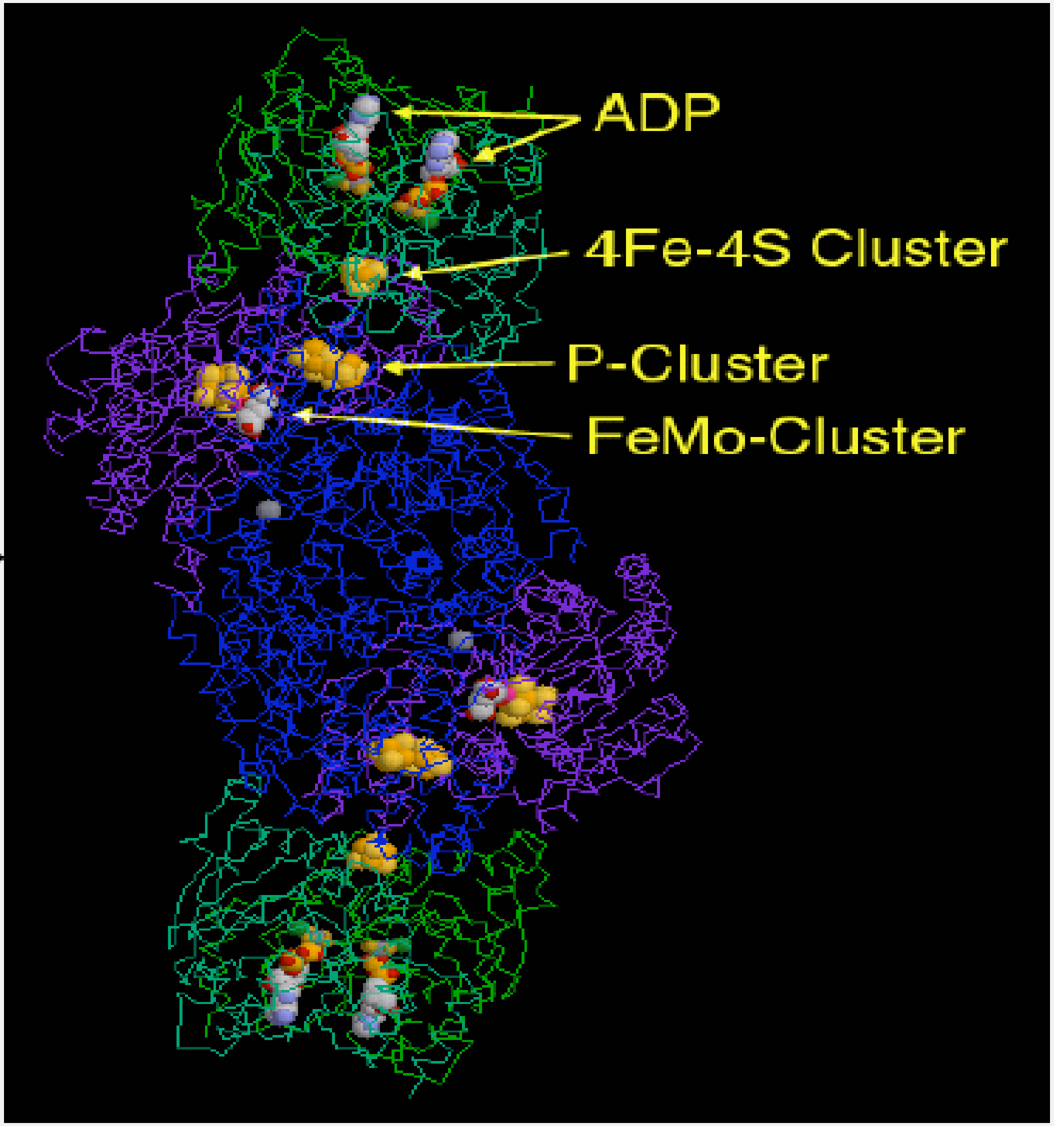
- ATP supplies the energy to drive the transfer of electrons from the Fe protein to the MoFe protein. The reduction potential of each electron transferred to the MoFe protein is sufficient to break one of dinitrogen's chemical bonds.
- ATP is hydrolysed and the two proteins disassociate to begin another cycle of reduction and avoid back electron transfer. Only 6 electrons used in the useful reduction, another two are wasted to make H_2, which can back react with N_2H_2.

2 ATP bind to the Fe-protein before it binds to the MoFe-protein.

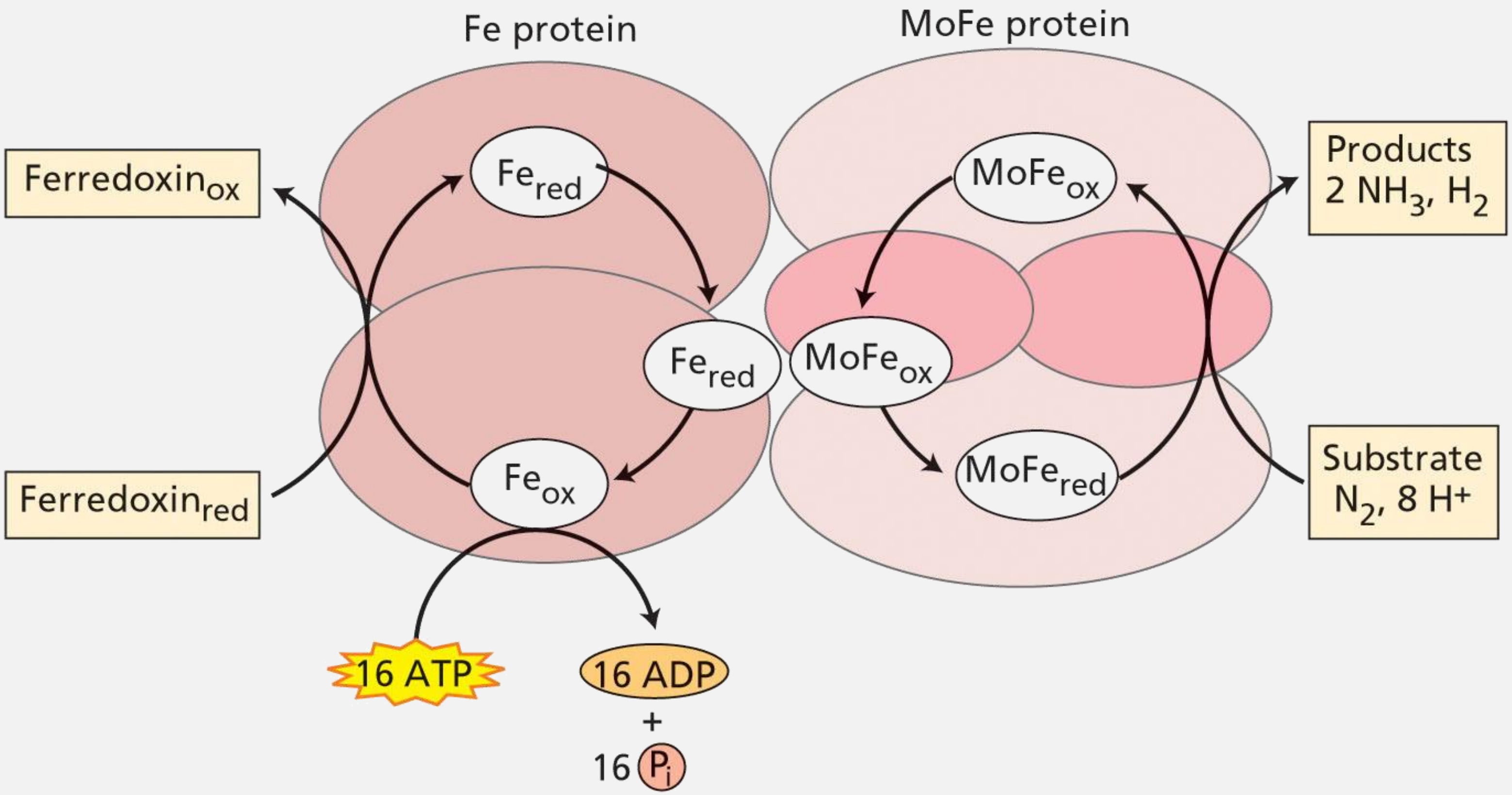
Fe-protein

MoFe protein

Fe-protein



Nitrogenase enzyme complex



Fe protein

MoFe protein

Ferredoxin$_{ox}$

Ferredoxin$_{red}$

Fe$_{red}$

Fe$_{red}$

Fe$_{ox}$

MoFe$_{ox}$

MoFe$_{ox}$

MoFe$_{red}$

Products
2 NH_3, H_2

Substrate
N_2, 8 H^+

16 ATP

16 ADP
+
16 P_i

Role of Mo

- the Mo atoms affect the reactivity of the cofactor that facilitates efficient nitrogen fixation.
- The Mo atoms slow the protonation of the cluster at the active site, which suppresses dihydrogen production.
- It also maximize the possibility of dinitrogen binding to that active site.
- Mo-Fe-S cluster protonate slowly than Fe-S cluster and it has also been shown that Mo containing Fe-S clusters have greater substrate affinity as compared to Fe-S clusters
- Thus the role of Mo as cofactor is justified and it has been used in in vitro nitrogen fixation as well.

Bell et al. Angew. Chem. Int. Ed. 2003, 42, No. 10

Sensitivity of nitrogenase

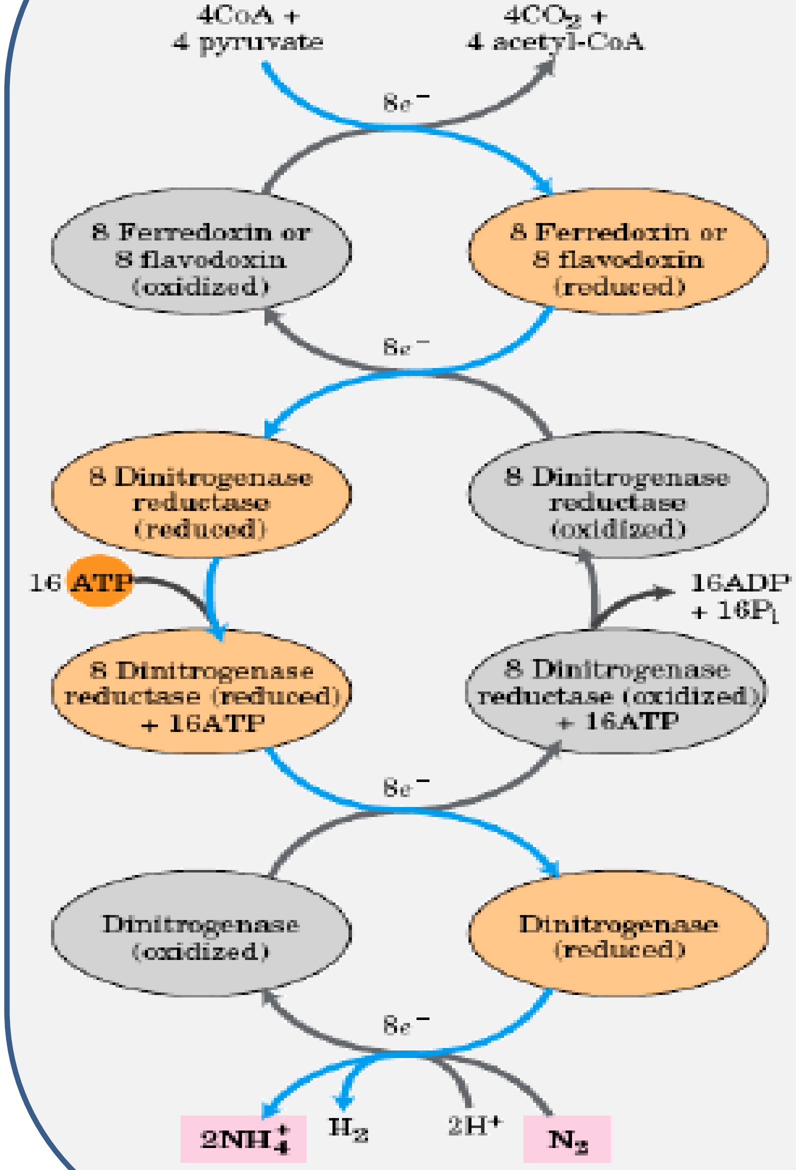
- Nitrogenase is extremely sensitive to oxygen as it oxidizes Fe-S cluster. Therefore N_2 fixation can proceed only at very low oxygen concentrations. Infact nitrogen fixation is an anaerobic process.

- Free-living bacteria that fix nitrogen cope with this problem in a variety of ways. Some live only anaerobically or repress nitrogenase synthesis when oxygen is present.

- Other species solve this problem via the symbiotic relationship, especially between leguminous plants and the nitrogen-fixing bacteria.

- To solve the oxygen-toxicity problem, plants produce a protein called **leghemoglobin** which binds all available oxygen and efficiently delivers it to the bacterial electron-transfer system.

Electron flow



- The process requires eight electrons: Six for the reduction of N_2 and two to produce one molecule of H_2 as an obligate step

- All electrons are transferred one at a time The process:(repeated 8 times to transfer eight electrons)

- First, Reducatse is reduced by ferrodoxin then reduced reductase binds 2 ATPs and change its conformation

- Reducatse (+2ATP) binds to the dinitrogenase and transfers a single electron to it release ADP and becomes oxidized

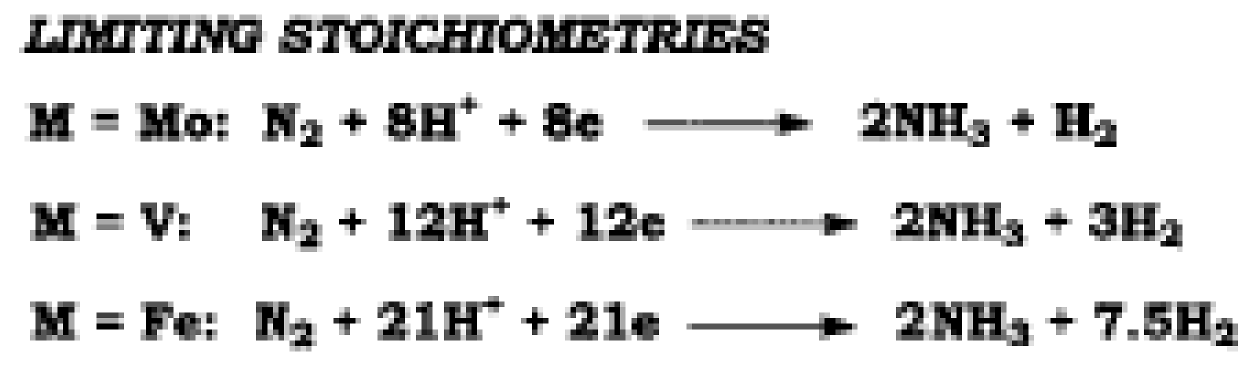
- Highly reduced Dinitrogenase then carries out nitrogen fixation and generates NH_3 & H_2

Rate of nitrogen fixation

N_2 fixing system	**Nitrogen Fixation (kg N/hect/year)**
Rhizobium-legume	200-300
Cyanobacteria- moss	30-40
Rhizosphere associations	2-25
Free- living	1-2

Vanadium nitrogenase

- In 1980, a second (alternative) nitrogen-fixing enzyme was hypothesized to exist in ***Azotobacter vinelandii (Bishop et al***.1980). It was predicted that this enzyme was expressed during Mo starvation, a condition that would not favor the synthesis of the conventional (Mo-containing) enzyme.
- In place of Mo and Fe it contains V and Fe with a V:Fe ratio of 1 : 13
- There is one more kind of nitrogenase i.e iron only nitrogenase which contains only iron and not any other transition metal.
- Among three nitrogenase, molybdenum nitrogenase is the most efficient in terms of nitrogen fixation owing to less waste of energy.



LIMITING STOICHIOMETRIES

M = Mo: $N_2 + 8H^+ + 8e \longrightarrow 2NH_3 + H_2$

M = V: $N_2 + 12H^+ + 12e \longrightarrow 2NH_3 + 3H_2$

M = Fe: $N_2 + 21H^+ + 21e \longrightarrow 2NH_3 + 7.5H_2$

Recent advances

- Apart from the Fe-Mo and Fe protein , a third metal binding site has gained considerable importance in the Mo-Fe cluster. This binding site was initially supposed to contain Ca^{2+} ion but lately Zhang et al. found that the sixteenth iron in the Nitrogenase Fe-Mo protein is Fe only.

- Bellenger et al (2014) recently demonstrated the enhanced efficiency of Vanadium nitrogenase as compared to what has been proved earlier by comparing R ratio.

- Yang et al (2013) has shown that at turnover conditions and high pH, Mo-Fe nitrogenase enzyme deactivation occurs which suggest a mechanism based transformation that may provide a new probe of nitrogenase catalysis.

Biological nitrogen fixation

$$\mathbf{N_2} + 8\ \text{flavodoxin}^- + 8H^+ + \boxed{16\ \text{MgATP}^{2-}} + 18\ H_2O \xrightarrow{\text{nitrogenase}}$$
$$\boxed{\mathbf{2NH_4^+}} + 2OH^- + 8\ \text{flavodoxin} + 16\ \text{MgADP}^- + 16H_2PO_4^- + H_2$$

1. Rare, extremely energy consuming conversion because of stability of triply bonded N_2
2. Produces fixed N which can be directly assimilated into N containing biomolecules

Biological nitrogen fixation

a. What is it?

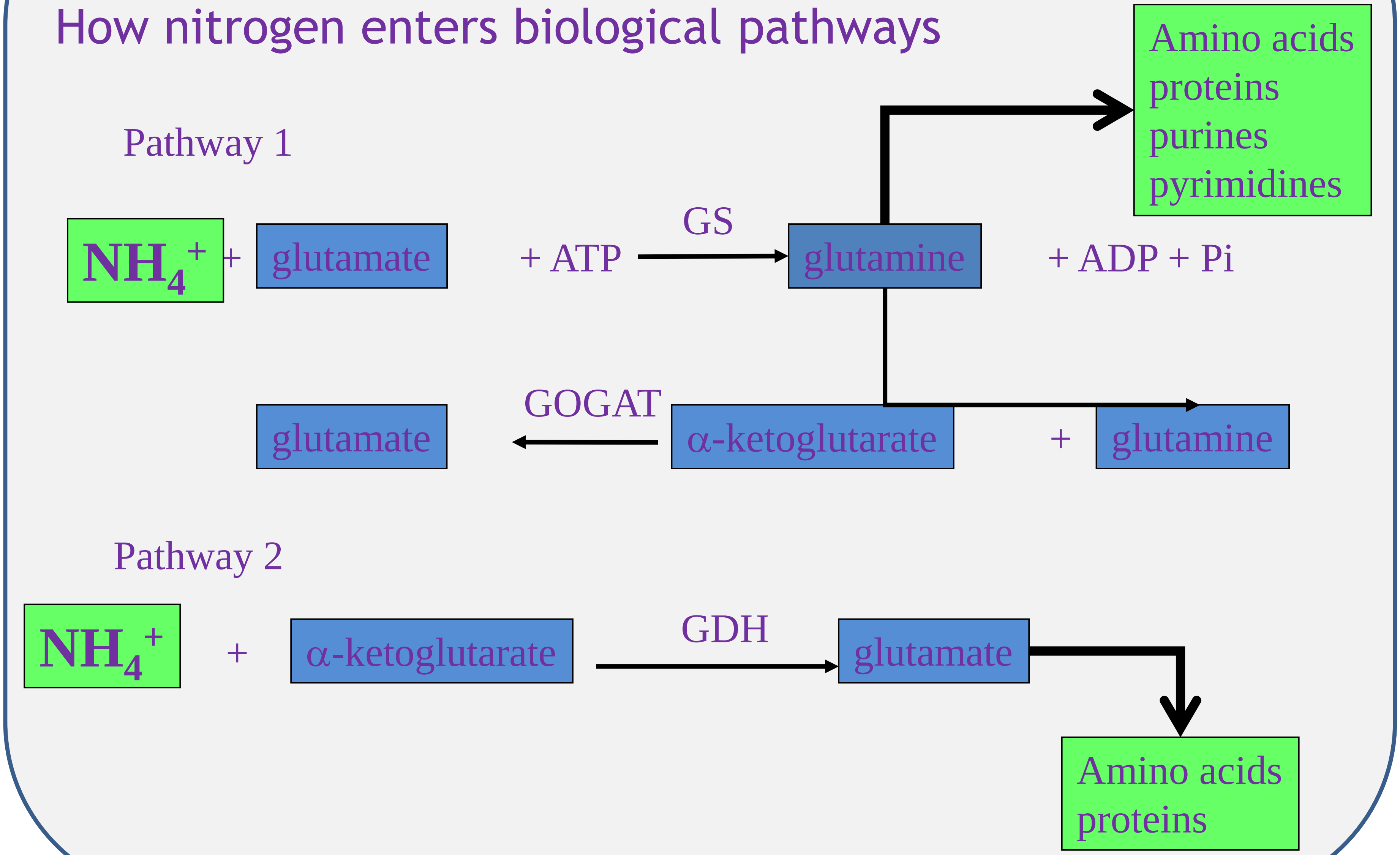
- Conversion of atmospheric N_2 to NH_4^+ (actually, amino acids)
- Under natural conditions, nitrogen fixation is the main pathway by which new, available nitrogen enters terrestrial ecosystems

Paradox of Nitrogen limitation

- Nitrogen is the element that most frequently limits terrestrial NPP
- N_2 is the most abundant component of the atmosphere
- Why doesn’t nitrogen fixation occur almost everywhere?
- Why don’t N fixers have competitive advantage until N becomes non-limiting?

Ammonia assimilatory cycle

How nitrogen enters biological pathways



Pathway 1

NH_4^+ + glutamate + ATP $\xrightarrow{GS}$ glutamine + ADP + Pi

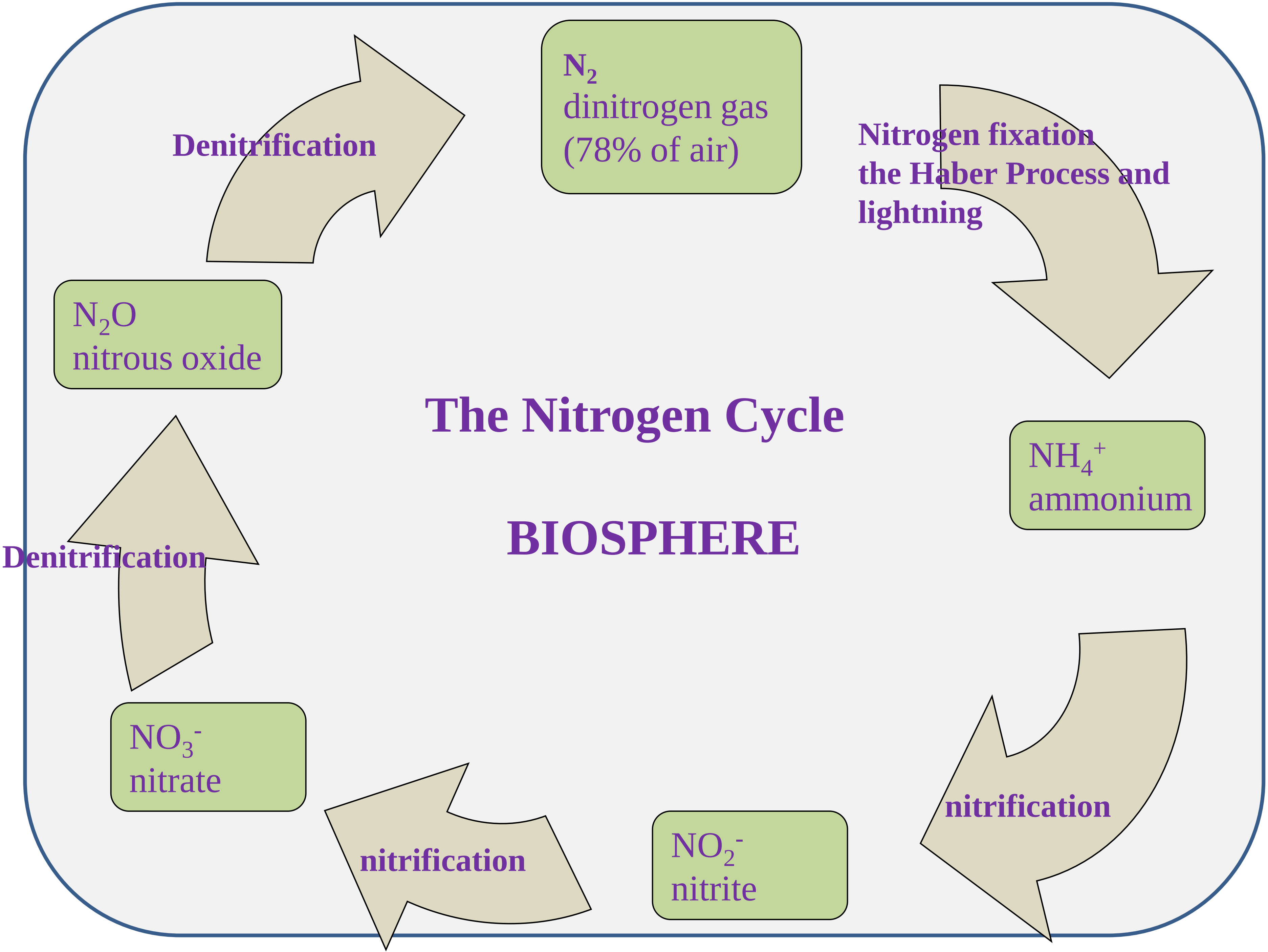
Amino acids
proteins
purines
pyrimidines

glutamate $\xleftarrow{GOGAT}$ α-ketoglutarate + glutamine

Pathway 2

NH_4^+ + α-ketoglutarate $\xrightarrow{GDH}$ glutamate

Amino acids
proteins



The Nitrogen Cycle

BIOSPHERE

N_2
dinitrogen gas
(78% of air)

Nitrogen fixation
the Haber Process and
lightning

NH_4^+
ammonium

nitrification

NO_2^-
nitrite

nitrification

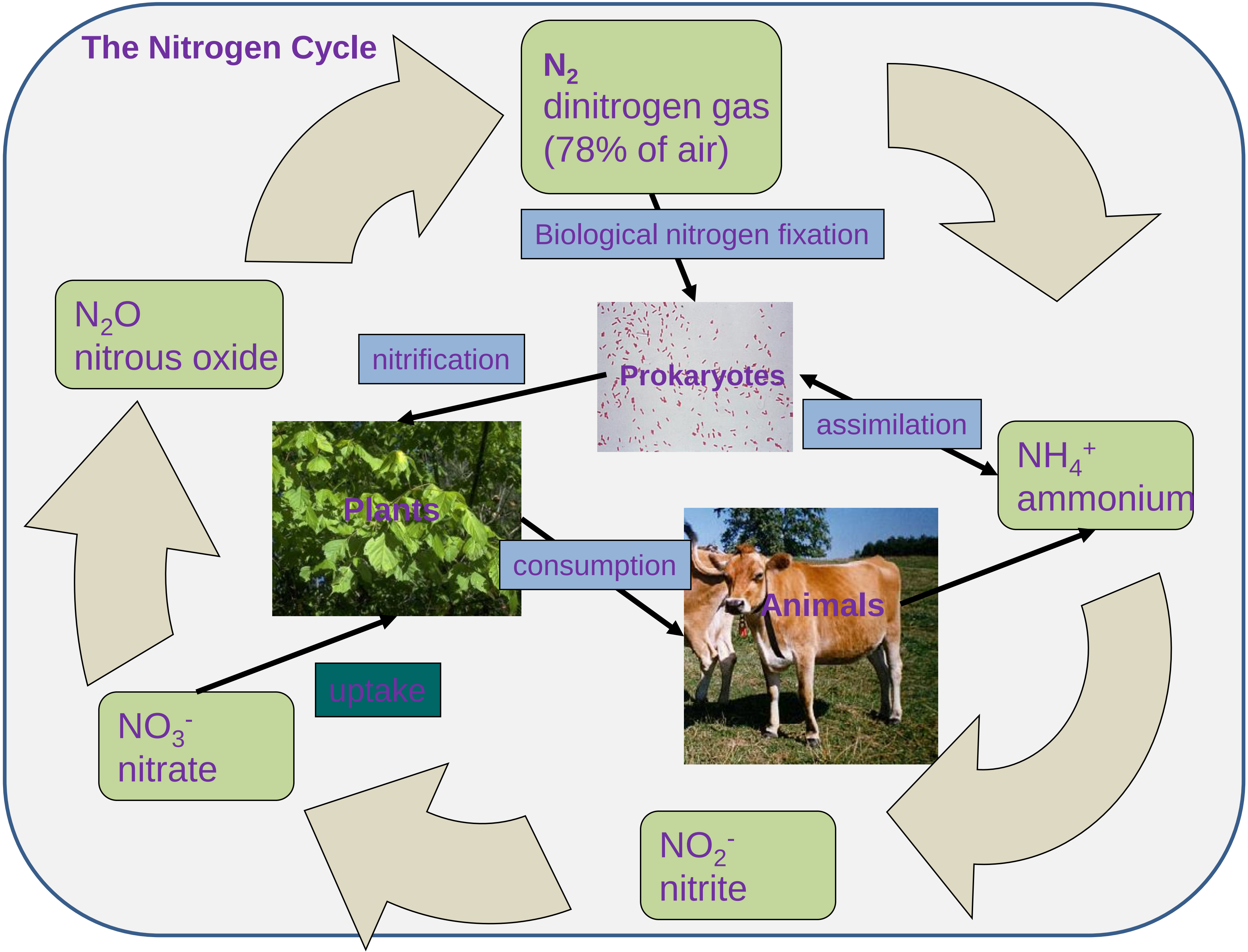
NO_3^-
nitrate

Denitrification

N_2O
nitrous oxide

Denitrification

The Nitrogen Cycle



N_2
dinitrogen gas
(78% of air)

Biological nitrogen fixation

Prokaryotes

assimilation

NH_4^+
ammonium

Animals

consumption

Plants

nitrification

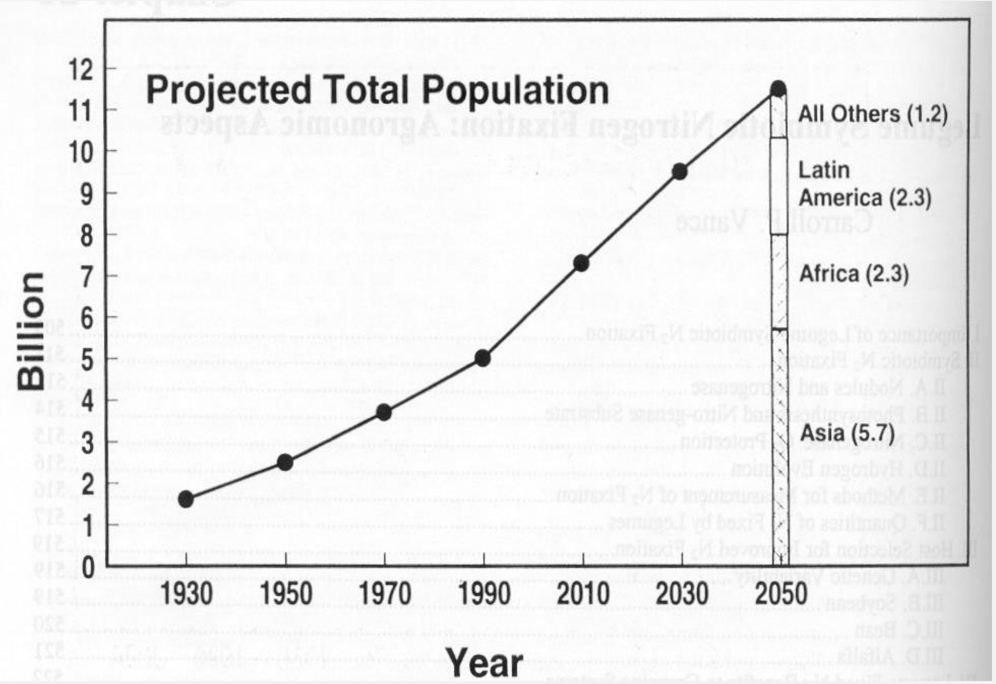
N_2O
nitrous oxide

NO_3^-
nitrate

uptake

NO_2^-
nitrite

A growing population must eat!

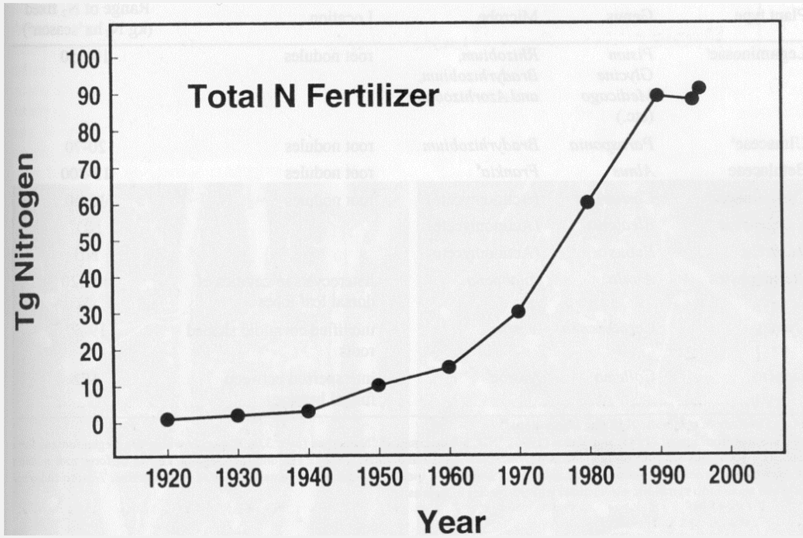


- Combined nitrogen is the most common limiting nutrient in agriculture

- Estimated that 90% of population will live in tropical and subtropical areas where (protein-rich) plant sources contribute 80% of total caloric intake.

- In 1910 humans consumed 10% of total carbon fixed by photosynthesis, by 2030 it is predicted that 80% will be used by humans.

Why chemical fertilizers aren't the answer



The Haber-Bosch process

$$N_2 + H_2 \xrightarrow{\hspace{6cm}} 2NH_3$$

300 to 1000 bar pressure

Consumes 1.4% of total fossil fuels annually

400 to 600 C

Catalyst

Electrical discharge

$$N_2 + O_2 + \text{lightning} \longrightarrow NO_x$$

$$NO_x + H_2O \longrightarrow HNO_2 \text{ or } HNO_3$$

- Production of nitrogenous fertilizers has "plateaued" in recent years because of high costs and pollution

- Estimated 90% of applied fertilizers never reach roots and contaminate groundwater

Non-symbiotic nitrogen fixation

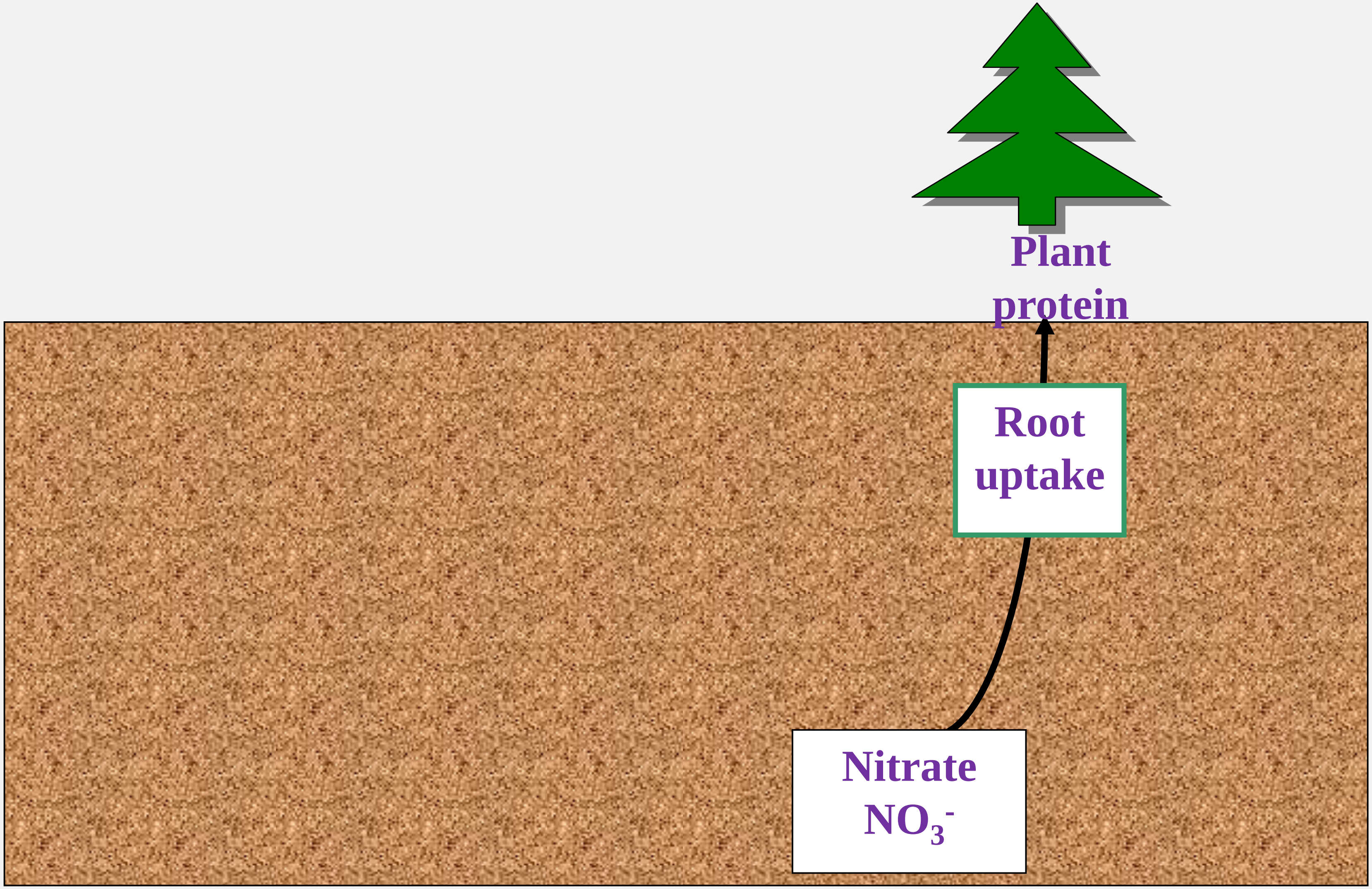
Aquatic:

Cyanobacteria
Anabaena
Nostoc

Terrestrial and rhizosphere-associated:

Azospirillum
Azotobacter
Acetobacter
Klebsiella
Clostridium

Nitrates are essential for plant growth

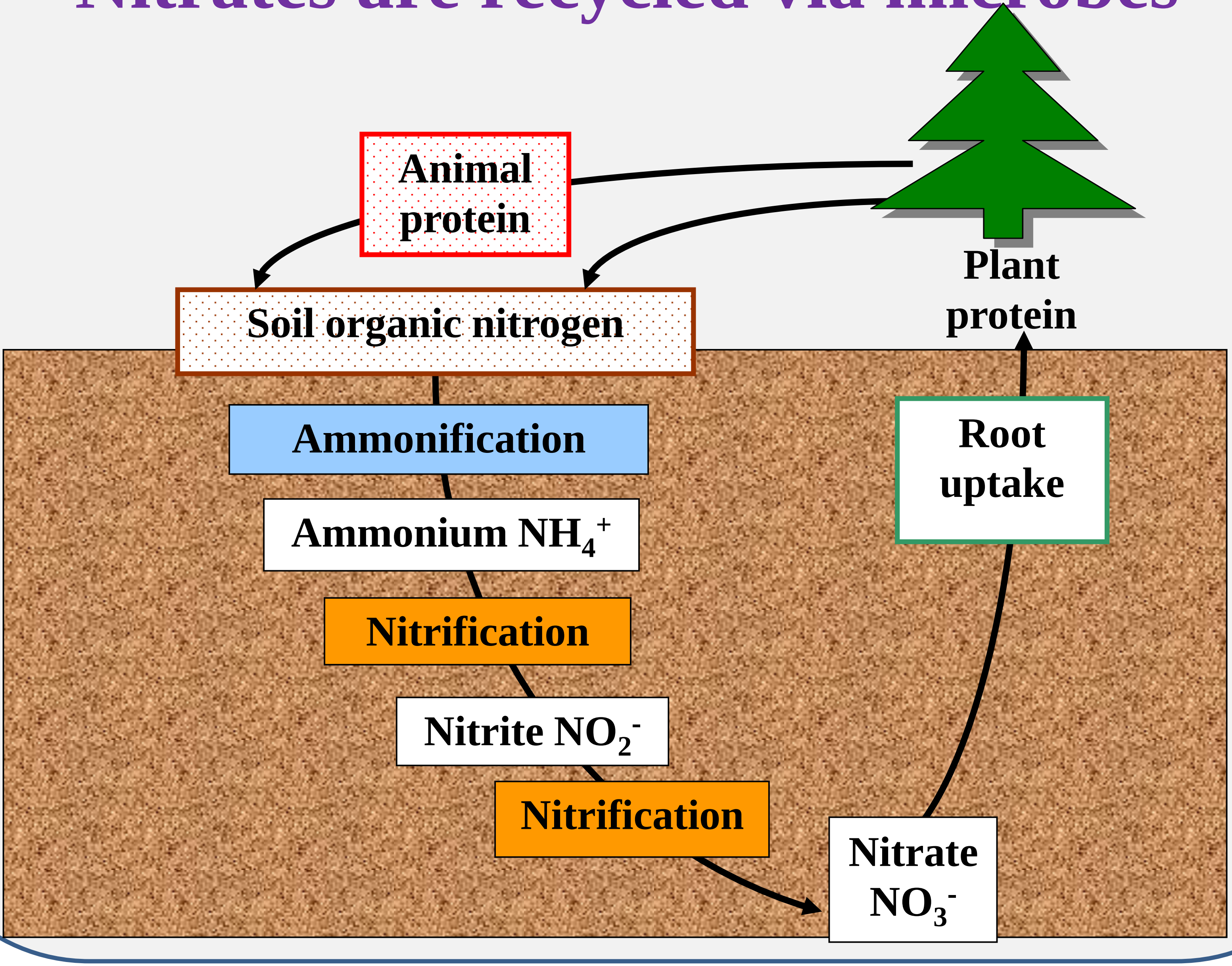


Plant protein

Root uptake

Nitrate NO_3^-

Nitrates are recycled via microbes



Animal protein

Soil organic nitrogen

Ammonification

Ammonium NH_4^+

Nitrification

Nitrite NO_2^-

Nitrification

Nitrate NO_3^-

Root uptake

Plant protein

Ammonification

- Nitrogen enters the soil through the decomposition of protein in dead organic matter

Amino acids + $1^1/_2O_2 \rightarrow CO_2 + H_2O + NH_3$ + 736kJ

- This process liberates a lot of energy which can be used by the saprotrophic microbes

Nitrification

- This involves two oxidation processes
- The ammonia produced by ammonification is an energy rich substrate for ***Nitrosomas*** bacteria

They oxidise it to nitrite:

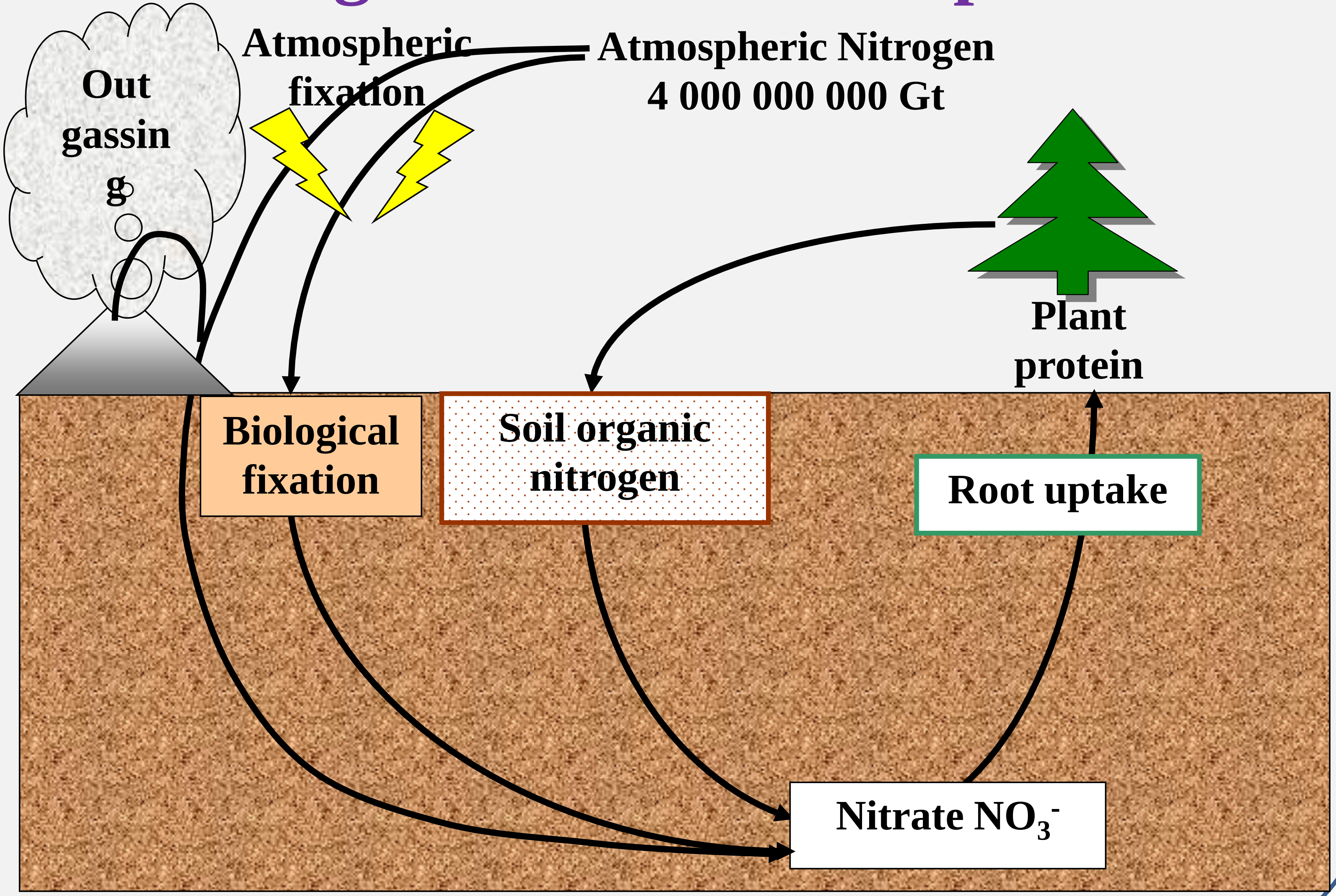
$$NH_3 + 1^1/_2O_2 \rightarrow NO_2^- + H_2O \qquad + 276kJ$$

This in turn provides a substrate for ***Nitrobacter*** bacteria oxidise the nitrite to nitrate:

$$NO_3^- + {}^1/_2O_2 \rightarrow NO_3^- \qquad + 73\ kJ$$

- **This energy is the only source of energy for these prokaryotes**
- **They are chemoautotrophs**

Nitrogen from the atmosphere



Out gassing

Atmospheric fixation

Atmospheric Nitrogen
4 000 000 000 Gt

Plant protein

Biological fixation

Soil organic nitrogen

Root uptake

Nitrate NO_3^-

Atmospheric nitrogen fixation

- Electrical storms
- Lightning provides sufficient energy to split the nitrogen atoms of nitrogen gas,
- Forming oxides of nitrogen NO_x and NO_2

Atmospheric Pollution

- This also happens inside the internal combustion engines of cars
- The exhaust emissions of cars contribute a lot to atmospheric pollution in the form of NO_x
- These compounds form **photochemical smogs**
- They are **green house gases**
- They dissolve in rain to contribute to **acid rain** in the form of nitric acid
- The rain falling on soil and running into rivers
- They contribute to the **eutrophication** of water bodies

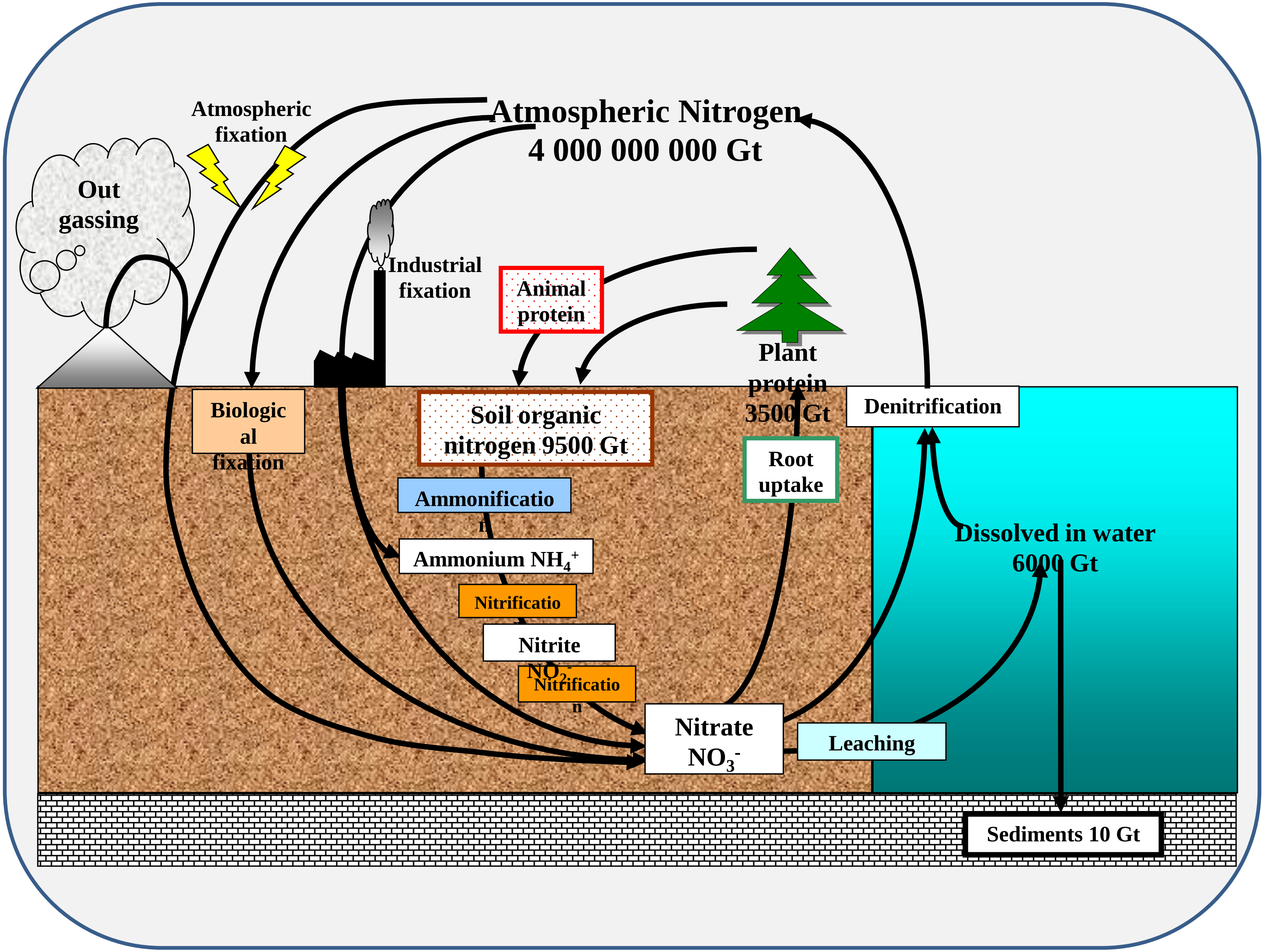
Return to the atmosphere: Denitrification

- Nitrates and nitrites can be used a source of oxygen for ***Pseudomonas*** bacteria
- Favourable conditions: Cold waterlogged (anaerobic) soils

$2NO_3^- \rightarrow 3O_2 + N_2\uparrow$ providing up to 2385kJ

$2NO_2^- \rightarrow 2O_2 + N_2\uparrow$

- The liberated oxygen is used as an electron acceptor in the processes that oxidise organic molecules, such as glucose
- These microbes are, therefore, **heterotrophs**



Atmospheric Nitrogen
4 000 000 000 Gt

Atmospheric fixation

Out gassing

Industrial fixation

Animal protein

Plant protein 3500 Gt

Biological fixation

Soil organic nitrogen 9500 Gt

Ammonification

Ammonium NH_4^+

Nitrification

Nitrite NO_2^-

Nitrification

Nitrate NO_3^-

Root uptake

Denitrification

Dissolved in water 6000 Gt

Leaching

Sediments 10 Gt