Co-digestion of Pretreated Chicken - Goat and Untreated Cow Manure at Different Substrate to Inoculums Ratios and Total Solids for Biogas Production

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ABSTRACT

Biogas production can be greatly affected by inoculum addition and total solids. The effect of substrate to inoculum ratios and total solids of chicken, goat and cow manure on biogas production was studied using a 0.15m³ laboratory scale batch digester at a constant temperature of 35°C. Feed stocks were mechanically minced to 3 mm effective particle sizes prior to co-digesting with untreated cow manure from a free-range dairy rearing system. Different amounts of cow substrate inoculum were used at ratios of 2:1, 3:1, 4:1, 5:1 and 6:1, while total solid levels between (7.5% and 10.5%) at intervals of 0.5% were used to study their effects on biogas production. Increasing inoculums and total solids resulted in increased biogas production with peaks at a substrate to inoculum ratio of 4:1 (20% inoculum addition) and 9% total solids. Biogas production rates of 0.61 and 0.63m³/m³d were realized respectively.

Keywords: Biogas Production, Chicken-Goat-Cow Manure, Substrate to Inoculum Ratios, Total Solids

INTRODUCTION

Countries are moving away from fossil to renewable fuel-based economies because of increased negative environmental effects of the latter, thus biomass production and consumption chains are being promoted in generation of eco-friendly and sustainable fuels [1].

Substrate to inoculum (S/I) ratio is the quantitative relation of the amount of volatile solids in the substrate per the amount of volatile solids contained in the inoculum at the start of each batch digestion process [2; 3]. Each feed stock has its suitable substrate to inoculum ratio [4], because of the material-specific quantity of volatile fatty acids and its capacity to buffer against the VFAs that accumulate throughout its biodegradation process. Chicken-goat-cow manure mixture can provide the right C/N ratio for anaerobic digestion and re-feeding of spent slurry (contains washed out microbes) into the digester can also be a way of improving biogas production [5].

Total solids (TS) content is the dry matter of a feedstock or substrate and includes both volatile and dissolved solids. The role total solids (TS) on activities of anaerobic bacteria are always important in order...
to increase the efficiency of the anaerobic digestion process [6] as too much TS leads to clogging of the system while too much dilution decreases biogas digestion. A variation in TS content influences changes in microbial activity and therefore affects the amount of gas produced [7; 8; 9]. Information on the optimal total solids in the chicken-goat-cow manure substrate is, therefore, critical and useful in determining an efficient biogas production system from these materials.

Co-digestion is the anaerobic bio-degradation of a homogenous multi-mixture substrate. It leads to low-cost biogas production and may encompass a more efficient use of digesters [10]. The multi components should provide a complementary growth factor to give a balanced C/N ratio [11]. Co-digestion of the chicken, goat and cow manure can offer a suitable C/N [12] ratio for system stability and improve biogas production.

Most studies focused on the effect of inoculum levels alone on gas yield; however, this research sought to find the optimum substrate to inoculum ratio and corresponding total solids for anaerobic co-digestion of chicken, goat and cow manure.

MATERIALS AND METHODS

Substrate Preparation

Preparation of 120 litres of influent was done by mixing mechanically pretreated (3mm effective particle size) chicken, goat and fresh cow manure (at ratios 1:1:1 volatile solid basis) [13, 14] with spent cow dung slurry at different ratios. Inoculum was passed through a 2 mm sieve to eliminate any biodegradable material remnants prior to preparation of substrate to inoculum (S/I) ratios.

(a) Substrate to inoculum ratios

The effect of substrate to inoculum ratios on biogas production was investigated at 2:1, 3:1, 4:1, 5:1 and 6:1, for 10 days each. Substrate to inoculum ratios were distributed around an optimal ratio of 4:1 reported in literature when fresh cow manure [15] was used in the co-digestion of food waste and rice husks, anaerobically digested cow manure was used to inoculate wheat straw digestion [16] and pig slurry effluent to inoculate the biodegradation of pig slaughterhouse wastes [3]. The base S/I ratio is within the optimal range reported by Dennis [17] for cow manure and rumen inoculum.

Table 1 presents volumes of substrate and inoculum used to prepare digester influent at different ratios.

| Table 1: Influent preparation at different substrate to inoculum ratios |
| --- |
| **Substrate** | **Inoculum added** |
| **Substrate to Inoculum Ratio** | **%** | **Volume (litres)** | **%** | **Volume (litres)** |
| 2:1 | 66.67 | 80.00 | 33.33 | 40.00 |
| 3:1 | 75.00 | 90.00 | 25.00 | 30.00 |
| 4:1 | 80.00 | 96.00 | 20.00 | 24.00 |
| 5:1 | 83.33 | 100.00 | 16.67 | 20.00 |
| 6:1 | 85.71 | 102.85 | 14.29 | 17.15 |
(b) Total solids

Substrate for digester feeding was prepared by separately diluting different weights of pretreated chicken, goat and fresh cow manure with computed quantities of tap water to attain different influent total solids. This was because pretreated chicken, goat and untreated cow manure had average total solids of 88.97%, 33.53% and 14.72% respectively, hence required separate dilution to predetermined influent total solids prior to their mixing.

The effect of total solids on biogas production was investigated at 7.5%, 8.0%, 8.5%, 9.0%, 9.5%, 10.0 % and 10.5%; each for 10 days. The choice of total solids for the study were guided by Budiyono et al. [20], Abbassi-Guendouz et al. [21]; Orhorhoro et al. [6] and Paramaguru et al. [22] who reported a TS range of 8 % to 10% as optimal for biogas production from most feed stocks. Preparation of influent at every TS level was done using equations 1 and 2

\[
Feedstock = \left( \frac{F_{ts}}{I_n} \right) \times TI
\]

\[
Water = \left( 1 - \frac{F_{ts}}{I_n} \right) \times TI
\]

where; Fts = Final total solids of the substrate (%), Its = Initial total solids of fresh feed stock (%), TI = Total substrate, comprising of water and feed stock

Table 2 represents individual feed stock to water ratios used in their dilution to attain the different total solids in the final influent as per equations 1 and 2.

| Total solids (%) | Feed stock to water ratio |
|------------------|--------------------------|
|                  | Chicken manure | Goat manure | Cow manure | Total substrate |
| 7.5              | 1:10.7         | 1:3.5       | 1.0:1      | 1:2.4           |
| 8.0              | 1:10.0         | 1:3.2       | 1.2:1      | 1:2.2           |
| 8.5              | 1:9.3          | 1:2.9       | 1.4:1      | 1:2.0           |
| 9.0              | 1:8.7          | 1:2.7       | 1.6:1      | 1:1.8           |
| 9.5              | 1:8.3          | 1:2.5       | 1.8:1      | 1:1.7           |
| 10.0             | 1:7.8          | 1:2.4       | 2.1:1      | 1:1.5           |
| 10.5             | 1:7.4          | 1:2.2       | 2.5:1      | 1:1.4           |

Key: F = Feed Stock, W = Water

RESULTS AND DISCUSSION

Effect of Substrate to Inoculum Ratios on Biogas Production

Data on biogas production rates and trends from different inoculum levels of pretreated chicken-goat and untreated cow manure, is presented in Table 3, Figures 3 and 4. It is evident that increasing the amount of inoculum (Cow substrate inoculum – CSI) significantly increased biogas yield up to an optimal ratio of 4:1 (20% inoculum addition) with a production rate of 0.61m$^3$/m$^3$d. Further addition of inoculums resulted in reduced biogas production ($\alpha = 0.05$, LSD = 0.02) (Table A in the Appendix)
Table 3: Biogas production rates from different Substrate to Inoculum ratios

| Day | Gas yield from different Substrate to Inoculum ratios | Production per unit digester volume (m³/m³ d) |
|-----|------------------------------------------------------|---------------------------------------------|
|     | Substrate to Inoculum ratio                         | 6    | 5    | 4    | 3    | 2    | Mean |
| 1   |                                                      | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2   |                                                      | 0.41 | 0.42 | 0.44 | 0.43 | 0.42 | 0.42 |
| 3   |                                                      | 0.45 | 0.55 | 0.57 | 0.54 | 0.47 | 0.51 |
| 4   |                                                      | 0.53 | 0.58 | 0.68 | 0.59 | 0.54 | 0.58 |
| 5   |                                                      | 0.60 | 0.65 | 0.72 | 0.66 | 0.62 | 0.65 |
| 6   |                                                      | 0.67 | 0.70 | 0.75 | 0.72 | 0.67 | 0.70 |
| 7   |                                                      | 0.61 | 0.60 | 0.66 | 0.64 | 0.62 | 0.63 |
| 8   |                                                      | 0.56 | 0.55 | 0.58 | 0.56 | 0.56 | 0.56 |
| 9   |                                                      | 0.53 | 0.54 | 0.55 | 0.54 | 0.54 | 0.54 |
| 10  |                                                      | 0.47 | 0.50 | 0.52 | 0.52 | 0.48 | 0.50 |
| Cumulative |                                            | 4.83 | 5.09 | 5.46 | 5.19 | 4.92 | 5.10 |
| Mean |                                                      | 0.54d | 0.57cb | 0.61a | 0.58b | 0.55dc | 0.57 |

Means followed by the same letter(s), (a, b, c, d), are not significantly different at α = 0.05, LSD = 0.02.

This is attributable to a better balance offered by the amount of substrate and inoculum in the digester at 20% (S/I = 4:1) inoculum addition whereas the other combinations received setbacks of unbalanced substrate to inoculum ratio [21]. It may also be due to excessive proportions of inoculum occupying more digester volume hence reducing its organic loading [22]. Brown and Li [23] also observed that gas yield from the co-digestion of food yard waste and food waste decreased with increasing S/I ratios.

Plots of different biogas yield trends obtained for the different S/I ratios are shown in Fig. 3. Biogas from all the experimental runs began on the 2nd day throughout the entire period to the set 10 days of AD, with peak production on the 6th day. Higher gas yields at lower S/I ratios (more inoculum addition) may be attributed to the increased inoculums for more methanogenic bacteria that facilitated the effective conversion of volatile fatty acids into biogas [24; 25]. Biodegradation of inoculum volatile solids might be occurring simultaneously with that of the substrate in the reactor.

With an R² value of 0.878 (within the range R² = 0.75- 1.0) [24], 87.8% of the variation in actual data represented a good fit and could be predicted by and represented the model. Biogas production exhibits an inverse relationship with increase inoculums [27; 28] as lower S/I ratios might cause system instability hence low gas output [29]. Excessive S/I ratios can lead to VFA accumulation, inhibition and incomplete feedstock degradation and hence lower biogas yield [18; 30], therefore careful compromise on the ratios is key, given the type of substrate and inoculum.

Therefore, the relationship in equation (3) can be developed for different substrate conditions for future universality and use by biogas plant operators to predict gas production from their plants at different ratios, depending on the availability of substrate contents and cow manure inoculum.
Figure 3: Biogas production at different substrate to inoculum ratios

Average production rate from co-digesting the three feed stocks at various substrate to inoculum ratios can be predicted (as guided figure 4) by using equation 3;

\[
y = -0.0134x^2 + 0.1108x + 0.3652 \\
\text{where } y = \text{Average biogas production rate (m}^3/\text{m}^3 \text{d)} \\
x = \text{Substrate to Inoculum ratio}
\]

Figure 4: Average biogas production rate from different Substrate to Inoculum ratios
Effect on Total Solids on Biogas Production

Co-digesting pretreated chicken-goat and untreated cow manure at different total solids resulted in biogas production data presented in Table 4 and Figure 5.

| Day | Gas yield from different Total Solids Production per unit digester volume ($m^3/m^3d$) |
|-----|----------------------------------------------------------------------------------------|
|     | Total solids (%)                                                                       |
|     | 7.50 | 8.00 | 8.50 | 9.00 | 9.50 | 10.00 | 10.50 | Mean |
| 1   | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00  | 0.00  | 0.00 |
| 2   | 0.41 | 0.42 | 0.44 | 0.43 | 0.43 | 0.41  | 0.42  |      |
| 3   | 0.46 | 0.55 | 0.57 | 0.57 | 0.56 | 0.45  | 0.51  |      |
| 4   | 0.53 | 0.68 | 0.68 | 0.69 | 0.68 | 0.58  | 0.62  |      |
| 5   | 0.60 | 0.70 | 0.71 | 0.71 | 0.71 | 0.66  | 0.59  | 0.67 |
| 6   | 0.67 | 0.73 | 0.75 | 0.76 | 0.76 | 0.68  | 0.66  | 0.72 |
| 7   | 0.62 | 0.66 | 0.68 | 0.70 | 0.70 | 0.64  | 0.62  | 0.66 |
| 8   | 0.58 | 0.62 | 0.63 | 0.65 | 0.63 | 0.60  | 0.58  | 0.62 |
| 9   | 0.50 | 0.56 | 0.56 | 0.61 | 0.55 | 0.56  | 0.50  | 0.55 |
| 10  | 0.43 | 0.47 | 0.53 | 0.54 | 0.53 | 0.51  | 0.43  | 0.49 |
| Cumulative | 4.81 | 5.39 | 5.55 | 5.67 | 5.55 | 5.11  | 4.75  | 5.26 |
| Mean | 0.53d | 0.60b | 0.62ba | 0.63a | 0.62ba | 0.57c | 0.53d | 0.58 |

Means followed by the same letter(s), (a, b, c, d), are not significantly different at $\alpha = 0.05$, LSD = 0.0231

Mean biogas production rate significantly increased ($\alpha = 0.05$, LSD = 0.02) (Table B in Appendix) with an increase in total solids up to 9% where maximum yield rate of $0.63 m^3/m^3d$ was realized. Continued increase in the total solids being fed to the digester exhibited an inverse relationship with biogas production. A slight increase in total solids marginally improves gas yield up to some point (7.5% to 9.0%), after which further increase in total solids no longer results in increased biogas production (9.0% to 10.5%), as reported in previous works by Igoni et al. [7] and Masinde et al. [31].

This is attributable to higher organic loading rates and volatile fatty acid in the digester that lead to mesophilic bacteria decline [32; 9], and digester clogging [7]. Total solids of 7.5% to 9.0% might have provided more moisture content that enhanced mass transfer during the process [24; 25] whereas the lower amounts of water at higher total solids above 9.0% reduced microbial activity, hence lower biogas production Igoni et al. [7].

From figure 5, average production rate from co-digesting three feed stocks at different total solids can be predicted using equation 4:

$$y = -0.043x^2 + 0.775x - 2.832$$  \(4\)

where $y$ = Average biogas production rate ($m^3/m^3d$), $x$ = Total solids (%)
The model took care of 97.2% ($R^2 = 0.972$) of variations in actual data hence can be used by biogas plant operators to predict biogas production from their plants when using chicken-goat-cow manure substrate, with the best total solids loading being 9.0%. This result is within the range of 8 – 10% for most feed stocks as reported by Budiyono et al. [18], Abbassi-Guendouz et al. [19]; Orhorhoro et al. [6] and Paramaguru et al. [20].

Inadequate microorganisms (low inoculum volume) for substrate degradation may derail the methane production process [32, 33] whereas too high inoculum levels may lead to low cumulative biogas yield due to inadequate organic nutrients for the microorganisms leading to their decline. A careful integration between total solids and inoculums is required to get high production rates from the three feed stocks to enhance microbial activity, facilitate mass transfer and offer nutrient stability to the methanogenic bacteria. Therefore, an effective way of improving biogas yield from the three feed stocks would require optimizing the S/I ratio and TS of the influent.

CONCLUSION AND RECOMMENDATIONS

From the research, biogas yield rate increased with the addition of inoculums up to a maximum of $0.61 \text{m}^3/\text{m}^3\text{d}$ at $S/I = 4:1$ (20% inoculum addition), after which further increase in inoculum (lower S/I ratios) resulted in lower gas production. Biogas production increased steadily with increase in total solids from 7.5% to 9.0% and declines as total solids increase further above 9.0%. Therefore, 20% inoculum addition and total solids of 9.0% would be ideal for the co-digestion of chicken, goat and untreated cow feed stocks.

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**APPENDIX**

**Table A-1:** Dependent Variable - Substrate/Inoculum ratio Biogas yield rate

| Source       | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|--------------|----|----------------|-------------|---------|--------|
| Model        | 12 | 0.31118111     | 0.02593176  | 45.59   | <.0001 |
| Error        | 32 | 0.01820145     | 0.00056880  |         |        |
| Corrected Total | 44 | 0.32938256     |            |         |        |

**R-Square**

| Coeff Var | Root MSE | TS_Biogas_yield_rate Mean |
|-----------|----------|---------------------------|
| 0.944741  | 4.211184 | 0.023849                  |
|           | 0.000569 | 0.566336                  |

| Source       | DF | Type III SS | Mean Square | F-value | Pr > F |
|--------------|----|-------------|-------------|---------|--------|
| Rep          | 8  | 0.28416508  | 0.03552063  | 62.45   | <.0001 |
| Var          | 4  | 0.02581920  | 0.00645480  | 11.35   | <.0001 |

**Table A-2:** t Tests (LSD) for Inoculums level biogas yield rate

| Alpha                              |
|------------------------------------|
| 0.05                               |

| Error Degrees of Freedom | 32 |
|--------------------------|----|
| Error Mean Square        | 0.000569 |
| Critical Value of t      | 2.03693 |
| Least Significant Difference | 0.0229 |

| t Grouping | Mean  | N  | Var |
|------------|-------|----|-----|
| A          | 0.60660 | 9  | L   |
| B          | 0.57661 | 9  | M   |
| BC         | 0.56513 | 9  | K   |
| CD         | 0.54698 | 9  | N   |
| D          | 0.53636 | 9  | J   |

Means with the same letter (s) (a, b, c, d) are not significantly different.

Key: S/I for J = 6:1, K = 5:1, L = 4:1, M = 3:1 and N = 2:1
Table B-2: Dependent Variable - TS Biogas_yield_rate

| Source       | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|--------------|----|----------------|-------------|---------|--------|
| Model        | 14 | 0.60809841     | 0.04343560  | 73.30   | <.0001 |
| Error        | 48 | 0.02844444     | 0.00059259  |         |        |
| Corrected Total | 62 | 0.63654286  |             |         |        |

R-Square 0.955314  Coeff Var 4.166322  Root MSE 0.024343  TS_Biogas_yield_rate Mean 0.584286

| Source | DF | Type III SS | Mean Square | F-value | Pr > F |
|--------|----|-------------|-------------|---------|--------|
| Rep    | 8  | 0.51280000  | 0.06410000  | 108.17  | <.0001 |
| Var    | 6  | 0.09529841  | 0.01588307  | 26.80   | <.0001 |

Table B-2: t Tests (LSD) for Total Solids biogas yield rate

| Alpha  | 0.05 |
|--------|------|
| Error Degrees of Freedom | 48 |
| Error Mean Square | 0.000593 |
| Critical Value of t | 2.01063 |
| Least Significant Difference | 0.0231 |

| t Grouping | Mean | N | Var |
|------------|------|---|-----|
| A          | 0.63000 | 9 | M   |
| BA         | 0.61667 | 9 | N   |
| BA         | 0.61667 | 9 | L   |
| B          | 0.59889 | 9 | K   |
| C          | 0.56778 | 9 | P   |
| D          | 0.53333 | 9 | J   |
| D          | 0.52667 | 9 | Q   |

Means with the same letter (s) (a, b, c, d) are not significantly different.

Key: J = 7.5%, K = 8.0%, L = 8.5%, M = 9.0%, N = 9.5%, P = 10.0%, Q = 10.5% total solids