

Article

Biogas Production and Energy Balance in a Two-Stage Anaerobic Digestion of Fruit and Vegetable Waste: Thermophilic versus Mesophilic

Pham Van Dinh ^{1,*} and Takeshi Fujiwara ²

¹ Department of Environmental Technology and Management, Hanoi University of Civil Engineering, 55 Giai Phong Road, Hanoi City 11616, Vietnam

² Department of Environmental Science and Technology, Graduate School of Environmental and Life Science, Okayama University, 3-1-1 Tsushima, Kita, Okayama 7008530, Japan

* Correspondence: dinhpv@huce.edu.vn or dinh88.nuce@gmail.com; Tel.: +84-782328999

Abstract: This study aimed to investigate biogas production and energy balance in a two-stage anaerobic digestion system of fruit/vegetable waste under thermophilic and mesophilic conditions. Firstly, the feedstock was hydrolyzed and acidified in an acidic reactor at 37 °C with a retention time of 5 d. Then, the liquid hydrolysate was collected and pumped into an up-flow methane reactor under a mesophilic temperature with a retention time of 5 d and a thermophilic condition with a retention time of 3 d. The experimental results showed that in the thermophilic methane reactor, the COD removal, biogas yield, and methane concentration were 96.3%, 492 mL/g-VS, and 70.4%, respectively. These values were 3%, 10%, and 3% higher, respectively, than those obtained in the mesophilic methane reactor. In terms of energy, the mesophilic and thermophilic methane reactors consumed the same thermal energy demand for temperature control. They were much lower than the heat values produced by the power engine. The two-stage anaerobic digestion system using a thermophilic methane reactor obtained a gross energy of 11.20 kJ/g-VS and a net energy of 9.83 kJ/g-VS. These values were 13.2% and 14.8% higher, respectively, than those obtained by the system with a mesophilic condition. Moreover, the use of a thermophilic reactor helped reduce the reactor volume by 40%, leading to significant investment cost savings.

Keywords: energy balance; mesophilic; thermophilic; two-stage anaerobic digestion; vegetable waste



Citation: Dinh, P.V.; Fujiwara, T. Biogas Production and Energy Balance in a Two-Stage Anaerobic Digestion of Fruit and Vegetable Waste: Thermophilic versus Mesophilic. *Fermentation* **2023**, *9*, 601. <https://doi.org/10.3390/fermentation9070601>

Academic Editor: Yutuo Wei

Received: 28 May 2023

Revised: 19 June 2023

Accepted: 21 June 2023

Published: 27 June 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

An enormous amount of fruit and vegetable waste (FVW) is generated every year around the world, which causes great health and environmental concerns [1,2]. It is often discarded at landfill sites. Due to its high biodegradability, this kind of waste inevitably causes natural degradation, impacting the environment and human health by causing odor pollution, greenhouse gas emissions, groundwater pollution, surface water pollution, and disease spread [3]. Most studies have shown that anaerobic digestion (AD) is considered the best solution for handling the biodegradable fraction of solid waste, including FVW [2,4]. This technology has several unique advantages compared to others, including low energy consumption, a low sludge residue volume, a high organic load, and residual products that can be utilized as bio-fertilizer. It especially brings benefits to energy recovery and greenhouse gas emission control [1,2,5]. Anaerobic digestion is not a new technology but is still being studied further to make it more perfect.

One of the most popular and simplest techniques is single-stage anaerobic digestion (SAD), which is designed to perform all digestion steps (hydrolysis/acidogenesis, acetogenesis, and methanogenesis) in one reactor. It is widely applied to deal with biodegradable municipal solid waste (BMSW). Compared to BMSW, FVW has many distinctive characteristics, including a very high moisture content (>90%) and a high organic content

(VS = 80–90% TS) with carbohydrates as the main components (74–97% VS) [1,6]. These substances are rapidly hydrolyzed and acidified to volatile fatty acids (VFAs) during the anaerobic digestion process, leading to a sharp decrease in the pH of the substrate. Meanwhile, methane-fermenting anaerobes are inhibited under low pH conditions (inhibited at $\text{pH} < 6.2$) or when exposed to VFAs at high concentrations (inhibited at $\text{VFAs} > 4 \text{ g/L}$) [1,4,7]. Thus, it is much more difficult to produce methane with FVW in the SAD system. In fact, single-stage anaerobic digestion of FVW can rarely occur at high organic loading rates (OLR). Babæe and Shayegan [8] studied the effect of OLR (1.4–2.7 $\text{kg-VS/m}^3/\text{d}$) on methane production from SAD of FVW, and they suggested an OLR of 1.4 $\text{kg-VS/m}^3/\text{d}$ as a criteria. Ji et al. [1] reviewed a series of FVW digestions and showed that applying SAD was often successfully at an OLR below 3 $\text{kg-VS/m}^3/\text{d}$. Wang et al. [9] performed SAD of FVW at both the lab scale and pilot scale. The authors showed that the SAD system only ran well with maximum OLR of 3.5 $\text{kg-VS/m}^3/\text{d}$. To solve the problem mentioned above, many studies suggested the solution of co-digestion, which means mixing FVW and other substrates such as dairy manure [10], horse manure [11], food waste [12], household garbage, agricultural wastes, and sludge [1]. The purpose of co-digestion is to achieve a balance of nutritional and pH conditions in a methane reactor [1]. However, Hegde and Trabold [13] reported that conducting co-digestion helped increase the performance of SAD, but the system could not be stable at an OLR above 4 $\text{kg-VS/m}^3/\text{d}$. Shen et al. [12] also found that the efficiency of the SAD system for co-digestion of FVW and food waste was significantly reduced when the OLR was higher than 3.5 $\text{kg-VS/m}^3/\text{d}$. Therefore, some studies proposed to use the two-stage anaerobic digestion (TAD) technique for degrading FVW. The TAD separates acidogenesis and methane fermentation in two different reactors. As a result, the process will be more stable and more productive. In fact, compared to SAD, TAD can not only operate at higher loads but also provides many advantages, including the biogas quality, the biogas quantity, the kinetic rate constant, flexibility, and stability [2,4,5,12,14]. Although TAD systems are often to be operated at mesophilic temperatures, recent studies have found that performing TAD with a thermophilic methane reactor (T-MR) can provide many benefits such as a higher methane yield, higher solid destruction, a higher OLR, and a lower RT [15–17]. However, these studies were performed with feedstock of BMSW or food waste, so the use of FVW is still limited in the literature.

Therefore, this study aimed to evaluate the use of a TAD system to deal with FVW under thermophilic conditions. In addition, a comparative assessment analysis between a T-MR and a mesophilic methane reactor (M-MR) based on the biogas quality, biogas quantity, and energy balance were also carried out.

2. Materials and Methods

2.1. Chemical Analysis

According to the guideline for standard methods [18], the measurements of total solid (TS) and volatile solid (VS) of samples were conducted at 105 °C and 350 °C, respectively. The pH condition was detected with a pH meter (Total Meter—Taiwan); the carbon (C) content of the FVW was measured directly by using an SSM-500 module [19]; the nitrogen (N) content of the FVW was extracted and converted to a soluble solution in accordance with EPA method 1687 and then was analyzed with a TN module. Both the SSM-500 and TN modules were attached to a TOC-Vcph analyzer (Shimadzu—Japan); the chemical oxygen demand (COD) and soluble COD (SCOD) of the liquid samples were measured with a spectrophotometer (DR890, HACH—USA) in which SCOD was performed after being filtered (0.45 μm).

The biogas composition was determined with a gas chromatograph (GC-2014, Shimadzu—Japan) equipped with a conductivity detector and a packed column (Shin-carbon ST 60/80). Operation: Injection temperature 250 °C; carrier gas He, 40 mL/min; detector temperature 200 °C; column temperature program rate 40~200 °C (10 °C/min).

The VFA components were analyzed with a GC-2014 (Shimadzu, Japan) equipped with a flame ionization detector and a capillary column. Samples were centrifuged for

15 min at 8000 rpm. The supernatant was collected and then combined in a 1:1 ratio with TCA 10%. To ensure the protein precipitation reactions, the mixture was placed in the ice for a full hour. After that, it was centrifuged at 4 °C and 8000 rpm for another 10 min to completely remove all suspended materials. One μL of the supernatant was collected to determine VFA components by GC-2014.

2.2. Fruit and Vegetable Waste

The FVW was collected in a traditional market near the campus. After that, it was cut into even pieces of less than 5 mm and blended into a slurry mixture with a blender. The mixture was stored in the conditions of 0–4 °C to prevent degradation. Characteristics of the FVW were determined (see Section 2.1) and are presented in Table 1. Among these parameters, the C/N ratio is a relative measure of the nutritional balance in the feedstock [7]. When the C/N ratio of the substrate is too high, the substrate will be deficient in nitrogen, which is needed for the growth of the microorganism. If the C/N ratio is too low, the degradation of the feedstock releases a high concentration of free ammonia formation, and this is toxic to the anaerobes [7,20]. In general, the optimal C/N ratio is recommended to be between 15 and 30 [1,4,21,22]. To obtain the optimal C/N ratio, a co-digestion method has been proposed [1,7,10]. However, the C/N of the FVW in this study was in the optimal range, so it was not necessary to mix the FVW with another substrate.

Table 1. Characteristics of the Feedstock ($n = 3$).

Characteristics	Average
TS (%)	8.33 ± 0.58
VS (%TS)	91.87 ± 1.12
C (%TS)	44.81 ± 0.53
N (%TS)	2.54 ± 0.01
C/N	17.64 ± 0.19

2.3. Experimental Model and Operation

The two-stage anaerobic digestion system shown in Figure 1 included one acidogenic reactor (AR) and one methanogenic reactor (MR). A review of TAD systems by Dinh et al. [4] showed that the acidogenesis of biodegradable solid waste under mesophilic conditions was successfully performed with a retention time (RT) in the range of 2–5 days. For safety priority, this study also operated the AR at 35–37 °C with a five-day RT. The AR was a continuous stirred tank. The MR was an up-flow reactor with an anaerobic organism layer at the bottom. The MR could be maintained at a mesophilic temperature (35 °C) or a thermophilic condition (55 °C) with a hot water jacket equipped with a temperature controller. The MR was operated with a food-to-microorganism ratio (F/M) of $0.8 \text{ g-COD/g-VS.d}^{-1}$, which was referenced from a study by Shin et al. [23].

After being acidified in the AR at pH 6.5, the substrate was diluted with water to a 1:1 ratio and then passed through a 1 mm filter to remove particles. Afterward, the liquid was pumped into the MR for the methane-forming process, in which the retention time for the mesophilic and thermophilic conditions was 5 d and 3 d, respectively.

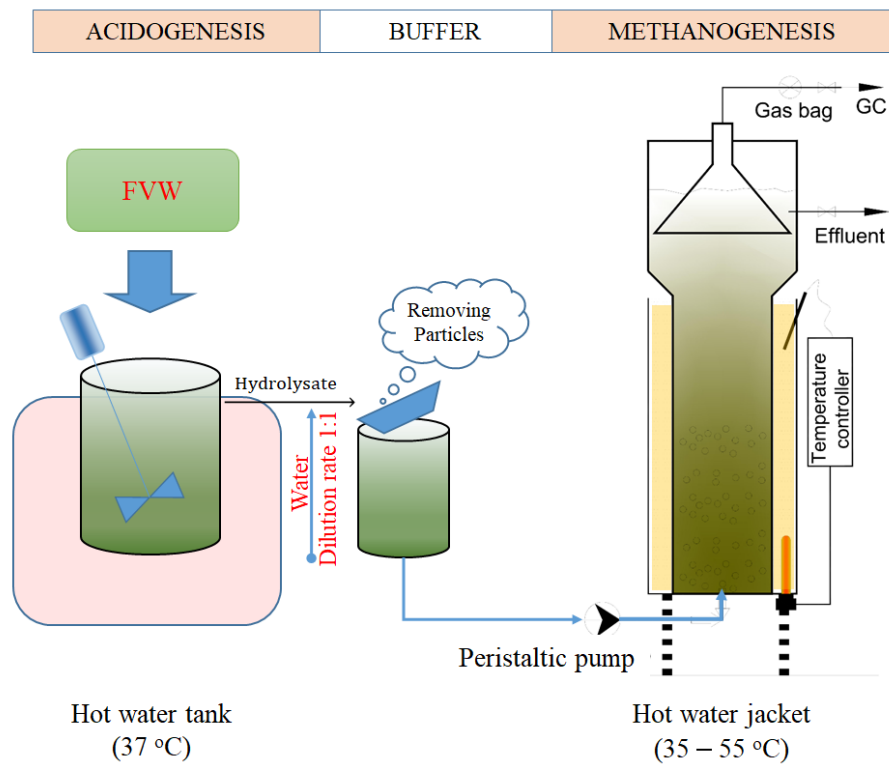


Figure 1. Diagram of the experimental system.

2.4. Energy Balance Modeling

Energy calculations of the TAD fed with FVW were conducted to compare the benefits between the mesophilic and thermophilic conditions and also evaluate their scalability. In order to make such assessments, the capacity of TADs should be scaled up to a real plant. According to previous studies [17,24], a scale-up system was considered for a mass flow rate of 100 m³/d. To apply for an AR with an RT of 5 d, the parameters for the scale-up were a volume of 500 m³, a diameter of 8 m, and a height of 5.5 m. The volume of 500 m³ of the M-MR (RT of 5 d) was divided into two reactors with a 5 m diameter and a 26 m height; the height-to-diameter ratio of the up-flow reactor followed López and Borzacconi [25]. For the T-MR (RT of 3 d), the volume, diameter, and height were 300 m³, 6 m, and 22 m, respectively. It was assumed that the biogas yield, biogas quality, and VS removal of the scaled-up reactors were the same as those obtained in the test. The energy calculation included the input, output, and energy recovery, in which the input energy was divided into the electricity and heat demands (as shown in Figure 2).

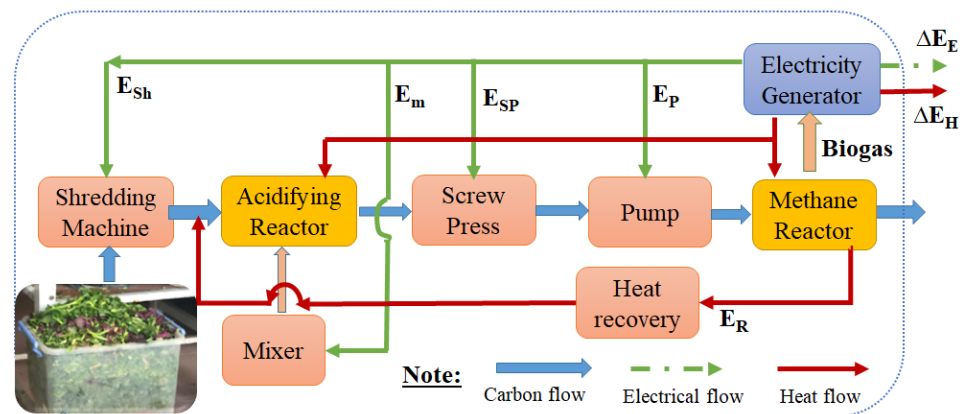


Figure 2. Diagram of energy balance model.

The feedstock is first ground using a shredding machine, and it was then fermented for five days with agitation in a warm fermentative reactor. The hydrolysate is then put through a screw press to remove the solids. The collected liquid hydrolysate is pumped with a retention time to the methane tank. The combined heat and power equipment uses the biogas produced by the methane tank to generate both heat and electricity. Other system components are kept functioning in part by the electricity and heat collected. The output energy of the system was calculated from the biogas yield and methane concentration according to Equation (1):

$$E_o = P_{Biogas} \cdot \xi \cdot \eta_m \tag{1}$$

where E_o : output energy (kJ/g-VS); P_{Biogas} : biogas yield (m³-biogas/g-VS); ξ : the lower heating value of the biogas (kJ/m³-Biogas) (this value varies with methane concentration in biogas [26]); and η_m : energy conversion factor of methane (0.9).

The input electricity demand (including shredding (E_{Sh}), mixing (E_m), screw pressing (E_{SP}), and pumping (E_P)) was estimated using Equations (2)–(6) [17,27,28]:

$$E_{Sh} = \frac{17.91 \times U \times X_o^{-0.9} \times 3600}{VS} \tag{2}$$

$$E_m = \frac{V \cdot \omega_m}{Q \cdot VS} = \frac{RT \cdot \omega_m}{VS} \left(\frac{kJ}{g} - VS \right) \tag{3}$$

$$E_{SP} = \frac{3600 \cdot \omega_{SP}}{VS} \left(\frac{KJ}{g} - VS \right) \tag{4}$$

$$E_P = \frac{\theta}{VS} \left(\frac{KJ}{g} - VS \right) \tag{5}$$

where X_o : size of the solid waste (cm); $U = 0.5$: soft coefficient of FVW; and VS : volatile solids content (g/ton of waste). In the SAD, the maximum X_o could be set at a large particle; for example, 3 cm for the Valorga system, 5 cm for the Kompogas system, and 4 cm for the Dranco system [4]. However, for wet TAD systems, the raw material should be shredded to less than 1.5 cm to accelerate the hydrolysis process [4]; therefore, we set $X_o = 1.5$ cm; V (m³): the volume of the hydrolytic reactor; Q (ton/d): the flow of waste; RT (day): the retention time in the reactor; ω_{SP} : the electricity consumption for drying with the screw press (1.1 kW·h/ton of waste) [28]; ω_m : the electricity consumption for mixing (300 kJ/m³_{reactor}·d); and θ : the electricity consumption for pumping (1800 kJ/m³) [17].

The input heat demand included heat energy to increase the influent temperature to the digestion temperature for the hydrolytic reactor $E_{h,a}$ (kJ/g-VS) and methane reactor $E_{h,m}$ (kJ/g-VS) as well as the input heat for compensating for the heat losses for the hydrolytic reactor $E_{hl,a}$ (kJ/g-VS) and methane reactor $E_{hl,m}$ (kJ/g-VS):

$$E_{h,a} = \frac{\rho \cdot Q \cdot \gamma (T_a - T_i)}{Q \cdot VS} (KJ/g - VS) \tag{6}$$

$$E_{h,m} = \frac{\rho \cdot Q \cdot \gamma (T_m - T_a)}{Q \cdot VS} \left(\frac{KJ}{g} - VS \right) \tag{7}$$

$$E_{hl,a} = \frac{(A_{wa}(T_a - T_{air})k_w + A_{fa}(T_a - T_e)k_f + A_{sa}(T_a - T_{air})k_s)}{Q \cdot VS} \tag{8}$$

$$E_{hl,m} = \frac{(A_{wm}(T_m - T_{air})k_w + A_{fm}(T_m - T_e)k_f + A_{sm}(T_m - T_{air})k_s)}{Q \cdot VS} \tag{9}$$

where A_{wa} is the wall area of the AR, A_{fa} stands for the floor area, and A_{sa} stands for the surface area of the same reactor; A_{wm} is understood as the wall area, A_{fm} as the floor

area, and A_{sm} as the surface area of the MR; k_w ($0.8 \text{ w/m}^2 \cdot \text{°C}$), k_f ($1.7 \text{ w/m}^2 \cdot \text{°C}$), and k_s ($1.0 \text{ w/m}^2 \cdot \text{°C}$) represent the heat transfer coefficient for the wall, floor, and cover of the reactors, respectively [17]; and T_a , T_m , T_{air} , T_e , and T_i are the temperature of the acid reactor, methane reactor, air ambient, earth, and the influent ($^{\circ}\text{C}$), respectively. It was assumed that T_e and T_i were equal to T_{air} with an average observed value of 28.7 °C [29].

The heat recovery (E_R —kJ/g-VS) was estimated as Equation (10) based the heat of the effluent and percentage of heat recovered ($\theta = 60\%$) without considering the heat of biogas [17]:

$$E_R = \frac{\rho \cdot Q \cdot \gamma (T_m - T_i) \cdot \theta}{Q \cdot VS} \tag{10}$$

The net energy (ΔE —kJ/g-VS) (including net electricity (ΔE_E) and net heat energy (ΔE_H)) were calculated based on Equation (11):

$$\Delta E = \Delta E_E + \Delta E_H = (0.35 \cdot E_o - E_{Sh} - E_m - E_{SP} - E_P) + (0.65 \cdot E_o - E_{h,a} - E_{h,m} - E_{hl,a} - E_{hl,m} + E_R) \tag{11}$$

3. Results and Discussions

3.1. Acidogenesis

The solid part of the hydrolysate measured 33 g/L ; its characteristics are shown in Table 2. This meant a significant amount (24.93%) of the initial total solid could not be converted in the anaerobic digestion. This result could be compared to those reported by Dinh et al. [3], Wu et al. [30], and Ganesh et al. [31], who showed that non-hydrolyzable material accounted for 25%, 30%, and 24% of the initial total solids, respectively. This could be explained by the FVW containing a large amount of lignocellulose (24–25% initial TS) [1,31], which could not be hydrolyzed in several days [32,33]. Therefore, removing non-hydrolysate would save a significant volume of the methane reactor designed to store this material. It will provide a series of benefits such as reducing the reactor construction costs, pumping costs, and heating costs.

Table 2. Characteristics of the solid hydrolysate (n = 3).

Characteristics	Average
TS (%)	31.36
VS (%TS)	78.42 ± 0.22
C (%TS)	41.03 ± 0.49
N (%TS)	1.76 ± 0.01
C/N	23.27 ± 0.27

The liquid part of the hydrolysate contained 370 mg-VFA/g-VS , in which acetic acid and butyric acid were dominant components, as shown in Figure 3. The same phenomenon also was observed by Dinh et al. [5], Li et al. [34], Ravi et al. [14], and Schievano et al. [16] when acidifying FVW. This could be explained by the fact that FVW is mainly composed of carbohydrates (such as cellulose and starch) [1,6], which are readily fermented into hexoses and then converted to acetic acid and butyrate with a central branching point of pyruvate [7,35].

The yields of VFA were significantly different in the reports mentioned above. This was attributed mainly to the effects of the digestion time and pH condition of the AR. It tended to have a positive correlation with the pH and retention time. Ravi et al. [14] obtained $215.3 \text{ mg-VFAs/g-VS}$ and $347.8 \text{ mg-VFAs/g-VS}$ under conditions of 4 d of digestion (at pH 5.5) and 6 d of digestion (at pH 6.0), respectively. Schievano et al. [16] only acquired total VFAs of 136.5 mg/g-VS at pH 5.5 with 3 d of RT. Dinh et al. [5] carried out the acidogenesis of FVW in the same pH conditions as the current study but with a longer RT (9 d), and they obtained a much higher VFA yield ($484.4 \text{ mg-VFA/g-VS}$). Li et al. [34] also showed that the VFA concentration increased with the digestion time; however, the acidification rate increased insignificantly after five days of decomposition.

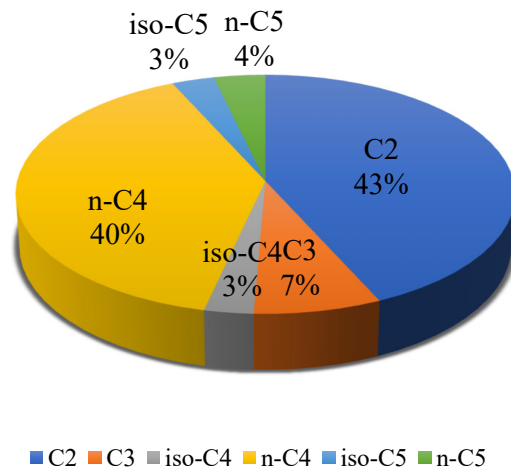


Figure 3. VFA composition in the liquid hydrolysate. Notes: C2, acetic acid; C3, propionic acid; C4, butyric acid; C5, propionic acid.

3.2. Methanogenesis

During methanogenesis, the organic concentration of the influent was maintained stable at $33,085 \pm 278$ mg-TCOD/L with 68% of the substance in soluble form throughout the experiments. The results of the COD removal at both mesophilic and thermophilic conditions are shown in Figure 4. The mesophilic methane reactor (M-MR) rapidly reached steady status, with the effluent having a TCOD of 1459 mg/L, a removal of 93.2%, and a pH of 7.41. Meanwhile, the thermophilic methane reactor (T-MR) needed 8 d to reach a stable state; it seemed slightly more sensitive than the M-MR. However, operation at the thermophilic temperature helped improve the reactor’s effectiveness significantly (COD removal of 96.3%) with the effluent having a TCOD of 1234 mg/L and a pH of 7.65. The higher pH in the effluent of the T-MR indicated that a lower acid concentration was obtained. Overall, the performance of the T-MR in removing organic matter was much better than that of the M-MR.

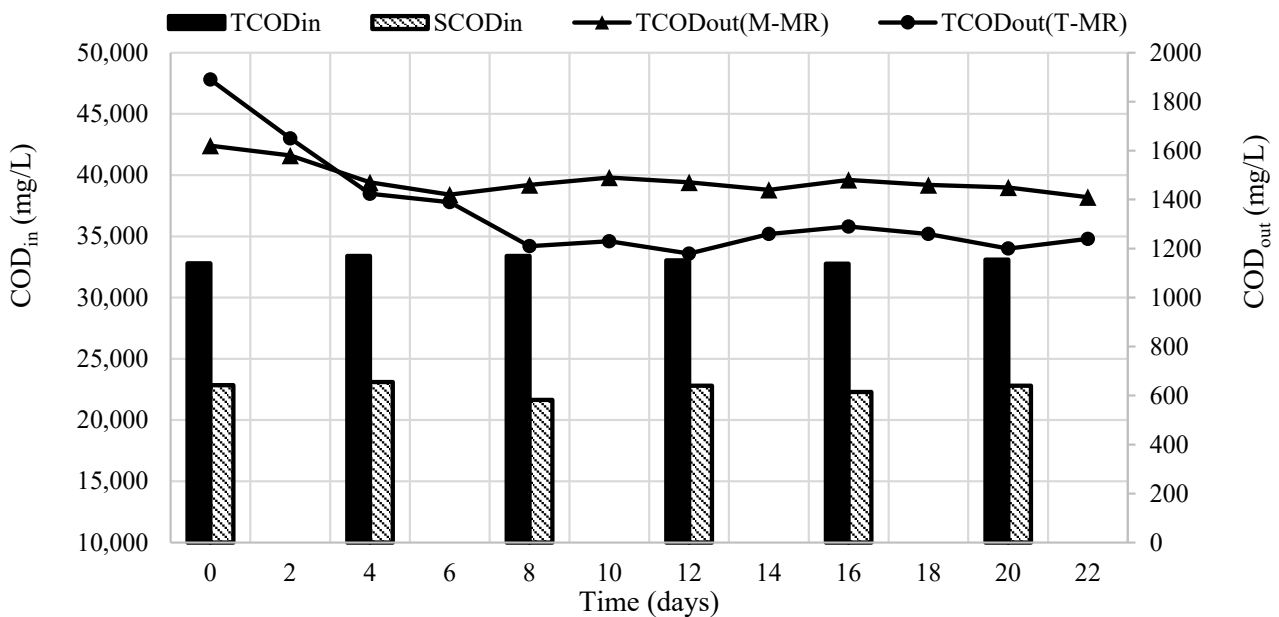


Figure 4. Variation in COD concentration in methane reactors.

The results for the quality and quantity of biogas production by the T-MR and M-MR are shown in Figure 5. For both cases, the biogas production gradually increased because

the microorganisms needed time to acclimate. In contrast, the methane concentrations gradually decreased to steady states. Dinh et al. [5] explained that in a shock status, the hydrogenotrophic methanogens (convert CO_2 to CH_4) are more robust in acclimating to environmental change than acetotrophic methanogens (which convert acetate to $\text{CH}_4 + \text{CO}_2$) in the methane-conversion chain.

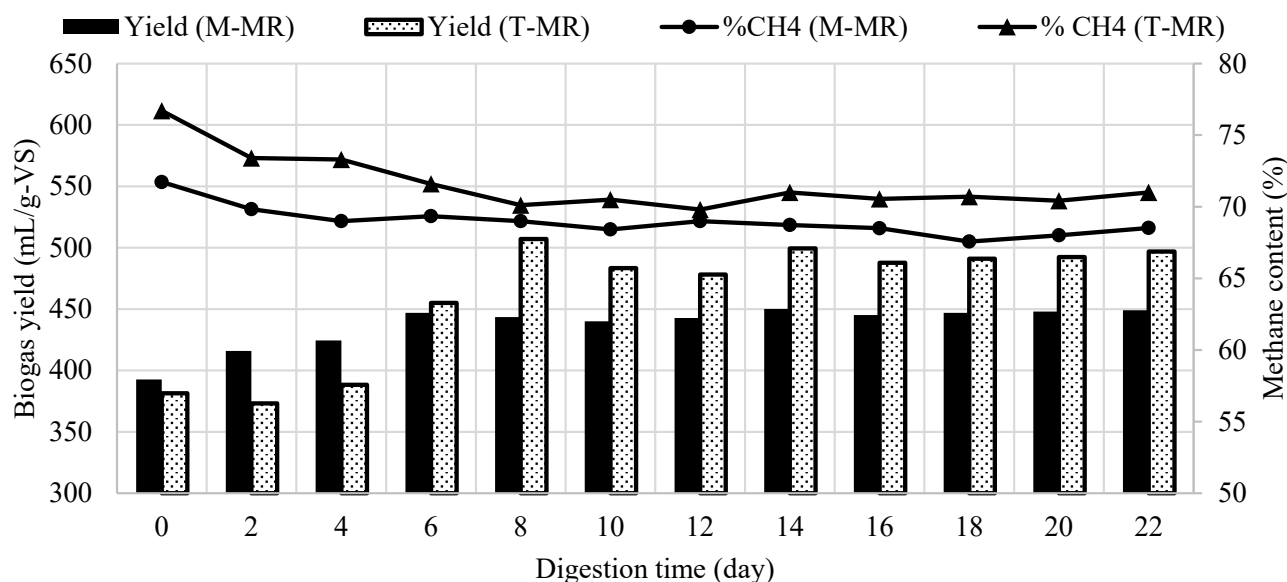


Figure 5. Biogas yield and methane content observed in methane reactors. Yield (M-MR) and Yield (T-MR) were the biogas yield (mL/g-VS) collected in the mesophilic and thermophilic methane reactors, respectively; %CH₄ (M-MR) and %CH₄ (T-MR) were the methane content in the mesophilic and thermophilic methane reactors, respectively.

In a mesophilic condition, operation with an RT of 5 d led to an organic loading rate (OLR) of the M-MR of 8.3 kg-TS/m³/d (7.6 kg-VS/m³/d). The biogas production reached a stable yield of 446 mL/g-VS (68.6% CH₄) after four days. This result could be compared to that reported by Ganesh et al. [31], who operated a semi-continuous M-MR at an OLR of 7 kg-VS/m³/d and obtained a methane yield of 300 mL-CH₄/g-VS. At a higher OLR (10 kg-VS/m³/d), they found that an accumulation of VFA in the reactor was causing a significant reduction in the biogas production. At In another study, Monserrat et al. [2] only obtained a yield of 231 mL-CH₄/g-VS when running at a much higher OLR (13 kg-VS/m³/d) compared to those studies. Obviously, increasing the OLR solved the processing volume problem but significantly reduced the processing efficiency. Therefore, M-MRs have been often recommended to be operated with an OLR below 10 kg-VS/m³/d. On the other hand, a low OLR could result in a high biogas yield; however, using a very low OLR led to a high investment cost (large reactor), which was not economical.

In another operation mode and configuration, Smith and Almquist [11] employed a batch two-stage system and observed a result of up to 510–560 mL-CH₄/g-VS at a very low OLR (0.9 kg-VS/m³/d) and a very long retention time (33 d). Raynal et al. [36] used two-stage sequencing batch reactors and obtained a yield of 350 mL-CH₄/g-COD at an OLR of 2.6 kg-COD/m³/d. Obviously, the effectiveness of a TAD for FVW also depends quite a lot on the type of reactor and the operating mode.

In the thermophilic condition, the methane reactor was operated with a RT of 3 d, equivalent to an OLR of 13.8 kg-TS/m³/d (12.7 kg-VS/m³/d). At the acclimation stage, the T-MR had a lower biogas yield and required a longer time (6–8 days) to become stable compared to the M-MR. Firstly, this could be explained by its operation at a much higher OLR. Secondly, the thermophilic bacteria were more sensitive than the mesophilic ones, as shown in various previous reports such as Gerardi [7] and Dinh et al. [4]. In the stable period, the biogas generation in the thermophilic methane reactor was 492 mL/g-VS

(70.4% CH₄), which was much higher than that of the mesophilic one. Similarly, Trisakti et al. [37] performed two-stage AD of palm milk and obtained a biogas yield of 37 mL/g-VS in a thermophilic condition compared to 31.7 mL/g-VS in a mesophilic condition. These results indicated that the thermophilic methanogen boosted productivity much more than the mesophilic one. The results of the current study for the T-MR could be compared to the report of Verrier et al. [38], who operated a two-stage AD system with CSTR reactors to treat vegetable waste in thermophilic conditions and obtained a biogas yield of 420 mL/g-VS (75% CH₄) at an OLR of 5.65 g-VS.m⁻³.d⁻¹.

3.3. Energy Analysis

Under thermophilic conditions, the yield of biogas obtained was 446 mL/g-VS with 68% CH₄, equivalent to a gross energy of 9.89 kJ/g-VS (in which the electrical energy accounted for 35% and the heat energy remained 65%). This value was significantly lower than the one obtained in the TAD system with a T-MR (11.20 kJ/g-VS). While the total electric consumption in both cases was the same (0.357 kJ/g-VS), the total heat energy consumption was 0.97 kJ/g-VS and 1.01 kJ/g-VS for the M-MR and T-MR, respectively. Therefore, the net energy difference mainly came from biogas production. Actually, using the T-MR helped increase the gross energy by 13.2%, the net energy by 14.8%, and the net electricity by 14.7%. Details of the calculations are shown in Table 3.

Table 3. Result of Energy Analysis.

No.	Item	AR	M-MR	T-MR
1	E_{SH} (kJ/g-VS)	0.270		
2	E_m (kJ/g-VS)	0.018		
3	E_{SP} (kJ/g-VS)	0.048		
4	E_P (kJ/g-VS)	0.022		
5	E_h (kJ/g-VS)	-0.418	-0.000	-0.907
	E_{hl} (kJ/g-VS)	-0.149	-0.655	-0.332
	E_R (kJ/g-VS)		+0.251	+0.795
6	E_o (kJ/g-VS)	0.000	9.446	11.196
7	$\Delta E_{\text{electricity}}$ (kJ/g-VS)		3.104	3.561
8	ΔE_{heat} (kJ/g-VS)		5.456	6.266

In the operation of the M-MR, the current study obtained a net energy of 8.56 kJ/g-VS and consumed 13.4% of the energy for heating and mechanical equipment. This result could be compared to those obtained by Bohn et al. [39] and Parawira et al. [40]. Bohn et al. [39] performed two-stage AD of crop residues using two continuous stirred tank reactors operated at a temperature of 30 °C and an OLR in the range of 0.5–4.1 kg-VS/m³/d. As calculated, they gained an energy yield in the range of 8.3–10 kJ/g-VS; however, only mixing and heating were counted among the items that required energy consumption. Parawira et al. [40] carried out hydrolysis in a stirred tank reactor and methanogenesis in a biofilm reactor (in mesophilic conditions) to treat a mixture of beet and potato. They obtained a gross energy in the range of 2.1–3.4 kW·h/g-VS (equivalent to 8.3–12.2 kJ/g-VS).

Compared to the M-MR tank, the T-MR required a considerable amount of energy for heating the temperature from 37 to 55; however, the T-MR tank had a short retention time that made the volume much smaller, leading to the results of much less heat loss due to heat transfer through the walls, roof, and bottom. In addition, heat recovery from the T-MR tank was also higher than that from the M-MR tank. Therefore, the heating demands for the M-MR and T-MR were similar. In general, the amount of heat consumed in the two processes, although different, was still much lower than the heat generated from the combined heat power engine. Therefore, heat consumption might not be a concern in the TAD system.

4. Conclusions

In the fermentation of FVW, the non-hydrolyzed material accounted for a large amount of the initial total solids. For the soluble part of the hydrolysate, acetic acid and butyric acid were the predominant products.

In the methanogenesis, the M-MR was slightly more stable than the T-MR was; however, the use of the T-MR provided many advantages, including a much lower retention time, a higher methane yield, and a higher gross energy recovery.

Author Contributions: P.V.D. conducted the experiments and wrote the original draft. T.F. supervised the experiments and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Hanoi University of Civil Engineering (HUCE) under grant number 07-2020/KHXD-TĐ.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data generated for this study are available on request to the corresponding author.

Acknowledgments: We would like to thank Okayama University for supporting the project.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ji, C.; Kong, C.-X.; Mei, Z.-L.; Li, J. A review of the anaerobic digestion of fruit and vegetable waste. *Appl. Biochem. Biotechnol.* **2017**, *183*, 906–922. [[CrossRef](#)] [[PubMed](#)]
2. Monserrat, M.G.M.; Esteban, V.C.S.; Josefina, P.V.; Gabriela, Z.J.; Alejandra, V.P.; Kiskey, C.S.C. Two-stage anaerobic digestion of fruit and vegetable wastes. *J. Biotechnol. Res.* **2019**, *10*, 29–37.
3. Dinh, P.V.; Fujiwara, T.; Giang, H.M.; Phu, S.P. The fate of carbon in two-stage anaerobic digestion of vegetable waste. *IOP Conf. Ser. Earth Environ. Sci.* **2019**, *307*, 012019. [[CrossRef](#)]
4. Dinh, P.V.; Fujiwara, T.; Tho, B.L.; Toan, P.P.S.; Minh, G.H. A review of anaerobic digestion systems for biodegradable waste: Configurations, operating parameters, and current trends. *Environ. Eng. Res.* **2020**, *25*, 1–17. [[CrossRef](#)]
5. Dinh, P.V.; Takeshi, F.; Minh, G.H.; Phu, S.T.P. Comparison between single and two-stage anaerobic digestion of vegetable waste: Kinetics of methanogenesis and carbon flow. *Waste Biomass Valorization* **2020**, *11*, 6095–6103. [[CrossRef](#)]
6. Singh, A.; Kuila, A.; Adak, S.; Bishai, M.; Banerjee, R. Utilization of vegetable wastes for bioenergy generation. *Agric. Res.* **2012**, *1*, 213–222. [[CrossRef](#)]
7. Gerardi, M.H. *The Microbiology of Anaerobic Digesters*; Wiley-Interscience: Hoboken, NJ, USA, 2003; p. 188.
8. Babæe, A.; Shayegan, J. Effect of organic loading rates (OLR) on production of methane from anaerobic digestion of vegetables waste. In Proceedings of the World Renewable Energy Congress-Sweden, Linköping, Sweden, 8–13 May 2011; pp. 411–417. [[CrossRef](#)]
9. Wang, L.; Shen, F.; Yuan, H.; Zou, D.; Liu, Y.; Zhu, B.; Li, X. Anaerobic co-digestion of kitchen waste and fruit/vegetable waste: Lab-scale and pilot-scale studies. *Waste Manag.* **2014**, *34*, 2627–2633. [[CrossRef](#)]
10. Xu, H.; Yun, S.; Wang, C.; Wang, Z.; Han, F.; Jia, B.; Chen, J.; Li, B. Improving performance and phosphorus content of anaerobic co-digestion of dairy manure with aloe peel waste using vermiculite. *Bioresour. Technol.* **2020**, *301*, 122753. [[CrossRef](#)]
11. Smith, D.B.; Almquist, C.B. The anaerobic co-digestion of fruit and vegetable waste and horse manure mixtures in a bench-scale, two-phase anaerobic digestion system. *Environ. Technol.* **2014**, *35*, 859–867. [[CrossRef](#)]
12. Shen, F.; Yuan, H.; Pang, Y.; Chen, S.; Zhu, B.; Zou, D.; Liu, Y.; Ma, J.; Yu, L.; Li, X. Performances of anaerobic co-digestion of fruit & vegetable waste (FVW) and food waste (FW): Single-phase vs. two-phase. *Bioresour. Technol.* **2013**, *144*, 80–85. [[CrossRef](#)]
13. Hegde, S.; Trabold, T.A. Anaerobic digestion of food waste with unconventional co-substrates for stable biogas production at high organic loading rates. *Sustainability* **2019**, *11*, 3875. [[CrossRef](#)]
14. Ravi, P.P.; Lindner, J.; Oechsner, H.; Lemmer, A. Effects of target pH-value on organic acids and methane production in two-stage anaerobic digestion of vegetable waste. *Bioresour. Technol.* **2018**, *247*, 96–102. [[CrossRef](#)]
15. Kasinski, S. Mesophilic and Thermophilic Anaerobic Digestion of Organic Fraction Separated during Mechanical Heat Treatment of Municipal Waste. *Appl. Sci.* **2020**, *10*, 2412. [[CrossRef](#)]
16. Schievano, A.; Tenca, A.; Scaglia, B.; Merlino, G.; Rizzi, A.; Daffonchio, D.; Oberti, R.; Adani, F. Two-stage vs single-stage thermophilic anaerobic digestion: Comparison of energy production and biodegradation efficiencies. *Environ. Sci. Technol.* **2012**, *46*, 8502–8510. [[CrossRef](#)]

17. Xiao, B.; Qin, Y.; Wu, J.; Chen, H.; Yu, P.; Liu, J.; Li, Y.-Y. Comparison of single-stage and two-stage thermophilic anaerobic digestion of food waste: Performance, energy balance and reaction process. *Energy Convers. Manag.* **2018**, *156*, 215–223. [[CrossRef](#)]
18. USEPA. Total, Fixed, and Volatile Solids in Water, Solids and Biosolids. In *Method 1684*; EPA-821-R-01-015; USEPA: Washington, DC, USA, 2001.
19. *DIN ISO 10694:1995*; Soil Quality–Determination of Organic and Total Carbon after Dry Combustion (Elementary Analysis). International Organization for Standardization (ISO): Geneva, Switzerland, 1995.
20. Zhang, C.; Yun, S.; Li, X.; Wang, Z.; Xu, H.; Du, T. Low-cost composited accelerants for anaerobic digestion of dairy manure: Focusing on methane yield, digestate utilization and energy evaluation. *Bioresour. Technol.* **2018**, *263*, 517–524. [[CrossRef](#)]
21. Huang, X.; Yun, S.; Zhu, J.; Du, T.; Zhang, C.; Li, X. Mesophilic anaerobic co-digestion of aloe peel waste with dairy manure in the batch digester: Focusing on mixing ratios and digestate stability. *Bioresour. Technol.* **2016**, *218*, 62–68. [[CrossRef](#)]
22. Zhang, C.; Xiao, G.; Peng, L.; Su, H.; Tan, T. The anaerobic co-digestion of food waste and cattle manure. *Bioresour. Technol.* **2013**, *129*, 170–176. [[CrossRef](#)]
23. Shin, H.-S.; Han, S.; Song, Y.; Lee, C. Performance of UASB reactor treating leachate from acidogenic fermenter in the two-phase anaerobic digestion of food waste. *Water Res.* **2001**, *35*, 3441–3447. [[CrossRef](#)]
24. Gray, D.; Suto, P.; Peck, C. *Anaerobic Digestion of Food Waste: Funding Opportunity*; EPA-R9-WST-06-004; US Environmental Protection Agency Region 9: Hawthorne, CA, USA, 2008.
25. López, I.; Borzacconi, L. Anaerobic digestion for agro-industrial wastes: A Latin American perspective. *Int. J. Eng. Appl. Sci.* **2017**, *4*, 71–76.
26. Ludington, D. *Calculating the Heating Value of Biogas*; DLtech, Inc.: New York, NY, USA, 2006.
27. Fitzgerald, G.C.; Themelis, N.J. Technical and economic impacts of pre-shredding the MSW feed to moving grate WTE boilers. In Proceedings of the North American Waste-to-Energy Conference, Chantilly, VA, USA, 18–20 May 2009; pp. 237–243. [[CrossRef](#)]
28. Karunanithi, A. *System Analysis of De-Watering Process for Treating Biogas Digestate*; Linköping University: Linköping, Sweden, 2014.
29. Xuan, L.T.T.; Egondi, T.; Ngoan, L.T.; Toan, D.T.T.; Huong, L.T. Seasonality in mortality and its relationship to temperature among the older population in Hanoi, Vietnam. *Glob. Health Action* **2014**, *7*, 23115. [[CrossRef](#)] [[PubMed](#)]
30. Wu, Y.; Wang, C.; Liu, X.; Ma, H.; Wu, J.; Zuo, J.; Wang, K. A new method of two-phase anaerobic digestion for fruit and vegetable waste treatment. *Bioresour. Technol.* **2016**, *211*, 16–23. [[CrossRef](#)] [[PubMed](#)]
31. Ganesh, R.; Torrijos, M.; Sousbie, P.; Lugardon, A.; Steyer, J.P.; Delgenes, J.P. Single-phase and two-phase anaerobic digestion of fruit and vegetable waste: Comparison of start-up, reactor stability and process performance. *Waste Manag.* **2014**, *34*, 875–885. [[CrossRef](#)] [[PubMed](#)]
32. Przemieniecki, S.W.; Kosewska, A.; Kosewska, O.; Purwin, C.; Lipiński, K.; Ciesielski, S. Polyethylene, polystyrene and lignocellulose wastes as mealworm (*Tenebrio molitor* L.) diets and their impact on the breeding condition, biometric parameters, metabolism, and digestive microbiome. *Sci. Total Environ.* **2022**, *832*, 154758. [[CrossRef](#)] [[PubMed](#)]
33. Wang, K.; Yun, S.; Xing, T.; Li, B.; Abbas, Y.; Liu, X. Binary and ternary trace elements to enhance anaerobic digestion of cattle manure: Focusing on kinetic models for biogas production and digestate utilization. *Bioresour. Technol.* **2021**, *323*, 124571. [[CrossRef](#)]
34. Li, Y.; Zhang, X.; Xu, H.; Mu, H.; Hua, D.; Jin, F.; Meng, G. Acidogenic properties of carbohydrate-rich wasted potato and microbial community analysis: Effect of pH. *J. Biosci. Bioeng.* **2019**, *128*, 50–55. [[CrossRef](#)]
35. Ungerfeld, E.M. Metabolic hydrogen flows in rumen fermentation: Principles and possibilities of interventions. *Front. Microbiol.* **2020**, *11*, 589. [[CrossRef](#)]
36. Raynal, J.; Delgenès, J.-P.; Moletta, R. Two-phase anaerobic digestion of solid wastes by a multiple liquefaction reactors process. *Bioresour. Technol.* **1998**, *65*, 97–103. [[CrossRef](#)]
37. Trisakti, B.; Irvan, M.; Turmuzi, M. Effect of temperature on methanogenesis stage of two-stage anaerobic digestion of palm oil mill effluent (POME) into biogas. *IOP Conf. Ser. Mater. Sci. Eng.* **2017**, *206*, 012027. [[CrossRef](#)]
38. Verrier, D.; Roy, F.; Albagnac, G. Two-phase methanization of solid vegetable wastes. *Biol. Wastes* **1987**, *22*, 163–177. [[CrossRef](#)]
39. Bohn, I.; Björnsson, L.; Mattiasson, B. The energy balance in farm scale anaerobic digestion of crop residues at 11–37 °C. *Process Biochem.* **2007**, *42*, 57–64. [[CrossRef](#)]
40. Parawira, W.; Read, J.S.; Mattiasson, B.; Björnsson, L. Energy production from agricultural residues: High methane yields in pilot-scale two-stage anaerobic digestion. *Biomass Bioenergy* **2008**, *32*, 44–50. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.