Interval Analysis of Volatile Solid and Biochemical Oxygen Demand in Batch Anaerobic Fermentation of Selected Agrowaste

ABSTRACT

This study investigated the degradation of volatile solids (VS) and biochemical oxygen demand (BOD) during the anaerobic digestion of selected agro-waste, specifically cassava peels, maize husks, pig slurry, and the composite. The objective was to evaluate the biodegradability of these feedstocks, in line with their potential for biogas production. The Research was conducted at the National Centre for Energy Research and Development (NCERD) laboratory, University of Nigeria Nsukka (UNN). Batch reactors were used to conduct experiments over a period of 45 days. The digesters were operated under controlled conditions, and samples were analyzed at three (3) different points: 24 hours, peak of gas production, and at the end of the digestion. The results demonstrated that all the substrates exhibited a reduction in VS and BOD in the course of digestion. However, the most significant reduction in VS and BOD occurred around the peak of gas production. This entails that this period is critical for microbial activity and biogas yield. Corn husks had high initial VS and BOD values of 4.73±0.22 and 92.80±3.26 respectively. However, that did not translate to higher biogas production compared with the composite that had initial values of 4.34±0.19 and 88.00±5.50 respectively. By the end of the digestion, an average VS reduction of 65% and a BOD reduction of 70% were observed across all substrates, indicating a substantial degradation of organic material. Biogas production was better in the composite (72.580±4.53), an indication that higher VS and BOD do not automatically translate to higher gas yield. Thus, factors like multiple substrate interaction affect gas yield and process efficiency.

KEYWORDS; *Volatile solid (VS), Biochemical oxygen demand (BOD), anaerobic digestion, batch fermentation, agrowaste, biogas yield, parameter measurement.*

1. INTRODUCTION

Anaerobic digestion is a biological treatment process that recovers valuable products, energy, and nutrients, from organic waste streams in usable forms. Energy is recovered in the form of biogas, typically a mixture of methane (CH_4), and carbon dioxide (CO_2), with few other gases (Rittmann & McCarty, 2021). Small-scale anaerobic digestion in rural developing countries can positively impact the quality of life of its communities or an individual family's quality of life. Anaerobic digestion addresses issues as; energy production in the form of methane, which can be used as a cooking fuel, unsustainable deforestation due to the collection of wood for use as a biomass cooking fuel, mitigation of methane and carbon black emissions into the atmosphere, treatment of animal and/or human waste, reduced amount of biosolids to be disposed, produces nutrient-rich digestate that could be used as a fertilizer (Demirbas, 2018).

Biomethanation of fermentable organic materials, such as cattle dung, kitchen waste, poultry droppings, and agricultural waste, is one of the major processes of anaerobic digestion in the presence of methanogenic bacteria. The process occurs inside anaerobic biodigesters. The digested slurry from biogas plants is available for its utilization as bio/organic manure in agriculture, horticulture, and pisciculture as a substitute/supplement to chemical fertilizers (Tricase&Lambardi, 2019). In other words, biogas (anaerobic digestion) plants provide a three-in-one solution for gaseous fuel generation, organic manure production, and wet biomass management (White, 2017). Methane content varies from 50~75% to 25~50% of carbon dioxide, with trace amounts of hydrogen sulfide, hydrogen, oxygen, and nitrogen (Ahring, Sandberg &Angelidaki, 2015). However, biogas can be further treated to increase the methane content according to the demand and use (Liu, Olsson & Mattiasson, 2014).

In anaerobic fermentation, organic waste undergoes a series of microbial transformations to produce methane (CH_4) and carbon dioxide (CO_2). The success of this process depends largely on the composition and characteristics of the substrate, microbial community structure, and operational conditions such as pH, temperature, and hydraulic retention time (HRT). Understanding the behavior of key parameters such as volatile solids (VS) and biological oxygen demand (BOD) over time is crucial for optimizing the fermentation process.

2. MATERIALS AND METHODS

2.1 Sample Collection

Selected feedstocks (cassava peels, corn stalk and pig slurry) and cow rumen were sourced from the university farm. Sacks were used to gather cassava peels and corn stalks, while airtight buckets with surface sterilization were used to gather pig slurry and cow rumen contents. They were all brought to the lab for additional processing, examination, and use.

2.2 Sample Treatment

Plant substrates were physically pretreated by being shred into tiny pieces of approximately 2 mm in size, in order to improve their surface area. The shredded substrates were immersed in large water baths with a precisely determined amount of sodium hydroxide, a chemical process that aids in the breakdown of cellulose and lignin (Lansing et al., 2018). In order to achieve a desirable moisture composition, shredded samples were turned into slurry. Tap water was added and mixed in order to accomplish this. To maximize the methanogens engaged in methane production, 0.5 kg of cow rumen was added for every 10 kg of treated substrate (prior to loading).

2.3 Physicochemical Analyses

Substrate compositions were determined before and during digestion. Total solids and chemical oxygen demand were analyzed using the standard method for each, as described by the Association of Analytical Chemists (AOAC, 2010).The pH of the slurry was measured using a pH meter. Organic carbon(C) was determined by the wet oxidation method (Page et al, 2008). The temperature was maintained at a mesophilic level (30-40^{0}C), in order to maximize the microbial activities. There were intervals sampling of digesters content, to ascertain the levels of these parameters. Hydraulic retention time (HRT) was maintained at 45 days.

<u>2.3.1 Determination of Biochemical Oxygen Demand (BOD)</u>

BOD was determined by dilution method, as reported by Edelmann et al. (2020). The dissolved oxygen (DO) concentration of the digestate will be measured, before and after the incubation period of 5 days, following appropriate dilution. Three milliliters of the digestate sample will be placed in a 300ml of incubation bottle and then appropriately diluted with dilute water to fill the bottle (1/100 dilution). The bottle will be properly covered and sealed with masking tape, then stored in the dark at 20^{o}C for 5 days, to prevent dissolved oxygen production via photosynthesis. BOD_5 will be calculated using the formula;

$$BOD_5\ (mg/L) = (DO_0 - DO_5)P \qquad \text{Equ.ation 1}$$

Where; DO_0 is the dissolved oxygen on day zero (0), DO_5 is the dissolved oxygen on day five (5), P is the decimal dilution factor.

<u>2.3.2 Determination of Volatile Solids</u>

The volatile solid is the true organic matter available for bacterial action during digestion. The method of Edelmann et al (2020) was used. The solid residue from the total solid determination was heated in a muffle furnace at 600^{o}C for 2 hours. The heated residue was cooled in a desiccator and weighed.

$$\text{Volatile solid (VS)} = \frac{B-C}{g} x \frac{100}{1} \qquad \text{Equation 2}$$

B = Weight of dried residue from total solid determination, C = Weight of residue after further heating at 600°C, g = Original weight of sample.

2.4 Working Principles of Used Digesters

Figure 1 represents the working principle of used digesters in the course of the research. Measured quantities of the substrates were fed in using the inlet. The pressure valve allowed excess pressure to be released from the vessel. Stirrer was used to agitate the vessel content, promoting mixing, and preventing the formation of clumps. The gas outlet ensured the release of produced gas, while the sludge outlet was used to empty the digester after fermentation. In the batch fermentation process, feedstocks were added to the vessels, the anaerobic digestion was allowed to proceed until all the desired level of fermentation was achieved at day 45. Digestate was then removed, and the vessels were made clean and ready for another process.

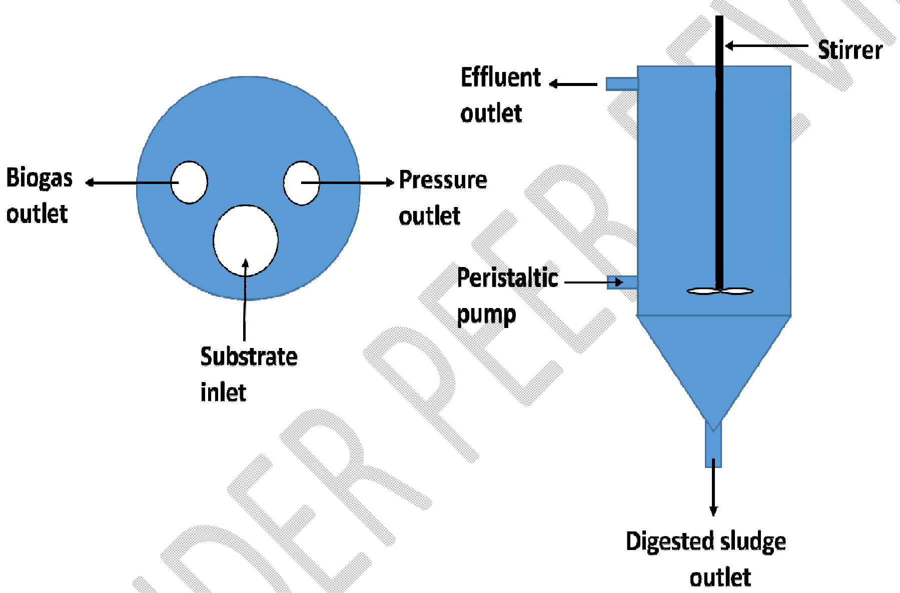

Fig. 1. Working principles of used digesters
Source: Ugwu et al 2021

2.5 Sample Feeding

To allow for the build-up of gas and pressure, 20L of each prepared slurry was cautiously added to the 30L digester capacity (for all substrates). A mechanical agitator was used to stir the mixture for five minutes after the required measurements were taken each day. Stirring was necessary to avoid clogs. The composite volume ratio was 1:1:1.

2.6 Gas Volume Measurement

A gas analyzer from the Aero-qual (500 series) was used to measure the methane yield during digestion (Model No.: DO-5509). In accordance with Wagentrist et al. (2017) methodology, this was completed by first putting the gas detection probe into the biogas digester nozzle and then unlocking it to collect data. After that, statistical analysis was performed on the readings of all the fermenters to identify discrepancies.

3. RESULTS AND DISCUSSION

3.1 Physicochemical Characteristics

Total solids (TS) and volatile solids (VS) are indicators of the organic matter content in the samples, the latter (VS) represents the portion of the total solids that is organic and biodegradable (Igoni, 2016). In other words, VS includes the organic matter that can be broken down by microbial activity into gases, primarily methane (CH_4) and carbon dioxide (CO_2), during anaerobic digestion. The relatively high TS and VS values suggest that the samples were rich in organic matter. Though cassava peel had a high VS value, its gas production could be lower at times. Itodo and Phillips (2011) attributed that to the presence of phenol. Phenol and other chemicals were also implicated by the works of Ahamefule et al, (2024) and Ali et al, (2022) to suppress the activity of methanogenic microbes.

Most values (e.g., chemical oxygen demand) as observed in this study were within range and not harmful to the environment. This is a confirmation of the organic nature of the substrates. It is also an indication that the substrates contained suitable nutrients required for anaerobic digestion (Alao et al, 2021).

From the findings, pig slurry has the highest initial pH value. Shah et al, (2014) attributed it to the significant amount of ammonia, primarily derived from the breakdown of nitrogen-containing compounds in pig waste. Ammonia is a weak base that can contribute to the alkalinity of the slurry when it reacts with water. Urine and other nitrogenous waste in pig slurry contain urea, which can undergo hydrolysis, leading to the release of ammonia. This process contributes to the overall alkalinity of the slurry (Glanpracha& Annachhatre, 2016). The high pH in the composite was contributed by pig slurry.

All samples exhibited similar TS values, ranging from 11.29% to 11.87%. This indicates that the solid content in these feedstocks is relatively consistent. COD, a measure of the organic matter present, varied across the samples. Pig slurry had the highest COD value of 234.4 mg/L, suggesting it contained the most organic matter, compared to cassava peels (213.6 mg/L).

Table 1. Physicochemical Results of Sample Contents at 24hrs of Setup

PARAMETERS	CASSAVA PEELS	CORN HUSKS	PIG SLURRY	COMPOSITE
TS	11.56 ±0.72	11.87 ±0.74	11.84 ±0.74	11.29 ±0.71
VS	4.19 ±0.26	4.73 ±0.30	4.13 ±0.26	4.34 ±0.27
COD	213.6 ±13.34	202.4 ±12.64	234.4 ±14.64	219.2 ±13.69
BOD	86.4 ±5.40	92.80 ±5.80	83.20 ±5.20	88.00 ±5.50
pH	7.80 ±0.49	9.80 ±0.61	11.50 ±0.72	9.51 ±0.59

Values are means of duplicate determinations with standard deviations (Mean±SD). Note that values; Total Solid (TS) and Volatile Solid (VS), are in percentage (%). Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) are in mg/L, pH (no unit).

3.2 Interval Analysis of Volatile Solid (VS) and Biochemical Oxygen Demand (BOD) of Digester Contents

At the beginning of the anaerobic digestion process, the volatile solid concentration was high. This is because the feedstocks contained a significant amount of organic matter that could be readily converted into biogas and digestate. As anaerobic digestion proceeds, the organic matters in the influent are broken, converting the volatile solids into biogas. This breakdown of organic materials results in a reduction of the VS content in the digester, which is a key indicator of the effectiveness of the digestion process (Ugwu et al 2021). Table 2 obeyed the aforementioned narration, where VS at the end of a digestion tended lesser than the initial value.

Understanding the variations in BOD during anaerobic digestion is important for monitoring and controlling the process. It can also be helpful in optimizing the system's performance, maximizing biogas production, and minimizing environmental impacts (Igoni, 2016). The initial BOD values are usually higher and could increase in the first few days of the fermentation because the complex organic compounds in the biomass are being broken down into simpler, soluble compounds, which can increase the BOD of the mixture (Wagentrist, et al, 2016). As the anaerobic digestion process continues, the BOD typically decreases. This is a result of the conversion of the organic matter into biogas and stable organic residues. The breakdown of complex organic compounds into simpler, less biodegradable forms, and the conversion of these substances into biogas and digestate, reduces the BOD.

Throughout the anaerobic digestion process, there could be fluctuations in BOD, powered by other factors. Factors like temperature and pH were maintained at a fair constant during this research. However, as the fermentation process nears completion, the BOD stabilizes at a lower level. This is an indication that the organic matter has been effectively converted into biogas and stable organic residues. The stabilized BOD level is typically much lower than the initial BOD of the feedstock (Rabii et al, (2019).

Understanding how the VS content and BOD change over time would help an operator to optimize the process and monitor its stability (Hadiyanto et al, 2023). VS and BOD decreased continuously from the state of charging to the end of the research, for batch fermentation. In continuous processes, BOD reduces during the reaction but could increase at the end of the experiment. This was reported by Ali et al, (2022) and Nwosu et al, (2020). The fact that new substrates are continuously fed into the digesters contributes to an increase in BOD in continuous fermentation.

Table 2. Interval Analysis of Volatile Solid (VS) and Biochemical Oxygen Demand (BOD) of Digester Contents

PARAMETERS	CASSAVA PEELS	CORN HUSKS	PIG SLURRY	COMPOSITE
VS (at 24 hrs)	4.19 ±0.21	4.73 ±0.22	4.13 ±0.20	4.34 ±0.19
VS (mid)	3.20 ±0.13	3.59 ±0.11	3.09 ±0.10	3.33 ±0.11
VS (end)	2.80 ±0.12	3.09 ±0.12 ±0.17	2.86 ±0.16	2.93
BOD (at 24 hrs)	86.4 ±3.77	92.80 ±3.26	83.20 ±2.92	88.00 ±5.50
BOD (mid)	64.0 ±4.00	60.8 ±3.80	70.4 ±4.40	65.6 ±4.10
BOD (end)	51.20 ±2.23	41.60 ±1.46	32.00 ±1.12	41.60 ±2.60

Values are means of duplicate determinations with standard deviations (Mean±SD). VS (volatile solid, BOD (Biochemichal oxygen demand).

3.3 Gas Yield Analysis

Table 3 presents the relationship that existed between methane (CH_4) and carbon dioxide (CO_2) concentrations in the generated biogas. In anaerobic digestion, CH_4 and CO_2 are the two primary gases produced, and their proportions can vary depending on several factors, including the feedstock. As observed in other works of Alao et al. (2024), and Mondal and Chatterjee (2016), this relationship between CH_4 and CO_2 in biogas is typically inversely proportional. In other words, as the CH_4 concentration increases, the CO_2 concentration tends to decrease, and vice versa. The ratio of CH_4 to CO_2, often referred to as the methane content or methane yield, is an essential indicator of biogas quality.

Co-digestion, the process of digesting multiple substrates together, often results in a higher gas yield compared to mono-digestion, where a single substrate is used (Ahamefule et al, 2024). Table 3 proved that the composite, an equal combination of cassava peels, maize husks, and pig slurry, co-digested together yielded the highest gas among others. According to Karakashev et al. (2015), the combination of different substrates in codigestion can result in synergistic degradation, where certain compounds in one substrate can facilitate the breakdown of complex components in another. This synergistic effect enhances the overall degradation efficiency, leading to increased gas production from the combined substrates. It shows that substrates interactions (composite) is more in precedence, than the initial values of VS and BOD, in terms of gas yield.

Table 3 Generated Biogas

GAS CONTENTSPEELS	CASSAVA	CORN HUSKS	PIG SLURRY	COMPOSITE
Methane	54.187 ±3.38 ±3.62	69.373 ±4.33	69.057 ±4.53	72.580
CO_2	38.742 ±1.69	25.743 ±1.60	26.588 ±0.90	23.120 ±1.44

Values are means of duplicate determinations with standard deviations (Mean±SD). CO_2 represent carbon-dioxide. Values are expressed in percentage (%).

4 Conclusion

This research analyzed the volatile solid (VS) and biochemical oxygen demand (BOD) levels in digester contents derived from various feedstocks. The study found that feedstock type significantly influenced the VS and BOD in the digester. Cassava peels were characterized by lower VS and BOD levels compared to the others. Corn husks, on the other hand, exhibited higher VS and BOD levels, especially in the initial stages of digestion. The composite demonstrated intermediate values, suggesting that combining different feedstocks can balance the organic matter content and biodegradability (Aichinger, 2015).

These findings have important implications for the design and operation of anaerobic digesters. The choice of feedstock can affect the digestion efficiency and subsequent production of biogas. Therefore, it is possible to optimize digester performance and maximize biogas yield, by understanding the VS and BOD characteristics of the substrates. There is also a need to monitor VS and BOD regularly, together with other parameters, during anaerobic digestion. This can help identify any operational issues and ensure optimal substrate digestion. The study established that high values of VS and BOD of a substrate do not automatically translate to high gas yield in fermenting such substrates, as there are other parameters and factors (e.g. codigestion) at play in the overall success of anaerobic digestion.

References

1. Rittmann, B. E., & McCarty, P. L. (2021). Environmental biotechnology: Principles and applications (International ed.). Singapore: McGraw Hill. Pp. 58-72.

2. Demirbas, A. (2018). *Biofuels sources, biofuel policy, biofuel economy and global biofuel projections. Energy Conversion Management, 49, 2106-2116.*

3. Tricase, C. & Lombardi, M. (2019). State of the art and prospects of Italian biogas production from animal sewage: technical-economic considerations. Renewable Energy. 11, 445-458.

4. White, J. (2017). Biogas Generation Potential of Coconut Copra in the Anaerobic Digestion Process. New Zealand: University of Canterbury. Pp. 12-18.

5. Ahring, B. K., Sandberg, M. & Angelidaki, I. (2015). Volatile fatty acids as indicators of process imbalance in anaerobic digesters. Applied Microbial Biotechnology, 43, 559-565.

6. Liu, J., Olsson, G. & Mattiasson, B. (2004). Control of an anaerobic reactor towards maximum biogas production. Water Science Technology, 50(11), 189-198.

7. Lansing, S., Martin, J., Botero, R. B., Da-Silva, T. N. & Da-Silva, E. D. (2018). Methane production in low-cost, unheated, plug-flow digesters treating swine manure and used cooking grease. Bioresource Technology, 101, 4362-4370.

8. AOAC, (2012). Official Method of Analysis: Association of Analytical Chemists. 19th Edition, Washington DC, 121-130.

9. Page, D. I., Hickey, K. L., Narula, R., Main, A. L. & Grimberg, S. J. (2008). Modeling Anaerobic Digestion of Dairy Manure Using the IWA Anaerobic Digestion. Water Science and Technology, 58(3), 1-8.

10. Edelmann, W., Engeli, H. & Gradenecker, M. (2020). Co-digestion of organic solid waste and sludge from sewage treatment. Water Science and Technology, 41(3), 213-221.

11. Ugwu, T. N,. Nwachukwu, A. A., Ogbulie, T. E., &Anyalogbu, E. A. (2021). Compositional assessment of selected plant-based substrates for biogas production. Journal of Advances in Biology & Biotechnology, 24(3):1-6.

12. Wagentrist, H., Schreiner, M. & Zollitsch, W. (2017). Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations. Bioresource Technology, 98, 3204–3212.

13. Igoni, A. H. (2016). Design of anaerobic bio-reactors for the simulation of biogas production from municipal solid waste. Chemical Engineering Journal, 426, 131-132.

14. Itodo, I. N. & Phillips, T. K. (2011). Determination of suitable material for anaerobic biogas digesters. Proceedings of the 2nd international conference and 23rd Annual General Meeting of the Nigerian Institution of Agricultural Engineers, 23,437-441.

15. Ahamefule, A. K., Ogbulie, T. E., & Ezeji, E. U. (2024). Comparative evaluation of methane yield from continuous and batch fermentative processing of selected agrowaste. Journal of Advances in Biology and Biotechnology, 27(10),1017-25.

16. Ali, S. S., Elsamahy, T., Abdelfattah, A., Mustafa, A., Khalil, M. A., Mastropetros, S. G., et al. (2022). Exploring the potential of anaerobic co-digestion of water hyacinth and cattle dung for enhanced biomethanation and techno-economic feasibility. Fuel, 329(1),125-197.

17. Alao, K. T., Gilani, S. I., Sopian, K., Alao, T. O., Oyebamiji, D. S., & Oladosu, T. L. (2024). Biomass and organic waste conversion for sustainable bioenergy: A comprehensive bibliometric analysis of current research trends and future directions. International journal of Renewable Energy Development, 13(4),750-782.

18. Shah, F. A., Mahmood, Q., Shah, M. M., Pervez, A. & Asad, S. A. (2014). Microbial Ecology of Anaerobic Digesters: The Key Players of Anaerobiosis. The Scientific World Journal, 20,1-21.

19. Glanpracha, N. & Annachhatre, A. P. (2016). Anaerobic co-digestion of cyanide containing cassava pulp with pig manure. Bioresource Technology, 214,112-21.

20. Rabii, A., Aldin, S., Dahman, Y. & Elbeshbishy, E. (2019). A Review on Anaerobic Co-Digestion with a Focus on the Microbial Populations and the Effect of Multi-Stage Digester Configuration. Energies, 12(6), 1106; https://doi.org/10.3390/en12061106

21. Hadiyanto, H., Octafalahanda, F. M., Nabila, J., Jati, A. K., Christwardana, M., Kusmiyati, K., &Khoironi, A. (2023). Preliminary Observation of Biogas Production from a Mixture of Cattle Manure and Bagasse Residue in Different Composition Variations. International Journal of Renewable Energy Development, 12(2), 390-395.

22. Nwosu-obieogu, K., Aguele, F.O., Onyenwoke, A. & Adekunle, K. (2020). Kinetic Model Comparison for Biogas Production from Poultry Manure and Banana Peels. European Journal of Sustainable Development Research, 4(2), 1-5.

23. Mondal, C., Das, A. & Chatterjee, S. (2016). A time-lag model for biogas production by anaerobic digestion. Journal of Renewable and Sustainable Energy, 8(6), 1-14.

24. Karakashev, D., Batstone, D. & Angelidaki, I. (2015). Influence of Environmental Conditions on Methanogenic Compositions in Anaerobic Biogas Reactors. Applied and Environmental Microbiology, 71(1), 331-338.

25. Aichinger, P. (2015). Synergistic co-digestion of solid-organic-waste and municipal-sewage-sludge: 1 plus 1 equals more than 2 in terms of biogas production and solids reduction. Water Resources, 87, 416-423.