

## Assessment of Biodegradation Effects of Agro-Wastes (Poultry Droppings and Cow Dung) on Some Physico-Chemical Properties of Their Effluents

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**Abstract** The biodegradation effects of agro-wastes (poultry droppings and cow dung) on some physico-chemical properties of the effluents were assessed. Triplicate slurries (1:3 w/v) of five ratios of poultry droppings and cow dung: (0:1, 1:3, 1:1, 3:1 and 1:0 as treatments A, B, C, D and E) were separately fed into 13.6L locally constructed digesters, under strict anaerobic condition. They were kept for eight weeks retention period. Separate fractions of the undigested and digested treatments were subjected to standard assay procedures to determine their C, N, heavy contents, chemical oxygen demand(COD). The average weekly temperature and the pH before and after digestion of the media were measured. The cumulative biogas yield was in the order of treatment C (2961.0ml) > D(2241.7ml) > E(2197.9ml) > A(2079.0ml) > B(2031.1ml), based on the following mixing ratios 1:1>3:1>0:1>1:0>1:3. The gas production was affected by weekly temperature variation, which peaked at the mesophilic range (40.5±0.3–44.1±0.3<sup>o</sup>C). The resultant pH of the digestates was in the order of 1:3 > 3:1 > 1:1 > 0:1 > 1:0. There was a general reduction in heavy metal contents for all treatment digestates, except Cu, with 200.00, 35.82 and 7.34% as %increases in treatments A, E and C respectively. All treatments indicated reduction in C :N ratios, ranging from 7.93–13.02, in the order of 3:1>1:1>1:3>0:1>1:0. Similarly, there was decrease in COD contents for all treatments due to AD. Consequently, the percentage COD reduction (%CODR) was in the order of treatment D(53.70%) > E(34.15%) > C(29.63%) > A(25.81%) > B(19.23%). The biodegradation process had provided an effective means of alternative energy production, agricultural waste management initiative, which would ensure bioremediation, sustainable public health and environmental management.

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

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### I. Introduction

There is a growing need for alternative energy production globally today. Renewable energy feedstock looks promising and potential option that could be explored to meet this need. Biological conversion of organic substrates to methane (CH<sub>4</sub>) by anaerobic digestion processes powered by microorganisms generate biogas mainly methane and other trace byproducts [1]. The process has many useful benefits, including municipal and industrial waste management, environmentally sustainable and economically viable energy alternative, waste volume reduction, bio-fertilizer source, carbon-emission reduction, prevention of the transmission of pathogenic organisms from surface organic waste dumps [2, 3]. Livestock waste generations have increased with increasing demand for livestock products. Inadequate management strategies and indiscriminate disposal of these wastes and their direct applications on farmlands often pose socio-economic and environmental health risks, as they constitute ugly scenes, generate nauseating odors, breeding ground for pathogenic microbes, rodents and flies [4, 5], as well as sources of underground and surface water pollution, ammonia and GHGs emissions, Phosphorus and heavy metal contaminations of soil and water [6]. Although some heavy metals are required for life's physiological processes by microorganisms (components of metalloenzymes), their excessive accumulation in the living system via the food production chain, often lead to detrimental effects on human health and metal-contaminated environment [7]. The heavy metal composition of the digestates depends on the waste characteristics used for the process as well as the microbial consortiums [8], and their bioremediating capacity [8]. According to [9], the essential parameters required for bioremediation (nature of pollutants, soil structure, temperature, pH, moisture content, hydrogeology, the nutritional state, redox-potential, and microbial diversity), would invariably determine the resultant concentrations of the heavy metals of the digestates [10]. Reduction of heavy metal concentrations often characterized biogas effluents. [11], reported that bioremediating tendency could have accounted for the reduction of Fe, Zn, Pb, Mn and Cu assayed from resultant digestates. This study

therefore focuses on the quantity of biogas produced, from different ratios of mixture of poultry droppings and cow dung, while comparing the heavy metal composition of the digestates.

## II. Materials And Methods

### Preliminary treatment of Experimental Substrates

Poultry droppings and cow dung collected from the poultry farm and the research farm of the Federal College of Forestry, Jos–Nigeria, were subjected to pre-anaerobic digestion treatments, after air drying. The substrates were thereafter mixed in five predetermined ratios (w/w)(Table 1), separately packed into sterilized black polythene bags and stored in a cool dry place below 20°C [12].

**Table 1: Treatment description**

Treatment	Description	Ratio(w/w)
A	PD + CD	0:1
B	PD + CD	1:3
C	PD + CD	1:1
D	PD + CD	3:1
E	PD + CD	1:0

PD = poultry droppings, CD = Cow dung

### Slurry Preparation, Loading and Biogas Measurement

Triplicate of each of the five selected sample ratios were made into slurries by mixing 1.0kg of each with sterile distilled water in a 1:3 ratio w/v, [2]. Each of the resultant fifteen (15) experimental units (slurries) was separately fed into 13.6L capacity sterilized digesters with a thermometer and a gas delivery pipe fittings, and made air-tight to ensure anaerobic condition. They were arranged using completely randomized design (CRD) under a uniform temperature condition within an experimental cubical. Homogenous condition was maintained by one minute manual agitation of each digester daily at a regular interval, for a 56 day retention time. Weekly volume (dm<sup>3</sup>/kg) of biogas production was measured by the method downward displacement of water for eight weeks [13].

### Determination of Iron, Copper, Zinc, Manganase and Lead Contents of Substrates Before and After Anaerobic Digestion

Into separate 250ml conical flasks was 2.0g of each of the non-digested pulverized treatment units separately weighed. A mixture of concentrated nitric, perchloric and sulphuric acids [14] in a ratio of 5:1:1 respectively was used to digest and solubilize it by heating on a hot plate in fume cupboard to dryness at 100°C [15]. 1.0ml of HNO<sub>3</sub> and 3.0ml of HCl (aqua regia) were added to the digestate [16]. After cooling and leached with 5ml of 2M HCl, the resulting extracts was used for the determination of Fe, Cu, Zn, Mn and Pb, with the aid of atomic absorption spectrophotometer (AAS) [17]. The same procedure was followed for the digestates resulting from each of the experimental units after 8(WOD).

### Analytical Methods

The chemical oxygen demand (COD) content of each experimental units before and after anaerobic digestion(AD) was determine using the method of [18], which Incorporated Spectrophotometer DR 2800, according to 8000-Reactor.

The Kjeldahl method [14].was used to determine the nitrogen content of the experimental units before and after AD. It was calculated using the formula:-

$$\% \text{ nitrogen} = \frac{(a-b) \times 0.01 \times 14 \times c}{d \times e}$$

Where:-a = titre value for digested sample; b = Titre value for the blank; c = Volume to which the digest was made up with distilled water; d= Aliquot distilled; e = Weight of dried sample.

The organic carbon (OC) composition was assessed based on standard procedure of [19]. While temperature variation during anaerobic digestion (AD) was measured using a mercury in glass thermometer (range 0 to 100°C) [20] and pH(before and after AD) were measured with a digital pH meter (Model 526,Germany).

## III. Results

### Effects of Anaerobic Digestion on Biogas Yield and Temperature and pH,

Biogas generation steadily increased within the first six weeks, after which a sudden decline was observed at the 7<sup>th</sup> and 8<sup>th</sup> weeks of digestion (WOD), in all the digesters. During this period, treatment C(1:1- poultry droppings + cow dung) maintained a steady lead(621.0ml/kg), followed by treatments D(3:1- poultry droppings + cow dung ), E(1:0 - poultry droppings + cow dung) B(1:3 - poultry droppings + cow dung), while A(0:1 - poultry droppings + cow dung) generated the least(393.0ml/kg). Cumulative yield was in the order of

treatments C(2961.0 ml/kg) > D(2241.7 ml/kg) > E(2197.9 ml/kg) > A(2079.0 ml/kg) > B(2031.1 ml/kg) (Table 2).

During the digestion period, there was a gradual rise in average weekly temperature, which peaked at the mesophilic range (40.5±0.3 - 44.1±0.3°C), with a sudden decline during the last two weeks of digestion (Table 3). These variations affected the biogas yield pattern, depending on nature of and ratio of treatment mixtures (Figure 1).

**Effects of Anaerobic Digestion on Some Heavy Metals**

Before anaerobic digestion (AD), treatment E had the highest copper (Cu, 33.50mg/kg), manganese (Mn, 296.00mg/kg) and zinc(Zn, 846.50mg/kg), while treatment C was in iron(Fe, 1988.50mg/kg) and lead(Pb, 147.25mg/kg) respectively. Treatment A recorded the least contents of Cu, Mn, Zn, and Fe, while D had the least of Pb. After AD, there was a general reduction in contents of the metals for all treatment digestates, except Cu in treatments A, E and C with percentage increase of 200.00, 35.82 and 7.34% respectively (Table 4).

**Effects Of Anaerobic Digestion On Carbon – Nitrogen Ratio and Chemical Oxygen Demand (COD) Contents of the Digestates**

The organic carbon(OC) and total nitrogen (TN) content of the treatments were A(35.75% and 1.94%), B(45.33% and 2.12%), C(31.92% and 2.49%), D(51.71% and 2.51%) and E(37.03% and 2.59), giving a carbon – nitrogen (C/N) ratios in the order of B(21.38) > D(20.60) > A(18.43) > E(14.30) > C(12.82). However, after digestion all treatment digestates recorded a remarkable reduction in the C/N ratio ranging from 7.93 to 13.02. Digestate of treatment D and E had the highest (59.27%) and least(12.94%) %C/N reduction respectively (Table 5). All the mixed substrates indicated higher values of %C/N reduction than the single substrates in the following order 3:1> 1:1 > 1:3 > 0:1 > 1:0.

There was a general reduction in chemical oxygen demand (COD) contents of all substrates after anaerobic digestion (AD). The AD effected a decrease in COD contents from a range of 26x10<sup>3</sup> to 54x10<sup>3</sup> before digestion to 19x10<sup>3</sup> to 27x10<sup>3</sup> after AD. Consequently, the percentage COD reduction (%CODR) was in the order of treatment D(53.70%) > E(34.15%) > C(29.63%) > A(25.81%) > B(19.23%). Between the two single substrates, poultry droppings (E) had a better %CODR than the cow dung (A) (Table 5).

**Table 2: Mean Biogas Production (ml/kg) During Eight Weeks of Anaerobic Digestion**

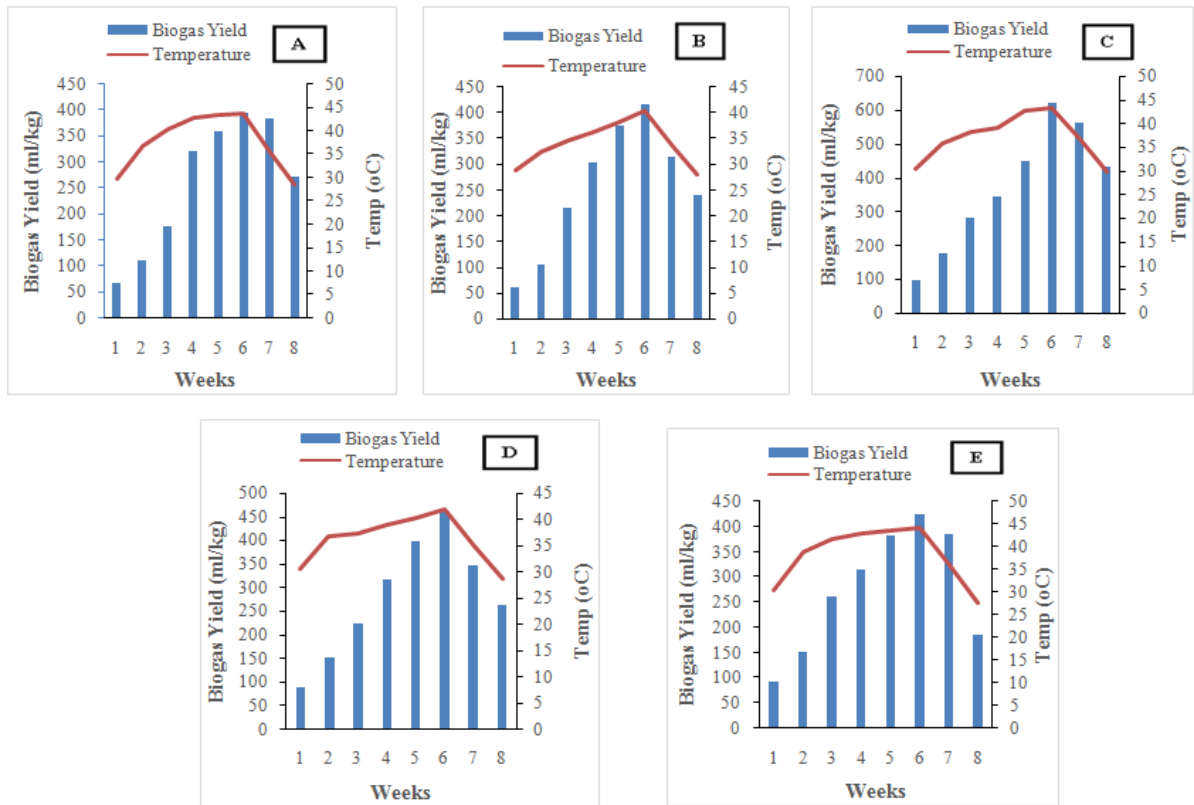
Tmt	Week								Total
	1	2	3	4	5	6	7	8	
A	66.7 <sup>d</sup>	110.0 <sup>c</sup>	177.3 <sup>a</sup>	320.7 <sup>b</sup>	358.0 <sup>a</sup>	393.0 <sup>a</sup>	381.3 <sup>b</sup>	272.0 <sup>b</sup>	2079.0
B	62.0 <sup>e</sup>	105.0 <sup>d</sup>	214.0 <sup>d</sup>	304.7 <sup>d</sup>	376.7 <sup>d</sup>	415.7 <sup>d</sup>	314.0 <sup>c</sup>	239.0 <sup>c</sup>	2031.1
C	98.3 <sup>a</sup>	176.7 <sup>a</sup>	280.3 <sup>a</sup>	345.7 <sup>a</sup>	447.3 <sup>a</sup>	621.0 <sup>a</sup>	562.0 <sup>a</sup>	429.7 <sup>a</sup>	2961.0
D	86.7 <sup>c</sup>	150.0 <sup>b</sup>	221.7 <sup>c</sup>	315.7 <sup>c</sup>	396.7 <sup>b</sup>	462.3 <sup>b</sup>	345.3 <sup>c</sup>	263.3 <sup>b</sup>	2241.7
E	93.3 <sup>b</sup>	150.7 <sup>b</sup>	262.7 <sup>b</sup>	316.3 <sup>c</sup>	382.3 <sup>c</sup>	423.3 <sup>c</sup>	385.0 <sup>b</sup>	184.3 <sup>d</sup>	2197.9
Σ	407.0	692.4	1156.0	1603.1	1961.0	2315.3	1987.6	1388.3	11510.7

Means along each column bearing different superscripts are significantly different (P < 0.05) at 5% level by Duncan’s New Multiple Range Test; Tmt = Treatment

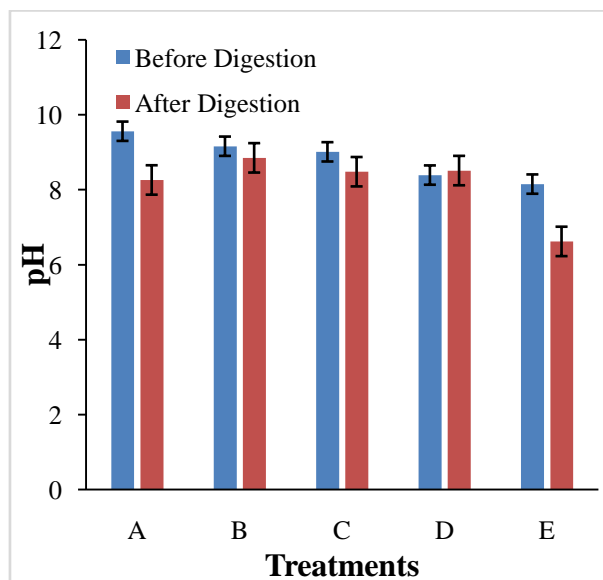
**Table 3: Temperature (°C)Variation of Samples during Eight Weeks of Anaerobic Digestion**

Tmt	Week							
	1	2	3	4	5	6	7	8
A	29.8±0.3	36.6±1.5	40.3±1.9	42.7±0.1	43.2±0.4	43.5±0.3	35.7±0.6	28.5±0.3
B	28.7±0.5	32.3±1.4	34.6±0.9	36.3±0.8	38.4±0.2	40.5±0.3	33.8±0.1	27.9±0.2
C	30.4±0.7	35.7±0.4	38.2±0.4	39.0±0.5	42.5±0.3	43.2±0.3	36.6±0.2	29.8±0.3
D	30.4±0.2	36.8±0.9	37.3±0.5	38.9±0.4	40.4±0.3	42.0±0.7	34.9±0.6	28.6±0.5
E	30.3±0.1	38.9±0.8	41.5±1.1	42.8±0.4	43.5±0.3	44.1±0.3	36.4±0.2	27.6±0.2
MAT(°C)	24.3±2.0	27.0±3.0	27.0±1.3	26.3±2.1	25.0±3.3	22.6±1.1	24.9±2.04	28.7±1.8

Tmt = treatment; MAT = Mean Ambient Temperature



**Figure 1:** Effects of Temperature Variation on Volume of Biogas Production from treatment substrates A(0:1-poultry droppings+ cow dung); B(1:3-poultry droppings + cow dung); C(1:1-poultry droppings+ cow dung); D (3:1-poultry droppings+ cow dung) and E(1:0 -poultry droppings + cow dung)



**Figure 2:** pH of Substrates before and after Digestion

**Table 4: Effects of Anaerobic Digestion on some Heavy Metal (mg/kg) Contents**

Tmt		Cu	Mn	Zn	Fe	Pb	
A	BAD	11.25	157.00	443.75	1053.00	84.75	
	AAD	33.75	0.11	0.04	2.37	11.25	
	%EAD	200.00	-99.93	-99.99	-99.77	-86.73	
B	BAD	18.00	250.00	533.25	1404.25	129.25	
	AAD	15.25	0.04	0.15	0.89	16.00	

	<b>%EAD</b>	-15.28	-99.99	-99.97	-99.94	-87.62
<b>C</b>	BAD	27.25	253.50	675.00	1988.50	147.25
	AAD	29.25	0.04	0.36	2.23	21.00
	<b>%EAD</b>	7.34	-99.98	-99.95	-99.89	-85.74
<b>D</b>	BAD	28.25	277.50	825.25	1724.25	50.00
	AAD	26.50	0.04	0.30	0.89	22.00
	<b>%EAD</b>	-6.19	-99.99	-99.96	-99.95	-56.00
<b>E</b>	BAD	33.50	296.00	846.50	1782.25	113.50
	AAD	45.50	0.07	0.61	2.31	48.25
	<b>%EAD</b>	35.82	-99.98	-99.83	-99.87	57.49

Tmt = treatment; BAD = Before Anaerobic Digestion; AAD = After Anaerobic Digestion; %EAD = Percentage Effects of Anaerobic Digestion

**Table 5: Carbon/Nitrogen Ratios of Experimental substrates Before and After Anaerobic Digestion**

*Tmt	OC <sub>BAD</sub>	TN <sub>BAD</sub>	OC <sub>AAD</sub>	TN <sub>AAD</sub>	C/N <sub>BAD</sub>	C/N <sub>AAD</sub>	%C/N <sub>Red</sub>	COD <sub>BAD</sub> (x 10 <sup>3</sup> )	COD <sub>AAD</sub> (x 10 <sup>3</sup> )	CODR (%)
<b>A</b>	35.75	1.94	23.70	1.82	18.43	13.02	29.35	31	23	25.81
<b>B</b>	45.33	2.12	22.14	1.91	21.38	11.59	43.65	26	21	19.23
<b>C</b>	31.92	2.49	15.54	1.96	12.82	7.93	38.38	27	19	29.63
<b>D</b>	51.71	2.51	16.27	1.94	20.60	8.39	59.27	54	25	53.70
<b>E</b>	37.03	2.59	22.65	1.82	14.30	12.45	12.94	41	27	34.15

OC= Organic Carbon, TN = Total Nitrogen, C/N = Carbon/Nitrogen ratio, %C/N<sub>Red</sub> = Percentage Carbon/Nitrogen reduction due to anaerobic digestion, AD = anaerobic digestion; COD<sub>BAD</sub> = Chemical Oxygen Demand Before Anaerobic Digestion; COD<sub>AAD</sub> = Chemical Oxygen Demand After Anaerobic Digestion; %CODR=Percentage Chemical Oxygen Demand reduction

#### IV. Discussion

The progressive increase in biogas production with time corroborated the findings of [21], who inclined it to availability of digestible fraction at the onset, which declined with rise in gas production, [22], reported similar steady increase in gas production up till the 6<sup>th</sup> week of digestion,(WOD), which was followed by a sharp decreased at the 7<sup>th</sup>. They attributed this to depletion in the quality of substrate in the reactor and the rate of fermentation. [23], suggested the effects of depletion of soluble biodegradable fractions, accumulation of volatile fatty acids and a low pH, on the yield, after an initial increase. [24], opined that High concentrations of ammonia due to low C/N ratio could lead severe inhibition of methanogenesis.[25], reported that methanogenesis inhibition depending on the nature of the substrates, inoculum, temperature, pH, and acclimation periods. The effects of mixing ratios engendered significantly (p< 0.05) high biogas production over single substrates. This according to [26], was attributed to a dilution effect of chemical oxygen demand and volatile fatty acid concentrations to optimal range, as well as reduction of the lag phase period of methanogenises. Biogas yields based on the mixing ratio was in the order of 1:1>3:1>0:1>1:0>1:3. This agreed with [27], who reported a significantly (p<0.05) effect of co- digestion with mixing ratio 1:1on gas yield. [28], maintained that co-digesting different livestock wastes with cassava peels at a mixing ratio of 1:1 had significant effect in increasing average cumulative biogas yield. [21], reported biogas yields was in the order of 3:1, 1:1 and 1:3 when cattle dung was co-digested with maize cob. [29], pointed that co-digestion provides positive synergisms, mainly attributed to more balanced nutrients and increased buffering capacity, bacterial diversities in different wastes and the supply of missing nutrients by the co-substrates. [30], noted other physicochemical properties like high volatile solids and sufficient pH(6.5 to 8.0) and optimization strategies provided by co-digestion to improve biogas production [31, 32].

The effects of temperature on biogas yield have been studied by many workers. [33]. Who posited a positive correlation between rise in temperature and yield. They reported a 43% higher methane yield at 35°C relative to 25°C. [34], reported 65.3%, 64.0% and 62.0% as methane contents in the biogas at digestion temperature of at 35°C, 30°C and 25°C, respectively. They opined that decrease in temperature had a negative effect on the microbial metabolic rate, leading to sharp COD decreased, 40% and 39% drop in gas production and volatile solids (VS) removal. This effects of sudden temperature drop was observed in the present study at the 6<sup>th</sup> week, which considerably affected the gas yield pattern across the treatments (Figure 1). This corroborated the findings of [33], who described similar scenario as breakpoint, the beginning of biological stress, beyond which the methane production rate decreased sharply [12].

The average pH value of the treatment slurries ranged from  $8.15 \pm 0.03$  to  $9.56 \pm 0.02$  before AD, with treatments A and E having the highest and lowest values respectively. After AD, all treatment digestates recorded a reduction in resultant pH values, ranging from  $6.62 \pm 0.04$  to  $8.85 \pm 0.01$  except treatment D. All co-substrates had higher resultant pH values than the mono-substrates (Figure 2). Different workers have reported different pH ranges, depending on the types of substrates and other operating conditions [35, 36]. The drop in pH values was attributed to increased volatile fatty acids production by acidogenic bacteria, during hydrolysis and acidogenesis [29, 37]. The reduction inhibits the acidification, destroy methanogenic bacteria activity and leads to failure of digester ultimately. The increase in pH as observed in digestate of treatment D, has been attributed to rapid metabolic degradation of organic acids and intense proteolysis which releases ammonia [38]. Similar pattern of pH profile was obtained by [39]. [33], opined that pH depression, which suppresses biogas formation could be enhanced by buffering to raise the pH to around neutral or alkalinity, usually preferred by methanogens [40]. The resultant pH of the digestates was in the order of  $1:3 > 3:1 > 1:1 > 0:1 > 1:0$ , suggesting superior buffering effects of co-digestion on degradation media, making it conducive for the methanogens and methanogenesis [41]. [41], showed that co-digestion of organic wastes enhances a reduction in carbon-to-nitrogen (C/N) ratio, which engenders ammonia build-up, increase pH and methanogenic activity.

The reduction in the levels of the heavy metals occasioned by anaerobic digestion, agrees with the findings of [42], indicating that these elements were needed for normal growth of bacteria involved in anaerobic digestion (AD) of organic wastes. Insufficient supply of these nutrients in the digestion medium should therefore be compensated for by applying smaller nutrient loads otherwise a reduced efficiency of the system would result [43]. The increase in Cu level after AD, corroborated the reports of [44], who related the increase to the nature of the organic substrates. [45], have found that methanogens are more inhibited when exposed to heavy metals than acidogens, while some heavy metals are more toxic to anaerobic bacteria than others during acidogenesis and methanogenesis. [46], found Cu to be the most toxic metal to anaerobic bacteria, among six different metals studied while Pb was the least. Manganese is required by microbes for the formation of manganese peroxidase, an enzyme which aids in the Lignin and lingo-cellulosic degradation [47]. [7], reported that microbial community under co-digestion could experience selective inhibition by heavy metal due to different tolerant levels leading to stratification of the community structurally and functionally. This, as stressed by [48, 49], could disrupt some microbial pathways, making them more sensitive to some metals than others, resulting in selective inhibition. This could result in decline of both numbers and diversity of organisms relying on those pathways [50].

The C/N ratios obtained for the substrates prior to digestion were similar to [42], stressing that an excessively high C/N ratio would increase acidity of the medium which retards methanogenesis. Excessive N obtained from very low C/N ratio is converted to ammonium-N at a faster rate than it can be assimilated by the methanogens, leading to  $\text{NH}_3$  poisoning. On the contrary, a very high C/N ratio meant higher acidity of digesting medium which retards methanogenesis. Co-digestion condition has been reported to balance the negative effects arising from both extremes (too high and too low) C/N ratios [29]. This is due to its capacity to provide buffering conditions for the degradative microbes. This could have informed the pattern of results in the study.

The efficiency of the anaerobic process was described in terms of biological conversion of the substrates based on volatile solids (VS) or COD removal [51]. Thus, the quantitative differential of COD before and after AD, is indicative of its removal or reduction, which invariably is equivalent to organic material converted into biogas by methanogens [52]. Co-digestion has been reported to give higher CODR than single substrates [53, 51]. Although the current study gave a CODR favored by high substrate mixing ratio (3:1), however, the ratio 1:1 generated the highest cumulative biogas. This indicated that other prevailing factors in the digesting medium might have resulted in low gas production. [53], reported similar scenario, which was described as antagonism condition, where co-substrates condition(s) intended to work against inhibition rather promoted it, resulting into relative reduction in gas production. This could be attributed to delay in recovery from shock due to sudden drop in temperature.

## V. Conclusion

The biodegradative capacity of co-digested poultry droppings and cow dung to generate biogas reflected the process efficiency. Cumulative biogas production was in the order of treatments  $C(2961.0 \text{ ml/kg}) > D(2241.7 \text{ ml/kg}) > E(2197.9 \text{ ml/kg}) > A(2079.0 \text{ ml/kg}) > B(2031.1 \text{ ml/kg})$ . The gas production was affected by temperature variation, while the resultant pH of the digestates was in the order of  $1:3 > 3:1 > 1:1 > 0:1 > 1:0$ . There was a general %reduction in heavy metal contents of the digestates, except Cu. The process effected various % reductions in C/N ratio and COD. Co-substrates had higher C/N reduction in the order of  $3:1 > 1:1 > 1:3 > 0:1 > 1:0$ , while CODR was in the order of  $3:1 > 1:0 > 1:1 > 0:1 > 1:3$ . The anaerobic digestion co-digested of poultry droppings and cow dung has engendered reduction in heavy metal, thereby revealing its bioremediating potential..

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