

## Performance Test of Biogas Production from Tapioca Wastewater: Effect of HRT and Immobilized Media

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**ABSTRACT:** Tapioca starch industry wastewater contains high concentrations of organic matter, offering potential for renewable energy generation via biogas production. This study evaluates the effects of hydraulic retention time (HRT) variation and Lampung natural zeolite as an immobilization medium on biogas production in an anaerobic reactor. Two reactor configurations were compared: one with zeolite as the microbial immobilization medium and a control reactor without any immobilization media. Zeolite improved the sCOD degradation efficiency up to 93% compared to the non-immobilized reactor. VFA conversion to methane remained more stable with zeolite, producing up to 520 mL of biogas at a 10-day HRT, with an average biogas yield of 61.97 mL/g dry substrate, much higher than 4.15 mL/g in the non-immobilized reactor. This research confirms the effectiveness of Lampung natural zeolite for enhancing microbial retention, accelerating methanogenesis, and improving overall anaerobic reactor performance. These results have substantial implications for developing efficient, sustainable, and environmentally friendly treatment technologies for tapioca starch wastewater, offering simultaneous solutions for pollution control and renewable energy generation.

**Keywords:** biogas; tapioca wastewater; anaerobic reactor; hydraulic retention time; Lampung natural zeolite.

### 1. Introduction

Lampung Province has significant agricultural potential, particularly as Indonesia's largest cassava producer, with a total output of 6.7 tons in 2023 (Dinas Kominfo Provinsi Lampung, 2023). One product of cassava processing is tapioca flour, a major carbohydrate source that ranks fourth among staple food crops in developing countries, after rice, corn, and wheat (Krishnakumar et al., 2021). The rapid expansion of the tapioca flour industry, however, has raised serious environmental concerns due to the large volume of wastewater generated during processing, particularly from washing and starch separation stages (Banowati et al., 2018; Trihatmoko et al., 2025).

Tapioca wastewater is characterised by extremely high organic content, with chemical oxygen demand (COD) ranging from 7,000-30,000 mg/L and biological oxygen demand (BOD) between 3,000-7,500 mg/L (Katayama-Hirayama et al., 2011). If discharged without proper treatment, this wastewater can degrade water quality, generate malodorous compounds, and produce harmful

gases such as methane and hydrogen sulfide (Racho et al., 2025; Tsilo et al., 2022). Due to its high concentration of biodegradable organic compounds, tapioca wastewater has strong potential as a substrate for anaerobic digestion (AD) (Hasanudin et al., 2023; Insan et al., 2018). The process of AD is a sustainable technology that enables simultaneous organic waste treatment and renewable energy generation in the form of biogas (Kahar et al., 2018; Ripoll & Toledo, 2021). However, system performance is strongly influenced by operational parameters, particularly hydraulic retention time (HRT), which governs microbial stability, substrate utilisation, and methane production (Q. Huang et al., 2021; Li et al., 2018; Szaja et al., 2021; Yang et al., 2024). Improper HRT can lead to microbial washout or inefficient reactor performance, especially under high organic loading conditions (Pu et al., 2026; Yuan et al., 2026).

To address microbial washout and enhance digestion efficiency, the use of microbial immobilization media such as natural zeolite has gained increasing attention due to its high surface area, microporous structure, and ability to support microbial attachment (Mutiaru et al., 2021; Senila et

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al., 2022; Viradiansyah & Nurkhamidah, 2024). Although previous studies have demonstrated the benefits of zeolite addition in anaerobic digestion of various organic waste, limited research has focused on the combined effect of natural zeolite and HRT variation in the anaerobic digestion of tapioca wastewater (Damayanti et al., 2018; Montalvo et al., 2020; Soeprijanto et al., 2020) Similarly, Spyridonidis *et al* demonstrated that incorporating natural zeolite into the anaerobic digestion of chicken manure leachate increased methane production by adsorbing inhibitory compounds and creating a favourable environment for microbial activity, thereby stabilizing the digestion process and improving biogas yield (Spyridonidis et al., 2022). Therefore, this study aims to investigate the potential of tapioca wastewater as a substrate for biogas production using natural zeolite as a microbial immobilization medium. The novelty of this research lies in evaluating the performance of an anaerobic digestion system under different HRT conditions with an zeolite addition, focusing on key indicators such as sCOD removal, reduction of volatile fatty acid (VFA) accumulation, and methane concentration. Through this analysis, the research aims to identify the optimal reactor operating conditions for maximizing the efficiency of tapioca wastewater conversion into biogas.

## 2. Materials and Methods

### 2.1. Materials

In this study, three primary materials will be utilized: reactor substrate, inoculum, and immobilization. The substrate used in this study was tapioca starch wastewater from PT Bumi Waras (BW) located in Kabupaten Lampung Tengah, Lampung. The characteristics of waste from PT Bumi Waras (BW) are shown in Table 1.

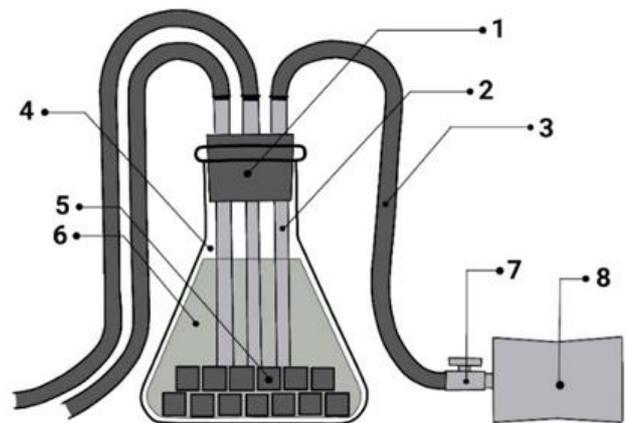
**Table 1.** Content of PT Bumi Waras (BW) Tapioca Starch Waste Water.

| Compound                       | Value    | Unit |
|--------------------------------|----------|------|
| Water                          | 99.46    | %    |
| Protein                        | 0.198    | %    |
| Ash                            | 0.0735   | %    |
| Lipid                          | 0        | %    |
| Carbohydrate                   | 0.2685   | %    |
| Soluble Chemical Demand (sCOD) | 1,170    | mg/L |
| Chemical Oxygen Demand (COD)   | 10,950   | mg/L |
| Total Solid (TS)               | 6,580    | mg/L |
| Volatile Solid (VS)            | 8,766.66 | mg/L |

An inoculum refers to a population of microorganisms or cells introduced into a fermentation medium or other suitable environment. The inoculum used in this study was mesophilic sludge collected from a reactor at PT. Bumi Waras (PT. BW) in Lampung Tengah, Lampung, with 26.92 mg/L total solids and 27.45 mg/L volatile solids. *Zeolite alam Lampung* was used as the immobilization medium. The zeolite used as the immobilization medium was sourced from CV Minatama, located in Bandar Lampung, Lampung.

### 2.2. Methods

The stage of this research included activation of the immobilization media and a performance test. Lampung natural zeolite, used as an immobilization medium, was activated with 3 M NaOH solution (Merck, pellet p.a.). After base activation, the zeolite was neutralized by rinsing with water until the pH reached 7. The zeolite was then dried in an oven (Merck Memmert, Germany) at 110°C for 3 hours, followed by calcination in a furnace (Thermo Scientific FB310M-33) at 400°C for 3 hours to enhance the pore surface area. The activated ZAL was subsequently placed into a carbon fiber textile bed.



**Figure 1.** Apparatus of Immobilized Media Anaerobic Reactor. Annotation: 1) Rubber stopper, 2) Glass pipe, 3) Rubber tube, 4) Erlenmeyer, 5) Zeolite, 6) Substrate 7) Valve, 8) Gas bag

In the performance test, two anaerobic reactors were employed to identify the optimal biogas yield. The reactors were used 2-liter Erlenmeyer flasks, as depicted in Figure 1. Reactor RA was applied with zeolite as the immobilization medium, while Reactor RB was operated without any immobilization media. Additionally, ZAL with carbon fiber textile will be introduced into the reactor at a composition ratio of  $130 \text{ g}_{\text{zeolite}}/1.5 \text{ L}_{\text{inoculum}}$ . After the acclimatization phase, the organic substrate and HRT will be altered. The organic loading rate (OLR) corresponding to each HRT was calculated using the influent sCOD concentration, reactor working volume, and influent flow rate.

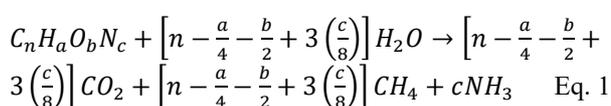
OLR values are expressed as g COD/L.day and are presented together with reactor performance data. Both reactors were tested under varying HRT conditions, specifically from 60 days to 10 days. HRT adjustments were performed only after microbiological stability was achieved, indicating that the growth of key microbial populations responsible for degrading organic matter had been established. This condition was reflected in consistent and stable biogas production. Consequently, no changes to HRT were made during the initial adaptation phase (Van et al., 2020).

### 2.3. Analysis Methods

The surface morphology, particle size, and elemental composition of the zeolite were examined using Scanning Electron Microscopy equipped with an Energy-Dispersive X-ray spectrometer (SEM-EDX, JEOL JSM 6510). Fourier Transform Infrared Spectroscopy (FTIR, Agilent CARY 630) was used to identify the functional groups present in the zeolite within a wavenumber range of 4000–600  $\text{cm}^{-1}$ . The surface area and pore characteristics were determined using the Brunauer–Emmett–Teller (BET) method (QUADRASORB-evo). The phase composition and crystal structure of the zeolite were analyzed using X-Ray Diffraction (XRD, Bruker D8 Advance).

The measurements of the substrate's protein, fat, carbohydrate, ash, moisture content, Total Solids (TS), Volatile Solids (VS), and Soluble Chemical Oxygen Demand (sCOD) are the main objectives of this research. TS refers to the total amount of solid material remaining in a sample after all moisture has been evaporated. TS were analyzed using the APHA 2540b method (Baird et al., 2017), determined by drying the sample in an oven (Merck Memmert, Germany) at 110°C for 1 hour. In contrast, VS represents the fraction of solids remaining after oven drying. VS were analyzed using the APHA 2540e method (Baird et al., 2017), which is subsequently ignited in a furnace (Thermo Scientific FB310M-33) at approximately 600°C for 3 hours. To determine sCOD, a digestion solution containing potassium dichromate (as the oxidizing agent) and sulfuric acid was added to a test tube containing the substrate or inoculum. The test tube was then tightly sealed and gently shaken until homogeneous, then digested in a sCOD reactor. The sCOD value was measured using a spectrophotometer (Hanna Instruments, USA) at 600 nm.

During the anaerobic digestion process, further analyses were conducted, including the measurement of sCOD, VFA concentrations, and biogas volume. VFA and sCOD concentrations were measured using the APHA 5560C method (Baird et al., 2017). Samples were collected 3 times a week. Each sample was first centrifuged using a centrifuge (Gemmy PLC, Taiwan) to separate the solid fraction, then distilled to isolate volatile fatty acids from other organic compounds, and finally titrated to determine the concentrations of VFA and sCOD.



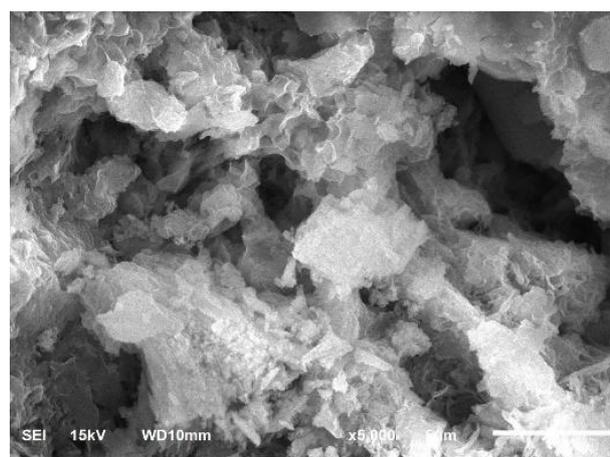
Volume of biogas collected every day and measured by a 60 mL syringe (One Med). Methane composition was analyzed periodically using Gas Chromatography (GC) (Shimadzu 2014, Japan) at representative steady-state conditions for each HRT. The analysis followed the procedures outlined in IKM 7.2.2.25 for  $\text{CH}_4$ , and IKM 7.2.2.27 for  $\text{CO}_2$ . Gas samples were injected into a GC system equipped with a Porapaq-type capillary column. The theoretical biogas production potential from tapioca wastewater was estimated using the empirical equation

presented in Equation (1). The empirical molecular formula employed in this estimation was derived from the proximate analysis data summarized in Table 1.

## 3. Results and Discussion

### 3.1. Characterization of Immobilization Media

The morphological structure of the activated natural zeolite in Figure 2 appears to have more cavities and large pore sizes. This structure is formed during alkaline activation with NaOH, which facilitates deagglomeration of the zeolite. After the NaOH treatment, the particles appear to become irregular (Ates, 2018; Morentera et al., 2022) Activation using NaOH on zeolite notably affects the overall surface, particularly the pore size and volume (Table 2), which are crucial for the immobilization of microorganisms in anaerobic processes. Based on the BET characterization results, the activated natural zeolite exhibited a surface area of 33.208  $\text{m}^2/\text{g}$ , a pore volume of 0.088  $\text{cc}/\text{g}$ , and a pore diameter ranging from 16 to 152 Å (0.0016–0.0152  $\mu\text{m}$ ). This structure provides more extensive surface area for microorganisms to adhere to the zeolite and allows greater contact with the substrate, thereby enhancing the degradation process (Cahyono et al., 2018).



**Figure 2.** Morphology Analysis of Alkaline Activated Zeolite at Magnifications of 5000x (b)

Alkaline activation treatment not only affects the surface area and pore size but also changes the crystallinity of the activated zeolite. This change in crystallinity was analysed using XRD. The peak intensity of ZAL is relatively low, reflecting a low crystallinity phase and a high content of amorphous silica. The crystallinity value obtained is 21%, indicating that the majority of ZAL consists of an amorphous structure where atoms or molecules are arranged randomly. XRD analysis shows that alkaline activation modifies the degree of crystallinity of natural zeolite while preserving its crystal structure.

Table 2 describes the results of FTIR analysis between natural zeolite and activated zeolite in the wavelength range of 4000 to 650  $\text{cm}^{-1}$ . Based on the study, the Si-O-Si functional group was observed in both zeolites. In natural

zeolite, this group was detected at 1013.8 cm<sup>-1</sup>, shifting to 998.9 cm<sup>-1</sup> after activation with NaOH. The observed spectral shift is attributed to dealumination and desilication processes occurring within the natural zeolite. This shift in wavenumber indicates a substantial decrease in water content, which in turn results in the displacement of vibrational bands toward higher frequencies (Ates & Hardacre, 2012),

### 3.2. Performance Test of Anaerobic Digestion

#### 3.2.1. sCOD removal

At the start of the process, as shown in Figure 3a, the substrate in the reactors had an initial sCOD concentration of 10,950 mg/L, which then increased significantly to a peak of 15,000 mg/L in both reactors. During this period, microorganisms undergo an adaptation phase, involving enzymatic adjustments and the selective development of microbial populations specifically suited for processing tapioca wastewater (Montalvo et al., 2020).

This condition can lead to a transient accumulation of sCOD, often due to the incomplete breakdown of organic compounds and the accumulation of intermediate metabolites such as VFAs (Kpata-Konan et al., 2020; Magdalena et al., 2019). Following the initial increase in sCOD, a notable reduction was observed on day 31 after the reactor conditions were adjusted to an HRT of 50 days and a feed rate of 50 mL/day. This change indicates that the microorganisms have effectively acclimatized to their environment. The decrease in sCOD during the anaerobic digestion occurs as complex organic materials are broken down into simpler compounds, primarily VFA and gases such as hydrogen (H<sub>2</sub>) and carbon dioxide (CO<sub>2</sub>) (Y. Huang et al., 2019).

during digestion, likely due to the relatively short HRT combined with a high OLR, which limited the microorganisms' ability to degrade organic matter efficiently. Yousefi *et al.* demonstrated that increasing the HRT substantially enhanced the sCOD removal efficiency in anaerobic reactors. Their findings highlighted that shorter HRTs were associated with reduced sCOD removal rates, underscoring the necessity of sufficient retention time to allow microbial communities to stabilize and efficiently degrade organic substances (Yousefi et al., 2018).

In addition to the short HRT, an OLR exceeding the microbial community's capacity can overwhelm the microorganisms, leading to incomplete degradation of organic matter. Elevated concentrations of organic matter may inhibit specific bioconversion pathways, particularly those mediated by methanogens, resulting in the accumulation of VFAs and an increase in sCOD. This accumulation occurs because microorganisms are unable to process the excess organic matter efficiently, leaving unutilized substrates in the system. Yang et al. reported that increasing the organic loading reduced the average sCOD removal rate, indicating that high organic loads can adversely affect microbial growth and activity. They also observed that elevated salinity and organic load levels further hindered microbial performance, resulting in decreased sCOD removal efficiency (Y. Yang et al., 2018).

#### 3.2.2. VFA produced

Total VFA was measured in this study, while individual volatile fatty acid species (e.g., acetate, propionate, and butyrate) were not separately quantified. Consequently, the discussion of VFA-related inhibition is based on total VFA accumulation rather than specific acid profiles. Reactor pH was not directly monitored during this study.

**Table 2.** Analysis Result of Zeolite Characterization

| Method | Parameter Measured    | Result  |  | Interpretation   |
|--------|-----------------------|---|--|--|
|        |                       | Natural ZAL   | Activated ZAL                                      |  |
| SEM    | Surface morphology    | More solid, agglomerative, small pores.               | Porous, irregular, rough surface                   | Indicates high surface area for microbial attachment.                  |
| EDS    | Elemental composition | Si: 28.61%<br>Al: 5.62%                               | Si: 28.89%<br>Al: 7.48%                            | Increase the Si and Al composition and lower the Si/Al ratio.          |
| BET    | Pore size and volume  | N/A   | 16-152 Å<br>0.088 cc/g                             | Consistent with mesoporous material                                    |
|        | Surface area          | N/A   | 33.208 m <sup>2</sup> /g                           | High surface area supports microbial immobilization.                   |
| XRD    | Crystalline structure | N/A   | 21% crystallinity,<br>79% amorphous                | The atoms or molecules are randomly arranged without a regular pattern |
| FTIR   | Functional groups     | Bands at 1013.8, 790.2, 1625.1, 3623 cm <sup>-1</sup> | Bands at 998.9, 790.2, 1640, 3623 cm <sup>-1</sup> | Asymmetric Si–O and Al–O. Shift due to dealumination/desilication.     |

The sCOD removal efficiency initially decreased and then increased again when the reactor conditions were altered to an HRT of 10 days with a feed rate of 150 mL/day. This fluctuation was attributed to microbial instability

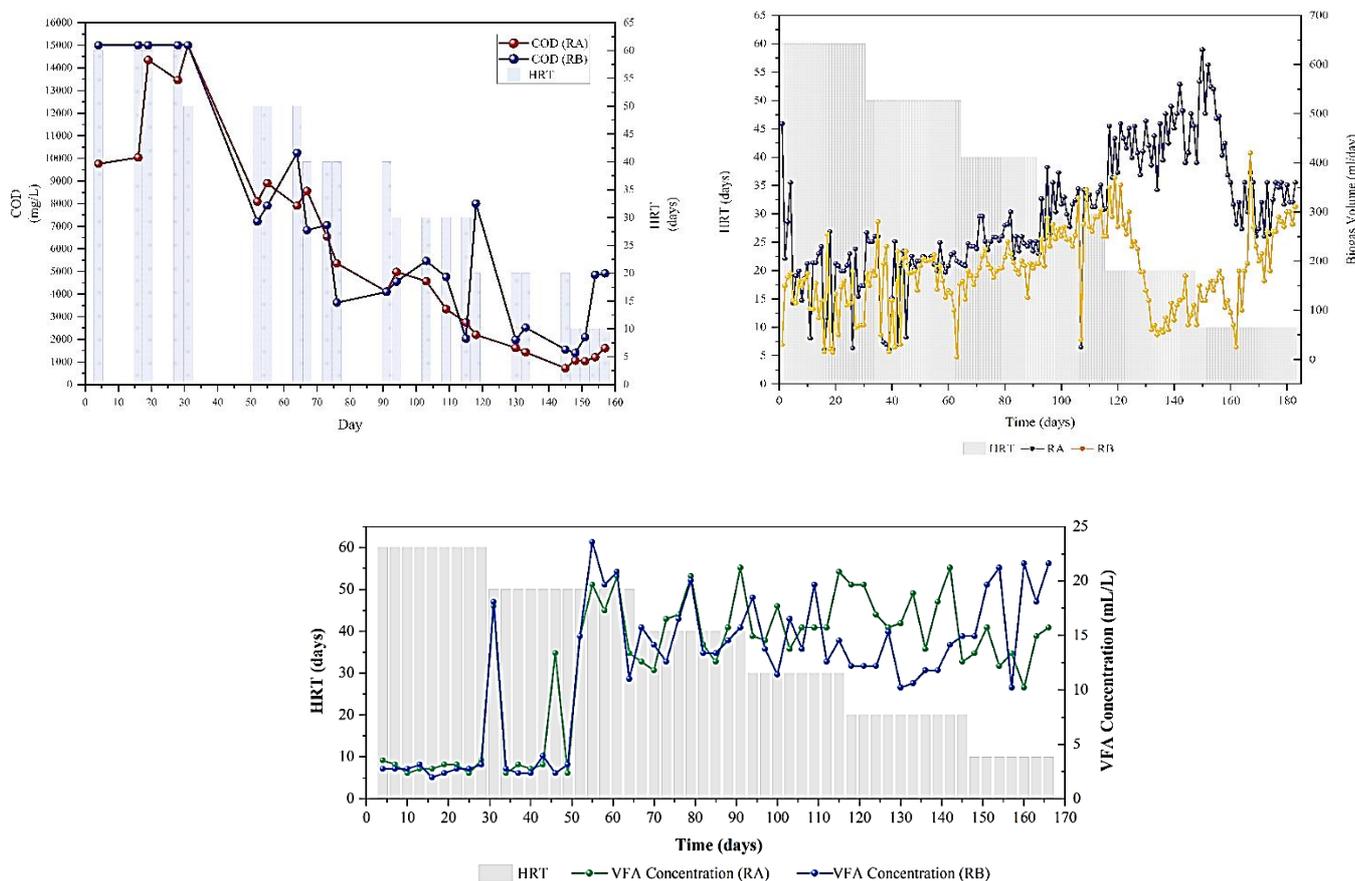
However, the observed accumulation of volatile fatty acids at short HRTs indicates a high acidogenic activity relative to methanogenic conversion. The dynamics of VFA changes are illustrated in Figure 3c. VFA concentrations

steadily increased when the HRT was decreased from 50 days to 20 days from day 28 to 142 in the RA bioreactor and from day 49 to 127 in the RB bioreactor. At this HRT, VFA concentrations notably increased along with a higher feed flow rate. The total VFA concentrations, along with methane production performance, indicate that the RA bioreactor achieved a higher methane production. Notable peaks were observed around day 60 and day 150, when VFA concentrations reached their maximum levels.

In the initial phase (0–30 days), methane production was very low, while VFA concentrations remained high. This observation is consistent with the sCOD measurements in Figure 3a, which show an increase in sCOD during the same period. During this phase, microorganisms had not yet adapted to the reactor conditions, preventing them from effectively degrading organic matter. In the final phase (days 120–166), under reactor conditions with a hydraulic retention time (HRT) of 10 days and a feed rate of 150 mL/day, VFA concentrations continued to fluctuate.

methane. In contrast, VFA levels in RB tended to increase, while biogas volume decreased, indicating VFA accumulation. This is further supported by Figure 3a, which shows a marked increase in sCOD levels in RB during this period.

The biogas production in RA was notably higher, attributed to the immobilization media, which helped maintain microbial stability (Ni et al., 2020). The incorporation of zeolite as a support structure in anaerobic digestion systems demonstrates substantial benefits in enhancing microbial performance (Montalvo et al., 2012). Zeolite enhances syntrophic interactions between microbial populations, which are crucial for the efficient degradation of VFAs into methane (Paritosh et al., 2020). In the context of short HRT, biofilms help retain microorganisms by preventing their washout due to strong fluid flow, thereby maintaining an active microbial population within the system (Baltrėnas et al., 2019).



**Figure 3.** Effect of hydraulic retention time on (a) sCOD removal efficiency, (b) biogas volume production, and (c) total VFA concentration

Nevertheless, methane yields in RA and RB differed notably. In RA, VFA concentrations tended to decrease, accompanied by an increase in biogas volume (Figure 3b), indicating that VFAs were successfully converted into

methane. In contrast, the RB system exhibited declining performance at an HRT of 10 days, leading to reduced methane production. This imbalance was primarily due to the shortened HRT, which limited the residence time

available for microbial degradation of the substrate (Wang et al., 2014). As a result, VFA degradation was hindered, leading to acid accumulation in the fermentor slurry, as microbial proliferation could not keep pace with the substrate loading rate (Park et al., 2019). Excessive VFA accumulation causes a drop in pH, which can inhibit the metabolic activity of methanogenic archaea. This condition disrupts the anaerobic process equilibrium, reduces methane production efficiency, and lowers the overall performance of the bioreactor (Wong et al., 2019).

### 3.2.3. Biogas production

Overall, the biogas produced by the two reactors exhibited substantial performance differences. The average data on the volume of biogas produced under each HRT variation and six feed flow rates: 25, 30, 37.5, 50, 75, and 150 ml/day, are shown in Table 3.

**Table 3.** Average volume of biogas at various HRT

| HRT (days) | Total biogas (mL) |                      |
|------------|-------------------|----------------------|
|            | Immobilized (RA)  | Non-immobilized (RB) |
| 60         | 178.83            | 114.7                |
| 50         | 177.7             | 155.94               |
| 40         | 241.33            | 184.18               |
| 30         | 304.95            | 253.33               |
| 20         | 443.78            | 170.5                |
| 10         | 520               | 141.0                |

Figure 3b displays a graph illustrating the relationship between HRT and biogas production. Biogas production increased with higher flow rate and tended to stabilize at HRTs of 40 days with 37.5 ml/day and 30 days with 50 ml/day. This aligns with previous research (Zhao et al., 2020). Indicating that fixed-bed reactors perform well under high-flow-rate conditions. However, there is a difference between RA and RB in biogas volume, with RA producing more. This is due to the addition of immobilization media in RA, which is more effective in enhancing biogas production (Ayu et al., 2017).

In addition to having lower production than RA, RB experienced a decrease in biogas production on the 116th day, when the flow rate was increased to 75 ml/day. Although the feedstock supply is proven to increase biogas and methane yields, on the other hand, it would lead to severe process imbalance if the proportion of degradable carbohydrates exceeds the acid degradation capacity limit inside the reactor (Terboven et al., 2017). This decline in productivity occurred because the number of microorganisms in the system was insufficient to digest the provided organic material, and mass-transfer resistance increased as the flow rate increased. This situation arises during propionate-type fermentation, which controls anaerobic digestion at a higher flow rate. Propionic acid is not easily digested by methanogens, leading to increased system resistance and hindered biogas production due to the accumulation of intermediate products (Liu et al., 2018).

The lowest biogas production occurred at an HRT of 50 days with a flow rate of 25 ml/day. Following this, the HRT increased to 30 days with a flow rate of 50 ml/day, resulting in increased stability in reactor production up to the final variation in HRT, which was 10 days with a loading rate of 150 ml/day. The graph shows that the highest biogas volume produced by RA was at an HRT of 10 days, reaching 520 mL. However, after reaching this peak, biogas production performance declined on the subsequent day. This decline was caused by the short HRT, which led to process instability because the microorganisms did not have sufficient time to proliferate and effectively digest the substrate.

This observation is supported by the study conducted by (Parajuli et al., 2022), which demonstrated that reducing HRT while increasing flow rate can lead to substantial decreases in methane production and process stability. As HRT decreases, biogas production becomes unstable, and VFA accumulate, disrupting the biochemical balance in the anaerobic digester. In reactor B, the highest volume was produced on the first day, at an HRT of 20 days and a loading rate of 75 ml/day. Similar to RA, after reaching the peak biogas volume, productivity performance declined on the following day. This indicates that the operating conditions in RB were no longer stable, likely due to the excessively long HRT and the increased flow rate. With excessive HRT, the substrate remains in the reactor for too long, leading to the formation of byproducts, such as VFAs and ammonia nitrogen (Ali et al., 2023).

### 3.2.4. Methane concentration of biogas

The methane concentration measured in this study was relatively low, with maximum values of 14.65% in reactor RA and 2.33% in reactor RB. These values are substantially lower than those typically reported for fully stabilized anaerobic digestion systems treating starch-rich wastewater. This indicates that the anaerobic process did not reach complete methanogenic stabilization under the applied operational conditions. The comparison of methane concentrations between the RA and RB reactors is presented in Table 4, further highlighting the immobilized media's effectiveness in promoting methanogenesis and improving overall biogas production.

**Table 4.** Theoretical and actual methane concentration

| HRT (day) | Theoretical Volume (mL/g Dry substrate) | Actual (mL/g Dry Waste) |       |
|-----------|---|-------------------------|-------|
|           |   | RA                      | RB    |
| 60        | 498.08                                  | 195.33                  | 25.33 |
| 50        | 498.08                                  | 217.03                  | 32.74 |
| 40        | 498.08                                  | 271.25                  | 32.07 |
| 30        | 498.08                                  | 351.55                  | 43.48 |
| 20        | 498.08                                  | 458.96                  | 30.66 |
| 10        | 498.08                                  | 341.77                  | 12.07 |

The results indicate that at an HRT of 20 days, the process using immobilized media yielded the highest biogas

production, approximately 458.963 mL/g. In contrast, the non-immobilized media process produced a much lower biogas volume, measuring only 43.481 mL/g at an HRT of 30 days. This stark contrast in biogas yields underscores the substantial enhancement in methane production achieved by utilizing immobilized media. The data strongly support the superior efficiency of the immobilized system, highlighting its potential to notably improve the overall performance of anaerobic digestion processes.

The integration of zeolite as a support structure in anaerobic digestion systems notably enhances microbial performance by providing a high surface area that promotes microbial colonization and stabilizes community dynamics. This environment is particularly advantageous for methanogenic archaea, such as *Methanosaeta*, which exhibit higher abundance and activity when immobilized on zeolite compared to suspension in the feedstock. Additionally, zeolite facilitates syntrophic interactions among microbial populations, improving the conversion of VFAs into methane. By creating a stable microhabitat, zeolite not only supports the metabolic activity of methanogens but also enhances the overall efficiency and robustness of the anaerobic digestion process (Ciezkowska et al., 2020; Paritosh et al., 2020).

### 3.3. Carbon Mass Balance and Stoichiometric Interpretation

Although the sCOD removal efficiency reached approximately 93%, the measured methane concentration remained relatively low, with maximum values of 14.65% in reactor RA and 2.33% in reactor RB. Based on anaerobic digestion stoichiometry, 1 g COD theoretically yields about 0.35% L CH<sub>4</sub> at standard condition, corresponding to a predicted methane potential of 498.08 mLCH<sub>4</sub>.g<sup>-1</sup> dry substrate (Table 4). However, the actual methane production was significantly lower, particularly in reactor RB, indicating that only a fraction of the removed carbon was converted into methane. This discrepancy suggests that a substantial portion of the consumed sCOD was diverted toward alternative carbon sinks, including microbial biomass formation and carbon dioxide generation during acidogenic and acetogenic metabolism (Pu et al., 2026; Schuchmann & Müller, 2016).

The low methane fraction further indicates that the anaerobic process did not reach complete methanogenic stabilization and was likely constrained at the acidogenic or acetogenic phase. This limitation was especially evident in the non-immobilized reactor (RB), where methane recovery remained below 10% of the theoretical potential across all HRTs. In contrast, reactor RA showed markedly higher methane conversion efficiency, achieving up to 458.96 mL.g<sup>-1</sup> dry waste at an HRT 20 days, highlighting the role of immobilized media in enhancing microbial retention and syntrophic interaction. These results confirm that high sCOD removal alone is insufficient to ensure efficient methane recovery and emphasize the importance of reactor configuration in directing carbon flux toward

methanogenesis rather than biomass accumulation or intermediate product (Tor et al., 2023).

## 4. Conclusions

The addition of zeolite as an immobilization medium notably improved sCOD removal efficiency compared to the system without immobilization. Furthermore, the zeolite positively influenced the conversion of Volatile Fatty Acids (VFA) to methane, as methanogenic microorganisms exhibited greater stability and efficiency in converting VFA to methane gas than in the non-immobilized reactor. The total volume of biogas produced also showed a notable increase, with the reactor containing zeolite generating 458.963 mL/g dry substrate, substantially higher than the 43.481 mL/g dry substrate produced in the non-immobilized reactor. Additionally, variations in zeolite immobilization media influenced the methane content of the biogas, with a higher methane concentration observed than in the system without immobilization. These findings confirm that zeolite creates a more stable environment for anaerobic microbial communities, enhances sCOD degradation efficiency, accelerates VFA conversion to methane, and improves total biogas production and methane concentration in biogas. Therefore, the use of activated Lampung natural zeolite presents substantial potential for wastewater treatment in the tapioca industry as a more efficient, sustainable, and environmentally friendly technology.

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## Statement

During the preparation of this manuscript, the author utilized ChatGPT version 3.5 and Grammarly to enhance the English language and assist with proofreading. All content was subsequently reviewed and revised by the authors, who take full responsibility for the published version.

## CRedit authorship contribution statement

**Andri Sanjaya:** Conceptualization and Writing – original draft. **Jabir Shoji Arhab:** Resources, Visualization, and Software. **Dwinda Salsabila:** Resources, Formal analysis and Visualization. **Damayanti Damayanti:** Validation and Funding acquisition. **Yunita Fahni:** Formal analysis, Data curation, and Data curation. **Deviany Deviany:** Writing – original draft, Validation, and Methodology. **Wika Atro Auriyani and Putri Agustriyani:** Writing – original draft and Visualization. **Desi Riana Saputri:** Funding acquisition, Writing – review & editing, and Project administration

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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