Enrichment of Biogas Manures with Beneficial Microorganisms

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A B S T R A C T

The beneficial microorganisms used in the present study (\textit{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.

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

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 microorganisms.

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\textsubscript{1} 250 g cow dung + 500 g press mud + 1500 ml water (1:2:6), T\textsubscript{2} 250 g cow dung + 500 g poultry litter + 1500 ml water (1:2:6), T\textsubscript{3} 250 g cow dung + 500 g kitchen waste + 1500 ml water (1:2:6), T\textsubscript{4} 250 g cow dung + 500 g maize stalks + 1500 ml water (1:2:6), in T\textsubscript{5} 250 g cow dung + 500 g fruit waste + 1500 ml water (1:2:6) and T\textsubscript{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\(^{\circ}\)C 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 \(^{\circ}\)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\textsubscript{1} (Cow dung + Press mud) \(6.0\times10^{3}\) CFU g\(^{-1}\) compared to T\textsubscript{6} (Cow dung alone) \(3.0\times10^{3}\) CFU g\(^{-1}\), T\textsubscript{2} (Cow dung + Poultry litter) \(3.0\times10^{3}\) CFU
organisms and \(20.2 \times 10^3\) 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 \(3.1 \times 10^9\) 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) \(4.8 \times 10^9\) CFU g\(^{-1}\) compared to T\(_1\) (Cow dung + Press mud) \(3.2 \times 10^9\) CFU g\(^{-1}\), T\(_2\) (Cow dung + Poultry litter) \(2.8 \times 10^9\) CFU g\(^{-1}\), T\(_3\) (Cow dung + Kitchen waste) \(1.0 \times 10^9\) CFU g\(^{-1}\), T\(_5\) (Cow dung + Fruit waste) \(0.9 \times 10^9\) CFU g\(^{-1}\) and less in T\(_4\) (Cow dung + Maize stalks) \(0.8 \times 10^9\) 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) \(1.2 \times 10^9\) CFU g\(^{-1}\) (\(4.8 \times 10^9\) CFU g\(^{-1}\), on the first day to \(6.1 \times 10^9\) CFU g\(^{-1}\), at the end of fourth week) and less in T\(_4\) (Cow dung + Maize stalks) \(0.6 \times 10^9\) CFU g\(^{-1}\) (\(0.8 \times 10^9\) CFU g\(^{-1}\), on the first day to \(1.6 \times 10^9\) 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) \(6.1 \times 10^9\) CFU g\(^{-1}\) at the end of fourth week to \(3.0 \times 10^8\) CFU g\(^{-1}\) at the end of sixth week and less reduction in population was observed in T\(_5\) (Cow dung + Fruit waste) \(1.9 \times 10^9\) CFU g\(^{-1}\) to \(0.8 \times 10^8\) CFU g\(^{-1}\) (Table 3.3). Finally the viability was significantly more in T\(_5\) (Cow dung +
Fruit waste) $0.9 \times 10^9$ CFU g$^{-1}$, on the first day to $0.5 \times 10^7$ CFU g$^{-1}$ at the end of tenth week and comparatively less viability was observed in T$_2$ (Cow dung + Poultry litter) $2.8 \times 10^6$ CFU g$^{-1}$ on the first day and $0.8 \times 10^7$ 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 $3.0 \times 10^9$ 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) $3.2 \times 10^7$ CFU g$^{-1}$ compared to T$_2$ (Cow dung + Poultry litter) $2.4 \times 10^6$ CFU g$^{-1}$, T$_1$ (Cow dung + Press mud) $1.8 \times 10^6$ CFU g$^{-1}$, T$_4$ (Cow dung + Maize stalks) $1.6 \times 10^6$ CFU g$^{-1}$, T$_3$ (Cow dung + Fruit waste) $1.1 \times 10^9$ CFU g$^{-1}$ and less in T$_3$ (Cow dung + Kitchen waste) $1.0 \times 10^9$ 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) $2.6 \times 10^9$ CFU g$^{-1}$ ($1.0 \times 10^9$ CFU g$^{-1}$ on the first day to $3.6 \times 10^9$ CFU g$^{-1}$ at the end of fourth week) and less in T$_2$ (Cow dung + Poultry litter) $1.6 \times 10^6$ CFU g$^{-1}$ ($2.4 \times 10^9$ on the first day to $4.0 \times 10^9$ 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) $3.7 \times 10^9$ CFU g$^{-1}$ at the end of fourth week to $2.1 \times 10^8$ CFU g$^{-1}$ at the end of sixth week and less reduction was observed in T$_2$ (Cow dung + Poultry litter) $4.0 \times 10^9$ CFU g$^{-1}$ at the end of fourth week to $2.5 \times 10^8$ 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) $1.0 \times 10^9$ CFU g$^{-1}$ on the first day to $2.0 \times 10^7$ CFU g$^{-1}$ at the end of tenth week and comparatively less viability was observed in T$_4$ (Cow dung + Maize stalks) $1.6 \times 10^9$ CFU g$^{-1}$ on the first day to $1.4 \times 10^7$ 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 $2.6 \times 10^9$ 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) $2.8 \times 10^9$ CFU g$^{-1}$ compared to T$_1$ (Cow dung + Press mud) $2.2 \times 10^9$ CFU g$^{-1}$, T$_2$ (Cow dung + Poultry litter) $2.0 \times 10^9$ CFU g$^{-1}$, T$_3$ (Cow dung + Kitchen waste) $1.7 \times 10^9$ CFU g$^{-1}$, T$_5$ (Cow dung + Fruit waste) $1.2 \times 10^9$ CFU g$^{-1}$ and less in T$_4$ (Cow dung + Maize stalks) $1.0 \times 10^9$ 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) $1.6 \times 10^9$ CFU g$^{-1}$ ($2.2 \times 10^9$ CFU g$^{-1}$ on the first day to $3.8 \times 10^9$ CFU g$^{-1}$ at the end of third week) and less in T$_3$ (Cow dung + Fruit waste) $1.2 \times 10^9$ CFU g$^{-1}$ ($1.2 \times 10^9$ CFU g$^{-1}$ on the first day to $1.4 \times 10^9$ 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) $2.2 \times 10^9$ CFU g$^{-1}$ at the end of third week to $2.5 \times 10^8$ CFU g$^{-1}$ at the end of fourth week and less reduction was in T$_1$ (Cow dung + Press mud) $3.8 \times 10^9$ CFU g$^{-1}$ to $4.4 \times 10^9$ 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 (CFU g⁻¹) |
|--------------------------------|----------------------|-----------------------|-----------------------|------------------------|
| Cow dung + press mud (T₁)      | 46.0×10³             | 40.0×10³              | 12.0×10³              | 20.0×10³               |
| Cow dung + Poultry litter (T₂) | 32.0×10³             | 44.0×10³              | 42.0×10³              | 12.0×10³               |
| Cow dung + Kitchen waste (T₃)  | 6.1×10³              | 4.6×10³               | 4.6×10³               | 18.0×10³               |
| Cow dung + Maize stalks (T₄)   | 3.1×10³              | 4.0×10³               | 2.0×10³               | 2.2×10³                |
| Cow dung + Fruit waste (T₅)    | 5.1×10³              | 3.0×10³               | 3.1×10³               | 2.8×10³                |
| Cow dung alone (T₆)            | 34.0×10³             | 46.0×10³              | 42.0×10³              | 24.0×10³               |

T₁ = Cow dung (250 g) + Press mud (500 g) + water (1500 ml) – 1:2:6
T₂ = Cow dung (250 g) + Poultry litter (500 g) + water (1500 ml) – 1:2:6
T₃ = Cow dung (250 g) + Kitchen waste (500 g) + water (1500 ml) – 1:2:6
T₄ = Cow dung (250 g) + Maize stalks (500 g) + water (1500 ml) – 1:2:6
T₅ = Cow dung (250 g) + Fruit wastes (500 g) + water (1500 ml) – 1:2:6
T₆ = 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)    | 3.1×10⁹      |
| King’s B broth (Pseudomonas)                | 3.0×10⁹      |
| Azotobacter glucose broth (Azotobacter)     | 2.6×10⁹      |
| Potato infusion broth (Azospirillum)         | 2.6×10⁹      |
Table 3: Population of *Rhizobium* 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) \(\times 10^9\) CFU g\(^{-1}\) | At the end of eighth week (56th day) \(\times 10^9\) CFU g\(^{-1}\) | At the end of tenth week (70th day) \(\times 10^9\) CFU g\(^{-1}\) |
|-------|------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| \(T_1\) | 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.

| Treatment (T)          | 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) (\(\times 10^9\)CFU g\(^{-1}\)) | At the end of sixth week (42\(^{nd}\) day) (\(\times 10^7\)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) (\(\times 10^7\)CFU g\(^{-1}\)) |
|------------------------|--------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|-----------------------------------------------------------------------|-----------------------------------------------------------------------|-----------------------------------------------------------------------|
| T\(_1\) Cow dung (250 g) + Press mud (500 g) + water (1500 ml) – 1:2:6 | 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\(_2\) Cow dung (250 g) + Maize stalks (500 g) + water (1500 ml) – 1:2:6 | 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\(_3\) Cow dung (250 g) + Poultry litter (500 g) + water (1500 ml) – 1:2:6 | 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\(_4\) Cow dung (250 g) + Fruit wastes (500 g) + water (1500 ml) – 1:2:6 | 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\(_5\) Cow dung (250 g) + Kitchen waste (500 g) + water (1500 ml) – 1:2:6 | 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\(_6\) Cow dung (750 g) + water (1500 ml) – 3:6 | 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  \(T_2\) = Cow dung (250 g) + Maize stalks (500 g) + water (1500 ml) – 1:2:6  \(T_3\) = Cow dung (250 g) + Poultry litter (500 g) + water (1500 ml) – 1:2:6  \(T_4\) = Cow dung (250 g) + Fruit wastes (500 g) + water (1500 ml) – 1:2:6  \(T_5\) = 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 (×10^9 CFU g⁻¹) | At the end of first week (7th day) (×10^9 CFU g⁻¹) | At the end of second week (14th day) (×10^9 CFU g⁻¹) | At the end of third week (21st day) (×10^9 CFU g⁻¹) | At the end of fourth week (28th day) (×10^9 CFU g⁻¹) | At the end of sixth week (42nd day) (×10^8 CFU g⁻¹) | At the end of eighth week (56th day) (×10^7 CFU g⁻¹) | At the end of tenth week (70th day) (×10^6 CFU g⁻¹) |
|---|--------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| T₁ | 2.23 (0.80)              | 3.03 (0.37)                                  | 3.40 (0.46)                                  | 4.46 (0.60)                                  | 2.03 (2.43)                                  | 3.13 (1.71)                                  | 3.16 (2.80)                                  |
| T₂ | 2.03 (0.73)              | 2.76 (0.40)                                  | 3.16 (0.40)                                  | 4.03 (0.47)                                  | 1.56 (2.47)                                  | 2.36 (1.32)                                  | 2.23 (2.13)                                  |
| T₃ | 1.66 (0.47)              | 2.13 (0.53)                                  | 2.66 (0.37)                                  | 3.63 (0.60)                                  | 1.3392 (3.0)                                 | 2.16 (1.11)                                  | 1.40 (2.00)                                  |
| T₄ | 1.03 (0.40)              | 1.43 (0.60)                                  | 2.03 (0.20)                                  | 2.56 (0.33)                                  | 1.03 (1.53)                                  | 2.26 (0.80)                                  | 1.13 (2.14)                                  |
| T₅ | 1.26 (0.57)              | 1.83 (0.57)                                  | 2.23 (0.40)                                  | 3.03 (0.60)                                  | 1.13 (1.90)                                  | 2.00 (0.90)                                  | 1.26 (1.87)                                  |
| T₆ | 2.83 (0.73)              | 3.56 (0.47)                                  | 4.03 (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 |

*T₁ = Cow dung (250 g) + Press mud (500 g) + water (1500 ml) – 1:2:6 T₂ = Cow dung (250 g) + Maize stalks (500 g) + water (1500 ml) – 1:2:6 T₃ = Cow dung (250 g) + Poultry litter (500 g) + water (1500 ml) – 1:2:6 T₄ = Cow dung (250 g) + Fruit wastes (500 g) + water (1500 ml) – 1:2:6 T₅ = Cow dung (250 g) + Kitchen waste (500 g) + water (1500 ml) – 1:2:6 T₆ = 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.

| Treatment | Initial (×10⁹ CFU g⁻¹) | At the end of first week (7th day) (×10⁹ CFU g⁻¹) | At the end of second week (14th day) (×10⁹ CFU g⁻¹) | At the end of third week (21st day) (×10⁹ CFU g⁻¹) | At the end of fourth week (28th day) (×10⁹ CFU g⁻¹) | At the end of sixth week (42nd day) (×10⁸ CFU g⁻¹) | At the end of eighth week (56th day) (×10⁷ CFU g⁻¹) | At the end of tenth week (70th day) (×10⁶ CFU g⁻¹) |
|-----------|-------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| T₁        | 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)                                    |
| T₂        | 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)                                    |
| T₃        | 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)                                    |
| T₄        | 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)                                    |
| T₅        | 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)                                    |
| T₆        | 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                                         |

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

T<sub>₁</sub> = Cow dung (250 g) + Press mud (500 g) + water (1500 ml) – 1:2:6  
T<sub>₂</sub> = Cow dung (250 g) + Poultry litter (500 g) + water (1500 ml) – 1:2:6  
T<sub>₃</sub> = Cow dung (250 g) + Kitchen waste (500 g) + water (1500 ml) – 1:2:6  
T<sub>₄</sub> = Cow dung (250 g) + Maize stalks (500 g) + water (1500 ml) – 1:2:6  
T<sub>₅</sub> = Cow dung (250 g) + Fruit wastes (500 g) + water (1500 ml) – 1:2:6  
T<sub>₆</sub> = Cow dung (750 g) + water (1500 ml) – 3:6
Finally the viability was significantly more in T₆ (Cow dung alone) 2.8×10⁹ CFU g⁻¹ on the first day to 4.1×10⁶ CFU g⁻¹ at the end of tenth week and comparatively less viability was observed in T₃ (Cow dung + Kitchen waste) 1.7×10⁹ CFU g⁻¹ on the first day to 1.4×10⁷ 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 2.6×10⁹ 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 T₆ (Cow dung alone) 2.6×10⁹ CFU g⁻¹ compared to T₁ (Cow dung + Press mud) 2.4×10⁹ CFU g⁻¹, T₂ (Cow dung + Poultry litter) 2.0×10⁹ CFU g⁻¹, T₃ (Cow dung + Kitchen waste) 1.4×10⁹ CFU g⁻¹, T₄ (Cow dung + Maize stalks) 1.0×10⁹ CFU g⁻¹ and less in T₅ (Cow dung + Fruit waste) 0.6×10⁹ 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 T₅ (Cow dung + Fruit waste) 1.7×10⁹ CFU g⁻¹ (0.6×10⁹ CFU g⁻¹ on the first day to 2.3×10⁹ CFU g⁻¹ at the end of fourth week) and less in T₄ (Cow dung + Maize stalks) 1.1×10⁹ CFU g⁻¹ (1.0×10⁹ CFU g⁻¹ on the first day to 2.1×10⁹ 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 T₂ (Cow dung + Poultry litter) 3.4×10⁸ CFU g⁻¹ at the end of fourth week to 2.0×10⁸ CFU g⁻¹ at the end of sixth week and less reduction was observed in T₄ (Cow dung + Maize stalks) 2.16×10⁸ CFU g⁻¹ to 2.03×10⁸ CFU g⁻¹ (Table 3.6). Finally the viability was significantly more in T₃ (Cow dung + Fruit waste) 0.6×10⁹ CFU g⁻¹ on the first day to 1.3×10⁶ CFU g⁻¹ at the end of tenth week and comparatively less viability was observed in T₄ (Cow dung + Maize stalks) 1.0×10⁹ CFU g⁻¹ on the first day to 1.0×10⁶ 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 (13.3×10⁷ CFU g⁻¹ at 30 days and 2×10⁷ 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 80.5×10⁵ bacteria, 124×10³ fungi, 12×10² actinomycetes, 15.3×10³ phosphate solubilise micro organisms and 20.2×10³ 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 1×10⁷ 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 T3 (Cow dung + Fruit waste) for Rhizobium and Azospirillum, whereas in T3 (Cow dung + Kitchen waste) for Pseudomonas and more in T6 (Cow dung alone) for Azotobacter.

References

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