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CO-DIGESTION OF COW DUNG WITH SOME CEREAL WASTES FOR BIOGAS PRODUCTION

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ABSTRACT

Co-digesting different ratios of cereal parts with cow dung to produce biogas was investigated. Four cereal components gotten from local traders in Ido-Ekiti, Ekiti State, Nigeria, were used for anaerobic digestion. The cereal parts used were millet shaft, sorghum shaft, corn cob, and rice husk. They were ground evenly for homogeneity and were mixed in the ratios of 4:1:3:2, and 2:3:4:1 respectively. Cow dung obtained from Federal University of Technology, Akure, Nigeria farm was soaked for seven days and was used as fungal inoculum by mixing it with the two biomass ratio of varied concentrations. Control experiment was set up without adding cow dung. Four anaerobic digesters were used for the anaerobic digestion of the cereal for thirty days. Physicochemical parameters, namely, pH, temperature, pressure, total solids and proximate composition were determined. The temperature varied from 28 ^oC to 44 ^oC throughout the digestion process; the pressure varied from 0-500 kpa; while the pH varied from 3.7 to 6.8. Microbial population of the cereal was analyzed, before, during and after the digestion process. Bacteria and fungi populations in the digesting materials ranged from 10 x 10^6 to 80 x10^6cfu/ml and 0 x 10^6 and 7 x 10^6 sfu/ml respectively. Twenty two microorganisms were isolated before, during and after the digestion process. These included *Lactobacillus delbrueckii, Micrococcus luteus, Streptococcus pneumonia, Coryne bacterium diptheriae, Clostridium perfringes, Lactobacillus fermenti, Staphylococcus aureus, Bacillus cereus, Escherichia coli, Salmonella enterica, Enterobacteraerogenes, Proteus vulgaris* and *Methanosarcinabarkeri.* The fungi isolated included *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Rhizopusstolonifer, Penicilium italicum, Mucormucedo, Fusariumgram inearum, Mouldmonalia, Rhizopusoryzae,* and *Aspergillusoryzae.* The proximate composition result from the least to the highest value were: ash content, which ranged from 28.84% to 40.22%, moisture content, from 10.77% to 85.70%, fat content, from 1.80% to 9.13%, fibre content, from 4.11% to 32.69%, protein content, from 0.59% to 11.17%, while the carbohydrate content was from 2.77% to 29.07%. The

Sodium content was from 3.84% to 16.88%, Potassium content ranged from 3.29% to 89.46%, Calcium content, 0.01% to 52.37%. Magnesium content, 0.66% to 81.60%, while the Phosphorus content was from 11.41% to 168.33%. The two digesters which contained cow dung had the highest methane content of 65.159% and 65.143% respectively, while the two control experiments had the lowest methane content of 58.49% and 57.36% respectively. These results showed that cow dung has significant impact on the co-digestion with the cereals.

Key words: Agricultural residues, anaerobic condition, biogas, cereal wastes, cow-dung and ligno-cellulosic.

INTRODUCTION

Global depletion of fossil fuels has led to the search for alternative sources of energy. Biomass has the largest potential and can only be considered as the best option for meeting the demand and insurance of future energy (biofuel) supply in a sustainable manner [1].

Biogas is generated when bacteria degrade biological material in the absence of oxygen, in a process known as anaerobic digestion. Biogas is a renewable fuel. It is a mixture of methane (about 65%), carbon dioxide (CO_2), and impurities of hydrogen sulphide (H_2S), and water [2].

The concept of co-digestion with centralized facility had been employed at different corners of the world to make digestion cost-effective because the volume of organic waste wastes generated at a particular site may not be feasible for a treatment plant [3]. Callaghan *et al.* [4] studied co-digestions of cattle manure slurry with chicken manure at 7.5% and 15% total solid content, fish offal, fruit and vegetable wastes, brewery sludge and dissolved air floatation sludge.

Co-digestion of different materials may enhance the anaerobic digestion process due to better carbon and nutrient balance [5, 6]. Das and Mondal conducted a study and observed that there are few data published on the co-digestion of energy crops with manure [3]. Lower methane yield has been reported in co-digestion of manure with straw compared with digestion of manure alone [7], whereas another study reported high methane yield from co-digestion of cow manure and 40% of wheat straw of total solid content of 52. Lertluck *et al.* [8] investigated effect of crop to manure ratio for methane production by co-digestion of energy crops and crop residues with manure, and observed that there is volumetric methane yield increased by 16% in reactors fed with 30% VS of sugar beet tops, grass silage and oat straws along with manure compared to manure alone fed in reactors at a similar loading rate.

Consequently, one of the approaches for improving cost-benefit of cow-dung digesters is to increase their biogas production rate through co-digestion with more biodegradable wastes.

Therefore, this research was aimed at exploring this potential for possible increase in biogas production through the co- digestion of some locally available lignocellulosic agricultural cereal wastes residues and cow-dung mixture under anaerobic condition.

MATERIALS AND METHODS

Collection of Samples

Cow dung used for this study was collected from the Livestock section at the Teaching and Research Farm of the Federal University of Technology, Akure. Similarly the cereals, which comprised of rice husk, maize cob, millet shaft and sorghum wastes, used in this study were all gotten from local traders in Ido-Ekiti, Ekiti-State, Nigeria. Both sample were collected in sterile polythene bag and were transported to the laboratory for further processing and usage.

Digester Construction

The construction of the biodigesters used in this study was carried out at Victory life Enterprises, Akure, Ondo State, Nigeria. Four biodigesters were fabricated, each of height 31 cm, volume of 35 L and with three outlets. Attached to it, is a pressure gauge, a thermometer, and a control knob as shown in Plate 1. The gas pressure variation was measured via the pressure gauge.

Plate 1: Biodigester Set Up for Production of Biogas

Preparation of Waste Materials

Four biodigesters were constructed. Each biodigester has a thermometer and pressure gauge attached to it. About 10 kg of each biowaste were mixed thoroughly with 25 L of water and used to fill the biodigester with each having different mixtures of biowastes, all in ratio 1:4. The ratio of the biowastes and cow dung are enumerated below:

A= Rice husk: Maize cob: Millet husk: Sorghum shaft (4:1:3:2) + 4 kg Cow dung + 25 L Water

B= Rice husk: Maize cob: Millet husk: Sorghum shaft (2:3:4:1) + 4 kg Cow dung+ 25 L Water

AC= Rice husk: Maize cob: Millet husk: Sorghum shaft (4:1:3:2) + 25 L Water

BC= Rice husk: Maize cob: Millet husk: Sorghum shaft (2:3:4:1) + 25 L Water

Each biodigester mixture was mixed thoroughly into a slurry form before pouring into the biodigester. The cereal mixture (AC and BC) without cow dung represents the control experiment.

Isolation of Microorganisms

A total of 22 microorganisms were isolated and characterized before, during and after the digestion process following method of Olutiola *et al.*[9]. Table 1 shows the occurrence of bacteria isolated.

Physicochemical Analyses

The pH of the digesting wastes was determined, before, during and after digestion, using a digital pH meter, (Hanna ECI pH meter, Hanna scientific, USA). Five grams of the waste were collected in a beaker before, during and after digestion, and then a calibrated pH meter was dipped into the samples and readings taken. Similarly, the daily temperature was taken with the aid of a mercury thermometer, while the pressure variation was measured with the aid of pressure gauge (Heise CM dial pressure guage, Minnesota) both attached to the bio-digester as shown in the diagram above.

Proximate and mineral analyses

The proximate compositions of the combined substrates at the initial stage of digestion, the final stage of digestion and the individual substrates were determined by the standard of AOAC [10] and were expressed in percentages. The mineral analyses were carried out by Atomic Absorption Spectrophotometry (AAS) machine (ICE 3300 ACE AAS, Thermofisher scientific, Massachusetts).

Composition of biogas determination

For the collection of the biogas, a small volume of n-Hexane was dispensed in a small glass bottle and the bottle was gently put at the tip of the gas valve of the digester, which was then opened, to collect the biogas sample. The sample was transferred into 20 ml vial glass container with no addition of reagents carried out. The vials were capped with hand tool capper made of aluminum materials. The sample in the glass vials were placed in the sample holder of the HP 7694 Headspace sampler (Gentech scientific, New York). The temperature of the operation of the Headspace sampler was 300 ºC. The Headspace sampler was connected to the Gas

Chromatography (GC 2010 PRO, Shimadzu Corporation, USA) for the injection into the GC column in an automated manner after completing the operational cycle. The standard gas mixture in the glass vials was placed in the Headspace sample hole. The connection with the GC was activated in an automated manner. The standard mixture and samples were analyzed under the same conditions.

RESULT AND DISCUSSION

Bacteria and Fungi populations in the digesting materials are from 1.0×10^{7} to 8.0×10^{7}cfu/ml and 0×10^{6} and 7×10^{6}sfu/g respectively as shown in Tables 1 and 2. The bacterial load decreased during the digestion and later increased towards the end of the digestion process. This could be due to the relatively high count at the end of the digestion and could be traced to factors such as air tight environment, optimum temperature, pH, and various reactions and interactions that would have occurred among methanogens, non-methanogen as also inferred by Okareh *et al*. [11]. The fungal load is reduced compared to the bacteria load in all the digesting materials which can be attributed to the moisture content in the digester which favoured bacteria growth than the fungi growth as described by Henz *et al*. [12].

The twelve bacteria isolated were *Lactobacillus delbrueckii*, *Micrococcus luteus*, *Streptococcus pneumonia*, *Corynebacterium diptheriae*, *Clostridium perfringes*, *Lactobacillus fermenti*, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Salmonella enterica*, *Enterobacteraerogenes*, *Proteus vulgaris* and *Methanosarcinabarkeri*. The Fungi isolated are *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Rhizopus stolonifer*, *Penicilium italicum*, *Mucormucedo*, *Fusarium graminearum*, *Mouldmonalia*, *Rhizopus oryzae*, and *Aspergillus oryzae* in the four biodigesters set up (Tables 3 and 4).

The bacteria isolated are all mesophilic, and thermophilic anaerobic bacteria, same was reported by Al-Kayiem *et al*. [13]. Of interest is the succession of bacteria that takes place during the digestion process. For example *Bacillus cereus* and *Micrococcus luteus* were found at the start of the bio-digestion process as also reported by Al-Kayiem *et al*. [13]. The organisms might have also contributed to the biogas production. There was microbial degradation of the substrate by the bacteria involved which accounts for their succession.

Table1: Bacterial load of the digesting materials

Samples	Before digestion (cfu/ml)	During digestion (cfu/ml)	After digestion (cfu/ml)
A	6.0×10^{7}	1.1×10^{7}	5.7×10^{7}
B	5.0×10^{7}	1.0×10^{7}	8.0×10^{7}
AC	1.5×10^{7}	5.0×10^{7}	6.0×10^{7}
BC	1.0×10^{7}	1.5×10^{7}	1.5×10^{7}

Table 2: Fungal load of the digesting materials

Samples	Before digestion (sfu/ml)	During digestion (sfu/ml)	After digestion (sfu/ml)
A	6.0×10^{6}	2.0×10^{6}	7.0×10^{6}
B	3.0×10^{6}	4.0×10^{6}	5.0×10^{6}
AC	1.0×10^{6}	1.0×10^{6}	3.0×10^{6}
BC	2.0×10^{6}	0.0×10^{6}	2.0×10^{6}

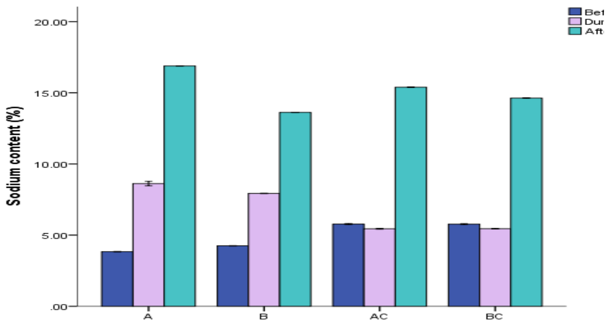
KEY:
A: Rice husk:corncob:millet shaft:sorghum shaft (4:1:3:2) + Cow dung
B: Rice husk:corncob:millet shaft:sorghum shaft (2:3:4:1) + Cow dung
AC: Rice husk:corncob:millet shaft:sorghum shaft (4:1:3:2) without Cow dung (Control)
BC: Rice husk:corncob:millet shaft:sorghum shaft (2:3:4:1) without Cow dung (Control)

Table 3: Occurrence of bacteria in the digesting materials

A			B			AC			BC			Suspected
B	D	A	B	D	A	B	D	A	B	D	A	Bacteria
+	+	-	+	+	-	-	-	-	-	-	-	*L.delbrueckii*
+	+	-	+	-	-	-	-	-	-	-	-	*M .luteus*
-	+	-	+	+	-	-	+	+	-	+	+	*S.pneumonia*
+	-	-	+	-	-	+	-	-	+	-	-	*C.diptheriae*
+	+	-	+	+	-	+	+	-	+	+	-	*C.perfringes*
+	+	-	-	+	-	-	+	-	-	+	-	*L. fermenti*
+	+	-	+	+	-	+	-	-	+	-	-	*S.aureus*
+	+	-	+	+	-	+	-	-	+	-	-	*B. cereus*
+	+	+	+	+	+	+	+	+	+	+	+	*E. coli*
+	-	-	+	-	-	+	-	-	+	-	-	*S.enteric*
-	+	+	-	-	-	-	-	-	-	-	-	*E.aerogenes*
-	-	+	-	+	+	-	-	+	+	+	-	*P. vulgaris*
-	-	+	-	-	+	-	-	+	-	-	+	*M .barkeri*

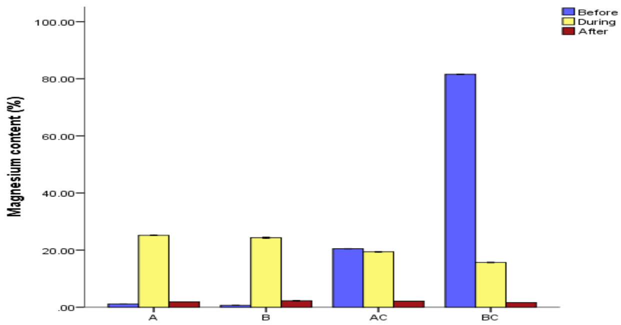
KEY:
A: Rice husk:corn cob:millet shaft:sorghum shaft (4:1:3:2) + Cow dung
B: Rice husk:corn cob:millet shaft:sorghum shaft (2:3:4:1) + Cow dung
AC: Rice husk:corn cob:millet shaft:sorghum shaft (4:1:3:2) without Cow dung (Control)
BC: Rice husk:corn cob:millet shaft:sorghum shaft (2:3:4:1) without Cow dung (Control)
+ : presence, - : absence

Among the four bio-digesters, *Bacillus subtilis, Proteus mirabilis, Micrococcus luteus* and *Clostridium rectum* took part through the process of hydrolysis by secreting enzymes, and hydrolyzing polymeric materials into monomers. There was also change in the aerobic state of the biodigester to an anaerobic stage during the digestion process. During the acidogenic stage, *Lactobacillus fermenti*, *Escherichia coli*, and *Staphylococcus aureus* helped out, while the acetogenic stage was completed by *Clostridium perfringes*. *Methanobacterium barkeri* which was found at the later stage of the digestion process was responsible for the reduction of carbon iv oxide and subsequently the production of biogas.



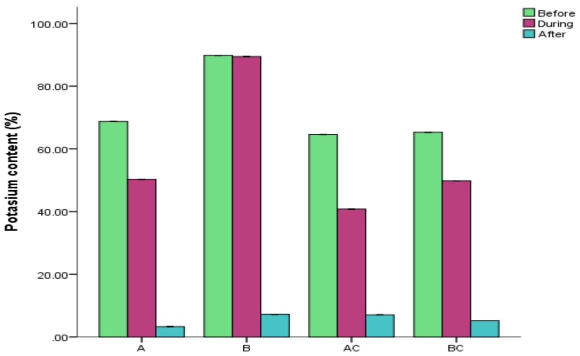
Substrate of different ratios

Fig. 1: Sodium content of the digesting material



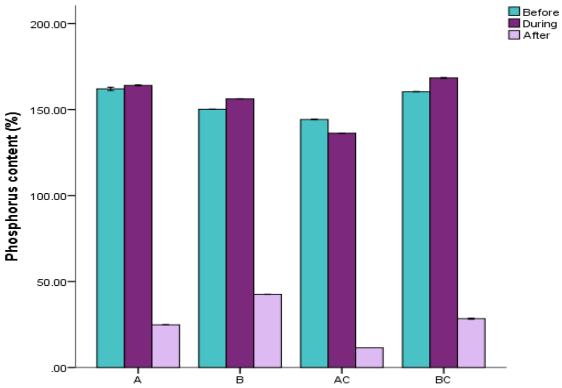
Substrate of different ratios

Fig 4: Magnesium content of the digesting material



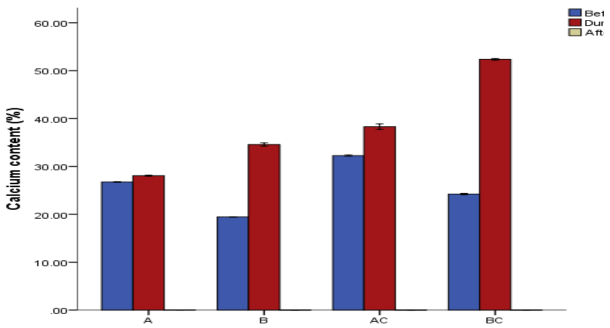
Substrate of different ratios

Fig. 2: Potassium content of the digesting material



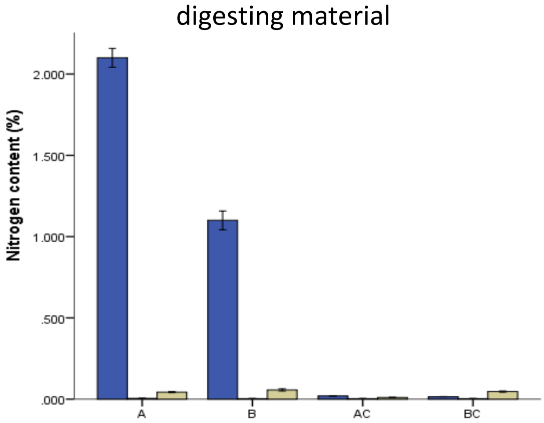
Substrate of different ratios

Figure 5: Phosphorus content of the digesting material



Substrate of different ratios

Fig.3: Calcium content of the digesting material



Substrate of different ratios

Figure 6: Nitrogen content of the digesting material

KEYS

A: Rice husk:corn cob:millet shaft:sorghum shaft (4:1:3:2) + Cow dung

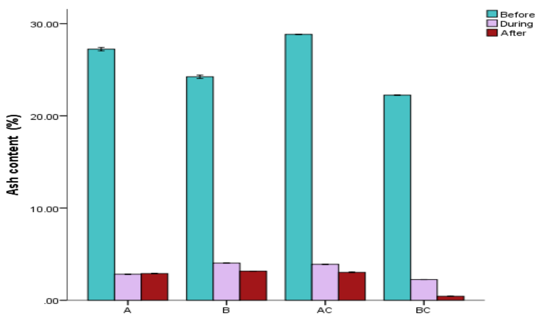
B: Rice husk:corn cob:millet shaft:sorghum shaft (2:3:4:1) + Cow dung

AC: Rice husk:corn cob:millet shaft:sorghum shaft (4:1:3:2) without Cow dung (Control)

BC: Rice husk:corn cob:millet shaft:sorghum shaft (2:3:4:1) without Cow dung (Control)

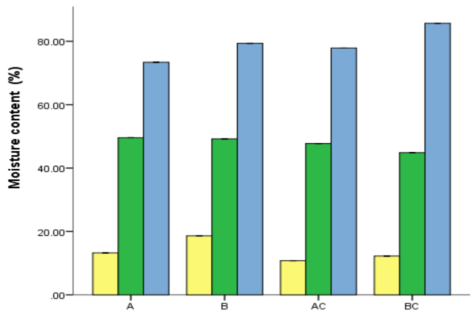
The result of the proximate analyses of the waste showed that the digesting materials contain more nitrogen, phosphorus and potassium. Cow dung helped in the quick degradation of the substrates by acting as spoilage fungi. There was gradual decrease in the protein content of the digesting waste (Figure 13). Nitrogenous compounds in the organic waste are usually proteins which are converted to ammonium by anaerobic digestion [14]. The fibre content also decrease after the digestion process this is in line with the work of Nwodo and Obinna [15] and it was because during digestion fiber is converted to glucose and is utilized by bacteria for their own energy production. The carbohydrate content decrease during the digestion process was due to starch hydrolysis to sugars. The ash content decreased as the digestion progresses. This shows depletion of minerals as a result of microbial degradation by the spoilage fungi to a form that can be readily utilized for energy production, Vincent *et al.* [16]. The fat content increased in digester A, B, and AC, because during anaerobic digestion, bacteria utilized the vegetable waste as feed and converted them into volatile fatty acid thus increasing the fat content, this is also in line with the findings of Sakhawat *et al*. [17].

The high moisture content result is important for anaerobic digestion as reported by Hernandez-Berriel *et al.* [18] as it provides enabling environment for the anaerobic digestion. The moisture content after digestion was greater than before anaerobic digestion due to the fact that during anaerobic digestion in the digester, solid break down occurs in the presence of bacteria which increase the moisture contents especially as seen in digester AC with a moisture content of 85.70



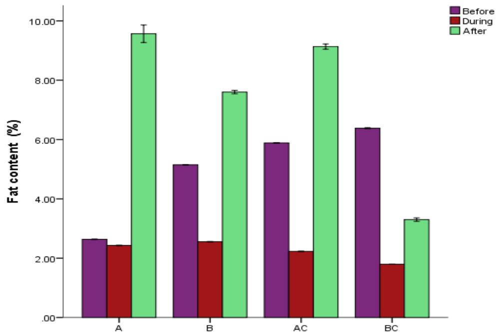
Substrate of different ratios

Figure 7: Ash content of the digesting material



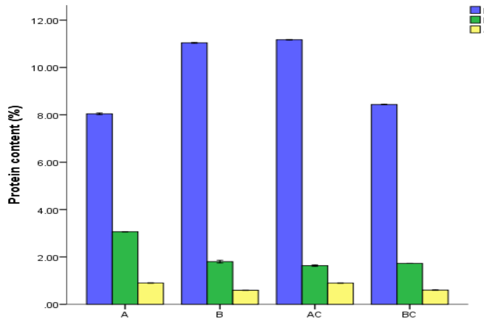
Substrate of different ratios

Figure 8: Moisture content of the digesting material



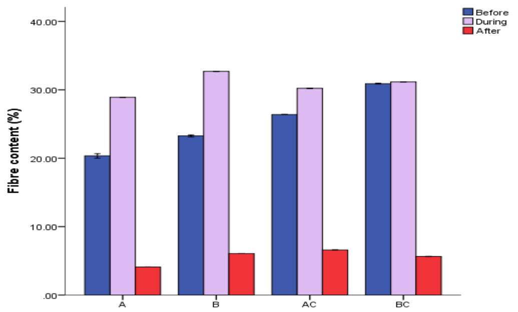
Substrate of different ratios

Figure 9: Fat content of the digesting material



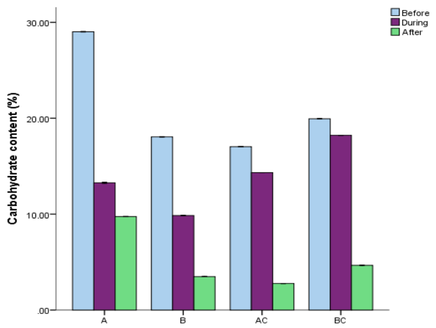
Substrate of different ratios

Figure 11: Fibre content of the digesting material



Substrate of different ratios

Figure 10: Fibre content of the digesting material



Substrate of different ratios

Figure 12: Carbohydrate content of the digesting material

KEYS

A: Rice husk:corn cob:millet shaft:sorghum shaft (4:1:3:2) + Cow dung
B: Rice husk:corn cob:millet shaft:sorghum shaft (2:3:4:1) + Cow dung
AC: Rice husk:corn cob:millet shaft:sorghum shaft (4:1:3:2) without Cow dung (Control)
BC: Rice husk:corn cob:millet shaft:sorghum shaft (2:3:4:1) without Cow dung (Control)

Digester A and B had the highest biogas production rate of 400 kpa and 500 kpa, and it could be due to co-digestion with cow dung. Lertluck *et al.* [8] reported similar result stating that co-digestion of substrates with animal manure produces higher methane yield and biogas production.

The temperature value (ranged from 27 ºC to 42 ºC) obtained from this study is in line with El-Mashad *et al.* [19] who reported that anaerobic digestion of biomass wastes could occur both at mesophilic (25–37 °C) and thermophilic (55–65 °C) temperature ranges. Bjornsson [20] likewise reported that temperature range of 32 ºC and 35 ºC has proven to be most efficient for stable and continuous methane production. The changes in temperature may be due to effects on methanogenic bacteria, since these appear to exhibit similar optimal regions (and also there was increase in temperature as observed in digester A, B, and AC, which was indicative of high rate of microbial activities. Also, Adegunloye *et al.* [21] reported that the high level of temperature usually recorded during biogas production is as a result of activities of methanogens leading to methane production.

The pH value increased and later decreased during the digestion process. This was in agreement with previous study by Adegunloye *et al*. [21] who attributed it to the amount of organic acid produced by the acid bacteria at first. The pH variation may also be due to the action of acetogenic methanogens as they break down sulphur containing organic acid and inorganic compounds as well as the formation of fatty acids [22]. The increase in pH in sample D could be as a result of low fatty acid formation by the organisms as shown in the analysis of lipid content.

Figure 14 shows value of the pressure observed during the digestion process, indicating the rate of biogas production. It was observed that biodigester A and B had the highest biogas production rate of 1300 kpa and 500 kpa respectively. The high value observed in digester A could be due to the reported similar result stating that, co-digestion of substrates with animal manure produces higher methane yield and biogas production.

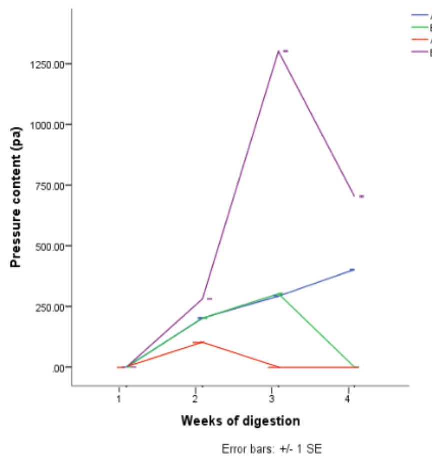


Figure 13: Pressure content of the digesting materials

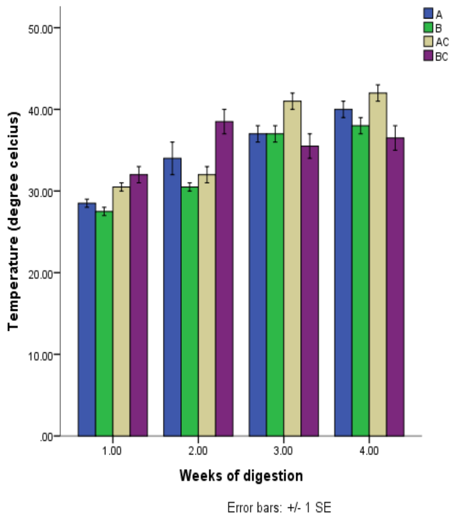


Figure 15: Temperature (^{0}C) of the digesting materials

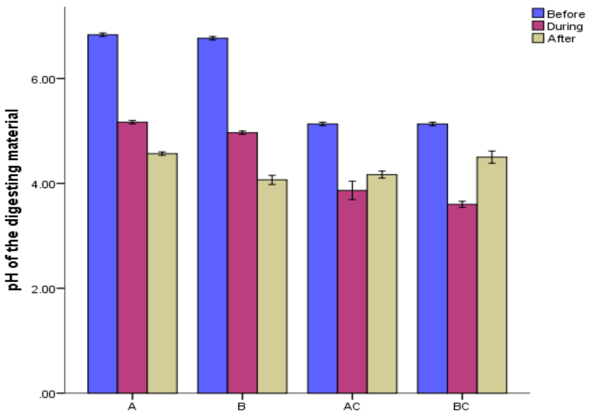


Fig. 14: pH of the digesting materials

KEY:

A: Rice husk:corncob:millet shaft:sorghum shaft (4:1:3:2) + Cow dung
B: Rice husk:corncob:millet shaft:sorghum shaft (2:3:4:1) + Cow dung
AC: Rice husk:corncob:millet shaft:sorghum shaft (4:1:3:2) without Cow dung (Control)
BC: Rice husk:corncob:millet shaft:sorghum shaft (2:3:4:1) without Cow dung (Control)

Percentage composition of the biogas produced as shown in Table 4, revealed that biogas produced consists of ammonia, methane, carbon (iv) oxide, hydrogen sulphide, carbon monoxide. Digesting materials which had cow dung innoculum, showed the highest methane composition of 65.591% and 65.143% respectively as compared to the other two digesters without cow dung innoculum58.499% and 57.316% as shown in Table 7. The high methane content seen in the two digesters is as a result of co-digestion with cow dung due to the supply of suitable microorganisms and missing nutrients by the cow dung [23], The mixing of various substrates i.e concurrent presence in the same anaerobic reactor of different organic wastes in the digesting systems facilitated microbial actions to decompose the waste efficiently [24] and consequently high methane content.

Cow dung has been established by researchers as being superior in quality biogas production over other animal dungs [25]. Due to the high Carbon to Nitrogen C: N ratio in cow dung, it is necessary for the production of biogas. The substrate has very good biogas potential. The percentage methane content and the overall composition, conforms to a typical biogas composition [26]. The methane content of digesters A and B (65.591% and 65.143% respectively) exceeds the methane content of the domestic gas from a commercial gas vendor which has a value of (61.3%) methane, making them suitable to be used as domestic gas.

Table 4: Percentage Constituents of biogas produced

Groups	Methane (CH_4) %	Ammonia (NH_3) %	Carbon Monoxide (CO) %	Hydrogen Sulphide (H_2S) %	Carbon (Iv) Oxide (CO_2) %
A	65.591	0.1867	0.9561	0.4976	32.768
Ac	58.499	0.1036	0.8889	0.6123	39.896
B	65.143	0.0878	0.6324	0.4488	33.687
BC	57.316	0.127	0.920	0.7195	40.917
DG	61.370	0.009	2.056	3.036	33.520

A: Rice husk: corn cob: millet shaft:sorghum shaft (4:1:3:2) + Cow dung
B: Rice husk: corn cob: millet shaft:sorghum shaft (2:3:4:1) + Cow dung
AC: Rice husk: corn cob: millet shaft:sorghum shaft (4:1:3:2) without Cow dung (Control)
BC: Rice husk: corn cob: millet shaft:sorghum shaft (2:3:4:1) without Cow dung (Control)
DG: Domestic gas

CONCLUSION

The study investigated the co-digestion of various ratios of different cereals with cow dung, revealing improved biogas production. It has also revealed that cereals are good substrate for biogas production. Synergies are established during the anaerobic digestion, because nutrients that are lacking in the cereal waste are supplied from the cow dung and vice-versa. Co-digestion of cow dung is, therefore, one way of addressing the problem of lack of enough feedstock for biogas production. Methane which is the major component of biogas can be produced through the co-digestion of cow dung with cereals and also in percentage that meets a typical biogas composition, suitable for domestic use. The effective use of dung would contribute to increase energy security and reduce environmental degradation and greenhouse gases.

RECOMMENDATIONS

Despite the success recorded in this study using co-digestion of Cow dung and ligno-cellulose biomass, there is still that need to further optimize the physicochemical conditions affecting biogas production for possible high production. Finally, other animal and lingo-cellulosic substrate waste should be explored as a possible source of energy.

Alternative energy source can be produced for domestic use from the arrays of cereals wastes generated daily from human activities. This reduces environmental pollution, and produce environmental friendly energy source. Co-digestion appears to be a potential and viable option for generation of alternative renewable source of energy to substitute the fossil fuels. The use of different cereals and cow dung is a promising way of biogas generation, hence this and other types of biomass co-digested with cow dung and the best mixture proportions that could generate more biogas should be studied.

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