

Full Length Research Paper

# Cost effective pilot scale production of biofertilizer using *Rhizobium* and *Azotobacter*

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We standardized the protocol for pilot scale production of *Rhizobium* and *Azotobacter* biofertilizer technology using region specific and environmental stress compatible strains isolated from various agro-climatic regions of Odisha, India. The cost benefit of biofertilizer production through a cottage industry is also presented. With an investment of \$5000 as fixed cost, recurring expenses approximately \$460 per year, indirect cost towards salary and wages \$4800, 24000 packets of biofertilizer can be produced. By selling the biofertilizer \$0.5 per pack, the net benefit is \$6000 per year. If the production capacity is quadrupled, the profit would be about \$30000 per year. This cost benefit calculation showed the possibility of entrepreneurship on microbial biofertilizers through cottage industry in rural areas in India as well as other developing countries.

**Key words:** *Rhizobium*, *Azotobacter*, biofertilizer, agro-climatic regions.

## INTRODUCTION

The contamination of the soil due to excessive use of chemical fertilizers and pesticides is one of the major problems facing agriculture (Bushby and Marshall, 1977). As a result of continuous use of agrochemicals, soil becomes degraded year after year, making it difficult to sustain the soil fertility. These chemicals not only cause immense damage to soil health but also create a chain of ecological and economic problems. Hence the alternative is use biological nitrogen fixation technology for maintenance of soil health and sustainable agriculture (Parr *et al.* 1990).

*Rhizobium* and *Azotobacter* are important plant growth promoting rhizobacteria (PGPR) microorganisms used as biofertilizer. They fix atmospheric dinitrogen under free-living condition and promote plant growth activities like phosphate solubilization, production of plant growth hormones like auxins, gibberellins, cytokinins, vitamins

and aminoacids (Kloepper and Schroth 1978, Sen and Palit 1988). Improvement in crop production due to *Rhizobium* and *Azotobacter* inoculation has been reported extensively (Kannaiyan *et al.*, 1980; Paul and Verma, 1999; Sattar *et al.*, 1997, Sethi and Adhikary, 2009 a, b). At present biofertilizers using these microorganisms are produced in large scale industrially and are available for field application (Bhattacharyya and Singh, 1992). Biofertilizer production technology comprises three important steps: 1) development of strains, 2) upscale of biomass, and 3) preparation of inoculants. Since the beginning of biofertilizer production in India, pure bacterial broth with high cell count is blended aseptically with sterilized carrier such as peat, charcoal and/or lignite so as to obtain a moist powdered formulation having high population of desired microbes (Gulati and Seth, 1973; Jauhari and Subba, 1984; Somasegaran and Hoben, 1985; Jauhari, 1988; Mishra and Dadhich, 2010). It is generally recommended that product free from contaminants and having a microbial load of approximately  $10^7$  cells per gram carrier can give

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optimum results of plant growth promotion in designated crop following recommended method of application (ISI, 1986).

The main bottleneck in the biofertilizer production by commercial units is that bacterial strains are usually developed and maintained by research laboratories but not by them. Further, in order to use efficient strains, focus is required to be paid to obtain region, soil and crop specific strains and make them easily available to production units as the up scaling of biomass is done in industries by entrepreneurs (Burton, 1981; Motsara and Bhattacharyya, 1994). As biofertilizer are live microbial preparation of very high cell count, the desired organisms are carefully monitored during the production process so as to obtain contamination free microorganisms. These problems in biofertilizer production technology have been addressed by server workers (Sen and Palit, 1988; SubbaRao, 1993; Sattar et al. 1997), however, proper procedure with cost effective calculation of biofertilizer production and marketing, especially at small scale level in rural areas has not been worked out to attract entrepreneurs to adopt biofertilizer technology as agri-business. In the present communication cost effective production of biofertilizer using *Rhizobium* and *Azotobacter* in rural areas of India through cottage industry taking into consideration of the operating cost and the wage of workers is presented.

## METHODOLOGY

For mass production of *Rhizobium* and *Azotobacter* in pilot scale biofertilizer technology the following equipments are required: (1) Twenty liter fabricated fermenter fitted with an air pressure pump, (2) horizontal rotary shaker, (3) vertical autoclave with pressure regulator, (4) incubator, (5) refrigerator, (6) inoculation chamber (with provision of UV-C), (7) distillation apparatus, (8) microscope, (9) polythene sealer and (10) air-conditioning provision.

Region specific and environmental stress compatible bacterial strains isolated from various agroclimatic regions are obtained from authentic sources, grown in slants and transferred to liquid broth in the rotary shaker to prepare mother culture. The cultures are grown in large scale in the fermenter for up to five days, harvested in batch culture mode and then mixed with unsterile forest soil: charcoal in a ratio of 1:3. The shade dried bacteria and the carrier

are mixed and packed, 200 g each in polythene pack, and stored for use as biofertilizer for the desired crop.

## RESULTS AND DISCUSSION

For limited scale production of biofertilizer two glass fermenters of 10 L capacity are used for each batch. For one litre of bacterial culture 5 kg of carrier material is required for biofertilizer preparation. Thus 100 kg of carrier based biofertilizer is produced from two fermenters in each batch or 500 packets of biofertilizer, each of 200 g. From one time harvest in a week (in average) 500 packets of carrier based biofertilizer can be produced. Thus in a month, production will be 2000 packets and in a year it will be 24000 packets (4800 kg from 960 l of broth).

For production the above quantities of biofertilizer one skilled and one unskilled worker are required. Following the above protocol the cost benefit of commercial production of bacterial biofertilizer (*Rhizobium* as well as *Azotobacter*) is as follows.

### Step-wise calculation of pilot scale production cost of *Rhizobium* and *Azotobacter* biofertilizers (as per existing cost of utilities, wages and labor cost etc. in eastern regions of India):

- (i) Fixed cost: It includes capital investment on equipments.
- (ii) Variable cost: Raw material cost, carrier material, broth (that is, chemicals), polythene bags for package of biofertilizer.
- (iii) Indirect cost:
  - (a) Salary of the staff; marketing cost: Transport cost, power consumption per packet; publicity cost; marketing margin; whole sale and retail, subsidy or commission if any, risk coverage against unsold packets.
  - (b) Miscellaneous expenses.

### Fixed cost (non-recurring)

Equipment	Cost (\$)
Glass fermenter (fabricated with aerator), 2 nos.	3000
Autoclave (one electrically operated), 1 no. 20 L capacity	600
Glass double distillation set 5 L/hr capacity, 1 no.	200
Incubator, 1 no.	400
Compound microscope (binocular), 1 no.	300
Refrigerator, 1 no.	200
Inoculation chamber with UV. 1 no. (fabricated)	100
Polythene sealer, 1 no.	100
Chemical balance, 1 no.	100
Total	5000

**Variable cost per year (recurring)**

Material	Quantity	Cost of the material (\$)
Carrier material	10 quintal	80
Broth	1000 L, \$0.25 per litre	250
Polythene bag	30000, \$1 per 1000 bags	30
Recurring expenses per year (consumables) plastic/ polypropylene bottle + plastic wares + glass wares (conical flask, pipettes, test tube, measuring cylinder, beaker etc.)		100
<b>Total cost</b>		<b>460</b>

**Requirements of chemicals in the production process:** For production of *Rhizobium* broth per 1000 packets require 40 L of broth, say an example Himedia chemical cost with VAT as per 2009 to 2010 price lists as follows:

Chemical required	For 40 L (g)	Total cost for 40 L (\$)
Mannitol	400	7.5
Yeast extract	16	1.0
NaCl	4	0.5
MgSO <sub>4</sub> . 7H <sub>2</sub> O	8	1.0
K <sub>2</sub> HPO <sub>4</sub>	20	1.0
<b>Total cost</b>		<b>11.0</b>

So for 40 l broth the cost of required chemicals = \$11  
For 1 l = \$11 ÷ 40 = \$0.275

**Indirect cost:** Salary and wages per annum (as per the existing wages in rural areas of eastern regions of India):

production of bacterial biofertilizer is = \$6000 or \$500 per month.

Salary component	Value (\$)
One Microbiologist (skilled) per month	200
One production assistant (unskilled) per month	100
Miscellaneous expenses per year (Maintenance of equipment, fuel charge, office expenses etc.)	100
<b>Total cost</b>	<b>500</b>

Thus for 24000 packets of the biofertilizer the production cost is the variable cost + indirect cost = \$5260 + 10% depreciation on fixed cost \$500 = \$5760 or say \$6000. Therefore, per packet production cost is Rs. \$6000 ÷ 24000 packets = \$0.25

Price per packet of biofertilizer is calculated as follows:  
Production cost per packet = \$0.25  
Power consumption (0.046 KW/packet) = \$0.05  
Profit margin = \$0.2  
So sale price per packet is = \$ 0.5

**Return:** Net sale value of 2000 packets per month of biofertilizer using 80 L of broth in four batches (one batch per week) is \$1000 or per annum = \$12000. By subtracting the \$6000 towards investment for the production the net profit per year through this pilot scale

**Alternatively**

Alternatively if the production capacity will be multiplied by four times using eight fermenters; 10 L capacity each, the cost benefit calculation is as follows:

**Fixed cost:** The cost would be \$12000 (including cost of six more fermenters) + \$200 for other equipments = \$14000. So from eight fermenters production of biofertilizer is 96000 packets of 200 g each.

**Variable cost:** The cost of carrier material, broth and polythene will be four times more than the total cost which is \$1840.

**Indirect cost:** The additional man power required with a salary component is as follows:

1 microbiologist, \$240 per month = \$2880  
1 production assistant, \$160 per month = \$1920  
1 marketing and sale personnel, \$160 per month = \$1920  
1 laboratory attendant cum peon, \$80 per month = \$960  
Miscellaneous expenses = \$2000  
Total cost = \$7680

**Return:** Net sale value of  $24000 \times 4 = 96000$  packets of biofertilizer per year with a price of \$0.5 per packet = \$48000

Investment for production: 10% depreciation against fixed cost (\$1400) + variable cost (\$1840) + indirect cost (\$7680) = \$10920

Gross profit per year = \$48000 – \$10920 = \$37080

After deduction of the cost of advertisement in print and electronic media of about 20% from the gross profit the net profit is = \$37080- \$7416 = \$29664 per year or say \$30000 or \$2500 per month.

In the above production system, unsterilized carrier (forest soil: charcoal) in the dried form after grinding is used to avoid the natural population of microorganisms to compete with the inoculated bacterial broth. However, if sterile carrier is used the cost would be higher. From our experience it was found that the brown forest soil available in most of the central and southern regions of Odisha State and India is free from microbial load, hence is used as a carrier even in non-sterile conditions.

### Quality control

Quality of inoculants in the biofertilizer pack is one of the most important factors resulting in their success or failure and acceptance or rejection by the farmers. Basically the quality means the presence of right type of microorganism in active form and in desired numbers. The stages requiring quality control are during mother culture stage, during carrier selection, during broth culture stage, while mixing of broth with carrier, during packing and during storage. Testing of the culture is usually done by taking a sample from the finished product for comparison with standard specification at the time of mixing of broth with carrier. In India, the Indian Stand Institution has developed standard for *Rhizobium* and *Azotobacter* (ISI, 1986). The standard prescribed is that the inoculants shall contain a minimum  $10^7$  cells per gram of carrier on dry mass basis within 15 days before the expiry date marked on the packet when the inoculants is stored at 25 - 30°C (Jauhari 1988, Subba Rao, 1993, Matsara and Bhattacharyya 1994). If hazards imposed by harsh storage and transport conditions under tropical climatic regime are minimized, either by decentralization of manufacturing units or by rapid transportation to farmers at the time of showing/planting, the quality of biofertilizers reaching the end users can be improved to a greater extent.

From the above cost benefit of *Rhizobium* and *Azotobacter* biofertilizers production through cottage industry, entrepreneurship can be developed which would be highly beneficial for sustainable agriculture, and in addition, can lead to additional income generation of the farming community in rural areas of India and other developing countries.

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### REFERENCES

- Bhattacharyya P, Singh T (1992). Simplified methods of mass production and application of biofertilizer. Proc. Natl. Sem. Organic farming. Edited by Rai MM, Verma LN, JNKVV and IGKVV, Indore, India, pp. 166-178.
- Burton JC (1981). *Rhizobium* inoculants for developing countries. Trop. Agric. 58: 291-295.
- Bushby HVA, Marshall KC (1977). Some factors affecting the survival of root- nodule bacteria on desiccation. Soil Biol. Biochem. 9: 143-147.
- Gulati SL, Sethi VK (1973). An improvised portable fermentor for small scale production of *Rhizobium* culture. Curr. Sci. 42: 394-395.
- ISI (1986). Indian standard specification for *Rhizobium* inoculants. Standard: IS 8268-1986.
- Jauhari KS (1988). Dilution optima of broth cultures for maximizing production of carrier based inoculants. Curr. Sci. 57: 507-509.
- Jauhari KS, Subba Rao NS (1984) Simple glass fermentor for *Rhizobium* culture production. Curr. Sci. 53: 984-985.
- Kannaiyan S, Govindarajan K, Lewin HD (1980). Effect of foliar spray of *Azotobacter chroococcum* on rice crop. Plant Soil. 56: 487-490.
- Kloepper JW, Schroth MN (1978). Plant growth promoting rhizobacteria on radish. In: Proc. of the 4th Int. Conf. on Plant pathogenic bacteria. pp- 879-882. INRA, Angers, France. Apte R, Shende ST, Zbl. Bakt. II, 136, pp. 548-554.
- Motsara MR, Bhattacharyya P (1994). Status of biofertilizer and onstraints in their large scale commercialization. In. Commercialization of biotechnology for agriculture and aquaculture, edited by. Srivasatava P, Chandrasekhar KS, Oxford and IBH Publ. Co. pp. 285-298.
- Mishra BK, Dadhich SK (2010). Methodology of nitrogen biofertilizer production. J. Adv. Dev. Res. 1: 3-6.
- Parr JF, Stewart BA, Hornick SB, Singh RP (1990). Improving the sustainability of dryland farming systems: A Global perspective. Adv. Soil Sci. 13: 1-5.
- Paul S, Verma OP (1999). Influence of combined inoculation of *Azotobacter* and *Rhizobium* on the yield of Chickpea (*Cicer arietinum* L.). Ind. J. Microbiol. 39: 249-251.
- Sattar MA, Khanam D, Ahmad S, Haider MR, Podder AK, Bhuiyan MA (1997). In Extending nitrogen fixation research to farmers fields, edited by Rupela OP, Johansen CC, Herridge DF (ICRISAT, Patancheru, India), pp. 227-234
- Sen P, Palit P (1988). Biofertilizers – potentialities and problems. Naya Prokash, Calcutta, pp. 51-56.
- Sethi SK, Adhikary SP (2009a). Vegetative growth and yield of *Arachis hypogea* and *Vigna radiata* in Response to Region Specific *Rhizobium* Biofertilizer Treatment. J. Pure Appl. Microbiol. 3: 295-300.
- Sethi SK, Adhikary SP (2009b). Efficacy of region specific *Azotobacter* strain on vegetative growth and yield of *Solanum melongena*, *Lycopersicon esculentum* and *Capsicum annum*. J. Pure Appl. Microbiol. 3: 331-336.
- Somasegaran P, Hoben HJ (1985). Methods in legume *Rhizobium* technology. NifTAL, MIRCEN, Univ. Hawaii, Paia, USA, p. 367.
- Subba Rao NS (1993). Biofertilizers in Agriculture and Forestry, edited by Subba Rao NS (Oxford and IBH Publishing Co, New Delhi), p. 242.