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Anaerobic co-digestion of food waste and sewage sludge under mesophilic and thermophilic conditions: Focusing on synergistic effects on methane production



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ABSTRACT

Globally, food waste (FW) and sewage sludge (SS) are among major organic wastes that needs to be properly handled. Anaerobic digestion (AD) has been a widely accepted method to treat those wastes and simultaneously recover energy and nutrients. The objective of this study was to elucidate synergistic effects in co-digestion of FW and SS by incubating at mesophilic and thermophilic conditions. The results demonstrated temperature did not significantly affect the cumulative methane yields (*CMY*); instead, higher temperature resulted in accelerated methane production. Although not clearly shown on CMYs (less than 10% increase), positive synergistic effects on methane production rates were characterized by a co-digestion impact factor (CIF) during the early phase of co-digestion. This early synergism (up to 24% increase) was primarily attributed to the accelerated hydrolysis due to addition of readily degradable FW, which may have practical implication for selection of retention time in order to optimize digestion process.

1. Introduction

Food waste (FW) constitutes 30-50% of municipal solid waste (MSW) and globally 1.4 billion tons of FW was produced in a year (Kiran et al., 2014; Wong et al., 2018). The Food and Agriculture Organization (FAO) of the United Nations estimated that more than 2.2 billion tons of FW would be generated worldwide by 2025 (Mehariya et al., 2018). In China alone, approximately 60 million tons of FW was produced in a year (Li et al., 2013), and the FW production is expected to keep increasing due to social-economic and population growth. In addition, the generation of sewage sludge (SS) in China has also remarkably increased in recent years. From 2007 to 2015, the quantity of SS produced by wastewater treatment plants (WWTPs) had an average growth of 13% per year (Li et al., 2018). Approximately 30-40 million tons of SS were generated in 2015 (Liu et al., 2015; Xu et al., 2018), and China is expected to produce more than 60 million tons in 2020 (Xiao et al., 2018). Therefore, significant effort is needed to handle ever-increasing FW and SS in order to avoid detrimental environmental and social impacts.

Among technologies that may be used to treat FW and SS, anaerobic digestion (AD) has been well recognized as a promising way for waste

stabilization and simultaneous energy recovery in the form of methanerich biogas (Mata-Alvarez et al., 2000; Levis and Barlaz, 2011; Zhou et al., 2017). While a variety of physical, chemical, and biological pretreatments prior to digestion have been investigated for their roles to improve methane generation during AD processes (Yin et al., 2016; Zhang et al., 2016; Chen et al., 2018; Gupta et al., 2019), co-digestion alone has been often reported to improve specific methane yields and methane production rates upon comparison with mono-digestion, due to superior nutrient availability, toxicity dilution, robust and synergistic microbiomes, etc. (Mata-Alvarez et al., 2000; Mehariya et al., 2018). Furthermore, unlike physical, chemical or biological pretreatment, codigestion does not require additional energy, chemicals and installation input; therefore, it has been considered as a cost effective way to improve digestion efficiency.

Recent years have witnessed an increasing trend to investigate anaerobic co-digestion of FW and SS. The majority of existing studies indicated that co-digestion lead to increased methane production compared with mono-digestion of single substrates. For example, Adelard et al. (2015), Kim et al. (2017), Xie et al. (2017), and Pan et al. (2019) found co-digestion exhibited improvement on substrates' specific methane yields. However, contrary results did exist. No synergistic

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effect on methane yields resulting from co-digestion was reported by Dai et al. (2013), Koch et al. (2015) and Liu et al. (2016). The varied observations on synergistic effects in the literature can be attributed to different substrate/inocula mixture composition, incubation temperature, and batch/continuous reactors used, etc. In addition, studies that focusing on co-digestion of FW and SS have often limited their investigation at either mesophilic or thermophilic temperature. The operating temperature is a key factor influencing AD performance. The findings of Caporgno et al. (2015) showed the higher operating temperature did not improve biogas yields from co-digestion of SS and microalgae. However, a study by Zamanzadeh et al. (2016) reported conflicting results: the methane yield of FW under mesophilic conditions was higher than that under thermophilic conditions. Nevertheless, a systematic elucidation of synergistic effects during co-digestion of FW and SS at different operating temperatures is lacking in the literature.

In this study, two series of anaerobic batch experiments were conducted under mesophilic and thermophilic conditions with different mixing ratios of FW and SS. The objectives of this study were to (a) investigate the co-digestion of FW and SS using different mixing ratios to identify whether an improved methane production can be obtained, (b) assess the relationship between any apparent synergistic effects and digestion time and (c) explore whether different operating temperatures affect the synergistic effects of co-digestion on methane production.

2. Materials and methods

2.1. Preparation of substrates and inocula

Synthetic FW was formulated on a percent wet basis as follows: rice (60%), vegetables (25%), lean meat (10%) and oil (5%). This synthetic formulation was prepared according to a FW characterization study performed in Chongqing City, China (He et al., 2014). Once mixed, the FW was milled, homogenized and stored at 4 °C in a refrigerator before use. SS (a dewatered mixture of primary and excess sludge) was collected from a local municipal wastewater treatment plant that was operated to treat wastewater with an anaerobic-anoxic-oxic (A²O) process. The SS sample was also stored at 4 °C before use.

The inocula were obtained from two FW anaerobic digesters, which were operated at mesophilic (37 °C) and thermophilic (55 °C) temperatures, respectively. Once collected, both inocula were sieved to remove large particles (> 1 mm) and then pre-incubated for approximately 22 days and 17 days at their respective operating temperatures to remove the residual biodegradable organic matter. The characteristics of the substrates and inocula used in this study are presented and discussed in Section 3.1.

2.2. Anaerobic digestion tests

2.2.1. Experimental design

Modified biochemical methane potential (BMP) tests were conducted according to the original protocol described by Owen et al. (1979), except the elimination of adding external nutrients/trace elements, as FW and SS were considered to contain sufficient nutrients/ trace elements. The BMP tests were performed in two different experimental groups, which included one group exposed to mesophilic conditions (M group) and one group exposed to thermophilic conditions (T group). Each group was prepared using a substrate to inoculum ratio (S/I) of 1:1 on the basis of volatile solids to provide a balance between having a sufficient amount of microbial biomass while avoiding acid inhibition. A positive control (PC, cellulose) and a blank control (BC, no substrate) were used to evaluate inoculum performance and provide baseline correction for methane production from inocula. The FW:SS mixing ratios (on volatile solids basis) were selected as 100:0, 75:25, 50:50, 25:75 and 0:100. Each sample group was set up in duplicates with the exception of PC and BC, which were set up in triplicates.

2.2.2. Experimental set-up

The digestion process was carried out by two sets of automatic methane potential measurement system (RTK-BMP, RTKINS, China) with 18 reactors each set. Each reactor consists of a 500 mL reaction bottle, a mechanical mixing rotor, a carbon dioxide absorption flask and a gas measurement system with microbubble counter. A recorder installed inside the system can automatically control the mixing and record the gas generation with an accuracy of 0.1 mL after carbon dioxide is adsorbed by the sodium hydroxide solution.

After the addition and subsequent mixing of substrates with corresponding inocula in reactors according to the experimental design, deionized (DI) water was added to increase the working volume up to 450 mL. The headspace of each reactor was purged with N₂ for 5 min to create an anaerobic environment. All reactors were stirred for one minute at 50 r/min every six hours using mechanical mixing. The reactors were then incubated at $37(\pm 1)^{\circ}$ C and $55(\pm 1)^{\circ}$ C, respectively. The BMP tests were terminated when methane generation in reactors had reached a plateau, i.e., daily methane generation during three consecutive days was less than 1% of the accumulated methane yield according to the protocol proposed by the Anaerobic Biodegradation, Activity and Inhibition (ABAI) Task Group of the International Water Association (IWA) (Holliger et al., 2016).

2.3. Analytical methods

The total solids (TS) and volatile solids (VS) were measured according to standard methods (APHA, 2005). The pH was measured by a pH meter (PHS-3E, YoKe, China). The elemental analysis (C, H, O and N) of substrates and inocula was performed using an elemental analyzer (Vario EL III, Elementar, Germany).

2.4. Data analysis

2.4.1. Methane yield and biodegradability

The cumulative methane yield $(CMY_b \text{ mL CH}_4 \text{·gVS}^{-1})$ of substrates refers to the measured methane yield by time t of the experiment. When test is terminated, the BMP of substrates was evaluated based on the ultimate cumulative methane yield $(CMY_u, \text{ mL CH}_4 \text{·gVS}^{-1})$. The theoretical methane yield $(TMY, \text{ mL CH}_4 \text{·gVS}^{-1})$ of substrates was estimated using the Buswell formula (Buswell and Mueller, 1952), which was based on the elemental composition of a substrate (Eqs. (1) and (2)). The biodegradability index (*BI*, %) is defined as the ratio of *CMY_u* to *TMY*, which was calculated using Eq. (3).

$$C_{n}H_{a}O_{b}N_{c} + \left(n - \frac{a}{4} - \frac{b}{2} + \frac{3c}{4}\right)H_{2}O \rightarrow \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4} + \frac{3c}{8}\right)C$$
$$O_{2} + \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} + \frac{3c}{8}\right)CH_{4} + cNH_{3}$$
(1)

$$TMY(mL \cdot gVS^{-1}) = \frac{22.4 \times \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8}\right) \times 1000}{12n + a + 16b + 14c}$$
(2)

$$BI = 100\% \times CMY/TMY \tag{3}$$

where *n*, *a*, *b* and *c* represent the number of moles of C, H, O, and N, respectively. The value of 22.4 is the volume (in L) occupied by an ideal gas at standard temperature and pressure; the value of 1000 corresponds to the volume conversion factor required for converting from L to mL; and 12, 1, 16 and 14 are the molecular weights $(g-mol^{-1})$ of C, H, O, and N, respectively.

The weighted methane yield (*WMY*, mL $CH_4 \cdot gVS^{-1}$) of mixed substrates was calculated as the weighted sum of the *CMY* values of each substrate (Li et al., 2013), with an assumption that the two substrates, FW and SS, would not interfere with each other on its methane yield during the co-digestion process.

2.4.2. Kinetic analysis

The measured methane yield was fitted using the following modified Gompertz model:

$$CMY(t) = P \times \exp\{-\exp[(R_m \times e \times (\lambda - t))/P + 1]\}$$
(4)

where *CMY(t)* represents the cumulative methane yield by time t of the experiment (mL CH₄·gVS⁻¹), *P* is the predicted value of the ultimate methane yield, R_m stands for the maximum methane yield rate (mL CH₄·gVS⁻¹ d⁻¹), λ refers to the lag phase time (d), *t* is indicative of the digestion time (d), and *e* is Euler's number (≈ 2.71828).

2.4.3. Synergistic effects analysis

A few studies have demonstrated that the co-digestion of FW and SS produced synergistic effects on methane generation, which included increased methane yields, accelerated biodegradation processes or a combination of both (Zhen et al., 2016; Xie et al., 2017). However, fewer studies also provided controversial evidence that co-digestion only resulted in an additive effect instead of a positive synergistic effect (Liu et al., 2016). Therefore, to explore whether and extent of synergistic effects in co-digestion of FW and SS, a co-digestion impact factor (*CIF*) was used to indicate if synergistic or additive effects exist as described by Ebner et al. (2016) in Eq. (5):

$$CIF = CMY_{mix}/WMY_{mix}$$
(5)

Given the nature and uncertainty in BMP measurements, the following threshold values were proposed as criteria for evaluation of synergistic effects of co-digestion on methane generation: CIF > 1.1was indicative of a positive synergistic effect, CIF < 0.9 was indicative of a negative synergistic effect, and $0.9 \le CIF \le 1.1$ indicated only an additive effect existed, or no synergistic effect.

2.4.4. Statistical analysis

Significance of variance tests were performed using an analysis of variance (ANOVA) with a significance level of 0.05. Data processing and figure preparation were performed using OriginPro 8.0 (Origin Lab, USA).

3. Results and discussion

3.1. Characterization of substrates and inocula

As shown in Table 1, the two substrates, FW and SS, had high moisture content close to 80%. The moisture level of the synthetic FW corresponded well with previously reported values associated with its specific content (Capson-Tojo et al., 2017; Parra-Orobio et al., 2018), which consisted primarily of cooked rice and vegetables. The TS of sludge sample was similar to that of dewatered sludge reported by Liu et al. (2016). While the TS content of two substrates were similar, the VS content of FW was 99.2%, much higher than that of SS (51.5%). This trend may be explained because the primary components of FW were



Fig. 1. Cumulative methane yields as a function of time in mono- and co-digestion of food waste (FW) and sewage sludge (SS) under mesophilic (a) and thermophilic (b) conditions. (Symbols represent the experimental data, and solid lines represent the modified Gompertz model fit; PC, positive control.)

organic materials (including carbohydrates, lipids and proteins), while the SS contained some proportion of inorganic components.

The elemental analysis indicated that the carbon-to-nitrogen ratios (C/Ns) of FW and SS were barely within the optimal C/N range (25–30) for anaerobic biodegradation (Kayhanian and Dan, 1996). However, the methane yields and *BI* data, which will be discussed in the following sections, showed that the sub-optimal C/N ratios did not affect the biodegradation of FW and SS. This is likely because the inocula provided sufficient nutrients for microbial growth.

Table 1

Characteristics of food waste (FW), sewage sludge (SS), mesophilic inoculum (M-inoculum) and thermophilic inoculum (T-inoculum) used in batch experiments.

Parameter	Unit	Substrates		Inocula		
		FW	SS	M-inoculum	T-inoculum	
TS	% (w.b.) ^a	24.9 ± 0.2	20.6 ± 0.1	2.36 ± 0.01	5.9 ± 0.4	
VS	% TS	99.2 ± 1.8	51.5 ± 0.5	37.82 ± 0.9	36.63 ± 6.4	
pH	pH units	_b	-	8.36 ± 0.1	9.13 ± 0.2	
С	% TS	47.7	28.4	-	-	
Н	% TS	8.6	3.4	-	-	
0	% TS	42.6	22.6	-	-	
N	% TS	0.86	1.4	-	-	
C/N	g·g ⁻¹	55.4	21.0	-	-	

^a Wet basis.

^b Not measured.

The mesophilic and thermophilic inocula contained less than 6% TS, with similar VS content. Although the pH of thermophilic inoculum (T-inoculum) was around 9.13, which exceeded the suggested pH range (7.0–8.5) for BMP tests by Holliger et al. (2016), it did not seem to affect its performance in digestion tests.

3.2. Effect of temperature on AD performance

3.2.1. Cumulative methane yield

As shown in Fig. 1, the mesophilic and thermophilic experiments were performed for 31 days and 20 days, respectively, according to the termination criteria described in the Experimental Design section.

At mesophilic and thermophilic temperatures, the mean CMY_{ij} of PC (cellulose) were 364.9 (\pm 7.1) and 326.2 (\pm 27.9) mL CH₄·gVS⁻¹, respectively, which were 88% and 79% of its TMY (415 mL CH₄·gVS⁻¹). These values met or were near the effective criteria recommended by Holliger et al. (2016) for BMP test data. In addition, after methane production ended, the final pH values in reactors under mesophilic and thermophilic conditions ranged from 7.98 to 8.06 and 7.63 to 7.75, respectively (data not shown). Those pH values were lower than initial pH values of their respective inocula (Table 1) and suitable for methanogenesis. These results evidenced that the activity of the inocula were acceptable and serious acidification inhibition was unlikely to occur during the test. This could have occurred because the two types of inocula used in this study were collected from well-operated mesophilic and thermophilic AD plants and were incubated at their respective operating temperatures prior to tests, therefore, resulting in suitable seeding performance.

A comparison of the CMY_u values of identical substrate group tested at two operating temperatures showed that the CMY_u values of most test groups under mesophilic conditions were, to some extent, lower than those under thermophilic conditions (deviation: 0.1–11.0%, data not shown). However, the variance analysis of CMY_u obtained from identical substrate groups at different operating temperatures indicated no significant differences existed (p > 0.05), suggesting that temperature had minimal to zero effect on cumulative methane yields.

In general, the *CMY* development of the substrate groups were consistent at both operating temperatures. As reaction time increased, the *CMY* experienced a short stagnation period, which was followed by a rapid increase and a final plateau. At the end of the experiment, the *CMY*_u of test groups declined in an order as follows: FW:SS = 100:0 > 75:25 > 50:50 > 25:75 > 0:100 and the *CMYs* of mixed substrate groups increased as the FW fractions increased, which indicated the degree of FW degradation to generate methane was much higher than that of SS. These results were similar to previous observations (Park et al., 2016; Xie et al., 2017; Pan et al., 2019), as FW was typically rich in easily degradable carbohydrates, proteins and lipids, while SS contained more complex polymeric substances, e.g., extracellular polymeric substances (EPS).

3.2.2. Anaerobic biodegradability

The *BI* (Eq. (3)) is used to reflect the degree to which substrates are converted to methane. Under mesophilic conditions, the average *BI* values of 3 out of 5 test groups were lower than those under thermophilic conditions (Fig. 2). However, the statistical analysis results showed these observed differences were not significant among different test groups (p > 0.05). Therefore, the different operating temperatures used in this study did not significantly affect substrate biodegradability, which was consistent with the CMY_u results. FW alone (FW:SS = 100:0) was almost completely biodegraded to methane, and its CMY_u achieved more than 94% of its TMY (533 mL CH₄·gVS⁻¹). As the proportion of FW increased, the degradation of mixed substrates increased and was higher than that of SS only (FW:SS = 0:100).

3.2.3. Methane production rate

As discussed above, although the temperature did not show effect



Fig. 2. Biodegradability of food waste (FW), sewage sludge (SS), and their mixtures incubated under mesophilic and thermophilic conditions.



Fig. 3. Methane production rates as a function of time in mono- (a) and codigestion (b) of food waste (FW) and sewage sludge (SS) under mesophilic conditions (M group, open symbols) and thermophilic conditions (T group, solid symbols).

on cumulative methane yields, it did clearly influence the rates of methane production. On the one hand, as shown in Figs. 1 and 3, the thermophilic test groups completed methane production in about 20 days, remarkably shorter than that occurred under mesophilic conditions (31 days). On the other hand, when the operating temperatures

varied, the occurrence time and intensity of peaks in methane production were significantly different for identical substrate groups (Fig. 3).

When incubated under mesophilic conditions, the mono-digestion of FW and SS (Fig. 3(a)) exhibited peak methane production of 58.3 and 29.1 mL CH₄: $gVS^{-1}\cdot d^{-1}$, respectively, on Days 12 and 13; the co-digestion groups (FW:SS = 75:25, 50:50 and 25:75, Fig. 3(b)) showed maximum production of 32.6 to 48.7 mL CH₄: $gVS^{-1}\cdot d^{-1}$, on Days 10 and 11. In contrast, when incubated under thermophilic conditions, the peak methane production of both mono- and co-digestion groups occurred much earlier (on Days 3 and 4), with higher values of 86.9–129.2 mL CH₄: $gVS^{-1}\cdot d^{-1}$. The reason for generation of two or three gas production peaks under mesophilic conditions was likely due to the different degradation rates of carbohydrate, protein and lipid components in FW. In contrast, the differences in degradation rates of those FW components were less pronounced under thermophilic conditions, as the degradation kinetics were boosted at higher temperature.

Notably, the majority of test groups under mesophilic conditions exhibited low methane production during the initial stage of the experiment (1–9 days). The methane production rates were small (< 40 mL CH₄·gVS^{-1·d⁻¹) at first, and then they gradually increased. In contrast, the corresponding test groups under thermophilic conditions did not have a beginning period with low methane production rates, indicating short lag-phase in methane production. These results evidenced that the operating temperature had a significant impact on the start-up of the methane production process, which was likely related to accelerated hydrolysis at higher temperature. For example, Arras et al. (2019) discovered higher temperature increased hydrolysis rates during AD start-up, although minimal impact of temperature on the final hydrolysis efficiency was observed.}

3.2.4. Kinetic features

The kinetic parameters have been often used to evaluate and predict the anaerobic degradation characteristics of various organic substrates. In this study, the modified Gompertz model was used to fit the methane production results in order to obtain those kinetic parameters under mesophilic and thermophilic conditions (Table 2).

In general, the modified Gompertz model exhibited a high accuracy for fitting the anaerobic mono- or co-digestion process data ($\mathbb{R}^2 \ge 0.98$), indicating its suitability to simulate the methanogenic behaviors of FW, SS, and their mixtures under batch conditions. In most cases, the differences between predicted ultimate methane yields (*P*) and measured methane yields (*CMY*_u) were less than 5%, suggesting the biodegradation of substrates was nearly complete when test termination occurred.

 R_m and λ are two parameters indicative of methane production rates. Higher R_m and lower λ values obtained from T group were consistent with the fact that methane production occurred faster at thermophilic temperature. The lag time, λ , for test groups under thermophilic conditions, was less than 1 d; while the lag time, λ , for corresponding test groups under mesophilic conditions, ranged from 2.7 to 4.2 d. The maximum methane production rates, R_m , under thermophilic conditions (59.2–94.4 mL CH_4 'gVS^{-1·d⁻¹) were two- to three-fold of those under mesophilic conditions (23.2–30.4 mL CH_4 'gVS^{-1·d⁻¹).}}

3.3. Evaluation of synergistic effects on methane yields and production rates

3.3.1. Effects on methane yields

The effects of co-digestion of FW and SS relative to mono-digestion of each substrate were evaluated using the *CIF*, which was defined as a ratio of the measured methane yield of the mixed substrates (CMY_{mix}) to the weighted average methane yield (WMY_{mix}), calculated based upon the measured methane yields of each substrate and its proportion in sample mixture. The results are presented in Table 3.

As indicated by CIF values greater than 1.0, under both temperature conditions, most measured *CMY* values of co-digestion test groups were slightly higher than the *WMY* values calculated using Eq. (5). However, no significant difference was observed between *CMY* and *WMY* values (*CIF* range: 0.99–1.10), suggesting that co-digestion did not result in a clear boost in methane yields. In other words, additive effects, rather than positive synergistic effects, occurred on the methane yields in co-digestion of FW and SS. Dai et al. (2013) and Koch et al. (2015) obtained similar results by comparing the anaerobic mono- and co-digestion of FW and SS, in which the amount of biogas increased as the proportion of FW in the substrate mixture increased, but no synergistic effect between two substrates was observed on gas yields.

3.3.2. Effects on methane production rates

To further explore if synergistic effects existed on rates of methane production, the *CIF* values were calculated at different time points during the experiment and plotted in Fig. 4. Clearly, although at the end of experiment, *CIF* values for all test groups, were close to 1.0, indicative of no positive synergistic effects on cumulative methane yields, *CIF* values experienced significant variations during the experiments on both temperature conditions, indicative of potential synergistic effects on methane production rates.

Under mesophilic conditions, the CIF change over time can be generally divided into three phases. During the early phase (Days 1-3), CIFs of those co-digestion test groups increased sharply to peak values of 1.43, 1.71, and 1.89, corresponding to FW proportion of 25%, 50% and 75%. After those short peaks, CIFs declined sharply and increased to reach broader peaks on Days 9 and 10 with values of 1.50, 1.71, and 1.93. Subsequently, those CIFs decreased and gradually stabilized with final values close to one. In contrast, the development of CIFs under thermophilic conditions was characterized by just one single broad peak between Days 1 and 7, with peak values of 1.15, 1.28, and 1.35 on Day 3, corresponding to FW proportion of 25%, 50% and 75%. After Day 7, CIFs quickly stabilized around a constant value of 1.0. These observations were consistent with those of Poulsen and Adelard (2016), who observed a somewhat similar pattern of relative increase in methane yields for a set of 95 biomass mixes comprising vegetable waste, pig manure, cow dung, chicken manure and grass clippings, etc.

Table 2

Modified Gompertz model parameters obtained by fitting the methane production data under mesophilic (M group) and thermophilic conditions (1 group)	р).
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Parameter	Unit	M group –	M group – FW:SS mixtures					T group – FW:SS mixtures				
		0:100	25:75	50:50	75:25	100:0	0:100	25:75	50:50	75:25	100:0	
CMY	mL CH ₄ ·gVS ⁻¹	280.4	368.7	402.7	466.5	505.4	315.1	361.5	418.1	465.8	511.8	
Р	mL CH_4 ·gVS ⁻¹	278.9	374.9	413.8	485.9	550.2	302.1	350.1	406.9	462.4	513.5	
Difference ^a	%	0.54	1.7	2.7	4.2	8.9	4.1	3.2	2.7	0.74	0.35	
R_m	mL CH ₄ ·gVS ⁻¹ ·d ⁻¹	23.2	30.1	29.6	30.4	26.6	59.2	74.1	86.1	94.4	88.2	
λ	Day	3.9	3.6	3.1	2.7	4.2	0.52	0.59	0.59	0.60	0.76	
\mathbb{R}^2	_b	0.99	0.99	0.99	0.99	0.98	0.99	0.99	0.99	1.00	1.00	

^a Calculated using |(CMY - P)|/CMY * 100%.

^b Dimensionless quantity.

Table 3

Parameter	Unit	M group – FW:SS mixtures			T group – FW:SS mixtures			
		25:75	50:50	75:25	25:75	50:50	75:25	
CMY WMY Difference ^a CIF	$ \begin{array}{c} mL \ CH_4 \cdot gVS^{-1} \\ mL \ CH_4 \cdot gVS^{-1} \\ \% \\ _^b \end{array} $	368.7 336.7 8.7 1.10	402.7 392.9 2.4 1.02	466.5 449.2 3.7 1.04	361.5 364.3 0.76 0.99	418.1 413.5 1.1 1.01	465.8 462.6 0.69 1.01	

^a Calculated using |(CMY - WMY)|/CMY * 100%.

^b Dimensionless quantity.



Fig. 4. Co-digestion impact factor (*CIF*) as a function of time under mesophilic conditions (M group, open symbols) and thermophilic conditions (T group, solid symbols). The vertical dash lines represent T_{90} , when 90% of the *CMY*_u was achieved.

It is worth noted that the peak *CIF* values of mesophilic test groups were clearly higher than those of thermophilic test groups for each substrate mixtures, suggesting more pronounced synergistic effects on methane production rates under mesophilic conditions. The reason for the observation was likely due to the accelerated hydrolysis caused by high temperature was more pronounced than the enhanced hydrolysis caused by co-digestion, which will be further discussed in the following section.

3.3.3. Implication

As presented in Fig. 4, a reference T₉₀ was set by vertical dash lines for co-digestion test groups, denoting the time required to achieve 90% of the CMY_u for each substrate mixture. At those time points (Days 16, 18, and 20), CIF values for test groups operated under mesophilic conditions were 1.21, 1.21 and 1.24, respectively, indicating that 21-24% improvement on methane yields in co-digestion of dual substrates, relative to mono-digestion of single substrate by those specific time points. This improvement has been observed in the literature, and likely resulted from improved hydrolysis of SS due to addition of readily degradable FW (Koch et al., 2015). In comparison, at T₉₀ (Days 7 and 8), CIF values for test groups operated under thermophilic conditions were 1.00, 1.05 and 1.09, respectively, barely deviated from 1.0, suggesting limited improvement on methane production due to co-digestion. This limited improvement observed under thermophilic conditions could be explained by that the enhanced effect of co-digestion on hydrolysis was less important compared with the effect of higher temperature to accelerate hydrolysis. Thus, the positive synergistic effects of co-digestion on gas production kinetics are more pronounced at mesophilic conditions.

Overall, those positive synergistic effects observed in early phases of methane production in co-digestion tests may implicate that when a short retention time is selected in practice, an enhanced methane production may be achieved due to co-digestion, especially under mesophilic conditions. It should be noted that batch tests conducted in this study do not allow testing of substrates or nutrients deficiency effects, as those effects only occur in long-term, continuous AD operation. Therefore, further experiments with continuous AD operation should follow to verify those findings of this study.

4. Conclusions

The temperature did not significantly influence the methane yields of mono- and co-digestion of FW and SS, although higher temperature resulted in accelerated methane production. The synergism on methane yields was initially observed but not significant at the end of experiments, which suggested that the co-digestion of FW and SS did not enhance the extent of degradation, but accelerated the degradation process. The T_{90} analysis results showed that if an appropriate retention time was selected, the conversion of FW and SS to methane could be improved in co-digestion, especially under mesophilic conditions, which could have implications for engineering application.

CRediT authorship contribution statement

Juan Gu: Methodology, Formal analysis, Data curation, Writing original draft, Visualization. Rui Liu: Methodology, Formal analysis, Data curation, Writing - original draft, Visualization. Yi Cheng: Methodology, Investigation. Nemanja Stanisavljevic: Writing - review & editing. Lei Li: Writing - review & editing. Djordje Djatkov: Writing review & editing. Xuya Peng: Supervision. Xiaoming Wang: Conceptualization, Methodology, Writing - review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

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

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