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Dynamic behaviors of batch anaerobic systems of food waste for methane production under different organic loads, substrate to inoculum ratios and initial pH

Wanli Zhang,[1,*] Lintong Li,[1] Wanli Xing,[1] Bin Chen,[1] Lei Zhang,[2] Aimin Li,[2] Rundong Li,[1] and Tianhua Yang[1]

School of Energy and Environment, Key Laboratory of Clean Energy (Liaoning Province), Shenyang Aerospace University, No. 37 Daoyi South Avenue, Shenyang 110136, PR China[1] and Key Laboratory of Industrial Ecology and Environmental Engineering (Ministry of Education), School of Environmental Science and Technology, Dalian University of Technology, Linggong Road 2, Dalian 116024, PR China[2]

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This study characterized dynamic behaviors of batch anaerobic digesters treating food waste in terms of methane production, organics destruction and process stability under different organic loads (OLs), substrate to inoculum (S/I) ratios [on volatile solid (VS) basis] and initial pH. The results showed that OL, S/I ratio and initial pH significantly affected batch anaerobic process. Methane yield was proved to be inversely proportional to OL and S/I ratio. Digester with lowest OL (5 g VS/L) obtained greatest methane yield (551.4 mL/g VS), highest organics removal (94.1%) and good stability. Enhancing OL to 10 g VS/L was recommended for satisfactory stability and higher volumetric methane productivity. When OL was designated as 10 g VS/L, digester with low S/I ratio (1/2) achieved satisfactory methane yield (539.3 mL/g VS), high organics removal (92.3%) and stable performance. When OL was relatively high (20 g VS/L), adjusting initial pH to 7.5 contributed to stable performance via enhancing buffering capacity against volatile fatty acids (VFA) disturbance. Strong VFA inhibition occurred under high OL (40 g VS/L) or great S/I ratio (2/1) or acidic initial pH (6.5). In this case, acetate was dominant VFA, followed by butyrate. However, when digester was stable, acetate was main VFA, followed by propionate. This study provided practical guidance on process configurations for batch digesters of food waste needed to achieve satisfactory performance and stability.

[**Key words:** Anaerobic digestion; Food waste; Biogas; Methane; Organic load; Substrate to inoculum ratio; Initial pH]

Nowadays, anaerobic digestion of food waste (FW) is one hot research topic in the field of biofuel production from biomass waste. More and more researchers and engineers have devoted themselves to fundamental research and engineering application of anaerobic biotechnology treating FW for methane production (1). FW is a great bio-waste stream that contains huge bioenergy. FW is generated throughout almost all steps of food supply chain from food harvest to storage and transportation then to processing or cooking and sale or eating and so on, which makes FW an easily available resource of biomass (2,3). It is reported that at present about 1.3 billion tons of food is wasted every year and this value will continuously rise along with the increase of global population and economic development in the future (4). FW contains abundant biodegradable organics and could be easily converted to biogas by biochemical reactions of anaerobic microorganisms, which ensures its high methane production potential and the high organic removal of anaerobic process (5). All of above provide significant conditions for sustainable development of anaerobic digestion of FW.

The driving force of development of anaerobic digestion of FW also derived from serious shortage of fossil fuels and the urgent demand for renewable and sustainable alternative fuels. According to BP Statistical Review of World Energy 2018 (6), in 2017 global oil and coal consumption reached 4621.9 and 3731.5 million tons oil equivalent, respectively, and their share of total primary energy consumption reached 61.8%. While renewables (wind, geothermal, solar, biomass and waste) consumption was just 486.8 million tons oil equivalent, accounting for only 3.6% of global primary energy consumption (6). Unfortunately, now global proved reserves of oil and coal are 1696.6 billion barrels and 1035.0 billion tons, which are only sufficient to meet 50.2 and 134 years of global production, respectively (6). What is worse is that burning of coal and oil released too many gaseous pollutants, particulate matters and greenhouse gases to environment and caused serious air pollution and global warming (5). These factors lead to a move towards renewable, sustainable and cleaner energy sources, especially biofuel from biomass waste (7,8). Anaerobic biotechnology is one of the most efficient methods of converting FW to energy (7). It was reported that methane yield of FW reached 400–600 mL/g volatile solid (VS), which was much higher than those of other biomass waste such as sewage sludge, lignocellulosic waste and livestock manure (9–11). Methane produced from FW via anaerobic digestion could certainly replace some coal and oil consumption with lesser emissions. Besides, the digestate rich in nutrients is also attractive, which could be used as organic fertilizer for agricultural

* Corresponding author. Tel./fax: +86 24 89728889.
E-mail address: zhangwanli@sau.edu.cn (W. Zhang).

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application (12). It is clear that anaerobic digestion playing as a bridge connects a closed ecological chain from FW to energy and resource mainly in the form of methane and digestate fertilizer.

In recent years, many scholars are focusing on anaerobic digestion of FW and have carried out plenty of researches from lab-scale experiments to full-scale applications (3). However, the real methane yields of FW reported in literature are much lower than their methane production potentials (13–17). One main reason is that poor understanding of anaerobic process results in digester upsets, especially in the case when the basic substrate (typical for FW) was too easy to degrade into organic acids but methanogenesis could not keep pace with acidogenesis and subsequent acetogenesis (3,17). In fact, poor performance of anaerobic system of FW was often reported (18). As a result, anaerobic system could not release all potential abilities of producing methane and even might be inhibited by low pH and accumulated volatile fatty acid (VFA), which greatly limited the commercial application of anaerobic digestion of FW. So far there is still a lack of comprehensive and systematic understanding concerning characterization of batch anaerobic digestion of FW under different process configurations, which is important to achieve maximum methane yield and optimal process performance. Organic load, substrate to inoculum (S/I) ratio (on VS basis) and pH are often reported as important factors governing process performance of batch anaerobic digesters (4,19–22). Kawai et al. (19), Zhou et al. (23) and Raposo et al. (24) investigated effects of S/I ratio on batch anaerobic digestion of food waste, bean curd waste and sunflower oil cake, respectively. Li et al. (25) examined process performance of batch anaerobic system treating kitchen waste under organic loads of 10 and 20 g VS/L. Wang et al. (26) investigated the methane yield of batch anaerobic digestion of kitchen waste and fruit/vegetable waste under the organic load of 16.5 g VS/L. Yang et al. (27) explored effects of pH adjustment (to 7.0, 8.0 and 9.0) on high-solids thermophilic anaerobic digestion of food waste in batch mode. Montañés et al. (28) investigated effects of initial pH control on batch mesophilic anaerobic digestion of sugar beet pulp. Most of previous researches examined effects of single operating parameter on batch anaerobic digestion of food waste, while compound effects of organic load, S/I ratio and pH adjustment have been less studied. In the present study, dynamic behaviors of batch anaerobic digesters treating FW under different conditions were comprehensively and systematically investigated. Emphasis was focused on effects of three key operating parameters including organic load, S/I ratio and initial pH on methane production, organics destruction and process stability. This research could provide practical guidance on appropriate process configurations for batch anaerobic system of FW needed to achieve good performance and stability by sustaining high metabolic activity of anaerobic microorganisms (especially methanogens) thus avoiding VFA inhibition and maintaining high methane yield.

MATERIALS AND METHODS

Materials and sample preparation procedures FW obtained from a student restaurant of Dalian University of Technology in China was used as anaerobic digestion substrate. FW composed of rice, meat, vegetables and fruits was first sorted by hand to remove unwished impurities (mainly bones, napkins, plastic bags, beverage bottles and one-off chopsticks), then was minced into some fragments of 0.5–1.0 cm with a domestic meat mincer, finally was homogenized and screened using a kitchen blender and a 14-meshes screen (1.4 mm) as shown in Fig. 1. FW samples were stored at −20 °C and were unfroze at 5 °C for 12 h before experiment. The anaerobic sludge taken from an anaerobic digester treating sewage sludge in a municipal sludge treatment plant of Dalian in China was used as inoculum. The pH of inoculum was 7.45 ± 0.12 and its total solid (TS) content was 68.5 ± 1.6 g/L with a VS to TS ratio of 46.1%.

Experimental procedures In order to evaluate the biodegradability and methane production potential of FW, the biochemical methane potential (BMP) tests were carried out in 100-mL serum bottles (with silica gel stoppers) in triplicate. Every bottle had a working volume of 50 mL filled with 45 mL of inoculum and 5 mL of substrate. The organic load was set as 1 g VS/L. In order to eliminate the effect of background methane production of inoculum, the control was filled with 45 mL of inoculum and 5 mL of distilled water. Nitrogen gas was injected into every bottle for 1 min to discharge the air and create anaerobic condition. Then bottles were incubated in a shaker at 150 rpm under mesophilic condition (37 °C). The BMP tests finished when no more methane was produced.

In order to investigate dynamic behaviors of batch anaerobic digesters under different conditions, a series of batch trials were carried out. Several 500-mL Schott Duran bottles with silica gel stoppers were used as digesters, which had a working volume of 300 mL. The experimental design are summarized in Table 1. In group 1 (organic load trials), in order to investigate effects of organic load on behaviors of batch anaerobic systems, organic loads of R1–R4 were designated as 5, 10, 20 and 40 g VS/L, respectively. Because the volume of inoculum in each bottle was constant (240.2 mL) in group 1, the corresponding S/I ratios (on VS basis) of R1-R4 were 1/5.06, 1/2.53, 1/1.27 and 1/0.63, respectively. Due to the low pH value of FW, the initial pH values in R1-R4 gradually decreased from 7.41 ± 0.03 to 7.19 ± 0.01 with the increasing organic load as shown in Table 1. In group 2 (S/I ratio trials), in order to examine performance and stability of batch anaerobic systems under varying S/I ratios, S/I ratios of R5-R7 were set as 1/2, 1/1 and 2/1, respectively, but organic loads maintained a same value of 10 g VS/L. It meant that the volume of inoculum in each reactor was different as well as the initial pH. It should be pointed out that in groups 1 and 2 the initial pH of digesters varied with different organic loads and S/I ratios and were not artificially adjusted to a same value. This was exactly to keep the real state of anaerobic system of FW without human intervention on pH, as the low pH value of FW substrate was a point that should be taken into account. The low pH of FW was considered as a potential limiting factor that might have an adverse effect on anaerobic process stability. In group 3 (initial pH trials), in order to explore the responses of batch anaerobic digesters to different initial pH values when the organic load was relatively high. The organic loads and S/I ratios of R8–R11 were designated as 20 g VS/L and 1/1.37, respectively. The initial pH values of R8–R11 were respectively adjusted to 6.5, 7.5, 8.0 and 8.5 using HCl (12 mol/L) or NaOH (4 mol/L) solution. Every experiment was carried out in triplicate and lasted for 50 days. The methods of injecting nitrogen gas and operating parameters of the shaker were the same as BMP tests. The control filled with inoculum and distilled water was incubated under the same conditions for background methane production of inoculum.

Analytical methods Moisture, TS and VS were analyzed according to the standard method (29). The pH value was measured using a pH meter (PB-10, Sartorius, Göttingen, Germany). Total chemical oxygen demand and soluble chemical oxygen demand were determined using a microwave digestion method (30). Elemental contents of carbon (C) and nitrogen (N) were measured by an elemental analyzer (Vario EL III, Elementar, Hanau, Germany). A Soxhlet extractor (SXT-06, Benon, Shanghai, China), a Kjeldahl apparatus (KDY9820, Rebon, Beijing, China) and a spectrophotometry (DR-5000, Hach, Loveland, CO, USA) were respectively employed to determine contents of lipid, total Kjeldahl nitrogen (TKN) and ammonia. Protein content was approximately calculated by multiplying the organic nitrogen value (TKN subtracted by ammonia) by 6.25 reported by Ahn et al. (31). Carbohydrate content was estimated by subtracting lipid and protein from VS (15). The composition of biogas was determined by a gas chromatograph (GC-7800, Purui, Beijing, China) with a thermal conductivity detector and a packed column (TDX-01). The volumes of methane and carbon dioxide were calculated on the basis of the change of molar ratio of methane or carbon dioxide to nitrogen gas (7,32). Another gas chromatograph (GC-7900, Techcomp, Shanghai, China) with a flame ionization detector and a capillary column (DB-FFAP, 30 m × 0.25 mm × 0.25 μm) was employed to analyze concentrations of six

FIG. 1. Preparation procedures of FW samples.

TABLE 1. Summary of experimental design of batch anaerobic experiments.

Batch anaerobic trials	Digester	Organic load (g VS/L)	S/I ratio (on VS basis)	Initial pH	FW (g)	Inoculum (mL)
Group 1: organic load trials	R1	5	1:5.06	7.41 ± 0.03	7.2	240.2
	R2	10	1:2.53	7.36 ± 0.02	14.4	240.2
	R3	20	1:1.27	7.28 ± 0.03	28.9	240.2
	R4	40	1:0.63	7.19 ± 0.01	57.8	240.2
Group 2: S/I ratio trials	R5	10	1:2	7.33 ± 0.02	14.4	189.9
	R6	10	1:1	7.24 ± 0.01	14.4	94.9
	R7	10	2:1	7.16 ± 0.01	14.4	47.5
Group 3: initial pH trials	R8	20	1:1.37	6.5	28.9	260.1
	R9	20	1:1.37	7.5	28.9	260.1
	R10	20	1:1.37	8.0	28.9	260.1
	R11	20	1:1.37	8.5	28.9	260.1

kinds of VFAs including acetate, propionate, iso-butyrate, butyrate, iso-valerate, and valerate. The operating parameters of above two gas chromatographs and preparation procedures of VFA samples were described by Zhang et al. (15) in detail. Theoretical methane potential (TMP) of FW was estimated on the basis of contents of carbohydrate, protein and lipid using an empirical equation as follows (13):

$$\text{TMP}\left(\frac{\text{mL}}{\text{g}}\text{VS}\right) = 1014 \times \%\text{lipid} + 496 \times \%\text{protein} + 415 \times \%\text{carbohydrate} \quad (1)$$

where %lipid, %protein and %carbohydrate refer to percentages of lipid, protein and carbohydrate in VS, respectively.

RESULTS AND DISCUSSION

Characteristics of FW substrate Table 2 presents characteristics of FW substrate used in this study as compared to literature reports (16,33–38). A low pH of FW (4.6 ± 0.1) showed that it began to acidify and decompose within a few hours after sampling. It was due to the composition of FW including rice, meat, vegetable, meat and oil, which were all easily biodegradable and perishable (2,20). This feature was the main reason of environmental pollution caused by FW as well as a potential limiting factor that might have an adverse effect on anaerobic process stability. The high VS content (20.77 ± 0.13 wt.%) and COD concentration (353.3 ± 1.7 g/L total chemical oxygen demand and 162.5 ± 1.4 g/L soluble chemical oxygen demand) indicated that FW was a typical high-concentration organic waste stream. The high VS/TS ratio (82.7%) confirmed the findings of Zhang et al. (16) and Vrieze et al. (36) that most solids of FW were organics. The moisture content of FW (74.9%) was moderate for anaerobic digestion as FW exhibited a semifluid state after homogenized. It was found that FW was rich in organic nutrients. The contents of carbohydrate, protein and lipid reached 113.8, 39.8 and 54.1 g/L, accounting for 54.79%, 19.16% and 26.05% of VS. Among them, carbohydrate mainly from starch, sugar and cellulose of cereals, fruits and vegetables was main organic component. The TKN concentration of FW reached 6.5 ± 0.3 g/L while ammonia concentration was only 130 ± 10 mg/L. The result suggested that nitrogen mainly existed in the form of organic nitrogen like protein. Ammonia mainly resulted from hydrolysis of protein. Above characteristics of FW were in agreement with the findings of others (16,33–38). The relatively low C/N ratio (14.5) of FW used in this study was in accordance with the results of Zhang et al. (16) (13.2 ± 0.2), Han and Shin (33) (14.7) and Zhang et al. (34) (14.8), but was very different from those of Zhang et al. (37) (24.5) and Zhang et al. (38) (21.1). The reason might be that different dietary habits led to diverse components and properties of FW in different regions. The C/N ratio of FW in this study was not within the optimal range (20–30) for the growth and metabolism of anaerobic microorganisms reported in literature (20). However, in fact, the optimal C/N ratio was still controversial. Thus, effects of this level of C/N ratio of FW substrate on anaerobic process should be further investigated.

BMP and TMP were used for evaluating biodegradability and methane production potential of FW. It was found that BMP and TMP of FW were 593.6 and 586.6 mL/g VS, respectively, and reached an extremely high level. The result confirmed the satisfactory anaerobic biodegradability and methane potential reported by others (13–15). Fig. 2 exhibits BMP values of general organic waste streams including municipal solid waste (MSW) (FW, yard waste and waste paper) (13–15,39–41), agricultural biomass (9,42–44), algae (45–48) and other biomass (waste sludge and manure) (43,49,50) reported in literature. It was found that BMP values of FW from different regions ranged from 434.0 to 593.6 mL/g VS, which were much higher than those of other organic solid wastes reported in selected literature. This comparative results agreed with previous observations of Zhang et al. (20) and Li et al. (51). It might be connected with the chemical composition of FW. Plenty of carbohydrate, protein and lipid from easily biodegradable cereals, vegetables, fruits, eggs, oil, milk and meat constituted the VS of FW and had methane potentials of 415, 496 and 1014 L/kg, respectively (13). Among them, lipid was regarded as an attractive substrate for anaerobic digestion owing to its much higher methane potential than protein and carbohydrate. Compared to other biomass wastes, FW contained more lipid, which reached 26.05% of VS and contributed more methane yield. With regard to lignocellulosic biomass, in many cases lignin and cellulose are limiting factors for anaerobic degradation due to poor biodegradability and rigid structure (43). In addition, hydrolysis of waste sludge is considered to be the limiting stage of anaerobic process due to the great difficulty of disrupting sludge floc structure, which greatly affects its methane production (40). Thus, more easily biodegradable compositions of FW than other wastes contributed to higher methane potential. Although the BMP data of food waste presents a few variances due to different experimental methods and materials from different origins, above comparative results still clearly show the high feasibility of anaerobic digestion of FW.

Dynamic behaviors of batch anaerobic systems under different organic loads In order to enhance current understanding concerning batch anaerobic process of FW, dynamic behaviors of batch anaerobic digesters under different organic loads, S/I ratios and initial pH values were characterized from three aspects of methane production, organics degradation and stability based on multiple performance parameters including methane production rate, cumulative methane and biogas yields, average methane content, VS removal, pH, total VFA (TVFA) and specific VFA concentrations. In organic load trials, organic loads of R1–R4 were designated as 5, 10, 20 and 40 g VS/L, respectively. Fig. 3 shows process performance and stability of R1–R4. As shown in Fig. 3A, methane production rates of R1–R3 exhibited similar changes. In initial period, methane production rates of R1–R3 grew fast and reached respective peaks of 153.7, 98.3, and 51.1 mL/g VS·day on

TABLE 2. Characteristics of FW substrate as compared to literature reports.

Moisture (%)	pH	SCOD (g/L)	TCOD (g/L)	TS (wt.%)	VS (wt.%)	VS/TS (%)	C (% TS)	N (% TS)	C/N ratio	Lipid (% VS)	Protein[a] (% VS)	Carbohydrate[b] (% VS)	TKN (g/L)	Ammonia (mg/L)	Reference
74.9 ± 0.4	4.6 ± 0.1	162.5 ± 1.4	353.3 ± 1.7	25.10 ± 0.10	20.77 ± 0.13	82.7	46.5 ± 0.1	3.2 ± 0.1	14.5	26.05	19.16	54.79	6.5 ± 0.3	130 ± 10	This study
79.5	–	–	–	20.5	19.5	95	51.4	3.5	14.7	–	–	–	–	–	33
69.1	–	–	–	30.90 ± 0.07	26.35 ± 0.14	85.3	46.78 ± 1.15	3.16 ± 0.22	14.8	–	–	–	–	973 ± 571	34
81.9	–	106.0 ± 5.3	238.5 ± 3.8	18.1 ± 0.6	17.1 ± 0.6	94	46.67	3.54	13.2 ± 0.2	13.63	19.24	67.13	5.42 ± 0.26	160 ± 40	16
76.26	4.71 ± 0.01	–	–	23.74 ± 0.08	21.71 ± 0.09	91.44	–	–	–	–	–	–	8.12 ± 0.01	–	35
74.5	–	–	260.0 ± 47.0	25.5 ± 0.4	24.0 ± 0.6	94.12	–	–	–	–	–	–	11.93 ± 1.1	384 ± 4	36
76.9	4.2 ± 0.2		–	23.1 ± 0.3	21.0 ± 0.3	90.9	56.3 ± 1.1	2.3 ± 0.3	24.5	–	–	–	–	–	37
81.5	5.2 ± 0.3	–	–	18.5 ± 0.1	17.0 ± 0.1	91.9	46.5 ± 1.5	2.2 ± 0.3	21.1	–	–	–	–	–	38

SCOD, soluble chemical oxygen demand; TCOD, total chemical oxygen demand; TS, total solid; VS, volatile solid; TKN, total Kjeldahl nitrogen.
[a] Protein = (TKN − ammonia) × 6.25.
[b] Carbohydrate = VS − lipid − protein.

day 2, 4, and 9. As reaction further proceeded, methane production rates of R1-R3 gradually decreased and finally dropped to zero on day 15, 21 and 25, respectively, which meant the end of trials. However, the situation of R4 with a extremely high organic load (40 g VS/L) was quite different. R4 only produced a bit of methane (5.6 mL/g VS·day) on the first day, then went into a long stagnation period without methane production. Until day 30, methane production rate of R4 began to increase but was still at a extremely low level (about 2–7 mL/g VS·day). Until day 50, no obvious increase of methane yield was observed in R4, which implied the failure of anaerobic process. Hence, the experiment of R4 was forced to end on day 50. The results indicated that organic load had a significant effect on batch anaerobic system of FW. The digester with a lower organic load achieved a earlier and higher peak of methane production rate, a higher reaction rate and a shorter reaction period. Compared to R1, the time to reach the peak of methane production rate in R2 and R3 were prolonged by 2 and 7 days, and the reaction time were also lengthened by 6 and 10 days, respectively. The reason might be that processing capacity of anaerobic system mainly depended on biomass. In the case when the biomass was constant, higher organic load meant more time needed to degrade more organics (like R2 and R3). However, if organic load was further enhanced and exceeded the processing capacity of anaerobic microorganisms (especially methanogens), anaerobic system would lose its balance (like R4). As shown in Fig. 3B, cumulative methane yields of R1 and R2 were 551.4 and 546.8 mL/g VS_{added}, reaching 92.9% and 92.1% of BMP of FW and were much higher than those of R3 (509.5 mL/g VS_{added}) and R4 (98.0 mL/g VS_{added}). With the increase of organic load from 5 to 40 g VS/L, methane yield showed an obvious declining trend. The cumulative biogas yields and average methane contents of R1-R4 also exhibited similar trends. As shown in Fig. 3C, cumulative biogas yields of R1-R4 were 847.1, 862.0, 814.2 and 282.9 mL/g VS_{added} with average methane contents of 65.1%, 63.4%, 62.6% and 34.6%, respectively. It should be noted that both R1 and R2 exhibited satisfactory methane production rate, but the volumetric methane productivity of R2 was double of that of R1 as organic load of R2 was 2-folds of R1. The result suggested that it was feasible to enhance the organic load to a moderate extent on the premise of not affecting methane production.

VS removals shown in Fig. 3D were used for evaluating organics degradation of R1-R4. It was found that VS removals of R1 and R2 reached 78.6% and 66.9%, respectively, on day 5, which indicated most organics were already degraded. While at this moment VS removals of R3 and R4 were only 19.7% and 1.2%, respectively. Methane production derived from organic degradation and conversion, so higher VS removal meant higher methane yield. Then, VS removals of R1–R3 gradually increased and finally reached 94.1%, 93.6% and 87.8%, respectively. While VS removal of R4 raised very slowly and was only 21.5% at the end. The result indicated that most organics in R4 were not converted to biogas. R1 and R2 showed best organics destruction followed by R3, and R4 was worst.

VFAs, main intermediate products of anaerobic digestion of organics, were reported to be sensitive indicators for process stability (15). The pH value had a significant effect on metabolic activity of anaerobic microorganisms and was considered to be one key process parameter of anaerobic system (18). Changes of pH and TVFA concentrations of R1–R4 are presented in Fig. 3E and F. It was found that both pH and TVFA concentrations of R1–R3 exhibited similar trends. In the initial 3 days the pH of R1–R3 sharply dropped to about 7.0 as plenty of organic acids resulting from fast hydrolysis and acidification of organics consumed the alkalinity of anaerobic system. At this moment, methanogenesis was the limiting stage due to its lower reaction rate than acidogenesis and acetogenesis.

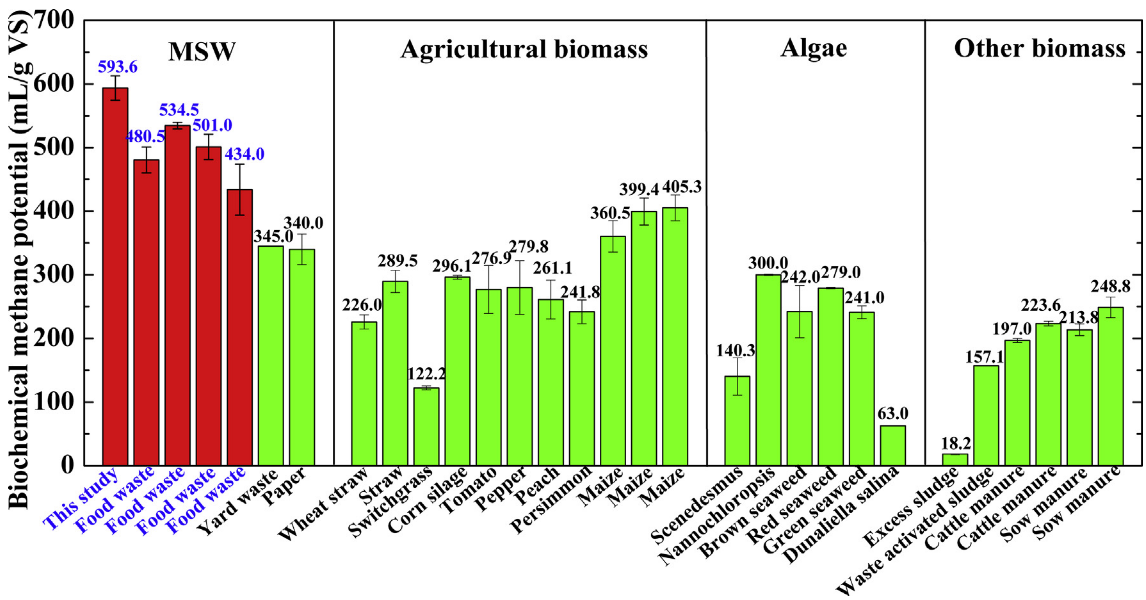

FIG. 2. BMP of general organic waste streams including MSW (13–15,39–41), agricultural biomass (9,42–44), algae (45–48) and other biomass (43,49,50) reported in literature as compared to the FW in the present study.

The TVFA concentrations of R1-R3 rapidly increased and accumulated to the peaks of 1544.2, 4982.7 and 10923.1 mg/L on day 2, 2 and 3, respectively. Then, the speed of VFAs degradation increased with the improvement of methanogens activity, which resulted in decreases of TVFA concentrations in R1–R3. As a result, the pH of R1–R3 gradually rose up to above 7.5 on day 4, 5 and 9, respectively. Then, as VFA concentrations in R1–R3 further declined, the corresponding pH values reached above 8.0 from day 20. Finally, almost all of VFAs of R1–R3 were converted to biogas via acetogenesis and methanogenesis, as indicated by the extremely low VFA concentration in digesters in the end. The result demonstrated that R1–R3 ran steadily and were not obviously inhibited by VFA. While, R4 with the highest organic load exhibited a quite different status. In the initial stage, hydrolysis and acidogenesis of FW produced plenty of VFAs (above 25,000 mg/L) and led to the sharp drop of pH to below 6.0 in R4. The low pH and the high-concentration TVFA in R4 greatly inhibited methanogenesis, as indicated by the extremely low methane yield. Although methanogens activity recovered slightly after day 30, TVFA still maintained a high concentration and exerted a strong inhibition of methane production. There was no doubt that R4 was overloaded.

In order to provide a deep insight into organic acids production and conversion during anaerobic digestion, changes of acetate, propionate, iso-butyrate, butyrate, iso-valerate, and valerate concentrations in R1–R4 are characterized and presented in Fig. 3. It was found that the organic load also greatly affected the pathway of organics degradation. In R1–R3, acetate appeared as dominant VFA, followed by propionate, However, in R4 acetate was main VFA, followed by butyrate. The result suggested that acetate fermentation was the dominant pathway of organics degradation, but the proportion of butyrate fermentation rose when organic load was relatively high and the pH was relatively low. This result was in agreement with the findings of Wang et al. (52), Horiuchi et al. (53) and Appels et al. (54) that acetate and butyrate were the main VFAs under acidic pH condition.

In short, organic load exerted a significant influence on process performance and stability of anaerobic system of FW. When the organic load was not very high ($\leq$ 10 g VS/L), anaerobic system exhibited better performance and stability and a moderate enhancement of organic load on the premise of not affecting methane production was recommended (like R2). When organic load was so high that exceed the processing capacity of anaerobic system (like R4), methanogenesis was greatly inhibited by VFA accumulation and the resulting pH drop.

Dynamic behaviors of batch anaerobic systems under different S/I ratios In S/I ratio trials, R5–R7 were operated under varying S/I ratios of 1/2, 1/1 and 2/1, respectively, and an unified organic load (10 g VS/L). Fig. 4 presents process performance and stability of R5–R7. It was found that the digester with a lower S/I ratio exhibited better performance. The methane production rates of R5 and R6 first increased and reached respective peaks of 105.7 and 68.0 mL/g VS·day on day 5 and 11, then gradually dropped and finally reduced to zero on day 15 and 50, respectively. Although methane production rate of R7 slowly increased from day 30, it was still at a low level during the whole reaction period, which resulted in an unsatisfactory cumulative methane yield of 293.0 mL/g VS_{added}, accounting for only 49.4% of BMP of FW. While cumulative methane yields of R5 and R6 reached 539.3 and 489.7 mL/g VS_{added}, which were 1.84- and 1.67-folds of that of R7. The results clearly showed that with the increase of S/I ratio from 1/2 to 2/1, methane yield gradually decreased. R5 achieved the highest methane yield. The results were in agreement with the findings of Kawai et al. (19) that highest methane yield of food waste was observed in the lowest S/I ratio. Haider et al. (55) also suggested that a low S/I ratio was beneficial to a high methane yield when food waste combined with rice husk was digested in a batch digester. Alzate et al. (22) used microalgae as substrate of batch anaerobic digester and achieved a same result that the optimal S/I ratio was 0.5. The VS removal of R5 reached 90.4% on day 10, then gradually increased to 92.3% and maintained this value until the end. Meanwhile, TVFA concentration of R5 rapidly accumulated to the peak of 4873.7 mg/L on day 3, then gradually declined to 126.5 mg/L on day 10 as shown in Fig. 4F. The results indicated that in the initial 10 days most organics in R5 were decomposed into VFAs then were further converted to biogas. The high VS removal and the high production and conversion rates of VFAs confirmed the high

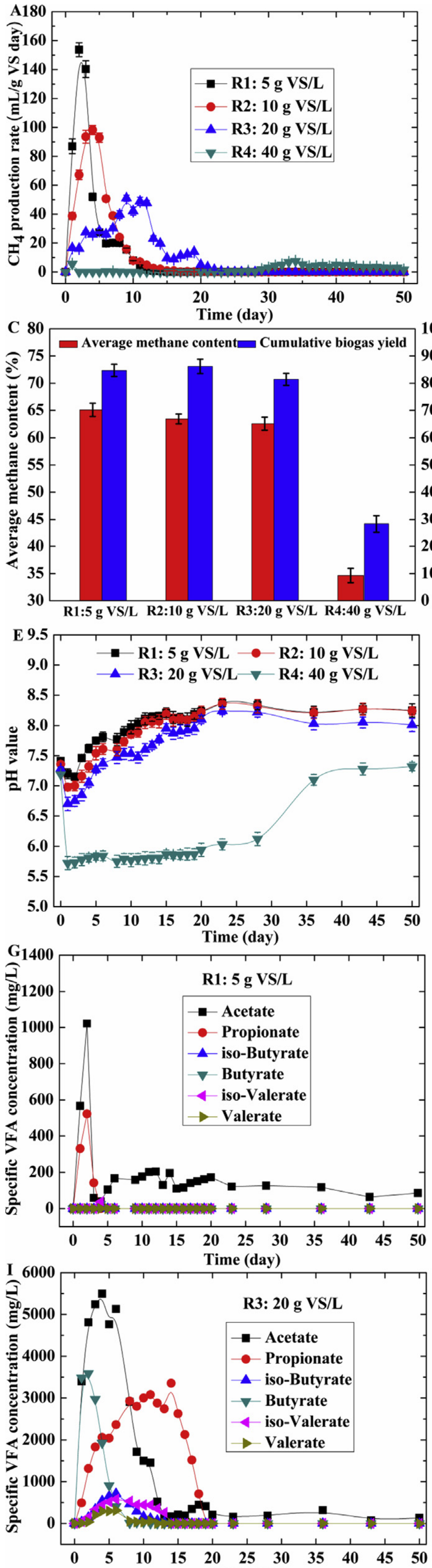

FIG. 3. Process performance and stability of batch anaerobic systems under different organic loads: CH_4 production rate (A); cumulative CH_4 yield (B); cumulative biogas yield and the average CH_4 content (C); VS removal (D); pH (E); TVFA concentration (F); specific VFA concentrations in R1 (G); specific VFA concentrations in R2 (H); specific VFA concentrations in R3 (I); specific VFA concentrations in R4 (J).

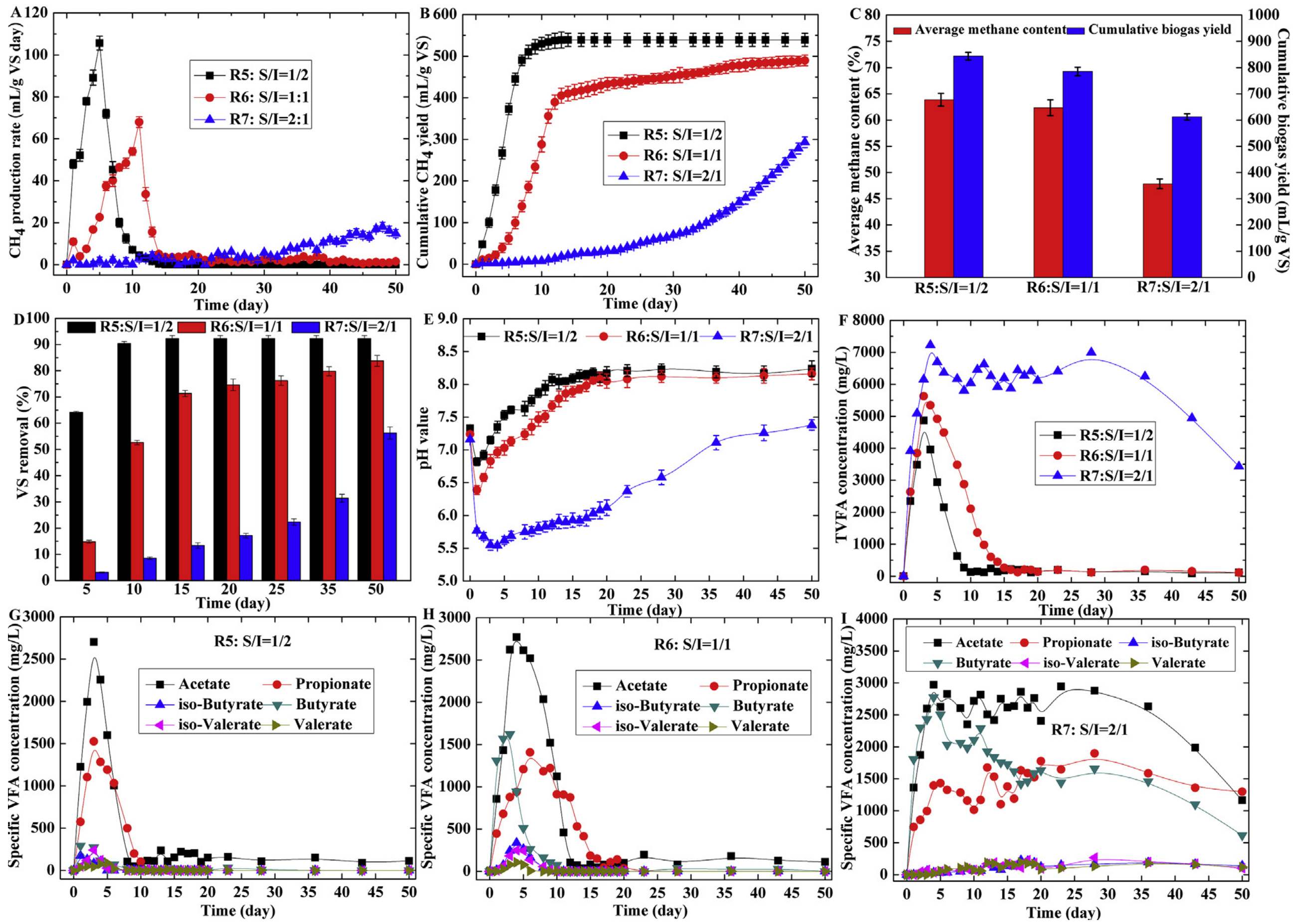

FIG. 4. Process performance and stability of batch anaerobic systems under varying S/I ratios: CH_4 production rate (A); cumulative CH_4 yield (B); cumulative biogas yield and the average CH_4 content (C); VS removal (D); pH (E); TVFA concentration (F); specific VFA concentrations in R5 (G); specific VFA concentrations in R6 (H); specific VFA concentrations in R7 (I).

methane yield of R5 in the initial 10 days. The VS removal of R6 exhibited a similar trend as R5 but was obviously lower than that of R5. In the initial 3 days, R6 seemed to be slightly affected by VFA accumulation (up to 5630.3 mg/L on day 3) and the acidic pH (below 6.5), as indicated by a short lag period of methane production from day 1–3. Then, the improvement of methanogenic activity resulted in the rapid degradation of VFAs, which dropped to below 200 mg/L on day 20. On the contrary, during the whole operating period, methane production in R7 was strongly inhibited by VFA accumulation (above 6000 mg/L) and the acidic pH (below 6.0). At the end of the experiment on day 50, VS removal of R7 was only 56.3%, and TVFA concentration was still as high as 3432.5 mg/L. The results implied that the degradation of organics in R7 was much slower than those of R5 and R6. It was found that process performance and stability of batch anaerobic system deteriorated with the increase of S/I ratio from 1/2 to 2/1. The reason was that when the organic load was identical, higher S/I ratio meant fewer biomass. Especially for methanogens which had a much lower growth rate than acidogens, the low methanogenic biomass meant a high risk of VFA inhibition. In addition, the high sensitivity of methanogens to environmental conditions was also one reason why methanogens were susceptible to pH disturbance in the initial stage. Interestingly, it was found that the S/I ratio of R7 (2/1) was higher than that of R4 (1/0.63) but its methane yield (293.0 mL/g VS_{added}) was much higher than that of R4 (98.0 mL/g VS_{added}), which showed a special phenomenon. The reason was that the organic load of R4 (40 g VS/L) was 4-folds of that of R7 (10 g VS/L). In R4, the fast hydrolysis and acidogenesis of organics produced plenty of VFAs, and TVFA concentration (above 25,000 mg/L) greatly exceeded its inhibitive threshold to methanogens and was also much higher than those in R7 (below 7000 mg/L). At this moment, the biomass in R4 was far from enough even it had a lower S/I ratio than R7. The determinant factor governing process performance became VFA inhibition and the resulting pH drop. Thus, when the organic loads of digesters were greatly different (typical for R4 and R7), above conclusion obtained from R5–R7 operated under an unified organic load (10 g VS/L) might be not applicable.

Fig. 4 also shows changes of specific VFA concentrations in R5-R7. It was observed that acetate was the dominant VFA in all three digesters. However, with the increase of S/I ratio, butyrate fermentation gradually surpassed propionate fermentation as the secondary pathway of organic degradation. Although the pH of R7 gradually grew up to above 7.0 from day 35, accumulated VFA (reached 6244.2 mg/L) mainly composed of 2631.2 mg/L of acetate, 1584.5 mg/L of propionate and 1458.7 mg/L of butyrate still exerted a strong inhibition to methanogens. Thus, the recovery of methanogenic activity in R7 was slow. The results showed that the acidic pH was not the solo inhibitory factor. The inhibition was a result of comprehensive effects of acidic pH and VFA accumulation. It was concluded that S/I ratio also had a great effect on batch anaerobic system of FW. With the increase of S/I ratio from 1/2 to 2/1 in R5–R7, process performance and stability of anaerobic system

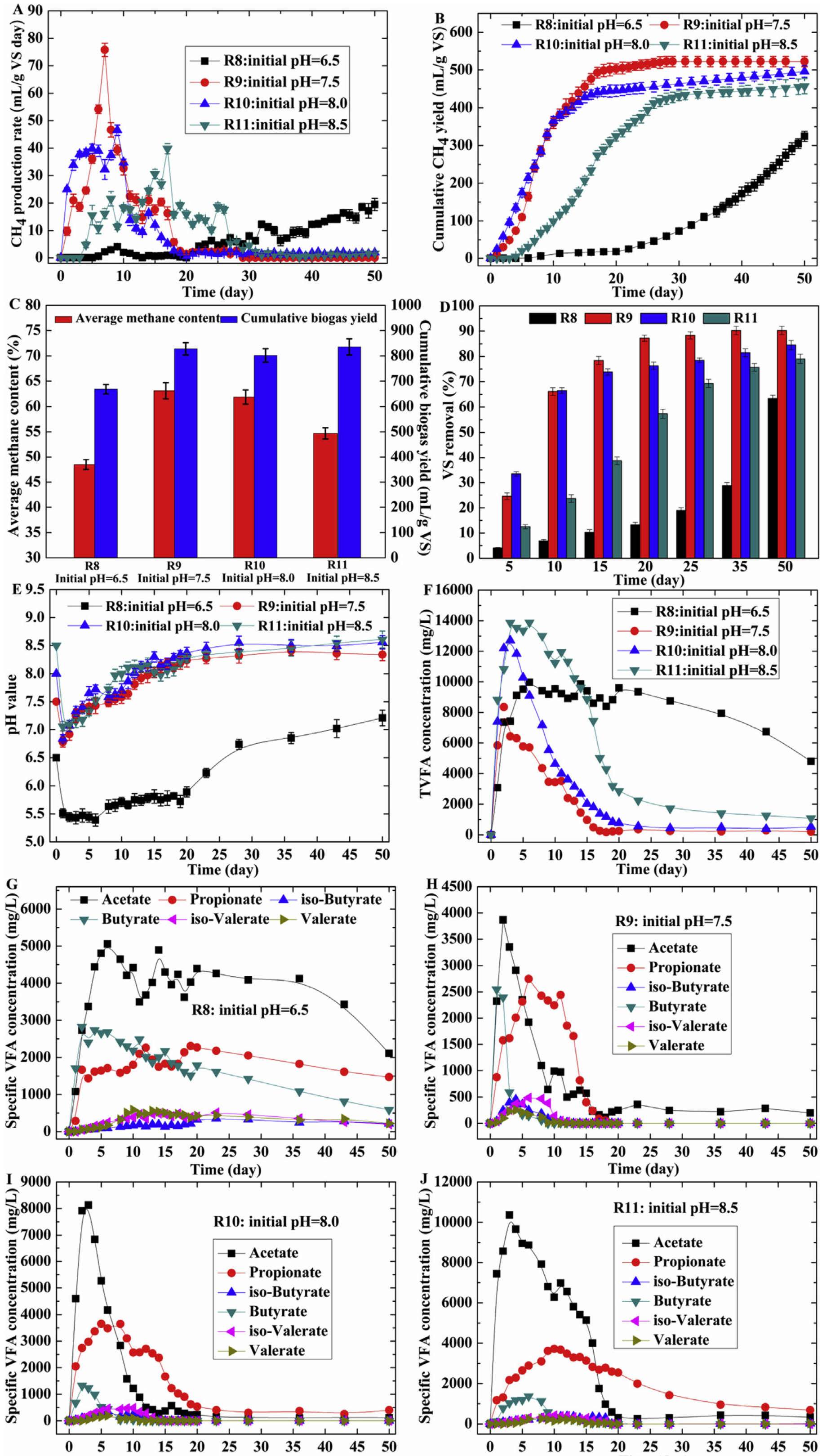

FIG. 5. Process performance and stability of batch anaerobic systems under varying initial pH values: CH_4 production rate (A); cumulative CH_4 yield (B); cumulative biogas yield and the average CH_4 content (C); VS removal (D); pH (E); TVFA concentration (F); specific VFA concentrations in R8 (G); specific VFA concentrations in R9 (H); specific VFA concentrations in R10 (I); specific VFA concentrations in R11 (J).

gradually deteriorated. Among them, R7 was greatly inhibited by VFA accumulation and the resulting acidic pH. R5 with the lowest S/I ratio (1/2) exhibited the best process performance and stability, as indicated by the highest methane yield (539.3 mL/g VS_{added}), VS removal (92.3%), suitable pH and no VFA inhibition. In R7, acetate was the main VFA followed by butyrate under acidic pH.

Dynamic behaviors of batch anaerobic systems under different initial pH In initial pH trials, initial pH values of R8–R11 were adjusted to 6.5, 7.5, 8.0 and 8.5, respectively, to investigate effects of initial pH and to verify the feasibility of enhancing process performance via adjusting initial pH, especially when the organic load was relatively high (20 g VS/L) or when there was the potential risk of VFA inhibition. Fig. 5 presents process performance and stability and specific VFA concentrations of R8–R11. It was found that anaerobic digesters exhibited quite different performance under different initial pH. R8 with an acidic initial pH (6.5) was greatly inhibited by VFA. Its methane production maintained a low level during the whole operating period although a slight increase was observed from day 30. R8 produced fewest methane (324.2 mL/g VS_{added}) and degraded fewest organics with a lowest VS removal (63.4%) and lowest pH value. When initial pH was adjusted to 7.5 in R9, methane production rate reached the peak of 75.8 mL/g VS·day on day 8, which was higher than that of R3 (51.1 mL/g VS·day on day 9). No obvious lag period of methane production was observed in R9 as compared to R3, which had a lag period during day 3–6. R9 obtained a satisfactory methane yield of 522.5 mL/g VS_{added}, which reached 88.0% of BMP of FW and was also higher than R3 (509.5 mL/g VS_{added}). The average methane content (63.1%) and VS removal (90.2%) of R9 were also higher than those of R3. The comparative results clearly showed the positive effect of adjusting initial pH to 7.5 on process performance of anaerobic system when organic load was 20 g VS/L. However, when initial pH was further adjusted to higher values such as 8.0 in R10 and 8.5 in R11, process performance of anaerobic systems unexpectedly got worse. The cumulative methane yields of R10 and R11 were 496.0 and 457.1 mL/g VS_{added}, which were lower than that of R3 or R9, so were average methane contents and VS removals. The results suggested that the moderate adjustment of initial pH (to 7.5 in R9) was beneficial to address the challenge of process disturbance resulting from VFA accumulation and pH drop due to fast hydrolysis of organics during start-up period, while both over high (R10 and R11) and over low (R8) initial pH had negative effects. As shown in Fig. 5, butyrate concentration in R8 with an acidic initial pH was much higher than those in R9–R11 with alkaline initial pH. Although acetate always appeared as the dominant VFA, the proportion of butyrate obviously increased under a relatively low pH. This finding confirmed the results of R4 and R7 that butyrate fermentation became another important organic degradation pathway when anaerobic system was unstable and had a low pH value. Thus, initial pH value also had important effects on process performance and stability of anaerobic digestion of FW. Enhancing initial pH to a moderate value (such as 7.5 in R9) could improve the ability of anaerobic system against VFA accumulation and alkalinity drop during start-up period, especially in the case when the substrate (typical for FW) could be easily hydrolyzed into VFAs. The results were in line with the findings of Montañés et al. (28) that initial pH control (to 7.5) exhibited positive effects on batch anaerobic digestion of sugar beet pulp for methane production. Yang et al. (27) also concluded that adjusting the pH of anaerobic system to a weak alkaline value could greatly improve the methane yield of high-solids thermophilic batch anaerobic digestion of food waste.

Fig. 6 summarizes methane yields of different digesters under varying organic loads (5, 10, 20 and 40 g VS/L), S/I ratios (1/2, 1/1 and 2/1) and initial pH values (6.5, 7.5, 8.0 and 8.5). Within above ranges of process parameters designated in this study, methane yield was inversely proportional to organic loads and S/I ratios, but it first rose then fell with the changes of initial pH from acidic to alkaline. These three parameters had significant effects on process performance and stability of anaerobic digestion of FW. Among these experiments, R1 achieved highest methane yield of 551.4 mL/g VS_{added}. While after doubling organic load to 10 g VS/L, methane yield of R2 (546.8 mL/g VS_{added}) was also satisfactory and was comparable to that of R1. The results implied that biomass was so adequate that most organics were converted to biogas when organic load was not more than 10 g VS/L. In this case, enhancing organic load to a moderate extent was beneficial to make the best use of anaerobic microorganisms to obtain higher volumetric methane productivity. This point was significant to enhance methane production capacity and equipment utilization rate in commercial practice. R4 with highest organic load (40 g VS/L) produced fewest methane as the amount of organics already exceeded maximum processing capacity of anaerobic system, which implied that it was overloaded. Whether the biomass was adequate was often determined by S/I ratio. The lower the S/I ratio, the more adequate the biomass, the higher the methane yield, as

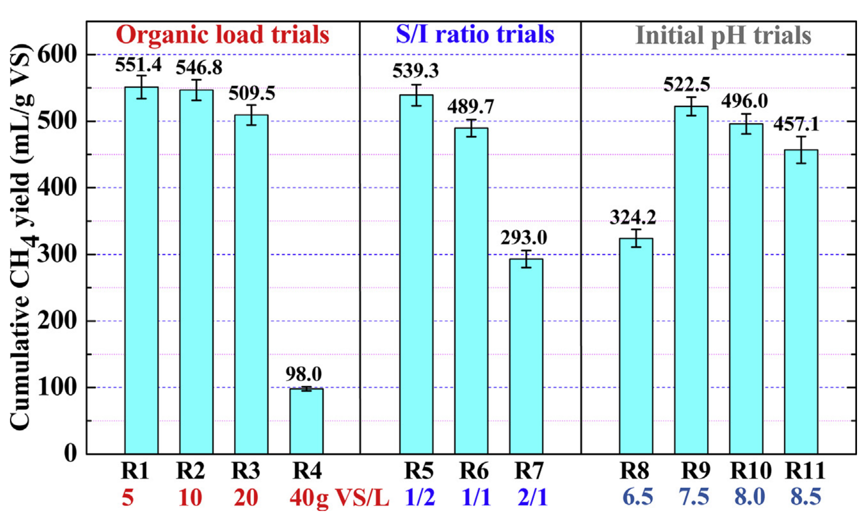

FIG. 6. Comparison of methane yields of different digesters under varying organic loads, S/I ratios and initial pH values.

indicated by the results of R5-R7. It was clear that VFA accumulation and pH drop during start-up period had great effects on subsequent reaction. The objective of adjusting initial pH was exactly to enhance the buffering capacity against pH drop and alkalinity depletion. It was found that adjusting initial pH to 7.5 was enough to counteract pH drop, which not only ensured the pH not lower than the optimal pH range (6.8−7.2) for anaerobic microorganisms reported in literature (56), but also not led to over high pH under the influence of ammonia−nitrogen generated from proteolysis during subsequent period. This study enhanced current understanding concerning dynamic behaviors of batch anaerobic systems of FW under different organic loads, S/I ratios and initial pH values and provided practical guidance on appropriate process configurations needed to achieve good performance and stability by sustaining high metabolic activity of anaerobic microorganisms thus avoiding VFA inhibition and maintaining high methane yield.

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