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Research Article

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Physicochemical Parameters and Microbial Community in Anaerobic Digestion of Organic Wastes

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Abstract

The demand for an alternative source of energy and challenge of increase in wastes pollution initiates the need for renewable energy and management of waste using anaerobic digestion (AD). Anaerobic digestion is an effective and efficient method of waste treatment and energy generation. The study focused on investigating the physicochemical parameters and microbial community in anaerobic digestion of organic wastes and 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 fifteen liter (15L) fabricated digesters labeled D1, D2 and D3 at 37OC. The pH, volatile fatty acid (VFA), moisture content (MC), total ammonia, total solid, volatile solid, alkalinity was assessed before and after digestion while the microbial community diversity was analyzed using 16S rRNA amplicon-based next-generation sequencing (NGS).

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 at day 42 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 microbial community identified phylum Bacteroidetes, Firmicutes, proteobacteria, Tenericutes, Verrucomicrobia, Actinobacteria, Euryarchaeota among others. The study shows that physicochemical properties and microbial community diversity are useful tools to indicate digester performance and also to enhance anaerobic digestion process.

Keywords: Anaerobic Digestion, Biogas, Microbial Community, Physicochemical Properties, Retention Time, Substrate.

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 with resultant negative effect on the environment (Amin, 2013). 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 greenhouse gases among others (Amin, 2013; Iacovidou et al., 2012). Stringent environmental rules on waste management have led to the application 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 (Weiland, 2011; Iacovidou et al., 2012). 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 (Chynoweth et al., 2001; Weiland, 2011). 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 landfilling which results in greenhouse gas emissions and leaching of nutrients into the environment (Holm-Nielsen et al. 2009; Ferguson et al.,2014).

Anaerobic digestion includes four main steps hydrolysis, aci-

dogenesis, acetogenesis and methanogenesis, the first three steps are completed by bacteria and the final one by archaea (O'Flaherty et al., 2006). Each of the steps function effectively with relationship with physicochemical conditions and microbial consortium. Several studies have shown parameters such as pH, volatile fatty acid, volatile solid, moisture and organic loading rate among others can influence the digester performance(biogas production) and the dynamics of the microbial community (Rincón et al.,2008; Chen et al.,2012).Study carried out by SivaKumar et al., (2012) on the effect of pH on biogas production from spoiled milk conducted with substrate of different pH values (5-8) reported a better digester performance with substrate with 7 pH. Krakat et al., (2011) reported a correlation between organic loading rate and bacterial community structure while Lerm et al., (2012) 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 according to Lerm et al., (2012) 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 (Yadav et al.,2014). Therefore, knowledge of the microbial diversity and operational parameter properties require to determine the anaerobic digestion process, 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 investigate the physicochemical parameters and microbial community in anaerobic digestion of organic wastes (chicken waste and food waste).

Materials and Methods

Sample Collection

The chicken waste used in this study was obtained from Premium poultry farm located at Kuje Federal Capital Territory, Abuja, Nigeria. The food wastes were 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.

Sample Preparation

The chicken wastes were prepared by sorting out non-degradable material (feathers, stone, wood) while the food wastes were reduced in size using Binatone blender.

Digester Design

The three-digester used for this study was constructed at a metal fabricating (welding) workshop at Zuba mechanic village Abuja, Nigeria. A fifteen-liter semi-continuous capacity aluminum fabricated digester (Plate 1) was used for the study. 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 an attached thermometer to read the average temperature within the digester as well as an attached substrate collector (H-26cm, D-17.5cm). The substrate collector has an inlet to collect the substrate which feed the digester and an outlet to remove the digested slurry. The digester also has an attached gas 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.

Plate 1 Fabricated Anaerobic Digester (Modified Mohan et al., 2013).

Schematic Diagram of the Digester Design

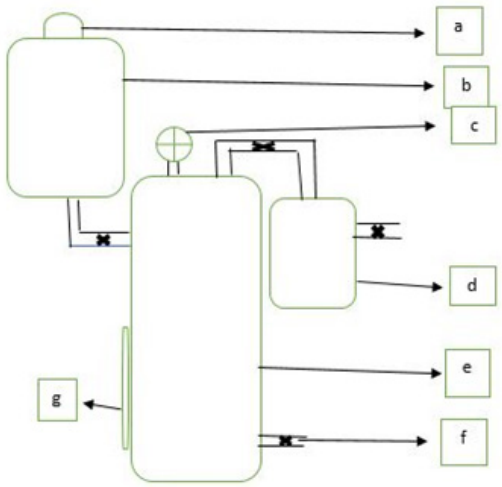

Figure 1: Schematic Diagram of the Fabricated Digester (a. Inlet, b. Substrate collector, c. pressure gauge, d. Gas collector, e. Digester, f. Outlet, g. Thermometer).

Experimental Procedure

Twelve (12) kilogram each of the fresh chicken waste (CW) in a ratio of 3:1 of waste to water was introduced into three sterile containers and mixed homogenously by stirring to form slurry. The slurry 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. At day 21 of the anaerobic digestion, two kilograms (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 while the microbial community was identify using 16S rRNA amplicon-based next-generation sequencing (NGS).

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., (2011).

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:

$$TS = (M_1 – M_2)/V$$

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:

$$VS = (M_1 – M_3)/V$$

With

M_1: mass of crucible dish after drying at 105 °C (mg)

M_3: Mass of crucible dish after ignition at 550 °C (mg))

V: Volume of sample (L))

Moisture Content

The moisture content of the substrate was determined by weighing 10g of substate 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.

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

ΔSB = weight of dish and sample before drying.

ΔSA = 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 (Greenberg et al.,1985; Lin et al., (2011).

Microbial Community Diversity Analysis (Modified Klindworth et al.,2013)

Thirty grams (30 g) aliquot of the sample was collected using aseptic techniques and dispensed into 20 ML of sterile LB broth. The added mixture was incubated for twenty hours (24h) before the total community DNA was extracted using the Qiagen Dneasy Blood and Tissue Kit (cat. 69506). The growth from the broth were pelletized in a well labelled 1.5mL microcentrifuge tubes, 200µL Buffer AL (lysis buffer to break open cells) was added to each of the tubes and mixed by vortexing. The tubes were then incubated at 56°C for 10 minutes after which 200µl of ethanol (96–100%) was added and mixed thoroughly by vortexing. The mixture was pipetted into a DNeasy Mini spin column in a 2 ml collection tube and centrifuged at 6000 x g (8000 rpm) for 1 min. The flow-through and collection tube were discarded. The spin columns were placed in new 2 ml collection tubes.500µl of Buffer AW1(wash solution buffer) was added to the spin column and centrifuged for 1 minute at 6000 x g. The process was repeated with the addition of 500µl of Buffer AW2 (wash solution buffer) and centrifuged for 3 minutes at 20,000 x g (14,000 rpm). The flow-through and collection tube

were discarded and the spin columns were carefully removed to avoid contact with the flow-through. The spin columns were then transferred into new 1.5 ml or 2 ml microcentrifuge tubes of which 200µl of Buffer AE was added to the centre of the spin column for elution of the genomic DNA. The eluent was then Incubated for 1 min at room temperature and centrifuge for 1 min at 6000 x g. DNA quality and concentration were checked by running 2µl of the diluted DNA sample on 1% agarose gel. Accurate DNA quantification was carried out using a NANODROP®2000 spectrophotometer (Thermo Scientific Inc.)

PCR was carried out in a total volume of 25µl containing 100ng of genomic DNA, 2.5µl of 10× PCR buffer, 1µl of 50mM $MgCl_2$, 2µl of 2.5mM dNTPs (Thermo Scientific), 0.1µl Taq polymerase (Thermo Scientific), 1µl of DMSO, 1µl each of forward and reverse primer and 11.3µl of H_2O. Touch-down PCR was used for amplification as follows: initial denaturation step of 5mins at 94°C, followed by 9 cycles each consisting of a denaturation step of 20sec at 94°C, annealing step of 30sec at 65˚C, and an extension step of 72°C for 45sec, this was followed by another 30 cycles each consisting of a denaturation step of 20sec at 94°C, annealing step of 30sec at 55˚C, and an extension step of 72°C for 45sec. Resulting amplicons were gel purified, end repaired and illumina specific adapter sequence were ligated to each amplicon (NEBNext Ultra II DNA library prep kit).

Following quantification, the samples were individually indexed (NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1), and another AMPure XP bead-based purification step was performed. Amplicons were then sequenced on illumina's MiSeq platform, using a MiSeq v3 (600 cycle) kit. For each samples 20Mb of data (2x300bp long paired end reads) were produced for each sample. The BLAST-based data analysis was performed using an Inqaba in-house developed data analysis pipeline.

Data Analysis

Data generated were analyzed using Analysis of variance (ANOVA) with multiple error terms to test for significant difference between means at significant level of ($P<0.05$)

Results and Discussion

Physicochemical Parameter Analysis of Substrate before and after Digestion.

The physicochemical parameter of the organic substrate was analyzed before and after digestion to measure specify amount. Table 1 represents the results of the physicochemical parameter of the chicken wastes before digestion. A pH value of 7.91±0.04 was recorded for the fresh chicken wastes (CW), 7.00±0.05 for the dry chicken waste (CW*) and 7.46±0.08 for food wastes (FW). Total solid (TS) was observed to have a percentage of 56.40±0.6, 38.40±1.60, 21.90±0.70 for CW, CW* and FW respectively. The result of moisture content indicates a positively significant differences among the substrates. The variation clearly is attributed to the number of solid materials in the substrate. The food waste used in this study is composed of cooked food, fruits and vegetables which according to research carried out on the nutritional value contain higher percentage of water when compared to fresh chicken waste and dry chicken waste which are by-products of droppings, bedding materials such as straws, sawdust, wood shavings or rice hulls among others, dead birds, hatchery wastes, feathers (Moreki et al.,2013).

Table: 1 Physicochemical Properties of Substrate before Digestion

Parameters	CW	CW*	FW
pH	7.91 ± 0.04^{c}	7.00 ± 0.05^{a}	7.46 ± 0.08^{b}
ALK (mg/l)	23.70 ± 0.40^{a}	315.00 ± 6.00^{b}	528 ± 12.00^{c}
TS (%)	56.40 ± 0.6^{c}	38.40 ± 1.60^{b}	21.90 ± 0.70^{a}
VS (%)	64.70 ± 0.7^{c}	48.80 ± 0.25^{a}	54.30 ± 0.70^{b}
OM (%)	5.05 ± 0.17^{ab}	4.60 ± 0.40^{a}	5.99 ± 0.04^{b}
TC (%)	2.86 ± 0.16^{ab}	3.21 ± 0.03^{b}	2.57 ± 0.13^{a}
COD (mg/l)	17.01 ± 0.10^{b}	4.61 ± 0.11^{a}	17.90 ± 0.50^{b}
NH_4^+-N (mg/l)	0.35 ± 0.02^{b}	0.28 ± 0.3^{ab}	0.18 ± 0.04^{a}
MC (%)	27.70 ± 0.50^{b}	19.80 ± 0.35^{a}	40.02 ± 1.33^{c}

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

	RT/Days	
Parameters	**21**	**42**
pH	7.43±0.08[b]	6.65±0.12[a]
ALK (mg/l)	18.50±1.54[a]	428.00±5.00[b]
TS (%)	9.64±0.39[b]	4.04±0.08[a]
VS (%)	55.70±1.20[a]	54.30±1.60[a]
OM (%)	1.92±0.12[b]	1.24±0.06[a]
TC (%)	1.04±0.04[a]	0.89±0.03[a]
COD (mg/l)	14.7±0.05[a]	17.9±0.15[b]
NH_4^+-N (mg/l)	0.31±0.02[b]	0.14±0.01[a]
MC (%)	96.4±3.60[a]	98.9±2.65[a]
VFA(g/l)	69.75±1.33[a]	72.17±1.42[a]

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_4^+-N=Ammonia-Nitrogen, COD=Chemical oxygen dissolved, MC: Moisture content, D1: CW+FW.

Table: 3 Physicochemical Properties of Substrate after Digestion in D2

	RT/Days	
Parameters	**21**	**42**
pH	8.01±0.13[b]	7.27±0.13[a]
ALK (mg/l)	17.4±0.45[a]	300±7.00[b]
TS (%)	13.8±0.70[b]	6.27±0.13[a]
VS (%)	59.2±1.05[b]	40.8±1.20[a]
OM (%)	1.18±0.04[b]	0.89±0.11[a]
TC (%)	0.98±0.03[a]	0.88±0.11[a]
COD (mg/l)	14.04±0.71[a]	24.5±0.63[b]
NH_4^+-N (mg/l)	0.26±0.02[a]	0.40±0.04[a]
MC (%)	88.6±2.45[a]	92.3±1.81[a]
VFA(g/l)	44.75±0.60[a]	58.35±2.58[b]

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, NH_4^+-N=Ammonia-Nitrogen, COD=Chemical oxygen dissolved, MC: Moisture content.

Table: 4 Physicochemical Properties of Substrate after Digestion in D3

	RT/Days	
Parameters	**21**	**42**
pH	6.85±0.15^{ab}	6.43±0.27^{ab}
ALK (mg/l)	22.9±0.82^{a}	223±18.00^{b}
TS (%)	11.2±1.20^{b}	3.33±0.12^{a}
VS (%)	60.3±1.73^{b}	39.9±1.70^{a}
OM (%)	1.92±0.04^{b}	0.52±0.14^{a}
TC (%)	1.06±0.06^{a}	1.00±0.08^{a}
COD (mg/l)	9.30±0.30^{a}	10.1±0.90^{a}
NH_4^+-N (mg/l)	0.38±0.04^{a}	0.28±0.03^{a}
MC (%)	92.0±0.80^{a}	96.4±3.60^{a}
VFA(g/l)	55.89±1.35^{b}	40.56±0.38^{a}

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, NH_4^+-N=Ammonia-Nitrogen, COD=Chemical oxygen dissolved, MC: Moisture content.

The result of the physicochemical parameter during digestion (day 21) and after digestion (day 42) as shown in Table 2,3 and 4 recorded a pH of 7.43±0.08 at day 21 and 6.65±0.12 at day 42 for D1 (co-digestion of FW and CW) 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.

The pH fluctuated slightly and was maintained in the range of 6.4–8.0 during the whole digestion process. The reduction in pH may be attributed to volatile fatty acids concentration (72.17±1.42, 58.35±2.58 and 40.56±0.38 g/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 (Veeken et al.,2000). This is ruinous for methanogens and cause reduction in their population and metabolic activities. The pH value obtained from D1 at day 21 in this research is almost in consonance with the study conducted by Zhai et al (2015) 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 (Molinuevo-Salces et al.,2010; Holmes et al.,2016). The increase in pH observed in the reacting material of D2 at day 21 as compared to pH value of the substrate before digestion indicate consumption and conversion of metabolic products of acetogenesis by acetoclastic methanogens. Methanogens are more sensitive to pH. The pH recorded in this study, is within the pH range for efficient digestion necessary to activate the growth of methanogens and for process performance (Aremu et al., 2013).

The study revealed decrease in total solid (TS) 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 (2020) and Dupade et al., (2013) that observed a TS of 40.8% and 45%. After digestion at day 21, the TS value for D1 was 9.64±0.39 and 4.04±0.08 at day 42 while that of D2 was 13.8±0.70 and 6.27±0.13 at day 21 and 42 and D3 was 11.2±1.20 and 3.33±0.12 at day 21 and 42 respectively. The decrease in the percentage TS between the range of 3-13% 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 before digestion 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 at day 21 and 42. 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 (Sadaka et al.,2003; Leh-Togi et al., 2018).

Volatile solids (VS) removal indicates a reduction in D1, D2 and D3 with D1 having the slightest reduction rate of 54.30±1.60 % (Table 2) at day 42. The slight reduction observed in D1 may have

ensued from co-digestion with food wastes and chicken waste at day21 when compared to D2 which was a mono-digestion of chicken waste and D3 batch system without substrate addition. The variation in the amount of VS removal observed in D3 compared to VS removal before digestion was clearly attributed to complete degradation of the organic fraction of the substrate. Volatile solid is the amount of organic solid in a substrate and its degradability which also implies possible biogas production (Dinh et al, 2020). According to Somashekar et al (2014) 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 (El Hadj et al.,2009) Therefore, the upturn of NH_4^+-N concentration recorded 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(Garcia et al.,2009). 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±0.04 mg/l NH_4^+-N 58±2.34%VS) and contribute to the vital nutrients for microbial growth (Rajagopal et al., 2013). The attribute of the result suggests that, the concentration of NH_4^+-N can either hint at digester stability or failure.

The chemical oxygen demand (COD) of the chicken wastes before digestion was 17.01±0.10mg/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 (Chen et al., 2008; Angelidaki et al.,2016)

Percentage total carbon reduction occurred in the slurry during the digestion. The TC fed into the digester before digestion was 2.86 ± 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 CO2, Volatile fatty acids and H_2 by facultative microorganisms or the conversion of carbon to CH4 by methanogens (Hobson et al., 1976).The continuous process of feeding with TC of 2.57 ± 0.13% for food wastes and 3.21 ± 0.03% for dry chicken was also efficient in removing carbon content which explains the reduction in D1, D2.

The result of VFA (Table 2,3,4) showed an increase of 69.75 ± 1.33 g/l, 44.75 ± 0.60g/l and 55.89 ± 1.35g/l for D1, D2 and D3 at day 21 with a biogas production of 441±35.00 kpa pH 7.43±0.08, 600±12.00kpa pH 8.01±0.13 and 326±19.00kpa pH 6.85±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 (Lukitawesa et al., 2020). 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 D2 and D3 across the row 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 (Hori et al.,2006).

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 ± 35.00 kpa, 600 ± 12.00 kpa, 326 ± 19.00kpa with D2 having the highest buildup of biogas. At day 42, D1, D2 and D3 recorded a biogas yield of 686±17.00, 700±11.00 and 521±21.00 respectively.

Table: 5 Cumulative Biogas Production (Kpa) and Temperature (^OC)

Substrates				
RT /(days)	D1	D2	D3	Temperature (OC)
0	$0.00±0.00_a^a$	$0.00±0.00_a^a$	$0.00±0.00_a^a$	36
21	$441±35.00_b^b$	$600±12.00_b^c$	$326±19.00_b^a$	37
42	$686±17.00_c^b$	$700±11.00_c^b$	$521±21.00_c^a$	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 the digesters and the differences in the quantity of gas produced from the digesters may have ensued from microbial adaptation, degradation of the substrate, microbial community diversity and ecology in the biodigester (Baggi et al., 2007; Banks et al., 2012, Werner et al., 2012).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 (Ozbayram et al., 2018).

Leh-Togi et al. (2018) and Forhad et al., (2013) 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+) (Zahan et al., 2017).

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(Rincón et al., 2008) 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 Relationship between cumulative biogas production from the different digesters

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

**. 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

Substrates				
Component	D1	D2	D3	Avg GC
CH_4	$46.11\pm1.11^{a}_{d}$	$52.4\pm1.05^{b}_{d}$	$50.31\pm1.33^{ab}_{d}$	49.61±1.14e
CO_2	$28.41\pm1.79^{b}_{c}$	$17.42\pm1.24^{a}_{c}$	$16.68\pm0.70^{a}_{c}$	20.84±1.33d
H_2	$4.44\pm0.44^{b}_{b}$	$2.24\pm0.78^{ab}_{a}$	$1.29\pm0.04^{a}_{a}$	2.66±0.64b
O_2	$5.68\pm0.30^{b}_{b}$	$7.02\pm0.02^{c}_{b}$	$2.87\pm0.25^{b}_{ab}$	5.19±0.21c
H_2S	$1.99\pm0.09^{b}_{a}$	$1.23\pm0.17^{a}_{a}$	$1.11\pm0.11^{a}_{a}$	1.44±0.10a

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 percentage composition of methane recorded in the digesters agrees with that reported by Nasir et al. (2013). According to Demirbas et al. (2016) biogas is made-up of CH_4 (55-75%), CO_2 (25-45%), H_2S (0-1%), and O_2 (0-2%). The characterization of the biogas produced reveals consistency with data obtained from previous study.

Microbial Community of Digested Substrate

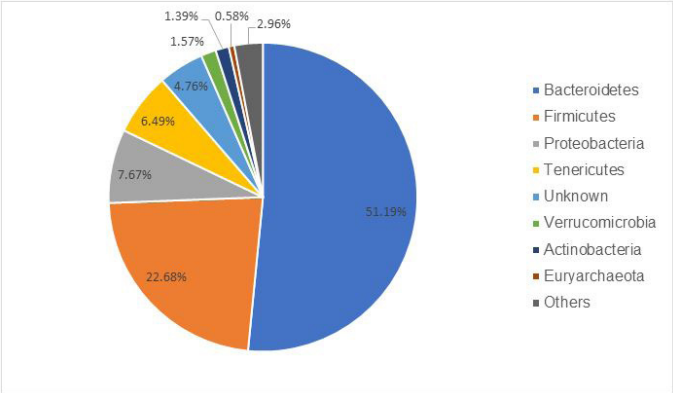

Figure 2: Distribution of Top Phylum classification observed at day 21 based on Percentage OTUS Abundance.

Figure 2 represent the major microbial community diversity in anaerobic digestion of chicken wastes at day twenty-one. The result recorded a total sequence of 214,321 which was clustered using 97% identity threshold to calculate Operational Taxonomic Units (OTUs). The OTUs were regularize into total number of read counts of the microbial community recovered. The digested slurry at day 21 recorded a total of 23,482 bacterial reads consisting of 98.69% of the quality reads, 175 read counts of unknown organisms (0.74%) and Archaeal 137 read count containing 0.58%. 51.19% of the bacterial phyla identified were Bacteroidetes, 22.68% Firmicutes, 7.67% proteobacteria ,6.49 were Tenericutes 1.57% Verrucomicrobia, 1.39% Actinobacteria and 0.58% were Euryarchaeota. The result clearly identifies Bacteroidetes as the most abundance phylum in contrast to previous studies that identify Firmicutes as the most abundance (Kröber et al.,2009; Werner et al.,2012). The most abundance Archaeal diversity identified belong to the class of Methanomicrobia (0.31%) and Thermoplasmata (0.22%). which include the family of Methanocorpusculaceae (0.19%), Methanosarcinaceae (0.14%) and Thermoplasmataceae (0.28%). The Archaeal population was limited in number which probably may have resulted from the changing environmental condition. Methanocorpusculaceae and Methanosarcinaceae are acetogenic methanogens which suggest that the digester condition consist of acetic acid. Thermoplasmata was also identified. Thermoplasmata are thermophilic organism that thrives in acidic environment at a pH below 5 and temperature above 370C. In this study anaerobic digestion was carried out in mesophilic condition which therefore suggest that their presence maybe attributed to increase volatile fatty acid or a shift in the environmental condition to thermophilic.

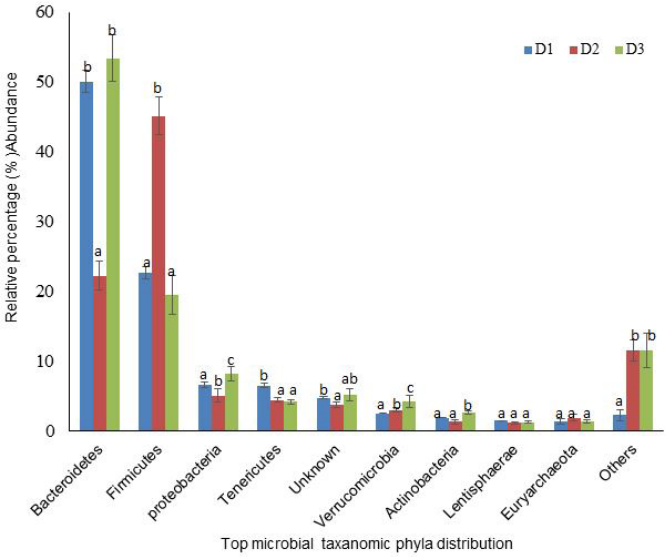

Figure 3: Frequency of occurrence of phyla in the different substrates at day 42

Bars with different alphabets within each phylum are significantly different at $p<0.05$

Figure 3 shows the major microbial phylum identified and their frequency at day 42. D1 recorded Percentage relative abundance of 50.06%, 22.68%, 6.64%, 1.30%, for Bacteroidetes, Firmicutes, Proteobacteria and Euryarchaeota while D2 and D3 indicate an abundance richness of 22.24%, 45.14%, 5.06%, 1.89% and 53.37%, 19.46%, 8.19%1.35% for Bacteroidetes, Firmicutes, Proteobacteria and Euryarchaeota. As shown in the result D2 indicates richness of Firmicutes (45.14%) for bacteria and Euryarchaeota (1.89%) for Archaea with increasing biogas yield of 700±11.00kpa pH7.27±0.137 in contrast to D1 and D3 which were more abundant in Bacteroidetes. The predominant of phylum Firmicutes in D2 is in agreement with previous studies reported by Kröber et al. (2009) and Werner et al. (2012).

The Relationship between Physicochemical Parameters and Biogas Yield from the Different Digester

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					
BGY	0.952 (0.003)			0.859 (0.028)	
VFA					0.939 (0.006)
NH4 -N					0.876 (0.022)
Digester three					
BGY			-0.922 (0.009)	-9.24 (0.008)	0.890 (0.018)
NH4 -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 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 (Karim et al.,2005; EL-Mashad et al.,2004). In this the study, the upsurge in biogas with corresponding increase in VFA may be 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 are in good agreement with the study conducted by Hill et al. (2000) who also reported increase methane yield with increase VFA. The result therefore clearly demonstrates that concentration of VFA 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 which also suggest reduction in these parameters, increase biogas yield (39.9±1.70%VS, 10.1 ± 0.90 mg/l COD, 521 ± 21.00kpa).

Conclusion

The study physicochemical parameters and microbial community in anaerobic digestion of organic wastes shows that different parameters and microbes affects not just anaerobic digestion performance (biogas production) but also the system (process, stability among others). 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.

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

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Authors Contributions

All authors contributed to the study conception and design. Ma-

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