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.

Very often microorganisms are not as efficient in natural surroundings as one would expect them to be and therefore artificially multiplied cultures of efficient selected microorganisms play a vital role in accelerating the microbial processes in soil.

Use of biofertilizers is one of the important components of integrated nutrient management, as they are cost effective and renewable source of plant nutrients to supplement the chemical fertilizers for sustainable agriculture. Several microorganisms and their association with crop plants are being exploited in the production of biofertilizers. They can be grouped in different ways based on their nature and function.

S. No.	Groups	Examples
N2 fixing Biofertilizers		
1.	Free-living	*Azotobacter, Beijerinkia, Clostridium, Klebsiella, Anabaena, Nostoc,*
2.	Symbiotic	*Rhizobium, Frankia, Anabaena azollae*
3.	Associative Symbiotic	*Azospirillum*
P Solubilizing Biofertilizers		
1.	Bacteria	*Bacillus megaterium* var. *phosphaticum, Bacillus subtilis Bacillus circulans, Pseudomonas striata*
2.	Fungi	*Penicillium sp, Aspergillus awamori*
P Mobilizing Biofertilizers		
1.	Arbuscular mycorrhiza	*Glomus sp.,Gigasporasp.,Acaulospora sp.*, *Scutellospora sp. & Sclerocystis sp.*
2.	Ectomycorrhiza	*Laccaria sp., Pisolithus sp.*, *Boletus sp.*, *Amanita sp.*
3.	Ericoid mycorrhizae	*Pezizellaericae*
4.	Orchid mycorrhiza	*Rhizoctonia solani*
Biofertilizers for Micro nutrients		
1.	Silicate and Zinc solubilizers	*Bacillus* sp.
Plant Growth Promoting Rhizobacteria		
1.	Pseudomonas	*Pseudomonas fluorescens*

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2. Different types of biofertilizers

Rhizobium

Rhizobium is a soil habitat bacterium, which can able to colonize the

legume roots and fixes the atmospheric nitrogen symbiotically. The morphology and physiology of Rhizobium will vary from free-living condition to the bacteroid of nodules. They are the most efficient biofertilizer as per the quantity of nitrogen fixed concerned. They have seven genera and highly specific to form nodule in legumes, referred as cross inoculation group. *Rhizobium* inoculant was first made in USA and commercialized by private enterprise in 1930s and the strange situation at that time has been chronicled by Fred (1932).

Initially, due to absence of efficient bradyrhizobial strains in soil, soybean inoculation at that time resulted in bumper crops but incessant inoculation during the last four decades by US farmers has resulted in the build up of a plethora of inefficient strains in soil whose replacement by efficient strains of bradyrhizobia has become an insurmountable problem.

Azotobacter

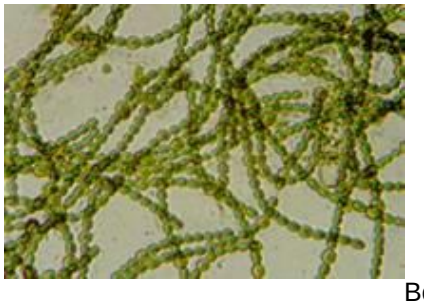
Of the several species of *Azotobacter*, *A. chroococcum* happens to be the dominant inhabitant in arable soils capable of fixing N2 (2-15 mg N2 fixed /g of carbon source) in culture media. The bacterium produces abundant slime which helps in soil aggregation. The numbers of *A. chroococcum* in Indian soils rarely exceeds 105/g soil due to lack of organic matter and the presence of antagonistic microorganisms in soil.

Azospirillum

Azospirillumlipoferum and *A. brasilense* (*Spirillum lipoferum* in earlier literature) are primary inhabitants of soil, the rhizosphere and intercellular spaces of root cortex of graminaceous plants. They perform the associative symbiotic relation with the graminaceous plants. The bacteria of Genus *Azospirillum* are N2 fixing organisms isolated from the root and above ground parts of a variety of crop plants. They are Gram negative, *Vibrio* or *Spirillum* having abundant accumulation of polybetahydroxybutyrate (70 %) in cytoplasm.

Five species of *Azospirillum* have been described to date *A. brasilense*, *A.lipoferum*, *A.amazonense*, *A.halopraeferens* and *A.irakense*. The organism proliferates under both anaerobic and aerobic conditions but it is preferentially micro-aerophilic in the presence or absence of combined nitrogen in the medium. Apart from nitrogen fixation, growth promoting substance production (IAA), disease resistance and drought tolerance are some of the additional benefits due to *Azospirillum* inoculation.

Cyanobacteria



Both free-living as well as symbiotic cyanobacteria (blue green algae) have been harnessed in rice cultivation in India. A composite culture of BGA having heterocystous *Nostoc*, *Anabaena*, *Aulosira etc*. is given as primary inoculum in trays, polythene lined pots and later mass multiplied in the field for application as soil based flakes to the rice growing field at the rate of 10 kg/ha. The final product is not free from extraneous contaminants and not very often monitored for checking the presence of desiredalgal flora.

Once so much publicized as a biofertilizer for the rice crop, it has not presently attracted the attention of rice growers all over India except pockets in the Southern States, notably Tamil Nadu. The benefits due to algalization could be to the extent of 20-30 kg N/ha under ideal conditions but the labour oriented methodology for the preparation of BGA biofertilizer is in itself a limitation. Quality control measures are not usually followed except perhaps for random checking for the presence of desired species qualitatively.

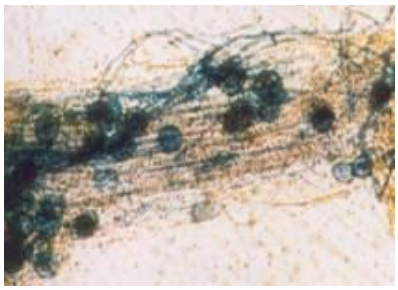
Azolla

Azolla is a free-floating water fern that floats in water and fixes atmospheric nitrogen in association with nitrogen fixing blue green alga *Anabaena azollae*. *Azolla* fronds consist of sporophyte with a floating rhizome and small overlapping bi-lobed leaves and roots. Rice growing areas in South East Asia and other third World countries have recently been evincing increased interest in the use of the symbiotic N2 fixing water fern *Azolla* either as an alternate nitrogen sources or as a supplement to commercial nitrogen fertilizers. *Azolla* is used as biofertilizer for wetland rice and it is known to contribute 40-60 kg N/ha per rice crop.

Phosphate solubilizing microorganisms(PSM)

Several soil bacteria and fungi, notably species of *Pseudomonas, Bacillus, Penicillium, Aspergillus etc*. secrete organic acids and lower the pH in their vicinity to bring about dissolution of bound phosphates in soil. Increased yields of wheat and potato were demonstrated due to inoculation of peat based cultures of *Bacillus polymyxa* and *Pseudomonas striata*. Currently, phosphate solubilizers are manufactured by agricultural universities and some private enterprises and sold to farmers through governmental agencies. These appear to be no check on either the quality of the inoculants marketed in India or the establishment of the desired organisms in the rhizosphere.

AM fungi



The transfer of nutrients mainly phosphorus and also zinc and sulphur from the soil *milleu* to the cells of the root cortex is mediated by intracellular obligate fungal endosymbionts of the genera *Glomus, Gigaspora, Acaulospora, Sclerocysts* and *Endogone* which possess vesicles for storage of nutrients and arbuscles for funneling these nutrients into the root system. By far, the commonest genus appears to be *Glomus*, which has several species distributed in soil. Availability for pure cultures of AM (Arbuscular Mycorrhiza) fungi is an impediment in large scale production despite the fact that beneficial effects of AM fungal inoculation to plants have been repeatedly shown under experimental conditions in the laboratory especially in conjunction with other nitrogen fixers.

Silicate solubilizing bacteria (SSB)

Microorganisms are capable of degrading silicates and aluminum silicates. During the metabolism of microbes several organic acids are produced and these have a dual role in silicate weathering. They supply H+ ions to the medium and promote hydrolysis and the organic acids like citric, oxalic acid, Keto acids and hydroxy carbolic acids which from complexes with cations, promote their removal and retention in the medium in a dissolved state.

The studies conducted with a Bacillus sp. isolated from the soil of granite crusher yard showed that the bacterium is capable of dissolving several silicate minerals under *in vitro* condition. The examination of anthrpogenic materials like cement, agro inputs like super phosphate and rock phosphate exhibited silicate solubilizing bacteria to a varying degree. The bacterial isolates made from different locations had varying degree of silicate solubilizing potential. Soil inoculation studies with selected isolate with red soil, clay soil, sand and hilly soil showed that the organisms multiplied in all types of soil and released more of silica and the available silica increased in soil and water. Rice responded well to application of organic sliceous residue like rice straw, rice husk and black ash @ 5 t/ha. Combining SSB with these residues further resulted in increased plant growth and grain yield. This enhancement is due to increased dissolution of silica and nutrients from the soil.

Plant Growth Promoting Rhizobacteria (PGPR)

The group of bacteria that colonize roots or rhizosphere soil and beneficial to crops are referred to as plant growth promoting rhizobacteria (PGPR).

The PGPR inoculants currently commercialized that seem to promote growth through at least one mechanism; suppression of plant disease (termed Bioprotectants), improved nutrient acquisition (termed Biofertilizers), or phytohormone production (termed Biostimulants). Species of *Pseudomonas* and *Bacillus* can produce as yet not well characterized phytohormones or growth regulators that cause crops to have greater amounts of fine roots which have the effect of increasing the absorptive surface of plant roots for uptake of water and nutrients. These PGPR are referred to as Biostimulants and the phytohormones they produce include indole-acetic acid, cytokinins, gibberellins and inhibitors of ethylene production.

Recent advances in molecular techniques also are encouraging in that tools are becoming available to determine the mechanism by which crop performance is improved using PGPR and track survival and activity of PGPR organisms in soil and roots. The science of PGPR is at the stage where genetically modified PGPR can be produced. PGPR with antibiotic, phytohormone and siderophore production can be made.

Despite of promising results, biofertilizers has not got widespread application in agriculture mainly because of the variable response of plant species or genotypes to inoculation depending on the bacterial strain used. Differential rhizosphere effect of crops in harbouring a target strain or even the modulation of the bacterial nitrogen fixing and phosphate solubilizing capacity by specific root exudates may account for the observed differences. On the other hand, good competitive ability and

high saprophytic competence are the major factors determining the success of a bacterial strain as an inoculant.

Studies to know the synergistic activities and persistence of specific microbial populations in complex environments, such as the rhizosphere, should be addressed in order to obtain efficient inoculants. In this regards, research efforts are made at Agricultural College and Research Institute, Madurai to obtain appropriate formulations of microbial inoculants incorporating nitrogen fixing, phosphate- and silicate- solubilizing bacteria and plant growth promoting rhizobacteria which will help in promoting the use of such beneficial bacteria in sustainable agriculture.

Liquid Biofertilizers

Biofertilizers are such as *Rhizobium, Azospirillum* and Phosphobacteria provide nitrogen and phosphorous nutrients to crop plants through nitrogen fixation and phosphorous solubilization processes. These Biofertilizers could be effectively utilized for rice, pulses, millets, cotton, sugarcane, vegetable and other horticulture crops. Biofertilizers is one of the prime input in organic farming not only enhances the crop growth and yield but also improves the soil health and sustain soil fertility. At present, Biofertilizers are supplied to the farmers as carrier based inoculants. As an alternative, liquid formulation technology has been developed in the Department of Agricultural Microbiology, TNAU, Coimbatore which has more advantages than the carrier inoculants.

Benefits

The advantages of Liquid Bio-fertilizer over conventional carrier based Bio-fertilizers are listed below:

- Longer shelf life -12-24 months.
- No contamination.
- No loss of properties due to storage upto 45º c.
- Greater potentials to fight with native population.
- High populations can be maintained more than 109 cells/ml upto 12 months to 24 months.
- Easy identification by typical fermented smell.
- Cost saving on carrier material, pulverization, neutralization, sterilization, packing and transport.
- Quality control protocols are easy and quick.
- Better survival on seeds and soil.
- No need of running Bio-fertilizer production units through out the year.
- Very much easy to use by the farmer.
- Dosages is 10 time less than carrier based powder Bio-fertilizers.
- High commercial revenues.
- High export potential.
- Very high enzymatic activity since contamination is nil.

Characteritistics of different liquid Bio-fertilizers

Rhizobium

This belongs to bacterial group and the classical example is symbiotic nitrogen fixation. The bacteria infect the legume root and form root nodules within which they reduce molecular nitrogen to ammonia which is reality utilized by the plant to produce valuable proteins, vitamins and other nitrogen containing compounds. The site of symbiosis is within the root nodules. It has been estimated that 40-250 kg N / ha / year is fixed by different legume crops by the microbial activities of *Rhizobium*. The percentage of nodules occupied, nodules dry weight, plant dry weight and the grain yield per plant the multistrain inoculant was highly promising Table-2 shows the N fixation rates.

Quantity of biological N fixed by Liqiud *Rhizobium* in different crops

Host Group	*Rhizobium* Species	Crops	N fix kg/ha
Pea group	*Rhizobium leguminosarum*	Green pea, Lentil	62- 132
Soybean group	*R.japonicum*	Soybean	57- 105
Lupini Group	*R. lupine orinthopus*	Lupinus	70- 90
Alfafagrp.Group	*R.mellilotiMedicago Trigonella*	Melilotus	100- 150
Beans group	*R. phaseoli*	Phaseoli	80- 110
Clover group	*R. trifoli*	Trifolium	130
Cowpea group	*R. species*	Moong, Redgram, Cowpea, Groundnut	57- 105
Cicer group	*R. species*	Bengal gram	75- 117

Physical features of liquid Rhizobium

- Dull white in colour
- No bad smell
- No foam formation, pH 6.8-7.5

Azospirllium

It belongs to bacteria and is known to fix the considerable quantity of nitrogen in the range of 20- 40 kg N/ha in the rhizosphere in non- non-leguminous plants such as cereals, millets, Oilseeds, cotton etc. The efficiency of *Azospirillium* as a Bio-Fertilizer has increased because of its ability of inducing abundant roots in several pants like rice, millets and oilseeds even in upland conditions. Considerable quantity of nitrogen fertilizer up to 25-30 % can be saved by the use of *Azospirillum* inoculant. The genus *Azospirillum* has three species viz., *A. lipoferum*, *A. brasilense* and *A. amazonense*. These species have been commercially exploited for the use as nitrogen supplying Bio-Fertilizers.

One of the characteristics of *Azospirillum* is its ability to reduce nitrate and denitrify. Both *A. lipoferum*,and *A. brasilense* may comprise of strains which can actively or weakly denitrify or reduce nitrate to nitrite and therefore, for inoculation preparation, it is necessary to select strains which do not possess these characteristics. *Azospirlliumlipoferum* present in the roots of some of tropical forage grasses uch as Digitaria, Panicum, Brachiaria, Maize, Sorghum, Wheat and Rye.

Physical features of liquid *Azospirillum*

- The colour of the liquid may be blue or dull white.
- Bad odours confirms improper liquid formulation and may be concluded as mere broth.
- Production of yellow gummy colour materials comfirms the quality product.
- Acidic pH always confirms that there is no Azospirillum bacteria in the liquid.

N2 fixing capacity of Azospirillum in the roots of several plants and the amount of N2 fixed by them.

Plant	Mg N2 fixed /g of substrate

Oryza sativa (Paddy)	28
Sorghum bicolour (Sorghum)	20
Zea mays (Maize)	20
Panicum sp.	24
Cynodondactylon	36
Setariasp	12
Amaranthus spinosa	16

Production of growth hormones

Azospirillum cultures synthesize considerable amount of biologically active substances like vitamins, nicotinic acid, indole acetic acids giberllins. All these hormones/chemicals helps the plants in better germination, early emergence, better root development.

Role of Liquid *Azospirillum* under field conditions

- Stimulates growth and imparts green colour which is a characteristic of a healthy plant.
- Aids utilization of potash, phosphorous and other nutrients.
- Encourage plumpness and succulence of fruits and increase protein percentage.

Sign of non functioning of *Azospirillum* in the field

- No growth promotion activity
- Yellowish green colour of leaves, which indicates no fixation of Nitrogen

Azotobacter

It is the important and well known free living nitrogen fixing aerobic bacterium. It is used as a Bio-Fertilizer for all non leguminous plants especially rice, cotton, vegetables etc. *Azotobacter* cells are not present on the rhizosplane but are abundant in the rhizosphere region. The lack of organic matter in the soil is a limiting factor for the proliferation of *Azotobaceter* in the soil.

Field experiments were conducted in 1992, 1993 and 1994 during the pre-kharif wet seasons to find out the influence on rice grain yield by the combined use of N- fixing organisms and inorganic nitrogen fertilizer which recorded increase in was yield.

Physical features of liquid *Azotobacter*

The pigmentation that is produced by Azotobacter in aged culture is melanin which is due to oxidation of tyrosine by tyrosinase an enzyme which has copper. The colour can be noted in liquid forms. Some of the pigmentation are described below-

- *A. chroococcum*: Produces brown-black pigmentation in liquid inoculum.
- *A. beijerinchii*: Produces yellow- light brown pigementation in liquid inoculum
- *A. vinelandii*: Produces green fluorescent pigmentation in liquid inoculum.
- *A. paspali*: Produces green fluorescent pigmentation in liquid inoculum.
- *A. macrocytogenes*: Produces, pink pigmentation in liquid inoculum.
- *A. insignis*: Produces less, gum less, grayish-blue pigmentation in liquid inoculum.
- *A. agilies*: Produces green-fluorescent pigmentation in liquid inoculum.

Role of liquid *Azotobacter* in tissue culture

The study was conducted by Dr. Senthil et al (2004) on sugarcane variety CO 86032 in Tissue culture Laboratories of Rajashree Sugars and Chemicals Ltd, Varadarajnagar, Theni, Tamilnadu. The liquid bioinoculants were provided by Dr. Krishnan Chandra, Regional Director, RCOF, Bangalore to

evaluate their growth promoting effects on sugarcane micropropagation. He recorded Biometric observations like Plant height, leaf length, width, root length, no of roots. Chemical parameters – Protein, Carbohydrates, N, P,K total biomass and concluded as follows:

- The performance of *Azotobacter* liquid inoculant was c
- omparatively better than all the treatments in 10 % MS medium followed Azospirillum.
- The performance of *Azotobacter* liquid inoculant was comparatively better than all the treatments followed by Azosopirillum for the growth of the polybag sugarcane seedlings.

Role of liquid Azotobacter as a Bio-control agent

Azotobacter have been found to produce some antifungal substance which inhibits the growth of some soil fungi like *Aspergillus, Fusarium, Curvularia, Alternaria, Helminthosporium, Fusarium* etc.

Acetobaceter

This is a sacharophillic bacteria and associate with sugarcane, sweet potato and sweet sorghum plants and fixes 30 kgs/ N/ ha year. Mainly this bacterium is commercialized for sugarcane crop. It is known to increase yield by 10-20 t/ acre and sugar content by about 10-15 percent.

Effect of liquid *Acetobacter diazotrophicus* on sugarcane

In South India use of Azospirillum and Phospho-bacterium on the cash crop sugarcane is a regular practice for the past few years with a saving of nearly 20 % of chemical nitrogen and phosphate applications. Now, it has been reported that a bacteria *Acetobacter diazotrophicus* which is present in the sugarcane stem, leaves, soils have a capacity to fix up to 300 kgs of nitrogen. This bacteria first reported in brazil where the farmers cultivate sugarcane in very poor sub-soil fertilized with Phosphate, Potassium and micro elements alone, could produce yield for three consecutive harvests, without any nitrogen fertilizer. They have recorded yield 182- 244 tones per ha. This leads to the assumption that active nitrogen fixing bacteria has associated within the plant.

Do's and Don't for Entrepreneurs, Dealers and farmers

Do	Don't
Keep Bio-fertilizers bottles away from direct heat and sunlight. Store it in cool and dry place.	Don't store Bio-fertilizers bottles under heat and sunlight
Sell only Bio-fertilizers bottles which contain batch number, the name of the crop on which it has to be used, the date of manufacture and expiry period.	Don't sell Bio-fertilizers bottles after their expiry period is over.
If the expiry period is over, then discard it as it is not effective.	Don't prick holes into the bottles or puncture them to pour the content
Keep Bio-fertilizers bottles away from fertilizer or pesticide containers and they should not be mixed directly.	Do not mix the Bio-fertilizers with fungicides, insecticides, herbicides, herbicides and chemical fertilizers.

Liquid Bio-fertlizer application methodology
There are three ways of using Liquid Bio-fertilizers

1. Seed treatment
2. Root dipping
3. Soil application

Seed Treatment

Seed Treatment is a most common method adopted for all types of inoculants. The seed treatment is effective and economic. For small quantity of seeds (up to 5 kgs quantity) the coating can done in a plastic bag. For this purpose, a plastic bag having size (21" x 10") or big size can be used. The bag should be filled with 2 kg or more of seeds. The bag should be closed in such a way to trap the airs as much as possible. The bag should be squeezed for 2 minutes or more until all the seed are uniformly wetted. Then bag is opened, inflated again and shaked gently. Stop shaking after each seeds gets a uniform layer of culture coating. The bag is opened and the seed is dried under the shade for 20-30 minutes. For large amount of seeds coating can be done in a bucket and inoculant can be mixed directly with hand. Seed Treatment with *Rhizobium*, *Azotobacter, Azospirillum*, along with PSM can be done.

The seed treatment can be done with any of two or more bacteria. There is no side (antagonistic) effect. The important things that has to be kept in mind are that the seeds must be coated first with *Rhizobium, Azotobacter* or *Azospirillum*. When each seed get a layer of above bacteria then PSM inoculant has to be coated as outer layer. This method will provide maximum number of each bacteria required for better results. Treatments of seed with any two bacteria will not provide maximum number of bacteria on individual seed.

Root dipping

For application of *Azospirillum*/ /PSM on paddy transplating/ vegetable crops this method is used. The required quantity of *Azospirillum*/ /PSM has to be mixed with 5-10 litres of water at one corner of the field and the roots of seedlings has to be dipped for a minimum of half-an-hour before transplantation.

Soil application

Use 200ml of PSM per acre. Mix PSM with 400 to 600 kgs of Cow dung FYM along with ½ bag of rock phosphate if available. The mixture of PSM, cow dung and rock phosphate have to be kept under any tree or under shade for over night and maintain 50% moisture. Use the mixture as soil application in rows or during leveling of soil.

Dosage of liquid Bio-fertilizers in different crops

Recommended Liquid Bio-fertilizers and its application method, quantity to be used for different crops are as follows:

Crop	**Recommended Bio-fertilizer**	**Application method**	**Quantity to be used**
Field crops Pulses Chickpea, pea, Groundnut, soybean, beans, Lentil, lucern, Berseem, Green gram, Black gram, Cowpea and pigeon pea	*Rhizobium*	Seed treatment	200ml/acre
Cereals Wheat, oat, barley	*Azotobacter/Azospirillum*	Seed treatment	200ml/acre
Rice	*Azospirillum*	Seed treatment	200ml/acre
Oil seeds Mustard, seasum, Linseeds, Sunflower, castor	*Azotobacter*	Seed treatment	200ml/acre
Millets Pearl millets, Finger millets, kodo millet	*Azotobacter*	Seed treatment	200ml/acre
Maize and Sorghum	*Azospirillum*	Seed treatment	200ml/acre
Forage crops and Grasses Bermuda grass, Sudan grass, Napier Grass ,ParaGrass, StarGrass etc.	*Azotobacter*	Seed treatment	200ml/acre

Other Misc. Plantation Crops Tobacco	*Azotobacter*	Seedling treatment	500ml/acre
Tea, Coffee	*Azotobacter*	Soil treatment	400ml/acre
Rubber, Coconuts	*Azotobacter*	Soil treatment	2-3 ml/plant
Agro-ForestRY/Fruit Plants All fruit/agro-forestry (herb,shrubs, annuals and perennial) plants for fuel wood fodder, fruits,gum,spice,leaves,flowers,nuts and seeds puppose	*Azotobacter*	Soil treatment	2-3 ml/plant at nursery
Leguminous plants/ trees	*Rhizobium*	Soil treatment	1-2 ml/plant

Note:
Doses recommended when count of inoculum is 1 x 108 cells/ml then doses will be ten times more besides above said Nitrogen fixers, Phosphate solubilizers and potash mobilizers at the rate of 200 ml/ acre could be applied for all crops.

Equipments required for Biofertilizer production

In biofertilizer production industry, equipments are the major infrastructure, which involves 70 percent of capital investment. Any compromise on the usage of the following mentioned equipments may finally decline in the quality of biofertilizer.After studying the principle behind the usage of all instruments, some of the instruments can be replaced with a culture room fitted with a U.V.Lamp. Autoclaves, Hot Air Oven, Incubators and sealing machines are indigenously made with proper technical specifications. The correct use of equipments will give uninterrupted introduction with quality inoculum.

Essential equipments
Autoclave

It is an apparatus in which materials are sterilized by air free saturated steam (under pressure) at a temperature above 100OC. If the steam pressure inside the autoclave is increased to 15 psi, the temperature will rise to 121°C. this is sufficient to destroy all vegetative cells. Normally all growth medium are sterilized in the autoclave.

Laminar air flow chamber

Laminar air flow chamber provides a uniform flow of filtered air. This continuous flow of air will prevent settling of particles in the work area.Air borne contamination is avoided in this chamber. Culture transfers and inoculation can be done here.

BOD incubators

Incubators providing controlled conditions (light, temperature, humidity, etc.) required for the growth and development of microorganisms. Multiplication of starter culture can be done in this instrument.

Rotary shaker

It is used for agitating culture flasks by circular motion under variable speed control. Shaking provides aeration for growth of cultures. Shakers holding upto 20-50 flasks are generally used. The capacity of the shaker may be increased if it is a double- decker type.

Hot air oven

Hot air oven is meant for sterilizing all glassware materials. Dry heat is used in this apparatus to sterilize the materials. Normally 180OC is used for two hours for sterilizing glasswares.

pH meter

An instrument for measuring pH of the solution using a 0-14 scale in which seven represents neutral points, less than seven is acidity (excess of H‘ over OH-) and more than seven is alkality (excess of OH- over H‘) useful in adjusting the pH of the growth medium.

Refrigerator

This equipment is used preserving all mother cultures used for biofertilizer production. The mother culture is periodically sub-cultured and stored in the refrigerator for long- term usage.

Fermentor

A fermentor is the equipment, which provides the proper environment for the growth of a desired organism. It is generally a large vessel in which, the organism may be kept at the required temperature, pH , dissolved oxygen concentration and substrate concentration. Different models of fermentors are available depending upon the necessity. A simple version model contains steam generator, sterilization process devices and agitator. A sophisticated fermentor contains pH regulator, oxygen level regulator, anti-foam device, temperature controller, etc.

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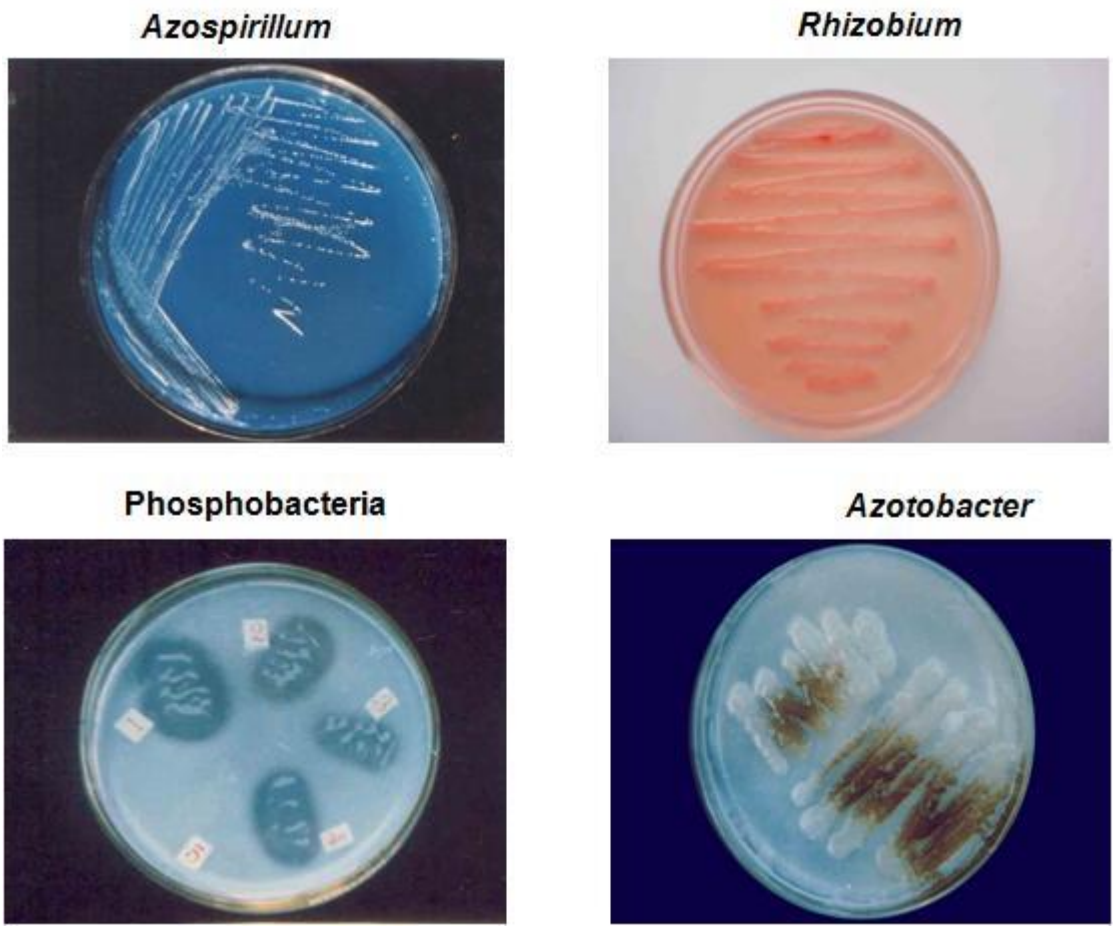
3. Mass production of Bacterial Biofertilizer



Biofertilizers are carrier based preparations containing efficient strain of nitrogen fixing or phosphate solubilizing microorganisms. Biofertilizers are formulated usually as carrier based inoculants. The organic carrier materials are more effective for the preparation of bacterial inoculants. The solid inoculants carry more number of bacterial cells and support the survival of cells for longer periods of time.

- 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*, *Azospirillum*, *Azotobacter* and phosphobacteria.
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.*

Azospirillum :Dobereiner's malic acid broth with NH4Cl (1g per liter)

Composition of the N-free semisolid malic acid medium

Malic acid	-	5.0g
Potassium hydroxide	-	4.0g
Dipotassium hydrogen orthophosphate	-	0.5g
Magnesium sulphate	-	0.2g
Sodium chloride	-	0.1g
Calcium chloride	-	0.2g
Fe-EDTA (1.64% w/v aqueous)	-	4.0 ml
Trace element solution	-	2.0 ml
BTB (0.5% alcoholic solution)	-	2.0 ml
Agar	-	1.75 g
Distilled water	-	1000 ml
pH	-	6.8
Trace element solution		
Sodium molybdate	-	200 mg
Manganous sulphate	-	235 mg
Boric acid	-	280 mg
Copper sulphate	-	8 mg
Zinc sulphate	-	24 mg
Distilled water	-	200 ml

Waksman medium No.77 (N-free Mannitol Agar Medium for *Azotobacter*)

Mannitol	:	10.0 g

Ca CO3	:	5.0 g
K2HPO4	:	0.5 g
Mg SO4.7H2O	:	0.2 g
NaCl	:	0.2 g
Ferric chloride	:	Trace
MnSO4.4H2O	:	Trace
N-free washed Agar	:	15.0 g
Ph	:	7.0
Distilled Water	:	1000 ml

Phosphobacteria :Pikovskaya's Broth

Glucose	10.0 g
Ca3(PO4)2	5.0 g
(NH4)2SO4	0.5 g
KCl	0.2 g
MgSO4. 7H2O	0.1 g
MnSO4	Trace
FeSO4	Trace
Yeast Extract	0.5 g
Distilled Water	1000 ml

The broth is prepared in flasks and inoculum from mother culture is transferred to flasks. The culture is grown under shaking conditions at 30±2°C as submerged culture. The culture is incubated until maximum cell population of 1010 to 1011 cfu/ml is produced. Under optimum conditions this population level could be attained with in 4 to 5 days for *Rhizobium*; 5 to 7 days for *Azospirillum*; 2 to 3 days for phosphobacteria and 6-7 days for *Azotobacter*. The culture obtained in the flask is called **starter culture**. For large scale production of inoculant, inoculum from starter culture is transferred to large flasks/seed tank fermentor and grown until required level of cell count is reached.

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.

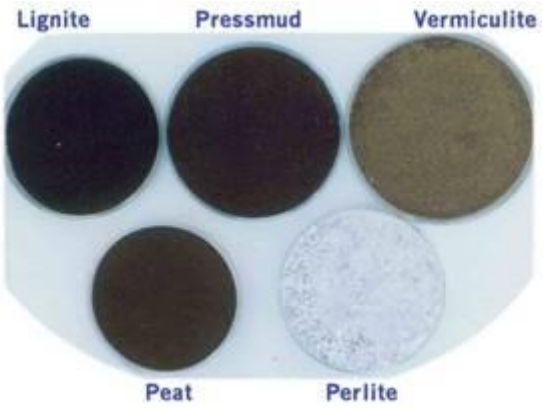
Processing of carrier material

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



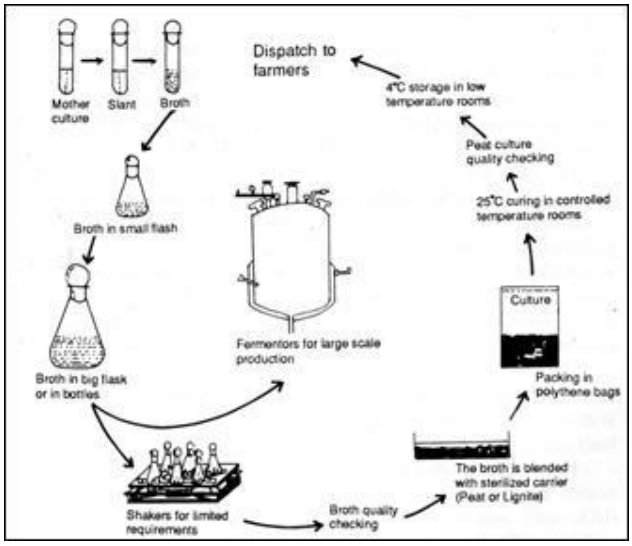
- The carrier material (peat or lignite) is powdered to a fine powder so as to pass through 212 micron IS sieve.
- The pH of the carrier material is neutralized with the help of calcium carbonate (1:10 ratio) , since the peat soil / lignite are acidic in nature (pH of 4 - 5)
- The neutralized carrier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

Specification of the polythene bags

- The polythene bags should be of low density grade.
- The thickness of the bag should be around 50 – 75 micron.
- Each packet should be marked with the name of the manufacturer, name of the product, strain number, the crop to which recommended, method of inoculation, date of manufacture, batch number, date of expiry, price, full address of the manufacturer and storage instructions etc.,

Storage of biofertilizerpacket

- The packet should be stored in a cool place away from the heat or direct sunlight.
- The packets may be stored at room temperature or in cold storage conditions in lots in plastic crates or polythene / gunny bags.
- The population of inoculant in the carrier inoculant packet may be determined at 15 days interval. There should be more than 109 cells / g of inoculant at the time of preparation and107 cells/ g on dry weight basis before expiry date.