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BIOFERTILIZERS : AN INDISPENSABLE COMPONENT OF SUSTAINABLE AGRICULTURE

1. INTRODUCTION

Role of soil health in boosting crop productivity has been recognized by man since the dawn of settled agriculture around 10,000 years ago. Agricultural systems persisted a long, over many centuries without much change until the augmentation of industrial and scientific revolution during the 18th century in Europe when it was given more scientific basis to put check on recurrence of disastrous famines. Over hundred years have passed since the entry of chemical fertilizers in agriculture. Chemical fertilizers have established their credibility globally and dominate in all crop production systems.

Indiscriminate use and faulty practices of applications of chemical fertilizers today have jeopardized the future of intensive agriculture. Approximately one billion ton input of chemical fertilizers into the arable lands threatens the human existence because of its contribution to global warming, ground water pollution and soil health deterioration. Moreover, their production process is based on fossil fuel energy, which is limited. In addition their intensive use is not going to be economical because of its cost implications. Despite spectacular progress in use of fertilizers, now a need is being felt for more sustainable and ecofriendly crop production systems.

Biofertilizers being ecological component and based on renewable sources of energy, offer an economical, environment friendly, low investment requiring and non-bulky plant nutrient source.

1.1. Importance

Much consumption of non –renewable energy sources has become the global concern whileas after green revolution even several Asian countries have escalated the use of chemical fertilizers indicating further push in consumption of fossil fuel energy. Bio-fertilizers can relieve this pressure to some extent by becoming the component of integrated nutrient supply system. They can contribute to crop production by following means:

i. They can act as potential supplementary source to meet partial to complete nutrient requirement of crops.
ii. Phosphatic biofertilizers help in increasing the solubility and availability of phosphorus fixed in the soils.
iii. They suppress the incidence of diseases caused by soil borne pathogens.
iv. They enhance crop growth by liberating growth promoting substances.
v. Biofertilizers can increase crop yield by 10 to 50%
vi. They are cheaper, economical and pollution free.
vii. They improve the soil tilth, physical as well as chemical properties.
viii. Nutritional benefits are also passed on to the companion as well as succeeding crops.
ix. Stabilize the soil ecological balance by encouraging soil flora and fauna growth.
x. Biofertilizers promote retention of nutrients and reduce percolation losses.

2. DEFINITIONS

"Microbial inoculants containing active strains of selective micro-organisms (like bacteria, algae, fungi alone or in combination) applied to the seed or soil for enhancing soil & crop productivity by N-fixation, enhancing solubility, mobility & availability of plant nutrients or plant growth stimulation

(1) Biofertilizers as it literary means nutrient source for plants of biological origin. **Accordingly they include FYM, compost, Crop residue, green manure, vermicompost and all microbial inoculants.** (2) **However, more specifically the term is restricted to microorganisms, which have the capability of fixing atmospheric nitrogen, enhancing, solubility, mobility and availability of plant nutrients.**

(3) **Biofertilizers have been defined variously as the living organisms which augment plant nutrients supply in one way or the other** (Motsara *et al*_1995). (4) Gupta (1991) termed biofertilizers as microbial inoculants which contain live or latent cells of efficient strains of nitrogen fixing, phosphate solubilizing or cellulytic microorganisms used for application to seed or to composting (soil) areas with the objective of increasing the number of such microorganisms and accelerating those microbial processes which augment the availability of nutrients. (5) Kaushik (1990) defined biofertilizers as microbial inoculants containing active strains of selective microorganisms like bacteria, algae, fungi alone or combination which help in increasing soil and crop productivities by way of enhanced biological nitrogen fixation, solubilization of insoluble fertilizers, stimulating plant growth or accelerated decomposition of plant residues. Hence, biofertilizers are preparations of live microorganisms, which help in improving plant nourishment and soil health. Thus, they pave a way for sustainable crop production.

2.1 Synthetic fertilizers vs. biofertilizers

Synthetic fertilizers are chemicals that have characteristics of quick supply of nutrients to plants. It may be attributed to their high concentration in soil upon application or easily assimilation by plants or both. Synthetic fertilizers as well as biofertilizers both are source of nutrients for plants but they differ in respect of many features. (Table1)

3. BIOFERTILIZER INDUSTRY

Carrier based microbial inoculants in viable state are used as biofertilizers. Either seed or soil application is recommended. Adverse effects due to use of chemical fertilizers on soil, plant and human health have attracted the global attention and the need for biofertilizers application is becoming an eco-environmental and political compulsion.

Even after more than one hundred year, a US patent on production of *Rhizobium* inoculant was granted (Nobble and Hitler1896) only 170 organizations in 24 countries are involved in commercial production of biofertilizers. Demand and production are still irregular and illusive and do not exhibit an encouraging trend. While research on effective application of bio fertilizers is going on in several countries and area under biofertilizers has increased in USA, Australia, China, Japan and India but poor response of farming community remains the main bottleneck. Reasons could be.

i. Biofertilizers are living organisms. Their cultivation, proliferation, transport and storage need complete know-how which must be transferred to producers, dealers and users.
ii. Slow response to biofertilizer applications by plant and soil.
iii. Supply of poor quality culture of biofertilizers. It may be overdated in some cases while in other cases material might have lost viability because of poor storage facilities.
iv. Sole application of biofertilizers cannot maintain higher levels of crop productivity.
v. Phenotypic plasticity is less in biofertilizers compared to host plant species.
vi. Biofertilizers are less popular with the farmers. It necessitates formulation of subsidized price policy and launching of participatory research in biofertilizer usages.
vii. Inadequate marketing network.
viii. Limited number of demonstrations of biofertilizers as a component of integrated plant nutrient supply system.
ix. Ecological based application technology is still lacking.

Table 1. Difference in synthetic fertilizers and biofertilizers.

Synthetic fertilizers	Biofertilizers
1. Manufacture process makes use of non-renewable energy.	Biofertilizers make use of renewable energy.
2 Oirgin is synthetic chemicals.	Origin is bio-organism products.

3 Contain no living cells.	They are the living organisms,
4 Quick and short effect on plant growth.	Slow and long effect on plant growth.
5 Production and action processes are less affected by environmental factors.	Being living structures, they are affected by environment.
6 Ecological impact negative.	Ecological impact positive.
7 Synthetic fertilizers are of recent origin.	Biofertilizers have evolved in the course of evolution since long.
8 Percolation, run-off and volatilization losses are more causing pollution.	Percolation, run-off and volatilization losses are less so pollution effects are not known.
9 Can be used in comparatively diverse range of environments.	Range of adaptation is limited.
10 Usually crop plant non-specific.	Relatively crop plant specific.
11 Interdependence does not exist.	Interdependence association exists in many cases.
12 Lethal concentration levels are easily attained.	Lethal concentration levels have not been yet reported.
13 Manufacturing sector is well established.	Biofertilizers industry is still in infancy.
14 They are bulky.	They are not bulky.

India ranks top in terms of biofertilizers consumption in Asia. National Biofertilizer Development Center, Ghaziabad worked out national requirement to be 5.1 lakh ton for nitrogen fixing biofertilizers and 2.55 lakh ton for phosphorous solubilizing biofertilizers. At present there are 68 commercial production biofertilizer units in the country, which have annual capacity of 3,000 tons. *Rhizobium* is the common biofertilizer. The current level of biofertilizers production is 1500 –2000 ton annually. Hence a large gap between demand and supply continue to exist. Production units extend over 17 states with Madhya Pradesh being the largest producer (table 2).

Table 2. Biofertilizers production units in India

State	Production units	Capacity ton / year
Andhra Pradesh	4	165
Assam	2	90
Bihar	2	20
Delhi	2	240
Gujarat	2	200
Haryana	2	125
Himachal Pradesh	1	10
Karnatka	6	150

Madhya Pradesh	4	450
Maharashtra	5	250
Manipur	1	50
Orissa	2	125
Punjab	1	75
Rajasthan	2	100
Tamil Naidu	8	350
Uttar Pradesh	13	300
West Bengal	4	150
Total	61	2850

4. TYPES OF BIOFERTILIZERS :

Various microorganisms that can act as biofertilizer may be free living, associative or symbiotic. They include bacteria, fungi and algae and are classified as under:

A) NITROGEN FIXING MICROORGANISMS

a) Bacteria :

i) Symbiotic: *Rhizobium*, *Frankia*, and bacteria

ii) Non- symbiotic bacteria:

- a) Aerobic: *Azotobacter*, *Azomonas*, *Azospirillium* and *Mycobacteria*.
- b) Anaerobic: *Closteridium*, *Chlorobium* and *Chromatium*.
- c) Facultative anaerobes: *Bacillus*, *Enterobacter*, *Escherichia* and *Klebsiella*, Rhodospirillum.

b) Algae:

iii) Blue green algae: *Anabaena*, *Anabaenosis*, *Nostoc*, *Tolyphotrix*.

c) Azolla (fern plant) : Anabaena azollae

B) PHOSPHATE SOLUBLIZING MICRO-ORGANISMS

- a) Bacteria: *Bacillus polymyxa*, *Pseudomonas striata*.
- b) Fungi: *Aspergillus awamori*; *Penicillium digitatum etc*.

C) ORGANIC MATTER DECOMPOSERS

- a) Cellulotytic: *Trichoderma virids*, *Trichoderma harzianum*.
- b) Lignolytic: *Clavaria sp*; *Cephalosporium sp*; *Humocola sp*.

D) NITROGEN FIXING FERN: AZOLLA IN ASSOCIATION WITH BLUE GREEN ALGE *ANABAENA AZOLLAE*.

Some important species which have the potential as biofertilizers are summarized in table 3 with economic importance.

4.1. Rhizobium

Legumes are well known for their character of enriching soil fertility. It is also now established that nodules on legume roots are the sites where nitrogen fixing bacterium '*Rhizobium*' is housed. The association, which is known as symbiosis, contributes 40 to

200 kg of nitrogen per ha per year depending on crop species, soil and season out of which 20-65 kg N/ha to succeeding or associated crops. *Rhizobium* is a gram negative soil bacterium. It infects legume roots and develops nodules. The process is strain or species specific. Thus, a strain may be fast growing, slow growing or ineffective. Fast growers double in 2-4 hours while slow growers take 6-12 hour for doubling.

4.1.1. Nitrogen fixation

Of the total 139 millions tons of nitrogen fixed biologically in various terrestrial ecosystems annually, contribution by *Rhizobium* legume symbiosis stands around 35 million tons. Under best compatibility and crop management conditions 80 to 90 % nitrogen requirement of the crop is met by *Rhizobium*. Wide range of variation is observed in amount of nitrogen fixed by *Rhizobium* within and between different crop species (table 4). Crops for its growth use much part of it. ~~However various fertility management studies in pulses have indicated contribution of 20 to 65 kg nitrogen/ha to succeeding or associated crops under favorable environment for rhizobial population activity~~ (Pareek *et. al.* 2002).

Table 4. Amount of nitrogen fixed by Rhizobia in symbiosis with different legumes.

Legume crop	Nitrogen fixed (Kg N/ ha.)
Alfalfa	100-200
Black gram	21-140
Chickpea	3-141
Cowpea	9-201
Clover	100-150
Common bean	0-125
Green gram	9-112
Groundnut	50-60
Fababean	53-330
Lentil	10-192
Pea	17-244
Soybean	60-80
Pigeonpea	7-235

Sources: Peoples *et. al.* (1995); Motsara *et. al.* (1995)

4.1.2. Legume- Rhizobium symbiosis

An efficient association of legume and Rhizobium at least involves three stages

i. *Rhizobium* ecology in soil i.e., its occurrence, growth and survival outside the host, particularly in unfavorable environments such as drought, acidity, alkalinity, submergence, high N available status of soil and nutrient deficiency.

ii. Host- *Rhizobium* biochemical signaling to initiate infection, formation and development of nodules in roots.

iii. Functioning of nodules, that is nitrogen fixation.

Numerous factor display their role in fixation of nitrogen in rhizobia. These are environmental, genotypical, nutritional, and their interactions. This leads to complexity of situation.

① 4.1.2.1 Temperature

Genetic variation for tolerance to temperature exists in *rhizobia*. Some generalizations are:

- i. Isolates from tropical legumes possess relatively more tolerance to elevated temperature with maximum growth in the range of 30-42°C.
- ii. Strains withstand high temperature in heavy and organic soils than light textured soils.
- iii. Slow glowing strains are more prevalent in higher temperatures than fast growing.
- iv. Tolerance is more in dry soils than in moist soils.
- v. The optimum growth temperature for fast growing rhizobia is 25°C.

② 4.1.2.2 Light

Effects of light have not been much studied but it may have adverse effect on root nodulation.

③ 4.1.2.3 Soil moisture

Optimum moisture conditions are essential for survival, nodulation and N_2 fixation activity of rhizobia. Drought and flooding both are unfavorable for rhizobia infection, nodule initiation, growth and nitrogenase activity. Nonetheless, rhizobia have good ability to tide over adverse conditions of drought and flood.

④ 4.1.2.4 Soil type

The medium textured loam soil is suitable for legume cultivation. It promotes the process of symbiosis as well.

⑤ 4.1.2.5. Soil aeration

Rhizobia are aerobic and require air for growth. Even low concentration of oxygen ($<$0.01percent) supports good growth. Access to increased aeration favors proliferation as well as fixation ability.

⑥ 4.1.2.6. Soil pH

Rhizobium trifolii and R. meliloti can tolerate wide range of pH from 4.5 to 8.5. *R. legminosarum* has optimum pH range of 6.5 to 8.0. All *Rhizobia* strains work better at ph 6.5 to 7.5.

⑦ 4.1.2.7. Soil salinity

Rhizobia are sensitive to salt stress (salinity). *R. meliloti and R. trifolii* are more salt tolerant than other species. Seed pelleting with calcium carbonate, gypsum or calcium humate provides protection to rhizobia against salinity and alkalinity. Ram *et.al.* (1989) reduction in nodule number, nodule mass and N_2 fixation in chickpea under salt stress conditions.

(8) 4.1.2.8 Organic matter

Rhizobia in absence of leguminous host live in soil as saprophytes for long period. Multiplication in soil organic matter reveals that *rhizobia* use components from it as source for carbon as well as energy. Humic acid and fulvic acid content of soil organic matter improve rhizobial growth, Greater population of *R. legminosarum and R. meliloti* are observed in soil rich in organic matter and better survival of *rhizobial* cells in peat based inoculants. These reflect beneficial effects of organic manuring.

(9) 4.1.2.9. Inorganic nutrients

(i) N

Nitrogen content of soil has not much influence on survival of *Rhizobium.* It may hinder the process of biological nitrogen fixation. There is need to develop *Rhizobium* strains tolerant to high NO'_3 levels. Nitrogen delay nodulation, reduce nodule number and size. However small starter dose of nitrogen (20 to 25 Kg N /ha in case of pulses) at sowing and 2% urea spraying or nitrogen application at flowering show positive effect on nodule dry weight and grain yield of legumes.

(ii) Phosphorus increases rhizobia population, more nodule number and higher nitrogenase activity. Potassium, calcium, magnesium, iron, molybdenum and zinc contribute towards better *rhizobial* growth, nodulation and nitrogen fixation. Mo is an integral part of the nitrogenase enzyme

X 4.1.3.Future strategies for harvesting more nitrogen through Rhizobium-legume symbiosis

4.1.3.1 Improved strains of Rhizobium

Improved strains of *rhizobia* can be defined as the ones, which are able to compete with the native strains and have higher nitrogenase activity. Competitivenese has also to be evaluated under various abiotic stresses. It would need screening of thousands of strains for nitrogen fixing ability. More genetic manipulations are possible by latest techniques of biotechnology.

4.1.3.2 Selection of better compatible host varieties

Genetic variation of host exists towards establishment of symbiosis with *Rhizobium*. It is recognized in the form of high nodulating lines, low nodulating lines or non-nodulating lines. High nodulating lines in chickpea yielded more than low nodulating lines (Dudeja *et.al.* 1997). Similarly large variation for NO'_3 tolerance has been reported in soybean by Belts and Herridge (1987). Super nodulating genotypes have been also identified in soybean but they were yielding 20-33 % less grain yield compared to parents (Wu and Harper 1991).

4.1.3.3. Rhizosphere management

Host-microbes interaction display very complex environment around rhizosphere. Inoculated rhizobia have to compete with native strains, plant pathogens, rhizobacteria and VAM fungi. In depth research on role of these microorganisms will help in better management of rhizosphere for exploitation of biological nitrogen fixation. Following aspects demand immediate redressal

i. Systematic research on role of rhizobacteria and VAM fungi in the process of symbiosis in legumes

ii. Phosphorus solubilizing microorganisms and rhizobium interaction in plant nutrition.

iii. Development of combined technique of beneficial microorganisms.

iv. A reliable method of accurate measurement of nitrogen fixed by the process of symbiosis.

4.2.Azospirillum

Azospirillum lipoferum (C_4 crops)
Azospirillum brasilense (C_3 crops)

Azospirillum is a gram negative, aerobic soil bacterium and belongs to family spirillaceae. *Azospirillum* spp. are found associated with roots and rhizosphers of many members of family Poaceae. Species-specific association has been also reported in *Azospirillum* (Baldani and Dobereiner 1980). A maize root inhabits predominantly *A. lipoferum* while rice and wheat roots were preferential host for *A.brasilense*. Some other important facts of azospirilla are:

i. *Azospirillum* association is more frequent in tropical regions than temperate ones.

ii. Alluvial soils favor more *Azospirillum* activity than eroded soils.

iii. Nitrogenase activity of *Azospirillum* is not observed without roots.

iv. *Panicum maximum* exhibits more efficient association with azospirilla compared to other grasses.

v. *A.lipoferum* shows preference for C_4 plants except sugarcane while. *A.brasilense* for C_3 plants.

4.2.1 Nitrogen fixation

Azospirilla can fix 20-40 kg N /ha /year under low nitrogenous fertilizers application. Highly variable results have been reported with its inoculation but most of the time positive (Lehri and Tewari 1990). Increase in grain yield ranges between 15-30%. Besides nitrogen, Azospirilla secrete growth-promoting substances such as IAA, GA and vitamins.

These promote germination and healthy growth of seedlings. *Azospirillum* show synergistic effects with PSM and VAM fungi. Starter dose of nitrogen at the rate of 20kg/ha has positive effect on functioning of Azospirillum. Nitrogenase activity of *Azospirillum* with different grasses is shown in table 5.

Table 5. Nitrogenase activity of different grasses and pure cultures of Azospirillum isolated from them.

Species	Nitroginase activity of	
	Roots	Pure Culture
Cenchrus biflorus	22.5	34.8
Cenchrus ciliaris	45.8	24.9
Cenchrus setigerus	16.9	84.8
Cyanodon dactylon	1262.5	185.6
Cyper rotundus	40.3	22.2
Desmostachia bipinnata	899.6	206.7

Eragrostis tremula	2768.5	185.2
Panicum antidotale	23.4	-
Pennisetum americanum	46.2	71.1
Saccharum munja	74.2	79.3
Cymbopogon marginatus	52.0	76.9
Heteropogon contortis	89.2	108.6

4.2.2. *Azospirillum* association with the host

Plant growth responses after inoculation with Azospirillum may be accounted to hormonal production, besides nitrogen, by the bacterium. Brown (1974) suggested that yield increases on application of *Azospirillum* to crops are attributable to microbial population changes in the rhizosphere, suppression of disease causing organisms and production of growth promoting substances. These activities were observed at a reasonable starter dose of nitrogen (20 to 60 kg/ha) and are acknowledged to show positive effects as under:

i. Increased *Azospirillum* population in the rhizosphere and reduced the population of undesirable microorganisms.

ii. Hormonal secretions by Azospirillum accelerated root growth, which in turn enhanced nutrient uptake.

iii. Nitrogenase activity and nitrogen transformation rate increased.

Response of *Azospirillum* inoculation to different crops compiled by Lehari and Twari (1990) revealed that out of 23 studies, five either showed non-significant or negative effect while in rest of the studies inoculation had significantly positive effect on crop performance. Nitrogen fixation by azospirilla is beyond doubt and has been confirmed by acetylene reduction assay (ARA) and isotopic enrichment method involving $^{15}N_2$ yet its actual potential as biofertilizer needs further research.

4.2.3. Factors affecting N_2 fixation ability

Azospirillum spp. are aerobic and require low oxygen level for nitrogenase activity. Because of conformational protection and location of enzyme nitrogenase within the cell, azospirilla can fix nitrogen in air. This is known as micro-aerophily. Salts of malic, succinic, lactic and pyruvic acids support vigorous growth. A correlation between organic acids and *Azospirillum* accumulation in roots of C_4 grasses has been advocated by Dobereiner and Day (1976). However N_2 fixing ability increased with low oxygen level and decreased carbon supply besides many ecological factors.

i. **Temperature:** Azospirillum nitrogenase functions well at as high temperature as 32 to 40°C, which indicates its adaptability to tropical conditions. While the activity of nitrogenase declines sharply below 18°C revealing its susceptibility to low temperature.

ii. **pH:** The optimum pH for azospirilla nitrogenase activity is 6.8 to 7.8.

iii. Salts: ammonia has repressive effects on nitrogenase activity. Rao and Venkateswarlu(1985a) reported that bicarbonates were more toxic to *Azospirillum* followed by chlorides and sulphates.

iv) Root exudates

Root exudates are consisted of various biologically active compounds such as organic acids, sugars and amino acids. They help in multiplication and faster establishment of association of rhizo-microflora with the host roots. Nitrogenase activity shows direct relationship with amount of organic carbon in the root exudates as in most of the cereals.

v) Effect of other microorganisms

Synergistic effects of *Azospirillum* in combined inoculation with other biofertilizers indicate that the microorganisms have more commercial potential in mixed culture. For instance, *A.brasilense* in combination with *Azotobacter chrococcum* produced more root mass than either of individual treatment in rice and maize. Similarly mixed inoculation of nitrogen fixing *Pseudomonas sp.* And *A. lipoferum* to rice seedlings promoted tillering per cent (Watanbe and Lin 1984). Favorable associate effects have been reported for *A. brasilense* and VAM fungi mixed inoculation to barley and pearlmillet (Subba Rao et al. 1985b). Additive beneficial effects have been also reported in combined inoculation of *Azospirillum* and *Rhizobium* on cowpea; *Azospirillum* and *Glomus macrocarpum* (VAM) on Panicum virgatum and Azolla + *Azospirillum* on rice.

Mixed inoculation shows better results even under unfavorable eco- environmental conditions inferring more scope of synergism in biofertilizer technology.

4.3. Azotobacter

Azotobacters are free living, gram negative, polymorphic, aerobic bacteria capable of fixing atmospheric nitrogen as non-symbiont. They belong to family Azotobacterrioaceae and important species are: *Azotobacter beijerinckii, A. chroococcum, A. paspali, A. vinelandii, Azomonas insignis, A. macrocyotgenes, Azotococcus agillis, Beijerinckia derxii, B. indica, B. glumensis, B. mobilis, Derxia gummosa, Xanthobacter autotrophicus, X. flavus.* Beneficial effects of Azotobacter inoculation have been observed in number of field crops including cereals, vegetables and ornamentals. Under laboratory it fixes nitrogen 20-40 mg per g of carbon, which is equivalent to 20-40 kg of nitrogen per ha. per year In addition, it secretes growth-promoting substances and suppresses the growth of pathogenic bacteria such as *Aspergillus* and *Fusarium.*

4.3.1. Nitrogen fixation

Azotobacters are non-symbiotic nitrogen fixers. They are recommended for wide variety of plants where supplementation of nitrogen to the tune of 40kg /ha is possible. Its application gave an yield advantage of 5-31% in rice, 16-30% in wheat, 9-38% in sorghum, and grain and stover yield advantage of 3.5 and 10.0% in pearlmillet. Increase in yield has also been reported in cabbage, greengram, sesame, bamboo, Chickpea, tomato, sugarcane, ragi, kale, sugarbeet, potato and cotton. Some observations taken on *Azotobacter* inoculation to crops at National Biofertilizer Development Center are presented in table 6. Many plus points with *Azotobacter* as biofertilizer are described here under;

Table 6. Crop yield advantage in response to Azotobacter inoculation.

Crop	Yield increase %
Potato	3.4-11.5
Onion	18-22
Tomato	11-19
Cabbage	20-40
Cauliflower	28-33.8
Brinjal	2-5
Cotton (Seed yield)	15.8-38.5
Wheat	5-12

Source: Pandey and Kumar (2002)

i. It can act as a supplemental source of nitrogen to wide variety of host plants.

ii. Anti- microbial activity towards soil pathogens indicates azotobacters potential in biological control of soil borne diseases.

iii. It has the capacity of colonizing rhizosphere in diverse ecological conditions.

iv. It is effective both with and without nitrogen application as basal dose.

v. Adds nitrogen to the soils even under fallow conditions.

4.3.2. Factors affecting nitrogen fixation

Free-living bacteria have earned special interest of agricultural scientists because of their wide range of host association. Azotobacter ranks top in potential and it is essential to study the ecological requirements of the bacterium for maximum biological nitrogen fixation. However ecological determinants for Azotobactor and other free-living microorganisms have not been yet fully elucidated.

i) Temperature

The lowest toleratable temperature is 16°C and highest being 37°C.

ii) Soil moisture

Azotobacter show a heavy demand for moisture with limiting pF values between 3.6and 4.4 corresponding to wilting point of higher plants.

iii) Aeration

Azotobacter being aerobic needs oxygen for better metabolism but efficiently fix nitrogen at low oxygen tension.

iv) pH

The bacterium flourishes around pH 7.2 to 7.6. Below pH 6.0 their growth and nitrogen fixing ability is drastically reduced.

v) Organic manures

A high degree of correlation between Azotobacter population and humus content exists. This may be due to easily and enough availability of utilizable carbon. Rather than proteinaceous wastes decomposting materials with no nitrogen create more conducive environment for Azotobacter.

vi) Chemical nutrients

High dose of nitrogenous fertilizers tend to slow down nitrogen fixation. *Azotobacter chroococcum* show marked high demand for *phosphorus*. *Molybdenum* is essential being an integral part of nitrogenase enzyme. The element is required at concentration of 0.1to 1.0 ppm during the process of nitrogen fixation. Iron is required for synthesis of nitrogenase (ferredoxin component). The requirement is in the range of 2-10 ppm. *Calcium* is needed by Azotobacters for growth and nitrogenase activity. *Aluminium* ions are toxic to *Azotobacter*.

4.4. Blue Green Algae (BGA)

Blue green algae are potent source of organic nitrogen for low land paddy. They promote paddy crop growth by supplying fixed nitrogen through exudation and microbial degradation of dead algal cells. 20-30 kg nitrogen per ha is fixed and an increase in yield in the range of 15-20% is observed. Besides, beneficial effects are also enhanced by way of producing indole acetic Acid (IAA) and Gibbrellic Acid (GA).

Blue green algae or cyanobacteria are the photosynthetic prokaryotes comprises 8 families which contain 27 genera, 489 species: Out of 489 species only 101 are known to fix nitrogen. Important genera are *Anabaena, Aulosira, Nostoc, Calothrix, Tolypothrix, Scytonema, westelliopais, Anabaenopsis, Cylindrospermum, Plectonema* and *Gloecocapsa*. *Anabaena azollae* forms symbiosis with fern Azolla. As blue green algae utilize the most inexhaustible energy source that is solar radiation for nitrogen fixation so they deserve priority for development of ecofriendly plant nutrient supply system.

4.4.1. Nitrogen fixation

Wide variety of blue green algae can fix nitrogen. Some are heterocystous in which the oxygen sensitive nitrogenase is protected in specialized non- oxygen evolving cells. Others fix nitrogen under microaerophilic or anaerobic conditions. It is observed that in the well-established field, nitrogen addition at the rate of 30 kg/ha is possible in a cropping season. Nutrient supply is through both exudation and microbial decomposition of algae. Increase in rice yield by algae inoculation has been reported to the tune of 15-25% (Kaushik 1995). Benefits realized from algalization are also implicated to various growth promoting substances produced by algae in addition to nitrogen. Moreover, presence of niacin, pantothenic acid, folic acid and various amino acids besides hormones substantiate the broad-spectrum effects of blue green algae on crop and soil. These may be summed up as:-

i. Improvement in soil aggregation.
ii. Increase on organic carbon of soil.
iii. Availability of phosphorus enhanced.
iv. Algalization brings significant decrease in pH, electrical conductivity and exchangeable sodium in saline and alkaline soils.
v. Soil biological activities are accelerated
vi. Higher grain yield with improved quality (Shukla and Gupta 1967).

4.4.2. Ecological determinants of blue green algae

Blue green algae have worldwide distribution but eco-physiological requirements have been studied in respect of rice fields.

i) Temperature

Wide adaptation to thermal range being found in polar region at -5°C to hot spring regions at 90°C is characteristic feature of BGA. Lowland paddy fields provide conducive environment of 25-35°C,mostly devoid of extreme of temperatures.

ii) Light

BGA are photosynthetic so light is the major requirement for their growth. Normal light intensities are sufficient for continuous nitrogenase activity. Their existence in deep soil, rhizosphere and phyllosphere explains myxotropicity nature of BGA. Some species can grow in shade while some other in dark (Chemoheterotrophs).

iii) Moisture

The optimum soil moisture content for the nitrogen fixing blue green algae has been found to be 80-100 per cent of soil dry weight. They are capable of withstanding droughts etc. because of high desiccation tolerance provided by thick mucilaginous sheaths.

iv) Aeration

Low oxygen levels enhance the nitrogenase activity.

v) pH

They occur in wide range of pH but optimal range is neutral to slightly alkaline.

vi) Organic manures

Addition of FYM, crop residues and green manures encourage blue green algae growth and proliferation.

vii) Inorganic nutrients

Nitrogen does not have repressive effect except at very high dose. Blue green algae show nitrogen-fixing activity in presence of nitrate nitrogen as well as ammonium nitrogen.

viii) Phosphorus

It has stimulating effect on nitrogen fixation ability of BGA.

ix) Others

BGA are also responsive to molybdenum, iron, cobalt and magnesium.

4.5. Azolla

Azolla is an aquatic fern widely distributed in tropical and temperate fresh water ecosystems. It harbors ponds, ditches, canals and rice fields. Interesting feature has been that the fern in symbiosis with blue green algae *Anabaena azollae* is quite efficient in nitrogen fixation on swampy lands including rice fields. *Anabaena azolloe* resides in dorsal lobes of leaves of Azolla and is mainly responsible for nitrogen fixation. The genus *Azolla* belongs to order Salviniales, family Azollaceae. The genus is divided into two sub-genera Euazolla and Rhizosperma. Important species are *Azolla caroliniana, A.filiculoides, A.*

microphylla, A. mexiana, A. pinnata and *A. nilotica*. The symbiosis can supply 20 to 80 kg of nitrogen per ha.

In addition to it Azolla serves as green manure and imporves soil quality characterstics. Azolla's green cover on soil surface suppresses weeds. It can also be used as supplemental feed source for birds and animals.

4.5.1. Nitrogen fixation

Nitrogen fixation by *Azolla-Anabaena* symbiosis is reported sufficient to meet the requirement of rice crop. Though combined use of chemical fertilizers and Azolla fern is suggested for sustaining high rice productivity. A great variation for nitrogenase activity has been reported among the different species. Overall nitrogen yielding potential is determined from growth rate and maximum biomass. Doubling time is 2-3 days in most of the species. Highest amount of nitrogen fixation in India is reported from *A. pinnata* that is 840kg N per ha per year. However highest estimate of 1000 kg N/ha/ year has been reported from Vietnam (Singh and Singh 1995). Watanbe (1982) recorded 2.8 to 3.4 kg N per ha per day in rice field.

Some useful facts about biofertilization with Azolla are as under: -

The incorporation of *Azolla* at the rate of 8-10 ton /ha is as effective as 30-40 kg N/ ha of ammonium sulphate.

i. Release of nitrogen from *Azolla* compost is much slower.
ii. In the rice fields split applications of chemical nitrogen have less adverse effect on *Azolla*.
iii. *Azolla* green manure increases the number and weight of the panicles, number of grains per panicle and nitrogen uptake and decreases percent spikelet sterility.
iv. A lower water level of 5-10 cm is desirable for fern growth as it increases mineral availability to soil.
v. Azolla multiplies vegetatively and can be maintained round the year.

4.5.2. *Azolla -Anabaena* symbiosis: Ecological determinant

Azolla can be used as a green manure fresh, dry or as compost and as a dual crop with rice. Total nitrogen content is 4 to 5 percent on dry weight basis. It contributes to physical, chemical and biological improvement of soil health. It is of recent consideration in India while its use in China and Vietnam has been centuries old. Yet role of ecology in its efficient performance is not fully understood.

i) Temperature

On the basis of the response to thermal environment Azolla forms are of several types (Table.7). Adoptability varies with place, season and isolate.

Table 7. Thermotypes in Azolla.

S. No.	Type	Azola sp.	Thermal range	Optimum (Distribution)
1.	Cold tolerant & heat sensitive	*A. filiculoides* *A. rubra*	-5 to 40°C	25°C (Temperate region)

2.	Heat tolerant & cold	*A. microphylla* *A. mexicana*	5 to 45°C	30°C (Tropical region)
3.	Cold and heat tolerant	*A. caroliniana* *A. pinnata var. imbricata*	-3 to 45°C	30°C (temperate dry)
4.	Non- tolerant to cold and heat	*A. nilotica* *A. pinnata var. pinnata*	1 to 40°C	25°C

ii) Light

Azolla flourishes in natural light intensity of 50 to 60 Klux.

iii) Moisture

For good growth, shallow water depth when the roots of Azolla just touch the soil is desirable. More water level leads to blowing off by wind and results in killing.

iv) pH

Azolla tolerates wide range of pH i.e., 3.5 to 10 but the optimum pH for it is 5to7.

v) Inorganic nutrients

Low application of chemical fertilizers has boosting effects on Azolla growth. Higher doses reduce nitrogenase activity. Phosphorus enhances both growth and nitrogenase activity. Other elements whose deficiency adversely influences *Azolla* functioning are molybdenum, iron, calcium, magnesium, potassium, manganese, cobalt, zinc, copper and boron. Their threshold levels are 1μg, 20μg, 0.5mmol, 20μg, 25μg, 0.04 mmol, 20μg, 30μg, 0.6μg and 20μg per litre respectively for nitrogenase activity.

4.6. Frankia

Biological nitrogen fixation has been observed in many woody species. The microorganisms associated with the process belongs to genus *Frankia*, family Frankiaceae and order Actinomycetales. The biofertilizer role of Frankia-nodulated plants has been recognized in improving the forest productivity and maintenance of woodland ecology. The bacterium contributes to soil nitrogen by establishing symbioses with more than 200 plant species mostly woody in nature. The ecological impact of the actinomycete nodulated plants can be enumerated in following points.

i. Nitrogen fixed in the root nodules is made available to the host for its own growth.

ii. Deciduous nitrogen fixing trees enhance soil fertility by shedding leaves in winter. Decomposition of leaves makes nutrients available to associated plant communities.

iii. Nitrogen is accreted in large quantity by actinomycete-nodulated nitrogen fixing woody plants (table.8). Through root exudates it is available to the roots of adjoining non-nitrogen fixing trees.

Table8. Some important actinorhizal plant species.

Species	**Nitrogen accretion kg N/ ha / year**	**Stand age**
Alnus crispa	62	0-40
Alnus glutinosa	125	0-8
Alnus rubra	140-209	7-30
Alnus rugosa	170	18
Alnus incana	50	0-30
Casuarina equisetifolia	58	13
Ceanothus sp.	100	0-12
Ceanothus velitunus	56	15
Coriaria arborea	150	14-20
Datisca glomerata	30	-
Dryas drummondi	12	0-25
Myrica cerifera	120	-
Myrica gale	34	-
Hippophae rhamnoides	179	13-16

Source: Rodriguez-Barrueco and Moiroud(1990)

iv. Relatively high nitrogen content of the fallen leaves (1.8 to 3%) lowers carbon nitrogen ratio in litter and accelerate decomposition in soil. Amount of total leaf-fall in a year from alder plant is 2.5 ton/ha while it is 14 ton /ha from *Elaeagnus orientails.*

✓ v. Leaf litter decomposition rate is low that leads to slow release of nitrogen and Consequently small nutrient losses. Alder leaf litter takes 1 to 4 years to decompose. Pine needles take 7 to 8 years while leaf liter of *Myrica gale* takes more than 10 years for complete decomposition.

vi. Actinorhizal plants throng well in desertic saline, degalaciated and unproductive soils.

Seabuckthorn (*Hippophae rhamnoides*) dominates cold deserts of India and may accumulate 60-80kg N /ha /year. These plants can play important role in vegetationalization of barren lands of the world.

4.6.1. Biological nitrogen fixation

Frankia nodulates a number of species ranging from trees to shrubs. Those trees which have established economic importance by way of stimulating growth of non-nitrogen fixing species are *Alnus* and *Casuarina*. Alnus covers temperate region while Casuarina is dominant in tropical and sub-tropical regions. Other genera that are used to enrich soils with nitrogen are *Coriaria, Myrica, Ceanothus, Purshia* and *Elaeagnus*. Non- nitrogen fixing trees, which show increased productivity in community of nitrogen -fixing symbiotic systems are *Populus, Fraxirus, Acer, Pinus, Liriodendrum, Platanus, Pseudotruga* and *Picea*. Long-term association of Populus with *Alnus crispa* exhibited higher biomass by

22.5 times and similarly Douglas fir interplanted with alder showed better height and diameter than pure plantation. Soil under mixed plantation possessed more organic matter and nitrogen. Annual nitrogen acceration by some actinomycete-nodulated nitrogen fixing species is given in table 8. *Alnus rubra* and *Hippophae rhamnoids* comes in the higher range. Thus, the bacterium Frankia symbiosis with actinorhizal plants has role as biofertilizer in sustainable silvicultural practices. Further the socio-economic and ecological significance of this association stems from their following features: -

i. A number of actinorhizal contributes nitrogen to the soil in range of 40-60 kg/ha/year that improves timber quality of interplanted trees.
ii. Actinorhizal pure stands are good enough to supply fuel wood, poles, tool handles and timber for light furniture.
iii. Many of them are used to improve marginal soils, to check soil erosion and stabilization of sand dunes i.e., use of *Hippophae* sp. in Nobra valley of Ladakh region of India

They can act as windbreak. Few species are good cover plants and soil binders. Thus, are used to check soil erosion due to wind and water.

4.6.2. Factors affecting symbiotic performance

The significance of Frankia–nodulated plants as biofertilizer is well recognized in forest plant communities. They have role in sustaining woodlands productivity by maintaining soil nitrogen economy, checking soil erosion and rejuvenation of wastelands. However many aspects of Frankia-plant association are still elusive or poorly understood. Frankia prevalence, infection and nodule development are not fully understood. Role of host and endophyte traits in establishing symbiosis has not been clearly established. Nonetheless evidence is growing to accept the Frankia-actinorhizal plant symbioses as nitrogen providers to the biosphere. Rodriguez- Barrueco and Moiroud (1990) proposed that for a more efficient actinorhizal system to identify thorough understanding of host, microsymbiont and environment is necessary.

4.6.2.1. Endophyte (Frankia)

i. Frankia strains classified as Sp+ nodule type produce sporangia and spores within the nodules. Nodules containing these structures have infectivity 100-1000 times greater than those lacking them.
ii. Ineffective or low nitrogen fixing strains compete for nodulation in soils with more efficient strains.
iii. Selection of efficient strains and inoculation with them is solution to the problem.
iv. Many a time Frankia population does not proliferate in accordance with range of host plant area. In such situations artificial inoculation is pre-requisite for symbiosis to occur.

4.6.2.2. Host plant

Frankia establishes symbiosis with a number of woody species with varying degree. *Alnus* has been reported most preferred host by *Frankia*. Host has to attract, encourage infection by symbiont. It should be also in position to supply required amount of energy through photosynthate translocation for growth of the Frankia as well as for nitrogenase activity. Gover *et.al.* (1987) identified the role of host in symbiosis at the following stage:

Recognition of the symbiont
Root hair invasion
Formation of infection threads
Nodule differentiation
Morphogenesis of Frankia in root tissue.

The physiological, biochemical and molecular basis of these interactions are still poorly understood. However preferred host species can be selected and in combination with efficient strains, the phenomenon can be utilized in sustaining the productivity of forest communities.

4.6.2.3. Environment

Like other micro-symbionts, Frankia growth, development and performance is affected by several environment parameters such as carbon source, oxygen levels, minerals, vitamins and association of other microorganisms. The use of nodulated actinorhizal plants in afforestation and successful plantation in marginal and poor fertile soils indicates that the symbiosis has broad spectrum adaptability covering various diverse and adverse situations. Individual factor analysis has not been largely carried, may because of problems in handling Frankia culture *in vitro*.

i. More nitrogen in external medium has repressive effect on nitrogenase activity.
ii. Introduction of third symbiont, particularly VAM fungus has been also suggested to boost the symbiotic performance.
iii. High variability to salinity among Frankia strains is observed. *Casuarina equisetifolia* with *Frankia* maintains N_2 fixation rates even at 200mM NaCl level.
iv. Some *Frankia* nodulated plants do well at extreme acidic pH of 3.1 to 3.5 with liming while *Frankia* strains *in vitro* tolerated a pH of 4.2.
v. *Frankia* spp. are abundant in cold environments but in *in vitro* conditions fail to show growth below 15°C.

⇒ 4.7. Vesicular Arbuscular Mycorrhizas (VAM) (from crop physiology notes of S.R. Arora)

Vesicular arbuscular mycorrhizas are obligate symbionts and exist in mutualistic association with roots of higher plants including agriculturally important crop plants.

They belong to family Endogonaceae with genera: *Acaulospora, Complexipes, Endogone,. Entophosphora, Gigospora, Glaziella, Glomus, Modicella and Sclerocystis.*

VAM are characterized by vesicles and arbuscules, which are used for storage of nutrients and channelizing these nutrients to the host root system respectively. VAMs' occurrence on wide variety of flora from bryophytes to pteridophytes, gymnosperms and angiosperms in diverse ecological conditions indicate their large range of adaptation.

VAM's association with increased uptake of phosphorus has been confirmed by radioactive studies with ^{32}P labeled phosphates. They may act both phosphorus solubilizers as well as mobilizers besides uptake of nitrogen, copper, zinc and sulpher etc. is also increased. In addition to nutrition other benefits to the host are:

i. Better survival of host under low moisture conditions,
ii. Equipping host with resistance against root disease.
iii. Increased growth and yield.
iv. Sustainable crop production because of improvement in soil structure.
v. Additive effects of benefits to host in mix culture of associative microbes.

4.7.1. VAM and host nutrition

Phosphorus nutrition to host through VAM fungi has been established. Mycelia network around roots increases contact area with soil so root hairs become capable of getting phosphorus from larger area through this network. Arbuscules are the main sites of transfer of phosphates from fungus to plant. This fungus-host interface phosphate transfer is mediated by an active transport mechanism involving membrane bound ATPase activity.

In addition phosphorus supply from labile pool, various VAM plants have been reported to produce Phosphatase, which converts insoluble phosphorus forms into soluble forms that are available to plants (See Bhandari *et. al.* 1990).

VAM enhance nitrogen uptake by assimilating ammonia via glutamate synthetase pathway while some VAM has nitrate reductase enzyme and can assimilate nitrate. VAM infection also favors uptake of Zn, Cu, Mn and Fe and also to limited extent hyphal translocation of sulphur.

Further VAM fungi may assist in water uptake in stress. The also synthesize growth-promoting substances like auxins, gibbrellins, cytokinins. Contribution to host performance through these aspects needs more credentials.

4.7.2. Crop responses to VAM

VAM improve host performance by affecting several yield contributing characters. Increase in yield results from more root /shoot ratio, more number, area and thickness of leaves and total dry matter production. In alfalfa increase in plant dry weight accompanied more total uptake of nitrogen and phosphorus. Effect of VAM inoculation on some agricultural crop plants is presented in table 9. Some important observations are:

Table9. Crop yield advantage due to VAM inoculation

Crop	VAM inoculant	Per cent yield increase over Check
Soybean	*Glomus fasciculatum*	26.40
Flooded	*Glomus fasciculatum*	1.90
Finger millet	*Glomus fasciculatum*	8.26
Groundnut	*Glomus fasciculatum*	20.90
Citrus	*Gogaspora sp.*	34.80
Onion	*Glomus fasciculatum*	39.60
Carrot	*Glomus fasciculatum*	42.00

Garlic	*Glomus fasciculatum*	36.80
Chilli	*Glomus fasciculatum*	5.70
Chickpea	*Glomus fasciculatum*	11.00

Source: Pandey and Kumar (2002).

(i) VAM inoculations are more advantageous in phosphrous deficient soils.

(ii) Mixed (dual) inoculation is more beneficial than individual culture such as *Glomus fasciculatum+Rhizobium japonicum* in soybean, *Glomus mossae+ Rhizobium* sp in faba bean. *Glomus fasciculatum+* native *Rhizobium* sp. in Luecaena, *Azospirillum brasilense+* VAM in barly and pearlmillet, *Rhizobium+ Glumus fasciculatum* in urd, moong and chickpea.

(iii) Addition of VAM fungi can replace the application of soluble phosphate.

(iv) VAM inoculation depresses the growth of soil borne pathogens.

4.7.3. Factors affecting VAM functioning

Functioning of VAM is related to species or its strain, host species or its variety and prevailing environmental conditions.

i. VAM fungi efficiency is related to competitiveness of the inoculated strain with native population and compatibility with the host.

ii. Plants differ with respect to mycorrhizal dependency. This difference also exists at varietal level. It may be due to differential phosphorous requirement of the host species.

iii. Pesticides, nitrogen, phosphorus and potassium applications have negative effects.

iv. Organic manures have favorable effects on VAM survival and proliferation

v. Dual inoculations (with Rhizobium or Azospirillum) boost the VAM activities.

4.8. Phosphorus Solubilizing Microorganisms (PSM)

Phosphorus solubilizing microorganisms are perhaps the most important biofertilizers with ample socio-economic perspectives. Phosphorus is the second most important plant nutrient and to optimize its availability, phosphatic fertilizers are added regularly. Only 15-25% of it applied P is utilized by plants. And rest is fixed in the soils in the form of iron or aluminium phosphates in acid soils or calcium phosphate in alkaline soils. Many microorganisms can solubilize inorganic phosphates into absorbable from (ortho-phosphate). Promising cultures are *Pseudomonas striata, Bacillus polymyxa, Aspergillus awamori, Penicillium digitatum, and Pseudomonas rathonls, Fusarium* sp.and *Trichoderma* sp.

Bacteria Fungi

The process of phosphate solubilization by these microorganisms is brought about by production of organic acids such as malic acid, glyoxalic acid, succinic acid, fumaric acid, citric acid and alpha-ketogluconic acid. Humic acid and folic acid react with aluminum and iron phosphates while citric, lactic and 2-ketogluconic acids are strong chelators for calcium.30-50kg of P_2O_5 per ha can be saved with an yield advantage of 10 to 20%. Besides solubilization of phosphorus, PSM also enhance uptake of P by the crops. They also benefit crops by producing growth promoting substances, too. Phospho-fungi do well in acidic soils while phospho-bacteria are active in neutral to alkaline soils.

4.8.1. PSM and plant nutrition

PSM have multifaceted role in plant nutrition. They reduce phosphate fixation in soil, enhance phosphorus solubilization and mobilization. Dephosphorylating process is carried by various organic acids and enzymes produced by microorganisms as a result of metabolisms and decomposition of organic matter. Rock phosphate, bone meal, di-and tricalcium phosphates, iron and aluminium phosphates etc. are converted into available P (orthophosphate) by the PSM. In barley uptake of phosphorus has been 24% higher in presence of microflora mineralizing fixed phosphates. The microflora is more concentrated in rhizosphere, revealing its role in phosphorus nutrition of host plants. The increase in yield has been reported in barley, soybean, berseem, maize, wheat, cowpea and rice. However yield increases in many cases were not significant but nutritional contribution of PSM was observed from better nutritional status of soil and improved quality yield of the crops National biofertilizer Development Center, Ghaziabad has studied response of crops to different PSMs (table10). The results are encouraging but further studies are needed to determine the parameters that accelerate the process of mineralization from fixed phosphates and rock phosphate by the PSMs.

Table 10. Increase in crop yield by PSM inoculation

Crop	Yield (kg/ha)		Increase in yield (%)
	Uninoculated	Inoculated	
Rice	2050	2300	12.2
Wheat	3616	4066	12.5
Gram	2038	2350	15.3
Cotton	2002	2322	23.2
Soybean	1400	1650	12.8
Onion	3400	4000	17.6
Sunflower	2250	2450	11.5
Mustard	2100	2300	9.2
Tomato	5000	6000	20.0
Pea	2600	2720	4.6

4.8.2. Factors affecting PSM activities

Various bacteria and fungi species are involved in the process of mineralization of phosphorus from insoluble phosphates. The microorganisms are found concentrated around rhizosphere. These microbes are also active during the process of composting. Further good quality compost in short period is possible by addition of efficient strains of *Penicillium* or *Aspergillus* spp. along with *Azotobacter*. Some of the factors that show relation with PSM activities are:

i. Glucose increases P solubilization activity of fungi as well as bacteria. PS fungi prefer glucose, sucrose, arabinose, mannitol and xylose. Bacteria prefer glucose and Sucrose

ii. Ammonium nitrate, asparagines, potassium nitrate, urea are the best sources of nitrogen.

iii. PS bacteria prefer neutral to slightly acidic pH while fungi works better at low pH of 4.0 to 5.0.

iv. Optimum temperature of PSM is around 25±5°C. Their activity is hampered at 35°C or above.

v. Magnitude of phosphorus solubilization property is species specific. PS fungi are generally more efficient than bacteria, actionmycetes and yeast. The variation for P solubilization also exists within the species.

vi. Synergistic effects of VAM fungus *Endogone* with PSM, *Agrobacterium* sp. and *Pseudomonas* sp. have been reported.

5. MECHANISMS OF BIOLOGICAL NITROGEN FIXATION

Biological nitrogen fixation (BNF) has been known since long but yet it awaits its exploitation for ecofriendly crop production. It is also fact that BNF is not restricted to rhizobia and the legumes.26 genera of bacteria including both photosynthetic and non-photosynthetic are now known to fix nitrogen. Rhizobia have received much attention and 14,000 sp. of legumes have been examined for symbiosis. The actinomycete Frankia are main nodulators on non-legumes and fix nitrogen for longer time.

Nitrogen fixation in microorganisms is carried by the enzyme nitrogenase whose activity is strongly ATP dependent. Molybdenum is component of all nitrogenases isolated from different microorganisms barring few exceptions. Fe protein and a Mo-Fe protein, a Fe –Mo cofactor, a strong reductant and Mg ATPase are the other components of nitrogenase system. Stoichiometry of nitrogen fixation is as under:

$$N_2+8H+8e+16ATP \longrightarrow 2NH_3+H_2+16ADP+16Pi.$$

It is clear that there is considerable requirement of ATP for N fixation. Hytler *et al* (1985) study of nitrogen fixation in soybean indicated that about 12g of carbohydrates are used for one gm of nitrogen fixation in soybean through symbiosis. Studies have also revealed that hydrogen evolution is associated ability of nitrogenase and 25% of ATP energy is used for this purpose .

Other proteins participating in the process of biological nitrogen fixation are leghemoglobin and nodulins. Leghemoglobin is a heme protein and unique to nodules formed by rhizobia or by *Frankia*. Its synthesis is controlled both by plant and bacteria that is protein component (globulin) by plants and heme component by the bacterium. The major function of leghemoglobin seems to be regulation of oxygen supply to bacteriods by facilitated diffusion. Nodulins are synthesized in response to infection and nodule development. They are thought to take part in ammonia assimilation or could be enzymes involved in ammonia assimilation.

The genetic map of nif regulon given by Brooks *et al* (1985) from the study of bacterium *Klebsiella pneumoniae* has a chromosomal location. It comprises 17genes in 7 operon. On the basis of functions of nif genes, they are classified as :

i. Structural genes for synthesis of nitrogenase (nif HD and K).
ii. Genes for electron transport proteins (nif F & J).
iii. Genes for synthesis of Fe-Mo cofactor (nif QBNE).
iv. Genes for processing of nitrogenase (nif USVM).
v. Genes synthesizing regulatory proteins (nif L & A).

The organization of nif genes has revealed a more complex system in other organisms. In symbiotic as well as free-living rhizobia, nif genes are located on plasmids. The number and size of these plasmids vary among species. These plasmids may be eliminated at elevated temperature, making the microorganism unable to fix nitrogen.

Functioning on nif genes is partially understood as it appears to be controlled by various factors besides its own cascade of controlling elements such as regulation by LA proteins, oxygen, temperature, molybdenum and post-transcptional pattern. However successful symbiosis that involves invasion, nodule development and establishment of host bacterium interaction indicates the role of several host genes also.

The much of information on BNF is gathering now from both symbiotic as well as free-living nitrogen fixing microorganisms. The mechanisms operates in many microorganisms under highly variable ecological condition. It offers good scope for its further improvement but still it demands more information on following aspects to be become a socio-economic and ecological tool for sustainable crop productivity.

i. There is limited knowledge on working of nif genes under different ecological factors such as salt stress, nutrient stress, moisture stress, temperature and oxygen supply.
ii. Microbial interactions within the species, between the species, or with other flora and fauna are least understood. Various reports on synergistic effects of rhizobia with phosphorus solubilizing bacteria have enlarged the commercial prospects of biofertilizers.
iii. Instability of nif plasmids in rhizobia is witnessed, particularly at high temperatures. The reasons for loss of plasmids could be internal or alteration in host or bacterium or both behavioral response which need to be elucidated to make *Rhizobium* applications more dependable.
iv. More genetical physiological and biochemical investigations required to be conducted on functioning of nif genes in working soil environment, which is obviously complex and challenging.
v. Genetic engineering of both the host plant and the symbiotic bacterium should be undertaken to develop more specificity in relationship to reduce the association with undesirable strains or species.

6. CROP RESPONSES TO BIOFERTILIZERS

Cereal-cereal cropping systems continue to dominate in Indian agriculture both under rainfed as well as irrigated production systems. Out of 250 double cropping systems; top ten that covered 20 million ha area, 16.91 million ha is being allocated to one or the other cereal. Soil productivity and fertility both are declining because of such monotonous and unsustainable cropping patterns. A need is shooting up to seek technological intervention to make the prevalent crop production systems more sustainable and ecofriendly. Needless to say biofertilizers are the potential area to be explored to meet the aspirations of degrading agro-ecological systems.

P-solubilizing bacteria

Many microorganisms inhabit the rhizosphere of agricultural flora. Barring few, their association with plants has been mutual benefiting. ① Acharya and Biswas (2002) observed significant increase in grain yield of chickpea with PSB treatment, which increased further, with addition of 30kg of P_2O_5/ha. Phosphorus uptake in grain and stalk was more by 22% and 25%. Seed protein content was enhanced by 5.74%. Thus both quantity and quality of plant product were improved (table11). ② Singh and Pareek (2003) reported that in mungbean combined inoculation of *Rhizobium*+PSB was superior to either of the individual treatments. But addition of 45 P_2O_5kg per ha to it showed significant interactive effects in terms of increased grain yield, seed protein content and uptake of nitrogen as well as phosphorus.

Table11. Response of chickpea to Phosphorus Solubilizing Bacteria

Treatment	Seed yield (Kg /ha) Grain	Phosphorus (Kg/ha) Stalk	uptake	Protein content (%)
Control (No P)	1555	5.42	6.52	19.86
PSB	1790	6.65	8.20	21.00
30 kg P_2O_5 /ha	2082	7.62	9.53	22.49
30 kg P_2O_5 /ha + PSB	2205	8.77	10.51	22.74
60 kg P_2O_5	2280	8.70	10.53	22.67
60 kg P_2O_5 /ha + PSB	2397	9.33	10.62	22.42
C D at 5 %	104	0.60	0.68	0.69

Source: Acharya and Biswas (2002)

③ Increase in wheat yield by 10-15% has been reported by inoculation with PSB (*Pseudomonas striata*) and VAM (*Glomus fasciculatum*) in sandy loam soil with neutral pH (Panda *et.al.* 2003). ④ Sorghum, pearl millet and finger millet, responded to *Azospirillum* inoculation with yield enhancement by 5-50% and resulting into saving of 15-40 kg of nitrogen per ha (table 12).

Table 12. Effect of Azospirillum application on some cereals

Cereal	Yield increase (%)	Nitrogen saving (Kg/ha)
Sorghum	9-30	10-40
Pearlmillet	4-83	13-20
Fingermillet	2-31	13-20

Source: Subba Raò (1988).

Varietal specific response to VAM inoculation has been reported in wheat (Al-Karaki and Al Reddar 1997). It was associated with increased uptake of phosphorus, zinc, manganese copper and iron (table 13). Several legumes including pulses and forages show stimulated growth by mixed inoculation of VAM and Rhizobium.

Table 13. Effect of Rhizobium inoculation and P application on chickpea yield and residual effect on succeeding crop.

Treatment	Chickpea (kg /ha)	Sorghum green Fodder	Chickpea (kg/ha)	Maize (kg/ha)
Control	1433	3240	1588	2195
Inoculation	2238	4300	2521	2773
40 kg P_2O_5 /ha	1953	3800	2188	2426
Inoculation + 40 kg P_2O_5 /ha	2427	4650	2708	2940
30 kg N /ha	1866	3920	2142	2130
120 kg N /ha	1744	3700	1971	2343
CD at 5 %	177	42	133	123

Source: Sharma *et. al.* (1995)

Effects of biofertilizers have been found benefiting to companion as well as succeeding crops. Sharma *et al* (1995) on their study on chickpea observed Rhizobium inoculation gave a grain yield advantage of 56%, and yield advantage of 32.7% was extended to succeeding sorghum green fodder crop. Highest benefit in host and succeeding crop was realized with treatment of Rhizobium+40kg P_2 O_5 per ha. In the same study sequence of chickpea and maize exhibited superiority over control by 58.7% and 26% as main and succeeding crops respectively (table 14)

Table 14. Effect of VAM on nutrient uptake in wheat

Treatment	Wheat	Uptake of nutrients				
		P(%)	Zn(ppm)	Mn(ppm)	Cu(ppm)	Fe(ppm)
No VAM	CR057	1.32	448	170	70	677
	CR006	1.34	327	172	63	645
VAM inoculation	CR057	2.22	528	262	106	1054
	CR006	2.40	480	245	102	864

Source: Al-Karaki and Al-Reddar (1997)

Crop responses to biofertilizers have been reported with many positive, favorable influences on plant, soil and environment. It is speculated that these microorganisms can play a pivotal role in maintenance of soil ecology and lastable increases in plant productivity per unit area are expected.

7. CONSTRAINTS IN BIOFERTILIZER APPLICATION

In spite of many salubrious effects on soil, plant and environment, biofertilizers are still not very popular with the farmers. And the actual problem has been reproducibility of the results. In the era of chemical fertilizers, farmers have become prone to quick response of these chemicals shown by crops.

Biofertilizers effects begin with small quantum, additive in action and last for longer time. At the same time being living organisms, their activities are governed by physical, chemical and biological environments surrounding them. Some issues of immediate consideration are listed below: -

i. Precise environment for growth and development is not fully known. Being soil flora, biofertilizers *in vivo* studies are difficult.

ii. Highly variable results with low reproducibility.

iii. Native strains of low efficiency compete with introduced strains and decrease establishment frequency.

iv. Nutritional deficiencies are also one of factors responsible for poor performance of biofertilizers. Nitrogen fixing organisms show high demand for phosphorus. Molybdenum is an integral part of nitrogenae enzyme.

v. Antagonistic soil organisms reduce activity of biofertilizers. (i) Rhizobia are destroyed by soil borne bacteriophages, amoebae, flagellates and nematodes. (ii) Antibiotic producing microorganisms attack Azotobactor. (iii) Pests infest Azolla and BGA. Phosphorus has unfavorable effects on VAM population (Panda *et. al.* 2003)

vi. Technical constraints comprise, isolation, mass multiplication and quality maintenance.

vii. Farmers participation in production and application technologies lacking.

viii. Non-availability of suitable packing material, transport and storage facilities.

ix. Inadequate efforts to motivate farmers through demonstrations by technically qualified personnels.

x. Proper marketing network from manufacturers to farmers is either lacking or ineffectively existing.

xi. For reproducible results, more understanding of phenomenon of biological nitrogen fixation is needed.

xii. Compatible combinations of efficient strains and efficient hosts need to be developed.

xiii. Dual or triple inoculation technologies of synergistic microorganisms should be evolved to make the biofertilizer applications more result productive and reproducible.

xiv. Multidisciplinary research coordination from plant breeding, microbiology, agronomy and soil science is highly wanted.