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Enhanced Biogas Production via Anaerobic Digestion of Organic Waste with Rice Water: Methane Yield and Substrate Interactions

Sudarman[1], Roro Heni Hendaryati[1], Achmad Fauzan Hery Soegiharto[1], Dyanu Moulana Ismail[1], Rizki Raihan Ramadhan[1], Willy Satrio Nugroho[2], Yepy Komaril Sofi'i[1]*

[1] *Department of Mechanical Engineering, Faculty of Engineering, University of Muhammadiyah Malang, Raya Tlogomas Street No. 246, Malang, 65144, INDONESIA*

[2] *Department of Mechanical Engineering, Faculty of Engineering, University of Brawijaya, Mayjend Haryono Street No. 167, Malang, 65145, INDONESIA*

*Corresponding Author: yepykomarils@umm.ac.id
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Methane yield, anaerobic digestion, organic waste, rice water, co-substrate synergy

Abstract

The global energy crisis caused by fossil fuel depletion has prompted a shift toward renewable energy sources. This study investigates the effect of rice water as a co-substrate on methane production via anaerobic digestion of fruit (Artocarpus heterophyllus), vegetable (Lactuca sativa), and grass (Cenchrus purpureus) waste. To understand their chemical and structural properties, organic substrates were characterized using Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), and Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM-EDX). Experiments were conducted using a 10 L custom reactor over 15 days. The combination of jackfruit and lettuce produced the highest methane concentration (637.75 ppm), followed by jackfruit alone (479.53 ppm), representing a 32.96% increase due to co-substrate addition. In contrast, grass and lettuce yielded lower concentrations (430.68 and 540.59 ppm, respectively). The high carbohydrate and microbial content in rice water enhanced hydrolysis and acidogenesis, accelerating methane formation. Statistical analysis confirmed that substrate type significantly influenced methane output ($p < 0.05$), while fermentation duration showed no significant effect ($p > 0.05$). This study concludes that rice water substantially boosts methane yield in mixed organic waste digestion, particularly with jackfruit and lettuce, offering a simple, low-cost enhancement for small-scale biogas systems.

1. Introduction

Adequate energy availability is a prerequisite for human civilization's progress (Pan et al., 2023). However, the overexploitation of fossil energy sources such as oil and natural gas has triggered a global energy crisis, resulting in resource scarcity, rising energy prices, and significant environmental degradation (Hassan, Algburi, et al., 2024; Hassan, Viktor, et al., 2024; Listiani Sukmawati et al., 2025; Mokhtar et al., 2021). These challenges underscore the urgent need to develop alternative energy sources that are both sustainable and environmentally friendly (Owusu & Asumadu-Sarkodie, 2016).

Biogas, a gas produced from the anaerobic decomposition of organic materials, offers substantial potential as a renewable energy source with multiple environmental and health (Bista et al., 2023; Sidi Habib et al., 2024). For instance, biogas production can mitigate the greenhouse effect by replacing fossil fuels emitting high greenhouse gas emissions (GHGs) (Staples et al., 2017). However, a potent GHG, the methane (CH_4) emissions from biogas are considerably lower than those from other sources, like decomposing waste in landfills (Mar et al., 2022; Staples et al., 2017). Additionally, biogas technology can significantly reduce unpleasant odors and the spread of diseases associated with improper waste management practices (Kabeyi & Olanrewaju, 2022; Yadav et al., 2024).

The production of biogas can utilize a variety of organic materials, including human waste, livestock, and household waste (Jameel et al., 2024). However, conventional waste disposal methods often lead to environmental problems such as air and groundwater pollution due to the emission of methane, nitrous oxide, ammonia, and volatile organic compounds (Atelge et al., 2020; Jameel et al., 2024). Anaerobic digestion (AD) offers a sustainable solution to these issues by transforming waste into clean energy while reducing environmental pollution (Kasinath et al., 2021).

Anaerobic digestion (AD) relies on synergistic microbial consortia to degrade proteins and complex organic compounds into simpler molecules through a sequential process involving hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Raja Ram & Nikhil, 2022; Tshemese et al., 2023). While numerous studies have investigated the effects of substrate types, dilution ratios, fermentation durations, and reactor configurations on biogas yield (Kamilia et al., 2021; Murjani et al., 2020; Ritonga et al., 2021; Subagyo & Wijaya, 2017; Yuwanda et al., 2019). The specific role of rice water as a co-substrate in AD remains poorly explored.

Rice water is an abundant and underutilized co-substrate, the starchy effluent produced from rinsing or soaking rice. In contrast to conventional co-substrates such as whey, fruit peels, or wastewater sludge, rice water contains high concentrations of soluble carbohydrates (mainly starch and glucose), amino acids, vitamins, and natural enzymes (Hegde & Trabold, 2019; Nabayi et al., 2021). These compounds provide readily digestible energy sources that can accelerate the hydrolysis and acidogenesis phases, enhancing microbial activity and methane production (Li et al., 2019). Moreover, rice water contains lactic acid bacteria (LAB) that contribute to microbial diversity and fermentative efficiency, offering a biological advantage over nutrient-deficient co-substrates. Despite these advantages, rice water remains largely overlooked in biogas studies, particularly in combination with various types of organic waste. Therefore, a deeper investigation into its biochemical interactions and comparative performance is essential for optimizing its application in AD systems.

In particular, the molecular interactions between organic waste and rice water during fermentation and their impact on methane yield are poorly understood. Furthermore, limited research has been done on optimizing biogas production by considering organic waste and rice water mixtures. Addressing these gaps is crucial for developing more efficient biogas production methods, particularly in regions like Indonesia, where raw material availability and technological efficiency remain significant challenges. This study aims to fill these gaps by conducting experimental research on biogas production using organic waste, including fruits (Artocarpus heterophyllus), vegetables (Lactuca sativa), and grass (Cenchrus purpureus) mixed with rice water. The research will investigate the effectiveness of this mixture in producing methane gas (CH_4). This study seeks to contribute to developing more sustainable and efficient biogas technologies by addressing the underexplored areas in biogas production.

2. Methods

2.1 Methodology

This research was conducted in several stages: biogas reactor design, material characterization, fermentation process, data collection, and analysis. The biogas reactor was designed using a modified drum to create an optimal anaerobic environment conducive to the growth of biogas-producing microorganisms. The specific dimensions, an outer diameter of 40 cm, an inner diameter of 32 cm, and a height of 62.5 cm, were selected based on a balance between maximizing the volume-to-surface area ratio and maintaining a manageable reactor size. This ratio enhances microbial contact with the substrate, critical for efficient biogas production. The drum material was chosen for its durability and ease of modification, ensuring it could withstand the pressure changes during fermentation without compromising structural integrity. Additionally, a gas valve, pipe, water trap, and manometer were incorporated into the reactor design (see Fig. 1 and Table 1). These components were included to monitor and regulate gas accumulation, ensuring consistent fermentation performance. The design choices were informed by previous studies that demonstrated the effectiveness of similar configurations in small-scale biogas production systems, particularly in enhancing microbial activity and methane gas yield.

The substrate used in this research consisted of domestic organic waste, including fruits, vegetables, and grass. Specifically, jackfruit (Artocarpus heterophyllus), lettuce (Lactuca sativa), and grass (Cenchrus purpureus) were selected due to their availability, biodegradability, chemical characteristics, and potential for methane generation in biogas production through anaerobic digestion. It was hypothesized that the optimal combination of these substrates would enhance biogas production efficiency. Each organic substrate was chopped into small pieces to increase surface area and facilitate microbial degradation. For each substrate and its combinations, two treatment groups were prepared. The first group received rice water as a co-substrate, while the second group used distilled water and functioned as the control. In both groups, the substrates were mixed with liquid at a fixed ratio of one part organic material to four parts liquid by volume (1:4), ensuring consistent moisture conditions across all treatments. The selection of rice water was supported by preliminary trials and literature indicating that carbohydrate-rich liquids enhance microbial hydrolysis and acidogenesis by stimulating microbial enzymatic activity (Hegde & Trabold, 2019; Mishra & Upadhyay, 2021). Each anaerobic batch reactor was filled to 10 liters to ensure sufficient fermentation space and maintain consistent operational conditions. This experimental setup enabled a direct comparison of methane production in the presence and absence of rice water under controlled and identical conditions.

While the microbial community structure was not directly analyzed, the experimental design was intended to indirectly assess the influence of rice water on microbial activity through variations in methane yield and biogas production trends. Rice water contains soluble carbohydrates, amino acids, vitamins, and lactic acid bacteria (LAB), which can act as enzymatic precursors and microbial enhancers (Mishra et al., 2022). These components are hypothesized to enrich fermentative and acidogenic microbial populations, thereby accelerating the hydrolysis and acidogenesis stages of anaerobic digestion (Li et al., 2019; Nabayi et al., 2021). Observable effects were inferred from the biogas production profiles and methane concentrations over time, particularly in mixed-substrate combinations with rice water. The analysis focused on comparing the trends between treatment and control groups to identify potential shifts in biochemical reaction rates and substrate utilization efficiency.

Before the fermentation process, the organic materials, including jackfruit (Artocarpus heterophyllus), lettuce (Lactuca sativa), and grass (Cenchrus purpureus), were characterized to understand their chemical and structural properties. Three analytical techniques were employed:

- Fourier Transform Infrared Spectroscopy (FTIR): FTIR analysis was performed using an IRPrestige 21 (Shimadzu, Japan) to identify the functional groups in the organic materials. This method provided insights into the chemical bonds and compounds within the samples, crucial for understanding the substrates' biodegradability and potential to produce methane.
- X-ray Diffraction (XRD): XRD patterns were obtained using an X'PertPRO diffractometer (Panalytical, Netherlands) to determine the crystalline structures in the materials. The degree of crystallinity and the types of crystalline phases identified helped predict the rate of anaerobic decomposition and methane production, as more crystalline materials tend to decompose more slowly.
- Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM-EDX): SEM-EDX was conducted using an FEI (USA) system to analyze the samples' surface morphology and elemental composition. The detailed images of the waste's microstructure and the quantification of elemental distribution provided insights into the physical barriers to microbial activity and the overall efficiency of methane production.

These analyses supported the understanding of substrate biodegradability and helped explain variations in methane yield among treatments.

The prepared substrate mixture was transferred into the reactor and sealed tightly to maintain anaerobic conditions. The fermentation process was conducted over approximately 15 days, with the temperature controlled between 30-37°C using an external heat source to ensure optimal mesophilic conditions. The pH was regularly monitored using a digital pH meter, with adjustments made using buffering agents as necessary to maintain a neutral pH range of 6.8-7.2. These conditions were meticulously maintained to support methanogenic bacteria's growth and metabolic activity, as deviations from these parameters can significantly impact methane production.

The volume of methane gas produced was measured daily in parts per million (ppm) using an MQ-4 methane gas sensor connected to a data logger for continuous monitoring. Measurements were taken until gas production reached a stable point or showed a significant decrease, indicating the end of the active fermentation phase. Data were analyzed using statistical methods, including ANOVA, to determine the influence of the variables (substrate type and anaerobic fermentation time) on biogas production. The statistical analysis was performed using Statistical Software Suite (SPSS), with assumptions of normality and homogeneity of variances checked before analysis. Significance levels were set at $p < 0.05$.

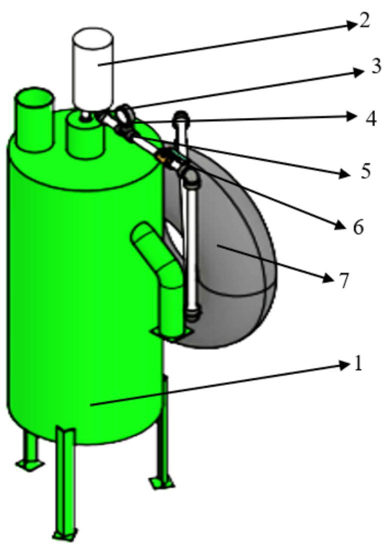

Fig. 1 *Experimental apparatus*

Table 1 *Biogas reactor specifications*

No.	Item	Specifications	Function
1	Biogas reactor	Height: 62.5 cm Outer diameter: 40 cm Inner diameter: 32 cm	Processing organic waste
2	Methane gas sensor gas	MQ-4 sensor	Measuring methane gas
3	Pipe	PVC ½“	Channeling methane gas to storage
4	Pressure gauge	50 Psi	Measuring methane gas pressure
5	T – joint		Connect the pipe with a pressure gauge.
6	Elbow	PVC ½“	Connecting pipes to storage
7	Methane Storage	Volume: 4.2 L	Storing methane gas

3. Results And Discussion

3.1 Rice Water Characterization

The characterization of rice water revealed relevant physical, chemical, and microbial parameters essential for understanding its contribution to the anaerobic digestion process and methane production. The slightly acidic pH of 6.53 and electrical conductivity of 372.83 µS cm^{-1} indicate a moderate ionic content, which could influence microbial activity during fermentation (Nabayi, Sung, et al., 2021). The fluctuations in viscosity observed over time suggest changes in the molecular composition, likely due to microbial activity and the breakdown of complex carbohydrates (Marto et al., 2018). The high carbohydrate content, mainly starch and glucose, provides a readily available energy source for microorganisms, which could enhance the hydrolysis stage of anaerobic digestion. The presence of lactic acid bacteria (LAB) and other beneficial organisms in the rice water further supports its role in accelerating the fermentation process by enhancing microbial diversity and activity (Larasati et al., 2022; Marto et al., 2018; Nabayi, Sung, et al., 2021).

3.2 Organic Waste Characterization

Organic waste comprising fruits, grass, and vegetables was characterized using Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM-EDX), and X-ray Diffraction (XRD) to gain insights into their chemical and structural properties. FTIR was employed to identify the functional groups in the organic matter, revealing the types of chemical bonds and compounds within the waste. XRD was used to determine the crystalline structure of the materials, offering insights into the mineralogical composition and any crystalline phases present. Together, these techniques provided a comprehensive understanding of the organic waste's physical, chemical, and structural properties, facilitating its potential application in processes like methane production or composting. SEM-EDX provided detailed images of the waste's surface morphology and allowed for the elemental composition analysis, highlighting the distribution of elements like carbon, oxygen, and other trace elements.

3.2.1 Fourier Transform Infrared Spectroscopy (FTIR)

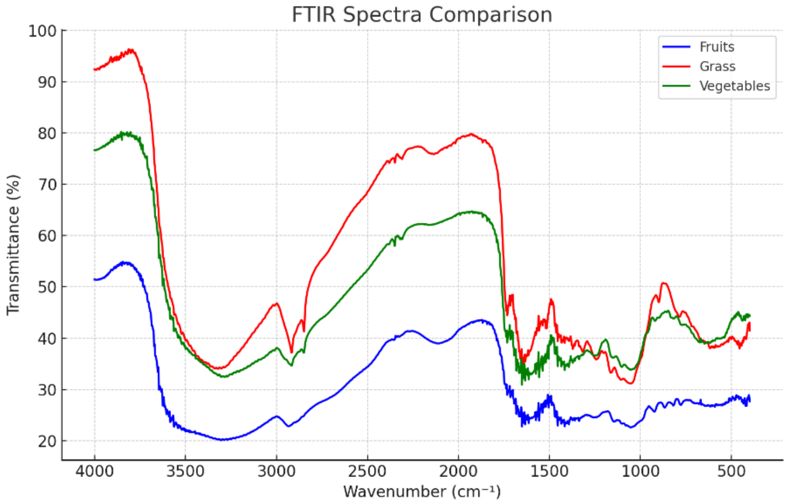

Fig. 2 *FTIR test results of fruits, grass, and vegetables*

As shown in Fig. 2, the FTIR spectra provide valuable information regarding the functional groups present in the samples:

The fruit samples' FTIR spectrum displays characteristic peaks around 3300 cm^{-1}, indicating O-H (Oxygen-Hydrogen) stretching, commonly associated with alcohols or phenols. Peaks near 2900 cm^{-1} suggest C-H (Carbon-Hydrogen) stretching from alkanes. The fingerprint region (1500-500 cm^{-1}) shows complex absorption patterns indicative of C-O, C-C, and C-H bending vibrations, suggesting the presence of carbohydrates and organic acids.

The FTIR spectrum for grass samples reveals significant peaks near 2920 cm^{-1} and 2850 cm^{-1}, corresponding to C-H stretching vibrations from aliphatic chains. A peak around 1730 cm^{-1} suggests the presence of ester carbonyl groups, which are typical in lipids. The region around 1030 cm^{-1} shows C-O stretching, commonly associated with cellulose or hemicellulose, indicative of the grass's structural components.

The spectrum of vegetables shows prominent peaks around 3400 cm^{-1}, pointing to O-H stretching, possibly due to water content or hydroxyl groups. Peaks around 1650 cm^{-1} are associated with C=O stretching, which could indicate amides or carboxylic acids. The fingerprint region further supports the presence of polysaccharides and proteins, as indicated by C-O and C-N stretching vibrations.

As revealed by the FTIR spectra, the chemical composition analysis indicates distinct differences among the organic materials studied. Fruits exhibit a significant presence of carbohydrates, particularly in simple sugars, and a variety of organic acids, which are crucial for their metabolic processes and energy storage. This composition likely contributes to their rapid biodegradation and potential for high methane yield in anaerobic digestion. In contrast, vegetables are characterized by a higher water content, which correlates with the presence of hydrated compounds. Additionally, they contain notable amounts of proteins and polysaccharides, including pectin and starch, which play critical roles in their structural integrity and nutritional value. These components may influence both the rate and extent of biogas production. On the other hand, grass is distinguished by its high content of structural carbohydrates, mainly cellulose and hemicellulose, which are integral to its rigid structure. The FTIR spectra also reveal the presence of lipids, which, although present in smaller quantities, contribute to the overall energy content of the biomass. The complex structure of grass may pose challenges for biodegradation, but it also suggests a slower, more sustained release of methane during anaerobic digestion.

The FTIR spectra of the samples revealed distinct functional groups, which have implications for their biodegradability and methane production potential. Fruits showed a significant presence of carbohydrates and organic acids, which are quickly degraded during anaerobic digestion, potentially leading to a higher methane yield. Vegetables with higher water content and complex polysaccharides may degrade more slowly, resulting in lower methane production. Grass, with its high content of structural carbohydrates like cellulose and hemicellulose, may also decompose more slowly but could provide a more sustained methane release over time.

3.2.2 X-Ray Diffraction (XRD)

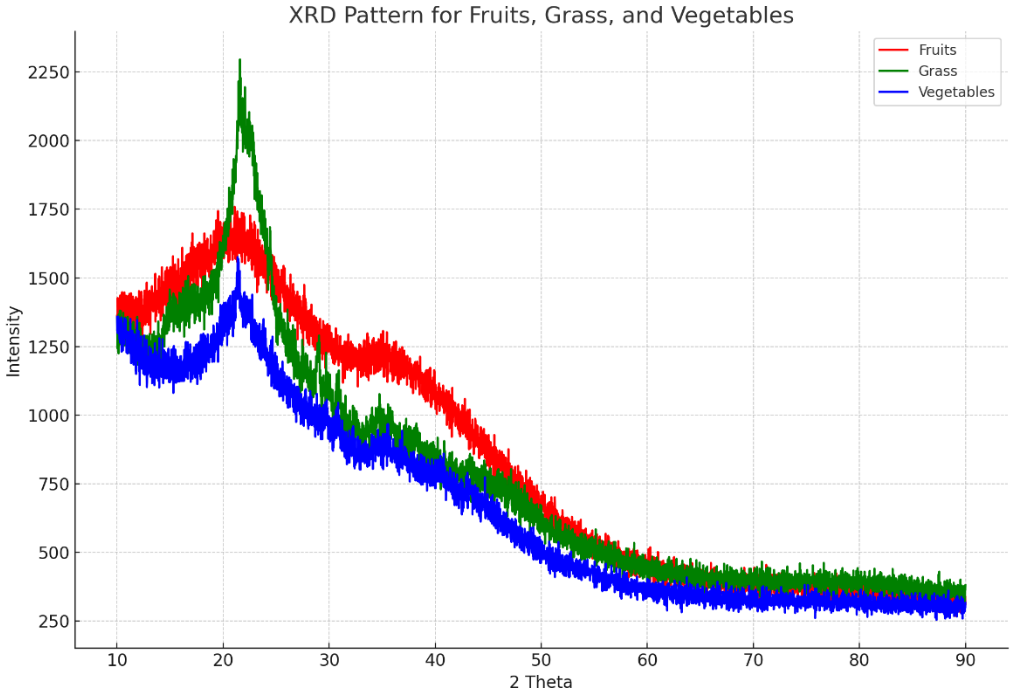

Fig. 3 *XRD Pattern of fruits, grass, and vegetables*

Based on the Fig. 3, the XRD results show that:

Crystalline phase identification:

- Fruits: The presence of higher intensities and sharper peaks in the XRD patterns of fruits suggests a higher degree of crystallinity, likely due to crystalline cellulose or other organic molecules with well-defined structures.
- Grass: The XRD pattern of grass, characterized by lower and broader peaks, indicates a lower degree of crystallinity, consistent with the higher lignocellulosic content dominated by amorphous lignin.
- Vegetables: Vegetables exhibit an intermediate diffraction pattern between fruits and grass, indicating a mixture of crystalline and amorphous phases, including pectin (amorphous) and cellulose (crystalline).

Implications for methane production:

- Fruits: The higher crystallinity in fruits could lead to a slower anaerobic decomposition process, as crystalline structures are typically more resistant to microbial breakdown, potentially reducing the methane production rate.
- Grass: Due to its lower crystallinity and higher proportion of amorphous components, grass is likely to decompose more readily, possibly resulting in a faster methane production rate.
- Vegetables: The mixed crystalline and amorphous phases in vegetables suggest an intermediate decomposition rate, with methane production potential that varies depending on the specific composition of the vegetable sample.

In summary, the crystallinity of the organic material influences its decomposition rate during anaerobic digestion, thereby affecting the overall methane production. Fruits, with their higher crystallinity, may produce methane more slowly, while grass, with its lower crystallinity, may lead to faster methane generation. Vegetables, with a mix of crystalline and amorphous phases, are likely to exhibit methane production rates that fall between those of fruits and grass.

3.2.3 Scanning Electron Microscopy-Energy-Dispersive X-Ray Spectroscopy (SEM-EDX)

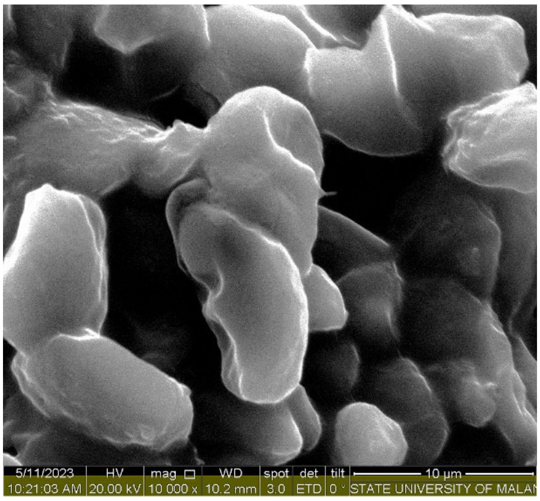

Fig. 4 *SEM of fruits*

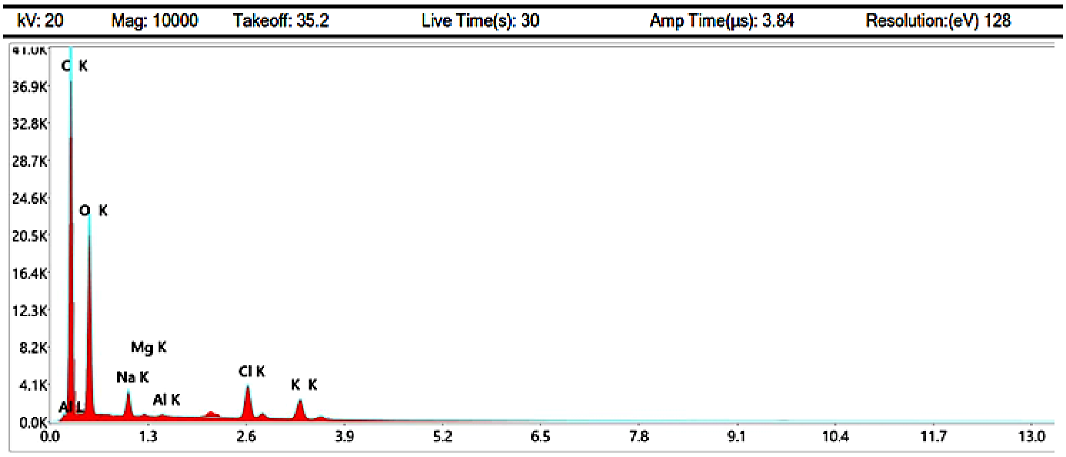

Fig. 5 *EDX of fruits*

As shown in Fig. 4, at a magnification of 10,000×, the fruit samples exhibited a relatively homogeneous morphological structure with surfaces that appeared less porous and denser at the observed scale. Carbon content was highly dominant in these samples (66.0% by weight), with oxygen being the second most abundant element (30.9% by weight). Minor elements such as sodium (Na), chlorine (Cl), and potassium (K) were present in small quantities (see Fig. 5). The homogeneous and denser morphology could be associated with the high carbon content, which typically reflects more complex organic structures such as polysaccharides or cellulose (Tudu & Samanta, 2023). This dense structure stores more carbon-rich organic compounds, primarily precursors in methane formation through anaerobic fermentation (Pandey et al., 2022).

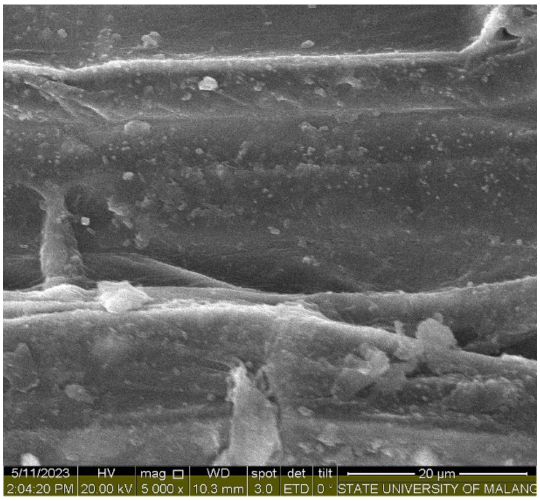

Fig. 6 *SEM of grass*

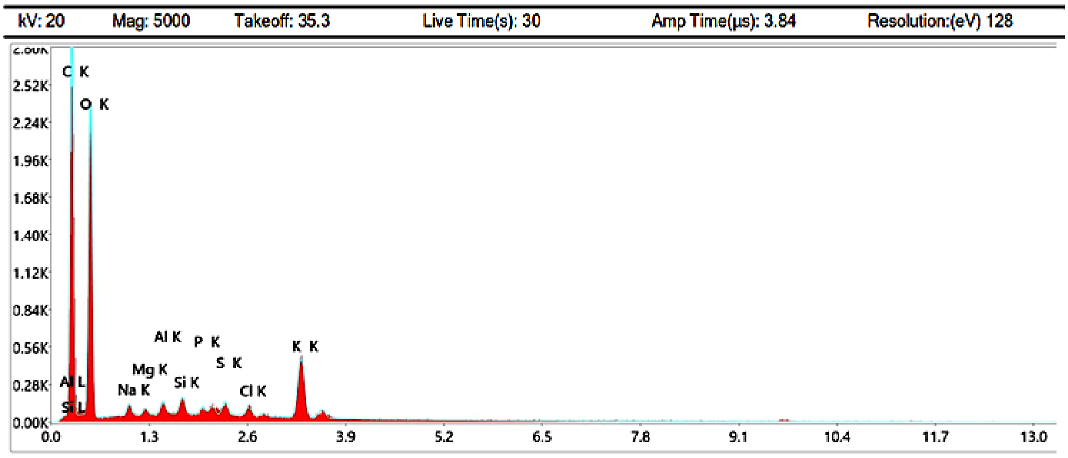

Fig. 7 *EDX of grass*

At a magnification of 5000×, the grass samples exhibited a more varied morphology, with more fibrous and porous structures than fruits and vegetables, as shown in Fig. 6. Based on Fig. 7, grass had a slightly lower carbon content (57.9% by weight) than fruits and vegetables, while the oxygen content was higher (37.9% by weight). Additionally, there were small amounts of mineral elements such as sodium (Na), magnesium (Mg), aluminum (Al), silicon (Si), phosphorus (P), sulfur (S), chlorine (Cl), and potassium (K). The grass's more fibrous and porous structure may be related to the higher oxygen and lower carbon content. This morphology may indicate the presence of more complex lignocellulosic structures, which tend to be more challenging for anaerobic microorganisms to degrade (Norfarhana et al., 2024).

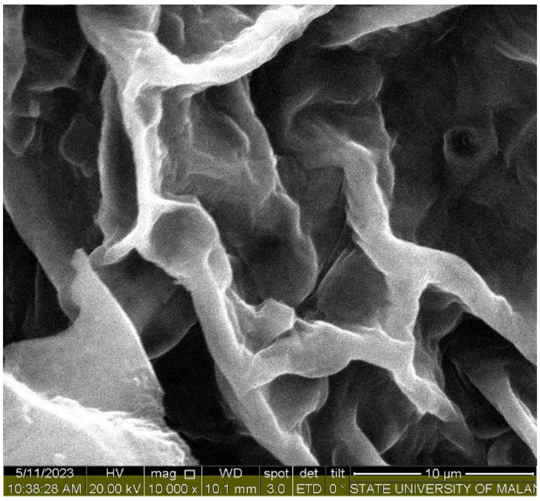

Fig. 8 *SEM of vegetables*

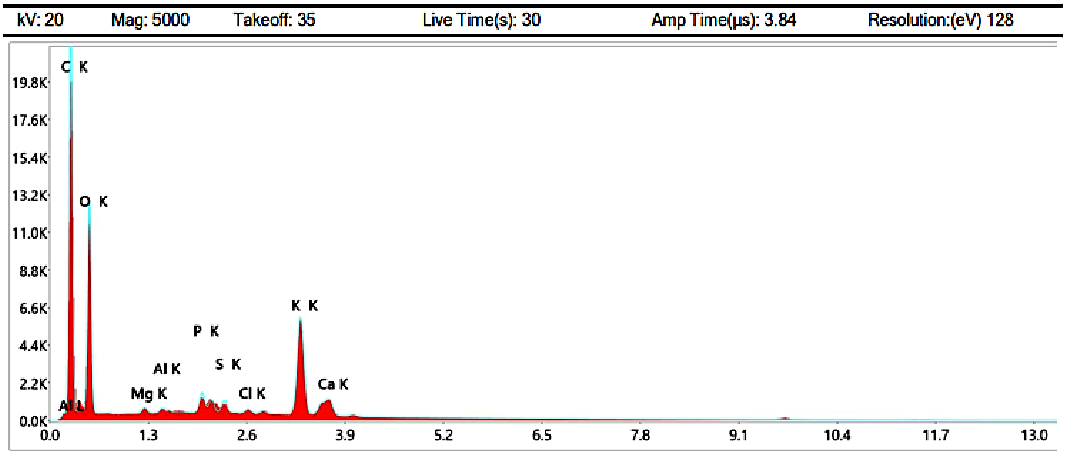

Fig. 9 *EDX of vegetables*

Based on Fig. 8, at a magnification of 10,000×, the vegetable samples displayed a somewhat heterogeneous morphological structure, with pore size and distribution variations, which might reflect the diversity in tissue composition within the vegetables. Fig. 9 shows that the carbon content in vegetables was also high (61.5% by weight), though slightly lower than in fruits. Oxygen was the second most abundant element (32.3% by weight). Additionally, there were minor elements such as magnesium (Mg), phosphorus (P), sulfur (S), chlorine (Cl), potassium (K), and calcium (Ca).

The heterogeneity in morphology may reflect the diversity in chemical composition, with some regions possibly being richer in specific compounds. For example, areas with more complex or porous structures may contain more minerals, such as calcium and phosphorus, which could alter the pathways of organic decomposition and influence methane production. The variations in morphology also suggest potential barriers in the anaerobic degradation process.

3.3 Chemical Reaction

The anaerobic fermentation process is the biological conversion of organic matter into biogas, primarily methane (CH_4), under anaerobic (oxygen-free) conditions. In this research, the anaerobic fermentation reactions were examined using three organic substrates: fruits (glucose—$C_6H_{12}O_6$), grass (phenylpropanoid acid—$C_9H_{10}O_2$), and vegetables (ascorbic acid—$C_6H_8O_6$) in the presence of rice water. Rice water introduces additional microbes and nutrients, enhancing fermentation (see Equation 1-9).

However, we have simplified the system for chemical reaction modeling by substituting rice water with H_2O in the reaction equations. This simplification allows us to focus on the fundamental anaerobic processes while acknowledging that the complex nutritional profile of rice water, including vitamins, minerals, and residual starch, has a significant role in the actual fermentation process. Although H_2O provides a generalized view of anaerobic fermentation, it is essential to recognize that the results derived from this model may differ from those observed in experiments where rice water is utilized due to the additional microbial and nutrient contributions that rice water provides.

- Anaerobic Fermentation of Fruits (Glucose - $C_6H_{12}O_6$)

When glucose ($C_6H_{12}O_6$) from fruits undergoes anaerobic fermentation with rice soaking water, it passes through several stages, including hydrolysis, acidogenesis, and methanogenesis. Below are the detailed reactions for each stage:

Hydrolysis $$C_6H_{12}O_6 + H_2O \rightarrow 2C_3H_4O_3\,(Pyruvate) \quad (1)$$

Acidogenesis $$C_3H_4O_3 + H_2O \rightarrow C_2H_5OH\,(Ethanol) + CO_2 \quad (2)$$

Methanogenesis $$C_2H_5OH + CO_2 \rightarrow CH_4 + H_2O \quad (3)$$

These reactions demonstrate the stepwise breakdown of glucose into simpler molecules, eventually leading to methane production. In the presence of rice water, the hydrolysis stage is accelerated due to the additional microbial activity. However, these reactions do not reach static equilibrium; they are dynamic, with continuous consumption and production of reactants and products.

- Anaerobic Fermentation of Grass (Phenylpropanoic Acid - $C_9H_{10}O_2$)

The fermentation of phenylpropanoic acid ($C_9H_{10}O_2$) from grass involves similar stages, though the complexity of the molecule leads to different intermediate products.

Hydrolysis $$C_9H_{10}O_2 + H_2O \rightarrow C_6H_5COOH\,(Benzoic\ Acid) + CH_4 + CO_2 \quad (4)$$

Acidogenesis $$C_7H_6O_3 + H_2 \rightarrow CH_4 + CO_2 + H_2O \quad (5)$$

Methanogenesis $$C_7H_6O_3 + H_2 \rightarrow CH_4 + CO_2 + H_2O \quad (6)$$

In this process, the presence of rice water aids in breaking down the lignocellulosic structure of the grass, facilitating hydrolysis and subsequent fermentation stages. As with glucose, the reactions are dynamic and do not achieve a static equilibrium.

- Anaerobic Fermentation of Vegetables (Ascorbic Acid - $C_6H_8O_6$)

Ascorbic acid ($C_6H_8O_6$) found in vegetables undergoes anaerobic fermentation with rice soaking water, resulting in the following chemical transformations:

Hydrolysis $$C_6H_8O_6 + H_2O \rightarrow C_6H_6O_3 + 2H_2 \quad (7)$$

Acidogenesis $$C_6H_6O_3 + H_2O \rightarrow C_2H_5OH + CO_2 \quad (8)$$

Methanogenesis $$2C_2H_5OH + CO_2 \rightarrow CH_4 + H_2O \quad (9)$$

Rice water enhances microbial activity, particularly in hydrolysis, leading to more efficient breakdown of ascorbic acid. Like the other substrates, the reactions are dynamic and proceed as long as substrates are available without reaching static equilibrium.

3.4 Methane Gas Sensor Calibration

The methane gas concentration was measured using an MQ-4 sensor connected to an Arduino Uno microcontroller. A volume of 3 mL of methane gas was injected into a 330 mL vessel, and the sensor, with 20 repetitions, measured the concentration. The data obtained from these measurements are presented in Fig. 10.

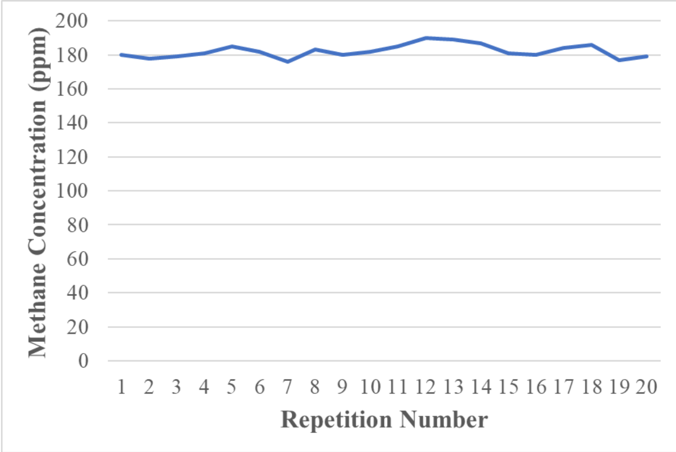

Fig. 10 *MQ-4 sensor calibration*

Descriptive statistical analysis and data uniformity tests were performed to evaluate the accuracy of the methane gas concentration calibration data. The study revealed a low coefficient of variation of approximately 2.13%, indicating that the data variation relative to the mean is minimal, indicating good measurement consistency. Subsequently, a normality test was conducted to determine whether the data follow a normal distribution with a Shapiro-Wilk statistic of 0.967 and a p-value of 0.681, well above the standard significance level of 0.05. There is insufficient evidence to reject the null hypothesis that the data follow a normal distribution. This indicates that the methane concentration measurement data follow a normal distribution. Overall, the methane gas calibration data exhibit low variation and a normal distribution, indicating that the sensor functions correctly and that the measurement results are reliable.

Fig. 11 presents the results of methane gas composition through the anaerobic digestion of organic waste. The experiment was conducted with three replicates. The organic waste used in the study consisted of fruits, grass, vegetables, combinations of fruits and grass, fruits and vegetables, and vegetables and grass. The anaerobic process involved the use of rice water and organic waste. The mixture included 8 kg of rice water and 2 kg of organic waste.

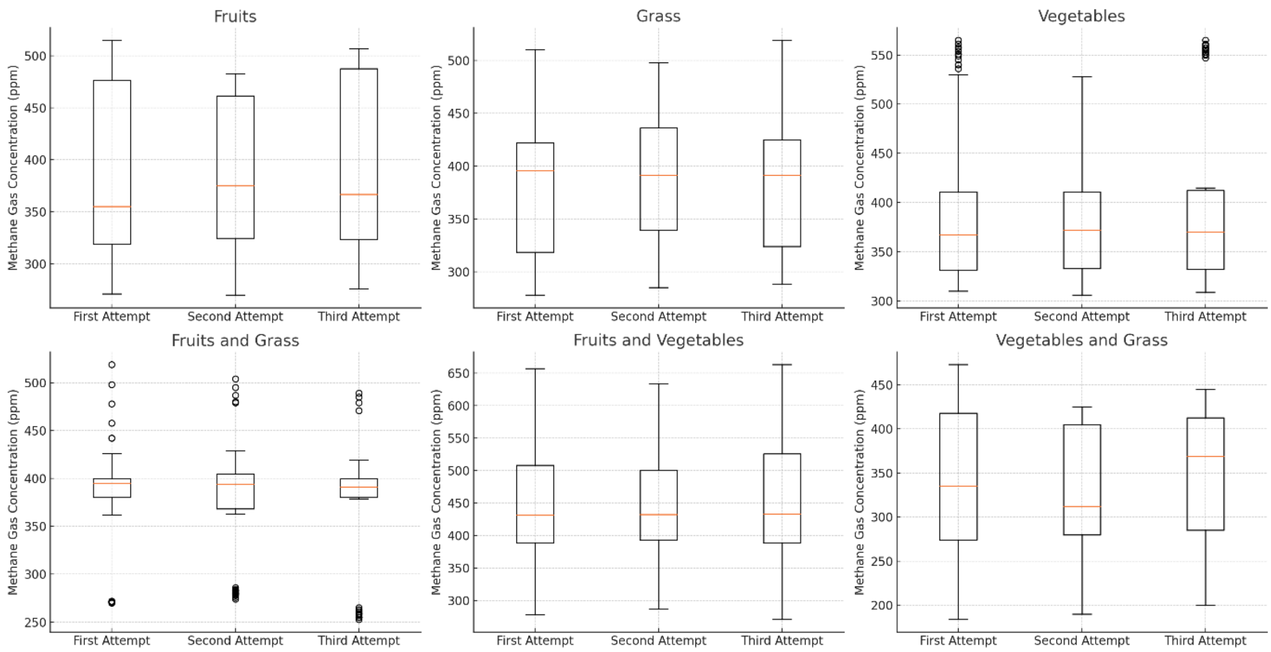

Fig. 11 *Methane gas composition through anaerobic digestion of organic waste over three replicates*

The significance between Experiments I, II, and III for each material variation was determined using a One-Way ANOVA statistical test. The results of the One-Way ANOVA are presented in Table 2.

Table 2 *One-way ANOVA results comparing attempts I, II, and III for each material variation*

Waste Organic Variation	F-statistic	p-value
Fruits	0.335737485	0.715173034
Grass	0.413296913	0.661973745
Vegetables	0.206275229	0.813764878
Fruits and Grass	0.274126966	0.7604925
Fruits and Vegetables	0.188815976	0.828071674
Vegetables and Grass	0.235890214	0.79006511

The results of the One-Way ANOVA for each waste organic variation indicated p-values considerably greater than 0.05. This suggests that there are no statistically significant differences between Experiments I, II, and III for any of the material variations. Thus, the data from all three replicates were averaged to present the representative methane concentration in Fig. 3.

Table 3 *Methane concentration on the 9th day*

Substrate Composition	Methane Concentration (ppm) Without Rice Water	Methane Concentration (ppm) With Rice Water
Fruits	392.00	477.37
Fruits_Grass	359.00	426.11
Fruits_Vegetables	375.74	637.75
Grass	317.00	380.44
Vegetables	342.00	374.84
Vegetables_Grass	333.00	436.46

Table 3 presents the methane concentration measured on the 9th day of anaerobic digestion for each substrate composition, both with and without rice water. Adding rice water increased methane concentration across all substrate types, with the fruits and vegetables mixture showing the most significant increase of 69.7%. Among the tested combinations, the fruits and vegetables waste mixture produced the highest methane concentration (637.75 ppm) when supplemented with rice water, compared to only 375.74 ppm without rice water. This result supports the hypothesis that rice water, rich in soluble carbohydrates and micronutrients, can significantly stimulate microbial activity during the early stages of digestion.

Biochemically, rice water contains starch, simple sugars, amino acids, vitamins, and lactic acid bacteria (LAB) (Mishra et al., 2022), which can act as enzymatic cofactors and microbial growth enhancers. These components likely facilitated the early stages of anaerobic digestion by accelerating hydrolysis, where complex organics are broken down into simpler molecules, and acidogenesis, where these products are converted into volatile fatty acids (VFAs). The enhanced production of VFAs increases substrate availability for methanogens in the subsequent methanogenesis stage. The observed increase in methane concentration suggests that rice water improved the rate and extent of organic matter conversion, particularly in combinations involving high-carbon fruit waste and complementary vegetable components.

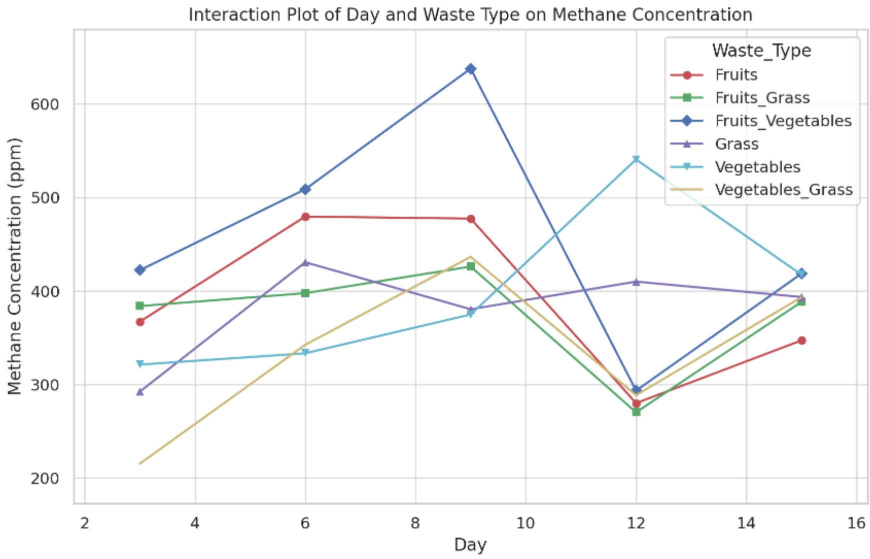

Fig. 12 *Interaction plot of day and organic waste type on methane concentration*

Fig. 12 illustrates the significant variation in methane gas concentration patterns produced by different types of organic waste compositions over time. Although the interaction between organic waste type and time did not exhibit statistically significant effects, a visual examination of the plot suggests that certain combinations of organic waste have more potential for generating higher methane gas concentrations than others.

The analysis consistently shows that the combination of fruits and vegetables yields higher methane gas concentrations than other types of waste throughout the observation period. This finding indicates that this combination has more potential for biogas production. The optimal time for achieving the highest methane concentration with this composition appears to be between the 6th and 12th days of the anaerobic process, where a significant increase in methane concentration is observed before stabilizing or declining.

Based on these results, it is recommended to utilize a combination of organic waste comprising fruits and vegetables, with an optimal fermentation period ranging from 6 to 12 days, to achieve the highest methane concentration for more efficient biogas production. Overall, the interaction plot indicates that while there are differences in the methane concentration patterns among different types of waste, the effect of time on methane concentration does not differ significantly between waste types. This finding aligns with the Repeated Measures ANOVA results, which demonstrate that time does not substantially influence methane concentration (see Table 5). This indicates that the type of organic waste plays a more dominant role in determining the outcome compared to the duration of the process (see Table 4).

The combination of fruits and vegetables yields the highest methane concentration. This is likely due to the high carbon content in both types of materials (see Figs 5 and). Although vegetables alone produce lower amounts of methane, when combined with fruits, they may complement each other regarding nutrients and structure, thereby supporting the more effective activity of methanogenic microbes. When combined with fruits, minor vegetable elements may also play a catalytic role in accelerating the anaerobic fermentation process.

On the other hand, although vegetables possess a relatively high carbon content, their low methane production can be attributed to their more complex morphology and the presence of minor elements such as Mg, P, S, Cl, K, and Ca, which may disrupt or inhibit the anaerobic fermentation process. The heterogeneous morphological structure and these minor elements create suboptimal conditions for methane production, making vegetables the lowest methane producers among the samples tested.

While the crystalline phase identification suggests that fruits may have a slower decomposition rate due to their higher crystallinity (see Fig. 3), the actual methane production results indicate that a mixture of fruits and vegetables yields the highest methane production. This could be due to the complementary nature of the organic materials in the mix. Combining the more easily decomposable amorphous components in vegetables with the crystalline structures in fruits might create a balanced environment that optimizes microbial activity and methane production.

When fruits are used alone, their higher crystallinity might slow the decomposition process, but they still generate a significant amount of methane due to their organic content. On the other hand, a mixture of vegetables and grass, with their more amorphous structures, likely leads to a moderate rate of methane production, as the decomposition process is faster but may not be as efficient in methane yield as the fruits-vegetables mixture. This suggests that the interplay between crystalline and amorphous phases and the specific combination of organic materials is crucial in optimizing methane production during anaerobic digestion.

Fruits, rich in carbohydrates, straightforward sugars, and organic acids, are highly biodegradable and rapidly convert into volatile fatty acids (VFAs) during anaerobic digestion, resulting in efficient methane production (Pandey et al., 2022). On the other hand, despite having a higher water and protein content that may initially slow down degradation, vegetables still contribute significantly to methane production when combined with fruits due to their polysaccharides and organic acids that complement the simple sugars from fruits (see Fig. 2). The combination of fruits and vegetables thus creates a balanced substrate, wherein the quick breakdown of carbohydrates from fruits and the additional organic content from vegetables ensures a sustained and enhanced production of VFAs, leading to the highest observed methane production.

On the other hand, vegetables produce the lowest methane concentration during anaerobic digestion due to their chemical composition, as revealed by FTIR analysis. The high water content in vegetables, indicated by strong O-H stretching peaks around 3400 cm^{-1}, dilutes the organic content available for digestion, reducing methane production efficiency and slowing the digestion process by creating a less favorable environment for the breakdown of organic matter into volatile fatty acids (VFAs). Additionally, the presence of complex organic matter, shown by significant C=O stretching peaks near 1650 cm^{-1}, suggests that vegetables contain amides and carboxylic acids, which are more challenging to degrade during anaerobic digestion, leading to a lower overall methane yield. Furthermore, vegetables have a lower availability of easily degradable carbohydrates than fruits, containing more complex polysaccharides and proteins that are less readily converted into VFAs, thus making the methane production process less efficient when vegetables are digested alone.

Table 4 *Analysis of variance (ANOVA)*

Day	F-statistic	p-value
3	124.9585	$2.23 x10^{-37}$
6	1394.81	$3.15 x10^{-79}$
9	126.5419	$1.4x10^{-37}$
12	2249.425	$9.19x10^{-83}$
15	12.23135	$4.7x10^{-9}$

As shown in Table 4, the very small P-values ($p < 0.05$) across all days indicate significant differences in methane gas concentrations produced by various types of organic waste. This result suggests that organic waste significantly affects methane production, with different waste types generating varying methane concentrations.

Table 5 *Repeated measures ANOVA*

Statistic	Value
F Value	1.270726
Num DF	5
Den DF	20
Pr > F	0.315021

The Repeated Measures ANOVA aimed to determine whether time (measurement days) significantly affected methane gas concentration while accounting for the differences in organic waste types. As indicated in Table 5, a P-value greater than 0.05 (i.e., 0.315) suggests no significant effect of anaerobic process duration on methane concentration after considering the type of organic waste. In other words, while the type of organic waste influences methane concentration, the duration of the anaerobic process does not significantly affect the variation in methane concentration.

3.5 Comparison of The Recent Study With The Previous Study

Table 6 *Comparison of the gas concentration of the recent study with previous research*

No.	Substrates	Total Substrate Volume (Liter)	Gas Concentration (ppm)	References
1	Tempe waste	150	6738.15	(Kamilia et al., 2021)
2	Yeast and cow dung	0.2	206.76	(Subagyo & Wijaya, 2017)
3	Sargassum Sp. seaweed	15	177525.17	(Murjani et al., 2020)
4	Cowdung	120	125823.26	(Ritonga et al., 2021)

No.	Substrates	Total Substrate Volume (Liter)	Gas Concentration (ppm)	References
5	Organic waste	19	2650	(Yuwanda et al., 2019)
6	Fruit	10	479.53	Recent Study
	Grass		430.68	
	Vegetables		540.59	
	Fruits and vegetables		637.75	
	Fruits and grass		426.11	
	Vegetables and grass		436.46	

As shown in Table 6, in the study by Kamilia et al., which utilized tempeh waste as a substrate, the methane gas concentration achieved was 6738.15 ppm. This study demonstrated that variations in the ratio between liquid and solid tempeh waste affected the start-up time and methane production. However, the results were still lower compared to studies using other substrates. The research conducted by Subagyo et al., which employed a mixture of cow dung and yeast, produced a much lower methane concentration of 206.76 ppm with a smaller substrate volume, indicating that this combination was less effective in enhancing methane production. Conversely, the study by Murjani et al., which used Sargassum sp. seaweed, achieved a very high methane concentration of 177525.17 ppm. Although these results were significant, using seaweed may not be practical on a larger scale, particularly in regions far from marine resources. The study by Yuwanda et al. explored methane production from organic waste, reaching an optimal concentration of 2650 ppm on the eighth day of the fermentation process. This study highlighted the potential of organic waste as a substrate, but the methane yield was relatively modest compared to other studies. The study by Ritonga et al. focused on cow dung as the primary substrate. It demonstrated that activated charcoal and zeolite could significantly increase methane concentration, reaching 125823.26 ppm after purification. However, this research primarily focused on gas purification after biogas production and did not prioritize optimizing the substrate.

Compared to other co-substrates investigated in previous studies, such as whey combined with livestock waste, up to 84 % improvement in methane production has been observed (Vivekanand et al., 2017). In another case, co-digestion of food waste and active sludge yielded a 24.6 % increase in methane production (and 10.1 % VS reduction) (He et al., 2024). In our study, the maximum increase in methane concentration reached ~69.7 % with rice water supplementation, comparable to or surpassing many co-substrate systems reported in the literature.

The combination of fruits and vegetables produced the highest methane concentration (637.76 ppm), outperforming single-substrate digestion. This indicates a synergistic interaction, where fruits provide easily degradable carbohydrates while vegetables contribute essential micronutrients and buffering capacity. Adding rice water further enhanced this synergy by supplying fermentable organics and microbial growth factors, creating a more favorable environment for microbial metabolism. Although microbial profiling was not conducted, the observed methane trends suggest that rice water accelerated hydrolysis and acidogenesis, consistent with findings from previous studies involving LAB-rich and carbohydrate-rich additives (Li et al., 2019; Nabayi et al., 2021). These results support the role of rice water as an effective co-substrate in anaerobic digestion (AD), enhancing microbial kinetics and methane yield. Compared to substrates like *Sargassum sp.*, which produce higher methane concentrations but have limited scalability, household organic waste with rice water offers a more practical and sustainable solution for decentralized biogas production.

4. Conclusions

This study demonstrated that incorporating rice water as a co-substrate significantly enhances methane yield in the anaerobic digestion of organic waste. Using a 1:4 substrate-to-rice water ratio, the combination of jackfruit (Artocarpus heterophyllus) and lettuce (Lactuca sativa) produced the highest methane concentration at 637.75 ppm, representing a 32.96% increase compared to jackfruit. This enhancement is attributed to rice water's high carbohydrate and microbial content, accelerating hydrolysis and acidogenesis during fermentation. Material characterization through FTIR, XRD, and SEM-EDX confirmed distinct biochemical and structural profiles among substrates, corresponding to differences in methane yield. Statistical analysis confirmed that substrate type significantly influenced methane concentration ($p < 0.05$), whereas fermentation duration did not ($p > 0.05$). These findings underscore the importance of substrate formulation in optimizing biogas production efficiency.

Although the 1:4 ratio yielded promising results, future research should explore a range of substrate-to-rice water ratios to identify the optimal formulation. Additionally, extending the investigation to other types of organic waste, such as household food scraps, market waste, and agricultural residues, would broaden the applicability of this approach. Microbial community analysis using metagenomic tools is also recommended to better understand the biological mechanisms driving methane enhancement. Finally, scaling up this method and evaluating its operational performance over time will be crucial for practical implementation in decentralized waste-to-energy systems.

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Conflict of Interest

The authors declare no conflict of interest regarding the paper's publication.

Authors Contribution

The authors confirm contribution to the paper as follows: ***study conception and design:*** *Sudarman, Roro Heni Hendaryati, Achmad Fauzan Hery Soegiharto;* ***data collection:*** *Dyanu Moulana Ismail, Rizki Raihan Ramadhan;* ***analysis and interpretation of results:*** *Willy Satrio Nugroho, Yepy Komaril Sofi'i;* ***draft manuscript preparation:*** *Yepy Komaril Sofi'i. All authors reviewed the results and approved the final version of the manuscript.*

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