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Effort to Mitigate Volatile Fatty Acid Inhibition by Using Mixed Inoculum and Compost for the Degradation of Food Waste and the Production of Biogas

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Abstract: Food waste is a rich organic matter that can potentially be converted into biogas as a source of renewable energy. The limitation in energy production lies in the presence of volatile fatty acid (VFA) during the anaerobic digestion of food waste due to the high degradation rate. The accumulation of VFA leads to a decrease in pH that exceeds the optimal pH range of 6.8–7.6 for methanogens, thus inhibiting methanogenesis and affecting biogas production. In the present study, a symbiotic culture of bacteria and yeast (SCOBY) and kombucha mixed inoculum and compost was applied as an alternative treatment method to alleviate inhibition. The digestion efficiency was evaluated on pH, total alkalinity (TA), total volatile fatty acid (TVFA), total solid (TS), and volatile solid (VS) throughout the digestion period of 80 days to analyse the stability of the system. The results revealed that SCOBY and kombucha mixed inoculum caused system instability, inducing inhibition at TVFA of 12,874.1 mg/L, while the pH dropped to 5.23. The inhibition in the digestion system with only the SCOBY inoculum occurred at TVFA of 11,908.3 mg/L, and the pH dropped to 5.67. The biogas and methane yield quantified from the mixed inoculum is 8.792 $\times$ 10^{-4} L/L d, comparatively lower than the ethanol pre-fermentation treatment method. These findings indicate that the addition of compost improved the pH, VS, and TVFA.

Keywords: biogas; food wastes; factor; volatile fatty acid; compositing

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1. Introduction

Food constitutes its biodegradable characteristics. Waste accounts for 31 to 45% of municipal solid waste, and 80% of this waste is discharged to the landfill, despite their biodegradable properties [1]. The decomposition of food waste in landfills causes a high emission of CH_4 and CO_2 into the open air. Food waste decomposition is caused by insufficient oxygen and chemical digestion occurs in landfills. The process escalated into a serious environmental issue, such as CH_4 production, which is associated with greenhouse gas emissions and accelerates climate change. One of the limitations of the application of food waste composting for biogas production is the accumulation of volatile fatty acids (VFA) which inhibit the methanogenesis process in anaerobic digestion due to

the decrease in pH range of 6.8–7.6 required for methanogens. Therefore, studies in the literature used a symbiotic culture of bacteria and yeast (SCOBY), kombucha, and compost to alleviate inhibition, since SCOBY contains many active microbes that convert organic matter to biogas while providing pH balance to the substrate. Yeast in SCOBY converts organic matter into neutral ethanol in the acidogenesis phase to avoid a drastic pH drop, while other bacteria such as *Acetobacter* spp. and *Lactobacillus* spp. accelerate food waste degradation during pre-fermentation aerobically [2,3]. Kombucha is made by cultivating SCOBY in an aerobic sugar tea. The diversity of microbes in kombucha depends on the origin and change of fermentation. The most common bacteria reported in kombucha is *Acetobacter* spp., *Komagataeibacter* spp., Gluconacetobacter spp., and *Lactobacillus* spp. [4–6]. The yeast species include *Saccharomyces* spp., *Schizosaccharomyces* spp., *Zygosaccharocyces* spp., *Bretanomyces* spp., *Candida* spp., *Torulospora* spp., *Koleckera* spp., *Picha* spp., *Mycotorula* spp., and *Mycoderma* spp.

Composts are used as limestone alternatives to raise soil pH for agricultural plantations. Studies of dairy fertilizer compost have shown similar pH buffering effects in compost and limestone treatments [7]. Therefore, in this study, we use compost to address the pH drop problem instead of limestone. The response of compost to acidification is dependent on the pH buffering capacity [8]. The available microbial consortium in compost enables bioaugmentation with an increase of 6% gas yield and a shift towards hydrogenotrophic methanogen in methanogenic community [9]. Methanogens such as *Methanosarcina thermophila, Methanoculleus thermophilus,* and *Methanobacterium formicicum* have been reported in compost [10]. These microbes allow compost to be a potential alternative method to alleviate acid inhibition. Therefore, compared to traditional methods of using inorganic compounds such as sodium hydroxide, sodium carbonate, potassium carbonate, and calcium hydroxide, compost is a cheaper and easily available alternative. However, these claims must be investigated to confirm their effectiveness in reducing the operational cost of biogas production.

Anaerobic digestion is affected by the source of the inoculum, the degradation rate, the composition of biogas, the digestion time, and the stability of the reactor [11]. Methanogens require a strict living condition of pH 6.5–7.6 to convert VFA into biogas. High levels of VFAs in biogas production from food waste are a common occurrence that leads to a drastic pH drop in the digestion system, directly inhibiting methanogenesis. Moreover, the cultivation period and substrate–inoculum ratio are considered among the main factors which contribute effectively to the increasing or decreasing of the biogas production [12,13]. Hence, it is critical that each of the parameters, pH, organic loading rate (OLR), volatile fatty acid (VFA), and total alkalinity (TA), are within the acceptable range to ensure the stability of digestion system. In the current study, the application of compost, kombucha, and SCOBY in biogas production to alleviate acid inhibition was explored. The efficiency of these independent factors in the production of biogas as well as classification for each factor role was analyzed. The study aimed to determine the effects of the mixed inoculum on the biogas yield and microbial community in food waste degradation, and to determine the effectiveness of compost as an alternative biomaterial source for alkalinity stabilization and pH buffer, and this emphasizes the novelty of the current work.

2. Materials and Methods

2.1. Experimental Design

The laboratory experiment is set up to determine the effects of compost, SCOBY, and kombucha on substrate condition in a semi-continuous mono-digestion system. SCOBY is a commercial product (bio-starter) consisting of lactic acid bacteria (LAB), acetic acid bacteria (AAB), and yeast. The overall research focusses on exploring effective methods to alleviate VFA inhibition in food waste anaerobic digestion. Preliminary experiments were conducted on the mixed compost feed substrate to study the pH buffering effect of compost in the substrate; then, the experiments were carried out on anaerobic digestion of food waste using SCOBY and kombucha inoculum. The methane produced from the digestion of SCOBY and

kombucha mixed inoculum substrate was quantified. The digestion performance is then compared to that of the VFA alleviation method of using ethanol pre-fermentation, with the addition of ethanol produced by activated yeast. Compost was added after acidification occurs, to restore the system concurrently and understand the pH buffering capacity and alkalinity. TA, pH, total solid (TS), volatile solid (VS), total volatile fatty acid (TVFA), and VFA were investigated during the digestion process for 80 days. The collected data were subjected to classification analysis based on discriminant analysis (DA) to determine the effect of the interaction between the independent variables.

2.2. Effect of pH Buffering of Substrate Composting

A preliminary study on a compost mixed substrate was conducted to study the pH buffering capacity of dry compost and compost tea on the substrate. The dry compost was selected to further study the alleviation of VFA inhibition in food waste. Three separate batches of 500 mL of substrate were prepared by mixing food waste with three different substrates: tap water, dry compost, or compost tea in a 1:1 ratio (Figure 1). The pH and TA of each substrate were analyzed according to the electrometric method 4500-H + APHA [14] and the titration method 2320 B, every 1–3 d, to compare the buffering effect.

Figure 1. Substrate mixture to study compost as a pH buffering biomaterial.

2.3. Substrate Preparations and Pre-Fermentation

The method of kombucha preparation was modified according to the method used by Jayabalan et al. [15]. Kombucha was first brewed with 2 L of water, 67 g of SCOBY, 200 g of sugar, and 4 bags of green tea. Fermentation was conducted semi-anaerobically instead of aerobically as studied by Jayabalan et al. [15] for eight days by opening the cap for one hour a day. This is to provide a suitable condition for yeast to produce ethanol and less acetic acid produced by AAB. Food waste was collected from Fajar and Bakti cafeteria in USM for 5 consecutive days. Subsequently, the kombucha was ready, and the food waste was defrosted to discard nonbiodegradable substances such as tissue, plastics, paper, and bones. The food waste and SCOBY are then blended.

The substrate was designed with a liquid-to-solid ratio of 1:1. For reactor 1 (R1), 2 L of kombucha, 67 g of SCOBY, 21 kg of food waste, and 13 L of water were mixed to form the substrate, while the substrate for reactor 2 (R2) was prepared by mixing 67 g of SCOBY, 21 kg of food waste, and 21 L of water. Both mixtures were left to pre-ferment anaerobically for a day.

2.4. Setup and Operation of the Anaerobic Reactor

After a day of pre-fermentation, the substrates are added to R1 and R2, respectively. Details of the operation setting are presented in Table 1 and Figure 2 with a schematic

diagram of the reactor. The substrates were allowed to undergo the acclimatization phase in the first 7 days. No feeding was carried out. This is to allow the microbial consortium to adapt to the new environment and grow. After acclimatization, sampling was carried out every three days, except during public holidays. During the anaerobic digestion stage, the substrate of both reactors was eluted and replenished with the same amount of feedstock to maintain 5 g-VS·L^{-1}·d^{-1} OLR. The eluted substrate was collected as samples for pH, TA, TVFA, and VFA composition, and VS and TS analysis. Biogas yield and composition are measured when gas was produced. On day 56, 11 L of substrate in both reactors are eluted and replenished with the same amount of compost mixture to alleviate acidification.

Table 1. Operation setting of R1 and R2.

Properties	Value
Maximum OLR	5 g-VS·L^{-1}·d^{-1}
C:N ratio	18.00 for R1, 17.08 for R2, and 17.05 for feedstock
Mixing	60 rpm for 15 min every 2 h
Temperature	37 °C
Initial pH	7.4 for R1 and 7.03 for R2

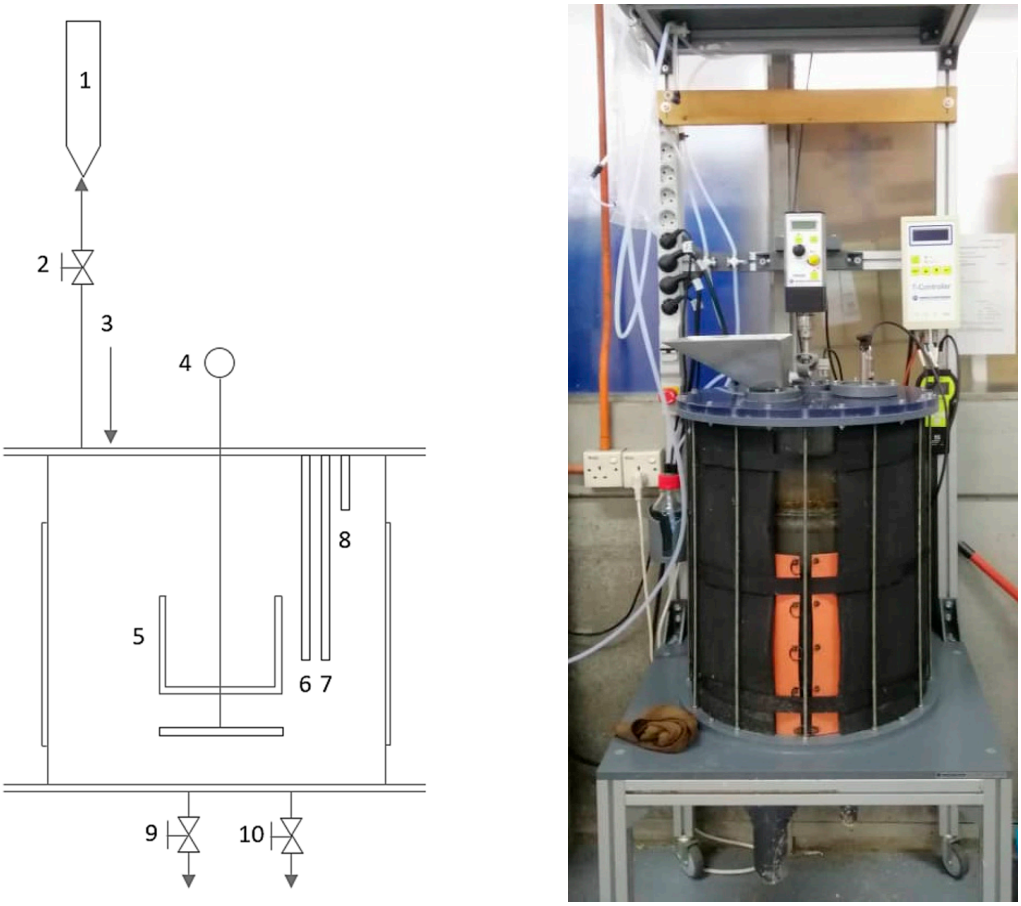

Figure 2. Schematic diagram and photo of Umweltleistungen biogas test plant standard (BTP2). "1" refers to gas bag, "2" refers gas outlet valve, "3" refers to substate inlet, "4" is meter, "5" is mixer blade, "6" is pH meter, "7" thermometer for substrate, "8" thermometer for gas, "9" large substrate outlet, and "10" small substrate outlet.

2.5. Analytical Methzod for Methane Yield and Substrate Parameters

The investigated parameters were analyzed according to APHA [14] (Table 2). The detailed procedure of APHA standard method for all parameters tested are de-scribed in Supplementary Materials. Data for pH and TA were collected by measuring the pH of

the substrate and then titrating with standard sulfuric acid until pH 4.5. Ac-id used was recorded for calculation of alkalinity.

Table 2. Analytical methods, and instruments or apparatus used for the respective analysis.

Parameters	Method	Apparatus and Instruments
pH and TA	APHA (Method 4500-H^+. Electrometric, Method 2320 B. Titration)	HACH sension3 pH meter
TS and VS	APHA (Method 2540 B and 2540 E. Gravimetric)	Binder oven and Carbolite muffle furnace
TVFA	APHA (Method 5560 C. Distillation)	Rotary evaporator and titration set
VFA composition	APHA (Method 5560 D. GC-FID)	Shidmadzu GC-2010 Plus
Biogas composition	Handheld biogas analyzer	MRU instruments Optima 7 biogas
Biogas yield	Water displacement method	Measuring cylinder and beaker
C:N ratio	CHNS elemental analyzer	Perkin Elmer 2400 Series II

Concentration of TS was quantified through the process of filtering, drying, and weighing, where the samples were dried under 105 °C, then burnt under 550 °C. The TS concentration was calculated as the difference in weight before and after drying, whereas the VS concentration was calculated based on the difference in weight before and after burning. The concentration of TVFA was quantified through distillation and then titration. The first step for analysing composition of VFA was sample preparation by acidifying and filtering the sample. Standard VFA solutions were prepared for the determination of VFA concentration, while blanks were prepared for quality assurance. Samples, standard solutions, and blanks were simultaneously analysed for gas chromatography with flame-ionization detection (GC-FID) analysis. Total biogas yield was measured though water displacement method as used by various previous studies [16,17]. The percentage gas composition was analyzed using handheld biogas analyzer. Methane yield and carbon dioxide yield in mL/d were then calculated based on the total biogas yield (Equation (1)).

$$\text{Methane yield (mL/d)} = \frac{\%\ gas\ composition\ x\ total\ biogas\ yield}{days\ of\ gas\ production}. \quad (1)$$

Initial C:N ratios of both substrates in R1, R2, and feedstock stored for a week are analysed by collecting 10 mL of sample and dried under 40 °C. The dried samples are then pulverized with mortar and pestle. Two mg of powdered samples are measured and sealed in tin capsules before entering the CHNS elemental analyzer. The C:N ratio was calculated based on composition of carbon and nitrogen.

Gram staining was performed to determine Gram-negative and Gram-positive bacterial diversity of Gram-negative and Gram-positive bacteria in food waste substrate. Morphological characteristic of bacteria colonies grown after incubation such as the shape, margin, form, and elevation are observed and recorded.

3. Results and Discussion

3.1. pH Value and TA Concentrations during the Composting

The classification of pH values during the process of digestion of food waste in tap water for 30 d is presented in Figure 3. It was noted that 73.3% of the samples (n = 31) recorded a pH of more than 5.64, while 26.3% of the samples recorded a pH of less than 4.59. In the composite reactor, 63.2% of the samples (n = 31) recorded a pH of more than 6.71, while 36.8% of the samples recorded a pH of less than 5.32. In the supernatant medium, 84.2% of the samples (n = 31) recorded a pH of more than 5.38, while 15.8 % of the samples recorded a pH of less than 4.31.

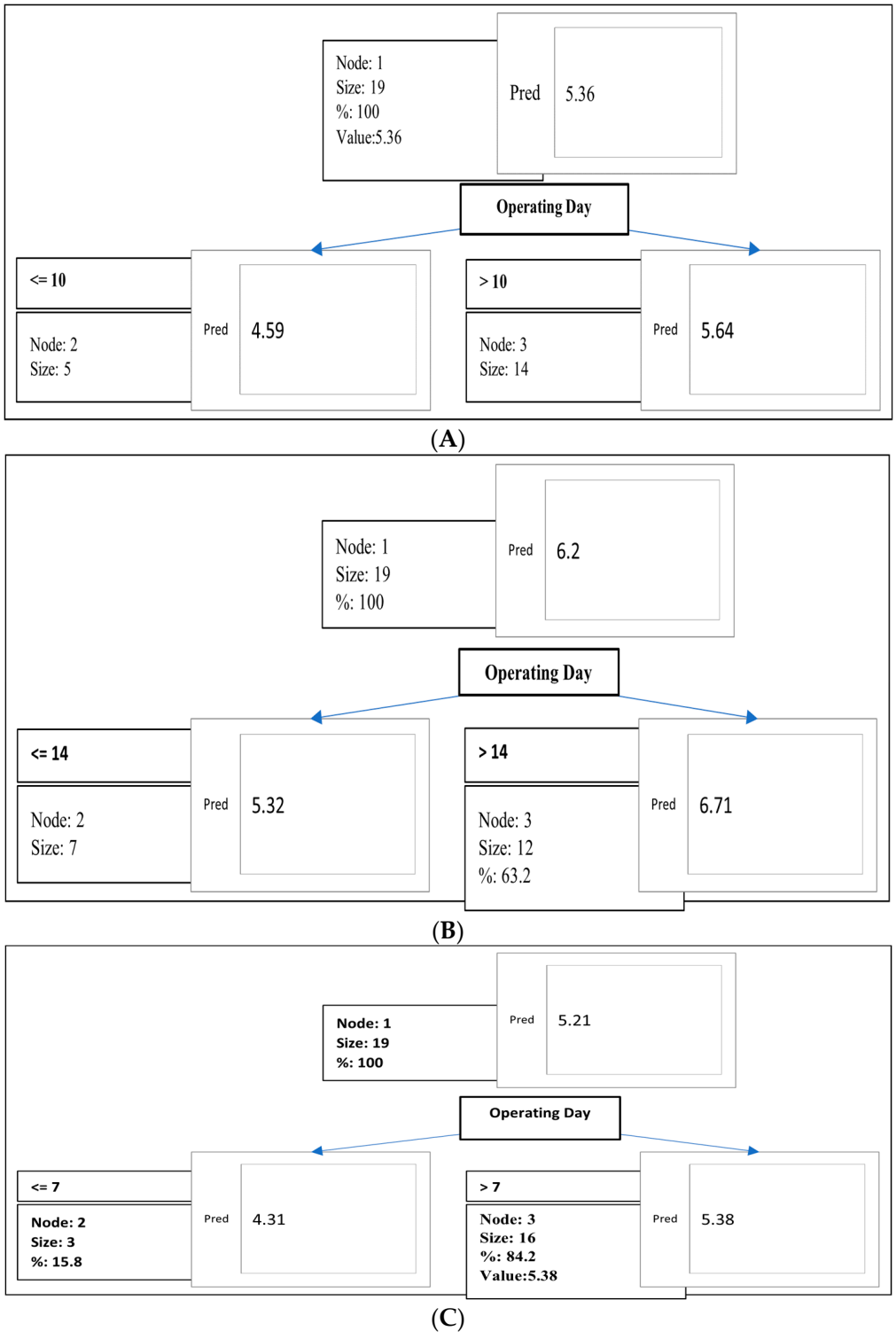

Figure 3. Classification of pH values in three compositing substrates based on discriminant analysis (DA): (**A**) Tap water medium; (**B**) dry composting; (**C**) SCOBY medium.

The behavior of TA on the substrate of food waste mixed with tap water and composite was classified into four classes: in tap water, 10.5% of the samples (n = 31) recorded TA concentrations greater than 10,283.33 mg/L, 31.6% recorded between 5961.11 and 8194.44 mg of TA/L (Figure 4), and 26.3% of the samples recorded 283.53 mg/L of TA, while the average concentration of TA was 5627.25 mg/L. In the substrate mixed with dry compost, the results revealed that 21.1% of the samples recorded 19,616.67 mg TA/L, 31.6%

recorded between 6715.97 and 15,833.8 mg TA/L, while 15.8% achieved 2946.09 mg TA/L. In comparison, the food waste substrate mixed with SCOBY was classified into three classes: 52.6% of the experimental runs achieved 7380 mg TA/L, 21.1% achieved 5841.67 mg TA/L, and 26.3% achieved 228.86 mg TA/L. These findings indicated that the substrate mixed with dry compost showed a significant increase in pH value and TA, compared to those with tap water and SCOBY. Compost can provide the optimal level of alkalinity and buffer pH during acidification. In response to this result, compost has also had a pH buffering effect in peat moss [18]. However, dry compost has a high viscosity, which could lead to interrupted operations, such as mixing and flow.

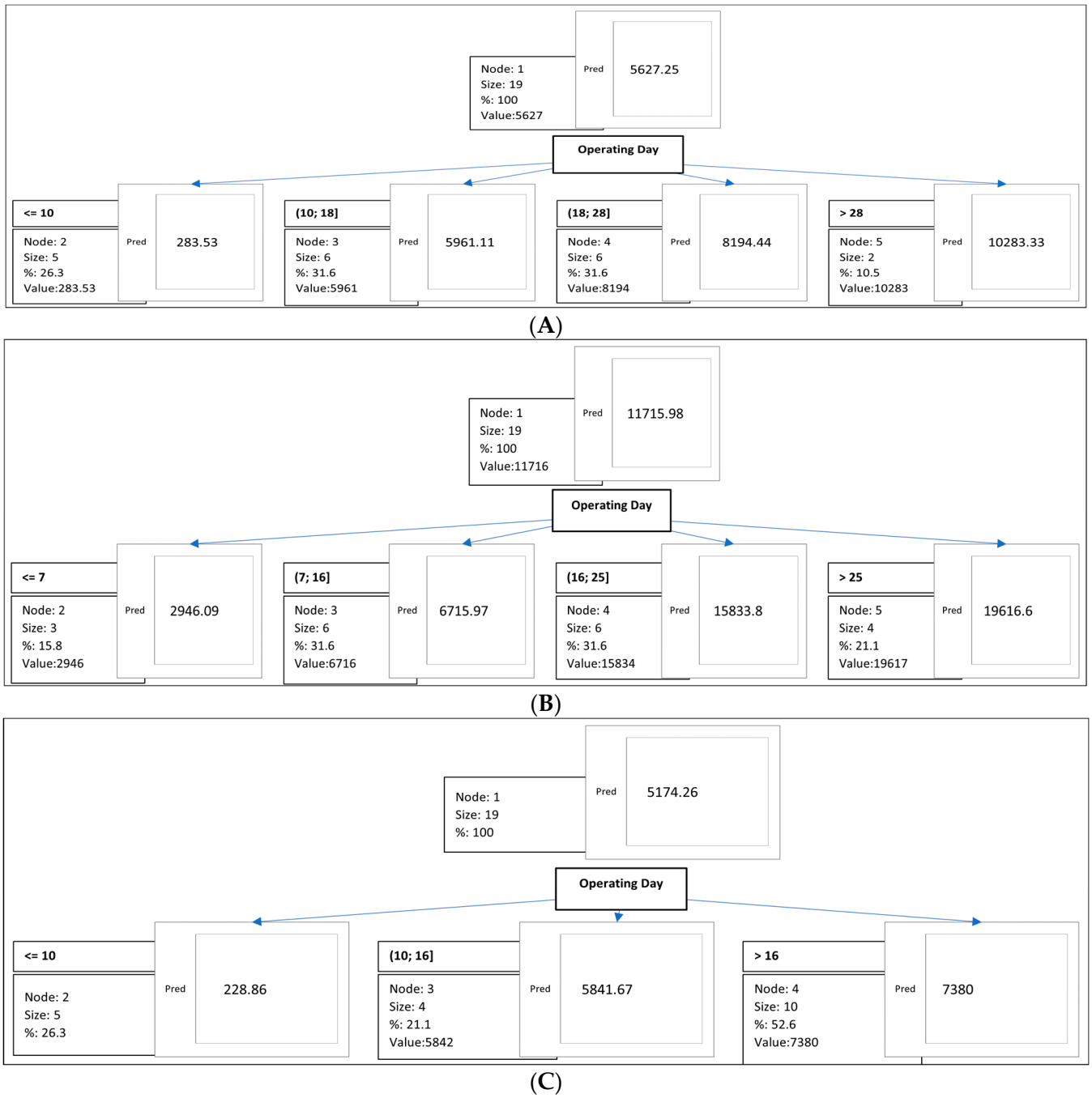

Figure 4. Classification of TA concentrations in three compositing substrates based on discriminant analysis (DA): (**A**) Tap water medium; (**B**) dry composting; (**C**) SCOBY medium.

Based on Figure 5, the substrate mixed with dry compost showed a significant increase in TA and pH value, where the pH value is 4.64–7.54, and TA managed to reach 20,333.33 mg/L, 96.8 % higher than the TA of the tap water substrate mixture. SCOBY exhibits lower TA and pH compared to those of the tap water mixed substrate. This shows that dry compost contains a higher concentration of sodium carbonate or sodium bicarbonate that contributes to alkalinity and is not completely dissolved in compost tea. Low pH and TA in the first seven days in the compost tea substrate are due to decomposition of organic matter in compost to release various amino acids, acid-forming compounds, and micro-organisms [19]. Based on the result of this preliminary study, dry compost was added to R1 and R2 to study the inhibition capability of VFA.

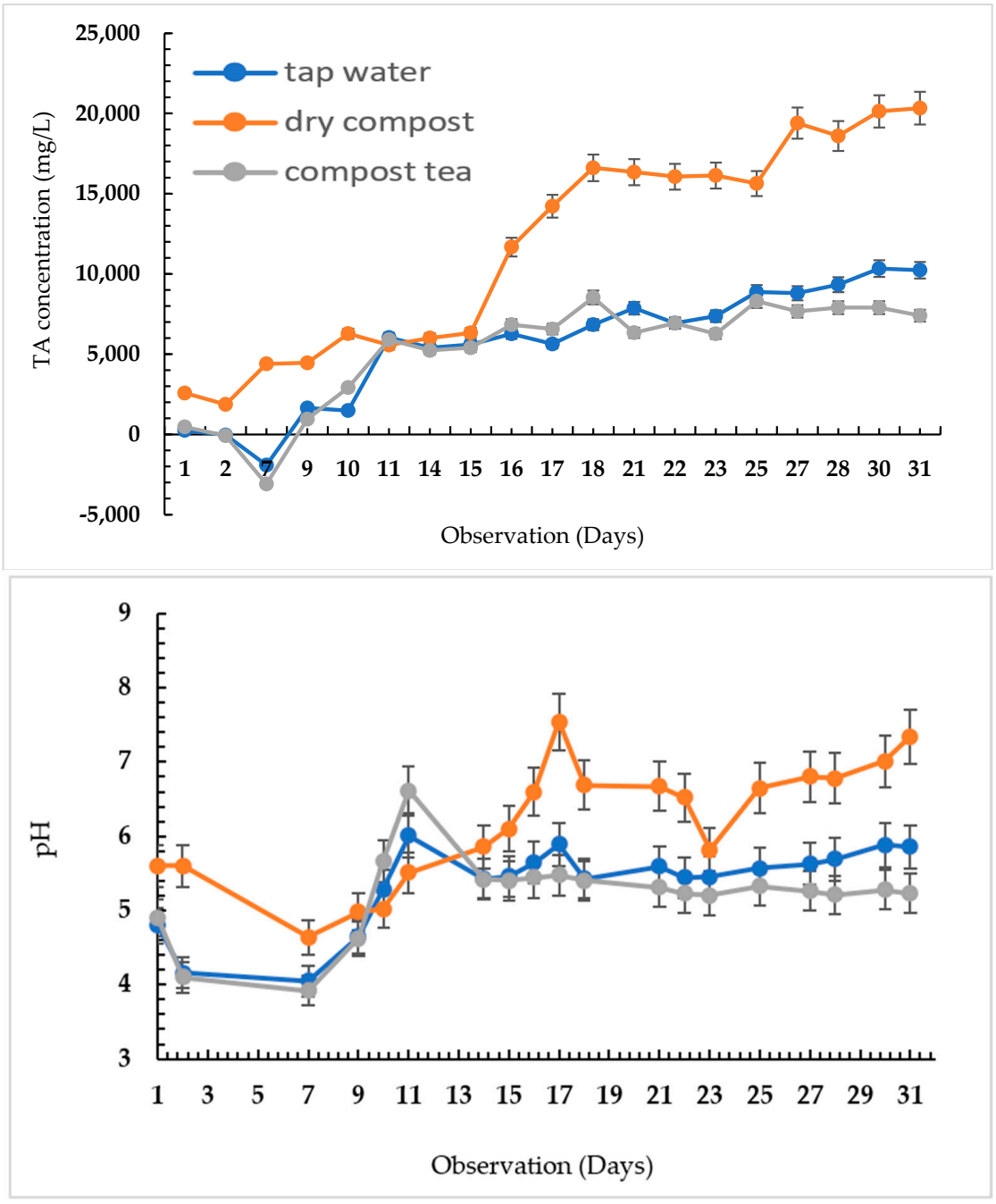

Figure 5. TA concentrations and pH value of food wastes compositing with tap water, dry compost, and compost tea substrate mixture (SCOBY).

3.2. Methane Yield for Food Waste Digestion with Mixed Inoculum

The C:N ratio for R1, R2, and feedstock is calculated from the result of the CHNS analysis as presented in Table 3. The C:N ratio of the feedstock and substrate in R1 and R2 differs by ±1.08, showing that the feedstock storage method can reduce degradation of the food waste, and the composition of the food waste is similar between R1 and R2.

Table 3. Elemental composition of feedstock, R1 and R2 substrate.

Sample	Weight (mg)	Carbon %	Hydrogen %	Nitrogen %	Sulfur %	Others %
Feedstock	1.951	46.04	6.92	2.70	0.59	43.75
R1	2.163	24.31	2.19	1.35	0.30	71.85
R2	1.863	49.02	9.19	2.87	0.81	38.11

Based on Figure 6a, a spike in CO_2 yield was observed on day 13, indicating the occurrence of carbonation due to the metabolic activity of SCOBY [20]. The CO_2 was converted to CH_4 through hydrogenic methanogenesis, as observed on day 26 where CH_4 yield peaked together with hydrogen sulphide, Not only H_2S concentration but also CO_2 concentration has reduced. The generation of H_2S indicated the decomposition of organic matter. The highest concentration of H_2S was 914 ppm, which is within the usual range of 35.87–3587.15 ppm H_2S in biogas plant [21]. Methane production was inhibited after day 30 due to acidification and has remained low after the addition of compost to alleviate the inhibition. According to Figure 6b, the methane yield is 1347.895 mL or 8.792×10^{-4} L/L/d. It is comparatively lower than the ethanol preference method conducted by Ma et al. [22] with a cumulative maximum methane production of 1.26 L/L/d and 1.99 L/L/d conducted by Wu et al. [23]. This is due to the instability of the system caused by the accumulation of VFA in the early stages, as observed by monitoring the substrate parameters in Figure 6.

The overall result of the substrate monitoring showed that the mixed inoculum of SCOBY and kombucha failed in alleviating the inhibition of VFA. In Figure 7, inhibition occurred as TVFA increased to 12,874.1 mg/L and 11,908.3 mg/L for R1 and R2, respectively.

The TVFA concentration in R^2 is significantly lower than R1 ($p < 0.05$), showing that the presence of kombucha causes a higher TVFA level due to the presence of AAB and lactic acid bacteria [24]. Acetic acid is the major contributor of TVFA as shown in Figure 8 where R1 is 96.25 % higher in acetic acid concentration than R2. Various studies have shown that early introduction of TVFA into the digestion system causes instability and lower biogas production. The TVFA inhibition of this study corresponds to the study of acetic acid pre-treatment of rice straw waste by Budiyono et al. [25] and lactic acid pre-fermentation on food waste by Zhao et al. [26] for biogas production. Butyric acid remained constant at lower concentrations for both reactors during operating days, indicating that longer chain fatty acids such as butyric acid are not produced in a large amount and are not used effectively in the digestion process.

The pH value which has a direct correlation with TVFA is significantly lower in R1 compared to the pH in R2, thus accelerating VFA inhibition. The pH in R1 gradually decreases from the initial pH of 7.4 to pH 5.23–5.30 in the acidification stage, while the pH in R2 falls from 7.03 to pH 5.67–5.74. TA decreases across the time as TVFA exhausts the alkalinity to avoid a drastic drop of pH. VS is seen steadily rising during the biogas production stage, showing signs of bacteria growth, but later decreases in the acidification stage indicating that the survival of bacteria was affected. VFA/TA as an indicator of system stability shows rapid instability in both reactors, since the ratio exceeded 0.3 in the early stages, then steadily increasing to 1.04 for R1 and 0.96 for R2 in the acidification phase.

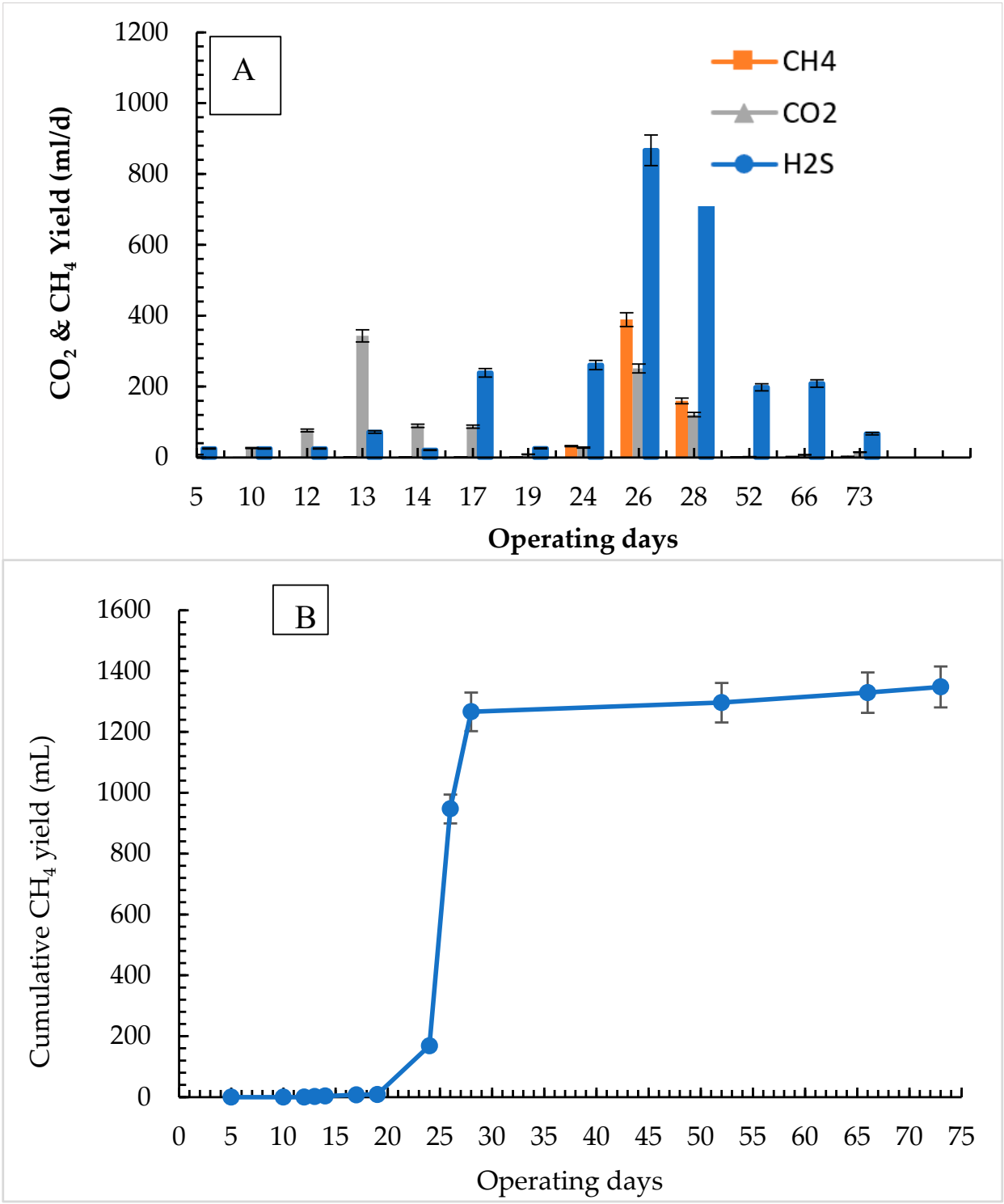

Figure 6. Biogas yield in R1 (**A**). Cumulative methane yield in R1 (**B**).

The changes in TVFA, TA, VFA/TA, VS, pH, and TS are monitored across three stages: biogas production, acidification, and compost addition as shown in Figure 7. Parameters R1 and R2 consistently show the same trend in all substrate parameters. The biogas production lasted for 30 days until acidification occurred due to VFA accumulation. The significant differences in parameters for the presence of kombucha and addition of compost as independent variables are shown in the Mann–Whitney U test and two-way ANOVA in Table 4.

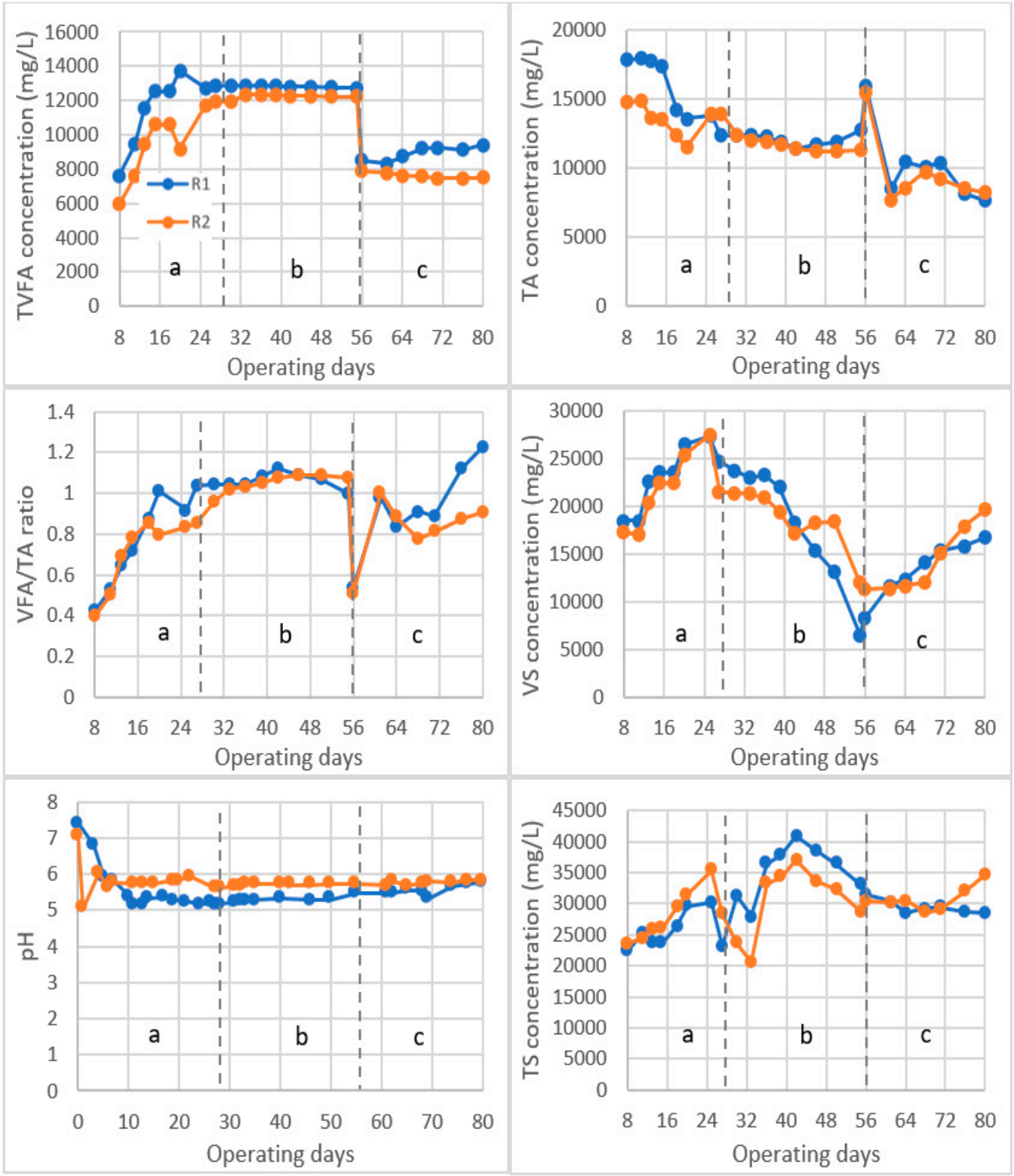

Figure 7. Parameter changes of Reactor 1 (R1, blue colour) and Reactor 2 (R2, red colour) across 3 stages. "a" refers to biogas production stage, "b" refers to acidification stage, "c" refers to compost addition stage.

Table 4. Mann–Whitney U Test and two-way ANOVA among parameters with presence of kombucha and addition of compost as independent variables. Bolded value indicates $p < 0.05$; thereby, there is significant difference.

Independent Variables	Dependent Variables	*p*-Value
Presence of kombucha	pH	**0.000**
	TA	0.138
	TVFA	**0.003**
	TS	0.403
	VS	0.947
	VFA/TA	0.524
Addition of compost	pH	R1 = **0.002**, R2 = **0.043**
	TA	R1 = **0.021**, R2 = **0.021**
	TVFA	R1 = **0.001**, R2 = **0.001**
	TS	R1 = **0.021**, R2 = 0.643
	VS	R1 = 0.083, R2 = **0.021**
	VFA/TA	R1 = 0.165, R2 = **0.002**
Presence of kombucha * Addition of compost	pH	0.129
	TA	0.951
	TVFA	**0.000**
	TS	**0.036**
	VS	0.936
	VFA/TA	0.350

Note: * High significant correlation.

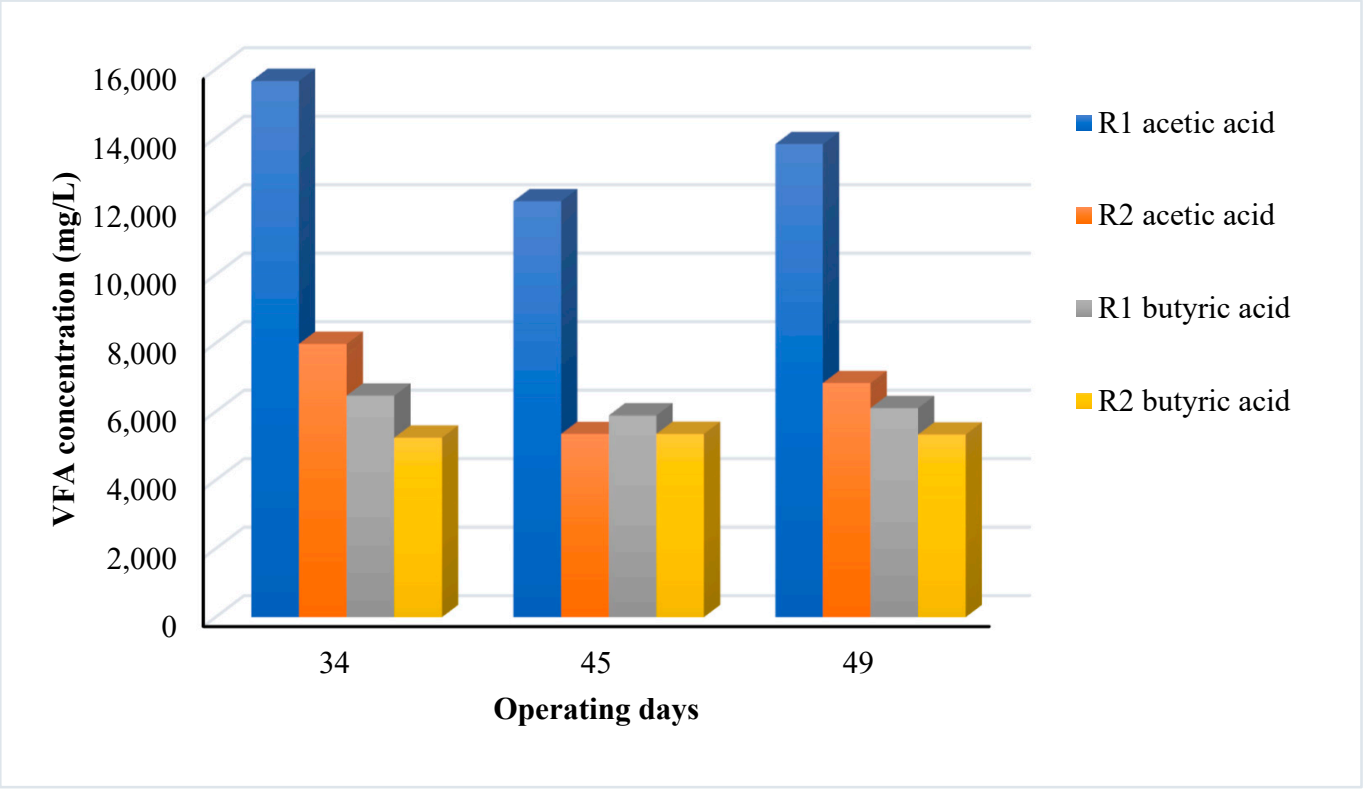

Figure 8. Acetic acid and butyric acid concentration on day 34, 45 and 49.

Comparison of parameters between the acidification stage and compost addition stage shows significant changes in all parameters except TVFA/TA and VS in R1 and TS in R2. This shows that the compost exhibits a significant pH buffering effect by bringing the pH up from 5.46 to 5.78 in R1 and from 5.69 to 5.85 in R2. However, that is not sufficient to bring the pH to the optimal level of 6.8–7.6 in this limited duration of the experiment.

Improvements are shown in VS and TVFA after the addition of compost, but the system is still unstable due to the decrease in TA. Signs of instability in the system can also be observed through the low methane yield and even higher VFA/TA ratio after compost addition. Based on the result of two-way ANOVA analysis, the effect of compost addition on substrate parameters does not depend on the effect of the presence of kombucha, except for TS and TVFA.

3.3. Enumeration of Bacteria on Substrate with SCOBY and Kombucha Inoculum

Based on the result of morphological characteristic and Gram staining in Table 5, FW and SCOBY contain mostly Gram-positive bacteria. A shift in the microbial community to Gram-negative bacteria is observed after the mixture is mixed with the substrate and fermented. This can be due to microbial competition with acidogenic bacteria such as Gram-negative *Enterobacteriaceae* during acidogenesis. *Enterobacteriaceae* including pathogens such as *Salmonella* sp., *E. coli*, and *Shigella* sp. are facultatively anaerobic bacteria and present in many foods. They anaerobically ferment glucose into acids (acetic acid, lactate, succinic acid, and formate), carbon dioxide, hydrogen, and ethanol [27].

Table 5. Gram staining and morphological classification of bacteria in substrate.

Samples	Gram Stain Test	Shape	Elevation	Form	Margin
SCOBY	Gram +ve	bacilli	Convex	Circular	Entire (even)
Kombucha	No colony found				
Food waste	Gram +ve	bacilli	Convex	Circular	Entire (even)
Food waste, kombucha, SCOBY	Gram −ve Gram −ve	rod rod	Flat Convex	Circular Circular	Undulate (wavy) Entire (even)
Food waste, SCOBY	Gram −ve Gram −ve	rod rod	Flat Convex	Circular Circular	Undulate (wavy) Entire (even)
Fermented food waste, kombucha, SCOBY	Gram −ve Gram −ve Gram +ve	rod rod Cocco- rod/rod	Flat Convex Convex	Circular Circular Punctiform	Undulate (wavy) Entire (even) Entire (even)
Fermented food waste, SCOBY	Gram −ve Gram −ve	rod bacilli	Flat Convex	Circular Circular	Undulate (wavy) Entire (even)

Most pathogens are Gram-negative bacteria because of the presence of the outer lipid membrane that is drug resistant, compared to Gram-positive bacteria, where the outer lipid membrane is absent. Therefore, Gram-negative bacteria could cause various infections and diseases, commonly associated with gastrointestinal infections and wound infections. Hence, the microbiological safety of digestate and treated sludge are critical since they affect human health and are biohazardous to the environment [28]. Furthermore, handling raw feedstock materials and exposure to pathogens pose a danger to operators. The degree of pathogen removal achieved during the anaerobic digestion of organic wastes is influenced by the interaction of substrate characteristics and operational conditions. In the mesophilic condition, *Enterobacteriaceae* is more likely to grow as it provides the ideal temperature for growth. Low hydraulic retention time aids in reducing the time of pathogen exposure to the temperature and conditions of the anaerobic digestion reactor, resulting in pathogen reduction. Moreover, mixing can improve pathogen destruction [29].

4. Conclusions

SCOBY and kombucha mixed inoculum are not suitable for use as a method to alleviate VFA inhibition in food waste anaerobic digestion due to the early introduction of acetic acid. This caused instability in the system, as indicated by substrate parameters of VFA/TA higher than 0.3 and a rapid drop of pH exceeding the range 6.8–7.6 in the early stage. The results revealed that acidification occurred where biogas production is inhibited at TVFA 12,874.1 mg/L and 11,908.3 mg/L, with and without the presence of kombucha, respectively.

Biogas production from food waste with SCOBY and kombucha mixed inoculum produces a maximum methane yield of 8.792 $\times$ 10^{-4} L/L-d, comparatively less than the ethanol pre-fermentation method and chemical treatment. The addition of compost showed a significant pH buffering effect bringing up the pH while reducing TVFA and increasing VS. However, the treatment method with compost is time consuming and its dense solid structure could cause mixing problems. Balance in the amount of compost added and TS concentration needs to be achieved. Microbial consortium in the substrate of this study is dominated by Gram-negative bacteria which are commonly pathogenic due to suitable conditions for the growth of bacteria such as acidogen *Enterobacteriaceae*. Further treatment such as disinfection is required if the substrate is to be used as fertilizer after digestion, since the microbiological analysis revealed the presence of some bacterial species belonging to *Enterobacteriaceae*.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su15021185/s1, Section S1: APHA Standard Method for Substrate Parameters Monitoring; Section S2: Gram Staining Result for Bacteria Enumeration; Section S3: Standard Curve for VFA Analysi; Section S4: GC-FID Chromatogram.

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