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Production of Biofuel from Cow Dung and Food Waste using locally made Anaerobic Digester

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Abstract: *Energy crisis due to unsustainable nature of fossil fuels and environmental pollution, especially effluent, food and other organic wastes are main big challenges the world is currently facing. To avoid further aggravation of the problems; renewable energy should be developed to increase the energy reserves in the country. The current research was conducted to generate biogas (or biofuel) from cow dung and food wastes by blending to get homogenates using locally made anaerobic digester. Food wastes (corn cobs, and potato peels at the ratio of 1;1) were collected from the Kano State Polytechnic canteen and fresh cow dung from Abattoir, Fagge LGA slaughter house as feedstock for the anaerobic digestion system to produce biogas energy. This design was to combined feedstock with high caloric content and anaerobic microbes. The volume of the biogas generated was measured using calibrated cylinder with paraffin oil displacement arrangement. The proximate analysis revealed that the various feedstock contained energy yielding nutrients but at varying concentrations. The study revealed that the pH decreased possibly as the bacteria produce acids in the digester. Hence, the volume of biogas production decreases in respect of number of days under the various experiments. Biogas production increased in the earlier days of the experiment and then started decreasing as acid concentration increased as shown by the decrease in pH. Thus, the combined waste slurry (cow dung and food waste) produced more gas at 15^th^ day (36.55 mL) than the cow dung and food waste alone (20.30 mL and 12.25 mL). This may be attributed to the fact that food waste contained more carbohydrates and less protein contents than the dung. The total aerobic and anaerobic bacterial counts ranged from 6.5×10^7 to 2.7×10^8 and 1.7×10^7 to 2.5×10^7 cfu/ml, respectively. The microorganisms isolated from the digester during anaerobic digestion include Bacillus sp. and Pseudomonas sp. The research has proven that using combined feedstock (cow dung and food wastes), the efficiency of biogas production can be enhanced and increased. Therefore, biogas technology is a good way of providing solution to waste management and disposal problems apart from the generation of biofuel or clean energy from renewable energy sources.*

Keywords: *Cow dung, Food waste, Biogas, Anaerobic digestion, waste management.*

1.0 INTRODUCTION

Currently the world is facing major challenges, which includes energy supply alongside environmental management and control amongst others, due to rapid population growth and industrialization. This has lead to a worldwide increase in energy demand and increasing rate of consumption of fossil fuels. Consequently, the focus is on renewable sources of energy such as biogas which is obtained from a controlled anaerobic digestion. Anaerobic digestion is the treatment of organic wastes by a series of biological process and recovery of bio-energy in the form of biogas, which consists mainly of CH4 and CO2 (Clemens *et al*., 2006). Biogas production from biomass is of growing importance as it offers extensive environmental and economic benefits such as energy production, recycling of nutrients within agriculture and waste management etc., (Ojo *et al*., 2007). Livestock manure has been established as major source of microbes while food wastes have high caloric and nutritive values for growth and sustainability of microbes (Uzodinma *et al.,* 2007; Ukpabi and Akubugwo, 2011). Biogas technology is distinct because of its potentials of using organic material to generate energy and at the same time producing superior nutrient compost or digestate as fertilizer. This process produces gases principally methane (CH4) and carbon (IV) oxide (CO2) and has positive environmental impact due to the reduction of CO2 and CH4-the cause of global warming (Clemens *et al.,* 2006; Zahariev *et al.,* 2014; Stephen *et al.,* 2013).

In Nigeria, biogas production does not have any geographical limitations or requires advanced technology for producing energy, nor is it complex or monopolistic. However, due to instabilities in set-up and operation, a large number of factors affect biogas production efficiency including quality of feedstock. In this study attempt was made to evaluate and compare the quality and quantity of biogas production from animal dung and combination of animal dung and foods wastes as earlier reported in Ukpabi *et al.* (2017).

In practice, the biogas production process from the cow manure digester takes time because the microorganisms that convert cow manure into biogas are limited. In addition, the process is conducted only by microorganism that requires anaerobic digester substrate. Microorganisms' activity influences the rate of biogas production in the anaerobic digester (Dewi *et al.*, 2016). The higher the action of microorganisms, the higher the rate of biogas production in the anaerobic digester. Different proportions of methane gas were obtained from the addition of isolated bacteria grown on NA (Nutrient Agar) and RGCA(Rumen fluid-glucose-cellobiose agar) media, which were tested using a Gas Chromatography (GC) tool (Lestarie *et al.*, 2016). Therefore, proportion of methane gas is influenced by the biomass of methanogenic bacteria contained in it.

Biogas emits very negligible amount of smoke or sometimes smokeless, hygienic and more convenient in comparison to other solid fuels (Gopinath *et al.*, 2014). Industrial and kitchen wastes, commonly known as garbage or trash, such as product packaging, grass clippings, furniture, clothing, bottles, food residues, newspapers and other appliances from hospitals, homes, schools and businesses are all reliable substrates for biogas production UNEP (2010). The biological treatment processes of biogas plant have advantages over the other energy such as;

(i) Economically available and attractive source of investment,
(ii) Operated easily without sophistication and safe to installation,
(iii) A renewable source of electricity and heat stable, resulting in a reduction of CO2 emissions
(iv) Methane emissions are less from manure storage (Gopinath *et al.,* 2014).
(v) Improved fertilizer quality when used as a source of manure (Akbulut, 2012),
(vii) Odourless with reduced solid content when the substrate has been digested due to the degradative action of microorganisms.
(viii) That the digested sludge is been conserved in the digestion process resulting in the enhancement of the fertilizer value.
(ix) That pathogenic microorganisms such as *Salmonella sp.* and *Brucella sp.* as well as weed seeds are destroyed during the anaerobic digestion process (Ghaly and Hattab, 2011).

The anaerobic digestion of public waste can have positive environmental value since it can combine waste removal and stabilization with net fuel (Biogas) production. The solid or liquid residue can further be used as feed or as biomass briquette for cooking (Gupta et al., 2016; Brown and Li, 2013). Therefore, this research aimed at isolation, identification and characterization of some bacteria associated with biogas production through anaerobic digestion using cow dung and food waste in Kano State, Nigeria.

2.0 MATERIALS AND METHODS

3.1 Research Area and Period

The study was conducted at the Biology Laboratory, Department of Science Laboratory Technology, Kano State Polytechnic, Kano State, Nigeria between the month of August and September, 2025 at Kano City is in the North central part of Nigeria.

3.2 Sample collection and preparation

Fresh cow dung was collected from Kano main abattoir along IBB road, Fagge LGA, Kano state, Nigeria. The sample was collected in large clean plastic container and transported to the laboratory for further analytical work. The sample was air dried and pounded into powder using pistol and mortar. Fresh food wastes (corn cobs and potatoes peels) were also collected from Kano State Polytechnic canteen within the campus. The two samples were stored in the refrigerator. The Nutrient agars used for the study were prepared according to manufacturer's instructions and stored in the refrigerator for further use.

The food wastes were crushed with mixing grinder while the animal dung was mixed with water thoroughly by stirring with rod. The cow dung served as inoculums. The samples were weighed and poured into a 40L metal jacket digester based on the experimental design. After the digester was kept at ambient temperature with constant agitation for proper homogenization of the waste in the digester for the first 24hrs, gas production was checked and quantifies subsequently using standard method.

3.3 Biogas Production

The experimental studies were carried out on batch system using the fabricated digester. In the single dung experiment, the digester was fed with 200g of cow dung will be mixed with water at the ratio of 1:1 respectively. In the combined digester experiment, 200g of the premix mixed dung and food waste will be introduced into the digester at the same ratio. The combined waste (feedstock) experiment consisted of 100g of cow dung and 100g of food waste were mixed with water at the ratio of 1:1, respectively. The digester was provided with suitable arrangement for feeding, gas collection and draining residues. The digester was connected to a calibrated measuring cylinder with paraffin oil displacement arrangement. The digester was fed by opening the cover of the dome and remnant was evacuated via the outlet device at the bottom of the dome. A shaft was created to drive the slurry in the digester with the aid of a pulley bell and electric motor.

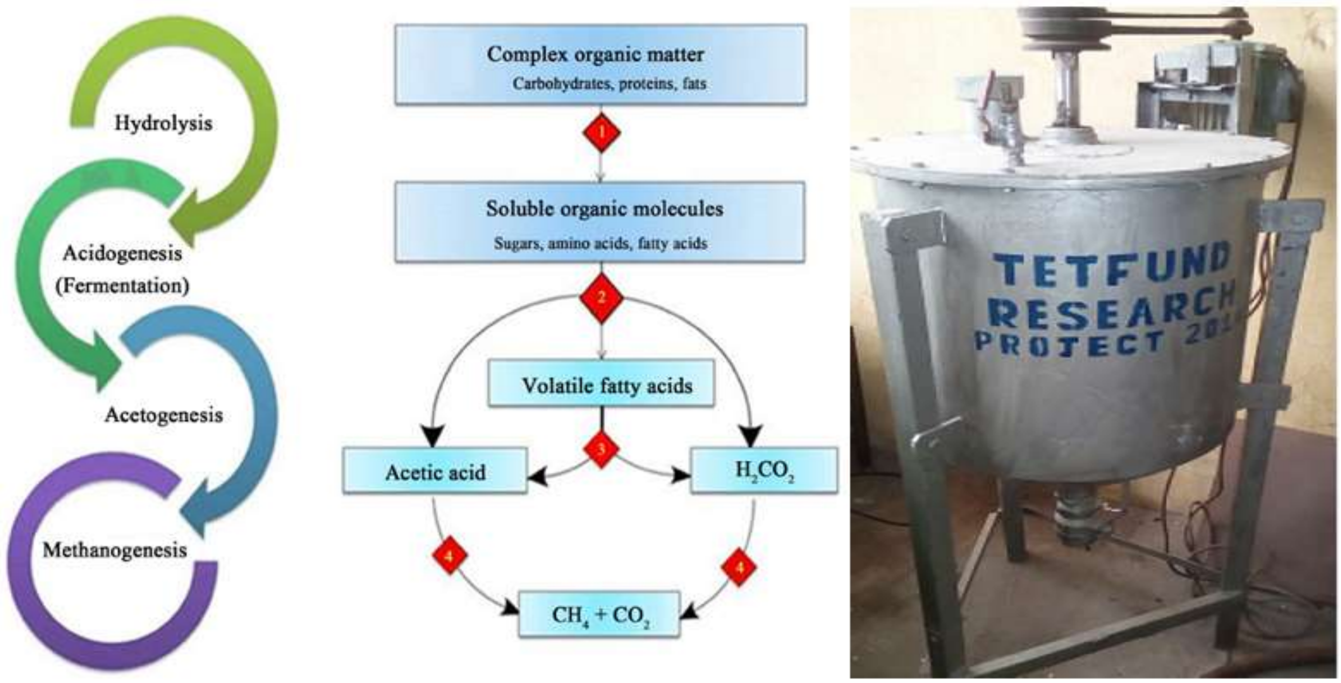

Figure1: Flow chart of anaerobic digestion process (Left) and locally made Anaerobic digester (Right)

3.4 Tests for the Presence of Methane

Methane gas (CH4), which was the major component of the biogas that has combustible characteristic was tested by lighting a match on a Bunsen burner connected to the digester.

3.5 Proximate analysis and pH determination

Proximate composition of the dung was carried out according to the method of AOAC (1984) as described in Ukpabi*et al* (2012). The dung pH was determined electrometrically using glass electrode pH meter.

3.6 Microbiological analysis of biogas producing bacteria from mixture of cow dung and food waste

3.6.1 Total Bacterial Count

Preparation for fresh cow dung and cow dung slurry after mixed up with food waste for bacterial analyses will be done according to the methods described in reported literature (Mohammed, 2015). Cow dung was prepared using serial dilution method. Pour plate technique was employed for the isolation biogas producing bacteria. Before plating, the sample will be incubated at 37 for 30-40 min in incubator for activation of microorganism. Nine millilitres (9 ml) of sterilized distilled water will be used in the test tubes assembled for the serial dilutions. One millilitre (1 ml) of standardized solution will be transferred aseptically into test tube 1 and 1 ml into test tube 2 and the procedure was repeated for each dilution in order to reduce the bacterial load from each test tube. The last 1ml was discarded, while the 0.1 ml of the second and the fourth dilutions was used for the inoculation of the sample and was dispensed in the prepared medium (Nutrient agar) plate, which was properly spread and incubated at 37 for about 24-48 h under *aerobic and anaerobic* conditions to obtain total aerobic and anaerobic bacterial counts. After incubation, the numbers of emergent colonies on each plate were counted using colony counter and the results were expressed as colony forming unit per milliliter (cfu/ml).

3.6.2 Identification and characterization of bacterial isolates

The previously inoculated plates were checked for bacterial growth; the plates with viable culture were picked and sub-cultured on another media to obtain pure cultures of the colony. The isolates were classified based on their colony morphology during digestion notably: *colour, shape, size, surface, edges, margins, elevation and gram staining* as described in previous reports (Gupta*et al.*, 2016; Baki, 2004). The isolates were further identified by comparing and referring to the identification manual of known taxa using the schemes reported in literature (Cowan *et al.,* 1993).

3.6.2 Spore Staining Test and Biochemical Characterization of the Isolates

The morphological examinations of the isolates, some spore staining and biochemical tests were carried out on the isolates with the intention to characterize and identify the organisms. The biochemical tests were; *indole, Coagulase, Urease, Oxidase, Methyl red, Voges- Proskauer, Catalase, Citrate utilization, Hydrogen sulphide* according to Cheesebrough (2006).

3.0 RESULTS AND DISCUSSION

Recent studies revealed that production of biofuel is usually dependent on pH and the volume of the slurry in the anaerobic digester (Suyog, 2022; Meres *et al.,* 2004). Thus, during the course of experimental study, much attention was given to the pH within the digester, with the avoidance of much volume of the slurry in the bio-digester. Generation of gas was measured and recorded on daily basis through the connection of calibrated measuring cylinder with paraffin oil displacement arrangement, while the pH values were also measured from small slurry taken from the outlet device of the digester. The composition of the food waste and the cow dung were presented in Table 3.1 below. The results indicated that the two feedstock contained energy yielding nutrients but at varying concentrations. The carbohydrate values of the food waste (corn cobs 64.50±0.58 g/kg and potatoes peels 58.25±1.60 g/kg) were significantly (p=0.05) higher than the cow dung (25.10±0.50 g/kg). Crude proteins were higher in the cow dung (5.80±0.54 g/kg) than the food wastes (0.10±0.50 g/kg and 0.21±1.41 g/kg). This may suggest the reason for the production of more acids in the combined animal slurry than the combined waste. Likewise, the significant concentrations of carbohydrates in the food wastes may explain that food waste use as feedstock would provide energy more for the microbes to live and sustain the process of biogas production.

Table 3.1: Shows Results of Proximate composition of the substrates

Parameter (g/kg)	Corn cobs	Potatoes peels	Cow dung
Moisture	32.35 ± 0.15	30.50 ± 1.15	20.57 ± 0.02
Ash	1.55 ± 1.20	3.97 ± 0.68	11.10 ± 0.58
Crude Fiber	0.59 ± 0.10	1.88 ± 0.02	38.20 ± 0.20
Crude Proteins	0.10 ± 0.50	0.21 ± 1.41	5.80 ± 0.54
Crude Fats	0.61 ± 0.45	6.10 ± 0.12	4.20 ± 0.40
Carbohydrates	64.50 ± 0.58	58.25 ± 1.60	25.00 ± 0.50

Values are mean ± SD, n=5

The data also shows that the pH decreased possible as bacteria produce more acids in the digester. The decrease in pH was observed more in the combined slurry as it is recorded acidic at the 4^{th} day than the waste slurry which was recorded acidic at 12^{th} day (Table 3.2). This revealed that acids were produced which caused decrease in pH of the slurry. It can be concluded that biogas production increased in the earlier days of the experiment and then started decreasing as acid concentration increased. However, following complete anaerobic degradation, the amount of biogas increases in the second week in all the samples used (Table 3.3).

Table 3.2: pH Values obtained during Biogas production

Day	Cow Dung	Combined Waste
1	6.10	6.58
4	5.95	6.00
8	5.60	6.55
12	5.43	5.20
15	5.90	5.55

The biogas produced was examined and established that the biogas was combustible (containing methane) with a bluish flame that lasted for 50s. The experimental findings of the current study support earlier reports by many other authors who reported similar findings (Bahtiyar, 2013; Kobra *et al.,* 2014). These observations were more pronounced in the combined cow dung with food waste than the food wastes and fresh cow dung slurry as it became acidic on the 4^{th} day as compared to the combined waste which became acidic on the 6th day. This may be attributed to the high contain of crude proteins. The results supported the observation that acid concentration greatly affects the biogas production. The results also indicated that the combined waste produced considerable amount of biogas at the end of the study (18.80 mL) compared to the food waste (12.43 mL) and the cow dung as the least (5.72 mL). The observed findings can be attributed to the organic content of the various waste and possibly, the activities of microbial population of the waste in the degradation process. Thus, the combined waste slurry produces more gas (36.55 ml) than food waste (12.25 ml) and cow-dung slurry alone (20.30 ml) because, the combined waste slurry contains more nutrients compared to them all as it works synergistically for significant production of the biogas. The biogas can be used as a fuel, for cooking at homes or other purposes and the solid residue can be used as organic compost for the production of organic fertilizer.

Table 3.3: Daily Biogas production (mL) from different wastes

Day	Cow Dung	Food waste	Combined (Waste and Dung)
1	1.15	0.80	2.80
2	4.00	1.80	4.95
3	8.20	2.10	7.85
4	9.50	2.85	11.30
5	10.58	3.50	14.80
6	11.20	3.98	15.60
7	11.95	4.90	18.95
8	13.85	5.30	20.25
9	14.50	5.35	23.65
10	14.90	6.90	24.80
11	15.30	7.80	25.75
12	16.32	8.50	26.50
13	16.97	9.55	27.35
14	17.80	10.20	20.95
15	20.30	12.25	36.55
Av. Gas produced	**12.43**	**5.72**	**18.80**

The total bacterial count before and during the digestion process is presented in Table 3.4. The count of aerobic organisms shows a decrease trend from 2.7×10^8 cfu/ml in the first day of digestion to 6.5×10^7 cfu/ml in the 15th day. The anaerobic count was found to have an increasing trend from 1.7×10^7 cfu/ml in the first day of digestion to 2.5×10^7cfu/ml in the last 15th day as seen in (Table 3.4).

Table 3.4: Total Bacterial Counts Before and During the Anaerobic Digestion Process

Days of Digestion	TAC (cfu/ml)	TANC (cfu/ml)
0	2.7×10^8	1.7×10^7
7	2.0×10^8	2.3×10^8
15	6.5×10^7	2.5×10^7

Key: TAC = Total aerobic bacterial counts; TANC = Total anaerobic bacteria count; cfu = Colony forming unit; ml = millilitres

The morphological characterization of isolates from fresh cow dung slurry during anaerobic digestion results are shown in Table 3.5 below. Gram staining and Biochemical identification of isolates from fresh cow dung slurry during anaerobic digestion result is also shown in Table 3.6 below. The results revealed that during anaerobic digestion, two bacterial species were isolated which includes; *Pseudomonas* spp and *Bacillus* spp.

Table 3.5: Morphological characterization of bacteria isolated from fresh cow dung slurry during anaerobic digestion

Characters observed	1st isolate	2nd isolate
Form of colony	Circular	Weakly irregular

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Translucency/opacity	Opaque	Opaque
Elevation of colony	Convex	Undulate
Surface of colony	Rough	Rough
Pigmentation	Pearlescent	Reddish
Cell shape	Bacillus	Pearlescent Bacillus

Table 3.6: Gram-staining and Biochemical identification of isolates from fresh cow-dung slurry during anaerobic digestion

Isolate Code	GR	Coag	Ure	Ind	Oxi	Cat	Glu	Mot	H_2S	Gas	Mb	Cit	VP	SP	Bacteria Isolated
1st	+	-	-	-	+	-	-	+	-	-	+	+	-	-	*Pseudo* spp.
2nd	+	-	-	-	-	+	+	+	+	-	-	+	+	+	*Bacillus* spp.

Key: + = positive reaction, = negative reaction, GR = Gram stain, Coag = Coagulase, Ure = Urease, Ind = Indole, Oxi = Oxidase, Cat= Catalase, Glu = Glucose, Mot = Motility, H_2S = Hydrogen Sulphite, Mb = Methyl blue, Cit = Citrate, VP = Voges-Proskeur, SP = Spore, *Pseudo.* =*Pseudomonas, Staph.* = *Staphylococci*

Therefore, current research shows that *Pseudomonas* spp. and *Bacillus* spp. were isolated from cow dung slurry during anaerobic digestion. This is similar to have been reported by Pratik 20, patrik 30, patrik 40, patrik 50, which were later identified as *Pseudomonas* sp, and VBC 1, VBC 2, and VBC 3 identified as *Bacillus* spp. (Pratiksha and Gishesh, 2012). Furthermore, the two bacteria isolated from cow dung slurry in this research during anaerobic digestion which included *Pseudomonas* spp. and *Bacillus* spp, also shows similarity in accordance with the study where non methanogenic microorganisms from waste matter including *Listeria, Arthrobacter, Pseudomonas* sp., *Citrobacter* sp., *Bacillus* sp., *Escherichia coli, Staphylococcus, Lactobacillus, Flavobacterium,* and *Micrococcus* were isolated from cow dung slurry (Gore *et al.,* 1979). These microbes may be accounted for the breaking down of the composite organic substances to intermediates such as unstable fatty acids which were finally changed to biogas. The isolation of *Bacillus licheniformis* and *Escherichia coli* from biogas digesters were also reported Rabah *et al.,* 2010; Baki 2004). *Bacillus, Yersinia* and *Pseudomonas* species were also responsible for biogas production in cow dung slurry (Oluyega *et al.,* 2006).

4.0 CONCLUSION AND RECOMMENDATIONS

4.1 Conclusion

Conclusively, the potential of biogas production from cow dung and food waste using locally made anaerobic digester have been demonstrated as a good, cheap and alternative source of bio-fuel or clean energy. The production of the gas started on the 7^{th} day and continued to increase till the 15^{th} day of the research work. The results also revealed that the acidity of the feedstock is largely affected the biogas production, especially in feedstock with high protein contents. Also, the rate of gas production could be attributed to the presence of the bacteria isolated during anaerobic digestion which includes: *Pseudomonas* spp. and *Bacillus* spp, Therefore, biogas production when generated in commercial quantity provides an alternative source of sustainable energy, that can serve as a means of waste management and its disposal issues for the whole world particularly, Nigeria.

4.2 Recommendations

i. Since, biogas can be used as a fuel, for cooking at homes or other purposes and the solid residue can be used as organic compost to be utilized for biofertilizer, there is need for comparative analyses of the biogas generated from various feedstock.
ii. The methanogenic bacteria involved in biogas generation should be isolated, identified and characterized at molecular stage to strain levels and then used selectively for biogas production.
iii. Further research should be carried out to access the role of anaerobic digestion on microbial pathogens generally.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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