e-ISSN :3027-2068:Print ISSN:3026-9830

https:www.ijbsmr.com

Int. J.Bio. Sc. M ol. Res. Vol.1(1)54-66 March,2023

Comparative Assessment of Biogas Yield from Two Animal Wastes Co-Digested with Guinea Grass (*Panicum maximum* Jacq.)

[1]Obaji*, D. G and [2]Oamen, H.P.
[1]Biotechnology Department, Alex Ekwueme Federal University Ndufu Alike Ebonyi State, Nigeria).
[2]Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria

*Corresponding author: Obaji Deborah G., email: ginny.udeh@yahoo.com, GSM: 08063208258

Abstract

Biogas, a renewable energy source, can form a good alternative to energy from fossil fuels which are non-renewable. This study was carried out to ascertain the biogas-generating efficiency of selected animal wastes digested with guinea grass (*Panicum maximum* jacq.). Moisture content and the total solid content of samples were determined. Volatile solids of grass samples, fresh cow dung, fresh pig dung, and inoculi were also obtained. Twelve reactors were used; six of the reactors contained inoculi and substrate while the remaining six reactors contained inoculi only: three for each of the two different dungs. Daily reading of the biogas yield was taken for one month. Cellulose, hemicellulose, and lignin content of the substrate were analyzed in addition to the nutrient (carbon, ammonium, sulfur, and nitrogen) and microbial content of the inoculi for cow and pig dung after the experiment. The result shows that pig dung contained more organic nutrients and a higher concentration of microorganisms, thus implying that pig dung has more potential for biogas yield. Furthermore, a rapid yield of biogas from cow dung was observed but with greater potential for biogas production from pig dung if retention time was increased. The research also showed that biogas yield was enhanced by co-digestion with grass because the reactors containing inoculi and grass generated more biogas. The results obtained from this study support the need to fully harness the potential of grasslands and properly utilize animal wastes to meet the energy demand of society.

Keywords: Anaerobic Digestion, Animal waste, Biogas, Grass, Reactor

1 Introduction

Energy is one of the most important factors in the global economy. It contributes immensely to human civilization and it can be obtained from nuclear sources, fossil fuels, biomass, etc. However, the global increase in population we experience today and the recent increase in oil and natural gas prices have led to an imperative search for alternative energy sources. Energy demand is a critical reason for extensive climatic change, resource exploitation, and restriction of human living standards (Li *et al.*, 2007). The dependence on fossil fuels as a primary source of energy contributes to environmental degradation, changes in climatic conditions, and deterioration in human health (Budiyono *et al*., 2010). Nations' economic prosperity and standard of living of people are inadvertently linked to the countries' per-capita energy consumption which is a great determinant and indicator of economic development (Rajendran *et al*., 2012). The release of carbon dioxide from the burning of fossil fuels such as petroleum and coal is considered a major factor contributing to global warming and a driving force for the search for additional or alternative carbon-neutral energy sources.

Methane, the desired component of biogas is a colorless, blue-burning gas used for cooking, heating, and lighting (Itodo *et al.*, 2007). Biogas is a clean, efficient, and renewable source of energy that can be used as a substitute for fossil fuels that are also used for energy and heat generation but are highly implicated in greenhouse effects. Biogas is a good substitute for firewood and crop residue that can meet the needs of the rural population. According to Martins *et al*. (2009), 1.0 m^3 of purified biogas has the same amount of energy as 0.97 m^3 of natural gas, 1.1 liters of gasoline, and 1.7 liters of bioethanol. In the year 2040, the world's population is predicted to be 9-10 billion people and they must be provided with energy and materials (Okkerse and Berkum, 1999) because energy is an integral part of any socio-economic development for raising the standard of living of people.

In Nigeria, the status of biogas technology remains minimal. Although biogas technology is not common in Nigeria, various research works on the science, technology, and policy aspects of biogas production have been carried out by various scientists in the country. Some significant research has been done on reactor design by some Nigerian scientists that would lead to process optimization in the development of anaerobic digesters. For instance, the Usman Danfodio University, Sokoto, designed a simple biogas plant (with an additional gas storage system) that could produce 425 L of biogas per day which could be sufficient to cook meals for one person (Dangogo and Fernando, 1986). Igoni *et al*. (2008) provided a synthesis of the key issues and analyses concerning the design of a high-performance anaerobic digester. Ezekoye and Okeke, (2006) designed and constructed a plastic biodigester and used it to produce biogas from spent grains and rice husk mixed together. The digestion of the slurries was undertaken in a batch operation and good biogas production was reported. About 21 pilot demonstration plants with a capacity range of between 10m3-20m3 have been sited in different parts of the country, still, biogas technology has remained at the level of institutional research work and pilot schemes in Nigeria (Ngumah *et al*., 2013). Waste

generally constitutes environmental pollution which is detrimental to health. Uncontrolled discharge of waste causes serious environmental, social, and health problems, thus it is necessary to reduce the risk following the current legislation (Molinuevo-salces *et al.*, 2010)

Grasslands have different applications such as pasture for livestock, cultural landscapes, recreational centers, etc but recently, grass has been greatly used as a substrate for biogas production. The energy use of grass depends on the characteristics of the grass. Some parameters such as grass species, time of harvest or cutting period, method of storage, processing, and management intensity determine the biogas yield. There is a huge expanse of unused grasslands in Nigeria, hence the need to exploit this natural resource for alternative energy production.

2 Materials and Method

2.1 Collection, Description and Preparation of Samples

The samples used for the experiments included guinea grass and animal dung, specifically, cow and pig dung. Grass samples of average height 161.25 cm, were harvested in April 2013 from the fallow land behind mushroom building in Plant Biology and Biotechnology department of Faculty of Life Sciences, University of Benin. Grass samples collected were in their flowering stage, chopped up with kitchen knife to about 1 cm and dried in the oven at 85 ˚C for 16 hours. The animal dung obtained was collected from NIFOR [Nigerian Institute for Oil Palm Research], Benin City, Edo State. A portion from each of the dung samples (cow and pig dung) was mixed with water at the ratio of 1:4 to form the inoculi.

2.2 Determination of Moisture Content (MC) and Total Solid (TS) of samples

After sample size reduction to about 1 cm, fresh weights of grass samples were obtained before drying in the oven at 85 ˚C for 16 hrs. After drying, the weights of dried grass sampleswere obtained and subtracted from the weight of fresh samples. The moisture content was calculated using Equation 1 below:

$$MC = \frac{\text{weight of fresh sample} - \text{weight of dry sample}}{\text{Weight of fresh sample}} \times 100 \ldots\ldots\ldots\ldots \text{- Equ (1)}$$

The Total Solids for the substrate was obtained by subtracting the value of Moisture Content from 100 as shown in eq (2) below:

$$Total\ Solids = 100 - Moisture\ Content$$

--Equ (2)

A portion was collected from both the fresh cow and pig dung. The weights of the dung samples and the inoculi (animal dung mixed with water) were noted before drying in the oven for 16 hrs at 85 ˚C. The weights of the dried samples were also obtained and moisture content and total solids were calculated for the samples using the above formula. The results of the experiments are shown in Table 1.

2.3 Determination of Volatile Solid (VS) of the Samples

Two grams (2g) each of the dried samples of grass, fresh cow dung, fresh pig dung, and inoculi {Mixed pig and cow dungs} were obtained and put into different crucibles of known weights, and then loaded into a muffle furnace which operated at 500 ˚C for 3hrs. The Muffle furnace was allowed to cool down for about 12 hrs before the samples were brought out and the weights determined. The volatile solid was calculated for the different samples using equation (3) below and the results were shown in Table 1.

$$\%\ \text{Volatile solids} = \frac{\text{weight of oven dried sample} - \text{weight of ash}}{\text{Weight of oven-dried sample}} \quad \text{------- Equ (3)}$$

2.4 Set-up of reactors for biogas assay

Twelve (12) different setups were used for the experiment in replicates of three (3) for each group with a total of four groups as shown in Table 2. Each setup was made up of a measuring cylinder, conical flask, delivery tube, water trough, beehive shelf, bung, retort stand, clamp, and holder. The total working volume in each reactor was 400 mL. Each reactor exceptthe controls contained dung and grass in proportions as presented in Table 2. Control contained only inoculum. The delivery tube connected the reactors containing the samples to the measuring cylinder for delivery of the gas produced into the cylinder, where the reading will be taken. After 24 hrs, the volume of gas produced was measured by directly taking the reading from the measuring cylinder. The temperature of the environment was also measured using a mercury thermometer. Subsequently, daily gas yield and temperature were measured every day for 32 days.

2.5 Determination of Nutrient Content of the Inoculi

2.5.1 Nitrogen

The nitrogen content analysis was carried out by the routine semi-micro Kjedahl technique (Kjedahl, 1883). A measured amount (0.5 g) of the inoculi was weighed carefully into the Kjeldahl digestion tubes, and 1 Kjeldahl catalyst tablet was added in addition to 10 mL of Conc. H_2SO_4. These were set in the appropriate hole of the digestion block heaters in a fume cupboard. The digestion was left on for 4 hours; a clear colorless solution was left in the tube. The digest was then cooled and transferred into 100 mL volumetric flask. The solution was distilled using

Markham Distillation Apparatus. 5 mL portion of the digest was pipette into the apparatus. To this was added 5 ml of 40% (w/v) NaOH. The mixture was steam-distilled for 2 minutes into a 50 mL conical flask containing 10 mL of 2 % Boric acid plus mixed indicator solution placed at the receiving tip of the condenser. The solution then changed color from red to green. This was then titrated against 0.01N HCL. The percentage of nitrogen in this analysis was calculated using the formula:

$$\%N = Titre\ value\ x\ Atomic\ mass\ of\ Nitrogen\ x\ Normality\ of\ HCL\ used\ x\ 4$$ –Equ (4)

2.5.2 Sulphur

The sulphur content was determined using turbidity method (Tabatabai, 1970). 0.6 g of gelatine was dissolved in 200 mL of hot distilled water, cooled, and allowed to stand in the refrigerator for 16hrs and this was brought to room temperature. To this, 2 g of $BaCl_2$ was added and mixed. The resulting solution was stored in the refrigerator for another 5hrs and was again brought to room temperature. 25 mL of the stock solution was pipetted into a 50 mL flask and made up with distilled water. 10 mL of the sample was pipetted into a 50 mL flask. Distilled water was added to bring the volume to about 20 mL. 1 mL of the gelatine-barium chloride reagent was added and the solution was made up to mark with distilled water. The resulting solution was thoroughly mixed and left to stand for 30 minutes. The turbidity was read at 420 nm. A calibration curve was prepared from the spectrophotometer reading. The absorbance of the sample and the standard was measured against the blank and the concentration of the SO_4^{2-} in the sample was determined from the calibration curve with the concentration calculated as:

$$SO4\ (ppm) = \frac{\text{Instrument reading x slope reciprocal x column volume}}{\text{Volume taken}}$$ ----- equation (5)

2.5.3 Ammonium Nitrogen (NH4-N)

Five milliliters (5 mL) of samples and ammonium standard stock solution were pipette into 50 mL flasks, 2.5 mL of alkaline phenol, 1 mL of sodium tartrate, and 2.5 mL of sodium hypochlorite were added and properly mixed in between each addition. This was then read using a spectrophotometer at a wavelength of 636nm (Koroleff, 1966). Calculation

$$NH4\ N = \frac{IR \times SR \times Colour\ Developer \times Extract\ Ratio}{Weight\ of\ Sample \times Aliquot\ taken}$$ -----equation (6)

2.5.4 Carbon

One hundred milligrams (100 mg) samples were weighed into five 50mL digestion tubes. 1.00 ml of sucrose standard solutions was added into the 5 digestion tubes. 5 mL of $K_2Cr_2O_7$ solution and 10 mL of concentrated H_2SO_4 were also added. This was capped with a rubber

stopper and swirled on a vortex mixer until the sample was completely dispersed and placed in a digestion block preheated to 135˚C for exactly 30 minutes. T0on (7)

2.6 Microbial Analysis

Serial dilution of the sample was made by weighing 0.10 mL of the sample into Eppendorf tubes containing 0.9µL of sterile water. Volume of 10^{-3} and 10^{-6} mL were measured into Eppendorf tubes containing 0.9 µL of sterile petri dishes. The mixtures were pour plated using PCA and left to gel. They were then incubated at 37 ˚C for 24 hours and then counted. Growth was observed after a few days and the number of colonies counted. The formula below was used for counting the number of colonies and it is called a colony-forming unit [CFU].

$$CFU = \frac{\text{number of colonies x dilution factor } \{10^{-3} \text{ or } 10^{6}\}}{\text{Volume of inoculums [i.e. 0.1uL]}}$$ ------ equation (8)

2.7 Substrate Analysis

2.7.1 Determination of lignin content of the substrate

Ten milligrams (10mg) of the grass sample were weighed, dissolved in 10 mL (25%) acetyl-bromide, and allowed to stand for 30 min. 25 mL of acetic acid; 4.5 ml NaOH and 0.5 mL hydroxylamine were mixed in 100 mL flask. Then the dissolved sample was transferred into the 100 mL mixture and made up to mark. This was made to stand for 20mins and read at an absorbance of 280nm using a spectrophotometer. The blank being, glacial acetic acid. (Hatfield, 1999)

2.7.2 Determination of cellulose content of the substrate.

Using the Paloheimo method (Paloheimo *et al*., 1962), 2 g of the sample was weighed into a 100 mL beaker, and 16.4 g Acetic–Nitric digestion acid was added and boiled for 20 minutes. The heating was stopped; 20 mL of 95 % ethanol was added and then filtered. The sample was washed in a filter paper, using 20 mL of hot 95 % ethanol, 20 ml of hot benzene, 20 mL of hot ethane, and 20 mL of ether respectively. This was then dried at 100°C overnight, cooled, weighed (W1) into a crucible, and ash in a furnace at 500^{o}C for 2 hours. The sample was allowed to cool and the weight was obtained (W2). The loss in weight was calculated on ignition as cellulose.

$$\% \text{ Cellulose} = \frac{W1 - W2}{\text{Weight of a sample}}$$ --- equation (9)

2.7.3 Determination of Hemicellulose content of the substrate

Two gram (2 g) of the sample was weighed into a 100 mL beaker. Susequently, 16.4 g cetic – Nitric digestion acid was added and the mixture was boiled for 20 minutes, after which the heating was stopped. Twenty ml of 95 % ethanol was added and then filtered. The sample was washed in a filter paper, using 20mL of hot 95% ethanol, 20mL of hot benzene, 20 mL of hot ethane, and 20 mL of ether respectively. This was then dried at 100°C overnight, cooled, weighed (W1) into a crucible, and ash in a furnace at 500°C for 2 hours. The sample was allowed to cool and the weight was obtained (W2). The loss in weight was calculated on ignition as hemicellulose. (Patton & Gieseker, 1942)

$$\% \text{ Hemicellulose} = \frac{W1 - W2}{\text{Weight of a sample}} \quad \text{------ equation (10)}$$

3 Results

The grass and inoculi were analyzed; moisture content, total solid, and volatile solid of the samples were calculated. The result shown in the table below depicts that the moisture content of cow dung was higher than the moisture content of pig dung, conversely, the total solid and volatile solid of pig dung was higher than that of cow dung.**Table 1: Moisture content, Total solid and Volatile solid of inoculum and substrates.**

Sample	Moisture Content (%w/w)	Total Solid (% w/w)	Volatile Solid (% w/w)
Grass	69.04 ± 0.52	30.96 ± 0.52	28.79 ± 1.19
Cow Dung	97.28 ± 0.40	2.72 ± 0.46	2.44 ± 0.27
Pig Dung	94.96 ± 0.85	5.04 ± 0.85	4.08 ± 0.28

Table 2: Parameters of substrate and inoculi before charging into digester

Sample ID	Volatile Solid of inoculi. (%w/w)	Volume of inoculi (mL)	Volatile Solids of grass. (% w/w)	Amount of grass. (g)	Volatile Solid in grass addition
Grass+ CD	2.4	384	28.8	16.000	4.608
Grass+ PD	4.1	373.4	28.8	26.579	7.655
Cow Dung	2.4	400	NIL	NIL	NIL
Pig Dung	4.1	400	NIL	NIL	NIL

The volume of inoculi and quantity of grass sample used for the biogas setup was calculated using the volatile solid of the samples and the values are shown in Table 2.

3.1 Substrate Analysis

The grass sample was analyzed for cellulose, hemicelluloses, and lignin content. It was observed that the grass sample contained 14.19% of cellulose, 14.34% of hemicellulose, and 3.1% of lignin. The hemicellulose content was slightly higher than the cellulose content of the grass sample. The lignin content was significantly low.

3.2 Inoculi Analysis

The inoculi were analyzed for microbial constituent and the results are shown thus

Table 3: Total bacteria count in the inoculi

Dung	Concentration (cfu/mL)	Bacteria
Pig dung	5.7×10^4	*Baccillus spp, Pseudomonas spp, Staphylococcus spp*
Cow dung	5.3×10^4	*Baccillus spp, Pseudomonas spp, Staphylococcus spp*

The result of the analysis shows that the mean counts of microorganisms were higher in pig dung than in cow dung and similar bacteria were detected in both dungs.The nutrient content of the inoculi was also analyzed. The organic nutrient in pig dung was found to be generally higher than in cow dung. The percentage of nitrogen was quite higher than every other nutrient, followed by sulphur, ammonium, and organic carbon respectively in both cow and pig dung. This result is presented in Table 3.

Table 4: Nutrient content of the inoculi

Organic nutrients	Pig dung	Cow dung
Nitrogen (%)	57.14	19.29
NH_4-N(ppm)	0.054	0.012
Carbon (%)	0.011	0.01
Sulphate (ppm)	0.136	0.082

Test of significance: The calculated F-value is lower than the critical F-value at 0.05 probability level. The null hypothesis is accepted i.e. there is no significant difference in biogas production between cow dung and pig dung

3.3 Biogas Assay Result

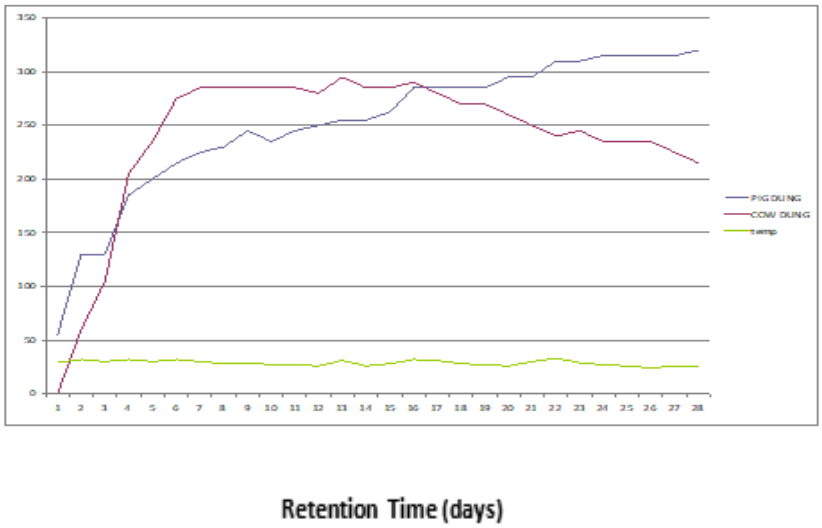

Figure 1: Specific cumulative gas volumes of cow and pig dung

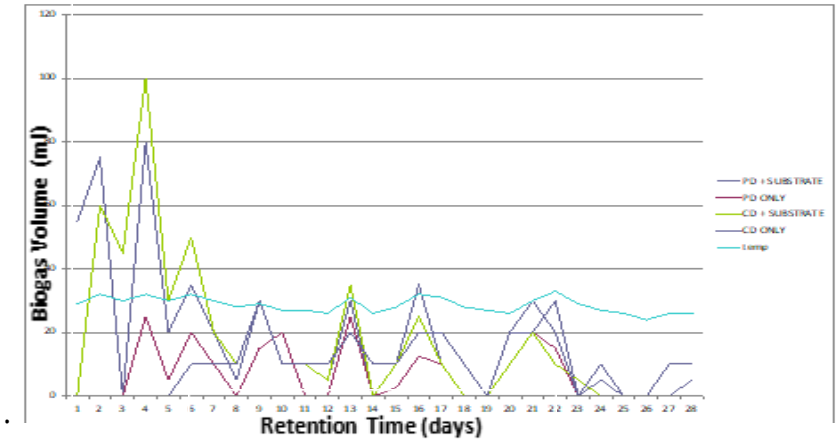

Figure 2: The average daily gas volumes of all the treatments.

4 Discussion

Nizami *et al*. (2009) stated that cellulose, hemicellulose, and lignin are the three main types of polymers that constitute lignocellulosic materials like grass and these polymers were found in the analyzed grass samples. Sa´nchez (2009) described cellulose and hemicellulose are macromolecules consisting of the same or different carbohydrate units, while lignin is an aromatic polymer made by phenyl propanoid precursors. The lignin content of the substrate was low because it was harvested during the early stage of maturity which corresponds with the fact that lignin content starts increasing with plant maturity and growing season, i.e., after anthesis as reported by Nizami *et al*.(2009).

In this experiment, the substrate was chopped into small pieces of about 1cm to enhance biogas production because substrate particle size affects the gas yield significantly as it influences the ratio between surface and volume for each organic particle. According to Mshandete *et al.* (2006), methane yield increased when the size of the particles reduced from 100 to 2mm whereas the threshold limit of particle size, particularly for grasses, was set at 0.40 mm by Sharma *et al.* (1988)

The anaerobic digestion process is a complex process requiring the presence of several different microorganisms. It is of great importance to find appropriate inoculum containing the necessary microorganisms for the degradation process to proceed (Angelidaki and Sanders, 2004). The analyzed inoculi showed the absence of some basic organisms necessary for biogas production such as methanogenic bacteria which had been used up during the experiment for biogas production. The organic nutrient content of the inoculi was quite low especially in cow dung because it had been utilized for biogas production.

The cumulative biogas production during the study period showed that biogas production for cow dung was slow at the beginning and toward the end. This may be probably due to the microorganisms' adaptation to the system at the beginning and spent materials towards the end of the production of auto-toxic substances by the microbes (Oyewole, 2010). During the 1st and 2nd days of the experiment, there was less biogas production for cow dung due to the lag phase of microbial growth; afterward, there was a substantial increase due to the exponential growth of microbes. On the 7th to 11th day of the experiment, there was no change in the biogas yield because of the effect of low temperature and probably the stationary phase of microbial growth. Towards the end of the experiment, there was a gradual decrease in biogas yield from cow dung due to the microbial phase of decline in biogas production.

According to Prochnow *et al.* (2009), grass tends to float upon the fluid surface in the digester, leading to increased stirring expenses. But due to the nature of the set-up, the digester content was not stirred, this could be one of the reasons for the gradual decrease in the biogas yield towards the end of the experiment. And it could also be the reason for sub-zero values that were recorded during the experiment because the biogas yield from the system without substrate was higher than the yield from the system with a substrate on such days, due to the fact that the floating substrate required stirring.Table 4 depicts that the organic nutrient present in pig dung is significantly higher than the nutrient present in cow dung and this encouraged the growth of microbes which explains the higher microbial content of pig dung. Hence the greater tendency for more biogas yields from pig dung if the retention time was increased and this was clearly shown in figure 1, where the microbial stage of the pig dung is still at the early period of stationary phase; which will still complete the stationary phase and decline phase with time. There was rapid production of biogas from cow dung and this was why previous studies have

shown that cow dung is the best substrate for biogas production (Garba *et al*., 1998), but pig dung has greater potential for biogas yield though the process is gradual.

Most of the biogas produced was within the first 10 days of the experiment, even the highest yield was recorded on the 2nd and 4th day for the pig dung and cow dung respectively. Much of the biogas production is in the initial phase showing that a retention time of 20 days is sufficient to generate the majority of the biogas for this substrate (Asam *et al.*, 2011).There was an increase in daily biogas yield whenever there was a significant increase in temperature and decreased yield on days with lower temperature, in accordance with Lawal et al. (2001); that biogas production is favored with an increased temperature and as temperature drops, so the rate of biogas production declines. The ambient temperature affects the rate of digestion due to the fact that the system absorbs or loses heat depending on the temperature gradient between the system and its immediate environment (Ukpai *et al.*, 2012) this implies that seasons affect the rate of heat loss or gain which in turn affects the microbial activities in the system.

Co-digestion is the simultaneous digestion of a homogenous mixture of two or more substrates. The most common situation is when a major amount of a basic substrate (e.g. manure or sewage sludge) is mixed and digested ==-..0together with minor amounts of a single, or a variety of additional substrates. This experiment showed that the systems with substrate had higher yields than the ones without substrate because methane production is improved by co-digesting grass silage with manure (Romano and Zhang, 2008). In several studies, co-digestion had a higher methane yield compared to mono-substrate digestion. The use of co-substrates usually improves the biogas yields from anaerobic digester due to positive synergisms established in the digestion medium and the supply of missing nutrients by the co-substrates (Mata-Alvarez *et al.*, 2000).

Conclusion

In conclusion, the energy demands of Society are increasing rapidly and this research work is a step in the right direction because of the obvious gains that investing in biogas production holds, both economically and environmentally. During the research, biogas production from different animal waste was investigated comparatively and the experiment revealed that biogas production from animal waste was immensely increased when co-digested with grasses, hence the need to fully harness the potential of grasslands and proper utilization of animal wastes. It was also deduced from the experiment that pig dung has a greater potential for biogas yield. These wastes are readily available in the environment and can be used as an alternative source of energy, coupled with the fact that it is cost effective and ecologically friendly.

References

Angelidaki, I. & Sanders, W. (2004). Assessment of the anaerobic biodegradability of macropollutants. *Reviews in Environmental Science and Biotechnology*, 3,117–129.

Asam, Z., Poulsen, T.G., Nizami, A., Rafique, R., Kiely, G. & Murphy, J. D. (2011). How can we improve biomethane production per unit of feedstock in biogas plants. *Applied Energy*, 10, 10-16.

Budiyono, B., Widiasa, I. N., Johari, S. & Sunarso, O. (2010). The kinetic of biogas production rate from cattle manure in batch mode. *International Journal of Chemical and Biological Engineering* , 3(1), 2010.

Dangogo, S. & Fernado, C. (1986). A simple biogas plant with additional gas storage system. *Nigerian Journal of Solar Energy*, 5,138-141

Ezekoye, V.A. & Okeke, C.E. (2006). Design, construction and performance evaluation of plastic biodigester and the storage of biogas. *The Pacific Journal of Science and Technology*, 7, 176-184.

Garba, B., Atiku, A.T. & Aliyu, M. (1998). *Proceedings of the World Renewable Energy Congress* V, Florence, Italy, 20-25.

Hatfield, R. D., Grabber, J., Ralph, J. & Brei, K. (1999). Using the Acetyl Bromide Assay To Determine Lignin Concentrations in Herbaceous Plants: Some Cautionary Notes. *Journal of Agricultural and Food Chemistry*, 47 (2), 628-632.

Igoni, A. H., Ayotamuno, M. J., Eze, C. L., Ogaji, S.O.T. & Probert, S.D. (2008). Designs of anaerobic of digesters for producing biogas from municipal solid-waste. *Applied Energy* 85, 430-438.

Itodo, I. N., Agyo, G.E. & Yusuf, P. (2007). Performance evaluation of a biogas stove for cooking in Nigeria. *Journal of Energy Sources in Africa*. 18, 14–18.

Kjeldahl, J. (1883) "Neue Methode zur Bestimmung des Stickstoffs in organischen Körpern" (New method for the determination of nitrogen in organic substances). *Zeitschrift für analytische Chemie*, 22 (1), 366-383.

Koroleff, F. (1966), Direct spectrophotometric determination of ammonia in precipitation. *Tellus*, 18: 562-565.

Li, G.Z., Niu, S. W. & Liang, Y.H. (2007). Estimate on the ecological and economic benefits of rural household biogas construction project in loess hilly region, china. In proceedings of *international conference on wireless communications: networking and mobile computing*, 1(15), 5075–5078.

Lawal, A.K., Ajuebor, F.N. & Ojosu, J. O. (2001). Characteristics of Piggery waste feedstock for determination of design parameters of biogas digester plant. *Nigerian journal of Research and Review in Science*, 2, 193-198.

Martins das Neves, L.C., Converti, A. & Vessoni Penna, T.C. (2009). Biogas Production: New trends for alternative energy sources in rural and Urban zones. Chemical, Engineering Technology, 32(17), 1147-1153.

Mata-Alvarez, J., Mace, S. & Llabres, P. (2000). Anaerobic digestion of organic solid wastes: An overview of research achievements and perspectives. *Bioresource Technology*, 74, 3-16.

Molinuevo-Salces, B., Garcia-Gonsalez, M.C., Gonselez-Fernandez, C., Cuetos, M.J., Moran, A. & Gomez, X. (2010). Anaerobic co-digestion of livestock wastes with vegetable processing waste: A statistical analysis. *Bioresource Technology*, 101, 9479-9485

Mshandete, A., Bjornsson, L., Kivaisi, A.K., Rubindamayugi, M.S.T. & Mattiasson, B. (2006). Effect of particle size on biogas yield from sisal fibre waste. *Renewable Energy***,** 31(14), 2385– 2392.

Ngumah, C., Ogbulie, J., Orji, J. & Amadi, E. (2013). Potential of Organic waste for biogas and biofertilizer production in Nigeria. *Environmental Research, Engineering and Management*, 63, 60-66.

Nizami, A., Korres, N.E & Murphy, J.D. (2009) Review of the integrated process for the production of grass biomethane. *Environmental Science Technology* 43, 8496-8508.

Oyewole, O. A. (2010). Biogas production from chicken droppings. *Science World Journal,* 5(4), 1597-6343.

Okkerse, C. & Bekkum, H. V. (1999). From fossil to green. Green Chemicals, 1, 107-114

Paloheimo, L., Herkola, E., & Kero, M.L. (1962). A method for cellulose determination. *Agricultural and Food Science*, 34(1), 57–65.

Patton, A.R., & Gieseker, L. (1942). Seasonal Changes in the Lignin and Cellulose Content of Some Montana Grasses. *Journal of Animal Science*, 1, 22-26.

Prochnow, A., Heiermann, M., Plochl, M., Linke, B., Idler, C., Amon, T. & Hobss, P.J. (2009). Bioenergy from permanent grassland. *Bioresource energy*, 100, 4931-4944.

Rajendran, K., Aslanzadeh, S. & Taherzadeh, M.J. (2012). Household biogas digesters—A review. *Energies,* 5, 2911-2942.

Romano, R.T. & Zhang, R.H (2008). Co-digestion of onion juice and wastewater sludge using an anaerobic mixed biofilm reactor. *Bioresource Technology*, 99 (3), 631–637.

Sa´nchez, C. (2009). Lignocellulosic residues; biodegradation and bioconversion by fungi. *Biotechnology Adv,* 27(2), 185– 194.

Sharma, S.K., Mishra, I.M., Sharma, M.P. & Saini, J. S. (1998). Effect of particle size on biogas generation from biomass residues. *Biomass* 17(4), 251–263.

Tabatabai, M.A. & Bremner, J.M (1970). A Simple Turbidimetric Method of Determining Total Sulfur in Plant Materials. *Agron. J.* 62, 805–806.

Ukpai, P.A. & Nnabuchi, M.N. (2012). Comparative study of biogas production from cow dung, cow pea and cassava peeling using 45 litres biogas digester. *Advances in Applied Science Research*, 3(3), 1864-1869.

Walinga, I., Kithome, M., Novozamsky, I., Houba, V. J. G. & van der Lee, J. J. (1992) Spectrophotometric determination of organic carbon in soil. *Communications in Soil Science and Plant Analysis*, 23:15-16, 1935-1944