

## Effect of Anaerobic Co-digestion on Microbial Community and Biogas Production

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A study was carried out to explore the biogas production potential of co-digestion using chicken droppings as inoculum and different substrate combinations. The various substrate combinations were mixed with the chicken droppings in the ratio 1:1 and subjected to anaerobic digestion using fabricated laboratory scale biodigesters, immersed in water bath and set at 37°C for a period of 30 days. The initial and final pH values of the digesters were recorded. The amount of biogas generated was measured by the method of downward displacement of water from a measuring cylinder. The bacteria and fungi associated with the production of biogas were isolated and then characterized using standard microbiological techniques. The microbial population isolated from the biodigesters include species of *Bacillus*, *Clostridium*, *Escherichia*, *Pseudomonas*, *Yersinia*, *Methanosarcina*, *Methanobacterium*, *Penicillium* and *Aspergillus*. It also indicated a slight shift from an alkaline medium to a slightly acidic environment in all the digesters. The result shows that all the substrate combinations demonstrated potentials for biogas production.

With growing concern about change in climate, quality of air, energy import dependence and fossil fuel depletion, the yearning for renewable fuels is increasing and biogas is one of the versatile renewable fuels (Rasiet *al.*, 2011). Biogas is produced by anaerobic digestion of biological wastes such as poultry and sheep droppings, vegetable wastes, cattle dung, municipal solid waste, industrial waste water and land fill to give mainly methane (50-70%), carbon dioxide (20-40%) and traces of other gases such as nitrogen, hydrogen, ammonia, hydrogen sulphide, water vapour, etc (Gopinath *et al.*, 2014). Basically, it can be used to generate energy-heat, electricity and cooking.

Compared to other solid fuels, biogas is smokeless, hygienic and more convenient to use (Gopinath *et al.*, 2014). Wastes, more commonly known as garbage or trash consist of everyday items used and discarded, such as product packaging, grass clippings, furniture, clothing, bottles, food residues, newspapers and other appliances from hospitals, homes, schools and businesses (US EPA, 2015).

Refuse dump sites, due to poor and ineffective management has turned to a source of health hazard to people living in the vicinity of such dumps (Ogunrinola *et al.*, 2012). These 'mountain' of rubbish in landfills and open waste dumps are sources of harmful microorganisms and are usually

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covered with flies, thus serving as breeding grounds for rodents and mosquitoes which are disease vectors (Ozturk, 2013).

In a bid to examine the link between environmental pollution arising from waste dumps and public health, the United Nations Environmental Programme (UNEP) conducted a pilot study of the Dandora Waste Dump in Kenya. The study, as tentative as it was, showed that a link exists between environmental pollution and public health (UNEP, 2010). The extensive tests carried out on the soil and water around the dump site in comparison with samples from other sites as well as medical tests carried out on humans living around the dumpsite shows evidence of infections from water, land and air pollution. The leachates generated in the landfills and open dumpsites are sources of pollution which is inimical to public health (UNEP, 2010).

The process of anaerobic digestion involves the decomposition of organic matter by a microbial consortium in an environment free of oxygen (Ward *et al.*, 2008). Arguably, biogas is the most valuable product of anaerobic digestion. However, it is not the only useful product (Park *et al.*, 2012). Biogas is produced in the process and also slurry- biologically known as digestate. The digestate can be applied directly on farmland and used as fertilizers due to their excellent N-content (Olufunmi, 2014). Anaerobic digestion is a process where microorganisms break down organic matter, such as waste and manure, in the absence of oxygen and it has a series of metabolic reactions (Khalid *et al.*, 2011). The metabolic reactions produce biogas from a three-phase process namely, hydrolysis, acid-forming and methane-forming phases. Microbes and their enzymatic pathways ensure these transformations.

Co-digestion is a method of treating waste that involves various waste being mixed and treated together (Khalid *et al.*, 2011). This technique is mostly used to increase methane production (EPA, 2012).

Comparing single waste digestions with co-digestion of combined wastes, it is seen that co-digestion results in higher methane gas yields, with a positive impact on the quality and quantity (CH<sub>4</sub> content) of biogas produced (Kangle *et al.* 2012). For instance, an optimization protocol for maximizing production of biogas by anaerobic

co-digestion of several wastes was carried out by (Alvarez *et al.* 2015). Pig manure, tuna fish waste and biodiesel wastes were considered in order to validate the process. The results obtained showed that the highest methane production rate (16.4 L CH<sub>4</sub>/kg COD) was obtained by a mixture containing 88% pig manure, 4% fish waste and 8% bio- diesel waste as compared to single digestion. Also, in studies done by (Ozturk, 2013), co-digestion of cattle manure and cheese whey yielded a higher rate of biogas production (0.72 min<sup>-1</sup>m<sup>-3</sup>) as compared to digestion of only cattle manure (0.63 min<sup>-1</sup>m<sup>-3</sup>). (Park *et al.* 2012), in evaluating the effect of co-digestion on methane yield and macronutrient degradation, co-digested algae biomass residue with lipid-rich fat, oil, and grease waste (FOG). The corresponding result was an increase in methane production. Aragaw *et al.* (2013), asserted that anaerobic co-digestion strategies are needed to enhance biogas production when treating certain residues such as cattle/pig manure. They co-digested food waste with animal manure or other feedstock with low carbon content and found that this can improve process stability and methane production. In their study, anaerobic digestion and co-digestion of cattle manure with organic kitchen waste using rumen fluid as inoculum was experimentally tested to determine the biogas potential, and co-digestion substantially increased the biogas yields by 24 to 47% over the control (organic kitchen waste and dairy manure only). Ugwu. (2012) studied 5 batch digesters containing varying ratios of mixture of chicken droppings and cow dung for a period of 30 days at ambient temperature for biogas production. Results from this study showed that co-digestion of chicken droppings and cow dung increased biogas yield as compared to pure samples of either chicken droppings or cow dung. To crown it all up, Wei Wu in the article titled “Anaerobic Co-digestion of Biomass for Methane Production: Recent Research Achievements”, included numerous studies which demonstrates that co-digestion improves biogas yields, with special attention being paid to the anaerobic co-digestion of animal waste, crop and crop residues, municipal solid waste, as well as municipal sewage sludge. This he attributed to positive synergisms established in the digestion medium and the supply of missing nutrients by the co-substrates.

The bacteria that have been isolated in biogas production from chicken droppings include: *Methanococcus* spp. *Methanobacterium*spp, *Pseudomonas aeruginosa* and *Bacillus subtilis*. The fungi that have been isolated in biogas production from chicken droppings include: *Mucormucedo*, *Aspergillusniger*and *Penicilliumnotatum*(Oyewole, 2010).In addition, co- digestion offers several possible ecological and economic advantages as nowadays, refuse is no longer considered as waste but rather something that must be recovered or reused as potential resources (Wei, 2007).To this effect, efficient planning for municipal solid waste management system requires accounting for the entire cycle of wastes (Emery *et al.*, 2006).Thus, to achieve a functioning yet stable process with high production of methane, it is necessary to create and maintain a beneficial environment for the activity of bacterial consortia of suitable specie (Kankalet *al.*, 2012). This study is thereforeaimed at determining the effect of co-digestion on microbial community during biogas production with a view of comparing the biogas productivity of the different feedstock composition.

**MATERIALS AND METHODS**

**Biodigester**

Three 1L, laboratory-scale digesters were constructed from 1000cm<sup>3</sup>non-corrosive pipes with internal diameters of 6cm. 3cm screw taps were tightly fitted towards the base of the biodigesters, which served as the influent and effluent ports. Gas cylinder valves were tightly fitted at the top of each digester and a gas meter attached to the valve to measure biogas production. The gas meter was calibrated at the University of Jos Chemistry Laboratory.

**Sample Collection**

The inoculum (Chicken droppings) was gotten from a commercial poultry and the substrates: Energy crops (Vegetables), Fat, oil and grease [FOG] (Animal fat) and Sugar source (Sugarcane juice, Waste yoghurt and Waste fruit) were gotten from Angwanrukuba market, Faringada market and Chobe market. The substrates were dried and sorted into the different combinations as shown below:

**Combination of substrates used**

1. Chicken droppings (Inoculum)

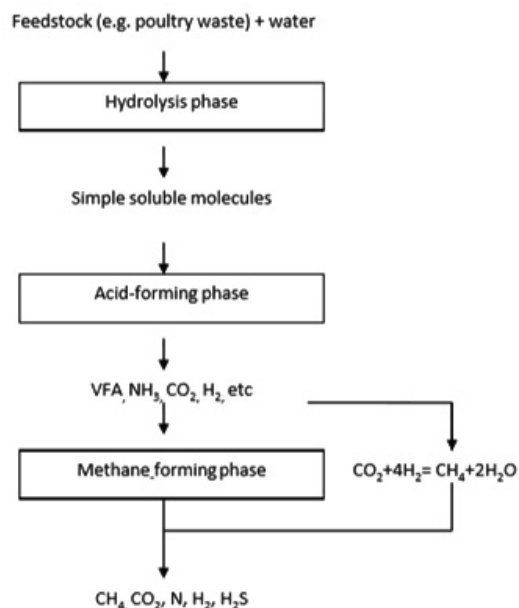
2. Vegetables
3. Animal fat
4. Sugar source [Sugarcane juice (A), Waste yoghurt (B), Waste fruit (C)]

The segregates were then crushed into fine particles using a mortar and pestle. Slurry of each segregate was prepared in the ratio 1:1:1:1 of inoculum, vegetable, animal fat and sugar source. All the samples used were collected in Jos North LGA of Plateau State in tightly sealed sterile polythene bags and isolation, identification and characterization of microorganisms was carried out at Central Diagnostic Laboratory (CDL) of National Veterinary Research Institute (NVRI), Vom.

**Experimental Design**

The experimental design used for the production of biogas using anaerobic co-digestion involved the use of various combinations of substrates and chicken droppings, which served as an inoculum. The design is shown in Table A.

The prepared slurry was placed in each digester in the ratio of 1:1 of chicken droppings and the various substrates in a water bath set at 37°C.The biodigester was tightly fitted to prevent the entry of oxygen using a stopper. The top of the biodigester was tightly fitted with valves and a gas meter attached to it. Air tightness was ensured by the use of gas in sealing the biodigester to prevent



(Asgari, 2011).

leakage. Stirring was done during and after feeding the biodigester to avoid the formation of scum (Usacket *et al.*, 2014). The experimental set up was left for 30 days and a record of the amount of biogas produced was taken. Polyvinyl chloride tubes (PVC tubes) was fitted to the valve of the gas meter and the unattached end was channeled to an inverted measuring cylinder in a filled water trough. The measuring cylinder was firmly held using a retort stand. The rate of downward displacement of water in the measuring cylinder served as a measure of determining the amount of biogas generated. This was set up as the gas meter was unable to give readings of the amount of biogas generated.

#### Isolation of Microorganisms

Bacteria was isolated using nutrient agar and modified methanogenic agar medium under anaerobic condition at 37°C for 24 hours while fungi were isolated using Sabouraud dextrose agar under anaerobic condition at 28°C for 3-5 days after which total plate count was done.

#### Procedure

1g of digestate from each of the biodigesters was inoculated in 10mls of Reinforced Clostridial Medium (RCM) and incubated for 24 hours at 37°C to enable easy isolation of strict anaerobes and 1g of digestate was also inoculated in 10mls of Brain-Heart Infusion Broth (BHIB) to isolate facultative aerobes. This served as the stock. The digestate from the Reinforced Clostridial Medium (RCM) was serially diluted to 10<sup>-4</sup> in Reinforced Clostridium Medium (RCM) and the digestate from the Brain-Heart Infusion (BHIB) was serially diluted to 10<sup>-4</sup> in peptone water. 0.2 ml of the diluent was inoculated on Modified Methanogenic Agar Medium (MMAM) and Nutrient Agar (NA) and incubated for 24 hours at 37°C anaerobically (in an anaerobic jar) for RCM and aerobically for BHIB and sub-cultured on MacConkey agar and Blood agar to obtain pure isolates. Fungi was inoculated

on Sabouraud Dextrose Agar and incubated at room temperature for 9 days.

#### Development of Modified Methanogenic Agar Medium

A Modified Methanogenic Agar Medium was developed in the laboratory to stimulate the standard methanogenic agar as described by Uwaezuoke, (1998).

#### Identification of Isolates

The bacterial isolates were identified based on their Gram reaction and biochemical tests. Anaerobic organisms were cultured and subcultured on Modified Methanogenic Agar Medium while other organisms were cultivated on conventional Nutrient agar, Blood agar and MacConkey agar media. The various bacterial isolates were repeatedly subcultured and subjected to morphological, microscopic and biochemical characterization which included production of coagulase, catalase, urease, oxidase, citrate utilization test, H<sub>2</sub>S production and carbohydrate fermentation as described by Cheesbrough, (2006) and Egbere, (2008) while fungal isolates were identified based on their morphological characteristics and microscopic appearance and then compared with the fungal atlas.

## RESULTS

Table 1 shows bacteria isolated from the biodigesters. The result obtained in Table 2 and Table 3 shows a pattern of bacteria and fungi respectively isolated from the inoculum and digestate. The organisms isolated from the inoculum (chicken droppings) were *Camphylobacter*spp, *Bacillus cereus*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Rhizopus*spp and *Aspergillus*spp while *Yersinia enterocolitica*, *Clostridium*, *Methanosarcina*, *Methanobacterium*, *Aspergillus* and *Penicillium* species were

**Table A.** Experimental Design for the Production of Biogas using Anaerobic Co-digestion

	Chicken dropping (g)	Vegetable (g)	Animal fat (g)	Sugar source (g)
Digester 1	150	150	150	150 (A)
Digester 2	150	150	150	150 (B)
Digester 3	150	150	150	150 (C)

Table 1. Isolation And Characterization Of Bacterial Isolates From The Biogas Digesters

Isolate Code	Shape	Gram reaction	Catalase	Sucrose	Urease	Glucose	Acid production	Gas	Coagulase	Maltose	Mannitol	Citrate	Dulcitol	Indole	Motility	Oxidase	Lactose	Bacteria isolated
Isolate 1	Rod shaped	+	-	*	*	+	+	+	+	*	*	+	+	+	+	+	+	- Clostridium spp
Isolate 2	Round end rods	+	+	+	+	+	+	+	+	*	*	+	+	+	+	+	+	- Bacillus megaterium
Isolate 3	Cocci	+	+	-	-	+	+	+	+	*	*	+	+	+	+	+	+	- Staphylococcus aureus
Isolate 4	Short rods	-	-	+	+	+	+	+	+	*	*	+	+	+	+	+	+	- Escherichia coli
Isolate 5	Single rods	-	-	+	+	+	+	+	+	*	*	+	+	+	+	+	+	- Pseudomonas spp
Isolate 6	Rod shaped	+	+	+	*	+	+	+	+	*	*	+	+	+	+	+	+	- Yersinia enterocolitica
Isolate 7	Irregular cocci	-	+	+	*	+	+	+	+	*	*	+	+	+	+	+	+	+ Methanosarcinaspp
Isolate 8	Long rods	+	+	-	*	-	-	-	-	*	*	+	+	+	+	+	+	- Methanobacterium spp

Key: + = Positive, - = Negative, \* = Not tested

predominant in the digestate in all biogas digesters. The result of the percentage occurrence of bacteria and fungi isolated from the biogas digesters is presented in Figure 1. *Methanobacterium* spp and *Methanosarcina* spp were the methanogens isolated. *Methanobacterium* spp had the highest frequency of occurrence with 52% while *Methanosarcina* spp had a frequency of occurrence of 17%. The fungi isolated were *Aspergillus* spp and *Penicillium* spp with *Aspergillus* spp having a frequency of occurrence of 11% and *Penicillium* spp having a frequency of occurrence of 8%. Other bacteria isolated had 12% frequency of occurrence. Figure 2 depicts the cumulative volumes of biogas produced from the different biogas digesters. Biogas digester 3 produced the highest amount of 21 cm<sup>3</sup>, biogas digester 2 produced 13 cm<sup>3</sup> and biogas digester 1 produced 11 cm<sup>3</sup>. The result of the pH of the various digesters before and after biogas production is presented in Table 4. The result indicates that there was a general decrease in the pH values with digester 1 yielding the highest percentage decrease in the pH value and digester 3 yielding the lowest.

### DISCUSSION

There was a general decrease in the pH values of each digester showing a slight shift from an alkaline medium to a slightly acidic environment in all digesters. Digester 1 had the highest percentage decrease in pH (22.37%) while digester 3 had the lowest (13.35%). The decrease in pH values is obviously due to the acid production, a second stage of biogas production (acidogenesis) in the production of biogas. The pH values fell within the optimum pH range as reported by Gashaw. (2014) for the production of methane, indicative of the presence of methanogens in the digestate. The high percentage decrease in pH in digester 1 could be attributed to the fermentation of sugarcane juice, as this was reported to lead to a reduction in pH by Pound *et al.* (2011) as opposed to other high sugar sources.

Digester 3 had the highest amount of biogas with 21 cm<sup>3</sup> generated compared to the other two digesters. This could be due to adequate moisture content provided by the waste fruit as Kigozi *et al.* (2014) reported that adequate moisture content in feedstock combination is required for excretive and other essential metabolic processes of

the microorganisms, which leads to the production of more gas. It can also be attributed to the presence of indigenous microorganisms associated with waste fruit. High availability of nutrients makes microorganisms more active producing gas at a higher rate (Garbaet *et al.*, 2009).

Less production in digester 1 could be due to the low final pH of 6.7, which is indicative of

an acidic environment and Kangleet *et al.* (2012) and other sources have reported that methanogens are sensitive to acid concentrations within the digester and their growth is inhibited by acidic conditions thus, in such an environment, biogas production will be at a lower rate.

*Aspergillus*spp and *Penicillium*spp isolated from the digesters are efficient physical

**Table 2.** Bacterial Isolates in the Inoculum and Digestate

Isolate	<i>Campylobacter</i> spp		<i>Bacillus cereus</i>		<i>Bacillus megaterium</i>		<i>Staphylococcus epidermidis</i>		<i>Staphylococcus aureus</i>		<i>Escherichia coli</i>		<i>Pseudomonas</i> spp		<i>Clostridium</i> spp		<i>Yersinia enterocolitica</i>		Methanogens	
	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A
Digester 1	+	-	+	-	+	+	+	-	+	-	+	+	-	-	-	+	-	+	-	+
Digester 2	+	-	+	-	-	-	+	-	+	+	+	-	-	-	-	+	-	+	-	+
Digester 3	+	-	+	-	-	-	+	-	+	-	+	-	-	+	-	+	-	+	-	+

Key: + Present; B – Before fermentation (Inoculum)  
- Absent ; A – After fermentation (Digestate)

**Table 3.** Fungal Isolates In The Inoculum And Digestate

Isolates	<i>Rhizopus</i> spp		<i>Aspergillus</i> spp		<i>Penicillium</i> spp	
	B	A	B	A	B	A
Digester 1	+	-	+	+	-	+
Digester 2	+	-	+	+	-	+
Digester 3	+	-	+	+	-	+

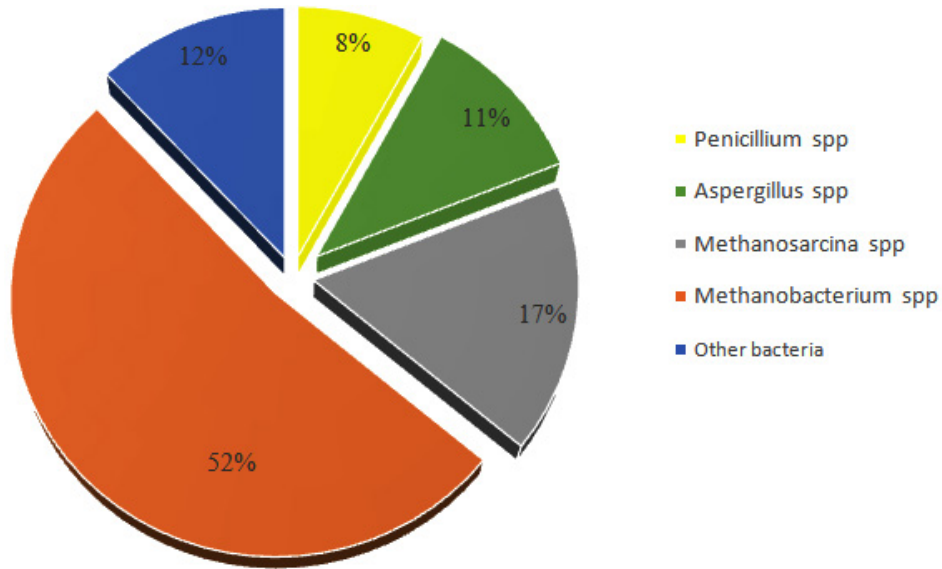
Key + Present; B – Before fermentation (Inoculum)  
- Absent; A – After fermentation (Digestate)

**Table 4.** pH of the Digesters before and after Biogas Production

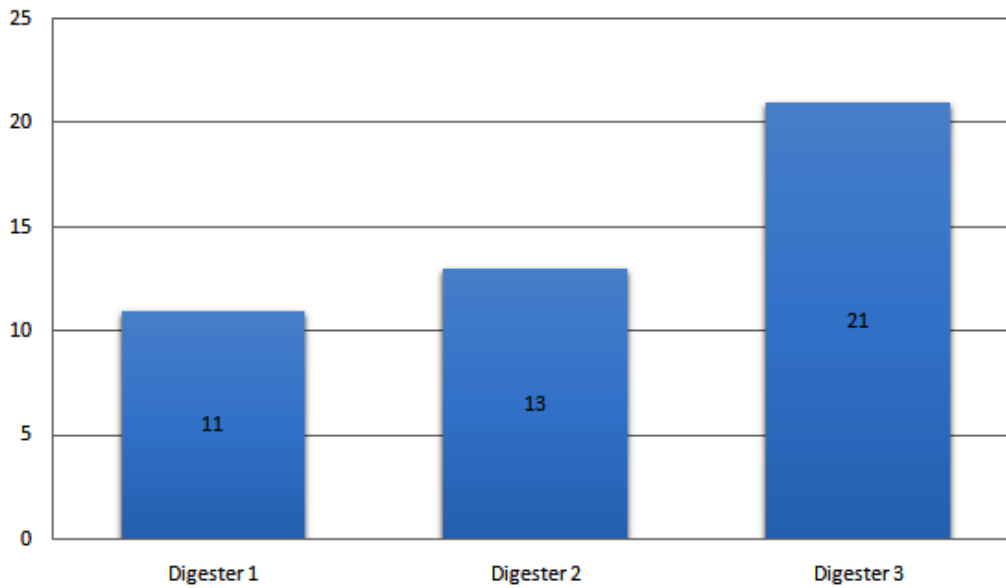
	Digester 1	Digester 2	Digester 3
Duration of Fermentation (Days)	32	32	32
Initial pH	8.67	8.52	9.14
Final pH	6.73	7.14	7.92
% decrease in pH	22.37	16.20	13.35

and enzymatic degraders of lignocellulose-rich substrates and they make biogas production more efficient. Owamah *et al.* (2014) reported a similar result when they isolated *Aspergillus*spp and *Penicillium*spp from the co-digestion of food waste and human excreta.

Bacteria and fungi isolated from the inoculum and digestate indicates that the strict and facultative anaerobes thrived in the digestate



**Fig. 1.** The % Occurrence of Bacteria and Fungi Isolated from the Biogas Digesters



**Fig. 2.** The Volume (Cm<sup>3</sup>) Of Biogas Produced In The Various Biodigesters

because the pH and temperature of the digesters during biodigestion favoured their growth and as oxygen was depleted in the biodigesters, obligate aerobes that were in the inoculum died off and the methanogens utilized the resultant CO<sub>2</sub> to generate methane.

The two groups of bacteria isolated from the digester during the anaerobic co-digestion include the acid-formers (*Bacillus*, *Escherichia* and *Clostridium* species) and methane formers (*Methanosarcina* and *Methanobacterium* species). These findings are in line with that of Egberet *et al.*

(2011), Rabahet *et al.* (2010) and Kankalet *et al.* (2012). The presence of both groups of microorganisms determines the successful operation of anaerobic digesters for biogas production as reiterated in the study done by Owamah *et al.* (2014).

### CONCLUSION

This study has shown that different segregates of municipal solid waste can be used in combination for the generation of biogas. The active microorganisms present during the biodigestion include *Bacillus megaterium*, *Escherichia coli*, *Pseudomonas* spp., *Yersinia enterocolitica*, *Methanosarcinas* spp., *Methanobacterium* spp., *Penicillium* spp and *Aspergillus* spp. Digester 3 (Chicken droppings, Vegetable, Animal fat and Waste fruit) produced the highest amount of biogas from the different substrate combinations. Biogas technology will transform waste into resources, generate income and provide a pollution-free environment. The selective and strategic adoption of biogas technology in Nigeria will solve the problems of solid waste management confronting the nation presently.

This study recommends further studies should be carried out, using the various substrate combinations to produce biogas in large quantities. The methanogenic bacteria involved in biogas production should be isolated and characterized to species and strain levels and then used selectively for biogas production. Cultural techniques gives far less accuracy compared to culture-independent techniques as it is difficult to cultivate methanogens at culture level; Molecular characterization should therefore be adopted in further researches to give a true picture of the microorganisms implicated in biogas production. The health hazards associated with indiscriminate dumping of wastes around residential areas and other ecological sensitive areas such as rivers, streams and arable lands cannot be underestimated. Nigeria should therefore direct her effort towards the treatment of wastes before disposal so as to minimize the health hazards associated with dumping of wastes.

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