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The Material Flow and Stability Performance of the Anaerobic Digestion of Pig Manure after (Hyper)-Thermophilic Hydrolysis Is Introduced: A Comparison with a Single-Stage Process

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Abstract: Slow hydrolysis persistently affects the anaerobic digestion of animal manure. Thermophilic and hyper-thermophilic treatments introduced into a two-stage anaerobic process treating pig manure were investigated, with a single-stage mesophilic process as a control. The results from the 100-day experiment showed the thermophilic-mesophilic system had the highest removal efficiency of volatile solids at 60.8%, 18% higher than the single-stage process. The thermophilic and hyper-thermophilic hydrolysis reactors contributed 23.5% and 21.7% solubilization of chemical oxygen demand (COD), respectively. The hydrolysis efficiency achieved in the single process was 49.7%, which was lower than the hydrolysis in the two-stage processes. Approximately 60% of COD was distributed in the solid fraction in the first stage, and more than half of the particle COD continued to hydrolyze in the subsequent second stage. The mass balance of COD and volatile solids removal performance illustrated the advantages of the temperature-phased process. Comparatively, the three mesophilic reactors all had strong stability.

Keywords: anaerobic digestion; pig manure; thermophilic and hyper-thermophilic; mass balance; process stability

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1. Introduction

Pig husbandry produces a large amount of manure which may cause environmental risks; therefore, the management of pig manure is vital for the sustainable development of the pig industry [1]. Pig manure is an organically rich biomass, and suitable for treatment in anaerobic digestion (AD). However, the slow hydrolysis significantly limits the overall efficiency of anaerobic digestion. Pig manure has a high organic matter content, averaging 70–80% volatile solids (VS) [2,3]. However, pig manure has low methane production due to its high percentage of suspended solids. Consequently, pretreatment that aims to enhance the hydrolysis of particulates in manure has been developed to improve methane production efficiency. Through the adoption of a thermophilic or hyper-thermophilic bio-hydrolysis under a short hydraulic retention time (HRT), particulates can be largely decomposed to enhance methane formation in the subsequent mesophilic stage [4,5], and as a result, the hydrolysis and acidogenesis rates are significantly improved. Moreover, with thermophilic/hyper-thermophilic pretreatment, the inactivation of culturable pathogens can be greatly increased [6]. Considering energy recovery and sanitation, introducing a thermophilic and/or hyper-thermophilic bio-hydrolysis is a suitable approach for treating pig manure. Nevertheless, in comparison to the widely reported previous studies on the enhancement of bio-hydrolysis in the treatment of sludge, food waste, and co-substrate, it is still not known whether the same methods can be adopted for pig manure.

Methane production enhancement for manure with thermal pretreatment has been reported in batch tests for methane yield potential [7]. However, contradictory results have indicated that the high temperature only provides a limited enhancement for biogas production and organics decomposition in the long-term operation of a continuous anaerobic reactor [8]. Furthermore, it has been reported that even thermophilic hydrolysis pretreatment does not induce the desired performance enhancement [9]. To further increase the benefits of pretreatment, hyper-thermophilic (normally around 70 °C) bio-hydrolysis has been adopted in previous studies. However, whether high-temperature hydrolysis can produce a better performance during the treatment of pig manure requires further investigation. Increasing the temperature would require higher energy input, but a comparative investigation could show the advantages obtained from such a high-temperature process. Material balance analysis is an effective method to evaluate the operational status of the anaerobic system, and the material flow reflects the operational efficiency of the whole anaerobic system. In particular, the material flow between the hydrolysis pretreatment and the subsequent methane fermentation can informatively illustrate the advantages of the process. Evaluation of the available literature shows that little is known about the flow distribution of chemical oxygen demand (COD), and the mass conversion of pig manure in the AD process is still not yet fully understood.

The core objective is the stability of the anaerobic process, and this is even more important than degradation performance in the AD system. For pig manure, the unstable conditions encountered when using a slightly shorter hydraulic retention time is a persistent challenge [10]. Theoretically, when the manure is pre-hydrolyzed, the receiving methane formation reactor should have a stronger buffer capacity to accept the flowing acidified materials, including much higher fatty acids. In each feeding cycle, the methanogens may have increased pressure to manage the elevated concentration of acids. To reveal the stability performance, the long-term operation of anaerobic digestion after the introduction of hydrolysis pretreatment is essential, and this should be directly compared to the widely used single-stage process.

Therefore, this study aims to overcome the slow hydrolysis during the AD of pig manure by introducing (hyper)thermophilic hydrolysis, and compare the materials flow and stability performance with a single-stage process to illustrate the effects of (hyper)thermophilic hydrolysis pretreatment.

2. Materials and Methods

2.1. Design and Operation of Single-Stage and Two-Stage Reactors

Five continuous stirred-tank reactors (CSTRs) were used in this study and every CSTR had a working volume of 4 L (total volume of 6 L). The five reactors, which were maintained under the operating temperatures by water baths (TMK-2K, Osaka, Japan), consisted of three sets of operation processes: a single-stage M_{37} (37 ± 1 °C) system with an HRT of 30 d as control; a two-stage of T_{55} + $M_{37\leftarrow55}$ (55 ± 1 °C & 37 ± 1 °C) system with an HRT of 5 d + 25 d; and a two-stage of H_{70} + $M_{37\leftarrow70}$ (70 ± 1 °C & 37 ± 1 °C) system with an HRT of 5 d + 25 d. In the two-stage systems, the effluent of the first stage was the feeding of the second stage. The five reactors were not started up at the same time. Based on the T_{55} and H_{70} reactors' startup time, the M_{37} and $M_{37\leftarrow70}$ reactors were started up on the 7th day, and the $M_{37\leftarrow55}$ was started up on the 32nd day. During the whole experiment (100-day period), pH was not adjusted, and no external reagent was added. The three systems were illustrated in the Figure 1 and parameters were shown in Table 1.

The measured total solids (TS) and VS of raw pig manure from a farm in Beijing were 31.9% ± 0.6% and 24.9% ± 0.3%, respectively. The pig manure was homogenized using a multi-function pulverizing machine (JJ2BS, Fujian, China) and diluted using tap water. And the final TS was around 10% for feeding. Obtained feedstock pig manure was stored at a low temperature (4 °C) until use. The inoculum sludge was from a mesophilic anaerobic pilot plant in the Beijing Drainage Group, and each reactor was inoculated with

4 L of the seed sludge. The characteristics of inoculum sludge have been described in a previous study [6].

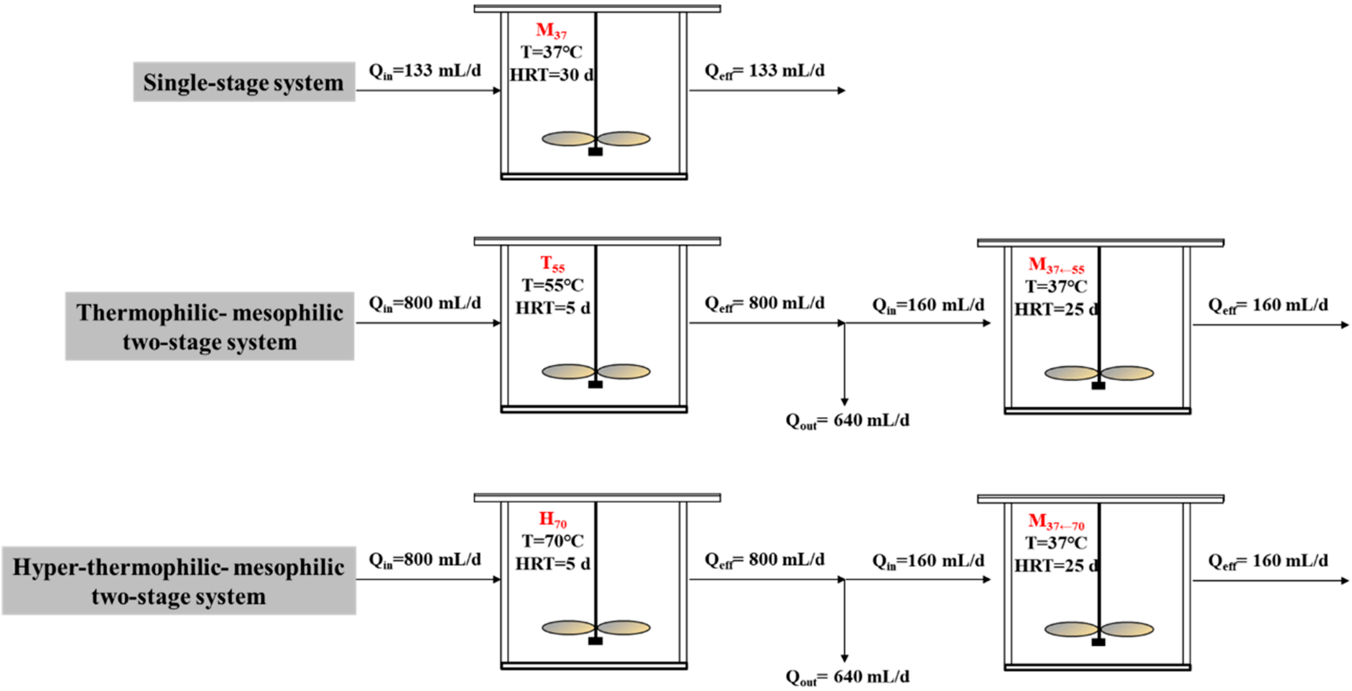

Figure 1. Experimental set-up used in the study.

Table 1. Parameters of experimental set-up in the study.

Parameters	Single System	T_{55} + $M_{37\leftarrow55}$ System		H_{70} + $M_{37\leftarrow70}$ System	
	M_{37}	T_{55}	$M_{37\leftarrow55}$	H_{70}	$M_{37\leftarrow70}$
Temperature (°C)	37	55	37	70	55
HRT (d)	30	5	25	5	25
Total volume (L)	6	6	6	6	6
Work volume (L)	4	4	4	4	4
Daily feed input (mL)	133	800	160	800	160
Daily feed output (mL)	133	800	160	800	160

2.2. Analytic Methods

Biogas from each reactor was collected in plastic gasbags. The measurement of gas compositions, biogas volume, pH, TS, VS, volatile fatty acids (VFA), NH_4^+-N, soluble chemical oxygen demand (SCOD), and total chemical oxygen demand (TCOD) were in reference to a previous study [6]. A high performance liquid chromatography (HPLC) was used to determine the concentration of lactic acid (LA) in the T_{55} and H_{70} reactors, as in a previous study [11].

2.3. Calculations

The removal efficiencies of the systems were calculated using Equation (1).

$$R_X = \frac{X_0 - X_2}{X_0} \times 100\%, \quad (1)$$

In the two-stage process, every stage had a removal efficiency and contributed to the system removal. The contribution rates of the first stage and the second stage were calculated using Equations (2) and (3) [4].

$$R_1 = \frac{X_0 - X_1}{X_0 - X_2} \times 100\%, \quad (2)$$

$$R_2 = \frac{X_1 - X_2}{X_0 - X_2} \times 100\%, \quad (3)$$

where X_0 is the TS/VS/TCOD concentration in the influent, g/L; X_1 is the TS/VS/TCOD concentration in the effluent from the first stage, g/L; X_2 is the TS/VS/TCOD concentration in the effluent from the second stage, respectively, g/L. R_X means the TS/VS/TCOD removal efficiencies. R_1 and R_2 represent the contributions to the total removal by the first and second stage, respectively.

The conversion efficiencies of the four steps in anaerobic digestion were calculated using Equations (4)–(7) [12].

$$\text{Hydrolysis\%} = \frac{SCOD - SCOD_{in} + COD_{CH_4}}{TCOD_{in} - SCOD_{in}}, \quad (4)$$

$$\text{Acidogenesis\%} = \frac{COD_{VFA} - COD_{VFA_{in}} + COD_{CH_4}}{TCOD_{in} - COD_{VFA_{in}}}, \quad (5)$$

$$\text{Acetogenesis\%} = \frac{COD_{C_2} - COD_{C_{2_{in}}} + COD_{CH_4}}{TCOD_{in} - COD_{C_{2_{in}}}}, \quad (6)$$

$$\text{Methanogenesis\%} = \frac{COD_{CH_4}}{TCOD_{in}}, \quad (7)$$

$TCOD_{in}$, $SCOD_{in}$, COD_{VFAin}, and COD_{C2in} were the total COD, SCOD, VFA, and acetate of the influent calculated by COD. SCOD, COD_{VFA}, and COD_{C2} were the SCOD, VFA and acetate concentrations of the effluent calculated by COD. COD_{CH4} was calculated based on the principle of 350 mL-CH_4/g-COD under standard conditions.

Considering the proliferation of microorganisms, the COD conversion efficiency (η) was calculated using Equation (8) [13].

$$\eta\% = \frac{COD_{CH_4} + COD_M}{COD_{in}} \times 100\%, \quad (8)$$

where COD_{CH4} is the COD of methane, g; COD_M is the COD converted to microorganism, g, the conversion factor is 0.08 g-VSS/g-COD according to the reference [14]; COD_{in} is the COD in the influent, g.

2.4. Statistical Analysis

The methane yields, pH values and VFA concentrations of the three systems were carried out statistical analysis. When the data conform to the normal distribution, One-way ANOVA was used; while when the data do not conform to the normal distribution, Kruskal–Wallis ANOVA was used. OriginPro 2021 was used for statistical analysis.

3. Results and Discussion

3.1. Methane Production Performance

The performance in terms of gas production and methane content in the biogas within the five reactors is illustrated in Figure 2. After 60 d, all reactors were considered to have entered a steady period. The following data results calculated are all based on the data from the stable period. Table 2 summarizes the gas performance results in the three systems. The volumetric biogas production rates of the three systems were all over 1.2 L/L/d, and highest one appeared in the H_{70} + $M_{37\leftarrow 70}$ system with 1.44 L/L/d. In the case of each reactor, the $M_{37\leftarrow70}$ obtained the highest volumetric biogas production rate of 1.71 L/L/d, while the volumetric biogas production rate of the H_{70} was lowest (0.07 L/L/d). The biogas yields in the T_{55} + $M_{37\leftarrow55}$ and H_{70} + $M_{37\leftarrow70}$ systems were, respectively, 9.4% and 14.3% higher than those in the M_{37}. However, the three systems had similar methane yields. One-way ANOVA showed a significant difference was not present between the T_{55} + $M_{37\leftarrow55}$ and H_{70} + $M_{37\leftarrow70}$ processes in terms of methane yield, yet when compared to the single-stage process, the two-stage process showed a higher methane yield. The result confirms previous research demonstrating that a thermophilic-mesophilic two-stage anaerobic system is conducive to increasing methane production [15]. The M_{37} system had the highest methane content of 65.7%, approximate to the average methane content of 65% in a biogas plant of pig manure [16]. The methane contents of the T_{55} + $M_{37\leftarrow55}$ and H_{70} + $M_{37\leftarrow70}$ systems were 63.7% and 59.4%, respectively. On the basis of those results, it can be concluded that a high-temperature treatment increased biogas production in the two-stage process, with better effects being observed in the thermophilic condition. However, when contrasted with single-stage process, the methane content did not increase. By comparing the methane content of the three mesophilic reactors, it was found that a high-temperature treatment decreased the methane content, indicating that the higher the temperature, the lower the methane content. This may have been due to the difference in the concentration of various organic compounds in the hydrolysates. In addition, the shifts in microbial community may be another reason. The effluent of the first stage was as the feeding of the mesophilic reactor, with a large number of hydrolytic bacteria and acidifying bacteria entered the mesophilic reactor, which may change the microbial community.

Table 2. The biogas composition and yield in the three systems during the steady states.

Performance	Unit	Single System	T_{55} + $M_{37\leftarrow55}$ System			H_{70} + $M_{37\leftarrow70}$ System		
		M_{37}	T_{55}	$M_{37\leftarrow55}$	Total	H_{70}	$M_{37\leftarrow70}$	Total
Volumetric biogas production rate	L/L/d	1.22	0.32	1.57	1.36	0.07	1.71	1.44
CH_4	%	65.7	36.6	64.9	63.7	0	59.9	59.4
CO_2	%	30.3	51.0	32.5	33.5	28.6	39.1	39.1
Biogas yield	L/g-VS_{in}	0.456	0.021	0.567	0.499	0.004	0.523	0.521
Methane yield	L/g-VS_{in}	0.30	0.01	0.37	0.32	0	0.32	0.31

Table 3 shows the comparison between previous researches on the anaerobic digestion of pig manure. In the single CSTR, the methane yield was in the 188–320 mL/g-VS range. In this study, the methane yield was 293 mL/g-VS, consistent with the literature report. The different properties of feed pig manure and process operation parameters may be the reason for the difference in exact methane yield. As for the two-stage CSTRs, this study showed a near methane yield in the T_{55} + $M_{37\leftarrow55}$ (317 mL/g-VS) and the H_{70} + $M_{37\leftarrow70}$ (313 mL/g-VS), 6–8% higher than the single M_{37}. The research of Hu et al. also proved that the hyper-thermophilic-mesophilic two-stage process has better methane yield than the single-stage process.

Table 3. Comparison of previous research on pig manure.

Process	T (°C)	HRT (d)	Operation Time (d)	Feeding TS (%)	Methane Yield (mL/g-VS)	References
Single CSTR	37	15	~64	/	188	[17]
Single CSTR	37	15	49	7.2	217	[18]
		25.3	39/504 ^{b}	6.6	267	
Single CSTR	50~52	21	54/504 ^{b}	7.1	272	[19]
		21	61/504 ^{b}	7.2	320	
Single CSTR	37	30	91	10.6	293	This study

Table 3. *Cont.*

Process	T (°C)	HRT (d)	Operation Time (d)	Feeding TS (%)	Methane Yield (mL/g-VS)	References
Single CSTR	35	41	~67/~225 [b]	23.6	199	[20]
Two-stage CSTRs	70 [a]–35	3 + 29	~47/~225 [b]	23.6	298	
	70 [a]–35	3 + 41	~67/~225 [b]	23.6	315	
Thermal treatment + batch	55–35	1 + 23	24	22.4–10	193	[21]
	65–35	3 + 23	26	22.4–10	206	
Two-stage CSTRs	55–37	5 + 25	100	10.6	317	This study
Two-stage CSTRs	70–37	5 + 25	100	10.6	313	

Note: [a] raw pig manure was pretreated for 3 days at 70 °C; [b] x/y, x means the duration of this phase; y means the total operation time.

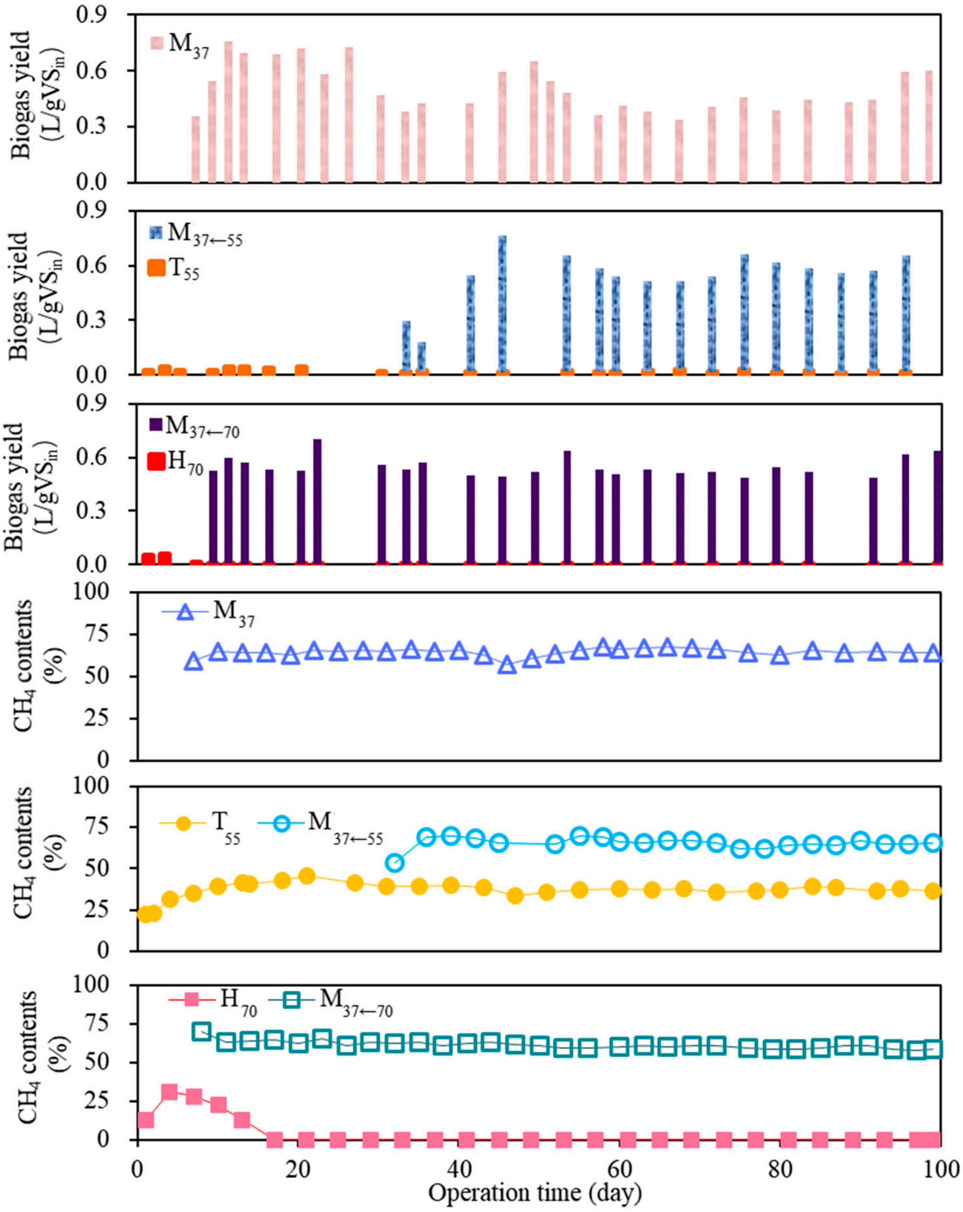

Figure 2. The biogas yield and methane content i in the three systems.

3.2. Decomposition Performance and Mass Balance

During the AD of the pig manure, the changes in TS, VS, and TCOD reflected the degradation and transformation of organic matter. The organic matter removal efficiency is shown in Figure 3a–c. In the M_{37}, the average removal efficiencies of TCOD, VS, and TS were 56.9%, 52.3%, and 39.2%, respectively. The removal efficiencies of the $M_{37 \leftarrow 70}$ were not much different from that of the M_{37}. However, the $M_{37 \leftarrow 55}$ had the highest removal efficiencies in comparison with the M_{37}, with 9.9% higher TS removal efficiency, 1.9% higher VS removal efficiency, and 2.6% higher TCOD removal efficiency. Among the reactors, the H_{70} reactor had the lowest removal efficiencies of TCOD, VS, and TS. These were observed to be lower than 6.5%, indicating that only a small part of the organic matter was degraded in the H_{70}, and the absence of methanogenesis in this reactor resulted in this low rate of degradation. The rate of contribution of T_{55} in the T_{55} + $M_{37 \leftarrow 55}$ system in terms of TCOD and VS removals was 13.4% and 28.1%, respectively. Furthermore, the rate of contribution of H_{70} to the H_{70} + $M_{37 \leftarrow 70}$ system during TCOD and VS removals was 7.4% and 8.3%, respectively.

TS and VS balance are shown in Figure 3d,e.

In the M_{37} system, 39.7% of TS was converted to biogas, which was consistent with the removal efficiency of TS. In the T_{55} + $M_{37 \leftarrow 55}$ and H_{70} + $M_{37 \leftarrow 70}$ systems, 51.6% and 40.8% of TS were converted to biogas, respectively. The VS removal efficiencies in the M_{37}, T_{55} + $M_{37 \leftarrow 55}$ and H_{70} + $M_{37 \leftarrow 70}$ systems were 52.3%, 61.5%, and 53.0%, respectively. One-way ANOVA shows there was a significant difference. In addition, the TCOD removal efficiencies in the M_{37}, T_{55} + $M_{37 \leftarrow 55}$ and H_{70} + $M_{37 \leftarrow 70}$ systems were 56.9%, 62.8%, and 58.0%, respectively. The T_{55} + $M_{37 \leftarrow 55}$ system had the best organic matter removal efficiency, and the two-stage AD was able to degrade more organic matter than the single-stage AD.

COD mass balance was calculated based on the experimental results, and Figure 3f shows the summarized results. In feedstock pig manure, SCOD and particle chemical oxygen demand (PCOD) constituted the TCOD, which was calculated as 100%. In the T_{55} and H_{70} reactors, as a result of hydrolysis and acidification, the PCOD content decreased and the SCOD concentration increased. Furthermore, the H_{70} had a higher SCOD concentration than the T_{55} due to the absence of methanogenesis. There was similar SCOD content remaining in the effluent of the M_{37} (5.7 $\pm$ 0.8 g/L), $M_{37 \leftarrow 55}$ (5.6 $\pm$ 0.7 g/L), and $M_{37 \leftarrow 70}$ (5.8 $\pm$ 0.9 g/L), while a significant difference was present by One-way ANOVA. The lowest amount of PCOD + SCOD (34.5%) remained in the T_{55} + $M_{37 \leftarrow 55}$ system, which corresponded with the highest CH_4 yield. Meanwhile, the single-stage system had the lowest CH_4-COD (54.4%), corresponding with the highest PCOD + SCOD (40.3%).

The degradation of organic matter depends on the action of microorganisms. One part of the degraded organic matter is used for the proliferation of microorganisms, and the other part is eventually converted into methane. The biomass distribution and COD conversion efficiency of the three systems are presented in Figure 4, with an influent COD of 100%. In the single AD, about 61% of the organic matter could have been converted by microorganisms, with 56.1% converted to methane, and 4.9% used for the proliferation of anaerobic microorganisms. In the T_{55} + $M_{37 \leftarrow 55}$ system, only 9.2% of the organic matter was converted by microorganisms in the T_{55}. The remaining undecomposed organic matter went into the mesophilic reactor, and about 59.5% of this organic matter could have been converted by microorganisms, with 54.7% converted to methane, and 4.8% used for the proliferation of anaerobic microorganisms. In the H_{70} + $M_{37 \leftarrow 70}$ system, more undecomposed organic matter (94.4%) in the H_{70} reactor went into the mesophilic reactor, and about 57.8% of the organic matter could have been converted by microorganisms, with 53.2% converted to methane, and 4.6% used for the cell proliferation. The final undecomposed organic matter in the three systems was M_{37} (39%) > H_{70} + $M_{37 \leftarrow 70}$ (37%) > T_{55} + $M_{37 \leftarrow 55}$ (32%), which was consistent with the organic matter removal results (Figure 4).

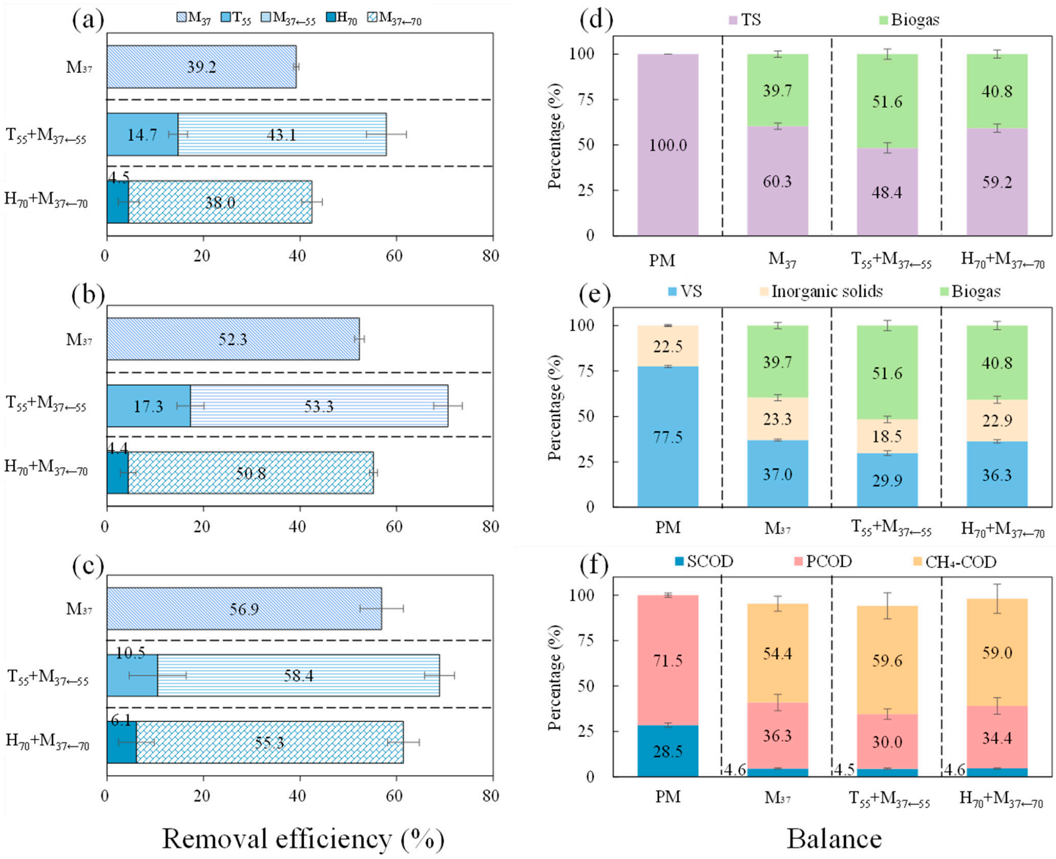

Figure 3. Organic matter removal and mass balance of the three anaerobic digestion systems: (**a**) TS removal efficiency; (**b**) VS removal efficiency; (**c**) TCOD removal efficiency; (**d**) TS balance; (**e**) VS balance; (**f**) COD balance.

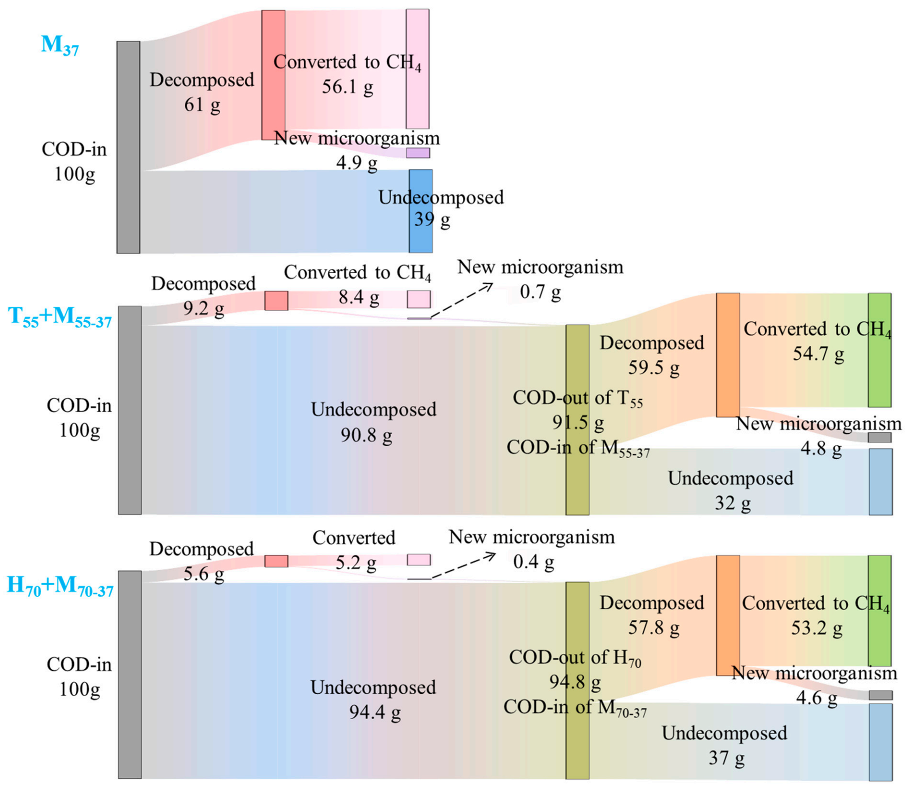

Figure 4. Biomass distribution and COD conversion efficiency of the three systems.

3.3. Characterization of COD Flow in the Three Systems

Balancing the acidogenesis and methanogenesis metabolism is crucial for an anaerobic process [22]. With the high-temperature hydrolysis pretreatment, the COD flow may have experienced changes because higher VFAs went to the methanogenic reactor. The conversion efficiencies for the four steps of AD in the reactors/systems are shown in Table 4. In the T_{55} + $M_{37\leftarrow 55}$ system, around 56.3% of suspended solid in pig manure was hydrolyzed, which was higher than the 49.7% observed in the M_{37} system. When considered alongside the next three steps, the conversion efficiencies of the T_{55} + $M_{37\leftarrow 55}$ system were 5.7%, 5.1%, and 4.8% higher than the M_{37} system during the acidogenesis, acetogenesis, and methanogenesis steps. However, in the T_{55} reactor, the conversion efficiencies during the four steps were far less than those in the M_{37} and $M_{37\leftarrow 55}$ reactors, recording a hydrolysis ratio of 14.5%, and an acidogenesis ratio of 12.1%. The low hydrolysis ratio in the T_{55} reactor could be attributed to the acidic pH (6.21 $\pm$ 0.05) and high TVFA (23.1 $\pm$ 1.8 g-acetate/L), which led to an adverse environment for functional microorganisms, thus inhibiting the hydrolysis of organic matter. In the T_{55}, 1.4% methanogenesis indicated that the consumption rate of VFA was slow, while the generation rate of VFA from the acidogenesis of pig manure was higher, which led to the VFA accumulation.

The H_{70} + $M_{37\leftarrow 70}$ system accomplished conversion efficiencies, approximately 4–6% higher than those in the M_{37} reactor. In the H_{70} reactor, the conversion efficiencies were extremely low, with a hydrolysis ratio of 12.8%, and an acidogenesis ratio of 0.9%. Low pH (5.89 $\pm$ 0.16) and total VFA (TVFA) (13.9 $\pm$ 2.0 g-acetate/L) could have been the cause. In the H_{70} reactor, acidogenesis appeared to be the rate-limiting step. When comparing the conversion efficiency with the four steps in the T_{55} + $M_{37\leftarrow 55}$ and H_{70} + $M_{37\leftarrow 70}$ systems, the former was about 1% higher than the latter, suggesting that the thermophilic-mesophilic AD contributed more to enhancing hydrolysis than the hyper-thermophilic-mesophilic AD. Acidogenesis and acetogenesis conversion efficiencies also reflected the conversion of acid. There were a few residual VFAs (0.5–0.6 g-COD/L) in the second stage and the removal efficiency reached 96.6–97.6%. The change of acetic acid was similar in the second stage, at 96.2–97.6% removal efficiency.

Table 4. COD conversion efficiencies (%).

Performance	Single System	T_{55} + $M_{37\leftarrow 55}$ System			H_{70} + $M_{37\leftarrow 70}$ System		
	M_{37}	T_{55}	$M_{37\leftarrow 55}$	Total	H_{70}	$M_{37\leftarrow 70}$	Total
Hydrolysis	49.7	14.5	55.3	56.3	12.8	53.3	55.4
Acidogenesis	53.9	12.1	59.4	59.6	0.9	61.8	58.5
Acetogenesis	57.5	5.0	65.7	62.6	0.6	65.1	61.8
Methanogenesis	59.1	1.4	68.3	63.9	0.0	66.6	63.1

Based on hydrolysis and methanogenesis conversion efficiencies, Figure 5 shows the COD flow of the three systems. The 100 g of feed pig manure (as TCOD) contained 28.5 g SCOD and 71.5 g PCOD. In the M_{37}, 49.7% of PCOD was converted into SCOD; thus, the hydrolysis conversion efficiency was 49.7%. Supposing this portion of the hydrolyzed COD was eventually completely converted to methane, then 23.6% methanogenesis conversion efficiency would be attributed to the contribution of SCOD in feed pig manure. Lastly, the methanogenesis conversion efficiency (59.1%) was higher than the hydrolysis conversion efficiency. In the two two-stage systems, 9.2–10.4% PCOD was converted into SCOD during the first stages and the 53.3–55.3% remaining PCOD was converted into SCOD in subsequent mesophilic reactors. Thus, it can be seen that the ability to convert PCOD to SCOD in the three mesophilic reactors was in this order: $M_{37\leftarrow 55}$ > $M_{37\leftarrow 70}$ > M_{37}. Thermophilic and hyper-thermophilic conditions in the first stage hydrolyzed solid organic matter by a small margin; therefore, the enhancement effect of high temperature on hydrolysis was reflected in both the first and second stages. Additionally, the thermophilic condition was better than the hyper-thermophilic condition. In the T_{55} + $M_{37\leftarrow55}$ system, 25.5 g out of 28.5 g SCOD in pig manure was converted into methane (supposing the COD hydrolyzed during the two stages was completely converted to methane). In the H_{70} + $M_{37\leftarrow70}$ system, 24.2% methanogenesis conversion efficiency was attributed to the contribution of SCOD in feed pig manure. Thermophilic-mesophilic AD had a better effect on hydrolysis and methane production.

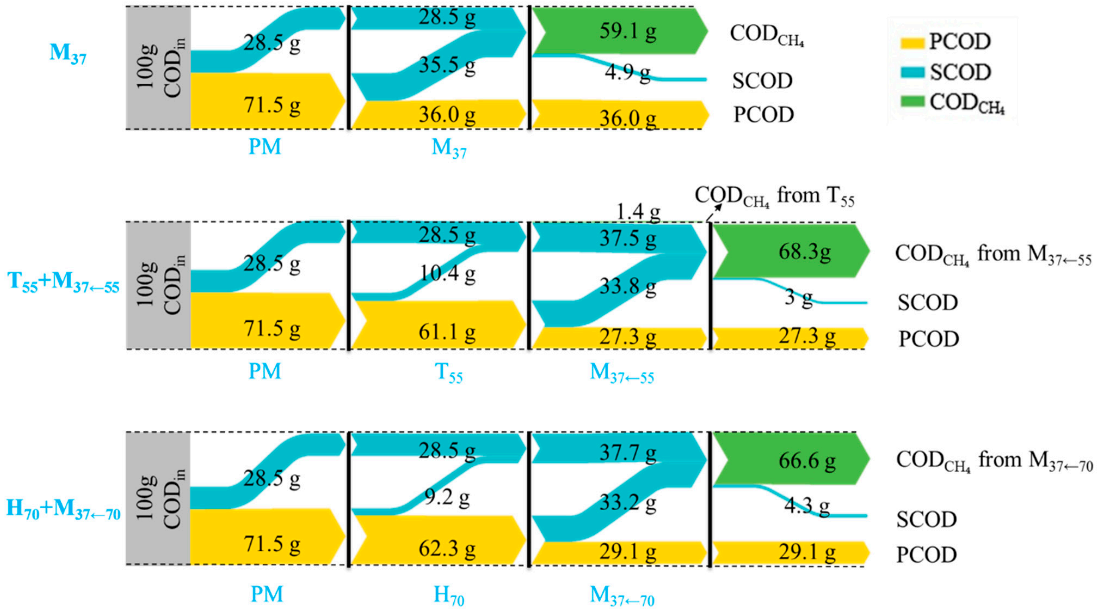

Figure 5. COD flow of the three systems.

3.4. Stability Performance in the Three Processes

Figure 6 summarizes the pH and VFA characteristics of feedstock pig manure and the sludge in the five reactors. The pH of pig manure fluctuated between 5.55~6.53. However, the pH of the thermophilic and hyper-thermophilic reactors stabilized at 6.14~6.30 and 5.69~6.10, respectively, although it did experience a rapid decline in the initial period. Unlike those two reactors, there were no distinct escalations or drops in the other three reactors during the initial period, and the pH ranges in the entire operation period were 7.69~8.06 (M_{37}), 7.58~8.01 ($M_{37\leftarrow55}$) and 7.64~7.99 ($M_{37\leftarrow70}$), respectively. Neutral to slightly alkaline conditions are optimal for methane-producing bacteria. A general understanding is that methanogenic bacteria have their optimum pH value within the range of 6.7~7.4 [23]. However, it has been reported that the anaerobic digestion process can tolerate a pH range from 6.5 up to 8.0 [24]. The pH ranges in the above three reactors were within the limits suitable for the growth and metabolism of methanogens, under conditions that required no pH adjustment. In the five reactors, the average pH values were 7.86 ± 0.08 (M_{37}), 6.21 ± 0.05 (T_{55}), 7.89 ± 0.08 ($M_{37\leftarrow55}$), 5.89 ± 0.16 (H_{70}), and 7.77 ± 0.05 ($M_{37\leftarrow70}$). Kruskal–Wallis ANOVA showed that there were distinctive differences ($p < 0.05$) in the pH values between the T_{55} and H_{70} reactors, between the $M_{37\leftarrow70}$ and M_{37} reactors, and between the $M_{37\leftarrow70}$ and $M_{37\leftarrow55}$ reactors. There were distinctive differences ($p < 0.01$) in the pH values between the T_{55} and H_{70} reactors, and between the $M_{37\leftarrow55}$ and $M_{37\leftarrow70}$ reactors. However, the M_{37} and $M_{37\leftarrow55}$ reactors had indistinctive differences ($p = 0.199 > 0.05$). The pH value of the H_{70} was lower than 6.2, which is a value under which methanogenic activity is considerably inhibited [25]. It arises from the toxic effect on the methanogenic archaea of some intermediates, and this could explain the reason for the H_{70} reactor not producing methane (Figure 2).

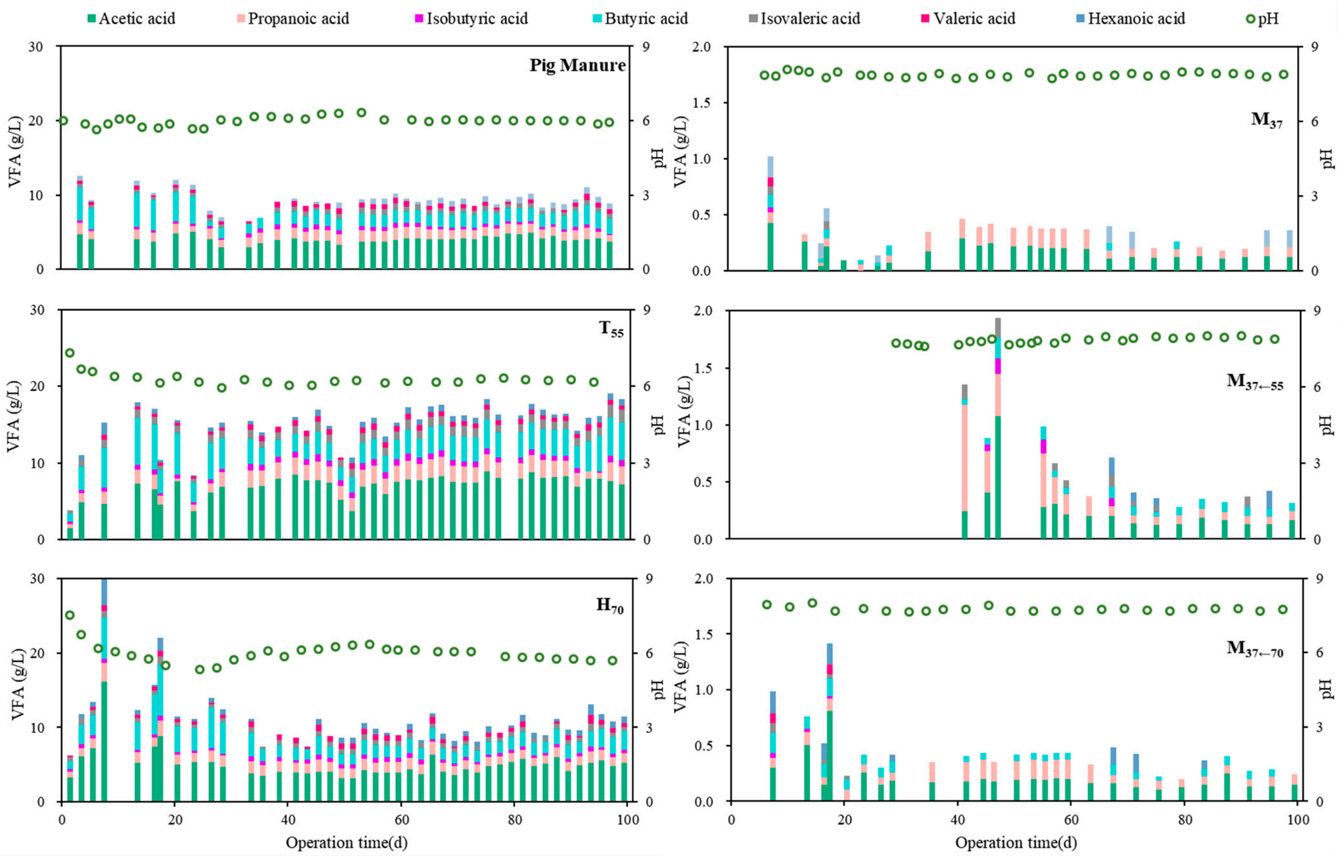

Figure 6. The variation of pH and VFA in the three anaerobic digestion systems.

Table 5 summarizes the main VFA and LA concentrations in the reactors. TVFA is the acidizing product and direct/indirect substrate for methanogenesis in anaerobic digestion, and the accumulation of VFAs should be avoided for a stable anaerobic process. A 13.1 $\pm$ 1.1 g-acetate/L high TVFA concentration was observed in the pig manure, and the concentrations of TVFA in the T_{55} and H_{70} were 23.1 $\pm$ 1.8 and 13.9 $\pm$ 2.0 g-acetate/L, respectively. A higher TVFA concentration in the T_{55} than in the H_{70} indicated that it was more beneficial to realize the acidification of organic matter in pig manure under thermophilic conditions. However, the T_{55} had a higher pH value than the H_{70}, which could be explained by lactic acid concentration. In the T_{55} and H_{70} reactors, the lactic acid concentrations were 5.6 $\pm$ 0.4 and 13.0 $\pm$ 0.5 g/L, respectively. This result was in accordance with a previous report that higher production of lactic acid was obtained under hyper-thermophilic conditions [26]. Soluble sugars are substrates available for the growth of lactic acid bacteria. Lactic acid can be degraded into acetic acid alone or acetic acid and propionic acid simultaneously [27]. According to a previous study, a pH range of 5–6 was the optimum for lactic acid production [28], explaining why additional lactic acid was produced at a hyper-thermophilic temperature. Another reason for the pH result could be attributed to a better buffer environment in the T_{55} due to the high concentration of ammonia nitrogen with 3.1 $\pm$ 0.2 g/L, whereas 1.8 $\pm$ 0.1 g/L ammonia nitrogen was measured in the H_{70}. At the same time, it also showed better proteolysis in the thermophilic reactor than in the hyper-thermophilic reactor. This may have been due to the lower acidic pH inhibiting protein degradation [29]. For pig manure with high nitrogen content, ammonia can lead to unstable anaerobic performance as a potential inhibitor [30]. However, in the second stage reactors, that situation was not serious. In the methane-producing reactors, low concentrations of VFA were observed. During the steady period, the TVFA in the M_{37}, $M_{37 \leftarrow 55}$, and $M_{37 \leftarrow 70}$ reactors were 0.4 $\pm$ 0.2, 0.6 $\pm$ 0.2, and 0.4 $\pm$ 0.2 g-acetate/L, respectively, which showed that the methanogenic reactors were in a good condition. One-way ANOVA showed that there was no significant difference in residual VFA of the three mesophilic reactors ($p > 0.05$).

Table 5. The summary of main VFA and LA concentrations.

	PM	M_{37}	T_{55}	$M_{37\leftarrow 55}$	H_{70}	$M_{37\leftarrow 70}$
TVFA(mg-acetate/L)	13.1 ± 1.1	0.4 ± 0.2	23.1 ± 1.8	0.6 ± 0.2	13.9 ± 2.0	0.4 ± 0.2
Acetic acid (g/L)	4.24 ± 0.32	0.13 ± 0.02	7.89 ± 0.48	0.13 ± 0.02	4.82 ± 0.75	0.15 ± 0.04
Propionic acid (g/L)	1.23 ± 0.17	0.09 ± 0.03	2.07 ± 0.44	0.09 ± 0.03	1.28 ± 0.17	0.09 ± 0.04
Butyric acid (g/L)	1.86 ± 0.28	0.01 ± 0.03	3.54 ± 0.64	0.01 ± 0.03	1.83 ± 0.32	0.05 ± 0.04
Lactic acid (g/L)	/	/	5.6 ± 0.4	/	13.0 ± 0.5	/

Note: / means the data was not measured.

In the five reactors, acetate, propionate, and butyrate were the main VFAs. The concentration of acetic acid in the T_{55} was the highest at 7.89 g/L, 64% higher than that in the H_{70} and 86% higher than that in pig manure. The concentration of propionic acid in the T_{55} was 2.07 g/L, 1.6 times that of the H_{70}. The concentration of butyric acid in the T_{55} was 3.54 g/L, 1.9 times that of H_{70}. In the T_{55} and H_{70}, acetic acid was the highest proportion of VFA, followed by butyric acid and propionic acid. This is in line with previous research which indicated that acetic acid and butyric acid were the dominant VFA at 55 °C and 80 °C when cellulose was used as the substrate of acidogenic fermentation [31]. As for the other three methane-producing reactors, the concentrations of acetic acid, propionic acid, and butyric acid were all below 0.2 g/L, which showed good stability.

4. Conclusions

The results of the two-stage process in a continuous experiment demonstrated an enhancement in the methane yield. Thermophilic and hyper-thermophilic treatment improved the COD conversion efficiencies compared to untreated pig manure and the thermophilic-mesophilic system received a better result. Nevertheless, the unaided thermophilic and hyper-thermophilic stages did not provide high COD conversion efficiencies. More than half of the particle COD was further degraded and converted in the second stage. Under an HRT of 30 days, the two-stage and single-stage AD of pig manure could steadily operate. The high concentration of VFA formed by thermophilic and hyper-thermophilic treatment had minimal effect on the stability of the subsequent methanogenic reactor. The two-stage processes showed some advantages over a single process for pig manure, and these could be improved further after optimizing the operation parameters.

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Abbreviations

AD	anaerobic digestion
COD	chemical oxygen demand
CSTRs	continuous stirred-tank reactors
HPLC	high performance liquid chromatography
HRT	hydraulic retention time
LA	lactic acid
PCOD	particle chemical oxygen demand
SCOD	soluble chemical oxygen demand
TCOD	total chemical oxygen demand
TS	total solids
TVFA	total volatile fatty acid
VFA	volatile fatty acids
VS	volatile solids

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