UNIT – 3 Soil Microbiology

Definition:

It is branch of science/microbiology which deals with study of soil microorganisms and their activities in the soil.

Soil:

It is the outer, loose material of earth's surface which is distinctly different from the underlying bedrock and the region which support plant life. Agriculturally, soil is the region which supports the plant life by providing mechanical support and nutrients required for growth. From the microbiologist view point, soil is one of the most dynamic sites of biological interactions in the nature. It is the region where most of the physical, biological and biochemical reactions related to decomposition of organic weathering of parent rock take place.

- **Components of Soil / Soil as an environment / Soil as a culture medium**

Soil is an admixture of five major components viz. mineral / inorganic matter, organic matter, soil-water. soil-air, and soil-microorganisms / living organisms. The amount / proportion of these components vary with locality and climate.

1. **Mineral / Inorganic Matter:** It is derived from parent rocks / bed rocks through decomposition, disintegration and weathering process. Different types of inorganic compounds containing various minerals are present in soil. Amongst them the dominant minerals are Silicon, Aluminum and iron and others like Carbon, Calcium, Potassium, Manganese, Sodium, Sulphur, Phosphorus etc. are in trace amount. The proportion of mineral matter in soil is slightly less than half of the total volume of the soil.
2. **Organic matter/components:** Derived from organic residues of plants and animals added in the soil. Organic matter serves not only as a source of food for microorganisms but also supplies energy for the vital processes of metabolism which are characteristics of all living organisms. Organic matter in the soil is the potential source of N, P and S for plant growth. Microbial decomposition of organic matter releases the unavailable nutrients in available from. The proportion of organic matter in the soil ranges from 3-6% of the total volume of soil.
3. **Soil-Water**: The amount of water present in soil varies considerably. Soil-water comes from rain, snow, dew or irrigation. Soil-water serves as a solvent and carrier of nutrients for the plant growth. The

microorganisms inhabiting in the soil also require water for their metabolic activities. Soil-water thus, indirectly affects plant growth through its effects on soil and microorganisms. Percentage of soil-water is 25% total volume of soil.

4. **Soil-air (Soil-gases):** A part of the soil volume which is not occupied by soil particles i.e. pore spaces are filled partly with soil-water and partly with soil-air. These two components (water & air) together only accounts for approximately half the soil's volume. Compared with atmospheric air, soil is lower in oxygen and higher in carbon dioxide, because CO_2 is continuous recycled by the microorganisms during the process of decomposition of organic matter. Soil-air comes from external atmosphere and contains nitrogen, oxygen Co_2 and water vapour (CO_2 > oxygen). Co_2 in soil air (0.3-1.0%) is more than atmospheric air (0.03%). Soil aeration plays important role in plant growth, microbial population, and microbial activities in the soil.
5. **Soil-microorganisms:** Soil is an excellent culture media for the growth and development of various microorganisms. Soil is not an inert static material but a medium pulsating with life. Soil is now believed to be dynamic for living system.

Soil contains several distinct groups of microorganisms and amongst them bacteria, fungi, actinomycetes, algae, protozoa and viruses are the most important. But bacteria are more numerous than any other kinds of microorganisms. Microorganisms form a very small fraction of the soil mass and occupy a volume of less than one percent. In the upper layer of soil (top soil up to 10-30 cm depth i.e. Horizon A), the microbial population is very high which decreases with depth of soil. Each organisms or a group of organisms are responsible for a specific change / transformation in the soil. The final effect of various activities of microorganisms in the soil is to make the soil fit for the growth & development of higher plants.

Living organisms present in the soil are grouped into two categories as follows.

1. Soil flora (micro flora) e.g. Bacteria, fungi, Actinomycetes, Algae and
2. Soil fauna (micro fauna) animal like eg. Protozoa, Nematodes, earthworms, moles, ants, rodents.

Relative proportion / percentage of various soil microorganisms are: Bacteria-aerobic (70%), anaerobic (13 %), Actinomycetes (13%), Fungi /molds (03 %) and others (Algae Protozoa viruses) 0.2-0.8 %. Soil organisms play key role in the nutrient transformations.

Brief account and definition of microbial interaction with examples

a) Symbiosis: -

The term symbiosis, or "together-life," can be used to describe many of the interactions between microorganisms, and also microbial interactions with higher organisms, including plants and animals. These interactions may be positive or negative.

Or

It is a condition in which the individuals of a species live in close association with individuals of another species.

Or

Living together of two different organisms.

Or

It is the coexistence of two different forms of life in an intimate ecological relationship, which may be of long or short duration and requires close physical contact.

e.g. i) Association or living together of nitrogen fixing bacteria in the roots of plants.

ii) Living of bacteria in the rumen of animals.

iii) Normal flora in the intestine of human.

Endosymbiosis: -

Symbiotic association in which one partner is actually inside a cell or tissue of the other.

Ectosymbiosis: -

Symbiotic association in which one partner is external to the other.

Microbial associations may be ---->

i) Neutral association: - e.g. a) Neutralism

ii) Positive or beneficial; - e.g. a) Mutualism b) Syntrophism c) Commensalism

iii) Negative or detrimental: - e.g. a) Antagonism b) Competition c) Parasitism d) Predation

i) Neutral association: - e.g. a) Neutralism

Two populations of microorganisms found in close association and not affect each other i.e. two organisms do not compete or show an inhibition even though they occupy the same area.

This condition probably exists between organisms, which are quite different in their requirements for growth hence; neither kind of organism affects the existence of the other.

ii) Positive or beneficial; - e.g. a) Mutualism b) Commensalism

In this association each organism benefits from the association i.e. it is beneficial for them.

a) Mutualism: -

Mutualism [Latin *mutuus*, borrowed or reciprocal] defines the relationship in which some reciprocal benefit accrues to both partners. This is an obligatory relationship in which the **mutualist** and the host are metabolically dependent on each other.

It is the symbiotic relationship in which each organism benefits from the association.

Examples

i) The protozoan-termite relationship is a classic example of mutualism in which the flagellated protozoa live in the gut of termites and wood roaches. These flagellates exist on a diet of carbohydrates, acquired as cellulose ingested by their host. The protozoa engulf wood particles, digest the cellulose, and metabolize it to acetate and other products. Termites oxidize the acetate released by their flagellates. Because the host is almost always incapable of synthesizing cellulases (enzymes that catalyse the hydrolysis of cellulose), it is dependent on the mutualistic protozoa for its existence.

i) **Lichens** are the association between specific ascomycetes (the fungus) and certain genera of either green algae or cyanobacteria. In a lichen, the fungal partner is termed the **mycobiont** and the algal or cyanobacterial partner, the **phycobiont.** Because the phycobiont is a photoautotroph—dependent only on light, carbon dioxide, and certain mineral nutrients—the fungus can get its organic carbon directly from the alga or cyanobacterium. The fungus often obtains nutrients from its partner by haustoria (projections of fungal hyphae) that penetrate the phycobiont cell wall. It also uses the O2 produced during phycobiont photophosphorylation in carrying out respiration. In turn the fungus protects the phycobiont from excess light intensities, provides water and minerals to it, and creates a firm substratum within which the phycobiont can grow protected from environmental stress.

b) Syntrophism / Satellitism: -

Syntrophism [Greek *syn*, together, and *trophe,* nourishment] is an association in which the growth of one organism either depends on or is improved by growth factors, nutrients, or substrates provided by another organism growing nearby. Sometimes both organisms benefit. This type of mutualism is also known as cross feeding or the satellite phenomenon.

It is the type of mutualism involving the exchange of nutrients between two species.

Examples

i) A very important syntrophism occurs in anaerobic methanogenic ecosystems such as sludge digesters anaerobic freshwater aquatic sediments, and flooded soils. In these environments, fatty acids can be degraded to produce H_2 and methane by the interaction of two different bacterial groups. Methane production by methanogens depends on **interspecies hydrogen transfer.** A fermentative bacterium generates hydrogen gas, and the methanogen uses it quickly as a substrate for methane gas production.

ii) Many microorganisms synthesize a particular essential nutrient in sub optimal amounts, others synthesize vitamins & amino acids in excess. These nutrients are required for other microorganisms; hence, certain combinations of species will grow together.

c) Commensalism: -

Commensalism [Latin *com*, together, and *mensa*, table] is a relationship in which one symbiont, the **commensal,** benefits while the other (sometimes called the host) is neither harmed nor helped.

It is the relationship between organisms in which one species benefits while other is not affected.

Examples

i) Commensalistic relationships between microorganisms include situations in which the waste product of one microorganism is the substrate for another species. An example is nitrification, the oxidation of ammonium ion to nitrite by microorganisms such as *Nitrosomonas,* and the subsequent oxidation of the nitrite to nitrate by *Nitrobacter* and similar bacteria. *Nitrobacter* benefits from its association with *Nitrosomonas* because it uses nitrite to obtain energy for growth.

ii) When oxygen is used up by the facultatively anaerobic *E. coli,* obligate anaerobes such as *Bacteroides* are able to grow in the colon. The anaerobes benefit from their association with the host and *E. coli,* but *E. coli* derives no obvious benefit from the anaerobes. In this case the commensal *E. coli* contributes to the welfare of other symbionts.

iii) Many fungi degrade cellulose into glucose. Many bacteria are unable to utilize cellulose, but they utilize the glucose produced from cellulose by fungi.

iv) Yeast can grow in extremely concentrated sugar medium due to which sugar concentration decreases. Now the growth of bacteria is possible in this medium.

iii) Negative or detrimental associations: -

In this association one species gets loss from the other.

E.g. a) Antagonism / Amensalism

b) Competition

c) Parasitism

d) Predation

a) Antagonism / Amensalism: -

Amensalism (from the Latin for *not* at the same table) describes the negative effect that one organism has on another organism. This is a unidirectional process based on the release of a specific compound by one organism which has a negative effect on another organism.

When an organism adversely affects the environment of the other organism, it is said to be antagonistic.

Examples

i) Penicillium spp produce penicillin antibiotic which inhibit or kill bacteria.
ii) Lactobacilli produce & tolerate acidic conditions in which other microorganisms may not be capable of growing at low pH.
iii) Bacteriocins produced by normal flora in intestine to control other pathogens.

b) Competition: -

Competition arises when different microorganisms within a population or community try to acquire the same resource, whether this is a physical location or a particular limiting nutrient

It is a association which may result from competition among species for essential nutrients. In such situations the best-adapted microbial species will predominant or eliminate other species, which are dependent upon the same limited nutrient substance.

Examples

i) Colonies of microorganisms, which are close together, are small, whereas more isolated colonies are much larger because close colonies microorganisms compete for space and nutrients.

c) Parasitism: -

This is a relationship in which one of a pair benefits from the other, and the host is usually harmed. This can involve nutrient acquisition and/or physical maintenance in or on the host.

It is defined as a relationship between organisms in which one organism lives in or on another organism.

The parasite feeds on the cells, tissues, or fluids of another organism, the host, which is commonly harmed in the process. The parasite is dependent upon the host and lives in intimate physical and metabolic contact with the host.

All major groups of plants, animals and microorganisms are susceptible to attack by microbial parasites.

Examples

i) Bacterial parasite of Gram-negative bacteria named ***Bdellovibrio bacteriovorus,*** which is widespread in soil and sewage. This unusual motile bacterium attaches to a host cell at a special region and eventually causes the lysis of that cell.

ii) There are also many strains of fungi, which are parasitic on algae and other fungi by penetration into the host.

iii) Viruses, which attack bacteria, fungi, and algae, are strict intracellular parasites since they cannot be cultivated as free-living forms.

d) Predation

Predation is a widespread phenomenon where the predator engulfs or attacks the prey. The prey can be larger or smaller than the predator, and this normally results in the death of the prey.

Some bigger microorganisms engulf smaller microorganisms is called as predation. The only true microbial predators are phagocytic microorganisms, which do not have rigid cell walls. Zooplankton in the sea feed on smaller fungi, algae and bacteria.

Examples

i) *Bdellovibrio* penetrates the cell wall and multiplies between the wall and the plasma membrane, a periplasmic mode of attack, followed by lysis of the prey and release of progeny.

ii) *Vampirococcus* attaches to the surface of the prey (an epibiotic relationship) and then secretes enzymes to release the cell contents.

iii) *Daptobacter* penetrates a susceptible host and uses the cytoplasmic contents as a nutrient source.

iv) Protozoa prey on the bacteria. Ciliates are excellent examples of predators that engulf their prey, and based on work with fluorescently marked prey bacteria, a single ciliate can ingest 60 to 70 prey bacteria per hour.

v) Protozoan ***Didinium*** preys on the protozoan ***paramecium.*** The only general requirement is that the prey should be smaller than the predator.

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I) Microbe – Microbe interactions
II) Plant – Microbe interactions
III) Animal – Microbe interactions

I) Microbe – Microbe interactions

a) ***Proteus vulgaris and Staphylococcus aureus***
b) ***Escherichia coli and Streptococcus faecalis***
c) ***Lichens*** **(**certain algae & fungi)

a) Between ***Proteus vulgaris and Staphylococcus aureus*** bacteria: -

Both these bacteria ferment lactose sugar producing acid but no gas. However if both species are inoculated into a tube of lactose broth, acid well as gas is produced.

Thus there are many similar examples in which combinations of microorganisms bring about results impossible in pure cultures.

b) Between ***Escherichia coli and Streptococcus faecalis*** bacteria: -

Mixed culture of these bacteria can produce **putrescine** compound from **arginine** amino acid but the transformation cannot be performed by the individual bacterium.

c) ***Lichens*** **(**certain algae & fungi): -

Lichens are the associations between **Ascomycetes fungus** and certain genera of either **Green algae or Blue green algae** (Cyanobacteria).

In the lichen, the fungal partner obtains nutrients from algae by projecting fungal hyphae that penetrate the cell wall of algae. Also fungus obtains O_2 produced during photosynthesis of algae.

In turn, the fungus protects algae from excess light intensities (heat), supplies water and minerals, provides a firm substratum within which the algae can grow protected from environmental stress.

II) Plant – Microbe interactions

a) Phyllosphere

b) Root nodules formation by *Rhizobia* bacteria in leguminous plants

a) Phyllosphere

A wide variety of microorganisms are found on and in the aerial surfaces of plants, called the **phyllosphere.** These include microorganisms that have complex interactions with the plant at various stages of development. The plant leaves and stems release organic compounds, and this can lead to massive development of microbes. The genera present on plant leaves and stems include *Sphingomonas,* which is especially equipped to survive with the high levels of UV irradiation occurring on these plant surfaces. This bacterium, also common in soils and waters, can occur at 108 cells per gram of plant tissue. *Sphingomonas* often represents a majority of the culturable species. Phyllosphere microorganisms play important roles in protection and possibly harm to the plant.

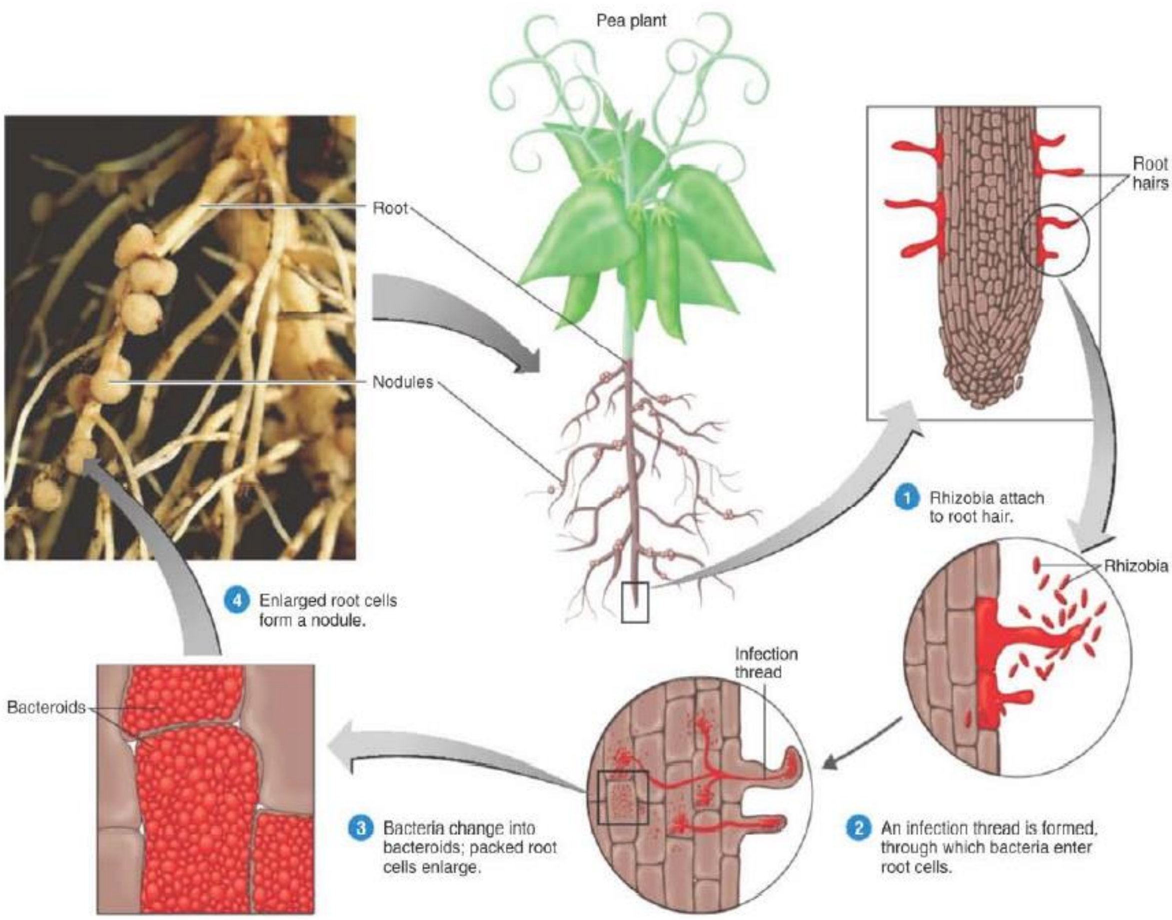
b) Root nodules formation by *Rhizobia* bacteria in leguminous plants

Several microbial genera are able to form nitrogen-fixing nodules with legumes. These include *Allorhizobium, Azorhizobium, Bradyrhizobium, Mesorhizobium, Sinorhizobium,* and *Rhizobium.*

The genus *Rhizobium* is a prominent member of the rhizosphere community. This bacterium also can establish a symbiotic association with legumes and fix nitrogen for use by the plant. *Rhizobium* infects and nodulates specific legume hosts.

a) Flavonoid inducers produced by the plant play a major role in this process by stimulating the *Rhizobium* to synthesize specific **Nod factors** that activate the host symbiotic processes necessary for root hair infection and nodule development.
b) After bacterial attachment, the root hairs curl and the bacteria induce the plant to form an **infection thread** that grows down the root hair.
c) The *Rhizobium* then spreads within the infection thread into the underlying root cells the *Rhizobium* actually enter the plant cytoplasm while it is in the infection thread. When the bacteria are released from the infection thread into the host cell, the *Rhizobium* is enclosed by a plant-derived membrane, called the peribacteroid membrane, to form a **bacteroid**.
d) Further growth and differentiation lead to the development of a nitrogen-fixing form, a structure called a **symbiosome** At this point, specific nodule components such as leghemoglobin, which protect the nitrogen

fixation enzymes from oxygen, are produced to complete the nodulation process. The symbiosomes within mature root nodules are the site of nitrogen fixation. Within these nodules, the differentiated bacteroids reduce atmospheric N_2 and form ammonia (the primary product) and alanine; these compounds are released into the host plant cell, assimilated into various other nitrogen-containing organic compounds, and distributed throughout the plant. Because reduced nitrogen is the nutrient most commonly limiting plant growth, biological nitrogen fixation, as exemplified by the *Rhizobium*-legume symbiosis, is of major importance to agricultural productivity.



III) Animal – Microbe interactions

a) **The Rumen ectosymbiosis**
b) **Bioluminescence**

a) The Rumen ectosymbiosis: -

It is the mutualism between ruminant animals and certain anaerobic microorganisms in which these microorganisms synthesize ***cellulases*** enzymes, which are used to digest cellulose-containing food by ruminant animals, and in turn rumen provides constant temperature and source of food for the growth of these microorganisms.

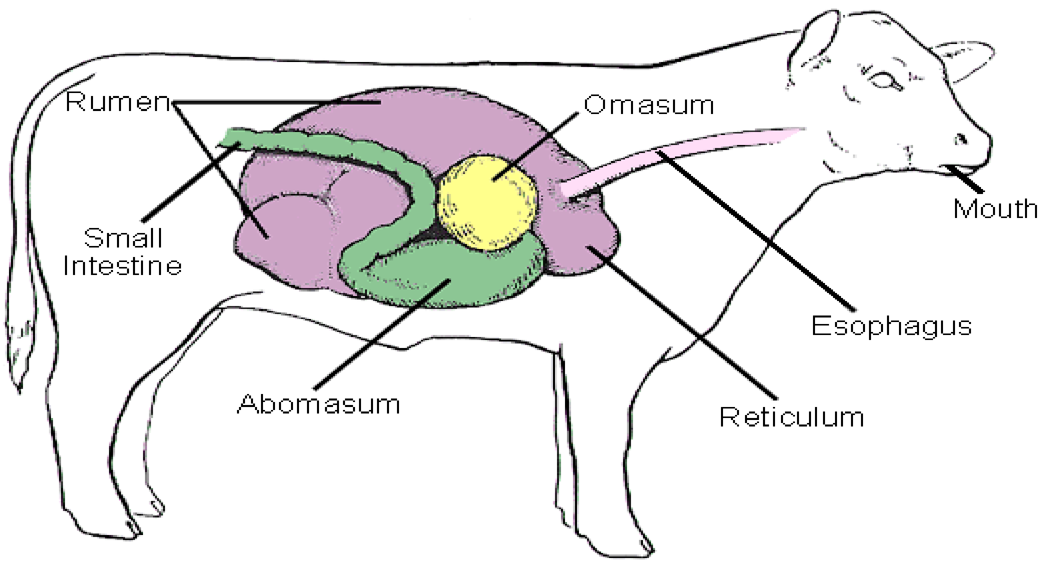
Ruminants are a group of herbivorous animals that have a stomach divided into four (4) compartments and chew a cud consisting of regurgitated, partially digested food.

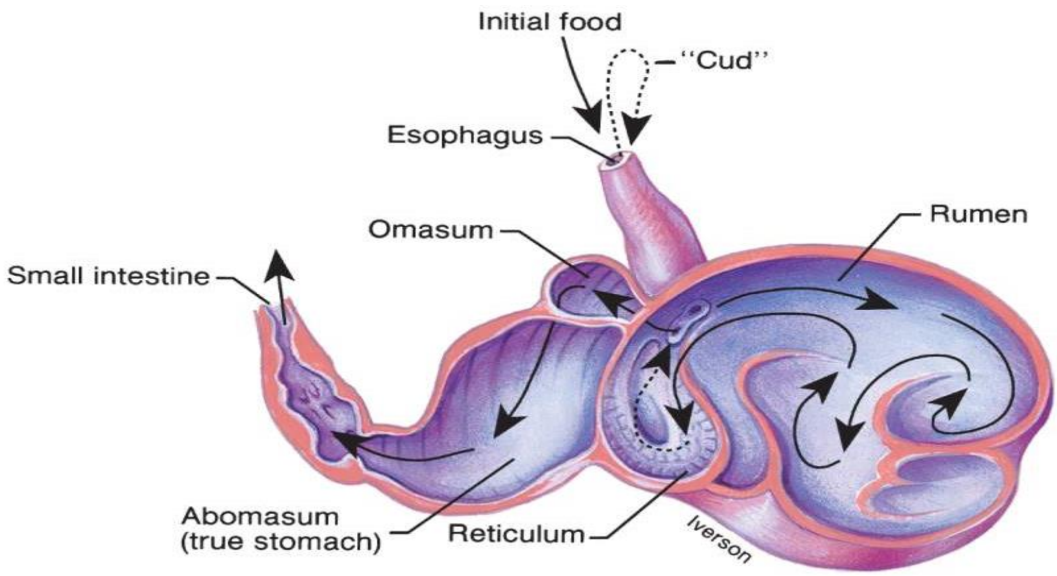
e.g. Cattle, Deer, camels, Buffalo, Sheep, Goats, and Giraffes.

This feeding method has evolved in animals that need to eat large amounts of food quickly, chewing being done later at a more comfortable or safer location.

Because ruminants cannot synthesize cellulases enzymes, they have evolved a symbiotic relationship with **anaerobic microorganisms** that produce these enzymes.

Cellulases hydrolyse the β (1 – 4) linkages between successive D—glucose residues of cellulose and release glucose, which is then fermented to organic acids. These organic acids (acetate, butyrate, propionate) are the true energy source for the ruminant.





<u>Ruminant Stomach</u>: - Stomach compartments of a cow. Arrows indicate direction of food movement.

The upper portion of a ruminant's stomach expands to form a large pouch called the **'Rumen'** and a smaller honeycomb like **'Reticulum'.** The bottom portion of the stomach consists of an antechamber called the **'Omasum'**, with the **'True stomach' (abomasum)** behind it.

The insoluble polysaccharides and cellulose eaten by the ruminant are mixed with saliva and enter the rumen. Within the rumen, food is churned in a constant rotary motion and eventually reduced to a pulpy mass. The rumen contains a large microbial population (10^{12} organisms per millilitre) that partially digests and ferments the food it is processing. Later the food moves into the reticulum. It is then regurgitated as a **'Cud'**, which is thoroughly chewed for the first time. The food is mixed with saliva, re-swallowed, and re-enters the rumen while another cud is passed up to the mouth. As this process continues, the partially digested plant material becomes more liquid in nature. The liquid then begins to flow out of the reticulum and into the lower parts of the stomach: first the omasum and then the abomasum (the true stomach). It is in the abomasum that the food encounters the host's normal digestive enzymes and the digestive process continues in the regular mammalian way.

Food entering the rumen is quickly attacked by the **cellulolytic anaerobic bacteria, fungi, and protozoa.** Microorganisms break down the plant material, as illustrated in figure. Because the oxidation-reduction potential in the rumen is **– 30 mV,** all indigenous microorganisms engage in anaerobic metabolism. Specifically the eubacteria ferment carbohydrates to fatty acids, carbon dioxide, and hydrogen. The archaeobacteria (methanogens) produce methane (CH_4) from acetate, CO_2, and H_2.

The dietary carbohydrates degraded in the rumen include soluble sugars, starch, pectin, hemicellulose, and cellulose. The largest percentage of each carbohydrate is fermented to volatile fatty acids (acetic, propionic, butyric, formic, and valeric), CO_2, H_2, and CH_4.

The fatty acids produced by the rumen organisms are absorbed into the bloodstream and are oxidized by the animal as its main source of energy. The CO_2 and methane, produced at a rate of 200 to 400 litres / day in a cow are released by eructation, a continuous, scarcely audible reflex process similar to belching. Energy trapped as ATP during fermentation is used to maintain the rumen microorganisms and to support their growth. These microorganisms in turn produce most of the vitamins needed by the ruminant. In the remaining two stomachs, the microorganisms having performed their symbiotic task, are digested by stomach enzymes to yield amino acids, sugars, and other products. These also are absorbed and used as nutrients by the ruminant.

b) Bioluminescence

Several species in the genera *Vibrio* and *Photobacterium* can emit light of a blue-green color. The enzyme luciferase catalyzes the reaction and uses reduced flavin mononucleotide, molecular oxygen, and a long-chain aldehyde as substrates.

$$\begin{aligned} FMNH_2 + O_2 + RCHO &\xrightarrow{\text{luciferase}} FMN \\ &+ H_2O + RCOOH + \text{light} \end{aligned}$$

The evidence suggests that an enzyme-bound, excited flavin intermediate is the direct source of luminescence. Because the electrons used in light generation are probably diverted from the electron transport chain and ATP synthesis, the bacteria expend considerable energy on luminescence. Luminescence activity is regulated and can be turned off or on under the proper conditions. Luminescent bacteria occupying the special luminous organs of fish do not emit light when they grow as free-living organisms in the seawater. Free-living luminescent bacteria can reproduce and infect young fish. Once settled in a fish's luminous organ, they begin emitting light, which the fish uses for its own purposes. Other luminescent bacteria growing on potential food items such as small crustacean may use light to attract fish to the food source. After ingestion, they could establish a symbiotic relationship in the host's gut.

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- **Major biogeochemical cycles:Carbon nitrogen, phosphorus, sulphur (cyclic turnover with microbiology).**

Soil microorganisms are the most important agents in the cycling / transformation of various elements (N, P, K, S, Iron etc.) in the biosphere; where the essential elements undergo cyclic alterations between the inorganic state as free elements in nature and the combined state in living organisms. Life on earth is dependent on the cycling of nutrient elements from their elemental states to inorganic compounds to organic compounds and back into their elemental states.

The microbes through the process of biochemical reactions convert / breakdown complex organic compounds into simple inorganic compounds and finally into their constituent elements. This process is known as "Mineralization".

Mineralization of organic carbon, nitrogen, phosphorus, sulphur and iron by soil microorganisms makes these elements available for reuse by plants. In the following paragraphs the cycling / transformations of some of the important elements are discussed.

The four most important cycles are mention below

A. Carbon Cycle
B. Nitrogen Cycle
C. Sulphur Cycle / Sulphur Transformation
D. Phosphorus Cycle / Transformation

A)Carbon Cycle

About half of the dry weight of living organisms is composed carbon. Ultimate source of organic carbon compound in nature is the Co_2 present in the atmosphere 0.03% Co_2 is the most oxidized state of carbon, and is reduced primarily by Photosynthesis.

Co_2 fixation

* Plant & algae photosynthesis:- In plant photosynthesis water serves as hydrogen donor and reduces co2 to sugar.
* Bacterial Photosynthesis: - In bacterial photosynthesis H_2S & other reduced compounds serve as hydrogen donor to reduce CO_2.
* Chemolithotrophs:- CO_2 derived from dissolution of carbonates and bicarbonates is utilized by certain Chemolithotrophs.
* $CO_2 + 2H_2O \longrightarrow (CH_2O)\ X + H_2O$

  Hetrotrophs:-CO2 fixation by heterotrophic in organs is common among bacteria.

  $CH_3\ COCOOH + CO_2 \longrightarrow HOOCCH_2\ CO\ COOH$

  Pyruvic acid Oxaloacetic acid

  Thus CO_2 in there processes is transformed into various cell components like organic carbon (carbohydrates, proteins, fats, nucleic acid etc). The photoautotrophs supply the organic nutrients to heterotrophs (animals & heteroprophic organismsms) Organic materials thus formed is utilized by primary, secondary & tertiary consumers

Organic carbon compound Degradation co2 release to atmosphere

Once the atmosphere carbon is fixed by photoautotrophs & others it will be exhausted from the atmosphere & is present in atmosphere only 0.03%.Therefore it should be recycled or returned to atmosphere.

The carbon is returned to atmosphere by way of respiration but mainly by decomposition of organic but mainly by decomposition of organic compounds by microbial activity. Decomposition of organic compounds which are resulted from waste products of living beings & after their death is done by aerobic & anaerobic processes. In aerobic decomposition organic compounds are oxidized to CO_2 & H_2O.

$$\text{Cellulose} \xrightarrow[\text{Cellulase}]{\text{enzyme}} \text{Cellobiose}$$

$$\text{Cellobiose} \xrightarrow[\beta\ \text{glucosidase}]{\text{enzyme}} \text{Glucose}$$

$$\text{Gluccose} \xrightarrow[\text{many m. orgms}]{\text{enzyme systems}} CO_2\ \ H_2O\ \text{\& other products}$$

Similar degradation pathways occur for the other major plant tissue substances such as hemicellulose lignin & pectin. CO_2 may also originate from the decarboxylation of amino acids as well as from the dissimilation of fatty acids all of these transformations occur in the soil.

Anaerobic decomposition:- of organic carbon yield end products such as CH_4 H_2 and CO_2 in addition to various organic acids & alcohols. The resulted CH_4 oxidized by & certain m orgms to CO_2 also carbon monoxide is resulted which is further oxidized to CO_2 by microorganisms some carbon is immobilized in the form of living matter, carbonates, limestone & related rocks which may be returned to the atmosphere by acids produced by microorganisms.

a) Decomposition of Cellulose: Cellulose is the most abundant carbohydrate present in plant residues/organic matter in nature. When cellulose is associated with pentosans (eg. xylans & mannans) it undergoes rapid decomposition, but when associated with lignin, the rate of decomposition is very slow. The decomposition of cellulose occurs in two stages: (i) in the first stage the long chain of cellulase is broken down into cellobiase and then into glucose by the process of hydrolysis in the presence of enzymes cellulase and cellobiase, and (ii) in second stage glucose is oxidized and converted CO_2 and water.

1. $\text{Cellulose} \xrightarrow[\text{Hydrolysis}]{\text{Cellulase}} \text{Cellobiose} \xrightarrow[\text{hydrolysis}]{\text{Cellobiase}} \text{Glucose}$

2. $\text{Glucose} \xrightarrow{\text{Oxidation}} \text{Organic Acids} \xrightarrow{\text{Oxidation}} CO_2 + H_2O$

The intermediate products formed/released during enzymatic hydrolysis of cellulose (eg. cellobiose and glucose) are utilized by the cellulose-decomposing organisms or by other organisms as source of energy for biosynthetic processes. The cellulolytic microorganisms responsible for degradation of cellulose through the excretion of enzymes (cellulase & Cellobiase) are fungi, bacteria and actinomycetes.

b) Decomposition of Hemicelluloses: Hemicelluloses are water-soluble polysaccharides and consists of hexoses, pentoses, and uronic acids and are the major plant constituents second only in quantity of cellulose, and sources of energy and nutrients for soil microflora.

When subjected to microbial decomposition, hemicelluloses degrade initially at faster rate and are first hydrolyzed to their component sugars and uronic acids. The hydrolysis is brought about by number of hemicellulolytic enzymes known as "hemicellulases" excreted by the microorganisms. On hydrolysis hemicelluloses are converted into soluble monosaccharide/sugars (eg. xylose, arabinose, galactose and mannose) which are further convened to organic acids, alcohols, CO_2 and H_2O and uronic acids are broken down to pentoses and CO_2. Various microorganisms including fungi, bacteria and actinomycetes both aerobic and anaerobic are involved in the decomposition of hemicelluloses.

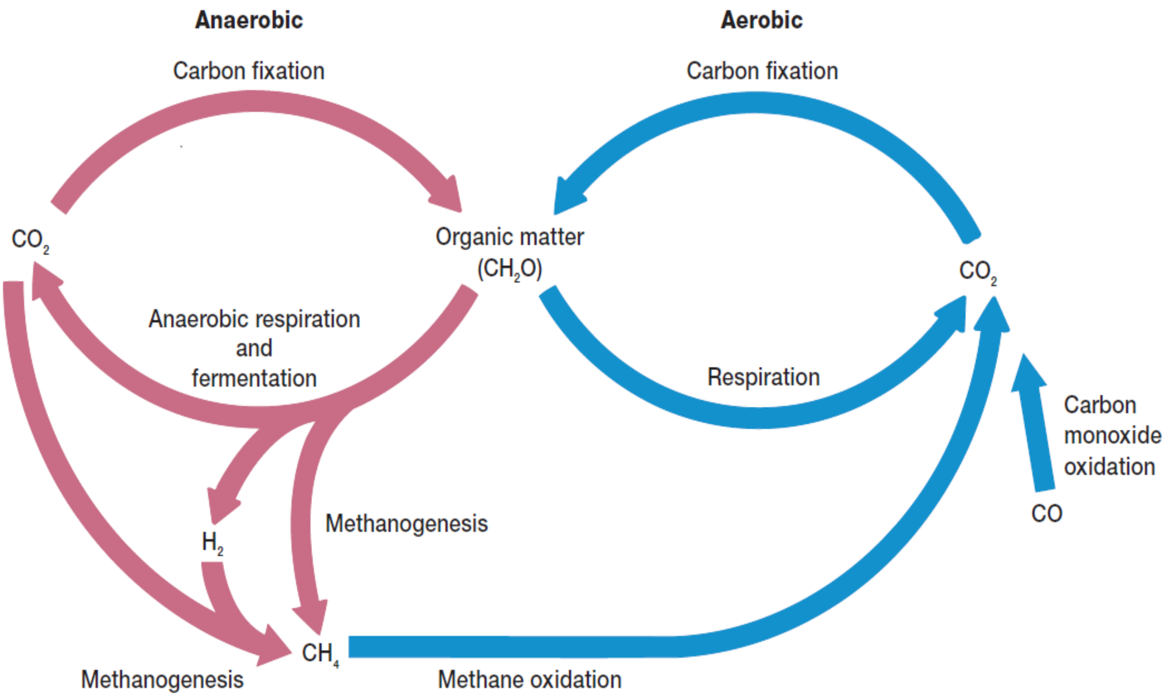
c) Lignin Decomposition: Lignin is the third most abundant constituent of plant tissues, and accounts about 10-30 percent of the dry matter of mature plant materials. Lignin content of young plants is low and gradually increases as the plant grows old. It is one of the most resistant organic substances for the microorganisms to degrade however certain Basidiomycetous fungi are known to degrade lignin at slow rates. Complete oxidation of lignin result in the formation of aromatic compounds such as syringaldehydes, vanillin and ferulic acid. The final cleavages of these aromatic compounds yield organic acids, carbon dioxide, methane and water.

<u>* Role of microorgms in Carbon compounds degradation</u>

Complex organic matter simple organic matter are decomposed by most of the soil microorganisms.

Compounds	**microorganisms**	**End products**
Simple organic Compound	Heterotrophic m orgms	Sugars, amino acids fatly a acid fats
Complex- Organmic matter Like cellulose Liqnin Hemicellulose	cellulomonas cellvibrio, clostridium, Pseudomonas, Actinomycetes & molds	Acids Co_2, CH_4 H_2
CH_4 forming-	Methanobacterium, Methanococcus, methanosarcina,	

	Clostridium	
*CH4 oxidizing	Pseudomonas, Methylomonas, methylocoaus capsulatus	
CO	Carboxydodmonas oligocaarbophila	CO_2
Hydrocarbons	methanomonas methanica	CO_2 +H_20
n- pentane, n- hexane,	methanomonas	CO_2
N- octance, paraffin aliphatic Compounds, petroleum, CH_4 fatty acids.	Anaerobic, purple Sulphar bacteria	CO_2



The carbon Cycle

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Nitrogen Cycle

Although molecular nitrogen (N_2) is abundant (i.e 78-80 % by volume) in the earth's atmosphere, but it is chemically inert and therefore, cannot be utilized by most living organisms and plants. Plants, animals and most microorganisms, depend - on a source of combined or fixed nitrogen (eg.

ammonia, nitrate) or organic nitrogen compounds for their nutrition and growth. Plants require fixed nitrogen (ammonia, nitrate) provided by microorganisms, but about 95 to 98 % soil nitrogen is in organic form (unavailable) which restrict the development of living organisms including plants and microorganisms. Therefore, cycling/transformation of nitrogen and nitrogenous compounds mediated by soil microorganisms is of paramount importance in supplying required forms of nitrogen to the plants and various nutritional classes of organisms in the biosphere. In nature, nitrogen exists in three different forms viz. gaseous / gas (78 to 80 % in atmosphere), organic (proteins and amino acids, chitins, nucleic acids and amino sugars) and inorganic (ammonia and nitrates).

Biological N_2 Fixation:

A. Symbiotic: Eg. Rhizobium (Eubacteria) legumes, Frankia (Actinomycete) and Anabaena (cyanobacteria) non - legumes

B. Non Symbiotic:

1. **Free Living:** eg. Azobacter, Derxia, Bejerinkia, Rhodospirillum and BGA.
2. **Associative:** eg. Azospirillum, Acetobacter, Herbaspirillim.

Nutritional categories of N_2 fixing Bacteria

A. **Heterotrops**
B. **Photoautotrophs**

Nitrogen cycle is the sequence of biochemical changes form free atmospheric N_2 to complex organic compounds in plant and animal tissues and further to simple inorganic compounds (ammonia, nitrate) and eventual release of molecular nitrogen (N_2) back to the atmosphere is called "nitrogen cycle".

In this cycle a part of atmospheric nitrogen (N_2) is converted into ammonia and then to amino acids (by soil microorganisms and plant-microbe associations) which are used for the biosynthesis of complex nitrogen-containing organic compound such as proteins, nucleic acids, amino sugars etc. The proteins are then degraded to simpler organic compounds viz. peptones and peptides into amino acids which are further degraded to inorganic nitrogen compounds like ammonia, nitrites and nitrates. The nitrate form of nitrogen is mostly used by plants or may be lost through leaching or reduced to gaseous nitrogen and subsequently goes into the atmosphere, thus completing the nitrogen cycle. Thus, the process of mineralization (conversion of organic form of nutrients to its mineral /inorganic form) and immobilization (process of conversion of mineral / inorganic form of nutrient elements into organic form) are continuously and simultaneously going on in the soil.

Several biochemical steps involved in nitrogen cycle are:

1. Proteolysis
2. Ammonification
3. Nitrification
4. Nitrate reduction
5. Denitrification.

1. Proteolysis:

Plants use the ammonia produced by symbiotic and non-symbiotic Nitrogen fixation to make their amino acids & eventually plant proteins. Animals eat the plants and convert plant proteins into animal proteins. Upon death, plant and animals undergo microbial decay in the soil and the nitrogen contained in their proteins is released. Thus, the process of enzymatic breakdown of proteins by the microorganisms with the help of proteolysis enzymes is known as “proteolysis".

The breakdown of proteins is completed in two stages. In first stage proteins are converted into peptides or polypeptides by enzyme "proteinases" and in the second stage polypeptides / peptides are further broken down into amino acids by the enzyme "peptidases".

$$\textbf{Proteins} \xrightarrow[\textbf{Proteinases}]{} \textbf{Peptides} \xrightarrow[\textbf{Peptidases}]{} \textbf{Amino Acids}$$

The amino acids produced may be utilized by other microorganisms for the synthesis of cellular components, absorbed by the plants through mycorrhiza or may be de animated to yield ammonia.

The most active microorganisms responsible for elaborating the proteolytic enzymes (Proteinases and Peptidases) are *Pseudomonas, Bacillus, Proteus, Clostridium Histolyticum, Micrococcus, Alternaria, Penicillium* etc..

2. Ammonification (Ammo acid degradation):

Amino acids released during proteolysis undergo deamination in which nitrogen containing amino (-NH_2) group is removed. Thus, process of deamination which leads to the production of ammonia is termed as "ammonification". The process of ammonification is mediated by several soil microorganisms. Ammonification usually occurs under aerobic conditions (known as oxidative deamination) with the liberation of ammonia (NH_3) or ammonium ions (NH_4) which are either released to the atmosphere or utilized by plants (paddy) and microorganisms or still under favorable soil conditions oxidized to form nitrites and then to nitrates.

The processes of ammonification are commonly brought about by *Clostridium* sp, *Micrococcus* sp, *Proteus sp.* etc. and it is represented as follows.

$$\underset{\text{Alanine}}{CH_3CHNH_2COOH} + 1/2O_2 \xrightarrow[\text{deaminase}]{} \underset{\text{Pyruvic acid}}{CH_3COCOOH} + \underset{\text{ammonia}}{NH_3}$$

3. Nitrification:

Ammonical nitrogen / ammonia released during ammonification are oxidized to nitrates and the process is called "nitrification". Soil conditions such as well aerated soils rich in calcium carbonate, a temperature below 30 ° C, neutral PH and less organic matter are favorable for nitrification in soil.

Nitrification is a two stage process and each stage is performed by a different group of bacteria as follows.

Stage I: Oxidation of ammonia of nitrite is brought about by ammonia oxidizing bacteria viz. *Nitrosomnonas europaea, Nitrosococcus nitrosus, Nitrosospira briensis, Nitrosovibrio* and *Nitrocystis* and the process is known as nitrosification. The reaction is presented as follows.

$$\underset{\text{Ammonia}}{2NH_3} + 1/2O_2 \longrightarrow \underset{\text{Nitrite}}{NO_2} + 2H + H_2O$$

Stage II: In the second step nitrite is oxidized to nitrate by nitrite-oxidizing bacteria such as *Nitrobacter winogradsky .Nitrospira gracilis, Nirosococcus mobiiis* etc, and several fungi *(eg. Penicillium, Aspergillus) and* actinomycetes *(eg. Streptomyces, Nocardia).*

$$\underset{\text{Nitrite ions}}{NO_2^{(-)}} + ½O_2 \longrightarrow \underset{\text{Nitrate ions}}{NO_3^{(-)}}$$

The nitrate thus, formed may be utilized by the microorganisms, assimilated by plants, reduced to nitrite and ammonia or nitrogen gas or lost through leaching depending on soil conditions. The nitrifying bacteria (ammonia oxidizer and nitrite oxidizer) are aerobic gram-negative and chemoautotrophic and are the common inhabitants of soil, sewage and aquatic environment.

4. Nitrate Reduction:

Several heterotrophic bacteria *(E. coli, Azospirillum)* are capable of converting nitrates to nitrites and nitrites to ammonia. Thus, the process of nitrification is reversed completely which is known as nitrate reduction. Nitrate reduction normally occurs under anaerobic soil conditions (water logged soils) and the overall process is as follows:

$$\underset{\text{Nitrate}}{HNO_3 + 4\,H_2} \xrightarrow{\text{Nitrate reductase}} \underset{\text{ammonium}}{NH4} + 3\,H_20$$

Nitrate reduction leading to production of ammonia is called "dissimilatory nitrate reduction" as some of the microorganisms assimilate ammonium for synthesis of proteins and amino acid.

5. Denitrification:

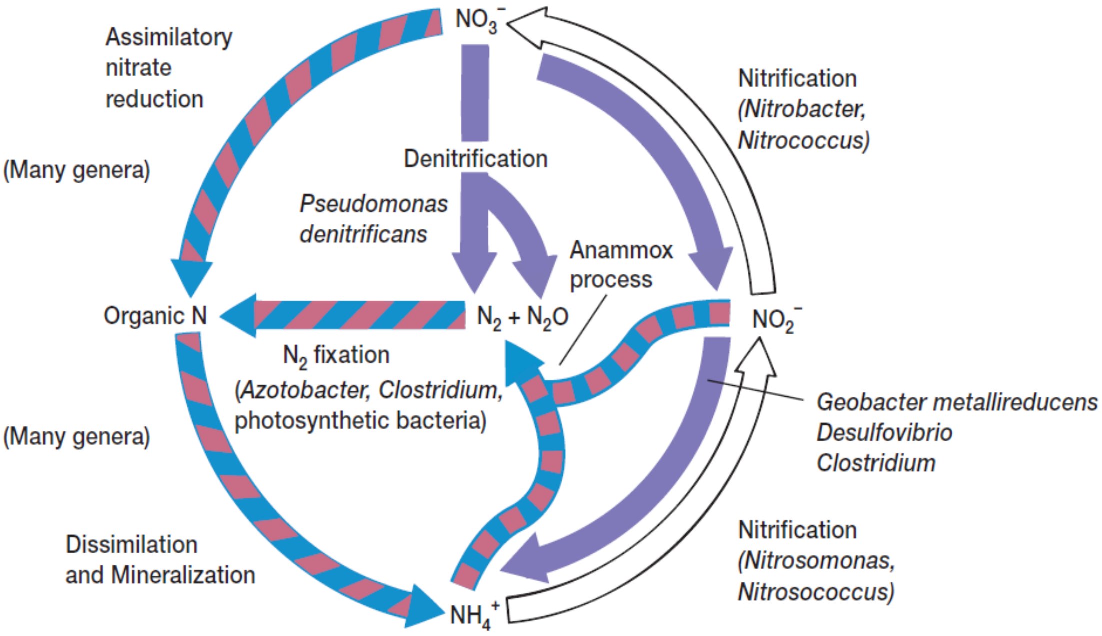
This is the reverse process of nitrification. During denitrification nitrates are reduced to nitrites and then to nitrogen gas and ammonia. Thus, reduction of nitrates to gaseous nitrogen by microorganisms in a series of biochemical reactions is called “denitrification". The process is wasteful as available nitrogen in soil is lost to atmosphere. The overall process of denitrification is as follows:

Nitrate -----> Nitrite ----> Nitric Oxide ----> Nitrous Oxide ------> Nitrogen gas

This process also called dissimilatory nitrate reduction as nitrate nitrogen is completely lost into atmospheric air. In the soils with high organic matter and anaerobic soil conditions (waterlogged or ill-drained) rate of denitrification is more. Thus, rice / paddy fields are more prone to denitrification.

The most important denitrifying bacteria are *Thiobacillus denitrificans, Micrococcus denitrificans,* and species of *Pseudomonas, Bacillus, Achromobacter, Serrtatia paracoccus* etc.

Denitrification leads to the loss of nitrogen (nitrate nitrogen) from the soil which results into the depletion of an essential nutrient for plant growth and therefore, it is an undesirable process / reaction from the soil fertility and agricultural productivity. Although, denitrification is an undesirable reaction from agricultural productivity, but it is of major ecological importance since, without denitrification the supply of nitrogen including N_2 of the atmosphere, would have not got depleted and No_3 (which are toxic) would have accumulated in the soil and water.



Nitrogen Cycle

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Sulphur Cycle / Sulphur Transformations

Sulphur is the most abundant and widely distributed element in the nature and found both in free as well as combined states. Sulphur, like nitrogen is an essential element for all living systems. In the soil, sulphur is in the organic form (sulphur containing amino acids-cystine, methionine, proteins, polypeptides, biotin, thiamine etc) which is metabolized by soil microorganisms to make it available in an inorganic form (sulphur, sulphates, sulphite, thiosulphale, etc) for plant nutrition. Of the total sulphur present is soil only 10-15% is in the inorganic form (sulphate) and about 75-90 % is in organic form.

Cycling of sulphur is similar to that of nitrogen. Transformation / cycling of sulphur between organic and elemental states and between oxidized and reduced states is brought about by various microorganisms, specially bacteria-Thus “the conversion of organically bound sulphur to the inorganic state by microorganisms is termed as mineralization of sulphur". The sulphur / sulphate, thus released are either absorbed by the plants or escapes to the atmosphere in the form of oxides.

Various transformations of the sulphur in soil results mainly due to microbial activity, although some chemical transformations are also possible (eg. oxidation of iron sulphide) the major types of transformations involved in the cycling of sulphur are:

1. Mineralization

2. Immobilization

3. Oxidation

4. Reduction

1. Mineralization: The breakdown / decomposition of large organic sulphur compounds to smaller units and their conversion into inorganic compounds (sulphates) by the microorganisms. The rate of sulphur mineralization is about 1.0 to 10.0 percent / year.

2. Immobilization: Microbial conversion of inorganic sulphur compounds to organic sulphur compounds.

3. Oxidation: Oxidation of elemental sulphur and inorganic sulphur compounds (such as H_2S, sulphite and thiosulphale) to sulphate (SO_4) is brought about by chemoautotrophic and photosynthetic bacteria.

When plant and animal proteins are degraded, the sulphur is released from the amino acids and accumulates in the soil which is then oxidized to sulphates in the presence of oxygen and under anaerobic condition (water logged soils) organic sulphur is decomposed to produce hydrogen sulphide (H_2S). H_2S can also accumulate during the reduction of sulphates under anaerobic conditions which can be further oxidized to sulphates under aerobic conditions,

a) $2S + 3O_2 + 2H_2O \longrightarrow 2H_2SO_4 \xrightarrow{\text{Ionization}} 2H(+) + SO_4$ (Aerobic)

b) $CO_2 + 2H_2S \xrightarrow{\text{Light}} (CH_2O) + H_2O + 2S$

$$\text{OR} \quad H_2 + S + 2\,CO_2 + H_2O \xrightarrow{\text{Light}} H_2SO_4 + 2\,(CH_2O) \text{ (anaerobic)}$$

The members of genus *Thiobacillus* (obligate chemolithotrophic, non photosynthetic) eg, *T. ferrooxidans* and *T. thiooxidans* are the main organisms involved in the oxidation of elemental sulphur to sulphates. These are aerobic, non-filamentous, chemosynthetic autotrophs. Other than *Thiobacillus,* heterotrophic bacteria *(Bacillus, Pseudomonas, and Arthrobacter)* and fungi *(Aspergillus, Penicillium)* and some actinomycetes are also reported to oxidize sulphur compounds. Green and purple bacteria (Photolithotrophs) of genera *Chlorbium, Chromatium. Rhodopseudomonas* are also reported to oxidize sulphur in aquatic environment.

Sulphuric acid produced during oxidation of sulphur and H : S is of great significance in reducing the PH of alkaline soils and in controlling potato scab and rot diseases caused by *Streptomyces* bacteria. The formation of sulphate / Sulphuric acid is beneficial in agriculture in different ways :

i) as it is the anion of strong mineral acid (H_2SO_4) can render alkali soils fit for cultivation by correcting soil PH.
ii) solubilize inorganic salts containing plant nutrients and thereby increase the level of soluble phosphate, potassium, calcium, magnesium etc. for plant nutrition.

4. Reduction of Sulphate:

Sulphate in the soil is assimilated by plants and microorganisms and incorporated into proteins. This is known as "assimilatory sulphate reduction". Sulphate can be reduced to hydrogen sulphide (H_2S) by sulphate reducing bacteria (eg. *Desulfovibrio* and *Desulfatomaculum)* and may diminish the availability of sulphur for plant nutrition. This is “dissimilatory sulphate reduction” which is not at all desirable from soil fertility and agricultural productivity view point.

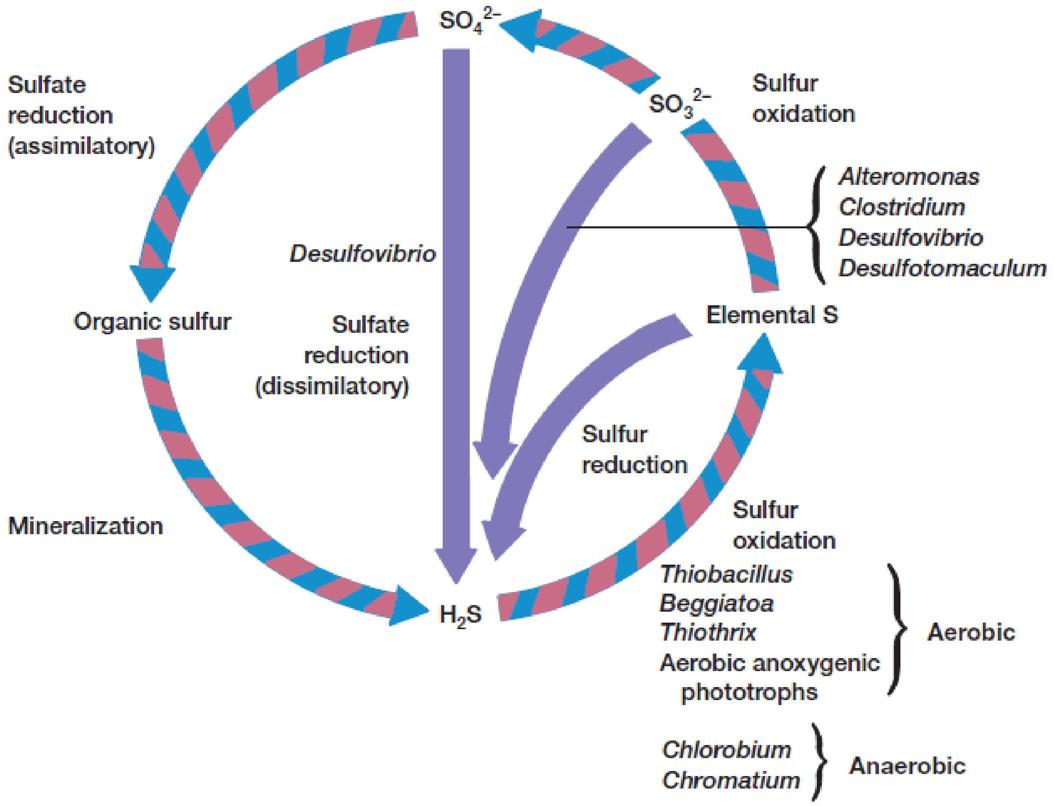
Dissimilatory sulphate-reduction is favored by the alkaline and anaerobic conditions of soil and sulphates are reduced to hydrogen sulphide. For example, calcium sulphate is attacked under anaerobic condition by the members of the genus *Desulfovibrio* and *Desulfatomaculum* to release H_2S.

$$CaSO_4 + 4H_2 \text{ ----------->} Ca(OH)_2 + H_2S + H_2O.$$

Hydrogen sulphide produced by the reduction of sulphate and sulphur containing amino acids decomposition is further oxidized by some species of green and purple phototrophic bacteria *(eg. Chlorobium, Chromatium)* to release elemental sulphur.

$$\underset{}{CO_2 + 2H_2 + H_2S} \xrightarrow[\text{Enzyme}]{\text{Light}} \underset{\text{Carbohydrate}}{(CH_2O)} + H_2O + \underset{\text{Sulphur}}{2\,S.}$$

The predominant sulphate-reducing bacterial genera in soil are *Desulfovibrio*, *Desulfatomaculum* and *Desulfomonas.* (All obligate anaerobes). Amongst these species *Desulfovibrio desulfuricans* are most ubiquitous, non-spore forming, obligate anaerobes that reduce sulphates at rapid rate in waterlogged / flooded soils. While species of *Desulfatomaculum* are spore forming, thermophilic obligate anaerobes that reduce sulphates in dry land soils. All sulphate-reducing bacteria excrete an enzyme called **“desulfurases” or "bisulphate Reductase"**. Rate of sulphate reduction in nature is enhanced by increasing water levels (flooding), high organic matter content and increased temperature.



Sulphur Cycle

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Phosphorus Cycle or Transformation

Phosphorus is only second to nitrogen as a mineral nutrient required for plants, animals and microorganisms. It is a major constituent of nucleic acids in all living systems essential in the accumulation and release of energy during cellular metabolism. This element is added to the soil in the form of chemical fertilizers, or in the form of organic phosphates present in plant and animal residues. In cultivated soils it is present in abundance (i.e. 1100 kg/ha), but most of which is not available to plants, only 15 % of total soil phosphorus is in available form. Both inorganic and organic phosphates exist in soil and occupy a critical position both in plant growth and in the biology of soil.

Microorganisms are known to bring a number of transformations of phosphorus, these include:

(i) Altering the solubility of inorganic compounds of phosphorus,
(ii) Mineralization of organic phosphate compounds into inorganic phosphates,
(iii) Conversion of inorganic, available anion into cell components i.e. an immobilization process and

(iv) Oxidation or reduction of inorganic phosphorus compounds. Of these mineralization and immobilization are the most important reactions / processes in phosphorus cycle.

Insoluble inorganic compounds of phosphorus are unavailable to plants, but many microorganisms can bring the phosphate into solution. Soil phosphates are rendered available either by plant roots or by soil microorganisms through secretion of organic acids (eg. lactic, acetic, formic, fumaric, succinic acids etc). Thus, phosphate-dissolving / solubilizing soil microorganisms (eg. species of *Pseudomonas, Bacillus, Micrococcus, Mycobacterium, Flavobacterium, Penicillium, Aspergillus, Fusarium* etc.) plays important role in correcting phosphorus deficiency of crop plants. They may also release soluble inorganic phosphate (H_2PO_4), into soil through decomposition of phosphate-rich organic compounds.

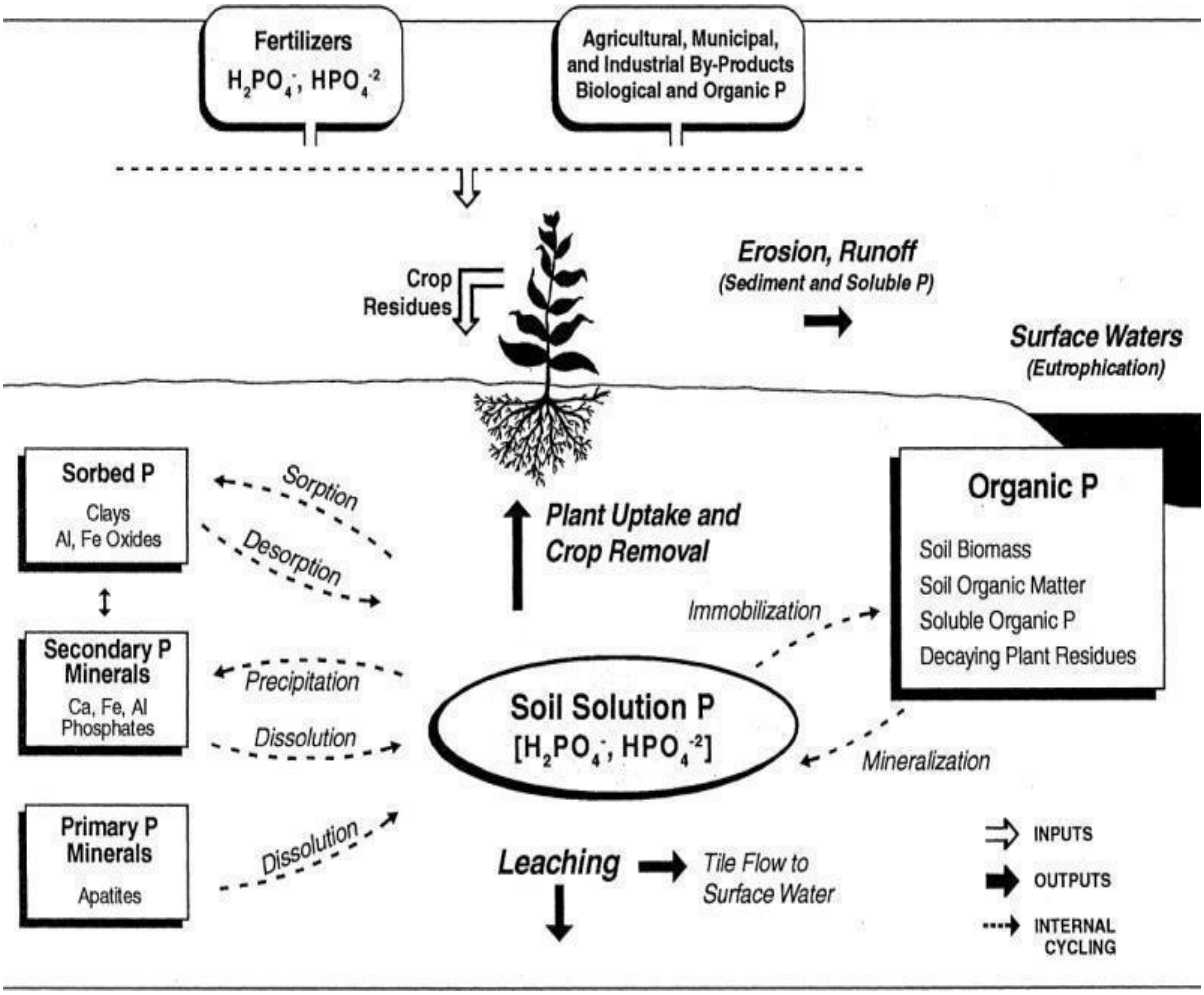
Solubilization of phosphate by plant roots and soil microorganisms is substantially influenced by various soil factors, such as PH, moisture and aeration.

In neutral or alkaline soils solubilization of phosphate is more as compared to acidic soils. Many phosphates solubilizing microorganisms are found in close proximity of root surfaces and may appreciably enhance phosphate assimilation by higher plants.

By their action, fungi bacteria and actinomycetes make available the organically bound phosphorus in soil and organic matter and the process is known as mineralization. On the other hand, certain microorganisms especially bacteria assimilate soluble phosphate and use for cell synthesis and on the death of bacteria, the phosphate is made available to plants. A fraction of phosphate is also lost in soil due to leaching. One of the ways to correct deficiency of phosphorus in plants is to inoculate seed or soil with commercial preparations (eg. Phosphobacterin) containing phosphate - solubilizing microorganisms along with phosphatic fertilizers.

Mineralization of phosphate is generally rapid and more in virgin soils than cultivated land. Mineralization is favored by high temperatures (thermophilic range) and more in acidic to neutral soils with high organic phosphorus content. The enzyme involved in mineralization (cleavage) of phosphate from organic phosphorus compound is collectively called as **“Phospatases"**.

The commercially used species of phosphate solubilizing bacteria and fungi are: *Bacillus polymyxa, Bacillus megatherium. Pseudomonas strita, Aspergillus, Penicllium avamori and Mycorrhiza*.



Phosphorus cycle

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General account of microbes used as biofertilizers

Biofertilizers are microbial inoculants or carrier based preparations containing living or latent cells of efficient strains of nitrogen fixing, phosphate is solublizing and cellulose decomposing microorganisms intended for seed or soil application and designed to improve soil fertility and plant growth by increasing the number and biological activity of beneficial microorganisms in the soil.

The objects behind the application of Biofertilizers /microbial inoculants to seed, soil or compost pit is to increase the number and biological / metabolic activity of useful microorganisms that accelerate certain microbial processes to augment the extent of availability of nutrients in the available forms which can be easily assimilated by plants. The need for the use of Biofertilizers has arisen primarily due to two reasons i.e. though chemical fertilizers increase soil fertility, crop productivity and production, but increased / intensive use of chemical fertilizers has caused serious concern of soil texture, soil fertility and other environmental problems, use of Biofertilizers is both economical as well as environment friendly. Therefore, an integrated approach of applying both chemical fertilizers and Biofertilizers is the best way of integrated nutrient supply in agriculture.

Organic fertilizers (manure, compost, vermicompost) are also considered as Biofertilizers, which are rendered in available forms due to the interactions of microorganisms or their association with plants. Biofertilizers, thus include i) Symbiotic nitrogen fixers *Rhizobium sp.* ii) Non-symbiotic, free living nitrogen fixers *Azotobacter*, *Azospirillum* etc. iii) BGA-inoculants *Azolla-Anabaena,* iv) Phosphate solubilizing microorganisms (PSM) *Bacillus Pseudomonas, Penicillium Aspergillus* etc. v) Mycorrhiza vi) Cellulolytic microorganisms and vii) Organic fertilizers.

Nobbe and Hiltner (1895, USA) produced the first *Rhizobium* biofertilizer under the brand name “Nitragin” for 17 different legumes.

Definition of Biofertilizers

Bio-fertilizers are the fertilizers containing microorganisms which when added in the soil; mineralize essential elements like Nitrogen, Sulphur, and Phosphorous, which are used by the plants and thus increase the crop yield.

Advantages of biofertilizers

Biofertilizers are known to play a number of vital roles in soil fertility, crop productivity and production in agriculture as they are eco friendly and can not at any cost replace chemical fertilizers that are indispensable for getting maximum crop yields. Some of the important functions or roles of Biofertilizers in agriculture are:

1. They supplement chemical fertilizers for meeting the integrated nutrient demand of the crops.
2. They can add 20-200 kg N/ha year *(eg. Rhizobium sp* 50-100 kg N/ha year ; *Azospirillum , Azotobacter* : 20-40 kg N/ha /yr; Azolla : 40-80 kg N/ha; BGA :20-30 kg N/ha) under optimum soil conditions and thereby increases 15-25 percent of total crop yield.
3. They can at best minimize the use of chemical fertilizers not exceeding 40-50 kg N/ha under ideal agronomic and pest-free conditions.
4. Application of Biofertilizers results in increased mineral and water uptake, root development, vegetative growth and nitrogen fixation.

5. Some Biofertilizers *(eg, Rhizobium BGA, Azotobacter* sp) stimulate production of growth promoting substance like vitamin-B complex, Indole acetic acid (IAA) and Gibberellic acids etc.
6. Phosphate mobilizing or phosphorus solubilizing Biofertilizers / microorganisms (bacteria, fungi, mycorrhiza etc.) converts insoluble soil phosphate into soluble forms by secreting several organic acids and under optimum conditions they can solubilize / mobilize about 30-50 kg P2O5/ha due to which crop yield may increase by 10 to 20%.
7. Mycorrhiza or VA-mycorrhiza (VAM fungi) when used as Biofertilizers enhance uptake of P, Zn, S and water, leading to uniform crop growth and increased yield and also enhance resistance to root diseases and improve hardiness of transplant stock.
8. They liberate growth promoting substances and vitamins and help to maintain soil fertility.
9. They act as antagonists and suppress the incidence of soil borne plant pathogens and thus, help in the bio-control of diseases.
10. Nitrogen fixing, phosphate mobilizing and cellulolytic microorganisms in bio-fertilizer enhance the availability of plant nutrients in the soil and thus, sustain the agricultural production and farming system.
11. They are cheaper, pollution free and renewable energy sources
12. They improve physical properties of soil, soil tilth and soil health in general.
13. They improve soil fertility and soil productivity.
14. Blue green algae like *Nostoc, Anabaena, and Scytonema* are often employed in the reclamation of alkaline soils.
15. Bio-inoculants containing cellulolytic and lignolytic microorganisms enhance the degradation/ decomposition of organic matter in soil, as well as enhance the rate of decomposition in compost pit.
16. BGA plays a vital role in the nitrogen economy of rice fields in tropical regions.
17. *Azotobacter* inoculants when applied to many non-leguminous crop plants, promote seed germination and initial vigor of plants by producing growth promoting substances.
18. *Azolla-Anabaena* grows profusely as a floating plant in the flooded rice fields and can fix 100-150 kg N/ha /year in approximately 40-60 tones of biomass produced,
19. Plays important role in the recycling of plant nutrients.

General account and types of microbes used as bio-fertilizers

Most of the mineral supply of soil is dependent upon microorganisms. The plants are unable to use the gaseous nitrogen present in the atmosphere or sulphur or phosphorous present in the soil. Such essential elements are to be converted into usable forms by plants **(Mineralization).**

The bio-fertilizers do not directly increase soil fertility but they usually initiate or accelerate the process of mineralization.

The bio-fertilizers include—

I) Nitrogen fixing microorganisms
II) Phosphate solubilizing microorganisms

III) Sulphur oxidizing microorganisms

IV) Organic matter decomposing microorganisms

Bio-fertilizers are also known by the names—

- ➔ Microbial preparations
- ➔ Microbial cultures
- ➔ Microbial inoculants

I) Nitrogen fixing microorganisms

A large number of microorganisms are known to have the ability to reduce atmospheric nitrogen into nitrogenous compounds. **E.g. Ammonia**.

Nitrogen fixation: - The reduction of atmospheric gaseous nitrogen to ammonia is called as nitrogen fixation.

This nitrogen fixation can be studied under three headings—

A) Non-symbiotic N_2 fixation
B) Associative N_2 fixation
C) Symbiotic N_2 fixation

A) Non-symbiotic N_2 fixation: -

In this type, N_2 fixation is done by those microbes, which live freely and independently in the soil.

E.g. **Bacteria: -** *Azotobacter, Bacillus, Clostridium, Chlorobium, Chromatium, Desulfovibrio, Enterobacter, Escherichia coli, Klebsiella, Rhodospirillum, Thiobacillus spp etc.*

Cyanobacteria (Blue-green algae): - *Anabaena, Nostoc*

B) Associative N_2 fixation: -

In this type of association no nodules are formed like symbiotic bacteria. The bacteria grow in the **rhizosphere** in close contact with the roots; some times invade the outer cortical regions of the roots and fix nitrogen.

Azospirillum brazilense bacterium discovered by Brazilian microbiologist: J. Dobereniner (1978) is the best-studied bacterium forming associative symbiosis with the cereal roots. Others are—

Bacillus, Enterobacter, Klebsiella, Pseudomonas azotogensis.

These microbes fix nitrogen in association with the roots of grasses and cereal plants.

C) Symbiotic N_2 fixation

There are 3 types—

1. **Through nodule formation in legumes**
2. **Through nodule formation in non-legumes** (Actinomycetes symbiosis)
3. **Through non-nodulation** (Mycorrhizal symbiosis)

1. Through nodule formation in legumes

Symbiotic nitrogen fixation is accomplished by ***Rhizobium spp***, which occur upon the roots of ***leguminous plants.*** Before these bacteria can fix nitrogen, they must establish themselves in the root cortical cells of the host plant ultimately forming **'root nodules'.**

2. Through nodule formation in non-legumes (Actinomycetes symbiosis)

Nitrogen fixing symbiosis between ***non-leguminous trees*** and ***actinomycetes*** occur in almost **140** species woody perennial shrubs and trees belonging to **17** genera in **8** families. **'Alnus'** species in tem regions and **casuarina** species in the tropics and subtropics are capable of fixing atmospheric nitrogen by virtue of symbiotic relationship established between roots of tree and the soil microorganism **frankia.**

3. Through non-nodulation (Mycorrhizal symbiosis)

Mycorrhizal association is the most widely spread symbiosis among plants and most nitrogen fixing trees form abundant mycorrhizae on their roots.

Thus mycorrhiza is mutualistic symbiosis between roots of higher plants and fungal hyphae. In this association plants are benefited by uptake of **phosphorous and other inorganic nutrients,** enhance growth of tree.

II) Phosphate solubilizing microorganisms

After the decomposition of organic phosphorous containing compounds, inorganic compounds like phosphoric acid which combines with soil bases and produce salts of **calcium, magnesium, iron** are produced. These salts are less soluble and less available to plants.

These salts are converted into soluble form by the acids like **sulphuric and nitric acid** by the microorganisms. The **Tri-calcium phosphate** is transformed first into mono-phosphates and then into phosphates, which are absorbed by roots of plants.

These phosphate-solubilizing microbes are used as bio-fertilizers e.g.

Aspergillus, Flavobacterium, Penicillium, Pseudomonas, Micrococcus, Mycobacterium spp. Etc.

III) Sulphur oxidizing microorganisms

Sulphur oxidizing bacteria oxidise sulphur containing compounds to sulphates which are utilized by plants. Also these bacteria convert sulphide into sulphuric acid, which act on insoluble soil compounds like Calcium carbonate, Magnesium carbonate, Calcium silicate, Tri-calcium phosphate and bring them in soluble state. These soluble compounds are then absorbed by the plants.

E.g. *Thiobacillus spp.*

IV) Organic matter decomposing microorganisms

The organic matter in soil consists of carbohydrates, proteins, lipids, and other materials. Organic matter makes up from 2 to 10 % most agriculturally important soils.

All organic matter in soil is derived from the remains of microorganisms, plants, animals, their waste products and the biochemical activities of various microorganisms. A great portion of organic matter is of plant origin mostly dead roots, wood, bark and leaves. A second source of organic matter is the vast number of bacteria, fungi, algae, protozoa and viruses, which can total billions per gram of fertile soil. These organisms break down organic substances producing and maintaining a continuous supply of inorganic substances that plants and other organisms require for growth. Much of the organic matter in soil is ultimately decomposed to inorganic substances such as ammonia, water, carbon dioxide and various compounds of nitrates, phosphates, and calcium. This conversion of organic compounds into simple inorganic compounds or into their constituent elements is called **mineralization.**

A considerable part of this organic matter in soil occurs as **Humus.** This dark material consists of partially decomposed organic matter, chiefly materials that are relatively resistant to decay.

The addition of organic matter either completely or partially decomposed is essential to the fertility of soil; moreover these spongy organic materials loosen the soil and thereby prevent the formation of **heavy crusts** and increase the pore spaces. The addition of pore spaces in turn increases aeration and water retention.

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Rhizosphere Concept and It's Historical Background

The root system of higher plants is associated not only with soil environment composed of inorganic and organic matter, but also with a vast community of metabolically active microorganisms. As living plants create a unique habitat around the roots, the microbial population on and around the roots is considerably higher than that of root free soil environment and the differences may be both quantitative and qualitative.

1. Rhizosphere: It is the zone/region of soil immediately surrounding the plant roots together with root surfaces, or it is the region where soil and plant roots make contact, or it is the soil region subjected to influence of plant roots and characterized by increased microbial.

2. Rhizoplane: Root surface along with the closely adhering soil particles is termed as rhizoplane.

Historical Background:

Term "Rhizosphere" was introduced for the first time by the German scientist **Hiltner** (1904) to denote that region of soil which is subjected to the influence of plant roots. The concept of "Rhizosphere Phenomenon" which shows the mutual interaction of roots and microorganisms was came into existence with the work of **Starkey et al (1929), Clark (1939) and Rauath and Katznelson (1957).**

H. Katznelson (1946) suggested the **R:S ratio** i.e. the ratio between the microbial population in the rhizosphere (R) and in the soil (S) to find out the degree or extent of plant roots effect on soil microorganisms. R: S ratio gives a good picture of the relative stimulation of the microorganisms in the rhizosphere of different plant species.

R: S ratio is defined as the ratio of microbial population per unit weight of rhizosphere soil (R), to the microbial population per unit weight of the adjacent non-rhizosphere soil (S)

Microorganisms in the Rhizosphere and Rhizosphere Effect

The rhizosphere region is a highly favorable habitat for the proliferation, activity and metabolism of numerous microorganisms. The rhizosphere microflora can be enumerated intensively by microscopic, cultural and biochemical techniques. Microscopic techniques reveal the types of organisms present and their physical association with the outer root tissue surface / root hairs. The cultural technique most commonly followed is "serial dilution and plate count method" which reveal the quantitative and qualitative population of microflora. At the same time, a cultural method shows the selective enhancement of certain categories of bacteria. The biochemical techniques used are designed to measure a specific change brought about by the plant or by the microflora. The rhizosphere effect on most commonly found microorganisms viz. bacteria, actinomycetes, fungi, algae and protozoa is being discussed herewith in the following paragraphs.

A. Bacteria:

The greater rhizosphere effect is observed with bacteria (R: S values ranging from 10-20 or more) than with actinomycetes and fungi. Gram-negative, rod shaped, non-sporulating bacteria which respond to root exudates are predominant in the rhizosphere *(Pseudomonas, Agrobacterium).* While

Gram-positive, rods, Cocci and aerobic spore forming *(Bacillus, Clostridium)* are comparatively rare in the rhizosphere. The most common genera of bacteria are: *Pseudomonas, Arthrobacter, Agrobacterium, Alcaligenes, Azotobacter, Mycobacterium, Flavobacter, Cellulomonas, Micrococcus* and others have been reported to be either abundant or sparse in the rhizosphere. From the agronomic point of view, the abundance of nitrogen fixing and phosphate solubilizing bacteria in the rhizosphere assumes a great importance. The aerobic bacteria are relatively less in the rhizosphere because of the reduced oxygen levels due to root respiration. The bacterial population in the rhizosphere is enormous in the ranging form 10^8 to 10^9 per gram of rhizosphere soil. They cover about 4-10% of the total root area occurring profusely on the root hair region and rarely in the root tips. There is predominance of amino acids and growth factors required by bacteria, are readily provided by the root exudates in the region of rhizosphere.

B. Fungi:

In contrast to their effects on bacteria, plant roots do not alter / enhance the total count of fungi in the rhizosphere. However, rhizosphere effect is selective and significant on specific fungal genera *(Fusarium, Verticillium, Aspergillus* and *Penicillium)* which are stimulated. The R:S ratio of fungal population is believed to be narrow in most of the plants, usually not exceeding to 10. The soil / serial dilution and plating technique used for the enumeration of rhizosphere fungi may often give erratic results as most of the spore formers produce abundant colonies in culture media giving a wrong picture / estimate *(eg Aspergilli* and *Penicillia).* In fact the mycelial forms are more dominant in the field. The zoospore / forming lower fungi such as *Phytophthora, Pythium, Aphanomyces* are strongly attracted to the roots in response to particular chemical compounds excreted by the roots and cause diseases under favorable conditions. Several fungi *eg Gibberella* and *fujikurio* produces *phytohormones* and influence the plant growth.

C. Actinomycetes, Protozoa and Algae:

Stimulation of actinomycetes in the rhizosphere has not been studied in much detail so far. It is generally understood that the actinomycetes are less stimulated in the rhizosphere than bacteria. However, when antagonistic actinomycetes increase in number they suppress bacteria. Actinomycetes may also increase in number when antibacterial agents are sprayed on the crop. Among the actinomycete, the phosphate solublizers (eg. *Nocardia, Streptomyces)* have a dominant role to play.

As rule actinomycetes, protozoa and algae are not significantly influenced by their proximity to the plant roots and their R: S ratios rarely exceed 2 to 3: 1 and around roots of plants, R: S ratio for these microorganisms may go to high. Because of large bacterial community, an increase in the number or activity of protozoa is expected in the rhizosphere. Flagellates and amoebae are dominant and ciliates are rare in the region.

Associative and Antagonistic activities in the Rhizosphere

In natural environments (eg. Soil, Air, Water etc.) a number of relationships exist between individual microbes, microbial species and between individual cells. The composition of microflora of any habitat (soil / rhizosphere) is governed by the biological equilibrium created by the associations and interactions of all individuals found in the community. In soil and rhizosphere region, many microorganisms live in close proximity and their interactions with each other may be associative or antagonistic.

A. Associative interactions / activities in rhizosphere: / Significance for fertility

The dependence of one microorganism upon another for extra-cellular products (eg. amino acids & growth promoting substances) can be regarded as an associative activity / effect in rhizosphere. There is an increase in the exudation of amino acids, organic acids and monosaccharide by plant roots in the presence of microorganisms. Gibberellins and gibberellin- like substances are known to be produced by bacterial genera viz *Azotobacter, Arthrobacter, Pseudomonas,* and *Agrobacterium* which are commonly found in the rhizosphere. Microorganisms also influence root hair development, mucilage secretion and lateral root development. Fungi inhabiting the root surface facilitate the absorption of nutrient by the roots.

Mycorrhiza is one of the best known associative / symbiotic interactions which exist between the roots of higher plants and fungi. This mycorrhizal association has been found to improve plant growth through better uptake of phosphorus and zinc from soil, suppression of root pathogenic fungi and nematodes. Another example is association between the bacterium *Rhizobium* and roots of legumes and *Azospirillum* with cereal crops (wheat, rye, bajara, maize etc).

B. Antagonistic interactions / activities in rhizosphere:

The biochemical qualities of root exudates and the presence of antagonistic microorganisms, plays important role in encouraging or inhibiting the soil borne plant pathogens in the rhizosphere region. Several mutualistic, communalistic, competitive and antagonistic interactions exist in the rhizosphere. The number and qualities of antagonistic microorganisms in the rhizosphere could be increased through artificial means such as fertilizer application, organic amendments, foliar spraying of chemicals etc.

Antagonistic microorganisms in the rhizosphere play an important role in controlling some of the soil borne plant pathogens. Stanier et al (1966) discovered the bacterial strain *Pseudomonas fluorescens* and the fluorescent pigments of this species in biological control of root pathogens. Strains of *P. fluorescence* are collectively called as **"Fluorescent Pseudomonads"**. They produce variety of biologically active compounds such as plant growth substances, cyanides, antibiotics and iron chelating substances called **"Siderophores"** Rovira and Campbell (1975) , showed that bacterial strains of *P fluorescens* could lyse the hyphae of *Gaumannomyces graminis var. Tritici,* the causative agent of take-all disease of wheat. Fluorescent pseudomonads *(P. fluorescens, P. putida)* are known to produce iron chelating substances called Siderophores. These are low molecular weight, extra cellular, iron-binding agents produced by pseudomonads in response to low iron stress or when Fe3 is in short supply. Thus, iron stress triggers the formation of iron-binding ligands called siderophores. Siderophores contains the pigments Pyovirdin (Fluorescent) and Pyocyanin (non-Fluorescent) having iron chelating properties. Another pigment "Pseudobactin" is a fluorescent chelator of iron which is known to promote plant growth and inhibition of pathogenic bacteria in the rhizosphere. An antibiotic called "Pyrrolnitrin" reduces damping-off disease in cotton caused by *Rhizoctonia solani.* Several species of *Bacillus* are known to cause mycolysis in the rhizosphere. *eg. Fusarium oxysporum* hyphae are known to undergo lysis in soil due to these bacterial metabolites.

The successful antagonists among fungi are *Trichoderma* sp (T. viride and *T. harzianum, T. hamatum)* and *Gliocladium virens* which parasitize, lyse or kill the phytopathogenic fungi in the soil. Antifungal and antibacterial actinomycetes in the rhizosphere play an important role in controlling pathogenic fungi and bacteria, for example *Micromonospora globosa* is a potent antagonist of *Fusarium udum* causing wilt of pigeon pea. Amoebae are also known to play an antagonistic role in controlling soil fungi, eg. control of take-all disease of wheat caused by *Gaumannomyces graminis* through the use of Myxamoebae. There can also occur antagonisms between two fungi producing metabolite and interfering the growth of the other fungus as in case of *Peniophora* antagonizing *Heterobasidium.*

Rhizosphere in relation to Plant Pathogens

Plant root exudates influence pathogenic fungi, bacteria and nematodes in various ways. The effect may be in the form of attraction of fungal zoospores, or bacterial cells towards the roots; stimulation of germination of dormant spores and hatching of cysts of nematodes. Root exudates may contain inhibitory substances preventing the establishment of pathogens. The balance between the rhizosphere microflora and plant pathogens and soil microflora and plant pathogens is important in host-pathogenic relationship. In this context, the biochemical qualities of root exudates and the presence of antagonistic micro-organisms plays an important role in the proliferation and survival of root infecting pathogens in soil either through soil fungi stasis, inhibition or antibiosis of pathogens in the rhizosphere.

Some of the most common interactions between plant roots and plant pathogenic microorganisms in the rhizosphere are discussed herewith.

A. Zoospore attraction: Amino acids, organic acids and sugars in the root exudates stimulate the movement and attraction of zoospores towards root of the plants. For example attraction of zoospores has been reported in *Phytophthora citrophthora* (Citrus roots), *P. parasitica* (tobacco roots) and *Pythium aphanidermatum* (pea root).

B. Spore germination: The spores or conidia of many pathogenic fungi such as *Rhizoctonia, Fusarium, Sclerotium, Pythium, Phytophthora* etc. have been stimulated to germinate by the root exudates of susceptible cultivars of the host plants. There are some reports on the selective stimulation of *Fusarium, Pseudomonas* and root infecting nematodes in the rhizosphere region of the respective susceptible hosts. This stimulus to germination is especially important to those plant pathogens which are not vigorous competitors and remain in resting stage due to shortage of nutrients or fungistasis. As a rule, germination and subsequent hyphal development are promoted by non host species and also by both susceptible and resistant cultivars of the host plants. The quantity and quality of microorganisms present in the rhizosphere of disease resistant crop varieties are significantly different from those of susceptible varieties.

C. Changes in morphology and physiology of host plant: Changes in the physiology and morphology of host plant influence the rhizosphere microflora through root exudations. Hence, significant changes in the rhizosphere microflora of diseased plants were reported which are attributed to the nature and severity of the disease. Systemic virus diseases cause marked changes in the plant morphology and physiology to drastically alter the rhizosphere microflora.

D. Increase in antagonists activity: Root exudates provide a food base for the growth of antagonistic organisms which plays an important role in controlling / suppressing some of the soil borne plant pathogens. Generally, rhizosphere of the resistant plant varieties harboure moer number of *Streptomyces* and *Trichoderma* than that of susceptible varieties. For example in the rhizosphere of pigeon pea varieties resistant to *Fusarium udum,* the population of *Streptomyces* was found more which inhibited the growth of the pathogen. High density of *Trichoderma viride* in the rhizosphere of Tomato varieties resistant to *Verticillium* wilt has been reported with its ability to reduce the severity of wilt in susceptible plants.

E. Inhibition of pathogen: Root exudates containing toxic substances such as glycosides and hydrocyanic acid may inhibit the growth of pathogens in the rhizosphere. It has been reported that root exudates from resistant varieties of Flax (eg. Bison) excrete a glucoside which on hydrolysis produces hydrocyanic acid that inhibits Fusarium oxysporum, the flax root pathogen. Exudates of resistant pea reduce the germination of spores of Fusarium oxysporum.

In this light, the rhizosphere may be considered as a microbiological buffer zone in which the microflora serves to protect the plants against the attack of the pathogens.

F. Attraction of bacteria and nematodes: Root exudates attracts phytopathogenic bacteria and fungi in the rhizosphere for example *Agrobacterium tumefaciens* have been reported to be attracted to the roots of the host plants like peas, maize, onion, tobacco, tomato and cucumber.

Host root exudates also influence phytopathogenic nematodes in two ways: (i) though stimulation of egg-hatching process and (ii) attraction of larvae towards plant roots.