Proc Indian Natn Sci Acad **80** No. 2 June 2014 Spl. Sec. pp. 483-489

Review Article

Mass Production and Quality Control of Microbial Inoculants

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(Received on 10 Jan 2013; Revised on 10 June 2013; Accepted on 19 August 2013)

Since the commercialization of inoculants in India during late seventies, microbial inoculants popularly known as biofertilizers have come a long way. The journey that started with Rhizobium has now been diversified and various types of microorganisms have joined the race and are not only being exploited for nutrient mobilization or plant growth promotion but also playing a key role in plant protection. But, in spite of good going, the actual utilization is nowhere near the potential. Majority of the research work done in the field was on development of strains, their mode of action and on explaining the science behind their potentiality. Very little work has been done to convert the science into technology. Quality enforcement measures, which were missing till 2006, have been addressed to some extent by incorporation of some formulations under the provisions of Fertilizer (Control) Order 1985, but still a lot needs to be done. The present paper briefly summarizes the scenario of microbial inoculants (mainly biofertilizers like *Rhizobium, Azotobacter, Azospirillum,* Phosphate solubilizers, Potash solubilizers, Zinc solubilizers and mycorrhizal fungi) in terms of their mass production, technology involved, distribution, delivery of the product to the farmers and quality control issues.

Key Words: Mass Production; Quality Control; Biofertilizers; Microbial Inoculants; Liquid Formulations

Introduction

The commercialization of microbial inoculants popularly known as biofertilizers started in late seventies in India and currently they are being manufactured on a large scale. The journey of mass production of inoculants that started with *Rhizobium*, has now been diversified and various types of inoculants are commercially produced and utilized for nutrient mobilization and plant protection which play a key role in crop productivity. However, despite good potentiality of biofertilizer usage, the actual utilization is very low at about 2% of its potential. One of the initially introduced high potential biofertilizer, *Rhizobium* is losing the steam and is being gradually replaced by other inoculants such as phosphate solubilizing bacteria (PSB). With growing demand, many new inoculants are being launched and manufactured by the industry. Potash mobilizers, zinc solubilizers and consortia of biofertilizers are latest additions to the existing pool of biofertilizers manufactured in India [1].

Since the last 40 years, majority of the biofertilizer research work focused on development of strains, understanding their mode of action in the rhizosphere and on explaining the science of increasing biological nitrogen fixation. Very little scientific research has been done in the area of inoculant production to convert the science into technology but most of the inoculant producers in India continue to adopt the age-old practice of Burton technology for the preparation of inoculants. Automation technologies for carrier-based inoculants are not commonly adopted by the inoculant industry. Introduction of technology like use of sterile liquid

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inoculants with high microbial load for longer shelf life have contributed in addressing issues of increasing shelf life, contamination free products and tolerance to high temperature. However, it has yet to be adopted by majority of inoculant producers. Quality assessment measures, which were voluntary as per BIS specifications earlier, have been enforced by the government of India since 2006 under the provisions of Fertilizer (Control) Order 1985 (FCO 2006), so as to ensure production and distribution of quality biofertilizers in India. The present paper briefly summarizes the current scenario of mass production, technology used, distribution, and quality control standards of biofertilizers in India.

Production of Biofertilizers in India

As per the latest compilation on mass production [2, 3], India has about 225 biofertilizer production units with an installed production capacity of about 98,000 MT per annum. Against this, the actual production during the year 2010-11 was 37,997 MT. Out of various types of biofertilizers, PSB biofertilizers accounted for nearly 50% of total production and use. State wise details in respect of installed production capacity, actual production during the year 2010-11 and initial production figures for the year 2011-12 are given in Table 1. Share of different biofertilizers to total biofertilizer production is depicted in Fig. 1. Zone wise distribution of production is shown in Fig. 2. Remaining installed capacity is being used for the production of other microbial inoculants, mainly *Trichoderma*, *Pseudomonas fluorescens*, *Metarhizium anisopliae*, *Beauveria bassiana* and *Verticillium*, being traded as biopesticides. Share of biofertilizers to biopesticides in total production [2] is shown in Fig. 3.

Table 1: Installed production capacity and actual production of biofertilizers (including Mycorrhiza) during 2010-11 and 2011-12

S.No.	State	Installed production of capacity	Actual production of biofertilizers (MT) (MT/annum)	
			2010-11	2011-12
1	Andhra Pradesh	5825	999.60	1126.35
2	Arunachal Pradesh	0	0.00	0
3	Assam	225	130.00	68.33
4	Bihar	400	136.26	75.00
5.	Chhattisgarh	0	0	276.34
5	Delhi	2000	1205.00	1617.00
6	Gujarat	9550	6318.00	2037.35
7	Goa	1000	443.40	0
8	Haryana	775	6.53	914.41
9	Himachal Pradesh	25	9.00	1.29
10	Jharkhand	50	0.00	8.38
11	Karnataka	25488	6930.00	5760.32
12	Kerala	10400	3257.00	904.17
13	Madhya Pradesh	3750	2455.57	2309.06
14	Maharashtra	5315	2924.00	8743.69
15	Manipur	0	0.00	0
16	Mizoram	75	2.00	0
17	Meghalaya	75	0.00	0
18	Nagaland	150	21.50	13.00
19	Orissa	470	357.66	590.12
20	Punjab	575	2.50	692.22
21	Pondicherry	1900	783.00	509.45
22	Rajasthan	1500	819.75	199.78
23	Sikkim	0	0.00	0
20	Tamil Nadu	25265	8691.00	3373.81
24	Tripura	1000	850.00	1542.85
25	Uttar Pradesh	1690	1217.45	8695.08
26	Uttarakhand	550	45.00	263.01
27	West Bengal	300	393.39	603.20
	Total	**98353**	**37997.61**	**40324.21**

Distribution and Sale

Majority of the biofertilizers are being supplied to the farmers by the State Governments under different schemes with varied subsidy support ranging from 25% to 75%. Direct marketing of biofertilizers through dealers is yet to be adopted. Although many fertilizer companies claim to sell their biofertilizers through their direct network of dealers but it is under push strategy tagged with chemical fertilizers. Given

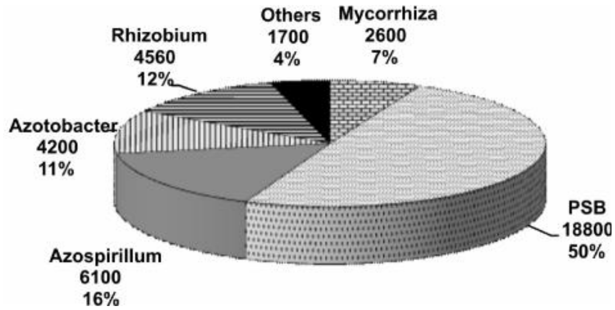

Fig. 1: Share of different biofertilizers to total production

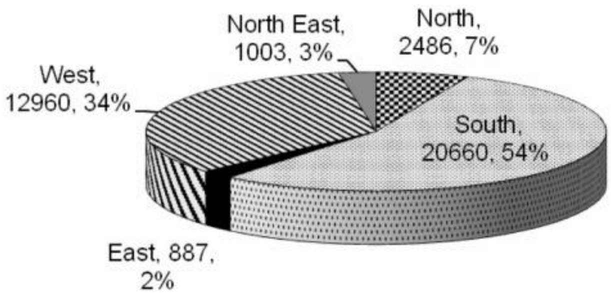

Fig. 2: Production of biofertilizers in different regions of the country

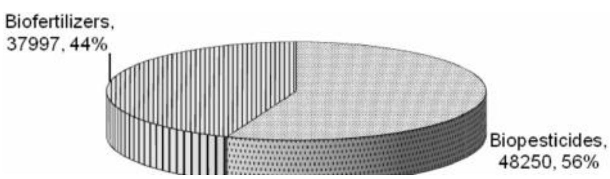

Fig. 3: Share of biopesticides and biofertilizers to total production of inoculants

the choice, majority of farmers is not willing to buy biofertilizers as part of their input package.

Poor acceptance among farming community is attributed mainly to their inconsistent response, short shelf life, temperature sensitiveness during storage and use, and poor quality in terms of total viable counts at the time of use.

Production Technology

Carrier based biofertilizer technology was disseminated by the research institutions in the country during late seventies till late nineties. Majority of the production units is still using the same technology of manual mixing of broth with lignite or charcoal as carrier material. In most cases, the carrier remains unsterile. The technology of biofertilizer production has two basic steps. In the first step, microbial biomass is up-scaled in a phased manner to desired microbial load and in the second phase, the pure bacterial broth is mixed with carrier material and is packed in polythene pouches under aseptic conditions. Although the basic technology is known for mass sterilization of carrier material before being impregnated with bacterial broth, but due to difficulties in sterilization of large volumes of carrier materials, majority of the production units ignore the carrier sterilization requirement. Handling of moist powder which was also sometimes lumpy and not free flowing also created hurdles in automation, resulting into practically no or very crude type of partial automation in final product handling and packing. Introduction of liquid formulations during early years of the 21st century provided some solutions to the problems of biofertilizer technology.

Liquid Formulations

Liquid formulations use liquid materials as carrier, which is usually water, oil or some solvents in form of suspension, concentrates or emulsions. Most popular liquid inoculant formulations [4] contain particular organism's broth 10-40%, suspender ingredient 1-3%, dispersant 1-5%, surfactant 3-8% and carrier liquid (oil and/or water) 35-65% by weight. Viscosity is adjusted at equal to the setting rate of the particles, which is achieved by the use of colloidal clays, polysaccharide gums, starch, cellulose or synthetic polymers.

Types of Microbial Inoculants (Biofertilizers) in Market

Rhizobium was the first microbial inoculant, which was introduced as biofertilizer during early seventies with the introduction of soybean into the country. *Azotobacter* and *Azospirillum* were added to the list in mid- nineties. Phosphate solubilising biofertilizer (PSB) was introduced in late nineties. During the same period, few more inoculants were added such as *Acetobacter* (or *Gluconacetobacter*), Potash mobilizer (*Frateuria aurantia* and *Bacillus* sp.), Zinc solubilizers and lately consortia of microorganisms comprising a mixture of *Azotobacter*, *Azospirillum*, PSB and *Pseudomonas fluorescens*. Mono cultures although continue to dominate the market but mixed cultures are picking up fast and may surpass the single strain inoculants in next 5 to 7 years.

Quality Control Initiative

Till 2006 although BIS standards were followed for assessment of quality for four types of biofertilizers, but it was voluntary in nature. As per the details available, only eleven manufacturers out of 135 (during 1999-2000) opted for BIS certification of their inoculants. Concerned with the continued quality issues, the Government of India brought four biofertilizers namely *Rhizobium*, *Azotobacter*, *Azospirillum* and PSB under the ambit of Fertilizer (Control) Order 1985 (FCO) during 2006. With the picking up of mycorrhizal biofertilizer production through tissue culture technique, the same was also brought under the FCO with separate specifications. Recently, two more biofertilizers, namely potash mobilizing and zinc solubilizing biofertilizers have also been incorporated under FCO [5]. Minimum specifications of these biofertilizers as specified in FCO are given in Tables 2 to 8.

Under the statutory provisions of FCO, biofertilizer production and its sales have been regulated and is a mandatory requirement of registration for every manufacturing unit with the State Fertilizer Controller (who is generally the Commissioner or Director of Agriculture Department). In every district, some officers of the Agriculture Department have been declared as Fertilizer Inspectors, who are authorized to inspect production and storage facilities and draw samples for quality analysis. National Centre of Organic Farming (NCOF), Ghaziabad and its six Regional Centres located at Bhubaneswar, Bangalore, Jabalpur, Nagpur, Hissar and Imphal have been declared as notified testing laboratories. Under the provisions of the act, State Governments can also develop their own quality control laboratories and notify them under the FCO 1985. So far, 11 State Governments have developed their quality control laboratories and notified their own biofertilizer testing laboratories.

For capacity building of personnel associated with quality control initiative, regular trainings are being organized by National Centre of Organic Farming and its Regional Centres. A ten-day training module for laboratory analysts and a five-day training module for field level officers and fertilizer inspectors have been designed.

Constraints Encountered

In spite of a remarkable growth of biofertilizer industry over the last 25 years in India, they are still far from their actual potential. Limited nutrient mobilization potential compared to their chemical counterparts and slow impact on crop growth are the major constraints. Inconsistent responses in the field under varied agro-ecological niches and cropping systems have also contributed to their low acceptance by farmers. Besides these, there are some technological constraints, which restrict the fast growth of biofertilizer industry. Some of the major constraints and limitations of the industry are as follows:

(a) susceptibility of strains to high chemical fertilizer use

(b) declining interest in scientific community on development of biofertilizer technologies

(c) dwindling number of culture collection banks leading to loss of hundreds of strains in the last 15 years

(d) deficiency in technology in respect of carrier suitability and product formulations

(e) lack of automation in product handling

(f) liquid inoculants are coming up as solution but the technology is still immature and not available in public domain

(g) distribution channels through Government agencies are not effective which are leading to cut throat competition among bidders, resulting in low cost poor quality inoculant production

Epilogue

Although biofertilizer technology has grown into a

Table 2: Specifications of *Rhizobium* biofertilizer

S.No.	Parameter	Requirements
(i)	Base	Carrier based* in form of moist/dry powder or granules, or liquid based
(ii)	Viable cell count	CFU minimum $5x10^7$ cells/g of powder, granules or carrier material or $1x10^8$ cells/ml of liquid
(iii)	Contamination level	No contamination at 10^5 dilution
(iv)	pH	6.5-7.5
(v)	Particles size in case of carrier based material	All material shall pass through 0.15-0.212mm IS sieve
(vi)	Moisture per cent by weight, maximum in case of carrier based	30-40%
(vii)	Efficiency character	Should show effective nodulation on all the species listed on the packet

*Type of carrier: The carrier materials such as peat, lignite, peat soil, humus, wood charcoal or similar material favouring growth of organism

Table 3: Specifications of *Azotobacter* Biofertilizer

S.No.	Parameter	Requirements
(i)	Base	Carrier based* in form of moist/dry powder or granules, or liquid based
(ii)	Viable cell count	CFU minimum $5x10^7$ cells/g of powder, granules or carrier material or $1x10^8$ cells/ml of liquid
(iii)	Contamination level	No contamination at 10^5 dilution
(iv)	pH	6.5-7.5
(v)	Particles size in case of carrier based material	All material shall pass through 0.15-0.212mm IS sieve
(vi)	Moisture per cent by weight, maximum in case of carrier based	30-40%
(vii)	Efficiency character	The strain should be capable of fixing at least 10 mg of nitrogen per g of sucrose consumed

*Type of carrier: The carrier material such as peat, lignite, peat soil, humus, wood charcoal or similar material favouring growth of the organism

Table 4: Specifications of *Azospirillum* Biofertilizer

S.No.	Parameter	Requirements
(i)	Base	Carrier based* in form of moist/dry powder or granules, or liquid based
(ii)	Viable cell count	CFU minimum $5x10^7$ cells/g of powder, granules or carrier material or $1x10^8$ cells/ml of liquid
(iii)	Contamination level	No contamination at 10^5 dilution
(iv)	pH	6.5-7.5
(v)	Particles size in case of carrier based material	All material shall pass through 0.15-0.212mm IS sieve
(vi)	Moisture per cent by weight, maximum in case of carrier based	30-40%
(vii)	Efficiency character	Formation of white pellicle in semisolid N-free bromothymol blue medium

*Type of carrier: The carrier material such as peat, lignite, peat soil, humus, wood charcoal or similar material favouring growth of the organism

Table 5: Specifications of Phosphate Solubilizing Bacterial Biofertilizer

S.No.	Parameter	Requirements
(i)	Base	Carrier based* in form of moist/dry powder or granules, or liquid based
(ii)	Viable cell count	CFU minimum $5x10^7$ cells/g of powder, granules or carrier material or $1x10^8$ cells/ml of liquid
(iii)	Contamination level	No contamination at 10^5 dilution
(iv)	pH	6.5-7.5 for moist/dry powder, granulated carrier based and 5.0-7.5 for liquid based
(v)	Particles size in case of carrier based material	All material shall pass through 0.15-0.212mm IS sieve
(vi)	Moisture per cent by weight, maximum in case of carrier based	30-40%
(vii)	Efficiency character	The strain should have phosphate solubilizing capacity in the range of minimum 30%, when tested spectrophotometrically
		In terms of zone formation, minimum 5mm solubilization zone in prescribed media having at least 3mm thickness

*Types of Carrier: The carrier material such as peat, lignite, peat soil, humus, wood charcoal or similar material favouring growth of the organism

Table 6: Specifications of Mycorrhizal Biofertilizers

S.No.	Parameter	Requirements
(i)	Form/base	Fine powder/tablets/granules/root biomass mixed with growing substrate
(ii)	Particle size for carrier based powder formulations	90% should pass through 250 micron IS sieve (60 BSS)
(iii)	Moisture content per cent maximum	8 -12%
(iv)	pH	6.0 to 7.5
(v)	Total viable propagules/g of product, minimum	100/g of finished product
(vi)	Infectivity potential	80 infection points in test roots/g of mycorrhizal inoculum used

Table 8: Specifications of Zinc Solubilizing Biofertilizers (ZSB)

S.No.	Parameter	Requirements
(i)	Base	Carrier based* in form of moist/dry powder or granules, or liquid based
(ii)	Viable cell count	CFU minimum $5x10^7$ cells/g of powder, granules or carrier material on dry wt basis or $1x10^8$ cells/ml of liquid.
(iii)	Contamination level	No contamination at 10^5 dilution
(iv)	pH	6.5-7.5 for carrier based in form of powder or granules and 5.0 – 7.5 for liquid based
(v)	Particles size in case of carrier based material	Powdered material shall pass through 0.15-0.212mm IS sieve
(vi)	Moisture per cent by weight, maximum in case of powder based	30-40%
(vii)	Efficiency character	Minimum 10 mm solubilization zone in prescribed media having at least 3 mm thickness

*Types of Carrier: The carrier material such as peat, lignite, peat soil, humus, wood charcoal or similar material favouring growth of the organism proven and assured biotechnological innovation, it is still struggling to get acceptability with the end users. There can be many factors behind this sluggish growth, but to ensure a sustained future and a sustainable and viable alternative to chemical approaches, biofertilizers need to play bigger role in the days to come. This would need not only a lot of refinement in the science and technology being employed, but also in creating awareness among users. An appropriate strain with proven efficiency record is the first and foremost requirement of science. Development of suitable carriers, refinement in formulation technology and integration of automation in product handling and packaging are the requirement of technology. Creation of dedicated storage and transport facilities with direct supply chain through a network of distributors and dealers, away from Government supported supply channel, will be the key to their success in the future.

With the growing awareness about and concerns for deteriorating soil health and mounting pressure for making plant nutrients available at affordable cost, efforts are being made to exploit microbial resources for crop nutrients biologically world over. Since early eighties biofertilizers are being promoted as important component of integrated nutrient management approach. With the emergence of organic farming, the contribution of biofertilizers has further gained importance. Industry and research fraternity have to share the responsibilities to overcome the constraints and offer appropriate, fail-proof biofertilizer input technologies to farmers.

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