UNIT-1

Biofertilizers Definition

"Biofertilizers are substances that contain microorganisms, which when added to the soil increase its fertility and promotes plant growth."

What is Biofertilizer?

Biofertilizers are the substance that contains microbes, which helps in promoting the growth of plants and trees by increasing the supply of essential nutrients to the plants. It comprises living organisms which include mycorrhizal fungi, blue-green algae, and bacteria. Mycorrhizal fungi preferentially withdraw minerals from organic matter for the plant whereas cyanobacteria are characterized by the property of nitrogen fixation.

Nitrogen fixation is defined as a process of converting the di-nitrogen molecules into nitrogen compounds. For instance, some bacteria convert insoluble forms of soil phosphorus into soluble forms. As a result, phosphorus will be available for plants.

Types of Biofertilizers

Following are the important types of biofertilizers:

Symbiotic Nitrogen-Fixing Bacteria

Rhizobium is one of the vital symbiotic nitrogen-fixing bacteria. Here bacteria seek shelter and obtain food from plants. In return, they help by providing fixed nitrogen to the plants.

Loose Association of Nitrogen-Fixing Bacteria

Azospirillum is a nitrogen-fixing bacteria that live around the roots of higher plants but do not develop an intimate relationship with plants. It is often termed as rhizosphere association as this bacteria collect plant exudate and the same is used as a food by them. This process is termed as associative mutualism.

Symbiotic Nitrogen-Fixing Cyanobacteria

Blue-Green algae or Cyanobacteria from the symbiotic association with several plants. Liverworts, cycad roots, fern, and lichens are some of the Nitrogen-fixing cyanobacteria. Anabaena is found at the leaf cavities of the fern. It is responsible for nitrogen fixation. The fern plants decay and release the same for utilization of the rice plants. Azolla pinnate is a fern that resides in rice fields but they do not regulate the growth of the plant.

Free-Living Nitrogen-Fixing Bacteria

They are free-living soil bacteria which perform nitrogen fixation. They are saprotrophic anaerobes such as *Clostridium beijerinckii*, Azotobacter, etc.

Among all the types of biofertilizers, Rhizobium and Azospirillum are most widely used.

Components of Biofertilizers

The components of biofertilizers include:

Bio Compost

It is one of the eco-friendly product composed of waste material released from sugar industries which are decomposed. It is magnified with human-friendly bacteria, fungi, and various plants.

Tricho-Card

It is an eco-friendly and nonpathogenic product used in a variety of crops as well as in horticultural and ornamental plants, such as paddy apple, sugar cane, brinjal, corn, cotton, vegetables, citrus, etc. It acts as a productive destroyer and antagonistic hyper parasitic against eggs of several bores, shoot, fruit, leaves, flower eaters and other pathogens in the field.

Azotobacter

It protects the roots from pathogens present in the soil and plays a crucial role in fixing the atmospheric nitrogen. Nitrogen is a very important nutrient for the plant and about 78% of the total atmosphere comprises of nitrogen.

Phosphorus

Phosphorus is one of the essential nutrients for plants growth and development. Phosphate solubilizing microorganisms, hydrolyze insoluble phosphorus compounds to the soluble form for uptake by plants. Many fungi and bacteria are used for the purpose such as *Penicillium, Aspergillus, Bacillus, Pseudomonas, etc.*

Vermicompost

It is an Eco-friendly organic fertilizer comprises of vitamins, hormones, organic carbon, sulfur, antibiotics that help to increase the quantity and quality of yield. Vermicompost is one of the quick fixes to improve the fertility of the soil.

Also refer: Vermicomposting

Importance of Biofertilizers

Biofertilizers are important for the following reasons:

- Biofertilizers improve soil texture and yield of plants.
- They do not allow pathogens to flourish.
- They are eco-friendly and cost-effective.
- Biofertilizers protect the environment from pollutants since they are natural fertilizers.
- They destroy many harmful substances present in the soil that can cause plant diseases.
- Biofertilizers are proved to be effective even under semi-arid conditions.

Applications of Biofertilizers

Following are the important applications of biofertilizers:

Seedling root dip

This method is applicable to rice crops. The seedlings are planted in the bed of water for 8-10 hours.

Seed Treatment

The seeds are dipped in the mixture of nitrogen and phosphorus fertilizers. These seeds are then dried and sown as soon as possible.

Soil Treatment

The biofertilizers along with the compost fertilizers are mixed and kept for one night. This mixture is then spread on the soil where the seeds have to be sown.

Discover more about what is biofertilizer, types of biofertilizers and applications of biofertilizers, only at

Rhizobium – Legume Symbiotic Relationship:

Rhizobia are diazotrophic bacteria that fix nitrogen after becoming established inside the root nodules of legumes (Fabaceae). To express genes for nitrogen fixation, rhizobia require a plant host; they cannot independently fix nitrogen.[1] In general, they are gram negative, motile, non-sporulating rods.

Rhizobia are a "group of soil bacteria that infect the roots of legumes to form root nodules".[2] Rhizobia are found in the soil and after infection, produce nodules in the legume where they fix nitrogen gas (N_2) from the atmosphere turning it into a more readily useful form of nitrogen. From here, the nitrogen is exported from the nodules and used for growth in the legume. Once the legume dies, the nodule breaks down and releases the rhizobia back into the soil where they can live individually or reinfect a new legume host

The first known species of rhizobia, *Rhizobium leguminosarum*, was identified in 1889, and all further species were initially placed in the *Rhizobium* genus. Most research has been done on crop and forage legumes such as clover, alfalfa, beans, peas, and soybeans.

Symbiotic relationship

Rhizobia are unique in that they are the only nitrogen-fixing bacteria living in a symbiotic relationship with legumes. Common crop and forage legumes are peas, beans, clover, and soy.

Nature of the mutualism

The legume–rhizobium symbiosis is a classic example of mutualism—rhizobia supply ammonia or amino acids to the plant and in return receive organic acids (principally as the dicarboxylic acids malate and succinate) as a carbon and energy source. However, because several unrelated strains infect each individual plant, a classic tragedy of the commons scenario presents itself. Cheater strains may hoard plant resources such as polyhydroxybutyrate for the benefit of their own reproduction without fixing an appreciable amount of nitrogen.[14] Given the costs involved in nodulation and the opportunity for rhizobia to cheat, it may be surprising that this symbiosis should exist at all.

Infection and signal exchange

The formation of the symbiotic relationship involves a signal exchange between both partners that leads to mutual recognition and development of symbiotic structures. The most well understood mechanism for the establishment of this symbiosis is through intracellular infection. Rhizobia are free living in the soil until they are able to sense flavonoids, derivatives of 2-phenyl-1.4-benzopyrone, which are secreted by the roots of their host plant triggering the accumulation of a large population of cells and eventually attachment to root hairs.[15][16] These flavonoids then promote the DNA binding activity of NodD which belongs to the LysR family of transcriptional regulators and triggers the secretion of nod factors after the bacteria have entered the root hair.[16] Nod factors trigger a series of complex developmental changes inside the root hair, beginning with root hair curling and followed by the formation of the infection thread, a cellulose lined tube that the bacteria use to travel down through the root hair into the root cells.[17] The bacteria then infect several other adjacent root cells. This is followed by continuous cell proliferation resulting in the formation of the root nodule.[15] A second mechanism, used especially by rhizobia which infect aquatic hosts, is called crack entry. In this case, no root hair deformation is observed. Instead the bacteria penetrate between cells, through cracks produced by lateral root emergence.[18]

Inside the nodule, the bacteria differentiate morphologically into bacteroids and fix atmospheric nitrogen into ammonium, using the enzyme nitrogenase. Ammonium is then converted into amino acids like glutamine and asparagine before it is exported to the plant.[15] In return, the plant supplies the bacteria with carbohydrates in the form of organic acids.[15] The plant also provides the bacteroid oxygen for cellular respiration, tightly bound by leghaemoglobins, plant proteins similar to human hemoglobins. This process keeps the nodule oxygen poor in order to prevent the inhibition of nitrogenase activity.[15]

Recently, a *Bradyrhizobium* strain was discovered to form nodules in *Aeschynomene* without producing nod factors, suggesting the existence of alternative communication signals other than nod factors, possibly involving the secretion of the plant hormone cytokinin.[15][19]

It has been observed that root nodules can be formed spontaneously in *Medicago* without the presence of rhizobia.[20] This implies that the development of the nodule is controlled entirely by the plant and simply triggered by the secretion of nod factors.

Mass Production of Rhizobium:

- 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

Culturing of Microorganisms

Although many bacteria can be used beneficially as a biofertilizer the technique of mass production is standardizedfor *Rhizobium*,
The media used for mass culturing are as follows:

Rhizobium : Yeast extract mannitol broth.

Growth on Congo red yeast extract mannitol agar medium

Mannitol	-	10.0 g
K2 HPO4	-	0.5 g
Mg So4 7H2 O	-	0.2 g
NaCl	-	0.1 g
Yeast extract	-	0.5 g
Agar		20.0 g
Distilled water		1000.0 ml

Add 10 ml of Congo red stock solution (dissolve 250 mg of Congo red in 100ml water) to 1 liter after adjusting the PH to 6.8 and before adding agar.

Rhizobium forms white, translucent, glistening, elevated and comparatively small colonies on this medium. Moreover, *Rhizobium* colonies do not take up the colour of congo red dye added in the medium. Those colonies which readily take up the congo red stain are not rhizobia but presumably *Agrobacterium*, a soil bacterium closely related to *Rhizobium.*

Inoculum preparation

- Prepare appropriate media for specific to the bacterial inoculant in 250 ml, 500 ml, 3 litre and 5 litre conical flasks and sterilize.
- The media in 250 ml flask is inoculated with efficient bacterial strain under aseptic condition
- Keep the flask under room temperature in rotary shaker (200 rpm) for 5- 7 days.
- Observe the flask for growth of the culture and estimate the population, which serves as the starter culture.
- Using the starter culture (at log phase) inoculate the larger flasks (500 ml, 3 litre and 5 litre) containing the media, after obtaining growth in each flask.
- The above media is prepared in large quantities in fermentor, sterilized well, cooled and kept it ready.
- The media in the fermentor is inoculated with the log phase culture grown in 5 litre flask. Usually 1 -2 % inoculum is sufficient, however inoculation is done up to 5% depending on the growth of the culture in the larger flasks.
- The cells are grown in fermentor by providing aeration (passing sterile air through compressor and sterilizing agents like glass wool, cotton wool, acid etc.) and given continuous stirring.
- The broth is checked for the population of inoculated organism and contamination if any at the growth period.
- The cells are harvested with the population load of 109 cells ml-1 after incubation period.

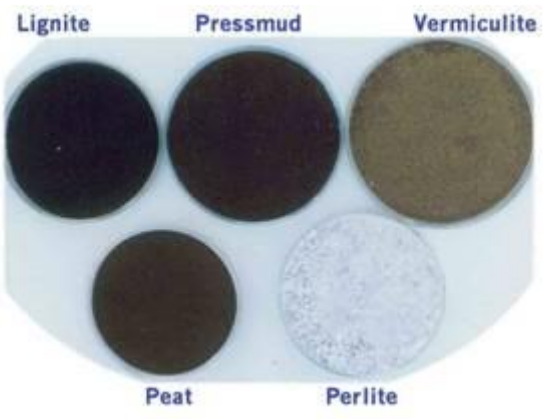
- There should not be any fungal or any other bacterial contamination at 10-6 dilution level
- It is not advisable to store the broth after fermentation for periods longer than 24 hours. Even at 4o C number of viable cells begins to decrease.

Processing of carrier material

The use of ideal carrier material is necessary in the production of good quality biofertilizer. Peat soil, lignite, vermiculite, charcoal, press mud, farmyard manure and soil mixture can be used as carrier materials. The neutralized peat soil/lignite are found to be better carrier materials for biofertilizer production The following points are to be considered in the selection of ideal carrier material.

- Cheaper in cost
- Should be locally available
- High organic matter content
- No toxic chemicals
- Water holding capacity of more than 50%
- Easy to process, friability and vulnerability.

Preparation of carrier material



- The carrier material (peat or lignite) is powdered to a fine powder so as to pass through 212 micron IS sieve.
- The pH of the carrier material is neutralized with the help of calcium carbonate (1:10 ratio) , since the peat soil / lignite are acidic in nature (pH of 4 - 5)
- The neutralized carrier material is sterilized in an autoclave to eliminate the contaminants.

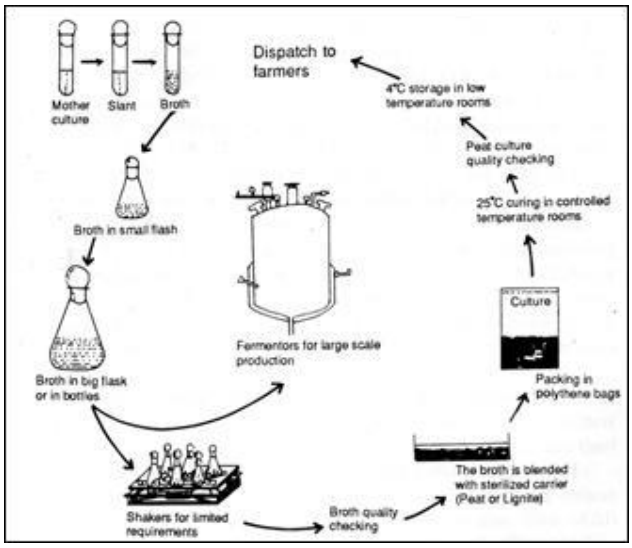
Mixing the carrier and the broth culture and packing

Inoculant packets are prepared by mixing the broth culture obtained from fermentor with sterile carrier material as described below:

Preparation of Inoculants packet

- The neutralized, sterilized carrier material is spread in a clean, dry, sterile metallic or plastic tray.
- The bacterial culture drawn from the fermentor is added to the sterilized carrier and mixed well by manual (by wearing sterile gloves) or by mechanical mixer. The culture suspension is to be added to a level of 40 – 50% water holding capacity depending upon the population.

- The inoculant packet of 200 g quantities in polythene bags, sealed with electric sealer and allowed for curing for 2 -3 days at room temperature (curing can be done by spreading the inoculant on a clean floor/polythene sheet/ by keeping in open shallow tubs/ trays with polythene covering for 2 -3 days at room temperature before packaging).



Schematic representation of mass production of bacterial biofertilizers