

# Characterization of Cassava Sludge and Tofu Wastewater as Anaerobic Digestion Substrates for Biogas Production

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## To cite this article:

Wulandari, D., Diana, R., Niti Mulyani, L., Alpa Harzon, J., & Aulia, M. (2026). Characterization of Cassava Sludge and Tofu Wastewater as Anaerobic Digestion Substrates for Biogas Production. *International Journal of Research in Vocational Studies (IJRVOCAS)*, 6(2), 23–30. <https://doi.org/10.53893/ijrvocas.v6i2.528>.

Received: 04 13, 2026; Revised: 05 24, 2025; Accepted: 08 11, 2026; Published: 08 22, 2026



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**Abstract:** The increasing generation of organic waste from the food processing industry has created opportunities for converting biodegradable residues into renewable energy. Cassava sludge and tofu wastewater contain biodegradable organic matter and may serve as substrates for anaerobic digestion. This study aimed to characterize the initial physicochemical properties of cassava sludge and tofu wastewater and assess their characteristics and endpoint gas profiles under different substrate formulations. Four formulations were evaluated: cassava sludge (S1), cassava sludge mixed with tofu wastewater (S2), tofu wastewater (S3), and cassava sludge supplemented with a microbial inoculum (S4). Activated Effective Microorganisms 4 (EM4) was applied to all formulations. Initial characterization included pH, temperature, total nitrogen (TN), total suspended solids (TSS), turbidity, chemical oxygen demand (COD), and biochemical oxygen demand (BOD). Reactor pH and temperature were monitored every three days for 21 days batch digestion period, followed by endpoint gas composition analysis. Cassava sludge exhibited the highest initial COD and BOD concentrations, at 19,409 and 8,640 mg/L, respectively, with TSS and TN concentrations of 680 and 270 mg/L. The cassava sludge tofu wastewater formulation showed COD and BOD concentrations of (13,355 mg/L and 6,028 mg/L), respectively, whereas tofu wastewater showed values of (6,644 mg/L and 2,974 mg/L). S4 exhibited lower initial COD and BOD concentrations of 389 and 175 mg/L, respectively, reflecting the initial characteristics of the formulation containing additional microbial inoculum. During the digestion period, temperatures ranged from (25.1–32.2 °C). while pH generally increased across the formulations. At the end of the 21 days digestion period, S4 showed the highest CH<sub>4</sub> reading (65.4%LEL), followed by S1 (9.7%LEL), S3 (4.7%LEL), and S2 (3.9%LEL). The observed results indicate differences in endpoint gas characteristics among the substrate formulations under the experimental conditions. Overall, the initial physicochemical characteristics and endpoint gas profiles provide a basis for assessing cassava sludge and tofu wastewater as substrates for anaerobic digestion.

**Keywords:** Biogas, Anaerobic Digestion, Cassava Sludge, Tofu Wastewater, Physicochemical Characterization, Gas Production

## 1. Introduction

The rapid expansion of agro industrial activities in Indonesia has contributed significantly to economic growth

while simultaneously increasing the generation of organic waste. The cassava processing and tofu manufacturing

industries are among the major agro industrial sectors producing substantial quantities of sludge and wastewater enriched with biodegradable organic matter [1]. If inadequately managed, these waste streams can pose serious environmental risks, particularly to aquatic ecosystems, due to their elevated concentrations of organic pollutants, as reflected by high chemical oxygen demand (COD) and biochemical oxygen demand (BOD) values. This challenge highlights the urgent need for sustainable waste management strategies that not only mitigate environmental pollution but also enhance resource recovery through the conversion of organic waste into renewable energy [2].

Anaerobic digestion has emerged as one of the most effective technologies for the sustainable valorization of organic waste. This biochemical process relies on the metabolic activity of anaerobic microorganisms to convert biodegradable organic matter into biogas under oxygen free conditions, with methane ( $\text{CH}_4$ ) and carbon dioxide ( $\text{CO}_2$ ) as the principal gaseous products. Beyond renewable energy production, anaerobic digestion substantially reduces the organic pollutant load of waste streams, thereby minimizing their environmental impact and improving waste management sustainability. The efficiency and stability of the anaerobic digestion process are governed by multiple operational and substrate related factors, including substrate characteristics, nutrient availability, pH, temperature, and the activity of the microbial community throughout the digestion process [3], [4], [5].

Cassava sludge, a solid by product generated during starch extraction, contains substantial amounts of residual starch, lignocellulosic fibers, and other biodegradable organic compounds, making it a promising feedstock for biogas production [6]. In contrast, tofu wastewater is rich in soluble proteins, carbohydrates, and dissolved organic compounds that can serve as readily available nutrients for anaerobic microorganisms [7]. The different physicochemical characteristics of these agro-industrial residues may influence substrate biodegradability and microbial conversion during anaerobic digestion. In addition to mono-substrate digestion, co-digestion of cassava sludge and tofu wastewater may provide complementary substrate characteristics and improve the availability of organic matter and nutrients required for microbial growth. Furthermore, the application of bioactivators such as Effective Microorganisms 4 (EM4) can provide additional microbial consortia to support the anaerobic degradation process, while the incorporation of an additional microbial inoculum may further influence microbial activity and the resulting gas characteristics [8], [9], [10]. Therefore, evaluating the physicochemical characteristics and anaerobic digestion behavior of cassava sludge and tofu wastewater, both individually and in combination with microbial supplementation, is important for assessing their potential utilization as substrates for biogas generation.

Despite the growing body of research on anaerobic digestion of agro industrial residues, studies focusing on the comparative physicochemical characterization of cassava sludge and tofu wastewater under different substrate formulations remain limited. Most previous studies have

primarily emphasized biogas production performance, while comparatively less attention has been given to the relationship between the initial substrate characteristics, process stability, and biogas quality. A comprehensive understanding of these relationships is essential for optimizing substrate selection and improving the efficiency of anaerobic digestion systems treating agro industrial waste.

Therefore, this study aims to characterize the physicochemical properties of cassava sludge and tofu wastewater and to evaluate the changes in process conditions and biogas composition during anaerobic digestion using different substrate formulations. The findings are expected to provide scientific evidence regarding the suitability of these agro industrial wastes as feedstocks for biogas production and contribute to the development of sustainable waste to energy technologies through the valorization of organic residues.

Substrate characterization is a fundamental step prior to the utilization of organic waste as a feedstock for biogas production. Comprehensive information on the physicochemical properties of the substrate is essential for assessing its suitability for anaerobic digestion and identifying the conditions that support efficient process performance. Key parameters, including pH, temperature, chemical oxygen demand (COD), biochemical oxygen demand (BOD), total suspended solids (TSS), total nitrogen (TN), and turbidity, are commonly employed to characterize the organic content and biodegradability of substrates intended for anaerobic digestion [11].

Although numerous studies have reported the utilization of cassava waste and tofu processing wastewater as feedstocks for biogas production, most have primarily focused on enhancing biogas yield, optimizing operational conditions, or improving reactor configurations. In contrast, the comprehensive physicochemical characterization of these substrates as a basis for evaluating their suitability for anaerobic digestion has received comparatively less attention. Moreover, integrated studies comparing the physicochemical characteristics of cassava sludge, tofu wastewater, their co-digestion mixture, and cassava sludge supplemented with a microbial inoculum under the same experimental framework remain limited. Such comparative information is essential for elucidating the relationship between substrate characteristics, process stability, and biogas production performance during anaerobic digestion.

Furthermore, continuous monitoring of pH and temperature throughout the digestion process is crucial for evaluating process stability, whereas biogas composition analysis provides valuable insight into gas quality and serves as an important indicator of methanogenic activity and overall anaerobic digestion performance [12], [13].

Based on the research gaps, this study aims to investigate the physicochemical characteristics of cassava sludge and tofu wastewater as potential feedstocks for biogas production through anaerobic digestion. Four substrate formulations were evaluated, namely cassava sludge (S1), a mixture of cassava sludge and tofu wastewater (S2), tofu wastewater (S3), and cassava sludge supplemented with a microbial inoculum (S4). Activated Effective Microorganisms 4 (EM4) was applied as a bioactivator in all treatments to promote microbial activity

and facilitate the initiation of the anaerobic digestion process. The physicochemical characterization included the determination of pH, temperature, total nitrogen (TN), total suspended solids (TSS), turbidity, chemical oxygen demand (COD), and biochemical oxygen demand (BOD). In addition, pH and temperature were continuously monitored throughout the anaerobic digestion process, while the composition of the produced biogas was analyzed using a portable gas detector. The findings of this study provide a scientific basis for evaluating the suitability of cassava sludge and tofu wastewater as anaerobic digestion feedstocks and improve the understanding of the relationships between substrate characteristics, process stability, and biogas quality. Furthermore, this study contributes to the development of efficient and sustainable waste to energy strategies through the valorization of agro industrial residues.

## 2. Research Methods

### 2.1. Sample Location and Preparation

Cassava sludge, cassava sludge containing microbial inoculum, and tofu wastewater were selected as substrates for anaerobic digestion. Fresh cassava sludge and cassava sludge containing bacterial inoculum were obtained from a commercial cassava starch industrial partner, while tofu wastewater was obtained from a local tofu processing enterprise. The samples were collected directly from the respective sources and transported to the laboratory in sealed plastic containers to minimize changes in their physicochemical properties prior to analysis. Upon arrival, the samples were thoroughly homogenized before initial physicochemical characterization and preparation of the substrate formulations for the anaerobic digestion experiments.

### 2.2. Initial Physicochemical Characterization of Substrates

Prior to the anaerobic digestion experiments, the substrates were subjected to initial physicochemical characterization. Four substrate formulations were prepared: cassava sludge (S1), a mixture of cassava sludge and tofu wastewater (S2), tofu wastewater (S3), and cassava sludge containing microbial inoculum obtained from industrial partner. (S4). For S2, cassava sludge and tofu wastewater were mixed at a 1:1 volumetric ratio. The microbial inoculum in S4 was inherent in the cassava sludge sample as received from the research partner and was not separately prepared or added by the researchers. Activated Effective Microorganisms 4 (EM4) was applied as a bioactivator in all treatments to stimulate microbial activity during the initial phase of anaerobic digestion.

The initial physicochemical characteristics of the substrates were determined by measuring chemical oxygen demand (COD), biochemical oxygen demand (BOD), total suspended solids (TSS), total nitrogen (TN), pH, temperature, and turbidity. All analyses were conducted in accordance with the Standard Methods for the Examination of Water and Wastewater [14], except where otherwise specified. These

measurements were performed at the beginning of the experiment to characterize the initial properties of each substrate formulation and assess their potential as substrates for anaerobic digestion.

### 2.3. Bioactivator Activation

Effective Microorganisms 4 (EM4) was used as the bioactivator in all anaerobic digestion experiments. Prior to its application, the bioactivator was activated by preparing 1 L of starter solution consisting of 90 mL of EM4 (equivalent to 0.75% of the substrate volume), 45 mL of molasses (50% of the EM4 volume), and 865 mL of clean water. The mixture was thoroughly homogenized and incubated in a sealed container at ambient temperature ( $28 \pm 2$  °C) for approximately 24 h to activate the microbial consortium, [8].

Following activation, 1 L of the activated EM4 starter solution was added to each reactor containing 12 L of substrate, resulting in a total working volume of 13 L. The activated EM4 starter solution was applied uniformly to all substrate treatments.

### 2.4. Anaerobic Digestion Process

Anaerobic digestion experiments were conducted using laboratory-scale batch digesters with a working volume of 13 L. One batch digester was used for each substrate formulation. Each digester was loaded with 12 L of the respective substrate and supplemented with 1 L of activated EM4 starter solution, resulting in a total working volume of 13 L. For treatment S2, the 12 L substrate consisted of 6 L of cassava sludge and 6 L of tofu wastewater, corresponding to a 1:1 volumetric ratio. Treatment S4 consisted of cassava sludge containing an additional microbial inoculum provided by the industrial partner. Activated EM4 was applied to all treatments.

The digesters were operated in batch mode for 21 days at ambient temperature. Following substrate loading, the digestion was tightly sealed to minimize air intrusion during the digestion period. The digestion process involved the activity of indigenous and inoculated microbial consortia in the degradation of biodegradable organic matter under anaerobic conditions [15], [4].

### 2.5. Monitoring the Anaerobic Digestion Process

The anaerobic digestion process was monitored by measuring the pH and temperature of each digester throughout the 21 days experimental period [16]. Measurements were conducted at three days intervals on days 0, 3, 6, 9, 12, 15, 18, and 21 to evaluate process stability under the different substrate formulations.

The pH and temperature were measured using a Smart Sensor pH Meter A8218. Prior to measurement, the instrument was calibrated in accordance with the manufacturer's instructions to ensure measurement accuracy and reliability. Before measuring each subsequent substrate sample, the probe was thoroughly rinsed with distilled water to remove residual sample and minimize cross contamination, followed by gentle drying before immersion into the next sample. Measurements were performed under consistent conditions for all treatments.

## 2.6. Biogas Composition Analysis

At the end of the 21 days anaerobic digestion period, gas parameters from each digester were measured using a SNDWAY BTYM-SW7500GT portable gas detector. The measurements were performed by directly connecting the detector to the gas outlet of each digester, and the readings were recorded after the instrument displayed stable values. The measured parameters included methane (CH<sub>4</sub>), oxygen (O<sub>2</sub>), hydrogen sulfide (H<sub>2</sub>S), and carbon monoxide (CO). According to the instrument output, CH<sub>4</sub> was reported in %LEL (*Lower Explosive Limit*), O<sub>2</sub> in %vol, and H<sub>2</sub>S and CO in µmol/mol. CH<sub>4</sub> was reported in %LEL according to the instrument reading and was not converted to CH<sub>4</sub> concentration in %vol.

Gas parameters were measured once at the end of the digestion period (day 21), and the resulting data represent the gas characteristics at the endpoint of the digestion process and were used to compare the gas profiles among the different substrate formulations [17].

## 2.7. Data Analysis

The initial physicochemical characterization data, and endpoint gas composition data were analyzed descriptively. The initial physicochemical parameters, including chemical oxygen demand (COD) using method SNI 6989.02:2019, biochemical oxygen demand (BOD) using method 6989.72:2009, total suspended solids (TSS) using method SNI 6989.03:2019, total nitrogen (TN) using method JIS K0102:2016 point 45.2, pH using method SNI 6989.11:2019, temperature using method SNI 06.6989.23:2005, turbidity using method SNI 06.6989.12:2004, were used to characterize the initial properties of the substrate formulations.

Temporal variations in pH and temperature during the 21 days digestion period were analysed to describe changes in the physicochemical conditions of the digestion process. The gas parameters measured at the end of the digestion period, including CH<sub>4</sub> (%LEL), O<sub>2</sub> (%vol), CO (µmol/mol), and H<sub>2</sub>S (µmol/mol) were compared descriptively among the substrate formulations to characterize their endpoint gas profiles. The CH<sub>4</sub> (%LEL) values were interpreted according to the instrument readings and were not converted to CH<sub>4</sub> concentration in %vol. The results were interpreted based on the observed values and temporal patterns among the substrate formulations. The experimental results are presented in tables and graphs to facilitate comparison among the substrate formulations [16], [18].

# 3. Result and Discussion

## 3.1. Physicochemical Characteristics of Cassava Sludge and Tofu Wastewater as Biogas Feedstocks

The initial physicochemical properties of cassava sludge and tofu wastewater were analyzed before anaerobic digestion to assess their suitability as biogas feedstocks. Parameters including pH, temperature, total nitrogen, total suspended solids (TSS), turbidity, chemical oxygen demand (COD), and biochemical oxygen demand (BOD) were measured because

they influence substrate biodegradability and anaerobic digestion performance.

Chemical Oxygen Demand (COD) is a parameter representing the amount of oxygen required for the chemical oxidation of oxidizable substances in wastewater, whereas Biochemical Oxygen Demand (BOD) indicates the oxygen demand associated with the biological degradation of biodegradable organic matter. COD values are generally higher than BOD values because COD encompasses a broader range of chemically oxidizable compounds, including organic compounds that may not be readily biodegradable. In anaerobic digestion, the presence of biodegradable organic matter is an important characteristic because it provides potential substrates that can be utilized by microbial consortia during anaerobic conversion [19],[20]. The results are presented in Table 1.

**Table 1. Initial Physicochemical Characteristics of Cassava Sludge and Tofu Wastewater**

| Parameter             | Cassava Sludge (S1) | Cassava Sludge + Tofu Wastewater (S2) | Tofu Wastewater (S3) | Cassava Sludge + Microbial Inoculum (S4) |
|-----------------------|---------------------|---------------------------------------|----------------------|------------------------------------------|
| pH                    | 3.41                | 3.84                                  | 3.53                 | 6.63                                     |
| Temperature(°C)       | 28.4                | 28.3                                  | 28.3                 | 28.2                                     |
| Nitrogen Total (mg/L) | 270                 | 221                                   | 91.3                 | 106                                      |
| TSS (mg/L)            | 680                 | 181                                   | 135                  | 80.5                                     |
| Turbidity (NTU)       | 840                 | 220                                   | 180                  | 69                                       |
| COD (mg/L)            | 19,409              | 13,355                                | 6,644                | 389                                      |
| BOD (mg/L)            | 8,640               | 6,028                                 | 2,974                | 175                                      |
| Rasio BOD/COD         | 0.45                | 0.45                                  | 0.45                 | 0.45                                     |

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Based on the results presented in Table 1, the four substrate formulations exhibited distinct initial organic characteristics, as indicated by their COD and BOD concentrations. Cassava sludge (S1) exhibited the highest initial COD and BOD concentrations, at 19,409 and 8,640 mg/L, respectively. These values indicate a relatively high initial organic matter load and the presence of a substantial biodegradable organic fraction that could potentially be utilized by anaerobic microorganisms. The co-substrate formulation (S2), consisting of cassava sludge and tofu wastewater at a 1:1 volumetric ratio, showed COD and BOD concentrations of 13,355 and 6,028 mg/L, respectively. The intermediate values observed in S2 reflect the combined initial characteristics of the two substrate sources following mixing.

Tofu wastewater (S3) exhibited COD and BOD concentrations of 6,644 and 2,974 mg/L, respectively, which were lower than those of S1 and S2. These values indicate a comparatively lower initial organic matter load, although the presence of biodegradable organic compounds still provides potential substrates for microbial activity under anaerobic conditions. Meanwhile, S4, consisting of cassava sludge supplemented with an additional microbial inoculum, exhibited the lowest initial COD and BOD concentrations, at 389 and 175 mg/L, respectively. These values represent the

initial characteristics of the S4 formulation prior to the anaerobic digestion process.

The differences in initial COD and BOD concentrations among S1–S4 indicate variations in organic matter availability and the biodegradable fraction of the substrate formulations. In general, higher initial COD concentrations indicate a greater overall organic matter load, whereas BOD provides an indication of the fraction of organic matter that can potentially be utilized through biological processes. Therefore, COD and BOD can be used as complementary parameters to characterize the initial organic properties and biodegradability potential of substrates intended for anaerobic digestion. However, these initial parameters describe the substrate characteristics before digestion and should not be interpreted as direct measures of biogas or methane production. The conversion of organic matter during anaerobic digestion is influenced by several factors, including substrate composition, biodegradability, microbial activity, and process conditions.

The other physicochemical parameters also demonstrated differences among the substrate formulations. S1 exhibited the highest Total Suspended Solids (TSS) and turbidity values, at 680 mg/L and 840 NTU, respectively, indicating a relatively high concentration of suspended materials. The Total Nitrogen (TN) concentration was also highest in S1, at 270 mg/L, whereas S3 exhibited the lowest TN concentration, at 91.3 mg/L. These differences indicate variations in the physical and nutritional characteristics of the substrate formulations, which may influence microbial growth and substrate utilization during anaerobic digestion.

The initial pH values ranged from 3.41 to 6.63. S1, S2, and S3 exhibited relatively acidic initial conditions, whereas S4 showed a pH of 6.63, which was closer to neutral. The initial temperature was relatively consistent among the formulations, ranging from 28.2 to 28.4°C, indicating comparable thermal conditions at the beginning of the experiment. These parameters provide a baseline description of the physicochemical conditions of each substrate before anaerobic digestion.

The BOD/COD ratio was 0.45 for all substrate formulations, indicating a relatively consistent proportion of biodegradable organic matter among the treatments. This ratio supports the presence of a biodegradable organic fraction within the substrates and their potential suitability for biological treatment under anaerobic conditions. Overall, the initial characterization demonstrates that S1, S2, S3, and S4 possessed different initial organic, suspended-solid, nutritional, and physicochemical characteristics. These initial characteristics describe the physicochemical conditions of the substrates prior to the anaerobic digestion process.

### 3.2. Changes in pH During Anaerobic Digestion

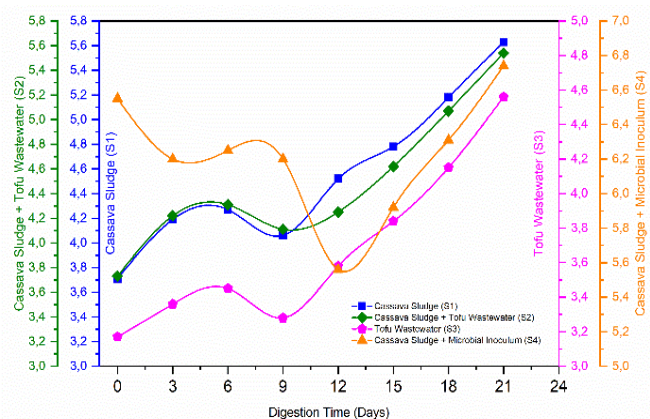
The pH of each substrate formulation was monitored throughout the 21-day anaerobic digestion period to characterize changes in process conditions. The results are presented in Table 2, and the corresponding pH profiles are shown in Figure 1.

**Table 2. Changes in pH During Anaerobic Digestion**

| Days | Cassava Sludge (S1) | Cassava Sludge + Tofu Wastewater (S2) | Tofu Wastewater (S3) | Cassava Sludge + Microbial Inoculum (S4) |
|------|---------------------|---------------------------------------|----------------------|------------------------------------------|
| 0    | 3.71                | 3.73                                  | 3.17                 | 6.55                                     |
| 3    | 4.19                | 4.22                                  | 3.36                 | 6.20                                     |
| 6    | 4.27                | 4.31                                  | 3.45                 | 6.25                                     |
| 9    | 4.06                | 4.11                                  | 3.28                 | 6.20                                     |
| 12   | 4.52                | 4.25                                  | 3.58                 | 5.56                                     |
| 15   | 4.78                | 4.62                                  | 3.84                 | 5.92                                     |
| 18   | 5.18                | 5.07                                  | 4.15                 | 6.31                                     |
| 21   | 5.63                | 5.54                                  | 4.56                 | 6.74                                     |

\*Smart Sensor pH Meter A8218 used for pH and temperature measurements

As shown in Table 2, all substrate formulations exhibited changes in pH throughout the anaerobic digestion process. At the beginning of the experiment, the pH values were generally within the acidic range, particularly for the cassava sludge and tofu wastewater treatments. This acidic condition indicates the occurrence of the initial stages of anaerobic digestion, namely hydrolysis and acidogenesis. S1, S2, and S3 exhibited initial pH values of 3.71, 3.73, and 3.17, respectively, whereas S4 showed a comparatively higher initial pH of 6.55. The initial pH values of S1–S3 were below the range generally considered favorable for methanogenic activity [12].



**Figure 1. Changes in pH During Anaerobic Digestion**

Throughout the 21-day digestion period, the pH of all treatments generally increased. The pH of S1 increased from 3.71 to 5.63, S2 from 3.73 to 5.54, and S3 from 3.17 to 4.56. S4 exhibited a comparatively higher pH profile and reached 6.74 on day 21. However, the final pH values of S1–S3 remained acidic and below the range favorable for methanogenic activity, indicating that the persistent acidic conditions may have constrained methanogenic activity during the experimental period. In contrast, the final pH of S4 approached the lower boundary of the favorable range, indicating comparatively more favorable acid–base conditions at the end of the digestion period.

Because volatile fatty acids (VFAs) and alkalinity were not measured, the observed pH variations were interpreted as changes in the overall acid–base conditions of the digesters rather than as direct evidence of VFA consumption or process stability. It should also be noted that the initial pH

characterization was conducted using the SNI 6989.11:2019 method, whereas pH monitoring during the digestion process was performed using a Smart Sensor pH Meter A8218. Therefore, differences between the initial and subsequent pH measurements should be interpreted in consideration of the different analytical methods and instruments employed.

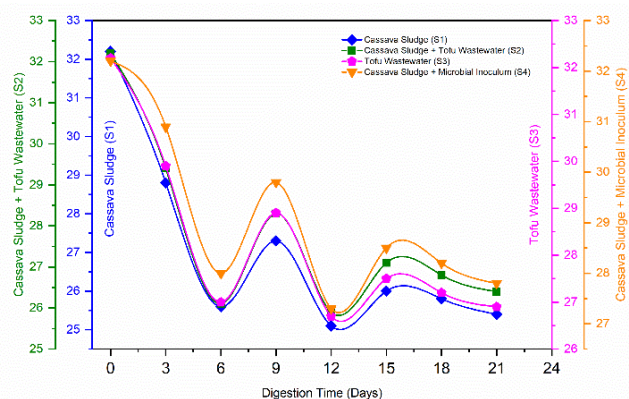
### 3.3. Changes in Temperature During Anaerobic Digestion

Temperature is a key operational parameter affecting microbial activity and anaerobic digestion performance. The reactor temperature was monitored throughout the 21-day digestion period. The measured temperature values are presented in Table 3, while the corresponding temperature profiles are shown in Figure 2.

**Table 3. Changes in Temperature During Anaerobic Digestion**

| Days | Cassava Sludge (S1) | Cassava Sludge + Tofu Wastewater (S2) | Tofu Wastewater (S3) | Cassava Sludge + Microbial Inoculum (S4) |
|------|---------------------|---------------------------------------|----------------------|------------------------------------------|
| 0    | 32.2 °C             | 32.2 °C                               | 32.2 °C              | 32.2 °C                                  |
| 3    | 28.8 °C             | 29.4 °C                               | 29.9 °C              | 30.9 °C                                  |
| 6    | 25.6 °C             | 26.1 °C                               | 27.0 °C              | 28.0 °C                                  |
| 9    | 27.3 °C             | 28.3 °C                               | 28.9 °C              | 29.8 °C                                  |
| 12   | 25.1 °C             | 25.9 °C                               | 26.7 °C              | 27.3 °C                                  |
| 15   | 26.0 °C             | 27.1 °C                               | 27.5 °C              | 28.5 °C                                  |
| 18   | 25.8 °C             | 26.8 °C                               | 27.2 °C              | 28.2 °C                                  |
| 21   | 25.4 °C             | 26.4 °C                               | 26.9 °C              | 27.8 °C                                  |

\*Smart Sensor pH Meter A8218 used for pH and temperature measurements.



**Figure 2. Changes in Temperature During Anaerobic Digestion**

The reactor temperature remained within the range of 25.1–32.2 °C throughout the 21-day anaerobic digestion period. The temperature was relatively higher at the beginning of the experiment and gradually decreased toward ambient conditions, followed by relatively minor fluctuations during the subsequent digestion period. This temperature pattern was observed across all substrate formulations.

Among the substrate formulations, S4 maintained comparatively higher temperatures during the later stage of digestion, reaching 27.8 °C on day 21, compared with 25.4 °C, 26.4 °C, and 26.9 °C for S1, S2, and S3, respectively. However, the temperature profile alone does not provide sufficient evidence to directly attribute these differences to

microbial activity or substrate degradation performance.

Overall, the recorded temperatures represented ambient and relatively low-temperature operating conditions, ranging from 25.1 to 32.2 °C. Although this range encompasses the lower end of the mesophilic temperature region, most measurements remained below the commonly applied mesophilic operating temperature of approximately 35 °C. Therefore, the observed temperature conditions should not be considered optimal for mesophilic anaerobic digestion. The relatively low operating temperatures may have influenced microbial activity and the progression of anaerobic digestion during the experimental period. A longer digestion period under controlled temperature conditions may provide more favorable conditions for the development of methanogenic activity.

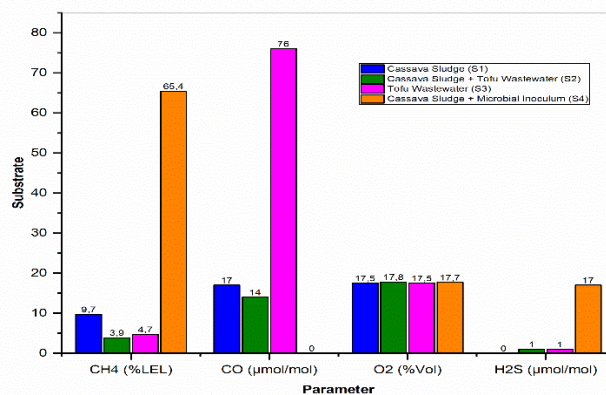
### 3.4. Implications of Substrate Characteristics for Biogas Production Potential

The composition of biogas produced after 21 days of anaerobic digestion was analyzed using a portable gas detector to evaluate the performance of the different substrate formulations. The measured gas components included methane (CH<sub>4</sub>), oxygen (O<sub>2</sub>), carbon monoxide (CO), and hydrogen sulfide (H<sub>2</sub>S). The results are presented in Table 4, while the comparison of methane (CH<sub>4</sub>) concentrations among the treatments is illustrated in Figure 3.

**Table 4. Biogas Composition Determined Using a Portable Gas Detector**

| Parameter                   | Cassava Sludge (S1) | Cassava Sludge + Tofu Wastewater (S2) | Tofu Wastewater (S3) | Cassava Sludge + Microbial Inoculum (S4) |
|-----------------------------|---------------------|---------------------------------------|----------------------|------------------------------------------|
| CH <sub>4</sub> (%LEL)      | 9.7                 | 3.9                                   | 4.7                  | 65.4                                     |
| CO (μmol/mol)               | 17                  | 14                                    | 76                   | 0                                        |
| O <sub>2</sub> (%vol)       | 17.5                | 17.8                                  | 17.5                 | 17.7                                     |
| H <sub>2</sub> S (μmol/mol) | 0                   | 1                                     | 1                    | 17                                       |

\*SNDWAY BTYM-SW7500GT four gas detector



**Figure 3. Biogas Composition Determined Using a Portable Gas Detector**

As shown in Table 4 and figure 3, the CH<sub>4</sub> (%LEL) readings for S1, S2, S3, and S4 were 9.7 %LEL, 3.9 %LEL, 4.7 %LEL, and 65.4 %LEL, respectively, with the highest reading observed for S4. These values represent readings on the Lower Explosive Limit (LEL) scale of the SNDWAY BTYM-SW7500GT detector and cannot be directly interpreted as CH<sub>4</sub> concentrations in %vol without a validated conversion factor.

or calibration basis. The higher CH<sub>4</sub> (%LEL) response observed in S4 indicates a greater combustible-gas response than the other treatments and may potentially be associated with enhanced substrate degradation during anaerobic digestion. However, this value should not be used as the sole indicator of improved biogas quality because S4 also exhibited the highest H<sub>2</sub>S concentration (17 µmol/mol). The presence of H<sub>2</sub>S at this level represents an undesirable gas characteristic because H<sub>2</sub>S is a toxic and corrosive contaminant. In addition, the relatively high and consistent O<sub>2</sub> concentrations across all treatments (17.5–17.8 %vol) are not fully consistent with the expected characteristics of anaerobic digestion gas and may indicate atmospheric-air dilution, leakage in the reactor or sampling system, headspace contamination, or limitations in the sampling procedure. Therefore, the O<sub>2</sub> results should be interpreted cautiously and should not yet be considered representative of the intrinsic gas composition generated under anaerobic conditions. CO<sub>2</sub>, which is one of the major components of biogas, was not measured because it was not available on the measurement channel of the detector used in this study. Consequently, the parameters presented in Table 4 represent the gas components detected by the available instrument and do not constitute a complete characterization of the biogas composition. These results indicate that S4 exhibited the highest combustible-gas response; however, comprehensive evaluation of biogas quality requires direct quantification of CH<sub>4</sub> and CO<sub>2</sub> together with gas-tight and validated sampling procedures.

## 4. Conclusion

The findings indicate that cassava sludge and tofu wastewater have potential for utilization as substrates for anaerobic digestion based on their initial physicochemical characteristics. Cassava sludge exhibited the highest initial organic matter load, with COD and BOD concentrations of 19,409 and 8,640 mg/L, respectively, while the BOD/COD ratio of approximately 0.45 across the substrate formulations indicated the presence of biodegradable organic fractions.

During the 21-day digestion period, pH generally increased across all substrate formulations. However, S1–S3 remained under acidic conditions at the end of the digestion period, whereas S4 reached a final pH of 6.74, indicating comparatively more favorable acid base conditions at the end of the process. The digestion process occurred under ambient temperature conditions ranging from 25.1 to 32.2 °C, with temperatures generally below the commonly applied mesophilic operating temperature of approximately 35 °C.

At the end of the 21 days digestion period, S4 exhibited the highest CH<sub>4</sub> reading (65.4%LEL), followed by S1 (9.7%LEL), S3 (4.7%LEL), and S2 (3.9%LEL). The observed differences indicate that the substrate formulations exhibited distinct endpoint gas characteristics under the experimental conditions. These findings provide an initial basis for assessing the utilization of cassava sludge and tofu wastewater as anaerobic digestion substrates and indicate that substrate formulation and microbial supplementation may influence the resulting gas characteristics.

## Acknowledgements

The authors gratefully acknowledge Politeknik Manufaktur Negeri Bangka Belitung for providing institutional support, laboratory facilities, and research assistance throughout this study. This research was financially supported by the Ministry of Higher Education, Science, and Technology of the Republic of Indonesia (Kementerian Pendidikan Tinggi, Sains, dan Teknologi Republik Indonesia) through the BIMA Research Program under the Penelitian Dosen Pemula (PDP) scheme. The authors also thank the commercial cassava starch processing plant and the local tofu processing enterprise for their cooperation in providing the substrates used in this research.

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