

Research Paper

BIOGAS PRODUCTION: AN ALTERNATIVE PANACEA FOR ENERGY CRISIS IN NIGERIA

ABSTRACT

Aim: Biogas research and subsequent production is a sine-quo-non to mitigating the possible depletion of crude oil and energy crisis, especially in Nigeria. This research paper was aimed at evaluating the biogas production capacity of water hyacinth, poultry droppings, cow dung and their combination.

Methods: One kilogram, two kilograms and three kilograms weight of these substrates were subjected to anaerobic digestion in starter and without starter cultures for 45 days at the interval of 5 days.

Results: Our results showed that heterotrophic bacteria and fungi counts were substrate-specific with poultry dropping fed-digester having the highest. Different bacteria and fungi were isolated including methane-producing bacteria such as *methanolreobacteria*, *methanoculleus bourgense*, *methanogenium cariaci*, *methanocorpusium parvum*, *methanosarcimon barkeri*, *methanoplanus lunicola*, *methanococcoides methyluteus* and *methanothrix soehngenii*. There were significant differences in the amount of biogas produced by the different substrates. However there was no significant differences ($P>0.05$) between the biogas produced by water hyacinth-fed digester (170.41mls) and poultry droppings-fed digester (182.88 mls). Combining all the substrates (WH+PD+CD) yielded the highest biogas (423.80 mls), which was followed by biogas production of cow dung (331.8 mls).

Conclusion: Explicitly, our present report showed that higher biogas yield can be achieved by the combination of different biogas feedstock.

KEY WORDS: Water hyacinth, agro-wastes, biogas production, energy crisis.

1. INTRODUCTION

The ever-increasing price and shortage of fossil fuel, coupled with global concerns on climate change and severe food insecurity has triggered the search for new natural materials that will be amenable to processing during extraction, thus reducing costs drastically and substituting fossil fuel. The anaerobic digestion of these organics has long been used to generate useful resources, which have

46 been harnessed for the use of mankind (Momoh and Nwaogazie,
47 2008).

48 Nigeria, indeed Cross River State has abundant, diverse and
49 unexploited renewable energy resources for the production of fuel,
50 which undoubtedly will help in no small measure to solving our energy
51 crisis and poverty (Itodo et al., 2007; Igoni et al., 2007; Mashandete and
52 Parawira, 2009). Importantly, Guruswamy et al. (2003), Alvarez and
53 Gunnar (2007) identified two challenges in the 21st century: firstly, the
54 development and use of renewable energy to decrease over-
55 dependence on fossil fuel and secondly, the management of the
56 waste generated by human activities. This obviously paints the real
57 picture of the developing countries of the globe such as Nigeria.

58 Critically, achieving the Millennium Development Goals (MDGs)
59 in Africa also requires a significant expansion of access to modern and
60 alternative renewable energy such as biogas, which is of growing
61 interest for the sustainable management of our waste and a major
62 breakthrough in the search for a renewable energy for the reduction in
63 over-dependence on non-renewable fossil fuel (Nagamani and
64 Ramasamy, 2003; Adeyanju, 2008). Biogas consists of 50 – 70%,
65 methane 30 – 40%, carbon dioxide 5 – 10%, hydrogen 1 – 2%, nitrogen 0
66 – 3% , water vapour and traces of hydrogen sulphide, carbon
67 monoxide and oxygen. It is colourless, relatively odourless and
68 flammable. It is also stable and non-toxic. It burns with a blue flame
69 and has a calorific value of 4500 – 6000kcal/m³ when its methane
70 content ranges between 60 – 70% (Adeyanju, 2008 and Igoni *et al*,
71 2008).

72 Water hyacinth (*Eichhornia crassipes*) has been reported to serve
73 as organic fertilizer (Gunnarsson and Peterson, 2007), therapeutic
74 agent (Bhattacharya and Kumar, 2010) and recently as a good
75 candidate for the biosynthesis of biofuel. This is however, premised on
76 the fact that it has high cellulose with low lignin content per unit
77 volume of dry matter (Poddar et al., 1991; Gressel, 2008), easily
78 degradable and will not compete with arable crops for space, light
79 and nutrients.

80 The option of using this plant as feedstuff for bio-fuel production
81 will solve the following problems: (a) saving the huge sums of money
82 spent in its control annually (Bhattacharya and Kumar, 2010), (b)
83 preventing marshes from becoming breeding grounds for various
84 disease-spreading insects and pests and (c) solving its interference
85 problems with irrigation channels, fishing and recreation activities.
86 Poultry droppings and cow dung though used as fertilizers constitute
87 environmental pollution (Long 1992, Lund et al., 1996) and thus the

88 need to search for an alternative measure for their being converted to
89 other useful ventures.

90 This research is aimed at determining the potentials of water
91 hyacinth, poultry droppings, cow dung and their combinations in
92 biogas generation.

93 **2. MATERIALS AND METHODS:**

94 **2.1 Sample collection and microbial screening of collected samples**

95 Fresh leaves of water hyacinth were obtained from Itigidi, Cross
96 River State, Nigeria while poultry droppings and cow dung were
97 obtained from University of Calabar poultry farm and abattoirs in
98 Calabar, Nigeria, respectively. Ten kilograms of each sample was put in
99 sterile polythene bags for analysis, which was done within 24 hours.
100 Leaves of water hyacinth were cut into pieces of about 2 to 5mm size,
101 while poultry dropping and cow-dung were well pulverized. Ten grams
102 of each were mixed with 90mls of sterile distilled water in 250mls
103 Erlenmeyer flask. The mixtures were vortexed and agitated thoroughly
104 and allowed to stand for ten minutes.

105 The supernatant was decanted and one milliliter volumes were
106 prepared in ten-fold serial dilutions. Dilutions of 10^{-5} to 10^{-7} were plated
107 on nutrient agar supplemented with $50\mu\text{gml}^{-1}$ Nystatin to prevent fungal
108 growth using surface plating. Plates were incubated for 24 – 48 hours at
109 35°C . Colony forming units per gram (cfug^{-1}) of bacterial growth
110 between 30 – 300 colonies were enumerated. For screening of fungi,
111 dilutions of 10^{-3} to 10^{-4} of the supernatant were plated on Sabouraud's
112 dextrose agar supplemented with 100mgml^{-1} streptomycin and
113 15mgml^{-1} of penicillin (to inhibit bacterial growth). This was incubated
114 for 72 to 96 hours. Plates with fungal colonies of between 30 to 300
115 were enumerated in cfug^{-1} . Methods of Ogundero (1981) and Hunter-
116 cevera *et al.* (1986) were employed for isolation and characterization
117 of fungi. All plating was done in triplicates.

118 119 **2.2 Preparation of starter culture**

120 The method of Geluk *et al.*, (1992) were employed. The support
121 activated carbon (charcoal) was washed 5 times with acetate buffer
122 pH (4-5) and finally re-suspended in the buffer overnight. Twenty
123 kilogram weights were placed in storage containers and kept at 10°C
124 in a refrigerator. Twenty kilograms weight of the slurry (residue w/v) of
125 an old but active cow dung digester was mixed with 20kg weight of
126 the pre -treated activated carbon and incubated at room
127 temperature in anaerobic condition for 40 days. The adsorbed cells
128 were used as crude starter culture for all digesting combinations. The
129 advantage of using the activated carbon as support for the

130 immobilisation was that it was relatively cheap and affordable, readily
131 available, mild and poses no problem of cell and enzyme inactivation.

132 **2.3 Preparation of substrates for biogas production**

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134 The methods of Sriramajayan *et al.*, (2007) and Chae *et al.*,
135 (2007) as modified by Asikong *et al.* (2012) was used. Leaves of water
136 hyacinth were cut into pieces of about 2-5mm size. The three
137 substrates, respectively were mixed in the ratio of 0.33:0.33:0.33;
138 0.66:0.66:0.66 and 1:1:1 weights to yield total weights of about 1kg, 2kg
139 and 3kg. Respective weights were mixed with water at the ratio of 1:3
140 and placed in the digesters. Duplicate of each weight were prepared,
141 one without starter culture and the other with 1kg weight of starter
142 culture from an old digester slurry mixed with charcoal. The digesters
143 were tightly corked with rubber stopper to create anaerobic condition
144 and connected to a gasometrical chamber. Biogas was monitored
145 and measured daily over a period of 45 days using the gasometrical
146 chamber with the displacement of paraffin wax. The total biogas yields
147 were determined by opening the outlet tap of the anaerobic digester
148 and the inlet tap to the graduated burette. The biogas generated was
149 released through the tube which then displaced the paraffin oil in the
150 graduated burette downward. The volume of gas yield was
151 determined by the volume of paraffin oil displaced, i.e gas yield was
152 directly proportional to paraffin oil displaced.

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154 **2.4 Data collection and analysis**

155 Data on total heterotrophic bacteria and fungi, biogas yield
156 were subjected to analysis of variance using PASW version 18.0 and
157 significant means were separated using Least Significant Difference
158 (LSD).

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3.

RESULTS

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161 Our result revealed that the highest bacteria count was
162 obtained in the culture containing poultry droppings- fed digester
163 (without starter culture), which differs slightly from those obtained in the
164 culture of cow dung. There were significant differences ($p < 0.05$)
165 observed in the bacteria and fungi counts before and after anaerobic
166 digestion, which was substrate dependent and with or without starter
167 culture –specific (Table 1). The following bacteria species were
168 identified and isolated from the culture; *E.coli*, *P. aeruginosa*, *B.*
169 *cereus*, *R. dacucus*, *C. freundii*, *K. pneumonia*, *P. vulgaris*, *S. dysenteriae*,
170 *Y. pestis*, *S. cholmesuios* and *S. aureus* while *Fusarium spp*, *Aspergillus*
171 *spp*, *Penicillium spp*, *Mucor spp*, *Aspergillum flavus* were the fungi
172 species that were identified and isolated (Tables 2 & 3).

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Additionally, other specific methane-producing bacteria were also identified and isolated such as *methanolreobacteria*, *methanoculleus bourgense*, *methanogenium cariaci*, *methanocorpusium parvum*, *methanosarcimon barkeri*, *methanoplanus lunicola*, *methanococcoides methyluteus* and *methanotherix soehngenii* (Table 4)

3.1 Biogas yield

There were significant differences in the amount of biogas produced by the different substrates. However there was no significant differences ($P>0.05$) between the biogas produced by water hyacinth-fed digester (170.41mls) and poultry droppings-fed digester (182.88 mls). Combining all the substrates (WH+PD+CD) yielded the highest biogas (423.80 mls), which was followed by biogas production of cow dung (331.8 mls) (Fig. 1). There were also differences recorded ($P<0.05$) among the different substrate inclusions. The biogas yield increased as the quantity of substrate increased. The 3kg weight produced the highest biogas (364.40mls) followed by 2kg (274.59mls) and then 1kg yielded the least (192.68 mls) the substrate type notwithstanding (Fig. 2).

Biogas production was also dependent on the use of either starter or without starter culture. The yield of biogas when starter culture was used was significantly higher (319.05 mls) than when starter culture was not used (235.40 mls)(Fig. 3). However, there were no interactions between the substrates, quantity of inclusion and with or without starter culture. It was observed that the yield of biogas was highly dependent on the duration of anaerobic digestion, the substrate notwithstanding. The trend was such that it gradually increased from 5 days of digestion and become optimal in the 15th and 20th day, especially for the combined substrate (653.33 and 695.83mls) before declining significantly from the 25th day to the 40th day (Fig. 4). However, the optimal biogas yield for cow dung was achieved at the 15th day of digestion (610.83 mls).

Table 1. Total viable bacteria and fungi counts from substrates slurry before and after anaerobic digestion

	Without starter culture					With starter culture				
	Control (water)	WH	PD	CD	WH+PD+CD	Control (water)	WH	PD	CD	WH+PD+CD
BCBD (cfug ⁻¹)	5.54 x10 ⁸	5.46 x10 ⁷	8.83 x10 ⁷	8.65 x10 ⁷	7.55 x10 ⁷	5.54 x10 ⁸	6.40 x10 ⁸	8.60 x10 ⁸	8.45 x10 ⁸	7.65 x10 ⁸
BCAD (cfug ⁻¹)	4.40 x10 ⁵	3.55 x10 ⁵	5.54 x10 ⁵	6.45 x10 ⁵	4.10 x10 ⁵	4.40 x10 ⁵	4.55 x10 ⁵	6.54 x10 ⁵	7.35 x10 ⁵	5.25 x10 ⁵
FCBD (cfug ⁻¹)	4.40 x10 ⁴	1.46 x10 ⁴	3.42x10 ⁴	3.55 x10 ⁴	2.35 x10 ⁴	4.40 x10 ⁴	2.46 x10 ⁴	4.42 x10 ⁴	4.55 x10 ⁴	3.35 x10 ⁴
FCAD (cfug ⁻¹)	3.15 x10 ²	1.20 x10 ²	2.26 x10 ²	2.25 x10 ²	1.20 x10 ²	3.15 x10 ²	2.20 x10 ²	3.26 x10 ²	3.25 x10 ²	2.20 x10 ²

BCBD = Bacteria counts before digestion; BCAD = Bacteria counts after digestion; FCBD = Fungi counts before digestion; FCAD = Fungi counts after digestion; WH = Water hyacinth; PD = Poultry droppings; CD = Cow dung; WH+PD+CD = Combined.

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Table 2. Characterization and identification of bacterial isolates

Code	Gram reaction	Morphology	Motility	Oxidase	Catalase	Indole	Citrate	Glucose	Lactose	Manitol	Urease	H ₂ S	Nitrate	V. Proskauer	Methyl Red	Coagulase	O ₂ requirement	Probable isolate
ABE ₁	-	Single rod	+	-	+	+	-	+	+	+	-	-	+	-	+	-	+	<i>Escherichia coli</i>
ABE ₂	-	Coccobacilli	±	±	±	-	+	-	-	-	-	-	+	-	-	-	-	<i>Pseudomonas aeruginosa</i>
ABE ₃	+	Straight rods	+	+	+	+	+	+	-	-	+	+	-	+	-	-	-	<i>Bacillus cereus</i>
ABE ₄	-	straight slightly curved	+	+	+	-	-	+	-	-		+	+		-	-	-	<i>Rhizobacter dacucus</i>
ABE ₅	-	straight rod	+	-	+	-	+	+	+	+	-	-	+	-	+	-	+	<i>Citobacter freundii</i>
ABE ₆	-	straight rod	-	-	+	+	+	+	+	+	NT	-	+	+	-	-	-	<i>Klebsiella pneumonia</i>
ABE ₇	-	straight rod	+	-	+	+	+	+	+	+	NT	-	-	+	-	-	-	<i>Proteus vulgaris</i>
ABE ₈	-	straight rod	+	-	-	-	+	+	+	+		-	+	+	±	-	±	<i>Serratia</i>
ABE ₉	-	straight rod	-	-	+	±	-	-	+	+	-	-		+	+	-	-	<i>Shigella dysenteriae</i>
ABE ₁₀	-	Straight rods	±	-	+	±	-	+	+	+	+	+	-	-	+	-	-	<i>Yersinia Pestis</i>
ABE ₁₁	-	Straight rods	-	-	+	-	+	+	+	+	+	-	+	+	-	+	-	<i>Salmonella choleraesuis</i>
ABE ₁₂	-	Spherical	-	-	+			-	+	+	+	ND	ND	+	ND	+	-	<i>Staphylococcus aureus</i>

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Table 3. Characterization and identification of fungal isolates

Colony code	Colour of hyphae	Macroscopic features	Probable organism
ABEF1	Pink wooly	Narrow septate hyphae, conidiophores occur singly and in groups, multicellular crescent shaped macronidia	<i>Fusarium</i> spp.
ABEF2	Black velvety	Septate and broad hyphae, large head entirely covered with phalides bearing chains of conidia.	<i>Aspergillus</i> spp.
ABEF3	Greenish velvety	Septate hyphae, conidiophore developed into branched phalides bearing chains of conidia brush-like appearance.	<i>Penicillium</i> spp.
ABEF4	White fluffy	Aseptate broad hyphae large spherical head without rhizoid	<i>Mucor</i> spp.
ABEF5	Brownish velvety	Septate hyphae, narrow hyphae, spherical head entirely covered with phalides bearing chains of conidia	<i>Aspergillus flavus</i>
ABEF6	Dark-brown (tan)	Aseptate, round sporangium	<i>Mucor</i> spp.
ABEF7	Black velvety	Septate and broad hyphae with large head entirely covered with phalides bearing chains of conidia	<i>Aspergillus</i> spp.
ABEF8	Greenish velvety	Septate hyphae, conidiophore developing into branched phalides bearing chains of conidia brush like appearance.	<i>Pericillium</i> spp.

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235 Table 4. Methane producing bacteria during the process of biogas production

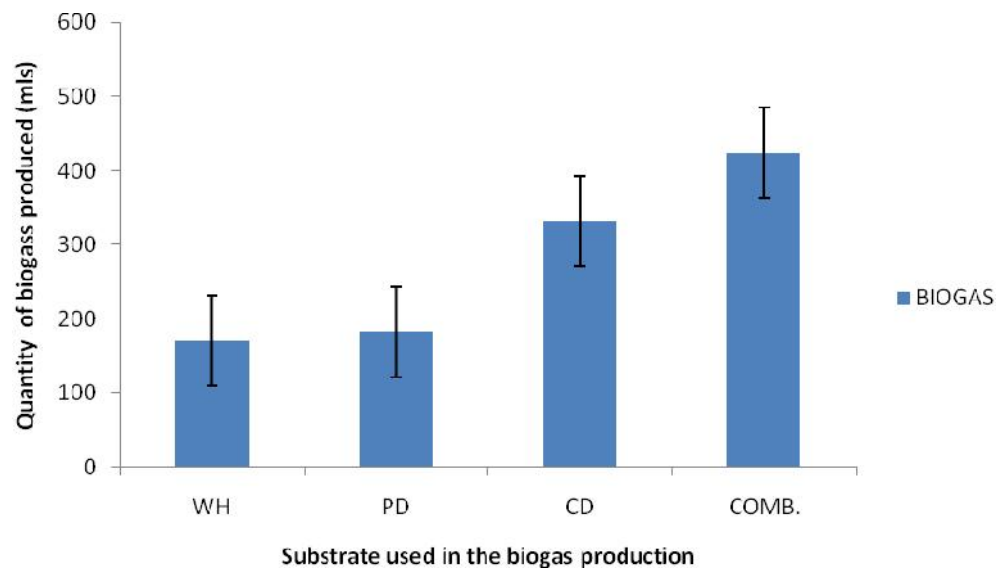
S/N	Morphology/shape	grams reaction	motility	catabol substrate H ₂ + CO ₂	formate acctate	Organic factor	pH	Temperatu re of growth	Isolates
1	Short rods in pairs or chain	+	-	+	++		6-7	37-40 °C	<i>Methanolreobacteria</i> SA
2	Irregular Cocci occurring single	+	-	+	++		6-8	35 – 40 °C	<i>Ruminantum</i> GIT
3	Irregular cocci	-	-	+	++		7.0	20 – 40 °C	<i>Methanoculleus bourgense</i> VSA GIT
4	Short curved rods	-	+	+	++		6.1 -69	40 °C	<i>Methanogenium cariaci</i> VSA GIT
5	Small irregular Cocci	-	-	+	++		6-7.5	30 -40 °C	<i>Methanocorpuslum Parvum</i> VSA AD
6	Irregular sphaeroid alone or group va aggregate		-	+	++		6-7.5	30 -40 °C	<i>Methanosarcinon barkeri</i> VSA
7	Very Irregular cocci plate shape	-	+	+	++		6.0-7.5	30 -40°C	<i>Methanoplanus lunicola</i> VA AOS
8	Extremely irregular cocci single or in pairs	-	-	+	+		7.0 -7.5	30 – 35 °C	<i>Methanococcoides methylutens</i>
9	large sheathed rods	-	-	+	+		7.1 -7.8	35 -40 °C	<i>Methanotherix sochnenii</i>

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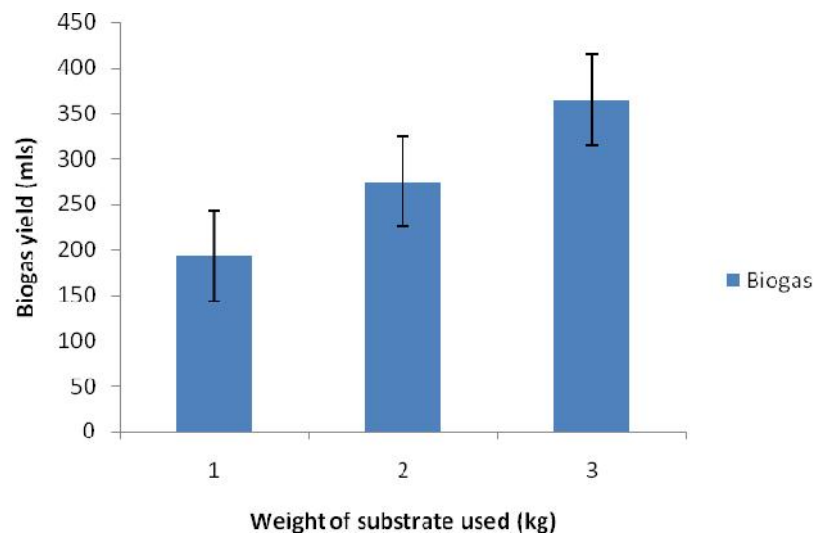
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243 Fig.1. Effect of substrates on biogas yield

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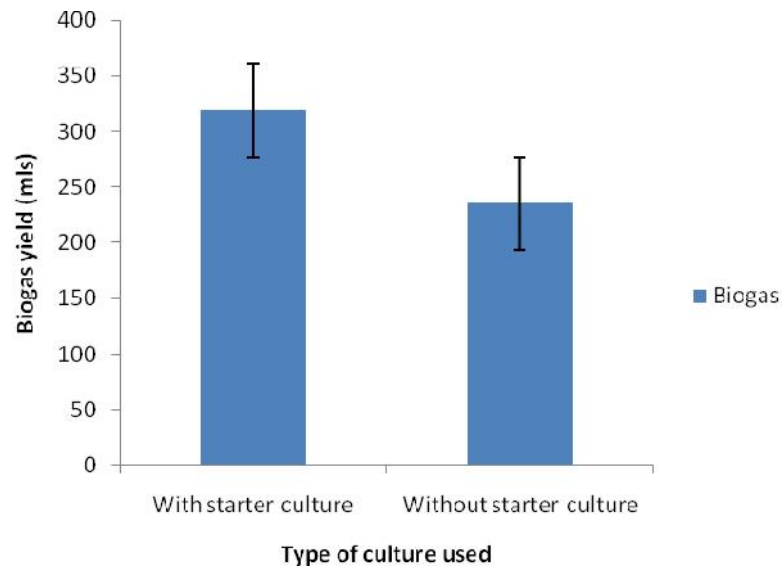
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247 Fig. 2. Effect of quantity of substrates on biogas yield

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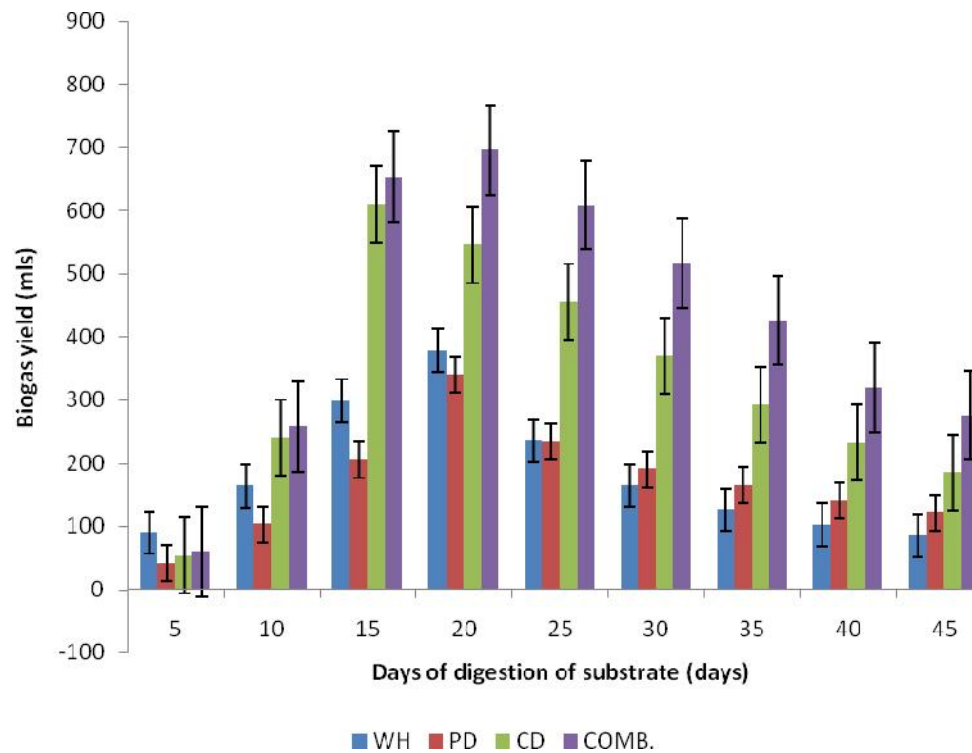
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Fig.3. Effect of with or without starter culture on biogas yield

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Fig. 4. Effect of digestion duration on biogas yield

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4.

DISCUSSION

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The worldwide shortage and rising price of fossil energy, together with climate and environmental protection aims, have led to enhanced development and utilization of alternative energy resources. This is predicated on the fact that the production of biogas from microbial degradation of organic matter is of great importance, since biogas is environmental friendly (Jaenick et al., 2011; Jaenick et al., 2011). Regrettably, though biogas production technology has established itself as a technology with great potential, which could exert major influence in the energy scene; however, it has not made any real impact on the total energy scenario despite the presence of about 1.8 million biogas digesters. This might be attributable to the nature of the substrate fed in the digester as this obviously, determines the type and extent of the fermentative bacteria present in the digester⁷ and the subsequent biogas yield.

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There are many microbial diversity in biogas digesters, which either act singly or synergistically to achieving high production of biogas. Interestingly, the microbial species that play crucial role in biogas production are substrate-specific. Though there was no systematic trend followed as regards bacteria and fungi counts before and after digestion, the counts were substrate-specific. I should be understood that different substrate contain varying amount of nutritive contents, which the microbes feed. This could be the underlying reason responsible for the high microbial counts before and after digestion recorded in the poultry and cow dung-fed digesters, with or without starter culture.

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According to Jaenick et al. (2011); Zakrzewski et al. (2012) members of the genus *Methanoculleus* frequently dominate methanogenic subcommunities in different anaerobic digester systems. Interestingly, it is observed that methanogenic sub-communities within biogas-producing consortia are crucial in the anaerobic degradation process for synthesis of methane. Many methanogens were identified and isolated in our present study. Dhevagi et al. (1992) used different feedstocks like cow dung, buffalo dung, dry animal waste, stray cattle dung, goat waste, and poultry droppings for their biomethanation potential and observed that poultry droppings showed higher gas production. Earlier Yeole and Ranade (1992) compared the rates of biogas yield from pig dung-fed and cattle dung-fed digesters and reported that the biogas yield was higher in the former. They attributed this higher biogas yield to the presence of native microflora in the

305 dung. Contrary to the reports of Dhevagi *et al.* (1992); Yeole and
306 Ranade (1992), the biogas yield of the combined substrate-fed
307 digester notwithstanding, cow dung-fed digester produced the highest
308 quantity of biogas. It is probable that the native microflora present in
309 the dung might be species-specific as the strain of the animal
310 producing the dung might be implicated.

312 Ramasamy *et al.* (1990) reported higher presence of
313 proteolytic organisms in cow dung-fed digesters and other animal's
314 waste-fed digesters. However, Preeti Rao and Seenayya
315 (1994) observed that while cow dung-fed digesters supported higher
316 amylolytic microorganisms, poultry waste-fed digesters showed higher
317 proteolytic population. This could answer why cow dung fed-digester
318 gave the highest quantity of biogas singly. It will make sense to observe
319 that most of the proteolytic bacteria isolated in this report might aid
320 delayed degradation since they produce toxic substances such as
321 phenols, ammonia, etc. Our result revealed that the combined
322 substrate - fed digester produced the highest quantity of biogas (Fig.
323 1). Undoubtedly, it will be proper to think that the different composites
324 should contribute their integral biogas yield to the combined pool. This
325 was succinctly the case in our report. The main problem in biogas
326 production is the capacity of the substrates to have a methane-
327 producing reservoir that will be subsequently converted into biogas by
328 methanogenic bacteria. It thus implies that cow dung has more of
329 methane producing reservoir comparing water hyacinth and poultry
330 droppings.

332 It was observed that the time of anaerobic digestion in the
333 substrate-fed digester affected biogas yield. Obviously, biogas
334 production was at its peak within 15 and 20 days of digestion, after
335 which the yield declines, the substrate notwithstanding. This might
336 probably be as a result of the particulate-bound bacteria that
337 predominate up to 20th day of initiation of biogas digester. There is
338 usually active degradation of substrate at the beginning of digestion.
339 However, as the degradation progressed, the source of energy
340 declines, leading to low microbial activity vis-a-vis biogas yield. The
341 implication is that there may not be any need to elongate digestion in
342 the digesters due to the cost effect of running the remaining days. It
343 might rather be necessary to increase the substrate quantity and
344 harvest biogas within these intervals of days. According to our report,
345 increase in the quantity of the substrate fed in the digester resulted to
346 increase biogas yield. This is not unconnected to the number of
347 microbes feeding on the energy source. The implication is the greater
348 the quantity of substrate, the higher the number of microbial
349 community, culminating to increase biogas yield. This was the case in
350 our research.

Presence of some metals influences biogas production. Seenayya *et al.* (1992); Geetha *et al.* (1990) ; Preeti Rao and Seenayya (1994) as they have been indicted for increasing methanogenic population. It seems to suggest that methanogenic bacteria produce enzymes that are metallo-specific, which are involved in biogas production. This might explain the differentials observed in the biogas producing capacity of the substrate used in the current report. Our result also revealed that biogas yield in the digester with starter culture was higher than the digester without starter culture. It thus therefore suggests that priming the degradation with inoculums (starter) will enhance biogas yield. The microorganisms identified and isolated, especially the methanogens are of immense importance. This is premised on the fact that they could be genetically manipulated to either increase their capacity to degrade or their genes cloned and moved into other microbes to confer on them the same capacity. It thus suggest that instead of allowing microorganisms that may not have any part in the pathway leading to biogas production to crowd the substrate, there will be more precise and target oriented anaerobic digestion. Worthy of note is the fact that manipulation of the identified and isolated microbes to enhance their capacity to degrade biogas substrates will be a good approach in the right direction.

However, the genetic manipulation of the substrate will undoubtedly, complement this venture for a holistic and improved biogas yield. Unfortunately, of the three substrates used in this research, water hyacinth is the only one that can be manipulated but disappointedly, produced the lowest quantity of biogas. This notwithstanding, the underlying factor(s) leading to the biogas yield recorded against poultry droppings and cow dung-fed digesters could be exploited, harnessed and optimized in water hyacinth genome. This becomes pertinent taking into cognizance the fact that poultry droppings and cow dung as well as other organic-like substrates may not be available in a good quantity that will yield the required biogas if the energy crisis, especially in Nigeria is anything worth solving.

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CONCLUSION

Explicitly, our present report showed that higher biogas yield can be achieved by the combination of different biogas feedstock. However, there are two most important criteria in the choice of selecting feed stocks for biogas production: economic considerations and biogas yield. It thus suggest that a second consideration should be made on water hyacinth giving its capacity to be manipulated genetically and fortified with metals that could increase microbial population, resulting in higher biogas yield.

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