Research article

Insights into the microbial community structure of anaerobic digestion of municipal solid waste landfill leachate for methane production by adaptive thermophilic granular sludge

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ABSTRACT

Background: The amount of municipal solid waste (MSW) gradually increased along with the rapid development of modern cities. A large amount of landfill leachate are generated with excessive chemical oxygen demand (COD), which create a great deal of pressure on the environment-friendly treatment process. Anaerobic digestion is an ideal technique to solve the above problem.
Results: A thermophilic granular sludge was successfully adapted for anaerobic digestion of MSW leachate (from an aging large-scale landfill) for methane production. The COD degradation efficiency improved by 81.8%, while the methane production rate reached 117.3 mL CH_4/(g VS d), which was 2.34-fold more than the control condition. The bacterial and archaeal communities involved in the process were revealed by 16S rRNA gene high-throughput pyrosequencing. The richness of the bacterial community decreased in the process of thermophilic granular sludge, while the archaeal community structure presented a reverse phenomenon. The bacterial genus, *Methanosaeta* was the most abundant during the mesophilic process, while *Methanobacterium*, *Methanoculleus*, *Methanosaeta* and *Methanosarcina* were more evenly distributed. The more balanced community distribution between hydrogenotrophic and acetotrophic methanogens implied a closer interaction between the microbes, which further contributed to higher methane productivity. The detailed relationship between the key functional communities and anaerobic digestion performances were demonstrated via the multivariate canonical correspondence analysis.
Conclusions: With the assistance of adaptive thermophilic granular sludge, microbial community structure was more evenly distributed, while both of COD degradation rate and methane production was improved during anaerobic digestion of MSW landfill leachate.

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1. Introduction

The amount of municipal solid wastes (MSWs) generated by a country gradually increases regardless of its level of development [1]. Landfill leachate is formed by the percolation of excessive rainwater and moisture from MSW. It is estimated that the generated amount of leachate from deposited landfills could reach 15–50% of its total volume [2,3]. Landfill leachate is the concentrated polluted essence of garbage, and is relatively highly toxic among all waste waters, which also has a strong representative trait. Especially in the early stages of landfills, many organic substances and intermediate metabolites are released, thus forming the complicated and high-concentration chemical oxygen demand (COD) leachate [2,4,5]. The excessive COD in leachate creates a great deal of pressure on the environment-friendly treatment process [6,7,8]. Therefore, effective and safe treatment of MSW leachate has

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attracted increasing attention. With the advantages of high-efficiency and lower investment and secondary pollution, anaerobic digestion was recognized as a feasible method for solving this problem [9,10,11,12,13].

Anaerobic granular sludge containing diverse microbial communities is the key factor during anaerobic digestion [14,15,16]. Various microorganisms are distributed in a certain way and are mutually beneficial to form an efficient and stable community structure [17,18]. It has been widely recognized that a microecosystem is vital to maintain stable and efficient anaerobic digestion [19,20,21], and ever-changing chemical parameters during anaerobic digestion could influence microbial metabolism. The different environment-adapting abilities of various microorganisms finally contribute to the unique community structure and function [12,22,23]. Occasionally, there are imbalances between different microbial communities such as the problem of faster hydrolysis/acidogenesis and slower methanogenesis, resulting in lower anaerobic digestion efficiency [24,25,26]. Therefore, there is an urgent need to investigate the composition and function of microbial communities during anaerobic digestion of sludge for further optimization of methane production from organic MSW leachate.

Hence, the aim of this study is to analyze the function of the microbial community structure during anaerobic digestion of MSW landfill leachate for methane production by using adaptive thermophilic granular sludge. In the medium and large cities, municipal solid wastes have basic components such as kitchen waste, paper, wood, textile, rubber, metal, glass, lime and masonry, etc. As one of the first-tier cities in China, Wuxi city's living leachate has a strong representative value as a research object. First, the different microbial morphologies of adaptive thermophilic granular sludge were studied using a scanning electron microscope (SEM). Second, the anaerobic digestion of MSW leachate for methane production was investigated, and factors such as kinetics of COD removal and methane accumulation, were also studied. Third, the bacterial and archaeal community of different anaerobic digestion processes was compared by pyrosequencing analysis. Lastly, the significant differences of microbial community interactions between different anaerobic digestion performances were further examined by a multivariate canonical correspondence analysis (CCA). Thus, these results would provide a theoretical basis to better understanding the relationship between microbial community structure and anaerobic digestion performance of MSW landfill leachate for methane production.

2. Materials and methods

2.1. MSW leachate and anaerobic sludge

Fresh leachate, used as the substrate in this experiment, was obtained from the regulating reservoir (30,000 m^3) of a municipal landfill in Wuxi, China (**Fig. S1**). The anaerobic granular sludge was collected from a municipal sewage plant in Wuxi, China. Granular sludge is black in color, spherical or ellipsoidal, and the particle size is 0.5–5.0 mm. The range of sedimentation rate is 30.0–100.0 m/h. The detailed characteristics of landfill leachate are shown in Table 1. The carbohydrate source of leachate (as COD form, 5625.0 ± 25.6 mg/L) was mainly from the lysis process of organic matter, while VFA was 1858.3 ± 15.8 mg/L. The total nitrogen was 1043.0 ± 12.1 mg/L, while NH + 4-N type nitrogen reached 845.2 ± 13.2 mg/L. In addition, some other ions such as Cl^{-1} were determined as 3120.0 ± 22.4 mg/L. The pH value of leachate was adjusted to 7.0 by 1 mol/L NaOH or 1 mol/L HCl solution before anaerobic fermentation. The ratio of C:N:P of the fermentation medium was kept at 266.6:49.4:1 to meet the microbial growth needs during anaerobic fermentation.

2.2. Adaptive process of thermophilic granular sludge

The landfill leachate was used as the fermented medium. Initially, the temperature-adaptive process was carried out, and then the adaptive ability with increased organic load was determined. The

Table 1
The main characteristic of landfill leachate and adaptive anaerobic granular sludge for anaerobic digestion experiments.

Types	Parameters	Units (mg·L^{-1})
Landfill leachate	COD	5625.0 ± 25.6
	Total nitrogen (TN)	1043.0 ± 12.1
	NH + 4-N	845.2 ± 13.2
	TP	21.1 ± 1.5
	VFAs	1858.3 ± 15.8
	Cl^{-1}	3120.0 ± 22.4
	pH	7.8 ± 0.1
Adaptive anaerobic granular sludge	Moisture content (%)	
	NS	84.10 ± 2.4
	MS	86.60 ± 4.3
	HS	85.10 ± 3.6
	VSS/SS (%)	
	NS	0.68 ± 0.02
	MS	0.72 ± 0.02
	HS	0.69 ± 0.03
	Ash content (%)	
	NS	5.00 ± 0.11
	MS	3.80 ± 0.15
	HS	4.80 ± 0.14

temperature-adaptive process was performed by increasing one degree every three days. The adaptive progress in the range of 35–41°C was smooth, whereas the activity of granular sludge began to greatly decrease from about 42°C. The adaptive process was maintained until the sludge activity was stable. Later, the organic load was gradually increased to strengthen the resistance of the leachate to high COD levels. The initial COD concentration was 2000.0 mg/L from days 1–4. Between days 5 and 8, the COD concentration was increased to 4000.0 mg/L. Subsequently, the COD concentration was increased further from days 9–12. The leachate with COD concentration of 8000.0 mg/L was used until the landfill leachate liquid could be steadily treated for two months. An adaptive thermophilic sludge (50°C-HS) was eventually developed.

2.3. Anaerobic digestion experiment of the MSW leachate

The schematic diagram of anaerobic digestion of the leachate is presented in **Fig. S1**. The detailed traits of the adaptive anaerobic granule sludge are listed in Table 1. The working volumes of anaerobic digester were 750 mL. The temperature of the substrate tank was controlled by a thermostatic heater. Each tank consisted of the anaerobic digester, a magnetic stirrer inside the digester, a liquid sampling port, and a biogas sampling port. Besides, an aspirator tube was set for creating an anaerobic environment to speed up the anaerobic digestion process and increase methane content. The inoculation process was performed as per the inoculated sludge quantity of 6 VSS/L. Wet anaerobic granular sludge (41.3, 46.9, and 44.6 g) was inoculated in the control (25°C; NS), mesophilic (35°C; MS) and thermophilic (55°C; HS) of leachates, respectively. During the entire digestion periods, 2 mL of leachate and 100 μL of biogas samples were collected for monitoring the concentrations of COD, VFAs, pH, cumulative biogas production and methane percentage.

2.4. Analysis of key biochemical parameters during anaerobic digestion

The pH value was measured with a pH meter (pHS-3TC, Sartorius, Germany). The concentration of COD, SS (suspended solids), VSS (volatile suspended solids), bicarbonate alkalinity, and NH$_4^+$-N was detected using a previously reported method [27]. The volatile fatty acids (VFAs) were estimated using the fast digestion-spectrophotometric assay. The cumulative volume of biogas produced from the reactor was quantified using a gas chromatograph equipped with an Innowax capillary column and a flame ionization detector. The methane percentage was quantified by the alkali absorption method with a

gas-tight syringe (1700 Series, Hamilton, Switzerland). The microbial morphology of the anaerobic granular sludge was observed by a scanning electron microscope (SEM, JSM-7401F, JEOL, Japan).

2.5. DNA extraction of granular sludge and polymerase chain reaction (PCR)

Three granular sludge samples from different anaerobic digestion processes were homogenized by vortexing, and washed thrice with dehydration buffer, PBS, and Na_2EDTA buffer. Then genomic DNA was extracted using an E.Z.N.A. Soil DNA Kit (OMEGA, USA). The DNA sample was stored at $-20°C$ until subsequent polymerase chain reaction (PCR) analysis. Hypervariable regions (bacterial V3–V4 and archaeal V3–V5) of 16S rRNA genes were amplified by PCR using universal primers. PCR conditions of bacterial sequences were as follows: Bac338F 5′-ACTCCTACGGGAGGCAGCA-3′; Bac806R 5′-GGAC TACHVGGGTWTCTAAT-3′; Arch344F 5′-ACGGGGTGCAGCAGGCGCGA-3′; Arch915R 5′-GTGCTCCCCCGCCAATTCCT-3′. The PCR condition of bacterial/archaeal sequences was listed as follows. Initial denaturation at 98°C for 3 min; annealing for 25 cycles at 98°C for 15 s, 55°C for 30 s, 72°C for 30 s; and a final extension at 72°C for 5 min (bacterial sequences PCR). PCR conditions for archaeal sequences were as follows: Initial denaturation at 98°C for 4 min; annealing for 27 cycles at 98°C for 30 s, 50°C for 45 s, 72°C for 1 min; and a final extension at 72°C for 7 min. The PCR products were purified according to the instructions of the QIAquick Gel Extraction Kit (BIOMIGA) and sequenced by the Roche 454 FLX+ pyrosequencing platform to generate 400-bp sequence reads.

2.6. Pyrosequencing and community analysis

The obtained sequences were sorted and qualified using the barcode sequence. The sequences within the scope of the threshold (>97%) were grouped together, while the chimeras were excluded [28]. The rarefaction curves tended to be flat after more than 110,000 sampling sequences, indicating that the sample sequencing volume was saturated. The coverage (Depth of Index) exceeded 99.0% at 97% similarity. The result indicated that the majority of three sludge bacterial communities satisfied the analytical requirements. The qualified 16S rRNA gene sequences from different samples were assigned into operational taxonomic units (OTUs) by using Usearch software (version 7.1 http://drive5.com/uparse/). Chao1, ACE and Shannon diversity indexes were used to evaluate the bacterial community richness and detailed community abundance. The multivariate canonical correspondence analysis (CCA), was carried out for assessing the relationship between abundance of bacterial/archaeal communities and anaerobic digestion performances based on taxonomic data using R software package with a vegan library.

3. Results and discussion

3.1. Microbial morphology differences of adaptive thermophilic granular sludge

The microbial morphology differences of adaptive thermophilic granular sludge (outer, middle and inner layers) were observed by SEM images (Fig. S2). The anaerobic granular sludge was filled with filiform, rod and spherical-shaped microorganisms with distinct stratification. Among them, the outer layer was abundant in different bacterial species, while the inner layer had less species abundance and mainly comprised spherical bacteria. It is suggested that anaerobic granular sludge is mainly composed of three types of microbial populations [15, 29]. The outer layer is mainly composed of acid-producing bacteria; hydrogen/acid-producing bacteria constituted the middle layer, while methanogens are located in the inner layer. In our study, various types of microbial populations both inside and outside of the granular sludge formed a micro-ecosystem that enhanced the collaboration for utilization of organic matter. Meanwhile, the microbial diversity also

gradually reduced along with the adaptive process. Compared with NS and MS, the distribution of bacteria in HS was more concentrated and the biodiversity was lesser. This might be because some microorganisms, sensitive to high temperature stress, were eliminated during the adaptive process, while only thermophilic microorganisms were retained. VSS/SS can reflect the microorganism content in the sludge; thus, the highest value of MS indicated that mesophilic environment is suitable to the microbial growth (Table 1). Additionally, the detailed microbial composition of the more diverse community in the outer layer and the homogeneous community in the inner layer can be revealed by the analysis of microbial diversity using pyrosequencing.

3.2. Anaerobic digestion of MSW leachate for methane production by adaptive thermophilic granular sludge

3.2.1. Effects of adaptive thermophilic granular sludge on pH and VFAs

The pH value fluctuated slightly and was maintained in the range of 6.4–7.4 during the whole digestion process (Fig. 1A). In the earl stage, the pH decreased to the range of 6.4–6.7 owing to the VFAs accumulation. However, the VFA accumulation could not cause a dramatic pH fluctuation due to the high alkalinity and ammonia content of MSW leachate (845.2 mg/L ammonia concentration). It is widely recognized that the hydrolytic acidogenic bacteria had a broad range of pH adaptation (5.0–8.5) during the anaerobic digestion [30]. In the later stage of methane production, the pH gradually increased to the range of 6.6–7.2 due to the consumption of VFAs by methanogens. Methanogens were more sensitive to pH fluctuation (6.5–7.5); therefore, the cell growth and metabolism of methanogens was inhibited and further caused the reactor [31]. In addition, the final pH of HS was the highest among all the treated sludge, which proved the higher methane production from another aspect.

The accumulation of VFAs during anaerobic digestion initially increased but then declined rapidly (Fig. 1B). In the early stage, most organic substances (acetic acid, propionic acid, butyric acid and valeric acid) were converted to VFAs by hydrolytic and acid-producing bacteria. However, the environment-adapting ability of methanogens was lower compared to acid-producing bacteria, resulting in the accumulation of VFAs. It is reported that, the generation time of acid-producing bacteria is only 10–30 min, far quicker than that of methanogens [17,30]. The concentration of accumulated VFAs reached 2875.7 mg/L in HS, the highest among the three processes. On performing community analysis, it was found that the result was also closely associated with the higher relative abundance of hydrolytic bacteria S1 and Clostridium. It is reported that higher digestion temperature increased the activities of key hydrolases and raised the proportions of bacteria involved in hydrolysis/acidification, resulting in higher VFA production [32]. We observed that with the maximum level VFAs, the VFA concentration dramatically decreased to 370.6 mg/L due to higher methane generation efficiency, while the level of NS was still maintained at 1150.3 mg/L. In the later stage of digestion, the accumulated organic acids were rapidly utilized by methanogens. Additionally, NH_4HCO_3, formed via the anaerobic digestion byproducts (CO_2 and NH_4^+), also provided a stronger buffering capacity and further maintained the stable acidic/alkaline equilibrium.

3.2.2. Effects of adaptive thermophilic granular sludge on COD removal efficiencies

The COD removal efficiencies during different anaerobic digestion are demonstrated in Fig. 1C. During the early period (0–24 h), hydrolytic and acid-producing bacteria (Anaerolinea in NS, Treponema in MS, and Clostridium in HS), played a pivotal function in converting the insoluble macromolecular organic substances (protein, lipid, starch) into easily degradable organic substances. Due to the increased content of small molecules and organic substances that can be easily degraded by the hydrolytic bacteria, the metabolism of hydrogen-producing acetogens was accelerated, especially at higher temperatures [7,32]. At 24 h, the degradation efficiency of COD in HS reached 28.2%, while the
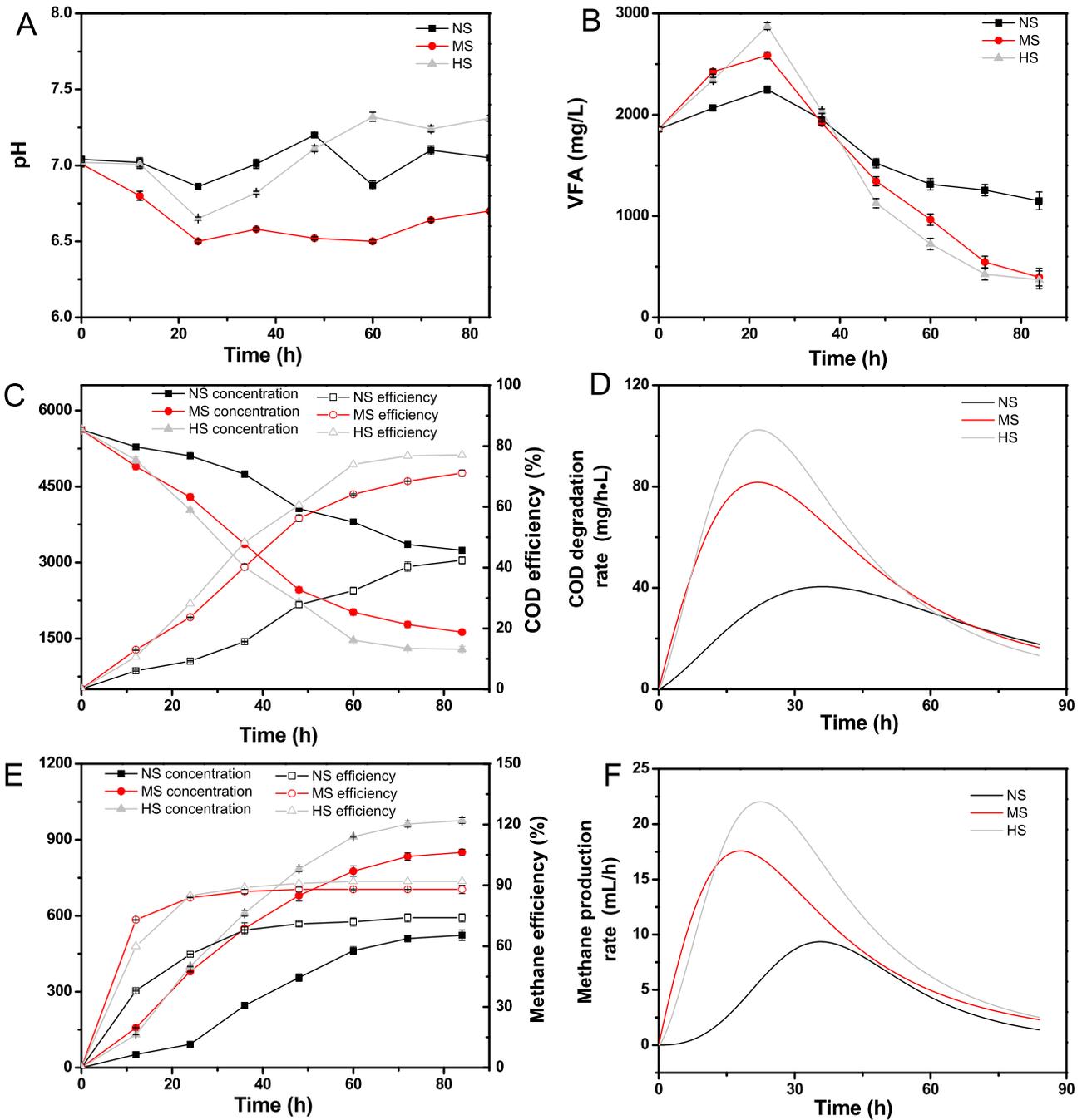
Fig. 1. Key parameter changes of anaerobic digestion of leachate for methane production. (A) pH; (B) VFAs; (C) COD concentration and removal efficiency; (C) COD concentration and removal efficiency; (D) COD degradation rate; (E) Methane production volume; (F) Methane production rate.

values were only 23.6% and 9.2% in MS and NS. Along with the depletion of carbon substrates, the COD degradation rate gradually slowed down. The final removal efficiency of HS reached 77.1%, higher than 42.4% and 71.1% in NS and MS, respectively. The COD kinetic degradation rate is presented in Fig. 1D. After the short lag phase, the COD degradation rate rapidly increased due to abundant nutrients and active microbial metabolism. The maximal COD degradation rate of NS was only 40.5 mg/(L·h^{-1}), while the values of MS and HS were 81.7 and 102.4 mg/(L·h^{-1}), respectively. The reaction times were also greatly diminished, from 36.2 h to about 24.0 h. The kinetic data was closely consistent with the above deduction.

3.2.3. Effects of adaptive thermophilic granular sludge on cumulative methane productions and methane percentages

After a brief delay in the lag stage, the methane accumulation raised rapidly and finally entered the stationary phase (Fig. 1E). With the increased accumulation of VFAs during the early stage, the methane began to generate by methane from 24 h. It is suggested that excessive accumulated VFA may cause higher content of carbon dioxide in the biogas [20]. In this study, the adaptive thermophilic granular sludge could utilize organic matter more thoroughly, resulting in higher methane production. The total accumulated production of methane was 976.0 mL, which was almost twice that of the NS system.

As shown in Fig. 1F, the highest values of methane production rate were 9.4 mL/h, 17.6 mL/h and 22.0 mL/h in NS, MS, and HS, respectively. However, the earliest appearance time of the maximum methane production was tested in the MS process, because the mesophilic condition was favorable for shortening the lag phase [32]. In HS process, more accumulated VFAs provided enough energy source for producing methane, thus the appearance time was consequently delayed. During anaerobic digestion process, if the accumulated VFAs cannot be degraded in time, and the facultative anaerobic microorganisms would use these organic matter to produce CO_2 owing to the small amount of oxygen in the beginning of anaerobic digestion. Thus, the methane percentage in total gas in the earlier phase was lower and gradually increased along with the deepening of anaerobic digestion process. In view of the methane proportion of biogas (NS-74%, MS-88% and HS-92%), the actual difference of methane production between NS (387.0 mL) and HS (850.0 mL) were further widened by 2.2-fold. Considering the total methane production and COD degradation, the amounts of methane produced per gram of COD were 229.0 mL (NS), 268.0 mL (MS) and 296.0 mL (HS), reaching 67.0%,

78.8% and 87.1%, respectively, of theoretical values (350.0 mL methane per gram COD). The maximum specific methane production rate was used to quantify the methanogenic activity of sludge. The maximum rate of specific methane production in HS reached 117.3 mL CH_4/(g VSS d), improving by 134.7% and 24.9% in NS and MS, respectively.

3.3. Bacterial community structure distribution

The optimized sequences from different fermentations were classified via Venn diagram at similar levels of 97% (Fig. 2). For NS, MS, and HS, 1541, 1389 and 1083 OTUs were obtained, respectively, while 652 OTUs (33.4%) were common to all the three groups. The Venn diagram analysis indicated that the three kinds of sludge had obvious differences in the bacterial community. These OTUs covered 14 fungi and 68.5% of bacteria belonging to *Firmicutes*, *Bacteroidetes*, *Chloroflexi* and *Proteobacteria*, and playing a critical role in anaerobic sludge. There were 1273 OTUs shared by the NS/MS, accounting for 43.0% of the total number of OTUs, while the values in MS/HS and NS/HS were 29.0% and 28.0%, respectively. The hierarchical cluster analysis of the three
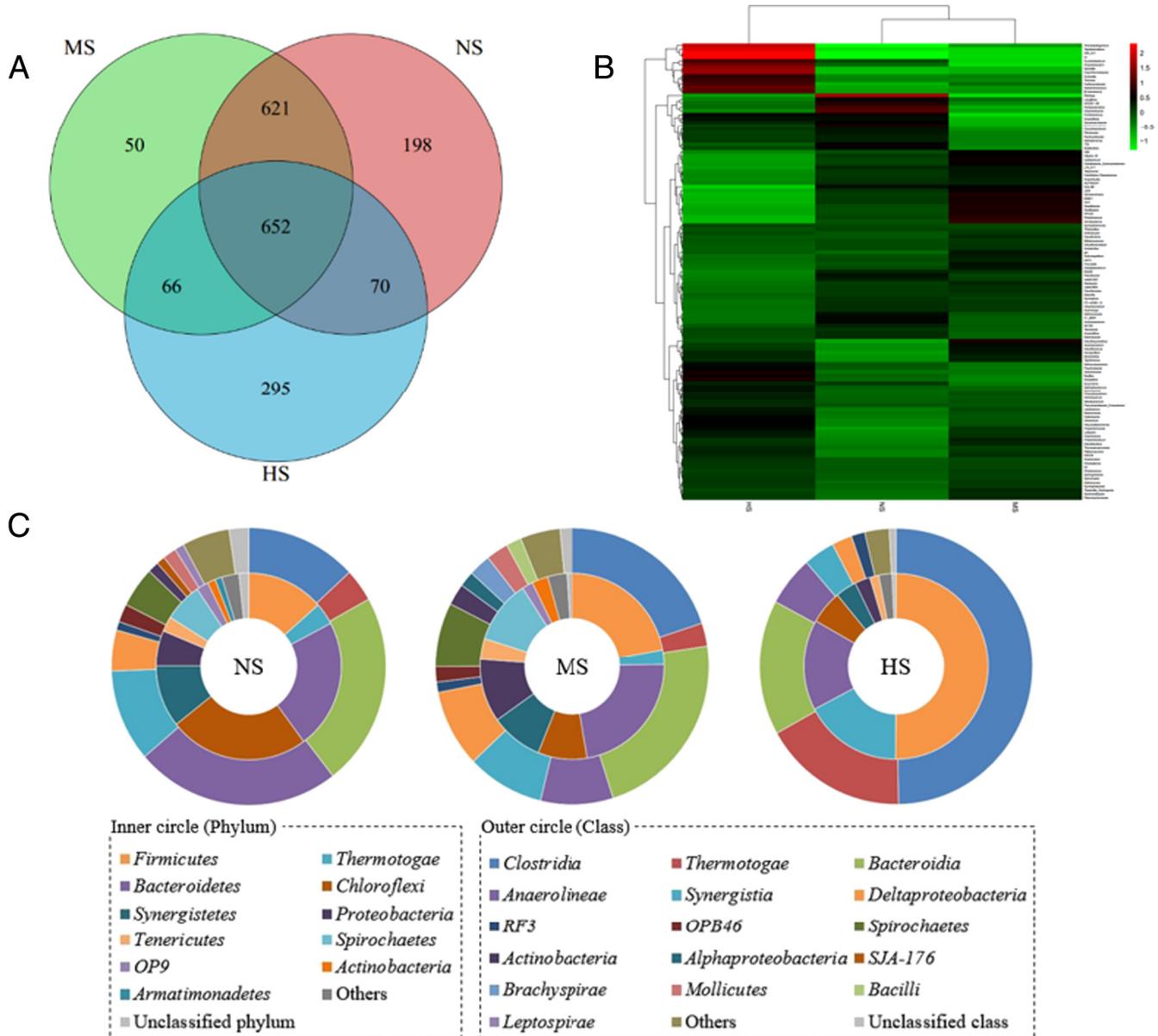
Fig. 2. The community structure analysis of bacterial flora from different anaerobic fermentations. (A) Venn diagram; (B) Hierarchical cluster analysis (genus level); (C) Taxonomic classification (phylum and class level).

bacterial communities also indicated that species distribution was more concentrated in HS than in NS/MS. Each sludge sample showed high bacterial community diversity; however they were significantly different from each other.

To clarify the phylogenetic structure of the bacterial flora, taxonomic distributions were analyzed at the phylum, class and genus levels (Fig. 2C). In NS, MS, and HS, 25, 21, and 14 phyla were detected, respectively. They accounted for 98.3%, 99.0% and 99.3% of the total sequences in NS, MS, and HS, respectively. Among all samples, >65% bacteria belong to *Chloroflexi*, *Firmicutes*, *Bacteroidetes* and *Proteobacteria*. The largest community of NS was *Chloroflexi* (24.4%), followed by *Bacteroidetes* (22.6%), *Firmicutes* (13.5%), and *Synergistetes* (10.7%). *Chloroflexi* bacteria, facultative anaerobes, uses light and chemical energy source, with the ability to hydrolyze carbohydrates. The dominant bacteria in MS belonged to *Bacteroidetes* (22.5%), followed by *Firmicutes* (22.1%), *Spirochaetes* (11.0%), and *Proteobacteria* (10.9%). The relative abundance of *Firmicutes* reached 50.1% in HS, followed by *Thermotogae* (17.2%) and *Bacteroidetes* (15.9%). *Firmicutes* belong to syntrophic bacteria, degrading VFAs into hydrogen. Other communities included *Chloroflexi* (5.8%), *Synergistetes* (3.7%), *Proteobacteria* (2.7%), and *Tenericutes* (1.6%), respectively. At the class level, the relative abundance of *Clostridia* (49.7%), belonging to phylum *Firmicutes*, was the highest in HS; while the relative abundance was only 13.1% and 19.9% in NS and MS, respectively. The class *Thermotogae*, gram-negative anaerobic thermophile, accounted for 17.2% in HS. However, its relative abundance in NS and MS was only 3.8% and 2.7%, respectively. It is previously suggested that raising anaerobic digestion temperature increases the abundances of *Thermotogae*, which further facilitates hydrolysis/acidification process [31].

Genus-level classification was conducted to further determine the phylogenetic discrimination and possible functions (**Table S1**). In NS, the genera *T78* and *Anaerolinea*, which are strictly anaerobic, possessed the predominant advantage with relative abundance of 5.2% and 5.0%, respectively. Both, *T78* and *Anaerolinea* belong to the phylum *Chloroflexi*; bacteria belonging to this phylum utilize carbohydrates to produce hydrogen and acetic acid. In MS, the dominant bacterial genus was *Treponema* (6.6%), which is a strict anaerobe and is grouped with acid-producing bacteria in phylum *Spirochaetes* [33]. The community of the strictly anaerobic desulfurizing-bacteria, *Desulfovibrio* (6%), depend strongly on the environmental temperature (>40°C), metabolizing acetic acid and hydrogen sulfide. The most dominant bacterial genus in HS was *S1* (16.8%), this community was not tested in NS and MS. Moreover, the hydrolytic bacteria *Clostridium* (8.1%) had community advantage, compared to the lower level in NS (1.9%).

3.4. Archaeal community structure distribution

The Venn diagram of the archaeal community presented at a similar level of 97% (Fig. 3A). NS, MS, and HS comprised 235, 260 and 237 OTUs, respectively. One thirty-one OTUs (26.4%) were common in NS/MS, 104 OTUs (20.9%) shared by MS/HS, while 99 OTUs were shared by NS/HS, accounting for 21.0%. The Venn diagram analysis implied that the archaeal community of the three processed were significantly diverse. The hierarchical cluster analysis of archaeal genera showed that the species distribution of archaeal communities in the sludge were dispersed with increased temperature. The highest diverse distribution of the archaeal community was found in HS (Fig. 3B).

The majority of archaeal sequences in NS (96.1%), MS (93.3%) and HS (96.4%) belonged to the phylum *Euryarchaeota*; while 3.2%, 5.7% and 3.1% in NS, MS, and HS, respectively, of the total sequences were grouped into *Crenarchaeota*. *Methanomicrobia* was the dominant species at a class level, accounting for 83.4%, 73.1%, and 61.4% in NS, MS, and HS, respectively, while *Methanobacteria* accounted for 9.6%, 15.5% and 31.3%, respectively. Among the three anaerobic digestion processes, 11 species (total 14 species) of both the phyla, *Euryarchaeota* and *Crenarchaeota* were detected in each process, while

other four species were dramatically different (Fig. 3C). *Methanosaeta*, which are obligate acetotrophic methanogens (acetic acid as the sole carbon/energy source), simultaneously dominated the community structure of NS (62.8%) and MS (75.9%). However, hydrogenotrophic (*Methanobacterium*/*Methanoculleus*) and acetotrophic methanogens (*Methanosaeta*/*Methanosarcina*) were evenly distributed with a higher abundance, indicating a close interaction between these two methanogens. It is recognized that microbial communities with a greater evenness have a more robust function in preserving the functional stability of an ecosystem [28,29]. The dominant community in HS was *Methanobacterium* (30.5%), while only 15.0% and 8.8% were present in MS and NS. The second dominant community was *Methanoculleus* in HS, accounting for 28.8%, while its proportion was very low in NS/MS (0.2–0.3%). Being hydrotrophic methanogens, the anaerobic digestion ability of *Methanobacterium*/*Methanoculleus* generally increased with higher temperature [32,34]. The third dominant community was *Methanosaeta* (18.1%), which had an absolute community advantage in MS/NS. It is reported that *Methanosaeta* uses acetic acid as the sole carbon/energy source to produce methane [35], and the utilization rate reaches up to 98.0–99.0% [30]. The fourth abundant genus in HS was *Methanosarcina* (9.5%), which was extremely low in NS/MS (0.1–0.2%). Both of *Methanosaeta* and *Methanosarcina* were acetotrophic methanogens, using acetic acid as energy source [19,30]. The results showed that the adaptive thermophilic process greatly inhibited the growth metabolism of *Methanosaeta*, while the community advantages of *Methanobacterium*, *Methanoculleus*, and *Methanosarcina* were strengthened. The more balanced community distribution between hydrotrophic and acetotrophic methanogens was beneficial to a better microbial interaction, which further enhanced the thorough utilization of the organic matter for methane production with better microbial interaction.

3.5. Relationship between dynamics of microbial communities and anaerobic digestion performance

The relationships among bacterial/archaeal community structures and anaerobic digestion performances (including VFA, COD removal and biogas production) were revealed by the CCA analysis (Fig. 4). The unrestricted permutation test indicated that adaptive thermophilic process could explain most variances in bacterial/archaeal community compositions. In the bacterial community, *S1*, *A55_D21* and *Clostridium* were dominant genera with an extremely high abundance in HS (**Table S1**) and were positively associated with methane production, while *Anaerolinea* and *Treponema* was negatively correlated with methane production. In the archaeal community, *Methanobacterium*, *Methanoculleus* and *Methanomassiliicoccus* were positively associated with methane production, while *Methanosaeta* and *Methanofollis* demonstrated a reverse correlation. The result was also closely associated with the community structure analysis shown in Section 3.4. A more balanced community distribution between hydrogenotrophic and acetotrophic methanogens was achieved in HS. It was proved that syntrophic interactions between hydrogen producers and hydrogenotrophic methanogens were pivotal for reducing the imbalance between the faster hydrolysis/acidogenesis and slower methanogenesis process [22,34]. The changing pattern of the relative abundance of related genera was in accordance with the traits of hydrogenotrophic methanogens. On the other hand, the dominance of obligate hydrogenotrophic methanogens in HS indicated that hydrogen was the electron transfer carrier between bacteria and methanogen. The generated hydrogen is known to be immediately consumed by methanogens, which further weakens the inhibitory effect of the accumulated substrate [29]. Other chemical parameters during anaerobic digestion did not contribute to the variability of the bacterial/archaeal community significantly. The comparison of the main traits between anaerobic digestion processes in this study is listed in **Table S2**.
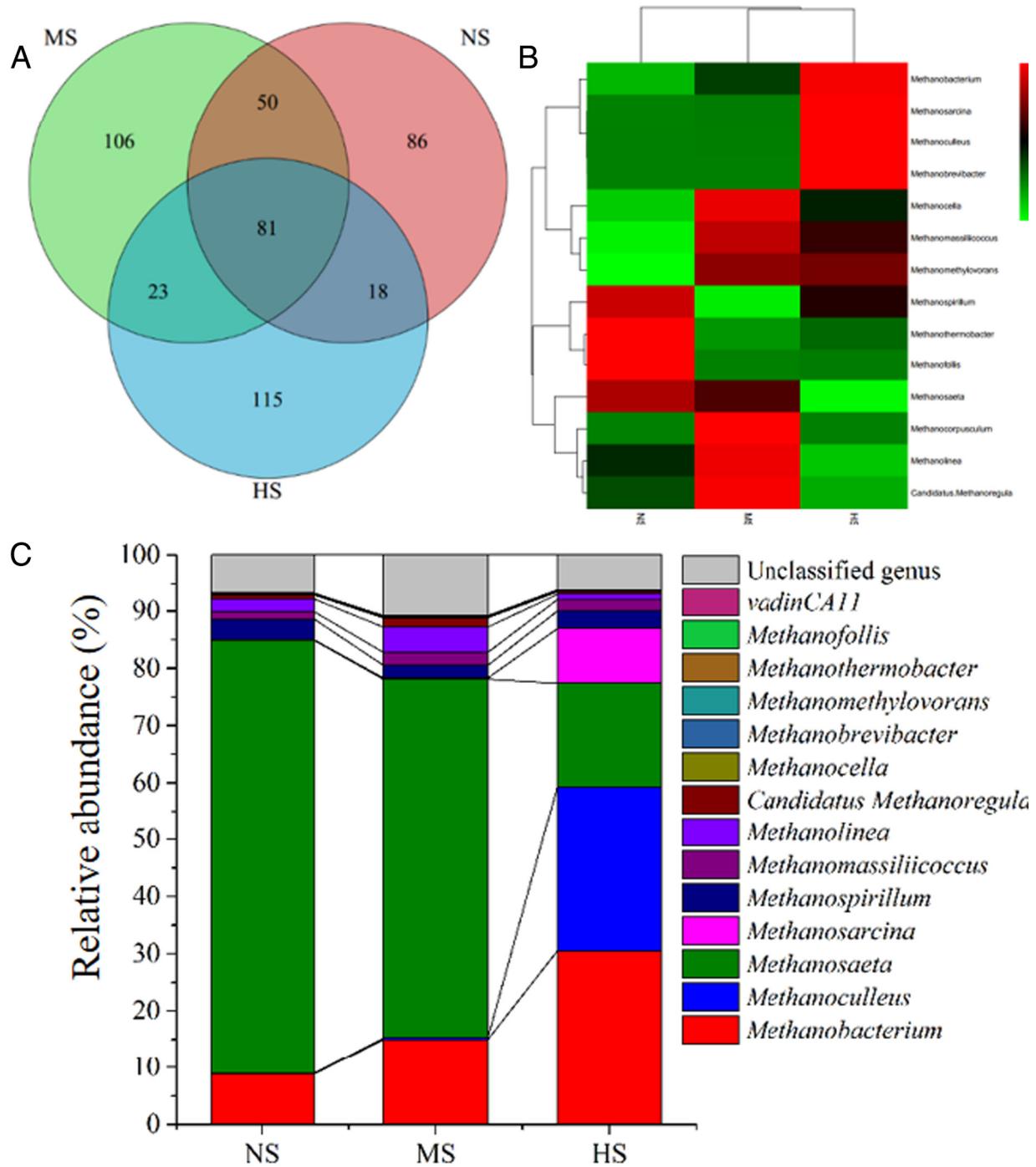
Fig. 3. The community structure analysis of archaeal flora from different anaerobic fermentation. (A) Venn diagram; (B) Hierarchical cluster analysis (genus level); (C) Taxonomic classification (genus level).

The practical values of knowledge about the shift in microbial community structure in this study can be summarized as following three aspects.

1) Economical utilization of excess waste heat from genset. The adapted thermophilic granular sludge (50°C) was used to treat the landfill leachate in this study. In the application of the large scale MSW landfill site, methane was produced from MSW and leachate via the anaerobic digestion, and tremendous heat were orderly released from the genset during the biogas power generation process. The heat energy can be economically recovered via and utilized for satisfying the higher temperature requirement during the anaerobic digestion process.

2) Reasonable dominant strain intervention. While the balance of community structure during anaerobic digestion process was not satisfied, we could solve the trouble problem via exogenous dominant strain intervention. The dominant strain such as *Methanobacterium*, *Methanoculleus*, *Methanosaeta* and *Methanosarcina* could be cultured outside of the system, and then the exogenous pure strain culture were added to artificially regulate the community structure to be more reasonable, which sequentially improved the methane production.

3) Exogenous substrate preference control. The substrate preference phenomenon exists objectively in different microorganism owing to various physiological and biochemical characteristics. Thus, various wastewaters from different sources can be regrouped and
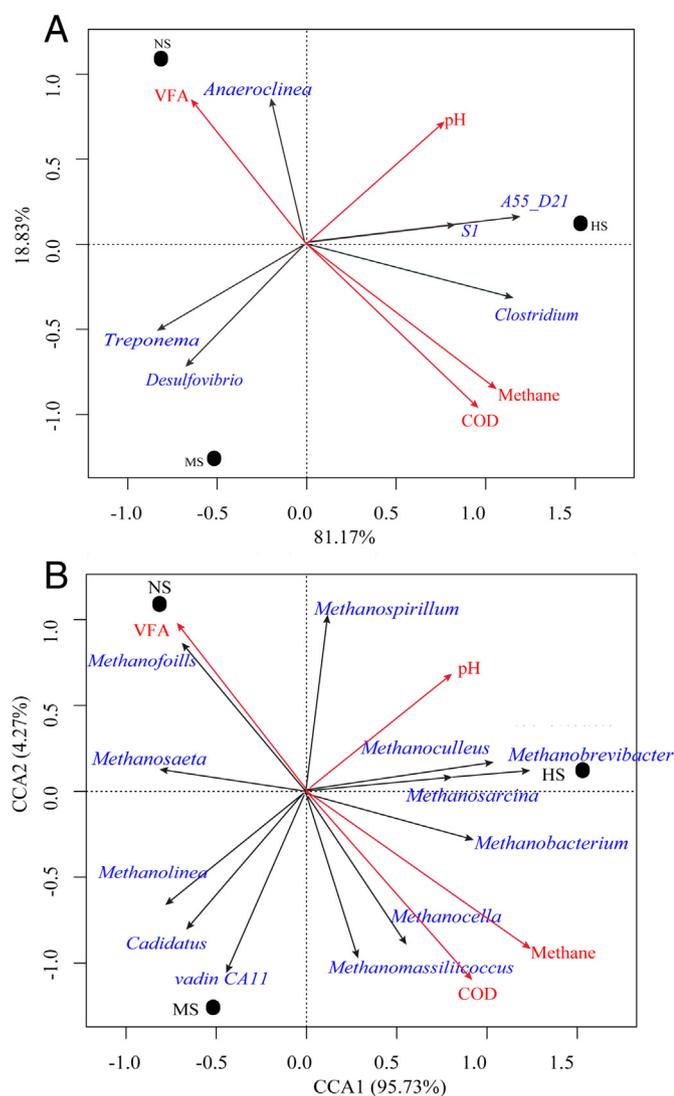
Fig. 4. Triplots of CCA ordination diagrams with community structures (at genus level, pyrosequencing data) and environmental conditions variables. (A) Bacterial flora; (B) Archaeal flora.

utilized for more favorably enhancing the community advantages of certain targeted strain during the anaerobic digestion process.

Taken together, these inferences expand our knowledge about the mechanisms of the microbial community of anaerobic fermentation, which will be helpful to further guide biotreatment of MSW leachate.

4. Conclusions

The adaptive thermophilic granular sludge was used to treat the landfill leachate from the large-scale MSW of Wuxi City's for methane production. The COD degradation rate was improved by 81.8% compared to the control, while the methane production rate reached 117.3 mL CH_4/(g VSS d), which is 2.34-folds more than the NS process. The high-throughput pyrosequencing analysis revealed that *Methanosaeta* has a greater dominant community in NS/MS, while hydrogenotrophic methanogens (*Methanobacterium*/*Methanoculleus*) and acetotrophic methanogens (*Methanosaeta*/*Methanosarcina*) were more evenly distributed in HS. The balanced community structure was closely associated with collaboration effects among these microbes. The detailed relationship between microbial community interactions

and anaerobic digestion performances was further corroborated by CCA analysis.

Conflicts of interest

There are no conflicts of interest to declare.

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Supplementary material

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