

Article

Anaerobic Co-Digestion of Food Waste and Microalgae at Variable Mixing Ratios: Enhanced Performance, Kinetic Analysis, and Microbial Community Dynamics Investigation

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Abstract: There is an urgent need for clean recycling strategies to address the increase in food waste (FW) and the harvesting of microalgae (MA). In this study, biogas production potential and operational stability were evaluated by testing combinations of FW and MA mixed at five different ratios. Co-digestion of FW and MA improved substrate biodegradability, achieving a decomposition rate of 0.45/d (FW/MA = 1:1), which is 1.25 to 1.55 times higher than that of MA or FW alone. Co-digestion of FW and MA resulted in a synergistic effect, improving biogas yield by 2.04–26.86%. Four mathematical models were applied to estimate biological degradation and biogas production kinetics, and the Cone model performed better than the other models in terms of reliability and accuracy. The abundance of Bacteroidetes, Firmicutes, and Synergistetes peaked at FW/MA = 1:1. At the same ratio, the genera *Methanospirillum*, *Methanocorpusculum*, and *Methanomethylovorans* were also found to have increased in abundance. The optimal ratio was found to be 1:1 for co-digestion of FW and MA, which is a feasible approach for simultaneous bioenergy production and biomass waste co-disposal.

Keywords: food waste; microalgae; anaerobic co-digestion; microbial community



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1. Introduction

Food waste (FW), a major fraction of municipal solid waste (37–62%), is complex, produced in large amounts, and perishable [1,2]. Globally, urbanization coupled with an increasing population has triggered the rapid generation of FW, reaching 1.3 billion tons annually [3]. This is a multifaceted issue due to FW's dual properties of being both a resource and a hazard [4]. FW can cause water quality deterioration and land pollution and can emit offensive odors [5]. With the increasing amount of FW, proper management strategies require clean recycling technologies. Currently, FW management is performed through landfill, fertilizer generation, composting, incineration, and anaerobic digestion [4,6]. Anaerobic digestion, a biochemical process that converts organic matter into renewable bioenergy (methane and hydrogen), is the most straightforward and feasible technology for FW disposal [7,8]. Volatile fatty acid (VFA) inhibition, rapid acidification, and low essential trace elements (Fe, Ni, Co, and Mo) set up a barrier for biogas generation from mono-digestion of FW [8,9]. To tackle this challenge, anaerobic co-digestion of FW with other substrates such as sludge [10], garden waste [11], paper waste [12], and animal manure [12] have been extensively investigated. Aiming to simultaneously improve local sustainability and reduce transport costs, anaerobic co-digestion seems to be an excellent alternative when co-feedstocks are generated on-site or nearby.

Microalgae (MA) are photoautotrophs that convert sunlight to chemical energy. They are characterized by a high concentration of organic matter and high productivity [13].

The excessive growth of MA in lakes has negative effects on aquatic ecosystems and even threatens the safety of drinking water [14]. Every day, thousands of tons of MA are harvested from Lake Taihu in China [15]. Further disposal of undesirable MA is urgent to avoid serious secondary pollution. Proteins, lipids, and carbohydrates are the main components of MA [16], making it a promising raw material for anaerobic digestion. MA production does not require fresh water resources or arable land and, therefore, has low levels of competition with the food market compared to other solid substrates [17]; however, mono-digestion of MA is hindered by low C/N ratios and cell wall degradability [18]. To address these obstacles, several studies have evaluated the feasibility of coupling MA with a variety of complex organic substrates, including diatomite [8], agri-industrial waste [19], and lipid waste [20]. Anaerobic co-digestion of MA could simultaneously enhance biogas production and microcystin biodegradation [21]. Because of this, co-digestion with other substrates has become an alternative strategy to recover bioenergy from MA more efficiently.

Recently, the combined use of FW and MA has become a new research field. The viability of anaerobic co-digestion FW with MA has been shown [22,23], and previous studies have demonstrated that FW mixed with diverse algae species (*Chlorella* sp. [24], *Spirulina platensis* [25], and *Dictyosphaerium* sp. [26]) facilitated the increased performance of anaerobic co-digestion for biogas production. The co-digestion of FW and MA has multiple advantages, including the ability to improve biodegradability, dilute toxic compounds, alleviate nutrient imbalances, maintain system stability, and enhance microbial synergistic effects [18]. Furthermore, the co-digestion effluent produced is rich in phosphorus and nitrogen and could be recycled and used as a nutrient source for MA farming [25], treating the waste stream as well as reducing cultivation costs. Although previous studies demonstrated some positive results for the co-disposal of FW and MA, the current knowledge in this field is far from complete [26]. Aside from optimizing operating parameters such as the mixing ratio, the synergistic effect and kinetics of biogas evolution as regulated by the heterogeneity of FW and MA need to be further studied. In addition, different mixing ratios likely shape different bacterial and archaeal communities in the co-digestion system, though this has seldom been investigated.

The objectives of the present study were to reveal the impact of the mixing ratio on biogas production performance during co-digestion of FW and MA, to evaluate the kinetic parameters and process stability under various mixing ratios, and to characterize the bacterial and archaeal community dynamics involved. These results could facilitate the optimization of process parameters, maximizing the synergistic effects for achieving simultaneous bioenergy production and biomass waste co-disposal.

2. Materials and Methods

2.1. Preparation of Substrates and Inoculum

FW was collected from the cafeteria of the Nanjing University of Finance and Economics (Nanjing, China), which mainly contained leftover rice, vegetables, fruits, noodles, meat, and fish. Indigestible materials such as napkins, plastics, eggshells, and bones were removed by hand. The FW was crushed and homogenized by a food crusher and sieved through a size 14 mesh screen (1.4 mm). The sieved FW was transferred into sealed bags and stored at 4 °C until feeding. The main characteristics of the pretreated FW were pH 6.20, total solids (TS) of 13.13 wt%, and volatile solids (VS) of 12.55 wt%. Due to its tendency to rot, the pretreated FW was used on the same day as collection.

MA biomass (>95% *Microcystis aeruginosa*) was harvested from the Zhushan Bay of Lake Taihu (31°24' N, 120°18' E) using a plankton net (64 µm). The freshly harvested algal biomass was stored in a portable refrigerator and transported to the laboratory immediately. Algal biomass was freeze-dried at −45 °C for 72 h because a high water content could potentially facilitate the decomposition of algal biomass residues. Freeze-dried algal biomass was passed through a 200-mesh sieve and homogenized. The freeze-dried samples

were ground into particles using a High-Speed Powder Grinder Machine, then sieved through a 0.075 mm mesh to obtain particles around 750 µm (200 mesh).

The anaerobic seed sludge used for co-digestion was obtained from an anaerobic digestion tank at Anhui Huibo Environmental Protection Technology Engineering Co., Ltd. (Hefei, Anhui, China). The seed sludge was pre-incubated at 35 °C for 2 weeks to reduce endogenous biogas before being mixed with the substrates under anaerobic conditions. The TS and VS of the seed sludge were 4.62 wt% and 3.25 wt%, respectively.

2.2. Batch Co-Digestion of FW and MA at Various Ratios

The batch co-digestion tests were carried out in a self-designed reactor (1000 mL) with an effective working volume of 800 mL. The ratio of inoculum and substrate was 2:1 based on VS; the mixed ratios of FW/MA (based on VS) were 0:1, 1:3, 1:1, 3:1, 1:0, and the total substrate VS mass was maintained at 6.49 g. All the tests were conducted in triplicate, and the blank group was used to account for endogenous methane. The initial pH value for each reactor was adjusted to 7.0 using a 1 mol/L hydrochloric acid and sodium hydroxide solution. Next, reactors were flushed with nitrogen gas (>99.99%, 1 L/min) for 5 min to create anoxic conditions. After, all reactors were tightly sealed using a rubber stopper with two ports that were both connected to a three-way stopcock. One port was used for biogas sampling and the other port was connected to an aluminum gas bag for biogas collection. Finally, all reactors were placed in thermostatic water bath shakers set to maintain a temperature of 35 ± 1 °C with a shaking speed of 120 rpm. The batch experiments were run for 35 days until the biogas production was <2% of the accumulated biogas produced, suggesting that biodegradation was completed [11]. The daily and cumulative biogas production with different mixing ratios were expressed at standard pressure and temperature.

2.3. Analytical Methods

TS and VS contents of the substrate, inoculum, and digested residue were determined according to standard methods (APHA, 2012) [27]. The pH of the system was continuously monitored and recorded using a pH meter (Dongguan Shenghui Instrument, Dongguan, China). The TC and TN contents of substrates were determined by a CN analyzer (MT-700, Kyoto, Japan) based on the dry combustion method. According to biogas production, 4 mL of fermentation broth was withdrawn for corresponding chemical and physical analysis. Volatile fatty acids (VFAs) were determined using high-performance liquid chromatography (Agilent 1200, Agilent Technologies, Santa Clara, CA, USA). Soluble chemical oxygen demand (SCOD) was determined as described by [28]. Ammoniacal nitrogen was determined using the Nash reagent method [14].

The microbial communities were analyzed at the end of batch digestion. The substrate in each vial was thoroughly mixed, and 1.5 mL from each was collected and stored at -80 °C for DNA extraction. The sampled DNA was treated with polymerase chain reaction using universal primers to amplify the bacterial 16S rRNA gene and archaeal 16S rRNA gene. The detailed information for DNA extraction, PCR amplification, and sequencing were the same as the methods reported in a previous study [29].

2.4. Statistical Analysis

The first-order model (Equation (1)), modified Gompertz model (Equation (2)), logistic model (Equation (3)), and Cone model (Equation (4)) were utilized to describe and compare the kinetics of biogas production during the anaerobic co-digestion of FW and MA [30,31].

$$Y(t) = P_0 \times [1 - \exp(-kt)], \quad (1)$$

$$Y(t) = P_0 \times \exp \left\{ -\exp \left[\frac{R_m \times e}{P_0} (\lambda - t) + 1 \right] \right\}, \quad (2)$$

$$Y(t) = \frac{P_0}{1 + \exp\left[4R_m \frac{\lambda - t}{P_0} + 2\right]}, \quad (3)$$

$$Y(t) = \frac{P_0}{1 + (kt)^{-n}}, \quad (4)$$

where $Y(t)$ is the cumulative biogas yield for a given time (mL/g VS), P_0 is the biogas production potential (mL/g VS), k is first-order decomposition rate constant (1/d), which indicates the rate of degradability of the substrate, t is the digestion time in days (d), R_m is the maximum biogas production rate (mL/(g VS day)), λ is the lag phase (d), e is the natural constant (2.72), and n is the shape factor.

To evaluate the antagonistic or synergistic effects of biogas production, the calculated biogas yield (CBY) and biogas potential improvement (BPI) were applied according to Equations (5) and (6) [32,33]:

$$\text{CBY} = \text{BP}_{\text{FW}} \times \alpha + \text{BP}_{\text{MA}} \times \beta, \quad (5)$$

$$\text{BPI} = (\text{EBY} - \text{CBY}) / \text{CBY}, \quad (6)$$

where BP_{FW} and BP_{MA} are the biogas potential of FW and MA obtained from 100% FW and 100% MA treatments, and α and β represent the corresponding percentages of FW and MA in each co-digestion mixture, respectively. EBY represents the experimental biogas yield.

Data processing was performed in Microsoft Excel 2020. The difference in cumulative biogas yield of different mixing ratios was analyzed by one-way ANOVA with the posthoc test at a significance level of $p < 0.05$ and $p < 0.01$. Graphing was conducted using Origin 2021 (Origin Lab, Northampton, MA, USA).

3. Results and Discussion

3.1. Effects of Mixing Ratios on Biogas Production

To assess the biogas yield potential from various combinations of FW and MA, the daily biogas yield and corresponding experimental and calculated biogas yield were evaluated (Figure 1). The daily biogas yield showed a similar profile across various ratios; it reached a peak after 1 to 2 days of digestion, then decreased significantly. Most of the biogas was produced in the first 7 days. The maximum and minimum values of daily biogas production were 213.25 and 40.07 mL/g VS for FW and MA as sole substrates, while the peak values were 165.43, 176.42, and 191.28 mL/g VS at FW/MA ratios of 1:3, 1:1, and 3:1, respectively. Biogas yield was accompanied by the degradation of organic matter in the substrate. These results confirmed that FW was rich in easy biodegradable substrates (protein and carbohydrate), which exhibited efficient biogas production. In contrast, the lower biogas yield seen for MA may be due to limitations caused by the rigidity of MA cell walls and the presence of cellulose [18]. This could be attributed to rich biodegradable substrates in FW, which were more easily hydrolyzed than the refractory substances (cellulose) surrounded by the rigid cell wall in MA [18]. Previous studies have also shown that cellulose content negatively correlates with biogas potential during anaerobic digestion [33,34]. Overall, the higher proportion of FW led to a greater biogas yield.

To further compare the co-digestion performance of FW and MA, the calculated biogas yield (CBY) and biogas potential improvement (BPI) were calculated and evaluated. Interestingly, the cumulative experimental biogas yield (EBY) was notably higher than the CBY at various ratios (Figure 1b). A 2.04–26.86% improvement in biomass yield was observed in the co-digestion system of FW and MA, with the higher BPI observed at the mixing ratio of FW/MA = 1:1 (9.03%) and FW/MA = 1:3 (26.86%). This suggests a positive co-metabolism (synergistic) effect for the co-digestion of FW and MA. Similarly, Zhang et al. [35] evaluated the optimal mixing ratio for the co-digestion of FW and MA (*Spirulina*) and reported the highest co-digestion synergy (8.7%) observed with a mixture of 75% MA and 25% FW. The performance enhancement obtained by the co-digestion

system could be explained by trace elements (Cu, Fe, Zn, Ca, etc.) being released after algal cell wall hydrolysis, where they subsequently serve as nutrients for the microbial communities [36]. In addition, co-metabolism was observed for MA and lipid waste [37], which could potentially enhance lipid degradation derived from FW. Taken together, these results further confirmed that MA are an excellent substrate for co-digestion with FW to achieve efficient waste disposal [32,38].

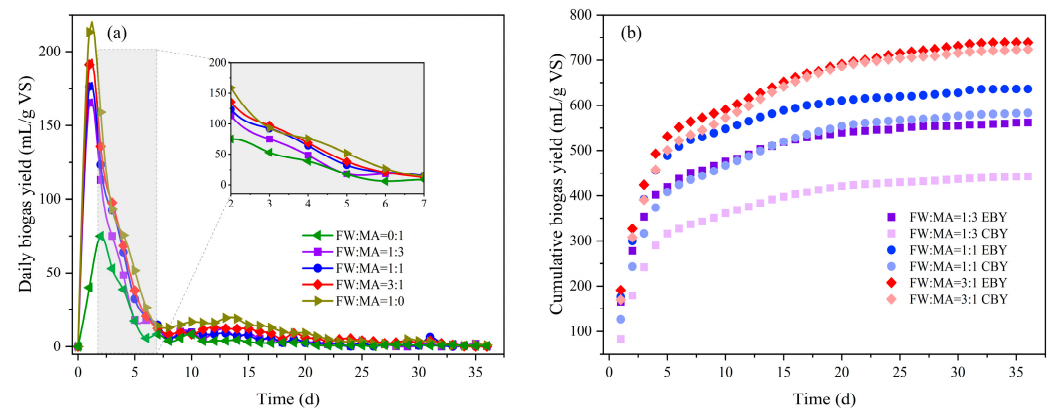


Figure 1. The effects of mixing ratios on biogas yields: (a) daily biogas yield and (b) cumulative biogas yield. EBY represents cumulative experimental biogas yield, and CBY represents calculated biogas yield according to the proportions of FW and MA applied in the co-substrates and their separate biogas yield during the mono-digestion process.

3.2. Estimation of Kinetic Parameters during Anaerobic Co-Digestion

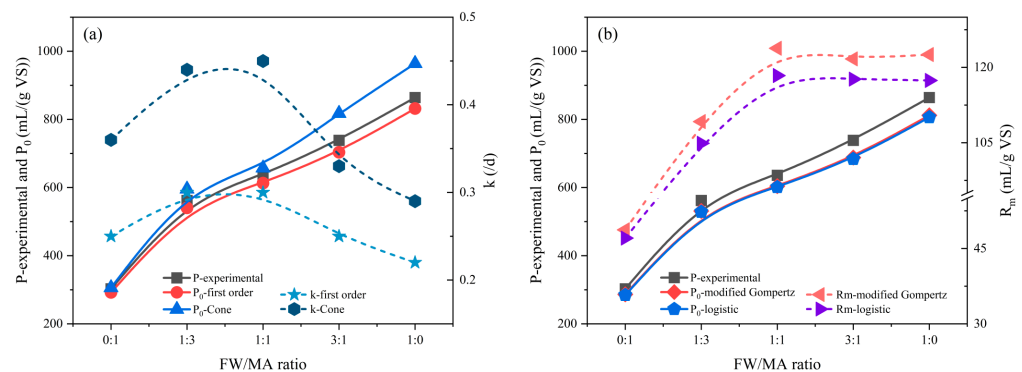
Apart from the cumulative biogas yield, the decomposition rate, lag phase, and biogas production rate are also important factors in assessing the efficiency of anaerobic co-digestion (AcoD) [39,40]. To evaluate these kinetic parameters, first-order, modified Gompertz, logistic, and Cone models were applied. The specific parameters are summarized in Table 1.

The biogas production potential (P_0) estimated for the four models increased with the growing proportion of FW, ranging from 284.78 to 964.00 mL/g VS at various mixing ratios of FW and MA; these results were in line with the result of Zhen et al. [41]. However, unlike Zhen et al. [41] who reported a P_0 range of 88.00–670.60 mL/g VS and the highest biogas production occurring in the FW/MA = 0.8:0.2 group, our experiment showed the highest biogas production at a ratio of 3:1. This discrepancy is primarily due to differences in the types of microalgae used. The observation was further verified in the previous study as a large amount of organic matter derived from FW promoted an increase in biogas production [32].

The decomposition rate (k) is another key indicator reflecting digestion efficiency and substrate biodegradability. The k values ranged from 0.22 to 0.45/d in this study, which is consistent with the k values for anaerobic digestion of FW reported in previous studies [40] (Browne & Murphy, 2013). Interestingly, k values of co-digestion systems were better than those for mono-digestion systems, particularly at the FW/MA mixing ratios of 1:1 and 1:3 (Figure 2a); this indicated that adding MA detritus to FW accelerated the total decomposition rate. Similarly, the maximum biogas production rate (R_m) was obtained at the FW/MA ratio of 1:1, indicating that appropriate mixing ratios could promote biogas production (Figure 2b). These results show that co-digestion of FW and MA had an impact on the organic matter biodegradability and conversion rate. In all cases, the estimated lag phase (λ) disappeared, which could be attributed to the readily available biodegradation components in the FW and MA [30]. This suggests that the large, easily biodegradable proportion of FW and MA was directly subjected to acidification and methanogenesis without the need for first undergoing solubilization and hydrolysis [41].

Table 1. Estimated kinetic parameters of first-order model, modified Gompertz model, logistic model, and Cone model used for co-digestion of FW and MA at various mixing ratios.

Models	Parameters	Units	Treatments (FW/MA Ratio)				
			0:1	1:3	1:1	3:1	1:0
First-order model	P-experimental	mL/g VS	302.69	562.08	636.13	738.62	864.24
	P_0 -potential yield	mL/g VS	292.30	540.29	613.09	703.59	832.09
	k	1/d	0.25	0.30	0.30	0.25	0.22
	R^2		0.98	0.97	0.98	0.96	0.96
Modified Gompertz model	P_0 -potential yield	mL/g VS	286.63	531.31	603.99	688.08	811.92
	R_m	mL/(g VS d)	48.72	109.22	123.81	121.68	122.55
	λ	d	0	0	0	0	0
	R^2		0.96	0.93	0.95	0.91	0.91
Logistic model	P_0 -potential yield	mL/g VS	284.78	528.75	601.29	683.78	806.42
	R_m	mL/(g VS d)	47.07	104.90	118.37	117.66	117.41
	λ	d	0	0	0	0	0
	R^2		0.94	0.91	0.93	0.89	0.88
Cone model	P_0 -potential yield	mL/g VS	306.06	595.36	657.19	817.59	964.00
	k	1/d	0.36	0.44	0.45	0.33	0.29
	n		1.41	1.01	1.16	0.90	0.93
	R^2		0.99	0.99	0.99	0.99	0.99

**Figure 2.** (a) Relationships between the experimental final biogas yield (P-experimental), the predicted biogas potential yield (P_0), the decomposition rate (k), and the FW/MA ratio. (b) Relationships between the experimental final biogas yield (P-experimental), the predicted biogas potential yield (P_0), the maximum biogas production rate (R_m), and the FW/MA ratio.

The reliability and accuracy of the four applied models were evaluated by comparing and calculating the correlation coefficients (R^2), the residual sums of squares (RSS), and the differences between experimental and model-predicted data (Dem). The Cone model outperformed the other models, with the highest R^2 in various mixing ratios (above 0.99); R^2 values of the first-order, modified Gompertz, and logistic models ranged from 0.96 to 0.98, 0.91 to 0.96, and 0.88 to 0.94, respectively. Additionally, the Cone model gave the lowest RSS and Dem, which further confirmed the agreement of predicted results with experimental data. Overall, the Cone model fit best in this study, which was highly consistent with the previous study [41,42], which examined co-digestion of FW and MA (*Scenedesmus* sp., *Chlorella* sp., *Spirulina platensis*).

3.3. Evolution of Chemical Parameters

The dry algal biomass had a TS, VS, total carbon (TC), and total nitrogen (TN) content of 96.43 wt%, 90.39 wt%, 514.98 mg/g TS, and 113.95 mg/g TS, respectively. To assess AcoD stability, the pH, VFAs, NH_4^+ -N, and SCOD of the system were also characterized as responses to mixing ratios of FW and MA. Variation in pH indicates solubilization or

decomposition of organic matter, whereas the variation in VFA concentrations indicates the hydrolytic–acidogenic stage [19].

As shown in Figure 3, VFA accumulation was apparent during the initial period as the VFA production rate was higher than the consumption rate; simultaneously, the pH value changed rapidly from 7.3 ± 0.1 to 6.4 ± 0.2 due to increased VFA accumulation. VFAs serve as intermediate products of AcoD; subsequently, they are consumed by acetophilic methanogenic bacteria and isotype acetogenic bacteria or are utilized in combination with substrates, resulting in a gradual decrease in total VFA concentrations during which the digestion system remains in good condition. It can be seen that co-digestion played a key role in alleviating the inhibition of VFA throughout the process. A pH rise was seen from the second to the third day before stabilizing. It has been reported that an appropriate pH range of 6.8 to 7.2 is required for efficient biogas production [43]. The co-digestion of FW and MA has been proven to adjust and buffer the pH, especially at mixing ratios of 1:1 and 1:3; these recovered and remained at $\text{pH } 7.2 \pm 0.1$, the same as the initial pH values.

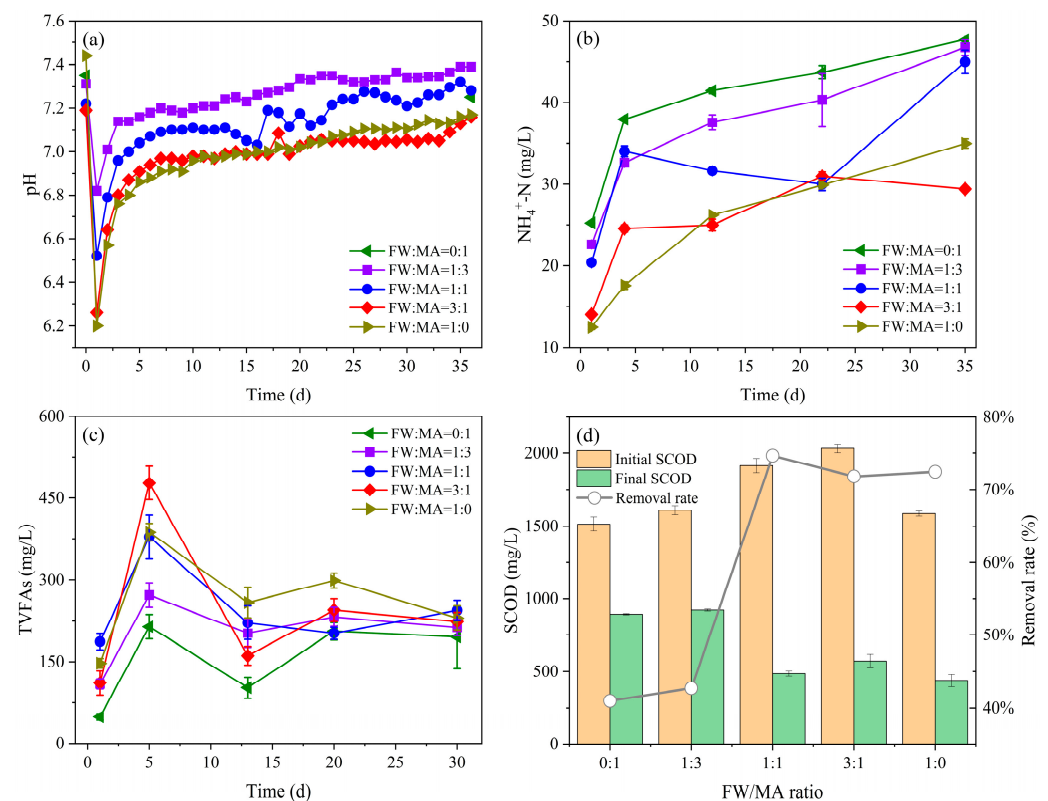


Figure 3. Variations in chemical parameters with various mixing ratios during the digestion period: (a) pH, (b) $\text{NH}_4^+\text{-N}$, (c) TVFAs, and (d) SCOD.

3.4. Response of Bacterial and Archaeal Communities

The type and composition of substrates shape microbial communities and have substantial impacts on AcoD performance. To demonstrate the response of the microbial community in the reactors to the mixing ratio, the structure and variation in bacterial and archaeal communities were analyzed. The Sobs and Ace indices were adopted to characterize the abundance of communities, while the Shannon and Simpson indices were applied to assess the diversity of communities.

As depicted in Table 2, the high coverage (>0.998) demonstrated the reliability and adequate depth of coverage of the results. It was found that an FW/MA mixing ratio of 1:1 had the highest Sobs and Ace indices (473 and 618.43, respectively), which were significantly higher than those obtained for mono-digestion of FW and MA. The Shannon and Simpson indices gradually increased with increasing proportions of FW (Table 2). This phenomenon indicated that the optimal ratio of FW and MA (1:1) increased the richness

of the microbial community and the proportion of FW addition enriched the diversity of the microbial community. Similarly, FW addition selectively enriched certain bacteria and the microbial community diversity varied remarkably with the mixed substrates [18]. The composition changes and abundance variation induced diversity increases, which enhanced metabolic functions and strengthened system stability [33].

Table 2. The alpha diversity and coverage in each digestion system.

Sample	Sobs	Ace	Shannon	Simpson	Goods Coverage
FW/MA = 0:1	415	557.73	3.27	0.77	0.9987
FW/MA = 1:3	409	530.78	3.58	0.84	0.9988
FW/MA = 1:1	473	618.43	3.66	0.83	0.9987
FW/MA = 3:1	468	617.15	3.83	0.86	0.9986
FW/MA = 1:0	450	599.47	3.94	0.88	0.9986

3.4.1. Effects of Mixing Ratio on Bacterial Community

The phylum-level composition of the bacterial community displayed remarkable heterogeneities among various mixing ratio groups, which indicated that the great change in the microbial community distribution was caused by the co-digestion of FW and MA compared with that found in mono-digestion systems. The dominant phyla were Chloroflexi, Bacteroidetes, Thermotogae, Synergistetes, Proteobacteria, Patescibacteria, and Firmicutes, which had >5% average relative abundance across sequenced samples (Figure 4a). The abundance of Chloroflexi increased with FW addition, and maximum relative abundance was identified in FW/MA = 1:0 (20.24%). Previous studies have revealed that Chloroflexi is a strict anaerobic multicellular filamentous and electroactive bacteria that can participate in glucose degradation and interspecies electron transfer [44]. The large quantity of easily biodegraded matter in FW would be beneficial for Chloroflexi proliferation. The abundances of Bacteroidetes, Firmicutes, and Synergistetes reached their maximums at FW/MA = 1:1 (10.80%, 6.37%, and 7.14%, respectively). Bacteroidetes and Firmicutes, two commonly typical acidogenic bacteria, are typically found as the dominant phyla in anaerobic digestion systems [33]; they can hydrolyze carbohydrates and proteins to produce VFAs [44]. The FW/MA = 1:1 group had a higher macromolecule degradation activity, which was consistent with the highest k value of 0.45/d that was also obtained in this co-digestion group (Figure 2). The remarkable enrichment of the aforementioned acidogenic bacteria could enhance the efficiency of acidogenesis in the anaerobic digestion process, especially during co-digestion of FW and MA.

To further illustrate the synergistic effect of FW and MA, the bacterial community structure at the genus level was compared. The genera Mesotoga, Longilinea, Syntrophobacter, Sulfurovum, Syner-01, Anaerolinea, and Flexilinea had >1% of average abundance in different mixing ratio systems (Figure 4b). A previous study reported that Mesotoga could efficiently degrade carbohydrates and short-chain fatty acids to acetic acid [34]. Inconsistently, the abundance of Mesotoga (7.30%) in FW/MA = 1:1 was markedly higher than in other digestion systems. With increasing added MA, the relative abundance of Proteiniphilum in the FW/MA = 0:1 group was 2.5 times higher than that of the FW/MA = 1:0 group. The genus Proteiniphilum was revealed to be responsible for the transformation of cellobiose and crude protein to acetic acid [3]. Overall, co-digestion of FW and MA could facilitate positive co-metabolism of mixtures of substrates (cellobiose, glucose, and starch). The apparent changes in dominant genera further confirmed that co-digestion of FW and MA reshaped the structure of the bacterial community. This may be an important reason explaining the improved biogas production performance seen with co-digestion.

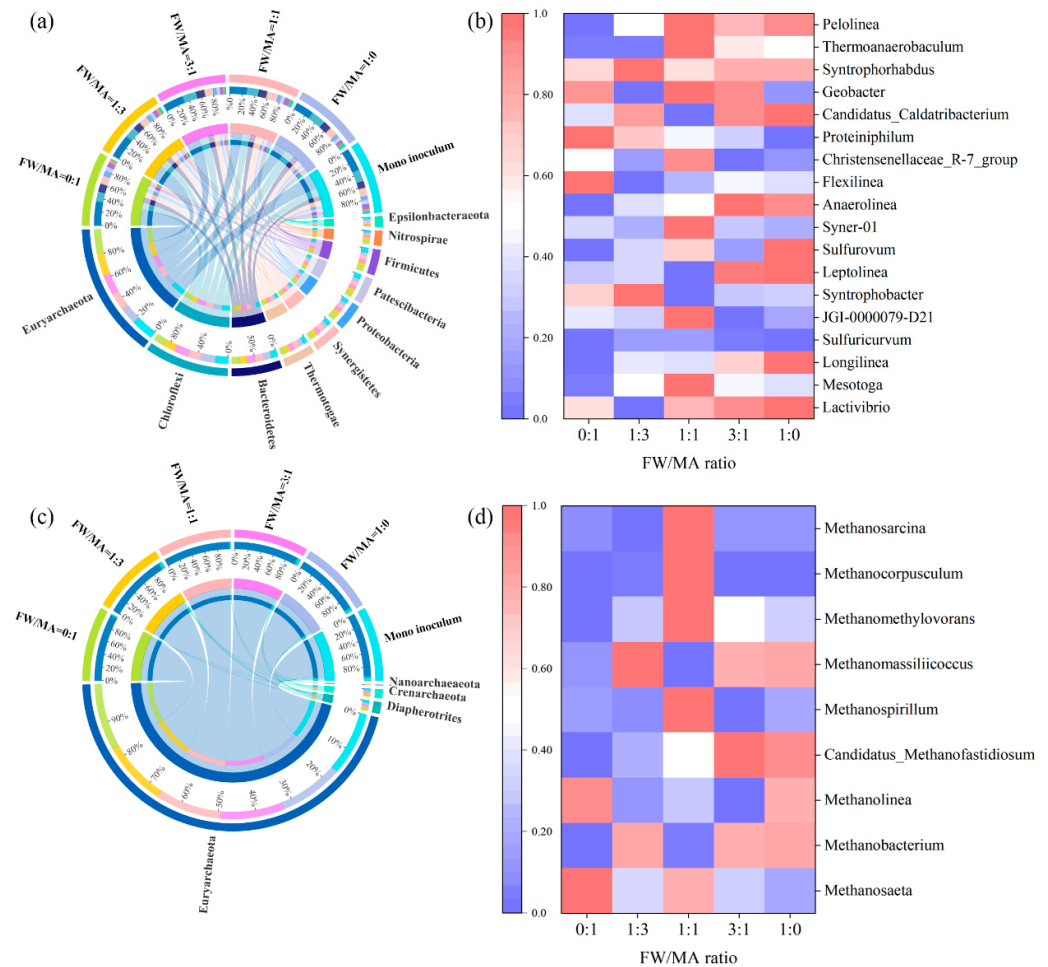


Figure 4. Microbial community analysis. (a) The microbial community distributions at the phylum level. (b) The relative abundance of bacteria at the genus level. (c) The archaeal community distributions at the phylum level. (d) The relative abundance of archaea at the genus level.

3.4.2. Effects of Mixing Ratio on Archaeal Community

Comparatively, the archaeal community had much lower richness and diversity compared to the bacterial community at both the phylum and genus levels. As depicted in Figure 4c, *Euryarchaeota* was the only dominant phylum of methanogens, and its relative abundance (93.61–97.85%) showed no observable differences among the various groups.

Due to the heterogeneities in various precursor substrates applied to produce biomethane, methanogenic pathways are divided into four types: acetoclastic, hydrogenotrophic, methylotrophic, and electron-dependent methanogenesis [43], all of which play crucial roles in biomethane production. To further clarify the methanogenesis pathways affected by different combinations of FW and MA, the genus distribution of the methanogenic community was analyzed. At the genus level, the predominant genera (>2%) identified in the archaeal community were *Methanosaeta* (41.16–73.31%), *Methanobacterium* (8.82–31.35%), *Methanolinea* (5.74–9.15%), *Candidatus_Methanofastidiosum* (1.28–5.17%), and *Methanospirillum* (2.08–6.49%), all of which exhibited significant changes in each group (Figure 4d). Notably, the abundance of *Methanospirillum* increased from 2.70% in the mono-digestion of MA (FW/MA = 0:1) and 2.92% in the mono-digestion of FW (FW/MA = 1:0) to 6.49% in the co-digestion of FW/MA = 1:1, indicating that it was selectively enriched during co-digestion. Interestingly, similar to *Methanospirillum*, the relative abundances of *Methanocorpusculum*, *Methanomethylovorans*, and *Methanosarcina* were also enriched in the co-digestion of FW/MA = 1:1 (Figure 4d). It is worth noting that most of these genera carry out hydrogenotrophic methanogenesis. Compared with mono-digestion, higher *Methanosaeta*

was seen in the FW/MA = 0:1 group (73.31%), and higher *Candidatus_Methanofastidiosum* was seen in the FW/MA = 1:0 group (4.87%), contributing to acetoclastic methanogenesis [36]. It is reasonable to conclude that the optimal mixture of FW and MA potentially changed the metabolic routes for biomethane generation, resulting in better anaerobic co-digestion performance.

4. Conclusions

The current study evaluated the AcoD of FW and MA combined at different mixing ratios. The co-digestion of FW and MA yielded an obvious synergistic effect in biogas production, especially at mixing ratios of 1:1 and 1:3, with maximum biogas potential improvements of 9.03% and 26.86%, respectively. In addition, chemical parameters (pH, VFAs, $\text{NH}_4^+\text{-N}$) confirmed that co-digestion of FW and MA regulates the stability and performance of AcoD. The microbial community analysis found the relative abundances of *Methanospirillum*, *Methanocorpusculum*, and *Methanomethylovorans* were selectively enriched in the co-digestion (FW/MA = 1:1) system, indicating that co-digestion of FW and MA induced better biomethane production performance. Given the increase in FW and frequency of MA blooms worldwide, millions of tons of these biomass wastes urgently need to be recycled and reutilized. Co-digestion of FW and MA is a promising potential strategy for biomass waste co-disposal and bioenergy production.

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