






Biogas generation from liquid waste of Beneng taro starch processing: The effect of initial pH

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ABSTRACT

The starch extraction process from Beneng taro generates a substantial amount of liquid waste, characterized by high concentrations of organic compounds. Because of its chemical oxygen demand (COD), the Beneng taro starch processing wastewater (BTSPW) is not permitted to be discharged directly to the environment. Anaerobic digestion (AD) is proposed to be applied to convert COD in the BTSPW to biogas. The aim of the current research was to examine the influence of initial pH on the AD performance in converting BTSPW to biogas. The initial pHs varied to 5.7 (control), 6.5, 7.0, 7.5, and 8.0. The substrate to inoculum ratio was 80:20%v/v. The results revealed that initial pHs of 5.7, 6.5, 7.0, 7.5, and 8.0 generated total biogas yields of 61.8, 113.62, 132.3, 103.0, and 52.1 mL/g-COD_{added}, respectively. Then, initial pHs of 5.7, 6.5, 7.0, 7.5, and 8.0 resulted in COD removals of 36.12, 39.85, 40.58, 36.86, and 36.78%, respectively. Furthermore, initial pHs of 5.7, 6.5, 7.0, 7.5, and 8.0 resulted in total solid (TS) removals of 17, 28, 32, 24, and 13%, respectively. Hence, the optimal initial pH in AD of BTSPW is 7.0. Through the modified Gompertz model, the initial pH of 7.0 had the highest P_m value (140.28 mL/g-COD_{added}) and the lowest λ value (3.79 days). The results of this study are in line with the hypothesis.

Keywords: Beneng taro starch, biogas, initial pH, liquid waste.

INTRODUCTION

Growing worldwide energy needs, coupled with the steady decline of fossil reserves, have raised serious issues regarding energy security and sustainability. Although fossil fuels are still the main contributor to the world's energy mix, they are finite and used much faster than they can naturally recover. Overdependence on these fuels speeds up resource depletion and increases the greenhouse gas output as well as other ecological problems. This reality shows the need to move toward energy sources that are cleaner and more sustainable. Renewable energy technologies are increasingly seen as a realistic solution because

they offer cleaner alternatives to conventional fuels. Considerable research has focused on the biogas generated via anaerobic digestion (AD) (Syaichurrozi et al., 2023). As a clean and flexible energy source, biogas is useful for heating, electricity generation, and as vehicle fuel (Aziz et al., 2020). The use of diverse organic wastes as feedstock not only ensures energy recovery, but also helps mitigate the environmental problems associated with improper waste management (Budiyo et al., 2021).

The sources of raw materials for biogas are very diverse, including agricultural waste, livestock manure, urban waste, and industrial liquid waste (Syaichurrozi et al., 2025). Another

potential but rarely studied source is the wastewater from the processing of Beneng taro starch. Beneng taro tubers are widely cultivated in various regions in Banten Province (Indonesia) due to their high starch content and nutritional value (Herawati et al., 2023; Suherna et al., 2023). In the process of Beneng taro starch from Beneng taro tubers, liquid waste is generated in a large amount that must be properly managed. If disposed of without treatment, this waste can cause environmental problems, such as foul odors, air pollution, and high chemical oxygen demand (COD). Processing it into biogas can minimize these negative impacts while also providing an additional renewable energy source. AD is superior to aerobic and chemical methods because it requires less energy, produces less sludge, and produces the digestate that can be reused as organic fertilizer. Furthermore, this process stabilizes organic compounds and significantly reduces pollution (Budiyono et al., 2021). The biogas produced from the AD process can be converted into electricity, which can then be used by the Beneng taro starch industry to partially replace its energy needs.

Initial pH is an fundamental factor in AD because it directly affects microbial activity and system stability. Several studies have examined the effect of initial pH on biogas production from various types of organic waste. Ali et al. (2021) reported that the use of corn waste with sheep rumen fluid inoculum under four pH conditions: 7, 6.5, 5.5, and 4.5 for 55 days of mesophilic digestion showed the best results at pH 7, with methane production reaching 17.8 g CH₄ (g/kg-VS) and a daily peak of 1425 mL on day 36. Conversely, low pH 4.5 yielded the lowest biogas production because of the accumulation of VFAs that suppress methanogen activity. Syaichurrozi et al. (2018) studied the co-digestion of *Salvinia molesta* with rice straw at ratios of 40:60 and 0:100 with initial pH variations of 6, 6.9, 7, and 8. The results showed that the 40:60 mixture was more stable and produced higher biogas (53.25–61.38 mL/g-TS) compared to the 0:100 ratio (45.98–51.20 mL/g-TS), with the highest performance at an initial pH of 6.138 mL/g-TS and CH₄ content 68.54%. Further research by Syaichurrozi et al. (2020) used the acid-treated *Salvinia molesta* and the addition of *Saccharomyces cerevisiae* at an initial pH of 5–8. The addition of yeast was shown to increase the biogas yield from 8.49–17.95 to 58.98–113.71 mL/g-VS, with optimum

conditions at an initial pH of 7 producing 113.71 mL/g-VS and a CH₄ content of 84.98%. Overall, these findings suggest that neutral pH conditions, especially with the addition of yeast, can accelerate hydrolysis and increase the efficiency of biogas production.

On the basis of the literature study, the majority of previous research has focused on the waste from other starch industries, resulting in limited information on the potential and characteristics of biogas from the Beneng taro starch processing wastewater (BTSPW). Bridgning this gap is a crucial step to ensure BTSPW suitability as a renewable energy source. This study focused on analyzing the effect of initial pH on biogas production from BTSPW, with the goal of identifying optimal conditions that yield the highest yield while supporting sustainable energy supply and reducing the environmental impact of waste disposal. The research hypothesis states that the initial pH plays a critical role in the digestion process of BTSPW. Neutral conditions are expected to optimize microbial activity, suppress the accumulation of volatile fatty acids, as well as increase the overall methane and biogas production. Therefore, BTSPW processing through AD has the potential to generate renewable energy while improving waste management.

MATERIALS AND METHODS

Materials

The primary substrate in this research was the BTSPW, collected from a warehouse in Cipocok Jaya District, Serang City, Banten Province. Before use, the wastewater was homogenized to ensure a consistent composition. The inoculum used was fresh rumen fluid, which was obtained from a slaughterhouse in Jombang District, Cilegon City, Banten Province. Rumen fluid was chosen, because it naturally contains a diverse microbial community, including hydrolytic, acidogenic, acetogenic, and methanogenic organisms that are essential for the different stages of AD. The physicochemical properties of both BTSPW and the rumen fluid (inoculum) were analyzed, and the results are presented in Table 1.

Table 1. Chemical characteristics of materials

Parameter	Unit	BTSPW	Inoculum
pH	-	6.33	4.24–4.71
COD (Chemical Oxygen Demand)	mg-O ₂ /L	5487.3	13266.7–20635.3
TS (Total Solids)	mg-dry matter/L	8500	32000–46500
TSS (Total Suspended Solids)	mg-dry matter/L	6000	8000–15000
TDS (Total Dissolved Solids)	mg-dry matter/L	2500	24000–31500
VFAs (Volatile Fatty Acids)	mg-acetic acid/L	1182.6	8056.5–8850.6

Experimental set-up

A batch digester was constructed using an Erlenmeyer flask having a total volume of 600 mL with a working volume of 500 mL. The digesters were operated in a batch system, meaning no inlet or outlet was provided during the AD. The substrate of BTSPW was combined with inoculum at a volumetric proportion of 80:20 (v/v). The biogas generated during digestion was directed through a saturated salt solution into a graduated cylinder for volume measurement. A silicone tube was attached to enable gas sampling, while a liquid sampling port was positioned at the bottom of the digester. The detailed schematic setup is presented in Figure 1.

Experimental design

The experimental design is shown in Table 2. First, the BTSPW and inoculum were mixed at a volume ratio of 80:20% v/v. Then, the 3 M NaOH solution was added to the mixture to adjust the liquid pH to 5.7 (control), 6.5, 7, 7.5, and 8. Furthermore, the mixture was fed into the digester. The digester was operated under ambient conditions (1 atm, 25–30 °C) in a batch system and shaken manually once every 3 days. The process was carried out until biogas production ceased.

Experimental procedure

In this study, AD was conducted for 21 days or until gas production had completely stopped. The experiments were performed under mesophilic conditions, with temperatures maintained at 25–30 °C and a pressure of 1 atm. Previous studies reported that the biogas produced during this time period can contain around 60% methane (Febrianti et al., 2024); other studies report methane levels of up to 68% (Mkhize et al., 2023).

At the start of the experiment, BTSPW was mixed with the inoculum and adjusted to various initial pH values before being transferred to the digesters. To preserve anaerobic conditions, the digesters were sealed with rubber stoppers and covered in aluminum foil, minimizing light interference that could inhibit methanogenic performance. Biogas production was recorded daily using the liquid displacement method (Syaichurrozi, Murtiningsih, et al., 2024). The cumulative gas volume is obtained by summing the daily results until no more biogas is produced. Every three days, approximately 10 mL of liquid sample was taken for pH measurement using a digital pH meter. The samples were then stored in a freezer for further analysis, including COD, volatile fatty acids (VFA), total solids (TS), total suspended solids (TSS), and total dissolved solids (TDS). At the same interval, 5 mL of gas samples were collected using a syringe, placed in a vacuum tube, and then analyzed for composition using Gas Chromatography based on a Thermal Conductivity Detector (GC-TCD).

Analyses

Chemical oxygen demand (COD)

COD analysis was performed using the closed reflux method and measured spectrophotometrically (Syaichurrozi, Murtiningsih, et al., 2024). In this procedure, 2 mL of liquid sample was placed in a test tube containing COD High-Range Plus reagent, shaken, then heated at 150 °C for two hours in a COD reactor. After cooling to room temperature, measurements were taken with a COD meter. The COD value was calculated using Equation 1, with *c* as the COD measurement result and *f* as the dilution factor.

$$\text{COD Concentration} = \left(\frac{\text{mgCH}_3\text{COOH}}{L} \right) = \frac{c}{10} \times f \quad (1)$$

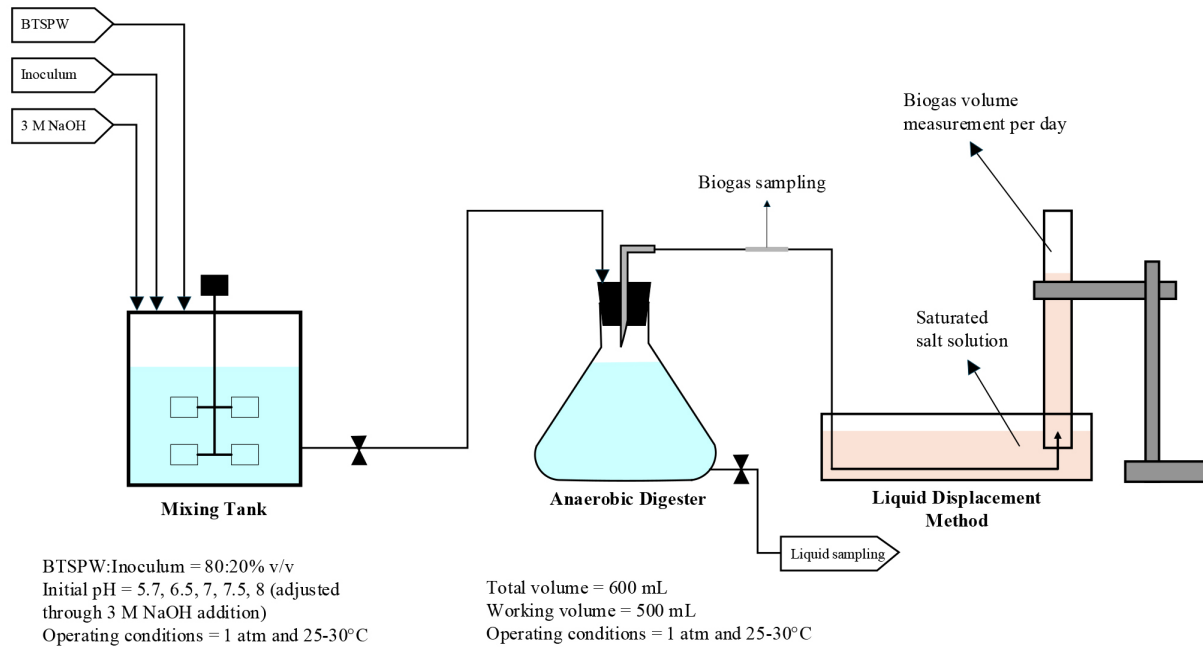


Figure 1. Laboratory-scale batch anaerobic digestion set-up

Table 2. Experimental design

Run	BTSPW volume (mL)	Inoculum volume (mL)	Initial pH
1	400	100	5.7 (Control)
2	400	100	6.5
3	400	100	7.0
4	400	100	7.5
5	400	100	8.0

Note: BTSPW – Beneng Taro Starch Processing Wastewater.

The COD removal was the difference between the initial COD (influent) and the final COD (effluent). The COD removal efficiency was quantified using Equation 2, which represents the reduction in organic matter concentration throughout the AD process.

$$\text{COD removal (\%)} = \frac{\text{COD}_{\text{influent}} - \text{COD}_{\text{effluent}}}{\text{COD}_{\text{influent}}} \times 100\% \quad (2)$$

Total solids (TS), total suspended solids (TSS), and total dissolved solid (TDS)

The empty crucible was pre-dried in an oven for 1 h and weighed to obtain its initial mass (W_1). Subsequently, a 10 mL liquid sample was added to the crucible and reweighed to determine W_2 . The crucible containing the sample

was dried in an oven at 105 °C until a constant weight was reached. Thereafter, it was cooled in a desiccator for 5 min and weighed using a digital balance. The final mass, representing the crucible plus dried solids, was recorded as W_3 . The determination of TS was carried out using the corresponding calculation formula, as presented in Equation 3.

$$\text{TS} \left(\frac{\text{mg}}{\text{L}} \right) = \frac{w_3 - w_1}{\text{Sample Volume}} \quad (3)$$

Prior to total suspended solid analysis, a Whatman filter paper (No. 42) was pre-dried in an oven and weighed to obtain its initial mass (W_1). A 20 mL sample was then filtered using the filter paper. The filter paper containing the retained wet solids was oven-dried at 105 °C for 6 h until its weight was constant, after which it was weighed again to obtain the final mass (W_2). The determination of TSS was carried out using the corresponding calculation formula, as presented in Equation (4).

$$\text{TSS} \left(\frac{\text{mg}}{\text{L}} \right) = \frac{w_2 - w_1}{\text{Sample Volume}} \quad (4)$$

The concentration of TDS was determined as the difference between TS and TSS, calculated using Equation 5.

$$\text{TDS} \left(\frac{\text{mg}}{\text{L}} \right) = \text{TS} \left(\frac{\text{mg}}{\text{L}} \right) - \text{TSS} \left(\frac{\text{mg}}{\text{L}} \right) \quad (5)$$

pH and VFAs

The pH analysis was carried out using a digital pH meter. A 10 mL liquid sample was withdrawn from the bottom section of the digester and subsequently measured for pH. Before the VFA analysis, a series of standardization procedures was conducted to ensure analytical accuracy. A total of 1.9079 g of sodium tetraborate heptahydrate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$) was accurately weighed and dissolved in distilled water to prepare 100 mL of borax primary standard solution, and its normality was calculated according to Equation 6. A 5 mL borax solution was then titrated with 0.1 N H_2SO_4 in the presence of 2–3 drops of methyl orange indicator, and the exact normality of H_2SO_4 was determined using Equation 7. A 5 mL volume of the standardized 0.1 N H_2SO_4 solution was mixed with 2–3 drops of phenolphthalein indicator and titrated with 0.05 N NaOH until a stable pink endpoint was reached. The normality of NaOH was then calculated using Equation 8.

$$\text{Normality of borax solution} = \left(\frac{\text{Mass of Borax}}{\text{Molecular weight of Borax}} \times \frac{1}{\text{Volume}} \right) \times \text{Borax purity} \times \text{Equivalent Valency} \quad (6)$$

$$\text{Normality of } \text{H}_2\text{SO}_4 \text{ solution} = \frac{\text{Normality of Borax} \times \text{Volume of Borax}}{\text{Volume of } \text{H}_2\text{SO}_4} \quad (7)$$

$$\text{Normality of NaOH solution} = \frac{\text{Normality of } \text{H}_2\text{SO}_4 \times \text{Volume of } \text{H}_2\text{SO}_4}{\text{Volume of NaOH}} \quad (8)$$

The recovery factor was determined through a two-step titration approach. In the first step, 2 mL of 0.7 N acetic acid was diluted with 50 mL of distilled water in a 250 mL Erlenmeyer flask. A few drops of phenolphthalein indicator were added, and the solution was titrated against 0.05 N NaOH until a faint pink color persisted. The NaOH volume at this point was recorded as the titrant volume of V_1 . For the distillation step, another 2 mL aliquot of the same acetic acid solution was diluted to 100 mL using a volumetric flask, transferred to a 500 mL Erlenmeyer flask, and mixed with 100 mL of distilled water plus 5 mL of 50% H_2SO_4 . The mixture was subjected to distillation under heating (600 W) with cooling water circulated through the condenser. The process continued until approximately 150 mL of distillate was collected. This distillate, after addition of phenolphthalein indicator, was titrated with 0.05 N NaOH to the same endpoint, and the

required volume was recorded as the titrant volume of V_2 . The recovery factor (F_{recovery}) was then determined based on the ratio between V_2 and V_1 , as expressed in Equation 9.

$$F_{\text{Recovery}} = \frac{V_2}{V_1} \quad (9)$$

Volatile fatty acids (VFAs) were quantified through a distillation–titration method. A 2 mL aliquot of the sample was first diluted to 100 mL in a volumetric flask, then transferred into a 500 mL Erlenmeyer flask and combined with 100 mL of deionized water and 5 mL of 50% H_2SO_4 . The solution was distilled on an electric heater until about 150 mL of distillate was obtained. This distillate was treated with a few drops of phenolphthalein indicator and titrated against 0.05 N NaOH until a faint pink color persisted, with the NaOH volume recorded as the titration value. To ensure accuracy, the apparatus was rinsed by re-distilling 200–250 mL of deionized water to collect an additional 150 mL of distillate. The VFA concentration in the original sample was then calculated using Equation 10.

$$\text{VFAs} \left(\frac{\text{mg}_{\text{acetic acid}}}{\text{L}} \right) = \frac{V_{\text{NaOH}} \times N_{\text{NaOH}} \times \text{BM}_{\text{Acetic acid}} \times 1000}{V_{\text{Sample}} \times F_{\text{Recovery}}} \quad (10)$$

Biogas yield

The biogas yield was quantified using a liquid displacement method based on the method of Syaichurrozi et al. (2024). The daily biogas volume was calculated and summed to obtain the total production. Biogas yield was expressed as the ratio of biogas volume to the initial COD of the BTSPW ($\text{mL/g-COD}_{\text{added}}$).

Kinetics

The modified Gompertz kinetic model with three main parameters, namely P_m , μ , and λ was used in this study. This model was chosen because it is capable of representing biogas accumulation during the AD batch process, where the maximum biogas potential, biogas production rate, and lag phase parameters are directly related to microbial activity. Its biological validity and reliability have been widely reported, making this model the most suitable for analyzing biogas production dynamics (Khedher et al., 2022).

The kinetic equation is listed in Equation 11 with the assumption that batch biogas production follows the microbial growth pattern. For optimization, the sum of squared error (SSE) was used as the objective function, which was calculated using Microsoft Excel, with the SSE equation shown in Equation 12.

$$P_t = P_m \cdot \exp \left\{ -\exp \left[\frac{\mu \cdot e}{P_m} (\lambda - t) + 1 \right] \right\} \quad (11)$$

$$SSE = \sum_{t=1}^n (P_i - \hat{P}_i)^2 \quad (12)$$

where: P_t is cumulative of biogas yield at time t (mL/g-COD_{added}), P_m is the maximum biogas yield that can be achieved (mL/g-COD_{added}), μ is the maximum biogas production rate (mL/g-COD_{added}/day), λ is lag time (days), e is 2.718282, t is the operating time (days), P_i is the experimental biogas yield, and \hat{P}_i is the modeled biogas yield.

RESULTS AND DISCUSSION

Biogas production and methane content

The organic components within the substrates underwent degradation through bacterial processes and were transformed into biogas throughout the AD process (Budiyo and Syaichurrozi, 2020). Figure 2 displays the daily and cumulative biogas yield patterns recorded during the AD process period. The daily biogas yield profile illustrated in Figure 2(a) shows the variation in daily biogas output across all experimental treatments. The daily biogas yield was strongly influenced by the difference in initial pHs. Figure 2(a) shows distinct patterns depending on initial pHs, with the highest productivity observed under neutral conditions. At an initial pH of 7.0, daily biogas yield reached its peak on day 11 with 40.23 mL/gCOD_{added} demonstrating the most efficient balance between acidogenic and methanogenic activity. A slightly alkaline condition at an initial pH of 7.5 also produced a high peak on day 10 with 27.04 mL/gCOD_{added}, although lower than at an initial pH of 7.0. Conversely, acidic conditions suppress methanogenesis, respectively at an initial pH 5.7 and 6.5, daily peaks were lower with 17.7 mL/gCOD_{added} and 36.37 mL/gCOD_{added}, with earlier fluctuations caused by rapid VFA

buildup further lowering the pH (Syaichurrozi et al., 2018). At an initial pH of 8.0, production was erratic, with a small peak on day 8 with 9.98 mL/gCOD_{added} but a rapid decline thereafter. However, this only lasted for a short time, before biogas production declined again until fermentation was complete. These dynamics are consistent with the findings that methanogens perform optimally at neutral initial pH and are inhibited under both acidic and strongly alkaline conditions (Syaichurrozi et al., 2019).

The cumulative biogas yield further confirms the superiority of neutral initial pH. Figure 2(b) shows the cumulative biogas yield. By day 21, the highest total yield was reached at an initial pH of 7.0 (132.3 mL/gCOD_{added}), followed by an initial pH of 6.5 (113.62 mL/gCOD_{added}), an initial pH of 7.5 (103.0 mL/gCOD_{added}), an initial pH of 5.7 (61.8 mL/gCOD_{added}), and an initial pH of 8.0 (52.1 mL/gCOD_{added}). The higher cumulative biogas yield at an initial pH of 7.0 reflects effective VFAs-to-biogas conversion by methanogens, preventing VFA excessive buildup that could suppress biogas production. Acidic treatments at initial pH 5.7 and 6.5 showed greater VFA accumulation, which depressed substrate pH and limited methane formation (Syaichurrozi et al., 2018). Conversely, at an initial pH 7.5 and 8.0, low VFA levels corresponded with reduced methane yield due to unsuitable initial pH. BTSPW has high starch and carbohydrate contents (Rostianti et al., 2018). These compounds undergo rapid hydrolysis into simple sugars, which are then fermented into VFAs. The abundance of readily degradable starch in BTSPW supports this observation, as it provides sufficient substrate for methanogenesis once the microbial consortium is fully acclimatized. Thus, the composition of the substrate is directly linked to the sharp increase in gas production during this period. In the initial phase of the process, pH tends to decrease due to the accumulation of VFAs resulting from carbohydrate decomposition. This occurs because the activity of acidogenic bacteria is faster than the growth of methanogens. On the other hand, the breakdown of nitrogen compounds produces ammonia (NH₃) or ammonium (NH₄⁺), which, if accumulated, can cause an increase in pH (Syaichurrozi et al., 2016). Therefore, an initial pH of 7 is ideal, because it maintains the balance of the process and the BTSPW can provide sufficient substrate to support the acidogenesis stage.

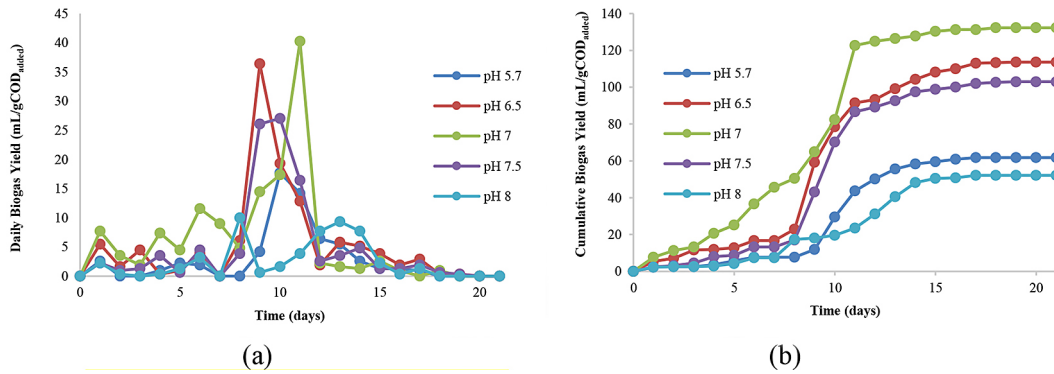


Figure 2. AD of BTSPW at various initial pHs: (a) daily biogas yield, (b) cumulative biogas yield

Table 3. Methane gas concentration at each initial pH variation

Sample	CH ₄ concentration (%)			
	Day-6	Day-9	Day-12	Day-15
pH 5.7 (Control)	NA	58.17	57.06	44.31
pH 6.5	NA	68.74	72.76	79.21
pH 7	68.02	64.66	58.88	45.77
pH 7.5	NA	67.78	65.29	60.99
pH 8	NA	57.91	53.51	43.97

Note: NA – not analyzed.

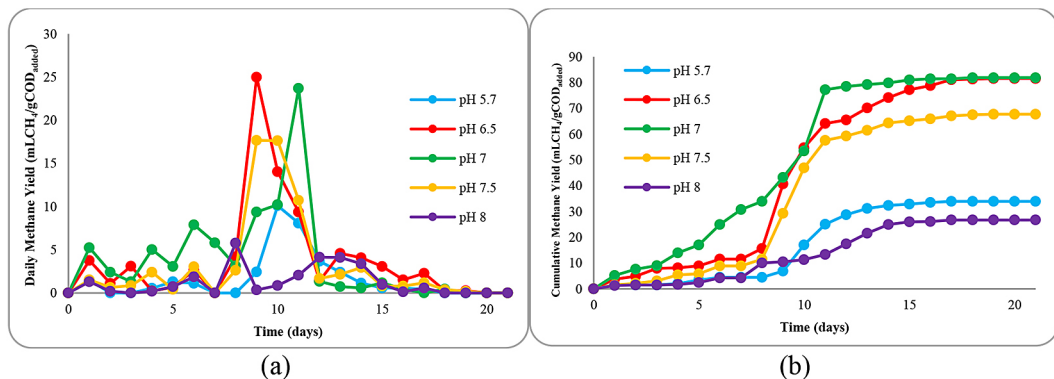


Figure 3. AD of BTSPW at various initial pHs: (a) daily methane yield, (b) cumulative methane yield

Methane concentration is often used as a key parameter for evaluating the quality of biogas, since the heating value is largely determined by the proportion of CH₄ in the gas mixture. The GC-TCD measurements in this study revealed that the methane content varied with the initial pH conditions. Table 3 shows the methane content in biogas on days 6, 9, 12, and 15 at various initial pHs. The methane content was then used to make the daily and cumulative methane yields, as shown in Figure 3.

In Figure 3(a), the daily methane data shows that the highest daily methane yields at initial pH 5.7, 6.5, 7.0, 7.5, and 8.0 was 10.10 mLCH₄/

gCOD_{added} on day 10, 25.00 mLCH₄/gCOD_{added} on day 9, 23.69 mLCH₄/gCOD_{added} on day 11, 17.67 mLCH₄/gCOD_{added} on day 9, and 5.78 mLCH₄/gCOD_{added} on day 8, respectively. The BTSPW undergoes rapid hydrolysis and acidogenesis into simple sugars and VFAs. At an acidic initial pH levels of 5.7, methanogens become stressed and cannot efficiently convert VFAs, leading to accumulation and decreased methane yield (Chen et al., 2008). At a neutral initial pH levels of 7.0, microbial activity is balanced, with hydrolytic and acidogenic bacteria producing VFAs, while methanogens efficiently consume them, resulting in stable and high

biogas production (Cornet, 2017). At slightly alkaline initial pH levels of 7.5 and 8.0, biogas can still be produced, but methanogenic activity gradually decreases, because many species are neutrophilic and less adaptable to higher pH levels, which reduces process stability and methane yield (Chen et al., 2008). Therefore, the daily pattern shows that an initial pH of 7 provides optimal conditions for the conversion of starch into methane (Syaichurrozi et al., 2018; Syaichurrozi et al., 2020).

Figure 3(b) shows that neutral conditions at an initial pH of 7 provide the most optimal methane accumulation, reaching $81.92 \text{ mLCH}_4/\text{gCOD}_{\text{added}}$, slightly higher than an initial pH of 6.5, which reached $81.60 \text{ mLCH}_4/\text{gCOD}_{\text{added}}$. Meanwhile, an initial pH of 7.5, 5.7, and 8 reached $67.73 \text{ mLCH}_4/\text{gCOD}_{\text{added}}$, $33.92 \text{ mLCH}_4/\text{gCOD}_{\text{added}}$, and $26.70 \text{ mLCH}_4/\text{gCOD}_{\text{added}}$, respectively. At acidic initial pH levels, at an initial pH of 5.7 and 6.5, substrate degradation produces high levels of VFAs, which lower the pH of the environment and inhibit methanogen growth (Syaichurrozi et al., 2018). As a result, the conversion of VFAs to methane becomes suboptimal. Under acidic and alkaline conditions at initial pH 5.7 and pH 8.0, a significant reduction in methane yield was observed, which can be attributed to the severe stress on methanogenic consortia as well as the consequent disruption of metabolic pathways and decrease in methane production. Microbial function stability and enzyme performance are maintained at initial pH levels of 6.5 and 7.5, resulting in higher methane production compared to when the process occurs under overly acidic or alkaline conditions (Qiu et al., 2020). An initial pH condition of 7.0 is considered ideal because acidogenic bacteria are able to produce VFAs in the quantities sufficient for methanogens, without causing excessive accumulation. Thus, VFAs can be efficiently converted into methane (Lay et al., 2013; Syaichurrozi et al., 2019).

The results of this study are consistent with research conducted by Mkhize et al. (2023), at an optimum initial pH of 7.0, mesophilic fermentation was found to produce up to 68% methane. This finding emphasizes that neutral conditions play a vital role in maintaining the balance of the microorganism community in the fermentation reactor. Therefore, controlling the initial pH is a key factor in improving biogas quality, not only in terms of production volume but also in terms of methane content, which determines energy value.

pH and volatile fatty acids (VFAs)

The biogas formation process is influenced by pH, because microbial enzyme activity is highly dependent on the acidity level of the environment (Febrianti et al., 2024). The pH dynamics throughout 21 days demonstrated a consistent decline across all treatments, as it is shown in Figure 4(a). At an initial pH of 5.7, pH values fell from 5.7 on day 0 to 4.6 on day 21, indicating acidification that suppressed methanogenic activity. At an initial pH 6.5, the decrease was from 6.5 to 5.43, while at an initial pH of 7.0, it fell from 7.0 to 5.26. In the alkaline range, initial pH of 7.5 dropped from 7.5 to 5.50, and an initial pH of 8.0 from 8.0 to 5.66. This downward trend is attributed to the rapid decomposition of BTSPW by hydrolytic and acidogenic bacteria, such as *Clostridium* sp. and *Clostridium sporogenes*, which convert carbohydrates into VFAs and total ammonia nitrogen (TAN) (Syaichurrozi et al., 2020). Because carbohydrates degrade more readily than proteins, the first phase of digestion typically shows a pH of substrate decline due to VFA accumulation, followed by stabilization when methanogens consume the VFAs. Methanogenic bacteria, however, are more sensitive to acidic conditions compared with acidogens, and prolonged $\text{pH} < 6$ severely inhibits their activity (Syaichurrozi et al., 2018). This explains why initial pHs of 5.7 and 6.5 yielded lower methane compared with an initial pH of 7.0. In the meantime, methanogens cannot adapt well to excessively high pH levels, which are initial pH 7.5 and 8, resulting in reduced methane production efficiency. Among all treatments, an initial pH of 7.0 proved to be the most favorable, balancing acidogenesis and methanogenesis, thus sustaining optimal biogas yield, consistent with the reports that neutral to slightly alkaline pH provides the best stability in AD (Lay et al., 2013).

The VFA profiles strongly correlate with pH behavior, showing that lower pH corresponds to higher VFA accumulation, while higher pH results in lower or slower VFA accumulation. In Figure 4(b). At an initial pH of 5.7, VFA concentrations rose from 2.883 mg-acetic acid/L on day 3 to 3.991 mg-acetic acid/L on day 21, the highest among treatments, reflecting rapid acidogenesis and poor conversion of VFAs into methane. At an initial pH of 6.5, VFAs increased more moderately, reaching 3.214 mg-acetic acid/L on day 21, indicating partial methanogenic activity. At an initial pH of 7.0, VFAs peaked at 3.259 mg-acetic

acid/L on day 12 and stabilized at 3.123 mg-acetic acid/L on day 21, showing effective turnover of VFAs into methane, which explains the highest cumulative gas production. At an initial pH of 7.5, VFAs ended at 3.123 mg-acetic acid/L, and methanogenesis was less efficient, resulting in lower gas yield. At an initial pH of 8.0, VFAs accumulation was lowest at 3.030 mg-acetic acid/L on day 21, but methane yield remained low due to methanogens experiencing physiological stress and being displaced within the microbial community. Under acidic conditions, namely initial pH 5.7 and 6.5, the concentration of VFAs tends to increase from the start of AD, because acidogenic bacteria continue to degrade starch and carbohydrates into volatile acids. However, methanogens, which play a role in converting VFAs into methane, do not work optimally at acidic initial pH, causing a continuous accumulation of VFAs. Meanwhile, at neutral and alkaline initial pHs, namely pH 7, 7.5, and 8, the VFA concentrations decrease briefly on the third day. This indicates that the VFAs formed during the acidogenesis stage are immediately consumed by methanogens that are more active at neutral pH, causing a temporary decrease. After that, the VFA concentrations increase again as fermentation progresses because VFA production by acid bacteria becomes dominant again, although some are still used for methane formation.

Mechanistically, VFAs are essential intermediates produced by acidogens (acetate, propionate, butyrate) and utilized by methanogens to generate methane (Syaichurrozi et al., 2024). However, excessive accumulation lowers substrate pH, disrupts biochemical equilibrium, and inhibits methanogens (Syaichurrozi et al., 2018). When VFAs exceed the metabolic capacity of methanogens, undissociated acids diffuse into cells, disturb proton balance, and inhibit key enzymes, thereby reducing methane production. Conversely, too little VFA accumulation under inhibitory conditions, as seen at an initial pH of 8.0, means less substrate is available for methanogenesis. Although the VFA profiles at an initial pH of 6.5, 7.0, 7.5, and 8.0 appear similar in Figure 4, an initial pH of 7 can still be considered the most favorable condition. Under excessively alkaline conditions, initial pH of 8.0, there is a gradual decline in methanogenic activity (Chen et al., 2024; Wormald et al., 2020). Conversely, the higher accumulation observed at an initial pH of 5.7 can exceed the tolerance limit for many methanogens,

leading to process instability and potential failure (Lackner et al., 2020). While comparable concentrations are observed across these ranges, at initial pH of 7, methanogens operate near their physiological optimum, which ensures that VFAs are metabolized efficiently and without hitting the levels that could cause inhibition, which is a balance that maximizes biogas production from carbohydrates (Syaichurrozi et al., 2020). The optimum initial pH for methanogens is typically cited as between 6.5 and 7.5 (Fotidis et al., 2013). This is supported by the findings showing that deviations from this range, especially above an initial pH of 8 or below an initial pH of 6.5, can cause a decrease in methanogenic efficiency and instability in the digestion process. BTSPW provides carbohydrate reserves that accelerate the hydrolytic activity of *Clostridium sp.* and promote higher glucose production. The abundant carbohydrate content also supports acid bacteria in converting glucose into acetic acid, butyric acid, and other organic acid compounds. Carbohydrates are more easily degraded than protein (Zhang et al., 2014).

This research aligns with the findings of Atasoy and Cetecioglu (2022), who examined AD at acidic pH of 5, neutral (without adjustment), and alkaline pH of 10. The results showed that neutral conditions at pH of 7 were proven to produce the highest biogas and methane. These results are supported by Ali et al. (2021), who recorded methane production reaching 17.8-g CH₄ (g/kg-VS) with a maximum daily volume of 1425 mL in corn waste mixed with sheep rumen inoculum at an initial pH of 7. Another study by Syaichurrozi et al. (2020) even showed that neutral initial pH, especially when combined with *Saccharomyces cerevisiae*, can significantly increase the methane content by up to 84.98%. These findings consistently indicate that neutral initial pH conditions are the most supportive environment for methanogen performance, while maximizing biogas production.

TS, TSS, TDS, and COD removals

The decrease in COD during AD of BTSPW indicates that microorganisms are utilizing dissolved organic matter to grow and produce biogas. Maintaining pH stability is essential to ensure environmental conditions support bacterial activity and growth throughout the process (Mohamed et al., 2024). On the basis of the experimental results shown in Figure 5(a), the COD removal efficiency varied across different pH

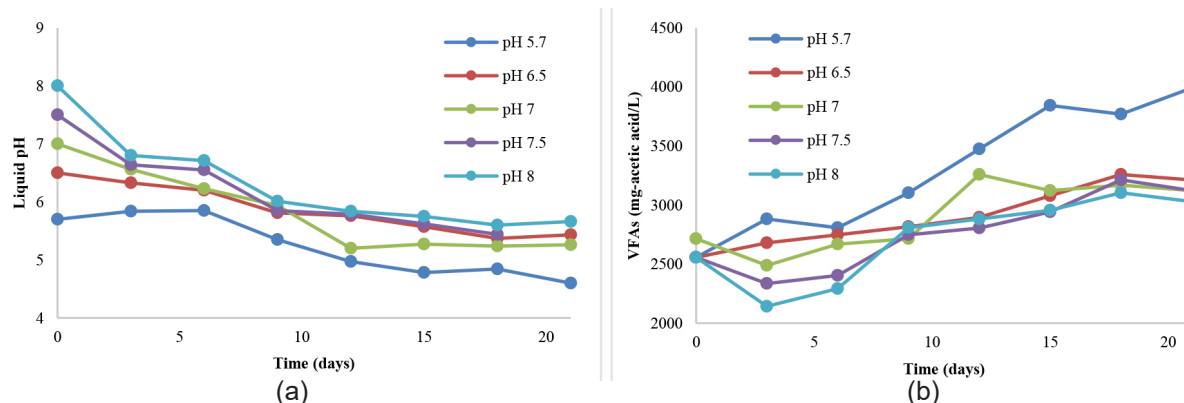


Figure 4. Profiles of (a) pH liquid, (b) volatile fatty acids (VFAs)

conditions. The highest removal was achieved at an initial pH of 7 with 40.68%, followed by an initial pH of 6.5 with 39.85%, and an initial pH of 7.5 with 36.86%. In contrast, initial pHs of 5.7 and 8 exhibited relatively lower COD removal efficiencies, 36.12% and 36.78%, respectively. The control condition an initial pH of 5.7 showed the lowest COD removal of 36.12%.

Theoretically, an initial pH is one of the most critical environmental factors in AD. The microbial activity in AD proceeds optimally within a pH range of 6.5–7.5 (Cremonez et al., 2021), 6.7–7.5 (Habib et al., 2024), and 6.9–7.4 (Kelly and Amagbor, 2019). At the initial stage of batch digestion, carbohydrates as carbon sources are converted into VFAs, leading to a decrease in liquid pH. Over time, the pH gradually increases as VFAs are converted into biogas and proteins are degraded into ammonia/ammonium. Anaerobic digestion in a single-stage system generally performs optimally under neutral pH conditions. Under excessively acidic conditions, with an initial pH of 5.7, the accumulation of VFAs can inhibit methanogenic activity, thereby limiting COD removal. Conversely, under alkaline conditions (initial pH of 8), microbial growth is also inhibited, which reduces substrate degradation (Zhou et al., 2024). These studies are consistent with the theoretical framework, demonstrating that initial pH of 7 achieved the highest COD removal along with the greatest biogas production, indicating a positive correlation between COD reduction and methane generation (Ramadhani et al., 2024).

TS removal followed a clear pH dependence, peaking at an initial pH of 7 with 32%, and declining on either side of neutrality. It can be shown in Figure 5(b) that initial pHs of 6.5 with 28%, 7.5 with 24%, 5.7 with 17%, and 8

with 13%. The maximum TS removal at neutral pH indicates that the overall solids reduction was most effective when the acidogenic and methanogenic consortia were not stressed by acidity or alkalinity. The pronounced drop at an initial pH of 8 with 13% suggests impaired microbial activity and/or increased solubilization of particulates under alkaline conditions, while the acidic value at an initial pH of 5.7 with 17% reflects acid stress on methanogenesis and incomplete conversion of dissolved intermediates.

TSS removal showed a clear variation across the tested initial pH values. The highest TSS reduction was observed at initial pHs of 7 and 7.5, both with 18%, followed by an initial pH of 6.5 with 14%, while the lowest value was obtained at an initial pH of 8 with 10% (Figure 5(c)). This shows that at an initial pH of 7, more complex organic compounds were successfully converted to simple organic compounds (such as VFAs, total ammonia nitrogen, etc.), so it achieved the highest TSS removal. Furthermore, the maximum TDS reduction occurred at initial pH of 7 with 45%, followed by initial pHs of 6.5 with 41%, 7.5 with 30%, and 8 with 15% (Figure 5(d)). The strong performance at an initial pH of 7 indicates efficient utilization and conversion of soluble intermediates, such as VFAs into methane under conditions favorable to methanogens. Meanwhile, the lower TDS removals at initial pHs of 7.5 and 8 reflect microbial stress and reduced efficiency in substrate conversion.

Since TS consists of both suspended and dissolved fractions, its pattern was largely determined by TDS removal. The highest TS reduction was obtained at an initial pH of 7 with 32%, driven by high TDS removal of 45% despite only moderate TSS removal of 18%. At an initial pH of

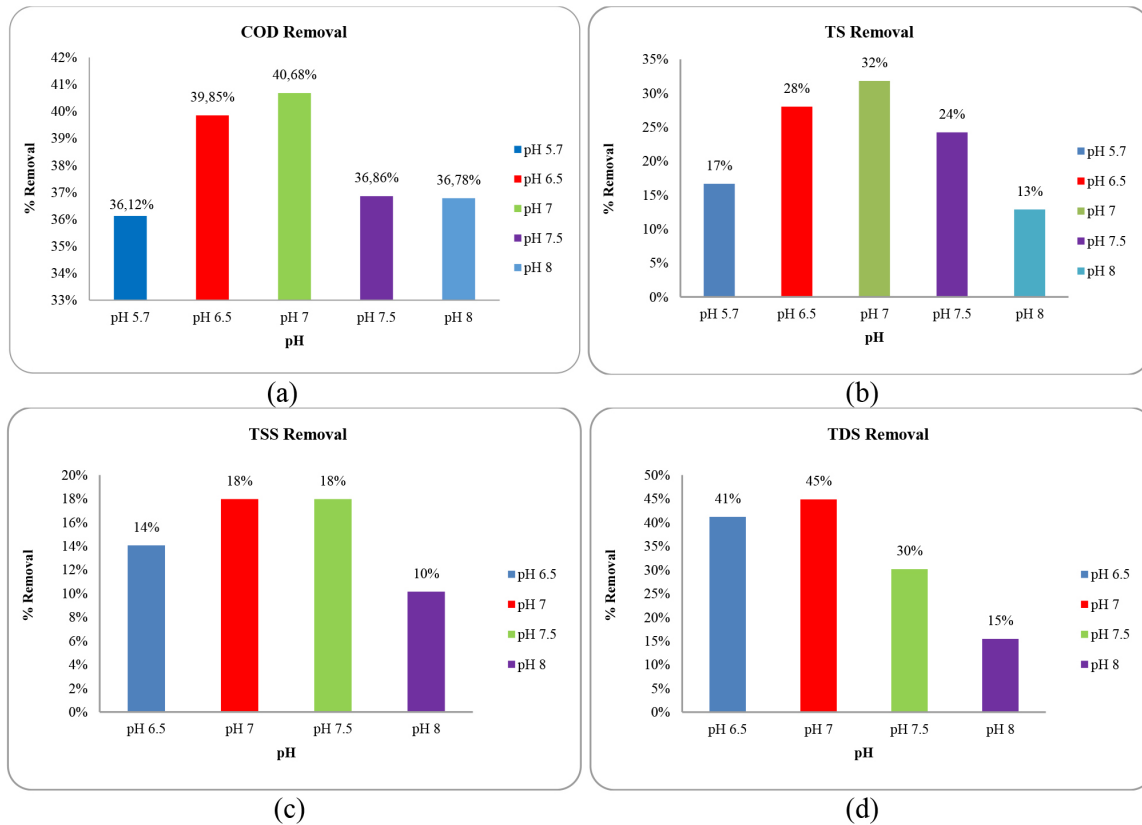


Figure 5. Organic and solid removal efficiency; (a) COD removal; (b) TS removal; (c) TSS removal; (d) TDS removal

6.5, substantial TDS removal of 41% combined with TSS removal of 14% resulted in a TS reduction of 28%. In contrast, at an initial pH of 8, the TDS removal was only 15%, which consequently limited the TS reduction to 13%. These findings indicate that the near-neutral range (pH 6.5–7.0) represents the most favorable condition for overall solids reduction.

Kinetic analysis

Biogas production was evaluated using a modified Gompertz model. The constant parameters derived from the model are presented in Table 4, while Figure 6 illustrates the comparison between the experimental results and the simulation

outputs. At different initial pH values 5.7, 6.5, 7.0, 7.5, and 8.0 had P_m values of 62.16, 114.04, 140.28, 102.33, and 58.21 mL/g-COD_{added}, respectively. It means the highest biogas production can be achieved at an initial pH of 7, followed by initial pHs of 6.5 and 7.5 with values of 114.04 and 102.33 mL/g-COD_{added}. These results indicate that a neutral atmosphere is able to significantly support the biogas formation process from BTSPW.

The neutral initial pH (6.5–7.5) also had a higher μ value than acidic or alkaline (Table 4). Initial pHs of 5.7, 6.5, 7.0, 7.5, and 8.0 had μ values of 13.95, 19.35, 15.48, 23.28, and 5.58 mL/g-COD_{added}, respectively. In other words, a neutral pH can stimulate microbial activity, thereby increasing the biogas production rate. This study

Table 4. Kinetic constant values

Constants	Units	pH 5.7	pH 6.5	pH 7.0	pH 7.5	pH 8.0
P_m	mL/g-COD _{added}	62.16	114.04	140.28	102.33	58.21
μ	mL/g-COD _{added} /day	13.95	19.35	15.48	23.28	5.58
λ	days	7.86	6.10	3.79	7.02	5.68
P_m (experiment)	mL/g-COD _{added}	62.1	113.7	138.1	102.3	55.4
Error	%	0.09	0.28	1.55	0.05	4.88

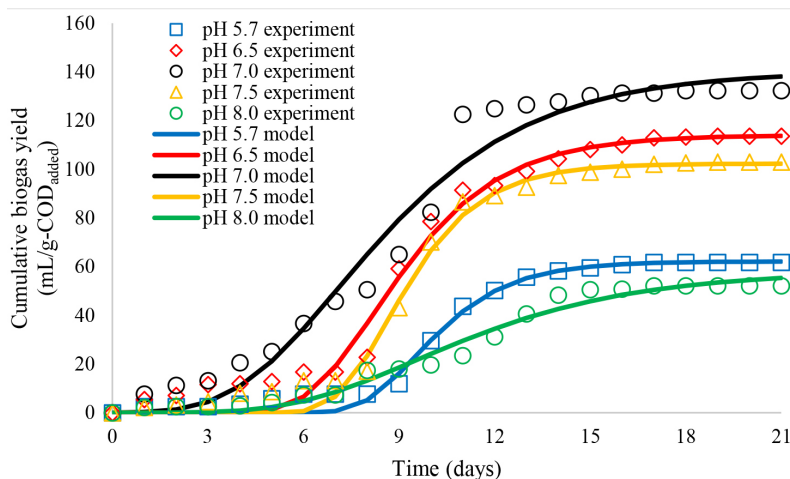


Figure 6. Plotting results using the modified Gompertz model

shows that an increase in the P_m is usually accompanied by an increase in the maximum biogas production rate (μ value). This means that the overall biogas production potential is predicted to increase with an acceleration in the production rate (Syaichurrozi, Murtiningsih, et al., 2024).

The lag phase λ reflects the adaptation time required by anaerobic microorganisms before methanogenesis begins (Syaichurrozi et al., 2016). Initial pHs of 5.7, 6.5, 7.0, 7.5, and 8.0 had λ values of 7.86, 6.10, 3.79, 7.02, and 5.68 days, respectively. It can be seen that the initial pH of 7.0 has the lowest λ value. It means that the initial pH of 7.0 provides the most comfortable conditions, so methanogenic microorganisms need the lowest time to adapt to the substrate (Syaichurrozi, Murtiningsih, et al., 2024).

CONCLUSIONS

This study demonstrated that the initial pH strongly influences the AD performance of BTSPW. Among the tested conditions, an initial pH of 7.0 achieved the highest biogas yield (132.3 mL/g-COD_{added}), the greatest COD (40.58%) and TS (32%) removals, and the most favorable kinetic parameters according to the modified Gompertz model ($P_m = 140.28$ mL/g-COD_{added}; $\lambda = 3.79$ days). These findings indicate that maintaining a neutral pH optimizes microbial activity, accelerates process initiation, and maximizes biogas production. Therefore, an initial pH of 7.0 is recommended as the optimal condition for efficient AD of BTSPW. The findings support the hypothesis that neutral to slightly alkaline pH provides

the best conditions for methanogens, resulting in higher methane production and more stable digestion, compared to acidic conditions. This study was carried out in batch experiments with a fixed substrate-to-inoculum ratio, so the results may not fully reflect large-scale or continuous processes. Other key factors, such as temperature, retention time, microbial interactions, and nutrient availability, were not considered here. For future work, it would be important to scale up the process, refine operating parameters, and evaluate how AD of BTSPW could be applied in real waste management systems. Exploring co-digestion with other agricultural or industrial residues may also improve methane yield and process stability.

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