
MICROBIAL POTENCY OF BIOGAS PRODUCTION FROM CO-DIGESTED WATER HYACINTH AND POULTRY LITTER, TREATED AND UNTREATED WITH WOOD ASH

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ABSTRACT

Iron fabricated bio-digesters A and B (32L each) were used to investigate microbial potency of biogas production from co-digested water hyacinth and poultry litter (5.76kg) mixed in the ratio of 1:5, using 0.4kg wood ash treatment for digester A and 18L of water for each digester. The digesters contents were stirred daily to ensure homogenous dispersion of constituents. Gas production, measured in liters was obtained by water displacement. Total microbial count, ambient and slurry temperatures, slurry pH, volume of gas produced and gas flammability readings were taken. Correlations, regression and t-test analyses were used at $p=0.05$. Results showed that digester A produced more gas over the retention time (32days), and had an earlier onset of flammability (4 days) compared with digester B. Both digesters had temperature range of 28°C–36°C. Ambient temperatures were lower than that of the digesters (28°C–34°C). Both digesters started with a slurry pH of about 6, which then dropped to 4, rose to 7, then sustained slightly below 6 for digester B, and slightly above 6 for digester A. Biogas production and total microbial count for digester A had a strong positive correlation, while those of digester B had a weak negative correlation. T-test analysis revealed significant difference ($p<0.05$) in biogas production, total microbial count and pH variation between the digesters. Gas composition analysis for both digesters showed methane concentrations to be above 60%. Pathogenic bacteria like *Staphylococcus* spp., *Klesiella* spp., *E. coli* and *Salmonella* spp. were isolated from both digesters. It was concluded that wood ash treatment significantly increased biogas production and enhanced the biogas production process from co-digested water hyacinth and poultry litter.

Keywords: Bio-gas, co-digested, microbial, production, poultry litter, treated, untreated and water hyacinth.

Introduction

1.1 Biogas production

Biogas is a methane-rich gas that is produced from anaerobic digestion of organic materials. It's a blue burning gas that can be used for cooking, heating and lighting. It has a heating value of 22MJ/m³ (Itodoet *al.*, 2007). Biogas consists of 50 – 70%, methane 30 – 40%, carbon dioxide 5

– 10%, hydrogen 1 – 2%, nitrogen 0 – 3%, water vapour and traces of hydrogen sulphide, carbon monoxide and oxygen. It is colourless, relatively odourless and flammable. It is also stable and non-toxic. It burns with a blue flame and has a calorific value of 4500 –6000kcal/m³ when its methane content ranges between 60 – 70% (Igoniet *al.*,2008; Mshandete and Parawira 2009).

Biogas production is a complex biochemical reaction found to take place under the action of delicately pH sensitive microbes mainly bacteria in the presence of little or no oxygen. Three major groups of bacteria (hydrolytic, acidogens/acetogens and methanogens) are responsible for breaking down the complex polymers in biomass waste to form biogas at anaerobic conditions and animal manure has been established as major sources of this gas (Boriet *al.*,2007).The anaerobic process that yields biogas is represented in fig.1.

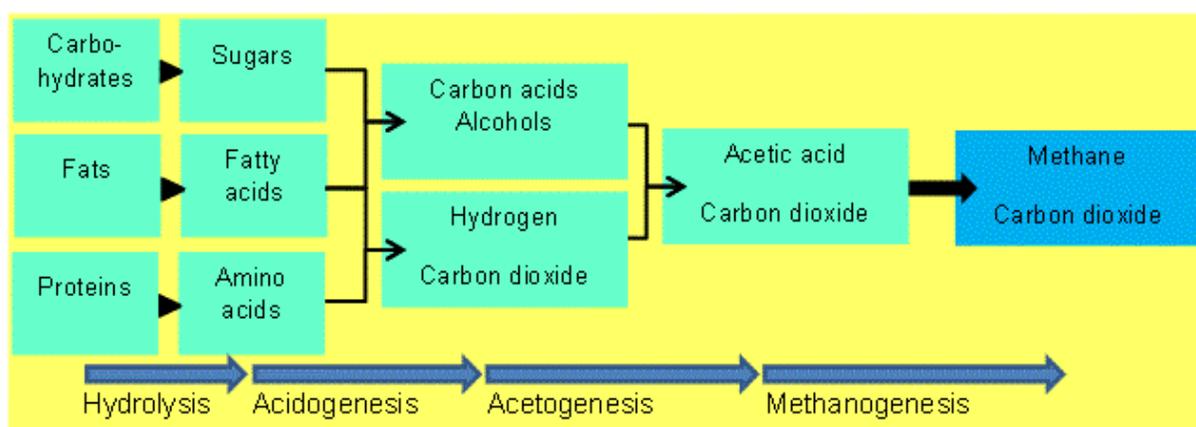


FIGURE 1: Observed Conditions/Changes in the Anaerobic Process BISIPLAN (2012)

1.2 Role of Microbes in Biogas Production.

WRAP (2010) have defined “anaerobic fermentation/digestion” which is responsible for biogas generation as ‘a process of controlled decomposition of biodegradable materials under managed conditions where free oxygen is absent, at temperatures suitable for naturally occurring mesophilic or thermophilic anaerobic and facultative bacteria and archaea species, that convert the inputs to biogas and whole dig estate’.

The anaerobic digestion of organic matter as has been stated occurs in three steps. The first step(hydrolysis), which is carried out by strict anaerobes such as Bactericides, Clostridia and facultative bacteria such as Streptococci etc, involves the enzyme-mediated transformation of insoluble organic material and higher molecular mass compounds such as lipids, polysaccharides, proteins, fats, nucleic acids, etc. into soluble organic materials, i.e. to compounds suitable for the use as source of energy and cell carbon such as monosaccharides,

amino acids and other simple organic compounds. In the second step (acidogenesis), another group of microorganisms ferments the break-down products of the first step into acetic acid, hydrogen, carbon dioxide and other lower weight simple volatile organic acids like propionic acid and butyric acid which are in turn converted to acetic acid. In the third step, these acetic acid, hydrogen and carbon dioxide formed from the second step are converted into a mixture of methane and carbon dioxide by the methanogenic bacteria which are acetateutilizers, like *Methanosarcinaspp.* And *Methanothrixspp.* and hydrogen and format utilizing species like *Methanobacterium*, *Methanococcus*, etc (Yadvika, *et al.*, 2004).

1.3 Water Hyacinth and Poultry Litter in Biogas Production

Water hyacinth (*Eichhorniacrassipes*) is one of the fastest growing aquatic weed known to man. They are free-floating perennial aquatic plants with broad, thick, glossy, waxy leaves with long, spongy and bulbous stalks (Jagdishet *al.*, 2012). Water hyacinth and its tendency of fast growth would have great potential if seen as a raw material for biogas production as it is rich in nitrogen, essential nutrients and has a high content of fermentable matter (Chankya *et al.*, 1993).

Anaerobic digestion has been identified as a well-established process for treating many types of organic waste, both solid and liquid. Poultry manure is seen to have a higher fraction of biodegradable organic matter when compared with other livestock wastes. As such, the digestion of poultry manure/liter and a range of other agricultural wastes have been successfully evaluated. Chicken manure is also an important waste for anaerobic digestion due to its biogas potential (Karaalpet *al.*, 2013).

The research “Microbial Analysis and Biogas Yield Of Water Hyacinth, Cow Dung and Poultry Dropping Fed Anaerobic Digesters” conducted by Asikong, *et al.*, (2014) revealed that higher biogas yield can be achieved by the combination of different biogas feedstock. The co – digestion of water hyacinth and poultry dung has been revealed to produce more biogas than individual digestion of the substrates (Jagdishet *al.*, 2012; Asikonget *al.*, 2014; Jagdishet *al.*, 2011).

1.4 Efficiency of Microorganisms in the Anaerobic Digestion Process

The anaerobic digestion process, having been identified as being microbiological breakdown or degradation (digestion) of organic materials in the absence of oxygen, consisting of several interdependent, complex sequential and parallel biological reactions in the absence of oxygen, during which the products from one group of microorganisms serve as the substrates for the next, resulting in transformation of organic matter (biomass) mainly into a mixture of methane and carbon dioxide (Parawira, 2004). This then means that the whole anaerobic digestion process is completely dependent on the performance of the microorganisms responsible for this process. The performance of microorganisms in the anaerobic digestion process was stated by Itodo,

(2007) to depend on the following factors; temperature, pH, total solids concentration of the slurry, digester type and design, presence of toxic ingredients in the waste stream and the carbon to nitrogen ratio of the slurry. Yadavika, *et al.*, (2004) opined that 'since it is carried out by a consortium of microorganisms and depends on various factors like pH, temperature, HRT, C/N ratio, etc., it is a relatively slow process'. Lack of process stability, low loading rates, slow recovery after failure and specific requirements for waste composition are some of the other limitations associated with it (Van der Berg and Kennedy, 1983). Anaerobic fermentation being a slow process, a large HRT of 30–50 days is used in conventional biogas plants. The effectiveness of microorganisms therefore depends on the chemical and environmental conditions of the bioreactor and slurry.

2.0 MATERIALS AND METHODS

2.1 Sample Collection; the samples used for this study were water hyacinth, poultry litter and wood ash.

Water hyacinth used for this study was obtained from River Benue in Makurdi, Benue State of Nigeria.

Overnight fresh poultry waste was collected from a poultry farm in Federal Low-cost Housing Estate in North bank, Makurdi, Benue State of Nigeria.

Wood ash was collected from a burned sawdust dump in a wood market in North bank, Makurdi of Benue state.

All samples were transferred in clean plastic bags. The bio digesters used were iron fabricated 32L capacity.

2.2 Experimental Set up

The whole water hyacinth (leaves, stem and root) on collection was dried under the sun for two weeks, chopped into smaller pieces and ground using a mortar and pestle.

5.76kg of the ground water hyacinth (0.96kg) and fresh overnight poultry litter (4.8kg), mixed at a ratio of 1:5 was treated by mixing with 0.48kg of wood ash and soaked in a plastic water bath for seven days to allow for partial decomposition of the waste by aerobic microbes which are known to be better at breaking down cellulose (Fulford, 1998).

5.76kg of untreated ground water hyacinth (0.96kg) and fresh overnight poultry litter (4.8kg) mixed at a ratio of 5:1 was also soaked in a plastic water bath and left for seven days.

2.2.1 Charging of the digesters

Thirty two liters (32L) iron fabricated digesters were used. The Water hyacinth/poultry litter mixtures were charged up to $\frac{3}{4}$ of the digesters leaving $\frac{1}{4}$ head space for collection of gas. The

treated water hyacinth/poultry litter mixture was mixed into digester A with 18L of water, while digester B contained untreated Water hyacinth/poultry litter and 18L of water. The ratio of water hyacinth/poultry litter to water was 1:3. The digester contents were stirred adequately and on daily basis to ensure homogenous dispersion of microbes in the mixture. Gas production measured in Liters was obtained by water displacement by gas, collected in an iron gas cylinder. The anaerobic digestion was batch operated for 32 days.

2.3 Analytical Factors

Total microbial count, ambient temperature, slurry temperature, volume of gas produced, slurry pH and gas flammability readings were taken daily throughout the retention period, and presented in tables and graphs.

2.3.1 Microbial analyses

The microorganisms in the waste were cultivated and identified using surface viable count method (Miles and Misra, 1938)

2.3.1.1 Isolation of bacteria in the waste

One gram of the waste was weighed and transferred into sterile test tubes. Sterile saline solution (10ml) was transferred to the test tubes containing the waste samples. The mixture was shaken to obtain uniformity. It was then allowed to set and the supernatant served as the inoculum. Using a sterile loop, a loop full of the supernatant was collected and streaked on the nutrient agar plate. The plates were incubated at 37°C for 48 hours. After the incubation period, the plates were carefully inspected for growth of bacteria.

2.3.1.2 Identification of bacteria

Some suspected colonies of pathogenic bacteria from the isolation above were identified with selective media. Gram-negative rods were grown on MacConkey agar, ceftrimide and desoxycollate citrate agar. Cocci shaped organisms were grown on mannitol agar. In this media, pathogenic bacteria present in each of the wastes were identified.

2.3.1.3 Isolation and Identification of Fungi

The same procedure adopted for isolation and identification of bacteria above was also used for that of fungi in wastes. But in place of nutrient agar, Sabouraud dextrose agar (SDA) was used. 1g of the raw waste was collected with the sterile loop and streaked on SDA plates. The plates

were incubated at 25 to 28°C for 48 hours. The fungi present in each of the waste were identified by microscopy.

2.3.1.4 Total Viable Count (Number of Living Micro-Organisms)

The method used was surface viable count. The suspension obtained from the isolation of bacteria was diluted with sterile distilled water using sterile pipette. The aim was to obtain a dilution that contained approximately 30 cells per 0.015ml or 0.015 volumes per drop. Agar plates were divided into eight segments with an indelible marker. A drop of the suspension was inoculated on each segment. These plates were then incubated for 24 hours at 37°C. Developed colonies were counted from the equation below

$$\text{Mean count} = \frac{\text{number of colonies in each segment}}{8}$$

$$\text{Total viable count} = \frac{\text{mean count} \times \text{dilution factor}}{\text{Vol. per drop}}$$

$$\text{Dilution factor} = 10^4$$

$$\text{Volume per drop} = 0.015\text{ml}$$

2.4 Storage of Gas Produced

Gas produced was analyzed with gas spectrometer and stored in an iron gas cylinder.

2.5 Statistics

1. The statistics used was correlation and regression analysis, to determine the relationship between biogas production and total microbial count for both digesters
2. t-test analysis was carried out to compare biogas production, pH variations and total microbial count for both digesters.

3.0 RESULTS

Represented on table 4.1 are the biogas productions and temperature variations of both digesters (A and B), so also ambient temperature variations, observed over the retention time period of 32 days. From the table, digester A had an earlier onset of flammability (11th day) while digester B started producing flammable gas on day 15. Digester A is seen to have produced more gas over all, while digester B produced more gas in the flammable phase. The temperature variation of both digesters is seen to be almost identical, yet slightly higher than the ambient temperature variation.

Graph 4.1 represents biogas produced by both digesters A and B against the retention time. The graph shows that digester A achieved a higher gas production peak of 15L, while digester B produced more gas after its peak compared with digester A which gas production dropped sharply after the peak.

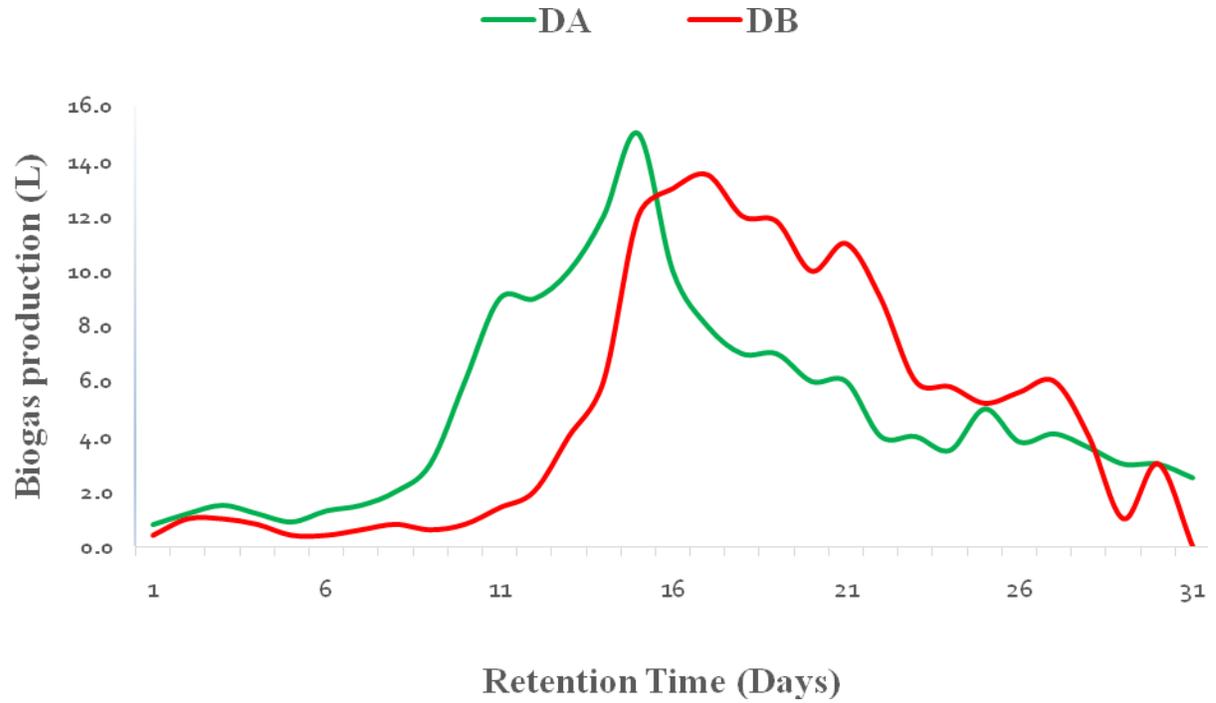
Graph 4.2 shows the ambient temperature variations, and temperature variations of digester A and B and plotted against the retention time. In the graph, digester A and B have almost identical temperature readings, while the ambient temperature is seen to have been relatively lower than those of digester A and B.

Graph 4.3 is the total microbial counts of digester B and B plotted against the retention time. Both digesters are seen to have experienced a steady increase in microbial count, until achieving a peak, after which they both experienced a steady decline. However, digester A achieved a higher peak.

Table 1: Gas production, temperature variation and flammability

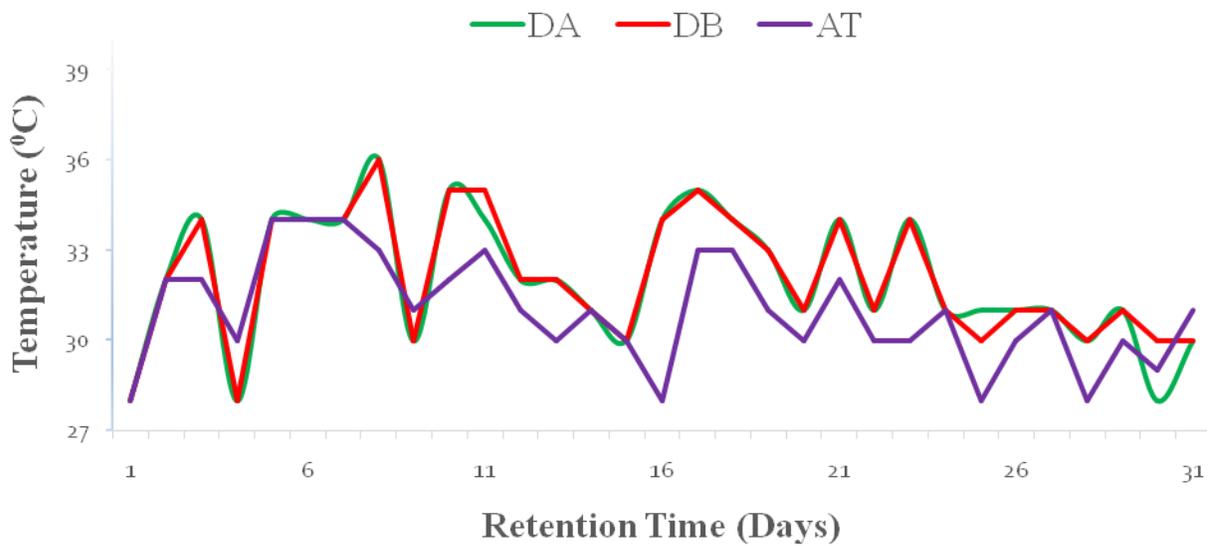
Retention time	Biogas Production (L)			Average Temperature (°C)						
Flammability Days	DA	DB	DA	DB	AT	DA	DB	AT	DA	
DB1	0.8	0.4		28	28	28		nil	nil	2
	1.2	1.0		32	32	32		nil	nil3	
1.5	1.0		34	34	32		nil	nil4		1.2
0.8		28	28	30		nil	nil5		0.9	0.4
34	34	34		nil	nil6			1.3	0.4	34
34	34		nil	nil7			1.5	0.6		34
34		nil	nil	8			2.0	0.8		36
33		nil	nil	9			3.0	0.6		30
31		nil	nil	10			6.0	0.8		35
32		nil	nil11			9.0	1.4		34	35
flame	nil12			9.0	2.0		32	32	31	flame
nil13			10.0	4.0		32	32	30		flame
	12.0	6.0		31	31	31		flame	nil15	
15.0	12.0		30	30	30		flame	flame16		
10.0	13.0		34	34	28		flame	flame17		
8.0	13.5		35	35	33		flame	flame18		
7.0	12.0		34	34	33		flame	flame19		
7.0	11.8		33	33	31		flame	flame20		
6.0	10.0		31	31	30		flame	flame21		
6.0	11.0		34	34	32		flame	flame22		
4.0	9.0		31	31	30		flame	flame23		
4.0	6.0		34	34	30		flame	flame24		
3.5	5.8		31	31	31		flame	flame25		
5.0	5.2		31	30	28		flame	flame26		
3.8	5.6		31	31	30		flame	flame27		
4.1	6.0		31	31	31		flame	flame28		
3.6	4.0		30	30	28		flame	flame		29
3.0	1.0		31	31	30		flame	flame		30
3.0	3.0		28	30	29		flame	flame		31
2.5	0.0		30	30	31		flame	-		

Keys: DA: Digester A, DB: Digester B, AT: Ambient temperature



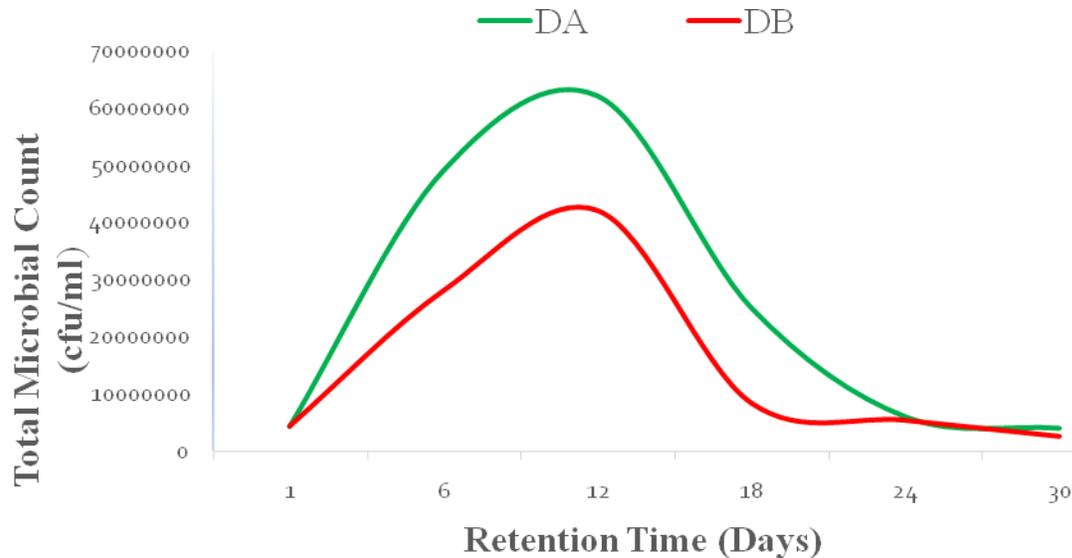
Keys:DA: Digester A, DB: Digester B

Figure2: Graph of Retention Time vs Biogas production of digesters A and B.



Keys: DA: Digester A, DB: Digester B, AT: Ambient temperature

Figure 3 : Graph of Retention Time vs Temperature variations of digesters and ambient temperature



Keys: DA: Digester A, DB: Digester B

Figure 4 : Graph of Retention Time vs Total Microbial Count of digesters.

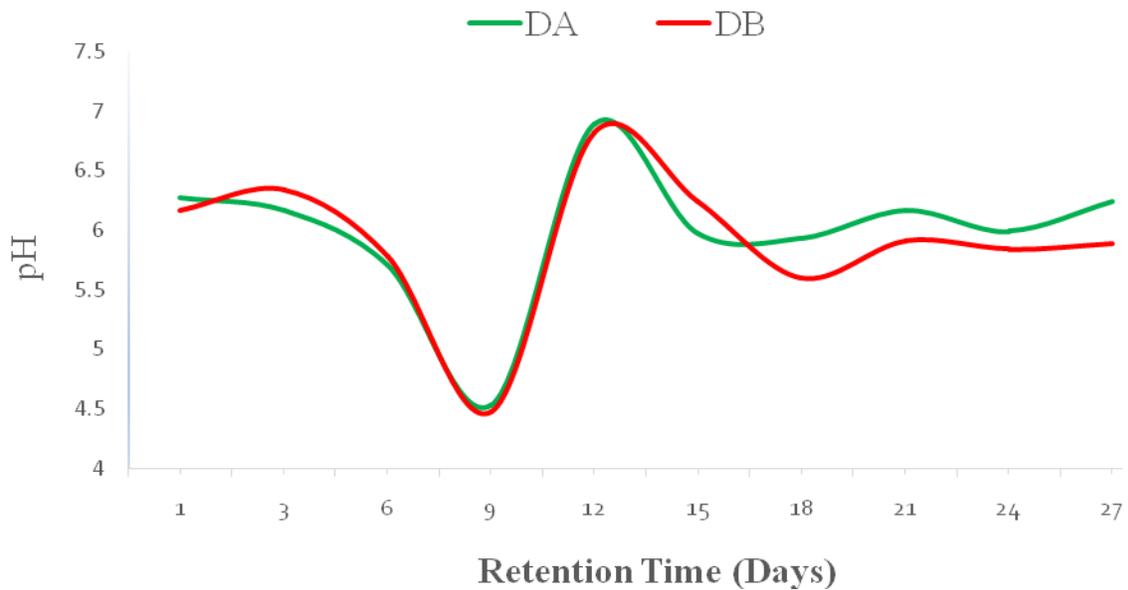
Figure 5 consists of the slurry pH of both digesters recorded over the period of the retention time. Both digesters are seen to have started with a slurry pH of just over 6, which then dropped to 4 (very acidic), rose to neutral position and then was sustained at just below 6 for digester B, and just above 6 for digester A.

Table 2 showed the correlation coefficient matrix between biogas production and total microbial count for both digesters. The biogas production and total microbial count for digester A are seen to have a strong positive correlation, while that of digester B is seen to have weak negative correlation.

Figure 6 is the regression line plot for biogas production vs total microbial count for digester A. It shows a positive curve, meaning an increase in microbial count results in an increase in biogas production.

Figure 7 represents the regression line plot for biogas production vs total microbial count for digester B. the graph is seen to have a slightly negative curve which implies that biogas production for digester B does not increase with increase in total microbial count, but rather reduces.

Table 3 shows the paired T-test values for biogas production, pH variations and total microbial counts compared between both digesters obtained over the period of the retention time. Both the T value and the probability values of the comparisons are listed. It is observed from the table that there was a significant difference in biogas production, total microbial count and pH variation between both digesters, with the most significant difference observed in biogas production, followed by pH variation and total microbial count having the least significant difference.



Keys:DA: Digester A, DB: Digester B

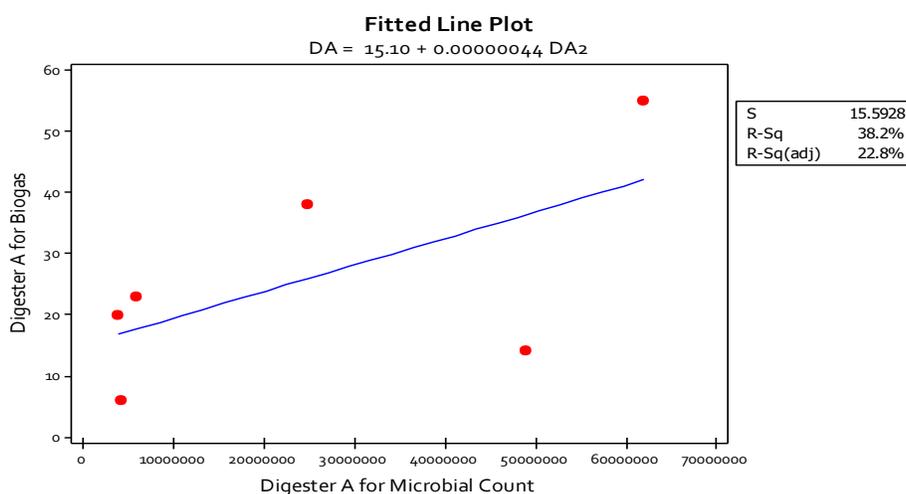
Figure: Retention Time vs pH variation of digesters

Table 2: Summarized correlation coefficient matrix

	A1	A2	B1	B2
A1	1	0.619	0.586	0.603
A2	0.619	1	-0.042	0.971
B1	0.586	-0.042	1	-0.165
B2	0.603	0.971	-0.165	1

Keys:A1 = Digester A biogas production, A2 = Digester A total microbial Count,

B1 = Digester B Biogas production, B2 = Digester B total microbial Count.

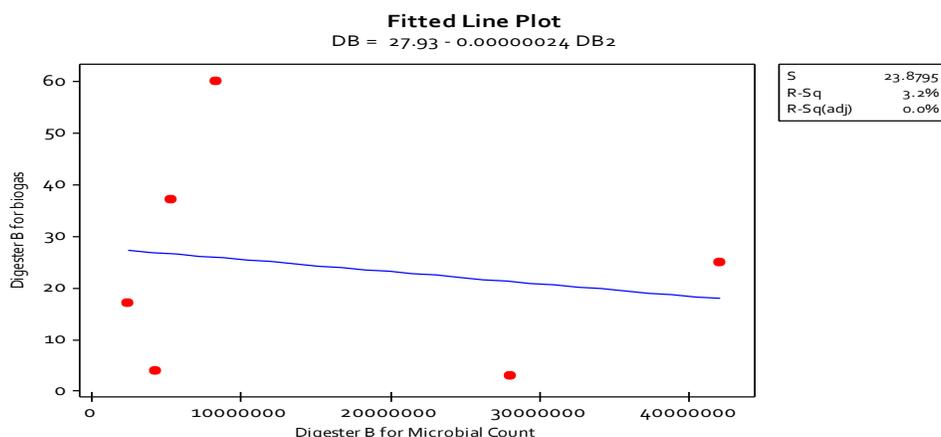


The regression equation $Y = a + bX$ is $DA = 15.1 + 0.00000044 DA2$

Keys:DA: Biogas production for digester A, DA2: Total microbial count for digester A

a: 15.1, b : 0.00000044

Figure 6: Regression line plot for digester A, (biogas production vs total microbial count)



The regression equation $Y = a + bX$ is $DA = 27.93 - 0.00000024 DA2$

Keys:DB: Biogas production for digester B, DB2: Total microbial count for digester B.

a: 27.93, b: - 0.00000024

Figure 7: Regression line plot for digester B, (biogas production vs total microbial count)

Table 3: Paired T-Test values for biogas production, total microbial counts and pH variations for both digesters

	BioGas production DA	Microbial Count DA&DB	pH variation DA& DB
T-Value	0.34	2.39	1.21
P-Value	0.738	0.063	0.259

Keys:DA: Digester A, DB: Digester B

Figure 8 is the pie chart representation of the composition of biogas produced from digester A. The methane level is seen to be about two times the volume of carbon dioxide, while other gases like hydrogen sulphide, carbon monoxide and ammonia all constitute a minute proportion in the composition.

Figure 9 is the pie chart representation of the biogas composition of biogas produced from digester B. methane value is seen to be above 60%, carbon dioxide is also observed to be about half of the methane volume while other gases occupy about 2% of the composition.

Table 4 shows the list of pathogenic bacteria and fungi isolated from the slurry from both digesters. It is observed that mostly yeasts were the fungi isolates while bacteria had a greater diversity.

Table 5 shows the cost of items used in the production of biogas from the substrates (water hyacinth and poultry litter). It is observed that the digester and pumping machine are responsible for the bulk of the costs.

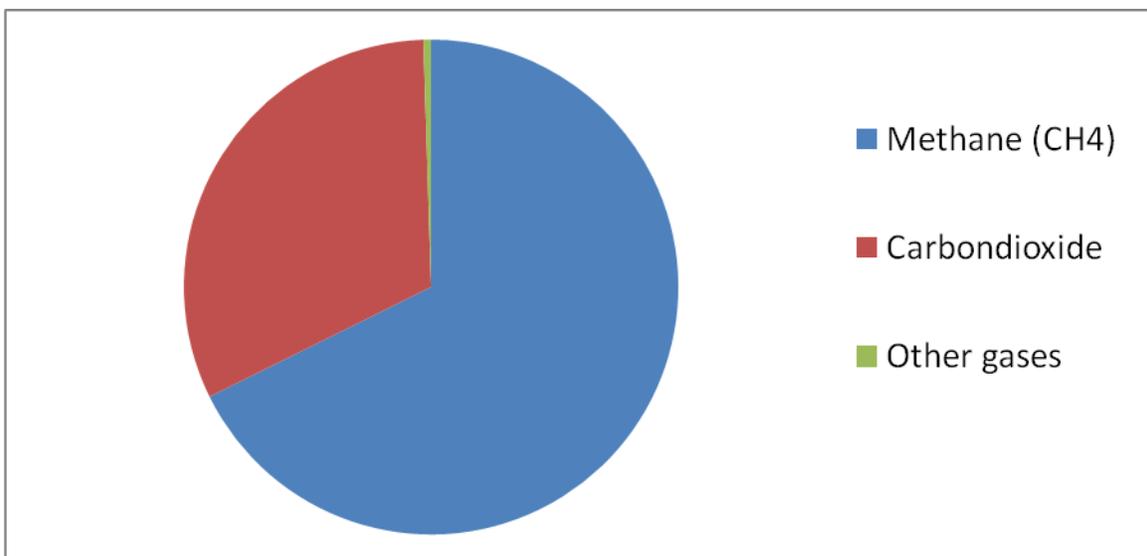


Figure 8: Pie Chat of biogas composition for digester A

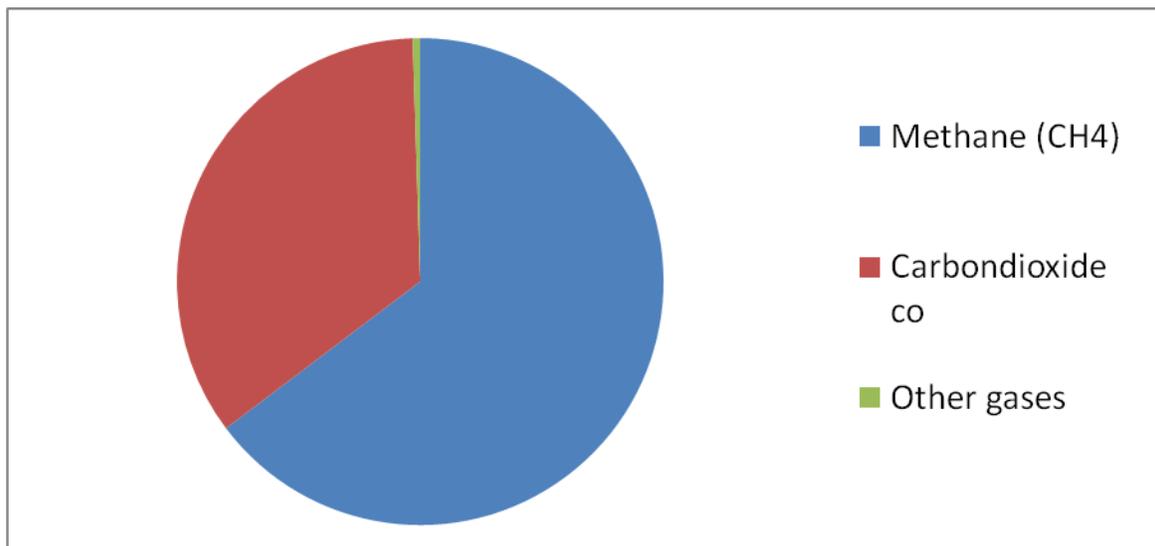


Figure 9: Pie Chat of biogas composition for digester B

Table 4: List of pathogenic bacteria and fungi, identified in slurry from both digesters.

Bacteria		Fungi	
<i>Pseudomasspp</i>		<i>Candida guller</i>	<i>Aerobacteraerogens</i>
	<i>Aspergilluspp</i>	<i>Escherichia coli</i>	
<i>Yeast</i>		<i>Salmonella spp</i>	
	<i>Klebsiella</i>		
<i>Staphylococcus spp</i>			

4.0 DISCUSSION

Digester A is seen to have an earlier onset of gas flammability (11th day) compared with digester B (15th day), this being an effect of wood ash treatment as observed by Itodoet *al.*, (1992) who observed that the use of wood ash as a medium material for seeding exhibited good characteristics in accelerating biogas yield. Digester A is also seen to have produced more biogas over the retention time. This is in line with observations of Adeyanju (2008) and Of omatahet *al* (2012) who all recorded increased biogas production due to ash treatment on substrate. From the table, it is observed that cumulative biogas produced from both digesters A and B are 157l and 148l respectively. This translates to 15l of biogas per kg of substrate for digester A and 14l of biogas per kg of substrate for digester B. Ntengweet *al.*,(2010) opined that 1000l of biogas is equivalent to 0.8l of petrol valued at ₦69. Hence, 66kg of ash treated co-digested water hyacinth and poultry dung at a ratio of 1:5 (respectively) an aerobically digested over 32 days will yield

biogas worth ₦69, while 71kg of untreated co-digested water hyacinth and poultry dung anaerobically digested for 32 days will yield biogas also equivalent to ₦69. This can then be used as a template for economical viable production of biogas from poultry dung from farms, co-digested with water hyacinth gotten from water ways in which they constitute a nuisance.

Digester A is observed to have achieved a higher gas production peak when compared with digester B, this can also be attributed to the effect of wood ash treatment considering that Ofomatahet *al.*, (2012) and Adeyanju (2008) all observed a higher biogas production peak from ash treated substrate. There is a more sustained biogas production over the retention time period for digester A, while digester A is also seen to have produced the bulk of biogas earlier (between days 10 and 21) compared with digester B which recorded highest biogas productions between days 14 and 28. These observations are also attributes of wood ash treatment as observed by Ofomatahet *al.*, (2012) who opined that the pH range obtained for the treated waste sample was favorable for microbial growth and therefore volume of biogas.

It is observed that both digesters experienced almost identical temperature variations which are seen to have been slightly higher than the ambient temperature within the same period, both of which also fall within the mesophilic temperature range for anaerobic fermentation. The environment is seen to have affected the slurry temperature for both digesters, seeing as both slurry temperatures are seen to have dipped and risen as the environmental temperature dipped and rose, even though both slurry temperatures stayed slightly higher than that of the environment. These fluctuations in the temperature value were due to the weather condition, which was cool on some days and warm on other days during the retention time. These findings were also observed by Adegunloye *et al.*, (2013) who also stated that “Since the temperature values remain within the mesophilic range, the growth and activities of methanogens will be enhanced, encouraging the production of biogas”.

The microbial load (Total viable count) was higher for digester A. However, the trend for both digesters was an initial increase of microbial load which peaked between days 9 and 12, and a gradual decrease till the end of the retention period. This observation (higher Total microbial count for A) is an effect of wood ash treatment in stabilizing the pH within the range that encourages methanogenic growth, hence the higher readings for digester A. Ofomatahet *al.*, (2012) also observed a higher microbial population (total viable count) for ash treated wastes when compared with those not treated with wood ash over the retention time. The trend of initial increase in microbial population followed by a gradual drop after a peak was achieved as observed in both digester was also replayed in the biogas production as observed. The rate of biogas production is then seen to be dependent on the rate of growth or proliferation of

microorganisms (in this case methanogens) which are responsible for producing biogas from their biochemical activities in the slurry, as also reported by Adegunloye, *et al.*, (2013).

The value for pH variation for both digesters during the retention time, both started at slightly above 6 and dropped to about 4.5 on day nine. This can be attributed to the amount of organic acid produced at the hydrolysis and fermentation stages of the anaerobic digestion process. Acetate and fatty acid produced during digestion tend to lower the pH of digesting material (Marchaim, 1986; Santhosh, *et al.*, 2012). The pH reading for both digesters is then seen to rise to a peak of 7 on day 12, digester A then was then observed to have had a sustained pH of slightly above 6 while digester B maintained a pH of just below 6 afterwards. This later pH reading for both digesters can be attributed to the activities of methanogenes which used up the acids formed earlier by other microorganisms to form biogas which reduces the acidity of the slurry, while digester A is seen to have a slightly higher pH due to the presence of wood ash which increases the alkalinity of the slurry which in turn resulted in both higher microbial counts and biogas yield for digester A. Ofomatah *et al.*, (2012) had observed that ash treatment on biogases had resulted in a pH range 7.2 to 7.8 while the untreated bagasse had a pH range of 4.8 to 5.8. This affected the production as the methanogens that convert wastes to flammable biogas are highly pH sensitive and operate optimally at a pH range of 6.5 to 8.5 (Anon, 1989). The pH range obtained for the treated waste sample was favorable for microbial growth hence volume of biogas.

The biogas production and total microbial count for digester A are seen to have a strong positive correlation, this means that increase in microbial population results in increase in biogas production for wood ash treated co-digested water hyacinth and poultry dung at the ratio used in this experiment. Digester B however is seen to have weak negative correlation which translates to slight decrease or no increase at all in biogas production when microbial population increases in untreated co-digested water hyacinth and poultry dung at the ratio used for this experiment. This shows that wood ash treatment helps in monitoring both microbial counts and rate of biogas production in an anaerobic digester.

The regression line plot for biogas production vs total microbial count for digester A showed a positive curve, meaning an increase in microbial count results in an increase in biogas production. This also provides the means of predicting the amount of gas produced for a particular population of microorganisms gotten from ash treated substrate, hence providing a model for applications of microbial counts and intended biogas production programs. However in digester B, the graph is seen to have a slightly negative curve which implies that biogas production for digester B does not increase with increase in total microbial count, but rather reduces slightly. This also means that decrease in microbial count of untreated substrate could also result in increase in biogas production.

There appears to be a significant difference (> 0.05) between both digesters for all the factors (biogas production, total microbial count and pH variation), the most significant difference however being observed in biogas production, followed by pH variation and total microbial count having the least significant difference. This goes to show that treatment with wood ash has a profound effect on the anaerobic digestion of co – digested water hyacinth and poultry dung by significantly increasing biogas production due to pH enhancement which in turn boost smethanogenic microbial activity.

The methane composition of the gas is significantly high (over 60%) which is adequate for effective flammability. Other gases are also observed to occupy a very small proportion of total biogas composition.

Methane value is also seen to be above 60%, carbon dioxide is also observed to be about the same. This shows that wood ash treatment does not affect the composition very much as it affects gas volume, while other gases occupy about the same composition.

Pathogenic bacteria and fungi are seen to have been isolated from both digesters. This was also the findings of Ofomatahet *al.*,(2012) and Adegunloye, *et al.*,(2013). These identified pathogenic microorganisms from the slurry can cause numerous diseases in human beings and animals. Among these diseases are skin infections, urinary tract infections, wound infections and food poisoning. The presence of these organisms means that handlers of spent slurry from anaerobic fermentation of co – digested water hyacinth and poultry dung should be careful observing proper hygienic practices.

5.0 CONCLUSION

This research work has shown that appreciable quantities of biogas can be produced from co-digested water hyacinth and poultry dung, and this process is an economically viable one. This work has also shown that wood ash treatment on co – digested water hyacinth and poultry dung results in a significant increase in biogas production, and that ash treatment also stabilizes the anaerobic process by enhancing biogas production rate control and observation. This work also described the relationship between microbial population of slurry and biogas production of co-digested water hyacinth and poultry dung, treated and untreated with wood ash.

The following are recommendations derived from this research work;

1. The co – digestion of water hyacinth and poultry dung is an economically viable and proffered method of disposing of water hyacinth and poultry dung.

2. That wood ash treatment is an effective method of enhancing biogas production from co-digested water hyacinth and poultry dung.
3. That further research should be carried out on the use of wood ash treatment on the anaerobic digestion of organic wastes to enhance biogas production from these wastes.
4. That anaerobic digestion is a proffered method of disposal of organic waste.

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