EFFECTS OF ANAEROBIC CO-DIGESTION OF ORGANIC WASTES ON BIOGAS YIELD AND SOME PROXIMATE CHARACTERISTICS OF THEIR BY- PRODUCTS

***[1]Chomini, M. S., [1]Peter, M. K., [1]Ayodele, A. O., [1]Mazeli, P.**
[1]Federal College of Forestry, Jos,
NIGERIA
* stevemchoms@gmail.com

ABSTRACT

The effects of anaerobic co-digestion of five ratios of mixtures of cow dung and maize cobs as treatments A, B, C, D and E respectively assessed on biogas yield and some proximate properties of their by-products. Triplicate slurries of these wastes (1:3 w/v) separately fed into 13.6L locally made digesters, under strict anaerobic condition, were monitored for eight weeks retention period. Separate treatment fractions were also subjected to standard methods to determine their proximate compositions before and after anaerobic digestion (AD). The biogas yield was in the order of treatment C (75:25-cow dung: maize cob) > D(50:50- cow dung: maize cob)>A(100:0 cow dung: maize cob) > E(25:75- cow dung: maize cob) > B(0:100 cow dung: maize cob), with treatments C(2522.40ml) and B(1713.20ml) having the highest and lowest average cumulative yields. All treatments recorded % increase in ash, crude protein (CP) and moisture contents(MC), with treatments B(287.91%), E(529.44%) and B(763.60%) showing the highest %increases in CP, MC and ash respectively. All co-digested substrates had percentage bioconversion efficiencies (%BE) greater than the single substrates for total solids (TS) and volatile solids(VS). Treatments D and B recorded the highest (24.75% and 53.12%) and lowest (6.37% and 29.84%) %BE for TS and VS reductions respectively. Similarly, treatments E(56.60%) and B(17.52%), and C(11.43%) and A(9.29%) recorded the highest and lowest %BE for COD and ME reductions respectively. The %reduction in C/N ratio was in the order of treatment A(81.80%)>E(72.39%) >D(62.17%) > A(29.35%) and C(10.41%). The agricultural waste management initiative had provided an effective means of alternative energy generation, veritable industrial biochemical production, which would guarantee sustainable public health and environmental management.

Keywords: Anaerobic, Biogas, Co-Digestion, Cow dung, and Maize Cob.

INTRODUCTION

Globally, the rise in volume of municipal solid wastes (MSW)(agricultural and industrial organics) generation is incommensurate to management strategies adopted. This consequently has engendered numerous socio-economic and environmental health challenges. The technology of anaerobic digestion provides a veritable strategy for organic waste management system, while producing an alternative fuel called biogas and eco-friendly, less toxic and useful effluent - bio-fertilizer (Muyiiya & Kasisira, 2009). The exploitation of agricultural organics for biogas production in Nigeria is still in its infancy (Gupta, *et al*., 2012). Present study therefore focuses on the effects of co-digestion of cow dung and maize cob on biogas yield and some proximate characteristics of their by- products.

MATERIALS AND METHODS

Source of Substrates and Preparation

Locally sourced and dried agricultural organic wastes (cow dung and maize cobs), were pulverized and subjected to preliminary treatments of homogenization and screening, before mixing in predetermined ratios (w/w) (table 1). They were parked in sterile black polythene bags and stored in a cool dry place below 20^{o}C, until use (Chomini, *et al.*, 2015).

Table 1: Treatment description

Treatment	Description	Ratio
A	Cow dung	100:0
B	Maize cob	0:100
C	A + B	75:25
D	A + B	50:50
E	A + B	25:75

Slurry Preparation, Loading and Biogas Measurement

The co-substrate treatments were separately mixed with sterile distilled water in a 1:3 ratio w/v to form different slurries (Ojolo *et al*, 2007). These were independently loaded into 13.6L capacity sterilized biogas reactors in triplicates and anaerobically conditioned, with mercury in glass thermometer and gas delivery pipe fittings. The fifteen (15) experimental units were arranged in a completely randomized design (CRD), under uniform temperature condition in an experimental chamber. A minute of daily manual shake was performed to ensure homogenous condition, and kept for an eight (8) week retention time. (Chomini *et al*., 2015). The biogas production (dm^3/kg) was measured weekly by downward displacement of water by the gas (Ofoefule *et al*., 2010), over an eight-week period.

Proximate characteristics analysis of substrates and spent slurry

Dried pulverized samples of raw and digested substrates (A to E) were analyzed for parameters such as moisture content (MC), crude protein (CP), crude fiber (CF), total fat (TF), nitrogen free extract (NFE), total ash (TA), total solid (TS) and volatile solid (VS), total nitrogen (TN) and total organic carbon (TOC) according to the standard procedure of AOAC (2005). While chemical oxygen demand (COD) was determined by the methods of APHA, (2005).

Metabolizable Energy (ME) Evaluation of the Experimental Substrates before and after Anaerobic Digestion

The prediction equation method of Pauzenga (1985) as reported Dairo and Egbeyemi, (2012), was deployed to determine the metabolizable energy (ME) of all the samples before and after digestion. This was done using the formula:

$$M.E. = 37 \times \%\,CP + 81.8 \times \%\,EE + 35.5 \times \%\,NFE.$$

Where: % CP = Percentage crude protein; % EE = Percentage ether ester (%lipid)
% NFE = Percentage nitrogen free extract (from proximate analysis)

RESULTS AND DISCUSSION

Effects of Anaerobic Digestion of Samples on Average Biogas Yields

The average production(ml) of biogas increased across the treatments, with retention time within the first six weeks of digestion (WOD), followed by sudden decreased at the 7th and 8th week.

The mixed treatment substrates recorded higher biogas yields than the single substrates, with treatment C(75:25, cow dung + maize cob), showing the highest average value (538.0ml) while treatment B(0:100, cow dung + maize cob) had the least (348.70ml) at 6 WOD. Treatment B (0:100, cow dung+ maize cob) gave the least average gas yield throughout the retention time, except at weeks 4 and 5 (Table 2). There was significant difference ($p<0.05$) of gas yield over the substrate type throughout the trial period. The cumulative mean of biogas yield was in the order of treatment C (75:25, cow dung+ maize cob) >D(50:50, cow dung+ maize cob) >A(100:0, cow dung+ maize cob) > E(25:75, cow dung+ maize cob) >B(0:100, cow dung+ maize cob) as shown on Table 2.

Effects of Anaerobic Digestion on Proximate Characteristics and Carbon-Nitrogen Ratio of Treatment Substrates and Spent Slurries

After anaerobic digestion (AD), the contents of moisture, crude protein and ash increased while total lipid decreased for all treatments. Treatments B(287.91%) and C(51.05%), E(529.44%) and B(140.65%), and B(763.60%) and A (55.93%) gave the highest and lowest percentage increases in CP, MC and ash respectively. AD effected % reduction in crude fiber content for all treatments except C with 21.94% increase. Similarly, only treatments B(18.12%) and E(23.29%) had % reductions in nitrogen free extract (NFE) due to AD (Table 2). All treatments showed a general reduction in total solids (TS), volatile solids (VS), chemical oxygen demand (COD), and metabolizable energy (ME) after AD. All mixed substrates indicated higher % bioconversion efficiencies (%BE) over the single substrates for TS and VS reductions, with 50:50 ratio (treatment D) having 24.75 and 53.12 as highest %BE for TS and VS reduction respectively. Treatments E(56.60%) and B(17.52%) gave highest %BE for %COD and %ME reduction, while treatments C(11.43%) and A(9.29%) had the least respectively(Table 3). Treatments B and A had 108.14 and 18.43 as the highest and lowest carbon – nitrogen (C/N) ratios before AD. After AD, all treatment substrates recorded varying % reductions in the order of B(81.80%) > E(72.39%) > D(62.17%) > A(29.35%) > C(10.41%).

Table 2: Mean Gas Production (ml/wk) During Eight Weeks of Anaerobic Digestion

Treatment/Weeks	One	Two	Three	Four	Five	Six	Seven	Eight	Total
A	66.7b	110.0c	177.3c	320.7c	358.0c	393.0b	381.3c	272.0d	2079.0
B	43.3a	78.3ba	134.3a	287.3b	321.3b	348.7a	303.3a	196.7a	1713.2
C	77.7e	120.7d	256.3e	329.3d	482.0e	538.0e	451.7e	266.7c	2522.4
D	76.7d	108.0c	188.0d	328.3d	421.7d	519.3d	437.3d	363.0e	2442.3
E	73.3c	105.3b	157.0b	246.7a	311.3a	427.3c	336.7b	255.0b	1912.6
∑	337.7	522.3	912.9	1512.3	1894.3	2226.3	1910.3	1353.4	**10669.5**

Means along each column bearing different superscripts are significantly different ($P < 0.05$) at 5% level by Duncan's New Multiple Range Test; A(100:0 cow dung: maize cob); B(0:100 cow dung: maize cob); C(75:25-cow dung: maize cob); D(50:50- cow dung: maize cob); E(25:75- cow dung: maize cob)

Table 3: Effects of Anaerobic Digestion on some Proximate properties of the Substrates

Treatment		AS	CL	CF	NFE	CP	MC
A	Before	23.71	13.81	29.85	15.95	12.13	4.55
	After	36.97	1.82	22.16	25.69	19.19	22.17
	%diff	55.93	-86.82*	-25.76	61.07	58.20	387.25
B	Before	2.83	9.54	36.98	43.26	3.06	4.33
	After	24.44	2.19	22.66	35.42	11.87	10.42
	%diff	763.60	-77.04	-38.72	-18.12	287.91	140.65
C	Before	16.41	12.28	14.95	40.16	11.38	4.82
	After	30.69	3.20	18.23	43.48	17.19	26.21
	% diff	87.02	-73.94	21.94	8.27	51.05	443.78
D	Before	15.99	9.31	29.33	32.35	8.44	4.58
	After	34.56	2.91	15.00	45.90	19.44	28.20
	%diff	116.14	-68.74	-48.86	41.89	130.33	515.72
E	Before	6.55	7.47	15.88	60.51	5.31	4.28
	After	30.95	3.19	9.18	46.42	17.33	26.94
	%diff	372.52	-57.30	-42.19	-23.29	226.37	529.44

* = negative (−) Percentage reduction due to anaerobic digestion; AS= ash; CL=crude Lipid; CF= Crude Fiber; NFE = Nitrogen Free Extract

- CP= Crude Protein; MC= Moisture Content; A(100:0 cow dung: maize cob); B(0:100 cow dung: maize cob); C(75:25-cow dung: maize cob); D(50:50- cow dung: maize cob); E(25:75- cow dung: maize cob)

Table 4: Effects of Anaerobic Digestion on TS, VS, COD and ME of the Substrates

Treatment		TS	VS	COD(x10^3)	ME
A	Before	95.45	71.74	31.00	2384.66
	After	77.83	40.86	23.00	2163.17
	%BE	-18.46	-43.04	-25.81	-9.29
B	Before	95.67	92.84	47.00	2533.72
	After	89.58	65.14	25.00	2089.74
	%BE	-6.37	-29.84	-46.81	-17.52
C	Before	95.18	78.77	35.00	2431.32
	After	73.79	43.10	31.00	2087.11
	%BE	-22.47	-45.28	-11.43	-14.16
D	Before	95.42	79.43	27.00	2475.29
	After	71.80	37.24	18.00	2074.49
	%BE	-24.75	-53.12	-33.33	-16.19
E	Before	95.72	89.17	53.00	2462.87
	After	73.06	42.11	23.00	2089.55
	%BE	-23.67	-52.78	-56.60	-15.16

– = Percentage reduction due to anaerobic digestion; TS= Total Solids; VS= Volatile Solids; COD= Chemical Oxygen Demand; ME= Metabolizable Energy; A(100:0 cow dung: maize cob); B(0:100 cow dung: maize cob); C(75:25-cow dung: maize cob); D(50:50- cow dung: maize cob); E(25:75- cow dung: maize cob)

Carbon/Nitrogen Ratios of Experimental Substrates Before and After Anaerobic Digestion

*Treatment	$C/N_{Before\ AD}$	$C/N_{After\ AD}$	$\%C/N_{Red}$
A	18.43	13.02	29.35
B	108.14	19.68	81.80
C	23.85	13.44	10.41
D	28.84	10.91	62.17
E	45.81	12.65	72.39

A(100:0 cow dung: maize cob); B(0:100 cow dung: maize cob); C(75:25-cow dung: maize cob); D(50:50- cow dung: maize cob); E(25:75- cow dung: maize cob)

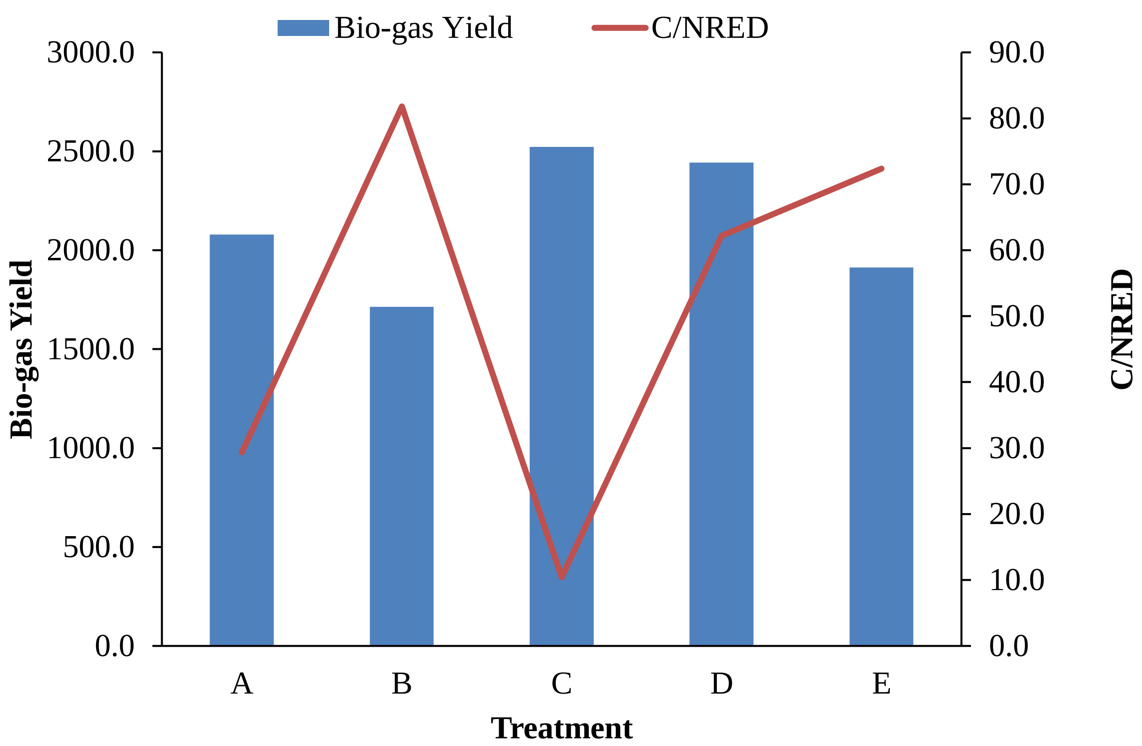

Figure 1: Effects of Anaerobic Digestion on C/N reduction
A(100:0 cow dung: maize cob); B(0:100 cow dung: maize cob); C(75:25-cow dung: maize cob); D(50:50- cow dung: maize cob); E(25:75- cow dung: maize cob)

DISCUSSION

Li et al. (2011), described the biogas yield pattern with digestion time as a function of availability of biodegradable organic matter and high load of microbial community in the substrates. All substrates recorded higher values of % chemical oxygen demand (%COD), prior to digestion, which became reduced at the end of the process. (Li *et al*., 2011). Jha *et al.* (2010), reported an inverse relationship between biogas yield and COD removal, indicating that the methanogenic consortium acclimated very well and consequently led to the depletion of organic matter (COD and VS). The drastic reduction in gas volume after an initial increase, corroborated previous findings of Xie *et al.* (2011). They attributed this to depletion of soluble biodegradable fraction of the substrates, low pH and high concentration of volatile fatty acids. The higher gas yield from the co-substrates especially from ratio 50:50 reiterated the findings of Lehtomaki *et al.* (2007), positing an optimal gas yield from 1:1 ratio of co-digested mixtures of cattle manure, grass silage, sugar beet tops and oat straw. In their views, the biogas yield was significantly ($p<0.05$) influenced by co-digestion as well as mixing ratio of the substrates. The cumulative average volume of biogas yield after 8 WOD is in the order of 75:25 (cow dung + maize cob) > 50:50(cow dung + maize cob) >100:0(cow dung + maize cob) >25:75 (cow dung + maize cob) > 0:100 (cow dung + maize cob)(Table 2).

Eze and Okonkwo (2013), posited that high moisture contents usually facilitate the anaerobic digestion. High water contents are likely to affect the process performance by readily dissolving degradable organic matter. It has been reported that the highest methane production rates occur at 60–80% of humidity (Bouallagui, Cheikh, Marouani & Hamdi, 2003). Hernandez-Berriel *et al*. (2008), found that the methanogenesis took place around day 70, at 70% - 80% moisture condition. However, bioreactors under the 70% moisture regime had a stronger leachate and consequently a higher methane production rate. The increase in crude protein content of all the substrates after AD, suggested that their initial values were adequate for the process (Ofoefule & Ibeto, 2010). Many workers (Sniffen, 1987; El Jalil, Faid & Elyachioui, 2001; Adeyemi & Familade, 2003), attributed this to the release of nitrogenous and non–nitrogenous fractions in addition to microbial single cell protein (Dairo *et al*., 2011), bioconversion of soluble carbohydrates fractions in the substrates to bacterial protein (Vijayan *et al*., 2009), coupled with the production of different enzymes and bio-molecules, which are proteinaceous (Hassan, 2003; Nwanna, 2003).

The reduction in total lipid content of all treatment effluents has been attributed to its metabolism during anaerobic digestion (Eze & Ezeudu, 2012). As a high energy source, lipid metabolism into short chain fatty acids, releases ATP for microbial growth, accounting for lower terminal % lipid (El-Mashad & Zhang, 2010). The reduction in % crude fiber content varied with treatment substrates, except for C. These results were connected to activities of cellulolytic microbes contained in the substrates, production of various enzymes during the vegetative and reproductive phases (Belewu & Belewu, 2005). Akinfemi *et al*. (2010), opined that the type of fungi species as well as nature of the fiber were major determinants for crude fiber fraction reduction. The digestion of fiber fraction had been linked to soluble sugar production, which increases the energy content, part of which is utilized for biogas production, with the residual converted to microbial protein to boost the protein fractions of the resultant effluents (Adenipekun & Okunlade, 2012). The process requires water for solubilization of lignin fraction at the vegetative and reproductive phases thus, necessitating a decrease in moisture content (Tamara *et al.*, 1996).

The high proportion of total solid(TS), volatile solids(VS), chemical oxygen demand(COD) and total organic carbon (TOC) fractions of influents depicted their biodegradable potentials and as an important determinant feedstock for biogas production (Jha, Li, Zhang, Ban, & Jin, 2013). Thus, the depletion is an indicative consumption by fermenting and methanogenic bacteria. The TS content of the wastes had been thought to be comprised of the ash and VS (biodegradable fraction of the organic substrate). The degradation of the VS fraction would have resulted in reduction of the TS of the spent slurries. Uzodinma and Ofoefule (2009), stressed that volatile solids of organic wastes decrease as anaerobes degrade them.

Jha *et al*. (2013), described the efficiency of degradation process in terms of biological conversion of the substrates, with volatile solids or chemical oxygen demand removal. This conversion implied reduction of organic waste simultaneously with production of biogas. Consequently, the differential between the initial and final values of TS, VS and COD reflects the level of removal, which is an index of the bioconversion efficiency (BE). This is reported to be directly proportional to the volume of biogas generated (Bagudo *et al*., 2008). VS and COD removal efficiencies of organic wastes can be enhanced under thermophilic condition than mesophilic temperature (Jha *et al*., 2013). The variations in values of the effluents on these parameters reflect the bioconversion efficiency. According to Umar, Firdausi, Sharifah, and Fadimtu (2013), VS removal efficiency is a vital parameter for determining biodegradation which directly signifies the metabolic status of most delicate microbe groups within the anaerobic system. This consequently denotes the process stabilization.

Macias-Corral *et al*., (2008), pointed out that the highest initial values of %BE for TS, VS and COD removal for mixed treatment (co-digested) substrates indicated apparent synergistic effect which improve nutrient and boost biodegradation. The bioconversion efficiency which is equivalent to TS and VS removal was in the order of treatment D(50:50– cow dung : maize cob) > E(25:75– cow dung : maize cob) > C(75:25– cow dung : maize cob) > A(100:0– cow dung : maize cob) > B(0:100 – cow dung : maize cob)(Table 3). This is similar to observations by Xie *et al*., (2011), who recorded highest volatile solids removal for 1:1 mixing ratio of pig manure blended with grass.

The initial higher metabolizable energy (ME) values of the substrates were considered adequate to effect reasonable biogas production (Ofoefule & Ibeto, 2010). This is used to power the preliminary processes (hydrolysis, acidogenesis and acetogenesis), which culminated in methanogenesis. According to Jha *et al*. (2013), considerable high energy input is required to maintain thermophilic temperature condition for biological activities within the digesters. Blummel, Makkar and Becker (1997), showed that initial low gas production was due to utilization of ATP (energy) for increased microbial growth. As the levels of acetate production increased more gas is produced, which in turn results in lower ATP production (acetogenesis). This is consequent upon the utilization of more of the energy component (total solids, volatile solids, total organic carbon, crude protein, and Lipid), accounted for lower terminal values and invariably, metabolizable energy reduction for all the treatments after digestion (Schafer, Letho &Teye, 2006). The carbon-to-nitrogen ratio (C/N) obtained for the substrates before digestion were in line with Ghasimi *et al*. (2009), stressing that an excessively high C/N ratio would increase acidity of the medium which retards methanogenesis by repressing microbial activities. But when co-digested with those of lower C/N ratio, increased methanogenesis (Karki, et al., 1994).. Co-digestion provides supplementary and complementary nutrient sources which trigger increase in digestion performance and methane yield, (Kacprzak, et al., 2010). This is because animal manure fraction of co-substrate provides high buffer capacity which mainly contains wide variety of nutrients necessary for optimal bacterial growth (Macias-Corral *et* al., 2008). It also promotes synergistic effects, which overcomes the nutrients imbalance.

Plant-based organic substrates is highly ligno-cellulosic, thus mixing with animal wastes would lowers the C/N ratio of the mixture, enhance their digestibility and producing more gas (Adelekan & Bamgboye, 2009). When the C/N ratio is too low, nitrogen is converted to ammonium-N at a faster rate than it can be assimilated by the methanogens, leading to NH_3 poisoning. This could have necessitated the pattern of yield for lower C/N treatments (D and E), despite their status as co substrates. The 75:25 mixing ratio (treatment C) had the highest biogas yield, which is attributed to its relative low lignin content, least C/N (Karki *et al*. 1994).

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CONCLUSION

The present study has revealed the biodegradative capacity of cow dung and maize cob to generate biogas at varying quantities. However, co-digested substrates ratio 75:25 had the optimal biogas production, while 0:100(cow dung : maize cob) had the least. The gas production is affected by C/N ratio and bio-conversion efficiency of total solids, volatile solids and chemical oxygen demand removal, which engenders metabolizable energy change. The anaerobic digestion of the cow dung : maize cob has also elucidated and enhanced some biochemical potentials of the wastes for industrial applications. Further studies should incorporate other indigenous agricultural and industrial organic wastes, under varying controlled conditions for process optimization with the view of improving biogas yields.

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