Asymbiotic N_2 fixation occurs without the activity of micro-organism. Soil derived N_2 is generally in the form of nitrate and atmospheric N_2 which must be reduced to ammonia to become available for aminoacid and protein synthesis. Enzyme nitrate reductase and nitrite reductase catalyze conversion of NO_3 into NH_4 and enzyme Nitrogenase, catalyse conversion of atmospheric N_2 into NH_4.

Since ammonia is toxic, it is rapidly assimilated in the glutamate-glutamine cycle with the help of enzymes glutamine ~~cycle~~ synthetase and glutamate synthetase. This is coupled with Aspartate. Aspartate system. Catalysed by asparagine synthetase and aspartate aminotransferase. Photosynthesis is used via TCA Cycle to generate carbon skeleton for the reaction. Asymbiotic N_2 Fixation are following:-

(i) By Free living Nitrogen Fixing Bacteria of Azotobacter and Clostridium groups:- Winogradsky discovered an anaerobic soil micro-organism, Clostridium pasteurianum which will fix free molecular nitrogen, when supplied with carbohydrate the amount of nitrogen fixed being roughly proportional to the amount of carbohydrate broken down. They were Azobacter chroococcum and Azobacter Agilis. The nitrogen fixing bacteria were found to differ strikingly in that clostridium is obligately anaerobic.

NH_3 is the key intermediate product in the

nitrogen fixing mechanism of all micro-organisms including Azobacter and Clostridium. The enzyme system responsible for nitrogen fixation has been identified and the whole enzyme system has once been termed as Nitrogenase. The individual enzyme first reacting with elementary nitrogen being called nitrogenase. Azotobacter also possess a Hydrogenase. Certain chemicals have been found to have a specific inhibitory effect on the other in fixing enzymes. We have seen that CO_2 is one of them and the other is gaseous hydrogen. However hydrogen has no marked inhibitory effect on nitrogen fixation by Clostridium or by photosynthetic bacteria. The key intermediate NH_4^+ must represent the end of fixation reaction and the start of assimilation of the fixed nitrogen into the organic molecules of the organism.

(22) By Free-Living Coloured Photosynthetic Bacteria:- Most interesting discovery has been that coloured photosynthetic bacteria are nitrogen fixing. All these bacteria are anaerobic organisms, carrying out photosynthesis only in complete absence of O_2. These bacteria are widely distributed in marine and also in fresh water habitats. The genera which are known to be definitely nitrogen fixing are Rhodospirillum, Rhodopseudomonas, Rhodomicrobium, belonging to the family Athiorhodaceae. These species cause inorganic sulphur compounds, they prefer organic reductants for assimilation of CO_2 in presence of light. They can best be described as facultative sulphur

bacteria, Green ~~bacteria~~ sulphur bacterium, ~~and the purple~~ ~~sulphur~~ such as chlorobium and chlorobacterium, and the purple sulphur bacteria chromatium are also N_2 fixers.

(iii) By Free-Living Colourless Sulphur Bacteria:- These are one of the most interesting groups of recently discovered sulphate reducing bacteria. These are obligate anaerobes. Instead of using O_2 oxidise their food, They use sulphate and as a by product the sulphide is formed and energy liberated. This exergonic energy could be utilised for the formation of the cell material of the bacteria in presence of carbonates which are available in the water in which the bacteria live. It is very recently established beyond all doubt that Desulphovibrio is also capable of fixing elementary nitrogen.

(iv) By Free-Living Yeast cells:- There have been many reports that certain yeasts and also other fungi are able To fix atmospheric nitrogen. Recently however conclusive evidence have been obtained by means of kjeldahl and other methods and also by use of isotopic Techniques with ^{15}N, of fixation of N_2 by ~~a~~ a variety of yeast, isolated from ~~teeth~~ heath soils.

Some free-Living N_2-fixing bacteria isolated from soil are Pseudomonas radiobacter and Flavobacterium fulvum in mixed culture.