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**ORIGINAL RESEARCH ARTICLE**

# VALIDATION OF WET CHEMISTRY METHOD AS ALTERNATIVE TO GAS CHROMATOGRAPHY FOR BIOGAS METHANE CONTENT DETERMINATION

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**ABSTRACT**

Anaerobic digestion technology has the potential for simultaneous waste treatment and biogas generation. Its rate of dissemination and adoption in most developing countries has however, slowed down for many years partly due to inadequate research facilities such as advanced gas measuring equipment, and associated cost of analyses. This study, therefore, tested and validated the use of classical wet chemistry analysis method (CWCAM) as a readily accessible and cheaper alternative to gas chromatographic method (GCM), for determining the proportion of methane ($CH_4$) in produced biogas. Biogas samples were simultaneously collected every week in 5 ml and 20 ml hypodermic syringes, from a digester in which cassava vinasse (CV) was being codigested with poultry droppings (PD), using ruminal fluids of cattle (RFC) as inoculum. While samples in 5ml syringes were analyzed using the GCM, the 20 ml samples were analyzed using the CWCAM, to determine the percentage of methane in the biogas samples collected each week. The corresponding volumes, corrected to standard temperature and pressure (STP) condition was then calculated. The results from both analytical methods were statistically analyzed and compared using the Data Analysis tool in Microsoft Office Excel. The difference in results from the two methods ranged from -3 to +2.9% with CWCAM giving slightly higher values of methane percentage. Overall, a relatively high coefficient of determination ($R^2$ = 0.9728) and low standard error (SE = 1.4) of the regression equation between the two sets of results indicate that the two methods may be used interchangeably.



## 1.0 Introduction

Anaerobic digestion technology has the potential for simultaneous waste treatment and the generation of biogas, a renewable energy source (del Real and Lopez-Lopez, 2012; Kafle and Chen, 2015). Biogas is a mix of many gases, mainly methane (50-75%) and carbon dioxide (25-45)% with other components such as hydrogen sulphide, nitrogen, ammonia, oxygen and water vapour, usually present in small amounts of 1-5% (Al Seadi *et al.*, 2013). Methane is the component responsible for the calorific quality of biogas and if less than 50%, the biogas will not burn (Siles *et al.*, 2011). As a result, monitoring the percentage of methane in biogas that is being produced during anaerobic digestion provide vital information on biogas quality, possible uses Awe *et al.*, (2017) and the overall

digestion processes and is, therefore, very necessary in biogas research. Gas chromatograph (GC) equipped with either a thermal conductivity or flame ionization detector and using either hydrogen or helium as the carrier gas, is the most widely used analytical method for this purpose (Subbarao et al., 2013; Adepoju et al., 2016; Canepa et al., 2017; Dubrovskis and Plume, 2017). In developing countries, biogas technology can support energy supply decentralization such that rural communities can produce biogas to meet their energy needs (Ray *et al.*, 2016). It can be used for cooking, lighting, crop drying, pumping water for irrigation, direct heating, running internal combustion engines and electric power generation applications (Rafiee *et al.*, 2021). Also, indoor air pollution (IAP) which is a serious health risk especially to women and children in the rural areas, is prevented by using biogas as cooking fuel instead of fuel wood, kerosene and charcoal (Abadi *et al.*, 2017).

Despite the numerous applications of biogas (Machunga-Disu and Machunga-Disu, 2012; Ray et al., 2016; Awe et al., 2017), the dissemination and adoption of its production technology in most developing countries has been very slow for many reasons, chief among which is the lack of necessary research facilities due to poor funding (Mukumba *et al.*, 2016; Patinvoh and Taherzadeh, 2019). Therefore, considering the significance of methane quantification in biogas research, limited access to such research facilities as the advanced gas measuring equipment like GC has prompted many researchers to investigate more affordable alternative tools/methods such as low cost self-assembled gas analyzers Yang *et al.*, (2019) and classical wet chemistry analytical methods (Beck, 1994; Raposo *et al*., 2011). The latter appears to be readily accessible especially to researchers in the rural areas as it only requires simple laboratory skills of volumetric analysis and measurements Anozie and Adeboye, (2009) while the former require skills in instrumentation. In an international inter-laboratory study to investigate the performances of different biochemical methane potential (BMP) protocols, Raposo *et al*. (2011) reported that one laboratory reliably measured methane volume directly after removing carbon dioxide ($CO_2$) by flushing the biogas through a NaOH solution. Abdel-Hadi (2008) however, went further in another study, to compare the results of biogas methane quantification using GC and the $CO_2$ absorption-volumetric analysis method with a view to test the precision of the latter as an alternative method that can be used to determine the proportion of methane in biogas samples produced in laboratories with limited access to advanced analytical equipment. Although the outcomes showed that the two sets of results were comparable, the alternative method was not validated. Method validation is required to provide scientific evidence that the alternative analytical method is reliable and consistent before it can be used in routine analysis (Belouafa *et al*., 2017). Linear regression is one of the most established and common tool used for this purpose (Twomey and Kroll, 2008).

The objective of this work therefore, was to compare and statistically determine the relationship and association between two alternative analytical methods of GCM and CWCAM, using linear regression analysis. This was with a view to validating the latter as a reliable, readily accessible and cheaper biogas analysis method that can stimulate biogas research especially in laboratories with no access to GC or other advanced gas measuring devices.

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## 2. Materials and Methods

### 2.1 Materials

The sources, collection and pretreatments of CV, PD and RFC used in this study have been described in a previous work (Ibrahim et al., 2021a). The digester system used was a modified floating drum model and is shown in Figure 1. Digestion takes place in the black-painted cylindrical reactor tank while the produced biogas flows into the attached piping assembly (that had been partly filled with acidified saline solution). It exerts pressure on, and displaces a quantity of the saline solution that is equivalent to its own volume into the adjacent tubes. Temperature and pressure inside the reactor were measured directly with the aid of a mercury-in-glass thermometer (0-100 $^{o}$C) and a pressure gauge (0-5 bar), respectively attached to the top of the tank. All reagents used in the study were of analytical grade.

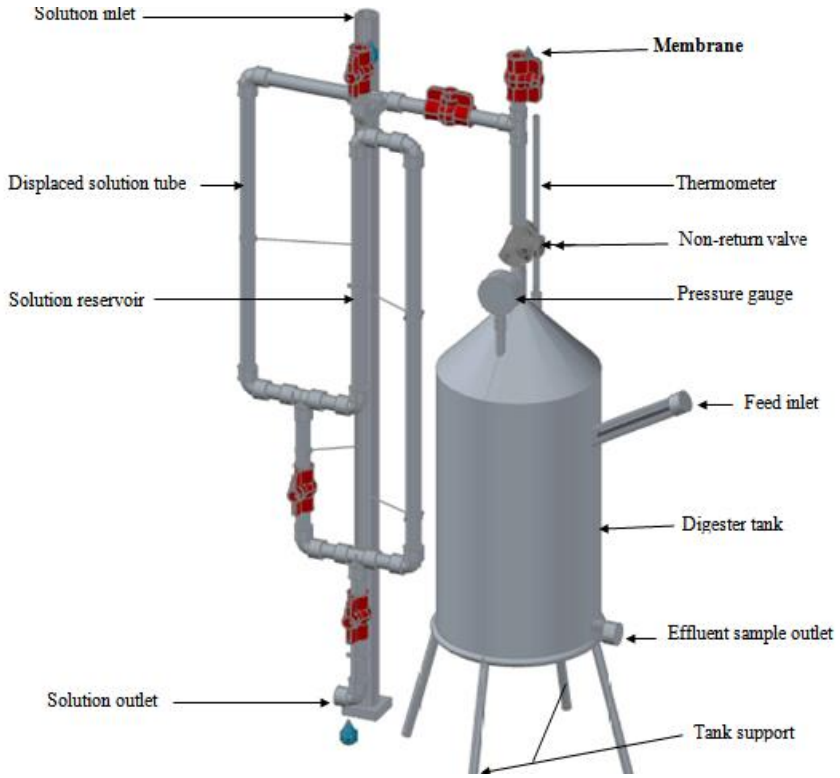


**Figure 1:** 15 l capacity digester system

### 2.2 Methods

#### *2.2.1 Digester Operation and Monitoring*

A CV and PD mix in the percentage ratio of 62.5:37.5 was loaded into the reactor tank of the digester, with a pre-degassed RFC as the inoculum (that was 25% of the total digester contents). This was obtained from the work of Ibrahim *et al*. (2021b) as the optimum mixing ratio for biogas production from CV. The system was operated in batch mode for a period of 10 weeks, at mesophilic temperatures. While pH and temperatures were daily monitored (Fleck *et al.,* 2017), biogas sample collection/measurement was carried out weekly according to Lami and Chimdessa (2016) and Dahunsi *et al.* (2018). Scum formation on slurry surface was prevented by gently shaking the digester for 1 min twice daily (Ghatak and Ghatak, 2018) and pH was controlled with the addition of a 0.05 M potassium phosphate buffer (Lei *et al.,* 2010).

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### *2.2.2 Biogas Methane Content Determination*

Biogas samples were simultaneously collected every week in 5 ml and 20 ml hypodermic syringes, via the membrane-plugged end of the pipe on top of the reactor, by slightly opening the connecting one-way valve. Meanwhile, the actual volume of biogas ($V_{biogas}$) produced each week was calculated from Equation (1) where 'h' is the height of the displaced acidified saline solution and 'A' is the cross-sectional area of the tube.

$$V_{biogas} = h \quad (1)$$

The 5ml syringe samples were analyzed in a GC (model HP5890) with an attached Hayesep Q column of dimension 13m x 0.5 m x 0.25 µm and a flame ionization detector. Its operation was according to the method used by Alfa *et al.* (2014). On the other hand, the biogas samples in the 20 ml syringe were analyzed for methane content by absorbing its $CO_2$ component in an alkaline solution and measuring the remaining gas volume, as described by Anozie *et al.* (2005) and Pham *et al.* (2013). About 15 ml of biogas sample in a 20 ml hypodermic syringe that was fitted with flexible hose (the syringe was initially filled with water and then emptied to reduce air contamination) and the free end of the tube was dipped into a 5 M NaOH solution. While excess gas was pushed out to have 7.5 ml gas sample ($V_1$) left in the syringe, approximately 7.5 ml of NaOH solution was drawn in with the end of the tube still submerged in the solution. The syringe was shaken for 30 s and pointed downwards as excess liquid was pushed out so that syringe plunger level reaches 7.5 ml. The small volume of liquid that was left in the syringe ($V_2$) equals the volume of $CO_2$ absorbed. Methane volume percent (z) was then calculated using Equation (2) while its actual volume was obtained from Equation (3), with the assumption that biogas components other than methane and $CO_2$ were only present in negligible amounts (Hafner *et al*., 2020). During the adjustment of NaOH solution level in the syringe, the biogas pressure ($P_o$) would have been equal to the atmospheric pressure (P) and so, the volume of methane at STP was calculated using the Ideal Gas Law (Equation (4)), for standardization.

$$z\% = 1 - \left(\frac{V_2}{V_1}\right) \quad (2)$$

$$V_{CH_4} = z_{CH_4} * V_{biogas} \quad (3)$$

$$V = \frac{V_o * T}{T_o} \quad (4)$$

Where,
V= gas volume at STP (ml);
$V_o$ = measured gas volume at room temperature (ml);
T = standard temperature (273 K);
$T_o$= room temperature in Kelvin;
$V_{biogas}$is the volume of biogas measured in the displacement unit;
$V_{CH_4}$is the volume of methane in the biogas

### *2.2.3 Statistical analysis of experimental data*

In this study, method of linear regression analysis as described by Leite and de Oliveira (2006) was applied to the output data (values of percentage of methane in biogas

samples) from GCM and CWCAM, with the aid of the Data Analysis tool in Microsoft Excel. The relationship between the two methods and the validity of CWCAM were then assessed at 95% confidence level, using indices such as the coefficient of determination ($R^2$), standard error and the p-value.

## 3. Results and Discussion

### 3.1 Digester Performance and Stability

The temperature profiles of digesting slurry for 10 weeks of anaerobic digestion are shown in Table 1. It shows that the average values of the morning and evening temperatures inside the digester naturally stayed within the mesophilic range of 25 to 34.6$^{o}$C respectively. Overall, the average daily temperature was estimated to be about 31 $^{o}$C. Even though, the loaded substrates-mix was conditioned to a pH of 7.2.

**Table 1:** Temperature profiles of the digesting slurry

| Weeks | Temperature ($^{o}$C) | |
|---|---|---|
| | Morning | Evening |
| 1 | 23.9 | 37.3 |
| 2 | 24.8 | 37.2 |
| 3 | 24.2 | 34.6 |
| 4 | 25.3 | 35.3 |
| 5 | 26.4 | 33.5 |
| 6 | 28.2 | 36.3 |
| 7 | 26.5 | 33.4 |
| 8 | 26.2 | 32.8 |
| 9 | 22.6 | 34.2 |
| 10 | 24.7 | 33.1 |
| *Average* | 25.28 | 34.77 |

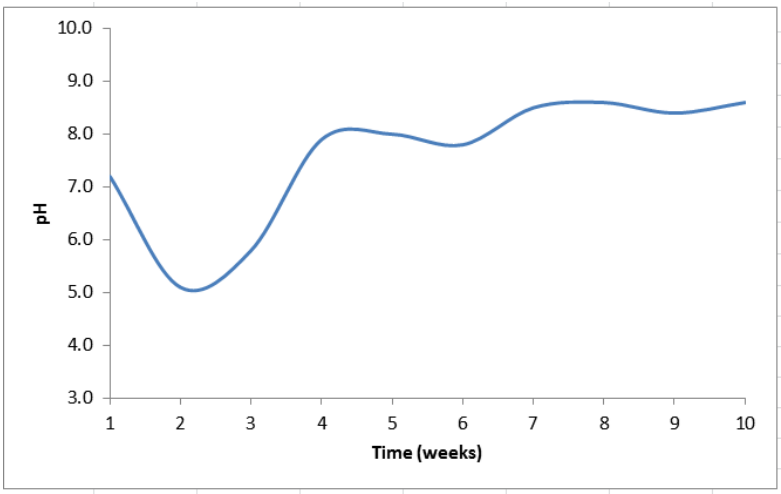


**Figure 2:** pH profiles of the digesting slurry

Figure 2 shows general fluctuations in pH within a range of ±2.1 during the retention period. This was due to the periodic accumulation of volatile fatty acids (VFA) and subsequent consumption by the methanogens (Macias-Corral *et al*., 2008). Highest pH drop was recorded at the start of digestion as the easily digestible fraction of organic

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matter was hydrolyzed and converted into VFA at the hydrolysis/acidogenesis stage (Lin *et al*., 2021). However, pH control efforts (addition of 1M NaOH and/or 1M $H_2SO_4$ as required) prevented digester failure and ensured biogas production throughout (Lei *et al*., 2010).

### 3.2 Biogas Production and its Methane Content

Figure 3 shows the daily production profiles of biogas, methane and carbon dioxide for the 10 week

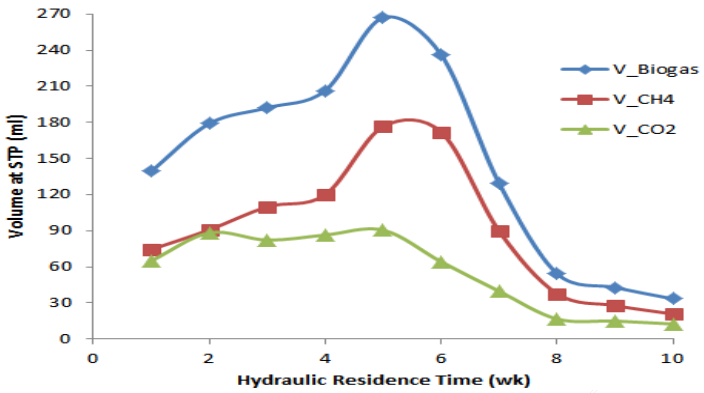


**Figure 3:** Biogas, methane and carbon dioxide production profiles based on CWCAM

digestion period. It was observed that after experiencing an initial lag phase, biogas production steadily increased from 139.5 ml in the first week to a maximum weekly production of 267 ml (which also corresponded with the highest methane volume) in the 5$^{th}$ week. Afterwards, it began to decrease steadily to 33.5 ml in the 10$^{th}$ week. The biggest drop in biogas production was observed to be 58% between the 8$^{th}$ and the 9$^{th}$ week. The steady decrease in production could be attributable to depletion of nutrients required for microbial activities (Baltrėnas *et* al., 2018). It was also observed that pH was generally alkaline from the 5$^{th}$ week and so, the drop in production could not have been due to imbalance between the hydrolysis/acidogenesis and the methanogenic phases of the digestion processes (Lin *et al*., 2021). Similar trends were also observed with the methane and $CO_2$ production profiles.

Digester stability and performance at any point during digestion was gauged by the quality of biogas being produced vis-à-vis its methane content and this is depicted in Figure 4. It shows that the best quality biogas had 72.8% methane and 27.2% $CO_2$ contents, obtained in the 6$^{th}$ week of digestion. This could be because the average temperature and pH at this time was respectively 31 $^oC$ and 7.8, in agreement with the report of Babaei and Shayegan (2019) which stated that the optimum condition for methanogenesis was 31-34 $^oC$ and 7.6-8 pH. Although, biogas production drastically dropped to 33.5 ml in the 10$^{th}$ week, digester stability remained good as indicated by the quality of produced biogas at 62.4% methane content.

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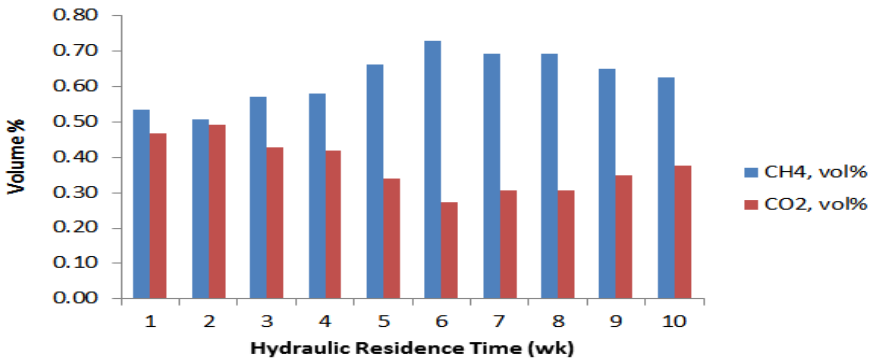


**Figure 4:** Methane and carbon dioxide contents in produced biogas (analysis based on CWCAM)

### 3.3 Comparison between the GCM and CWCAM Results for biogas Analysis

Results of the GCM and CWCAM performed to determine the percentage of methane in biogas samples collected during the anaerobic codiggestion of CV with PD are graphically shown in Figure 5. Both methods show that methane content generally increased with time but began to decline after about 6 or 7 weeks. Also, the values of methane percentage obtained from the CWCAM appear to be slightly higher than values from GCM with the highest variation being about 2.9%. This phenomenon could be attributed to the inability of the alkali solution to remove water, $H_2S$ and other trace components (Al Seadi *et al.*, 2013), along with $CO_2$ from the biogas. The closeness of the 2 datasets is clearly seen in the graph. Figure 6 is the plot of the regression equation (Equation (5)). It shows the linear relationship between the two analytical methods with a very high coefficient of determination ($R^2 = 0.9728$) and a low standard error (SE) of 1.40, which indicate that the two methods may be used interchangeably. At a p value of 1.5*E-7, the regression equation is statistically very significant ($p << 0.05$).

$$Y = 0.9061X + 6.1782 \quad (5)$$

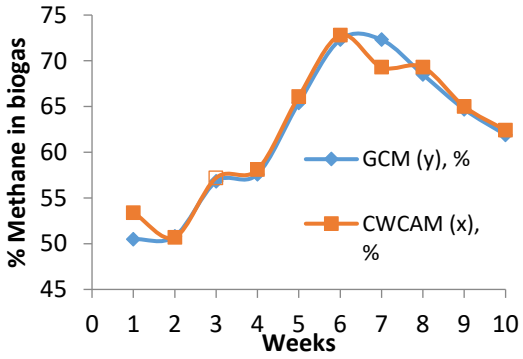


**Figure 5:** Comparisons between the GCM and CWCAM results for % methane determination.

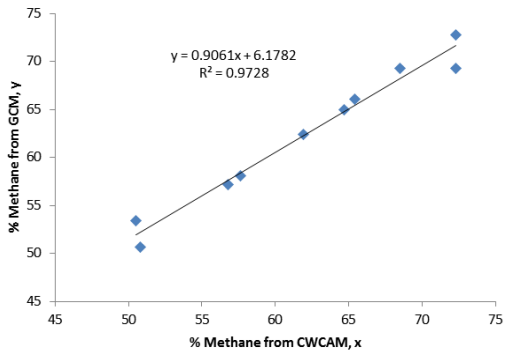


**Figure 6:** Correlation between the GCM and CWCAM results of % methane determination

## 4. Conclusion

Results from this work established that an accuracy that is comparable to that from gas chromatographic method of biogas analysis for methane content determination, can be achieved with the use of classical wet chemistry method. This indicate that the CWCAM

can be reliably used by researchers who have little or no access to modern gas measuring devices especially in the rural areas of developing countries.

## LIST OF ABBREVIATIONS

| | |
|---|---|
| BMP | Biochemical methane potential |
| $CH_4$ | Methane |
| $CO_2$ | Carbon dioxide |
| CV | Cassava vinasse |
| CWCAM | Classical wet chemistry analysis method |
| GCM | Gas chromatographic method |
| IAP | Indoor air pollution |
| PD | Poultry dopping |
| RFC | Ruminal fluid of cattle |
| STP | Standard temperature and pressure |
| $R^2$ | Coefficient of determibation |
| VFA | Volatile fatty acids |

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