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Introduction

- \times N₂ gas are found 78.084% on atmosphere of earth.
- × N_2 are found in molecular N_2 ($N \equiv N$) form in soil.
- X Dinitrogen are don't more active in chemically form.
- X Dinitrogen is more stable, so we need of nitrogen fixation.
- Nitrogen fixation is a process by which nitrogen of the Earth's atmosphere is converted into ammonia (NH3), nitrogen salts or other molecules available to living organisms.

Role of nitrogen in plant

- » Major substance in plants next to water
- » Building blocks
- » Constituent element of
 - »Chlorophyll
 - **»**Cytochromes
 - »Alkaloids
 - »Many vitamins
- » Plays important role in metabolism, growth, reproduction and heredity

Sources of nitrogen

- Atmospheric Nitrogen
 - -78.084 % 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.

Mechanism of nitrogen fixation

- Nitrogen fixation, natural and synthetic, is essential for all forms of life because nitrogen is required to biosynthesize basic building blocks of plants, animals and other life forms, e.g., nucleotides for DNA and RNA and amino acids for proteins.
- ➤ Nitrogen fixation is a process by which nitrogen in the Earth's atmosphere is converted into ammonia (NH₃) or other molecules available to living organisms. Atmospheric nitrogen or molecular dinitrogen (N₂) is relatively inert, it does not easily react with other chemicals to form new compounds.

Basic requirement of nitrogen fixation –

- Nitrogenase and hydrogenase enzyme.
- Constant supply of ATP.
- Hydrogen releasing system or electron doner
 [pyruvic acid or glucose /sucrose.]
- Co-enzyme and cofactors TPP, CoA, Inorganic phosphate and mg⁺²
- Cobalt and molybdenum.

Cont...

Biochemistry of nitrogen fixation –

- ✓ Nitrogen fixation process is given by Burris 1966.
- ✓ Nitrogen fixation in atmosphere N₂ is convert into ammonia or nitrates presence of nitrogenase enzyme.
- ✓ Nitrogen fixation in first stable compound is ammonia.
- ✓ Nitrogen convert into ammonia is a reduction.
- ✓ In this process, N_2 is spilt up into free N_2 atoms by breaking the triple bond with the help of enzyme nitrogenase.



This reaction is endergonic [energy consuming] it requires an input of nearly 160kcal energy.



$$N_2 + 160 \text{ kcal.}$$



2N

- ➤ Free nitrogen combines with hydrogen forming NH₃ presence of hydrogenase enzyme.
- ➤ The reaction is exergonic [energy releasing].[13kcal. Energy]



$$2N + 3H_2$$



$$2NH_3 + 13 \text{ kcal.}[\text{energy}]$$



Reduction process Dinitrogen 2H+, 2e-HN = HNDiimide 2H+, 2e-H₂N-NH₂ Hydrazine 2H+, 2e-HO2HN-NH2OH Hydroxyl amide 2H+, 2e-Ammonia 2 NH

According By :-Burris [1962]

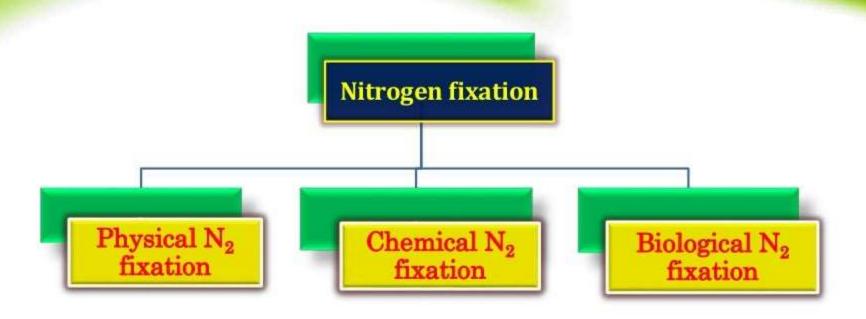
> BIOLOGICAL NITROGEN FIXATON MECHANISM





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

Types of Nitrogen fixation



Physical N₂ fixation

- ➤ Physical N₂ fixation are occurs by the natural factor such as :- lightening , thunder.
- ➤ It occurs in several steps and starts with combination atmospheric nitrogen with oxygen under the influence of electric discharge and thunder to produce nitric oxide.



 $N_2 + O_2$ Lightening and thunder Nitric oxide [2NO]

Cont...

The nitric oxide is then oxidized to nitrogen peroxide in presence of oxygen.



During rains, the nitrogen peroxide combines with rain water to form nitrous acid and nitric acid which come to ground along with rains.



$$2NO_2 + rain water \longrightarrow HNO_2 + HNO_3$$

Conti...

➤On ground the alkali radicals of the soil react with nitric acid to produce nitrites and nitrates which are soluble in water and which can be absorbed by the plant through roots.



Ca⁺⁺ or K⁺ salts + HNO₃ Soil Ca⁺⁺ or K⁺Nitrates



Chemical Nitrogen fixation

- Reduction of N₂ into NH₃ by the chemical process is called chemical nitrogen fixation.
- ➤ In the use of Haber's process.
- ➤ Haber's process in use of high pressure [~200Atm] and high temperature [~200A°C].

$$N_2 + 3H_2 \xrightarrow{200^{\circ}C} 2NH_3$$

The method is used in agriculture and industry.

Biological nitrogen fixation

- ➤ Fixation of atmospheric Nitrogen into nitrogenous salts with the help of micro-organisms.
- ➤ Biological nitrogen fixation (BNF) occurs when atmospheric nitrogen is converted to ammonia by an enzyme called nitrogenase.
- ➤ Biological Nitrogen fixation in all micro-organism are prokaryotes.
- > They micro-organism are called is diazotrophs.

Types of biological nitrogen fixers

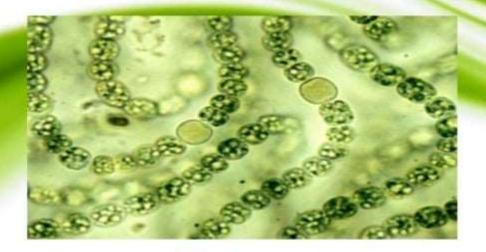
- Biological nitrogen fixers are classified based on fixing microorganism.
- ➤ Per year 60% part of total nitrogen fixing by biological nitrogen fixing on earth.
- Two types
 - -Symbiotic
 - -Non-symbiotic

Non-symbiotic

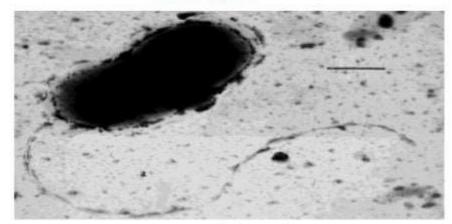
- They are free living nitrogen fixers.
- ➤ They inhabit both terrestrial & aquatic habitats.
- Aerobic, anaerobic and blue green algae
- ➤ Bacteria: special type (nitrogen fixing bacteria) types
 - o Free living aerobic : Azotobacter, Beijerenckia
 - o Free living anaerobic : *Clostridium*
 - o Free living photosynthetic: Chlorobium, Rhodopseudomonas

Cont...

- > Free living chemosynthetic : Desulfovibro, Thiobacillus
- ➤ Free living fungi: *Yeasts and Frankia*.
- ➤ Blue green algae:
 - o unicellular *Gloeothece, Synechococcus*
 - o Filamentous (non heterocystous) Oscillatoria, Plectonema.
 - o Filamentous (heterocystous) Anabaena, Nostoc



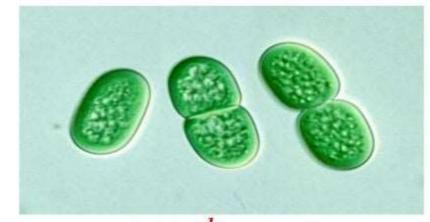
Nostoc



Desulfovibro



Oscillatoria



synechococcus

Symbiotic

- Fixation of free nitrogen by micro-organisms in soil living symbiotically inside
 - the plants
- 'Symbiosis' coined by De Bary
- > There are two type
 - ✓ Nodulated symbiosis -



Glycine max on rhizobium

- ➤ Nodule formation in leguminous plants- Rhizobium + Cicer aritetinum, Pisum sativum, Glycine max.
- ➤ Nodule formation in non-leguminous plants- Frankia + Casuarina , Alnus.
- ✓ Non nodulation- Cycas+Anabaena/Nostoc, Anthoceros + Nostoc



Nodule formation in leguminous plants Cicer aritetinum with " Rhizobium "



Nodule formation in non-leguminous plants

Alnus with "Frankia"

Factors affecting N₂ fixation

- > Presence of nitrate or ammonium.
- Presence of certain inorganic substances
- ➤ Ca, Co, Mo influence N₂ fixation along with P
- > pH:- Neutral- Azotobacter Acidic- Beijerinkia
- Soil moisture :- Adequate is good for fixation
- ➤ Temperature:- Mesophilic 30°C.

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Any questions?



Your questions is...

Q.1. which form of nitrogen present in atmosphere?

- Q.2. which enzyme is responsible for splitting $N \equiv N$ bond in N_2 Fixation.
- Q.3. Which process are used in chemical nitrogen fixation?

Q.4. Give a name of a nodulated symbiosis?

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