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Hydrogen Production by the Thermophilic Dry Anaerobic Co-Fermentation of Food Waste Utilizing Garden Waste or Kitchen Waste as Co-Substrate

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Abstract: Multicomponent collaborative anaerobic fermentation has been considered a promising technology for treating perishable organic solid wastes and producing clean energy. This study evaluated the potential of hydrogen production by thermophilic dry anaerobic co-fermentation of food waste (FW) with garden waste (GW) or kitchen waste (KW) as co-substrate. The results showed that when the ratio of FW to GW was 60:40, the maximum cumulative hydrogen production and organic matter removal rate reached 85.28 NmL g^{-1} VS and 63.29%, respectively. When the ratio of FW to KW was 80:20, the maximum cumulative hydrogen production and organic matter removal rate reached 81.31 NmL g^{-1} VS and 61.91%, respectively. These findings suggest that thermophilic dry anaerobic co-fermentation of FW using GW or KW as co-substrate has a greater potential than single-substrate fermentation to improve hydrogen production and the organic matter removal rate.

Keywords: dry anaerobic co-fermentation; hydrogen production; food waste; garden waste; kitchen waste

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1. Introduction

Food waste (FW) mainly refers to residual table waste produced in daily life, consisting of leftover rice, noodles, vegetables, meats, and other residual waste produced after the consumption of meals from restaurants and canteens [1]. According to the Food and Agriculture Organization (FAO), nearly 1.3 billion tons of FW are being discarded every year around the world [2,3]. Moreover, the annual production of FW in China has reached approximately 110 million tons [4]. With the development of the catering industry and improvement of human standards of living, the production of FW in China is exhibiting a rapid growth [5,6]. FW has high moisture, salt, oil, and fat contents, and it is easily degraded by rancidification, which produces unpleasant odors and pathogenic bacteria. These toxic and harmful substances not only cause environmental pollution, but also threaten human health [7–9]. Therefore, the efficient and effective treatment of FW has become an urgent issue.

Traditional FW disposal methods mainly include incineration, sanitary landfills, aerobic composting, and anaerobic fermentation. Among these methods, the cost of incineration is relatively high due to the high moisture content of FW, and pollutants, such as dioxins, are also generated during the combustion process [10,11]. Sanitary landfills consume extensive land resources and produce large amounts of unpleasant odors, which lead to secondary

environmental pollution. Aerobic composting produces large amounts of carbon dioxide, and the FAO estimates that every year, FW can produce the equivalent of approximately 3.3 billion tons of carbon dioxide greenhouse gas worldwide [12,13]. Therefore, anaerobic fermentation, which uses anaerobic microorganisms to degrade organic matter and convert it to biogas, is currently the mainstream technology for treating perishable organic solid wastes [14]. Compared with other treatment methods, anaerobic fermentation has lower risks of secondary pollution, and biogases, such as hydrogen and methane, are renewable, environmentally friendly, and clean biofuels that can achieve waste reuse [15].

Hydrogen is a product of the anaerobic fermentation process. Because of its high combustion calorific value and pollution-free characteristics, hydrogen can be used as a clean energy source [16]. Hydrogen production by anaerobic fermentation has the advantages of a wide range of available organic matter and a simple process. This approach has great development potential and is an ideal hydrogen production method. However, anaerobic fermentation still faces the problems of low organic matter bioconversion rates and low hydrogen production efficiencies. One reason is the imbalance of component proportions in perishable organic solid waste. For example, FW includes large amounts of salt and oil, which inhibit microbial growth, and high contents of easily degradable com-ponents, such as starch, that cause excessive rancidity and terminate the fermentation process. Moreover, the large amounts of protein present in FW lead to low C/N ratios, which are not suitable for microbial growth. Therefore, multicomponent synergistic anaerobic fermentation of FW and other materials can improve the potential and efficiency of hydrogen production and promote organic solid waste utilization by reducing salt and oil contents and adjusting the C/N ratio, the pH, and the ratio of easily degradable components to those difficult to degrade in substrate. Abreu [17] studied the co-fermentation of FW and GW for the anaerobic fermentation for biohydrogen production. The results showed that when the mass ratio of GW to FW was 90:10, the maximum cumulative hydrogen production was 51.06 mL g^{-1} VS. This value was increased by 82% compared with that of single FW fermentation, namely, 28 mL g^{-1} VS.

GW refers to waste that is generated by the natural littering or manual pruning of garden plants, including dead branches, fallen leaves, weeds, and other plant debris. The main features of GW are low moisture content and higher pH (~7) compared with FW (4~6) [18], and GW is a carbon-rich raw material with a high C/N ratio and a high content of carbohydrates, such as cellulose and hemicellulose, that are difficult to biodegrade [19]. Thus, when GW is used as a single raw material for anaerobic fermentation, there are problems of long fermentation times and low conversion rates of organic matter [17,20]. KW mainly refers to uncooked predinner leftovers from kitchens, including vegetable stems, eggshells, and raw meat. The difference between KW and FW is that KW is uncooked, predinner waste and FW is cooked, after-dinner waste. The main characteristics of KW are low fat contents, low salt contents, and high C/N ratios. FW is a nitrogen-rich raw material, and co-fermentation of FW with carbon-rich raw materials, such as GW or KW, to produce hydrogen can effectively adjust the C/N ratio and the ratio of easily degradable components to those that are difficult to degrade in a single raw material; this approach can also weaken the inhibitory effect of salt and oil on microbial growth, prevent excessive rancidity and ammonia nitrogen suppression, and enhance the adaptability of hydrogenogens to the fermentation substrate [21], thus improving the hydrogen yields and conversion rates of organic matter.

Another reason for the low hydrogen production efficiency of anaerobic fermentation is due to the total solid (TS) content of the substrate and the fermentation temperature. Generally, anaerobic fermentation can be divided into wet anaerobic fermentation (TS < 15% and temperature between 35–37 °C) and dry anaerobic fermentation (TS > 15% and temperature of 55 °C) [22]. Compared with wet anaerobic fermentation, dry anaerobic fermentation has many advantages, such as lower water consumption, higher gas production per unit volume, reduced biogas slurry, and lower moisture contents of biogas residues [23,24]. Chen [25] investigated the performance of anaerobic co-fermentation for

FW and green waste with a mixing ratio of 40:60 based on volatile solid (VS) content for TS contents of 5%, 10%, 15%, and 20% at 37 °C. The results showed that the volumetric gas production rate of dry anaerobic fermentation with TS 15–20% increased by 278–357%, compared with that of wet anaerobic fermentation with TS 5%. Furthermore, temperature is also an important factor for hydrogen production in anaerobic fermentation [26], which can affect enzyme activity and thereby affect the hydrolysis rates of organic matter and hydrogen yields. Wang [27] investigated the hydrogen production by anaerobic fermentation of FW under thermophilic (55 °C) and mesophilic (35 °C) conditions. The results showed that 126.50 mL g^{-1} VS of hydrogen yield was achieved under thermophilic (55 °C) conditions; by contrast, hydrogen yields were less than 80 mL g^{-1} VS under mesophilic (35 °C) conditions.

To improve the organic matter bioconversion rates and hydrogen production efficiencies of anaerobic fermentation, multicomponent material combinations and thermophilic dry anaerobic fermentation were conducted in this study. We analyzed the components of FW, GW, and KW, and then combined FW with GW or KW to balance the nutrition of the substrate. In addition, we used thermophilic dry anaerobic fermentation to evaluate fermentation performance and determined the optimal mixing ratio for future applications.

2. Materials and Methods

2.1. Substrate and Inoculum

The FW and KW were obtained from the cafeteria of Shanghai Advanced Research Institute, Chinese Academy of Sciences. The main components of the FW were identified as cooked rice, noodles, meat, and various vegetables. The KW was mainly composed of vegetable leaves, fruit peels and raw meat. The GW was also collected from Shanghai Advanced Research Institute, Chinese Academy of Sciences, and included leaves, roots, stems, seeds, and flowers. The collected materials were mechanically ground to obtain an even distribution. In addition, to improve GW hydrolysis, high-temperature pretreatment was performed at 120 °C for 20 min. All the materials were then subjected to physicochemical analysis. The inoculum was taken from the thermophilic dry anaerobic fermentation tank of the Shanghai Liming Resources Recycling Co., Ltd. (Shanghai, China), and the operating temperature of the reactor was 55 °C. The inoculum was starved at 55 °C in lab until no gas was produced before use to reduce the biogas produced by inoculum itself during the experiment. The physicochemical characteristics of each experimental material and inoculum are shown in Table 1.

Table 1. Characteristics of FW, GW, KW and inoculum.

Characteristics	FW	GW	KW	Inoculum
TS (%, wet basis)	28.83 ± 0.49	59.03 ± 0.81	26.19 ± 0.27	3.45 ± 0.08
VS (%, wet basis)	24.33 ± 0.44	46.77 ± 1.10	24.32 ± 0.67	2.15 ± 0.04
VS/TS (%)	84.41	79.23	92.90	62.32
TOC (%, dry basis)	39.67 ± 1.66	58.10 ± 1.23	33.48 ± 0.75	29.29 ± 0.58
TN (%, dry basis)	2.03 ± 0.04	1.04 ± 0.01	1.35 ± 0.07	4.36 ± 0.11
C/N	19.54	55.89	24.80	6.72
pH	5.51 ± 0.05	6.98 ± 0.09	4.80 ± 0.03	8.47 ± 0.06
Soluble salt (%, dry basis)	7.68 ± 0.74	1.25 ± 0.32	2.86 ± 0.88	1.18 ± 0.57
Starch (%, dry basis)	37.61 ± 1.91	0	27.33 ± 0.78	N.D.
Cellulose (%, dry basis)	7.52 ± 0.55	31.91 ± 1.23	10.45 ± 0.85	N.D.
Hemicellulose (%, dry basis)	4.52 ± 0.06	8.52 ± 0.15	6.40 ± 0.23	N.D.
Lignin (%, dry basis)	3.34 ± 0.12	28.67 ± 1.33	3.77 ± 0.06	N.D.
Crude fat (%, dry basis)	30.66 ± 2.11	1.40 ± 0.96	23.77 ± 2.07	N.D.
Crude protein (%, dry basis)	12.69 ± 0.93	6.50 ± 0.07	8.44 ± 0.16	N.D.

Note: TS: total solid; VS: volatile solid; TOC: total organic carbon; TN: total nitrogen; N.D.: not detected. Values indicate the means ± standard deviation based on triplicate determination.

2.2. Thermophilic Dry Anaerobic Fermentation

Batch experiments for the thermophilic dry anaerobic fermentation were carried out using automatic methane potential test system (AMPTS) (AMPTS II, Bioprocess, Sweden) according to the AMPTS Operation Manual. The AMPTS is mainly composed of four parts: (A) fermentation unit, which includes a water bath and fifteen 500-mL glass fermentation bottles; (B) CO_2 absorption unit, which includes fifteen 100-mL absorption bottles with NaOH solution as an absorbent for CO_2 and a small amount of H_2S; (C) gas volume measurement unit, which measures the volume of gas not absorbed by alkaline solution, such as hydrogen or methane; and (D) digital online monitoring unit, which obtains the daily biogas production and cumulative biogas production through the wireless network digital online monitoring system. The substrate for co-fermentation was composed of FW and GW (or KW) with different mixing ratios. As shown in Table 2, the mixing ratios of FW to GW (or KW) were 100:0, 80:20, 60:40, 50:50, 40:60, 20:80, and 0:100 based on VS content. The initial VS content of the co-fermentation substrate was controlled at 17.7% and consistent in all fermentation bottles. The inoculum substrate ratio (ISR) was 1:23 based on VS content. The working volumes of the above groups were adjusted to 400 mL with deionized water. Before starting the experiment, the materials were flushed with nitrogen gas for 15 min to ensure anaerobic conditions. The reaction temperature was 55 °C, the stirring speed was 60 r/min, and the fermentation time was 30 days. The volumes of hydrogen produced in this study are based on standard temperature and pressure (273 K and 1 atm). Daily hydrogen production and cumulative hydrogen production were normalized to the standard volume per gram initial VS added. All the experiments were carried out in triplicate, and the samples were collected for further analysis.

Table 2. Different mixing ratios of FW to GW as well as FW and KW for thermophilic dry anaerobic fermentations.

Item	Exp. I		Item	Exp. II	
	FW + GW	Inoculum/g		FW + KW	Inoculum/g
FG100:0	100% FW + 0% GW	120	FK100:0	100% FW + 0% KW	120
FG80:20	80% FW + 20% GW	120	FK80:20	80% FW + 20% KW	120
FG60:40	60% FW + 40% GW	120	FK60:40	60% FW + 40% KW	120
FG50:50	50% FW + 50% GW	120	FK50:50	50% FW + 50% KW	120
FG40:60	40% FW + 60% GW	120	FK40:60	40% FW + 60% KW	120
FG20:80	20% FW + 80% GW	120	FK20:80	20% FW + 80% KW	120
FG0:100	0% FW + 100% GW	120	FK0:100	0% FW + 100% KW	120

2.3. Analytical Methods

TS and VS of the samples were determined by the APHA method [28]. The TS was measured by comparing the mass changes of the samples before and after drying at 105 °C, while the VS was calculated by comparing the mass changes of the samples before and after ignition at 550 °C. The organic matter removal rate was calculated by Equation (1):

$$u(\%) = (VS_i - VS_e)/VS_i \times 100\% \quad (1)$$

where u is the organic matter removal rate (%), VS_i is the initial VS content of the co-fermentation substrate (g), and VS_e is the final VS content of the co-fermentation substrate (g).

TOC was measured with a TOC analyzer (Analytik Jena, Jena, Germany). TN was determined by the Kjeldahl method. The C/N ratios were calculated by dividing TN into TOC. pH was determined using a pH meter (PB-10, Sartorius). Soluble salt was measured by the NY/T 1121.1~1121.21 method [29]. The determination of starch content was based on the method of Holm et al. [30]. Cellulose, hemicellulose, and lignin were measured according to the NREL method [31,32]. Crude fat was analyzed as described by Thiex [33]. Crude protein was determined using the Kjeldahl method [34].

Volatile fatty acids (VFAs), including acetic acid, propionic acid, butyric acid, isobutyric acid, and pentanoic acid, were measured using a gas chromatography (GC) (2010 Plus, Shimadzu, Japan) equipped with a Rtx-Wax quartz capillary column (30 m × 0.25 mm × 0.25 µm, Shimadzu, Kyoto, Japan) and a hydrogen flame ionization detector (FID). The injector temperature and the detector temperature were set to 220 °C and 230 °C, respectively. The oven temperature was programmed at 80 °C for 2 min, increased to 200 °C at 20 °C/min, and held for 8 min. Nitrogen was used as a carrier gas at a flow rate of 30 mL/min [35].The hydrogen determination was analyzed by a GC (2010 Plus, Shimadzu, Kyoto, Japan) equipped with a TDX-01 stainless steel column (2 m × 2 mm, Shimadzu, Japan) and a thermal conductivity detector (TCD). The injector temperature and the detector temperature were all set at 150 °C, and the oven temperature was held at 120 °C for 10 min. The sample injection volume was 1.0 mL. Argon was used as a carrier gas at a flow rate of 40 mL/min.

The statistical analysis of data was calculated using paired comparison plot app (OriginPro 2022b, Northampton, MA, USA).

3. Results and Discussion

3.1. Hydrogen Production

3.1.1. Cumulative Hydrogen Production

FW mainly refers to residual table waste that is produced in daily life, and its main characteristics are high fat and salt contents, low C/N ratio, and low pH; GW refers to waste that is generated by natural littering or manual pruning of garden plants, and its main features are low moisture content, high C/N ratio, and high pH. Therefore, co-fermentation of FW and GW with different mixing ratios to produce hydrogen can effectively adjust the substrate nutrition and C/N ratio to satisfy the requirements for microbial growth. The cumulative hydrogen production values for the synergistic dry anaerobic fermentation of FW and GW are shown in Figure 1a.

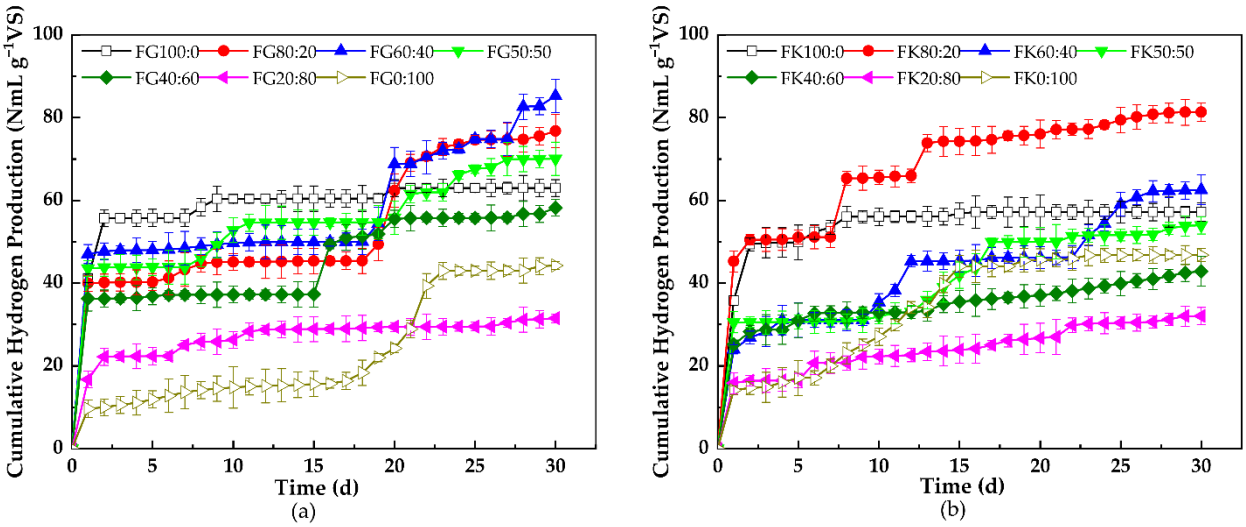

Figure 1. Cumulative hydrogen production for the thermophilic dry anaerobic co-fermentation of (**a**) FW + GW and (**b**) FW + KW.

When FW was used as a single substrate for dry anaerobic fermentation, it was prone to excessive rancidity and eventual fermentation failure, which were due to the high contents of starch, protein, and other easily degradable components in FW. It was clear that hydrogen production mainly occurred in the first nine days when using FW alone, after which only small amounts of hydrogen were produced. The cumulative hydrogen production of FW reached only 62.98 NmL g^{-1} VS during 30 days of dry anaerobic fermentation. Moreover, when GW was used as a single substrate for dry anaerobic fermentation, there was not much hydrogen production in the early stage, but large amounts of hydrogen were produced on approximately the 20th day, mainly because a long period was required for

the hydrolysis of the difficult-to-degrade components in GW, such as cellulose and hemicellulose. Although there was no rancidity during the fermentation process, the amount of hydrogen produced was relatively low, and the cumulative hydrogen production of GW reached only 44.22 NmL g^{-1} VS during 30 days of dry anaerobic fermentation due to the many organic substances that were difficult to biodegrade in GW. When the mixing ratio of FW to GW was 60:40, cumulative hydrogen production was highest ($p < 0.05$, shown in Figure 2a), namely, 85.28 NmL g^{-1} VS during 30 days of dry anaerobic fermentation. This production increased by 35.42% and 92.85% ($p < 0.05$, shown in Figure 2a), respectively, compared with that of single FW and GW. The results showed that synergistic dry fermentation with FW and GW could improve hydrogen production efficiency.

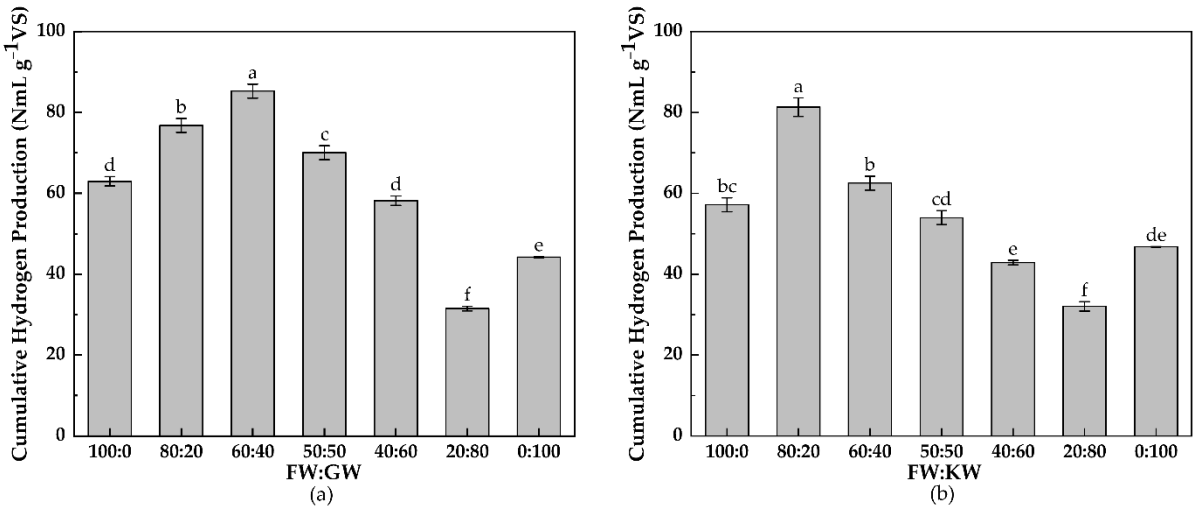

Figure 2. Cumulative hydrogen production on the 30th day for the thermophilic dry anaerobic co-fermentation of (**a**) FW + GW and (**b**) FW + KW. Letters indicate significant differences between the different mixing ratios ($p < 0.05$).

KW mainly refers to uncooked, predinner leftovers from kitchens and includes vegetable stems, eggshells, and raw meat. The main characteristics are low salt, low fat, and high C/N ratio. Anaerobic co-fermentation of FW and KW can weaken the inhibition of salt and oil on microbial growth. The cumulative hydrogen production levels for the synergistic dry anaerobic fermentation of FW and KW are shown in Figure 1b.

When FW was used as a single substrate for dry anaerobic fermentation, hydrogen production occurred only in the first eight days, after which only small amounts of hydrogen were produced. The cumulative hydrogen production of FW reached only 57.17 NmL g^{-1} VS during 30 days of dry anaerobic fermentation. When KW was used as a single substrate for dry anaerobic fermentation, hydrogen was gradually produced in the first 15 days, after which there was only a small amount of hydrogen produced. The cumulative hydrogen production of KW only reached 46.77 NmL g^{-1} VS during 30 days of dry anaerobic fermentation. When the mixing ratio of FW and KW was 80:20, the cumulative hydrogen production was highest ($p < 0.05$, shown in Figure 2b), namely, 81.31 NmL g^{-1} VS during 30 days of dry anaerobic fermentation. This value increased by 42.22% and 73.85% ($p < 0.05$, shown in Figure 2b), respectively, compared with that of FW or KW alone. The results showed that synergistic dry fermentation with FW and KW also improved the efficiency of hydrogen production.

3.1.2. Daily Hydrogen Production

The daily hydrogen production amounts from the synergistic dry anaerobic fermentation of FW and GW are shown in Figure 3a. After inoculation with the anaerobic digestion effluent, the hydrogen production of each fermentation group immediately increased and reached the maximum daily hydrogen production on the first day. This result could be

attributed to the VFAs in the system that were at high levels (3.1–6.4 g L^{-1}) on the first day [36], and to the fact that large amounts of VFAs were quickly decomposed by the microorganisms in the anaerobic digestion slurry to produce hydrogen and carbon dioxide [27]. When the ratio of FW to GW was 60:40, the daily hydrogen production was highest, namely, 47.01 NmL g^{-1} VS. This value was increased by 14.21% and 387.66%, respectively, compared with that of single FW and GW fermentations. The daily hydrogen production for the group with FW:GW of 60:40 reached a second peak of 14.44 NmL g^{-1} VS on the 20th day and a third peak of 7.78 NmL g^{-1} VS on the 28th day. However, during the whole process the fermentation of FW or GW as the single substrate, only one gas production peak appeared. Approximately 60% of total hydrogen production was generated within the first 10 days, after which the daily hydrogen production continued to decrease until the end of fermentation.

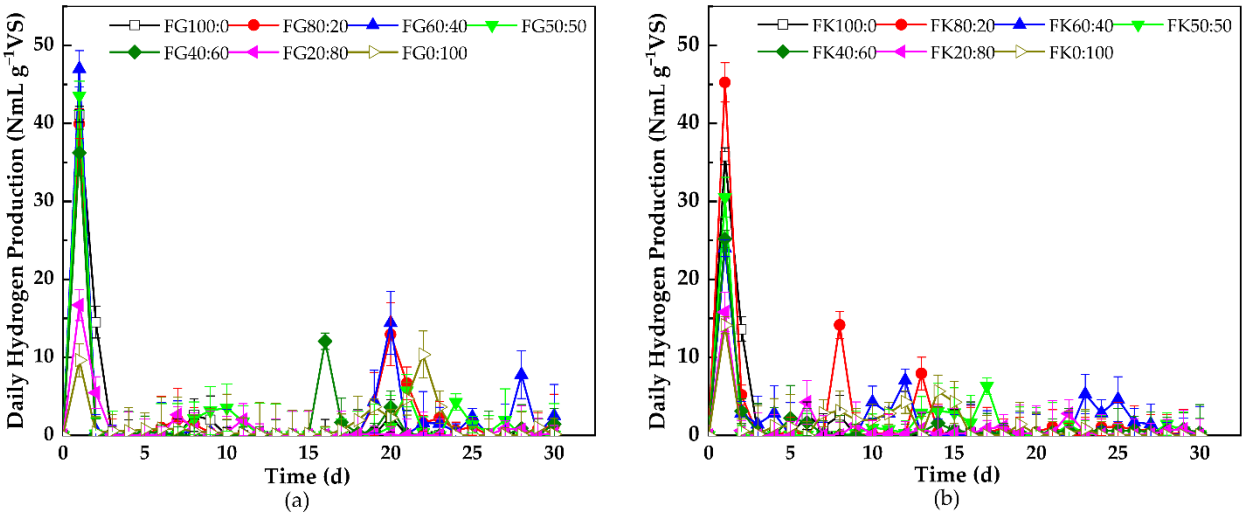

Figure 3. Daily hydrogen production for the thermophilic dry anaerobic co-fermentation of (**a**) FW + GW and (**b**) FW + KW.

The daily hydrogen production levels for the combined dry anaerobic fermentation of FW and KW are shown in Figure 3b. After inoculation with the anaerobic digestion effluent, the hydrogen production of each fermentation group also reached the maximum daily hydrogen production on the first day. When the ratio of FW to KW was 80:20, the daily hydrogen production was highest, namely, 45.26 NmL g^{-1} VS. This value was increased by 26.46% and 220.54%, respectively, compared with that of single FW and KW fermentations. The daily hydrogen production for the group with FW:KW of 80:20 reached a second peak of 14.17 NmL g^{-1} VS on the eighth day and a third peak of 7.93 NmL g^{-1} VS on the 13th day. However, during the whole process of fermentation with a single substrate, only one hydrogen production peak appeared.

3.2. Change in Organic Matter

3.2.1. VS Change

During dry anaerobic fermentation, biodegradable substrates undergo a series of reactions to produce VFAs and other substances that can be easily used by *hydrogenogens*. The VS and TS removal rates reflect the efficiency of substrate hydrolysis and VFAs utilization. The changes in VS contents during the dry anaerobic co-fermentation of FW and GW are shown in Figure 4a, and the VS content of each ratio showed a downward trend. The VS content of the 60:40 group decreased from 176.75 g L^{-1} to 64.89 g L^{-1} after 30 days of fermentation. The reductions in the VS content for the 60:40 group increased by 13.78% and 60.17% ($p < 0.05$), respectively, compared with that of single FW and GW; this was followed by the 80:20 group, for which the VS content decreased from 176.75 g L^{-1} to 66.32 g L^{-1}. The reduction in VS content for the 80:20 group increased by 12.33% and

58.12% ($p < 0.05$), respectively, compared with that of single FW and GW fermentations. Compared with single FW or GW fermentations, the VS contents after co-fermentation significantly decreased.

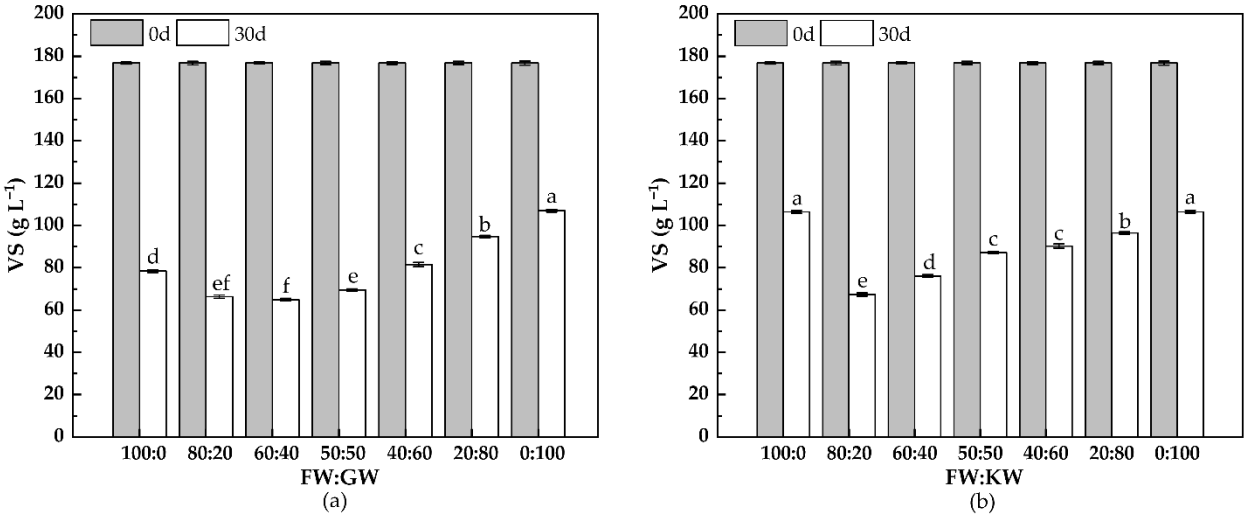

Figure 4. Changes in the VS contents for the thermophilic dry anaerobic co-fermentation of (**a**) FW + GW and (**b**) FW + KW. Letters indicate significant differences between the different mixing ratios ($p < 0.05$).

The changes in VS contents during the dry anaerobic co-fermentation of FW and KW are shown in Figure 4b. The 80:20 group exhibited the largest decrease in VS content. The VS decreased from 176.75 g L^{-1} to 67.32 g L^{-1} after 30 days of fermentation. The reduction in the VS content in the 80:20 group increased by 55.51% and 55.64% ($p < 0.05$), respectively, compared with that of the single FW and KW anaerobic fermentations; this result was followed by the 60:40 group, for which VS content decreased from 176.75 g L^{-1} to 76.11 g L^{-1}. The reduction in VS content for the 60:40 group increased by 43.02% and 43.14% ($p < 0.05$), respectively, compared with that of the single FW and KW fermentations. Compared with the single FW or KW fermentations, the VS contents after co-fermentation significantly decreased.

3.2.2. TS Change

The changes in the TS contents during the dry anaerobic co-fermentation of FW and GW are shown in Figure 5a. After 30 days of fermentation, the TS contents for each ratio also showed downward trends. The TS of the 60:40 group decreased the most, from 222 g L^{-1} to 100.11 g L^{-1}, i.e., 54.91% ($p < 0.05$) of the material was degraded; this result was followed by the 50:50 group, for which the TS contents decreased from 219.00 g L^{-1} to 102.63 g L^{-1}, i.e., 53.14% ($p < 0.05$) of the material was degraded.

The changes in the TS content during the dry anaerobic co-fermentation of FW and KW are shown in Figure 5b. The 80:20 group exhibited the greatest TS degradation, from 208.50 g L^{-1} to 82.74 g L^{-1}, i.e., 60.32% ($p < 0.05$) of the material was degraded; this was followed by the 60:40 group, for which the TS contents decreased from 204.80 g L^{-1} to 100.59 g L^{-1}, i.e., 50.89% ($p < 0.05$) of the material was degraded.

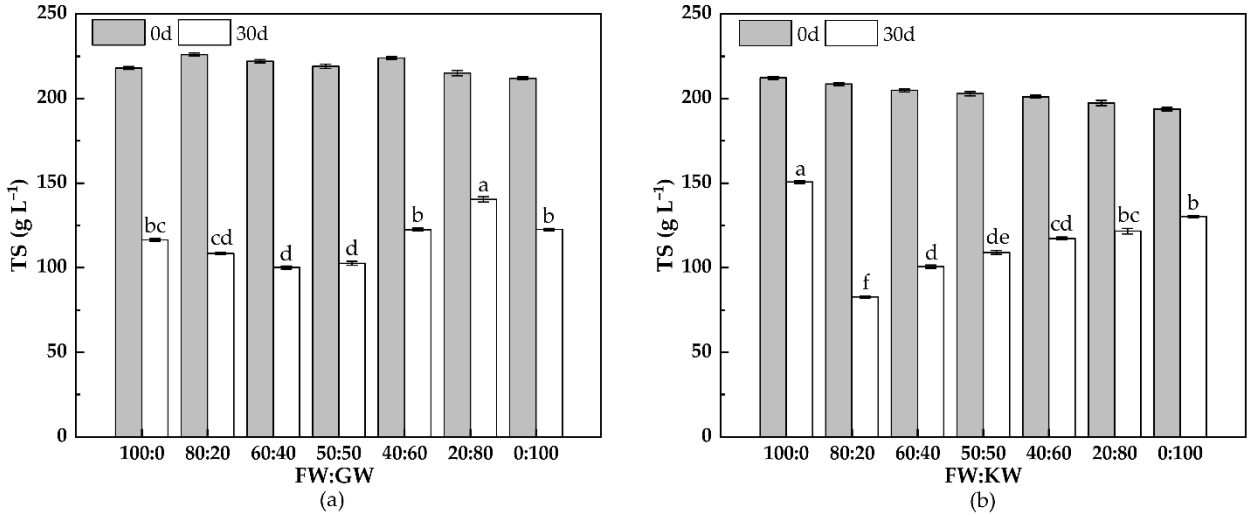

Figure 5. Changes in the TS contents for the thermophilic dry anaerobic co-fermentation of (**a**) FW + GW and (**b**) FW + KW. Letters indicate significant differences between the different mixing ratios ($p < 0.05$).

3.2.3. Organic Matter Removal

Figure 6a shows the organic matter removal rates for the collaborative thermophilic dry anaerobic fermentation of FW and GW. The dry anaerobic co-fermentation of FW and GW exhibited higher organic matter removal rates than those associated with the single dry anaerobic fermentation of GW, and the dry anaerobic co-fermentation of FW and GW with ratios of 80:20, 60:40, and 50:50 exhibited higher ($p < 0.05$) organic matter removal rates than those of the single dry anaerobic fermentation of FW. Compared with other groups, when the ratio of FW to GW was 60:40, the organic removal rate was highest at 63.29% ($p < 0.05$) after 30 days of fermentation, and this value was 1.14 times the organic matter removal rate of single FW (55.62%) and 1.60 times ($p < 0.05$) the organic matter removal rate of single GW (39.51%).

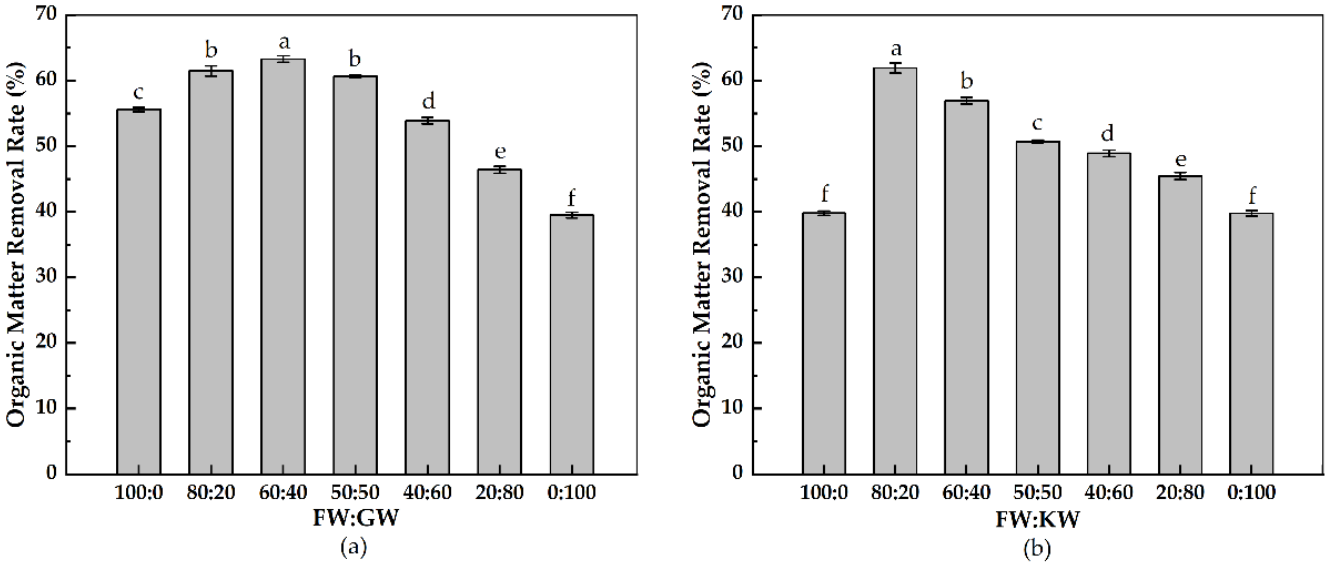

Figure 6. Organic matter removal rates for the thermophilic dry anaerobic co-fermentation of (**a**) FW + GW and (**b**) FW + KW. Letters indicate significant differences between the different mixing ratios ($p < 0.05$).

Figure 6b shows the organic matter removal rates for the collaborative thermophilic dry anaerobic fermentation of FW and KW. The dry anaerobic co-fermentation of FW and

KW had higher organic matter removal rates than the single dry anaerobic fermentations of FW or KW. Compared with other groups, when the ratio of FW to KW was 80:20, the organic matter removal rate was highest at 61.91% ($p < 0.05$) after 30 days of fermentation. This value was 1.55 times the organic matter removal rate of single FW (39.81%) and 1.56 times ($p < 0.05$) the organic matter removal rate of single KW (39.78%).

3.2.4. C/N Ratio

In anaerobic fermentation, carbon and nitrogen are essential elements for the growth and metabolism of microorganisms. Microbial population growth requires a suitable C/N ratio, while C/N ratios in the substrate that are too high or too low affect microbial growth and metabolism, resulting in a decreased anaerobic fermentation efficiency. FW has high biodegradability and its C/N ratio is relatively low, while anaerobic fermentation of only FW may encounter a variety of potential inhibitors, which include VFAs that are produced rapidly by starch degradation and ammonia nitrogen that is produced by protein degradation. Single fermentation of GW or KW also faces many problems, including unbalanced nutrient compositions and slow initiation; however, multicomponent synergistic anaerobic fermentation can improve the balance of nutrients and adjust the C/N ratio of materials, thereby increasing fermentation efficiency.

Figure 7a shows the C/N ratios for dry anaerobic co-fermentation of FW and GW. From the above results, the highest degradation potential occurred when the mixing ratio of FW to GW was 60:40, so the optimal C/N ratio of FW to GW for co-fermentation was 37.20, and after 30 days of fermentation, the C/N ratio terminated at 22.62. The results that explain the effect of C/N ratios on the anaerobic co-fermentation of FW and KW are explicitly presented in Figure 7b. Because the 80:20 group showed the highest degradation potential, the optimal C/N ratio for co-fermentation of FW and KW was 26.73. After 30 days of fermentation, the C/N ratio of the 80:20 group decreased the most to as low as 8.73.

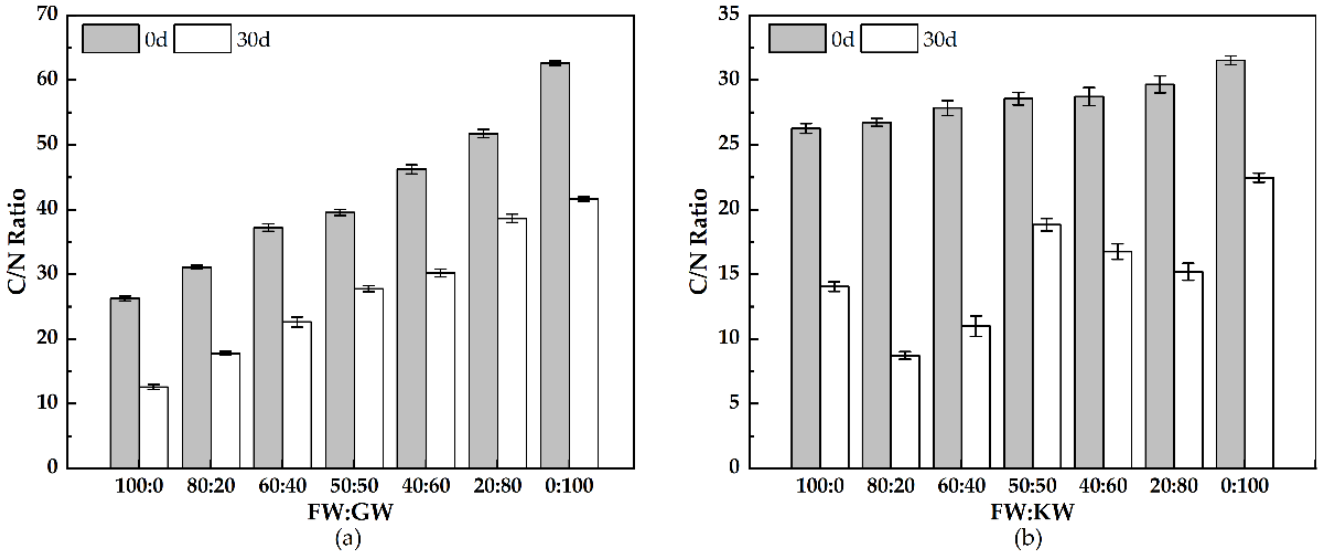

Figure 7. C/N ratios for the thermophilic dry anaerobic co-fermentation of (**a**) FW + GW and (**b**) FW + KW.

4. Conclusions

The thermophilic dry anaerobic co-fermentation of FW using GW or KW as co-substrate enhanced the cumulative hydrogen production by 35.42% and 42.22%, compared with that of single FW fermentation. Meanwhile, the organic matter removal rate of co-fermentation was 1.14 and 1.55 times that of single FW fermentation. Therefore, GW and KW would be ideal co-substrates for anaerobic fermentation of FW, owing to their high hydrogen production and excellent biodegradability via adjustments of C/N ratio,

salt content, and ratio of components difficult to biodegrade to easily degradable ones in substrate.

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