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## Biogas production by Co-digestion of pig dung and corn chaff

Abafor PU, Runde M and Shagal MH

**Abstract**

Sustainable energy is the desire of every country in the world and a lot have been invested in other to achieve that. In spite of the efforts vested on renewable energy, over 70% of the global population rely on non-renewable energy also called fossil fuels for their daily energy requirements. In some countries, solar, wind, hydropower, biomass and other municipal waste are being harnessed to generate energy but those country rarely make up to 20% of the world population. This work is channeled toward studying the energy generation by co-anaerobic digestion of pig waste and corn chaff. Three digesters of equal volumes were charged with three co-substrate ratios - pig dung to Corn chaff, 800:800, 1120:480 and 1400:200 equivalent to Carbon to Nitrogen ratio (C:N) of 21.5, 19.6 and 17.4 respectively. The 21.5 C:N digester gave a higher yield of biogas and ultimate bio methane. Co-digestion of energy crops like maize and its derivatives and organic manure like pig dung with C:N of 20:30 is thus preferred over mono-digestion. Carbon to nitrogen (C:N) ratio has been identified as the key parameter for improving the digestion of substrates. Higher bio-degradability, improvement in C:N ratio and environmental-friendly sludge production has been established as the hallmarks of co-digestion process.

**Keywords:** Biomass, municipal waste, anaerobic

**Introduction**

Over dependent on fossil fuel has created so many environmental challenges such as air pollution, greenhouse effect, oil spills and acid rains which mostly affect people proximal to oil producing and other mining companies <sup>[1]</sup>. The uprising atmospheric temperature can be linked to the burning of fossil fuels which produces heat-trapping gases <sup>[2]</sup>. It is now clearer that the sign for global warming which point at the fast melting of ice at the arctic sea. Despite that, oil companies are on the forefront in meeting up human needs for energy and even exploring virgin fields for oil production action which has devastating effects on the ecological set up <sup>[3]</sup>. There is also the threat of depletion of fossil fuel and its derivatives giving rise the need for alternative source of energy <sup>[4]</sup>.

Energy-rich fossil fuels are not readily regenerated and also take long time to form; hence is exhaustible and not renewable <sup>[5]</sup>. Attending solutions to possible future run out of fossil fuels and their current environmental challenges posed to the world, requires lasting potential approach for sustainable development <sup>[5]</sup>. Therefore, renewable energy resources are among the most efficient and effective solutions. <sup>[6]</sup>.

Biogas as an energy source is renewable and in many cases produces insignificant carbon by-products after used. Its main components are methane (CH<sub>4</sub>), carbon dioxide (CO<sub>2</sub>) and may have small amounts of hydrogen sulfide (H<sub>2</sub>S), moisture and siloxanes. Biogas is produced by anaerobic process leading to fermentation of mixture of organic waste matter <sup>[7]</sup>. The gases methane, hydrogen, and carbon monoxide (CO) are flammables and as such, released energy when oxidized <sup>[7]</sup>. This energy releasing characteristics makes biogas to be a useful fuel in cooking, heating and in a gas engine which convert the energy in the gas into electricity <sup>[8]</sup>. According to Abdulkareem, <sup>[4]</sup>, Biogas can be referred to a mixture of various gases produced by the breakdown of organic matter such as ones obtained from Agricultural waste, Animal manure, municipal waste, plant materials, sewages, green or food waste, which occur in the absence of oxygen. Biogas must be purified to the acceptable quality and must also contain the appropriate composition before it can be distributed for public consumption. Carbon dioxide, water, hydrogen sulphide, and particulates must be removed if present <sup>[9]</sup>.

## Statement of Problem

Currently, there are issues of expensive energy costs worldwide coupled with the lingering problems of environmental abuse, especially from farm houses and animal waste. The concept of bioenergy (Renewable energy) with respect to generating biogas from these wastes has been identified as a viable alternative source of energy and environmental clean-up. Furthermore, there is need to determine the quantity of biogas that can be obtain from the various sources as well as the quality of the gases in respect to the presence or absence of other trace gases like CO<sub>2</sub>, H<sub>2</sub>S, O<sub>2</sub>, N<sub>2</sub>. This study aims to analyze the quantity and quality of methane obtained from pigdung and corn chaff.

## Aim and Objectives

The aim of this research work is to compare the quality of bio-methane obtained from pig dung and corn chaff.

Its specific objectives include;

1. To generate biogas from co-digestion of pig dung (Animal manure) and corn chaff (Plant waste)
2. To analyzed the some qualities of biogas obtained from co-digestion of corn chaff and pig dung
3. To establish the standard carbon to nitrogen ratio for the production of biogas

## Significance of the Research

This research is significant because of the nature of the materials (organic waste materials) utilized in the production of useful gas. These waste materials were initially deemed no importance to the environment and which if left to decompose on their own would lead to the production of greenhouse gases known to depletes the ozone layer. Hence since biogas are environment friendly gases they serve as good alternative source of energy to fossil fuel. Also, the data generated from this research will give an informed choice of biological material that will be used for large scale biogas production.

## Justification

Several studies that analyze and compare the methane production potential of low number of different crop species already exist [10, 11, 12]. However, the optimal ratio selected and the species handled in this study has not been determined by any author.

## Limitations of the Research

This study is limited to renewable energy generation from biological sources (pig dung and corn chaff) which may not be readily available at all times and in all places. The study did not address the quality of the flame generated by combusting biogas in each of the sample analyzed.

## Materials and Methods

### Reagents

All reagents used are of analytical grade which includes 95% sulphuric acid, Sodium hydroxide, 1% boric acid-indicator mixture, 0.01N Hydrochloric acid, Sodium sulfate/copper sulfate, boric acid, boric solution, distilled water and other common laboratory reagents.

### Apparatus

Materials used in the course of this work include, anaerobic digesters, weighing balance, pH meter, plastic syringes, measuring cylinders, volumetric flask, beakers, titration

stand, round bottom flask, furnace, electric oven, mercury in glass thermometer (0-100°C), weighing balance (50kg capacity), electronic weighing balance, Bunsen Burner, micro Kjeldahl and a lots of other laboratory glass wares.

## Anaerobic Digesters

The construction of anaerobic digester was executed in accordance with the design of Ukpai and Nabuchi, [13] in which 5-liter plastic was utilize where charging point and main digestion chamber was provided also in the design, gas discharge/gas measuring point were included.

## Methods

### Sample collection

Freshly produced pig dung (Figure 1, Plate 1) was collected in a clean polyethene bag from Zamaki pig farm at Bajabure estate Yola and transferred to the chemistry laboratory for processing. Also corn chaff (Figure 1, Plate 2) were obtained from the local market in Girei LGA.

### Slurry preparation and design

Varied quantities of both pig dung and corn chaff were weighed out and mixed in a plastic water bath to make 3 mixture ratio of the co-substrate (Figure 2) as described by Runde, *et al*, [8]. Subsequently, 100g of solid manure mixture to a litre of water was then mixed to obtain the slurry. The mixture was thoroughly shaken to achieve homogeneity before charging into a 5-litre bioreactor. Table 1 shows the ratio of the pig dung to corn chaff used in the preparation of the different co-substrate slurry.

**Table 1:** Pig Dung to Corn Chaff Ratio Used In the Preparation of the Different Co-Substrate Slurry

Digester	Pig dung (%)	Corn chaff (%)
Digester 1	50	50
Digester 2	75	25
Digester 3	90	10



**Fig 1:** Pig dung before mixing (Plate 1) and Corn chaff before mixing (Plate 2)



**Fig 2:** Co-substrate Slurry Before Charging

### Charging the digester

The slurry was charged into the digester as adopted in the work of Runde, *et al*, [14] and properly stirred; its performance was monitored for fourteen days (Figure 4). The digesters were allowed to stand until anaerobic fermentation of the wastes by the action of various microorganisms took place and subsequent production of biogas in the absence of oxygen.

### Total Solid Analysis

Total solid of the substrates was analyzed using the method described by Igoni, *et al*, [14]. Using an evaporating dish, various weigh of the substrates was obtained at different intervals as shown below:

- Weigh of empty evaporating dis =  $W_1$
- Weigh of animal waste and evaporating dish before drying =  $W_2$
- Weigh of the evaporating dish and animal waste after drying =  $W_3$
- 

$$\%TS = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

TS = Total Solid

### Volatile Solid Analysis

The dried sample was transferred to the muffle furnace and ignited at 500°C for two hours.

The loss in weight was calculated after weighing which, represent the volatile solids.

$$\% \text{ total volatile solid} = \frac{(\text{weight of dish} + \text{dry sample}) - \text{dish}}{(\text{weight of dish} + \text{wet sample}) - \text{dish}} \times 100$$



Fig 3: Digesters on Stand During Digestion Process

### Determination of Crude Nitrogen of the substrate, using Micro-Kjedhal Method

Crude Nitrogen of the substrate was determined using Micro-Kjedhal method as Described by Persson, *et al*, [15]. Nitrogen in sample is converted to ammonium-nitrogen by digestion with sulphuric acid using a catalyst. The ammonia liberated when this digest is reacted with sodium hydroxide is removed by steam distillation and collected with 1% boric

acid-indicator mixture. This is then titrated with 0.01N HCl to give % nitrogen in the sample.

### Procedure

Two grams (2g) of the dried sample (pig dung/soil) was weighed and transferred into a Kjeldahl flask and 4g mixture of  $\text{Na}_2\text{SO}_4$  and  $\text{CuSO}_4$  was then added. About 25ml of concentrated sulphuric acid was also added to the flask, which was taken to the heater. After swirling, the mixture was heated gently at first, until frothing stopped, then more strongly, until a near clear solution resulted. The digest was cooled and transferred quantitatively into a 250ml volumetric flask and made up to mark. The mixture was shaken properly and 5ml of the digest was pipetted into the distillation unit. Exactly 10ml sodium hydroxide solution was added into the sample chamber and the liberated ammonia was collected with 10ml boric acid-indicator mixture in a conical flask placed at the condenser of the markham unit. The distillation of the mixture was stopped 5minutes after the boric acid-indicator mixture turned green. Thereafter, the conical flask was removed and was titrated with 0.01N HCl until the original colour of the boric acid-indicator mixture was restored.

$$\% \text{ Nitrogen} = \frac{T.V \times N \times 0.014 \times 100}{W_s \times V_a} \times 100$$

Where: T.V = Sample Titre Value, N = Normality  
 $W_s$  = weight of sample  $V_a$  = volume of sample distilled

### Determination of Carbon Content of the Substrate

Carbon content of the substrate was calculated using the Walkley and Black [16] method.

### Procedure

Two gram (2g) dried organic waste was weighed and transferred to a 500-mL Erlenmeyer flask. About 10ml of 0.167 M  $\text{K}_2\text{Cr}_2\text{O}_7$  was then added by means of a pipette and 20mL of concentrated  $\text{H}_2\text{SO}_4$  was added by means of a dispenser and was swirled gently to mix thoroughly, (avoiding excessive swirling that would result in organic particles adhering to the sides of the flask out of the solution) [16]. This mixture was allowed to stand for 30 minutes. The flasks were placed on an insulation pad during this time to avoid rapid heat loss. The suspension was diluted with 200 mL of water to provide a clearer suspension for viewing the endpoint. The 10 mL of 85%  $\text{H}_3\text{PO}_4$  and 0.2g of NaF were added using a suitable dispenser, (The  $\text{H}_3\text{PO}_4$  and NaF were added to complex  $\text{Fe}^{3+}$  which would interfere with the titration endpoint). Finally, 10 drops of ferroin indicator was added. The mixture was then titrated with 0.5 M  $\text{Fe}^{2+}$  to a burgundy end point. The colour of the solution at the beginning was yellow-orange but turned to dark green at the endpoint.

### Calculation

$$\% \text{ Organic carbon} = \frac{(V_1 - V_2) \times M \text{ of } \text{Fe}^{2+}}{S \times 0.39 \times \text{Mcf}}$$

Where

$V_1$  = mL of  $\text{Fe}^{2+}$  solution used to titrate blank

$V_2$  = mL of  $Fe^{2+}$  solution used to titrate sample

S = weight of sample in grams

M = molarity of ferrous sulphate solution from blank titration

Mcf = moisture correction factor

### Carbon/Nitrogen (C/N) Ratio

The C/N ratio was determined by dividing the total organic carbon content by the total nitrogen content, according to the following equation.

$$C/N = \frac{W_p \times C_p + W_c \times C_c}{W_p \times N_p + W_c \times N_c}$$

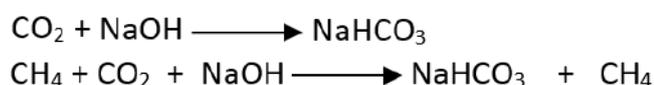
Where  $W_p$  and  $W_c$  were the weights of pig dung and corn chaff respectively in a single co-substrate mixture,  $C_p$  and  $C_c$  were the organic carbon content in each substrate and  $N_p$  and  $N_c$  were the nitrogen content in each substrate.

### Determination of Biogas Flammability

The flammability of the biogas produced was determined using a fabricated gas burner. The fabricated gas burner was connected to the digester's valve (tap); with a pipe hose, the valve was then open to allow the flow of gas through the hose to the gas burner, and was ignited.

### Determination of the Composition of Biogas Produced

The composition of biogas produced in each of the reactors was determined by absorbing  $CO_2$  in an alkaline liquid as described by [17]. An airtight 60ml hypodermic syringe containing 10 ml 0.1M NaOH was attached to the air outlet of the digester; the valve control of the digester was opened to allow 20 ml of the gas produced pass into the syringe. The airtight syringe-valve setup was swirled gently to allow the absorption of the  $CO_2$  present in the biogas, leading to a reduction of the volume of gas captured in the syringe. Change in gas volume represents the volume of  $CO_2$  in the biogas, while the remaining volume of gas represents the volume of bio methane Figure 4.



## Results and Discussion

This research tried to investigate the possibility of obtaining biogas by the anaerobic fermentation of pig dung and corn chaff co-substrate.

### Analysis of Pig Dung and Corn Chaff

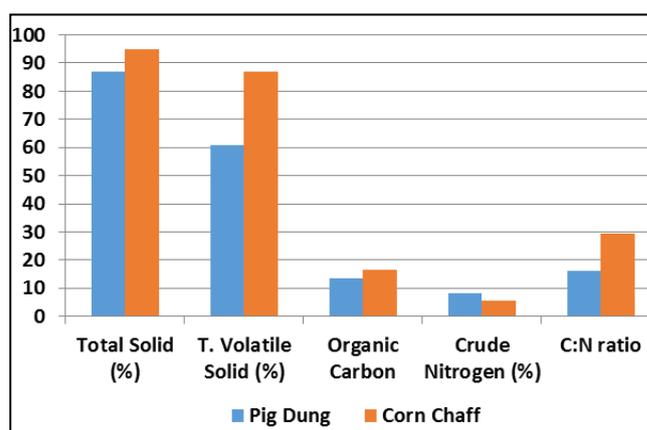
Table 2 shows the results of analysis of pig dung and corn chaff. The pig dung contained less total solid (87%), volatile solid (61%) and organic carbon content than the corn chaff which contain 95% and 87% total solid and volatile solid respectively. On the other hand, the pig dung contained more nitrogen content (8.30%) than the corn chaff (5.70%). C:N was also higher in corn chaff than in pig dung. Figure 5 gives the graphical description of the analysis of pig dung and corn chaff.

From the result of the analysis of the individual substrates pig dung and corn chaff (Table 2), it was observed that the pig dung with a high nitrogen content which will normally

inhibit the production of biogas in the case of a single substrate. The corn chaff which is higher in energy (carbon) content but lower in nitrogen serves as a better co-substrate to the pig dung as it would make up for the deficiency in energy content of the pig dung. This would if taken in proper ratio, give the optimum C:N ratio (20-30) for bio methane production as stated by Das and Mondal, [18]. This position is supported by a review done by Das and Mondal, [18]; where they submitted that more than 50% of the 3000 biogas plants in Germany has been using energy crops for energy recovery in the form of methane by the end of 2005. Most plants have been essentially utilizing maize in co-digestion with different manures and other organic materials [19], as was the case in the current study.

**Table 2:** Analysis of the Pig Dung and Corn Chaff

Parameters	Pig Dung	Corn Chaff
Total Solid (%)	87	95
Total Volatile Solid (%)	61	87
Organic Carbon	13.50	16.70
Crude Nitrogen content (%)	8.30	5.70
C:N ratio	16.20	29.40



**Fig 5:** Analysis of the Pig Dung and Corn Chaff

### Physical and Chemical Properties of Prepared Slurry

Table 3 shows the physical and chemical properties of the slurry prepared for the three digesters. The compositions of the co-substrate for the digesters were prepared in increasing mass of pig dung and decreasing corn chaff. Digesters 2 and 3 had 39 °C temperature higher than digester 1 with 38 °C. More water was required to form slurry in the digester 1 (800:800 Pg:Cc ration) than in the digesters 2 and 3 – 2.5 and 2.0 ltrs respectively. The C:N ratio of digester 1 – 21.5 was higher than that of digesters 2 and 3 – 19.2 and 17.4 respectively.

The assay on the physical properties of the individual slurry for the digesters Table 3, revealed that the co-substrate mixture ratio 1:1 Pd: Cc was the optimum for the biogas production. The mixture also has the C: N 21.5 that falls within the optimum C:N ratio for biogas production as stated by Das and Mondal, [16]. The  $VS_{pig\ dung} / VS_{corn\ chaff}$  for the mixture 1:1.5 however did not agree with what was established by Das and Mondal, [16] in their research where they established a  $VS_{manure} / VS_{corn\ chaff}$  of 1:3.

Water content of the slurry was also established to be directly proportional to corn chaff component of the slurry and to the biogas production. This is probably because of the high water retention capacity of the corn chaff. It is

however not established if the water composition of the slurry is critical to biogas production.

**Table 3:** Physical and Chemical Properties of Prepared Slurry

Parameters	Digester 1	Digester 2	Digester 3
Mass composition (Pg:Cc)	800:800	1120:480	1400:200
Temperature (°C)	38	39	39
Volume of water used (ltr)	3.5	2.5	2.0
Carbon to Nitrogen ratio	21.5	19.2	17.4

Since, the three digesters had temperatures within the mesophilic temperature range optimum for biogas production (20), temperature is then ruled out as a factor in biogas production in the current study. Thus, making the C: N and water content the factors that could have played the most critical role in the quality and quantity of biogas produced.

### Gas Flammability Test

Gas production in the digesters started on day 4, (Table 4). From the table below it was observed that the first gas produced did not burn. It also shows that when combustion began, it did not occur smoothly. This was because during the early period of biogas production, the content was mostly carbon (IV) oxide since the methane forming bacteria were not fully active yet. Hence, there was more of the acid phase taking place in the digester. This result collaborates the work of [21]. As the fermentation period progresses, the methane forming bacteria gains activity and more of the methane forming phase begin to occur. This led to increase in the percentage by volume of methane in the gas being produced and subsequently, the gas tendency to burn when ignited increases. As setup ages, the methane forming phase attains its maximum capacity with the methanogens acting on the substrate produced by the acid forming bacteria for maximum biogas production. Consequently, the digester continues to produce methane at its optimum capacity. At this stage, burning of the biogas occurs easily. The flame was observed to be blue and smokeless, which is a characteristic of methane gas [22]. There was also no appreciable deposition of soot on the burner.

**Table 4:** Flammability of Biogas Produced

Day of test after gas production	Flammability
1 – 4	No burning
5 – 8	Little Burning
9 – end	Proper burning

### Analysis of biogas produced in the bioreactors

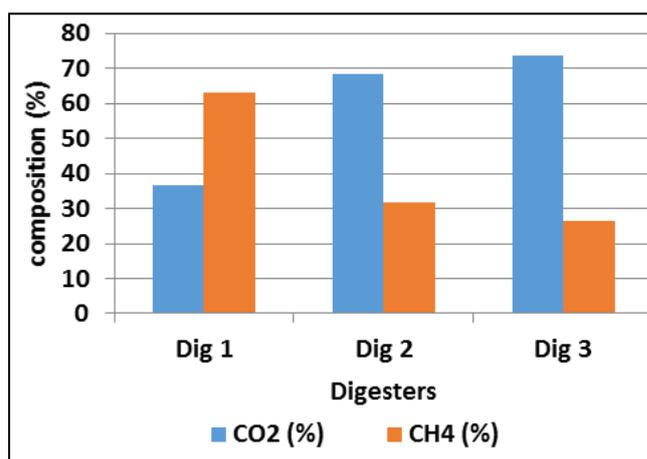
Table 5 presents the percentage composition of the component gasses – CO<sub>2</sub> and CH<sub>4</sub> present in the biogas produced. Composition of H<sub>2</sub>S and other constituent gasses were taken to be insignificant. Figure 6 is a graphical representation of the percentage composition of the gasses in the biogas. Table 4, shows that digester 3 contain greater composition of carbon dioxide this is because it contains the lowest C: N of 17.4 this ratio is due to the higher proportion in mass of the pig dung in the mixture and since pig substrate has a higher composition of nitrogen compared to its carbon, from experiment it has been observed that the microorganism responsible for the biodegrading of the waste consumes carbon roughly 30 times faster than

nitrogen. Presence of higher nitrogen leads to higher ammonia concentration in the digester which is considered as an inhibitory factor for methanogenic activity [18]. Moreover, the digester 1 with a C: N ratio of 21.5 gave the highest yield of bio methane. This is because it has a higher composition in nutrient of the bio-degradable material in the right proportion leading to the elimination of cases of ammonia inhibition and increased buffering capacity (caused by ammonia and volatile fatty acids), hence permitting a favourable environment for the methanogenic activity [23].

**Table 5:** Composition of the Component Gasses in the Biogas

Parameters	Dig 1	Dig 2	Dig 3
CO <sub>2</sub> (%)	36.80	68.40	73.70
Bio CH <sub>4</sub> (%)	63.20	31.60	26.30

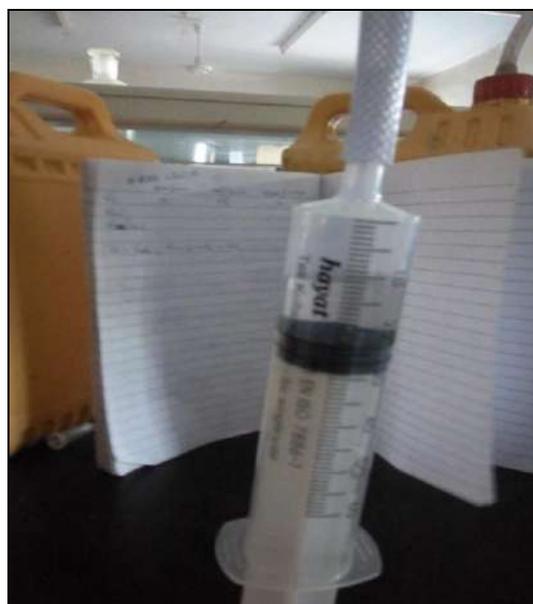
Dig = Digester



**Fig 6:** Percentage Compositions of the Gasses in the Biogas

### Biomethane content to C:N

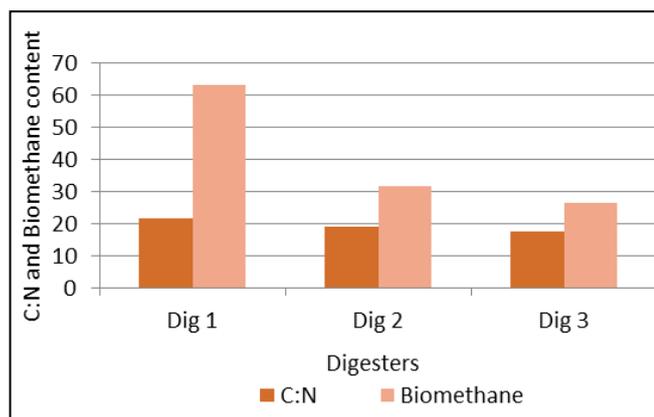
Table 6 shows the relationship between the compositions of biomethane in the biogas from the different digesters to the C:N in the feed substrate slurry. C:N was observed to be directly proportional to biomethane content of the biogas.



**Fig 4:** Determination of Biomethane Composition by CO<sub>2</sub> Absorption

**Table 6:** Relationship Between C:N and Bio methane Produced in The Digesters

Parameters	Dig 1	Dig 2	Dig 3
C:N	21.5	19.2	17.4
Bio methane	63.20	31.60	26.30

**Fig 7:** Relationship Between C:N and Bio methane Produced in The Digesters

### Conclusion

Biogas was successfully produced from the co-digestion of pig dung and corn chaff in the three different digesters with different mixing ratios. Digester 1 with the highest C: N of 21.5 produced a more qualitative biogas with a percentage bio methane yield of 63.5%. This is clearly seen in the burning characteristics of the gas with pale blue flame. Digester with the least C: N of 17.4 produced a less qualitative biogas with a percentage bio methane yield of 26.3% evident in its low combustion ability due to its high CO<sub>2</sub> content. Biogas yield and corresponding methane content in it was enhanced by co-digestion of energy crop (corn chaff) with organic waste (pig dung). Bio methane production and content of the biogas has been regarded as the key parameters for assessing the success of co-digestion process.

### Recommendation

The following are the recommendations drawn out during the course of this study;

1. Pig and corn chaff with higher C:N ratio should be investigated to establish the effect of higher C:N to ultimate bio methane production and methane production rate.
2. Construction of bio-digesters of higher volume is strongly advised to determine the prospect of commercializing this method as an alternative energy source.
3. The lag phase can be extended beyond the 14 days period used in the current study to establish maximum lag time and peak bio-methane production time.
4. Plastic was found to vessels of choice for bio-digesters than the metallic vessels because plastics are poor conductors of heat and thus will help maintain a stable mesophilic temperature within the system.

### References

1. Perera F. Pollution from Fossil-Fuel Combustion is the Leading Environmental Threat to Global Pediatric Health and Equity: Solutions Exist. *International*

*Journal of Environmental Research and Public Health* 2018;(16):1-17.

2. Lelieveld J, Klingmiller K, Pozzer A, Burnett RT, Haines A, Ramanathan V. Effects of fossil fuel and total anthropogenic emission removal on public health and climate. *Proceedings of the National Academy of Sciences of the United States of America* 2019;116(15):7192-7197.
3. Ranveer AC, Latake PT, Pawar P. The Greenhouse Effect and Its Impacts on Environment. *International Journal of Innovative Research and Creative Technology* 2015;1(3):333-337.
4. Abdulkareem AS. Refining Biogas Produced from Biomass: An Alternative to Cooking gas. *Leonardo Journal of Sciences* 2005, 1-8.
5. Nels WP, Cooper CJ. Implications of fossil fuel constraints on economic growth and global warming. *Science Direct Energy Policy* 2009;37:166-180.
6. Uzodinma EO, Ofoefule AU. Effect of Abattoir Cow Liquor Waste. *Scientific Research and Essay* 2008;3(10):473-476.
7. Angelidaki I, Ellegaard L. Anaerobic digestion in Denmark: Past, present and. Anaerobic digestion for sustainability in waste (water) treatment and reuse. *Proceedings of 7th FAO/SREN-Workshop 2002, 19-22. Moscow.*
8. Runde M, Shagal MH, Abba Y. Production and Purification of Biogas Generated by Co-digestion of Cow Dung and Kitchen Waste. *Communication in Physical Science* 2020;5(4):619-627
9. Hashimoto GVH. Factors affecting methane yield and production rate. *American Society of Agriculture Engineers (ASAE)* 1978, 1-5.
10. Song Y, Kwon S, Woo J. Mesophilic and thermophilic temperature co-phase anaerobic digestion compared with single-stage mesophilic- and thermophilic digestion of sewage sludge. *Water resources* 2004, 1652-1662.
11. Castillo E, Cristancho D, Arellano V. Study of the operational conditions for anaerobic digestion of urban solid wastes. *Waste Manage* 2006;26(5):546-556.
12. Dinsdale RM, Premier GC, Hawkes FR, Hawkes DL. Two-stage anaerobic digestion of waste activated sludge and fruit/vegetable waste using inclined tubular digesters. *bioresources technology* 2000;72:159-168.
13. Ukpai PA, Nabuchi MN. Comparative study of biogas production from cow dung, cow pea and cassava peeling using 45 liters biogas digester. *Advances in Applied Science Research* 2012;3(3):1864.
14. Igoni AH, Abowei MFN, Ayotamuno MJ, Eze CL. "Effect of Total Solids Concentration of Municipal Solid Waste on the Biogas produced in an Anaerobic Continuous Digester". *Agricultural Engineering International: the CIGR Ejournal. Manuscript EE* 2008;7(10):10.
15. Persson JA, Wennerholm M, O'Halloran S. *Handbook for Kjeldahl Digestion; FOSS, Hilleroed, Denmark* 2008.
16. Walkley A, Black IA. An examination of Degtjareff method for determining soil organic matter, and proposed modification of the chromic acid titration method. *Soil Science* 1934;37:29-38.
17. Raposo F, Fernández-Cegrí V, De la Rubia MA, Borja R, Béline F, Cavinato C. Biochemical methane

- potential (BMP) of solid organic substrates: evaluation of anaerobic biodegradability using data from an international inter-laboratory study. *Journal of Chemical Technological Biotechnology* 2011;86:1088-1098.
18. Das A, Mondal C. Biogas Production from Co-digestion of Substrates: A Review. *International Research Journal of Environment Sciences* 2016;5(1):49-57.
  19. Weiland P. Results and bottlenecks of energy crop digestion plants—required process technology innovations. *Proceedings of energy crops and biogas workshop 2005*.
  20. Vintilă T, Dragomirescu M, Crottoriu V, Vintila C, Barbu H, Sand C. Saccharification of lignocellulose - with reference to *Miscanthus* - using different cellulases. *Romanian Biotechnological Letters* 2010;15(4):5498-5504.
  21. Okonkwo UC, Onokpita E, Onokwai AO. Comparative study of the optimal ratio of biogas production from various organic wastes and weeds. *Journal of King Saud University – Engineering Sciences* 2016.
  22. Skorek A, Włodarczyk R. The Use of Methane in Practical Solutions of Environmental Engineering. *Journal of Ecological Engineering* 2018;19(2):172-178
  23. Chandra R, Takeuchi H. Methane production from lignocellulosic agricultural crop wastes: A review in context to second generation of biofuel production. *Renewable and Sustainable Energy Reviews* 2012;16(3):1462-1476.