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Role of Inoculum Source in Bio-Methane Production From Tea Waste and Fish Residues Using Methanogenesis Anaerobic Co-Digestion Process

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ABSTRACT

Fish residues and tea waste, which are often regarded as environmental pollutants due to their unsanitary nature, can be repurposed as effective biocatalysts to improve biogas production. This research explored the anaerobic co-digestion of fish residues (FR) and tea waste (TW) with cow dung (CD) under mesophilic conditions to enhance biogas yield. Substrates were combined with water in a 1:1 ratio, and biogas production was measured using the volumetric water displacement method. The findings revealed a significant improvement in biogas production rates through co-digestion. The highest biogas yields were observed at different substrate ratios: 190.25 mL/day on day 9 for FR:CD (1.5:1), 45.13 mL/day on day 23 for FR:TW:CD (1:1:0.5), 72.1 mL/day on day 21 for FR:TW:CD (1:1:1), and 35.18 mL/day on day 21 for TW:CD (1.5:1). In a subsequent phase, biogas production increased further, with maximum yields of 289.56 mL/day on day 10 for FR:CD (1.5:1), 246.95 mL/day on day 9 for FR:TW:CD (1:1:1), 205.67 mL/day on day 9 for FR:TW:CD (1:1:0.5), and 150 mL/day on day 14 for TW:CD (1.5:1). These results demonstrate the potential of co-digestion to optimize biogas production within a 40-day hydraulic retention time, providing a sustainable solution for energy generation and waste management while reducing environmental pollution.

INTRODUCTION

The increasing global demand for energy, combined with the rapid depletion of fossil fuel reserves, has driven the need for renewable and sustainable energy solutions (Alengebawy *et al.*, 2024). Anaerobic digestion has emerged as a viable technology for converting organic waste into biogas, which mainly consists of methane (CH_4) and carbon dioxide (CO_2) (Archana *et al.*, 2024). Biogas is both competitive and sustainable as a renewable energy resource due to its wide availability of inexpensive feedstocks and its diverse applications, including heating, electricity generation, and fuel production (Kabeyi & Olanrewaju, 2022). Furthermore, biogas production serves as a dual-purpose approach, addressing waste management challenges and mitigating environmental pollution through reduced greenhouse gas emissions. The overexploitation of natural resources and heavy reliance on fossil fuels have significantly contributed to global environmental changes, posing threats to human health and ecosystems. Industrialization, urbanization, and economic growth have further exacerbated the generation of solid waste (Suocheng *et al.*, 2011; Rosik-Dulewska *et al.*, 2011). Projections indicate that the global population may reach 9.7 billion by 2050, intensifying pressures on land and water resources for food and industrial goods production (United Nations, 2015). Ineffective waste management has become a critical environmental and socioeconomic challenge that demands innovative and sustainable solutions (Kostecka *et al.*, 2014; Jambeck *et al.*, 2015). In response, scientific efforts have focused on utilizing biological residues for energy production, particularly through biogas generation during anaerobic digestion (Ziauddin & Rajesh, 2015).

Biogas, which consists mainly of methane and carbon dioxide, is gaining recognition as a renewable energy source with multiple applications, including heating, cooking, power generation, and fertilizer production etc. (Andersson *et al.*, 2004; Themelis *et al.*, 2007). The anaerobic digestion process, typically conducted under mesophilic conditions in dome-shaped digesters, also produces digestate, which can be further utilized as a bio-fertilizer. Methane derived from biogas is particularly valuable, with applications ranging from energy production to industrial processes (Andersson *et al.*, 2004). Anaerobic digestion involves four key stageshydrolysis, acidogenesis, acetogenesis, and methanogenesis (Chen *et al.*, 1980; Hashimoto *et al.*, 1981, Sreekrishnan *et al.*, 2004 & Ward *et al.*, 2008) during which organic matter is broken down into simpler compounds, ultimately producing methane and carbon dioxide (Klass, 1984; Weiland, 2006; Yen & Brune, 2007). The potential of anaerobic digestion to process agricultural wastes, kitchen wastes, cow dungs, poultry, pig faeces, waste has been extensively explored (Riagbayire *et al.*, 2023). For instance, Dearman and Bentham (2007) demonstrated that tea waste could be anaerobically digested to enhance biogas production, while Pound *et al.* (1981) highlighted the importance of maintaining optimal C:N ratios to maximize methane yields. Research has also indicated the need for further studies on chemical modifications

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and microbial community dynamics to better regulate anaerobic processes (Ramchandra *et al.*, 2007). Biogas produced through this process typically contains 55–70% methane and 25–35% carbon dioxide (Ziauddin & Rajesh, 2015). Advanced purification method like adsorption, can increase the biomethane concentration to over 90% by removing carbon dioxide, thereby enhancing the calorific value of biogas (Maile *et al.*, 2017).

In addition to energy production, the anaerobic digestate, rich in nitrogen, phosphorus, potassium (NPK), and trace elements, is a valuable byproduct suitable for use as a bio-fertilizer. Compositions as high as 4.27% nitrogen, 0.66% phosphorus, and 4.71% potassium have been reported in biosolids (Makádi *et al.*, 2012). The anaerobic digestion process occurs under three distinct temperature regimes: ambient (<25°C), mesophilic (25°C to 35°C), and thermophilic (45°C to 60°C), with organic carbon being converted into methane and carbon dioxide through stepwise microbial activity (Angelidaki *et al.*, 2003). There are four stages to the biogas production process. Figure 1 depicts the biogas generation process.

Hydrolysis

Polymers cannot be used directly by fermentative microorganisms. During this phase, bacteria decompose complex carbohydrates, proteins, and lipids into their fundamental monomer units, including, sugars, amino acids, and long-chain fatty acids. Polymers are converted into soluble monomers, as demonstrated in Equation (1):

$n(C_6H_{10}O_5) + nH_2O \rightarrow n(C_6H_{12}O_6)$ ………............ (1)

Acidogenesis

In this stage, acidogenic bacteria breakdown the carbohydrates and amino acids to form alcohols and volatile fatty acids (VFAs). Various type of products (acetate, ethanol, and propionate) can be produced by fermenting the glucose shown in the Equations (2), (3), and (4) respectively (Angelidaki *et al.*, 2003).

$C_6H_{12}O_6 \rightarrow 3CH_3COOH$ ……………………… (2)

$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$ ………… (3)

$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O$ …(4)

Acetogenesis

Acetogenesis is the third stage of anaerobic digestion, during which specialized bacteria known as acetogens convert the organic acids formed in the preceding "acidogenesis" phase into acetate (acetic acid), hydrogen, and carbon dioxide. Equation (5) depicts the conversion process:

$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$ …. (5)

Methanogenesis

In this final stage, conversion of acetic acids to methane and CO_2 occurred by methanogenic bacteria. Methanogenesis is classified into two types based on the substrate used:

a) Hydrogenotrophic Methanogenesis: Methane is produced when hydrogen reacts with carbon dioxide, following the reaction:

$CO_2+4H_2 \rightarrow CH_4+2H_2O$ ……………………………(6)

b) Acetotrophic or Aceticlastic Methanogenesis: Methane is generated through the conversion of acetate, as represented by the reaction:

$2CH_3COOH \rightarrow CH_4+CO_2$ …………………(7)

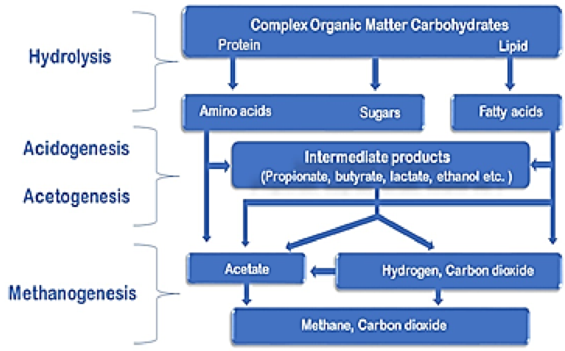

Figure 1: Flow diagram of anaerobic digestion process

There are several research available based on the production of biogas from solid waste such as cow dung, agricultural residues, and sewage sludge etc. The mixture of un-inoculated fruit and vegetable waste generated 300 mL of biogas, while the blank rumen produced 700 mL. In contrast, co-digesting the waste with rumen substrate resulted in 3500 mL of biogas (Mbugua *et al.*, 2024). However, it's rare to find notable research on the increase of bio-methane generation from fish residue and tea waste with inoculum sources via co-digestion. Research focused on trash reduction from tea shops and fish markets, as well as the possibilities of producing biogas from fish residue and tea wastes. This research focuses on obtaining the required quantity of bio-methane to offer as a replacement source of renewable energy by recycling fish residue and tea waste with cow dung to improve energy security and pollution control in order to develop an environmentally friendly waste management system.

MATERIALS AND METHODS

To determine the most effective approach for biogas production, meticulous care was taken to select the appropriate methods and procedures and ensure the validity of the data used. This chapter provides a detailed overview of the techniques and processes followed for biogas generation.

Raw Material Collection

The raw materials used in this study were sourced from various locations. Tea waste (TW) was obtained from Janapriyo Restaurant, located in Notun Bazar, Baluchar, Sylhet. Fish residue (FR) was sourced from Bandar Bazar fish market in Sylhet for the first phase of the experiment, while for the second phase, it was collected from Suhashini Das Hall dining at Sylhet Agricultural University. Cow dung (CD) was gathered from the Sylhet government dairy farm. After collection, the materials were stored at 4°C until use in the experiment.

Sample preparation

In preparation for fermentation, the collected fish residue (FR), tea waste (TW), and cow dung (CD) were weighed and combined in various proportions to form four different sample mixtures. Two mixtures were prepared in the ratios of fish residue (FR): tea waste (TW): cow dung (CD) [1:1:1] and fish residue (FR): tea waste (TW): cow dung (CD) [1:1:0.5]. The pH levels of the samples were measured, showing values of 6 and 6.6, respectively, for Phase 1 and 6.2 and 6.9, respectively, for Phase 2.

For optimal mixing, a combination of two types of waste was prepared. The first mixture consisted of fish residue (FR) and cow dung (CD) in a 1.5:1 ratio, while the second mixture was composed of tea waste (TW) and cow dung (CD) in a 1.5:1 ratio. These mixtures were properly diluted with water in a 1:1 (w/w) ratio to ensure a uniform consistency. The pH of these samples was measured as 6.9 and 5.6 in Phase 1, and 6.7 and 5.8 in Phase 2.

Fermentation Process

In the first phase of the experiment, four fermentation samples were prepared, each in a 2200 mL digester. Each digester was filled with 80% of the prepared mixture. After the feedstock was added, the anaerobic digesters were tightly sealed with rubber stoppers. The quantity of waste used in the reactors is detailed in Table 1. In the second phase, 90% of the digester was filled with the prepared mixture. After adding the feedstock, the digesters were sealed in the same manner as in Phase 1. The quantity of waste used for Phase 2 is shown in Table 2.

Table 1: Quantity of waste used in reactor.

Reactor	Waste (kg)	Water (kg)	Total weight of material (kg)
R1(tea +cow)	0.880	0.880	1.76
R2(tea+fish+cow)	0.880	0.880	1.76
R3(tea+fish+cow)	0.880	0.880	1.76
R4(fish+cow)	0.880	0.880	1.76

Table 2: Quantity of waste used in reactor.

Reactor	Waste (kg)	Water (kg)	Total weight of material (kg)
R1(tea +cow)	0.990	0.990	1.98
R2(tea+fish+cow)	0.990	0.990	1.98
R3(tea+fish+cow)	0.990	0.990	1.98
R4(fish+cow)	0.990	0.990	1.98

Experimental Setup

The lab-scale experimental setup utilized four 2200 mL digesters, each paired with a water chamber and a water collector for observation. The study was conducted within a temperature range of 16°C to 32°C. An 8 mm hose pipe was used to connect the digester to the water chamber, facilitating the flow of gas produced in the digester to the water chamber. This gas created pressure in the water chamber, displacing an equivalent volume of water, which was directed to the water collector through another 8 mm hose pipe.

One end of the gas pipe was attached to the top of the digester with a glass tube, while the other end connected to the top of the water chamber. Similarly, one end of the water pipe was connected to the water chamber, and the opposite end directed water to the water collector. A gas control valve was installed on the hose pipe to regulate the gas flow. Additional equipment included a thermometer, pH meter, glass tube, gas pressure gauge, gas flow control valve, graduated plastic bucket, beaker, and hose pipe. Biogas production was observed throughout the experiment, and data were collected over 40 days. A diagram of the experimental setup is presented in Figure 2.

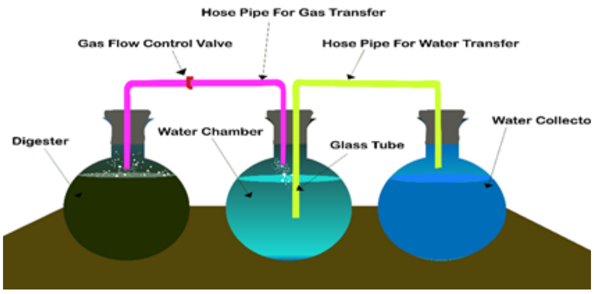

Figure 2: Graphical representation of experimental setup

Data Collection

The volume of gas produced during the experiment was determined using the water displacement method. Data collection occurred daily between 11:00 AM and 12:00 PM in the Agricultural and Biosystems Engineering Laboratory at Sylhet Agricultural University.

Observation

In both experiments (1 and 2), observations began after the digesters were filled with a mixture of fish waste, tea waste, and cow dung. Once the digesters were loaded, they were left to undergo anaerobic digestion. Gas production commenced on the 4^{th} and 7^{th} day of operation for the respective experiments and tapered off by the 40^{th} day. Water displacement method was used to measure the amount of produced biogas. The gas produced was directed through a pipe to a water chamber, displacing an equivalent volume of water into a collector via a hose pipe. The volume of expelled water was measured directly to determine the amount of gas produced. Observations continued until the displacement of water ceased, indicating no further gas production.

Analytical Method

A TP300 thermometer (China) and a PRUSMN digital food thermometer were used to monitor temperatures during the study, including the ambient environmental temperature. Both thermometers have a measurement range of -50°C to +300°C (-58°F to +572°F), with the PRUSMN thermometer featuring a resolution of 0.1°C (0.1°F) and an accuracy of ±1°C (±1°F). The PRUSMN thermometer includes buttons for ON/OFF, C/F, and Hold, and it is powered by a 1.5V LR44 / AG13 battery. It is constructed from 304 stainless steel and ABS plastic, with an auto power-off function that activates after 10 minutes of inactivity to save battery. Graphical analyses of the temperature data were conducted using Microsoft Excel 2010. The color of the thermometers may appear differently depending on monitor settings, and slight measurement discrepancies may exist due to manual measurement.

RESULTS AND DISCUSSION

Biogas production rates under mesophilic conditions are presented in Figures 3 through 10. By the end of the 40-day digestion period, all reactors showed minimal gas production.

Biogas Production Profile (Phase 1)

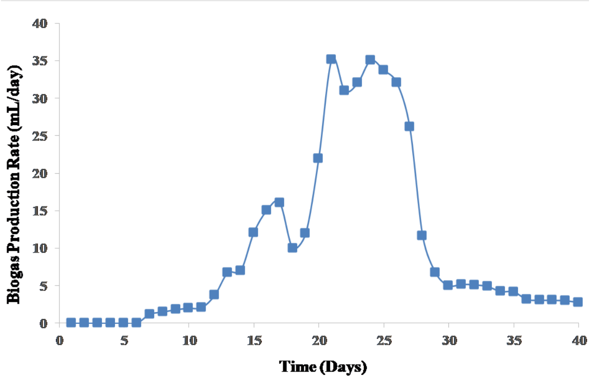

Figure 3: Daily Biogas Production Index for Cow Dung and Tea Waste (1:1.5)

Figure 3 shows the daily biogas production rate using a mixture of tea waste and cow dung. Over the 40-day digestion period, gas production began on the 7th day, attributed to the decomposition of undigested tea waste and cow dung. Production rates increased steadily between the 7th and 17th days before dropping abruptly on the 18th and 19th days. The production rate was influenced by factors such as methanogenic bacteria growth, temperature, and pH. Tea waste required a longer digestion time compared to fish residue. The highest gas production, 35.18 mL/day, was recorded on the 21st day, with an average production rate of 10.00 mL/day. The results indicate significant fluctuations in biogas production throughout the assimilation period.

Figure 4 highlights biogas production from a mixture of fish residue, tea waste, and cow dung. Production started on the 6th day and increased steadily between the 10th and 18th days. Temperature was a key factor affecting production rates. After the 28th day, production began to decline and continued to do so until the 40th day. The

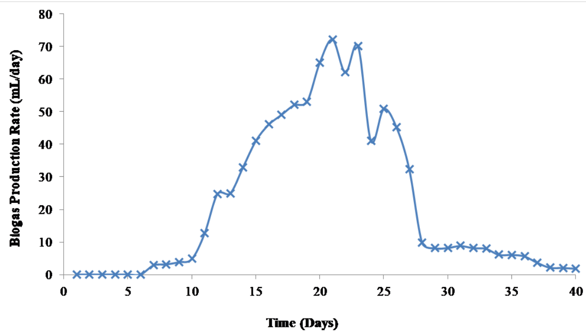

Figure 4: Daily Biogas Production Index for Fish Residue, Tea Waste, and Cow Dung (1:1:1)

peak production, 72.1 mL/day, occurred on the 21st day, with an average production rate of 21.78 mL/day. The results show higher biogas production for the 1:1:1 mixture compared to the 1:1:0.5 mixture of the same feedstocks.

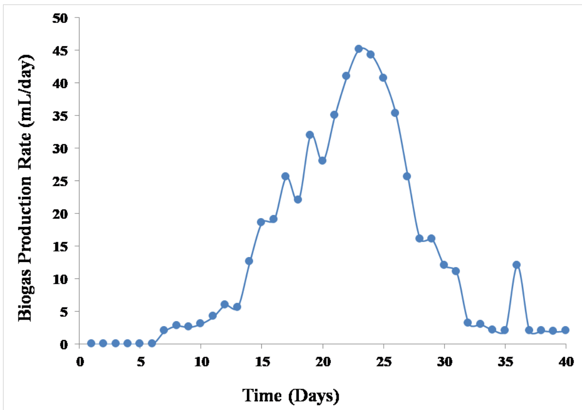

Figure 5: Daily Biogas Production Index for Fish Residue, Tea Waste, and Cow Dung (1:1:0.5)

Figure 5 depicts biogas production using fish residue, tea waste, and cow dung in a 1:1:0.5 ratio. Gas production started on the 5th day due to the rapid breakdown of undigested feedstock. Production rates varied with temperature and stabilized between the 37th and 40th days. The highest production, 45.13 mL/day, was observed on the 23rd day, with an average rate of 13.50 mL/day. Fluctuations in production were noted throughout the assimilation period.

Figure 6 illustrates biogas production from fish residue and cow dung in a 1.5:1 ratio. Gas production began on the 3rd day, attributed to the rapid degradation of undigested feedstock. The production rate fluctuated due to temperature variations. Among the mixtures tested, fish residue and cow dung produced more biogas than tea waste and cow dung. The highest daily production, 190.25 mL/day, was recorded on the 9th day, with an average rate of 67.35 mL/day over the 40 day retention period.

Biogas Production Profile (Phase 2)

Figure 7 presents the daily biogas production rate from a

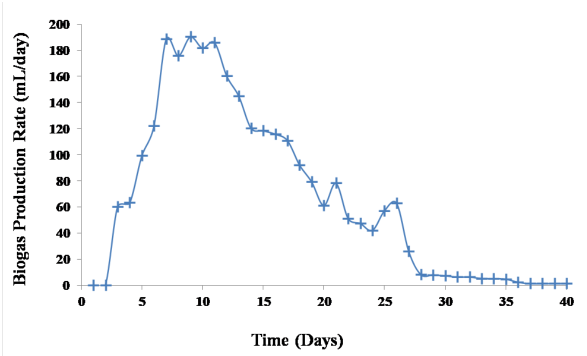

Figure 6: Daily Biogas Production Index for Fish Residue and Cow Dung (1.5:1)

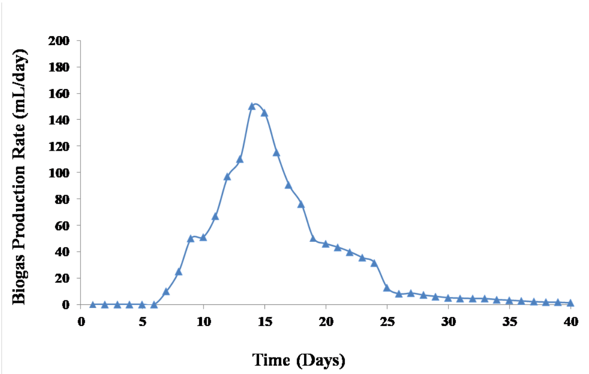

Figure 7: Daily Biogas Production Rate from Cow Dung and Tea Waste in a 1:1.5 Ratio

mixture of cow dung and tea waste in a 1:1.5 ratio. The production process began on the 5th day of the digestion period, as the breakdown of undigested feedstock took place. The production rate gradually increased, peaking around the 15th day at 150 mL/day. The pattern of biogas production is influenced by various factors, including the activity of methanogenic microorganisms, temperature, and pH, which are key to the anaerobic digestion process. The tea waste in the mixture, being relatively difficult to decompose, required more time to break down compared to other organic materials such as fish residue. The average biogas production rate observed throughout the study period was 32.96 mL/day, suggesting that while tea waste can contribute to biogas generation, its slower rate of decomposition may limit the overall production yield.

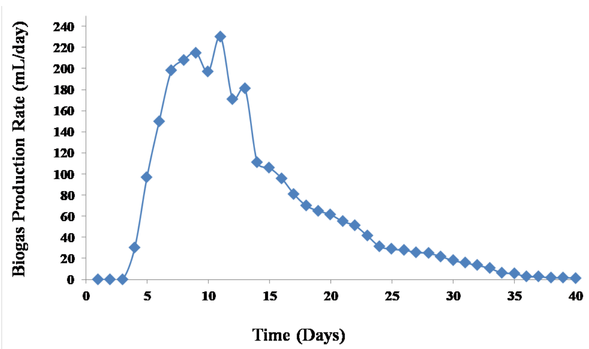

Figure 8: Daily Biogas Production Rate from Fish Residue, Tea Waste, and Cow Dung in a 1:1:1 Ratio

Figure 8 shows the biogas production rate from a mixture of fish residue, tea waste, and cow dung in a 1:1:1 ratio. Biogas production commenced on the 6th day and continued to increase until reaching a peak of 221 mL/day on the 11th day. Afterward, the rate began to decrease steadily until the 40th day. The overall average production rate for this mixture was 62.52 mL/day. The production fluctuations are closely linked to temperature variations, which have a significant impact on the metabolic activity of the microbial community involved in the digestion process. While the combination of these feedstocks showed notable biogas output, it was found to be less effective compared to a different mixture, as detailed in the following sections.

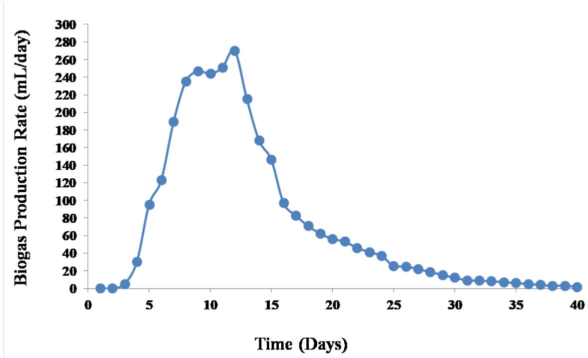

Figure 9: Daily Biogas Production Rate from Fish Residue, Tea Waste, and Cow Dung in a 1:1:0.5 Ratio

In Figure 9, the daily biogas production rate is shown for a mixture of fish residue, tea waste, and cow dung in a 1:1:0.5 ratio. Biogas production began on the 4th day, attributed to the rapid decomposition of fish residue. The highest production rate observed was 270 mL/day, recorded on the 12th day. Throughout the digestion period, the rate stabilized between the 8th and 10th days, as well as from the 38th to the 40th day. The average production rate for this combination was 71.20 mL/day, which was higher than the 1:1:1 mixture. These results emphasize the importance of balancing feedstock proportions to enhance biogas yield, with the 1:1:0.5 ratio proving more effective in generating biogas. Fish residue, in particular, accelerated the breakdown process and contributed to higher methane production.

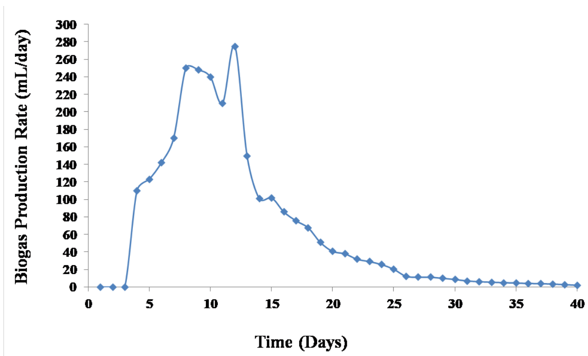

Figure 10: Daily Biogas Production Rate from Fish Residue and Cow Dung in a 1.5:1 Ratio

Figure 10 depicts the daily biogas production rate for a mixture of fish residue and cow dung in a 1.5:1 ratio. This combination showed the fastest rate of biogas production, starting on the 3rd day due to the quick decomposition of fish residue. The highest production of 289.56 mL/day was recorded on the 10th day, and the overall average production rate was 69.24 mL/day. Fluctuations in production were observed, primarily due to temperature changes that impacted microbial activity. This feedstock combination outperformed other mixtures, particularly those with tea waste, by producing the highest gas yields and demonstrating the potential of using fish residue as a primary feedstock in biogas production.

Cumulative Results Study

Figures 11 and 12 illustrate the cumulative biogas production for all digesters throughout the study period. In the initial 7 days of observation, biogas production showed a slight increase, though it remained relatively low. This slow start is typical in the early stages of anaerobic digestion as the microorganisms begin to acclimate to the feedstock and establish the necessary microbial populations to facilitate the breakdown of organic matter.

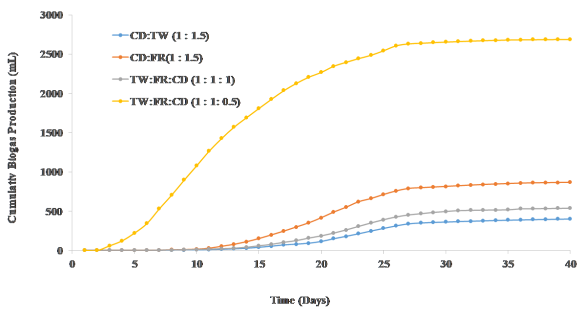

Figure 11: Cumulative Biogas Production for Different Feedstock Combinations in Phase 1

In Phase 1, Figure 11 displays the cumulative biogas production for the different feedstock combinations used in the study. These combinations included cow dung and tea waste, cow dung and fish residue, and tea waste, fish residue, and cow dung. Cumulative production values reached 400.36 mL for cow dung and tea waste, 869.36 mL for cow dung and fish residue, 539.71 mL for tea waste, fish residue, and cow dung, and 2693.67 mL for the final combination of tea waste, fish residue, and cow dung. These results highlight the variable biogas yields across different feedstock mixtures, with the combination of tea waste, fish residue, and cow dung yielding the highest cumulative biogas production. Notably, the majority of the cumulative production occurred within the first 15 days of the study period. This suggests that the initial digestion phase, which is characterized by rapid microbial growth and substrate degradation, contributed significantly to the overall biogas production. The gradual increase in production after the 7th day reflects the digestion process stabilizing as the microbial communities continued to mature and adapt to the feedstock.

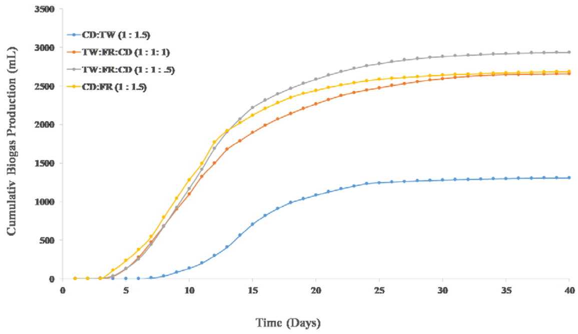

Figure 12: Cumulative Biogas Production for Different Feedstock Combinations in Phase 2

Figure 12 illustrates the cumulative biogas production rates for the same feedstock combinations in Phase 2. The cumulative production values were 1318.38 mL for cow dung and tea waste, 2500.93 mL for cow dung and fish residue, 2847.63 mL for the mixture of tea waste, fish residue, and cow dung, and 2769.43 mL for the second mixture of tea waste, fish residue, and cow dung. In Phase 2, a noticeable increase in cumulative production was observed compared to Phase 1. The results suggest that as the digestion process continued, particularly in the latter half of the study, there was a greater buildup of methane-producing bacteria and other microbial populations that contributed to enhanced degradation of the feedstock. As seen in Phase 1, the highest production was achieved in the first 15 days, with a steady increase in gas production in the following weeks. This pattern of early high production and gradual stabilization is characteristic of anaerobic digestion, where the system stabilizes after an initial burst of activity due to microbial acclimatization and feedstock availability. The results indicate that the mixture of tea waste, fish residue, and cow dung consistently produced higher biogas volumes than the simpler feedstock combinations, further supporting the importance of feedstock diversity in biogas production.

Temperature Profile Study

The temperature during the digestion process was consistently monitored, as temperature plays a critical role in the efficiency of anaerobic digestion. In both phases of the study, the temperature remained within the mesophilic range, which is typically between 20°C and 45°C and is optimal for the growth of methane-producing bacteria. The temperature fluctuations observed during the study were influenced by external environmental factors, which affected the overall digestion process.

In Phase 1, the lowest temperature recorded was 24°C on the 24th day of operation, while the highest temperature of 32°C was recorded on both the 10th and 26th days. The average temperature for Phase 1 was 28.63°C at the end of the 40 day retention period. This relatively stable temperature range is considered ideal for mesophilic digestion, supporting the optimal growth of microbial communities that are responsible for breaking down organic matter.

In Phase 2, the temperature fluctuated slightly more, with the lowest recorded temperature being 16°C, which occurred on the 1st, 2nd, 8th, 14th, and 29th days. The highest temperature recorded in this phase was 22°C on the 38th day. The average temperature for Phase 2 was 18.48°C, which is lower than in Phase 1. These lower temperatures in Phase 2 may have contributed to the slower rate of biogas production observed in this phase. Lower temperatures can reduce the metabolic activity of microbes, thereby slowing down the degradation process and leading to lower overall biogas production rates. However, despite these lower temperatures, Phase 2 still showed a gradual increase in biogas production, suggesting that the microbial communities were able to adapt to the cooler conditions over time.

Discussion

The present study investigates the biogas production at different feedstock mixing ratios and explores the role of temperature and pH in the anaerobic digestion process. Temperature, in particular, is a key factor influencing biogas generation. During the digestion phase, the daily average temperature was recorded. Throughout both phases of the study, the temperature remained within the mesophilic range, which is optimal for the microbial activity involved in anaerobic digestion. In Phase 1, the temperature ranged from a low of 24°C on the 24th day to a high of 32°C on the 10th and 26th days. After the 40 day hydraulic retention period, the average temperature in Phase 1 was recorded at 28.63°C.

In Phase 2, the temperatures ranged from a minimum of 16°C on the first, second, eighth, fourteenth, and twenty-ninth days to a maximum of 22°C on the 38th day. At the end of the 40-day retention period, the average temperature for Phase 2 was recorded at 18.48°C. The lower temperatures observed in Phase 2 contributed to a slower rate of biogas production during this phase. These findings are consistent with a study conducted (Rameshprabu and Yuwalee, 2016), where an increase in biogas and methane production was linked to rising temperatures. For example, a digester operating at 35°C produced a significantly higher volume of biogas and methane. Similar trends were noted (Uzodinma *et al.*, 2007) who found that biogas production increased continuously between the temperatures of 35°C to 40°C, with the highest cumulative gas yield occurring at 40°C.

Alongside temperature, pH is another crucial factor that affects biogas production. In this study, the pH levels of the sample mixtures were recorded as follows: 6.9, 6.6, 5.6, and 6 in Phase 1, and 6.7, 6.9, 5.8, and 6.2 in Phase 2. The sample with a pH of 6.9 consistently produced the highest biogas yield, followed by the other samples with lower pH values. These results align with previous findings (Jayaraj *et al.*, 2014), who observed that a pH of 7 led to higher biogas production and degradation capacity. Furthermore, (Jonas *et al.*, 2014) demonstrated that increasing the pH from 5.5 to 7.5 resulted in a reduction of acetic acid by 88.1% and a decrease in chemical oxygen demand (COD) by 18.3%, while the methane yield increased by 58%. These observations are in line with the current study, where the optimal methane production was achieved at temperatures between 30°C and 32°C, with a pH of 6.9.

Although both phases used identical digester volumes, the rate of biogas production in Phase 2 (winter) was higher than in Phase 1 (summer). This difference was attributed to the variation in feedstock input, leading to a higher inoculum percentage in Phase 2, which positively influenced biogas generation. Therefore, the results suggest that both temperature and pH play significant roles in optimizing biogas production, with the ideal conditions being a temperature of 30-32°C and a pH of 6.9 for maximum methane yield.

CONCLUSION

The global energy crisis, driven by depleting fossil fuel reserves and rising energy costs, underscores the urgent demand to transition toward renewable energy supplies. This study demonstrated the feasibility of utilizing fish residues, tea waste, and cow dung as substrates to produce biogas, providing a sustainable substitution to conventional fossil fuels. A batch digestion method was employed across two seasonal experiments. In the summer, the average biogas production was recorded as 10 mL/day for CD and TW (1:1.5), 21.78 mL/day for FR:TW:CD (1:1:1), 13.50 mL/day for FR:TW:CD (1:1:0.5), and 67.35 mL/day for FR:CD (1.5:1). During the winter, the respective biogas yields increased to 32.96, 62.5, 71.20, and 69.24 mL/day under the same substrate ratios. These results highlight that co-digestion with cow dung significantly enhances biogas production by acting as a natural catalyst in the bio-methanation process. This approach not only mitigates environmental pollution caused by organic waste but also offers an economical way of sustainable energy generation. Adopting such renewable strategies could play a crucial role in addressing the energy crisis while reducing dependency on fossil fuels.

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