Original Research Paper

Utilization of Rumen Content by Indigenous Microorganisms in a Modified Anaerobic Digester

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Abstract: The current work was designed to assess the effect of modification of model digester on the kinetics that affects efficiency in biogas generation using bovine rumen content as the feedstock. A biogas plant consisting of conventional and modified fixed-dome digesters (each with 2 m^3 capacity) was established; and bovine rumen content was used as feedstock. Standard methods were used to determine the kinetic (physicochemical and microbiological) parameters. Identification of fungal and bacterial species involved in the process was carried out by genomic study of the 18S rRNA and 16S rRNA regions, respectively; amplified using universal forward and backward primers for fungi (NS1/NS4), bacteria ((515F/926R) and archaea (Met86F/Met140R); submitted to GenBank and analysed using Blast Programme at National Centre for Biotechnology Information website. A mass balance approach was used to estimate the theoretical gas yield from the total solid/volatile sold lost. It was found that the temperature in both modified and conventional digesters was uniform throughout the hydraulic retention time, between 32 to 34.5°C, indicating a mesophilic range. The pH level was found to be lower in the modified digester compared to the conventional digester, indicating higher accumulation of organic acids. The mean total solid was found to drop from 12.49±0.53 to 3.82±0.21 in the modified digester after digestion, while this was from 13.30±0.4 to 6.69±0.16 in the conventional digester. The mean volatile solid drops from 66.67±1.62 to 36.13±0.27 in the modified digester after digestion, while it was from 69.94±1.54 to 54.23±1.33 in the conventional digester. The theoretical biogas yield was higher in the modified digester (87 mg/l) compared to the conventional one (65 mg/l). The microbial counts were observed to be affected by the kinetic parameters in both the digesters. The organisms identified were: *Lactobacillus acidophilus*, *Bacteroides nordii*, *Clostridium perfringens*, *Clostridium acetobutylicum*, *Proteus vulgaris*, *Methanosarcina sicilia*, *Methanosarcina mazei*, *Methanobrevibacter ruminantium*, *Fusarium solani*, *Fusarium graminearum*, *Aspergillus niger* and *Penicillium* specie. Conclusively, the modified digester, under a manual stirring at 10 round/min, 3 times a day at an interval of six hours, has appreciably generated higher theoretical biogas yield after 8 weeks hydraulic retention time. The agitation of the slurry using the improvised stirrer in the modified digester has significantly facilitated the utilization of the bovine rumen content by the indigenous microorganisms. Modification of digester to enhance mixing of the feedstock should therefore be encouraged in household anaerobic digesters.

Keywords: Anaerobic Digestion, Rumen Content, Indigenous Microorganisms, Modified Digester

Introduction

Over the past few decades, there has been a substantial increase in research and development in the area of biofuels. Many researchers around the world have dealt with environmental, economic, policy and technical subjects aspects relating to these studies (Aurélio, 2011). Biofuels are the potential and sustainable alternative sources of energy. Despite having tremendous efforts toward development of economically competitive biofuels, substantial move is yet to be made and the outcomes are still far from practical implementation globally (Chandel, 2019). One of the key issues in this respect is the conversion of the biodegradable feedstock to the desired biofuel. Anaerobic Digestion (AD) is one of the promising technologies for recovering energy from the organic fraction of municipal solid waste and other biodegradable waste. It is a series of biological processes that use a diversed population of microorganisms to break down organic materials into biogas, primarily methane and a combination of solid and liquid effluents, the digestate. It occurs in the absence of free oxygen (Sebola *et al.*, 2013). Such a conversion is driven by several factors including type and nature of feedstock, technology applied physicochemical parameters, microorganisms involved, etc. (Miertus, 2007).

Of the various factors that determine biogas yield, feedstock selection remains one of the most crucial steps in anaerobic digestion processes; digestibility and nutritional composition are considered in this respect. The use of bovine rumen content from slaughterhouse has been reported to contain a good level of both macro and micronutrients (trace elements). However, digestibility and nutritional composition might be different among bovines influenced by the (1) feeding regimen, (2) length of the holding time between feeding and slaughter, (3) season, (4) feed resource diversity and (5) selection of pastures by various animals in various areas (Cherdthong *et al.*, 2014).

The performance of anaerobic digesters is also affected by the retention time of substrate in the digester and the degree of contact between incoming substrate and a viable microbial population. These parameters are primarily a function of the hydraulic regime (mixing) in the reactors. The importance of mixing in achieving efficient substrate conversion has been noted by many researchers, although the optimum mixing pattern is a subject of much debate. Mixing of the substrate in the digester helps to distribute organisms uniformly throughout the substrate (Karim *et al.*, 2003).

Furthermore, agitation aids in particle size reduction as digestion progresses and in removal of gas from the mixture (Karim *et al.*, 2005). The two very important aspects of digester mixing are the intensity and duration of mixing. Most of the literature on anaerobic digestion emphasizes the importance of adequate mixing to improve the distribution of substrates, enzymes and microorganisms

throughout the digester (Parkin and Owen, 1986; Chapman, 1989; Lema *et al.*, 1991). However, the information available in the literature about the effect of the intensity and duration of mixing on the performance of anaerobic digesters are contradictory. Several studies indicated that a lack of sufficient mixing in low solids digesters dealing with municipal waste resulted in a floating layer of solids (Diaz and Trezek, 1977; James *et al.*, 1980; Stenstrom *et al.*, 1983). Chen *et al.* (1990) observed higher methane yield in the case of a 4.5 m³ digester under unmixed conditions than continuously mixed conditions. In another study, (Ben-Hasson *et al.*, 1985) observed 75% lower methane production rate from dairy cattle manure under continuously mixed conditions than unmixed conditions. On the contrary, (Ho and Tan, 1985) reported greater gas production for a continuously mixed digester than for an unmixed digester fed with palm oil mill effluents and (Hashimoto, 1983) found higher biogas production from beef cattle wastes under continuously mixed conditions than under intermittent mixing conditions. At the same time, (Dague *et al.*, 1970; Mills, 1979; Smith *et al.*, 1979) recommended intermittent mixing of anaerobic digesters over continuous mixing. It was with this view that the present study was designed to find out if modifying the household conventional anaerobic digester would improve biogas yield.

Materials and Methods

Establishment of Biogas Plant

The construction manual for modified (Georgia Gwinnett College [GGC] Model for Pakistan, 2009) was used in the present study to guide the construction of 2 m³ fixed-dome anaerobic digesters (Fig. 1). After construction, one digester was modified by improvising it with a mechanical stirrer, while the other was not; this was meant to compare the kinetics of biogas production. The construction was based on the locally available materials.

Feedstock Selection, Collection and Feeding of Digesters

Bovine rumen content was selected in this research to be used as feedstock; this was in consideration of its abundance at the abattoirs around Kano. A large quantity of fresh bovine rumen content was collected in large polypropylene bags (Nigerian Bag Manufacturing – BAGCO) from Kano main abattoir, along IBB Road, Dala Local Government Area, Kano State. The holding capacity of each digester was about 2000 litres and based on this, each digester was fed with fresh rumen content to occupy two-third of the total capacity; and clean water was introduced to fill up the remaining space to make a slurry with total solid of 11.8 and 12.3% in the modified and conventional digesters, respectively. A manual stirring at 10 round/min, 3 times a day at an interval of six hours was ensured.
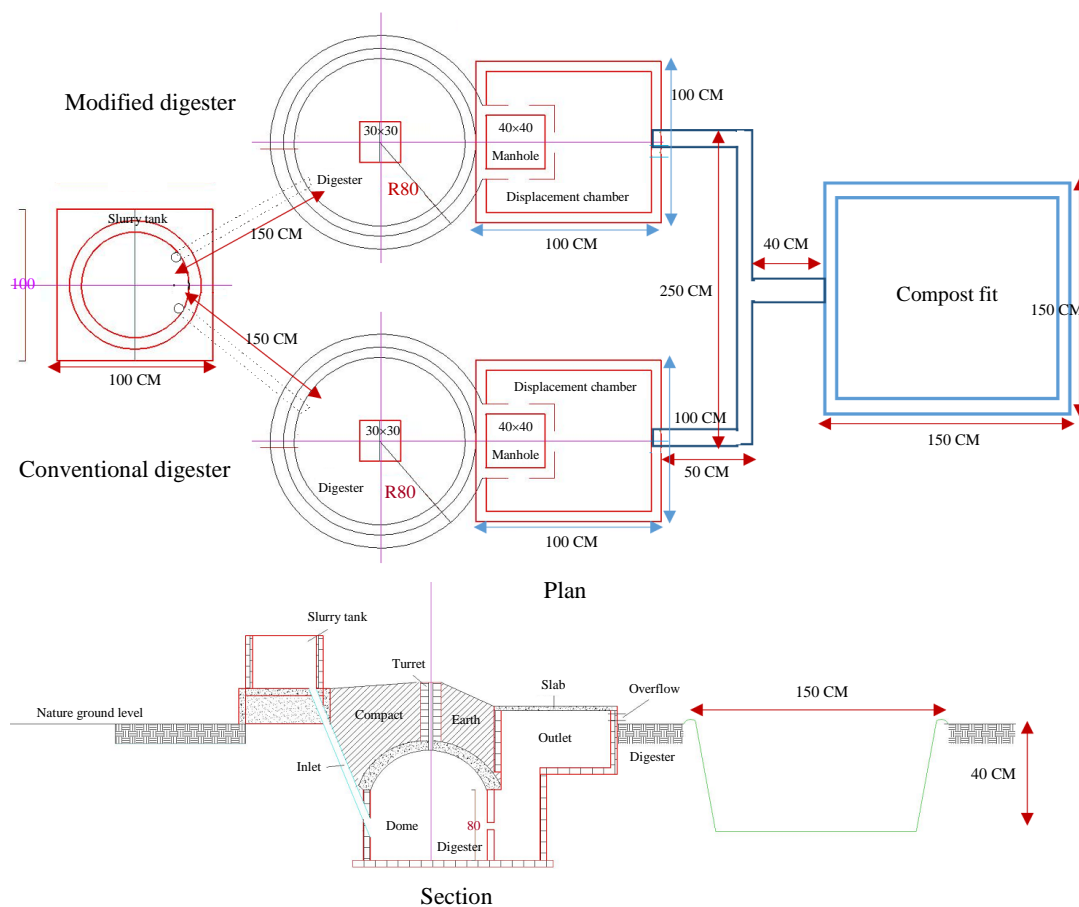
Fig. 1: Architectural plan of the biogas plant

Analytical Procedures

Physicochemical Analyses

Analyses started with the characterization of the rumen content, which was carried out immediately after collection. With exception of pH and temperature, all the analyses were carried out on weekly basis for eight (8) weeks; pH and temperature of the feedstock under digestion were recorded at an interval of 6 h. Total organic carbon was determined according to (Walkley and Black, 1934) as described by (Gelman *et al.*, 2011); Nitrogen was carried out using (Kjeldahl, 1883) method as described by (Flindt and Lillebø, 2005); total solid and volatile solid were determined according to (APHA, 1998); moisture content was calculated by subtracting %TS from 100 (Eckelman, 1996); ash content was determined using the ignition method in accordance with (APHA, 1998); a digital pH meter (JENWAY 3510 pH Meter) was used to determine the pH value; temperature was determined using thermometer (PM-K, PAMAENS, Shanghai, China). A mass balance approach was used to estimate the theoretical gas yield from the TS/VS lost. Initial TS/VS of the feedstock was determined before feeding into the

digesters and then on weekly basis for 8 weeks using the methods described above. The difference between the initial (mass of TS/VS fed) and the subsequent TS/VS (residual TS/VS in the digested feedstock) gave the quantity of TS/VS lost. It was presented as liters of biogas produced per gram TS/VS lost. This gave an index of process efficiency (Deressa *et al.*, 2015). The arithmetic was done by using the equation:

$$\frac{TS}{VS} Lost = Intial \frac{TS}{VS} - Subsequent \frac{TS}{VS}$$

Microbiological Analyses

Microbial Count

The anaerobic mesophilic bacterial count was carried out according to (Association of Official Analytical Chemists, 1990); nutrient agar (for bacteria) and Potato Dextrose Agar (PDA) media were used for this purpose. Following anaerobic incubation, distinct pure colonies obtained on the plates were counted with colony counter (Shine Scientific Instruments) and the microbial count was calculated as follows:

$$Number\ of\ Cell = \frac{\Sigma C}{\left(n_1 + 0.1 \times n_2\right)\left(V \times d\right)}$$

Where:

ΣC = Sum of colonies counted on all the dishes retained from two successive dilutions

V = The volume of inoculum applied to each dish

d = The dilution factor corresponding to the first counted dilution

n_1 = The number of dishes counted for the first counted dilution

n_2 = The number of dishes counted for the 1/10 dilution of the second counted dilution

The results of the number of the microbial counts from the modified digester were compared with that of conventional one. A microbial count was carried out on weekly basis for 8 weeks.

Identification of the Microbes

Isolation and identification of fungal and bacterial as well as archaeal species was carried out using molecular approach. The extraction of the genomic DNA was carried out using Nucleo-pore gDNA Fungal/Bacterial Mini Kit (Genetix Biotech Asia Manual, 2018) according to the manufacturer's instruction. After extraction, the purified DNA was subjected to PCR for amplification (Genetix Biotech Asia Manual, 2018).

PCR reagents from Genetix Biotech Asia Prv. Ltd. were used to amplify V3-V4 regions of the 16S rRNA with universal bacterial primers (515F/926R) and universal archaeal primers (Met86F/Met1340R); and 18S rRNA with universal fungal primers (NS1/NS4).

The amplified DNA fragments were separated on 1.5% agoarose gel. The SureTrap PCR Clean-up Gel Extraction kit (Genetix Biotech Asia Manual, 2018) was used to elute the amplicons from the gel. The purified PCR products were sequenced using both the forward and reverse primers (above). The sequencing was carried out by Sanger sequencing (Dideoxy Chain-termination) method with Big-Dye terminator kit using ABI Sequencers (Thermo Fisher Scientific, USA). The 16S rRNA (for bacteria and archaea) and 18S rRNA (for fungi) sequences were submitted to GenBank and analyzed using the BLAST program in GenBank at National Center for Biotechnology Information website (http://www.ncbi.nlm.gov/BLAST).

Results and Discussion

Results

This section presents the results of the research work. Figure 2 shows the biogas plant developed, which is comprised of two digesters (modified and conventional one), each with a displacement chamber, from which the digested slurry drains into a single compost pit; the two digesters also share a single slurry tank.

Results of Physicochemical Analyses

Table 1 shows some characteristics of rumen content used in the present research work. The rumen content was found to contain a C:N ratio of 34.07, suggesting its suitability in biogas production; this is in consideration of the parameters determined, which were within the recommended range and therefore supporting the microbial growth and metabolism.

Fig. 2: The layout of the biogas plant
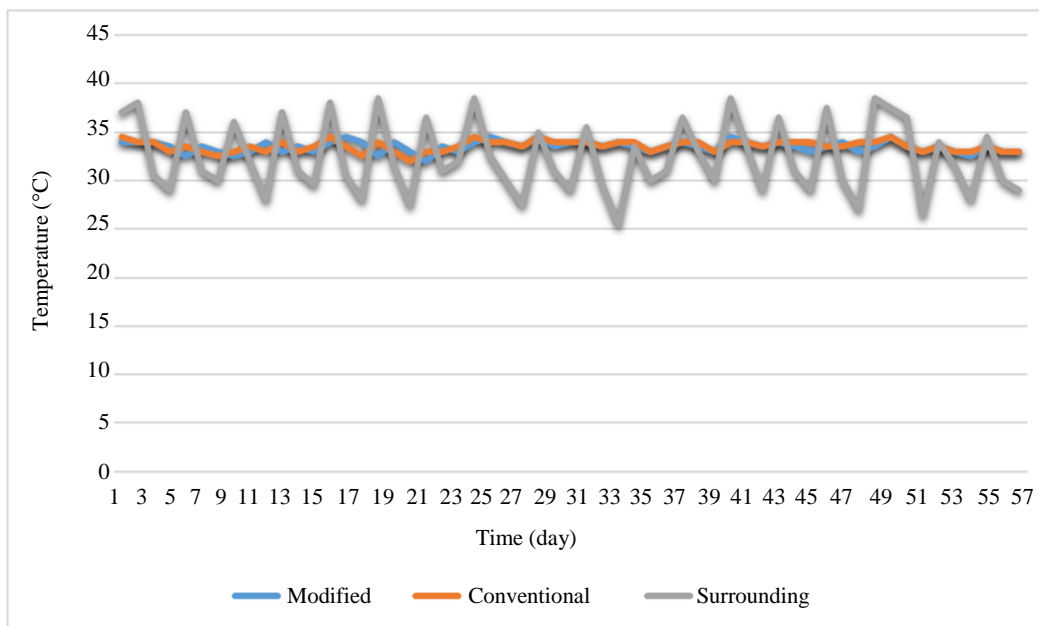
Fig. 3: Temperature monitoring in both modified and conventional digesters

Table 1: Characterization of Feedstock before Feeding

S/N	Parameters	Values	Recommended Value
1.	C:N	34.07	25-35 (Wellinger *et al*., 2013)
2.	Ash content	27.53%	<10% (Wellinger *et al*., 2013)
3.	Total solid	19.23%	8-16% (Marchaim, 1992)
4.	pH	6.67	6-7 (Wellinger *et al*., 2013)
5.	Temperature	27.5°C	20-45°C (Marchaim, 1992)

Figure 3 shows the mean temperatures of the modified and conventional digesters as well as the surrounding temperature. It was observed that, the fluctuation of temperature was highly minimized in both the digesters, ranging from 32 to 34.5°C, compared to the surrounding temperature, which shows remarkable fluctuation of temperature throughout the hydraulic retention time, ranging from 25 to 38.5°C.

Figure 4 presents the result of volatile acids production, which was qualitatively determined based on the detection of changes in pH of the slurry over a period covering the retention time. It was observed that there was a gradual drop in pH in the conventional digester throughout the retention time, which dropped from 6.67 on the feeding day to 5.57 after the 8th week. However, rapid drop in pH was observed in the modified digester from 6.67 on the feeding day to 4.95 on the fourth (4th) week and there was slight raise in pH to around 5.27 after 8 weeks of fermentation. It was noticed that the rapid drop in pH level in the modified digester was as a result of daily agitation of the feedstock.

Table 2 shows the result of the weekly determination of total solid and volatile solid in both the modified and conventional digesters. The Table reveals gradual

decrease in both the TS and VS in the digesters. The change in VS in the modified digester was from 66.67 to 36.13%, while in the conventional digester, VS drops from 69.94 to 54.23% after digestion. The TS in the modified digester was observed to drop from 12.49 to 3.82%, while in the conventional digester, TS drops from 13.30 to 6.96% after 8 weeks of digestion.

From Table 3, the change in TS/VS in the modified digester was slightly higher than that of the conventional digester. The initial TS/VS of the slurry was 0.196 and the final TS/VS in the modified digester was 0.111 implying a difference of 0.085, which was higher compared with 0.061 obtained in the conventional digester.

Figure 5 presents the graph of biogas yield at an interval of a week for 8 weeks. It can be seen that the graphs follow similar pattern, but with highest theoretical biogas yield (in mg/l) in the modified digester.

Results of Microbial Analyses

Table 4 shows a comparative analysis of the anaerobic mesophilic bacterial count between the modified and conventional digesters. In the first week, the highest mesophilic count was observed in the

modified digester with a count of 2.6×10^4, with corresponding count of 2.2×10^4 in the conventional digester. Decrease in counts was observed in the modified digester from the second week (2.4×10^4) to 4th week (1.6×10^4) and then started increasing in the 5th week (1.7×10^4) to 2.4×10^4 in the 7th week.

However, an increase in bacterial count (2.6×10^4) was recorded in the conventional digester in the second week, but with sharp drop (1.7×10^4) in the 3rd week; followed by gradual increase in the subsequent weeks, until the 8th week when decrease in count from 2.2×10^4 to 2.1×10^4 was observed.
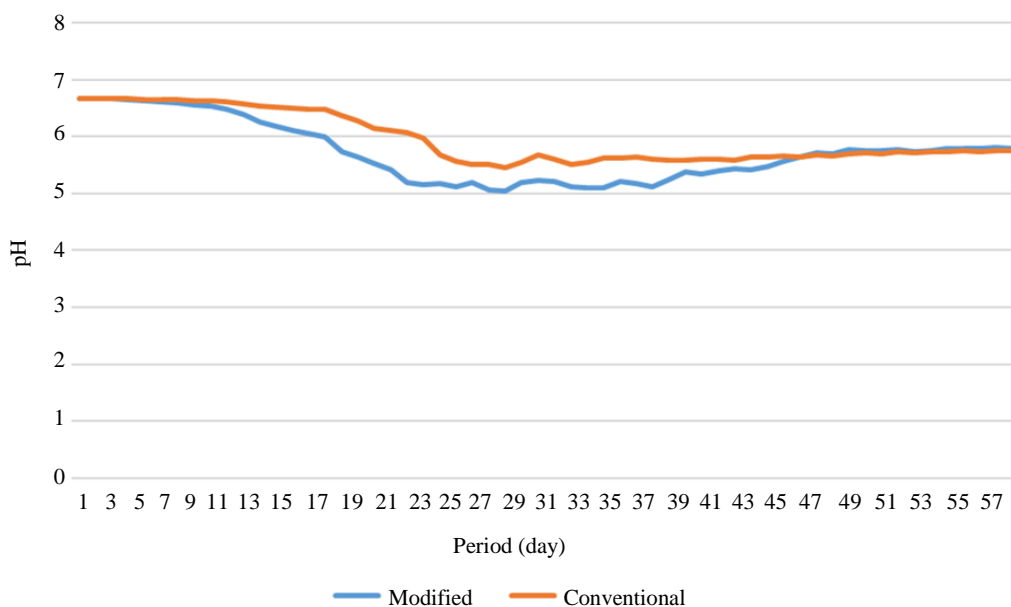
Fig. 4: Changes in pH in the modified and conventional digesters
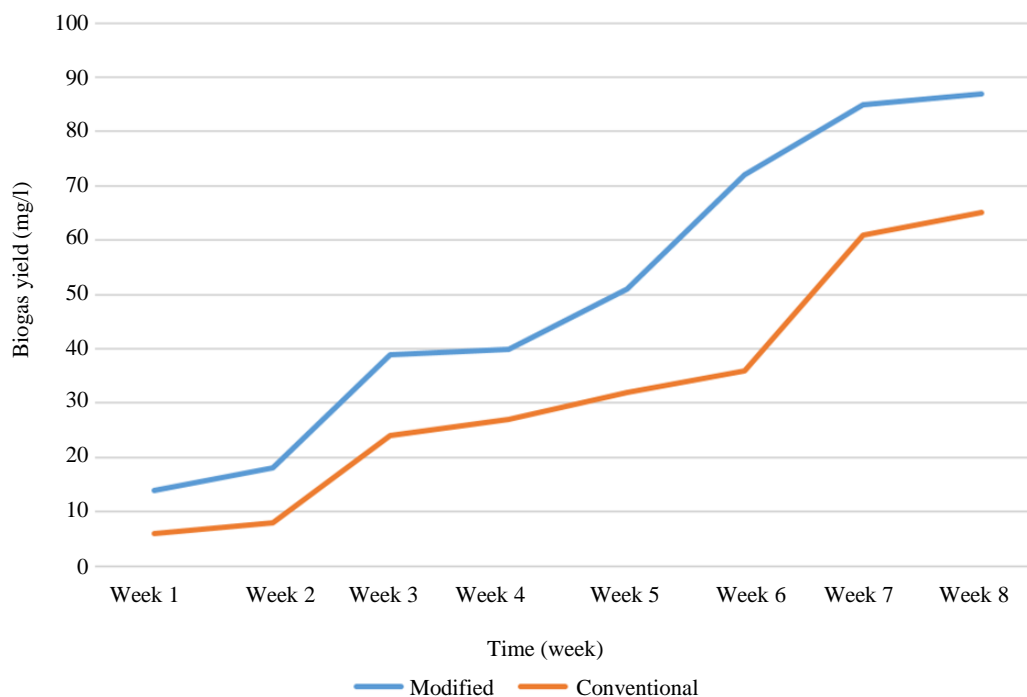
Fig. 5: Biogas yield per time in the modified and conventional digesters

Table 2: Mean total and volatile solids in both the modified and conventional digesters

Period (Week)	Modified digester			Conventional digester		
	Mean TS (%)	Mean VS (%)	TS/VS	Mean TS (%)	Mean VS (%)	TS/VS
Week 1	12.49±0.53	66.67±1.62	0.187	13.30±0.40	69.94±1.54	0.190
Week 2	11.56±0.53	64.93±1.00	0.178	12.26±0.23	65.24±0.81	0.188
Week 3	9.87±0.45	63.04±1.00	0.157	11.01±0.49	64.11±1.60	0.172
Week 4	9.84±0.37	63.17±1.26	0.156	10.73±0.47	63.46±1.01	0.169
Week 5	8.98±0.28	61.87±1.70	0.145	10.14±0.16	61.72±2.00	0.164
Week 6	6.85±0.66	55.33±4.17	0.124	9.39±0.38	58.67±1.36	0.160
Week 7	4.19±0.54	37.67±6.13	0.111	7.49±0.23	55.32±1.91	0.135
Week 8	3.82±0.21	36.13±0.27	0.109	6.96±0.16	54.23±1.33	0.131

Table 3: TS/VS lost in modified and conventional digesters (Initial TS/VS = 0.196)

Interval (Week)	Modified digester			Conventional digester		
	TS/VS	TS/VS Lost	TBY (mg/l)	TS/VS	TS/VS Lost	TBY (mg/l)
Week 1	0.187	0.014	14	0.190	0.006	6
Week 2	0.178	0.018	18	0.188	0.008	8
Week 3	0.157	0.039	39	0.172	0.024	24
Week 4	0.156	0.040	40	0.169	0.027	27
Week 5	0.145	0.051	51	0.164	0.032	32
Week 6	0.124	0.072	72	0.160	0.036	36
Week 7	0.111	0.085	85	0.135	0.061	61
Week 8	0.109	0.087	87	0.131	0.065	65

Table 4: Anaerobic Mesophilic Bacterial Counts (MAMBC) in modified and conventional digesters

Period (Week)	MAMBC in modified digester (cfu/mg)	MAMBC in conventional digester (cfu/mg)
Week 1	2.6×10^4	2.2×10^4
Week 2	2.4×10^4	2.6×10^4
Week 3	2.0×10^4	1.7×10^4
Week 4	1.6×10^4	1.8×10^4
Week 5	1.7×10^4	2.2×10^4
Week 6	1.9×10^4	2.2×10^4
Week 7	2.4×10^4	2.1×10^4
Week 8	2.3×10^4	2.1×10^4

Table 5: Anaerobic fungal counts in modified and conventional digesters

Period (Week)	Modified digester (cfu/mg)	Conventional digester (cfu/mg)
Week 1	4.0×10^2	4.0×10^2
Week 2	4.0×10^2	4.0×10^2
Week 3	4.0×10^2	4.0×10^2
Week 4	3.0×10^2	3.0×10^2
Week 5	3.0×10^2	3.0×10^2
Week 6	3.0×10^2	3.0×10^2
Week 7	3.0×10^2	4.0×10^2
Week 8	4.0×10^2	4.0×10^2

Table 6: Bacteria Isolated and Identified in the Feedstock before and after digestion

Microbial category	Accession Number	Organism
Bacteria	MK350333	*Lactobacillus acidophilus*
	MK350334	*Bacteroides nordii*
	MK350335	*Clostridium perfringens*
	MK350336	*Proteus vulgaris*
	MK355515	*Clostridium acetobutylicum*
Methanogenic Archaea	MK350337	*Methanosarcina sicilia*
	MK350338	*Methanosarcina mazei*
	MK355516	*Methanobrevibacter ruminantium*
Fungi	MK368534	*Fusarium solani*
	MK368535	*Fusarium graminearum*
	MK368536	*Aspergillus niger*
	MK368537	*Penicillium* specie

Table 5 presents the result of fungal count found in both the modified and conventional digesters carried out on weekly basis for a period of 8 weeks. The fungal counts were uniform in both the digesters throughout the span of the research and counts of 4.0×10^2 cfu/mg were obtained in both up to the third week; then dropped to 3.0×10^2 in the fourth to sixth week.

Table 6 presents the result of the identification of microorganisms in the study.

Discussion

The present study was carried out with the desire to test the hypothesis that the modification of some features of the conventional household fixed-dome anaerobic digester through the application of simple engineering design could improve its quality and enhance the microbial utilization of rumen content in biogas production. The study focuses on addressing the kinetics effects of manually stirring the mixture in the modified biodigester.

Physicochemical parameters observed in the present study were temperature, pH, TS and VS; these are the factors that affect microbial biodegradation of organic waste in anaerobic digestion system (Wellinger *et al.*, 2013) and change with change in mixing rate; this have overall effect on microbial growth (counts) and metabolism. The temperatures in both the modified and conventional digesters were relatively uniform and this relative uniformity had to do with the bigger size of the digester, which resists fluctuation due to changes in the surrounding temperature. The work of (Asikong *et al.*, 2016) have supported this view.

A decrease in pH was observed particularly during the 3rd to 4th week of digestion, which is attributed to CO_2 and high volatile fatty acids production during the fermentation process. The decrease in the pH level in the modified digester as compared to conventional digester was also attributed to daily agitation of the slurry using the improvised stirrer. These changes are attributed to the effect of modification of digester in the presence study. This finding agrees with the work of (Beevi *et al.*, 2013).

Microorganisms are the key producers of biogas and their number determines the favorability of condition in the anaerobic digester as well as the volume of biogas produced. Relatively low bacterial and fungal counts observed in the first week, particularly in the conventional digester, might be attributed to factors such as digestibility of the feedstock, high solid content and uneven distribution of microorganisms and nutrients in the digester. There was increase in bacterial count in the conventional digester in the second week when the bacteria started adapting to the new environment within the digester. The sudden decrease in bacterial count in the third week had to do with accumulation of organic acids, CO_2 as well as toxic bacterial metabolites; the count

stabilizes in the subsequent weeks. The count was relatively higher in the modified digester during the first week, but drops during the second week presumably due to rapid accumulation of organic acids enhanced by daily agitation, which resulted in the drop of pH level, thus inhibiting bacterial growth. Anaerobic fungal counts were uniform throughout the hydraulic retention time, suggesting high acidic resistance of the fungi. It was observed that, agitation enhances fermentation with resultant increased acids production. This increases the quantity of acids that undergo acetogenesis, with subsequent increase in the quantity of acetate that undergo acetoclastic methanogenesis and eventually results in high biogas yield.

With regard to yield in biogas production, reduction in volatile solid concentration determines the theoretical biogas yield. In this study, the VS in the modified digester reduced to 36.13% from the initial value of 66.67% with corresponding yield of 0.085 litre per gram (85 ml/g), which was higher when compared with conventional digester with VS reduction from 69.94 to 54.23% and a biogas yield of 65 ml/g. According to the statistical analysis (a paired t-test) conducted, the mean biogas yield (mean = 18.375; Standard Deviation = 9.054; N = 8) was found to be significantly greater than zero; t-Stat = 5.74; two-tail p = 0.001, providing evidence that the modification is effective in producing higher theoretical biogas. A 95% confidence interval about mean biogas yield was (10.805, 25.945). Moreover, the p-value (two-tail) was found to be less than the alpha level chosen (i.e., 0.05); and that, the t-value (5.74) was greater than the t-critical (two-tail) value (2.365). This led to the acceptance of the hypothesis that modification of the digester improves the biogas yield in anaerobic digestion of rumen content. The higher theoretical biogas yield in the modified digester was attributed to daily agitation of the slurry. This agrees with the work of (Klocke *et al.*, 2007), in which stirring was found to improved biogas production.

Conclusion

The microbial utilization of rumen content was found to be favoured by the effects of manual stirring of the mixture in a biodigester. The daily agitation in the modified digester facilitates the increase in the rate of fermentation in the modified digester; the rate of anaerobic digestion determined by the reduction in total solid and volatile solid, was also higher. Moreover, subsequent theoretical biogas production was higher in the modified digester (87 mg/l) compared to 65 mg/l produced in the conventional digester. The microorganisms identified were: *Lactobacillus acidophilus, Bacteroides nordii, Clostridium perfringens, Clostridium acetobutylicum, Proteus vulgaris, Methanosarcina sicilia, Methanosarcina mazei, Methanobrevibacter ruminantium, Fusarium solani,*

Fusarium graminearum, Aspergillus niger and *Penicillium* specie.

Optimization of biogas production in anaerobic digestion system should be geared towards the modification of digester with a view to improving the mixing rate through agitation at least once or twice a day to ensure uniform distribution of nutrients and microorganisms and also to break any scum that may prevent the release of gas from the slurry. Frequent stirring should be avoided to prevent accumulation of volatile acids which leads to inhibition of microbial growth and metabolism. Pretreatment approaches should be considered to improve microbial utilization of recalcitrant feedstocks such as rumen content and some plant materials in anaerobic digestion system.

Acknowledgment

Author's Contributions

All the authors in the present study have contributed a lot; as the research work is multidisciplinary in nature, so the authors; everyone has played his role diligently.

Ethics

This study is free of any ethical issue.

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