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AZOTOBACTER AS A SOURCE OF BIOFERTILIZER: FORMULATIONS AND ITS ADVANCES

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ABSTRACT

Biofertilizer consists of live microorganisms that enhances plant growth and development. They are cost-effective and eco-friendly bioinoculants that can provide nutrients to plant, secrete certain growth promoting substances, suppress phytopathogens, reduces the biotic and abiotic stress, detoxify the soil pollutants and improve the soil fertility. There is increased demand for chemical fertilizers and pesticides which in turn causes hazardous effect on environment so an alternative biofertilizers promises to be a sustainable technology. *Azotobacter* is most common free living, gram negative, cyst producing, aerobic and nitrogen fixing bacteria. It secretes indole acetic acid, cytokinins and gibberellin-like compounds. One of the major step of biofertilizer production is formulation. In formulation, the microbial strain is mixed with a carrier, which transfers them to target plant or soil. There are different types of formulation depending on the form of carrier used like solid, liquid and encapsulated. This paper reviews about the need of biofertilizer, characteristics of *Azotobacter* sp., biofertilizer production process and formulations types.

Keywords: Biofertilizer, Formulation, Bioinoculant, Microorganism

1. INTRODUCTION

A large population of a specific or a group of beneficial microorganisms that increases the soil productivity either by fixing the atmospheric nitrogen, by phosphorous solubilization or by secreting growth promoting substances that stimulates plant growth, is known as biofertilizer. Biofertilizers have definite advantage over

the chemical fertilizers as they are renewable energy sources, cost effective and ecofriendly. Biofertilizers stimulate plant growth by secreting growth promoting substances like hormones, vitamins, amino acids, etc. whereas chemical fertilizers supply only nitrogen **[1]**. In addition, chemical fertilizers portrait disadvantages such as pollution, it destroys beneficial microorganisms and friendly insects, and it makes the crop more susceptible to the attack of disease and also reduces the soil fertility. Thus, biofertilizers are considered as the most advanced biotechnology invention that supports to develop organic, sustainable, green and pollution- free agriculture. The biofertilizer not only increase the output but also improve the quality of agricultural products **[2]**. Biofertilizers are classified into different types on the basis of group of microorganisms they contain, such as Nitrogen fixing biofertilizers (includes Symbiotic, Free- living non photosynthetic, Free- living photosynthetic and associative nitrogen fixing bacteria), Phosphorus biofertilizers (Phosphorus solubilizing and phosphorus mobilizing biofertilizers), Potassium biofertilizers, Plant growth promoting biofertilizers (PGPB), Zinc solubilizing biofertilizers, Sulphur oxidizing biofertilizers and Silicate solubilizing biofertilizers **[3]**. Some of the common microorganisms used in biofertilizers are *Azotobacter, Rhizobium, Trichoderma, Pseudomonas striata, Bacillus megaterium, Bacillus subtilis*, Cyanobacteria (Blue green algae*), Azospirillium, Acetobacter, Penicillium*, etc. Biofertilizers supply nitrogen continuously whereas chemical fertilizers have to be provided repeatedly to the crops to replenish the loss of Nitrogen utilized by the crop for growth **[4]**. *Azotobacter* is a non- symbiotic, aerobic and free- living bacterium belonging to *Azotobacteriaceae* family. They can fix 40- 200 kg/ha nitrogen and contributes to 80- 90% of nitrogen requirement of crop. They can increase the plant yield up to 50%. The species *Azotobacter vinelandii, Azotobacter beijerinckii, Azotobacter chroococcum, Azomonas macrocytogenes* are found in the rhizosphere of crops like rice, maize, sugarcane, bajra and certain vegetables. Azotobacter can produce vitamin B and phytohormones that leads to inhibition of pathogenic strains **[5]**.

2. CHARACTERISTICS OF *AZOTOBACTER* SPECIES

The genus *Azotobacter* was 1st described in 1901 by Martinus Beijerinck and belongs to family *Azotobacteraceae/ Pseudomonadaceae* and class Gamma proteobacteria. *Azotobacters* are gram-negative, oval or pleomorphic shaped. They are large compared to other bacteria, about 1- 3 µm wide and 2- 10 µm long. They produce

yellow-green, or red- violet or brownish-black pigments. They are able to form cysts in harsh environments. *Azotobacters* can fix atmospheric nitrogen in the free- living state without any symbiosis with any plant **[6]**. Most of the microorganisms require anaerobic condition for nitrogen fixation whereas *Azotobacters* are able to tolerate oxygen. Cysts form of *Azotobacter* cannot fix nitrogen. Wang H.*et al.,* 2018, treated *Azotobacter chroococcum* with carbon dots to evaluate its effect on Nitrogen fixation activity. When 4 µg/mL carbon dots were applied to *Azotobacter chroococcum*, it altered nitrogenase structural configuration, resulting in enhanced biocatalytic activity of nitrogen fixation. Carbon dots were found to increase the nitrogen fixation process by 158% when compared to control **[7]**. Bageshwar, U. K. *et al.,* 2017, reported that *Azotobacter chroococcum* and *Azotobacter vinelandii* showed increased amount of indole acetic acid production in the presence of its precursor tryptophan **[8]**. In addition, *Azotobacters* are able to solubilize insoluble phosphates in soil as the plants assimilate only the soluble form of phosphates. Kumar, V. *et al*., 2001, developed the*Azotobacter* mutants which can release 1.5- 1.7 µg phosphate per mL of supernatant from tricalcium phosphate **[9]**. *Azotobacters* can suppress several fungi, bacterial and nematode infections in plants. *A. chroococcum* was found to suppress the effect of *Rhizoctonia solani* on potatoes **[10]**. Beniwal, M. S.*et al.,* 1996, found *A. chroococcum* reducing the effect of flag smut when the wheat seed were inoculated with this strain **[11]**. It also shows strong fungistatic activity against *Sclerotium sp., Fusarium sp., Cephalosporium maydis, Alternaria brassicola* and *Collectotrichum falcatum* **[12]**.

3. PRODUCTION OF *AZOTOBACTER* BIOFERTILIZERS

The development and production of any biofertilizer includes the following three steps: (1) development of selected microbial strain (2) upscale of biomass and (3) preparation of inoculants **[13]**. The above steps are achieved through various sub-steps, which are essential for large-scale production of biofertilizers. These steps include selection of suitable and efficient microorganism, selection of optimum growth conditions, selection of special propagation method, pilot-scale study, large-scale production and quality testing at each level. In addition, the selection of suitable carrier for biofertilizer formulation, packaging, storage and transport are also important parameters **[14]**. Each of the above mentioned step is important to obtain high quality and high quantity biofertilizer. The most important step in this process is the selection of proper microorganism, Azotobacter is one

such microbe that can competent the rhizosphere, fix atmospheric nitrogen and promote the plant growth. Azotobacter can be isolated from the soil of vegetable plants, bajra, rice, sugarcane, maize, etc. Ashby or Jensen media can be used for culturing the bacteria. For the commercial production of biofertilizers, cost-effective and easily available media are used, such as molasses, corn steep liquor, jaggery solution, wastewater sludge, etc. They provide the necessary nutrients for cultivation of Azotobacter species. For large-scale production of Azotobacter biofertilizer, the growth parameters like pH, aeration, agitation and temperature are needed to be optimized. For Azotobacter, pH between 6 and 8 (6.5-7.5), temperature around 28±2˚C and aerobic conditions are required. After the selection of suitable Azotobacter strain, suitable medium and optimum growth conditions at laboratory scale, the next step is scale-up, which is carried out in 2 steps: pilot-scale production andlarge scale production, using different size fermenters. Finally, the culture is processed for formulations. Mostly, either carrier-based formulation or liquid formulation is carried out.For carrier-based formulation, the scaled-up pure culture of Azotobacter speciesis mixed with appropriate carrier material like peat, charcoal lignite, vermiculite, etc. Sterilized carriers are used and then packed aseptically and supplied to farmers. For liquid formulations, the liquid solvents with the cell protectants are used as carriers. A study reported that when liquid biofertilizers of Bacillus, Azospirillium and Azotobacter were formulated with 2% polyvinyl pyrrolidone (PVP), 0.1% carboxymethyl cellulose (CMC) and 0.025% polysorbate, they promoted the growth and survival of the cells for a longer period of time **[15]**. After formulation, the final product is analyzed for the quality, stored and then dispatched to farmers.

4. FORMULATIONS

Biofertilizers consists of the live or latent microorganism. The formulation of biofertilizer is considered as a delivery vehicle, for the transport of live microbes to the field. The formulation is responsible for the long-term storage and effectiveness of biofertilizers. Thus, formulation is the major key factor for supplying the biofertilizer to their target plant or soil. The bioinoculant technology can be successful, depending on the following two factors-microbial strain and inoculant formulation. Therefore, formulation subjects potential success of inoculants **[16]**. The complete assembly of bioinoculants like N-fixers, P solubilizers, P mobilizers, biocontrol agents, PGPR, etc. along with the carrier and osmoprotectant, sticking agents, nutrients, etc. is called bioformulation. These additives are responsible for

stabilization and protection of microorganism during storage and transportation **[17]**. Also, formulations makes the biofertilizer simple to deal with and use by the farmers. They protect microbes from harsh environmental factors and helps to maintain good physiology for long time **[18]**. The types of formulation differs depending on the bioinoculant`s use, type of soil, type of plant, nature of application, availability of resources, etc. and so the understanding of bioformulation becomes necessary as it affect the abundance and performance of bioinoculants.

4.1 Solid formulations/ Solid-carrier based formulations

In solid formulations, the microorganisms are unified with carrier i.e. delivery vehicle which transfers them from laboratory to rhizosphere **[19]**. There is high variability of microenvironment in soil, so the carriers prevent microbial populations from declining by creating a temporary protective surface and by providing substrates that enhance microbial growth **[20]**. A carrier should be an inert material. Solid carrier materials increases the supply of phosphorus to plants, provides resistance to soil-borne pathogens and assist in biological degradation of organic pollutants, Hence they are proved to be advantageous **[21]**. A good carrier must be non-toxic to both inoculated strains and to the plants, should be easy to process, free of lump-forming materials, must have high organic matter, able to permit gas exchange and easy to sterilize by autoclaving or gamma-irradiation. Along with these, it must show some suitable physicochemical properties viz., a high water holding capacity, good pH buffering capability, easy adjustable pH and should be suitable for as many strains as possible **[22]**. Solid carrier based formulation is the oldest type of formulation, since the natural soil provides hostile environment to inoculant cells, so soil wasused initially as a carrier **[23]**. The solid formulation includes granules microgranules, wettable powders, wettable/water dispersible granules and dusts, and are prepared by addition of binder, dispersant, wetting agents etc. **[24]**.

4.1.1 Granules

Granules are dry particles. They are composed of ingredient, binder and carrier. Generally, the proportion of active ingredients is 5-20% **[25]**. Depending on the granule size, they are classified as coarse particles (having size range 100-1000 μm) and microgranules (having size range 100-600 μm). The qualities for granules are non-caking, non-dusty, free-flowing and should be easily disintegrable in the soil to release the active ingredient present inside it. Also, they should be safe to use causing no problem of inhalation. The most commonly used granules are

wheat meal granules, corn meal baits, granules prepared with gelatinized corn-starch or flour, gluten, cottonseed flour and sugars, gelatin or acacia gum, sodium alginate and diatomaceous earth **[26]**.

4.1.2 Wettable powders

One of the oldest formulations composed of 50-80% technical powder, 15-45% filler, 1-10% dispersant and 3-5% surfactant by weight. These formulations, as the name suggest are easily miscible with water (or any liquid carrier) before the application. Their shelf-life may exceed up to 18 months. This type of formulations are prepared from by-product of agricultural and industrial waste like wheat bran-sand mixture, sawdust-sand-molasses mixture, corn cob-sand-molasses mixture, bagasse-sand-molasses mixture, organic cakes, cow dung-sand mixture, compost/farm manure, inert charcoal, diatomaceous earth and fly ash **[27]**.

4.1.3 Wettable/Water-dispersible granules

Also known as dry flowables, contains high concentration of dispersing agent than in wettable powder, which makes them non-dusty, free-flowing granules rapidly dissolving in water. Their major role is to control nematode and occupies about 90% of the total market available for nematode-base products. They can be applied by spraying **[28]**.

4.1.4Dusts

Dusts are finely ground mix of the active ingredient with particle size of 50-100µm. Even though they are found effective in killing, they are being not much adapted because of handling and application problems **[29]**.

4.2 Liquid Formulation

Liquid formulations are not just composed of the desired microorganism and their nutrients but also contain special cell protectants and additives which promotes cell survival in storage and even after application to seed or soil **[30]**. Peat is the most preferred and extensively used carrier since many years, however its availability is limited and also it is being depleted at a very fast rate, thus researches are developing different liquid inoculants for all kind of biofertilizer. In seeding equipment, the liquid formulations is being adapted rapidly, as it can be sprayed onto the seed as it passes through the seed auger and dries before it travels into the seed bin on the planter **[31]**. The production of liquid formulation is comparatively simple. It is produced by a simple fermentation process, packed directly aseptically from fermentor and then stored. As there is no need for carrier sterilization, the process is cost-effective. There is no risk of contamination and also the inoculum quantity is less, so farmers can handle easily. Also, the liquid formulations promote the microbial cells to form spores

or cysts and thus increase their resistance to abiotic stress. Here, the selection of additives is the most important step, as it protects the microbe from high temperature, desiccation and seed chemicals on application on seeds. The additives are selected on the basis of their water solubility, non-toxicity, complex chemical nature, having good rheological properties and high water activities **[32, 33]**. A typical liquid formulation is composed of microorganism (10-40%), suspender ingredient (1-3%), dispersant (1-5%), surfactant (3-8%) and carrier liquid i.e. oil or water (35-65%) **[25]**. Liquid formulation are of the following types:

4.2.1 Suspension Concentrates

They are prepared by addition of solid active ingredient having poor solubility in water and satisfactory stability to hydrolysis. Their storage and solubility can be improved by the addition of surfactants and are diluted before use. This is more preferred compared to wettable powders as farmers can easily measure and pour into spray tank **[34]**.

4.2.2 Oil-Miscible Flowable Concentrate

In this type of liquid formulation, the active ingredient is diluted in an organic liquid before its use **[35]**.

4.2.3 Ultra low volume suspension

They are suspension ready for use by ultralow volume (ULV) equipment. ULV is an aerial or ground spray equipment which generates fire spray **[35]**.

4.2.4 Oil dispersion

As the name suggest, it is a stable suspension of active ingredient in water immiscible solvent or oil. Oil evaporates much less than water and so it remains in contact for greater time. Thus, it can be applied as an emulsion i.e. oil in water or invert emulsion i.e. water in oil. The commonly used high molecular weight additives for liquid formulation are polyvinyl pyrrolidone (PVP), methyl cellulose, polyvinyl alcohol, polyethylene glycol, gum Arabic, trehalose, glycerol, Fe-EDTA, sodium alginate, tapioca flour, etc. **[36]**. A study was conducted with different osmolytes in different concentration on *Azotobacter sp., Azospirillum sp., Acinetobacter sp., Bacillus sp.* and *Pseudomonas sp*. It showed that each microbe responds differently, such as *Pseudomonas sp*. and *Bacillus sp*. perform best with PVP K-15 at 2% concentration, whereas *Acinetobacter sp*. grows best with 2% PEG 2% glycerol supported growth of *Azotobacter sp.*, whereas PVP and PEG supported the growth of *Azospirillium* sp. **[37]**. Advantages of liquid formulations are less requirement of inoculant, transportation is easy, easy to produce and sterilize, no contamination and cost effective.

4.3 Encapsulation

In encapsulation, the microbial cells are entrapped or coated within a polymeric material to produce beads. These beads are permeable to nutrients, gases and metabolites to maintain the cell viability within the beads **[38]**. Encapsulation techniques provides good protection to the active ingredients from harsh environmental conditions. The polymers used commonly are gelatin, starch, cellulose, alginate, etc. Depending on the bead size produced, two techniques- macroencapsulation (mm to cm) and microencapsulation (1-1000 μm) are used **[39]**. The advantages of polymer trapped formulation are, it releases the microbes slowly into soil, it is non-toxic, can be manipulated easily as per the need, easy to produce and handle.

4.4 Fluid Bed Dry Bioformulation (FBD)

In fluid bed dryer, fluidized condition is created by maintaining the material suspended against gravity in an upward flowing air stream and electrical heaters are present which generate heat for drying the material. This hot air expands the bed of material at its terminal velocity so that it keeps hanging in air and thus creates turbulence in the product. This phenomenon is known as fluidization, in which the particle gets large surface area due to heated air. Because of the full agitation of solid particles, there is increased rate of heat transfer and uniform drying. This technique is commonly used in food industries to make instant coffee powder and for other drying procedures **[30]**. The Fluid-Bed dry method can be a better option for the inoculant industry.

CONCLUSION

Biofertilizers have the power to overcome the hazardous effects of chemical fertilizers. They are been widely used by farmers today. *Azotobacter*is one such effective microbe, which can help the plant to stimulate its growth and increase the productivity. The shelf life of microbe can be increased by selecting a proper formulation type.A bioformulation cannot be considered successful if it does not possess a positive effect in field conditions, market existence, reliability and cost-effectiveness. There are a number of technological challenges in biofertilizer production like fermentation process, formulation type, population microbe and delivery systems.

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