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# Energy generation from anaerobic co-digestion of food waste, cow dung and piggery dung

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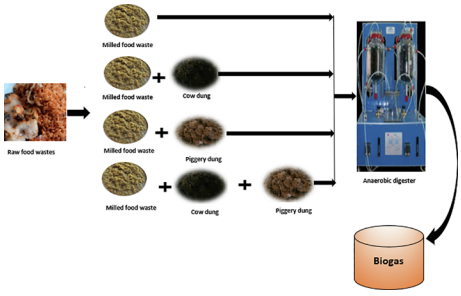
## GRAPHICAL ABSTRACT

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## ABSTRACT

The study investigated bioenergy generation from anaerobic co-digestion of food wastes (FW), cow dung (CD) and piggery dung (PD). The physicochemical parameters of the substrates were determined before and after digestion following standard procedures after mechanical pretreatment. Throughout the study, pH remained slightly alkaline while temperature varied between 26 and 32 °C. The highest cumulative biogas yield of 0.0488 L was recorded from the digestion of FW + CD + PD on the ninth day. After analyses, the highest methane content of 64.6 was obtained from the digestion of FW + PD while the lowest (54.0%) was from the digestion of FW only. Overall, cumulative biogas production for the four digestion regimes followed the order: FW + CD + PD, FW + PD, FW + CD and FW only respectively. Accumulation of VFAs was recorded at a slow rate during the digestions.

## 1. Introduction

Human population is increasing globally at an alarming rate thus calling for more energy availability and usage. Besides, there is depletion of non-renewable energy resources such as fossil fuels which has necessitated the search of clean and renewable sources of energy generation (Khalil et al., 2019). In addition, the over-dependence on the use of fossil-based fuel is regarded as the primary cause of gross pollution and degradation of the environmental and other adjoining issues such as through the release of greenhouse gases (GHGs) faced by

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humans (Naran et al., 2016). Hence, several bioenergy technologies including anaerobic digestion (AD) are considered veritable means of meeting the ever-increasing global energy need (Khalil et al., 2019). Also, biogas is preferred in comparison to other bioenergies because of the ease of production, among other factors like it is cheaper, eco-friendly and has direct applications such as fuel in internal combustion engines, generation of heat/electricity using boilers, generators or with Combined Heat and Power (CHP) units (Kadam and Panwar, 2017). Furthermore, biogas production is preferred since the residue/digestate is usually is nutrient-rich organic manure to improve soil nutrients and plant growth (Zhao et al., 2016).

Production of biogas is usually from organic wastes and other biodegradable resources by different groups of bacteria in an anaerobic condition (Chuichulcherm et al., 2017). Essentially, AD is a biochemical process that is greatly utilized for the treatment and energy recovery from different types of biomasses, particularly agricultural products and agro-industrial wastes (He et al., 2016). Anaerobic digestion leads to the production of biogas mixtures comprising mainly between 50 and 70% methane, 30 to 50% carbon dioxide and other gases including $H_2$, $NH_3$ and $H_2S$ contingent upon the nature of the organic matter/feedstock being employed (Saha et al., 2016). Many researches have demonstrated the use of different feedstock in AD which includes agricultural wastes and plant residues, food and other domestic wastes, solid and liquid wastes from municipalities and industries for the production of biogas (Dahunsi et al., 2016a,b, Dahunsi et al., 2017a,b,c,d; Oloko-Oba et al., 2018; Bala et al., 2019; Slorach et al., 2019).

There are reports of several adverse impacts of carbon dioxide, methane and nitrous oxide release into the milieu which could potentially be reduced by the use of bioresources as AD substrates (Kim et al., 2015). Cow dung (CD) is an important organic material in biogas generation (Franco et al., 2018). However, its biogas yield is comparatively low thus giving rise to its co-digestion with other biodegradable organic substrates for enhanced biogas yield (Ormaechea et al., 2018).

According to the 2019 report of the Food and Agriculture Organization (FAO) of the United Nations, up to 1.3 billion tons of food culminating in nearly 33% of total annual global production is turned to waste with each person generating an annual 250 to 300 kg average world per capita food wastes (FW) (Latha et al., 2019). Several processes involved in food production generate wastes and these include harvesting, processing, storage, distribution, marketing, cooking and serving (Food Waste Reduction Alliance 2016). Food waste contains highly biodegradable organic solids as such it is a suitable organic substrate for AD. Besides, the high moisture content and multiple organic nutrient contents in the FW make it is more suitable for AD (Latha et al., 2019).

Food waste is highly perishable with the characteristics of producing high quantities of volatile fatty acids (VFAs) and ammonium compounds which when accumulated often inhibit microbial activities and the rate of digestion when FW is digested alone (Naran et al., 2016). This can be more severe and even lead to complete failure of AD process if FW is digested at organic loading greater than 2.5 g VS/L/d and mostly at thermophilic temperature range. To overcome this challenge, FW is often co-digested with animal, lignocelluloses and sewage sludge as these will help to dilute the toxic compounds and boost nutrients balance as well as enhance microbial processes (Zhang et al., 2014). The co-substrates can supply micro-nutrients and alkalinity, and overcome the hindrances faced in mono-digestion of FW (Xu et al., 2018). Co-digestion of green biomass, such as crop residues and different parts of plants, has been shown to stimulate the AD of FW (Zhang et al., 2014). Another advantage of co-digesting FW with other organics is the neutralization of the toxicity caused by ammonia and sulfate thereby ensuring optimal ratio between carbon and nitrogen and carbon and sulfate (Chen, 2016). In some previous studies that involved the co-digestion of FW with fescue grass and differently with sewage sludge at an optimum co-digestion ratios (2:1 and 3:2), and organic loading rates of 10 and 15 gVS/Ld respectively, enhanced biogas yield coupled with higher removal of organic matter (0.290, 0.350 L/g$VS_r$) were reported (Hidaka et al., 2015). Dhamodharan et al. (2015) documented high methane yield of 3.47 and 3.36 L from the co-digestion of FW with CD and that of FW with PD at food to microorganism ratios of 2.0 and 15 respectively.

The mixing hydrodynamics in co-digestion methods in AD should be in the correct proportion so as to provide adequate contact surfaces between the digesting substrate and bacteria which will enhance maximum yield of biogas without disrupting the morphology of bacteria members of the archael group (Lindmark et al., 2014). If mixing of digesting substrates is inadequate, there will be formation of sediments and scums, production of foam, floating of frothing materials on the digesting slurry thereby hindering the rate of generation of gas (McMahon et al., 2001). On the other hand, if substrate mixing is done excessively, there will be high disruption of bacterial cells caused by shear stress, reduction in hydrogen pressure and the result will be reduced biogas yield or total failure of digestion if too adverse (Vavilin and Angelidaki, 2005).

This research sought to investigate biogas production from FW through AD with two livestock wastes i.e. CD and PD respectively using automated batch anaerobic reactors. The essence is to study the synergistic effect of substrate mixing on the overall reactor and yield of biogas. Even though biogas has been produced from the mono-digestion of each of the substrate, there were enormous limitations in terms of nutrient balance and gas yield. The physicochemical parameters of the substrates before and after digestion were analyzed and the methodologies used are not limited to the use of CD PD as the only livestock inocula. Even though CD in co-digestion with FW has been reported to produce the highest methane as against other different livestock inocula (Dhamodharan et al., 2015), Gaur and Suthar (2017) demonstrated that the combination of several inocula improves biogas production, hence this study.

## 2. Materials and methods

### 2.1. Collection of samples

Fresh CD and PD evaluated in this work were collected using sterile bags from the Teaching and Research farm at Ladoke Akintola University of Technology (LAUTECH), Ogbomosho, Oyo State, Nigeria. These were immediately kept under iced conditions before being moved to the research laboratory and stored at 4 °C until use. These wastes were later used both as co-substrates and as inocula in the developed assays to increase the methanogenic microorganisms in the reactors. Food waste was collected from the University's cafeteria 5 hourly, pooled together and delivered to the laboratory after which it was thoroughly homogenized using a wet grinder of 10-L capacity (Mechanical treatment) to obtain minimal particulate size of ≤3 mm mesh.

### 2.2. Description of reactor

The Computer controlled batch anaerobic reactor (EDIBON, United Kingdom) was employed in this study. It contains a pair of double-jacketed anaerobic chambers each with 10 L capacity equipped with sensors which regulates pH, flow of water, temperature, rate of mixing and production of gas. The anaerobic chambers/tanks are anoxic containing an auto-stirrer for mechanical mixing substrates and microorganism's distribution.

### 2.3. Analytical methods

For the determination of the chemical characteristics FW, CD, PD and the various combinations before and after digestion, an inductively coupled plasma mass spectrometry was used as earlier described

(Olayanju, 2003; Dahunsi, 2019a,b). For Chemical Oxygen Demand (COD) measurement, the standard method (APHA, 2017) was used. Concentrations of Volatile fatty acids (VFAs) were determined by gas chromatography (Clarus 580GC, PerkinElmer, USA) with an attached flame ionization detector. For total and volatile solids (TS) and (VS) and biochemical oxygen demand (BOD) determination, a standard method (Finnish Standard Association 1990) was used while a microtube test was used in determining the total phenolic contents of the samples followed by a 4-amino antipyrine colourimetric test (Monlau et al., 2012). The total concentration of Cr, Ni, Al, Cu, and Zn was determined using Flame Atomic Absorption Spectrometry (FAAS) model GBC 932 AA (Victoria, Australia) with deuterium lamp for background correction.

### 2.4. Biogas potential (BP) test

The BP test was done to determine the maximum amount of biogas that can be produced from the mixture of FW, CD and PD for biogas generation under constant condition. These were done in triplicate employing 250 mL batch mini-digesters attached to 500 mL eudiometer tubes with 10% ($m.v^{-1}$) VS. The BP in this study was determined at 37 °C following a standard procedure (Dahunsi et al., 2019a,b,c) also following the VDI 4630 (2006) standard for 30 days.

### 2.5. Digestion, monitoring of operational parameters and gas analysis

The different waste streams by weight were mixed together into slurry in the ratio 40:40:20 (2:2:1) for CD, PD and FW respectively in the combined experiment. Also, FW was equally digested with each of CD and PD in ratio 1:1 respectively while FW was also digested singly as the major substrate considered in this study. The slurry from each mixture was thoroughly mixed and introduced into the anaerobic chamber of the reactors filling ¾ of the volume leaving clear head space for gas production. The conditions of the reactors were monitored every day to ascertain stability. Reactors and ambient temperatures were taken twice every day using 2/1 °C Thermometers (England) with the average value taken. pH was measured weekly using pHS-2S meter (Shanghai Jinyke Rex, China). As the experiment progressed, biogas was constantly produced and collected using liquid displacement method previously reported (Dahunsi et al., 2019a). Determination of the biogas composition and quality in terms of methane, carbon dioxide and hydrogen sulfide were carried out using infrared and electrochemical sensors (BIOGASS5000, USA).

### 2.6. Analysis of microbial community

For microbial community analysis, 45 mL of samples were taken from all influents, mixed sludge and effluents of the digestion process on 5th, 10th, 15th, 20th and 25th experimental days and refrigerated at −20° C. Total genomic DNA of the samples were extracted (Vilchez-Vargas et al., 2013) and a Polymerase chain reaction (PCR) (Conventional) to target the entire bacteria present using specific primers (P338f and P518r) (Boon et al., 2002). To verify the quality and of the isolated DNA and products of the PCR, gel electrophoresis was conducted followed by another PCR (Real-time) with a StepOnePlus™ Real-Time PCR System (Applied Biosystems, Carlsbad, CA). This allowed all bacteria including methanogens to be analyzed. Methanogens include members of *Methanobacteriales, Methanomicrobiales* and *Methanosarcinaceae* (Desloover et al., 2015). Validation of the Real-time PCR product's quality was carried out by the examination of all parameters obtained from the software.

### 2.7. Statistical analyses

All statistical analyses were carried out using the Analysis of variance (ANOVA) while all the mean values were compared using the Tukey's test

**Table 1**
Physicochemical properties of selected wastes before digestion.

| Parameters | CD | PD | FW |
|---|---|---|---|
| Total Alkalinity (mg/L) | 240 ± 4.12[a] | 280 ± 6.41[a] | 260 ± 4.21[a] |
| pH | 7.74 ± 0.13[a] | 7.40 ± 1.13[b] | 7.32 ± 1.01[b] |
| Total Nitrogen (mg/L) | 25.6 ± 2.10[a] | 26.9 ± 3.11[a] | 26.8 ± 2.02[a] |
| Ammonium (mg/L) | 0.45 ± 0.01[a] | 0.70 ± 0.01[b] | 0.50 ± 0.01[a] |
| BOD (mg/L) | 180 ± 4.02[b] | 265 ± 7.04[a] | 256 ± 5.23[a] |
| COD (mg/L) | 450 ± 6.11[a] | 680 ± 8.22[b] | 560 ± 8.32[c] |
| Temperature (° C) | 26.7 ± 1.02[a] | 27.0 ± 1.12[a] | 26.9 ± 1.12[a] |
| Total Carbon (mg/L) | 224.7 ± 1.01[a] | 276.2 ± 2.01[b] | 290.1 ± 3.20[b] |
| Chromium (mg/L) | 0.34 ± 0.00[a] | 0.67 ± 0.01[b] | 0.39 ± 0.02[a] |
| Copper (mg/L) | 1.20 ± 0.12[a] | 3.10 ± 0.11[b] | 1.95 ± 0.32[c] |
| Nickel (mg/L) | 1.60 ± 0.02[a] | 5.20 ± 0.21[b] | 1.84 ± 0.12[a] |
| Zinc (mg/L) | 8.2 ± 1.10[a] | 25.0 ± 2.02[b] | 9.6 ± 1.11[c] |
| C/N | 9:1 | 10:1 | 11:1 |
| Weight of sample (g) | 1480 ± 11.20[a] | 1695 ± 14.02[b] | 1520 ± 11.31[a] |
| Total solids (%) | 14.4 ± 1.02[a] | 18.7 ± 1.11[b] | 16.83 ± 1.22[c] |
| Fixed solids (%) | 19.6 ± 2.11[a] | 16.8 ± 0.02[b] | 17.1 ± 1.21[b] |
| Volatile solids (%) | 80.4 ± 4.30[a] | 83.2 ± 4.03[a] | 82.9 ± 4.10[a] |
| Volume of sample ($cm^3$) | 1334 ± 9.21[a] | 1334 ± 8.12[a] | 1334 ± 9.26[a] |
| Moisture content (%) | 85.6 ± 5.02[a] | 81.3 ± 5.10[b] | 81.1 ± 3.10[b] |

Values shown in table are means of triplicate analyses; superscripts with same letters are statistically the same by the Tukey's test at 5%.

## 3. Results and discussion

### 3.1. Physicochemical properties of the substrates used

The physicochemical properties of the substrates (FW, CD and PD) used for this study are as shown in Table 1. In terms of TS content, PD was the densest in comparison with those of CD and FW. Also, PD had the highest VS contents though; there were slight variations in the VS content of the three substrates. In the same vein, there was a little variation in the nitrogen content of the substrates. Ammonium was highest in PD (0.70 mg/L) while CD had the lowest value (0.45 mg/L). Besides, for the mineral elements - Chromium, Copper, Nickel and Zinc, the highest values were documented for PD (0.67, 3.10, 5.20 and 25.0 mg/L) respectively. This could probably be due to the presence of these metals in the piggery feed as different materials are added to such feeds during production. The lowest values were however observed in CD (0.34, 1.20, 1.60 and 8.2 mg/L) respectively. Pig dung also had the highest COD value of 680 mg/L while the lowest (450 mg/L) was recorded in CD. The physicochemical characteristics of the three substrates used in this study i.e. FW, CD and PD are similar to those of poultry manure earlier reported (Dahunsi et al., 2019a,b,c,d). The bulky nature of the CD and PD could be attributed to the feed of the cow and swine especially the latter which often depend on varieties of food materials coupled with their ferocious eating nature. As shown in Table 2, mixing of these substrates before digestion had positive effects paramount among which was the increased C/N ratio across all four digestions. The values of 16 for FW + CD + PD and 15 for both FW + CD and FW + PD are very similar to the value (17) reported by Degueurce et al. (2016) by digesting spent animal beddings. The increase in the values of some elements as seen in the digestates is caused by the actions of microbes which ensured enormous breakdown of the large molecules of the substrates thereby yielding the monomers earlier locked up in their hence the increase values. Such trend had been reported for other substrates such as different biomass which include shoot of *Tithonia diversifolia* and *Chromolaena odorata*, fruit peels of *Arachis hypogeae* and *Telfairia occidentalis* as well as *Carica papaya* peels (Dahunsi et al., 2016a,b, 2017a,b,c,d). Table 2 shows the result of the physicochemical properties of the combined substrates i.e. FW + CD + PD, FW + CD and FW + PD.

Table 3 shows the results for physicochemical analyses of the

**Table 2**
Physicochemical properties of the combined substrates.

| Parameters | CD + PD + FW | FW + CD | FW + PD |
|---|---|---|---|
| Total Alkalinity (mg/L) | 310 ± 7.15[a] | 255 ± 4.19[b] | 321 ± 7.05[a] |
| pH | 7.49 ± 0.11[a] | 7.46 ± 0.10[a] | 7.66 ± 1.01[a] |
| Total Nitrogen (mg/L) | 22.2 ± 2.01[a] | 21.4 ± 2.01[b] | 23.5 ± 2.05[a] |
| Ammonium (mg/L) | 0.85 ± 0.01[a] | 0.67 ± 0.01[a] | 0.77 ± 0.01[a] |
| BOD (mg/L) | 270 ± 8.30[a] | 250 ± 6.05[a] | 287 ± 4.53[b] |
| COD (mg/L) | 894 ± 11.13[a] | 774 ± 9.12[b] | 796 ± 6.15[c] |
| Temperature (° C) | 27.2 ± 1.21[a] | 27.3 ± 1.02[a] | 27.8 ± 2.02[a] |
| Total Carbon (mg/L) | 348.6 ± 8.20[a] | 319.9 ± 5.21[b] | 347.4 ± 6.10[a] |
| Chromium (mg/L) | 0.86 ± 0.01[a] | 0.62 ± 0.02[b] | 0.89 ± 0.05[a] |
| Copper (mg/L) | 0.47 ± 0.01[a] | 1.20 ± 0.10[b] | 1.33 ± 0.32[c] |
| Nickel (mg/L) | 0.68 ± 0.01[a] | 1.01 ± 0.01[b] | 1.43 ± 0.11[c] |
| Zinc (mg/L) | 26.4 ± 2.11[a] | 28.0 ± 2.10[b] | 25.1 ± 1.04[a] |
| C/N | 16:1 | 15:1 | 15:1 |
| Weight of sample (g) | 4712 ± 12.22[a] | 4608 ± 12.01[b] | 4720 ± 13.01[a] |
| Total solids (%) | 22.6 ± 1.13[a] | 20.9 ± 2.01[a] | 21.81 ± 2.02 [a] |
| Fixed solids (%) | 14.2 ± 1.02[a] | 17.3 ± 1.02[b] | 17.4 ± 0.30[b] |
| Volatile solids (%) | 86 ± 4.11[a] | 88.6 ± 5.01[a] | 90.6.15 ± 4.10[a] |
| Volume of sample ($cm^3$) | 4002 ± 9.15[a] | 4101 ± 7.19[b] | 4141 ± 9.06[b] |
| Moisture content (%) | 77.44 ± 2.10[a] | 89.8 ± 6.11[b] | 91.8 ± 4.03[b] |

Values shown in table are means of triplicate analyses; superscripts with same letters are statistically the same by the Tukey's test at 5%.

substrates after digestion (Digestates). Across the four different digestion regimes, the values of elements such as nitrogen, ammonium, chromium, copper, zinc, total solids, fixed solids and moisture content increased in the digestates. It is significant to note that the digestion also had a significant impact on the COD values across the digestion as reductions of 46, 54, 57 and 53% were recorded for the digestions FW + CD + PD, FW + CD and FW + PD and FW alone respectively. However, the decrease in values of other parameters especially carbon is as a result of usage by the microbial community for cell wall formation and energy source during digestion. Significant among these was COD whose reduction shows that there was stabilization of organic matter during digestion and this agrees with a recent submission (Veroneze et al., 2019). The resulting digestates from the digestions in this study are highly useful as organic manure because of their enormous richness in virtually all the basic nutrients required by crop plants for their growth and wellbeing. These digestates are equally advantageous in their richness in diverse soil beneficial microorganisms and this shows their high potentials to boost the microbial as well as the nutrient status of nutrient-deficient or marginal soils when applied. In recent years, some researchers have documented findings that showed tha efficacy of such digestates as organic fertilizers and subtitutes for inorganic fertilizers across different agaricultural systems (Westphal et al., 2016).

The table also revealed that the substrate after digestion was bulkier than individual content of the 3 substrates in terms of TS and VS. The recorded values for the pH and temperature were within the designed ranges for the experiment. The pH remained slightly alkaline while temperature readings of the digester were between 26 and 32 °C throughout the experimental period.

The trend observed for pH during the digestions could be attributed to the chemical compositions of the substrates (Syaichurrozi et al., 2018). Also, the fluctuations might have been due to changes in metabolic activities of the mesophilic bacteria with regards to variations in the temperature and pH of the digester. This is so because, the performance of methanogenic microorganisms involved in bioconversion of substrates is to a large extent contingent upon the pH of the digester (Veroneze et al., 2019). Thus, in order to have maximal bioconversion of substrates by methanogenic microorganisms during AD, suitable pH must be maintained (Zahedi et al., 2016). The pH readings throughout the AD process in this study remained at slightly alkaline range and falls within the acceptable limit for efficient AD processes/maximal biogas yield. This corroborates earlier reports (Dahunsi et al., 2016a,b). It has also been documented that a pH of less than 6.5 or greater than 8 hampers the success of AD and could suppress methane production (Mirmohamadsadeghi et al., 2019).

Another key factor in the success of AD is temperature. This is so because; the different bacteria carrying out bioconversion of substrates are known to operate optimally at a specific temperature range (Mckennedy and Sherlock, 2015). Dahunsi et al. (2017a,b,c,d) affirmed that failure to establish such a temperature range could lead to complete breakdown of the AD system. Besides, mesophilic temperature influences substrates conversion rate/biogas production, provides a suitable environment for higher bacteria richness and efficiency and influences digestate quality (Mao et al., 2015).

### 3.2. Biogas potential (BP) results

In this study, one of the inocula (PD) produced more biogas than the other (CD) but both produced less than 10% of total generation from the actual co-digestion experiment and also lower than the potential of the standard i.e. microcrystalline cellulose. Production from the latter was higher by over 75% than the value obtained from the reference 650

**Table 3**
Physicochemical properties of the digestates.

| Parameters | CD + PD + FW | FW + CD | FW + PD | FW |
|---|---|---|---|---|
| Total Alkalinity (mg/L) | 290 ± 4.21[a] | 218 ± 3.12[b] | 231 ± 5.03[c] | 223 ± 5.12[c] |
| pH | 7.26 ± 0.12[a] | 7.63 ± 0.12[b] | 7.67 ± 1.00[b] | 7.62 ± 1.00[b] |
| Total Nitrogen (mg/L) | 25.8 ± 2.01[a] | 24.7 ± 2.20[a] | 26.2 ± 2.01[a] | 26.1 ± 2.01[a] |
| Ammonium (mg/L) | 0.89 ± 0.01[a] | 0.66 ± 0.05[a] | 0.56 ± 0.01[b] | 0.54 ± 0.01[b] |
| BOD (mg/L) | 190 ± 5.12[a] | 151 ± 4.02[b] | 147 ± 2.31[b] | 143 ± 3.04[b] |
| COD (mg/L) | 480 ± 7.20[a] | 358 ± 4.94[b] | 341 ± 4.02[b] | 261 ± 5.20[c] |
| Temperature (° C) | 32.0 ± 2.05[a] | 31.5 ± 1.12[a] | 32.2 ± 2.00[a] | 31.2 ± 0.12[a] |
| Total Carbon (mg/L) | 243 ± 5.12[a] | 222.1 ± 4.11[b] | 268.6 ± 4.05[c] | 211.6 ± 4.10[d] |
| Chromium (mg/L) | 0.94 ± 0.01[a] | 0.33 ± 0.02[b] | 0.91 ± 0.01[a] | 0.41 ± 0.01[b] |
| Copper (mg/L) | 0.48 ± 0.02[a] | 0.65 ± 0.10[b] | 1.36 ± 0.02[c] | 1.99 ± 0.02[d] |
| Nickel (mg/L) | 0.60 ± 0.05[a] | 0.81 ± 0.01[a] | 1.32 ± 0.01[b] | 1.81 ± 0.02[c] |
| Zinc (mg/L) | 28.5 ± 2.03[a] | 30.3 ± 1.40[b] | 27.0 ± 1.00[a] | 9.8 ± 0.10[c] |
| C/N | 9:1 | 9:1 | 10:1 | 8:1 |
| Weight of sample (g) | 4686 ± 6.12[a] | 4512 ± 10.11[b] | 4501 ± 10.02[b] | 3681 ± 9.05[c] |
| Total solids (%) | 29.8 ± 2.41[a] | 21.1 ± 0.51[b] | 22.13 ± 2.01[b] | 18.36 ± 1.05[c] |
| Fixed solids (%) | 15.2 ± 1.10[a] | 18.6 ± 2.12[b] | 18.2 ± 0.10[b] | 18.2 ± 0.04[b] |
| Volatile solids (%) | 44.8 ± 1.15[a] | 51.1 ± 3.02[b] | 48.1 ± 2.01[b] | 52.5 ± 2.12[b] |
| Volume of sample ($cm^3$) | 3920 ± 13.11[a] | 3456 ± 9.06[b] | 3244 ± 8.03[c] | 2532 ± 6.04[d] |
| Moisture content (%) | 79.73 ± 3.04[a] | 72.6 ± 4.04[b] | 75.3 ± 2.01[c] | 62.4 ± 4.02[d] |

Values shown in table are means of triplicate analyses; superscripts with same letters are statistically the same by the Tukey's test at 5%.

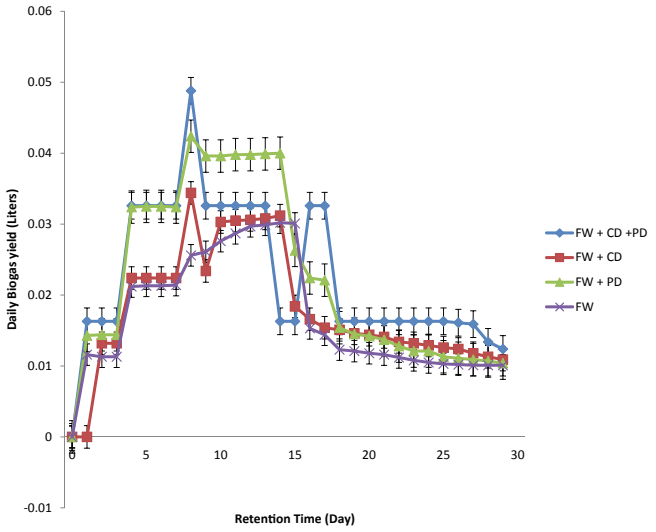


**Fig. 1.** Daily biogas yield from the digestion of FW, CD and PD.

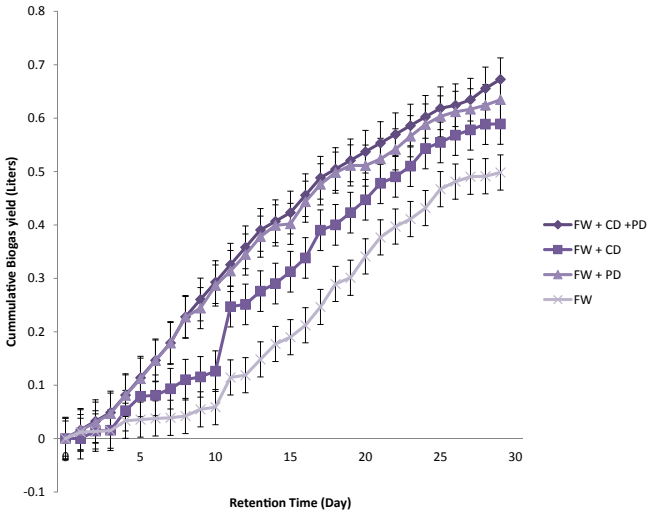


**Fig. 2.** Cumulative biogas yield from the digestion of FW, CD and PD.

LNbiogas.kg $VSad^{-1}$ produced by following the VDI standards 4630 (2006).

### 3.3. Performance evaluation and gas production

The pH value of the substrates could determine the performance of AD either when it is too much alkaline or acidic. Throughout the digestions in this study, pH remained at the alkaline range i.e. 7.26 to 7.67. Though initial fall to slightly acidic range was observed in the first five days of digestion across the four digestions, the pH switched back to alkaline range and remained throughout the digestion period. Similarly, the temperature of all reactors remained constant within the mesophilic range (26–32 °C) throughout the AD period.

The daily biogas produced from the AD of the substrates over a period of 30 days retention time (RT) is shown in Fig. 1. The results show that gas production started on the 2nd experimental day for digestions FW + CD + PD, FW + PD and FW alone while it commenced on the 3rd day for experiment FW + CD. Gas production remained constant until the 5th day. Biogas production reached a maximum (0.0488, 0.0344 and 0.0424 L) respectively on the 9th day (peak day) for digestions FW + CD + PD, FW + CD and FW + PD while the peak (0.0302 L) was reached on the 15th day for experiment involving FW only. A fall in gas production was observed from the 10th day and plateaued till the 14th day of the experiment in most cases. It further decreased on the 15th day but picked up on the 17th day. It dropped again on the 19th day and remained for the remaining days of the experiment probably because of the almost complete use up of the organic degradable compound. Low biogas production rate was recorded during the early digestion days i.e. the lag phase (days 1 to 4), probably due to oxygen that was trapped in the reactors during startup of digestion process (Deepanraj et al., 2017). This was similar to the report of Deepanraj et al. (2017) in which maximum biogas production was recorded on the 9th and 10th days in a mono-digestion of FW.

The percentage of $CO_2$ and $CH_4$ in the biogas was sampled and quantified at regular intervals. Results showed composition of 63.0, 55.0, 64.6 and 54.0% methane; 20.0, 22.5, 21.2 and 23.0% carbon dioxide and 12.0, 11.4, 13.1 and 13.4% for hydrogen sulfide for experiments FW + CD + PD, FW + CD, FW + PD and FW only respectively. Fig. 2 shows the cumulative biogas production for the 30 days retention time (RT). There were fluctuations in the volume of biogas production during the AD period as shown earlier in Fig. 1. Overall, cumulative biogas production for the four digestion regimes followed the order: FW + CD + PD, FW + PD, FW + CD and FW only respectively.

### 3.4. Microbial community

The major microbial groups in the substrates and the fermenting mixture and those of the digestate were identified. Aerobic and anaerobic bacteria belonging to genera Bacilli and Clostridia were seen to dominate. Prominent *Bacilli* includes *Bacillus stearothermophilus*, *Bacillus pantothenticus* and *Bacillus licheniformis* while *Clostridia* include *Clostridium clostridioforme* and *Clostridium histolytica*. Population distributions of other microflora show aerobic and anaerobic bacteria including *Klebsiella* spp, *Escherichia coli, Serratia ficaria, Proteus vulgaris, Fusobacterium mortiferum* and *Porphyromonas assacharolyticum* and methanogens of the genera *Methanococcus, Methanosarcinaceae, Methanobacteriales, Methanosaetaceae* and *Methanomicrobiales*. These various arrays of aerobic and anaerobic microbes implicated during the hydrolysis, acidogenesis and acetogenesis stages of the digestions showed robust microbial population and diversity as have been reported (Dahunsi et al., 2017a,b,c). Similarly, methane formers of the genera *Methanococcus, Methanosarcinaceae, Methanobacteriales, Methanosaetaceae* and *Methanomicrobiales* are known to be major players in the AD process in the current study, their sources are the CD and PD which are large reservoirs of these microorganisms responsible for methane formation while utilizing intermediate acids earlier produced in the reactors.

### 3.5. Dynamics of Volatile fatty acids

Due to the highly diversified and populated microbial group implicated in this study especially in the fermenting substrate which enhanced pronounced microbial activities, VFAs were produced and also accumulated in the reactors evidenced by the production of a number of intermediate acids known for inhibiting AD. The acid production and VFAs accumulation was due a slower rate of consumption by the reactor's microbial community which was not commensurate with the high VFAs production rate. Acetate, butyrate and propionate were the prominent VFAs implicated in this study (Fig. 3). Their concentrations peaked between the 11th and the 13th experimental days. The VFAs reported in this study are very similar to in Riggio et al. (2017). As seen in this study, neutrality was maintained in all reactors which was caused by ammonia buffering which resulted in stability of digestion coupled with enhanced gas generation especially in FW + CD + PD and FW + PD. Nitrogen inhibition was not recorded throughout the digestions because the value of nitrogen in all substrates as recorded in their analyses before digestion were very moderate. This had been earlier reported when food wastes and spent animal beddings were co-

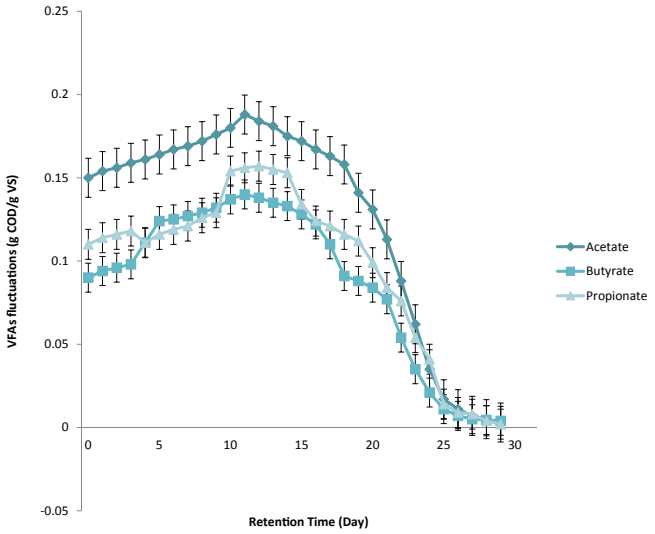


**Fig. 3.** Dynamics of VFSs during the digestion of FW, CD and PD.

**Table 4**
Stoichiometry and mass balance for one ton mixture of FW, CD and PD.

| Parameter | FW + CD + PD | FW + CD | FW + PD | FW Only |
|---|---|---|---|---|
| ***Input (Reactants)*** | | | | |
| CD + PD + FW (kg) | 1000 | 1000 | 1000 | 1000 |
| Volatile solids (VS) (kg) | 860 | 886 | 906 | 829 |
| ***Output (Products)*** | | | | |
| Methane ($CH_4$) (%) | 63.0 | 55.0 | 64.6 | 54.0 |
| Carbon dioxide ($CO_2$) (%) | 20.0 | 22.5 | 21.2 | 23.0 |
| Digestate (kg VS) | 448 | 511 | 481 | 525 |
| **Sum** | 531 | 588.5 | 566.8 | 602 |
| *Mass balance | 0.38 | 0.34 | 0.37 | 0.27 |
| % Volatile solids (VS) removal | 48 | 42 | 47 | 37 |

* = Input–output)/input (%).

digested (Riggio et al., 2017). A major factor influencing the production of VFAs is the high population of the facultative *Clostridia* organisms which are known to be very active during the acetogenesis and methanogenesis stages of AD causing decomposition of amino-acids in order to produce intermediates such as acetate, butyrate and propionates and the end-product being ammonia (Ghasimi et al., 2015; Degueurce et al., 2016; Dahunsi et al., 2018).

### 3.6. Stoichiometry and mass balance

The mass balance was evaluated in this study in order to assess the quantitative relationship between the reactants for each of the reactions (digestion) which in this case are FW, CD and PD and the products i.e. methane, carbon dioxide and the left over digestates (Table 4). These showed that considerable quantity of VS contents of each substrate was consumed owing to the diverse microbial diversity and population. Mass balances of 0.38, 0.34, 0.37 and 0.27 were obtained for the digestions FW + CD + PD, FW + CD, FW + PD and FW only respectively. These results are commensurate with the quantities of biogas produced from each of these digestions which further showed that the most balanced of the equations was FW + CD + PD which also has the highest molar ratio. This was followed by FW + PD and then FW + CD while the reaction involving FW only showed the least molar ratio. The table also shows the rate of VS consumption during the digestions with the removal efficiencies being 48, 42, 47 and 37 for the digestions FW + CD + PD, FW + CD, FW + PD and FW only respectively. This is also in tandem with the quantities of biogas produced as well as the digestates recovered. These values obtained for the stoichiometry and mass balance for the digested substrates in this study showed moderate balance between the reactants and the obtained products. Similarly, moderate consumption of the VS contents of the substrates was consumed. These trend have been previous reported (Dahunsi et al., 2017a,b,c,d).

## 4. Conclusion

This study has demonstrated that AD of FW with PD and CD is promising and suitable for energy generation. The highest cumulative biogas of 0.0488 L was produced by the co-digestion of FW + CD + PD whereas, the highest methane content of 64.6% was obtained from the co-digestion of FW + PD while the lowest i.e. 54.0% was from FW only. Based on this study however, further researches need to focus on reactor design, substrate mixing, organic loading, and feedstock pretreatment to enhance maximum biogas yield from FW and possibly in co-digestion with other substrates.

## CRediT authorship contribution statement

**Oladipupo S. Oladejo:** Conceptualization, Project administration, Supervision. **Samuel O. Dahunsi:** Funding acquisition, Supervision, Writing - original draft, Writing - review and editing. **Adekemi T. Adesulu-Dahunsi:** Resources, Methodology. **Samuel O. Ojo:** Data curation. **Adedoyin I. Lawal:** Formal analysis, Data curation. **Eunice O. Idowu:** Software. **Adewoye A. Olanipekun:** Validation. **Rotimi A. Ibikunle:** Visualization. **Christian O. Osueke:** Resources, Software, Validation. **Olusegun E. Ajayi:** Methodology. **Ngozi Osueke:** Writing - review and editing. **Ikponmwosa Evbuomwan:** Writing - original draft.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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