Int.J.Curr.Microbiol.App.Sci (2016) *5*(7): 242-252

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 7 (2016) pp. 242-252**
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas.2016.507.025

Enrichment of Biogas Manures with Beneficial Microorganisms

Y. Kavya[1*], A. Vijaya Gopal[1], R. Subhash Reddy[1] and M. Sreedhar[2]

[1]Department of Agricultural Microbiology and Bio energy, College of Agriculture, Acharya N. G. Ranga Agricultural University, Rajendranagar, Hyderabad-500030, India
[2]Quality Control Laboratory, Acharya N. G. Ranga Agricultural University, Rajendranagar, Hyderabad-500030, India
**Corresponding author*

A B S T R A C T

Keywords

Cow dung, Press mud, Poultry litter, Kitchen wastes, Maize stalks, Enrichment, Viability.

Article Info

Accepted:
12 June 2016
Available Online:
10 July 2016

The beneficial microorganisms used in the present study (*Rhizobium, Pseudomonas, Azotobacter, Azospirillum*) were collected from the department of Agricultural Microbiology, College of Agriculture, Rajendranagar, Hyderabad. All these four microorganisms were added to the biogas manure samples collected from biogas digesters set with six different substrates (cow dung, press mud, poultry litter, kitchen wastes, maize stalks and fruit wastes) after the gas production stopped. The beneficial microorganisms viability in the enriched biogas manures was monitored upto the end of tenth week and the increase in population was observed in all the biogas manures samples indicating that the biogas manure samples from different substrates support the beneficial microorganisms population for atleast 3-4 weeks duration.

Introduction

Organic manures in agriculture add much needed organic matter and minerals to the soil. The important manures used in organic farming are compost, vermicompost, biogas spent slurry, green manures and liquid organic manures like panchagavya, jeevamruth, etc. The beneficial effects of organic manures in agricultural production and soil fertility are known from many decades, but they are inadequate in nutrient supply and low in nutrient concentrations. The total nutrients recycled from organic matter decomposition are much less than the amount of nutrients utilized by the crop plants. This necessitates the enrichment of manures with beneficial microbial inoculants like free living nitrogen fixers, phosphate solubilizers *etc*. to improve the nutritional status of the manures. The enrichment of manures with beneficial microbial cultures results not only in improvement of nutritive value but also in higher growth and yield of crops. The microbial enrichment of organic manures will further contribute to the enhancement of phosphate solubilisation and nitrogen fixation (Hema *et al*. 2012). Hence, the present investigation was also aimed at enrichment of biogas manure with beneficial micro organisms.

Materials and methods

Collection of biogas slurry

Biogas slurry samples used in the present study were collected from biogas unit set in the lab scale with six treatments and three replication in the dept of Agricultural Microbiology and BioEnergy, College of Agriculture, Rajendranagar, Hyderabad. The six treatments include T_1 250 g cow dung + 500 g press mud + 1500ml water (1:2:6), T_2 250 g cow dung + 500 g poultry litter + 1500 ml water (1:2:6), T_3 250 g cow dung + 500 g kitchen waste + 1500 ml water (1:2:6), T_4 250 g cow dung + 500 g maize stalks + 1500 ml water (1:2:6), in T_5 250 g cow dung + 500 g fruit waste + 1500 ml water (1:2:6) and T_6 750 g cow dung + 1500 ml water (3:6). Biogas slurry samples were collected and dried under sun to 50 per cent moisture.

Microbial Analysis of Biogas slurry manure

The dried biogas slurry collected from different treatments was analysed for the presence of microorganisms i.e., *Rhizobium, Azotobacter, Azospirillum, Pseudomonas* were determined by serial dilution and plating on selective media as mentioned above. Replicates of the inoculated agar plates were incubated for 2 days at 37^0C for *Rhizobium* and *Pseudomonas*, 7 days for *Azotobacter* and *Azospirillum* after which the counts were taken.

Viability of added beneficial microbes in Biogas slurry manure

Collection of Beneficial micro organisms

Beneficial microorganisms *Rhizobium, Pseudomonas, Azotobacter, Azospirillum* cultures from the Agricultural Microbiology Department, College of Agriculture, Rajendranagar, were used in the experiment.

Enrichment of Biogas slurry

The dried biogas slurry samples collected from different treatments after drying were enriched with 4 types of biofertilizers *viz: Rhizobium, Azotobacter, Azospirillum* and *Pseudomonas* individually.

Microbial Analysis of Biogas slurry Enriched with Beneficial Microorganisms

Viable population of *Rhizobium, Pseudomonas Azotobacter, Azospirillum* were analyzed by the standard serial dilution plate count method (Vlassak *et al.*, 1992) at weekly interval in the first month and monthly interval from the second month by using different media *viz:* Yeast Extract Mannitol Agar with Congo Red for *Rhizobium*, Kings-B for *Pseudomonas* (King *et al*. 1954), Azotobacter medium for *Azotobacter spp*., Potato infusion agar medium for *Azospirillum spp*., and plates were incubated at 28±2 ^{o}C in an incubator in triplicates. The microbial colonies appearing after the stipulated time period of incubation were counted as Colony forming units (CFU) g^{-1} fresh weight of the sample. The microbial populations were expressed as number of colony forming units per gram.

Results and Discussion

Population of beneficial bacteria present in the biogas manures before enrichment

The population of *Rhizobium* in the biogas manure samples (50 per cent moisture) was significantly more in T_1 (Cow dung + Press mud) 46.0×10^3 CFU g^{-1} compared to T_6 (Cow dung alone) 34.0×10^3 CFU g^{-1}, T_2 (Cow dung + Poultry litter) 32.0×10^3 CFU

g^{-1}, T_3 (Cow dung + Kitchen waste) 6.1×10^3 CFU g^{-1}, T_5 (Cow dung + Fruit waste) 5.1×10^3 CFU g^{-1} and less in T_4 (Cow dung + Maize stalks) 3.1×10^3 CFU g^{-1} (Table 3.1).

The population of *Pseudomonas* in the biogas manure samples (50 per cent moisture) was significantly more in T_6 (Cow dung alone) 46.0×10^3 CFU g^{-1} compared to T_2 (Cow dung + Poultry litter) 44.0×10^3 CFU g^{-1}, T_1 (Cow dung + Press mud) 40.0×10^3 CFU g^{-1}, T_3 (Cow dung + Kitchen waste) 4.6×10^3 CFU g^{-1}, T_4 (Cow dung + Maize stalks) 4.0×10^3 CFU g^{-1} and less in T_5 (Cow dung + Fruit waste) 3.0×10^3 CFU g^{-1} (Table 3.1).

The population of *Azotobacter* in the biogas manure samples (50 per cent moisture) was significantly more in T_2 (Cow dung + Poultry litter) and T_6 (Cow dung alone) 42.0×10^3 CFU g^{-1} compared to T_1 (Cow dung + Press mud) 12.0×10^3 CFU g^{-1}, T_3 (Cow dung + Kitchen waste) 4.6×10^3 CFU g^{-1}, T_5 (Cow dung + Fruit waste) 3.1×10^3 CFU g^{-1} and less in T_4 (Cow dung + Maize stalks) 2.0×10^3 CFU g^{-1} (Table 3.1).

The population of *Azospirillum* in the biogas manure samples (50 per cent moisture) was significantly more in T_6 (Cow dung alone) 24.0×10^3 CFU g^{-1} compared to T_1 (Cow dung + Press mud) 20.0×10^3 CFU g^{-1}, T_3 (Cow dung + Kitchen waste) 18.0×10^3 CFU g^{-1}, T_2 (Cow dung + Poultry litter) 12.0×10^3 CFU g^{-1}, T_5 (Cow dung + Fruit waste) 2.8×10^3 CFU g^{-1} and less in T_4 (Cow dung + Maize stalks) 2.2×10^3 CFU g^{-1} (Table 3.1).

Hema *et al.* (2012) studied the influence of microbial enrichment on microbial population and nutrient status of organic manures. Microbial population in the slurry before enrichment was 80.5×10^5 bacteria, 124×10^3 fungi, 12×10^2 actinomycetes, 15.3×10^3 phosphate solubilise micro organisms and 20.2×10^3 free living nitrogen fixers.

Viability of the beneficial microorganisms inoculated individually into the biogas manure samples

Population of *Rhizobium* after the enrichment

The population of *Rhizobium* in the Yeast Extract Mannitol (YEM) broth was 3.1×10^9 CFU ml^{-1} (Table 3.2).

After the enrichment of the biogas manures with YEM broth, the population of *Rhizobium* on the first day was significantly more in T_6 (Cow dung alone) 4.8×10^9 CFU g^{-1} compared to T_1 (Cow dung + Press mud) 3.2×10^9 CFU g^{-1}, T_2 (Cow dung + Poultry litter) 2.8×10^9 CFU g^{-1}, T_3 (Cow dung + Kitchen waste) 1.0×10^9 CFU g^{-1}, T_5 (Cow dung + Fruit waste) 0.9×10^9 CFU g^{-1} and less in T_4 (Cow dung + Maize stalks) 0.8×10^9 CFU g^{-1} (Table 3.3).

There was an increase in the population of *Rhizobium* in all the six treatments until the end of fourth week. The rate of multiplication was significantly more in T_6 (Cow dung alone) 1.2×10^9 CFU g^{-1} (4.8×10^9 CFU g^{-1}, on the first day to 6.1×10^9 CFU g^{-1}, at the end of fourth week) and less in T_4 (Cow dung + Maize stalks) 0.6×10^9 CFU g^{-1} (0.8×10^9 CFU g^{-1}, on the first day to 1.6×10^9 CFU g^{-1}, at the end of fourth week). The decrease in population was observed after fourth week and significantly more reduction in the population was observed in T_6 (Cow dung alone) 6.1×10^9 CFU g^{-1} at the end of fourth week to 3.0×10^8 CFU g^{-1} at the end of sixth week and less reduction in population was observed in T_5 (Cow dung + Fruit waste) 1.9×10^9 CFU g^{-1} to 0.8×10^8 CFU g^{-1} (Table 3.3). Finally the viability was significantly more in T_5 (Cow dung +

Fruit waste) 0.9×10^9 CFU g^{-1}, on the first day to 0.5×10^7 CFU g^{-1} at the end of tenth week and comparatively less viability was observed in T_2 (Cow dung + Poultry litter) 2.8×10^9 CFU g^{-1} on the first day and 0.8×10^7 CFU g^{-1} at the end of tenth week (Table 3.3).

Population of *Pseudomonas* after the enrichment

The population of *Pseudomonas* in Kings B broth was 3.0×10^9 CFU ml^{-1}(Table 3.2).

After the enrichment of the biogas manures with King's B broth, the population of *Pseudomonas* on the first day was significantly more in T_6 (Cow dung alone) 3.2×10^9 CFU g^{-1} compared to T_2 (Cow dung + Poultry litter) 2.4×10^9 CFU g^{-1}, T_1 (Cow dung + Press mud) 1.8×10^9 CFU g^{-1}, T_4 (Cow dung + Maize stalks) 1.6×10^9 CFU g^{-1}, T_5 (Cow dung + Fruit waste) 1.1×10^9 CFU g^{-1} and less in T_3 (Cow dung + Kitchen waste) 1.0×10^9 CFU g^{-1} (Table 3.4).

There was an increase in the population of *Pseudomonas* in all the six treatments until the end of fourth week. The rate of multiplication was significantly more in T_3 (Cow dung + Kitchen waste) 2.6×10^9 CFU g^{-1} (1.0×10^9 CFU g^{-1} on the first day to 3.6×10^9 CFU g^{-1} at the end of fourth week) and less in T_2 (Cow dung + Poultry litter) 1.6×10^9 CFU g^{-1} (2.4×10^9 on the first day to 4.0×10^9 at the end of fourth week). The decrease in population was observed after fourth week and significantly more reduction in the population was observed in T_1 (Cow dung + Press mud) 3.7×10^9 CFU g^{-1} at the end of fourth week to 2.1×10^8 CFU g^{-1} at the end of sixth week and less reduction was observed in T_2 (Cow dung + Poultry litter) 4.0×10^9 CFU g^{-1} at the end of fourth week to 2.5×10^8 CFU g^{-1} at the end of sixth week (Table 3.4). Finally the viability was significantly more in T_3 (Cow dung + Kitchen waste) 1.0×10^9 CFU g^{-1} on the first day to 2.0×10^7 CFU g^{-1} at the end of tenth week and comparatively less viability was observed in T_4 (Cow dung + Maize stalks) 1.6×10^9 CFU g^{-1} on the first day to 1.4×10^7 CFU g^{-1} at the end of tenth week (Table 3.4).

Population of *Azotobacter* after the enrichment

The population of *Azotobacter* in *Azotobacter* glucose broth was 2.6×10^9 CFU ml^{-1} (Table 3.2).

After the enrichment of the biogas manures with *Azotobacter* glucose broth, the population of *Azotobacter* on the first day was significantly more in T_6 (Cow dung alone) 2.8×10^9 CFU g^{-1} compared to T_1 (Cow dung + Press mud) 2.2×10^9 CFU g^{-1}, T_2 (Cow dung + Poultry litter) 2.0×10^9 CFU g^{-1}, T_3 (Cow dung + Kitchen waste) 1.7×10^9 CFU g^{-1}, T_5 (Cow dung + Fruit waste) 1.2×10^9 CFU g^{-1} and less in T_4 (Cow dung + Maize stalks) 1.0×10^9 CFU g^{-1} (Table 3.5).

There was an increase in the population of *Azotobacter* in all the six treatments until the end of third week. The rate of multiplication was significantly more in T_1 (Cow dung + Press mud) 1.6×10^9 CFU g^{-1} (2.2×10^9 CFU g^{-1} on the first day to 3.8×10^9 CFU g^{-1} at the end of third week) and less in T_5 (Cow dung + Fruit waste) 1.2×10^9 CFU g^{-1} (1.2×10^9 CFU g^{-1} on the first day to 1.4×10^9 CFU g^{-1} at the end of third week). The decrease in population was observed after third week and significantly more reduction in the population was observed in T_4 (Cow dung + Maize stalks) 2.2×10^9 CFU g^{-1} at the end of third week to 2.5×10^8 CFU g^{-1} at the end of fourth week and less reduction was in T_1 (Cow dung + Press mud) 3.8×10^9 CFU g^{-1} to 4.4×10^9 CFU g^{-1} (Table 3.5).

Table.1 Population of beneficial bacteria present in the biogas manures before enrichment with beneficial microorganisms.

	Rhizobium (CFU g^{-1})	*Pseudomonas* (CFU g^{-1})	*Azotobacter* (CFU g^{-1})	*Azospirillum* (CFUg^{-1})
Cow dung + press mud (T_1)	46.0×10^3	40.0×10^3	12.0×10^3	20.0×10^3
Cow dung + Poultry litter (T_2)	32.0×10^3	44.0×10^3	42.0×10^3	12.0×10^3
Cow dung + Kitchen waste (T_3)	6.1×10^3	4.6×10^3	4.6×10^3	18.0×10^3
Cow dung + Maize stalks (T_4)	3.1×10^3	4.0×10^3	2.0×10^3	2.2×10^3
Cow dung + Fruit waste (T_5)	5.1×10^3	3.0×10^3	3.1×10^3	2.8×10^3
Cow dung alone (T_6)	34.0×10^3	46.0×10^3	42.0×10^3	24.0×10^3

$\mathbf{T_1}$ = Cow dung (250 g) + Press mud (500 g) + water (1500 ml) – 1:2:6
$\mathbf{T_4}$ = Cow dung (250 g) + Maize stalks (500 g) + water (1500 ml) – 1:2:6
$\mathbf{T_2}$ = Cow dung (250 g) + Poultry litter (500 g) + water (1500 ml) – 1:2:6
$\mathbf{T_5}$ = Cow dung (250 g) + Fruit wastes (500 g) + water (1500 ml) – 1:2:6
$\mathbf{T_3}$ = Cow dung (250 g) + Kitchen waste (500 g) + water (1500 ml) – 1:2:6
$\mathbf{T_6}$ = Cow dung (750 g) + water (1500 ml) – 3:6

*The values within the brackets in the table indicate the difference between the values of adjacent weeks.

Table.2 Microbial population in the broth

Medium	CFU ml^{-1}
Yeast extract mannitol broth (*Rhizobium*)	3.1×10^9
King's B broth (*Pseudomonas*)	3.0×10^9
Azotobacter glucose broth (*Azotobacter*)	2.6×10^9
Potato infusion broth (*Azospirillum*)	2.6×10^9

Table.3 Population of *Rhizobium* present in the biogas manures after the enrichment.

	Initial ($\times10^9$CFU $^{-1}$g)	**At the end of first week (7th day) ($\times10^9$CFU g^{-1})**	**At the end of second week (14th day) ($\times10^9$CFU g^{-1})**	**At the end of third week (21st day) ($\times10^9$CFU g^{-1})**	**At the end of fourth week (28th day) ($\times10^9$CFU g^{-1})**	**At the end of sixth week (42nd day) ($\times10^8$CFUg^{-1})**	**At the end of eighth week (56th day) ($\times10^7$CFUg^{-1})**	**At the end of tenth week (70thday) ($\times10^7$CFUg^{-1})**
T_1	3.06	3.20(0.14)	3.40(0.20)	3.66(0.26)	4.00(0.34)	1.93(3.80)	3.00(1.60)	2.16(0.84)
T_2	2.86	3.13(0.27)	3.26(0.13)	3.53(0.30)	3.66(0.13)	1.43(3.51)	2.03(1.22)	0.86(1.17)
T_3	1.00	1.33(0.33)	1.46(0.13)	1.66(0.20)	1.93(0.27)	0.80(1.85)	1.03(0.60)	0.36(0.67)
T_4	0.93	1.06(0.13)	1.33(0.27)	1.50(0.17)	1.66(0.43)	0.43(1.61)	0.73(0.35)	0.20(0.53)
T_5	0.93	1.26(0.33)	1.53(0.27)	1.66(0.13)	1.93(0.27)	0.86(1.84)	1.13(0.74)	0.46(0.67)
T_6	4.86	5.13(0.27)	5.33(0.20)	5.66(0.33)	6.06(0.40)	3.03(5.75)	4.43(2.58)	3.26(1.17)
S.E(m)	0.077	0.077	0.077	0.065	0.077	0.038	0.045	0.038
CD P=0.05	0.240	0.240	0.240	0.203	0.240	0.120	0.141	0.120

T_1 = Cow dung (250 g) + Press mud (500 g) + water (1500 ml) – 1:2:6 **T_4** = Cow dung (250 g) + Maize stalks (500 g) + water (1500 ml) – 1:2:6
T_2 = Cow dung (250 g) + Poultry litter (500 g) + water (1500 ml) – 1:2:6 **T_5** = Cow dung (250 g) + Fruit wastes (500 g) + water (1500 ml) – 1:2:6
T_3 = Cow dung (250 g) + Kitchen waste (500 g) + water (1500 ml) – 1:2:6 **T_6** = Cow dung (750 g) + water (1500 ml) – 3:6
*The values within the brackets in the table indicate the difference between the values of adjacent weeks.

Table.4 Population of *Pseudomonas* present in the biogas manures after the enrichment.

	Initial(× 10^9CFU g^{-1})	**At the end of first week (7^{th} day) (×10^9CFU g^{-1})**	**At the end of second week (14th day) (×10^9CFUg^1)**	**At the end of third week (21st day) (×10^9CFU g^{-1})**	**At the end of fourth week (28th day) (×10^9CFU g^{-1})**	**At the end of sixth week (42nd day) (×10^8CFU g^{-1})**	**At the end of eighth week (56th day) (×10^7CFU g^{-1})**	**At the end of tenth week (70th day) (×10^7CFU g^{-1})**
T_1	1.83	2.56(0.73)	3.03(0.47)	3.46(0.43)	3.73(0.27)	2.16(3.51)	3.33(1.82)	2.36(0.97)
T_2	2.36	2.86(0.50)	3.13(0.27)	3.63(0.50)	4.03(0.40)	2.56(3.77)	3.56(2.20)	3.06(0.50)
T_3	1.03	1.53(0.50)	2.66(1.13)	3.16(0.50)	3.63(0.47)	1.86(3.44)	2.26(1.60)	2.03(0.23)
T_4	1.56	2.03(0.47)	2.76(0.73)	3.03(0.27)	3.36(0.33)	1.26(3.23)	2.03(1.05)	1.43(0.60)
T_5	1.13	1.43(0.30)	1.73(0.40)	2.56(0.83)	3.03(0.47)	1.03(2.92)	1.73(1.62)	1.03(0.70)
T_6	3.16	4.00(0.84)	4.83(0.83)	5.33(0.50)	5.73(0.40)	3.56(5.37)	3.83(3.17)	3.36(0.47)
S.E(m)	0.041	0.065	0.053	0.047	0.047	0.047	0.047	0.041
CD (P=0.05)	0.127	0.203	0.164	0.147	0.147	0.147	0.147	0.127

T_1 = Cow dung (250 g) + Press mud (500 g) + water (1500 ml) – 1:2:6 **T_4** = Cow dung (250 g) + Maize stalks (500 g) + water+(1500 ml) – 1:2:6
T_2 = Cow dung (250 g) + Poultry litter (500 g) + water (1500 ml) – 1:2:6 **T_5** = Cow dung (250 g) + Fruit wastes (500 g) + water (1500 ml) – 1:2:6
T_3 = Cow dung (250 g) + Kitchen waste (500 g) + water (1500 ml) – 1:2:6 **T_6** = Cow dung (750 g) + water (1500 ml) – 3:6
*The values within the brackets in the table indicate the difference between the values of adjacent weeks.

Table.5 Population of *Azotobacter* present in the biogas manures after the enrichment.

	Initial ($\times 10^9$CFU g^{-1})	**At the end of first week (7^{th} day) ($\times 10^9$CFU g^{-1})**	**At the end of second week (14^{th} day) ($\times 10^9$CFU g^{-1})**	**At the end of third week (21^{st} day) ($\times 10^9$CFU g^{-1})**	**At the end of fourth week (28^{th} day) ($\times 10^8$CFU g^{-1})**	**At the end of sixth week (42^{nd} day) ($\times 10^8$CFU g^{-1})**	**At the end of eighth week (56^{th} day) ($\times 10^7$CFU g^{-1})**	**At the end of tenth week (70^{th} day) ($\times 10^6$CFU g^{-1})**
T_1	2.23	3.03(0.80)	3.40(0.37)	3.86(0.46)	4.46(0.60)	2.03(2.430	3.13(1.71)	3.16(2.80)
T_2	2.03	2.76(0.73)	3.16(0.40)	3.56(0.40)	4.03(0.47)	1.56(2.47)	2.36(1.32)	2.23(2.13)
T_3	1.66	2.13(0.47)	2.66(0.53)	3.03(0.37)	3.63(0.60)	1.3392.30)	2.16(1.11)	1.40(2.00)
T_4	1.03	1.43(0.40)	2.03(0.60)	2.23(0.20)	2.56(0.33)	1.03(1.53)	2.26(0.80)	1.13(2.14)
T_5	1.26	1.83(0.57)	2.23(0.40)	2.43(0.20)	3.03(0.60)	1.13(1.90)	2.00(0.90)	1.26(1.87)
T_6	2.83	3.56(0.73)	4.03(0.47)	4.23(0.20)	4.83(0.60)	3.03(1.80)	2.76(2.75)	4.06(2.35)
S.E(m)	0.047	0.041	0.038	0.033	0.033	0.047	0.045	0.051
CD (P=0.05)	0.147	0.127	0.120	0.104	0.104	0.147	0.141	0.159

T_1 = Cow dung (250 g) + Press mud (500 g) + water (1500 ml) – 1:2:6 **T_4** = Cow dung (250 g) + Maize stalks (500 g) + water (1500 ml) – 1:2:6
T_2 = Cow dung (250 g) + Poultry litter (500 g) + water (1500 ml) – 1:2:6 **T_5** = Cow dung (250 g) + Fruit wastes (500 g) + water (1500 ml) – 1:2:6
T_3 = Cow dung (250 g) + Kitchen waste (500 g) + water (1500 ml) – 1:2:6 **T_6** = Cow dung (750 g) + water (1500 ml) – 3:6
*The values within the brackets in the table indicate the difference between the values of adjacent weeks.

Table.6 Population of *Azospirillum* present in the biogas manures after the enrichment.

	Initial ($\times 10^9$CFU g^{-1})	**At the end of first week (7th day) ($\times 10^9$CFU g^{-1})**	**At the end of second week (14th day) ($\times 10^9$CFU g^{-1})**	**At the end of third week (21st day) ($\times 10^9$CFU g^{-1})**	**At the end of fourth week (28th day) ($\times 10^9$CFU g^{-1})**	**At the end of sixth week (42nd day) ($\times 10^8$CFU g^{-1})**	**At the end of eighth week (56thday) ($\times 10^7$CFU g^{-1})**	**At the end of tenth week (70th day) ($\times 10^6$CFU g^{-1})**
T_1	2.36	2.76(0.40)	3.23(0.47)	3.40(0.17)	3.63(0.23)	2.36(3.39)	1.66(2.10)	3.03(1.35)
T_2	2.03	2.60(0.57)	2.83(0.23)	3.03(0.20)	3.36(0.33)	2.03(3.15)	1.16(1.91)	2.76(0.88)
T_3	1.40	1.76(0.36)	2.06(0.30)	2.43(0.34)	2.73(0.40)	1.66(2.56)	1.00(1.56)	2.00(0.80)
T_4	1.03	1.16(0.13)	1.56(0.60)	1.83(0.27)	2.16(0.33)	2.03(1.95)	1.63(1.86)	1.03(1.52)
T_5	0.56	1.06(0.50)	1.76(0.60)	2.03(0.27)	2.33(0.30)	2.13(2.11)	1.43(1.98)	1.33(1.29)
T_6	2.63	3.13(0.50)	3.56(0.33)	3.80(0.24)	4.03(0.23)	3.03(3.72)	2.03(2.82)	3.36(1.69)
S.E(m)	0.030	0.045	0.041	0.027	0.047	0.047	0.038	0.038
CD P=0.05	0.095	0.141	0.127	0.085	0.147	0.147	0.120	0.120

T_1 = Cow dung (250 g) + Press mud (500 g) + water (1500 ml) – 1:2:6 **T_4** = Cow dung (250 g) + Maize stalks (500 g) + water (1500 ml) – 1:2:6
T_2 = Cow dung (250 g) + Poultry litter (500 g) + water (1500 ml) – 1:2:6 **T_5** = Cow dung (250 g) + Fruit wastes (500 g) + water (1500 ml) – 1:2:6
T_3 = Cow dung (250 g) + Kitchen waste (500 g) + water (1500 ml) – 1:2:6 **T_6** = Cow dung (750 g) + water (1500 ml) – 3:6
*The values within the brackets in the table indicate the difference between the values of adjacent weeks.

Finally the viability was significantly more in T_6 (Cow dung alone) 2.8×10^9 CFU g^{-1} on the first day to 4.1×10^6 CFU g^{-1} at the end of tenth week and comparitively less viability was observed in T_3 (Cow dung + Kitchen waste) 1.7×10^9 CFU g^{-1} on the first day to 1.4×10^7 CFU g^{-1} at the end of tenth week (Table 3.5).

Population of *Azospirillum* after the enrichment

The population of *Azospirillum* in the potato infusion broth was 2.6×10^9 CFU ml^{-1} (Table 3.2).

After the enrichment of the dried biogas manures with potato infusion broth, the population of *Azospirillum* on the first day was significantly more in T_6 (Cow dung alone) 2.6×10^9 CFU g^{-1} compared to T_1 (Cow dung + Press mud) 2.4×10^9 CFU g^{-1}, T_2 (Cow dung + Poultry litter) 2.0×10^9 CFU g^{-1}, T_3 (Cow dung + Kitchen waste) 1.4×10^9 CFU g^{-1}, T_4 (Cow dung + Maize stalks) 1.0×10^9 CFU g^{-1} and less in T_5 (Cow dung + Fruit waste) 0.6×10^9 CFU g^{-1} (Table 3.6).

There was an increase in the population of *Azospirillum* in all the six treatments until the end of fourth week. The rate of multiplication was significantly more in T_5 (Cow dung + Fruit waste) 1.7×10^9 CFU g^{-1} (0.6×10^9 CFU g^{-1} on the first day to 2.3×10^9 CFU g^{-1} at the end of fourth week) and less in T_4 (Cow dung + Maize stalks) 1.1×10^9 CFU g^{-1} (1.0×10^9 CFU g^{-1} on the first day to 2.1×10^9 CFU g^{-1} at the end of fourth week). The decrease in population was observed after fourth week and significantly more reduction in the population was observed in T_2 (Cow dung + Poultry litter) 3.4×10^9 CFU g^{-1} at the end of fourth week to 2.0×10^8 CFU g^{-1} at the end of sixth week and less reduction was observed in T_4 (Cow dung + Maize stalks) 2.16×10^9 CFU g^{-1} to 2.03×10^8 CFU g^{-1} (Table 3.6). Finally the viability was significantly more in T_5 (Cow dung + Fruit waste) 0.6×10^9 CFU g^{-1} on the first day to 1.3×10^6 CFU g^{-1} at the end of tenth week and comparatively less viability was observed in T_4 (Cow dung + Maize stalks) 1.0×10^9 CFU g^{-1} on the first day to 1.0×10^6 CFU g^{-1} at the end of tenth week (Table 3.6).

Shruthi *et al*. (2014) carried work on the survivability of *Bacillus megatherium* in different carrier materials for improved shelf life of biofertilizer. The microbial population was estimated once in 30 days upto 240 days of storage (13.3×10^7 CFU g^{-1} at 30 days and 2×10^7 CFU g^{-1} at 240 days). Maximum viability of the organism was observed in press mud based biofertilizer because it was a rich source of nutrients especially carbon which favours the microorganisms survivability, compared to vermicompost, then lignite and was lowest in cocopeat.

Hema *et al.* (2012) studied the influence of microbial enrichment on microbial population and nutrient status of organic manures. Microbial population in the slurry before enrichment was 80.5×10^5 bacteria, 124×10^3 fungi, 12×10^2 actinomycetes, 15.3×10^3 phosphate solubilise micro organisms and 20.2×10^3 free living nitrogen fixers.

Karmegam *et al.* (2012) conducted research on the enrichment of biogas slurry vermicompost with *Azotobacter chroococcum* and *Bacillus megatherium.* The inoculum level of *Azotobacter chroococcum* and *Bacillus megatherium* at rate of 35ml per 175g of vermibed substrate is sufficient to maintain 1×10^7 viable cells up to 160 days after harvesting of vermicompost. The inoculum of biofertilizer organisms in vermibed on 30th day showed increased viability rate and hence, the optimized inoculation of 35 ml of inoculum per 175 g of substrate on 30th day of vermicomposting is helpful for maintenance of sufficient viable population for more than five months in the enriched vermicompost.

In conclusion, the viability appears to be good upto the end of fourth week irrespective of treatments of the biogas manure samples for *Rhizobium*, *Pseudomonas,* the viability and *Azospirillum*. Whereas for *Azotobacter* the viability appears to be good only upto the end of third week irrespective of treatments of the biogas manures. Finally the viability was significantly more in T_5 (Cow dung + Fruit waste) for *Rhizobium* and *Azospirillum*, whereas in T_3 (Cow dung + Kitchen waste) for *Pseudomonas* and more in T_6 (Cow dung alone) for *Azotobacter.*

References

Hema, C.R., Sreenivasa, M.N., Hebsur, N.S., Shirnalli, G and Babalad, H.B. 2012. Influence of microbial enrichment on microbial population and nutrient status of organic manures. *Karnataka J. Agri. Sci.*, 25(4): 545-547.

Karmegam, N., Rajasekar, K. 2012. Enrichment of biogas slurry vermicompost with *Azotobacter chroococcum* and *Bacillus megaterium*. *J. Environ. Sci. Technol.*, 5(2): 91-108.

Shruthi, H., Krishna, Naik, L., Shivaprakash, M.K., Maina, C.C. 2014. Survivability of *Bacillus megatherium* in different carrier material for improved shelflife of biofertilizer. National conference on Productivity and Sustainability: Role of Agriculturally Important Microorganisms. University of Agricultural Sciences, GKVK, Banglore.

How to cite this article:

Kavya, Y., A. Vijaya Gopal, R. Subhash Reddy and M. Sreedhar. 2016. Enrichment of Biogas Manures with Beneficial Microorganisms. *Int.J.Curr.Microbiol.App.Sci.* 5(7): 242-252.
doi: http://dx.doi.org/10.20546/ijcmas.2016.507.025