LECTURE 27

Nitrogen Fixation

Biological nitrogen fixation is the bio-chemical process by which elemental ritrogen is combined in to organic forms. It is carried out by a number of organisms including several species of bacteria, a few actinomycetes and blue green algae.

The quantity of nitrogen fixed globally each year is enormous (175 million Mg), which is more than the nitrogen applied through chemical fertilizers.

Although nitrogen is fixed by a number of different organisms a common mechanism appears to be involved in all the cases.

 $N_2 + 6H + 6e^- \xrightarrow{Nitrogenas} (Fe \& Mo) \rightarrow 2NH_3$

NH₃ + organic acids ____ Amino acids ____ Protein

The site of nitrogen reduction is the enzyme nitrogenase, a two-protein complex consisting of a large ion, a molybdenum containing member, and a smaller companion containing iron.

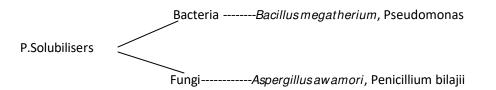
Nitrogen fixi	ng systems			
N- Fixing System	Organism Involved	Plant Involved	Site of fixation	Nitrogen kg N/ ha/ year
ymbiotic				
Legumes	Rhizobium bacteria	Legumes	Nodules	50-100
Non- legumes	Frankia (Actinomycetes)	Alnus	Nodules	50-150
	BGA(Anabaena)	Casuarinas Azolla(Fern)	Leaf	100-150
ssociative	Azospirillum	Non -legumes	Rhizosphere	5-30
<u>Non-</u> Symbiotic	BGA (Oscillatoria)	Not involved with plants	Soil & Water	10-50
	Azatobacter, Beijerinckia,			5-30
	(aerobic) Clostridium (anaerobic)			5-20

DENITRIFICATION : In flooded soils the oxygen supply to soil is virtually cut - off because very less diffusion rate. Under such conditions, the facultative anaerobes Viz: Thiobacillus denitrificans, Thiobacillus thioparus (autotrophic) , Pseudomonas, Micrococcus, Achromobacter and Bacillus, during respiration, utilize the NO₃⁻ as terminal acceptor and reduce it to NO, N₂O and finally to N₂. In this fashion the nitrate leached from oxidized layer and the nitrate nitrified at the root rhizosphere will be denitrified.

 $4NO_3^{-} + 5H_2O + 4H^{+} + 5C$? $2N_2$? $+ CO_2$? $+ 7H_2O$

The losses due to denitrification may range from 10 to 40 percent of the applied nitrogen. Of the denitrified gas, about 90 % is in the form of N_2 . N_2O is lost at pH below 7.0 and causes atmospheric pollution. Denitrification is influenced by high organic matter content, moisture regime, soil type, Eh of soil, high temperature, supply of nitrate and cropping.

Phosphorous Solubilisation : The phosphorous element which is released during mineralization is likely to be converted to an insoluble inorganic compound. Some of the bacteria and other micro organisms are known to solubilise these compounds and make the phosphorous available to plants.



P-Absorber ------VAM fungi (Versicular arbiscular mycorrhizae

Glomus fasciculatum and Gigaspora margarita.

Among different phosphorus solubilisers fungi is more efficient, but not exploited commercially, as they do cause some diseases to crop plants also.

VAM is useful to explore more area and utilize phosphorus like immobile elements.

Harmful Activities of Soil Organisms

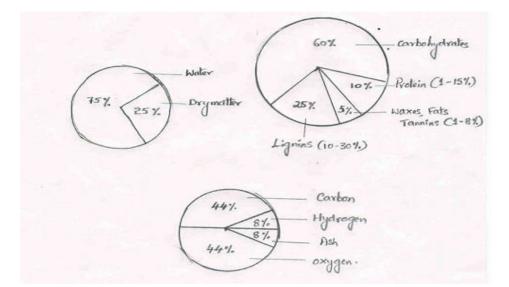
- Rodents, snails, slugs, termites, insect larvae and nematodes cause great
- damage to plants
- Mainly fungi, but bacteria and actinomycetes ,also, are responsible for
 - o many soil borne diseases of crop plants.

- Offer lot of competition to higher plants in utilizing the soil available nutrients, particularly in the presence of carbon sources in the soil.
- Under conditions of poor drainage, depletes the available oxygen and hampers the normal growth of plants.
- Under anaerobic conditions, bacteria transform some of the nutrients to unavailable form (k & Zn etc.) and some nutrients to be available in toxic levels (Fe & Mn etc.)

LECTURE 28

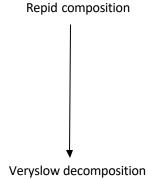
Soil Organic Matter

Sources of soil organic matter: The original source of soil organic matter is plant tissue (leaves, roots and left outs of harvested crops). Animals are the secondary sources of organic matter (their bodies when their life cycles are consummated)



DECOM POSABILITY OF PLANT RESIDUES

- * Sugars, Starches and simple proteins
 - 1 Crude proteins
- 2 Hemicelluloses
- 3 Cellulose
- 4 Fats, Waxes etc.,
- 5 Lignins



ORGANIC MATTER DECOM POSITION

When organic tissue is added to soil three general reactions take place.

- 1 The bulk of the material undergoes enzymatic oxidation with Co₂, H₂O and energy and heat as the major products.
- 2 The essential elements such as nitrogen, phosphorous and sulphur are released and / or immobilized by a series of specific reactions relatively unique for each element.
- 3 Compounds very resistant to microbial actions are formed either through modification of compounds in the original plant tissue or by microbial synthesis.

The native flora of soil i.e. bacteria, fungi and actinomycetes are involved in the decomposition of organic matter. Different soil enzymes (protein substances) produced by these microorganisms are directly responsible for the decomposition by reducing the activation energy, necessary to breakdown the bonds of different organic materials.

An enzyme is a substance, composed of proteins that are capable of lowering the activation energy of other selective compounds enough to allow the breaking of a particular bond under a particular environment.

The action of enzyme to make a split easier does not use up the enzyme. An activator that is not consumed or changed by such a process is called catalyst. Many different enzymes are produces by a single organism and many organisms produce the same enzyme.

The simple end products of decomposition of organic matter under aerobic soil situations are CO ₂, NH₄⁺, NO₃⁻, H₂PO₄⁻. SO₄²⁻, H₂O, resistant residues and various other essential plant nutrients like Ca ²⁺, Mg²⁺, Fe²⁺, Mn²⁺, Zn²⁺, Cu²⁺ etc.,. Under anaerobic conditions the end products are CH₄ (swamp gas), some organic acids (R-COOH) eg. lactic, propionic and butyric acid etc., NH₄⁺, various amine residues (R-NH₂), toxic gas H₂S and ethylene (H₂C=CH₂) and the resistant humus substance.

MINERALIZATION: Mineralization is the conversion of an element from an organic form to an inorganic as a result of microbial decomposition.

The heterogeneous group of heterotrophic soil micro-organisms takes part in mineralization of organic nitrogen and converts it to inorganic nitrogen. The reactions go uninterrupted, as long as carbon source is available for microbes.

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Enzymatic Hydrolysis *Enzymatic reaction* Polypeptides Protein

?Amino acids, Amines $+ CO_2 + Energy$

Ammonification : Process of conversion of amino acids to ammonia

 $\xrightarrow{EnzymaticHydrolysis} \rightarrow NH_3 + R - OH + Energy$ $RNH_2 + HOH$ -? $NH_4^+ + OH^-$ or $NH_3 + H_2CO_3 - --? 2NH_4^+ + CO_3^{2-}$

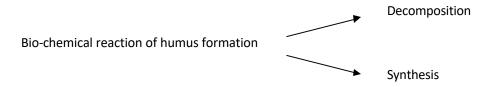
NH₃+H2O

IM M OBILIZATION: The conversion of the element from the inorganic to the organic form in microbial tissues or in plant tissues, thus rendering the element not readily available to other organisms or to plants, is called immobilization.

SOIL HUM US

Humus is a complex and rather resistant mixture of brown or dark brown amorphous and colloidal organic substances that results from microbial decomposition and synthesis and has chemical and physical properties of great significance to soils and plants

Synthesis of Humus : The formation of humus is strictly a bio-chemical process and rather complicated one. The compounds that make up humus have been synthesized by microorganisms in the soil from the products of the break down or alteration of the original tissue.



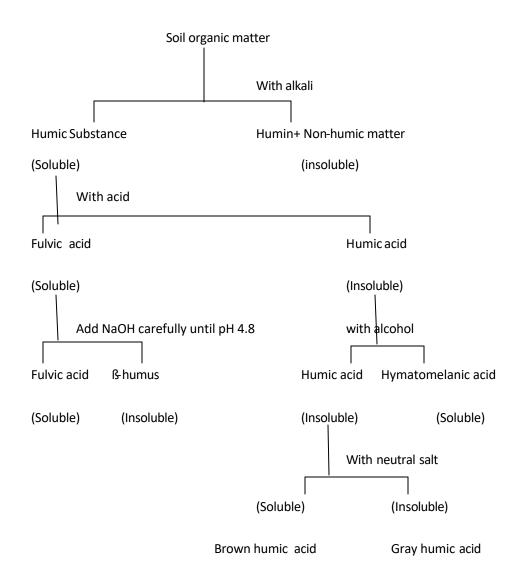
The simpler compounds resulted from break down of organic matter may take part immediately in human forming processes, a bio-chemical synthesis. These simpler compounds are metabolized in to new compounds in the body tissue of the soil organisms. The new compounds are subjected to further modification and synthesis as the microbial tissue is subsequently attacked by other soil micr oorganisms.

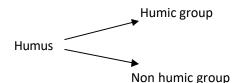
Additional synthesis reactions involve break down products of lignin as the phenols and quinones. These decomposition products, present initially as separate molecules called 'monomers' are enzymatically stimulated to join together in to polymers. By this process of polymerization (linkage) polyphenols and polyquinones are formed. These high molecular weight compounds interact with nitrogen containing amino compounds and give rise to a significant component of resistant 'Humus'.

Fractionation of Humus or Soil organic matter

Soil humus, the complex array of substances left after extensive chemical and biological break down of fresh plant and animal residues, makes up 60-70% of the total organic carbon in soil. Because of the complexity, humus is often divided through solubility separations.

Extraction and fractionation of organic matter or humus





Humic group : The humic substances makes up about 60-80% of soil organic matter and comprised of the most complex materials (Aromatic, ring type structures that include polyphenols and polyquinones) with no sharply defined physical and chemical properties. They are resistant to microbial attack. They are amorphous, dark in color and have high to very high molecular weights from a few hundred to several thousand.

On the basis of resistance to degradation and of solubility in aids and alkalis, humic substances are classified in to three chemical groupings.

- 1 Fulvic acid: lowest molecular weight, lightest in color, soluble in both acid and alkali and most susceptible to microbial attack.
- 2 Humic acid:- Medium in molecular weight and color, soluble in alkali but insoluble in acid and intermediate in resistance to degradation.
- 3 Humin:- Highest in molecular weight, darkest in color, insoluble in both acid and alkali and most resistant to microbial attack.

Non-Humic Group : Comprises 20-30% of the organic matter in soils. These are less complex and less resistant to microbial attack. They are comprised of specific organic compounds with definite physical and chemical properties. These include polysaccharides (polymers that have sugar like structures with general formula of $C_n(H_2O)_n$, sugar amines, nucleic acids, phospholipids, vitamins & sulfolipids. Polysaccharides in particular are helpful in cementing soil aggregates together.

LECTURE 29

IM PORTANCE OF SOIL ORGANIC MATTER / HUM US

Influence of Humus/ Organic matter on soil physical, biological and chemicalproperties.

- 1 Imparts dark color to soils
- 2 Supplies polysaccharides for binding soil particles for formation of aggregates (genesis of good soil structure)
- 3 Increases infiltration rate of water and provides better drainage

- 4 Increases water holding capacity
- 5 Reduces plasticity, cohesion, stickiness etc in clay soils
- 6 Reduces bulk density, there by influence porosity favorably
- 7 Through granulation, reduces wind erosion losses
- 8 Provides mulching (raw organic matter) and lowers soil temperature during summer. Acts as an insulator and retards heat movement between atmosphere and soil
- 9 Reduces alkalinity in soils by releasing organic acids and CO₂
- 10 With high adsorption capacity, it accounts for 30-90% of the adsorbing power of mineral soils (Carboxylic group 54%; phenolic & enolic groups 36%; imide group 10%)
- 11 Acts as a buffering agent and reduces the likelihood damage from acids and alkalis.
- 12 With its solubilising effect, increses the availability of nutrients
- 13 Acts as a store house for nutrients. Organic matter is the source of 90-95% of nitrogen in unfertilized soils. Also supplies available 'P', 'S' and micro nutrients like Fe, Mn, Cu and Zn etc.,
- 14 Adsorbs temporarily the heavy metal pollutants and cleans the contaminated waters.
- 15 Serves as a source of energy for macro and micro organisms in soils and helps in performing various beneficial functions in soils (N fixation, mineralisation etc.)
- 16 Acts as a chelate and increases the availability of micro nutrients
- 17 Various organic substances like vitamins, antibiotics and growth promoting substances namely auxins are produced by different micro organisms during decomposition of organic matter. Also some fungi-toxins are produced to control diseases.

Carbon : Nitrogen Ratio

Both mineralization and immobilization are accomplished by microbes under the influence of temperature, moisture and pH etc.,

These processes are more influenced by C/N, C/P and C/S ratios of decomposing plant residues.

An average proportion of C/N/P/S in soil humus is appropriately 140:10:1.3:1.3. The ratios recorded for C and P are some what more variable than for C and N, or C and S.

C:N Ratio	C:P Ratio	C:S Ratio	N.M	N.I
-20-1	<200.1	<200.1	Vec	No
<20:1	<200:1	<200:1	Yes	No
>30:1	>300:1	>400:1	No	Yes
20-30:1	200-300:1	200-400:1		Neither gain nor loss

C:N ratios of some organic materials

Material	<u>C:N ratio or C/N</u>
Microbial tissues	6-12
Sewage, Sludge	5-14
Soil Humus	10-12
Annual Manures	13-25
Legume residues and green manures	13-25
Cereal residues and straws	60-80
Forest Wastes	150-500

The quantity of carbon declines with the advancement of decomposition process. Similarly, the inorganic element released during mineralization process is also lost, by several means i.e. plant utilization, leaching, volatilization, conversion to insoluble compounds etc.,. It results in a stabilized C:N ratio to the soil i.e. 10:1 or 12:1

Significance of C: N ratio in Soil Fertility :

- 4 C: N ratio determines the microbial activity and their proliferation
- 5 C:N ratio of organic residues determines the rate of decomposition of organic residues.