The Impact of Physicochemical Parameter In Anaerobic Digestion of Organic Wastes

Stella Suanu Leh-Togi Zobeashia (✉ starbabet71@yahoo.com)  
Federal University of Technology Minna

Peter Olabisi Abioye  
Federal University of Technology Minna

Udeme Joshua Josiah Ijah  
Federal University of Technology Minna

Oluwafemi Adebayo Oyewole  
Federal University of Technology Minna

Research Article

Keywords: Anaerobic digestion, system, physicochemical properties, biogas, substrate, retention time

DOI: https://doi.org/10.21203/rs.3.rs-539720/v1

License: ☑️ ⬤ This work is licensed under a Creative Commons Attribution 4.0 International License.  
Read Full License
Abstract

The demand for an alternative source of energy, the challenge of increase in waste and pollution initiates the need for renewable energy and management of waste using anaerobic digestion (AD). AD is an effective and efficient method of waste treatment and energy generation. Certain operational parameters influence the anaerobic degradation of waste to energy. The impact of physicochemical parameters in anaerobic digestion was conducted using chicken wastes and food wastes as organic substrate under semi-continuous conditions at hydraulic retention time (HRT) of forty-two (42) days in a fifteen (15) litre fabricated digesters labeled D1, D2 and D3 at mesophilic temperature. The pH, volatile solid, percentage moisture, total ammonia, total solid, volatile fatty acid, alkalinity was assessed. The results indicated a pH value of 6.65 ± 0.12, 7.27 ± 0.13, 6.43 ± 0.27, volatile fatty acid of 72.17 ± 1.42, 58.35 ± 2.58, 40.56 ± 0.38, and moisture content of 98.9 ± 2.65, 92.3 ± 1.81, 96.4 ± 3.60 for D1 (Chicken waste and food wastes), D2 (Chicken wastes†), D3 (control) respectively. A collective biogas yield of 686±17.00 kpa for D1, 700±11.00kpa for D2 and 521±21.00 kpa for D3 were recorded. The characterization of biogas analyzed with non-dispersive infrared (NDIR) gas analyzer (gas board 3100p) revealed a percentage methane content of 46.11±1.11%, 52.4±1.05 %, 50.31±1.33 % for D1, D2 and D3 respectively. The study shows that physicochemical properties are useful tools to indicate digester failure/stability and also to enhance anaerobic digestion process.

Introduction

Increase in population along with the growing demand for livestock production have made animal husbandry a growing industry in many countries. This automatically results in immense livestock manure which will have negative effect on the environment [1]. Inadequate management of this manure leads to adverse environmental conditions such as ground and surface water contamination, spread of pathogens and disease-causing organisms, offensive odour and emission of green-house gases. Stringent environmental rules on waste management has led to the usage of anaerobic digestion method to livestock manure and an ample mixture of new wastes, including industrial wastes, food wastes, abattoir waste, municipal solid wastes (MSW), farm-house wastes, distillery and lipid rich wastes to cushion the effects of this wastes on the environment [2, 3].

Anaerobic digestion (AD) is a series of controlled biological degradation process in which microorganisms metabolize and stabilize biodegradable material in anaerobic conditions. It is an important renewable energy technology and has been assess as a well-organized, ecofriendly, environmentally and economically beneficial technology for waste management and conversion of wastes into energy [4]. In addendum to renewable energy production AD can be used to close the hoop between production and utilization of organic wastes by optimal recycling rather than landflling which results in greenhouse gas emissions and leaching of nutrients into the environment [5].
Anaerobic digestion includes four main steps hydrolysis, acidogenesis, acetogenesis and methanogenesis, the first three steps are completed by bacteria and the final one by archaea [6]. Each of the steps function effectively with relationship with physicochemical conditions. Several studies have shown the impacts of operational parameters such as pH, alkalinity, volatile fatty acid, volatile solid, moisture and organic loading rate has on biodegradation process, product and the succession of microbial community. Research carried out by Krakat et al.[7] reported a correlation between organic loading rate and bacterial community structure while Lerm et al.[8] observed a change from acetotrophic methanogen of genus Methanosarcina to the limited hydrogenotrophic methanogens Methanospirillum and Methanoculleus when organic loading rate was increased from 2.5 to 40 kg VS m$^{-3}$ day$^{-1}$. The shift in archaeal succession was attributed to significant increase in volatile fatty acid concentration which can also bring about changes in other parameters such as pH. Moisture content for instance supports the movement and growth of bacteria facilitating the dissolution and transport of nutrient and reduces the limitation of mass transfer of non-homogenous or particulate substrate. In general, the moisture content of the digestate increased with decrease in the amount of volatile solid and total solid thereby making these parameters accountable for biogas production [9]. Therefore, knowledge of the physicochemical properties of the waste is required to assess the quality of livestock substrate, determine the anaerobic digestion process and digester performance, eliminate the possibility of system failure and predict the condition of anaerobic system which automatically can help optimize the process and has the potential to radically improve the economic profitability of AD system. The aim of this research was to determine the impact of physicochemical parameters in anaerobic digestion of organic wastes (chicken waste and food waste).

**Materials And Methods**

**Collection and Processing of Samples**

The chicken waste used in this study was collected from Premium poultry farm located at Kuje Federal Capital Territory Abuja, Nigeria. The food wastes was collected from fast food vendors, Gwarimpa district, Federal Capital Territory Abuja, Nigeria. The samples were collected in a sterile container and transported to the Microbiology laboratory, Federal University of Technology Minna, Niger State, Nigeria.

**Digester Design**

The three-digester used for this study was constructed at a metal fabricating (welding) workshop at Zuba mechanic village Abuja, Nigeria. The digester is a 15litre capacity made up of internal structure of plastic and external structure of aluminum material. Its dimension consists of a height (H) of 46cm and diameter (D) of 28cm. It has cast, internal gas re-injecting agitating mechanism to stimulate mixing within the digester. It has attached thermometer to read the average temperature within the digester, attached to the digester also, is a substrate collector (H-26cm, D-17.5cm) with an inlet to collect the substrate and feed the digester and outlet to remove the digested slurry. Gas outlet/collector (H-17.5cm, D-15cm) to collect
the biogas, pressure gauge to measure the pressure within the reactor and highly resilient adhesive and plastic seals to prevent leakages.

**Experimental Procedure**

Twelve (12) kilogram each of the fresh chicken waste (CW) in a ratio of 3:1 of waste to water was fed into three (3) fabricated semi-continuous digesters labeled digester one (D1), digester two (D2) and digester three (D3) with working volume of 15Liters through the inlet and sealed properly to prevent air from entering. Anaerobic digestion of the substrate by microorganisms was allowed for a period of forty-two (42) days under mesophilic condition. The digesters was allowed for a period of twenty-one (21) days to adapt to the mono-substrate of chicken wastes. After the 21 days interval, two kilogram (2kg) of dry chicken wastes was added to D2 while D1 was co-digested with 2kg of food wastes (FW) and chicken wastes in a ratio of 1:1. The feed of D3 was kept unchanged to act as a reference. Within the retention time, biogas production and composition were monitored and recorded using non-dispersive infrared (NDIR) gas analyzer (gas board 3100p) and pressure gauge at two (2) days interval for 42days.

**Physicochemical Characterization**

Parameters such as pH, was determined using pHep pocket-sized pH meter (HANNA Instruments). Chemical oxygen demand (COD) was determined by Hanna Instruments HI 83224. Total alkalinity (TA) Volatile solid (VS), Total solid (TS) and moisture content was measured according to standard APHA methods while ammonium concentration, volatile fatty acid was analyzed as described previously by Lin et al.[10].

**Total solid**

The total solid content of the substrate was determined by drying a known volume of the substrate in a pre-weighed crucible dish at 105°C in a hot air oven for one hour. After which, it was left to cool at room temperature in a desiccator and weighed. The TS was computed using the following formula:

\[ TS = \frac{(M_1 - M_2)}{V} \]

with

- \( M_1 \): mass of crucible dish after drying at 105°C (mg)
- \( M_2 \): mass of initial crucible dish (mg)
- \( V \): Volume of sample (L)

**Volatile solid**
Volatile solids are the amount of solid that volatilizes when heated at 550°C. It was estimated by burning the total solid at 550°C for about 2 hours in a muffle furnace. The crucible was taken out and allowed to cool in a desiccator and then weighed. The VS was determined using the formula:

\[
VS = \frac{(M_1 - M_3)}{V}
\]

with

\[M_1: \text{mass of crucible dish after drying at 105°C (mg)}\]

\[M_3: \text{Mass of crucible dish after ignition at 550°C (mg)}\]

\[V: \text{Volume of sample (L)}\]

**Moisture Content**

The moisture content of the substrate was determined by weighing 10g of substrate into a converted dish previously dried at 98–100°C, it was allowed to cool in desiccator and weighed soon after reaching room temperature. The cover was loosened and heated at 98–100°C to constant weight. At the end of drying, the cover was tightened on the dish and transferred to desiccator and weighed soon after reaching room temperature.

\[
\text{Moisture}\% = \frac{\Delta SB - \Delta SA}{\text{Weight of sample}} \times 100
\]

\[\Delta SB = \text{weight of dish and sample before drying.}\]

\[\Delta SA = \text{weight of dish and sample after drying.}\]

**Ammonia concentration**

Ammonia concentration was determined by digesting the substrate with concentrated sulphuric acid at high temperature in the presence of a catalyst (CuSO₄) and a salt (K₂SO₄) until fumes started occurring. Mercury ammonium complex generated was decomposed by the addition of sodium thiosulfate/sodium hydroxide reagent after digestion. The flask used for digestion was connected to a steamed-out distillation apparatus, and the ammonia which has been generated from (NH₄)₂SO₄ by addition of hydroxide solution was distilled to a receiving flask containing a boric acid solution. Afterwards the distilled ammonia was determined by titration with standard solution of sulphuric acid[11, 10].

**Results**

**Physicochemical parameter analysis of substrate before and after digestion.**
Table 1 represents the results of the physicochemical parameter of the chicken wastes before and after digestion. A pH value of 7.91 ± 0.04 was recorded for the fresh chicken wastes, 7.00 ± 0.05 and 7.00 ± 0.05 for the dry chicken waste and food wastes. Total solid (TS) and volatile solid (VS) was observed to have a percentage of 56.40 ± 0.6, 38.40 ± 1.60, and 64.70 ± 0.7, 48.80 ± 0.25, 54.30 ± 0.70 respectively.

| Parameters     | CW       | CW*      | FW       |
|----------------|----------|----------|----------|
| pH             | 7.91 ± 0.04<sup>c</sup> | 7.00 ± 0.05<sup>a</sup> | 7.46 ± 0.08<sup>b</sup> |
| ALK (mg/l)     | 23.70 ± 0.40<sup>a</sup> | 315.00 ± 6.00<sup>b</sup> | 528 ± 12.00<sup>c</sup> |
| TS (%)         | 56.40 ± 0.6<sup>c</sup> | 38.40 ± 1.60<sup>b</sup> | 21.90 ± 0.70<sup>a</sup> |
| VS (%)         | 64.70 ± 0.7<sup>c</sup> | 48.80 ± 0.25<sup>a</sup> | 54.30 ± 0.70<sup>b</sup> |
| OM (%)         | 5.05 ± 0.17<sup>ab</sup> | 4.60 ± 0.40<sup>a</sup> | 5.99 ± 0.04<sup>b</sup> |
| TC (%)         | 2.86 ± 0.16<sup>ab</sup> | 3.21 ± 0.03<sup>b</sup> | 2.57 ± 0.13<sup>a</sup> |
| COD (mg/l)     | 17.01 ± 0.10<sup>b</sup> | 4.61 ± 0.11<sup>a</sup> | 17.90 ± 0.50<sup>b</sup> |
| NH₄⁺-N (mg/l)  | 0.35 ± 0.02<sup>b</sup> | 0.28 ± 0.3<sup>ab</sup> | 0.18 ± 0.04<sup>a</sup> |
| MC (%)         | 27.70 ± 0.50<sup>b</sup> | 19.80 ± 0.35<sup>a</sup> | 40.02 ± 1.33<sup>c</sup> |

Values are Mean±SEM of triplicate determinations. Superscript with different alphabets across a row are significantly different at p<0.05. CW: Fresh chicken wastes, CW*: Dry chicken waste. FW: Food wastes, ALK: Alkalinity, TS: Total solid, VS: Volatile solid, OM: Organic matter, TC: Total carbon Alkalinity, NH₄⁺-N=Ammonia−Nitrogen, COD=Chemical oxygen dissolved, MC: Moisture content.
Table 2
Physicochemical properties of substrate after digestion in D1

| Parameters   | RT/Days |          |          |
|--------------|---------|----------|----------|
|              | 21      | 42       |          |
| pH           | 7.43 ± 0.08<sup>b</sup> | 6.65 ± 0.12<sup>ab</sup> |          |
| ALK (mg/l)   | 18.50 ± 1.54<sup>a</sup> | 428.00 ± 5.00<sup>c</sup> |          |
| TS (%)       | 9.64 ± 0.39<sup>c</sup> | 4.04 ± 0.08<sup>a</sup> |          |
| VS (%)       | 55.70 ± 1.20<sup>b</sup> | 54.30 ± 1.60<sup>b</sup> |          |
| OM (%)       | 1.92 ± 0.12<sup>c</sup> | 1.24 ± 0.06<sup>ab</sup> |          |
| TC (%)       | 1.04 ± 0.04<sup>a</sup> | 0.89 ± 0.03<sup>a</sup> |          |
| COD (mg/l)   | 14.7 ± 0.05<sup>a</sup> | 17.9 ± 0.15<sup>b</sup> |          |
| NH<sub>4</sub>⁺-N (mg/l) | 0.31 ± 0.02<sup>ab</sup> | 0.14 ± 0.01<sup>a</sup> |          |
| MC (%)       | 96.4 ± 3.60<sup>a</sup> | 98.9 ± 2.65<sup>a</sup> |          |
| VFA (g/l)    | 69.75 ± 1.33<sup>a</sup> | 72.17 ± 1.42<sup>c</sup> |          |

Values are Mean±SEM of triplicate determinations. Superscript with different alphabets across a row are significantly different at p<0.05. CW: Fresh chicken wastes, CW*: Dry chicken waste. FW: Food wastes, ALK: Alkalinity, TS: Total solid, VS: Volatile solid, OM: Organic matter, TC: Total carbon Alkalinity, NH<sub>4</sub>⁺-N=Ammonia-Nitrogen, COD=Chemical oxygen dissolved, MC: Moisture content, D1: CW+FW.
Table 3
Physicochemical properties of substrate after digestion in D2

| Parameters       | RT/Days |          |          |
|------------------|---------|----------|----------|
|                  | 21      | 42       |          |
| pH               | 8.01 ± 0.13<sup>c</sup> | 7.27 ± 0.13<sup>b</sup> |          |
| ALK (mg/l)       | 17.4 ± 0.45<sup>a</sup> | 300 ± 7.00<sup>bc</sup> |          |
| TS (%)           | 13.8 ± 0.70<sup>c</sup> | 6.27 ± 0.13<sup>b</sup> |          |
| VS (%)           | 59.2 ± 1.05<sup>c</sup> | 40.8 ± 1.20<sup>a</sup> |          |
| OM (%)           | 1.18 ± 0.04<sup>c</sup> | 0.89 ± 0.11<sup>ab</sup> |          |
| TC (%)           | 0.98 ± 0.03<sup>a</sup> | 0.88 ± 0.11<sup>a</sup> |          |
| COD (mg/l)       | 14.04 ± 0.71<sup>a</sup> | 24.5 ± 0.63<sup>bc</sup> |          |
| NH<sub>4</sub>±-N (mg/l) | 0.26 ± 0.02<sup>a</sup> | 0.40 ± 0.04<sup>a</sup> |          |
| MC (%)           | 88.6 ± 2.45<sup>a</sup> | 92.3 ± 1.81<sup>a</sup> |          |
| VFA(g/l)         | 44.75 ± 0.60<sup>a</sup> | 58.35 ± 2.58<sup>b</sup> |          |

D2: CW+, Values are Mean±SEM of triplicate determinations. Superscript with different alphabets across a row are significantly different at p<0.05. CW: Fresh chicken wastes, CW*: Dry chicken waste. FW: Food wastes, ST: Substrate, ALK: Alkalinity, TS: Total solid, VS: Volatile solid, OM: Organic matter, TC: Total carbon Alkalinity, NH4+-N=Ammonia−Nitrogen, COD=Chemical oxygen dissolved, MC: Moisture content
After a total period of forty-two (42 days) of anaerobic digestion, D1 which was a co-digestion of food wastes and chicken waste recorded a pH of 7.43 ± 0.08, 6.65 ± 0.12 while that of D2 a mono addition series of chicken waste was 8.01 ± 0.13, 7.27 ± 0.13 and D3 (control) was 6.85 ± 0.15, 6.43 ± 0.27 respectively.

From the result, the pH values of the different stages, fluctuated slightly and was maintained in the range of 6.4–8.0 during the whole digestion process. In the initial stage D1 and D3 (Table 2, 4) recorded a minimal decrease in pH at day forty-two (42). The drop in pH to the range of 6.4–6.6 may be attributed to volatile fatty acids concentration (69.75 ± 1.33g/l and 55.89 ± 1.35g/l). The upsurge in VFA may be ascribed to the addition of substrate with reduced retention time which have been linked to buildup in the acidity of the substrate medium of the digester causing a fall in pH [12]. This is ruinous for methanogens and cause reduction in their population and metabolic activities. The range of pH obtained from D1 in this research is almost in consonance with the study conducted by Zhai et al [13] who reported a higher pH of 7.5 when animal manure was co-digested with food-waste.
Digester two (D2) also revealed a slight reduction in pH at day forty-two (Table 3). The slump in pH probably ensued from the type of substrate used. Chicken wastes is high in nitrogen, ammonium and have buffering capacity that yields alkalinity when digested by microbes during anaerobic degradation [14, 15]. The increase in pH observed in the reacting material indicate consumption of VFA by acetogenic bacteria and utilization of the product of the process by methanogens. Methanogens are more sensitive to pH. The pH recorded is within the pH range for efficient digestion necessary to activate the growth of methanogens and for process performance [16].

The study revealed decrease in total solid (%) of the slurry. Before digestion the fresh chicken wastes was 56.4% and the food waste was 21.9% which is below that recorded by Muhibbu-din et al [17] and Dupade et al [18] that observed a TS of 40.8% and 45%. The decrease in the percentage TS between the range of 2–13% suggest active digestion of the nutrient by microorganisms. Total solid determination shows the quantity of nutrient accessible by microorganisms. Decrease in TS also implies increase in digester performance which was noticed at day forty-two (Table 5) with an upswing in biogas generated (686 ± 17.00kpa, 700 ± 11.00kpa, 521 ± 21.00kpa) in D1, D2 and D3. The trifling variation in TS was observed to be significant (p-value < 0.05) and thus suggest that reduction in TS indicate biogas production.

Moisture content (%) increase from 27.70 ± 0.50 % chicken wastes and 40.02 ± 1.33% food wastes to 96.4 ± 3.60, 98.9 ± 2.65 for D1, 88.6 ± 2.45, 92.3 ± 1.81 for D2 and 92.0 ± 0.80, 96.4 ± 3.60 for D3 respectively. Moisture content play a pivotal role in anaerobic degradation process, it helps in the movement of water and microbial growth which improve the breakdown and access of nutrient. Moisture concentration reduce the limit of bulk transfer of non-homogenous substrate. In overall, the amount of moisture increases with increase in the amount of volatile solid and total solid reduction which implies substrate utilization by the microorganisms [19, 20].

Volatile solids (VS) reduction occurred with D3 having less 39.9 ± 1.70 % (Table 4) at day 42. The amount of methane generated depends on the quantity of volatile solid which is the amount of solids present in the waste and its degradability. According to Somashekar et al [21] volatile solids are responsible for methane production which makes domestic waste especially food wastes a high prospect of being use as raw materials for methane production.

Ammonia-Nitrogen (NH$_4^+$-N) in D1 and D2 showed decreased from the initial of 0.35 ± 0.02mg/l to 0.31 ± 0.03mg/l and 0.26 ± 0.02 mg/l at day 21 whereas D3 revealed a 0.03% increase at day twenty-one. After forty-two days of digestion, the change in substrate composition by addition of 2kg of dry chicken waste to D2 within a retention of 42 days indicated a maximum increase in NH$_4^+$-N (Table 3). Chicken waste consists of high level of organic nitrogen concentration when used as substrate for anaerobic digestion it results in high concentration of total ammonium ion plus free ammonia [22]. Therefore, the upturn of NH$_4^+$-N concentration recorded in D1 and D2 during the digestion process is attributed to not only the substrate concentration of nitrogen, digester loading, mono series addition of 2kg of CW, buffering capacity of the reacting substrate, temperature and pH [23]. In this research, the NH$_4^+$-N observed had significant effect (p < 0.05) on microbial activity and biogas production. The range recorded is not
inhibitory to the anaerobic system performance (700kpa $0.47 \pm 0.04$ mg/l $\text{NH}_4^+\text{-N}$ $58 \pm 2.34\%$VS) and contribute to the vital nutrients for microbial growth [24]. The attribute of the result suggests that, the concentration of $\text{NH}_4^+\text{-N}$ can either hint at digester stability or failure.

The chemical oxygen demand (COD) of the chicken wastes before digestion was $17.01 \pm 0.10$mg/l as compared to after digestion (D1, D2, D3) which reflects the content of readily available biodegradable organic matter. At day forty two D1, D2 and D3 observed an increase in COD (Table 2,3,4). The features of the results therefore indicate, estimation of COD prior to anaerobic digestion is not always vital indicator for assessing hydrolysable quality of the feedstock. The result is also a signpost for low organic matter availability or low hydrolysable quality matter which probably is due to the presence of non-degradable materials and may be a pointer to the amount of biogas produced [25, 26]

Percentage total carbon reduction occurred in the slurry during the digestion. The TC fed into the digester before digestion was $2.86 \pm 0.16\%$, after digestion TC found in D1, D2, D3 was within the range of 0.1-1% The decrease in total organic carbon by biological degradation processes was probably limited to the production of $\text{CO}_2$, Volatile fatty acids and $\text{H}_2$ by facultative microorganisms or the conversion of carbon to $\text{CH}_4$ by methanogens [27]. The continuous process of feeding with TC of $2.57 \pm 0.13\%$ for food wastes and $3.21 \pm 0.03\%$ for dry chicken was also efficient in removing carbon content which explains the reduction in D1, D2.

D1, D2 and D3 showed an increasing amount of VFA of $69.75 \pm 1.33$ g/l, $44.75 \pm 0.60$g/l, $55.89 \pm 1.35$g/l from day 21 with a biogas production of $441 \pm 35.00$ kpa pH $7.43 \pm 0.08$ and $600 \pm 12.00$kpa pH $8.01 \pm 0.13$, $326 \pm 19.00$kpa pH $6.85 \pm 0.15$. The progressive increase in VFA concentration in D1 and D2 was presumably the addition of substrate, the composition of the substrate and probably, the methanogenic reaction that utilize VFA progress at lesser rate as compared to the acidogenic reaction that produce the VFA [28]. D3 recorded gradual slump in VFA concentration, the drop recorded may be due to the conversion of the organic acids by acetoclastic methanogens to methane gas. The variation in D1, D2, D3 along the column was observed to be significant at $p < 0.05$ which suggest VFA is influenced by substrate composition and is linked to the system operation and performance [29]

**Cumulative Biogas Yield**

Table 5 represent the collective amount of biogas produced from digester 1,2 and 3 within a period of forty-two days at different temperature. After 21 days of anaerobic digestion, the collective biogas recorded in D1, D2 and D3 was $441 \pm 35.00$ kpa, $600 \pm 12.00$ kpa, $326 \pm 19.00$kpa with D2 having the highest buildup of biogas.
### Table 5
Cumulative Biogas Production (Kpa) and Temperature (°C)

| Substrates | RT(days) | D1        | D2        | D3        | Temperature (°C) |
|------------|----------|-----------|-----------|-----------|-----------------|
|            | 0        | 0.00 ± 0.00<sup>a</sup> | 0.00 ± 0.00<sup>a</sup> | 0.00 ± 0.00<sup>a</sup> | 36               |
|            | 21       | 441 ± 35.00<sup>b</sup> | 600 ± 12.00<sup>b</sup> | 326 ± 19.00<sup>b</sup> | 37               |
|            | 42       | 686 ± 17.00<sup>b</sup> | 700 ± 11.00<sup>b</sup> | 521 ± 21.00<sup>c</sup> | 35               |

Values are Mean±SEM of triplicate determinations. Different superscripts and subscripts across a row and along the column respectively are significantly different at p<0.05

The gradual increase noticed in D1, D2, D3 and the differences in the quantity of gas produced from the different digesters may have ensued from microbial adaptation, degradation of the substrate, microbial community diversity and ecology in the biodigester [30, 31, 32]. The speedy amount of gas production observed in D2 may have been due to the collective biodegradation activity of the methanogenic microorganisms present in the digester [33].

Leh-Togi <i>et al.</i>[20] and Forhad <i>et al.</i>[34] also reported maximum gas increase in the digestion of poultry dropping with other fermentable materials. The difference in the rate of biogas production observed in D2 at day forty-two as compared to D1 and D3 may have resulted from ammonia nitrogen concentration (0.40 ± 0.04). The biodegradation of chicken waste during anaerobic digestion process produces large amounts of ammonia (NH₃) and ammonium ions (NH₄⁺) [9]. It has been observed, in contrast to ammonium ion, ammonia can diffuse across the cell membrane and thus act as a definite toxic agent and inhibit anaerobic microbes particularly methanogens [35, 36, 37]. The rise in ammonia alters it to ionized ammonia (NH₄⁺) ratio that increases pH (which explains the 7.27 ± 0.13 observed in D2 as compared to that of D1 and D3). A surge in pH and ammonia may result in increased toxicity and inhibition to the digestion process, decrease in its overall performance and indicate impending digester failure [25, 38].

When D1 and D2 was co-digested with food wastes and chicken wastes at day twenty-one, the biogas production increased by 1% and 2% respectively for both D2 and D1. The increment in D2 at volatile solid of 40.8 ± 1.20% and D1 at VS of 54.30 ± 1.60 may denotes that more substrate needs to be added into D2[39] for auxiliary metabolic activity. While that of D1 therefore implies to digester operators as it suggests that changes in substrate composition may enhance digester performance in terms of biogas production and quality.

**Relationship Between The Cumulative Biogas Produced**
Table 6

|       | D1  | D2     | D3     |
|-------|-----|--------|--------|
| D1    | 1   | .982** | .999** |
|       |     | (.000) | (.000) |
| D2    | .999** | 1      | .980** |
|       | (.000) |        | (.001) |
| D2    | .999** | .980** | 1      |
|       | (.000) | (.001) |        |

**. Correlation is significant at the 0.01 level (2-tailed) Where values within the bracket represents the p-value < 0.05 while that outside is correlation (R)

Table 6 shows the correlation between the reacting substrate in D1, D2, D3 and biogas yield. There exists a positively strong significant correlation effect between the reacting substrate in the different digester and biogas yield at the 0.01 level which suggest, increase in substrate concentration leads to subsequent increase in biogas production.

Composition Of Biogas Produced

Table 7 Composition of biogas (%) from NDIR gas analyzer at Zero (0) minute

| Component | D1          | D2          | D3          | Avg GC       |
|-----------|-------------|-------------|-------------|--------------|
| CH₄       | 46.11 ± 1.11<sub>d</sub> | 52.4 ± 1.05<sub>d</sub> | 50.31 ± 1.33<sub>ab</sub> | 49.61 ± 1.14<sub>e</sub> |
| CO₂       | 28.41 ± 1.79<sub>c</sub> | 17.42 ± 1.24<sub>c</sub> | 16.68 ± 0.70<sub>a</sub> | 20.84 ± 1.33<sub>d</sub> |
| H₂        | 4.44 ± 0.44<sub>b</sub> | 2.24 ± 0.78<sub>a</sub> | 1.29 ± 0.04<sub>a</sub> | 2.66 ± 0.64<sub>b</sub> |
| O₂        | 5.68 ± 0.30<sub>b</sub> | 7.02 ± 0.02<sub>c</sub> | 2.87 ± 0.25<sub>ab</sub> | 5.19 ± 0.21<sub>c</sub> |
| H₂S       | 1.99 ± 0.09<sub>a</sub> | 1.23 ± 0.17<sub>a</sub> | 1.11 ± 0.11<sub>a</sub> | 1.44 ± 0.10<sub>a</sub> |

Values are Mean±SEM of triplicate determinations. Different superscripts and subscripts across a row and along the column respectively are significantly different at p<0.05

The characterization of the composition of biogas generated from the phase one of experimental procedure from NDIR gas analyzer detected CH₄, CO₂, H₂, O₂ and H₂S gases (Table 7). At 0.0 minute, the biogas composition revealed an average percentage content of CH₄, CO₂, H₂, O₂ and H₂S value as 49.61 ± 1.14, 20.84 ± 1.33, 2.66 ± 0.64, 5.19 ± 0.21, 1.44 ± 0.10.
The percentage composition of methane recorded in digesters agrees with that reported by Nasir et al. [40]. According to Demirbas et al.[41] biogas is made-up of CH$_4$ (55–75%), CO$_2$ (25–45%), H$_2$S (0–1%), and O$_2$ (0–2%). The characterization of the biogas produced reveals consistency with data obtained from previous study. **The relationship between physicochemical parameters and biogas yield from the different digesters**

| Table 8 | Relationship between physicochemical parameters and biogas yield |
|---------|---------------------------------------------------------------|
| **Digester one** | | |
| | VFA | pH | VS | COD | Time |
| BGY | 0.945 (0.005) | -0.828 (0.042) | | 0.906 (0.013) |
| VFA | | | | 0.842 (0.035) |
| pH | | 0.900 (0.15) | | -0.815 (0.048) |
| **Digester two** | | 0.859 (0.028) |
| BGY | 0.952 (0.003) | | | |
| VFA | | | | 0.939 (0.006) |
| NH$_4$-N | | | | 0.876 (0.022) |
| **Digester three** | | 0.890 (0.018) |
| BGY | | -0.922 (0.009) | -9.24 (0.008) | | |
| NH$_4$-N | | | | -0.944 (0.005) |
| COD | | | | -0.860 (0.028) |
| VS | | | | -0.859 (0.029) |

Where values within the bracket represents the $p$-value while that outside is the value of Pearson's correlation (R). $p$-value < 0.05 indicates a significant correlation.

Table 8 represent the relationship between the reacting substrate in the digester, physicochemical parameters and biogas yield. The D1 clearly demonstrate significantly positive correlation between biogas yield and and volatile fatty acid (72.17 ± 1.42g/l VFA 686 ± 17.00kpa). Increase in VFA leads to successive biogas yield. Contrarily to previous studies that reported increase in biogas resulting from decrease in VFA and vice versa [42, 43]. In this the study, the upsurge in biogas with corresponding increase in VFA maybe attributed to the dominant presence of acetoclastic methanogens, hydrogenotrophic methanogens, and some bacteria that convert the organic acids to methane gas. Statistically, the features of the result is in good agreement with the study conducted by Hill and Bolte[44] who also reported increase methane yield with increase VFA. The result therefore clearly demonstrate that concentration of volatile may not be signpost to system performance. The D1 from the result also
showed a negative significant relationship between pH and biogas yield suggesting that increase in pH leads to subsequent decrease in biogas production likewise decrease in pH can lead to increase in biogas yield.

D3 showed a significantly negatively correlation between volatile solid and COD. Reduction in this parameter, increase biogas yield (39.9 ± 1.70%VS, 10.1 ± 0.90 mg/l COD, 521 ± 21.00kpa).

**Conclusion**

The investigation of the effect of physical parameter on biogas production, shows that different parameter affects not just the production of methane gas but also the process. Since anaerobic digestion is totally reliant on the operational parameter and microbial community diversity which are also sensitive to the concentration of this parameter that is pivotal to bacterial activity thus the rate of biogas production. The study, does not show any symptoms of inhibition of the AD system but revealed reduced rate of performance of D3 at 521 ± 21.00kpa, 3.33 ± 0.12 %, 0.28 ± 0.03 mg/l TS and NH₄⁺-N concentration as compared to D1 and D2. Combination of substrate does not only result in substrate that are better balanced in terms of nutrient and degradation but also improve production of biogas.

**Declarations**

**Ethical Approval and consent to participate**

Not applicable

**Consent for publication**

All authors have approved the manuscript for its submission for publication.

**Availability of data**

All data generated or analyzed during this study are available from the corresponding author upon reasonable request.

**Competing interest**

Not applicable

**Funding**

No funding was received for conducting this study. This research was self-sponsored.

**Authors Contributions**
All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Stella Suanu Leh-Togi Zobeashia, Peter Olabisi Abioye, Udeme Joshua Josiah Ijah and Oluwafemi Adebayo Oyewole reviewed the protocol, supervised the data analysis and also explained the data. All authors read and approved the final manuscript.

References

[1] Amin Salehi F (2013). Modeling of environmental, technical and economical analysis of bagasse to energy in Iran [PhD thesis]. Tehran: University of Tehran.

[2] Weiland P (2011) Biogas production: current state and perspectives. *Applied Microbiology and Biotechnology* 85(4): 849-860.

[3] Iacovidou E, Ohandja DG, Voulvoulis N (2012). "Food waste co-digestion with sewage sludge–realising its potential in the UK." *Journal of Environmental Management* 112, 267-74.

[4] Chynoweth DP, Owens JM, Legrand R (2001). Renewable methane from anaerobic digestion of biomass. *Renewable Energy*, 22(1-3), 1-8.

[5] Holm-Nielsen JB, Seadi TAI, Oleskowicz-Popiel P (2009). "The future of anaerobic digestion and biogas utilization." *Bioresource Technology* 100(22), 5478-5484.

[6] O’Flaherty V, Collins G, Mahony T (2006). The Microbiology and Biochemistry of Anaerobic Bioreactors with Relevance to Domestic Sewage Treatment. *Reviews in Environmental Science and Biotechnology* 5, 39-55.

[7] Krakat N, Schmidt S, Scherer P (2011). "Potential impact of process parameters upon the bacterial diversity in the mesophilic anaerobic digestion of beet silage." *Bioresource Technology* 102(10): 5692-5701.

[8] Lerm S, Kleybocker A, Miethling-Graff R, Alawi M, Kasina M, Liebrich M, Wurdemann H (2012). "Archaeal community composition affects the function of anaerobic co-digesters in response to organic overload." *Waste Management* 32(3): 389-399.

[9] Zahan Z, Othman MZ, Muster TH (2017) Bioreactor performances in continuous anaerobic co-digestion of chicken litter with agro-industrial wastes: effect of C/N ratio, organic loading and lignocellulose fractionation. *5th IWA World Congress conference on Anaerobic Digestion (AD-15)*, 17-20.

[10] Lin J, Zuo J E, Gan L L, Li P, Liu F L, Wang K J, 2011. Effects of mixture ratio on anaerobic co-digestion with fruit and vegetable waste (FVW) and food waste (FW) of China. Journal of Environmental Sciences, 23: 1403-1408.

[11] Greenberg AE, Trussell RR, Clesceri LS, Franson MAH, (1985) Standard methods for the examination of water and wastewater, 16th ed. *American Public Health Association*, Washington, DC
[12] Veeken A, Kalyuzhnyi S, Scharff H, Hamelers B (2000) Effect of pH and VFA on hydrolysis of organic solid waste. *J. Environ. Eng. 126*, 1076–1081.

[13] Zhai N, Zhang T, Yin D, Yang G, Wang X, Ren G, Feng Y (2015) Effect of initial pH on anaerobic co-digestion of kitchen waste and cow manure. *Waste management*, 38, 126-131.

[14] Molinuevo-Salces B, García-González MC, González-Fernández C, Cueto MJ et al (20100 Anaerobic co-digestion of livestock wastes with vegetable processing wastes: A statistical analysis. *Bioresour. Technol. 101*, 9479–9485.

[15] Holmes DE, Smith JA (2016) Biologically produced methane as a renewable energy Source. *Advances in Applied Microbiology*, 97:161. https://doi.org/10.1016/bs.aambs.2016.09.001 PMid: 27926429.

[16] Aremu MO, Agarry SE (2013) Enhanced biogas production from poultry dropping using corn-cob and waste paper as co-substrate. *International Journal of Engineering Science and Technology* 5(2):247-253

[17] Muhibbu-din EL, Adebayo GB, Odedele OS (2020) Production and Characterization of biogas from domestic waste by Anaerobic digestion. 15(1):1-9.

[18] Dupade V, Pawar S (2013). Generation of Biogas from Kitchen Waste Experimental Analysis. *International Journal of Engineering Science Invention*, 2(10):15-19.

[19] Sadaka SS, Engler CR (2003). Effect of initial total solids on composting of raw manure with biogas recovery. *Compost Science and Utilization*. 11(4): 361-369

[20] Leh-Togi Zobeashia SS, Aransiola SA, Ijah UJJ, Abioye OP (2018) Anaerobic digestion and agricultural application of organic wastes. *Advances in Environmental Research* 7(2), 73-85.DOI: https://doi.org/10.12989/aer.2018.7.2.073.

[21] Somashekar RK, Rinku V, Manzoor AN (2014) Potential of biogas production from food waste in a uniquely designed reactor under lab conditions. *International Journal of Geology, Agriculture and Environmental Sciences*, 2(2):2348-0254

[22] El Hadj T.B, Astals S, Galí A, Mace S, Mata-Álvarez J (2009) Ammonia influence in anaerobic digestion of OFMSM. *Water Sci. Technol. 59*, 1153–1158

[23] Garcia ML, Angenent LT (2009) Interactions between temperature and ammonia in mesophilic digesters for animal waste treatment. *Water Res. 43*, 2373–2382

[24] Rajagopal R, Massé D, Singh G (2013) A critical review on inhibition of anaerobic digestion process by excess ammonia. *Bioresource Technology*, 143:632-41.

[25] Chen Y, Cheng JJ, Creamer KS (2008). "Inhibition of anaerobic digestion process: A review." *Bioresource technology* 99(10), 4044-4064.
[26] Angelidaki I, Ahring BK. (2016) Thermophilic anaerobic digestion of livestock waste: the effect of ammonia. *Applied Microbiology Biotechnology*. https://doi.org/10.1007/bf00242955

[27] Hobson PN, Shaw BG (1976) Inhibition of methane production by Methanobacterium formicicum. *Water Res.* 10, 849–852.

[28] Lukitawesa et al. (2020) Factors influencing volatile fatty acids production from food wastes via anaerobic digestion, *Journal of Bioengineering*. 11(1): 3952

[29] Hori T, Haruta S, Ueno Y, Ishii M, Igarashi Y (2006). “Dynamic transition of a methanogenic population in response to the concentration of volatile fatty acids in thermophilic anaerobic digester.” *Applied and Environmental Microbiology* 72(2), 1623-1630.

[30] Baggi D, Barate A, Hans G, Ludovico LA (2007), “Navigation interacting with notation and audio(NINA)”, *Proceedings of the 2nd International Workshop on Semantic Media Adaptation and Personalization*, London, UK, December.

[31] Banks CJ, Zhang Y, Jiang Y, Heaven S (2012). “Trace element requirements for stable food waste digestion at elevated ammonia concentrations.” *Bioresource Technology* 104(0), 127-135.

[32] Werner J J, Knights D, Garcia ML, Scalfone NB, Smith S, Yarasheski K, Cummings TA, Beers AR, Knight R, Angenent LT (2012) Bacterial community structures are unique and resilient in full-scale bioenergy systems. *Proceedings of the National Academy of Sciences of the United States of America* 108(10), 4158-4163.

[33] Ozbayram E, Ince O, Ince B, Harms H, Kleinsteuber S (2018). Comparison of Rumen and Manure Microbiomes and Implications for the Inoculation of Anaerobic Digesters. *Microorganisms*, 6(1), 15.

[34] Md. Forhad Ibne Al Imam, Khan MZH, Sarkar MAR, Ali SM (2013) Development of biogas processing from cow dung, poultry waste and water Hyacinth. *International Journal of Natural and Applied Sciences* 2(1):13 17

[35] Salminen E, Rintala J (2002) Anaerobic digestion of organic solid poultry slaughter house waste—a review. *Bioresource Technology*, 83(1), 13-26.

[36] Liu Z-G, Zhou X-F, Zhang Y-L, Zhu H-G (2012). Enhanced anaerobic treatment of CSTR-digested effluent from chicken manure: the effect of ammonia inhibition. *Waste Management*, 32(1), 137-143

[37] Zahan Z, Othman MZ, Muster TH (2016a). Characterization of agro-industrial wastes and their anaerobic digestion / co-digestion kinetic potential: a comparative batch study. *Sixth International Symposium on Energy from Biomass and Waste*, Great School of St. John the Evangelist, Venice, Italy. CISA Publisher, Italy
[38] Zahan Z, Othman MZ (2019). Effect of pre-treatment on sequential anaerobic co-digestion of chicken litter with agricultural and food wastes under semi-solid conditions and comparison with wet anaerobic digestion. *Bioresource technology*, 281, 286-295. https://doi.org/10.1016/j.biotech.2019.01.129

[39] Rincón BR, Borja JM, González MC *et al* (2008). "Influence of organic loading rate and hydraulic retention time on the performance, stability and microbial communities of one-stage anaerobic digestion of two-phase olive mill solid residue." Biochemical engineering journal 40(2): 253-261.

[40] Nasir I M, Ghazi TIM, Omar R, Idris A (2013) Anaerobic digestion of cattle manure: influence of inoculums concentration. – *International Journal of Engineering Technology* 10(1): 22-26.

[41] Demirbas A, Osman T, Durmus K (2016). Biogas production from municipal sewage sludge (MSS). *Energy Sources, Part A: Recovery, Utilization and Environmental Effects*, 38(20): 3027-3033

[42] Karim K, Klasson K, *et al* (2005) Anaerobic digestion of Animal waste: Effect of mixing . *Bioresource Technology*, 96, 1607

[43] EL-Mashad, Hamed M, *et al* (2004) Effect of Temperature and Temperature fluctuation on thermophilic Anaerobic Digestion of Cattle manure. *Bioresource Technology*, 95, 191

[44] Hill DT, Bolte JP(2000) Methane production from low solid concentration liquid swine waste using conventional Anaerobic fermentation . *Bioresource Technology, 74*, 241

**Figures**

![Fabricated Anaerobic Digester](image-url)

**Figure 1**

Fabricated Anaerobic Digester .
Schematic diagram of the digester design

Figure 2
Schematic diagram of the fabricated digester (a. Inlet, b. Substrate collector, c. pressure gauge, d. Gas collector, e. Digester, f. Outlet, g. Thermometer). (The fig 1 should be placed under the heading digester design)