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Evaluation of low cost and indigenous substrates as carriers of *Azotobacter* and phosphate solubilizing bacterial biofertilizers production

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Charcoal being an inert material is most popularly used as an ideal carrier in the production of various biofertilizers. Non-availability of charcoal in the powder form or even if available, its expensiveness increases the cost of biofertilizer production. Therefore, in the present experiment three locally available, low cost sunstrates carriers like charcoal (CC), flyash (FA) and peat soil (PS) alone and in combination with each other (1:1 ratio) were used to evaluate their suitability as carriers for *Azotobacter* and PSB biofertilizers. It was observed that CC+FA maintained maximum *Azotobacter* population (8.75×10^8 cells/g carrier) followed by CC+PS (8.25) and FA+PS (8.00×10^8 cells/g carrier) even after 120 days storage of. Whereas, the individual substrates recorded lesser population after the similar period of storage. Further, storage beyond 120 days after mixing (up to 210 days) however, caused significant decline in the population of both the types of bacterial cells. This indicates the combination of charcoal and fly ash is a better and cheaper alternative with an extended storage life for use as carrier for *Azotobacter* and the PSBs instead of charcoal only, the present carrier.

Key words : *Azotobacter*, PSBs, biofertilizers, low-cost carriers, bacterial count

INTRODUCTION

Improtance of biological N_2 fixation through the application of various diazotrophs as microbial manures has been greatly emphasized by various workers (Subba Rao, 1986; Sen and Palit, 1988; Sudhakar *et al.,* 2000). Carrier based microbial inoculants have almost completely replaced liquid cultures and other slope cultures which were so commonly used a few decades ago. Microbial inoculants as carrier-based preparations containing beneficial microorganisms in a viable state are intended for seed and soil applications and designed to inprove soil fertility and help plant growth by increasing the number and biological activity of desired microorganisms in the root environment. Starting from modest laboratory preparations in mid 30s of this century in USA, various base materials have been utilized as carriers of the microbes. Burton (1967) in USA utilized sedge peat to mix with rhizobial cell population of 10^9 cell/ml with a final moisture content of 35-40%. Date (1974) in Australia used milled dry peat as carrier. In India, peat like material available in the Nilgiri Valley (Iswaran *et al.,* 1969) and lignite (Kandasamy and Prasad, 1971) were utilized as carrier materials. Following the seccess of legume inoculants all over the world, carrier based *Azotobacter, Azospirillum* and phosphate solubilizing bacterial (PSBs) inoculants using powdered peat soil, lignite, farmyard manure (FYM) for various agricultural crops (leguminous and non-legume) have become increasingly popular during recent years (Subba Rao, 1986; Wani and Lee, 1992).

Tilak *et al.,* (1979) and Tilak and Subba Rao (1978) studied various indigenously available carriers for their ability to support the growth of *Azospirillum* and *Rhizobium* inoculants. They found charcoal alone and its combination with other materials as suitable carriers. However, non availability of charcoal in the required form (powdered), even if available being very expensive, is not only

hampering the production of biofertilizers but also escalating the cost of the same. Hence, search for other alternative indigenous and low cost carrier materials have become an imperative. During recent past large quantities of flyash, a burnt coal ash (95 mt/ year, Kumar *et al.*, 2001), is being produced by NTPC power plants which is spoiling the fertile soils andposing a safe disposal problem. Mission mode efforts supported by different funding agencies have been initialted to find out alternative uses of flyash such as through vermicasting (Chattopadhyay and Bhattacharya, 2000), as a carrier material in VA mycorrhizal production (TATA Industries) and as a neutralizing manure in the acid soils (Srivastava and Chhonkar, 2000, 2000a; Kumar *et al.*, 2001). Flyash being neutral in pH is almost having the similar characteristic features as that of charcoal and is available free of cost, hears great potential for use as a carrier in biofertilizer production which has not yet been tested in this field.

Hence, the present attempt was made to explore the possibility of utilizing flyash and other indigenous materials alone and in combination to each other to evolve a suitable carrier to economize the commercial production of *Azotobacter* and PSB biofertilizers at this institute during the year 2001-2002 as a pilot project.

MATERIALS AND METHODS

Five low cost and indigenously available substrates such as vermicompost, farmyard manure (FYM), peat soil (PS) collected from the bottom of ponds, charcoal (CC) and flyash (FA) were selected for the evaluation. The former 3 are rich in organic matter content and possess high microbial flora and the latter two are inert materials. All the carriers were dried and powdered finely as to pass through 200 mesh sieve. A preliminary suitability screening of the above materials was made through recording of natural bacterial and fungal flora and effect of sterilization by UV exposure autoclaving. On the basis of the above, 3 materials, viz. CC, PS and FA were selected for further study. The chemical characters of above 3 materials and their combination with each other in 1:1 ratio (w/w) was studied before and after mixing the bacterial broth. Six days old *Azotobacter* and phosphate solubilizing bacterial (PSBs) broth cultures with a population load of $>10^{10}$ cells/ml were blended with individual substrates and their combinations as per the requirement so as to adjust the moisture level to 50% of WHC of the carriers. The required quantity of the bacterial broth was determined for blending with different carriers of the basis of convenience for packaging.

After prepatation of both types of biofertilizers in different carriers, they were stored at room temperature (30°C). Viability of blended bacterial cells, other microbial contaminants and moisture content was nonitored from 0 to 210 days at intervals of 30 days by using DPT method on nutrient agar media plates.

RESULTS AND DISCUSSION

Screening of the materials

Though all the materials selected for the study have been lowcost and indigenous, still they varied in their natural content of microbial flora (Fig. 1). Vermicompost and farmyard manure (FYM) being organic in origin contained highest bacterial (48×10^7 and 28.5×10^6) and fungal population (25×10^5 and 18.5×10^4 cells/g). Charcoal and flyash being inert materials in nature contained lower populations (23.6×10^3 and 62×10^2 for bacterial and 6.5×10^3 and 68×10^2 cells/g for fungal). Peat soil contained the above populations in middle range. After sterilization through the exposure to UV radiation the microbial populations came down significantly in all the carriers but they could not be eliminated totally. The autoclaving had a better effect on all the materials. In the case of charcoal and flyash the bacterial population was reduced to about 11 cells/g with no fungal population. On the basis of these results vermicompost and FYM were dropped from further study. Other reasons for rejection has been their bulkiness, high labour cost required for making them powder and susceptibility to quick contamination having antagonistic effect on the desired microbes (Tilak and Subba Rao. 1978 and unpublished data of the authors).

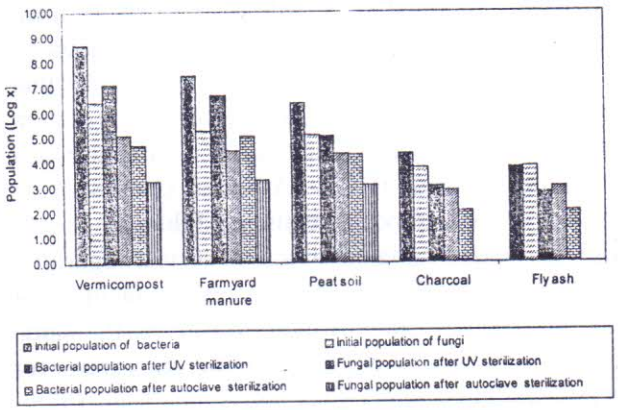

Fig. 1 : Microbial population in various substrates before and after UV/autoclave sterilization

Table 1 : Chemical characteristics of different carriers before and after blending with bacterial broth

Carriers	OC (%)	WHC (%)	pH		Quantity* of bacterial broth (ml/kg)	Moist content (%)
			Initial	After mixing with bacterial broth		
Charcoal (CC)	21.60	114.2	9.0	7.2	600	41.63
Fly ash (FA)	Trace	37.1	7.8	6.8	200	16.56
Peat soil (PS)	41.65	66.2	7.0	6.5	250	23.71
CC+FA	28.95	75.6	8.7	7.0	400	30.37
CC+PS	55.25	90.2	8.5	7.2	400	32.62
FA+PS	39.50	51.6	7.6	7.0	400	28.92

* Quantity of bacterial broth mixed with the carrier was standardized as per convenience of packeting.

Table 2 : Survival of *Azotobacter* and PSBs in various carrier based biofertilizers at different intervals of storage

Carriers	Bacterial population after blending with carriers (x/g)							
	0 days (× 10^{10})	30 days (× 10^{10})	60 days (× 10^{9})	90 days (× 10^{8})	120 days (× 10^{8})	150 days (× 10^{7})	180 days (× 10^{6})	120 days (× 10^{6})
1. *Azotobacter*								
Charcoal (CC)	5.62 (10.75)	0.56 (9.75)	3.16 (9.50)	31.62 (9.50)	0.18 (7.25)	0.89 (6.95)	5.62 (6.75)	3.16 (6.50)
Fly ash (FA)	3.16 (10.50)	0.32 (9.50)	1.00 (9.00)	0.56 (7.75)	0.18 (7.25)	0.03 (5.50)	0.32 (5.50)	0.18 (5.25)
Peat soil (PS)	0.32 (9.50)	5.62 (10.75)	3.16 (9.50)	31.62 (9.50)	0.56 (7.75)	0.56 (6.75)	3.16 (6.50)	0.18 (5.25)
CC+FA	5.62 (10.75)	0.56 (9.75)	10.00 (10.00)	31.62 (9.75)	5.62 (8.75)	3.98 (7.60)	31.62 (7.50)	1.78 (6.25)
CC+PS	10.00 (11.00)	5.62 (10.75)	5.62 (9.75)	56.23 (9.75)	1.78 (8.25)	0.79 (6.90)	5.62 (6.75)	3.16 (6.50)
FA+PS	5.62 (10.75)	5.62 (10.75)	10.00 (10.00)	56.23 (9.75)	1.00 (8.00)	1.78 (7.25)	3.16 (6.50)	1.78 (6.25)
Source	CD at 5%					SE ±		
Carriers	0.70					0.25		
Interval	0.81					0.29		
Carr. × Inter.	NS					0.71		
2. Phosphate solubilizing bacteria (PSBs)								
Charcoal (CC)	1.77 (10.25)	5.62 (10.75)	5.62 (9.75)	31.62 (9.50)	0.56 (7.75)	3.16 (7.50)	17.78 (7.25)	3.16 (6.50)
Fly ash (FA)	1.77 (10.25)	1.77 (10.25)	1.00 (9.00)	1.00 (8.00)	0.32 (7.50)	0.18 (6.25)	0.32 (5.50)	0.18 (5.25)
Peat soil (PS)	3.16 (10.50)	3.16 (10.50)	3.16 (9.50)	1.78 (8.25)	0.32 (7.50)	1.78 (7.25)	0.32 (5.50)	0.18 (5.25)
CC+FA	5.62 (10.75)	5.62 (10.75)	5.62 (9.75)	56.23 (9.75)	3.16 (8.50)	3.16 (7.50)	10.00 (7.00)	3.16 (6.50)
CC+PS	5.62 (10.75)	5.62 (10.75)	5.62 (9.75)	56.23 (9.75)	1.78 (8.25)	3.16 (7.50)	3.16 (6.50)	1.78 (6.25)
FA+PS	10.00 (11.00)	5.62 (10.75)	5.62 (9.75)	56.23 (9.75)	1.00 (8.00)	3.16 (7.50)	3.16 (6.50)	0.56 (5.75)
Source	CD at 5%					SE ±		
Carriers	0.76					0.27		
Interval	0.88					0.32		
Carr. × Inter.	NS					0.78		

* Figures in parenthiesis are Log transformations.

Chemical characteristics like organic carbon content (OC), water holding capacity (WHC), pH (before and after mixing the bacterial broth), and moisture content (after mixing of the culture broth) were also determined in above 3 parent materials and their combinations (Table 1). An ideal carrier should have neutral pH with sufficient OC and WHC for better multiplication of bacteria. The chemical analysis reveals adequate content of above attributes in the mixtures. Though CC had higher WHC but the water content was in non-available form for the bacteria. The added advantage with the mixtures was that they required lesser quantity of bacterial broth (400 ml/kg) to prepare the commercial grade of biofertilizers. As FA and PS consumed lesser quantity of bacterial broth (200-250 ml/kg) they were able to maintain low level of moisture content. CC absorded 600 ml/kg of bacterial broth indicating it to be uneconomic.

Evaluation of microbial viability in various carriers

On the basis of the above results, 3 parent materials (CC, FA and PS) and their combinations were taken up for the evaluation of viability of the bacterial cells of *Azotobacter and* PSBs over a period of time (0 to 210 days after mixing), besides the assessment of contaminants (saprophytic bacterial and fungal population).

a. ***Azotobacter population***

Population of *Azotobacter* in different carriers and between the intervals varied significantly but it was not significant in carrier x interval interaction. The initial *Azotobacter* population after mixing the broth with the carriers ranged from 0.32×10^{10} in PS to 10.00×10^{10} cells/g in CC + PS. The population decreased gradually with storage of the carrier based biofertilizers. In general, the bacterial population was higher in all the 3 carrier conbinations than their parent materials. Even after the storage of 210 days the similar trend was maintained with populations ranging from 1.78-3.16×10^6 in carrier combinations than the lone carriers ($0.18\text{-}3.6 \times 10^6$ cells/g). These findings are in conformity with other workers who have also found better maintenance of population in carrier combinations than their patent materials alone (Tilak and Subba Rao, 1978). For use of an ideal biofertilizer a population of $> \times 10^8$ is treated as standard (Subba Rao, 1986). Scrutinizing at that level, it was observed tha the required population was maintained by all the 3 carrier combinations at 120 days of storage (CC + FA-5.62, CC + PS - 1.78 and FA + PS 1.00×10^8 cells/g) (Table 2). In this study an added advantage was observed regarding viability of required bacterial population up to 4 months whereas other workers observed the same up to 3 months only while evaluating the cariers for *Rhizobium* inoculants (Tilak and Subba Rao, 1978).

b. ***Phosphate solubilizing bacterial (PSBs) population***

Further persual of the results in Table 2 reveals that an identical trend was exhibited by the PSBs, too. This indicates that the similar carrier combinations can be considered for the commercial production of PSB biofertilizers.

Selection of suitable carriers for commercial exploitation

On the basis of viability studies, the combinations of charcoal with flyash and peat soil and flyash with peat soil stand at par with each other. To determine the further suitability, a study on microbial contamination and moisture content at 120 days stage was conducted (Fig. 2). The unwanted fungal as well as the bacterial population, signifying contamination, was lowest in CC+FA. The combination of peat soil with CC and FA showed higher contaminations. The possible reason may be due to the presence of higher quantity of initial contamination in peat soil which multiplied during storage. This is one of the disadvantages attached with any carrier combination involving peat soil.

The importance of moisture content in biofertilizer prepatations lies in the fact that it should support the survival of bacterial cells during the storage. At the time of preparation of biofertilizers in this study an initial moisture level was maintained at 30 to 40%. The suitability of carriers depends upon their ability to maintain the moisture level nearer to the initial during storage. Moisture content study at 120 days stage revealed that it was 28.6% in CC + FA and 26.5% in CC + PS, with lowest value of 11.8%

in FA + PS. The reason for higher moisture content in charcoal based combinations, i.e. CC+FA and CC + PS, is due to the inherent capacity of charcoal for holding high H_2O content (114.2% WHC as compared to 37.1 and 66.2% of FA and PS, respectively). Besides, it is capable of improving the porosity and surface area of the carrier combinations with which it is mixed because of its own higher attributes. On above grounds both the carrier combinations involving charcoal stand superior than the rest.

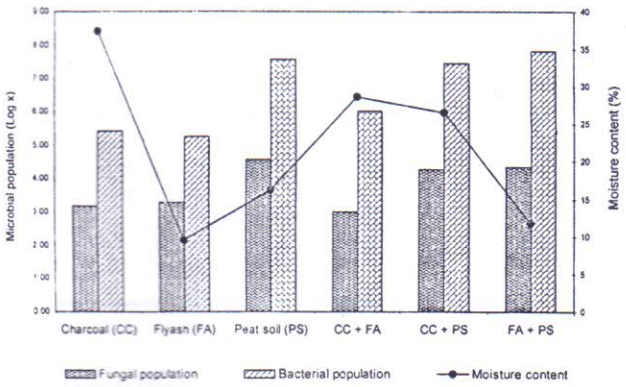

Fig. 2 : Microbial contamination and moisture content of carrier based biofertilizers after 120 days of storage.

All the carriers and their combinations were evaluated on their economic aspects also (Table 3). The economics was calculated on the basis of expenditure required for preparing the bacterial biofertilizers. On that basis it was found that the production cost of presently used popular charcoal based fiofertilizer was Rs. 13,200/mt. Though, the production cost of FA, PS and FA + PS was quite low (Rs. 2900 to 5125), they were unsuitable on other grounds. The rest two carrier combinations, found better on technical grounds, required expenditure of Rs. 8050 (CC + FA) and Rs. 8325 (CC + PS). However, combination of charcoal and flyash substrates (CC + FA) in 1:1 ratio, w/w) may be recommended for commercial exploitation as indigenous and low cost carrier for economic biofertilizer production. It provides a cost reduction of 39% over the traditional charcoal based biofertilizer. Moreover, use of flyash+charcoal carrier based biofertilizer, not only economizes the biofertilizer production cost but also gives an opportunity for gainful disposal of the flyash. Once applied in the soil it helps in soil amelioration by improving soil texture and WHC, increadeng bulk density, and, last but not the least, promotes the growth and yield of crops through the enhancement of nutrient uptake (Kumar *et al.,* 2001).

Table 3 : Economics of *Azotobacter* and PSBs biofertilizer of carrier based biofertilizers after 120 days of storage.

Carriers	Cost of raw materials (Rs./mt) Market price	Trans. expenses	Pulverization expenses	Total	Cost of bacterial broth consumed (Rs/mt)	Total input cost (5+6) (Rs/mt)	Reduction over charcoal biofertilizer (%)
1	2	3	4	5	6	7	8
Charcoal (CC)	6000	Nil	Nil	6000	7200	13200	—
Fly ash (FA)	Nil	500	Nil	500	2400	2900	78.03
Peat soil (PS)	Nil	50	1000	1050	3000	4050	69.3
CC+FA	3000	250	Nil	3250	4800	8050	39.0
CC+PS	3000	250	500	3525	4800	8325	36.9
FA+PS	Nil	275	50	325	4800	5125	61.2

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