Asian Journal of Chemistry Vol. 21, No. 1 (2009), 781-786

Improvement of Life Period of Biofertilizers by Addition of Humic Acid and Flyash

C. JAYANTHI
Department of Chemistry, Rajah Serfoji Government Arts College, Thanjavur-613 005, India
E-mail: jjayanthi21@yahoo.co.in

The aim of this work is to improve the storage life and quality of biofertilizers namely azospirilum, rhizobium and phosphobacteria by the inclusion of 1 % humic acid and 1 % flyash. The physico-chemical values, electrical conductivity, pH, percentage of carbon, organic matter, bulk density and water holding capacity of humic acid and flyash were determined. Then, the storage life and quality of the biofertilizers were tested in usual packing, packing with 1 % humic acid, packing with 1 % flyash and packing with both 1 % humic acid 1 % flyash of these biofertilizers for 5 months at 15, 30, 45, 60, 90, 120 and 150 d intervals. It was concluded that the inclusion of both 1 % humic acid and 1 % fly ash with usual packing of biofertilizers increased the life period of biofertilizers.

Key Words: Biofertilizers, Humic acid, Flyash, Colony counter.

INTRODUCTION

Biofertilizer is considered an important input for crop cultivation because of its low cost and its beneficial role in enhancing yield. At present, due to the rise in the cost of chemical fertilizers more emphasis is given for biofertilizer application which could meet part of fertilizer requirement and help the farmer to reduce the cost of cultivation. Normally, the self life period of biofertilizer is upto 3 months only. By improving the self life period a lot of research works have been conducted to increase the self life period of it depend upon the strain efficiency, carrier material storage condition and inclusion of quality improving substances. By keeping the above points in mind a study was carried out in the soil testing laboratory at Aduthurai, Tamil Nadu to identify the self life period of biofertilizers by the addition of 1 % flyash and 1 % humic acid.

EXPERIMENTAL

This study was conducted in year 2000. All the chemicals used were AR grade. Humic acid and fly ash were collected from Neyveli lignite corporation, Tamil Nadu, India.

Electrical conductivity and pH of humic acid and flyash were determined using automatic conductivity meter and pH meter, respectively[1,2]. Estimation of carbon[3] and organic matter in humic acid and flyash was done by Walkley and Black method[4]. About 10 g of well powdered sample was taken in 100 mL conical flask. Exactly 10 mL of the standard potassium dichromate was added from a burette and swirl gently. 20 mL of conc. H_2SO_4 was added rapidly into the solution and immediately mixed by swirling gently for 1 min. Then the flask was placed on asbestos sheet for 0.5 h. 20 mL of water and 10 mL of orthophosphoric acid were added and titrated against the 1 N ferrous sulphate solution. The colour was changed at the end point from dull green through turbid blue to a brilliant green for the diphenyl ammine indicator. Percentage of carbon was calculated by the following formula:

$$\% \text{ of carbon} = \frac{10(V_1 - V_2) \times 0.003 \times 100 \times 1}{V_1 \times W \times 0.77}$$

where V_1 = volume of ferrous ammonium sulphate (Blank), V_2 = volume of ferrous ammonium sulphate (sample), W = weight of sample taken, 0.003 for converting C (mg) into C (g), 0.77 is recovery factor for the oxidation.

$$\text{Organic matter} = \text{Amount of carbon} \times 1.724 \quad \text{(approx.)}$$

Bulk density and maximum water holding capacity of humic acid and flyash were determined using keen raczkowski box by weight basis[5]. The biofertilizers azospirilum, rhizobium, phosphobacteria packets were prepared as normal packing with 1 % humic acid, packing with 1 % flyash and packing with both 1 % flyash and 1 % humic acid using low density polythene bags and lignite used as the carrier material, all the packets were incubated in a BOD incubator for 5 months. The self life period of each packets were tested at 15, 30 45, 90, 120 and 150 d intervals.

Azospirilium: Azospirilum inoculant should contain a minimum of 10^8 viable azospirilium cells 1 g of the broth inoculant at the time of manufacture and 10^7 before the expiry date life period and population of azospirilium determined by most probable number method. 1 g of the inoculant was taken from the incubated packet and dissolved in sterile water to get 10^1 dilution then it was serially diluted from 10^1 to 10^{10} solution. Three sets of five tubes containing N-free bromothymol blue nutrient medium was prepared[7]. For testing the quality of broth and finished product 10^7, 10^8, 10^9 dilutions were taken as P_1, P_2, P_3, respectively. After transfer of 1 mL each dilution into sterilized nutrient medium the test tubes were labeled and kept for incubation at 23 ± 5 ºC for 3 to 5 d in the BOD incubator. After incubation the test tubes were examined for the growth. The development of azospirilum was showed in the top of the test tubes as white pellicles

and also change of the colour of the media from light yellowish green to blue. To calculate the growth of cells table of most probable number for use with 10 field dilution and 5 types per dilution was used (Table-1).

TABLE-1
PART OF TABLE SHOWN BELOW

P_1	P_2	Most probable number for indicated values of P_3					
		0	1	2	3	4	5
5	1	0.33	0.16	0.61	0.84	1.1	1.3
5	2	0.19	0.10	0.95	1.20	1.5	1.8
5	3	0.79	1.10	1.40	1.80	2.1	2.5
5	4	1.30	1.70	2.20	2.80	3.5	4.3
5	5	2.40	3.50	5.40	3.60	6.0	–

Rhizobium: Self life period and population of rhizobium was determined as per ISI standard, 1 g of inoculant taken from incubated packet and dissolved in 99 mL of sterile distilled water and shaken, make serial dilution upto 10^9. The 0.2 mL aliquot of 10^7 (P_1) 10^8 (P_2) 10^9 (P_3) was taken to delivered petri-dishes containing yeast extract monitor agar medium and spread it uniformly with glass spreads and labeled promptly place them in the incubator at 23 ± 5 °C to 3 to 5 d for fast growing rhizobial. After incubation colonies formed on the plates were counted with the aid of colony counter rhizobium colonies stand out as white translucent, glistering and elevated colonies. Quality status checked by using Table-2.

TABLE-2

No. of colonies	Dilution	Quality status
300	10^7	Passed
30	10^8	Passed
3	10^9	Passed

Phosphobacteria: The same procedure as that rhizobium was followed for the quality of phosphobacteria. According to above procedures the self life period and quality was determined for all the packets of mentioned biofertilizers.

RESULTS AND DISCUSSION

It was concluded that electrical conductivity (humic acid 62.36, flyash 41.57), pH (humic acid 8.2, flyash 7.9), % of carbon (humic acid 36.0, flyash 24.0), % of organic matter (humic acid 62.36, flyash 41.57), water holding capacity (humic acid 11.6, flyash 11.0) and bulk density (humic acid 0.64, flyash 0.70) of humic acid is higher than that of flyash. The quality data of all the three biofertilizers are presented in Tables 3-5.

The results (Tables 3-5) indicate the storage life of normal packing of biofertilizer is only up to 30 d. Packing with 1 % fly ash extend to 45 d. Packing with 1 % humic acid extend to 60 d. But the addition of both 1 % humic acid and 1 % fly ash with the normal packing increase the self life period up to 5 months. This is due to the presence of high carbon content and organic matter in humic acid and flyash. Carbon and organic matter facilitates the growth of microorganism than the usual carrier material of lignite.

TABLE-3
QUALITY STUDY OF AZOSPIRILUM

Days	Name of biofertilizer packets	No. of cells/g innoculant dilution			Remarks
		10^7	10^8	10^9	
15 days after preparation	Usual packet	5	5	5	Full growth
	Usual packet with 1% humic acid	5	5	5	Full growth
	Usual packet with 1% flyash	5	5	5	Full growth
	Usual Packet with 1% humic acid and 1% flyash	5	5	5	Full growth
30 days after preparation	Usual packet	5	5	5	Full growth
	Usual packet with 1% humic acid	5	5	5	Full growth
	Usual packet with 1% flyash	5	5	5	Full growth
	Usual Packet with 1% humic acid and 1% flyash	5	5	5	Full growth
45 days after preparation	Usual packet	5	5	2	5.4×10^8 Cells/g
	Usual packet with 1% humic acid	5	5	5	Full growth
	Usual packet with 1% flyash	5	5	5	Full growth
	Usual Packet with 1% humic acid and 1% flyash	5	5	5	Full growth
60 days after preparation	Usual packet	5	5	2	5.4×10^8 Cells/g
	Usual packet with 1% humic acid	5	5	2	5.4×10^8 Cells/g
	Usual packet with 1% flyash	5	5	5	Full growth
	Usual Packet with 1% humic acid and 1% flyash	5	5	5	Full growth
90 days after preparation	Usual packet	5	5	3	3.6×10^8 Cells/g
	Usual packet with 1% humic acid	5	5	2	5.4×10^8 Cells/g
	Usual packet with 1% flyash	5	5	2	5.4×10^8 Cells/g
	Usual Packet with 1% humic acid and 1% flyash	5	5	5	Full growth
120 days after preparation	Usual packet	5	5	3	3.6×10^8 Cells/g
	Usual packet with 1% humic acid	5	5	2	5.4×10^8 Cells/g
	Usual packet with 1% flyash	5	5	3	3.6×10^8 Cells/g
	Usual Packet with 1% humic acid and 1% flyash	5	5	5	Full growth
150 days after preparation	Usual packet	5	5	3	3.6×10^8 Cells/g
	Usual packet with 1% humic acid	5	5	3	3.6×10^8 Cells/g
	Usual packet with 1% flyash	5	5	3	3.6×10^8 Cells/g
	Usual Packet with 1% humic acid and 1% flyash	5	5	5	Full growth

TABLE-4
QUALITY STUDY OF RHIZOBIUM

Days	Name of biofertilizer packets	No. of cells/g innoculant dilution			Remarks
		10^7	10^8	10^9	
15 days after preparation	Usual packet	339	48	7	Passed
	Usual packet with 1% humic acid	410	45	8	Passed
	Usual packet with 1% flyash	375	40	5	Passed
	Usual Packet with 1% humic acid and 1% flyash	490	46	12	Passed
30 days after preparation	Usual packet	320	38	5	Passed
	Usual packet with 1% humic acid	408	45	9	Passed
	Usual packet with 1% flyash	368	39	6	Passed
	Usual Packet with 1% humic acid and 1% flyash	464	45	12	Passed
45 days after preparation	Usual packet	318	35	5	Passed
	Usual packet with 1% humic acid	386	40	8	Passed
	Usual packet with 1% flyash	343	32	6	Passed
	Usual Packet with 1% humic acid and 1% flyash	450	42	10	Passed
60 days after preparation	Usual packet	312	32	4	Passed
	Usual packet with 1% humic acid	382	37	8	Passed
	Usual packet with 1% flyash	330	30	5	Passed
	Usual Packet with 1% humic acid and 1% flyash	443	40	9	Passed
90 days after preparation	Usual packet	302	30	3	Passed
	Usual packet with 1% humic acid	385	31	8	Passed
	Usual packet with 1% flyash	315	31	4	Passed
	Usual Packet with 1% humic acid and 1% flyash	432	41	9	Passed
120 days after preparation	Usual packet	284	25	3	Failed
	Usual packet with 1% humic acid	382	31	8	Passed
	Usual packet with 1% flyash	308	31	7	Passed
	Usual Packet with 1% humic acid and 1% flyash	410	38	9	Passed
150 days after preparation	Usual packet	298	24	3	Failed
	Usual packet with 1% humic acid	379	30	7	Passed
	Usual packet with 1% flyash	300	30	6	Passed
	Usual Packet with 1% humic acid and 1% flyash	396	30	9	Passed

TABLE-5
QUALITY STUDY OF RHIZOBIUM

Days	Name of biofertilizer packets	No. of cells/g innoculant dilution			Remarks
		10^7	10^8	10^9	
15 days after preparation	Usual packet	320	35	6	Passed
	Usual packet with 1% humic acid	420	45	9	Passed
	Usual packet with 1% flyash	398	42	7	Passed
	Usual Packet with 1% humic acid and 1% flyash	520	45	9	Passed

Days	Name of biofertilizer packets	No. of cells/g innoculant dilution			Remarks
		10^7	10^8	10^9	
30 days after preparation	Usual packet	312	32	5	Passed
	Usual packet with 1% humic acid	418	92	8	Passed
	Usual packet with 1% flyash	380	35	6	Passed
	Usual Packet with 1% humic acid and 1% flyash	480	45	9	Passed
45 days after preparation	Usual packet	310	30	5	Passed
	Usual packet with 1% humic acid	390	40	7	Passed
	Usual packet with 1% flyash	378	33	6	Passed
	Usual Packet with 1% humic acid and 1% flyash	462	43	6	Passed
60 days after preparation	Usual packet	300	30	4	Passed
	Usual packet with 1% humic acid	380	35	7	Passed
	Usual packet with 1% flyash	365	30	5	Passed
	Usual Packet with 1% humic acid and 1% flyash	442	43	6	Passed
90 days after preparation	Usual packet	305	30	3	Passed
	Usual packet with 1% humic acid	368	39	6	Passed
	Usual packet with 1% flyash	350	32	5	Passed
	Usual Packet with 1% humic acid and 1% flyash	435	44	8	Passed
120 days after preparation	Usual packet	300	30	3	Passed
	Usual packet with 1% humic acid	340	35	5	Passed
	Usual packet with 1% flyash	325	30	5	Passed
	Usual Packet with 1% humic acid and 1% flyash	418	40	7	Passed
150 days after preparation	Usual packet	285	28	2	Failed
	Usual packet with 1% humic acid	320	35	5	Passed
	Usual packet with 1% flyash	300	30	5	Passed
	Usual Packet with 1% humic acid and 1% flyash	412	38	7	Passed

REFERENCES

1. B.M. Ganger, Amino Compounds in Acid Hydrolysates of Humic Acid (1970).
2. P. Dubach and N.C. Menta, The Chemistry of Soil Humic Substances, Soil and Fertilizers (1963).
3. E. Boyd, *Hydrobiologia*, **36**, 17 (1970).
4. W. Flaig, *Soil Sci.*, **111**, 19 (1971).
5. F.J. Stevenson and M.J. Mendez, *Soil Sci*., **101**, 383 (1967).
6. F.E. Brauns, Chemistry of Lignin, Academic Press (1952).
7. B. Bernier, *Biochem. J.*, **70**, 590 (1958).

(*Received*: 24 March 2008; *Accepted*: 5 September 2008) AJC-6830