JOURNAL OF RENEWABLE ENERGY AND SMART GRID TECHNOLOGY

Vol. 21, No. 2, July-December 2026



Biogas Production by Co-Digestion between Food Waste and Watermelon Rind on Biochemical Methane Potential and Pilot-Scale

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Article info:

Received: 22 April 2026
Revised: 25 July 2026
Accepted: 25 August 2026
DOI:
10.69650/rast.2026.267392

Keywords:

Anaerobic Digestion
Food Waste
Watermelon Rind
Biogas Production
Biochemical Methane Potential

ABSTRACT

This research aims to study the biogas production potential from anaerobic co-digestion of food waste (FW) and watermelon rind (WR) in biochemical methane potential (BMP) batch scale and semi-continuous pilot scale. At first part, BMP assay was conducted under mesophilic conditions (35 ± 2 °C) for 30 days. Substrate mixture between FW and WR were digested at different proportions of 1:1, 1:3, and 3:1. Cow manure was used as inoculum and substrate-to-inoculum ratio (SIR) was fixed at 1:2 on a VS basis. All conditions of co-digestion, the FW1:WR3 ratio achieved the highest cumulative biogas production (812.18 ± 24.42 NmL $g^{-1}VS_{add}$) and methane content (62.0 ± 2.0%). At the second part, based on the findings obtained from the BMP tests. The FW1:WR3 ratio was test in a pilot-scale semi-continuous anaerobic digester (total volume: 1,000 L; working volume: 800 L) at an organic loading rate (OLR) of 1 $kgCODm^{-3}d^{-1}$ and a hydraulic retention time (HRT) of 30 days. The system achieved stable biogas production, with a cumulative yield of 8,322.86 L and an average methane concentration of approximately 57%. The VFA/ALK ratio remained below 0.30, indicating stable process performance. In summary, the results demonstrated that co-digestion of FW and WR has potential for decentralized renewable energy generation and sustainable organic waste management in the future.

1. Introduction

The management of organic waste becomes a critical environmental challenge, particularly in urban areas where intensive food consumption generates large quantities of food waste [1, 2]. In Thailand, municipal solid waste generation reached approximately 26.95 million tons in 2024. Food waste accounted for about 10.24 million tons, or approximately 38%. This amount of food waste has made it the largest fraction of the waste stream [3]. Improper disposal of food waste has resulted in significant environmental impacts, including greenhouse gas (GHG) emissions, leachate contamination, and odor-related nuisances. These issues adversely affect public health and community well-being. When food waste was disposed of in landfills, anaerobic decomposition occurred. This process generated substantial amounts of methane (CH_4), a greenhouse gas with a global warming potential approximately 28 times higher than that of carbon dioxide (CO_2). The U.S. Environmental Protection Agency (EPA, 2023) reported that food waste contributed nearly 58% of fugitive methane emissions from municipal landfills [4]. Recent studies in Thailand have consistently shown that food waste accounts for approximately 39% of municipal solid waste. Landfill disposal largely remains a major contributor to national GHG emissions due to limited waste segregation and inefficient landfill gas recovery systems [5]. National inventories indicated that the waste sector accounted for approximately 22,173 $ktCO_2e$ in 2022. This figure represented to 5.75% of Thailand's total greenhouse gas emissions [6]. Similar trends have been observed in other developing countries, where inefficient food waste management significantly increases landfill-related methane emissions. Anaerobic digestion (AD) has been widely recognized as an effective and sustainable strategy for food waste valorization. AD enables both waste stabilization and renewable energy production. Through microbial degradation under oxygen-free conditions, AD converts organic matter into biogas, primarily composed of methane and carbon dioxide. Life-cycle assessment (LCA) studies consistently show that anaerobic digestion (AD) achieves higher energy recovery and lower greenhouse gas emissions than conventional disposal methods such as landfilling and incineration [7]. Moreover, biogas can be directly utilized for heat and electricity generation or upgraded to biomethane for transportation and grid injection.

Among the various feedstocks utilized for anaerobic digestion, food waste is increasingly favored due to its highly organic composition, rapid biodegradability, and superior biochemical methane potential [8]. Watermelon rind is a highly promising lignocellulosic residue that is generated in substantial quantities as agro-industrial waste [9]. It serves as a highly biodegradable substrate for anaerobic digestion. The high moisture content and abundance of readily biodegradable organic matter make it a highly suitable feedstock for anaerobic digestion and subsequent biogas generation [10]. While both substrates exhibit favorable characteristics, efficient methane

production also requires high biodegradability and sustains reactor stability throughout the digestion process. This further enhances its contribution to energy sustainability [11]. Mono-digestion of food waste exhibits high biodegradability and methane production potential. However, it is often associated with process instability. This is mainly attributed to rapid hydrolysis, the accumulation of volatile fatty acids, and limited buffering capacity. As a result, the pH may decline, methanogenic activity can be inhibited, and the digestion process may ultimately fail [12]. To overcome these challenges, co-digestion with complementary substrates has been widely adopted. This approach has been reported to enhance alkalinity, thereby promoting stable anaerobic digestion performance [13]. It also contributes to the stabilization of microbial consortia, thereby increasing methane yields. In this context, watermelon rind has emerged as a potential co-substrate for anaerobic digestion [14]. As an abundant agricultural by-product in Thailand, watermelon rind possesses high moisture content, low lignin concentration, and a carbohydrate-rich composition. These physicochemical characteristics facilitate rapid hydrolysis and acidogenesis. Although direct studies on methane production from watermelon rind remain limited, related research has demonstrated its strong fermentability and suitability for bioenergy applications. Elerakey et al. reported efficient biohydrogen production from watermelon peels, achieving a maximum yield of 108.3 mL H_2 g^{-1} VS under optimized anaerobic conditions [15]. Previous studies on anaerobic co-digestion further highlight the synergistic effects of watermelon rind when co-digested with food waste. A comprehensive review by Ibro et al. reported that co-digestion of food waste with fruit residues and agricultural by-products enhanced methane yields by 20–45%. It also improved volatile solids removal by up to 30%. These improvements were mainly attributed to better nutrient balance and enhanced buffering capacity [16]. Similar findings were observed by Al-Huqail et al. who demonstrated that anaerobic digestion of fruit peels, yielded 670.30 – 1422.76 mL of biogas and methane content 62.21 ± 0.13%. This process also generated digestate suitable for agricultural reuse. These results support circular-economy principles [17]. To maximize the benefits of co-digestion, it is essential to optimize key operational parameters, particularly the organic loading rate (OLR). OLR directly governs the daily input of organic matter into the digester and strongly influences microbial metabolism, VFA accumulation, and methane production. Several studies have demonstrated that increasing OLR within an optimal range enhances biogas yield, whereas excessive loading leads to acidification and process instability. Zhang et al. reported that increasing OLR from 2.0 to 4.5 g VS L^{-1} d^{-1} during thermophilic co-digestion significantly enhanced methane yield and stabilized methanogenic populations [18]. Conversely, studies on the anaerobic digestion of slaughterhouse and swine waste have reported that exceeding optimal organic loading rate (OLR) thresholds can lead to rapid accumulation of volatile fatty acids (VFAs), pH decline, and subsequent inhibition of methanogenic activity. These observations highlight the importance of maintaining an appropriate OLR balance, particularly in co-digestion systems involving highly biodegradable and carbohydrate-rich substrates such as food waste and watermelon rind.

Therefore, the present study aims to evaluate the biogas production potential of food waste co-digested with watermelon rind under BMP and pilot-scale anaerobic digestion conditions. The primary objective is to identify optimal operational conditions, with particular emphasis on organic loading rate, that maximize energy recovery while maintaining process stability. The findings are expected to contribute to the development of efficient, small-scale anaerobic digestion systems. These systems are suitable for community-level waste management and decentralized renewable energy production in Thailand.

2. Experimental details

The experiment consisted of two parts: biochemical methane potential (BMP) tests and semi-continuous operation.

2.1 BMP test

2.1.1 Substrate and inoculum preparation for batch scale

Prior to the BMP assays, food waste (FW) and watermelon rinds (WR) were collected from restaurants and fruit shops in communities surrounding Maejo University. Prior to use, the substrates were thoroughly homogenized to improve biodegradability. The experimental design evaluated three food waste to watermelon rind (FW: WR) mixing ratios (FW1 : WR1 , FW1 : WR3 , and FW3 : WR1 , w/w) under batch anaerobic digestion conditions. The inoculum was prepared by mixing dried cow manure obtained from the Faculty of Animal Science and Technology, Maejo University, with water at a 1:1 (w/w) ratio. Both substrates and inoculum were stored at temperatures below 4 °C prior to experimentation. The inoculum was pre-fermented under anaerobic conditions for 7 days and subsequently passed through a sieve with an aperture size not exceeding 10 mm to ensure uniform particle size prior to use. The substrate and inoculum were characterized based on total solid (TS), volatile solid (VS), pH, and VFA/Alk ratio. All analytical procedures were performed in accordance with standard method APHA 2005 shown in Table 1.

2.1.2 Experimental setup for BMP Batch assays

The experiment assessed the biogas and methane production potential of FW and WR following an adapted procedure based on the VDI 4630 standard. The study emphasized the influence of substrate proportion on biogas yield. A substrate-to-inoculum ratio (SIR) of 1:2 based on add volatile solids (gVS_{add}) was applied. The BMP assays were conducted in 1,000 mL serum reactors with a working volume of 400 mL, leaving approximately 600 mL of headspace for biogas accumulation.

Table 1 Characteristics of various substrates and inoculum.

Parameter	Cow manure (Inoculum)	Food waste (Substrate 1)	Watermelon rind (Substrate 2)
Chemical oxygen demand, COD (g/L)	38.29 ± 12.64	195.33 ± 11.73	28.96 ± 4.06
Total solid, TS (g/L)	47.12 ± 14.92	154.59 ± 9.21	28.91 ± 4.06
Volatile solid, VS (g/L)	27.35 ± 9.12	149.10 ± 8.98	24.44 ± 3.42
pH	7.00 ± 0.30	5.00 ± 0.40	5.40 ± 0.30
Volatile fatty acid to Alkalinity ratio, VFA/ALK	0.10± 0.05	0.45 ± 0.15	0.35 ± 0.10

The initial pH was adjusted to 7.0 ± 0.1. Anaerobic conditions were established by flushing both the liquid phase and headspace with nitrogen gas. The reactors were then sealed using rubber stoppers and aluminium crimps. A blank control containing only inoculum was included to correct for the background biogas production originating from the inoculum. This ensured that the reported biogas and methane yields reflected only the contributions from the added substrates. All digesters were maintained under mesophilic conditions (35.0 ± 2 °C) using a thermostatic water bath equipped with a water pump to ensure uniform temperature distribution [19] for 30 days.

2.1.3 Analytical methods of BMP

The physicochemical parameters analyzed in this study included chemical oxygen demand (COD), total solids (TS), volatile solids (VS), pH, and the volatile fatty acid-to-alkalinity (VFA/ALK) ratio. All analyses were performed in triplicate according to the Standard Methods for the Examination of Water and Wastewater (APHA, 2017). This analysis is essential for identifying the suitability and loading of substrates for optimal methane production [20]. The parameters were measured in three stages: Stage 1, analysis of the substrate and inoculum; Stages 2 and 3, analysis before entering the system and after exiting the system for all ratios. The volume of biogas produced was measured daily using a glass syringe, measured at the same time each day for data accuracy until the cumulative biogas production rate was constant. The biogas yield and biochemical methane potential were calculated in units of NmL $g^{-1}VS_{add}$ at standard temperature 0 °C. At atmospheric pressure 1 atm as shown in Equation (1).

$$BMP = \frac{\%CH_4 \times V_{STP,Biogas}}{m_{Substrate(gVS_{add})}} \quad (1)$$

Where $\%CH_4$ is the percentage of methane gas in the biogas, V_{STP}, Biogas is the volume of biogas at standard temperature 0 °C at atmospheric pressure 1 atm (NmL), $m_{Substrate}$ is the mass of substrate added to the system (gVS_{add}). After digestion, samples from each mixing ratio were collected for biogas analysis. Biogas composition was measured every 7 days using a GFM 406 series gas composition analyzer (Gas Data, United Kingdom) [21]. Based on the best results from the BMP tests which ratio has highest yield of biogas cumulative and methane composition was used to test in semi-continuous experimental.

2.2 Experimental semi-continuous pilot scale

As shown in Fig. 1, the pilot-scale semi-continuous completely stirred tank reactor (CSTR) biogas system comprised two main units.

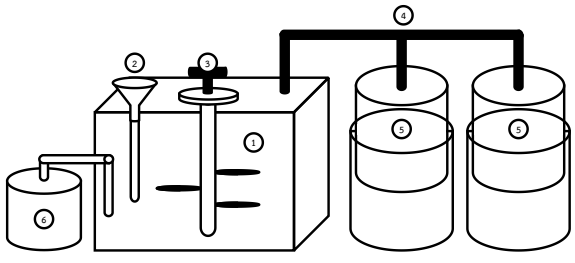


Fig. 1 Semi-continuous completely stirred tank reactor pilot scale (1) Reactor (2) Feeding inlet (3) Mechanical agitator (4) Gas pipeline (5) Biogas storage tank (6) Overflow tank.

2.2.1 Inoculum and substrates operation for semi-continuous pilot scale

The inoculum was anaerobic sludge collected from a community-scale biogas digester co-digesting cow manure and food waste. Before the experiment, the inoculum was pre-incubated at room temperature for 7 days to stimulate microbial activity and minimize background biogas production. Food waste and watermelon rinds collected from restaurants and fruit shops in Maejo area in Chiang Mai, Thailand, were manually sorted to remove non-biodegradable materials (e.g., bones, chopsticks, fruit peels, and other unsuitable items) prior to daily feeding into the digester. The reactor was operated at an organic loading rate (OLR) of 1 kg COD m^{-3} day^{-1} with a hydraulic retention time (HRT) of 30 days. The substrate ratio was determined based on the optimal results obtained from the previous batch-scale trials. To minimize variations in substrate moisture content, the daily food waste and watermelon rind were fed into the reactor within 1 h of collection.

2.2.2 Analytical methods of semi-continuous pilot scale

Daily analysis of parameters related to substrate input and digestate, including COD, pH, VFA/ALK, VS, and TS is performed at least every 5 days to assess the efficiency of dissolved organic matter removal and system stability during operation.

3. Results and discussion

3.1 Characteristics of substrate and inoculum

The chemical characteristics of the substrates are shown in Table 1. FW exhibited a higher COD (195.33 ± 11.73 g/L) than watermelon rind, which had a COD of 28.96 ± 4.06 g/L. Previous studies reported that food waste from municipal and industrial sources generally exhibited high organic loading. The COD values ranged from approximately 150 to 300 g/L. In contrast, fruit peel residues typically showed COD concentrations below 50 g/L. This was due to their lower organic density and higher moisture content [17]. These compositional differences suggest that the two substrates may exhibit distinct degradation kinetics and contribute differently to the anaerobic digestion process [16]. The high COD in food waste is primarily attributed to high concentrations of carbohydrates, proteins, and lipids. COD represents the bioavailable organic matter for microbial metabolism. Therefore, substrates with higher COD exhibit greater potential for biogas production. This is valid provided that operational parameters such as OLR, pH, and alkalinity are optimized.

However, while such high organic matter supports substantial methane potential, it may also increase the risk of rapid acidification if not properly balanced during digestion [22, 23]. Food waste exhibited high TS and VS contents of 154.59 ± 9.21 and 149.10 ± 8.98 g L^{-1}, respectively. The small difference between these values indicating that the solids were predominantly composed of biodegradable organic matter. The VS/TS ratio was approximately 0.96, indicating that food waste contained a high proportion of readily biodegradable substrates. Such high VS levels are generally associated with enhanced methane production potential, as volatile solids represent the organic fraction available for conversion to biogas by anaerobic microorganisms [23]. Consequently, food waste represents a highly viable feedstock for bioenergy production, due to its substantial organic load and inherent biodegradability. Therefore, combining FW with WR may provide a more balanced feedstock by integrating the high biodegradability of food waste with the dilution and stabilizing effects of fruit residues [16, 24].

In contrast, watermelon rind exhibited lower TS and VS contents, which are typical of fruit peel residues due to their high moisture content and lignocellulosic structure. Although the organic density of watermelon rind was lower than that of mixed food waste, its lignocellulosic composition resulted in slower hydrolysis. The slower degradation rate moderated acid formation and consequently reduced the risk of rapid acidification in anaerobic digestion systems [24, 25]. Consequently, the addition of watermelon rind as a co-substrate may enhance process stability by regulating substrate degradation and promoting balanced microbial activity during anaerobic digestion [26].

The initial pH values of food waste and watermelon rind were slightly acidic, with values of 5.00 ± 0.40 and 5.40 ± 0.30, respectively. Although these pH values were below the optimal range for methanogenic archaea (6.8–7.5), this initial acidity is commonly associated with substrates rich in readily fermentable carbohydrates, which are prone to rapid pre-acidification. Under these conditions, organic matter was rapidly degraded into volatile fatty acids (VFAs) via acidogenesis during the initial stages of digestion [24]. Although mildly acidic conditions promote hydrolysis and acidogenesis, excessive VFA accumulation can lead to the inhibition of methanogenic activity unless adequate buffering capacity is maintained [23, 25]. Therefore, adequate alkalinity is necessary to maintain pH stability and facilitate a balanced transition from acidogenesis to methanogenesis, thereby preventing process instability [25, 27].

The VFA/alkalinity (VFA/ALK) ratio serves as a critical indicator of the digestion stability of the substrates. Food waste exhibited a VFA/ALK ratio of 0.45 ± 0.15, whereas watermelon rind exhibited a lower ratio of 0.35 ± 0.10. Both ratios were within the range generally considered stable for the start-up phase of anaerobic digestion, as VFA/ALK ratios below approximately 0.5 signify adequate buffering capacity and a minimized risk of process inhibition [25, 27]. The higher ratio observed in food waste reflects its susceptibility to rapid acidification stemming from its high biodegradability; in contrast, the lower ratio in watermelon rind signifies a more robust acid–base balance. Co-digestion of food waste with watermelon rind mitigates organic overloading by balancing the substrate composition, which in turn catalyzes microbial metabolic pathways and ensures robust process stability alongside sustained methane yield [26, 27].

The utilized cow manure inoculum in this study demonstrated favorable characteristics for anaerobic digestion, featuring a COD concentration of 38.29 ± 12.64 g/L. This moderate organic load was sufficient to sustain microbial activity while mitigating the risk of excessive volatile fatty acid (VFA) accumulation and subsequent process instability [19, 28]. The initial pH was 7.00 ± 0.30, which falls within the optimal range for methanogenic archaea and indicates a favorable anaerobic environment for methane production. Moreover, manure-based inoculum generally provides inherent buffering capacity due to the presence of bicarbonate and ammonium ions. This helps to maintain pH stability and prevent process imbalance during anaerobic digestion [25, 29].

The inoculum also exhibited a low VFA/alkalinity ratio of 0.10 ± 0.05, indicating strong buffering capacity and stable anaerobic digestion performance. A VFA/alkalinity ratio of less than 0.2 signifies robust process stability, essential for avoiding rapid acidification and ensuring a smooth transition during the early phase of anaerobic co-digestion [25, 29]. Overall, the results demonstrate that food waste possesses superior biodegradability and methanogenic potential attributed to its high COD and VS contents, while watermelon rind acts as an effective buffering co-substrate to enhance process stability, The cow also manure inoculum exhibited favorable physicochemical properties—notably neutral pH and high buffering capacity—validating its efficacy as a robust microbial seed for anaerobic co-digestion.

3.2 Characteristics of Influent and effluent in Batch-Scale Experiment

All focused parameters are shown in Table 2. A COD removal efficiency of nearly 80% was achieved during a biochemical methane potential test. This represents high system performance, reflecting excellent substrate biodegradability, effective inoculum adaptation, and optimal anaerobic conditions. The FW1:WR3 ratio showed the highest removal efficiency at 79.61%. This finding highlights the advantages of co-digestion of food waste with watermelon rind. It helps balance the organic composition and physical properties. This, in turn, enhances microbial activity and improves the efficiency of organic matter degradation [30]. In comparison, the FW1:WR1 and FW3:WR1 ratios exhibited lower COD removal efficiencies of 67.59% and 78.04%, respectively. Higher COD removal is a key indicator of effective organic matter degradation during anaerobic digestion, as it signifies the successful conversion of both soluble and particulate organic pollutants into biogas [31]. The phenomenon in which excess organic matter leads to system acidification and reduced COD removal efficiency is well documented in anaerobic digestion. High organic loading can promote acidification, thereby limiting the efficiency of COD removal [18, 32, 33].

Table 2 Organic matter removal efficiency of batch scale.

Substrate ratio of Food waste to Watermelon rind		FW1:WR1	FW1:WR3	FW3:WR1
Chemical oxygen demand, COD (g/L)	Influent	62.91 ± 9.44	49.04 ± 7.36	76.77 ± 11.52
	Effluent	20.39 ± 2.04	10.00 ± 1.00	16.86 ± 1.69
Total solid, TS (g/L)	Influent	100.54 ± 10.05	58.29 ± 5.83	95.85 ± 9.59
	Effluent	45.00 ± 4.50	24.00 ± 2.40	43.00 ± 4.30
Volatile solid, VS (g/L)	Influent	95.27 ± 9.53	53.43 ± 5.34	91.15 ± 9.12
	Effluent	27.00 ± 2.70	13.50 ± 1.35	25.00 ± 2.50
pH	Influent	7.30 ± 0.30	8.10 ± 0.40	8.00 ± 0.30
	Effluent	7.74 ± 0.20	8.49 ± 0.20	8.13 ± 0.20
Volatile fatty acid to Alkalinity ratio, VFA/ALK	Influent	1.14 ± 0.23	1.11 ± 0.22	0.79 ± 0.16
	Effluent	0.86 ± 0.13	0.85 ± 0.13	0.65 ± 0.10

This suggests that the FW1:WR3 ratio may have provided a more favorable substrate balance, allowing more stable microbial conversion and improved organic matter stabilization. A biogas system maintains a stable pH range of 6.0–8.0 through the use of cow manure as the inoculum, which provides natural bicarbonate buffering, balanced microbial cooperation, and an appropriate feed-to-inoculum ratio. In addition, cow manure contains high levels of ammonium and alkalinity that neutralize the volatile fatty acids generated during anaerobic digestion. The underlying chemical mechanisms are presented in Equations (2) – (4).

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^- \quad (2)$$

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \quad (3)$$

When acidogenic bacteria produce volatile fatty acids (VFAs), these acids dissociate and increase the concentration of hydrogen ions (H^+) in the system. The bicarbonate ions (HCO_3^-) present in the reactor subsequently react with the released H^+ to form carbonic acid (H_2CO_3), which then dissociates into carbon dioxide (CO_2) and water (H_2O). This buffering reaction helps maintain pH stability and prevents a rapid decline in pH.

$$HCO_3^- + H^+ \rightleftharpoons H_2CO_3 \rightleftharpoons H_2O + CO_2 \quad (4)$$

As methanogenic microorganisms (methanogens) continuously consume the intermediates derived from volatile fatty acids (VFAs) for methane production, VFA accumulation within the system drops to zero. Consequently, the pH remains stable within the optimal range of 6.5–7.8, supporting the growth and activity of the microbial community in conjunction with the bicarbonate buffering system.

In terms of pH and the volatile fatty acid to alkalinity (VFA/Alk) ratio are fundamental and interdependent indicators of AD stability. These ratios are crucial for monitoring the health of methanogens which are highly sensitive to environmental fluctuations [26]. These parameters also reflect the balance between acid-forming and methane-forming microorganisms, which is essential for maintaining a stable digestion environment [25, 26]. The FW1:WR1, FW1:WR3, and FW3:WR1 ratios resulted in influent pH values ranging from 7.30 to 8.10, which are optimal for microbial activity in anaerobic digestion systems [26]. Considering the effluent pH, all substrate ratios exhibited an increase in values ranging from 7.74 to 8.49. This trend indicates that the digestion system remained stable throughout the batch experiment and developed favorable conditions for methane production. This increase further suggests that acid intermediates generated during hydrolysis and acidogenesis were effectively converted during the methanogenic phase, preventing excessive acid accumulation [22, 26]. The increase in pH reflects the consumption of volatile fatty acids by methanogenic microorganisms and the concurrent formation of bicarbonates, which enhances system alkalinity. The stable pH conditions observed across all substrate ratios likely contributed to the sustained methane production performance recorded during the digestive period. The FW1:WR1, FW1:WR3, and FW3:WR1 ratios resulted in influent VFA/ALK ratios ranging from 0.79 to 1.14. A VFA/Alk ratio greater than 0.3 to 0.4 is generally regarded as indicative of potential instability in anaerobic digestion systems, particularly under conditions of insufficient buffering capacity. Therefore, the analysis of VFA/Alk ratios should be considered together with pH trends, methane production, and overall digestion performance rather than as a standalone indicator [22, 34]. However, the VFA/Alk ratio in the substrate remained within the range of 0.65–0.86 across all effluent substrate ratios. This range is generally considered indicative of a potentially stressed or high-risk condition in anaerobic digestion systems, often associated with acid accumulation depending on substrate characteristics and operating conditions. In this study, the maintenance of favorable pH conditions despite increased VFA/Alk ratios suggests that the buffering capacity of the system remained sufficient to prevent severe acidification. Elevated VFA/ALK ratios exceeding 0.4 are commonly accepted as early indicators of process instability in anaerobic digestion. These ratios often signal impending imbalances like acid accumulation before a significant pH drop occurs. However, this threshold is not absolute, as high-loading systems, particularly those with strong buffering capacity, such as high protein-substrate digestion, can maintain stability with elevated levels. These findings suggest that increased VFA/Alk ratios did not necessarily translate into process failure under the tested co-digestion conditions [34].

In summary, these findings highlight that the optimization of substrate ratios is a key determinant of both efficient organic matter removal and enhanced methane bioconversion. These results further emphasize that the advantages of co-digestion depend not only on substrate selection, but also on achieving an appropriate mixing ratio that supports microbial balance and process efficiency [35, 36]. The results clearly indicate that the FW1:WR3 ratio provides the most favorable physiological environment for bioenergy recovery, promoting enhanced methanogenic activity and resulting in superior methane production. This favorable performance may be attributed to a more balanced substrate composition. This composition likely improved biodegradability, moderated acid accumulation, and supported more stable methanogenic conversion. These findings are consistent with previous studies, which suggest that optimizing substrate composition can improve methane production efficiency by promoting microbial interactions and maintaining process stability [32]. From a practical perspective, the use of watermelon rind as a co-substrate represents a promising strategy for improving the valorization of organic residues through anaerobic digestion.

3.3 Daily and cumulative biogas production during Batch-Scale Operation

The initial peak in daily biogas production can be attributed to the rapid hydrolysis and microbial conversion of highly biodegradable and readily accessible organic compounds, such as simple sugars and volatile fatty acids, into methane. As these readily degradable substrates become depleted, daily biogas production gradually declines because the remaining feedstock consists primarily of more complex and recalcitrant materials, such as cellulose and lignin, which require a longer time to decompose and convert into biogas [37]. Although all batch experiments followed a similar trend in daily biogas production, significant differences were observed among the substrate ratios. The FW1:WR3 ratio sustained relatively higher daily biogas production during the first two weeks of digestion, whereas the FW3:WR1 ratio showed the steepest decline after Day 10. Minor fluctuations observed during the later stage of digestion (Days 18–22) are likely associated with the slow biodegradation of complex organic matter, which continues to generate biogas at a reduced rate [39]. Cumulative biogas production increased steadily over the digestion period and reached a stable plateau after Day 25, indicating that the majority of biodegradable organic matter had been converted into biogas.

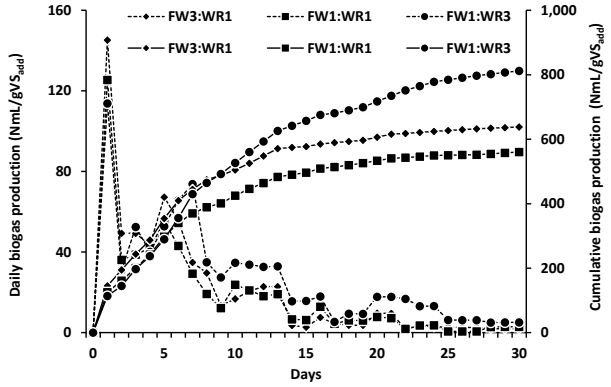


Fig. 2 Daily and cumulative biogas production.

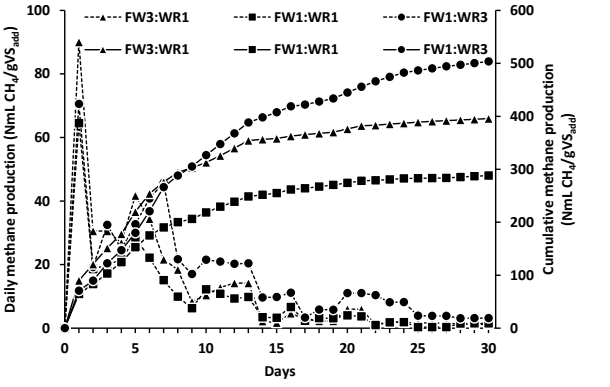


Fig. 3 Daily and cumulative methane production.

Among the tested substrate ratios, FW1:WR3 achieved the highest cumulative biogas yield (812.18 ± 24.42 NmL g^{-1} VS_{add}), followed by FW1:WR1 (638.04 ± 29.35 NmL g^{-1} VS_{add}) and FW3:WR1 (560.71 ± 24.54 NmL g^{-1} VS_{add}). Al-Huqail et al. reported a comparable biogas production, characterized by an initial phase of rapid biogas generation followed by a gradual decline, with cumulative biogas yields ranging from 670.30 to 1,422.76 mL depending on the type of fruit peel used [17]. In the present study, the cumulative biogas yield obtained from the FW1:WR3 ratio was within this reported range, further supporting the potential of watermelon rind as an effective co-substrate for anaerobic digestion. The enhanced biogas yield observed at the FW1:WR3 ratio can be attributed to the synergistic effects of co-digestion. The high moisture content and readily biodegradable carbohydrates present in watermelon rind improve substrate homogeneity, thereby facilitating microbial hydrolysis and enhancing biogas production. The co-digestion of watermelon rind with food waste likely resulted in a more balanced substrate composition, providing a favorable environment for microbial metabolism and supporting stable anaerobic digestion. It also helped reduce the risk of localized organic overloading. Conversely, the lower cumulative biogas yields observed at the FW1:WR1 and FW3:WR1 ratios may be attributed to the higher proportion of food waste. This condition could accelerate acidogenesis and promote the accumulation of intermediate metabolites, thereby inhibiting overall biogas production [39]. These results align with previous studies showing that co-digesting food waste with high-moisture fruit residues improves reactor stability and boosts biogas yield by balancing substrate characteristics and preventing acute acidification. For instance, Alves et al. observed that the anaerobic co-digestion of food waste with fruit and vegetable residues significantly increased methane yield, a result attributed to a more balanced nutrient and improved buffering capacity [40]. Similarly, Zamanzadeh et al. demonstrated that optimizing substrate combinations mitigates the operational challenges of food waste mono-digestion. This approach minimizes accumulation of volatile fatty acid and cultivates a more stable microenvironment for methanogenic communities [41]. These results demonstrate that watermelon rind provides favorable physicochemical properties for the anaerobic co-digestion of food waste, thereby enhancing process stability and overall methane yield.

3.4 Daily and Cumulative Methane Yields of Batch Scale Experiment

As shown in Fig. 3, the methane production for the FW to WR ratios of FW1:WR1, FW1:WR3, and FW3:WR1 showed distinct differences. These differences were observed in both daily methane production rates and cumulative methane production over the 30-day anaerobic digestion period.

All conditions showed a rapid increase in methane production within the first 2 to 3 days, indicating early activation of methanogenic archaea following the formation of intermediates during hydrolysis and acidogenesis [22, 34].

This early methane response suggests that readily biodegradable intermediates were rapidly converted into methane under all tested conditions. The FW1:WR1 ratio produced the highest initial peak in daily methane production, indicating rapid substrate conversion and strong early methanogenic activity. However, this peak was followed by fluctuations and a gradual decline in methane production. This pattern suggests potential instability in microbial activity or transient accumulation of inhibitory intermediates such as volatile fatty acids. Such a response may indicate that a substantial fraction of the readily degradable substrate was consumed rapidly during the initial phase, leaving less biodegradable material available for sustained methane generation. In contrast, the FW3:WR1 ratio showed moderate methane production during the initial stage but lower production during the later digestion period. This pattern might be attributed to high organic loading, which can disrupt the balance between acidogenic and methanogenic populations. High substrate concentrations might accelerate acid formation beyond the system's buffering capacity, thereby reducing methane conversion efficiency [18, 35, 36]. On the other hand, a more balanced degradation pattern appears to be more favorable for maximizing cumulative methane production over the entire digestion period [35]. Conversely, the FW1:WR3 ratio showed a more stable and sustained methane production pattern throughout the digestion period. Such a stable methane production is generally indicative of effective synchronization between acid-forming and methane-forming microbial communities [16, 24, 33]. Although the initial methane peak was lower than that observed for the FW1:WR1 ratio, methane production remained relatively stable throughout the digestion period. The reduced fluctuation in daily methane production suggests improved microbial interactions and more balanced metabolic pathways. These conditions support stable anaerobic digestion performance [34, 42]. Among the tested substrate ratios, the FW1:WR3 ratio demonstrated the best methane production performance, achieving the highest cumulative methane yield of 503.55 ± 15.14 NmL CH_4 g^{-1} VS_{add}, with a methane concentration of 62.0 ± 2.0%. These results indicate an enhanced biochemical methane potential compared with the other substrate ratios. The sustained methane generation suggests more progressive and complete degradation of biodegradable organic matter throughout the digestion period. Furthermore, the continuous increase in cumulative methane production reflects

efficient substrate conversion and stable methanogenic activity. These findings suggest that maintaining digester stability is a more important factor influencing overall methane yield than achieving high initial methane release rates [43]. These findings align with Wonyanya and Uzorka, who observed that balanced substrate mixing ratios improved biochemical methane potential by stabilizing pH and enhancing microbial activity during anaerobic digestion. The highest BMP (380 ± 14 mL CH_4 $g^{-1}VS_{add}$) were achieved using a balanced co-digestion mixture of cow dung, swine manure, and poultry droppings. In contrast, food waste alone exhibited a lower overall yield due to reactor acidification, despite its high inherent methane potential [44].

In comparison, the FW1:WR1 ratio achieved a moderate cumulative methane yield of 288.21 ± 12.61 NmL CH_4 g^{-1} VSadd, with a methane concentration of 51.4 ± 2.0%. This suggests that the FW1:WR1 ratio provided an intermediate digestion environment, although it was less favorable and balanced than that achieved by the FW1:WR3 ratio. The FW3:WR1 ratio exhibited a lower final cumulative methane yield compared with the FW1:WR3 ratio, reaching 395.59 ± 18.20 NmL CH_4 g^{-1} VS_{add}, with a methane concentration of 54.1 ± 2.0%. Although its methane yield was higher than that of the FW1:WR1 ratio, the lower methane concentration indicates reduced methane conversion efficiency compared with the FW1:WR3 ratio. This occurred despite the relatively high methane production rate observed during the initial digestion stage. The lower methane performance at the FW3:WR1 ratio may be associated with rapid substrate depletion and reduced process stability. These results demonstrate that high short-term methane production rates do not necessarily translate to a higher ultimate methane yield. Conversely, sustained, balanced digestion kinetics are essential to maximize ultimate methane recovery. Efficient methane recovery depends on the coordinated progression of hydrolysis, acidogenesis, acetogenesis, and methanogenesis rather than the rapid conversion of only the most labile substrate fractions [34, 42]. From an engineering perspective, cumulative methane production, stability of daily methane production, and resistance to inhibition are key indicators of biogas production performance. These performance indicators are particularly important because they directly influence reactor reliability, process controllability, and the feasibility of long-term system operation.

Based on these criteria, the FW1:WR3 ratio demonstrated the highest methane production potential, with stable daily methane production and the greatest cumulative methane yield. This finding was further supported by the improved organic matter removal and process stability parameters observed under the same condition. Therefore, the FW1:WR3 ratio appears to be the most suitable substrate combination for further evaluation in semi-continuous or continuous anaerobic digestion systems, particularly for maximizing energy recovery and maintaining long-term operational stability [33, 34]. However, further validation under semi-continuous or continuous feeding conditions is required to confirm its performance under dynamic loading conditions and long-term operational stress.

3.5 Organic Matter Removal Efficiency in Semi-Continuous Pilot-Scale Digestion

The organic removal efficiency of the semi-continuous pilot-scale anaerobic reactor was evaluated at an OLR of 1 kg COD m^{-3} day^{-1} to assess substrate degradation under stable operating conditions. This OLR was selected to facilitate microbial acclimation and prevent excessive volatile fatty acid accumulation during reactor start-up [45].

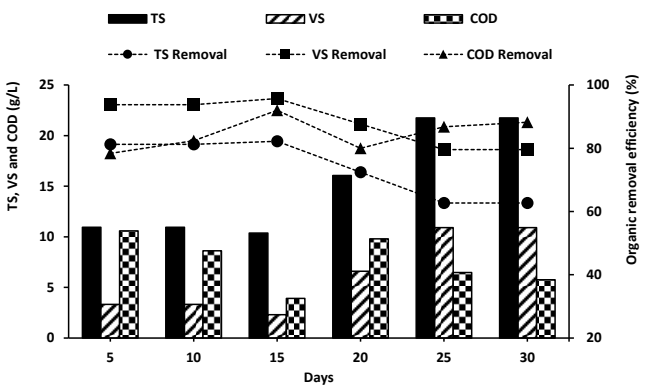


Fig. 4 Organic removal efficiency.

The removal efficiencies of COD, TS, and VS were used as key indicators of organic matter conversion and overall reactor performance.

Temporal variations in TS, VS, and COD, along with their respective removal efficiencies, reflect the progressive degradation of organic matter during anaerobic digestion (Fig. 4). These parameters serve as important indicators of substrate conversion efficiency and provide insight into the sequential breakdown of complex organic matter during digestion [46]. During the initial phase (days 5–15), moderate fluctuations in these parameters were observed, indicating active hydrolysis and solubilization of particulate organic matter. As anaerobic digestion progressed, TS and VS decreased continuously after day 15, reflecting the effective degradation of biodegradable solids. The reduction in TS and VS is also indicative of the effective conversion of biodegradable solids into gaseous end products, primarily biogas and methane [46]. Concurrently, COD exhibited a similar decline, indicating the conversion of soluble organic matter into intermediate compounds, which were subsequently transformed into methane via methanogenesis [44]. Accordingly, differences in the magnitude and rate of TS, VS, and COD reduction among substrate ratios can provide valuable insight into their relative digestion efficiency and process stability.

During the later stage of anaerobic digestion (days 25–30), TS, VS, and COD stabilized, indicating depletion of readily biodegradable substrates and the onset of substrate limitation. This stabilization phase likely reflects the predominance of more slowly degradable or recalcitrant organic fractions that require longer retention times for further conversion. This pattern is typical of stable anaerobic digestion systems, where rapid hydrolysis and acidogenesis in the early stage are followed by balanced methanogenic activity in the later phase. This pattern is commonly observed in stable anaerobic digestion systems, where rapid hydrolysis and acidogenesis in the early stage are followed by balanced methanogenesis in the later phase [46, 47]. The removal efficiencies of TS, VS, and COD exhibited distinct temporal patterns. VS removal peaked at over 85% around day 15, reflecting efficient biodegradation of the biodegradable fraction. The reduction in VS during this period is consistent with the active conversion of biodegradable organics into gaseous products, particularly methane [46]. COD remained consistently high at around 80–85%, indicating effective reduction of bioavailable organic matter. In contrast, TS was slightly lower and gradually decreased over time, likely associated with the progressive degradation of particulate solids. A short-term variation in removal efficiency around day 20 might be attributed to transient accumulation of metabolic intermediates or microbial adaptation, which briefly affected

system performance. Nevertheless, the rapid recovery of removal efficiencies reflects the stability of the microbial community and its capacity to maintain balanced metabolic pathways [46, 47]. Overall, these temporal trends confirm that the digestion process progressed efficiently from substrate hydrolysis to stable methanogenic conversion.

From a process engineering perspective, the high VS and COD removal efficiencies observed in this study reflect efficient organic matter conversion and favorable conditions for methane production. Sustained removal performance throughout the digestive period further indicates stable microbial activity and sufficient buffering capacity. These findings support process feasibility for enhanced biogas production and potential scale-up.

3.6 pH and VFA/Alkalinity Ratio in Semi-Continuous Pilot-Scale Digestion

As shown in Fig. 5. The stability of anaerobic digestion is governed by the dynamic balance between acidogenesis and methanogenesis. pH and the volatile fatty acid to alkalinity (VFA/Alk) ratio are widely used indicators of this balance. These parameters reflect the system's buffering capacity and its susceptibility to process imbalance [24, 29, 36]. Maintaining these parameters within an appropriate range is essential for preserving methanogenic activity, which is highly sensitive to changes in acid–base equilibrium. In this study, pH remained within a narrow alkaline range (8.3–8.9) throughout digestion, indicating a well-buffered system without significant acidification. This relatively stable alkaline condition likely helped prevent acid inhibition and supported the continued activity of methane-producing microorganisms [29, 45].

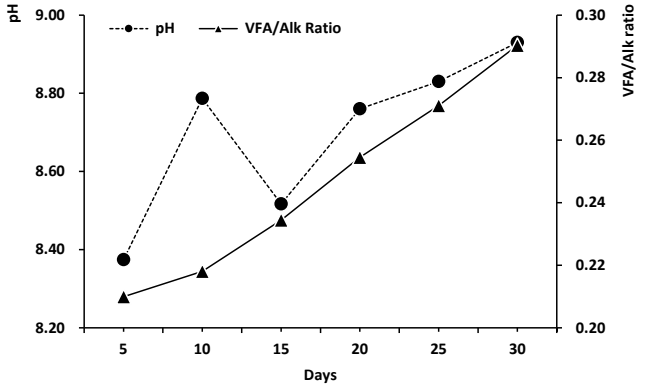


Fig. 5 The pH and the volatile fatty acid to alkalinity ratio.

A slight increase in pH was observed during the early stage (days 5– 10), followed by a moderate decline around day 15 and a gradual increase toward the end of digestion. The temporary mid-stage decrease may be attributed to intensified acidogenesis and transient VFA accumulation. However, the rapid recovery of pH suggests efficient conversion of these intermediates into methane by methanogens, thereby restoring system balance [24, 29]. The VFA/Alk ratio gradually increased from approximately 0.21 to 0.29 over the course of the experiment. Despite this increase, it remained below the widely accepted instability threshold (0.3–0.4), commonly used as an early warning indicator of acidification. Similar trends have been reported in stable anaerobic digestion systems [24, 36]. These stability trends are consistent with the sustained biogas and methane production observed during the digestion period, particularly under the more balanced substrate conditions.

From a process engineering perspective, the combination of a stable alkaline pH and subcritical VFA/Alk values reflect a strong buffering capacity and balanced microbial activity [29, 45].

These conditions indicate that the reactor operated under a stable biochemical regime, supporting sustained methane production and confirming its suitability for stable anaerobic digestion.

3.7 Biogas and Methane Production in Semi-Continuous Pilot-Scale Anaerobic Digestion

Fig. 6 shows cumulative biogas production (CBP), cumulative methane production (CMP), and methane content from the semi-continuous anaerobic reactor over a 30-day operational period. Both CBP and CMP increased steadily which indicates stable reactor performance under semi-continuous feeding. Such a production pattern is characteristic of a well-functioning semi-continuous system in which substrate input and microbial conversion remain effectively synchronized [45, 47].

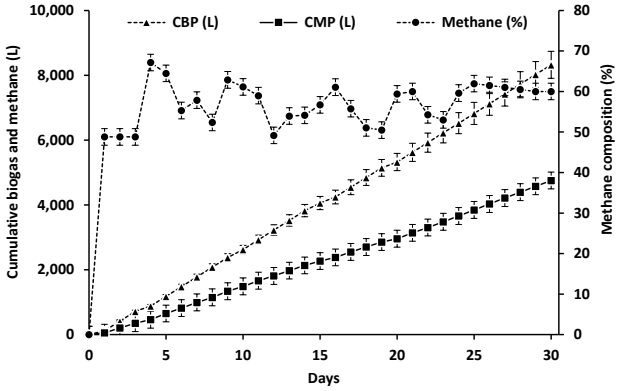


Fig. 6 Potential biogas and methane production.

By day 30, CBP reached 8,000–9,000 L and CMP 4,500–5,000 L (at standard temperature and pressure, STP). The near-linear increase in CBP and CMP suggests efficient organic substrate degradation and sustained microbial activity throughout the digestion process. The near-linear increase in CBP and CMP suggests efficient organic substrate degradation and sustained microbial activity throughout the digestion process. The near-linear increase in CBP and CMP suggests efficient organic substrate degradation and sustained microbial activity throughout the digestion process [46]. Methane content remained consistently high, reflecting effective methanogenesis under the tested conditions. Methane composition increased rapidly during the start-up phase, rising from negligible levels to approximately 65–70% within the first 4–5 days. This rapid increase in methane concentration indicates effective inoculum adaptation and early establishment of methanogenic activity under the selected operating conditions. After this acclimation period, methane concentration stabilized at 60–70%. These findings indicate the establishment of stable syntrophic interactions between acidogenic and methanogenic microbial communities. Minor fluctuations during operation likely resulted from variations in substrate characteristics or organic loading rate, which can influence volatile fatty acid dynamics and system buffering. Nonetheless, the sustained methane fraction above 60% confirms efficient conversion of intermediate metabolites to methane and indicates stable methanogenic activity [48].

In conclusion, the semi-continuous reactor exhibited sustained biogas production and stable methane composition. These findings reflect efficient substrate biodegradation and robust microbial activity, supporting its potential for application in continuous bioenergy recovery systems. Overall, these results demonstrate that the reactor maintained robust process performance under repeated feeding, supporting the feasibility of this substrate ratio for longer-term anaerobic digestion applications.

4. Conclusion

This study demonstrates that anaerobic co-digestion of food waste and watermelon rind significantly enhances biogas and methane production while maintaining process stability under mesophilic conditions. Among the substrate ratios tested, the FW1:WR3 ratio exhibited the best performance. This ratio achieved the highest methane production, superior organic matter removal, and stable operational parameters. The balanced substrate composition improved buffering capacity effectively controlled volatile fatty acid accumulation and supported sustained methanogenic activity. This performance can be attributed to improved substrate complementarity, which promoted balanced degradation kinetics and enhanced microbial conversion efficiency. The pilot-scale semi-continuous co-digestion of food waste and watermelon rind demonstrated stable biogas production, high methane content, consistently low VFA/alkalinity ratios, and robust microbial activity. These results confirm that integrating watermelon rind can mitigate instability associated with mono-digestion while enhancing energy recovery. The consistency between batch BMP performance and pilot-scale semi-continuous operation further strengthens the applicability of the selected substrate ratio under practical digestion conditions.

In conclusion, this study demonstrates the technical feasibility and environmental significance of co-digesting food waste with watermelon rind as a sustainable approach for community-scale organic waste management and renewable energy production in Thailand. Future studies should further evaluate long-term reactor performance, seasonal substrate variability, and economic feasibility to support full-scale implementation.

Acknowledgements

The authors gratefully acknowledge the financial support provided by the Graduate Program for the Production and Development of Graduate Competencies in Renewable Energy within ASEAN Countries, supported by the School of Renewable Energy, Maejo University, and the Maejo University Disciple Scholarship. The authors also extend sincere appreciation to the professors and staff of the Biofuel Laboratory for their valuable knowledge, technical support, access to laboratory equipment, and constructive recommendations, all of which contributed to the successful completion of this research.

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Ratchadaporn Puntharod: Methodology, Validation.
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Declaration of Interest Statement

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

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