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# Biogas generation from anaerobic co-digestion of tofu liquid waste and tapioca flour liquid waste at various initial pHs

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## ABSTRACT

Tofu liquid waste (TLW) and tapioca flour liquid waste (TFLW) are the two abundant wastewaters in Indonesia. The current research had a goal to examine the influence of the mixing ratio of TLW:TFLW=0:100, 50:50, 100:0v/v, and initial pHs of 5–8 on biogas production from TLW and TFLW. At an initial pH of 7, the TLW:TFLW ratios of 0:100, 50:50, and 100:0 resulted in biogas yields of 143.99, 341.13, and 93.00 mL/g-$COD_{added}$, respectively. It means that anaerobic co-digestion (AcoD) generated a higher biogas yield than anaerobic mono-digestion (AmoD). In addition, the biogas from AcoD contained a higher methane content (44.1–48.1%) than that from AmoD of TLW or TFLW. Furthermore, AcoD of TLW:TFLW=50:50 at initial pHs of 5, 6, 7, and 8 resulted in biogas yields of 187.76, 296.02, 341.13, and 165.21 mL/g-$COD_{added}$, respectively. Hence, an initial pH of 7 provided suitable conditions for microbes so biogas could be generated in a large amount. Initial pHs of 7 and 8 generated biogas with standard methane content (44.1–55.5%). The highest COD removal (55%) was achieved at AcoD (50:50) with an initial pH of 7. Therefore, the best condition was AcoD with an initial pH of 7. Based on the kinetic analysis, AcoD (50:50) with an initial pH of 7 had the highest biogas potential and the lowest adaptation time. The findings of the current study supported the hypothesis that the AcoD generated higher biogas yield and methane content than the AmoD, as well as the initial pH of 7 generated the highest biogas yield.

**Keywords:** biogas, co-digestion, methane, tapioca flour liquid waste, tofu liquid waste.

## INTRODUCTION

Biogas is one of the new and renewable energies (NREs) (Thuan et al., 2023). It can be used directly or converted into other forms such as electrical and heat energies. In Indonesia, the biogas generation process has been developed continuously because the Indonesian government has a goal to substitute fossil energy with NREs. In 2050, equal to or above 31% of the national energy need can be fulfilled by the NREs (Syaichurrozi et al., 2024b). On the other hand, in 2023, NREs only met around 13.29% of the national energy needs (Ministry of Energy and Mineral Resource Republic of Indonesia, 2024). Therefore, studies on renewable energy production should be conducted continuously until the target can be reached.

Biogas can result from the anaerobic digestion (AD) of several organic wastes (Budiyono et al., 2018a, 2018c, 2018b, 2017, 2014a, 2013, 2021a, 2019; Budiyono and Kusworo, 2011; Hadiyarto et al., 2015; Karemdabeh et al., 2025; Kavisa et al., 2020; Kusworo and Budiyono, 2012; Nugraha et al., 2020; Shitophyta et al., 2015; Sumardiono et al., 2022, 2018, 2017, 2015, 2013; Syafrudin et al., 2018, 2017, 2020; Syaichurrozi et al., 2015). Some abundant wastes in Indonesia are tofu liquid waste (TLW) (Budiyono et al., 2021b; Rahayu et al., 2018b, 2018a, 2015) and tapioca flour liquid waste (TFLW) (Budiyono et al., 2018b; Sunarso et al., 2010). The AD technology has several advantages, namely requiring a short residence time, producing high biodegradability, and producing methane which is a renewable energy (Syaichurrozi et al., 2024b). In addition, the effluent (digestate) of the AD process can be utilized as liquid organic fertilizer (Kowalczyk-Juśko et al., 2023; Ningsih et al., 2024). The TLW is abundant and contains a high organic matter. Commonly, the tofu industry will produce 32.6 tons of TLW for every ton of tofu produced. Every year, 2.56 million tons of tofu are produced in Indonesia. The chemical oxygen demand (COD) levels in TLW are quite high, ranging from 5,000 to 8,640 mg-$O_2$/L (Budiyono and Syaichurrozi, 2020). Meanwhile, the tapioca flour industry generates about 35.5 $m^3$ of TFLW to produce 1 ton of tapioca flour. Around 15–16 million tons of tapioca flour are produced each year. The COD levels in the TFLW range from 7,000 to 30,000 mg-$O_2$/L (Setyawaty et al., 2011). Due to their high COD levels, the two wastes are forbidden to be discharged directly to the environment. Through the AD process, the COD will be converted to biogas.

The ratio of COD to nitrogen (COD/N) is an essential affecting factor in the AD process. Substrates with too high COD/N easily generate volatile fatty acids (VFAs) which can drop liquid pH and disturb the methanogenesis stage. Meanwhile, substrates with too low COD/N easily generate ammonia/ammonium which can be toxic in a high concentration (Syaichurrozi et al., 2016). The COD/N ratio in the TLW and TFLW is about 42.7 (Syaichurrozi et al., 2016) and 96 (Neves et al., 2016), respectively. The suggested COD/N ratio in the biogas feedstock is about 71.4–85.7 (Syaichurrozi et al., 2013). Therefore, the two wastes can be mixed to get feedstock with a suitable COD/N ratio. It is called AcoD (Syaichurrozi et al., 2016). This concept is very interesting because it can treat two wastes at once, produces more biogas, and has less operating cost (Aljbour et al., 2021; Amelia et al., 2024; Korai et al., 2024; Lebiocka et al., 2019; Syaichurrozi et al., 2016; Szaja et al., 2022).

In addition, initial pH is an important factor in the AD process (Syaichurrozi et al., 2020, 2018). Initial pH is correlated with bacterial adaption time and bacterial cell acid-base equilibrium, both of which impact the rate of biogas production (Syaichurrozi et al., 2018). According to Metcalf (Metcalf and Eddy Inc. et al., 2003), Anderson and Yang (Anderson and Yang, 1992), and Speece (Speece, 2012), the optimal pH range for AD is 6.9–7.3, 6.4–7.6, and 6.5–8.2, respectively. The rate of biogas generation is poor if the pH level is either below or above the ideal range.

Previous studies conducted the AcoD to treat various waste, including TLW – vinasse waste (Syaichurrozi et al., 2016), TLW – rice straw (Budiyono et al., 2021b), TLW – cow manure (Sudarto et al., 2023), TLW – water hyacinth – cow manure (Sa'Diah and Putra, 2019), garden waste – food waste – tofu residue (Song et al., 2021), TLW – noodle waste – wingko waste (Suwandhi et al., 2024), microalgae (*Anabaena sp.* and *Chlorella sp.*) – manure (Alharbi, 2024), empty fruit bunch – decanter cake (Chanthong et al., 2024). Based on the information, the AcoD of TLW and TFLW has not been investigated by other researchers yet. Therefore, the main goal of the current research was to examine the impact of mixing ratio and initial pH on biogas generation from the AcoD of TLW and TFLW. This study hypothesizes that the AcoD of TLW and TFLW generates a higher biogas generation and methane percentage than the anaerobic mono-digestion (AmoD) of either TLW or TFLW. In addition, the initial pH of 7 is predicted the most suitable.

## METHODS

### Materials

The TLW was obtained from a small- and medium-sized tofu enterprise in Banten Province, Indonesia. The TFLW was collected from the settling process in the household scale tapioca flour production. The cow rumen fluid was utilized as inoculum. It was obtained from a slaughterhouse in Cilegon-Banten, Indonesia. The chemical characteristics of TLW, TFLW, and inoculum are displayed in Table 1.

### Experimental set-up

Digesters were created using modified Erlenmeyer flasks (Duran, Germany) with a 600 mL total volume. Via flexible plastic tubing, each digester was attached to a 100 mL reverse-measuring glass. There was saturated-salt liquid in the reverse-measuring glass. Figure 1 shows a comprehensive laboratory-scale experimental setup.

**Table 1.** Characteristics of TLW, TFLW, and inoculum

| Parameters | Unit | TLW | TFLW | Inoculum |
|---|---|---|---|---|
| pH | - | 3.51 | 3.59 | 6.68 |
| Total solid (TS) | mg-dry matter/L | 6500 | 12,500 | 10,000 |
| Total suspended solid (TSS) | mg-dry matter/L | 750 | 1000 | 1750 |
| Total dissolved solid (TDS) | mg-dry matter/L | 5750 | 11,500 | 8250 |
| COD | mg-$O_2$/L | 3113 | 3153 | 4909 |
| Volatile fatty acids (VFAs) | mg-acetic acid/L | 1466.6 | 1704.2 | 2059.3 |
| COD/N | - | 42.7[1)] | 96[2)] | - |

**Note:** [1)] (Syaichurrozi et al., 2016) and [2)] (Neves et al., 2016).

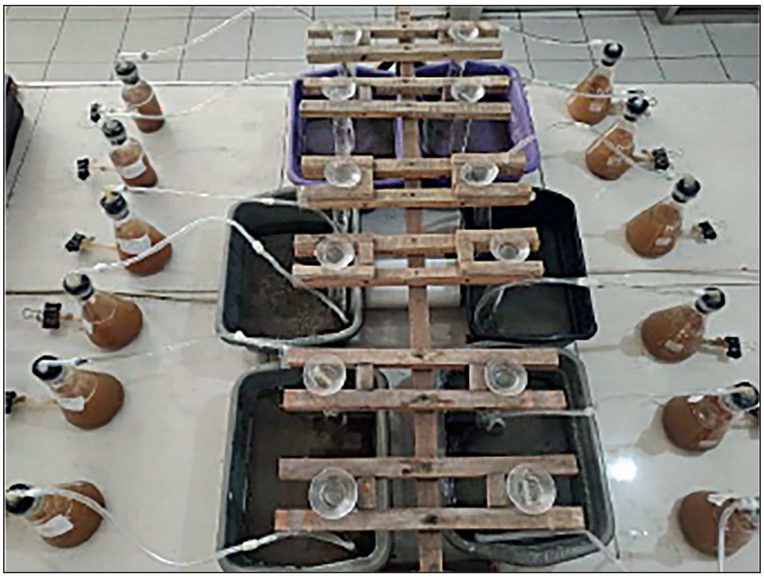

**Figure 1.** Experimental set-up

## Experimental design and procedures

The TLW and TFLW were mixed with mixing ratios of 0:100, 50:50, and 100:0 v/v with a total volume of 400 mL. The inoculum was added to the mixed substrate with a substrate:inoculum (S:I) ratio of 80:20 v/v (Paes et al., 2020). Then, the liquid pH was set to 5.0±0.1, 6.0±0.1, 7.0±0.1, and 8.0±0.1 with 5 N NaOH addition. The digesters were operated with a working volume of 500 mL in batch system under room conditions (25–30 °C, 1 atm). The resulting daily biogas volume was quantified using a liquid (saturated salt solution) displacement method. Meanwhile, the liquid (±10 mL) and biogas (±5 mL) were sampled per 4 days. The process of digestion continued until no more biogas was produced. The detailed experimental design can be seen in Table 2.

**Table 2.** Experimental design

| Run | Mixing ratio (v/v) | Initial pH |
|---|---|---|
| 1 | 50:50 | 5.0±0.1 |
| 2 | 50:50 | 6.0±0.1 |
| 3 | 50:50 | 7.0±0.1 |
| 4 | 50:50 | 8.0±0.1 |
| 5 | 0:100 | 7.0±0.1 |
| 6 | 100:0 | 7.0±0.1 |

## Analyses

### *Level of pH*

A digital pH meter with a Pen Type PH-009 model was utilized to measure the levels of pH in liquids (Syaichurrozi et al., 2024a).

### *Concentrations of TS, TSS, and TDS*

To determine TS, TSS, and TDS, this study followed procedures used by Syaichurrozi et al. (Syaichurrozi et al., 2024a) with a little modification by using Whatman 41 in determining TSS.

*Concentrations of COD and VFAs*

This study used a method of closed reflux-spectrophotometry to measure COD content in the liquid samples (Khomariah et al., 2024). The COD reactor with the specification of Hanna instrument HI839800 and the COD spectrophotometer with the specification of Hanna instrument-HI83399) were used. This study used a steam distillation method to measure VFAs (Khomariah et al., 2024; Syaichurrozi et al., 2024a).

*Methane content*

Methane percentage in the resulting biogas was analyzed using gas chromatography-thermal conductivity detection (GC-TCD, Shimadzu GC 8A, Japan). In this analysis, the $CH_4$ and $CO_2$ percentages were obtained. The presence of air in the headspace might affect the results of the GC analysis. Therefore, the methane content was recalculated using Equation 1.

$$\%CH_4 = \frac{\%CH_{4,GC}}{\%CH_{4,GC}+\%CO_{2,GC}} \times 100\% \quad (1)$$

## Calculations

*Biogas yield*

The volume of biogas was measured via the liquid (saturated salt solution) displacement method, which was in a unit of mL. Then, the biogas yield (mL/g-$COD_{added}$) was quantified by dividing the biogas volume (mL) by the initial COD in the substrate (mixture of TLW and TFLW). This calculation adapts to the calculation in prior research (Belibagli et al., 2024; Mekwichai et al., 2024; Syaichurrozi et al., 2024a, 2013; Vaez and Zilouei, 2020).

*COD removal efficiency*

The COD removal efficiency shows the amount of COD that can be degraded to become biogas. In this study, this parameter was calculated through Equation 2 (Syaichurrozi et al., 2024a).

$$COD\ removal\ (\%) = \frac{COD_{initial} - COD_{final}}{COD_{initial}} \times 100\% \quad (2)$$

## Kinetics

The study also conducted a kinetic analysis using the modified Gompertz model (Lihi et al., 2023; Sumardiono et al., 2021; Syaichurrozi et al., 2024a). The authors chose this kinetic model because it can result in more accurate predictions than the other models. (Syaichurrozi et al., 2023) declared that the modified Gompertz model resulted in a higher determination coefficient value ($R^2$) than the modified Logistic model and the modified first-order kinetic model. Then, another study (Syaichurrozi et al., 2024b) stated that the modified Gompertz model gave a more accurate prediction ($R^2$ = 0.9938) than the first-order model ($R^2$ = 0.9449) and the Cone model ($R^2$ = 0.9923). The modified Gompertz model had better prediction results than the Transference and Logistic models (Alharbi and Alkathami, 2024). The kinetic constants of the model can be used to understand more the impact of the mixing ratio and initial pH on biogas generation. The formula of the model is presented in Equation 3. Optimization was conducted using Microsoft Excel software with the objective function of Mean Absolute Percentage Error (MAPE) (Equation 4).

$$y(t) = ym.exp\left\{-exp\left[\frac{\mu.e}{ym}(\lambda - t) + 1\right]\right\} \quad (3)$$

$$MAPE = \frac{1}{n} \times \sum\left(\left[\frac{y-\hat{y}}{y}\right] \times 100\%\right) \quad (4)$$

where: $y(t)$ is the cumulative biogas yield at $t$ days (mL/g-$COD_{added}$), ym is the maximum biogas yield that can be reached (mL/g-$COD_{added}$), $\mu$ is the rate of biogas production (mL/g-$COD_{added}$/day), $\lambda$ is the lag time (days), $e$ is exp (1) or 2.718282, $t$ is the operating time (days), $R^2$ is the coefficient of determination, $\hat{y}$ is the modeled data, $y$ is the experimental data.

# RESULTS AND DISCUSSION

## Biogas production

*Comparison between co-digestion and mono-digestion*

In this study, the mixing ratio of TLW:TFLW was varied to 0:100, 50:50, and 100:0 (v/v) with an initial pH of 7. Daily and cumulative biogas yields are displayed in Figures 2 (A) and (B) respectively. Mixing ratios of 0:100, 50:50, and 100:0 resulted in peaks of daily biogas yields of 99.74, 140.96, and 43.14 mL/g-$COD_{added}$ achieved at days 1, 1, and 3, respectively. The more the TFLW fraction in the substrate, the lower the peak daily yield and the longer the peak time was achieved. The TLW and TFLW have a $BOD_5$/COD ratio of 0.526–0.759 (Hardyanti et al., 2023) and 0.458

(Thanwised et al., 2012), respectively. Because $BOD_5$/COD ratio in TLW was higher than that in TFLW, the former was easier to degrade. Therefore, the conversion rate of organic compounds to biogas decreased, thereby resulting in a lower peak daily biogas yield and longer peak time.

The TLW:TFLW mixing ratios of 0:100, 50:50, and 100:0 (v/v) generated total biogas yields of 143.99, 341.13, and 93 mL/g-$COD_{added}$, respectively. AcoD resulted in a higher biogas yield than AmoD. According to Table 1, the substrate at mixing ratios of 0:100, 50:50, and 100:0 contained COD/N ratios of 96, 59.3, and 42.7, respectively. It means that a COD/N ratio of 59.3 provided more suitable conditions for microbes than COD/N ratios of 96 and 42.7. In line with this study, a prior study revealed that the optimal COD/N in AcoD of vinasse waste and tofu-processing wastewater was 414/7 (or 59.1) obtained using the Ratkowsky model (Syaichurrozi et al., 2016). However, another study (Syaichurrozi et al., 2013) revealed that the optimal COD/N in AmoD of vinasse with urea addition was 600/7 (or 85.7). Then, another study (Dupont et al., 2023) found that the optimal COD/N in AcoD of swine manure and cassava bagasse was 29.4. The variations in the optimal COD/N could be caused by the varied characteristics of the inoculums and substrates.

### *Effect of initial pH*

In this section, AcoD with TLW:TFLW of 50:50 at initial pHs of 5, 6, 7, and 8 was conducted. Initial pH 5, 6, 7, and 8 resulted in peaks of daily biogas yields of 112.77, 112.77, 140.96, and 82.32 mL/g-$COD_{added}$ achieved at days 3, 1, 1, and 1, respectively (Fig. 2a). It means that microbes can adapt easily to the substrate at an initial pH of 7. Initial pH 5, 6, 7, and 8 resulted in total biogas yields of 187.76, 296.02, 341.13, and 165.21 mL/g-$COD_{added}$ (Fig. 2b). Thus, an initial pH of 7 obtained the most total biogas yield of all initial pHs.

The fraction of dissociated acids (DA) and non-associated acids (NDA) was influenced by pH. The NDA proportion in the system increases with decreasing pH. The microbial protein can be denatured by the NDA after it enters the cell. The microbial cell's pH is within the neutral range. Therefore, NDA penetration may upset the cell's acid-base balance (Syaichurrozi et al., 2020). Rumen liquid was employed in this investigation. The bacteria were placed in a different environment when the rumen fluid was added to the digester. Therefore, the microorganism can readily adjust to the system if the initial pH is in the neutral range (Budiyono et al., 2014b) which is correlated with the acid-base equilibrium of bacterial cells (Syaichurrozi et al., 2020).

This study found that the initial pH of 7 was the optimal condition in the AcoD of TLW and TFLW. This finding was similar to that in some previous studies. Zhang et al. (Zhang et al., 2015) revealed that the optimal initial pH was 6.81, generating 146.32 mL-biogas/g-VS. Then, in the AcoD of kitchen waste and cow manure, the optimal initial pH was 7.5, resulting in 8579 mL-methane (Zhai et al., 2015). However, a previous study (Syaichurrozi et al., 2018) found a higher optimal initial pH in the AcoD of *Salvinia molesta* and rice straw, namely 8 with a total biogas yield of 61.38 mL/g-TS. The different findings might be caused by the substrate and inoculum characteristics.

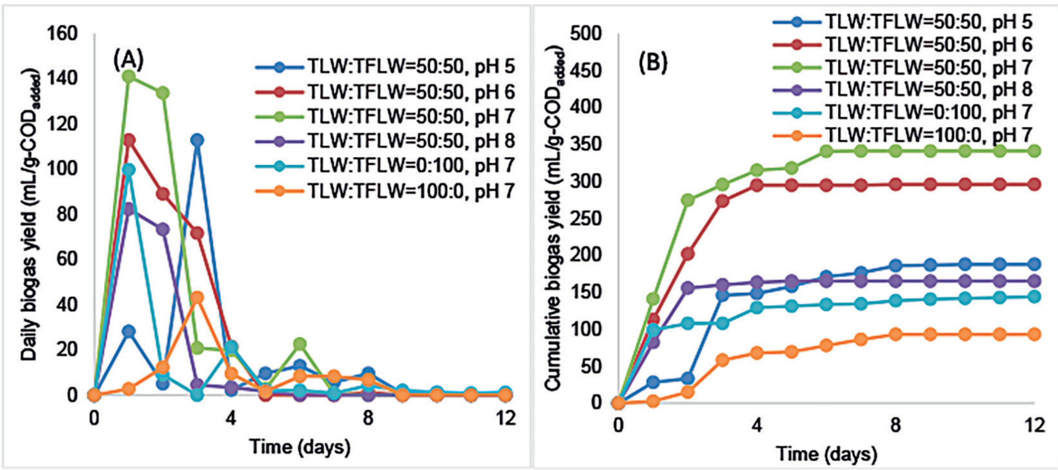


**Figure 2.** Biogas production (a) daily and (b) cumulative

## pH and VFAs

### *Comparison between co-digestion and mono-digestion*

Figure 3(A) displays the pH profiles during the experiment. At initial pH 7 and mixing ratios of TLW:TFLW of 0:100, 50:50, and 100:0 v/v, the pH changed from 7 to 5.27, 5.65, and 6.25, respectively. The substrate at mixing ratios of 0:100, 50:50, and 100:0 contained COD/N ratios of 96, 59.3, and 42.7. Thus, the lower the COD/N, the smaller the drop in pH. At TLW:TFLW ratios of 50:50 and 100:0, the pH values were still at an advantageous level for methanogenic microbes. In the opposite, the pH value at the TLW:TFLW of 0:100 was at a disadvantageous level for methanogenic microbes. Low pH can disrupt the delicate biochemical balance between acidogenic and methanogenic microbes, which is essential for the AD stability (Rajagopal et al., 2013). Methanogenic bacteria were more vulnerable to low pH than acidogenic bacteria. Methanogen metabolic activity would be considerably reduced at a low pH of ≤ 5.5 (Han et al., 2019). In the TLW:TFLW ratio of 0:100v/v, the microbes produced low biogas yield as a result of their poor growth. The current study's results were in line with those of a prior study (Syaichurrozi et al., 2023) stating that a pH condition of 5.7–7.4 was the optimal range for methane generation.

The decrease in pH was due to the generation of VFAs and total ammonia nitrogen. The profiles of VFAs during the AD are displayed in Figure 3(B). The final VFAs at the TLW:TFLW ratios of 0:100, 50:50, and 100:0v/v were 14,959, 7101, and 8521 mg-acetic acid/L, respectively. According to Karthikeyan and Visvanathan (Karthikeyan and Visvanathan, 2012), during the dry anaerobic digestion of biowaste, > 8 g/L VFAs had a detrimental effect on the entire methanogenesis process. Hence, the VFAs level in TLW:TFLW ratio of 50:50 was still at an advantageous level, so it generated a higher biogas yield than the AmoD of TLW or TFLW. VFAs can enter microbial cells and denature the protein therein, which can prevent the AD process by interfering with the methyl coenzyme M (CoM) reductase's activity (Deublein and Steinhauser, 2008). Methyl CoM reductase can make methane from methyl CoM via three distinct methane metabolic processes. These pathways of methane synthesis may be disrupted by a high concentration of VFAs (Xu et al., 2014).

### *Effect of initial pH*

The AcoD of TLW:TFLW=50:50 with initial pH of 5, 6, 7, and 8 experienced a change of pH from 5 to 5.16, 6 to 5.54, 7 to 5.65, and 8 to 5.81, respectively (Fig. 3a). As explained before, methanogen metabolic activity would be considerably reduced at a low pH of ≤ 5.5 (Han et al., 2019). Therefore, AcoD with initial pH of 5 and 6 was not recommended because the final liquid pH was ≤ 5.5. As a consequence, they generated a lower biogas yield than AcoD with an initial pH of 7. However, the initial pH of 8 generated a lower biogas yield than the initial pH of 7. The microbes can readily adjust to the system if the initial pH is in the neutral range (Budiyono et al., 2014b). It is associated with the bacterial cells' acid-base balance (Syaichurrozi et al., 2020). The initial pH of 7 was the most suitable in the current research. Figure 3b displays the VFA profiles during the experiment. The final VFA concentration at initial pHs of 5, 6, 7, and 8 was 8521, 8213, 7101, and 7186 mg-acetic

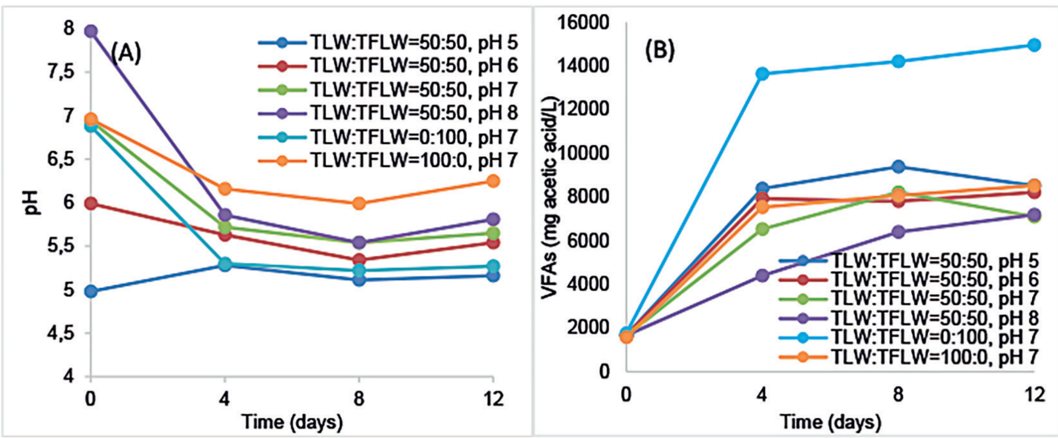


**Figure 3.** (a) pH profiles and (b) VFA profiles

acid/L. The VFAs of > 8 g/L had a negative impact on the entire methanogenesis process (Karthikeyan and Visvanathan, 2012). Hence, initial pHs of 5 and 6 generated a lower biogas yield than initial pH of 7. Furthermore, the initial pH of 8 also generated a lower biogas yield than the initial pH of 7 although it has good VFA concentration. It might be caused by the acid-base equilibrium of bacterial cells. The microbes could thrive in conditions with an initial pH of 7 rather than an initial pH of 8.

## Methane content

*Comparison between co-digestion and mono-digestion*

In this study, the biogas at days 4 and 8 were sampled and analyzed for methane content (%). The methane contents are presented in Table 3. According to Table 3, at an initial pH of 7, AcoD of TLW and TFLW (50:50) generated biogas with a higher quality than AmoD of TLW or TFLW. Biogas generated from AcoD had a higher methane content (44.1–48.1%). The AmoD of TLW (TLW:TFLW=100:0) resulted in biogas with a methane content of 14.7–34%. The AmoD of TFLW (TLW:TFLW=0:100) resulted in biogas with a methane content of 7–7.8%. It means that the methane content was impacted by the COD/N ratios. The substrate at mixing ratios of 0:100, 50:50, and 100:0 contained COD/N ratios of 96, 59.3, and 42.7. The TLW:TFLW of 0:100 or 100:0 contained too high or too low COD/N so the methane content was low. A prior study (Syaichurrozi et al., 2018) also revealed that a substrate with too high carbon generated biogas with low methane content (around 29%).

*Effect of initial pH*

The AcoD of TLW:TFLW=50:50 with initial pHs of 5, 6, 7, and 8 generated biogas with various methane content. Based on Table 3, AcoD at initial pHs of 5 and 6 generated low methane contents. These results were in line with an earlier study. Syaichurrozi et al. (Syaichurrozi et al., 2020) reported that a pH value of 5.0–5.7 throughout the anaerobic digestion process generated biogas with a very low methane content (6.6%). On the other hand, initial pHs of 7 and 8 resulted in higher methane content. Commonly, biogas contains 40–60% methane (Noori et al., 2020). Hence, the initial pHs of 7 and 8 resulted in biogas with standard methane content.

## Chemical oxygen demand

During the AD process, the organic matter was converted to biogas. The measurements of influent COD, effluent COD, and COD removal are shown in Figure 4. The AcoD of TLW:TFLW=50:50 at initial pHs of 5, 6, 7, and 8 generated COD removals of 36, 15, 55, and 54%. It shows that AcoD at an initial pH of 7 can degrade the highest COD concentration. AcoD at an initial pH of 7 generated the highest COD removal and the highest biogas yield. A prior study (Syaichurrozi et al., 2023) reported the same finding that an operating condition resulting in the highest biogas production gave the highest COD removal in the AD of distillery wastewater.

## Kinetic analysis

*Comparison between co-digestion and mono-digestion*

The fitting results on the experimental data using the modified Gomperz model for TLW:TFLW ratios of 0:100, 50:50, and 100:0 are shown in Figure 5. In addition, the kinetic constant values are displayed in Table 4. A kinetic constant of *ym* represents the maximum biogas yield that can be

**Table 3.** Methane content

| Run | Mixing ratio of TLW:TFLW (v/v) | Initial pH | Methane content (%) | |
|---|---|---|---|---|
| | | | Day 4 | Day 8 |
| 1 | 50:50 | 5.0±0.1 | 14.4 | 13.6 |
| 2 | 50:50 | 6.0±0.1 | 6.0 | n.a. |
| 3 | 50:50 | 7.0±0.1 | 44.1 | 48.1 |
| 4 | 50:50 | 8.0±0.1 | 55.5 | n.a. |
| 5 | 0:100 | 7.0±0.1 | 7.0 | 7.8 |
| 6 | 100:0 | 7.0±0.1 | 14.7 | 34 |

**Note:** n.a. – not analyzed.

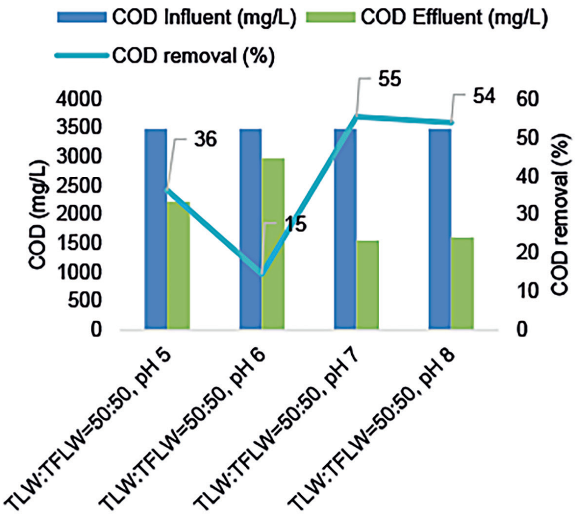


**Figure 4.** COD removal

reached. The TLW:TFLW ratios of 0:100, 50:50, and 100:0v/v had *ym* values of 134.3, 341.1, and 93.5 mL/g-$COD_{added}$, respectively. A TLW:TFLW ratio of 50:50 has the biggest *ym* because it resulted in the substrate with the most appropriate COD/N ratio. Then, the $\mu$ represents the biogas generation rate. A higher the $\mu$ value shows a faster biogas generation rate. Based on Table 4, the TLW:TFLW ratios of 0:100, 50:50, and 100:0 v/v had $\mu$ values of 108.3, 141.1, and 22.5 mL/g-$COD_{added}$/day, respectively. The substrate of TLW:TFLW of 50:50 was easier to decompose by microorganisms than others, so biogas can be generated in a higher amount. The $\lambda$ represents the time required by microbes to adapt to the system before generating biogas. Based on Table 4, the TLW:TFLW ratios of 0:100, 50:50, and 100:0v/v had $\lambda$ values of 0, 0, and 1.39 days, respectively. A higher TFLW fraction in the mixed substrate gave a higher $\lambda$ value. It was because the higher TFLW fraction lowered the $BOD_5$/COD ratio in the mixed substrates. A substrate with a lower $BOD_5$/COD ratio was more difficult to be degraded by microorganisms so it had a higher $\lambda$ value.

### *Effect of initial pH*

The AcoD of TLW:TFLW=50:50 at initial pHs of 5, 6, 7, and 8 had *ym* values of 190.0, 296.1,

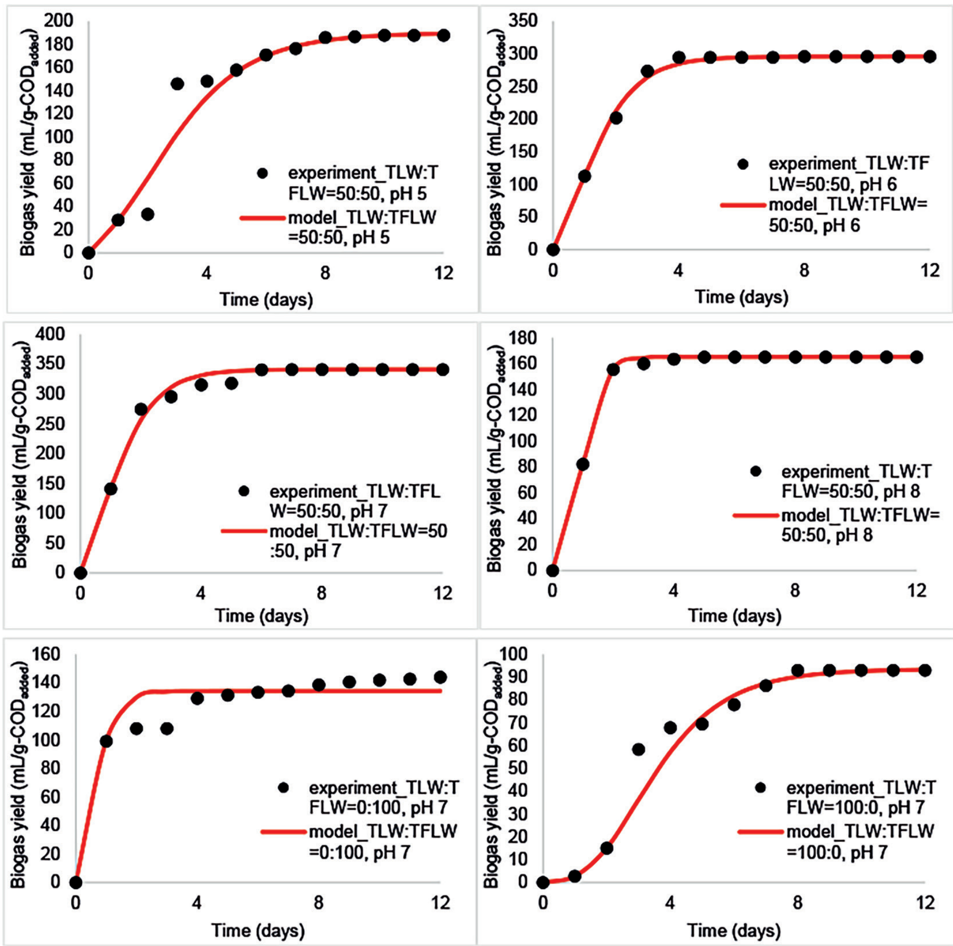


**Figure 5.** Simulation results

**Table 4.** Kinetic constants

| Constants | TLW:TFLW=<br>50:50 | TLW:TFLW=<br>50:50 | TLW:TFLW=<br>50:50 | TLW:TFLW=<br>50:50 | TLW:TFLW=<br>0:100 | TLW:TFLW=<br>100:0 |
|---|---|---|---|---|---|---|
| | pH 5 | pH 6 | pH 7 | pH 8 | pH 7 | pH 7 |
| ym<br>(mL/g-$COD_{added}$) | 190.0 | 296.1 | 341.1 | 165.2 | 134.3 | 93.5 |
| μ<br>(mL/g-$COD_{added}$/d) | 39.8 | 116.5 | 141.1 | 149.2 | 108.3 | 22.5 |
| λ (days) | 0.38 | 0.03 | 0.00 | 0.45 | 0.00 | 1.39 |
| MAPE (%) | 11.54 | 1.12 | 1.96 | 0.30 | 6.37 | 5.74 |

341.1, and 165.2 mL/g-$COD_{added}$, respectively. Hence, an initial pH of 7 had more value of *ym* than other initial pHs. It means the initial pH of 7 generated a larger maximum biogas yield (341.1 mL/g-$COD_{added}$) compared to others. The initial pH of 7 gave anaerobic bacteria favorable circumstances for metabolism. Furthermore, initial pHs of 5, 6, 7, and 8 had $\mu$ values of 39.8, 116.5, 141.1, and 149.2 mL/g-$COD_{added}$/day, respectively. Commonly, a higher value of *ym* would increase the value of $\mu$. However, the initial pH of 8 had a higher $\mu$ value than the initial pH of 7 although the former had a higher *ym* value. At an initial pH of 8, biogas was produced at a high rate from the first digestion time, but then it was very low. Therefore, even though the potential for biogas yield was lower at an initial pH of 7, the maximum rate of biogas production was higher at an initial pH of 8. That phenomenon was also found by a previous study (Budiyono et al., 2014b). Initial pHs of 5, 6, 7, and 8 had $\lambda$ values of 0.38, 0.03, 0.00, and 0.45 days. Hence, microbes at an initial pH of 7 required the shortest time to adapt (0.00 days), while microbes at other initial pHs needed a longer time. It shows that an initial pH of 7 was appropriate for anaerobic microbes to adapt to substrates.

### Limitations and future research

This study found important findings that the AcoD of TLW and TFLW had a positive effect on biogas yield. Besides that, the initial pH of 7.0 was found as the optimal initial pH in the AcoD. However, there are some limitations in this study. The VFA concentration was analyzed however the detailed composition of the VFAs was not detected. Hence, detailed VFA composition should be detected in the future. Furthermore, the mixing ratio of TLW and TFLW was varied to 0:100, 50:50, and 100 v/v. More mixing ratio values are needed to get the appropriate optimal mixing ratio. Therefore, the AcoD of TLW and TFLW with more varied mixing ratios, such as 0:100, 25:75, 50:50, 75:25, and 100:0 v/v, can be studied in the future. Techno-economy analysis should be conducted in the next research.

## CONCLUSIONS

The AD process for converting TLW and TFLW to biogas was successfully carried out. At an initial pH of 7, the mixing ratios of TLW:TFLW were varied to 0:100, 50:50, and 100:0v/v. The TLW:TFLW ratios of 0:100, 50:50, and 100:0 resulted in biogas yields of 143.99, 341.13, and 93.00 mL/g-$COD_{added}$, respectively. Hence, a TLW:TFLW ratio of 50:50 generated a higher biogas yield than other ratios (0:100 and 100:0v/v). Furthermore, the TLW:TFLW of 50:50 generated biogas with a higher methane content (44.1–48.1%) than other ratios. Then, in the AcoD process, the initial pH was varied to 5, 6, 7, and 8. The results showed that initial pHs of 5, 6, 7, and 8 resulted in biogas yields of 187.76, 296.02, 341.13, and 165.21 mL/g-$COD_{added}$, respectively. Hence, an initial pH of 7 provided suitable conditions for microbes so biogas could be generated in a large amount. Initial pHs of 7 and 8 generated biogas with standard methane content (44.1-55.5%). The highest COD removal (55%) was achieved at AcoD (50:50) with an initial pH of 7. Hence, the optimal condition was AcoD with TLW:TFLW=50:50 and an initial pH of 7. The evolution of biogas generation was successfully modeled via the modified Gompertz model. The AcoD (50:50) with an initial pH of 7 had the highest biogas potential and the lowest adaptation time. The study's findings confirmed the hypothesis that the AcoD produced a larger biogas yield and methane content than the AmoD, and that the highest biogas yield was obtained at an initial pH of 7.

The findings are expected can be used as the basis for future research using a larger-scale reactor at various affecting factors.

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