

Unit-II: Enzymes & Nitrogen Metabolism

classmate

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Enzymes :-

"Enzymes are proteins which catalyse specific biochemical reactions in living cells". i.e. "They are biological catalysts". The term 'enzyme' was first introduced by Kuhne (1878), derived from the Greek word meaning "yeast", because enzyme activity was first observed in fermentation activity carried out by yeast cells in 1857. J.C. Sumner (1926) first isolated the enzyme urease & crystallized it. He first proved that, the enzymes are proteins. He was awarded Nobel prize for his work.

Some enzymes act inside the cell in which they are synthesized. They are called endoenzymes. Some other enzymes are synthesized in ~~a~~ cell and sent out to carry out reactions outside the cell. Such enzymes are called exoenzymes.

Nomenclature & classification of enzymes :-

Nomenclature of Enzymes :-

"The naming of enzymes is called nomenclature of enzymes."

Many enzymes are named by adding ^{suffix} '-ase' to the name of substrate on which they act.

eg: Proteases, lipases, cellulases, pectinases, etc.

Certain enzymes are named by adding suffix '-ase' to the reaction which they catalyse.

eg: Hydrolase, oxidase, reductase, isomerase, etc.

Some enzymes are named on the basis of the substrate & type of reaction they catalyse.

eg: pyruvic decarboxylase, lactic dehydrogenase.

Classification of enzymes (IUB System) :-

The system of ~~class~~ enzyme classification is based on the recommendation of the "Commission on enzymes" of the "International Union of Biochemistry" (IUB) in 1961. It is based on the reaction type & reaction mechanism. In IUB system, enzymes are classified into 6 major groups, as follows :-

[Each & every enzymes has been designated with a 4 digit code, called Enzyme Code (E.C.). The first digit indicates type of reaction, second digit indicates reaction mechanism. The third ~~name~~ digit indicates the reactive group of the substrate & the fourth digit indicate the specific name of the enzyme.)]

(1) Oxidoreductases :- The enzymes which catalyse oxidation and reduction reactions are called as oxidoreductases.

a) Oxidases :- These enzymes cause oxidation with the help of molecular oxygen and transfer hydrogen from the substrate to molecular oxygen.

eg. : oxalic acid oxidase, ascorbic acid oxidase, etc.

b) Reductases :- These enzymes cause addition of hydrogen or an electron and remove oxygen.

eg. : Nitrate reductase.

(2) Transferases :- These enzymes which catalyse the transfer of ~~group~~ chemical groups from one compound to other are called transferases.

eg. : Transaminases, Kinases, etc

- (3) Hydrolases :- The enzymes which catalyse breakdown of the substrate molecule by addition of water, are called hydrolases.
eg.: Glycosylase, urease, proteases, etc.
- (4) Lyases :- The enzymes which catalyse addition or removal of a group from substrate without hydrolysis, are called lyases.
eg.: Aldolase, fumarase, etc.
- (5) Isomerases :- The enzymes which catalyse isomerization of molecules are called isomerases. They bring about intramolecular rearrangement of atoms in the substrate.
eg.: ^{xylose} isomerases, epimerases, etc.
- (6) Ligases (Synthetases) :- The enzymes which link together two molecules by establishing C-O, C-S, C-N, or C-C bonds between them, are called ligases or synthetases. They usually hydrolyse one ATP molecule during the ligation reaction.
eg.: DNA polymerase, Asparagine synthetase, etc.

Mechanism of Enzyme Action :-

The enzyme action involves following steps :-

(i) The enzyme molecule (E) combines with a suitable substrate molecule (S) to form an enzyme-substrate complex (ES).

(ii) The enzyme contains specific sites for attachment of substrates. These sites are called active sites.

(iii) The active sites loosen the chemical bonds in the substrate. This leads to the breaking of the substrate into end-products.

(iv) Finally, the enzyme dissociates from the end-products.

(v) The enzyme is now free to combine with another molecule of substrate.

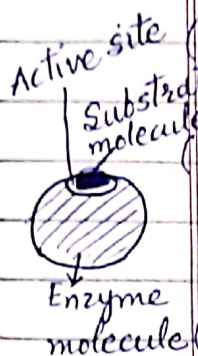
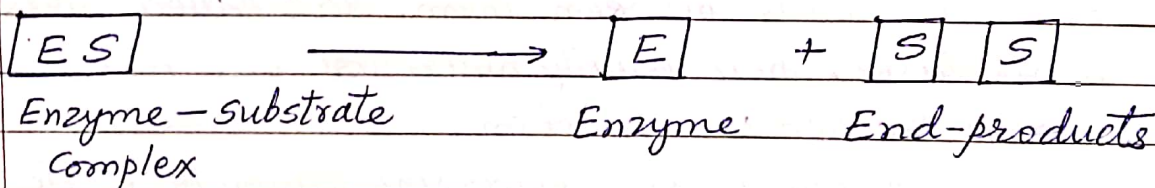
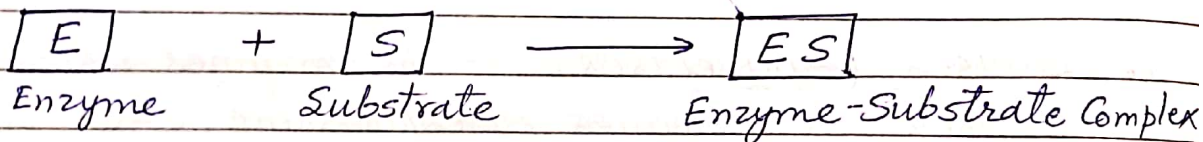


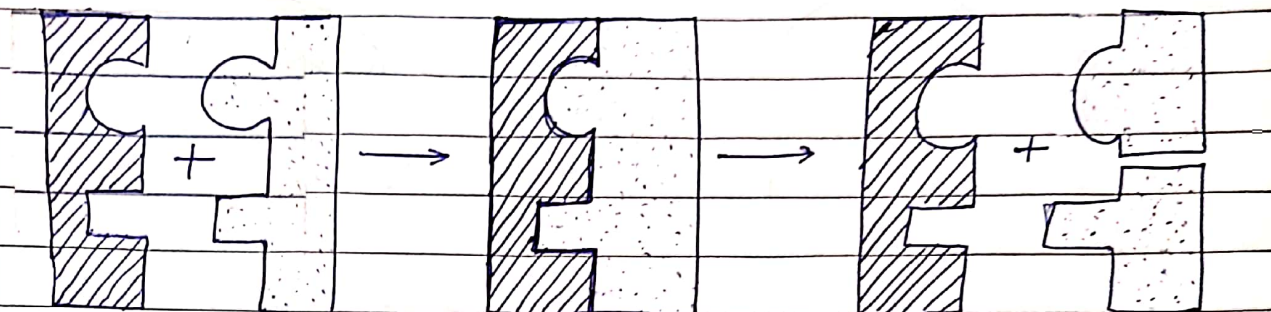
Fig: An enzyme with active site



There are two hypotheses to explain the mechanism of enzyme action (ie. formation of enzyme-substrate complex). They are -

- ① Lock and key theory, &
- ② Induced fit theory.

① Lock and Key theory :-



Enzyme Substrate Enzyme -
- Substrate
Complex Enzyme End-
- products

Fig. : Lock and Key theory of enzyme action

This theory was proposed by Fisher (1914). According to this theory, as a particular lock can be opened by a particular key, in the same way, particular enzyme acts on particular substrate.

The active site on enzyme molecule has a specific shape or surface geometry. Only a particular type of substrate molecule having matching or complementary shape can fit into the active site & form enzyme - substrate complex. This explains the high specificity of enzyme - substrate action and is called "lock and key mechanism".

According to this theory, the enzyme has a rigid unchanging structure of active site. But, this theory was found to be wrong. This theory is supported from the study of competitive inhibition of the enzyme activity. Competitive inhibitors have structural similarity with the substrate molecule, both of which compete for the same active site on the enzyme. If even some part of the active site is pre-occupied by competitive inhibitor, the substrate

... will not be able to combine with it & the activity of the enzyme is inhibited. Removal of competitive inhibitor clears the way for substrate molecule to combine with active site, so that enzyme activity is restored.

② Induced fit theory :-

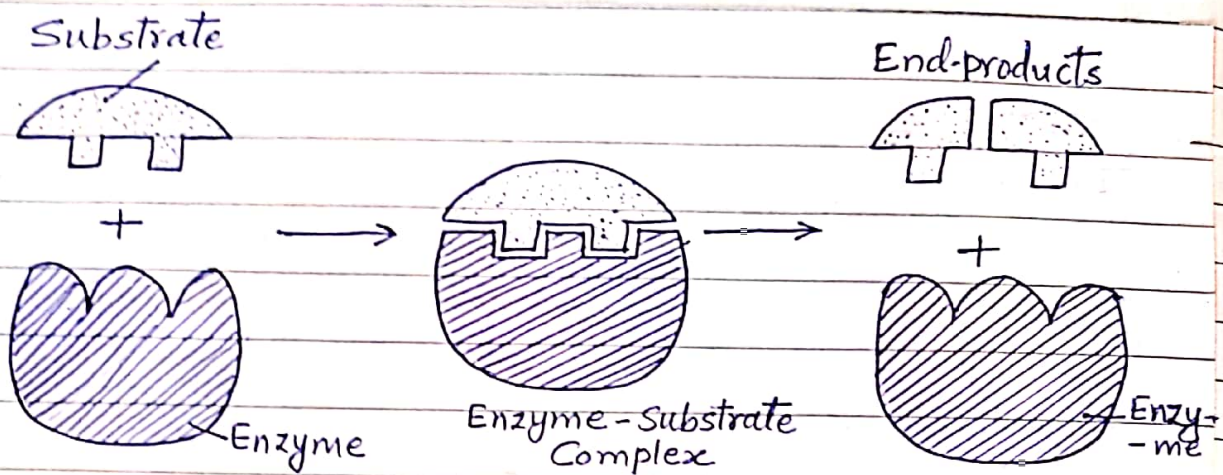


Fig.: Induced fit theory of enzyme action.

This theory was proposed by Koshland (1959). According to this theory, the active site of the enzymes does not have specific shape or surface geometry, but it is flexible. When a proper substrate molecule approaches the active site of the enzyme, the substrate induces certain changes in the active site of the enzyme. As a result, there is perfect fitting of the substrate molecule with the enzyme to form the enzyme-substrate complex. These changes induced in the enzyme are called as conformational changes and the mechanism is called as "induced fit mechanism". Recent investigations support this theory.

According to this theory, the enzyme in inactive condition has different shape. When substrate comes in its contact, it gets activated & acquires active form with active site for substrate attachment.

Concept of Holoenzyme :-

There are certain enzymes, which are entirely proteins. Many other enzymes, along with proteins, contain a non-protein group. The protein part of the enzyme is called ~~coen~~ apoenzyme and the non-protein part is called prosthetic group. The complete enzyme is called holoenzyme or conjugated protein.

When the prosthetic group is organic compound, the prosthetic group is called coenzyme. ~~eg.~~

eg. : NAD, NADP, ATP, Co-A, FMN, FAD, etc

When the prosthetic group is

The coenzyme is not firmly attached to the enzyme protein. It exists in a free state in the solution. It makes contact with the enzyme protein only at the time of enzyme action.

When the prosthetic group is metal ion, the prosthetic group is called activator.

eg. : Mg, Cu, Zn, D Fe, Mn, K, etc.

Allosteric inhibition / Feedback inhibition :-

Biochemical pathway in the living organisms is completed by a series of reactions & it is catalysed by a number of enzymes in sequence. Sometimes, the accumulation of the final end-product may cause inhibition in the activity of the first enzyme of the series. "This inhibition in the enzymatic reaction brought about by the final end-product, is called feedback inhibition & such an enzyme is called allosteric enzyme."

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The allosteric enzymes ~~are~~ the first enzyme in a series of enzymatic reactions of metabolic pathway.

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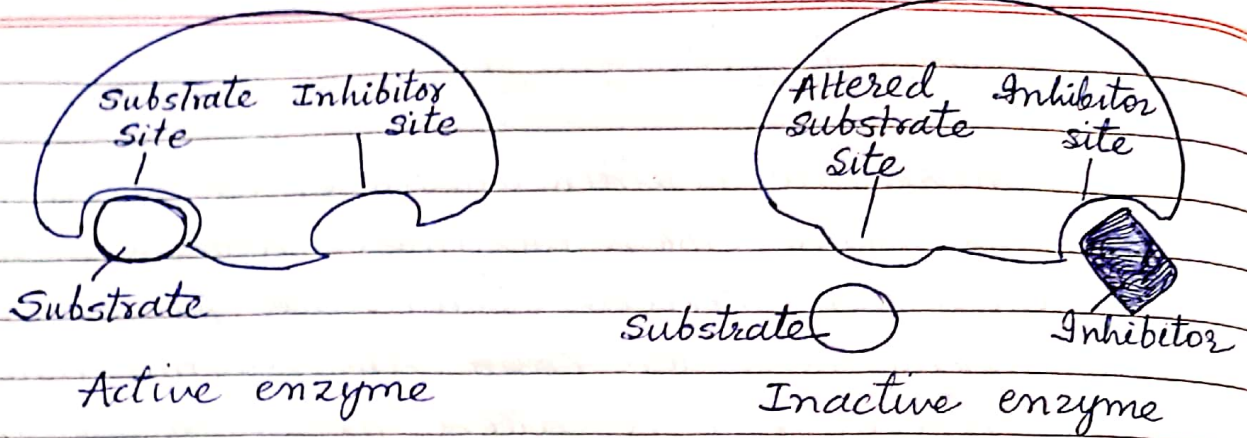


Fig.: Allosteric inhibition

The allosteric enzyme has two types of sites - active & allosteric. (Allo = other, stereos = space/site). The final end-product molecule combines with the allosteric site & brings about a change in shape of the enzyme, so that the active site of the enzyme becomes unfit to combine with the substrate. i.e. the end-product brings about a conformational change in the active site of the enzyme, resulting in the reduction of its catalytic activity. This phenomenon is also called as allosteric inhibition or allosteric transition.

The allosteric inhibition is reversible. When the concentration of the final end-product in the cell falls, it leaves the allosteric site, and the activity of the allosteric site enzyme is restored.

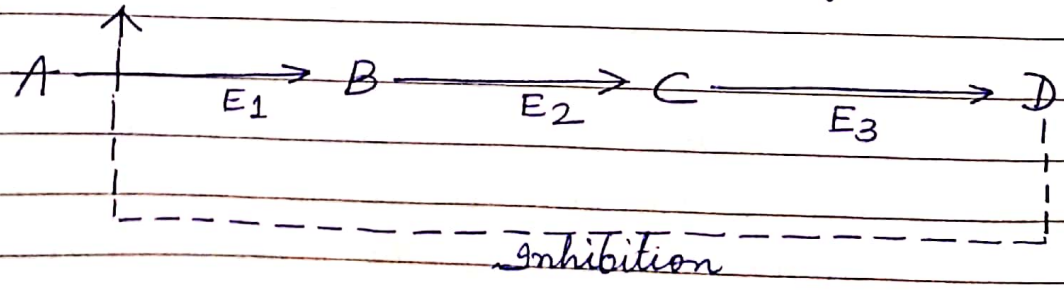


Fig.: Diagram showing the end-product acting as inhibitor for the first (allosteric) enzyme. E1, E2 and E3 are the enzymes

Example :

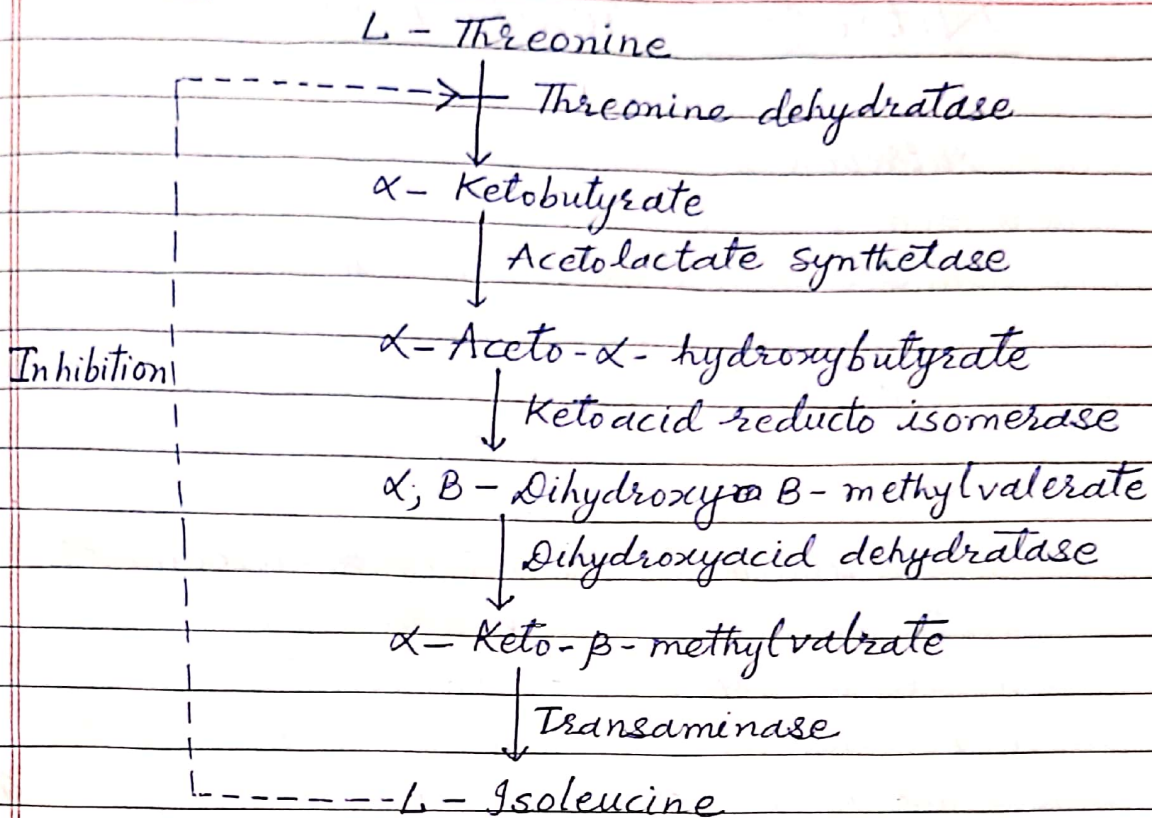


Fig.: Allosteric inhibition in E. coli.

In E. coli, during conversion of L-threonine. The best example of allosteric inhibition is conversion of L-threonine to into L-isoleucine by E. coli.

In this conversion, the first enzyme (threonine dehydratase) is inhibited by isoleucine which is the final end-product of this sequence. The activity of this enzyme is neither inhibited by any other intermediate of the sequence, nor is any other enzyme of this sequence is inhibited by isoleucine.

The inhibition of the first enzyme threonine dehydratase is reversible. When the concentration of isoleucine in the cell increases, the activity of this enzyme is decreased, so that production of isoleucine falls. But, when isoleucine concentration decreases, the activity of threonine dehydratase increases and the production of isoleucine in the cell is restored.

Nitrogen Metabolism :-

Nitrogen is very important element for plant and animal life. Nitrogen is essential for the synthesis of proteins, chlorophylls, cytochromes, alkaloids, nucleic acids & vitamins in the plants. So, nitrogen is essential for plant life. Nitrogen is present in the form of gas in the atmosphere and constitutes about 78% of the atmosphere. Plants are unable to fix nitrogen directly or indirectly from atmosphere and they have to depend for its supply ~~from~~ on soil & soil microbes. There are only few organisms which can make direct use of atmospheric nitrogen, such as Nostoc, Anabaena, Clostridium, etc.

Nitrogen metabolism can be defined as, "The series of biochemical changes taking place inside or outside the plant body which results in the construction of complex nitrogenous food from its simpler derivatives and the destruction of complex nitrogenous food into its components." It includes both anabolic (or constructive) and catabolic (or destructive) processes. The important anabolic processes include nitrogen fixation, protein synthesis & nucleic acid synthesis. The important catabolic processes include proteolysis, denitrification & nitrification. Because of both these anabolic & catabolic processes, of nitrogen metabolism, continuous supply of nitrogen to plants and animals other living organisms is maintained & at the same time, the percentage of nitrogen in the atmosphere is maintained ~~about~~ constant. (75-80%).

Sources of Nitrogen :-

- i) Molecular nitrogen which is available to only a few living organisms.
- ii) Nitrate which has to get converted into ammonia before entering the metabolism.
- iii) Ammonium compounds of which ammonium ions are absorbed directly by the roots of the plants along with the nitrate ions, &
- iv) Organic nitrogen present in the soil in the form of proteins or partially decomposed proteins & which can be absorbed by the plants as amino acids, ammonia or nitrate directly.

The higher green plants can utilize 4 kinds of compounds as sources of nitrogen, viz: nitrates, nitrites, ammonium salts and organic nitrogenous compounds.

Nitrogen fixation :-

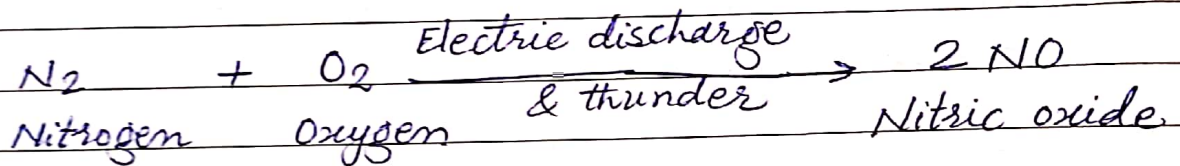
For centuries together, it was a well-known fact that, by growing leguminous crops, the reduced fertility of land can be increased again. The reason for this increase of fertility was not known for long. Later on, it was found that, the fertility of soil increases due to some bacteria, which are found in root nodules of leguminous crops & soil & these bacteria are capable of fixing atmospheric nitrogen.

Nitrogen fixation can be defined as, "the phenomenon of conversion of free nitrogen into nitrogenous salts to make it available for absorption by plants."

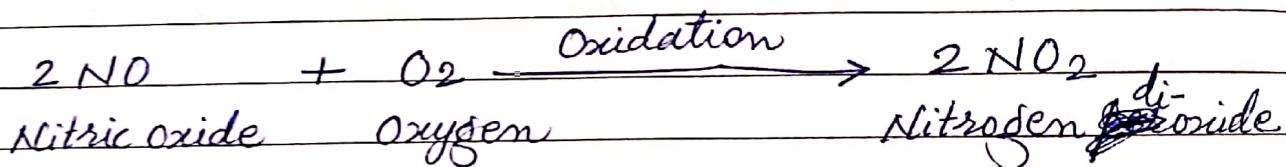
The nitrogen fixation is of two types, viz. physical nitrogen fixation & biological nitrogen fixation. Biological nitrogen fixation is more common in nature. About 200 million metric tonnes of nitrogen is fixed annually by natural process. Only 5% of this natural fixation occurs by physical process & about 95% occurs thro' biological process (ie. thro' certain microorganisms).

① Physical nitrogen fixation :-

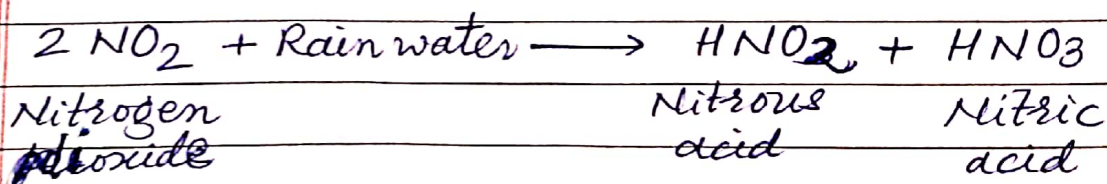
It occurs in several steps and starts with combination of atmospheric nitrogen with oxygen under the influence of electric discharge and thunder, to produce nitric oxide.



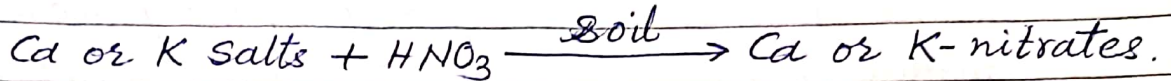
The nitric oxide is then oxidized to nitrogen dioxide in presence of oxygen.



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



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



② Biological nitrogen fixation :-

"The process of conversion of atmospheric nitrogen into nitrogenous ^(ammonia) salts by living organism is called biological nitrogen fixation."

Biological nitrogen fixation is of two types :-
viz : Symbiotic nitrogen fixation & non-symbiotic ^(Asymbiotic) nitrogen fixation.

(a) Symbiotic nitrogen fixation :-

"Symbiotic nitrogen fixation is the process in which the nitrogen-fixing organism (ie. bacteria) requires a host plant as a symbiont."

This kind of nitrogen fixation is carried out by bacteria ~~to~~ frequently found in the root nodules of leguminous plants. The most common bacterium is Rhizobium. It has two types of strains :

- a) fast growers - ~~ie~~ that include Rhizobium leguminosarum or Bacillus radicolica, R. faciolii, R. trifolii, R. mon malilotii, and
- b) Slow growers - that include Rhizopus japonicum & R. lupinii.

Rhizobia are commonly found in the soil of crop fields, where bacteria & plant both specifically affect each other's growth.

Nodule formation :- Nodule formation is initiated by the infection of root hair by free-living rhizobia. Since the bacteria digest can not digest cellulose, they enter the root hair from the tip region, where cellulose is nearly absent. The process of nodule formation is very peculiar. Legume plants release tryptophan in the soil, which is absorbed by the Rhizobium and is metabolised to produce indole acetic acid (IAA). The rhizobia produce another characteristic substance called root hair

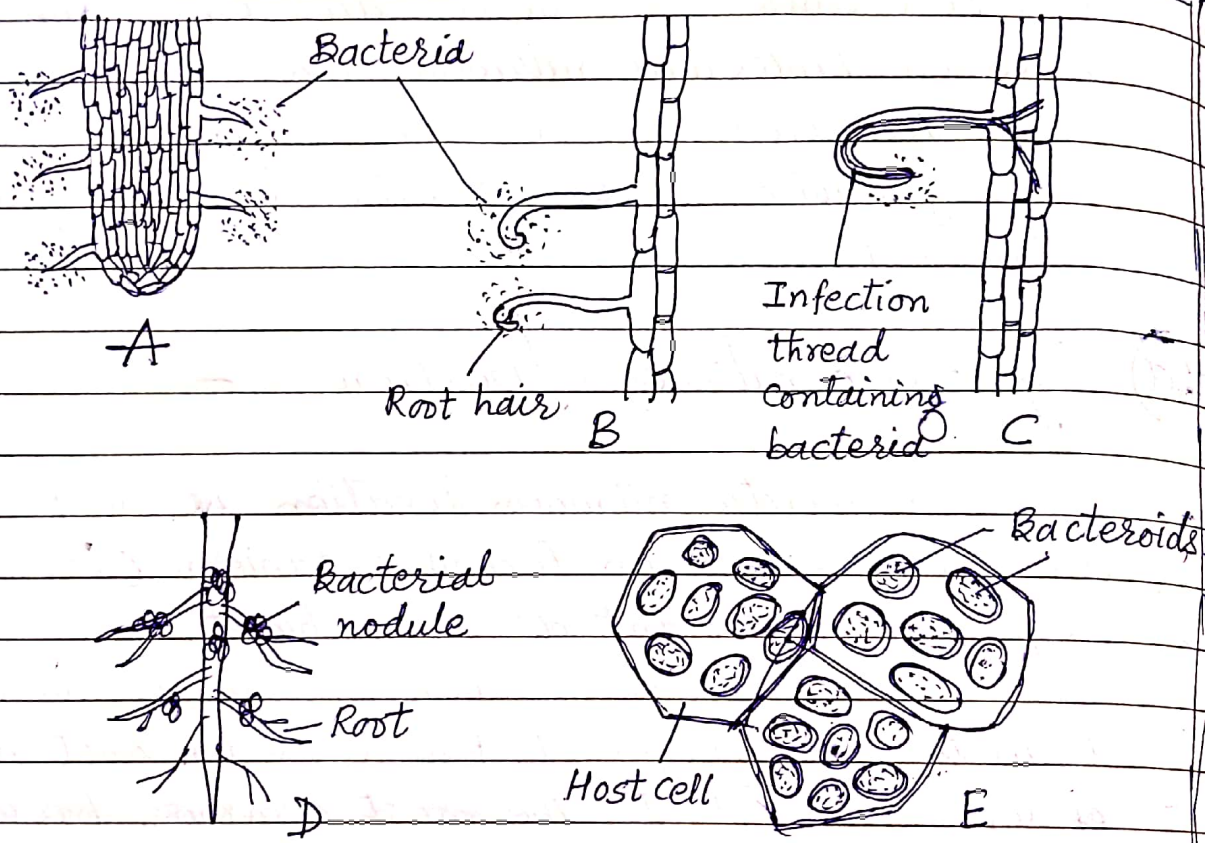


Fig.: Stages in the formation of root nodule.

- A - Rhizobia in soil near the root,
- B - Entrance of bacteria thro' root hairs tip of root hair,
- C - Infection thread formed by bacteria.
- D - Root containing root nodules.
- E - Bacteroids in membrane bound sacs in the host cells.

curling factor that causes deformation and twisting of root hairs. The root hair gets penetrated by large number of Rhizobia, which collectively convert into an infection thread lined by cellulose, which is provided by the host cells. The infection thread intrudes, settles & liberates its contents in ^{the} cortical cells. After the release of bacteria, the host cells undergo cell-divisions & ~~the final nodule consists of~~ resulting in the formation of a nodule. ~~It~~ The nodule structure consists of a central core containing Rhizobia, & the surrounding cortical area, in which vascular system is present. The cells in the nodule have twice the chromosome number. The cells of bacteria either individually or in small groups are enclosed by a membrane. ~~They~~ The bacteria enlarge, assume pleiomorphic shape & are called bacteroids. They ~~undergo~~ stimulate polyploid cell to undergo repeated divisions.

The nodule contains a pink-colored leghaemoglobin pigment, which like true haemoglobin, combines with O_2 ~~and~~ & gets oxidised into brown form with a trivalent iron. The development of leghaemoglobin and nitrogen-fixing capacity are outcome of symbiotic interaction, since individually neither the leguminous plants produce leghaemoglobin nor bacteria carry out nitrogen fixation. The symbiotic association is so essential that, in absence of Rhizobium & combined nitrogen in soil, leguminous plants do not grow.

~~Mechanism of nitrogen fixation~~ Nitrogenase :-

Nitrogen fixation is catalyzed by the enzyme nitrogenase, which is sensitive to oxygen. It is transcribed by nif Nif gene (Nitrogen fixing gene). Nitrogenase ~~is~~ reduces nitrogen to ammonia. The enzyme nitrogenase is

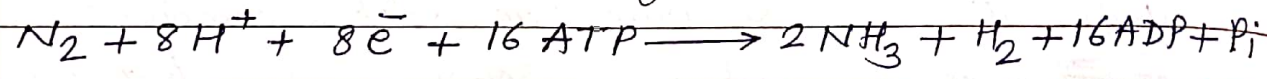
active in absence of oxygen & inactivated by oxygen. Nitrogenase consists of two proteins - an iron protein (Fe-protein) and one Molybdenum-Iron protein (Mo-Fe protein).

Absent in non-sym N₂ fixing organisms.

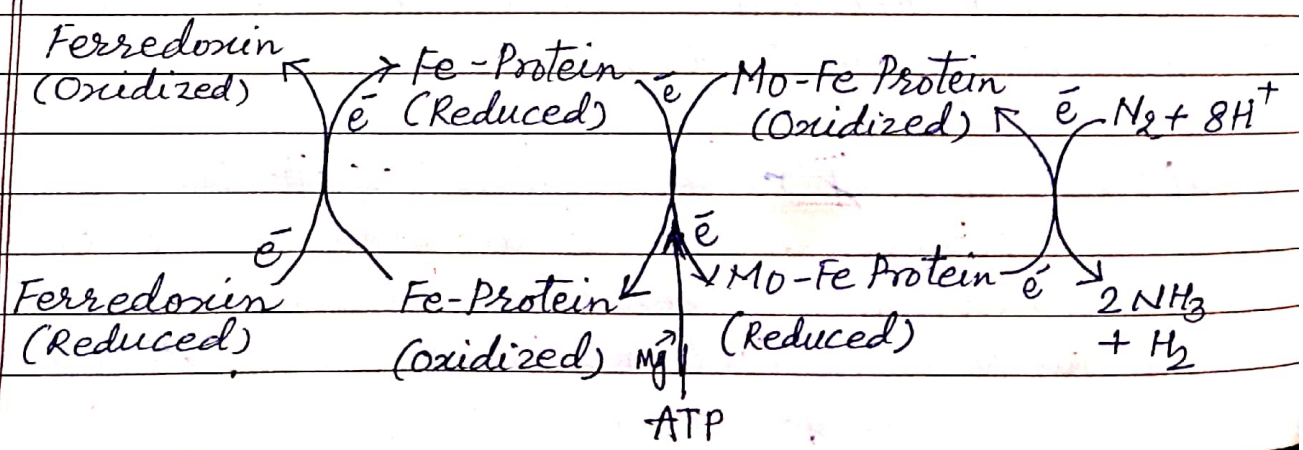
The leghaemoglobin regulates the course of O₂ and provides suitable atmosphere to the enzyme. It ~~is~~ The leghaemoglobin ~~is~~ very rapidly combines with the O₂ to avoid any inhibitory effect of it on enzyme. At the ~~same~~ same time, the pigment is able to release O₂ when required & thus makes available to the bacteroids for ATP production needed for N₂-fixation.

Mechanism of nitrogen fixation :-

The biological nitrogen fixation occurs in presence of enzyme nitrogenase. In addition to this enzyme, a source of reducing ~~reac~~ agents (Fd), ATP and protons are required. The overall redⁿ of biological nitrogen fixation is. — :



It is believed that, the nitrogen is bound to the enzyme surface and is not released until it is completely reduced to ammonia. Nitrogen bound to enzyme surface is reduced in stepwise reaction before N-N bond is ruptured.



The mechanism of non-symbiotic & symbiotic nitrogen fixation is the same & involves following steps:-

- i) The Fe-protein of nitrogenase receives electrons from ferredoxin or NADPH_2 & gets reduced.
- ii) The reduced Fe-protein accepts Mg-ATP and forms reduced Fe-protein-Mg-ATP complex. Mg activates Fe-protein.
- iii) The Mo-Fe subunit ~~complex~~ ^(RFP-MA) of nitrogenase binds with nitrogen to form nitrogenase-Nitrogen complex ^(NNC).
- iv) The reduced RFPMA complex binds to NNC to form an active nitrogenase complex, which accepts hydrogen ~~ions~~ ^{H^+} from cytoplasm.
- v) The hydrogen ^{H^+} is supplied to nitrogen & the nitrogen is converted into a diimide ($\text{HN}=\text{NH}$).
- vi) The diimide reacts with hydrogen ions to form hydrazine ($\text{H}_2\text{N}-\text{NH}_2$).
- vii) The hydrazine reacts with hydrogen ions to form two molecules of ammonia (NH_3).
- viii) The active nitrogenase complex dissociates into Fe-protein, Mo-Fe protein, Mg^{++} ions & ATP.
- ix) The enzyme is freed to reduce another nitrogen molecule.
- x) The ammonia is released in cytoplasm & converted into ammonium (NH_4^+). The ammonium is used in the biosynthesis of amino acids.

(Non-Symbiotic)
(b) Asymbiotic Nitrogen fixation :-

"Asymbiotic nitrogen fixation is the process in which the nitrogen-fixing organism does not require a host plant."

Many free-living bacteria & blue-green algae are capable to fix atmospheric nitrogen.

* Bacteria :-

i) Anaerobic forms - eg.: Bacillus polymyxa, Clostridium pasteurianum, Azospirillum brassiliense, Klebsiella pneumoniae.

ii) Aerobic forms - eg.: Azotobacter chroococcum, A. gills, A. azile, Azomonas, Beijerinckia.

* Fungi :- Pullularia & Yeasts.

* Blue-green algae :- Gloethece, Synechococcus, Oscillatoria erythroa, Lyngbya phormidium, Nostoc, Anabaena, Cylindrospermum, Calothrix, Tolypothrix, Alusira, Scytonema, Gloetrichia etc.

Nitrogenase :-

Same as symbiotic nitrogen fixation.

See Page No. 61 & 62. (Leghaemoglobin absent).

Mechanism of nitrogen fixation :-

Same as symbiotic nitrogen fixation.

See Page No. 62 & 63. (Leghaemoglobin absent).

* Ammonification, Nitrification & Denitrification:

① Ammonification :-

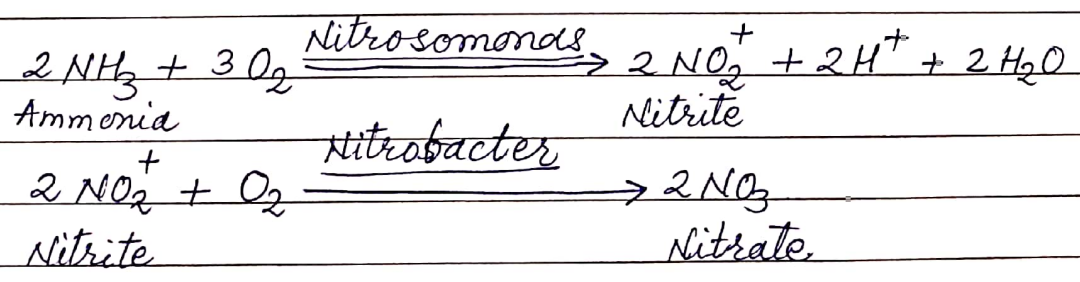
The process of formation of ammonia in soil from organic compounds is called ammonification & the ammoni bacteria which bring about ammonification are called ammonifying bacteria.

The soil contains large number of organic compounds in the form of plant remains & animal excreta. ~~These protein contents~~ ^{These} are decomposed into ammonia ~~amino acids~~ by some bacteria like Bacillus racemosus, B. mycoides & B. vulgaris. Many fungi and actinomy-cetes also help in this process.

Some of the ammonia goes into atmosphere, Ammonia can be ~~to~~ directly taken up by the plants or can be converted into δ nitrates by the process of nitrification.

② Nitrification :-

The process of conversion of ammonia into nitrite and then into nitrate is called nitrification & The bacteria which bring about nitrification are called nitrifying bacteria. Ammonia is oxidized to nitrite by Nitrosomonas and nitrite is further oxidized to nitrate by Nitrobacter.



Once nitrate is formed, it is taken up by the plants, where it gives rise to ammonia.

③ Denitrification :-

The process of conversion of nitrates and nitrites into molecular nitrogen is called denitrification & the bacteria which bring about denitrification are called as ~~denitrifying~~ denitrifying bacteria.

The molecular (free) nitrogen is then released into atmosphere. Such bacteria are therefore responsible for reducing nitrogen content of soil & therefore, fertility of soil is also reduced.

Denitrifying bacteria are Pseudomonas denitrificans, P. stutzeri, Bacillus subtilis, Thiobacillus denitrificans, Micrococcus, Clostridium, Azotobacter, etc.

Denitrification is necessary to maintain the balance of nitrogen in the atmosphere.

