A LABORATORY-BASED STUDY OF BIOMETHANATION BY THE CO-DIGESTION OF MUNICIPAL SOLID WASTE WITH DOMESTIC FOOD WASTE AND WASTEWATER SLUDGE

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ABSTRACT

The increasing global demand for sustainable alternative sources of energy has driven the need for studies in renewable energy. Biogas may serve to satisfy energy demands as a viable substitute to fossil fuel, thereby reducing indiscriminate deforestation. The large volume of waste generated in the form of food and other solids in urban areas could serve as suitable organic substrates in the production of biogas thus, providing a cheap and eco-friendly method of managing wastes. In this study, municipal solid waste was co-digested with Food Waste (FW) and Municipal Solid Waste (MSW) at different ratios to optimize the production of biogas. The anaerobic digestion process was conducted at ambient (room) temperature for three weeks for a hydraulic retention time. The performances of the wastes were assessed based on the volume and quality of the biogas produced. The highest amount of biogas produced (765 cm^3) was from the co-digestion of food waste and MSW in the ratio of 1.5:1.5. Methanogenic archaea isolated from the biogas digestate belonged to the following genera: *Methanobacterium*, *Methanococcus*, *Methanosarcina*, and *Methanoplanus*. In this study, the biogas produced from the co-digestion of food waste and municipal solid waste was better in terms of quality and quantity compared to that produced from the co-digestion of MSW with sludge. Thus, the findings of this study suggest that food waste has great potential as a substrate during the anaerobic digestion of municipal solid waste.

Keywords: *sludge, food waste, municipal solid waste, methanogens, biogas*

INTRODUCTION

In Municipal Solid Waste (MSW), food waste makes up most of the putrescible organic material. Food waste decomposes in landfills to generate methane, a greenhouse gas with a global warming potential 25 times that of CO_2 over a 100-year time scale [1]. By 2012, around 1300 Mt of municipal solid wastes (MSW) were generated annually over the world [2]. Similarly, food waste generation would increase 44 percent globally by 2025, according to a report by Ariunbaatar et al. [3]. By 2025, the MSW generation rate is expected to climb to approximately 2000 Mt, with more than 40% of that being organic [2],[4]. Population expansion, higher urbanization rates, industrialisation, economic growth, changing eating habits and consumption patterns are all variables connected with this trend [5],[6]. The United States (US) produced 254 million tonnes of solid garbage in 2013, with 167 million of those ending up in landfills [7]. Many problems, including inadequate roads, poor financial management, antiquated technology and equipment, and a lack of information regarding garbage collection dates, hinder the transfer and transportation of MSW in developing nations [8],[9]. As a result, gar generators resort to dumping rubbish in unapproved locations [10],[11],[12]. Low-income countries have a waste collection percentage of roughly 41%, compared to 98 percent in high-income countries, according to Hoornweg and Bhada-Tata [13]. Rapid urbanization, particularly in slums and other unplanned locations with insufficient infrastructure to support waste collection services, exacerbates poor MSW management in developing countries. In nations like Ghana and Nigeria, only around 20% of solid waste is effectively managed, with the remaining 80% disposed of through crude dumping [14]. Inadequate MSW disposal can affect people's health if they are exposed to it or eat contaminated foods [15],[16]. Poor solid waste management techniques, for example, have been related to diseases like diarrhoea, dengue fever, and malaria [17].

Composting or AD are suitable treatment techniques for resource recovery operations in poor countries due to waste's high organic and MC content. [18]. There are six types of waste in the waste stream worldwide, with organic waste portions accounting for 46% [13]. MSW has a great potential for resource recovery due to the larger amount of organics in the worldwide waste stream. In comparison to wastes in underdeveloped countries, MSW in industrialized countries has a high calorific content. Differences in waste sorting programs are to blame for these discrepancies. In wealthier countries, sorting systems are frequently used, while in developing countries, less sorting is done, leaving a lot of inert elements in MSW [19],[20]. Organic waste, such as grass clippings and food waste, makes up around 44% of municipal solid waste deposited in landfills. In landfills, the organic percentage

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is a source of uncontrollable greenhouse gas (GHG) emissions [21]. Leachate is also formed because of the decomposition of wastes deposited in them. Pollution of groundwater, surface water, and soil [22], is one of the potential environmental effects of landfill leachate, which creates long-term liability for landfill owners.

As a result, there is a need to develop cost-effective and technically feasible options [23],[24]. Aerobic composting or anaerobic digestion are the two most prevalent methods for dealing with organic waste (AD). Composting, on the other hand, can be prohibitively expensive because of the low product value. Furthermore, during the composting process, there are large nitrogen losses due to ammonia volatilization and uncontrolled air emissions. In these conditions, waste collection for the recovery of resources such as biogas and soil conditioners via Anaerobic Digestion (AD) operations may be a viable option. Furthermore, the recovered products can be used as a source of additional energy and fertilizer, and they can be sold to help defray the expenses of handling MSW [25]. Anaerobic digestion's application in waste management has attracted a lot of interests. One reason is that biogas use provides an on-site energy offset, as this technique maximizes the recycling and recovery of waste components. This biogas generated during the anaerobic decomposition of organic waste is regarded as a long-term energy source [26]. In an aquatic environment, AD is a microbial process that transforms biomass to energy. Biomass sources with less than 40% dry matter content can be used in the AD process. The organic matter is digested by a microbial consortium in an oxygen-free environment throughout the AD process. Various microbes carry out four stages of metabolic reactions during AD: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The creation of methane (50–75%), carbon dioxide (19–34%), and a minor amount of biohydrogen (1%) is the primary result of the AD process [27]. Recent studies show that, as compared to incineration technology, there is a greater possibility of improving AD systems [28],[29]. Due to the significant energy recovery associated with the process and the low environmental impact, AD is typically the most cost-effective waste management technology [30].

The organic part of MSW, agricultural wastes, energy crops, sewage sludge, and food wastes, are all good candidates for AD [31]. Although anaerobic digestion of food waste has been employed in the past for biogas production, the excessive amounts of organic acids produced at high organic loading rates (OLRs) may substantially impede anaerobic bacteria [32]. Food waste has a lot of organic matter and nutrients; therefore, it can be used to make a variety of high-value goods [33]. Food waste has a low total solid (TS) content, a high soluble organic content is easily degradable, and contains a high quantity of energy per dry mass. With anaerobic digestion of high solid content food waste, excess ammonia and Volatile Fatty Acid (VFA) accumulation is more likely [34].

Despite the fact that AD for bioenergy generation is becoming a realistic option [35], single substrate digestion of organic waste can result in low biogas yield due to its low organic content and high nitrogen concentration, which can cause inhibition and process instability [36]. Co-digestion, which is an opportunity to create a more proper balance between carbon and nitrogen by using substrates high in nutrients (N) and organic content (C), thereby regulating pH and avoiding ammonia inhibition concerns, could be a potential solution to these challenges [37]. Once the benefit of adjusting the C/N ratio was realized, co-digestion of food waste with other wastes in a single digester became increasingly popular [38]. Recent research has shown that anaerobic co-digestion of food waste is a practical and cost-effective method of increasing energy recovery [39]. Food waste co-digestion with other wastes has also been proposed and investigated [40]. Therefore, this study focused on co-digestion of municipal solid waste with domestic food waste and wastewater sludge for optimized biogas production.

MATERIALS AND METHOD

Sampling Area

All the biodegradable waste materials used in this study were collected from within Samaru, Zaria located on the coordinates 11.0855°N, 7.7199°E. The food waste substrates were obtained from waste disposal sites in Ahmadu Bello University, Zaria (main campus), and some restaurants within the University, respectively. On the other hand, the municipal solid waste was collected from different dumpsites in Samaru village, Zaria while, the sludge was collected from the Water Treatment Plant located at Ahmadu Bello University, Zaria.

Sample Collection

A total amount of three kilograms (3 kg) of the municipal solid wastes (MSW) and Food Wastes (FW) were collected in clean polythene bags from the dumpsites respectively, while three litres (3 L) of the sludge was carefully collected in a clean plastic container using gloves and face mask as shown in Figure 1. The samples were then transported to the laboratory in the Department of Microbiology, Ahmadu Bello University Zaria, Nigeria for further analyses.

Fabrication of Anaerobic digesters and the Experimental Setup

A total of six (6) digesters each for the digestion of a substrate for biogas generation were locally fabricated [41] from empty transparent plastic bottles of one-litre capacity each. A hole was drilled on the cover/lid of each bottle with a knife to fit the size of the flexible collection tubes used. The opening was drilled to enable the biogas produced to flow from the digester to the biogas collecting vessel. The tubes were firmly fixed into the opening and glued to avoid air entry into the anaerobic digester and escape the biogas out of the digester. The digesters were washed thoroughly before loading the substrate to remove any inhibitory substance which may hinder microbial growth and biogas generation.

(a)

(b)

Figure 1 (a) Site of collection of municipal solid waste in Samaru village (b) Site of collection of sludge at the university water treatment plant

Preparation of the Samples

The solid wastes were sorted out to separate the organic (degradable) from the non-degradable components. The surface area of the solid substrates was increased by reducing the particle size mechanically using laboratory mortar and pestle. This makes the pre-treatment and subsequent conversion easier and faster.

Pre-treatment of the Samples

All the municipal solid waste and food waste samples were separately pre-treated with 1 M NaOH (40 g of NaOH in 1000 ml of distilled water). A total of 300 g of municipal solid waste and food waste was separately mixed with two litres (2 L) of the freshly prepared 1M NaOH solution in a clean container and allowed to stand for three (3) days at room temperature. The pre-treated municipal solid wastes were then washed with distilled water to a neutral pH (7.0 ± 0.2).

Biogas Production

Preparation of Substrate Mixture

Total weight of 300g of the pre-treated municipal solid waste and food waste was loaded into well labelled clean containers respectively, and mixed with 600 mL of distilled water to obtain a slurry in the ratio of 1:2 according to the procedure described by Musa and Raji [42]. The untreated sludge samples were equally diluted with distilled water at the ratio of 1:2. Different combinations of the substrates were prepared in different proportions, and their performances in biogas production during co-digestion were compared as described by Haftu et al. [43]. The following were the various combinations used in this study and their proportions (Table 1).

Table 1 Various substrate combinations are used for co-digestion in biogas production

Substrate combinations	1.5:1.5	1:2	2:1
MSW + FW	150g + 150g	100g + 200g	200g + 100g
MSW + Sludge	150g + 150g	100g + 200g	200g + 100g

FW *food wastes* MSW *municipal solid wastes*

Loading of the Reactors and Purification Set-up

Each of the pre-treated substrates was then separately transferred to the digesters, labelled appropriately. The digesters were stoppered and sealed with gum to prevent gas leakage and connected via rubber tubing to a gas collecting jar (500 ml capacity measuring cylinder) inverted over a solution of 1 M NaOH. The gas was collected by 'upward delivery and downward displacement' of NaOH solution [42]. The alkaline NaOH solution helps dissolve acidic gases such as CO_2 and H_2S, which are considered impurities in biogas. There are various methods employed for the absorption of carbon dioxide (CO_2) and hydrogen sulphide (H_2S) from biogas. The most commonly used and convenient method is passing the biogas through concentrated alkaline solution such as sodium hydroxide, potassium hydroxide, and calcium hydroxide. An illustration of this reaction is shown in Equations 1 and 2 below. Methane was passed through the solution without reacting.

$$CO_{2(g)} + 2NaOH_{(aq)} \longrightarrow Na_2CO_3 + H_2O_{(l)} \quad (1)$$

$$H_2S_{(g)} + 2NaOH_{(aq)} \longrightarrow Na_2S + 2H_2O_{(l)} \quad (2)$$

Each experiment lasted for one complete cycle of 21 days. Gas production was measured at an interval of 24 hours by the volume displacement method, the initial temperature of each substrate (slurry) and the final temperature of the resulting sludge were measured using a thermometer [44]. The biogas production was carried out at an ambient temperature of 27-29°C. The gas obtained was also burned to test its combustibility.

Quantitative and Qualitative Assessment of the Biogas Produced

The parameters used for assessing the quantitative performance of the experimental substrates included the following: the commencement of gas production, daily gas yield, time to reach peak gas production, time when the production ceased, and the total volume of gas produced over the period of 21 days called the hydraulic retention time (HRT).

The quality of the biogas produced was on the other hand determined based on the degree of its flammability. This was carried out by lighting a match and passing the flame over the nozzle of the measuring cylinder (gas collection jar) in which the gas was collected [42].

Microbial (Methanogenic bacteria) Analysis

Preparation of isolation medium from basal (mineral) medium

The basal medium was prepared according to the manufacturer's instructions. A weighted amount of 0.564 g each was separately added into three different conical flasks labelled A, B, and C, each containing 60 mL of distilled water. The basal medium contained the micronutrients and macronutrients needed for the growth of the organisms. To the conical flask labelled A, 2 g of sodium formate was added to serve as the sole source of carbon. To each of the three conical flasks, 1.5% agar-agar was added [42]. The media was then brought to boil and was sterilized by autoclaving at 115°C for 20 minutes [45].

After sterilization, the media was allowed to cool to about 48°C, and Rifampicin (600 mg) was added to all the flasks to selectively isolate archaea (methanogens) and eliminate bacteria present. Sodium salt (0.57 g) was added to the sterile media contained in flasks A and B respectively, mixed, and then, 2 g of sodium nitrate and 1 mL of 0.01% resazurin solution were added to only the flask labelled B. While to the conical flask labelled C, 2 mL of methanol was added to the sterile media in the flask and mixed. The contents of each flask were then poured into sterile Petri-dishes and allowed to solidify.

Isolation of Methanogenic Archaea

A volume of 0.1 ml of the digestate from the completed biogas production plant was diluted up to 10^{-5} by ten-fold dilution, and 0.1 ml each from this final dilution was aseptically streaked on the surface of freshly prepared selective media as shown in Figure 2. The media was prepared according to the method employed by Musa and Raji [42]. To basal media (containing macronutrient

Figure 2 Experimental Set-up for Biogas Production

and micronutrient solutions, phosphate solution (KH_2PO_4) and sodium formate, which served as the organic source of carbon) 1% agar-agar was added, brought to boil, and then sterilized by autoclaving at 115°C for 20 mins at 15 lb/inch^2 was prepared the plates labelled A, supplemented with sodium formate without nitrate and methanol supported the growth of all the four genera which were identified presumably by Gram staining. For the plate labelled B, which was fortified with sodium nitrate in addition to the sodium formate, supported the growth of only *Methanobacterium* spp., while other members were even strictly inhibited. Being *Methanosarcina* spp., the only genus utilizing methanol as a source of carbon, the plate labelled C, fortified with methanol, revealed the growth of only *Methanosarcina* spp., identified by Gram- staining.

The plates were then incubated at 37°C for three to seven days in an anaerobic jar. After incubation for seven days, colonies were examined macroscopically, and results were recorded. Gram staining was carried for each of the isolates, and the microscopic characteristics were observed under 100x (oil immersion) objective lens as also described by Musa and Raji [42].

DISCUSSION

The production of biogas is generally known to be influenced by various factors at varying degrees. In this study, the initial pH (7.0 ± 0.2) and the initial temperature (room temperature ~ 298 K) were fixed. The performance of the co-digested substrates for biogas production was therefore solely dependent on the nature of the substrate and the organic loading rate (OLR). In all the experiments, varying proportions of OLR (1.5:1.5, 2:1 and 1:2) were assessed and the volume of biogas generated (quantitatively and qualitatively) was recorded. The final temperatures of the co-digestion of food waste and sludge with the municipal wastes were between 1-3°C higher than the initial temperatures, however, the increase was more evident or higher in the co-digestion of MSW with food waste (Table 2). Drosg [46] stated that temperature fluctuations of ±2-3°C will significantly affect the microbial community in the digester, and ultimately the quality of the biogas

Table 2 Assessment of biogas produced from co-digestion of food waste and municipal solid waste

Parameters	Ratio of municipal solid waste to food waste		
	1.5:1.5	1:2	2:1
Initial temperature (°C)	26	28	25
Day Production Started	3rd	3rd	5th
Day flammability started	10th	10th	5th
Flammability Test	+++	++	±
Day of peak Production	10th	10th	5th
Day Production Ceased	-----	-----	10th
Total gas produced	765	553	50
Final Temperature (°C)	28	30	28

Key: HRT= Hydraulic Retention Time; +++ = highly flammable ++ = flammable; = flammability fluctuates; --- = No cessation in production for the entire HRT.

produced. The sludge recorded the same temperature in some instances (Table 3). This might likely be due to the heat generated during the metabolic breakdown of the organic molecules in the substrate and their subsequent conversion into biogas.

Interestingly, this temperature increase might be responsible for the higher volume of biogas generated as it increased to near-optimal levels, especially for the bacteria which drive the preliminary stages of hydrolysis, acidogenesis and acetogenesis during biogas production. Generally, as the efficiency of these stages increases, the rate and volume of biogas generated are ultimately expected also increase. Additionally, the rise in temperatures might have decreased oxygen solubility in the reactors making it escape, thereby increasing the anaerobic process which favor the methanogens. On the other hand, the final temperature of the digestate from the co-digestion municipal solid waste and wastewater sludge was maintained nearly throughout the retention time. This might relate to the relatively lower nutritional richness of this substrate mixture compared to the co-digestion of food waste and municipal waste. This might therefore account for the lower biogas yield obtained. The amount of metabolizable nutrients in the substrate determines the type and amount of the end-product that will be obtained [47].

The highest volume (765 cm^3) of biogas obtained was from the co-digestion of food waste and municipal waste at the ratio of 1.5:1.5. Surprisingly, the lowest volume of 50 cm^3 obtained was also from the co-digestion of food waste and municipal waste, however, at 1:2. On the other hand, the co-digestion of municipal waste and sludge yielded the highest volume of 527 cm^3 at 1:2, which was about 31.1% lower than the highest yield obtained from the co-digestion of food waste and municipal waste at the ratio of 1.5:1.5. This difference might not be unconnected with the nutritional composition, which might include in addition to proteins and vitamins, other elements such as calcium and phosphorus, which are also required by the organisms involved in methanogenesis. These nutritional contents might also account for the continuous production of biogas during the co-digestion of food waste and municipal waste without cessation as compared to the co-digestion of municipal waste and sludge, which ceased earlier. The variation in the organic loading rate of the substrates might also account for this difference in biogas yield.

Figure 3 shows the weekly volume of biogas produced with the highest yield obtained during the second week of co-digestion of food waste and municipal waste at the ratios of 1.5:1.5 and 2:1. The weekly biogas yield from co-digestion of municipal waste and sludge are presented in Figure 4.

Table 3 Assessment of biogas produced from co-digestion of municipal solid waste and sludge

Parameters	Ratio of municipal solid waste to sludge		
	1.5:1.5	1:2	2:1
Initial temperature (°C)	28	27	28
Day production started	2nd	3rd	2nd
Day flammability started	7th	9th	5th
Flammability test	++	+	+++
Day of peak production	10th	5th	3rd
Day production ceased	15th	19th	10th
Total gas produced (cm3)	487	527	371
Final temperature (°C)	27	27	28

Key: +++ =highly flammable, ++= moderately flammable, + = low flammability.

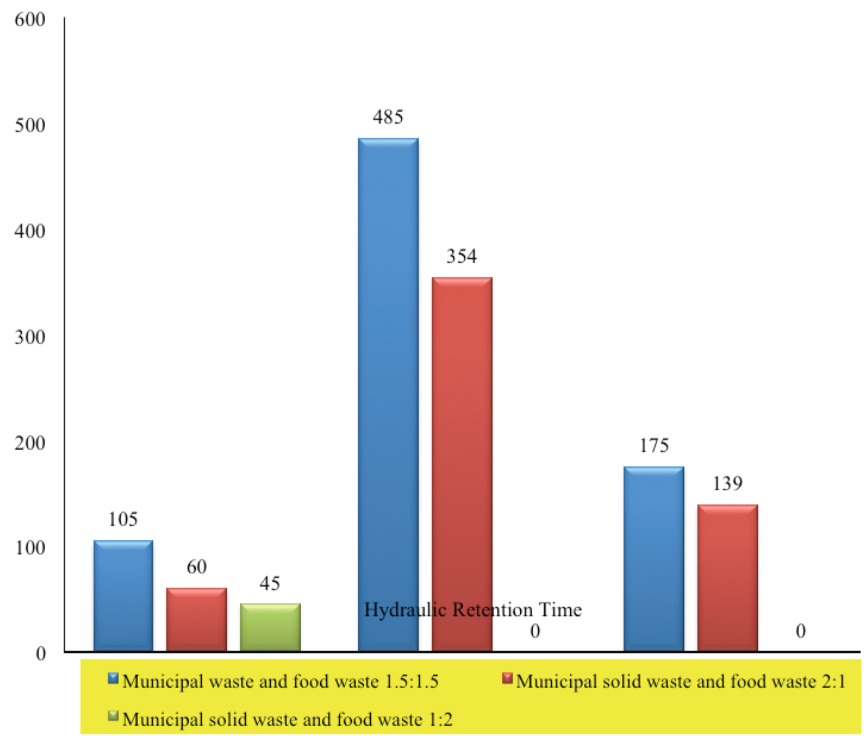

Figure 3 Volume of biogas produced from co-digestion of food waste and municipal solid waste against retention time

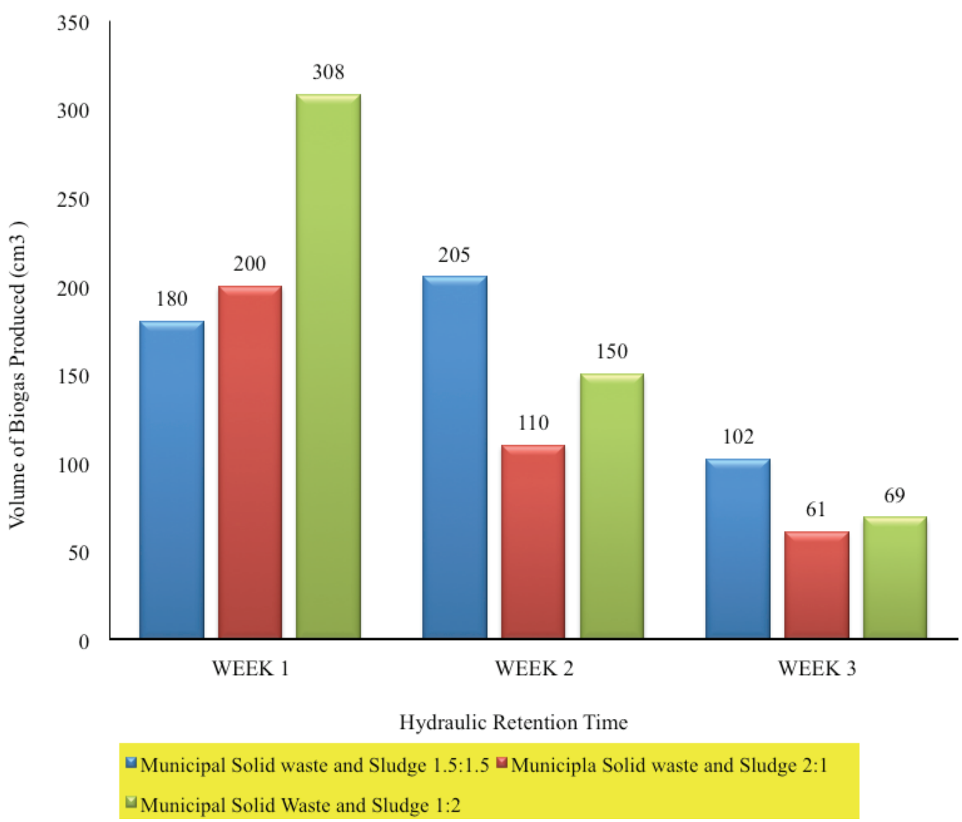

Figure 4 Volume of biogas produced from co-digestion of municipal solid waste and sludge against retention time

The flammability test in this study revealed that the biogas obtained from co-digestion of food waste and municipal waste at the ratio of 1.5:1.5 and from co-digestion of municipal waste and sludge at the ratio of 2:1 was the most flammable. The decrease in the flammability of the biogas with an increase in the amount of food waste in the substrate might be due to the high protein content, which resulted in an increased level of ammonia which is no-combustible. The high degree of flammability observed might be due to high methane content and/or low levels of such incombustible gases as carbon dioxide, oxygen, nitrogen, and ammonia as components of the biogas. This result corroborates with the finding of Jatau et al. [48].

Regarding the microbiological analyses (Tables 4 and 5), the isolates belonged to four genera belonging to the family, Methanobacteriaceae namely, *Methanobacterium*, *Methanococcus*, *Methanoplanus* and *Methanosarcina* since they are the only genera known to have been isolated in pure culture [42]. In this study, only the methanogenic archaea were the focus of the microbiological analyses, species of eubacteria such as *Clostridium*, *Shewanella*, *Ralstonia* and some Actinobacteria were not targeted. The reason for this is that while the consortium of microorganisms is responsible for the breakdown of macromolecules to simpler forms, the final stage during the anaerobic, which ultimately leads to methane production, is carried out by the methanogenic archaea. Due to the limitations in culturing methanogenic archaea and discovering novel strains of eubacteria with great potential in biogas production, omics technologies would provide more comprehensive information about the microbial community involved in the process. A study that highlighted the use of metagenomics and metatranscriptomics in studying the microbial community in a thermophilic biogas plant revealed that metagenomic analysis of 16S rRNA sequence data showed the presence of representatives of *Methanobacterium* and *Methanosarcina*, among other archaea [49]. In another study [50] which also focused on the metagenomic analysis of the 16S gene in an anaerobic digestate, identified the following dominant procaryotes: eubacteria (Clostridiaceae, Lachnospiraceae, Ruminococcaceae) and archaea (*Methanosarcina*, *Methanolobus*, *Methanosaeta*, and *Methanospirillum*).

The volume of municipal solid waste generated in the major cities in Nigeria has a great potential to produce biogas and biomethane, especially if co-digested with other wastes with higher organic content such as livestock and agricultural waste. However, considering the increased interest in all aspects of the agricultural sector, including aquaculture, livestock, and poultry farming, agronomy, there is potentially more waste generated, which could be channelled to biogas production in the country. The government can encourage research in this field by making available grants for academics and research staff in institutes to boost capacity building and form a strong basis for large-scale biogas projects. Biomethane offers an alternative source of power in the country especially because of the irregular power supply.

Table 4 Cultural and microscopic characteristics of methanogens isolated from digestate of MSW and food waste

	Macroscopic characteristics	Microscopic characteristics	Inference
A_1	Brownish, irregular, dry, small	Gram negative	*Methanoplanus* spp.
A_2	Brownish, small, dry, few	Gram positive cocci	*Methanococcus* spp.
A_3	Large, brownish, dry, irregular	Gram positive cocci	*Methanococcus* spp.
B_1	Pale green, dry	Gram positive rod	*Methanobacterium* spp.
B_2	Dry, pale green, small, few	Gram positive rod	*Methanobacterium* spp.
B_3	Dry, pale green, small, few	Gram positive rod	*Methanobacterium* spp.
C_1	Grey, dry, small	Gram positive cocci	*Methanosarcina* spp.
C_2	Grey, large, round	Gram positive cocci	*Methanosarcina* spp.

A: General medium for Methanogens; B: Selective for *Methanobacterium* spp.; C: Selective for *Methanosarcina* spp.
Subscripts $_{1,2,3}$ = Ratio of MSW to food waste; 1.5:1.5, 1:2, 2:1 respectively.

Table 5 Cultural and microscopic characteristics of methanogens isolated from digester containing MSW and sludge

	Colonial morphology	Microscopic characteristics	Inference
A_1	Light brown, dry, small	Gram positive cocci	*Methanoplanus* spp.
A_2	Light brown, small, dry	Gram negative cocci	*Methanoplanus* spp.
A_3	White, fluffy, small, dry	Gram positive cocci	*Methanococcus* spp.
B_1	Pale green, small, dry	Gram negative cocci	*Methanobacterium* spp.
B_2	Pale green, dry of varying sizes	Gram positive cocci	*Methanococcus* spp.
B_3	Green, small, dry	Gram negative cocci	*Methanococcus* spp.
C_1	White, small, dry	Gram negative cocci	*Methanosarcina* spp.
C_2	Green, dry of varying sizes	Gram positive cocci	*Methanosarcina* spp.

A: General medium for Methanogens; B: Selective for *Methanobacterium* spp.; C: Selective for *Methanosarcina* spp.
Subscripts $_{1,2,3}$ = Ratio of MSW to sludge; 1.5:1.5, 1:2, 2:1 respectively.

CONCLUSION

The findings of this research showed that a biogas generating plant could be easily constructed at a minimum cost since the materials used in constructing the digester were locally sourced. Based on the yield and quality of the gas produced, co-digestion of food waste and municipal waste at 1.5:1.5 was found to be the best substrate for biogas production. Similarly, one of the limiting parameters in biogas production at industrial scale is the inoculum, but this study is considered important since it showed that the inoculum could be obtained by culture using a selective laboratory medium which was found to support the growth of methanogens that principally mediate the biomethanation process.

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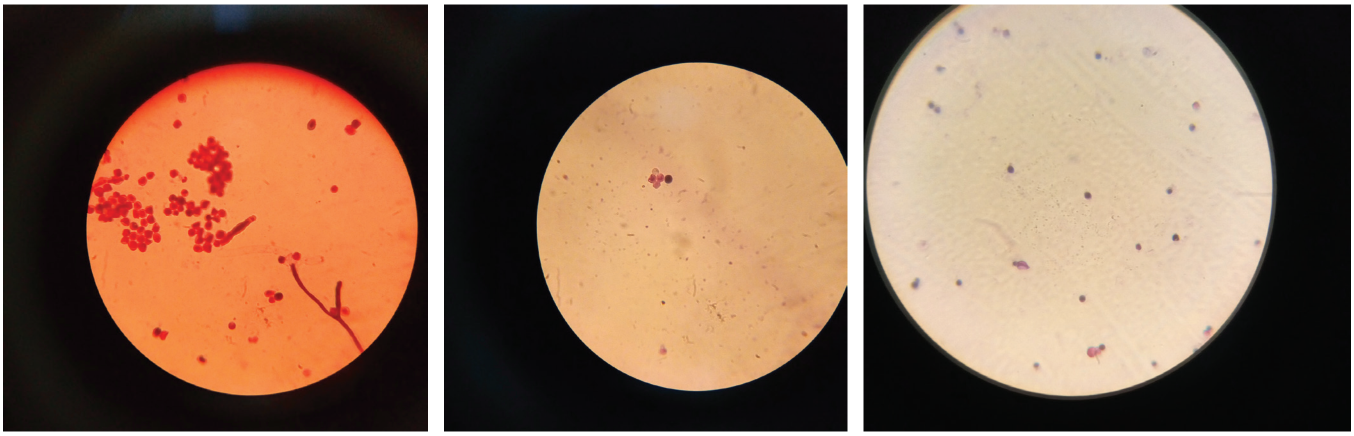
APPENDIX



(a) (b) (c)

(a) Photomicrograph of *Methanobacterium* spp. cultured on selective medium for *Methanobacterium* inoculated with digestate 1.5:1.5 (municipal solid waste and sludge)
(b) Photomicrograph of *Methanococcus* spp. isolated from digestate of 2:1 municipal solid waste and sludge.
(c) Photomicrograph of *Methanosarcina* spp. isolated from digestate of 1:2 municipal solid waste and sludge.