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RESEARCH PAPER

Biogas Production and Microbial Community Change during the Co-digestion of Food Waste with Chinese Silver Grass in a Single-stage Anaerobic Reactor

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Abstract Chinese silver grass (CSG), a potential subtropical energy crop, was investigated as a co-substrate to enhance the anaerobic digestion of food waste for municipal solid waste treatment. Results showed that 88.1% of food wastes were degraded using CSG as a co-substrate with 45 days of digestion, where the food waste, CSG, and sludge on VS/TS/working volume was 93.14 g/111.55 g/1 L, in which the average biogas production was at 429.3 L/kg solids, and the average methane content was around 60%. During the digestion, the concentrations of ammonium and free ammonia gradually increased to 1448.2 and 265.2 mg/L respectively, without any significant inhibitory effects on biogas production, which is probably due to the buffering effects of CSG. Microbial community analysis showed that microorganisms from the class of *Firmicutes* and *Bacteroidetes* were dominant during digestion, and that the microbial community diversity increased with active methanogenesis, suggesting that the addition of substrates contribute to the increase of microbial diversity, and could be beneficial for biogas production. Therefore, using CSG as a co-substrate in the single-stage food waste anaerobic digestion system is a potential simple method to convert CSG into renewable energy and to simultaneously improve food waste treatment.

Keyword: anaerobic co-digestion, food waste, Chinese silver grass, biogas production, bacterial community

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1. Introduction

Environmental pollution from municipal solid wastes (MSW) has become a worldwide concern, as developing countries, including China, have undergone rapid urbanization and continue to release large and increasing amounts of MSW at an unprecedented pace. In addition, the energy requirement for the production and treatment of MSW adds pressure to energy supply systems for many of these countries. The preferred disposal methods for MSW in developing countries usually involve sanitary landfills to minimize costs. However, sanitary landfills are not the most effective solution, because a large fraction of MSW in those countries are organic wastes which include food wastes causing groundwater contamination in the form of leachates [1]. Food wastes instead of paper and plastic constitute the largest fraction of MSW (-50% in dry weight) in China, compared with 20 to 30% in the United States and European countries [2]. MSW disposal in China is also predominantly by means of landfill because it is cost-effective and it can accommodate large fluctuations in the amount and type of waste. However, landfill process emit landfill leachate which has a high COD and heavy metal content, as well as being associated with unpleasant odors, and air pollution [3,4].

As a promising alternative to landfill of organic fraction of MSW (OFMSW), anaerobic digestion treats organic wastes and simultaneously produces energy. In anaerobic reactors, microorganisms convert OFMSW to biogas, which can contain up to 70% methane. In recent years, a number of anaerobic digestion systems, including two-stage and

single-stage anaerobic bioreactors have been developed for MSW treatment [5-7]. Single-stage systems have relatively simple designs and are easy to build and operate, and thus, they have been applied in nearly 90% of Europe's full-scale anaerobic digestion plants for organic waste treatment [8]. Numerous environmental factors reduce the performance of anaerobic digesters such as low pH, ammonia accumulation, and the accumulation of volatile fatty acids (VFAs), which stress and inhibit the activity of methanogenic bacteria [9,10]. The processes are usually inhibited if food wastes are the only substrates for anaerobic digestion because of the accumulation of ammonia, which is generated in food wastes with high nitrogen content [11,12]. At a low organic loading rate of 2.0 g volatile solid/L day, the performance of the digester was not stable when only food wastes were used as substrates [13]. Therefore, the co-digestion of different types of organic wastes was proposed as a possible disposal route, which can significantly improve waste treatment efficiency [12,14,15]. For instance, the single-waste digestion of OFMSW produced 37 m^3 methane/ton of dry waste, however, the co-digestion of OFMSW and cow manure resulted in 172 m^3 methane/ton of dry waste, in particularly, the co-digestion of solid waste utilizes the nutrients and bacterial diversities in various wastes, adjusts the carbon/nitrogen (C/N) ratio, and improves the pH buffering capacity to optimize digestion [16].

A number of agricultural or green wastes were investigated in previous studies to co-digest with food waste [10,17-19]. The present study aimed to understand the performance of co-digesting food waste with Chinese silver grass (CSG), with the aim of increasing biogas production and examining the relationships among the various parameters during digestion. CSG, a C4 energy crop which is characterized by rapid growth and efficient light energy utilization, has received significant attention as a renewable energy plant [20]. The ultimate goal is to efficiently convert CSG into bioethanol through a fermentation pathway, but this challenge is met with a number of technological barriers. No reports have addressed the co-digestion of food waste and CSG within anaerobic processes, which is a promising alternative to incineration or fermentation for the maximization of CSG value.

In this study, the variation of VFA concentrations, pH, ammonia, and biogas production and composition was determined. The structure and diversity of the bacterial community of the biogas slurry were investigated by 16S rDNA clone library analysis. This study can improve our understanding of the performance of the single-stage anaerobic reactor for the treatment of food waste when CSG waste is used as an addition. The study also provides information about the microbial community during anaerobic treatment of MSW when co-digested with CSG.

2. Materials and Methods

2.1. Characterization of food waste and garden waste

Food wastes were obtained from a local canteen. Both the leaf and the stem of the plant (CSG) were collected from a garden at Xiamen City, Fujian, China. The inoculated sludge was collected from a local wastewater treatment plant at Fujian, China. The total solid (TS) and the volatile solid (VS) contents, were 22.5 and 80% for food waste, 53.5 and 95.1% for CSG, and 23.0 and 48.5% for sludge, respectively. In addition, the basis characterizations of food wastes, CSG and sludge, such as COD, pH, carbon (%) and nitrogen (%), are also shown in Table 1. The CSG contained 46.55% C, 0.57% N and 0.19% S, respectively. The C content was close to that of food waste, but the N content was lower than that of food waste.

2.2. Co-digestion system

Fig. 1 shows the horizontal mid-scale anaerobic reactor system, which consists of a main reactor body, a temperature control unit, a gas–liquid separator, a gas purification unit, a wet biogas flow meter, and a biogas analyzer. The main body of the digester is made of opaque rigid polypropylene, with an inner diameter of 800 mm, a length of 1,000 mm,

Table 1. Basic characterization of different types of raw materials

Items	Types of raw materials		
	Food waste	China silver grass	Activated sludge
Total solid (wt, %)	22.5 ± 0.7	53.5 ± 2.7	23.0 ± 0.1
Volatile solid (wt,%, based on TS)	80.0 ± 7.6	95.1 ± 4.8	48.5 ± 0.2
pH	5.40	5.57	6.90
Carbon, C (%)	49.14 ± 1.26	46.55 ± 2.3	33.27 ± 0.09
Nitrogen, N (%)	2.83 ± 0.05	0.57 ± 0.03	3.82 ± 0.01
Sulfur, S (%)	0.30 ± 0.06	0.19 ± 0.01	3.30 ± 0.12
C/N ratio	17.36	81.67	8.71
$COD_{(TS=10\%)}$ (mg/L)	21,677	1,478	5,307

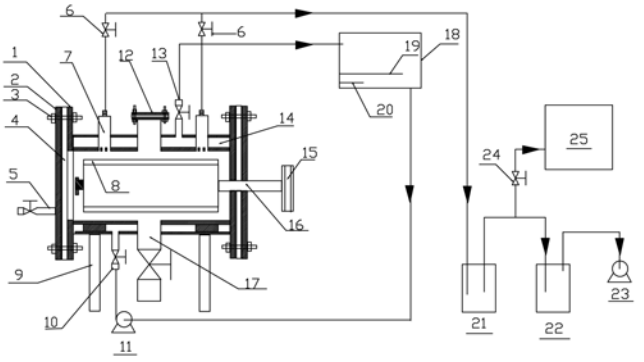

Fig. 1. Schematic of the anaerobic co-digester. (1) Inner flange; (2) Outer flange; (3) Bolt; (4) Sealing rubber pad; (5) Effluent export; (6) Valve; (7) Biogas export; (8) Stirrer; (9) Handcart; (10) Inlet of recycled water; (11) Pump; (12) Feed inlet; (13) Export of recycled water; (14) Jacket; (15) Motor; (16) Transmissiom bearing; (17) Discharge port; (18) Feed tank; (19) Heater; (20) Thermometer; (21) Gas liquid separator; (22) Desulphurization device; (23) Wet biogas flow meter; (24) Valve; and (25) Biogas analyzer.

and a total volume of 500 L. The digester is equipped with a stirrer to mix the materials, a warm water-jacket to maintain temperature stability, a substrate discharge port, a feed inlet, a sample port, and two biogas exports. The temperature control unit consists of an electric heater, temperature sensor, and a pump to circulate the water between the water feed tank and the jacket. Two filters are located in the biogas exports, and prevent clogging of biogas exports when the solid materials expand during co-digestion. The biogas and condensing water vapor are separated using a gas–liquid separator, and biogas flows to the purification unit for hydrogen sulfide removal. A wet biogas flow meter and a biogas collector are connected in turn within the purification unit. The co-digester is operated under mesophilic conditions (36 ± 1°C) by controlling the temperature of the feed tank.

2.3. Experiment design

In this study, the mixture of 50% food waste and 50% CSG (1:1), based on the dry weight basis added to the digester, was selected according to previously reported optimal ratios [18,19]. The mixture of wastes was added to the anaerobic digester (working volume of digester, 300 L; TS, 10%), and the total final solid weight was 30 kg. The sludge from the waste treatment plant was added as an inoculum, and the amount was calculated at 5% of the total weight based on a wet weight basis. Compared with dry anaerobic digestion (<80% moisture), it has generally been assumed that a higher biogas production can occur from wet (>90% moisture) to semi-dry conditions (80 ~ 90% moisture) during the anaerobic process [21,22]. Thus, the digester had a further 300 kg of tap water added to it to reach a 90% water content. Accordingly, the average organic loading rate was 2.1 g VS/L/day with a batch mode experiment. The pH vlaue was adjusted to about 7.0 and 0.8% calcium carbonate of the total weight was added as a pH buffer. The adjusted C/N ratio of the system was 24.0 by addition of CSG, and the concentration of mixture of CSG plus MSW and sludge on VS/TS/volume was 93.1 g /111.5 g /L. The concentrations of P, K, Na, Fe, Ca, and Zn were 17.91 g/L, 6.32 g/L, 4.85 g/L, 28.3 mg/L, 22.4 mg/L and 0.11 mg/L, respectively. The digester was started after the feed inlet was closed and the valve on the exhaust pipe was opened.

2.4. Analytical method

During the anaerobic co-digestion, the biogas yield, composition of biogas, chemical oxygen demand (COD), ammonia-N, VFAs, and pH were measured daily. The biogas yield was determined using a wet biogas flow meter, and the composition of biogas was analyzed using an *in situ* infrared methane gas analyzer (GASBOARD-3200L, Wuhan Sifang Instrument Factory, China). The VFA concentrations were determined by ion chromatography (IC) equipped with an AS11-HC column (DIONEX ICS 900, CA, USA), as described by Kleyböcker *et al.* [23]. 5 mMol Sodium hydroxide was used as the eluent. The flow rate and voltage were 1.3 mL/min and 20 mV, respectively. Analysis of micronutrients was performed using inductively coupled plasma-optical emission spectrometry (ICP-OES, Optima 7000DV, PerkinElmer, USA). Potassium and sodium were analyzed by flame atomic absorption spectrometry (FP640, China). The TS, VS, COD, total phosphorus (TP), and ammonium-N of the digested samples were determined according to the Standard Methods [24]. TS values were obtained by drying the samples at 105°C for 24 h in an oven, and the VS was then determined after calcination of the dry sample at 600°C for 2 h in a muffle furnace. The pH was measured using a pH meter (PHB-4).

2.5. Hydrolysis rate of solid waste and release of free ammonia

The hydrolysis rate of solid organic waste were calculated according to modified Eq. (1) [25]:

$$H = \mathrm{COD}_{\mathrm{CH_4}} + \mathrm{COD}_{i-1} - \mathrm{COD}_{\mathrm{i}}, \quad (1)$$

where H is the hydrolysis rate (mg/L COD/per day), $\mathrm{COD_{CH4}}$ is the COD for methane production (mg/L/per day), and $\mathrm{COD_{i\text{-}1}}$ and $\mathrm{COD_i}$ are the concentrations on days i-1 and i (mg/L), respectively.

The equilibrium of ammonia in the aqueous solution depends on pH and temperature. Free ammonia concentration is expressed by Eq. (2) below [26]:

$$[NH_3\text{–}N] = \frac{[NH_4^+\text{–}N]}{1+10^{pKa-pH}}, \quad (2)$$

where [NH_3-N] is the free ammonia concentration mg/L and [NH_4^+-N] is the ammonium concentration, mg/L. Meanwhile, the pKa is a decreasing function of the absolute temperature T (Kelvin temperature) in the range of 273 ~ 373 K. The value can be expressed as a function of temperature T by Eq. (3) below [27]:

$$pKa = 0.1075 + 2725/T \quad (3)$$

The combination of Eqs. (2) and (3) determines the free ammonia concentration.

2.6. Microbiological analysis

Microbial DNA samples were extracted according to the instructions provided in the MOBIO UltraClean® soil DNA isolation kit (catalog No. 12800-50) with 0.5 g pellets collected after centrifugation of the sampled digestion slurry at 11,000 rpm for 7 min. The extracted DNA was analyzed by gel electrophoresis and was then stored at -20°C. The extracted DNA was directly applied to a polymerase chain reaction (PCR). The forward primer in PCR amplification (27F, 5'-AGAGTTTGATCCTGGCTCAG-3') and the reverse primer (519r, 5'-CGTATTACCGCGGCTGCTGG-3') were used to amplify the variable V3 region of 16S rDNA. PCR amplification was performed using a PCR Thermal Cycler (Model: JC-96, China) at a final volume of 25 μL containing 12.5 μL of MasterMix (Tiangen Biotech Co., Ltd., China), 0.5 μL of each primer, 1 μL of DNA, and 10.5 μL ddH_2O. The thermal cycle was performed using an initial denaturizing step of 5 min at 94°C, followed by 10 cycles of 30 sec at 94°C, 45 sec at 65°C, and 72°C for 1 min. Subsequently, 20 cycles at 94°C for 30 sec, 55°C for 45 sec, and 72°C for 1 min were conducted, followed by a final step at 72°C for 7 min. A total of 80 clones and sequences were performed at the DNA Sequencing Facility, Shanghai Majorbio Bio-Pharm Technology Co., Ltd. The sequences were classified using the Ribosomal Database Project classifier software at an 80% confidence threshold [28]. In succession, the diversity index was estimated according to the Shannon–Wiener Index, and Chao1 was calculated using Fast group II [29].

3. Results and Discussion

3.1. Biogas yield and composition

Fig. 2 presents the daily temporal evolution of biogas and methane. After feeding the food waste and CSG into the anaerobic bioreactor, the biogas production rapidly peaked on day 2. However, the methane composition in the biogas was low, at less than 1%. The specific composition is due to the rapidly decomposing organic matter provided by the food waste and carbon dioxide production in the presence of oxygen. Thus, from days 3 to 10, the hydrolysis and fermentation of food waste were dominant, and biogas production was decreased from approximately 160 L/day to less than 20 L/day. After 11 days of co-digestion, biogas production gradually increased and reached the maximum value of 675 L/day on day 33. Fig. 3 shows the composition of the biogas, which is mainly composed of methane and carbon dioxide. For methane, the concentration gradually increased to the maximum value of 75.2% on day 19, and

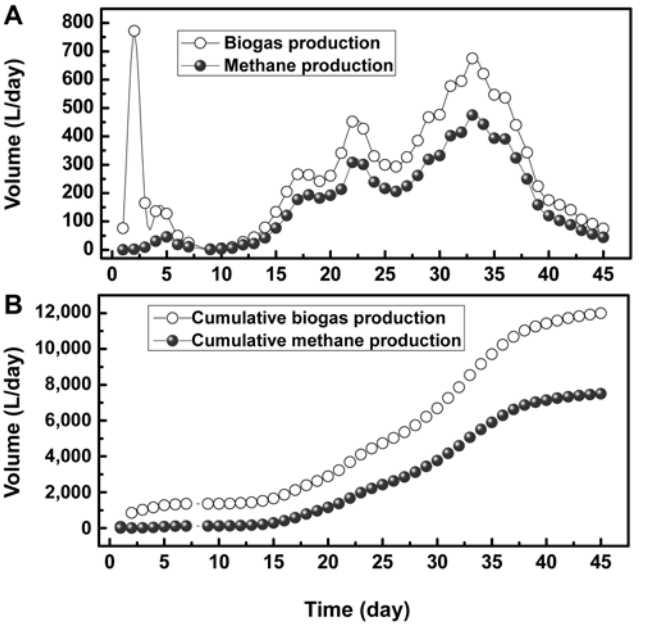

Fig. 2. Evolution of biogas yield per day and the accumulation of biogas during anaerobic co-digestion.

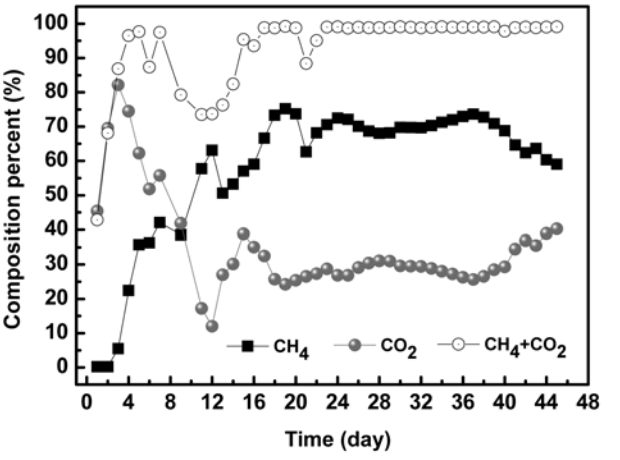

Fig. 3. Biogas composition during anaerobic co-digestion.

this concentration stabilized at about 70%. The concentration of CO_2 fluctuated by approximately 30% during the most stable stage. The changes in biogas composition indicated that the predominant fermentation step changed from hydrolysis/acidogenesis to methanogenesis.

Methane production exhibited a similar trend as that observed for biogas production (Fig. 2A). The methane production achieved a maximum of 475 L/day on day 33 and gradually decreased to 44.3 L/day on day 45. The accumulated biogas and methane production slowly increased in an almost linear manner from days 13 to 40 (Fig. 2B). Based on the working volume of the digester, the biogas production rates from day 13 to 40 varied between 0.15 and 2.25 L/L/day. Most biogas production rates were larger than those previously reported such as 1.48 L/L/day for the co-digestion of food waste and dairy manure at a ratio of 1:1 [17]. The total biogas and methane production were 429.3 and 268.4 L/kg VS added, respectively. In our previous study, Tang *et al.* [30] reported biogas production was 10 L/kg VS for food wastes alone under the same conditions over 63 days operation, and the total biogas production achieved 462 L/kg VS with the addition of herbal garden waste for anaerobic co-digestion of food wastes. Therefore, CSG addition in this study improved the performance and stability of food waste co-digestion in the single-stage anaerobic digester. In addition, Lin *et al*. [31] also reported that the anaerobic digestion process for food waste alone was inefficient due to the accumulation of acids accumulation at lower C/N ratios than those suggested in literature (20 ~ 30) for the stable operation of the anaerobic digester [32,33]. In the present study, the C/N ratio of the system was adjusted to 24.0 by the addition of CSG, and this C/N ratio ensured a good performance of the anaerobic digester.

3.2. Soluble COD variation during co-digestion

Fig. 4A shows the abrupt increase in soluble COD of the solution from 11,820 to 19,430 mg/L at day 4 of co-digestion. The easily decayed solid organic matter was rapidly decomposed by the microorganisms. After 13 days of digestion, the soluble COD reached the highest value at 21,480 mg/L. In succession, the soluble COD decreased to 10,150 mg/L on day 18, and then abruptly increased to 19,510 mg/L on day 19. As digestion time further increased from days 19 to 45, the COD continued to decrease until the value dropped as low as 2,555 mg/L on day 45. The final removal efficiency was 88.1%. The reason for these behaviors is that the particulate material is first converted into soluble organic compounds such as amino acids, long-chain fatty acids, and sugars, which increase the soluble COD concentration in the solution [33]. These soluble organic matters are then gradually converted into methane and carbon dioxide.

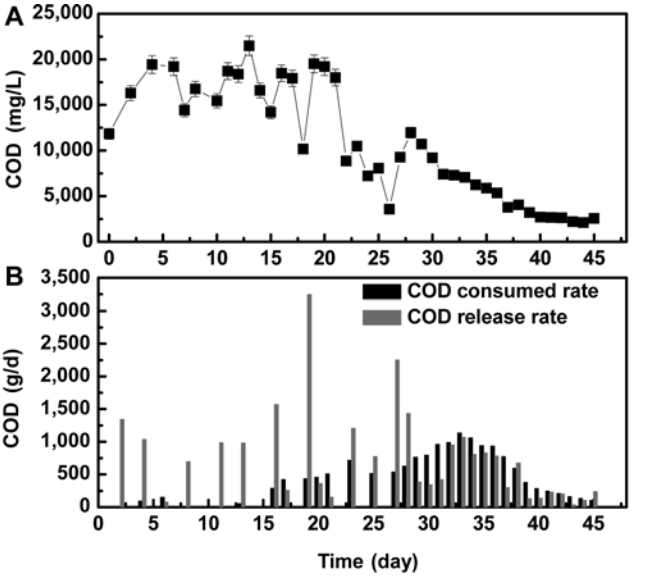

Fig. 4. Variation of soluble COD concentration of the biogas slurry (A) and release and utilization rates of COD during anaerobic co-digestion (B).

Fig. 4B shows the hydrolysis and utilization rates of COD. The hydrolysis/fermentation rate of solid organic matter is larger than the methanation rate from days 0 to 30, and the COD release rate achieved a maximum of 3243.4 g/day on day 19. As digestion time further increased, more COD was transferred to methane by methanogenic microorganisms, and the hydrolysis rate was nearly equal to the methanation rate. This observation demonstrated that balance is achieved between soluble organic matter and the utilization of microorganisms to produce biogas. Previous studies reported that hydrolysis is the first step and the rate limiting step in the anaerobic digestion of insoluble substrates [34]. This is particularly applicable to wastes containing a certain amount of relatively hard degradable components, such as cellulose and lignin, and thus, there is a need for a longer hydrolysis time in the first phase. However, the present study has no limitation for the hydrolysis of solid organic matter. At the end of the digestion, the hydrolysis/fermentation rate of solid organic matter was lower than the utilization rate of microorganisms to produce biogas.

3.3. VFA and pH variation during digestion

Fig. 5 shows the concentrations of major volatile fatty acids produced at various digestion times during the hydrolysis of food waste and CSG. The main compounds produced were acetic, propionic, and butyric acids, and the average sum of the three acids constituted 95% of the total acid concentration. Formic, pyruvic, and lactic acids were also detected at very low concentrations, constituting 5% of the

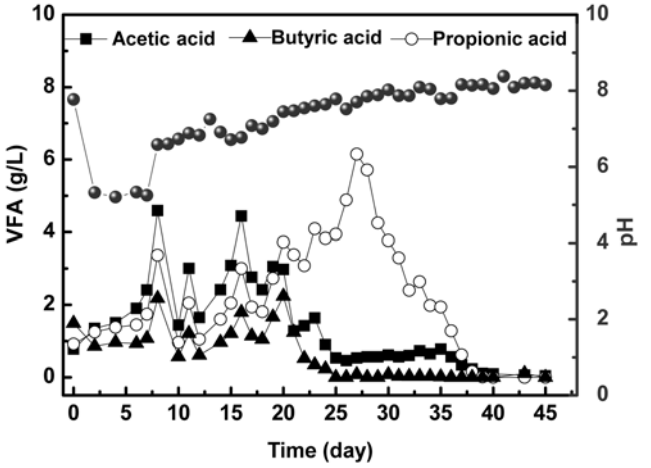

Fig. 5. VFA and pH variations during anaerobic co-digestion.

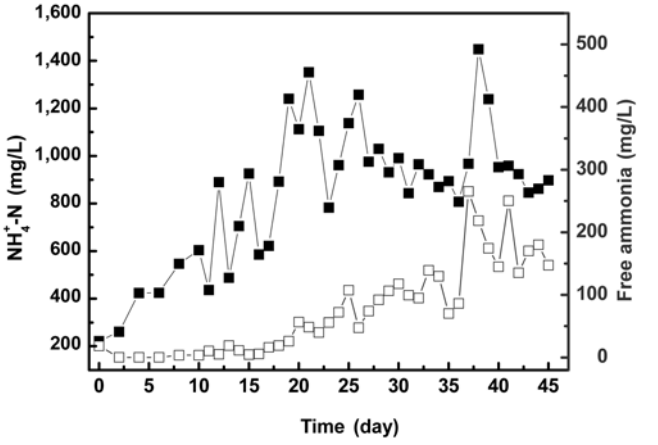

Fig. 6. Variations in ammonia-N and free ammonia concentration during anaerobic co-digestion of food waste and CSG.

total detected VFA. Within 20 days of the beginning of co-digestion, the concentration of the accumulated acetic acid was at its highest, followed by propionic and butyric acids. The maximum accumulated concentrations were 4.59, 6.15, and 2.24 g/L for acetic, propionic, and butyric acids on days 8, 27, and 20, respectively. As co-digestion time further increased, the concentration of VFA was gradually reduced, and the concentrations of some types of VFA dropped off to undetectable levels. In previous studies, the accumulation of VFA lead to the inhibition of anaerobic digestion, however, no such effect was observed in this study. This could be explained by the addition of CSA serving as a buffer to mitigate the toxicity of VFA [35,36].

Fig. 5 also shows the pH variation. The pH dropped rapidly at the beginning of the anaerobic digester startup. When the solution pH was lower than 5.25 on day 7, the pH was increased to more than 6.5 with sodium hydroxide solution to avoid the inhibition of methanogenesis. The reason for the adjustment is that the growth rate of fermentative bacteria is faster than that of acetogenic and methanogenic bacteria and thus, VFA production is faster than VFA conversion to methane. The methanogenic activity is more likely to proceed optimally within a narrow pH range, between 6.3 and 7.8, under anaerobic conditions [37]. After only one adjustment, the pH began to rise gradually and fluctuated between 6.6 and 8.4. The slight pH fluctuation is due to the periodic accumulation of VFA in the digester and the subsequent transfer and consumption of VFA by methanogenesis.

3.4. Ammonia-N variation during co-digestion

Fig. 6 presents the variation in the concentrations of NH_4^+-N and free ammonia. For NH_4^+-N, the concentration significantly increased to 1,351 mg/L on day 20, and the maximum concentration of 1448.2 mg/L was achieved on day 37. Ammonium nitrogen is a very important agent in all bioprocesses, particularly in anaerobic digestion. A high concentration of ammonium is toxic and inhibits biogas production during anaerobic co-digestion of waste [12,38]. Koster and Lettinga [39] reported that acidogenic populations in the granular sludge were hardly affected, whereas the methanogenic population lost 56.5% of its activity as ammonia concentrations increased from 4,051 to 5,734 mg/L. Liu *et al.* [40] reported that the ammonia nitrogen inhibition concentration ranges from 1,400 to 1,700 mg/L for the anaerobic digestion of kitchen wastes, especially for the ammonia nitrogen concentration rising to 2,000 mg/L. The anaerobic methanogenesis efficiency decreased sharply along with the VFA accumulation. However, in the present study, the NH_4^+-N concentration was lower than the inhibited concentration reported in previous studies, and did not inhibit methanogens during anaerobic digestion.

Free ammonia concentration consistently increased with the increase of pH, and the maximum concentration of 265.2 mg/L was obtained on day 37 (Fig. 6). The right amount of ammonia increased the buffer capacity of the methanogenic medium in the mesophilic anaerobic reactor. However, the high concentration of free ammonia directly inhibited the activities of methane-synthesizing enzymes. This behavior is due to the passive diffusion of hydrophobic free ammonia molecules into the cell, and the rapid conversion into ammonium, which exerts toxicity to the cell by intracellular pH alteration [41]. The free ammonia concentration in the entire process of anaerobic digestion was extremely lower than the inhibition concentration of 1,100 mg-N/L for the anaerobic digestion, as described in a previous study reported by Hansen *et al.* [38]. In addition, the amendment of CSG might mitigate the toxicity of free ammonia because the composition of CSG was enriched with adsorptive cellulose like materials. Thus, the accumulation

of free ammonia in the present study exhibited no negative effect on the performance of co-digestion. On the contrary, a sufficient buffer capacity of the anaerobic digester was ensured.

3.5. Bacterial community analysis

Food waste and CSG contain a lot of carbohydrates, *etc*., and the hydrolysis of these substances to VFAs is considered as the first step as well as the rate limiting step in the anaerobic digestion of insoluble substrates by bacteria. Therefore, the bacterial community was analyzed [34]. However, the data of the archaeal community was not obtained in the present study due to technical reasons, in which we suspect unknown compounds in the DNA samples of interfering with the PCR reaction using primers targeting the archaea community. Fig. 7A shows the diversity of the bacterial community present in the digester at different digestion times. The bacterial community changes in the biogas slurry samples significantly at different anaerobic digestion times during the co-digestion of food waste and CSG. Generally, the bacterial community diversity increased initially and then decreased with further increases in digestion time. For instance, the Shannon–Weaver index was calculated as 3.67 on day 0. However, compared with the initial bacterial diversity, the Shannon–Weaver index was 4.17 and 4.03 for bacterial diversity after days 21 and 41 of digestion, respectively. The bacterial diversity first increased and was then slightly decreased as digestion time increased. The increase of microbial diversity could relate to the addition of CSG, which diversified the substrate for varieties microbes and adjusted the C/N ratio to a level preferred by diversified microbial community [32,33,42].

Fig. 7B reveals the analysis of the composition and main microorganisms. On day 0, the major microbial groups were the *Firmicutes* (50/80, 50%), *Bacteroidetes* (23/80, 28.75%), and *Proteobacteria* (14/80, 17.5%), respectively. Members of *Spirochaetes* (1/80, 1.25%) and unclassified bacteria (2/80, 2.5%) were also detected. Compared with the sample at day 0, the microbial group increased significantly, and a small number of clones (fewer than 3.75% of the library) were also determined to be affiliated with *Synergistetes* (3/80, 3.75%), *Tenericutes* (2/80, 2.5%), *Spirochaetes* (1/80, 1.25%), and *Actinobacteria* (1/80, 1.25%) in addition to *Firmicutes* (33/80, 41.25%), *Bacteroidetes* (32/80, 40%), and unclassified bacteria (7/80, 8.75%) on day 21. The variation of the microbial structure was not obvious as operation time further increasing from day 21 to day 41.

However, the bacterial community responded to the production of VFAs and ammonia during digestion. For instance, the percentage of *Bacteroidetes* increased from 28.75 to 40.0%, which was accompanied by an increase in the total concentration of acetic, propionic and butyric acids,

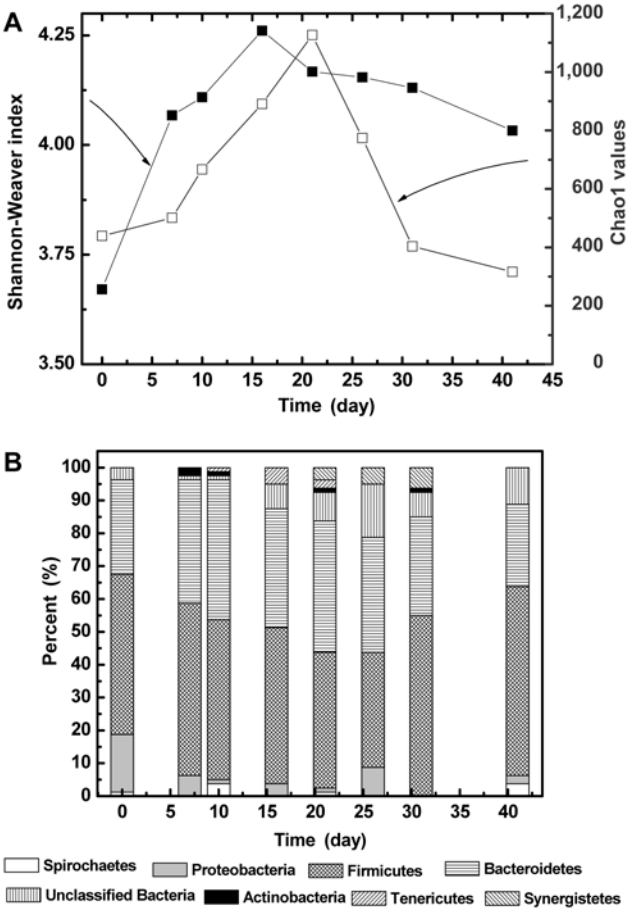

Fig. 7. Bacterial community changes at different digestion times during food waste and CSG co-digestion. (A) Diversity variation of microorganisms and (B) microbial composition.

from 3.2 to 5.9 g/L. While the percentage of unclassified bacteria increased to the peak 16.25% on day 26, the concentration of propionic acid peaked at 7.3 g/L on day 27, which might be explained by different acidogenic bacterial species producing different VFAs, and acetogenic bacterial species differing in their capacity to utilize them [43].

Proteobacteria, *Firmicutes*, and *Bacteroidetes* were the most abundant microorganisms during the entire process of digestion. Cardinali-Rezende *et al.* [44] observed that the presence of *Proteobacteria, Firmicutes* could improve biogas production during the digestion of fruit and meal scraps compared with anaerobic processes in the absence of these bacteria. Other studies have also found that members of *Firmicutes* and *Bacteroidetes* are abundant in different anaerobic reactors when applied to various waste treatments [45,47]. *Firmicutes* and *Bacteroidetes* are known to be resistant microorganisms capable of degrading complex organic compounds, such as vegetable and fruit residues [48]. These characteristics and behaviors of the members

of the abundant groups might have led to the successful anaerobic digestion in the present study. Thus, all results showed that the transition of the mesophilic bacterial community is consistent with the performance of the bioreactor during the co-digestion of food waste and CSG.

4. Conclusion

A single-stage system was developed for the co-digestion of food waste and CSG. The total biogas and methane production were 429.3 and 268.4 L/kg VS added, respectively. The methane content was above 60% of the total biogas produced at the stable stage. The addition of CSG adjusted the C/N ratio of the system, which prevented the accumulation of ammonium or free ammonia and their negative effects on the performance of the anaerobic bioreactor. The microorganisms that belong to *Firmicutes* and *Bacteroidetes* were dominant during the whole digestion period based, on 16s rDNA clone library analysis. In particular, the diversity of the microbial community diversity increased significantly during the active methanogenesis period. This behavior suggests that additional substrates could benefit methanogenesis by increasing the microbial community diversity. The CSG addition efficiently improved the food digestion performance of the anaerobic bioreactor for food waste digestion.

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