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Original Article

Experimental Investigation of Biogas Yielding Rate from Anaerobic Co-Digestion of Multiple Organic Feedstocks Using a Bio-digester

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Abstract

Background: The high cost of fossil fuels has driven the exploration of renewable energy alternatives. This study investigates the anaerobic co-digestion of waterleaf, cow dung, and food waste to optimize biogas production.

Methods: Four co-digestion experiments were performed using a prototype plastic bio-digester over 50 days. The combinations tested were: waterleaf with cow dung, waterleaf with food waste, food waste with cow dung, and a mix of all three feedstocks.

Results: Co-digestion of waterleaf and food waste yielded an average biogas output of 25%, with a pH of 7.2 and a carbon-to-nitrogen (C/N) ratio of 28. Combining waterleaf and cow dung produced a 31% yield, a pH of 7.2, and a C/N ratio of 29. The mixture of food waste and cow dung resulted in a 34% biogas yield, with a pH of 7.1 and a C/N ratio of 30. The highest yield, 46%, was achieved by co-digesting waterleaf, cow dung, and food waste, with a pH of 7 and a C/N ratio of 32. All feedstock combinations maintained neutral pH levels, benefiting from the unique properties of each component: waterleaf provided vitamins A and C, food waste supplied carbohydrates and proteins, and cow dung contributed anaerobic microbes essential for digestion. Additionally, temperature was a significant factor influencing biogas production.

Conclusion: Co-digesting waterleaf, cow dung, and food waste maximized biogas production, demonstrating the potential for enhanced renewable energy generation through optimized anaerobic digestion processes.

Keywords: Biogas, Anaerobic digestion, Energy, Organic feedstock, Co-digestion

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Introduction

The world is constantly experiencing a paradigm shift due to urbanization, rapid population growth, changing consumption patterns, and various cultural, economic, and social activities. These factors have led to an increase in the rate of waste generation. According to the 2012 World Bank report on solid waste, the annual municipal solid waste (MSW) generation was estimated at 1.3 billion tons per year. It projected a more than 40% increase in the annual generation rate by 2025 and a more than 300% increase by 2100 globally, with organic waste estimated to comprise the majority.[1]

The generation of waste is one of the most significant environmental problems affecting the well-being of populations, particularly in developing countries. Anaerobic digestion is widely utilized in treating the organic fraction of municipal waste, offering benefits such as odor reduction, biogas production, and organic fertilizer.[2,3] Recently, anaerobic digestion technology has been validated for organic waste treatment and biogas production, demonstrating its versatility across various applications.[4] The origins of anaerobic digestion technology trace back to the 10th century, when the Assyrians employed it to heat bath water. Although nearly extinct, the technology resurfaced in the 17th century with the recognition that decomposing organic matter produces flammable gases.[5] One of the earliest large-scale applications occurred in Exeter, United Kingdom, in the 1890s, where it was used in wastewater treatment. Since then, the technology has found numerous applications in stabilizing sewage sludge and treating organic solid waste. The first commercial-scale anaerobic digestion plant consisted of unmixed tanks, proving challenging to operate due to settlement of organic solids and scum formation. Its adoption remained limited until the 20th century. The modern iteration of the technology was first applied in China in 1929, serving as an alternative fuel gas for domestic and industrial use in rural areas.[6,7] Anaerobic digestion is a natural biological process that harnesses the energy content of organic matter by degrading them into simpler chemical compounds in the absence of oxygen.[8,9] It is sustainable, affordable, readily available, renewable, and a zero-carbon form of energy supply. Among the various forms of energy produced by the anaerobic digestion process, biogas is notable for its ability to serve both domestic and industrial purposes without negative environmental impacts, provided it is processed adequately. Without this process, methane would be released uncontrollably into the atmosphere due to the decomposition of organic matter in open dumps. It is estimated that decomposition of organic matter emits over 500-800 million tons of CH_4 into the atmosphere annually.[10] Energy serves as an essential commodity fostering growth and development in many nations, perceived as the backbone of industrial evolution in the world's economy. However, interest in renewable energy sources like biogas has surged in recent times due to environmental concerns and a growing awareness of the high costs and environmental impacts of fossil fuels.[11] Examples of organic materials suitable for biogas production include animal wastes (such as cow dung, poultry droppings, pig dung, sheep and goat droppings, etc), agricultural wastes (like maize silage, water hyacinth, oil cakes, rice bran, tobacco waste and seeds, wastes from fruits and vegetable processing, press mud from sugar, etc), and kitchen wastes such as food leftovers and peels, among others. Anaerobic digestion technology emerges as a superior technique for harnessing energy from biomass compared to other methods like combustion and pyrolysis, as it generates biogas with minimal emissions into the atmosphere.[12] The production of biogas through anaerobic digestion technology offers an economical means of accessing renewable energy for daily consumption. It replaces traditional fuels like firewood for cooking and kerosene for lighting and cooking, thereby contributing to saving trees from deforestation.[13-15] Additionally, it can help reduce greenhouse gas (GHG) emissions and mitigate the effects of global warming caused by emissions from open dumpsites. Biogas is generated anaerobically through the fermentation and degradation of biodegradable or organic matter by anaerobic microorganisms in the absence of oxygen. It typically consists of over 45%-65% methane (CH_4), 25%-35% carbon dioxide (CO_2), and 10%-20% traces of moisture, elements, and chemical compounds such as hydrogen sulphide (H_2S), siloxane, and hydrogen (H_2). Research indicates that dumpsites rank as the third-largest anthropogenic source of GHG emissions, contributing to about 13%-20% of global CH_4 emissions, equivalent to over 223 million metric tons of CO_2.[16] Notably, CH_4 is 21 times more potent than CO_2 when released into the atmosphere.[6,17] Recent studies focusing on maximizing biogas yield have highlighted the reliability of co-digestion of organic feedstocks in this application. Co-digestion offers various benefits, including the dilution of toxic compounds present in feedstock, adjustment of pH and moisture content, provision of adequate buffer capacity, full homogenization of substrates, and diversification of bacterial populations during the process.[10] Co-digestion involves combining different compositions of non-uniform feedstocks through anaerobic digestion for biogas production.[18] One of the primary advantages of employing co-digestion in organic waste processing is its ability to treat multiple organic wastes in one or a few batches of the co-digestion process. Ukpabi et al[19] studied the application of biogas produced from co-digestion of cow dung and food waste, finding that biogas can effectively be used as a cooking fuel while the remaining organic solid after digestion can serve as organic compost. Phetyim et al[20] studied the biogas yield rate from co-digestion of vegetable waste with dog manure and cow dung, noting a higher percentage of biogas yield from the co-digestion of vegetable waste with dog manure. Gashaw and Libsu[21] demonstrated that anaerobic co-digestion of food waste and cow dung improved biogas yield potential compared to using cow dung as a single feedstock. Hallaji et al[22] found that anaerobic co-digestion of waste activated sludge with mixed fruit waste and cheese whey significantly increased biogas yield and improved the quality of digested sludge compared to anaerobic digestion of waste activated sludge alone. Fitamo et al[23] showed that co-digesting food waste, grass clippings, and garden waste with mixed municipal sludge can enhance biogas yield by 35-48% while reducing hydraulic retention time (HRT) to 15 days. Zamanzadeh et al[24] investigated biogas yield rates from anaerobic digestion of food waste and co-digestion of cow dung and food waste at mesophilic and thermophilic temperatures. They observed the highest biogas yield (480 mL/g) in the mesophilic digester containing only food waste, while mesophilic co-digestion of food waste and cow dung yielded 26% more biogas than the sum of individual digestions of cow dung and food waste.

This study investigated the interplay between various organic feedstocks and their potential for biogas production through anaerobic digestion co-digestion processes. The experiment involved co-digesting different combinations of feedstocks, namely waterleaf and cow dung, waterleaf and food waste, food waste and cow dung, as well as waterleaf, cow dung, and food waste. Additionally, the study analyzed the impact of different anaerobic digestion parameters to gain a comprehensive understanding of the process.

Kinetics of Anaerobic Digestion Process

Anaerobic digestion, also known as bio-methanization, is a natural process characterized by the breakdown of biodegradable organic matter into bioenergy or biogas by microorganisms in the absence of oxygen. This complex biochemical process involves the biochemical decomposition of organic matter, yielding energy-rich biogas and effluents such as digestate and leachate. The anaerobic digestion process entails intricate interactions between microorganisms of different species and the organic feedstocks being digested. The major functional groups of bacteria are categorized according to their metabolic reactions.[25]

- Fermentation bacteria
- Hydrogen-producing acetogenic bacteria
- Hydrogen-consuming acetogenic bacteria
- Carbon (iv) oxide reducing methanogenesis
- Aceticlastic methanogens

In this study, anaerobic digestion process occurred in different phases such as hydrolysis, acidogenesis, acetogenesis and methanogenesis as follows:

Hydrolysis

Hydrolysis, also known as liquefaction, represents the initial and typically slowest stage in the anaerobic digestion process of organic matter. In this stage, insoluble complex organic matter (as illustrated in equation 1), such as cellulose, undergoes conversion into soluble molecules like sugars, amino acids, and fatty acids by fermentative bacteria.

$$\begin{gathered} Lipids \rightarrow Fatty\ acids \\ Polysaccharides \rightarrow Monosaccharides \\ Protein \rightarrow \text{Amino Acids} \\ Nucleic\ Acids \rightarrow Purines\ \&\ Pyrimidines \end{gathered} \quad (1)$$

During this stage, insoluble polymers such as proteins, lipids, nucleic acids, carbohydrates, and fats, along with water present in the waste stream, are broken down into soluble products such as sugar, glucose, glycerol, purines, and pyridines.[26] Equations 2-4 depict the hydrolysis reactions of carbohydrates, proteins, and lipids in organic waste.

Carbohydrate

Polysaccharide (starch) → Oligosaccharide → Disaccharide → Monosaccharide (glucose: $C_6H_{12}O_6$) (2)

Proteins

Proteins→Peptone→Polypeptide→Oligopeptide→Amino acids (aminoaceticacid: $NH_2 - CH_2 - COOH$) (3)

Lipids

Tri palmiticacid glycerel ester→Tri palmiticacid + Glycerol (4)

Acidogenesis

This biological process involves the further breakdown of the remaining components from hydrolysis by acidogenic bacteria. During this stage, volatile fatty acids (such as propionic, formic, lactic, butyric, or succinic acids), alcohols (e.g., ethanol, methanol, and glycerol), and acetone are produced, along with small amounts of carbon dioxide, ammonia, and hydrogen sulfide. The chemical reactions involved in acidogenesis include the conversion of glucose to ethanol (Equation 5), the conversion of glucose to acetic acid (Equation 6), and the conversion of glucose to propionate (Equation 7).

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \quad (5)$$

$$C_6H_{12}O_6 \leftrightarrow 3CH_3COOH \quad (6)$$

$$C_6H_{12}O_6 + 2H_2 \leftrightarrow C_2H_5COOH\,OH + 2H_2O \quad (7)$$

Acetogenesis

The major products of acetogenesis include acetate, CO_2, and H_2. Long-chain fatty acids, derived from the hydrolysis of lipids, are oxidized to acetate or propionate, with hydrogen generated in the process. Propionate and butyrate are the most significant acetogenic substrates.[27] Additionally, lactate, ethanol, methanol, H_2, and CO_2 undergo acetogenic conversion to acetate. The reactions for the production of acetic acid from propionic and butyric acids are illustrated in Equations 8 and 9.

Propionic Acid

$$CH_3CH_2COOH \rightarrow 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2 \quad (8)$$

Butyric Acid

$$CH_3(CH_2)_2COOH \rightarrow 2H_2O \rightarrow 2CH_3COOH + 2H_2 \quad (9)$$

The reaction that involves the conversion of propionate to acetate during the acetogenesis stage of the anaerobic digestion process is given by equation 10.

$$CH_3CH_2COO^- + 3H_2O \leftrightarrow CH_3COO^- + H^+ + HCO_3^- + 3H_2 \quad (10)$$

Other important reactions in this stage involve the conversion of glucose (Equation 11), ethanol (Equation 12) and bicarbonate (Equation 13) to acetate.

$$C_6H_{12}O_6 + 2H_2O \leftrightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (11)$$

$$CH_3CH_2OH + 2H_2O \leftrightarrow CH_3COO^- + 2H_2 + H^+ \quad (12)$$

$$2HCO_{3^-} + 4H_2 + H^+ \leftrightarrow CH_3COO^- + 4H_2O \quad (13)$$

Methanogenesis

In this stage, methanogenic bacteria, or methanogens, produce methane (CH_4), carbon dioxide (CO_2), and water (H_2O), collectively known as biogas, by consuming intermediate products such as acetic acid, hydrogen, and some carbon dioxide generated from previous stages. Approximately 66% of methane is formed from acetic acids via acetate decarboxylation, while the remaining 34% is produced from CO_2 reduction.[28] The chemical reactions for methanogenesis can be expressed as follows:

$$\underset{(Acetic\,acid)}{CH_3COOH} \rightarrow \underset{(Methane)}{CH_4} + \underset{(Carbon\,dioxide)}{CO_2} \quad (14)$$

$$\underset{(Ethanol)}{2CH_3CH_2OH} + \underset{(Carbon\,dioxide)}{CO_2} \rightarrow \underset{(Methane)}{CH_4} + \underset{(Acetic\,acid)}{2CH_3COOH} \quad (15)$$

$$\underset{(Carbon\,dioxide)}{CO_2} + \underset{(Hydrogen)}{4H_2} \rightarrow \underset{(Methane)}{CH_4} + \underset{(Water)}{2H_2O} \quad (16)$$

Equations 14-16 illustrate various products, by-products, and intermediates generated during the anaerobic digestion of organic matter before the final product, methane, is obtained.

Materials and Methods

Materials

The various materials used in the course of the experimental procedure in this study are stated as follows:

1. *Samples:* Different organic feedstocks, including food waste, waterleaf, cow dung, and distilled water, were utilized in the experiment. Various types of food waste were incorporated, such as soya beans, rice, cocoyam, cooked plantain, semovita, cooked banana, and fufu. Cow dung samples used in the experimental process were collected from a cattle range in Ovia North East Area, Benin City, Edo State. Food wastes were sourced from various cafeterias, restaurants, hotels, and households in the Benin City metropolis and surrounding areas.
2. *Apparatus:* The equipment utilized for the system includes a pH meter, stainless steel bowl, plastic pipes, rubber hose, bicycle tube ball valves, pressure gauge, a 50-L plastic vessel, digital thermometer, weighing scale, and a plastic bio-digester. Plastic was chosen as the material for the bio-digester due to its resistance to corrosion and chemical reactions during the bio-thermal disintegration process. Additionally, the bottom part of the bio-digester cover was insulated with foam to prevent leakage, minimize heat transfer, and stabilize temperature fluctuations.

The experimental set-up of the bio-digester is depicted in Figure 1. It comprises a 50-L capacity plastic bio-digester equipped with the following features:

- Inlet and outlet control valves (ball valves) for regulating the feeding and removal rate of organic substrate from the bio-digester. The feedstock is introduced into the bio-digester through the inlet valve, while the digested substrate is discharged through the outlet valve.
- A gas extraction hose (4-inch in diameter) is employed for evacuating biogas from the system.
- A pressure gauge is utilized for monitoring and measuring the gas pressure generated within the bio-digester.
- A thermometer is employed to measure temperature variations during the decomposition of organic feedstock inside the bio-digester.
- A pH meter is used for assessing the pH of the feedstock before, during, and after the digestion process.
- A deflated bicycle tube weighing approximately 500 g is utilized for storing the biogas generated by the system. The biogas flows into the rubber tube, causing it to inflate. When reaching saturated pressure, the biogas generated in the bio-digester flows through the gas extraction hose into the deflated tube, inflating it.

Figure 1. Experimental Set-up for Anaerobic Digestion

Methods

During the experimental process, biogas production was investigated through the co-digestion of three different types of feedstock: waterleaf, food waste, and cow dung. The experiment was conducted in four distinct batches, namely: co-digestion of waterleaf and food waste; co-digestion of waterleaf and cow dung; co-digestion of food waste and cow dung; and co-digestion of waterleaf, cow dung, and food waste. Distilled water was added to each batch of the experiment. Specific proportions of feedstocks were allocated to each batch, consisting of 10 kg each of food waste, 10 kg each of waterleaf, 10 kg each of cow dung, and 10 kg each of distilled water. The mix ratios utilized in each experimental batch resulted in the following feedstock fed into the bio-digester:1:1:1 = 10 kg of waterleaf + 10 kg of cow dung + 10 kg of water = 30 kg

1:1:1 = 10 kg of waterleaf + 10 kg of food waste + 10 kg of water = 30 kg

1:1:1 = 10 kg of food waste + 10 kg of cow dung + 10 kg of water = 30 kg

1:1:1:1 = 10 kg of waterleaf + 10 kg of cow dung + 10 kg of food waste + 10 kg of water = 40 kg

The experimental procedures were conducted as follows:

- Distilled water and all substrate samples were thoroughly mixed in a large bowl until a paste-like consistency was achieved.
- The total mass of each set of the four samples was measured using a weighing balance to determine the initial mass of the substrate.

- The mixture of distilled water and each set of samples was then transferred into the bio-digester through the inlet valve, which was subsequently closed.
- The pressure gauge was initially calibrated to 0.0 bar.
- The pH of the substrate was measured using a digital handheld pH meter. pH values were recorded before, during, and after the digestion process.
- A temperature device (thermometer) installed on top of the bio-digester cover was utilized to monitor internal temperature and thermal variations within the system.
- Biogas produced was directed via a pipe hose into a bicycle tube. The mass of the tube without biogas was subtracted from the mass of the tube with biogas to determine the actual quantity produced at each gas evacuation time.
- The same procedure was repeated for all four experimental samples.

C/N Ratio Measurement

In recent years, the development of advanced analytical instruments, such as feedstock CHN analyzers, has significantly enhanced the accuracy and speed of carbon-to-nitrogen (C/N) ratio measurements, crucial for optimizing biogas production rates. The C/N ratios of the feedstock in this study were determined using a CHN analyzer, specifically the Elementar Vario MACRO Cube elemental analyzer, following the procedure outlined in European Standard EN 15104:2011. Measurement of the C/N ratio is pivotal in biogas experiments as it offers valuable insights into the efficiency and stability of the anaerobic digestion process. The C/N ratio serves as a fundamental parameter influencing microbial activity, substrate degradation, and biogas production. The procedures employed in determining the C/N ratios of co-digested organic feedstock in this study are outlined as follows:

I. Sample Preparation

Collect representative samples of the substrate or feedstock used in the biogas experiment.

Ensure that the samples are homogenized and free from any contaminants or impurities.

Dry the samples at a suitable temperature to remove moisture content, ensuring accurate measurements.

II. Calibration of the CHN Analyser

Follow the manufacturer's instructions to calibrate the CHN analyser using certified reference materials.

Calibration ensures accurate and reliable measurements of carbon and nitrogen content in the samples.

III. Sample Analysis

Weigh a representative sample (approximately 5-10 mg) using an analytical balance.

Transfer the sample into a tin capsule or boat, ensuring it is tightly sealed.

Repeat the process for all samples and standards to maintain consistency.

Place the tin capsules into the autosampler tray of the CHN analyser.

IV. Running the Analysis

Set the appropriate analysis parameters on the CHN analyser, including combustion temperature and carrier gas flow rate.

Initiate the analysis, allowing the instrument to combust the sample and separate the resulting gases.

The CHN analyser quantified the carbon and nitrogen content in the sample by measuring the gases produced during combustion.

The instrument generates a report containing the C/N ratio and other relevant parameters.

V. Data Interpretation

Calculate the C/N ratio by dividing the carbon content by the nitrogen content obtained from the CHN analyser.

Compare the measured C/N ratio with the desired range for optimal biogas production.

A higher C/N ratio indicates a carbon-rich substrate, while a lower ratio suggests a nitrogen-rich substrate.

Adjust the feedstock composition if necessary to optimize the C/N ratio for efficient biogas production.

Results and Discussion

The results obtained from the anaerobic digestion of waterleaf, food waste and cow dung have been presented in this section. As stated earlier, each batch of the experiment was conducted with different combinations of the aforementioned organic feedstock with distilled water. Summary of the experimental results has been presented in Table 1.

Figure 2 shows the results obtained for biogas yield from co-digestion of water leaf and food waste, water leaf and cow dung, food waste and cow dung as well as waterleaf, cow dung and food waste at HRT of about 50 days duration. From Figure 2, it is observed that the result representing the co-digestion of waterleaf, cow dung, and food waste, which combines three feedstocks, yielded the highest amount of biogas with a cumulative yield of 14.36 kg, as shown in Table 1. The second feedstock that yielded a reasonable amount of biogas under anaerobic conditions following the previously mentioned feedstock was food waste and cow dung, with a cumulative yield of 12.1 kg, as shown in Table 1. Furthermore, the third feedstock that yielded a good amount of biogas under anaerobic conditions following the second feedstock was waterleaf and cow dung, with a cumulative yield of 9.54 kg, as shown in Table 1. Additionally, anaerobic co-digestion of waterleaf and food waste yielded an optimum amount of biogas under anaerobic conditions following the third feedstock, with a cumulative yield of 7.82 kg, as shown in Table 1. It is also observed in Figure 2 that biogas yield from each batch of the experiment using

Table 1. Summary of experimental results

Cumulative Parameters (Σ)				
Specimens	**Σ pH**	**Σ Temperature (°C)**	**Σ Pressure (Bar)**	**Σ Biogas Yield (kg)**
Waterleaf and food waste	223.5	1096.6	1.99	7.82
Waterleaf and cow dung	223.9	1168.3	1.79	9.54
Food waste and cow dung	222.8	1222.4	1.97	12.1
Waterleaf, cow dung and food waste	219.4	1346.4	1.98	14.36
Average Parameters				
Specimens	**pH**	**Temperature (°C)**	**Pressure (Bar)**	**Biogas Yield (kg)**
Waterleaf and food waste	7.2	35.3	0.06	0.25
Waterleaf and cow dung	7.2	37.6	0.05	0.30
Food waste and cow dung	7.1	39.4	0.06	0.39
Waterleaf, cow dung and food waste	7.0	43.4	0.06	0.46
Average Percentage by Mass (%)				
Specimens		Biogas yield		
Waterleaf and food waste		25		
Waterleaf and cow dung		30		
Food waste and cow dung		39		
Waterleaf, cow dung and food waste		46		
C/N Ratio				
Waterleaf and food waste		28		
Waterleaf and cow dung		29		
Food waste and cow dung		30		
Waterleaf, cow dung and food waste		32		

the four aforementioned sets of feedstocks commenced on the twentieth (20th) day, out of the 50-day HRT. The HRT represents the total duration in days where organic feedstocks remained in the bio-digester undergoing the anaerobic digestion process. In other words, it is the beginning and ending date when the substrate is charged in and out of the bio-digester. The results imply that waterleaf, cow dung, and food waste mixed together as one feedstock are the best combination of feedstock that can produce a large quantity of biogas. This is because the combination consists of complete requirements essential for the decomposition of organic matter. That is, cow dung already contains millions of microorganisms, waterleaf naturally contains the key healthy vitamins (A and C) to sustain the microbes, while food waste contains the nutritional elements (carbohydrates, protein, starch, etc) for microbial growth and development. This correlates with the findings of Ikpe et al,[29] which revealed that carbohydrate and protein-based feedstocks may yield a substantial amount of biogas than others. However, studies conducted by Atandi and Rahman[30] revealed that organic feedstocks higher in lipids and fats have the potential of yielding higher bio-methane compared to organic feedstocks with high carbohydrates and fats content. In addition, Esposito et al[10] affirmed that organic feedstocks composed of carbohydrates, cellulose, hemicellulose, proteins, and lipids are viable for optimum biogas yield.

The plot of bio-digester temperature from different organic feedstocks against HRT is shown in Figure 3. This indicates that bio-digester temperature plays a vital role in biogas production. Some anaerobic microorganisms cannot survive for too long under ambient temperature; therefore, they must be enclosed in a system with optimal temperature distribution for them to actively break down organic feedstock in the bio-digester as required.

From Figure 3, it is observed that the result representing the co-digestion of waterleaf, cow dung, and food waste, which combines three feedstocks in one batch of experiment, produced the highest bio-digester temperature with an average of 43.4 °C, as shown in Table 1. The second feedstock that produced reasonably high bio-digester temperature under anaerobic conditions following the previously mentioned feedstock was food waste and cow dung, with an average of 39.4 °C, as shown in Table 1. Furthermore, the third feedstock that produced a good bio-digester temperature under anaerobic conditions following the second feedstock was waterleaf and cow dung, with an average value of 37.6 °C, as shown in Table 1. Additionally, anaerobic co-digestion of waterleaf and food waste under anaerobic conditions produced an average bio-digester temperature of 35.3 °C following the third feedstock, as shown in Table 1. The bio-digester temperature is one of the important parameters upon which the rate of anaerobic digestion or decomposition of feed materials in the bio-digester depends.[31] Judging from the highest, intermediary, and least bio-digester temperature from each feedstock presented in Figure 3 and the highest, intermediary, and least biogas yield from each feedstock presented in Figure 4, it is observed that an increase in bio-digester temperature improves the rate of biogas yield. This is because methanogens are quite sensitive to temperature changes in bio-digesters, as their growth as well as metabolic activities are highly influenced by bio-digester temperatures.[32] Therefore, bio-digester temperature must be properly regulated to improve the rate of biogas yield in the methanogenic phase.[33] However, temperature may not be an issue in anaerobic digestion processes under mesophilic regime as their temperature oftentimes increase and decrease naturally, unlike the thermophilic temperature regime where the temperature source is regulated.

Bio-digester pressure is a crucial factor that must be carefully monitored during the anaerobic digestion of organic feedstocks for biogas production, as a continuous increase in bio-digester pressure without timely evacuation may lead to unforeseen explosions. This is because the variation in bio-digester pressure indicates that the conditions for anaerobic digestion process are favorable for microorganisms, and that the production of biogas is ongoing. Considering that the bio-digester is a closed system, the pressure gauge continues to rise

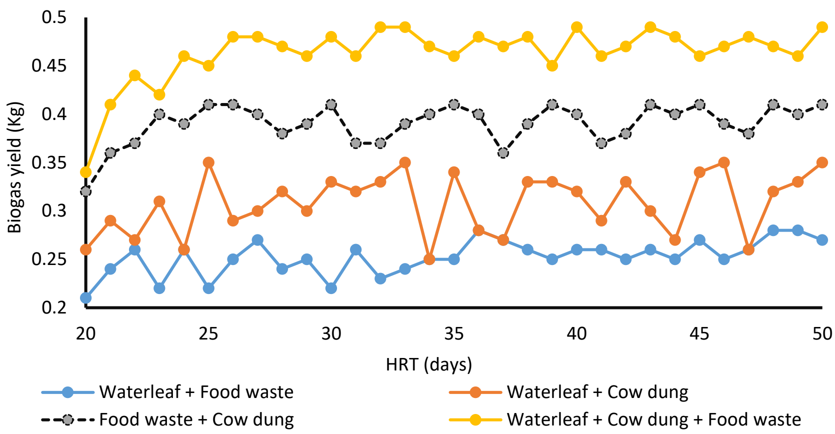

Figure 2. Plot of Biogas Yield From Different Organic Feedstocks Against HRT

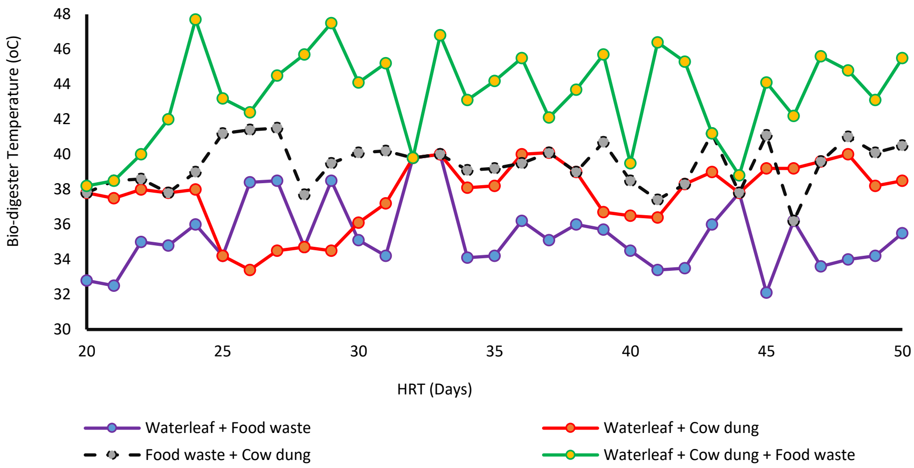

Figure 3: Plot of Bio-digester Temperature From Different Organic Feedstocks Against HRT

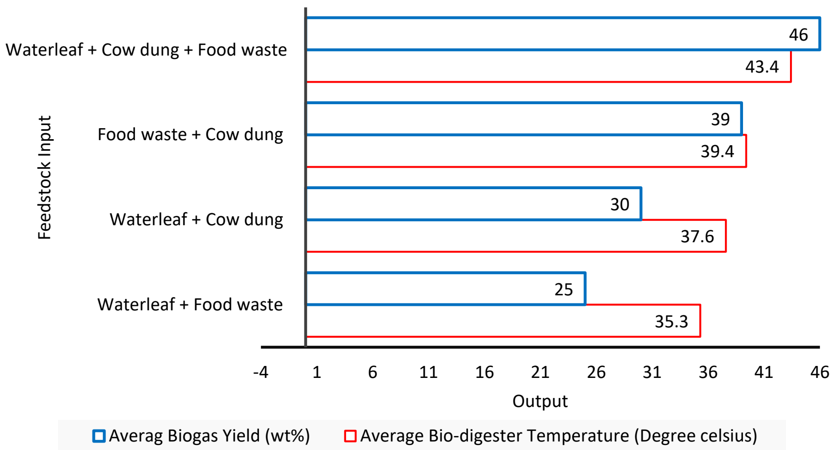

Figure 4. Average Percentage Yield of Biogas by Mass and Bio-digester Temperature

and accumulate as biogas is generated. If not evacuated on time to allow space for more gas to occupy, it can result in leakages around the monitoring probes and subsequent outbursts. This is in line with the general gas law, PV = nRT, where P represents the pressure of gas and n is the number of moles (quantity) of gas produced. The plot of bio-digester pressure from different organic feedstocks against HRT is presented in Figure 5. From the plot, it is observed that bio-digester pressure for all four categories of feedstock varies between 0.01 and 0.09 bar. Bio-digester pressure obtained by Ebunilo et al[34] ranged between 0.2 and 0.4 bar, while that of Ikpe et al[29] ranged between 0.11 and 0.17 bar. The difference in bio-digester pressure in this study may be due to the evacuation interval or the nature of feedstocks. The varying nature of the bio-digester pressure is a function of how long biogas yield occurred within each evacuation interval. In events where the gas generated in the system is not evacuated at

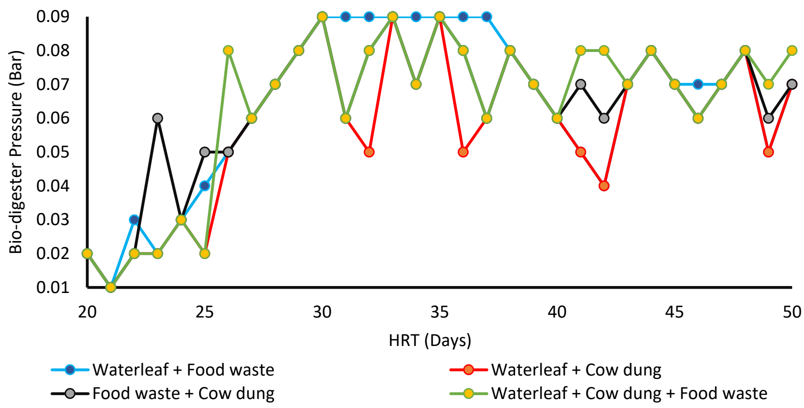

Figure 5. Plot of Bio-digester Pressure From Different Organic Feedstocks Against HRT

all, the pressure gauge continues to rise up to a maximum point where movement can no longer be noticed. At this stage, the anaerobic digestion process is at great risk due to excessive buildup and saturation of biogas in the bio-digester system. The variations in bio-digester pressure observed on the plot in Figure 5 are caused by settlement of the feedstock during decomposition or interruption in microbial activities, where the breakdown process of the feedstock by microorganisms is not in progress. However, in events where there is no increase, fluctuation, or variation, or a constant decrease from a higher point of pressure without any subsequent increase, it is likely that the process is interrupted.

On a standard scale, pH values from 0.0 to 6.8 are considered acidic, a pH of 7 is neutral, while pH values ranging from 7.1 to 14 are considered alkaline (basic). For biogas production, pH values from 0.0 to 6.7 are classified as acidic, from 6.8 to 7.4 as neutral, and from 7.5 to 14.0 as alkaline. The optimal pH range for biogas production from organic feedstock is between 6.9 and 7.2. Beyond this range, the feedstock's pH is either too alkaline or too acidic, negatively affecting biogas yield. Figure 6 graphically illustrates the percentage yield of biogas and average pH value from each feedstock category. Co-digestion of waterleaf, cow dung, and food waste yielded the highest percentage of biogas (46% by mass) with an average pH of 7. In contrast, co-digestion of waterleaf and cow dung yielded the least biogas (25% by mass) with an average pH of 7.2. Although other factors like the nutrients present in the feedstock matter, biogas recovery is significantly hindered if the pH of the feed substrates deviates from the neutral range. Figure 6 also shows that co-digestion of waterleaf and cow dung yielded more biogas (30% by mass) than waterleaf and food waste (25% by mass) despite having the same pH. This is because cow dung contains microorganisms essential for accelerating the breakdown of organic feedstock, while waterleaf provides key vitamins (A and C) to sustain microbial growth under anaerobic conditions. Cow dung is rich in essential anaerobic microorganisms. In contrast, food waste, while nutritionally rich (containing carbohydrates, protein, starch, etc.), requires time for microorganisms to develop. Reports indicate that food waste has a bio-methane yield potential of 0.44-0.48 m^2 CH_4/kg of added volatile solids.[35] If the microorganism development period is prolonged and there are no microbes to start decomposing the feedstock, the process can fail, resulting in no biogas recovery. The combination of waterleaf, cow dung, and food waste yielded the highest biogas because the mix provided an ideal environment for biogas production under anaerobic conditions. The impact of pH on anaerobic digestion of organic substrates was investigated by Orhorhoro et al[36] Their findings showed that pH values ranging from 5.2 to 9.6 are found in various samples, with 7.0 to 7.4 being optimal for biogas production. These results are consistent with the findings of this study and align with the observations of Ikpe et al[37] who noted that anaerobic co-digestion technology could meet growing energy demands, protect the environment from greenhouse gas emissions, and serve as an effective waste disposal alternative.

The C/N ratio serves as a crucial parameter in biogas experiments due to its direct impact on microbial activity and substrate degradation. A balanced C/N ratio is essential for optimal biogas production, as it provides an appropriate carbon source for methanogenic bacteria and sufficient nitrogen for protein synthesis. Monitoring the C/N ratio enables researchers to detect any imbalances in substrate composition and make informed decisions to improve the anaerobic digestion process. The preferred Carbon to Nitrogen ratios in anaerobic digestion typically range between 20 and 30. In simpler terms, the optimum C/N ratio for biogas production falls within the range of 20-30 carbon atoms to 1 nitrogen atom.[38] A C/N ratio higher than this range indicates an excess of carbon in the organic matter, leading to rapid consumption of nitrogen by methanogens, which can prolong decomposition, HRT, and the rate of biogas production. Conversely, a lower C/N ratio results in a malodorous substrate with high concentrations of ammonia and pH values above 8.5, which are toxic to methanogenic bacteria. Achieving optimum C/N ratios in the anaerobic digestion process often involves mixing organic materials with varying C/N ratios. For instance, combining organic solid waste with sewage or animal manure can help balance the C/N ratio.[39] The C/N ratios of some organic materials reported by Mittal[40] include 10, 24, 36, 26, 30, and 19 atoms of carbon to 1 atom of nitrogen for chicken dung, cow dung, yam peel, banana peel, plantain peel, and vegetable wastes, respectively. Studies have demonstrated that co-digestion of multiple organic feedstocks can yield C/N ratios within the optimum range. This is evident in Figure 7, which shows C/N ratios of 28 for the co-digestion of waterleaf and food waste, 29 for waterleaf and cow dung, 30 for food waste and cow dung, and 32 for waterleaf, cow dung, and food waste.

Augmentation of the Bio-digesters

Bio-digesters represent a sustainable solution for managing organic waste and producing renewable energy. However, to ensure their optimal performance, effective augmentation strategies are essential. These systems rely on anaerobic digestion to convert organic waste into biogas and nutrient-rich digestate. Augmenting bio-digesters offers several benefits, potentially leading to widespread adoption for organic waste management and energy recovery. To maximize their potential, bio-digesters require augmentation through the following strategies:

Selection of suitable feedstock: The choice of feedstock significantly influences bio-digester performance. Opting for feedstock with high organic content, such as animal manure, food waste, or agricultural residues, enhances biogas production and energy generation.[41]

Optimization of feedstock composition: Achieving an optimal feedstock composition is crucial. Maintaining a balanced mixture of carbon-rich and nitrogen-rich materials ensures an appropriate C/N ratio, ideally between 25:1 and 30:1.[42,43] Regular monitoring and adjustment of feedstock composition help maintain this ratio for improved performance.

Temperature Control: Maintaining the right temperature range within the bio-digester is vital for efficient anaerobic digestion. Most bio-digesters operate within the mesophilic range (around 35-40 °C), while some may require thermophilic conditions (around 50-60 °C). Implementing temperature control mechanisms, such as insulation or heating systems, ensures the desired temperature range, promoting enhanced performance.[44]

pH Regulation: The pH level within the bio-digester is critical for microbial activity. Maintaining the ideal pH range of 6.5 to 7.5 is essential. Regular monitoring and pH adjustment using buffering agents like lime or sodium bicarbonate help maintain optimal pH levels, improving bio-digester performance.[45]

Mixing and agitation: Proper mixing and agitation of bio-digester contents ensure uniform distribution of microorganisms and nutrients, facilitating efficient digestion. Mechanical or hydraulic mixing systems are employed to achieve adequate mixing and agitation.[46]

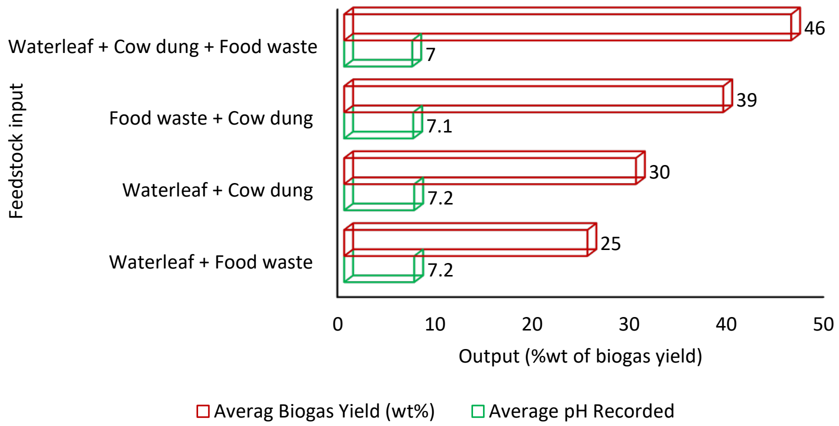

Figure 6. Average Percentage Biogas Yield by Mass and pH of Feedstocks

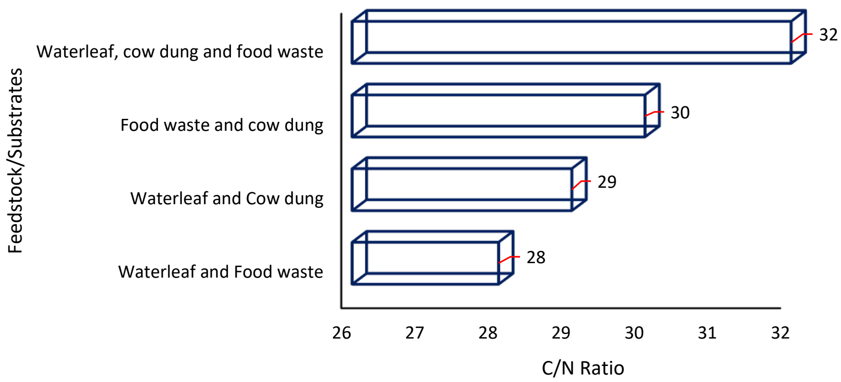

Figure 7. C/N ratios of Feedstock Used in the Study

Removal of inhibitory substances: Certain substances in the feedstock can inhibit microbial activity within the bio-digester, reducing performance. Pre-treatment methods like sieving, sedimentation, or filtration help remove inhibitory substances, enhancing bio-digester efficiency.[47]

By implementing these measures, bio-digester systems can achieve enhanced efficiency, leading to increased biogas production and improved waste management. Continued research and technological advancements in these areas will contribute to the sustainable development of bio-digester systems.

Conclusion

In this study, various organic feedstocks including combinations of waterleaf, cow dung, and food waste were co-digested in a locally assembled bio-digester to produce biogas via the anaerobic digestion process. While all feedstocks showed potential for biogas production, certain combinations outperformed others in terms of cumulative biogas yield. Notably, co-digestion of waterleaf, cow dung, and food waste yielded the highest cumulative biogas of 14.36 kg, accounting for an average percentage by mass of 46%. This feedstock also exhibited a higher average temperature (43.4 °C) compared to others and a neutral pH value of 7.0. This particular combination was advantageous due to its diverse microbial population, essential nutrients for microbial growth, and high content of volatile solids (VS) converted into biogas. Cow dung contributed millions of microorganisms, waterleaf provided key vitamins (A and C) to sustain microbial activity, while food waste supplied essential nutrients for microbial growth. The study underscores the importance of proper feedstock selection and monitoring of parameters such as pH and temperature to optimize biogas yield. Accurate measurement of the C/N ratio using a CHN analyser is crucial for assessing biogas production efficiency and stability. This data informs adjustments to feedstock composition to achieve an optimal C/N ratio for efficient biogas production. Additionally, considering the detrimental effects of impurities in biogas, incorporating filters into bio-digesters for effective purification is recommended. Purification of biogas is vital to prevent impurities from corroding equipment and posing health risks when combusted. The widespread adoption of augmented bio-digesters holds promise for addressing organic waste management challenges and energy crises. Governments, policymakers, and stakeholders should prioritize supportive policies and incentives to encourage the widespread adoption of biogas production technologies. This will not only mitigate environmental pollution but also contribute to sustainable energy generation and waste management practices.

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Competing Interests

None.

Ethical Approval

The authors declare that this research do not require any ethical committee approval or legal authorization. All authors have read and agreed to the publication of this research work.

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