

# Impact of volatile fatty acids to alkalinity ratio and volatile solids on biogas production under thermophilic conditions

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## Abstract

The study assessed the impact of volatile fatty acids (VFA) to total alkalinity (TA) ratio (VFA/TA), and percentage volatile solids (VS) reduction of batch and semi-continuous anaerobic co-digestion of palm nut paste waste (PNPW) and anaerobic-digested rumen waste (ADRW) on digester stability and biogas production under the environmental condition of  $50 \pm 1^\circ\text{C}$  and hydraulic retention time of 21 days for the batch studies and 14 days for semi-continuous co-digestion. The co-digestion ratios were based on percentage digester volume corresponding to 90%:10%, 75%:25% and 50%:50%. During batch and semi-continuous anaerobic co-digestion, VFA/TA of 0.32–1.0 and VS reduction of 53–67% were observed as the stable range at which biogas production was maximum. In terms of semi-continuous anaerobic digestion (AD), except for the 50%:50% ratio where biogas production progressed steadily from the first to fourteenth days, biogas production initially dropped from 180.1 to 171.3 mL between the first and third days of the 90%:10% reaching a maximum of 184 mL on the fourteenth day. Biogas production declined from 198.8 to 187.5 mL on the second day and then increased to  $198.8 \pm 0.5$  mL in the case of the 75%:25% with a significant difference between the treatment ratios at  $p < 0.05$ . Therefore, the study can confirm that the 50%:50% ratio (PNPW:ADRW) is a suitable option for managing crude fat-based waste under thermophilic AD due to its potential for rapid start-up and complete biodegradation of active biomass within a 21-day period. This presupposes that residual methane as greenhouse gas will be void in the effluent if disposed of.

## Keywords

Biogas, volatile fatty acids, total alkalinity, thermophilic, ammonium–nitrogen, digestate

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## Background

The palm oil industry plays a crucial role in the lives of most local economies in the African continent. Nonetheless, the industry generates huge quantities of waste whose disposal poses a Herculean task to the environment (Maniruzzaman et al., 2020). That is, in a conventional palm oil treatment facility, only 10% of the palm oil is extracted. This means about 90% of the biomass is classified as waste in the form of empty fruit bunches, fibres, shells and thick viscous liquid effluent. Typically, 600–700 kg of organic effluent containing crude fat is produced per tonne of fresh fruit bunch (Zafar, 2019). This makes the effluent resulting from the processing suitable for biogas production (Murphy et al., 2011). Conversely, its degradation slows down the hydrolysis process (Bah et al., 2014) as well as producing odorous and xenobiotic compounds (Gebreyessus and Jenicek, 2016).

Also, putrefaction of crude fat during anaerobic digestion (AD) start-up is often associated with production of volatile fatty acids (VFA) (Ohemeng-Ntiamoah and Datta, 2018). In effect, accumulation of VFA decreases pH resulting in acidification (Hyun et al., 2014). In this case, alkalinity forms the ultimate medium to neutralize the VFA generated in order to offset pH changes. For this reason, offsetting high VFA production during

AD requires that total alkalinity (TA) be maintained slightly above  $1.5 \text{ g CaCO}_3 \text{ L}^{-1}$ . Aside from alkalinity, other writers suggested co-digestion of organic waste containing 40% fat and 10% carbohydrate has the capacity to counterbalance acidification during AD (Kim and Kim, 2017). Aside this, thermophilic AD offers a faster biochemical reaction rate, deactivation of pathogens and a higher biogas production rate in a shorter retention time (Gebreyessus and Jenicek, 2016).

Moreover, current process indicators of early warning signs of AD include the VFA/TA ratio and the rate of constant production of biogas (Gomes, 2017). Therefore, VFA/TA preference during AD to pH is borne out of the fact that AD rarely returns to normalcy in the event of a drastic drop in pH, thus

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rendering pH less significant (Méndez-Acosta et al., 2010). In this case, alkalinity must be monitored alongside VFA to ascertain proper overview of anaerobic digester stability (Ahring and Angelidaki, 1997). Against this background a VFA/TA ratio of 0.23–0.3 constitutes a stable range for AD, and a quotient  $<0.23$  is an indication of an under-fed digester that requires more feeding whereas  $>0.3$  is an indication of indigestion and poor stability (Rosato, 2015). Similarly, VFA/TA quotient of  $\leq 0.4$  is a safe range for AD with less acidification risk whereas  $\geq 1.0$  produces carbon dioxide and hydrogen as the main decomposition products (Hernández et al., 2014). In contrast, VFA/TA quotients of 1.131–1.870 has been found to produce biogas between 13 and 19 days in an earlier study of carbohydrate-based waste (Kim and Kim, 2018). This study therefore assessed the impact of VFA/TA on digester stability and biogas production, as a means for managing biowaste containing crude fat other than carbohydrate waste.

## Materials and methods

The palm nut paste waste (PNPW) as substrate for the study was obtained from traditional palm oil processors in Ghana. In the traditional procedure of processing palm oil, the palm nuts are boiled at 100°C for 30 min and allowed to cool under room temperature. The boiled nuts are subsequently pounded in a mortar using a pestle with the addition of warm water. The mesocarp fibre is then separated from the kernels. After separation, the mesocarp fibre is thoroughly soaked in warm water and the resulting mixture decanted to obtain a paste-like fluid. This fluid is further boiled at a constant temperature of 100°C for another 30 min. This step thus allows the palm oil to gradually float to the top. The oil is then collected leaving behind the substrate PNPW as a major organic residue that is left on the environment with unpleasant odour.

Therefore, the PNPW was collected and preserved at 4°C to avoid biochemical activity and transported to the College of Science and Technology, the University of Rwanda within 6 h. Unlike the PNPW, the co-substrate, in this case rumen waste, was sourced from the abattoir located near the Gisozi sector office in the Gamposho Estate in the city of Kigali in Rwanda. This was carried in sterilized plastic containers and preserved in a refrigerator prior to use. Notwithstanding, the two substrates, that is, PNPW and anaerobic-digested rumen waste (ADRW) were independently characterized for total solids (TS), volatile solids (VS), crude fat, VFA, TA and  $\text{NH}_4\text{-N}$  in duplicates. The results are presented in Table 1.

## Experimental design

### Preparation of the co-substrate and mode of operation

The co-substrate was ADRW. This was prepared in a 1000-mL glass bottle at 50°C for a fortnight. At maximum biogas production, the substrate was withdrawn and co-digested with the

**Table 1.** Characterization of palm nut paste waste and rumen waste.

Parameters	Mean $\pm$ SE of PNPW	Mean $\pm$ SE of RW
Crude fat (% DM)	20.24 $\pm$ 0.01	ND
VS (wt%)	34.60 $\pm$ 0.85	17.10 $\pm$ 0.000
TS (wt%)	16.85 $\pm$ 0.74	19.21 $\pm$ 0.007
VS/TS ratio (%)	93.10 $\pm$ 2.80	89.06 $\pm$ 0.000
VFA (mg L <sup>-1</sup> )	390.90 $\pm$ 1.45	234.08 $\pm$ 0.000
TA (mg L <sup>-1</sup> )	479.40 $\pm$ 1.48	265.70 $\pm$ 0.019
VFA/TA ratio	0.81 $\pm$ 0.00	0.88 $\pm$ 0.000
pH	8.05 $\pm$ 0.04	8.81 $\pm$ 0.007
$\text{NH}_4\text{-N}$ (mg L <sup>-1</sup> )	2787.85 $\pm$ 0.92	4509.5 $\pm$ 0.403

DM: dry matter; ND: not determined; PNPW: palm nut paste waste; RW: rumen waste; SE: standard error; TA: total alkalinity; TS: total solids; VFA: volatile fatty acids; VS: volatile solids.

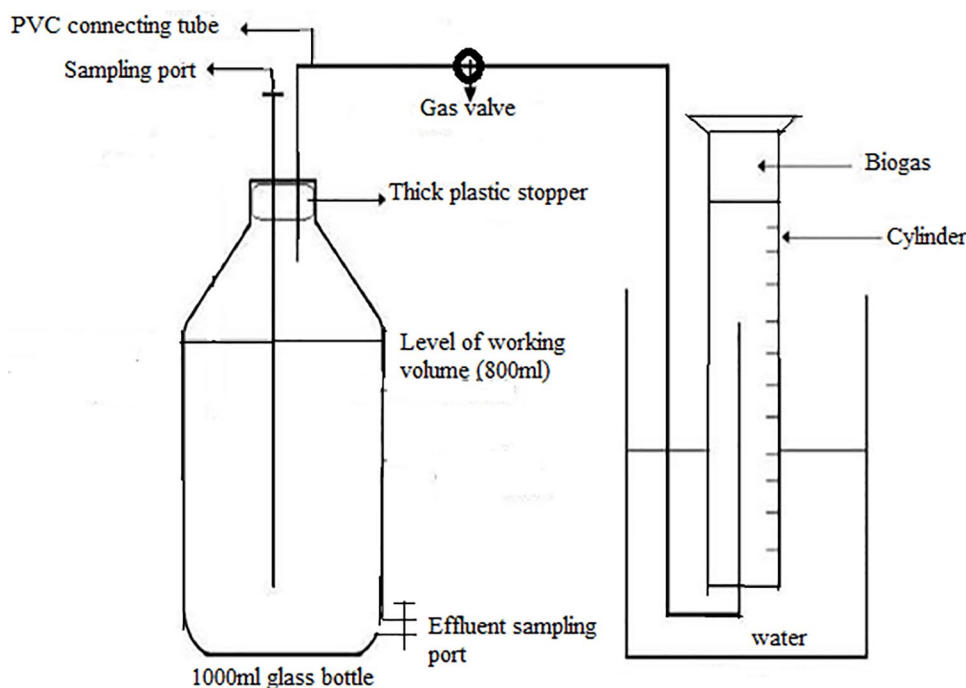
PNPW. All digesters for batch studies were operated at  $50 \pm 1^\circ\text{C}$  in a regulated incubator for 21 days. The batch mode operated with four digesters on the basis of percentage digester volume using the ratios PNPW:ADRW (100%:0%, 90%:10%, 75%:25% and 50%:50%). In each case, daily biogas was measured for 21 days whereas VFA and TA were assayed every two days from each digester using a syringe. The semi-continuous mode was operated by withdrawing 5% substrate daily from the batch digesters after the 21 days using a syringe and replacing with 2.5% PNPW and 2.5% ADRW and daily biogas measured for 14 days whereas VFA and TA were assayed every 2 days. The semi-continuous mode was introduced at maximum biogas production to assess constant gas production. Prior to withdrawing the substrate from the digesters for analysis of VFA/TA or withdrawing and injecting the substrate for the purpose of semi-continuous AD, the effluent sampling port was gradually opened to enable easy sample collection and quickly flushed with nitrogen gas for 30 s and closed.

### Biogas volume determination

Prior to AD, each of the 1000-mL bottles with a working volume of 800 mL were flushed with 100% nitrogen gas for 5 min to ensure anaerobic conditions. The digesters were firmly sealed with plastic caps with two pods for biogas and substrate sampling. The gas outlet was connected to a graduated cylinder to measure the volume of daily biogas produced by (liquid) downward displacement as in Figure 1.

## Assay procedures

TS was determined using the convection oven method. An aluminium dish was pre-dried at 105°C and weighed. A sample of 3.0 g was weighed into the dish and oven dried at 105°C for 4 h, cooled and re-weighed. TS were then calculated using equation (1) (Sluiter et al., 2008). Also, VS were evaluated by heating the sample at 550°C for 2 h.



**Figure 1.** Schematic diagram for biogas measurement.

$$\% \text{Total solids} = \left( \frac{\text{Weight}_{\text{dry pan plus dry sample}} - \text{Weight}_{\text{dry pan}}}{\text{Weight}_{\text{sample as received}}} \right) \times 100 \quad (1)$$

VFA/TA was assayed following a procedure outlined in Standard Methods for The Examination of Water and Waste Water (2017). A pH meter was first calibrated at two pH scales; that is 7.0 and 4.0. A 50-mL volume of supernatant was measured into a 100-mL beaker, stirred and the temperature and pH of the sample recorded. An initial burette reading was noted and the supernatant titrated with 0.10N  $\text{H}_2\text{SO}_4$  to a pH of 4.0. The sample was then boiled for 3 min, cooled and the sample titrated back to a pH of 4.0 with 0.05N NaOH. The burette reading was recorded and titrated again to pH 7.0. TA and VFA were then calculated using equations (2) and (3).

$$\text{Total alkalinity mg L}^{-1} = \frac{\text{Normality of } \text{H}_2\text{SO}_4 \times \text{mL } \text{H}_2\text{SO}_4 \times 50,000}{\text{mL of sample used}} \quad (2)$$

$$\text{Volatile fatty acids} = \frac{\text{Normality of NaOH} \times \text{mL NaOH} \times 50,000}{\text{mL of sample used}} \quad (3)$$

The crude fat was assayed by the Soxhlet extraction method. A 3.0-g sample was weighed into an extraction thimble. The thimble was placed in a Soxhlet apparatus. A dry, tarred flask was placed beneath the apparatus and connected to a condenser. The solvent petroleum ether (of boiling point 40–60°C) was added to the flask. Heat was applied at a rate that provided a condensation rate of five drops per second and extracted for 6 h. On completion, the thimble was removed and the ether recovered in a

boiling water bath. The flask was then dried at 105°C for 30 min, cooled in a desiccator and weighed. Percentage crude fat was then calculated using equation (4).

$$\text{Crude fat (\% of dry matter)} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100 \quad (4)$$

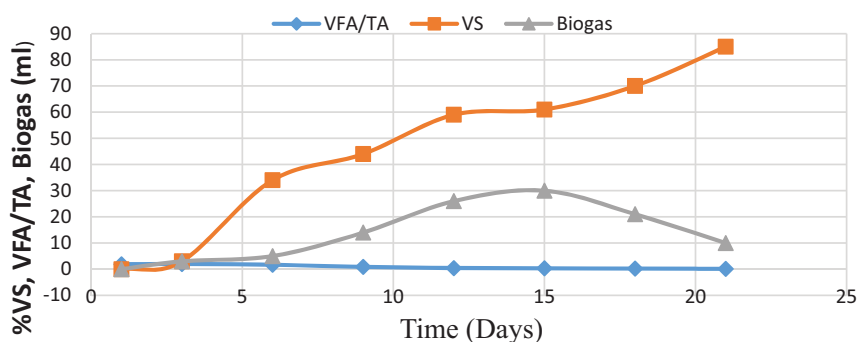
Also, the nitrogen content and crude protein were determined by the Kjeldahl method (Janssen and Koopmann, 2005). This was done by weighing 1.0 g of the sample into a digestion flask with 10 g of potassium sulphate, 0.7 g mercury oxide and 20 mL sulphuric acid. The flask was gently heated at an inclined angle until a clear solution was obtained without foaming. On cooling, about 90 mL distilled water was added and re-cooled with 25 mL sulphide solution added and mixed. Next 80 mL sodium hydroxide solution was added. The digestion flask was then immediately connected to a condenser and heated. The distillate was collected into 50 mL boric acid. The 50 mL distillate was then titrated against a standard solution of sulphuric acid to a violet end point. The percentage nitrogen content of the sample was calculated using equation (5) and the result multiplied by 6.25 to obtain crude protein: that is, percentage protein content = nitrogen content  $\times$  6.25.

$$\text{Kjeldahl nitrogen} = \frac{\text{ml acid} \times \text{Normality of standard acid}}{\text{Weight of sample}} \times 0.014 \times 100 \quad (5)$$

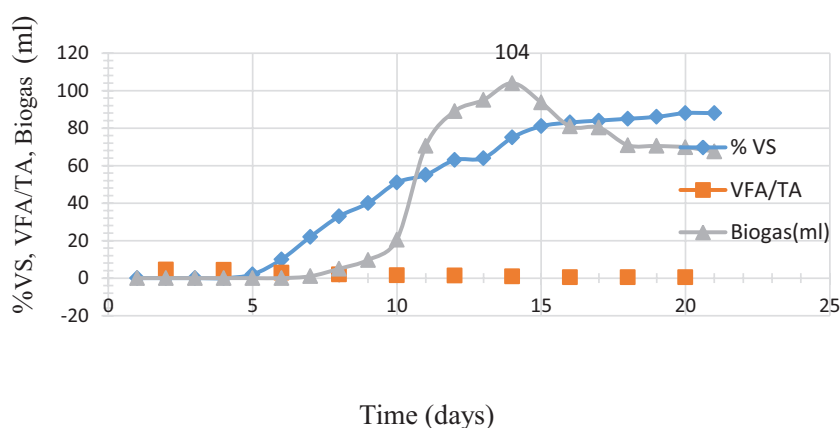
## Results and discussion

### *Mono-AD under batch conditions*

The anaerobic batch assay of mono-digestion of rumen waste under thermophilic conditions is shown in Figure 2. Biogas production



**Figure 2.** Anaerobic digestion of rumen waste (100%).  
TA: total alkalinity; VFA: volatile fatty acids; VS: volatile solids.



**Figure 3.** Mono-anaerobic digestion of palm nut paste waste.  
TA: total alkalinity; VFA: volatile fatty acids; VS: volatile solids.

showed rapid response from the third day due to the combined effect of naturally occurring methanogenic bacteria in the rumen waste (Hook et al., 2010; Kumar et al., 2009; Ozbayram et al., 2018) coupled with rapid biodegradation activity influenced by thermophilic reaction. The study also revealed that the VFA/TA ratio and percentage VS reduction were stable between 0.35 and 0.42 and between 59.0 and 61.0%, respectively, with a corresponding peak for biogas production of 30.0 mL on the fifteenth day. However, the 30.0 mL occurred specifically at a VFA/TA ratio of 0.35 and VS reduction of 61.0%. Biogas production nevertheless declined, reaching 10.0 mL on the twenty-first day with VS reduction of 85%. At this instance of 85% VS reduction, VFA/TA was 0.12 indicating that more substrate needed to be injected into the digester (Rosato, 2015) for further biochemical activity.

Conversely, biogas production from the AD of PNPW (that is 100% PNPW) started rather slowly until the seventh day as shown in Figure 3. The slow start-up process might be due to unavailability of active microbial consortia to degrade the crude fat layer and shorten the lag phase. In confirmation, it has been estimated that fat-based substrates slow down hydrolysis and delay the biogas production process (Bah et al., 2014). As the reaction proceeds, biogas production steadily increased to a maximum of 104.0 mL on the fourteenth day at 75% VS reduction and VFA/TA of 0.98. After the fourteenth day, biogas production declined reaching 67.5 mL at 88% VS reduction and VFA/TA of 0.41 on the twenty-first day. The threshold for AD to maintain

stability is estimated at 0.23–0.3 (Rosato, 2015). This study found stability of mono-digestion of PNPW at VFA/TA of 0.42–1.0 with maximum biogas production occurring at 0.98 on the fourteenth day. Likewise, Kim and Kim (2018) found stability of anaerobic digester and maximum biogas production at VFA/TA of 1.131–1.870 between 13 and 19 days.

### *Batch anaerobic co-digestion of PNPW and ADRW*

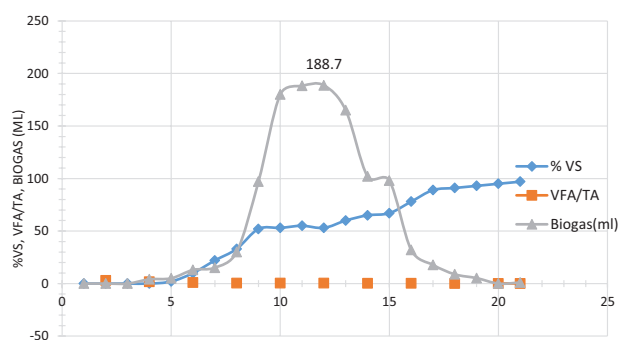
Anaerobic co-digestion of PNPW and ADRW was studied under batch conditions at 50°C. The ratios of co-digestion were based on percentage digester volume. The corresponding ratios of PNPW:ADRW as percentages were 90%:10%, 75%:25% and 50%:50%. The results of fermentation of the 90%:10% are shown in Figure 4. Biogas production started on the fourth day. This could be due to the ADRW that contributed active microbial consortia to facilitate the biochemical process. Biogas production nonetheless progressed steadily to 180.1 mL on the tenth day at VFA/TA of 0.35 to a peak of 188.7 mL on the twelfth day at VFA/TA of 0.31. During this period, percentage VS reduction at which the digester was stable ranged from 52% to 65%. Beyond this, biogas production declined reaching a minimum of 0.9 mL at 97% VS reduction and VFA/TA of 0.10.

In addition, fermentation of the 75%:25% ratio is shown in Figure 5. Unlike the 90%:10% ratio, biogas production in the

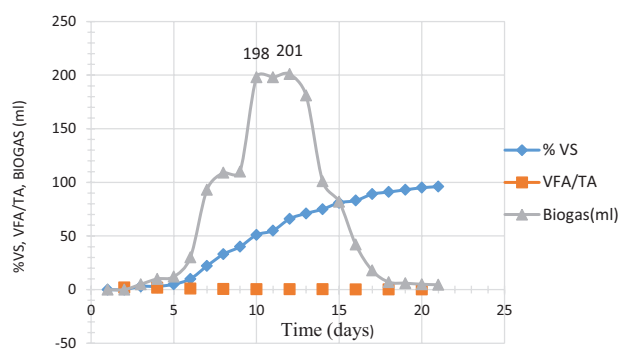


75%:25% ratio was faster, showing percentage VS reduction of 51–66% and peak biogas production of 201 mL on the twelfth day at VFA/TA of 0.32 before declining steadily to biogas production of 4.5 mL at 96% VS reduction and VFA/TA of 0.10. The VFA/TA of 0.32 is similar to the threshold of other workers' findings at 0.1–0.32 (Zickefoose and Hayes, 1976; Palacios-Ruiz et al., 2008). Moreover, the results of the 50%:50% co-digestion is shown in Figure 6. This ratio was the most rapid digester that responded to biogas production from the second day. This might be due to a well-balanced syndicate of microbial consortia of the archaea group contributed by the ADRW. However, biogas production progressed steadily

to a maximum range of 120–130 mL at stable VFA range of 0.32–0.35 and VS reduction of 52–67% between the ninth and thirteenth day. The peak of biogas production of 130 mL nonetheless occurred at VFA/TA of 0.32 and VS reduction of 67%. Despite the rapid biogas production start-up, the 50%:50% declined sharply reaching 0.0 mL on the twenty-first day due to complete utilization of the organic matter owing to the combined effect of high temperature and anaerobic microbes. Nevertheless, there were no significant differences between all the substrate ratios ( $p < 0.05$ , Table 2). These results affirm the contribution of the co-substrate in the co-digestion ratio that contributed active microbes to facilitate the breakdown of the crude fat layer in the PNPW.



**Figure 4.** Anaerobic co-digestion of palm nut paste waste and anaerobic-digested rumen waste in the ratio 90%:10%. TA: total alkalinity; VFA: volatile fatty acids; VS: volatile solids.

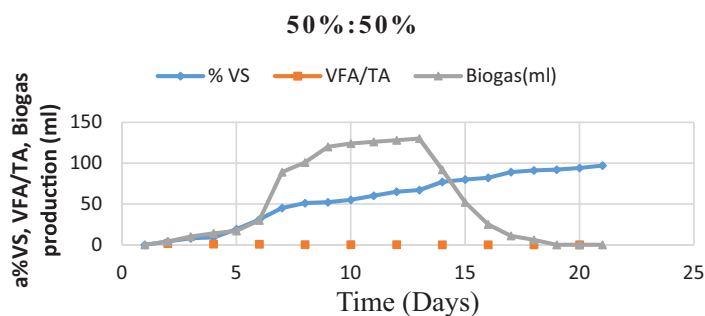


**Figure 5.** Anaerobic co-digestion of palm nut paste waste and anaerobic-digested rumen waste in the ratio 75%:25%. TA: total alkalinity; VFA: volatile fatty acids; VS: volatile solids.

### Semi-continuous AD

Following the batch scale anaerobic co-digestion, the ratios 90%:10%, 75%:25% and 50%:50% were *semi-continuously* fed at maximum biogas production. Feeding was done daily at a constant removal and replacement rate of 5% digester volume. The results are shown in Figure 7. For the 90%:10% mixture, daily feeding started at a stable range of VFA/TA of 0.32, VS reduction of 60% and 180.1 mL biogas production. Between the first and third days, biogas production dropped from 180.1 mL to 171.3 mL. The drop might be due to bacteria inability to adjust to the new feed introduced as a result of organic overload (Mshandete et al., 2004). The process subsequently stabilized maintaining constant biogas production of  $182.0 \pm 1$  mL reaching a maximum of 184.0 mL on the fourteenth day.

In the case of the 75%:25%, daily feeding started at VFA/TA ratio of 0.32, VS reduction of 55% and biogas production of 198.4 mL. The biogas production dipped to 187.5 mL on the second day of feeding and later increased from the 187.5 mL to 196.5 mL on the sixth day. Between the seventh and fourteenth days, biogas production further increased to  $198.8 \pm 0.5$  mL and stabilized. In contrast, biogas production in the 50%:50% ratio steadily increased from 130.0 mL from the first day of maximum biogas production to 156.0 mL on the fourteenth day. The digester maintained a VFA/TA ratio of 0.33 and VS reduction of 67%. Although the 50%:50% produced less biogas compared with the 90%:10% and 75%:25%, it maintained a constant biogas production rate from the seventh day to the fourteenth day at  $158 \pm 1$  mL. This might be due to effective microbial consortia belonging to the

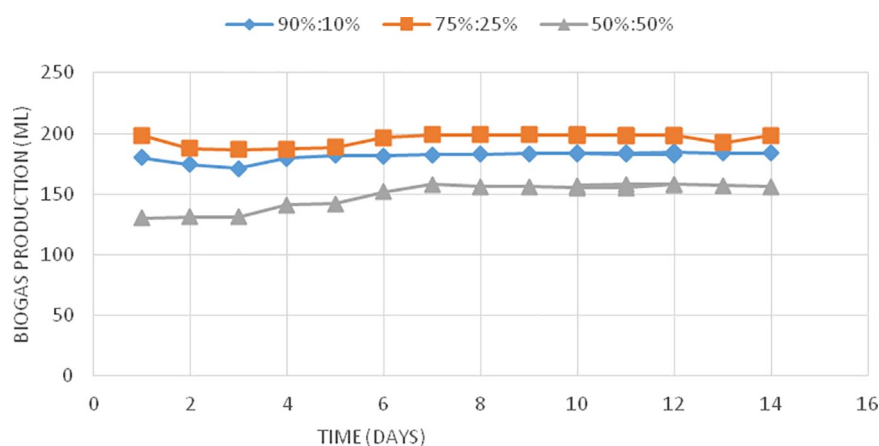


**Figure 6.** Anaerobic co-digestion of palm nut paste waste and anaerobic-digested rumen waste in the ratio 50%:50%. TA: total alkalinity; VFA: volatile fatty acids; VS: volatile solids.

**Table 2.** Tukey–Kramer method for comparison of biogas production between substrate ratios.

Comparison (%)	Absolute difference	Critical range	Results
100:0 vs. 90:10	28.118	78.003	Not significantly different
100:0 vs. 75:25	40.462	78.003	Not significantly different
100:0 vs. 50:50	24.586	78.003	Not significantly different
90:10 vs. 75:25	12.344	78.003	Not significantly different
90:10 vs. 50:50	3.532	78.003	Not significantly different
50:50 vs. 75:25	15.876	78.003	Not significantly different

If the absolute difference > the critical range, there is significant difference between the means.

**Figure 7.** Semi-continuous anaerobic digestion of different ratios of palm nut paste waste to anaerobic-digested rumen waste.**Table 3.** Tukey–Kramer method of comparing biogas production for different ratios under semi-continuous anaerobic digestion.

Comparison	Absolute difference	Critical range	Results
90:10 vs 75:25	13.282	7.903	Significant difference
90:10 vs 50:50	33.671	7.903	Significant difference
75:25 vs 50:50	46.953	7.903	Significant difference

Absolute difference > critical range indicates significant difference.  
 Absolute difference < critical range indicates no significant difference.

archaea domain that acted as perfect inoculum to maintain a balance for effective continuation of the process (Ma et al., 2019). However, the study found significant differences between the treatment ratios under semi-continuous conditions ( $p < 0.05$ , Table 3). The reason for the differences is that the co-substrate to bacteria (c-s/b) ratio during maximum biogas production varied for each of the treatment ratios and hence the synergistic action between the PNPW to c-s/b differs for each digester. This presupposes that the microbial population in the medium differs for any biochemical activity.

### Characteristics of the digestate

The residual solid after completion of the digestion process was analyzed. In terms of the batch scale reactor, the highest VS

removal of 97% occurred in the 90%:10% and 50%:50% ratios whereas the digester treating the 75%:25% recorded VS reduction of 96% (Figures 4 to 6). These reductions occurred on the twenty-first day. The characteristics of the digestate for the co-digestion ratios are presented in Table 4. In practice, the content of ammonium in the digestate is directly related to the total nitrogen content in the substrate (Drosg et al., 2015). In the case of co-digestion of PNPW and ADRW,  $\text{NH}_4\text{-N}$  ( $\text{mg L}^{-1}$ ) increased in the order of 50%:50% > 75%:25% > 90%:10% in the effluent with respect to the influent. The highest increase in  $\text{NH}_4\text{-N}$  in the 50%:50% ratio might be due to the highest proportion of rumen waste compared with the 75%:25% and 90%:10%. pH increased in the effluent with respect to the influent in the order 50%:50% (8.5) > 90%:10% (8.2) > 75%:25% (8.1). These pH values were slightly higher than established values of fresh digestate of 7.5–8.0 (Al-Seadi and Lakehurst, 2012; Vögeli et al., 2014). The higher pH values recorded in this study might be attributed to the characteristics of the co-digested substrate. That is, the ADRW has high nitrogen content compared with PNPW and as such has the potential of elevating the pH value in the effluent.

Moreover, the content of TS (known as dry matter) decreases during AD. There are reports suggesting that effluent from anaerobic origin contains 50–80% less TS compared with the influent substrate (Holm-Nielsen et al., 1997). This assertion nonetheless depends on the percentage TS in the influent. That is, 3–15% TS for wet state AD and 30% TS for dry state AD. It also depends on the availability of easily digestible organic matter. In this study,

**Table 4.** Digestate characteristics of co-digestion ratios.

Co-digestion ratios	pH			NH <sub>4</sub> -N (mg L <sup>-1</sup> )			Total solids (%)		
	In	Out	% increase	In	Out	% increase	In	Out	% decrease
90%:10%	7.2	8.2	13.89	2578.9	2634.3	21.48	16.1	4.1	74.5
75%:25%	7.9	8.1	25.30	3045.7	3112.7	22.99	17.4	5.3	69.5
50%:50%	8.1	8.5	4.94	4035.5	4267.9	57.59	19.1	9.1	53.3

the highest decrease in effluent percentage TS of 74.5% occurred in the ratio 90%:10%. This might be due to lowest and easy bio-degradable TS in the influent compared with 75%:25% and 50%:50% at 69.5% and 53.3%, respectively. The high TS in the influent of the 75%:25% and 50%:50% could be due to the contribution of organic matter of lignocellulose origin from the rumen waste.

## Conclusion

The AD of solely rumen waste and PNPW were conducted under thermophilic conditions. The study sought to objectively assess the impact of VFA/TA on digester stability and biogas production, as a means for managing biowaste containing crude fat. In the light of this, biogas production in relation to influencing indicators such as VFA/TA and VS reduction were strictly monitored. The study can conclude that the rumen waste responded faster to biogas production from the third day at VFA/TA of 0.32 and VS reduction of 61% compared with PNPW that commenced biogas production on the seventh day at VFA/TA of 0.9 and VS reduction of 88%. Although biogas production started in earnest with the rumen waste, the higher production occurred during AD of the PNPW at 104.0 mL compared with 30 mL for the rumen waste, possibly attributable to the crude fat layer of the PNPW. During batch studies of co-digestion of PNPW and ADRW at varying ratios of 75%:25%, 90%:10% and 50%:50%, VS reduction in all cases at which the digesters maintained stability and produced maximum biogas occurred between 52% and 67% and VFA/TA between 0.31 and 0.35. The response to biogas production nonetheless was rapid at the 50%:50% ratio on the second day. This was followed by 75%:25% on the third day and 90%:10% on the fourth day largely due to the difference in the proportions of the ADRW. Peak biogas production was highest in the 75%:25% followed by 90%:10% and 50%:50% at VFA/TA of 0.31–0.32. In terms of semi-continuous AD, biogas production dropped between the first and third days in the case of the 90%:10% and 75%:25% ratios due to initial organic overload before maintaining stability at VFA/TA of 0.32 and VS reduction of 60% and 55% respectively at the peak biogas production. Conversely, the 50%:50% ratio maintained a stable regime at VFA/TA of 0.33 uninterrupted from the seventh to fourteenth days. From day 21, biogas production in the 50%:50% ratio recorded 0.00 mL indicating void effluent residual methane. This finding suggests that a 50%:50% ratio might be a suitable strategy for managing fat-based waste

under thermophilic batch conditions within 21 days' retention time and VFA/TA of 0.32–0.9 whereas the 75%:25% is suitable for producing high volume biogas.

## Potential for further research

Although this work assessed the volume of biogas produced, it was limited in monitoring biogas composition. It therefore does not provide enough information to state whether the methane in the biogas produced could burn. A study on the biogas composition of this substrate is therefore imperative. Also, the 50%:50% ratio should be operationalized on the field to validate the practicality or otherwise of its performance.

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