

The effect of urea addition on biogas production from beneng taro starch wastewater

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ABSTRACT

Anaerobic digestion (AD) applied to Beneng taro starch wastewater (BTSW) presents an attractive route for combining wastewater treatment with renewable energy recovery. Nevertheless, biogas generation from this type of substrate is often constrained because carbohydrate-dominated wastewater tends to contain insufficient nitrogen to support optimal microbial function. Accordingly, this study was designed to determine how nitrogen supplementation through urea influences the performance of AD when BTSW serves as the substrate. The experiment was performed in 500 mL batch digesters using a substrate and inoculum mixture at a volumetric ratio of 80:20. Urea concentrations of 0, 100, 150, 200, and 250 mg/L were evaluated, while FeCl_3 was maintained at 60 mg/L. Among these treatments, the 150 mg/L urea dose gave the largest cumulative biogas output (187.86 mL/g-TS_{substrate}), roughly triple that of the untreated control, and also produced the greatest methane yield (54.39 mL CH_4 /g-TS_{substrate}), the smallest buildup of VFAs, and the strongest reductions in TS (32.69%), TSS (33.33%), and TDS (32.10%). Kinetic evaluation with the modified Gompertz equation further confirmed this outcome, showing a very close fit to the experimental data ($R^2 = 0.9979$) and the largest P_m value (187.90 mL/g-TS_{substrate}) among all treatments. Overall, these outcomes point to 150 mg/L as the most suitable urea dose for supplying nitrogen during BTSW digestion and for maximizing biogas output.

Keywords: anaerobic digestion, beneng taro starch wastewater, biogas, urea.

INTRODUCTION

Global demand for energy continues to be met largely by fossil resources, which remain non-renewable in nature. Projections suggest fossil fuels will still supply around 74% of global energy needs by 2030, only marginally lower than the 79% share recorded in 2019 (Goshe et al., 2025). This persistent reliance underscores the urgency of shifting toward cleaner and renewable alternatives, given the well-documented environmental costs of fossil fuel use, including greenhouse gas emissions and climate disruption. Among the available renewable energy sources, biogas has gained considerable attention because it can be produced from organic materials through anaerobic digestion (AD). As an environmentally

friendly energy source, biogas can be utilized for a wide range of applications (Budiyo et al., 2023). Agroindustrial wastewater is a particularly favorable feedstock for this process, since its high carbohydrate content is readily hydrolyzed into simple sugars and subsequently transformed into volatile fatty acids, acetate, hydrogen, and finally methane through microbial action (Mee-goda et al., 2018).

Beneng taro (*Xanthosoma undipes*) is a local crop grown mainly in Banten Province, and the expansion of its starch-processing industry has generated increasing volumes of organic-rich wastewater (Herawati et al., 2023). Left untreated, this wastewater poses a pollution risk given its heavy organic load. Channeling it through anaerobic digestion therefore offers a dual benefit

reducing pollution while generating renewable energy (Prasanna et al., 2024). AD is considered a technology that supports circular economy principles due to its ability to stabilize organic waste while generating biogas, which mainly contains methane and carbon dioxide, with relatively low energy consumption and minimal sludge production (Meegoda et al., 2018).

Despite the biodegradability of starch based wastewater, it typically has a skewed nutrient profile because carbohydrates dominate, the carbon-to-nitrogen ratio tends to be high, leaving insufficient nitrogen for microbial needs (Mir et al., 2016). Nitrogen is essential to microbial cell development, enzyme and protein synthesis, and overall metabolic function throughout digestion. A shortage of nitrogen therefore hampers microbial performance and methane formation, whereas adding too much nitrogen risks pushing ammonia concentrations to inhibitory levels that suppress methanogens and impair the digestion process (Yang et al., 2025). Achieving the right nitrogen balance is thus a key determinant of stable, efficient digestion.

Urea is a widely used nitrogen source for this purpose owing to its affordability, availability, high nitrogen content, and rapid conversion to ammonium usable by anaerobic microbes. Existing evidence indicates that moderate urea dosing benefits digestion by improving the C/N ratio and enhancing microbial activity, while overdosing triggers ammonia accumulation that suppresses methanogenesis (Yang et al., 2025). Urea supplementation has also been examined for boosting biogas yields from crop residues, animal manure, and various other organic feedstocks (Yuan et al., 2026). For instance, Ona et al., (2022) working with a rice straw and swine manure co-digestion system, found that a 1 g urea addition raised cumulative biogas output by 17.7% and 23.4% at substrate ratios of 1:1 and 1:2, respectively gains linked to more favorable pH conditions and improved nitrogen availability for methanogens. Their findings also showed that overly high urea doses became counterproductive once ammonia inhibition set in, reinforcing the need to tailor urea dosage to the substrate at hand.

Despite this body of work, no prior study appears to have examined how urea supplementation affects AD performance specifically for Beneng taro starch wastewater (BTSW). This study fills that gap by evaluating the effect of urea dosing on biogas production from BTSW,

with the underlying hypothesis that an optimal urea dose will enhance microbial activity, limit VFA buildup, and boost both methane and overall biogas output. The findings are intended to support more effective nitrogen management strategies for anaerobic digestion of carbohydrate-rich agroindustrial wastewater, contributing to both renewable energy development and sustainable liquid-waste handling.

MATERIALS AND METHODS

Materials

Beneng taro starch wastewater used as the substrate was supplied by a beneng taro starch processing facility in Cipocok Jaya District, Serang City, Banten, Indonesia. The inoculum used in this study consisted of cow rumen fluid, which was collected from an animal processing facility in Jombang District, Cilegon City, Banten, Indonesia. Technical-grade urea and FeCl_3 were purchased from a local chemical supplier in Cimuncang District, Serang City, Banten, Indonesia. Table 1 presents the chemical properties of BTSW and the inoculum.

Experimental set-up

The anaerobic digestion process was carried out using modified 600 mL Erlenmeyer flasks, each containing a working volume of 500 mL. All reactors were operated under batch conditions, with no influent addition or effluent withdrawal during the digestion period. Anaerobic digestion was carried out using a mixture of Beneng taro starch wastewater and cow rumen inoculum at an 80:20 (v/v) proportion. Biogas production was determined by the liquid displacement technique in a measuring cylinder containing saturated salt solution. The reactor was designed with separate ports for gas and liquid sampling, including a silicone tube for gas collection and a bottom outlet for liquid sampling. The details of experimental set-up are shown in Figure 1.

Experimental design and procedures

The procedure starts by setting up the experimental equipment, which includes a 100 mL graduated cylinder, a 500 mL modified Erlenmeyer flask, a gas tubing, wooden stands, a binder

Table 1. Chemical properties of materials

Parameters	Units	BTSW	Inoculum
pH	-	5.14	6.41
VFAs (volatile fatty acids)	mg-acetic-acid/L	824.67	7971.77
TS (total solids)	mg-dry matter/L	100000	38000
TSS (total suspended solids)	mg-dry matter/L	4500	19500
TDS (total dissolved solids)	mg-dry matter/L	5500	18500

Note: BTSW – Beneng Taro Starch Wastewater.

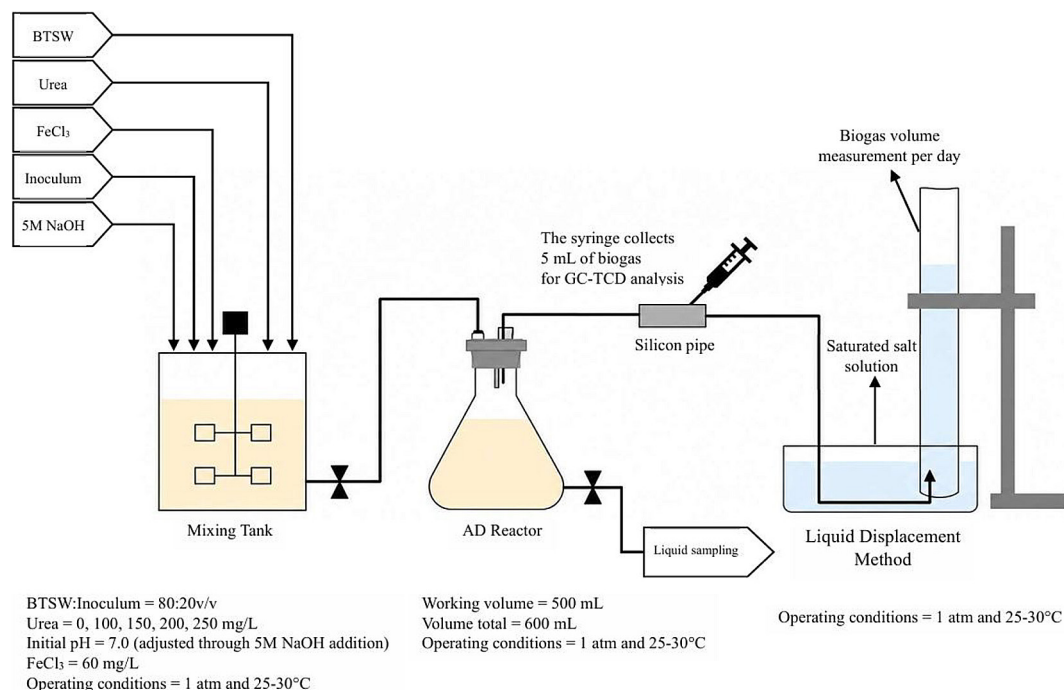


Figure 1. Experimental set-up of laboratory-scale batch anaerobic digestion

clip, and a container. The saturated salt solution is prepared by dissolving 1200 g of salt into 3 L of water. The substrate which is Beneng taro starch wastewater mixed with FeCl₃ (60 mg/L) and urea (0, 100, 150, 200, and 250 mg/L), then the pH is adjusted to 7.0 using 5M NaOH. The inoculum which is cow rumen is added at 20% of the total volume, and the pH is stabilized again at 7.0. The

prepared mixture was transferred into a modified Erlenmeyer flask, which was subsequently sealed tightly using hot glue and covered with aluminum foil to minimize exposure to oxygen and light. Every 3 days, 5 mL of biogas and 10 mL of liquid digester samples are collected for data analysis and modeling. Detailed experimental design is presented in Table 2.

Table 2. Experimental design

Digester	BTSW volume (mL)	Inoculum volume (mL)	Urea (mg/L)	FeCl ₃ (mg/L)	Process
1	400	100	0	60	AD
2	400	100	100	60	AD
3	400	100	150	60	AD
4	400	100	200	60	AD
5	400	100	250	60	AD

Note: BTSW – Beneng taro starch wastewater.

ANALYZES

Liquid pH and VFA concentration

Sample pH was measured using a digital pH meter, which was calibrated against a standard buffer solution beforehand to ensure the reliability and accuracy of the measurements. The steam distillation method was employed to determine volatile fatty acids (VFAs). For VFAs analysis, the liquid sample (2 mL) was diluted with distilled water in a 100 mL volumetric flask until the final volume was reached. Subsequently, 100 mL of distilled water and 5 mL of 50% H₂SO₄ were added to the diluted sample after it had been placed in a 500 mL flask. Distillation was performed using a 600 W heater, and the distillate was collected until its volume reached 150 mL. Titration of the collected distillate was carried out using 0.05 N NaOH, with phenolphthalein employed to indicate the endpoint. The VFA concentration was determined according to Equation (1) (Syaichurrozi et al., 2024).

$$VFAs \left(\frac{mg_{acetid\ acid}}{L} \right) = \frac{V_{NaOH} \times N_{NaOH} \times MW_{acetid\ acid}}{V_{sample} \times F_{recovery}} \quad (1)$$

Total solid, total suspended solid, and total dissolved solid concentrations

The oven-drying method was employed to determine total solids (TS). An empty crucible was first dried at 105–110 °C for 1 h and weighed (W₁). To determine TS, 10 mL of the liquid sample was first weighed in a crucible (W₂). The sample was then subjected to oven drying at the specified temperature until its weight remained constant. Once cooled to room temperature, the crucible was reweighed (W₃), and the TS value was obtained based on Equation 2.

$$TS = \frac{W_3 - W_1}{sample\ volume} \quad (2)$$

Total suspended solids (TSS) and total dissolved solids (TDS) were analyzed with Whatman Grade 42 filter paper. An oven-dried filter paper (105–110 °C, 1 h) was weighed before analysis (W_a). To separate suspended solids from the liquid phase, a 20 mL aliquot of the sample was filtered through filter paper. Following filtration, the residue retained on the filter paper was dried

at 105–110 °C for 2 h until no further change in weight was observed. The filter paper was then weighed (W_b), and the TSS and TDS values were calculated using Equations 3 and 4 (Lasalewo and Syaichurrozi., 2026).

$$TSS \left(\frac{mg}{L} \right) = \frac{(W_b - W_a)}{V} \quad (3)$$

$$TDS \left(\frac{mg}{L} \right) = TS \left(\frac{mg}{L} \right) - TSS \left(\frac{mg}{L} \right) \quad (4)$$

Biogas yield

The cumulative biogas obtained by adding up the daily biogas volume for 15 days. Biogas yield (mL/g-TS_{substrate}) was determined by dividing the cumulative biogas volume (mL) by the initial total solids content of Beneng taro starch wastewater. The cumulative biogas yield can be calculated using Equation 5 (Syaichurrozi et al., 2024).

$$Biogas\ yeild = \frac{Cumulative\ biogas\ volume\ (mL)}{Initial\ TS_{substrate}\ (g)} \quad (5)$$

Pollutant removal efficiencies

The removal efficiencies of TS, TSS, and TDS were determined using Equations 6–8 to evaluate the effect of anaerobic digestion (Lasalewo and Syaichurrozi., 2026).

$$TS\ removal\ (\%) = \frac{TS_{initial} - TS_{final}}{TS_{initial}} \times 100\% \quad (6)$$

$$TSS\ removal\ (\%) = \frac{TSS_{initial} - TSS_{final}}{TSS_{initial}} \times 100\% \quad (7)$$

$$TDS\ removal\ (\%) = \frac{TDS_{initial} - TDS_{final}}{TDS_{initial}} \times 100\% \quad (8)$$

Methane content

Biogas composition analysis was used a Gas Chromatography-Thermal Conductivity Detector (GC-TCD) to find the main contents of biogas, which are methane and carbon dioxide. The actual methane and carbon dioxide percentages were calculated using Equations 9 and 10 (Khomariah et al., 2025).

$$CH_4(\%) = \frac{\%CH_4}{(\%CH_4 + \%CO_2)} \times 100\% \quad (9)$$

$$CO_2(\%) = \frac{\%CO_2}{(\%CH_4 + \%CO_2)} \times 100\% \quad (10)$$

To calculate the methane yield, it was determined by multiplying the biogas yield by the CH_4 content as shown in Equation 11 (Lasalewo and Syaichurrozi., 2026).

$$\text{Methane yield} = \text{Biogas yield} \times \frac{\% \text{CH}_4}{100} \quad (11)$$

Kinetics analysis

The kinetics of biogas production were evaluated using the modified Gompertz model, which describes the behavior of anaerobic microorganisms through three kinetic parameters namely, P_m (maximum biogas production), μ (maximum rate of biogas production), and λ (lag phase duration). The mathematical expression of the model is presented in Equation 12. The agreement between the model predictions and experimental biogas production data was evaluated by optimizing the model parameters through minimization of the sum of squared errors (SSE), as presented in Equation 13. Model performance was further evaluated using the coefficient of determination (R^2), calculated with Microsoft Excel based on Equation 14 (Lasalewo and Syaichurrozi, 2026).

$$P_t = P_m \cdot \exp\left(-\exp\left(\frac{\mu e}{P_m}(\lambda - t) + 1\right)\right) \quad (12)$$

$$\text{SSE} = \sum_{i=1}^n (P_i - \hat{P}_i)^2 \quad (13)$$

$$R^2 = 1 - \frac{\sum_{i=1}^n (P_i - \bar{P}_i)^2}{\sum_{i=1}^n (P_i - \bar{P}_i)^2} \quad (14)$$

where: P_t is the cumulative biogas yield at time t (mL/g TS), P_m is the maximum biogas production potential (mL/g TS), μ is the maximum production rate (mL/g TS/day), λ is the lag phase duration (days), e is Euler's constant (2.718), and t is the digestion time (days). P_i , $\hat{P}_i$, and $\bar{P}_i$ are represent the experimental data (mL/g TS), the modelled data (mL/g TS), and the mean of the experiment data (mL/g TS), respectively.

RESULT AND DISCUSSION

Biogas production

The results of the biogas production experiments using liquid waste from BTSW, with varying urea additions via both daily and mixed

anaerobic digestion methods, yielded significant biogas yields over a 15-day period. Based on the experimental results, daily and cumulative yield profiles were created, as shown in Figure 2 (a and b). Figure 2a shows the data on the increase in daily biogas yield for each urea addition variation. Since urea serves as an additional nitrogen source, its supplementation influences the carbon-to-nitrogen (C/N) ratio, which is a key factor governing microbial activity and biogas production during anaerobic digestion (Nuhu et al., 2013). Since carbon is consumed more rapidly than nitrogen during anaerobic digestion, maintaining an appropriate C/N ratio is essential for efficient biogas production (Tanimu et al., 2021). As can be seen in Figure 2a, the data fluctuated from day 7 to day 10, with the peak values for each urea addition level reaching 23.85, 21.54, 66.16, 42.16, and 32 mL/g-TS_{substrate}, respectively, on days 8, 7, 7, 10, and 9 with urea additions of 0 mg/L, 100 mg/L, 150 mg/L, 200 mg/L, and 250 mg/L. At an addition of 0 mg/L, the daily biogas yield was low due to the absence of urea, which resulted in nitrogen levels insufficient to meet the microorganisms metabolic needs, as nitrogen acted as a limiting factor. Furthermore, at a 100 mg/L addition, the daily biogas yield remained in the low range, which can limit microbial growth during anaerobic digestion, resulting in a low substrate conversion rate for methanogenesis (Mir et al., 2016). At a 150 mg/L addition, the daily biogas yield provided an optimal nitrogen supply for anaerobic microorganisms under methanogenesis conditions. However, at 200 mg/L and 250 mg/L, the daily biogas yield decreased due to excessive urea addition, the time to reach peak production was delayed because the increased ammonia concentration inhibited the activity of methanogenic bacteria (Yang et al., 2025). However, the fluctuating increases at the peak of each variation were short-lived, lasting only a few days before declining again until the fermentation process ended. These findings are consistent with Opurum and Odibo (2018), who reported that urea supplementation enhanced biogas production by supplying sufficient nitrogen for microbial growth. However, the beneficial effect was limited to the optimum dosage, while excessive urea reduced biogas production due to inhibition of anaerobic microorganisms.

The cumulative biogas yield values for each urea addition variation are shown in Figure 2b. As can be seen in Figure 2b, the data show a

gradual increase from day 0 to day 15, with the highest cumulative biogas yield for each variation occurring on day 15 at 56.62 mL/g-TS_{substrate} for a urea addition of 0 mg/L, 65.08 mL/g-TS_{substrate} for a urea addition of 100 mg/L, 187.86 mL/g-TS_{substrate} for a urea addition of 150 mg/L, 87.93 mL/g-TS_{substrate} for a urea addition of 200 mg/L, and 73.08 mL/g-TS_{substrate} for a urea addition of 250 mg/L. The highest cumulative biogas yield at a urea addition of 150 mg/L indicates that this concentration provides an optimal C/N ratio for the activity of anaerobic microorganisms (Yuan et al., 2026). Similar results have been reported by Gopinathan et al. (2015) who observed that urea supplementation increased biogas production. This improvement was attributed to the role of urea in optimizing the C/N ratio, thereby supporting more efficient microbial activity during anaerobic digestion (Z. Yang and Han., 2013). In contrast, at urea additions of 200 mg/L and 250 mg/L, the cumulative biogas yield decreased due to excessive urea addition. These conditions may also be influenced by the use of the substrate taro starch wastewater which is characterized by high levels of carbohydrates (starch) and protein (Herawati et al., 2023). In the hydrolysis stage, these compounds are degraded into simple sugars. The resulting sugars are then converted by acidogenic bacteria into VFAs, alcohol, H₂, and CO₂. Subsequently, long-chain VFAs are converted into acetate, H₂, and CO₂, which are utilized by methanogenic microorganisms to produce biogas a process that has a relatively longer regeneration time, approximately 5–16 days (Prayitno and Rulianah., 2022). Therefore, daily and cumulative biogas yields generally do not reach their maximum values at the beginning of fermentation, which explains why the daily and cumulative biogas yield data from this study did not reach their peaks until days 7, 8, 9, and 10 of the process.

Methane content

Table 3 summarizes the GC-TCD analysis of biogas composition on day 9. Among the treatments, methane content peaked at 85.82% with the addition of 100 mg/L urea, whereas the smallest value (34.36%) was found at 150 mg/L urea. The addition of urea at a concentration of 100 mg/L can provide the nitrogen required for methanogenic microorganisms, thereby optimizing methane formation. Higher urea concentrations can reduce methane production because excessive urea can be converted into ammonia, which inhibits methanogenic microorganism. Ona et al (2022) also reported that nitrogen supplementation increased methane content, where as excessive urea addition could inhibit methanogens due to ammonia accumulation. In this study, the effect of urea addition on methane content did not show a linear pattern. This was also influenced by the conditions of the anaerobic digestion process. On the 9th day the reactor supplemented with urea concentration of 150 mg/L had the lowest pH at (5.85). Because methanogenic bacteria are highly sensitive to acidic conditions, a pH lower than 6 can significantly inhibit their activity and consequently decrease methane production. The optimal pH range for methanogenic activity and biogas production is 6.8–7.2 (Humphrey et al., 2024). A decline in pH is commonly associated with the accumulation of organic acids caused by organic overloading, whereas prolonged digestion in batch systems may further reduce methane production due to nutrient depletion and the accumulation of inhibitory metabolites (Vasmara et al., 2023).

In Figure 3 the highest cumulative methane yield was obtained with the addition of 150 mg/L of urea at (54.39 mL-CH₄/g-TS_{substrate}). Meanwhile, in Figure 4 the highest daily methane yield was obtained with the addition of 250 mg/L of

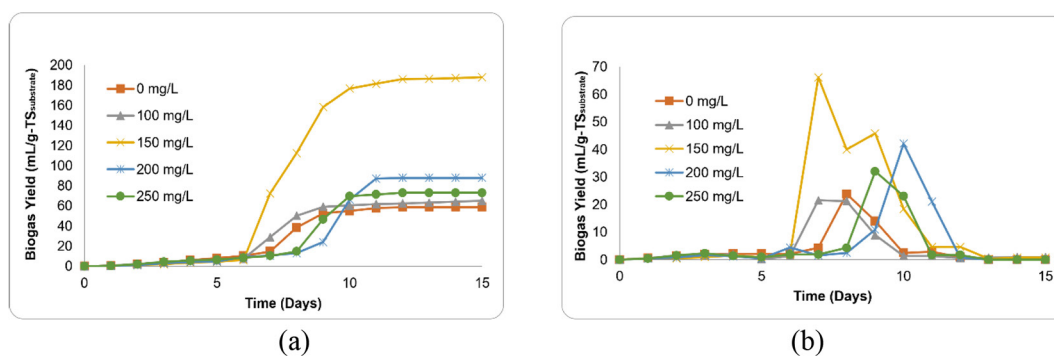
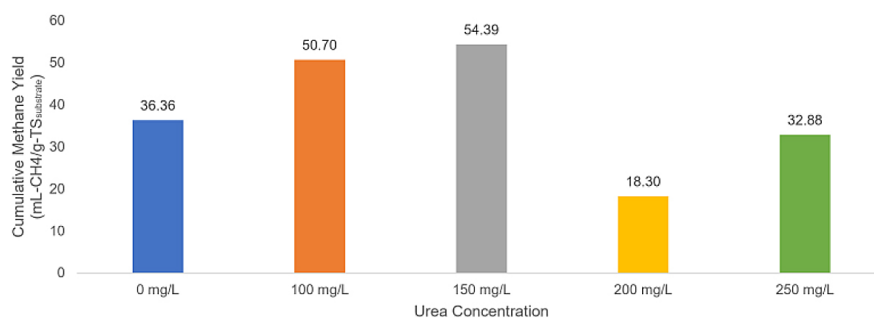
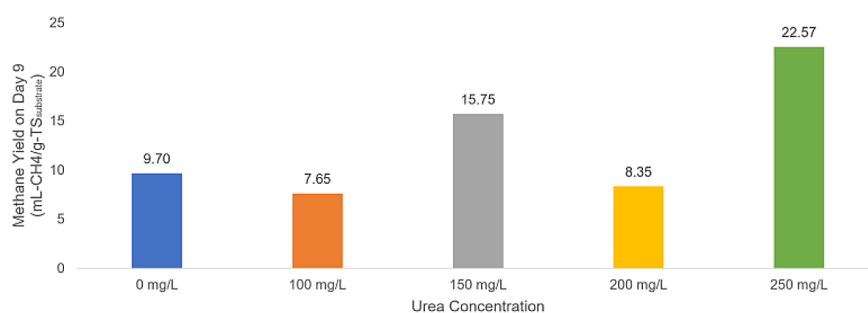


Figure 2. AD of BSW at various urea addition: (a) daily biogas yield; (b) cumulative biogas yield

Table 3. Methane content on 9th day

Urea concentration (mg/L)	Day	CH ₄ (%)	CO ₂ (%)
0	9	69.31	30.69
100		85.82	14.18
150		34.36	65.64
200		76.50	23.50
250		70.54	29.46

**Figure 3.** Cumulative methane yield on day 9 at different urea concentrations**Figure 4.** Daily methane yield on day 9 at different urea concentrations

urea at (22.58 mL-CH₄/g-TS_{substrate}). The methane yield results are influenced by the CH₄ content and the volume of biogas produced, so urea supplementation at an appropriate dose can improve the efficiency of substrate conversion into methane (Yuan et al., 2026).

pH and VFAs

In biogas production, pH is a factor that significantly influences the final outcome of the process, as methanogenic bacteria are highly sensitive to acidic environments, which can inhibit their growth and biogas production during anaerobic digestion (Mir et al., 2016). The pH data from the 15-day show a gradual decrease for each urea addition level, as illustrated in Figure 5a. The pH decreased throughout the 15 day of digestion period in all treatments. Initial pH values of approximately 7.0 declined to 6.38, 5.85, 5.83, 6.04, and 5.96 in the reactors supplemented with

0, 100, 150, 200, and 250 mg/L urea, respectively. This reduction reflects the formation and accumulation of VFAs during anaerobic digestion. Rapid carbohydrate degradation in BTSW may explain the observed reduction in pH, as the resulting simple sugars are further metabolized into VFAs during acidogenesis. The accumulation of VFAs lowers the pH, particularly during the early stage of AD. Although AD can proceed within a pH range of 5.5–8.5, methanogenic microorganisms are highly sensitive to pH fluctuations and exhibit optimum activity under near-neutral conditions (approximately pH 7). Methanogenic activity is also disrupted if the pH drops below 6.3 or rises above 7.8, as this can lead to failure in biogas production (Prasanna et al., 2024). Similarly, methane production has been reported to be highly dependent on pH and is generally most favorable under near-neutral conditions, with an optimum pH range of approximately 6.5–7.0 (Jafari et al., 2019). Although an initial pH of 7.0 is considered

favorable for methanogenesis, previous studies have shown that excessively high initial pH values (7.5–8.0) can impair methanogen adaptation and consequently reduce methane production efficiency (Syaichurrozi et al., 2026). It can be concluded that the addition of 150 mg/L of urea represents the optimal treatment, even though the pH value on day 15 fell within the lower range of the methanogenesis optimum. This condition is attributed to the fact that the highest biogas yield was achieved on day 7, meaning that methanogen activity proceeded more optimally before the pH began to decline that could have started to inhibit methanogen activity.

VFA and pH are interrelated, a high VFA concentration can cause the pH to decrease, and vice versa (Morales et al., 2018). As shown in Figure 5b, the VFA concentration varied with urea addition. At 0 mg/L urea, the concentration increased from 5039.63 to 6597.33 mg acetic acid/L between days 3 and 15, reflecting rapid acidogenesis that led to VFA accumulation and lower pH values. With the addition of 100 mg/L urea, the VFA concentration decreased compared to the previous treatment, reaching 5314.52 mg-acetide acid/L on day 15, indicating an increase in methanogenic microorganism activity, though it was not yet optimal. With the addition of 150 mg/L urea, the VFA concentration decreased compared to the previous treatment, reaching 4398.22 mg of acetic acid/L on day 15 indicating that the acidogenesis process produced VFA that could be immediately utilized by methanogenic bacteria as a substrate for methane formation. These results indicate that a balanced conversion of VFAs supported maximum biogas production, whereas inhibition of methanogenic activity promoted VFA accumulation (Wang et al., 2023). However, VFA concentrations increased at urea additions of 200 and 250 mg/L, reaching 4673.11 mg acetic acid/L on day 15, suggesting that excessive urea

inhibited the conversion of VFAs into methane. Therefore, excessive VFA accumulation should be avoided to sustain methane production during anaerobic digestion (Ghasimi et al., 2009). Therefore, the balance between the acidogenesis and methanogenesis stages is crucial to the success of the anaerobic digestion process. Excessive VFA accumulation has the potential to cause inhibitory (toxic) conditions if it hinders the anaerobic digestion process. Additionally, urea added as a nitrogen source can break down into ammonia at a rate of 34–80% during the hydrolysis stage (Prasanna et al., 2024). Excessive urea supplementation may promote ammonia accumulation, thereby inhibiting the activity of methanogenic microorganisms. It can be concluded, the VFA concentration at urea addition of 150 mg/L is the most ideal level because VFA accumulation is relatively low and yields the highest biogas production. Although other variations also produce biogas, they cannot yet be categorized as either ideal or toxic levels because they only result in a decrease in VFA efficiency, while the pH remains within a safe range.

TS, TSS, and TDS removal

TS removal efficiency was evaluated for each urea concentration, and the results are presented in Figure 6a, at 0 mg/L urea, the value was 16.67% at 100 mg/L urea, the value was 26.28% at 150 mg/L urea, the value was 32.69% at 200 mg/L urea, the value was 23.08% and at 250 mg/L urea, the value was 10.26%. Of these five data points, the maximum TS removal value was obtained at 150 mg/L urea, indicating that total solids removal was most effective at this concentration because there was sufficient nitrogen for the growth of anaerobic microorganisms during the hydrolysis stage. For urea concentrations of 0 mg/L and 100 mg/L, low removal

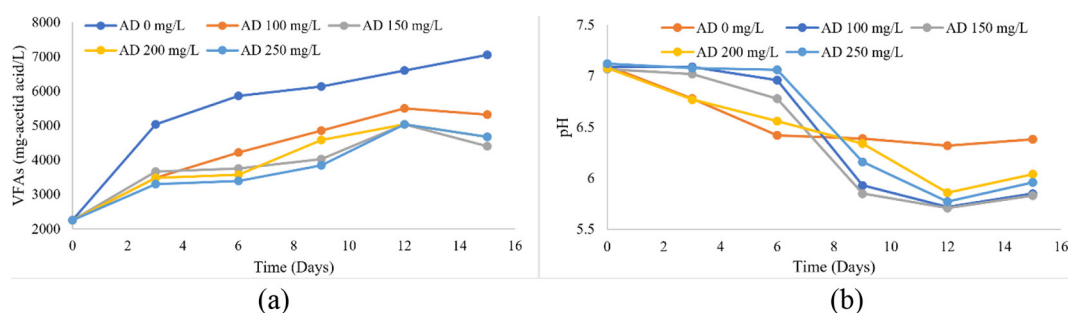


Figure 5. Profiles of: (a) pH liquid; (b) volatile fatty acids (VFAs)

values were observed because the amount of urea added was insufficient for optimal processing by methanogenic bacteria, resulting in only partial conversion. Meanwhile, urea additions of 200 mg/L and 250 mg/L led to the formation of ammonia during the hydrolysis stage due to excess urea, which can reduce the activity of methanogenic microorganisms.

The influence of urea concentration on TSS removal efficiency is presented in Figure 6b, at 0 mg/L urea, the value was 13.33% at 100 mg/L urea, the value was 26.67% at 150 mg/L urea, the value was 33.33% at 200 mg/L urea, the value was 20% and at 250 mg/L urea, the value was 10%. Of these five data points, the maximum TSS removal value was obtained at 150 mg/L urea, indicating that the mixing of the substrate-inoculum mixture and urea occurred optimally; this condition is supported by the previously obtained biogas yield and VFA concentration results. TDS removal was also calculated based on the urea addition in each variation, as shown in Figure 6c, at 0 mg/L urea, a value of 19.75% was obtained at 100 mg/L urea, a value of 25.93% at 150 mg/L urea, a value of 32.10% at 200 mg/L urea, a value of 25.93% and at 250 mg/L urea,

a value of 10.49%. Of these five data points, the maximum TDS removal value was obtained at 150 mg/L urea, indicating that at this concentration, the methanogenic bacteria achieved a balance between the rates of VFA production and consumption, which supports methanogenesis. The lower TSS and TDS removal results observed at urea concentrations of 0, 100, 200, and 250 mg/L indicate that methanogenic microbes were less efficient at converting the substrate; furthermore, urea concentrations that were either too low or too high could lead to VFA accumulation, resulting in ammonia production.

Therefore, it can be concluded that since total solids are a combination of suspended solids and dissolved solids, it can be demonstrated that changes in the TS value are influenced by both (Kiplagat et al., 2022). Upon adding 150 mg/L of urea, a TS value of 32.69% was obtained, supported by a TSS value of 33.33% and a TDS value of 32.10%. Although both fall within the 30% range, the TSS value contributes more significantly to total solids removal than TDS. Based on these findings, it can be concluded that the addition of 150 mg/L of urea represents the conditions most conducive to overall solids removal.

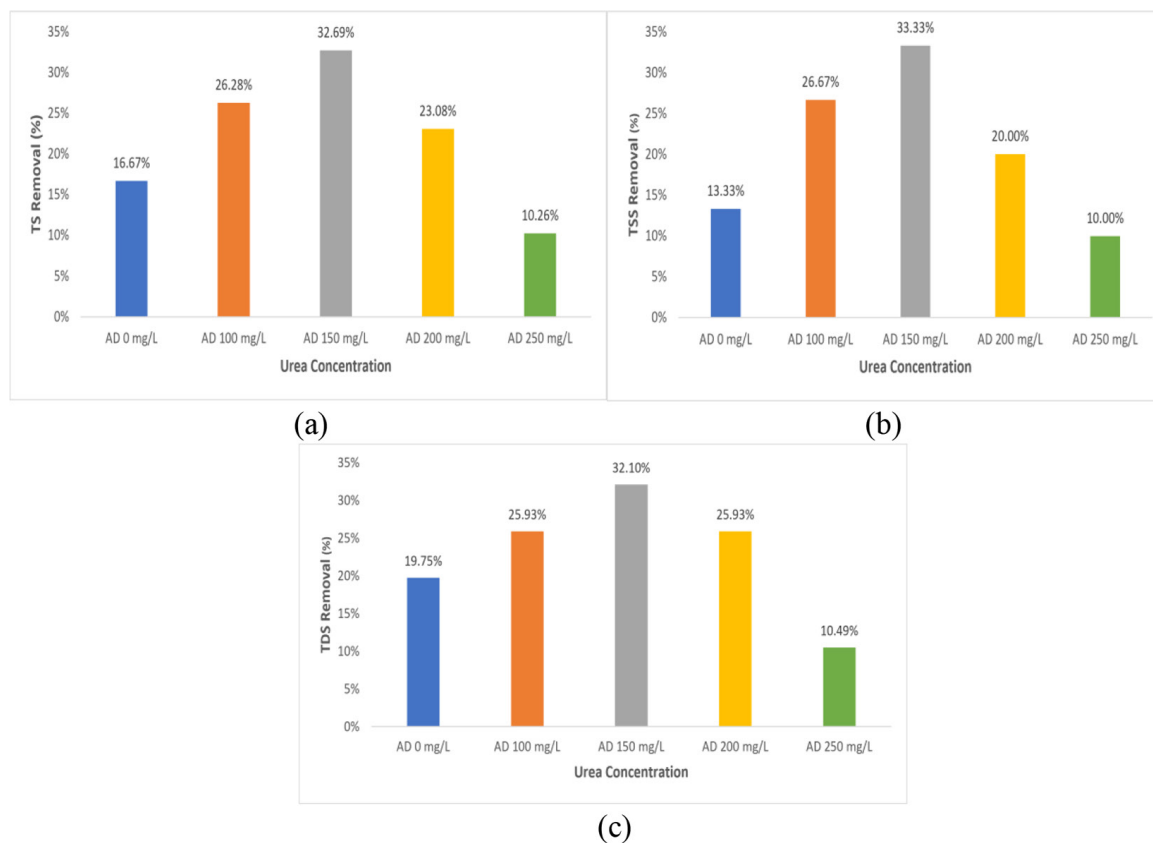


Figure 6. Organic and solid removal efficiency: (a) TS removal; (b) TSS removal; (c) TDS removal

Kinetic analysis

The kinetic behavior of biogas production was evaluated using the modified Gompertz model, with the estimated parameters presented in Table 4. The maximum biogas production value (P_m) increased with the addition of urea at 0, 100, and 150 mg/L, reaching 56.98, 63.57, and 187.90 mL/g-TS_{substrate}, respectively. Then, the value of P_m decreased with the addition of urea at 200 and 250 mg/L to 88.95 and 73.66 mL/g-TS_{substrate}. These results indicate that the addition of urea up to 150 mg/L is most optimal for enhancing biogas production potential. Higher P_m value indicates a greater potential for biogas production (Syaichurrozi and Rusdi., 2020).

The maximum biogas production rate (μ) increased with the addition of urea at 0, 100, and 150 mg/L, reaching 21.37, 24.98, and 61.37 (mL/g-TS_{substrate}/day), respectively. At urea concentrations of 200 and 250 mg/L, the μ values decreased to 49.52 and 37.04 (mL/g-TS_{substrate}/day). These results indicate that adding urea up to 150 mg/L can enhance the biogas production rate. The increase in μ value shows that biogas formation occurs rapidly to achieve maximum production (Syaichurrozi and Rusdi., 2020).

The lag phase constant (λ) represents the time required for anaerobic microorganisms to adapt

before initiating biogas production (Wang et al., 2024). The lag phase duration (λ) with the addition of urea at 0, 100, 150, 200, 250 mg/L was 6.28, 5.8, 5.96, 8.51, and 7.63 days, respectively. Among the treatments, microbial adaptation occurred most rapidly at 100 mg/L urea (5.80 days), while the longest adaptation period was observed at 200 mg/L (8.51 days). These findings indicate that an appropriate urea concentration supports microbial growth and shortens the lag phase, whereas higher urea concentrations prolonged microbial adaptation.

The comparison between the experimental results and the model predictions is presented in Figure 7. The addition of urea concentrations from 0–250 mg/L resulted in R^2 values of 0.9844, 0.9922, 0.9979, 0.9820, and 0.9822, respectively, with error values of 1.56, 0.78, 0.21, 1.8, and 1.78%, respectively. Among the treatments, 150 mg/L urea produced the best model fit, with an R^2 of 0.9979 and an error of 0.21%. In contrast, the 200 mg/L treatment showed the weakest fit, with an R^2 of 0.9820 and an error of 1.80%. The modified Gompertz model is capable of predicting biogas production potential effectively, as indicated by the coefficient of determination value (R^2 more than 0.90) (Syaichurrozi and Rusdi., 2020). Zou et al. (2021) also reported that the modified Gompertz model accurately fitted experimental methane production data.

Table 4. Kinetic constant values for modified Gompertz model

Urea concentration (mg/L)	P_m (mL/g-TS _{substrate})	μ (mL/g-TS _{substrate} /day)	λ (days)	R^2	Error (%)
0	56.98	21.37	6.28	0.9844	1.56
100	63.57	24.98	5.80	0.9922	0.78
150	187.90	61.37	5.96	0.9979	0.21
200	88.95	49.52	8.51	0.9820	1.80
250	73.66	37.04	7.63	0.9822	1.78

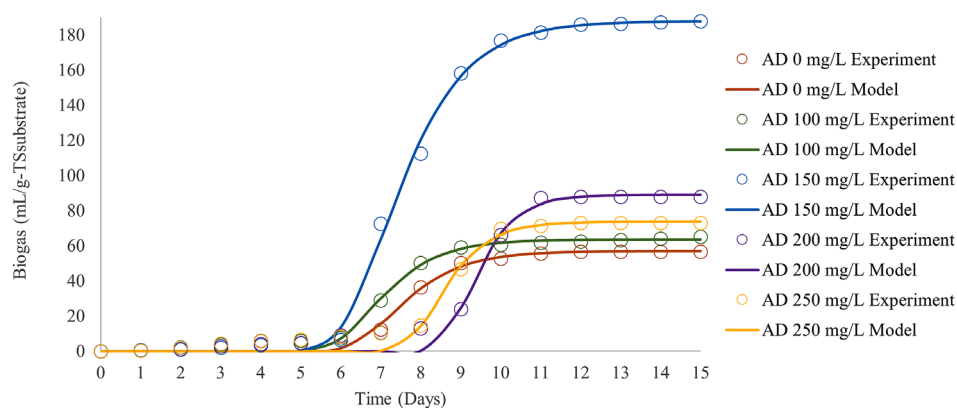


Figure 7. Plotting results using the modified Gompertz model

CONCLUSIONS

The findings confirm that urea dosage significantly affects AD performance when BTSW is used as the substrate. Among the five conditions examined, the 150 mg/L urea treatment consistently performed best, delivering the greatest cumulative biogas yield (187.86 mL/g-TS_{substrate}), the greatest cumulative methane yield (54.39 mL-CH₄/g-TS_{substrate}), the strongest solids-removal results (TS 32.69%, TSS 33.33%, TDS 32.10%), and the best kinetic fit under the modified Gompertz model ($R^2 = 0.9979$; error = 0.21%; Pm = 187.90 mL/g-TS_{substrate}). These results suggest that supplying 150 mg/L of urea establishes a nitrogen-to-carbon balance favorable to methanogenic activity and overall biogas formation. Interestingly, although the 100 mg/L treatment produced the highest methane content (85.82%), the highest cumulative biogas and methane yields were achieved at 150 mg/L despite its considerably lower methane content (34.36%). This suggests that nitrogen at 0–100 mg/L was still insufficient to fully meet microbial metabolic demand, whereas excessive nitrogen at 200–250 mg/L may have triggered ammonia inhibition. Because this study employed a laboratory-scale batch reactor using a constant proportion of substrate and inoculum, the findings cannot be directly extrapolated to large-scale or continuous anaerobic digestion systems. In addition, the effects of temperature and hydraulic retention time were not evaluated. Future work should therefore extend testing to larger operational scales and a broader range of process parameters, and further investigate BTSW's viability as a feedstock for sustainable waste-management systems including comparisons across different urea dosing strategies to identify the most effective and stable approach for maximizing biogas and methane recovery.

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