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Potentials of Biogas Generation from Mixture of Three Substrates, Water Hyacinth, Cassava Peels and Cow Dung - Wh+ Cp+ Cd

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ABSTRACT
The potentials of biogas generation from mixtures of three substrates, water hyacinth, cassava peels and cow dung was evaluated using standard microbiological techniques. The results revealed that the combination of the three substrates without starter culture recorded zero milliliter biogas production in the first 5 days with optimum yield of 300mls, 600mls and 715mls for 1kg, 2kg and 3kg weight of the mixture respectively within 25 days while the digester with starter culture yielded optimum biogas production of 475mls, 650mls and 820mls respectively in 1kg, 2kg and 3kg weight within 25 days. The total viable bacterial and fungal counts from the substrate slurry of the WH + CP + CD was $7.55 \times 10^8$ cfug$^{-1}$ and $2.35 \times 10^4$ cfug$^{-1}$ before digestion respectively and $4.10 \times 10^5$ cfug$^{-1}$ and $1.20 \times 10^2$ cfug$^{-1}$ after digestion respectively without starter culture. The digester with starter culture gave $7.68 \times 10^8$ cfug$^{-1}$ and $3.35 \times 10^4$ cfug$^{-1}$ for bacteria and fungi respectively before digestion and $5.25 \times 10^5$ cfug$^{-1}$ and $2.20 \times 10^2$ cfug$^{-1}$ respectively for bacteria and fungi after digestion. Optimum and total biogas produced from the treatments

KEY WORDS: BIOGAS, WATER HYACINTH, CASSAVA PEELS and COW DUNG

INTRODUCTION
Cross River State, Nigeria and indeed Africa are blessed with abundant, diverse renewable energy resources that are yet to be exploited for providing clean fuel to help end the energy crisis and poverty in Nigeria (Itodo et al., 2007; Mashandete and Parawira, 2009; Igoni et al., 2008). Guruswamy et al., (2003) and Alvarez et al., (2008) identified two significant and important challenges of the millennium and the twenty first century to include; the development and use of renewable energy to decrease dependence on fossil fuel and management of the waste generated by human activities as a result of agricultural activities, industrial growth and population explosion which are associated with waste generation. Achieving the Millennium Development Goals (MDGs) in Africa also requires a significant expansion of access to modern and alternative renewable energy such as biogas which is of growing interest for the sustainable management of our waste and a major breakthrough in the search for a renewable energy for the reduction in over-dependence on non-renewable fossil fuel (Nagamani and Ramasamy, 2003 and Adeyanju, 2008). Biogas is the product of organic matters decomposition under oxygen-free condition with microbial participation especially Methanogens. Biogas formation can occur naturally in swamps, marine sediments, and water logged soils, rice fields, deep bodies of water, sanitary landfills and even in the digestive system of ruminants; and termites. It can also be recovered from lagoons used for waste treatment. Biogas is also called; swamp gas, sewer gas, marsh gas, gobar gas and digester gas 'will O the wisp gas, natural gas, landfill gas and sewage gas. Biogas, a mixture of gasses consist 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. Generally, four different stages have been recognized in the production of biogas with several other intermediate products. These include; hydrolysis, acidogenesis, acetogenesis and methanogenesis. The efficiency, effectiveness and stability of anaerobic digestion and consequently biogas generation can vary significantly based on various operational factors such as; type of waste streams, digester design, temperature, moisture content, retention time, pH, agitation or mixing, bacterial species and organic loading rate. Presence of toxicants can also influence biogas production. Positive implications of biogas include; the reduction in environmental pollution, odour (Long 1992, Lung et al., 1996), and in the destruction of most pathogenic organisms, worms, ova, etc. Biogas can also serve as a clean alternative to fuel energy source to oil, electricity
and wood. This research is aimed at determining the potentials of biogas energy generation from a combination of water hyacinth, (which is a nuisance in aquatic environment), cassava peels and cow dung (which produce foul odour due to uncontrolled fermentation and thus constitute nuisance in our surroundings).

MATERIALS AND METHODS

Sample collection; Water hyacinth, cassava peels and cow dung

Ten kilogram (10kg) weight each of water hyacinth, (*Eichhornia crassipes*), cassava (*Manihot esculentum*) peels and cow dung were obtained from locations, 005° 53 04 N 008° 00 54 E, 005° 53 00N 008° 01’ 16 E, and 005° 53 00N 008° 01’ 16 E respectively in Cross River State, Nigeria. Samples were placed in sterile polythene bags and transported to the laboratory for analysis within 24 hours.

Preparation of substrates (Water hyacinth, cassava peels and cow dung) for biogas production

A modification of the methods of Sriramajayan *et al*., (2007) and Chae *et al*., (2007) where used. Leaves of water hyacinth were pounded in a mortar into pieces of about 2 – 5mm size. The cow dung and the cassava peels were screened to exclude other extraneous materials and well pulverized. The three substrates, respectively as above were mixed in the ratio of 0.33:0.33:0.33, 0.66:0.66:0.66 and 1:1:1 weights to yield total weights of about 1kg, 2kg and 3kg. Respective weights were mixed with water at the ratio of 1:3 and placed in the digesters. Duplicate of each weight were prepared, one without starter culture and the other with starter culture from an old digester slurry mixed with charcoal. The digesters were tightly corked with rubber stopper to create anaerobic condition and connected to a gasometrical chamber. Biogas was monitored and measured daily over a period of 45 days using the gasometrical chamber with the displacement of paraffin wax.

Preparation of starter culture

The methods of Geluk *et al*., (1992), were employed. The support activated carbon (charcoal) was washed 5 times with acetate buffer pH (4-5) and finally re-suspended in the buffer overnight. Twenty kilogram weights were placed in storage containers and kept at 10°C in a refrigerator. Twenty kilogram weight of the slurry (residue w/v) of an old but active cow dung digester was mixed with 20kg weight of the pre-treated activated carbon and incubated at room temperature in anaerobic condition for 40 days. The adsorbed cells were used as crude starter culture for all digesting combinations. The advantage of using the activated carbon as support for the immobilization was that it was relatively cheap and affordable, readily available, mild and posses no problem of cell and enzyme inactivation.

Innovation in digester design with gasometrical chamber

Biogas yield was measured daily using the gasometrical chamber which was an innovation, specially designed for this research. The chamber consisted of a gasometrical assembly which comprised of a graduated burette which was connected to the locally designed anaerobic digester through a rubber tube. The burette was also connected to a funnel with paraffin oil through a synthetic rubber tube (which could be transparent). The burette was linked to the tube from the anaerobic digester by a glass connector with two taps; the inlet and the outlet taps. The outlet tap was sealed with a flexible plastic tube with a strong clip (to avoid leakage). The total biogas yields were determined by opening the outlet tap of the anaerobic digester and the inlet tap to the graduated burette. The biogas generated was released through the tube which then displaced the paraffin oil in the graduated burette downward. The volume of gas yield was determined by the volume of paraffin oil displaced, i.e. gas yield was directly proportional to paraffin oil displaced (Figures l).
RESULTS AND DISCUSSION

Potentials of biogas generation by mixture of three substrates; water hyacinth, cassava peels and poultry dropping-WH+CP+PD.

The combination of three substrates; water hyacinth, cassava peels and cow dung without starter culture recorded zero biogas in the first 5 days with optimum yield of 300mls, 600mls and 715mls from the 1kg, 2kg and 3kg weights respectively within 25 days while the digesters with starter culture yielded optimum biogas of 475mls, 650mls and 820mls respectively in 1kg, 2kg and 3kg weights within 25 days as shown in table 2. Total yield obtained from the different treatment weights of 1kg, 2kg and 3kg was 948mls, 2690mls and 3685mls and 2065mls, 3335mls and 4394mls respectively without and with starter culture as shown in Table 2. Table 1 shows the total viable bacterial and fungal counts from substrates slurry before and after anaerobic digestion with and without starter culture. The production of biogas from water hyacinth, cassava peels and cow dung (WH + CP + CD) treatment combinations within intervals of 5 – 45 days were summarized in analysis of variance (ANOVA) Table 3. Figure 3 shows that there was negative correlation at early stage of the biogas generation while there was positive relation later in the digestion in both digesters with and without starter culture.

Combination of water hyacinth, cassava peels and cow dung

The optimal and total biogas yield was higher in combination of the three substrates than the single and double combinations. There was also reduction in the duration for optimal biogas yield than the respective individuals. Variations in biogas volume generated from WH + CP + CD treatment combinations showed significant difference without starter culture [F (2, 16) = 20.86, P < 0.001], but no significant difference with inclusion of starter culture [F (2, 16) = 1.18, P = 0.3314], (Table 3). There was also negative correlation between biogas production with and without starter culture inoculation. Thus, the volume of biogas from the above treatment combinations could be facilitated without inoculums while the presence of starter culture was capable of retarding the generation of biogas in the experiment. This is probably due to excessive production of acid by the cassava peels or experimental error in the course of mixing the substrates. Ofoefule and Uzodinma (2009) observed that combination of cassava peels and cow dung did not improve the biogas yield but only affected the onset of gas flammability. They concluded that there was low synergy existing between cow dung and cassava peels when compared to other wastes. Unfavourable fermentation environment for the methane bacteria could also result in low biogas and methane yield. This is because the bacteria that ferment organic matter into flammable biogas are highly pH sensitive and survive optimally within pH range of 6.6-7.6 and in some instances 8.5. Leakage could result in poor biogas yield.

Combination of water hyacinth, cassava peels and cow dung

From the ANOVA results, optimal and total biogas produced from the treatments showed significant difference without starter culture [F (2, 16) - 52.16, P < 0.001] and with starter culture [F (2, 16) = 58.03, P < 0.001] at the 1% level of significance (Table 3) as a result of the difference in weights and combination. Similar trend occurred for the duration of the experiment within intervals of 5 – 45 days with [F (2, 16) = 82.43, P < 0.001] or without starter culture [F (2, 16) - 54.59, P < 0.001]. Based on the results, volume of biogas produced varied widely with or without the inclusion of starter culture in the study. This implies that production of biogas from the prescribed treatment combinations can be facilitated with or without the use of starter culture. Thus, the cost of developing starter culture could be saved. The significant increase in biogas yield obtained could be due to synergy between the cassava peels, the water hyacinth and cow dung. The acidic effects of cassava peels may have been neutralized by the water hyacinth and cow dung.

Combination of water hyacinth, cassava peels and cow dung

The optimal, total and percentage biogas yield was higher in combination of the three substrates than the single and double combinations. There was also reduction in the duration for optimal biogas yield than the respective individuals. Variations in biogas volume generated from WH + CP + CD treatment combinations showed significant difference without starter culture [F (2, 16) = 20.86, P < 0.001], but no significant difference with inclusion of starter culture [F (2, 16) - 1.18, P = 0.3314], (Table 3). There was also negative correlation between biogas production with and without biogas production (Fig.3). Thus, the volume of biogas from the
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CONCLUSION

One of the major challenges of anaerobic digestion is the use of local technology to design a digester which will be sufficiently air tight to prevent leakage or introduction of air into it. This is because Methanogenic bacteria are highly sensitive to oxygen or air hence the entire system is destabilized and it takes a longer time to recover if ever it does. It is also obvious that higher temperature supports biogas generation at a shorter retention time than ambient temperature used in this study. There is the need to further research on a digestion model which will support biogas generation at ambient temperature since this conserves energy and can easily be applied by the rural dwellers. Methanogens naturally grow very slowly and this increases retention time, there is therefore the need for further study to screen novel bacteria and fungi which can grow faster with increased biogas generation. There is a further need to design a more effective way of storing the biogas generated for further use, especially by rural dwellers. Finally there is the challenge for sustainable research on biogas technology for it to create the expected impact as a source of renewable energy and a reliable alternative to the non renewable fossil fuel energy.
FIGURE 1: Anaerobic digester and gasometric chamber assembly showing flammable gas
TABLE 1

Total viable bacterial and fungal counts from substrates slurry before and after anaerobic digestion

<table>
<thead>
<tr>
<th>Culture mode</th>
<th>Raw substrates</th>
<th>Bacterial counts before digestion (BCBD) (cfug⁻¹)</th>
<th>Bacterial counts after digestion (BCAD) (cfug⁻¹)</th>
<th>Fungal counts before digestion (FCBD) (cfug⁻¹)</th>
<th>Fungal counts after digestion (FCAD) (cfug⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without starter culture</td>
<td>WH</td>
<td>5.46 x 10⁷</td>
<td>3.55x 10⁵</td>
<td>1.46x 10⁴</td>
<td>1.20 x 10²</td>
</tr>
<tr>
<td></td>
<td>PD</td>
<td>8.63 x 10⁷</td>
<td>5.54 x 10⁵</td>
<td>3.42 x 10⁴</td>
<td>2.26 x 10²</td>
</tr>
<tr>
<td></td>
<td>CD</td>
<td>8.65 x 10⁷</td>
<td>6.45 x 10⁵</td>
<td>3.55 x 10⁴</td>
<td>2.25 x 10²</td>
</tr>
<tr>
<td></td>
<td>WH+PD+CD</td>
<td>7.55 x10⁸</td>
<td>4.10 x 10⁵</td>
<td>2.35 x 10⁴</td>
<td>1.20 x 10²</td>
</tr>
<tr>
<td>With starter culture</td>
<td>PD</td>
<td>8.60 x 10⁸</td>
<td>6.54 x 10⁵</td>
<td>4.42 x 10⁴</td>
<td>3.26 x 10²</td>
</tr>
<tr>
<td></td>
<td>CD</td>
<td>8.45 x 10⁸</td>
<td>7.35 x 10⁵</td>
<td>4.55 x 10⁴</td>
<td>3.25 x 10²</td>
</tr>
<tr>
<td></td>
<td>WH+PD+CD</td>
<td>7.65 x10⁸</td>
<td>5.25 x 10⁵</td>
<td>3.35 x 10⁴</td>
<td>2.20 x 10²</td>
</tr>
<tr>
<td></td>
<td>Control (Water Only)</td>
<td>5.54 x10⁸</td>
<td>4.40 x 10⁵</td>
<td>4.40 x 10⁴</td>
<td>3.15 x 10²</td>
</tr>
</tbody>
</table>
FIGURE 2: Optimum biogas yield from combination of water hyacinth, cassava peels and cow dung-WH+CP+CD with and without starter culture
TABLE 2

Total biogas yield from combination of water hyacinth, cassava peels and cow dung- WH+CP+CD, with and without starter culture (milliliters).

<table>
<thead>
<tr>
<th>Digestion time (Days)</th>
<th>Volume of biogas (milliliters/5days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>without starter culture</td>
</tr>
<tr>
<td></td>
<td>1kg</td>
</tr>
<tr>
<td>5</td>
<td>0.0</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>15</td>
<td>184</td>
</tr>
<tr>
<td>20</td>
<td>205</td>
</tr>
<tr>
<td>25</td>
<td>300</td>
</tr>
<tr>
<td>30</td>
<td>91</td>
</tr>
<tr>
<td>35</td>
<td>70</td>
</tr>
<tr>
<td>40</td>
<td>54</td>
</tr>
<tr>
<td>45</td>
<td>34</td>
</tr>
<tr>
<td>Total</td>
<td>948</td>
</tr>
</tbody>
</table>

TABLE 3

Analysis of variance (ANOVA) summary results showing variations in volume of biogas produced from water hyacinth, cassava peels and cow dung with/without starter culture

<table>
<thead>
<tr>
<th>SOURCES OF VARIATION</th>
<th>Starter culture</th>
<th>DF</th>
<th>SIGNIFICANCE</th>
<th>MSS</th>
<th>F-CAL</th>
<th>F-VALUE</th>
<th>F-CRITICAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight Periods (Days)</td>
<td>Without</td>
<td>2</td>
<td>426509.60</td>
<td>213254.80</td>
<td>20.86***</td>
<td>3.49E-05</td>
<td>3.63</td>
</tr>
<tr>
<td></td>
<td>With</td>
<td>2</td>
<td>2999338</td>
<td>1499669</td>
<td>1.18ns</td>
<td>0.3314</td>
<td>3.63</td>
</tr>
<tr>
<td>Error (Without)</td>
<td>Without</td>
<td>8</td>
<td>725299.30</td>
<td>90662.42</td>
<td>8.87***</td>
<td>0.000127</td>
<td>2.59</td>
</tr>
<tr>
<td></td>
<td>With</td>
<td>8</td>
<td>14549487</td>
<td>1818686</td>
<td>1.44ns</td>
<td>0.2554</td>
<td>2.59</td>
</tr>
<tr>
<td>TOTAL</td>
<td>Without</td>
<td>16</td>
<td>163589.80</td>
<td>10224.36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>With</td>
<td>16</td>
<td>20262463</td>
<td>1266404</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ns = not significant

*** = Significant at 1% level

Source = Derived from Author’s experimental data (2008)
Figure 3: Relation between biogas production from water hyacinth, cassava peels and cow dung with and without starter culture
PLATE 1: Experimental Set-up used for biogas generation from combination of water hyacinth, cassava peels and cow dung- WH+CP+CD

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