Unit III: Air and Soil Microbiology

<u>Microbial analysis of air:</u>

Airborne bacterial and fungal cells and spores may be present in droplets as bioaerosols, minute particles that suspend in the air for longer periods of time. They can be an important source of infection in medical facilities and can contaminate manufacturing operations (food and dairy products, pharmaceuticals).For this regular monitoring of air is essential.

There are two methods of monitoring the microbial quality of air.

- Passive monitoring- settling or sedimentation plate technique
- Active sampling- Anderson air sampler

- Passive monitoring or **settle plate technique:**

In this technique, standard petri dishes containing appropriate culture media that are opened and exposed to the air for a given period of time and then incubated to allow visible colonies to develop and be counted.

<u>Advantages:</u>

Inexpensive, Easy to use, Requires no special equipment, Useful for qualitative analysis of airborne microbes, directly monitoring air borne contamination.

<u>Disadvantages</u>:

Monitors viable microbes only, Cannot sample specific volume of air so results are not quantitative , Vulnerable to contamination and interference by non air borne sources.

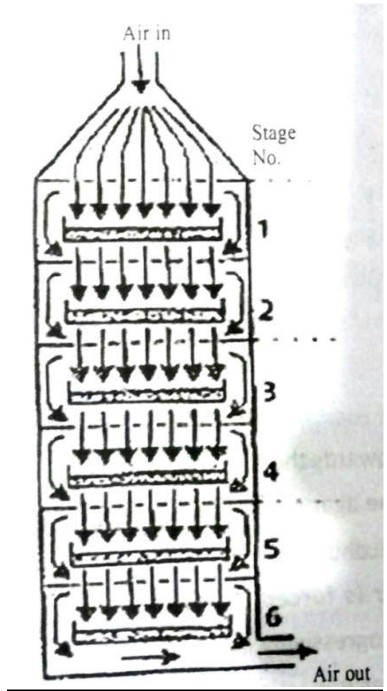
- Active monitoring or **Andersen sampler:**

Active monitoring requires the use of microbiological air sampler to physically draw a known volume of air over or through a particle collection device. One of the best known is the Andersen sampler(an impactor sampler), a multistage cascade sieve sampler that uses perforated plates with progressively smaller holes at each stage, allowing particles to be separated according to size. Impactor samplers use a solid or adhesive medium such as agar for particle collection. In this air is drawn into sampling head by a pump and accelerated through a perforated plate. This produces laminar air flow onto the collection surface. When a correct volume of air has been passed through the sampling head the agar plate(collection surface)can be removed and incubated directly without any furher treatment. After incubation counting the visible number of colonies gives direct quantitative estimate of the number of colony forming unites in the sampled air.

The Anderson sampler is an ingenious device for selectively trapping different sizes of particles according to their size (momentum). This sampler consists of a stack of 8 metal sections that fit together with ring seals to form an air-tight cylinder. Each metal section has a perforated base , and the number of perforations is the same in each section, but the size of these perforations is progressively reduced from the top of the column to the bottom. To use this sampler, open agar plates are placed between each metal section, resting on three studs.

When fully assembled (with an open agar plate between each unit) an electric motor sucks air from the bottom of the unit, causing spore-laden air to enter at the top and to pass down through the cylinder. When the sampler has run for 5-15 minutes or more, the metal plates are separated and the Petri dishes are removed for incubation to identify the colonies that develop. In this case the air sample contained spores from mouldy hay, and the agar plates were incubated at 37oC.

One of the interesting features of the Anderson sampler is that it mimics the deposition of spores (or other ariborne particles) in the human respiratory tract (see Figure). For example, relatively large fungal spores and pollen grains tend to be trapped on the mucus-covered hairs of our nostrils, where they can cause "hay fever" symptoms in sensitised individuals. Smaller particles are not trapped in the nostrils but instead are carried down into the bronchioles and alveoli. Here the air speed is very low, because the successive branching of the respiratory tract has reduced the air speed to a minimum. But spores of about 2-4 micrometres diameter can settle onto the mucosal surfaces of the alveoli. Some of these spores are important in initiating infections of the lungs. However, it is important to note that the underlying mechanisms of spore deposition in the Anderson sampler are entirely different from those in the human respiratory tract - the Anderson sampler traps spores by impaction, whereas spores are deposited in the human respiratory tract mainly by sedimentation.



Microbial Leaching

Definition: Bioleaching or microbial leaching is a process of "the dissolution of metals from their mineral source by certain naturally occurring microorganisms"

or

"the use of microorganisms to transform elements so that the elements can be extracted from a material when water is filtered trough it".

The Leaching Process in general:

In microbial leaching, low-grade ore is dumped in a large pile called the leach dump and a dilute sulfuric acid solution at pH 2 is percolated down through the pile. The liquid emerging from the bottom of the pile is rich in dissolved metals and is transported to a precipitation plant where the desired metal is precipitated and purified. The liquid is then pumped back to the top of the pile and the cycle repeated. As needed, acid is added to maintain an acidic pH.

Microorganisms involved in microbial leaching:

Acidithiobacillus ferrooxidans (Thiobacillus ferrooxidans),Leptospirillum ferrooxidans, Thiobacillus thiooxidans etc, etc.

$$4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O \text{ (Iron oxidizers)}$$

$$S_2O_3^{2-} + 2O_2 + 4H^+ \rightarrow 2SO_4^{2-} + 2H^+ \text{ (sulfur oxidizer)}$$

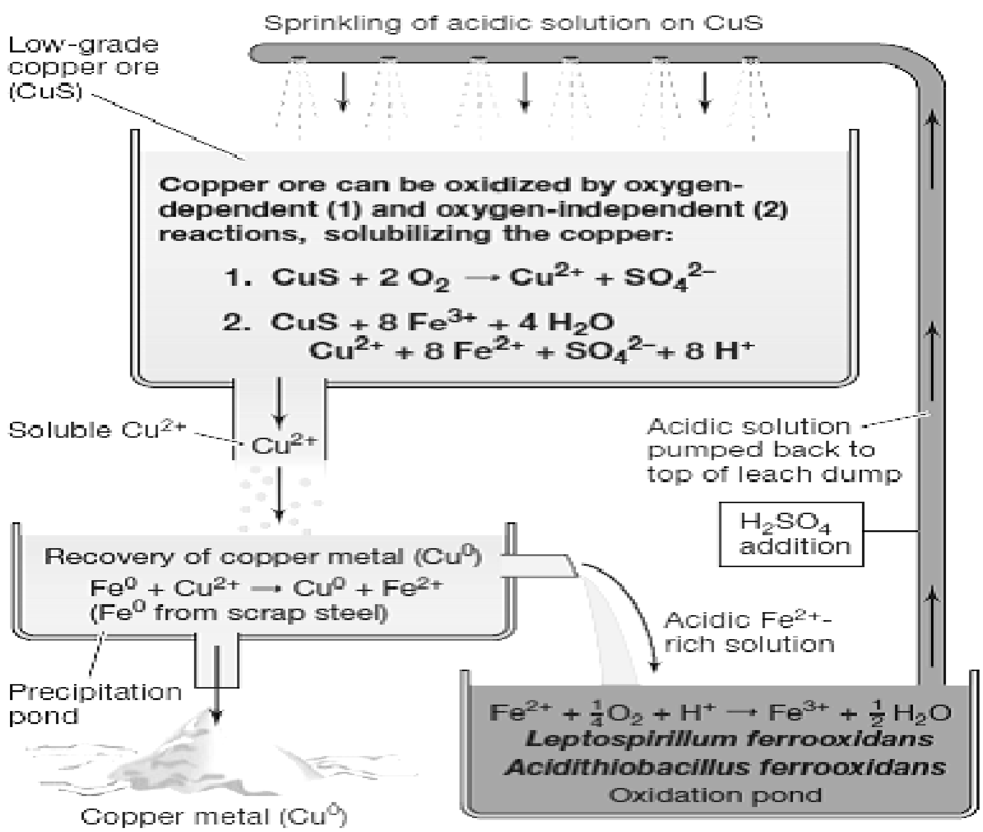
Microbial leaching of Copper:

In the case of copper, copper sulfide is microbially oxidized to copper sulfate and metal values are present in the aqueous phase. Remaining solids are discarded.

Microbial leaching of copper can be illustrated with the common copper ore CuS, in which copper exists as Cu2+. *A. ferrooxidans* oxidizes the sulfide in CuS to SO4 2-, releasing Cu2+. However, this reaction can also occur spontaneously. Indeed, the key reaction in copper leaching is actually not the bacterial oxidation of sulfide in CuS but the spontaneous oxidation of sulfide by ferric iron (Fe3+) generated from the bacterial oxidation of ferrous iron (Fe2+). In any copper ore, FeS2 is also present, and its oxidation by bacteria leads to the formation of Fe3+. The spontaneous reaction of CuS with Fe3+ proceeds in the absence of O2 and forms Cu2+ plus Fe2+; importantly for efficiency of the leaching process, this reaction can take place deep in the leach dump where conditions are anoxic.

Metal Recovery of Copper:

The precipitation plant is where the Cu2+ from the leaching solution is recovered. Shredded scrap iron (a source of Fe0) is added to the precipitation pond to recover copper from the leach liquid by the chemical reaction shown in the lower part of. This results in a Fe2+-rich liquid that is pumped to a shallow oxidation pond where iron-oxidizing chemolithotrophs oxidize the Fe2+ to Fe3+. This now ferric iron–rich acidic liquid is pumped to the top of the pile and the Fe3+ is used to oxidize more CuS. The entire CuS leaching operation is thus driven by the oxidation of Fe2+ to Fe3+ by iron-oxidizing bacteria. Temperatures rise in a leaching dump and this leads to shifts in the iron-oxidizing microbial populations. *A. ferrooxidans* is a mesophile, and when heat generated by microbial activities raises temperatures above about 308C inside a leach dump, this bacterium is outcompeted by mildly thermophilic iron-oxidizing chemolithotrophs such as *Leptospirillum ferrooxidans* and *Sulfobacillus.* At even higher temperatures (60–808 ^{0} C), hyperthermophilic Archaea such as Sulfolobus predominate in the leach dump.



Microbial Leaching of Uranium:

Bacteria are also used in the leaching of uranium (U) ores. In uranium leaching, *A. ferrooxidans* oxidizes U4+ to U6+ with O2 as an electron acceptor. However, U leaching depends more on the abiotic oxidation of U4+ by

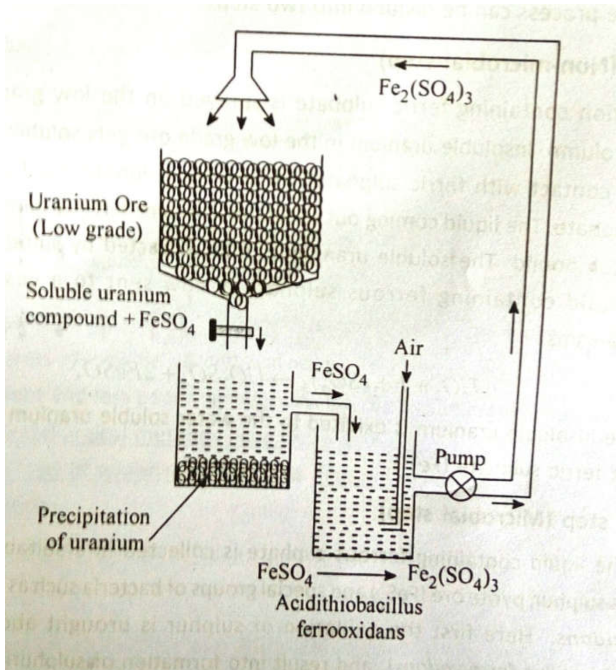
Fe^{3+} with *A. ferrooxidans* contributing to the process mainly through the reoxidation of Fe^{2+} to Fe^{3+}, as in copper leaching. The reaction observed is as follows: Unlike UO_2, the uranyl sulfate (UO_2SO_4) formed is highly soluble and is concentrated by other processes.

2FeS + H2O + 7 ½ O2 Fe(SO4)3 + H2SO4

T. Ferroxidans

U^{4+}O2 + Fe(SO4)3 U^{6+}O2SO4 + 2 FeSO4

Fe3+/H2SO4



Bioleaching of uranium

Biofertilizers

Biofertilizers are defined as preparations containing living cells or latent cells of efficient strains of microorganisms that help crop plants' uptake of nutrients by their interactions in the rhizosphere when applied through seed or soil. They accelerate certain microbial processes in the soil which augment the extent of availability of nutrients in a form easily assimilated by plants. Biofertilizers enhance the availability of nutrients viz., nitrogen by fixing atmospheric N_2 and phosphorus by solubilising soil phosphorus, to the crops.

Biofertilizers improve the health and quality of different types of soils that help the plants obtain the necessary nutrients. The soil becomes more nutritious and helps the seeds and roots grow to their full potential. Biofertilizers activate the microorganisms that are found in the soil, thus restoring the soils' natural fertility and protecting it against soil diseases and droughts, which stimulates the growth of plants.

Different types of biofertilizers:
1. Rhizobium
2. Azotobacter
3. Azospirillum
4. Cyanobacteria
5. Azolla
6. Phosphate solubilizing microorganisms (PSM)
7. Mycorrhiza

Rhizobium: Rhizobium spp. Are Gram negative soil bacteria capable of forming root nodule in most leguminous plants and some nonleguminous plants. Rhizobium cells contains genes for nitrogen fixation.
Azotobacter: Azotobacter uses the organic matter present in soil to fix nitrogen asymbiotically and occur in association with the roots of many plants, sugarcane, jowar, wheat, bajra,etc.
Cyanobacteria: These are photosynthetic, prokaryotic organisms, which fix N_2 aymbiotically.
Phosphate solubilising microorganisms: Many microorganisms in the soil are able to solubilize "unavailable" forms of calcium-bound P by excreting organic acids which either directly dissolve rock phosphate or chelate calcium ions to bring the P into solution.
Mycorrhiza: Mycorrhiza is a composite mutualistic association between a fungus and plant root. The fungus receives organic nutrients mainly carbohydrates and vitamins (P, Zn, S) from the plant and in return absorbs mineral salts and water which pass to plant root.

List of some important microorganisms with applications as biofertilizers.

Organism	Activity	Used in crops
Rhizobium leguminosarum, R. japonicum, R. phaseoli,etc.	N_2 fixation	Legumes(pulses, oil seeds, forage crops)
Azospirillum	N_2 fixation	Graminaceous crops(wheat, rice, sugarcane, jowar)
Azotobacter	N_2 fixation	wheat, rice, vegetables
Cyanobacteria(Anabaena, Nostoc, Plectonema,etc.)	N_2 fixation	Rice
Azolla-Anabaena complex	N_2 fixation	Rice
Phosphate solubilising bacteria(Thibacillus, Bacillus,etc.)	Phosphate solubilisation	Many crops
Mycorrhiza(Glomus)	Phosphate solubilisation	Many crops

Production of carrier based bacterial biofertilizers:

The mass production of carrier based bacterial biofertilizers involves three stages:

- **Culturing of microorganisms**
- **Processing of carrier material**
- **Mixing the carrier and the broth culture and packing**

Although many bacteria can be used beneficially as a biofertilizer the technique of mass production is standardized for Rhizobium, Azospirillum, Azotobacter and phosphobacteria.

Role of Bio-fertilizers in crop growth promotion:

1. Bio-fertilizers helps improve the soil fertility and the natural habitat, as well as increase the crop yield by 20% to 30%.
2. They supply the plants with 25 % of nitrogen and phosphorus by replacing the chemical fertilizers, which can deteriorate the environment and also cause harmful impacts on living beings. Ultimately, bio-fertilizers are environmentally friendly.
3. They not only help provide protection against drought and soil-borne diseases, but also prevent from damaging the natural sources, as well as cleansing the plant from precipitated chemical fertilizers .
4. Since bio-fertilizers have excellent buffering capabilities and they contain organic matters, they can balance the pH in the soil and reduce the acidity.
5. As they protect the soil from hardening, the organic matters that are added also enrich the air aeration, water, and nutrient retention capacity.
6. They have the ability to save water by retaining moisture and releasing it slowly. The microorganisms and the acids that are found in bio-fertilizers have the ability to improve the plants' health and hardiness when they enter the roots.
7. The organic materials and the acids that are found in the bio-fertilizers include humic and fulvic acids, organic fungi, and organic fertilizer nutrients. Humic acids are plant biostimulants that can increase soil fertility, enhance microbial activity and reduce water evaporation .
8.The bio-fertilizers can help by supplementing other fertilizers as well as aid other organisms and beneficial bacteria to grow and build soil. This will improve seed germination and produce thicker roots.
9. In plants, bio-fertilizers also help stabilize the chlorophyll, which remits in photosynthesis by providing darker green leaves and an increase in the carbohydrates content and the oxygen assimilation

Advantages of biofertilizers:

1. In the economy, bio-fertilizers are cost-effective and they also have low manufacturing costs, especially when it comes to nitrogen and phosphorus use.
2. Biolfertilizers lead to soil enrichment.
3. They are compatible with long term sustainability.
4. They are ecofriendly and pose no danger to the environment.

Disadvantages of Bio-fertilizers:

1. Sometimes bio-fertilizers are not readily accepted by the society primarily because they do not produce quick and impressive responses.
2. Some bio-fertilizer packets may have insufficient population of microorganisms as well as high levels of contaminants.
3. There may also be cases where the bio-fertilizers have insufficient amounts of phosphorus and nitrogen, which can change the way the plants grow.

Examples of biofertilizers:

Phosphate solubilizing bacteria (PSB):

Phosphorus (P) is one of the major essential macronutrients for biological growth and development. The concentration of soluble P in soil is usually very low. Only a small percentage of the total phosphorus (P) in a soil is in a form available - to plants, and an even smaller fraction in the soil solution. The remainder, excluding organically bound P, is in chemical forms that are, at best, only very slightly soluble.

Many microorganisms in the soil are able to solubilize "unavailable" forms of calcium-bound P by excreting organic acids which either directly dissolve rock phosphate or chelate calcium ions to bring the P into solution. The principal mechanism for mineral phosphate solubilisation is the production of organic acids, and acid phosphatases play a major role in the mineralization of organic phosphorous in soil.

Examples of PSB: Strains from the genera *Pseudomonas, Bacillus* and *Rhizobium.*

P bound to calcium P ⟶ solubilisation of P
(P unavailable to plants) PSB (P available to plants)

Mechanisms of phosphate solubilization

Mineral phosphate solubilization : The principal mechanism for mineral phosphate solubilization is the production of organic acids, and acid phosphatases play a major role in the mineralization of organic phosphorus in soil. It is generally accepted that the major mechanism of mineral phosphate solubilization is the action of organic acids synthesized by soil microorganisms. Production of organic acids results in acidification of the microbial cell and its surroundings. The production of organic acids by phosphate solubilizing bacteria has been well documented. Gluconic acid seems to be the most frequent agent of mineral phosphate solubilization. Also, 2-ketogluconic acid is another organic acid identified in strains with phosphate solubilizing ability.

Organic acid	Strains
Gluconic acid :	*Pseudomonas* sp., *Erwinia herbicola, Pseudomonas cepacia, Burkholderia cepacia*
2-Ketogluconic acid :	*Rhizobium leguminosarum, Rhizobium meliloti, Bacillus firmus*

Organic phosphate solubilisation: Soil contains a wide range of organic substrates, which can be a source of P for plant growth. To make this form of P available for plant nutrition, it must be hydrolyzed to inorganic P. Mineralization of most organic phosphorous compounds is carried out by means of phosphatase enzymes. Soil bacteria expressing a significant level of acid phosphatases include strains from the genus Rhizobium , Enterobacter, Serratia, Citrobacter, Proteus and Klebsiella, as well as Pseudomonas and Bacillus.

Chelating substances:

Chelating substances and inorganic acids such as sulphideric, nitric, and carbonic acid are considered as other mechanisms for phosphate solubilization. However the effectiveness and their contribution to P release in soils seems to be less than organic acid production.

Mycorrhizae:

Mycorrhiza is a composite mutualistic association between a fungus and plant root. The fungus receives organic nutrients mainly carbohydrates and vitamins from the plant and in return absorbs mineral salts and water which pass to plant root. The ectotrphic mycorrhiza forms a sheath around the root and penetrates into the intercellular spaces of cortex as in most forest trees. Endotrophic mycorrhiza also forms an intercellular network and appears to enter the cells. Root nodule formed by Rhizobium in leguminous plant is an example of mycorrhiza.

Mycorrhizae (derived from the Greek meaning "fungus root") are mutualistic relationships that develop between most plants and a limited number of fungal species. The fungus provides nutrients such as phosphorus from the soil to the plant, and the plant in turn transfers carbohydrates to the fungus.

Mycorrhiza infection area occurs only on the smallest order of secondary roots. These are the root tips that are still growing, elongating and increasing in girth. So we are talking about just a very small part of the root system of a plant which will be infected by the mycorrhizal fungus. This makes a great deal of sense since this is the only part of the root system that will absorb water and minerals. However, as I just mentioned, the fungus has a much more extensive growth in the soil.

In all mycorrhizae only the cortical cells of the root are invaded by the fungus. This is the area of the root between the epidermis and the vascular tissue of the root. If we look at the cross section of a young root, it would be here where these large somewhat circular cells are.

Examples of mycorrhizae:

The most thoroughly studied of these types are arbuscular mycorrhizae, ectomycorrhizae, ericoid mycorrhizae, arbutoid mycorrhizae and orchid mycorrhizae.

Mycorrhizae can be broadly classified as -

endomycorrhizae —those with fungi that enter the root cells, or as

ectomycorrhizae —those that remain extracellular, forming a sheath of interconnecting filaments (hyphae) around the roots.

Types of mycorrhizae recognized (can be divided into three categories):

1. Ectomycorrhizae: characterized by forming an external sheath of mycelium around the root tips and hyphal cells do not penetrate the cell walls (intercellular) although they may go between cells in the cortex (Hartig Net).
2. Endomycorrhizae: characterized by the lack of an external sheath around root tip and the penetration of cortical cells (intracellular) by the fungus mycelium.
3. Ectendomycorrhizae: mycorrhizal type that seems to be intermediate between ecto and endomycorrhizae. Mycelium sheath around root is reduced, or may even be absent, but Hartig Net is usually well developed as in ectomycorrhizae, but the hyphal cells may penetrate the cortical cells as in endomycorrhizae. However, because of similarities to ectomycorrhizae, they will not specifically be considered here.

Description of mycorrhizae types

Ectomycorrhizae: Ectomycorrhizae, sometimes abbreviated as EM, are not as widespread in nature as arbuscular mycorrhizae, occurring on the roots of about 5% of the world's plants. The most diagnostic feature of ectomycorrhizae is that the fungus never penetrates the cell walls of the plant and thus the exchange of nutrients must take place not only through the cell membrane but through both plant and fungal cell walls as well.

The ability to form ectomycorrhizae is found in many families of fungi, but most commonly among members of the class Agaricomycotina of the Basidiomycota, especially those producing mushrooms and boletes. Ectomycorrhizae dominate in the pine, oak, birch, willow, walnut and several other families. In the tropics these include the dipterocarps and large woody legumes.

It is referred to as "ecto-" because the fungal symbiont does not invade the cell protoplasm. However, the fungus does form a thick sheath around the root tip and mycelium also grows between the cells of the cortex forming the so-called Hartig net. The infected roots are very distinctive, forming short, paired, branches.

In this type of mycorrhiza, the fungal sheath, that forms around the secondary root tips, accumulate minerals from the decomposing litter, before they are able to pass into the deeper mineral layers of the soil where they would be unavailable to the roots. Thus, mycorrhizal fungi are also decomposers as well. Fungus does obtain simple carbohydrates that are produced by the plant, but not used by the plant. So it appears that these carbohydrates may be produced by the plant specifically for the fungus since they are not utilized by the plant.

The ectomycorrhizal root that is formed has a morphology that is distinct from that of uninfected roots. One distinctive characteristic of the infected root tips is that they lack root hairs. This is unusual because root hairs are normally presence, in abundance, in the young root. This morphology is in part due to the fungus secreting auxin, a plant hormone, that acts upon the root development and in the case of gymnosperms, form, thick dichotomous branches. Branching of the root system will differ with different plant families.

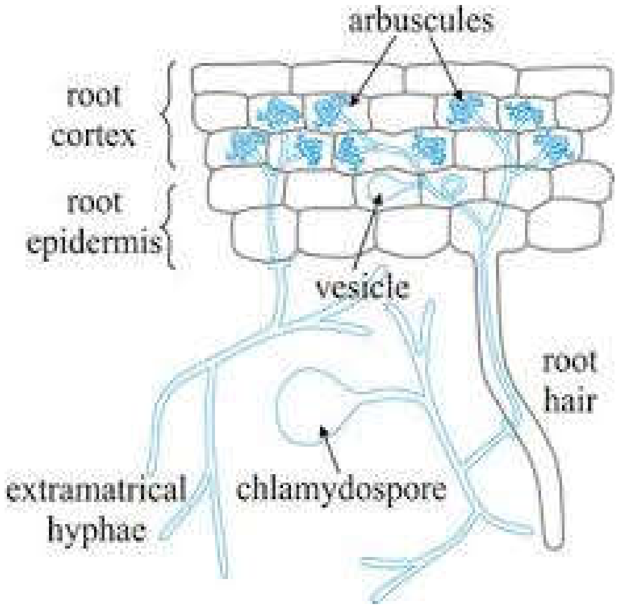
Ectendomycorrhizae :The ectendomycorrhizae morphology is like that of the ectomycorrhizae, i.e. presence of Hartig Net, same host range, etc. The only real morphological difference is that the host roots cells are penetrated by hyphal cell of fungus. Also, the fungi involved have not been identified.

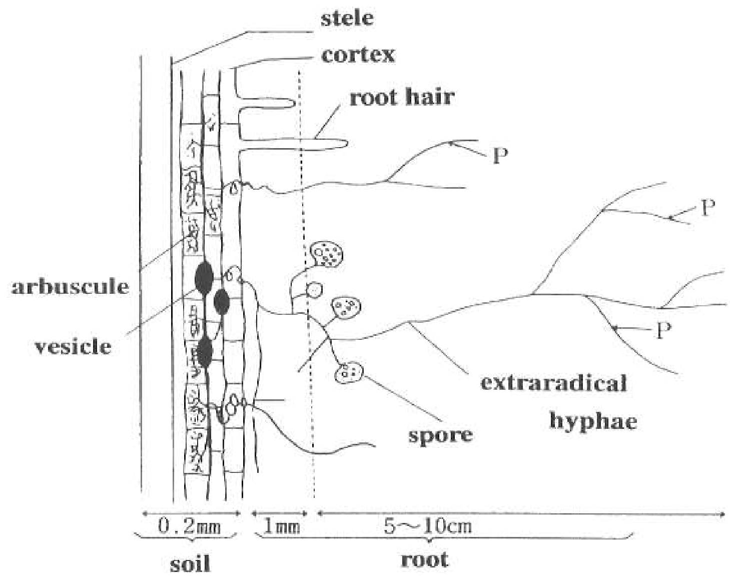
Endomycorrhizae: In endomycorrhizae, a part of the fungus becomes deeply embedded within the root tissue.There are several categories of endomycorrhizae. The only common feature that they all share is that the mycelium of the fungal symbiont will gain entry into the host, root cells by cellulolytic enzymes. Unlike the ectomycorrhizae, roots which are infected with mycorrhizal fungi do not differ morphologically from those that are not infected, i.e. root hairs are present and sheath is not formed around the root tip. However, the type of association that is formed between the host and fungus vary a great deal in the different categories of endomycorrhizae.

Arbuscular Mycorrhizae

This category of mycorrhiza can be found throughout the world, but more abundant in the tropics than in temperate regions, and is associated with more plants than any of the other categories of mycorrhizae. The name of this type of mycorrhizae comes from the distinct structures called arbuscules that can be seen inside the cells of infected roots. These structures can be recognized by their branched tree-like appearance. Another structure that can be frequently observed are the rounded vesicles. The vesicles and arbuscules contain the stored minerals that are needed by the plant. These structures lyse in the root cells and in this way the minerals become available to the plant. There is also extensive mycelium in the soil, but do not appear to be organized in any fashion.

The VAM fungi normally produce assorted types of spores which can be used in the identification of these fungi, i.e. zygospores, chlamydospores and azygospores. It was once thought that these fungi were nothing more than a rare curiosity. However, this was only because a technique was needed, which could more efficiently find VAM spores, than by simply sifting through the soil. Once this technique was found, this type of mycorrhiza was found to be the most common in nature. It is because VAM have a broad host range they were once considered to be a future tool in agriculture, i.e. fertilizer substitute. However, because these fungi cannot be grown in the absence of a host plant, individual inoculations would have to be done for each plant. This would be impractical for any grains grown as well as for most crops, but have been utilized in planting of fruit trees which are planted individually.





Benefits of Mycorrhizal Biofertilizer

Mycorrhiza plays a very important role on enhancing the plant growth and yield due to an increase supply of phosphorus to the host plant. Mycorrhizal plants can absorb and accumulate several times more phosphate from the soil or solution than non–mycorrhizal plants. Plants inoculated with endomycorrhiza have been shown to be more resistant to some root diseases.

Mycorrhiza increase root surface area for water and nutrients uptake. The use of mycorrhizal biofertilizer helps to improve higher branching of plant roots, and the mycorrhizal hyphae grow from the root to soil enabling the plant roots to contact with wider area of soil surface, hence, increasing the absorbing area for water and nutrients absorption of the plant root system. Therefore, plants with mycorrhizal association will have higher efficiency for nutrients absorption, such as nitrogen, phosphorus, potassium, calcium, magnesium, zinc, and copper; and also increase plant resistance to drought.

Benefits of mycorrhizal biofertilizers can be seemed as follows:

1. Allow plants to take up nutrients in unavailable forms or nutrients that are fixed to the soil. Some plant nutrients, especially phosphorus, are elements that dissolve were in water in neutral soil. In the extreme acidic or basic soil, phosphorus is usually bound to iron, aluminum, calcium, or magnesium, leading to water insolubility, which is not useful for plants. Mycorrhiza plays an important role in phosphorus absorption for plant via cell wall of mycorrhiza to the cell wall of plant root. In addition, mycorrhiza help to absorb other organic substances that are not fully soluble for plants to use, and also help to absorb and dissolve other nutrients for plants by storage in the root it is associated with.
2. Enhance plant growth, improve crop yield, and increase income for the farmers. Arising from improved water and essential nutrients absorption for plant growth by mycorrhiza, it leads to improvement in plant photosynthesis, nutrients translocation, and plant metabolism processes. Therefore, the plant has better growth and yield, reduce the use of chemical fertilizer, sometimes up to half of the suggested amount, which in turn increases income for the farmers. As in the trial involving mycorrhizal biofertilizer on asparagus it was observed that, when the farmers used suggested amount of chemical fertilizer together with mycorrhizal biofertilizer, it was found that the crop yield improved by more than 50%, and the farmers' income increased 61% higher than when chemical fertilizer alone was used.
3. Improve plant resistance to root rot and collar rot diseases. Mycorrhizal association in plant roots will help plant to resist root rot and collar rot diseases caused by other fungi.
4. It can be used together with other agricultural chemicals. Mycorrhiza are endurable to several chemical substances; for example; chemical pesticides, herbicides and chemical agents for plant disease elimination.

Biopesticides

Biopesticides, or also known as Biological Pesticides, are natural pest control agents that are obtained from natural substances. They can come from minerals, plants, and bacteria. Using biopesticides has advantages over using conventional pesticides, because biopesticides are less toxic to the environment and natural life. They play an important role in the protection of agricultural foods and protection against unwanted microbial organisms. Biopesticides include "naturally occurring substances that control pests (biochemical pesticides), microorganisms that control pests (microbial pesticides), and pesticidal substances produced by plants containing added genetic material.

Examples: Chitosan, Spinosad, Insect pheromones and other semiochemicals

Types of Biopesticides

(1)**Microbial pesticides** consist of a microorganism (bacterium, fungus, virus, protozoan or alga) as the active ingredient. Microbial pesticides can control many different kinds of pests, although each separate active ingredient is relatively specific for its target pest. For example, there are fungi that control certain weeds, and other fungi that kill specific insects. The microbial pesticides have the ability to control insects, weeds, fungi, and bacteria that can cause plant diseases.

(2) **Plant-Incorporated-Protectants (PIPs)** are pesticidal substances that plants produce from genetic material that has been added to the plant. They only target the pests that are living on the plants and not the natural enemies of it, such as beetles and birds.

(3) **Biochemical pesticides** are naturally occurring substances that control pests by non-toxic mechanisms. They naturally get produced by the plants and help control pests. These pesticides include plant growth regulators that may help the plant grow and mate, or interfere with those processes. These mechanisms also include substances that attract and repel pests, such as pheromones or scents that humans smell from the plants.

Advantages of biopesticides:

1. They are relatively cheaper.
2. Biopesticides are usually inherently less toxic than conventional pesticides.
3. Biopesticides generally affect only the target pest and closely related organisms. These do not harm non target species.
4. Biopesticides often are effective in very small quantities and often decompose quickly and do not leave harmful residues. This is beneficial to the environment because this results in avoiding the pollution problems, which most conventional pesticides cause.
5. To use biopesticides effectively, however, users need to know a great deal about managing pests.

Disadvantages of Biopesticides:

1. If bio-pesticides are ever used in large quantities, they can become capable of harming non-targeted organisms, including humans.
2. In order to even identify a specific pest or pathogen, multiple pesticides must be used. This is because bio-pesticides have a very high specificity and cannot be used on any plant without it being tested.
3. The constant use of pesticides can be very harmful for plants.

4. Since their reactions take place slowly, bio-pesticides would be very unsuitable if there is a pest-breakout .
5. If they don't work effectively at that time, there is a possibility of being an immediate threat to the crops.
6. Efficiency of biopesticides is influenced by other biotic and abiotic factors. Plus, if bio-pesticides, chemical or physical pesticides are constantly used, the targeted living organisms will also evolve to have an increased resistance to their control.

Examples of biopesticides:

Bacillus thuringiensis (Bt)

The most widely known microbial pesticides are varieties of the bacterium *Bacillus thuringiensis*, or Bt, which can control certain insects in cabbage, potatoes, and other crops. Bt produces a protein that is harmful to specific insect pests. Certain other microbial pesticides act by out competing pest organisms. Microbial pesticides need to be continuously monitored to ensure they do not become capable of harming non-target organisms, including humans. *Bacillus thuringiensis* is the most commonly used biopesticide globally. It is primarily a pathogen of lepidopterous pests like American bollworm in cotton and stem borers in rice. When ingested by pest larvae, Bt releases toxins which damage the mid gut of the pest, eventually killing it. Main sources for the production of BT preparations are the strains of the subspecies *kurstaki, galeriae and dendrolimus*.

Trichoderma

Trichoderma is a fungicide effective against soil born diseases such as root rot. It is particularly relevant for dryland crops such as groundnut, black gram, green gram and chickpea, which are susceptible to these diseases. Preparation of *Trichoderma* biopesticide is cheap and requires only basic knowledge of microbiology.