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Microbial Community Dynamics in Batch High-Solid Anaerobic Digestion of Food Waste Under Mesophilic Conditions

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Microbial community shifts, associated with performance data, were investigated in an anaerobic batch digester treating high-solid food waste under mesophilic conditions using, a combination of molecular techniques and chemical analysis methods. The batch process was successfully operated with an organic removal efficiency of 44.5% associated with a biogas yield of 0.82 L/g $VS_{removal}$. Microbial community structures were examined by denaturing gel gradient electrophoresis. *Clostridium* and *Symbiobacterium* organisms were suggested to be mainly responsible for the organic matter catabolism in hydrolysis and acidogenesis reactions. The dynamics of archaeal and methanogenic populations were monitored using real-time PCR targeting 16S rRNA genes. *Methanosarcina* was the predominant methanogen, suggesting that the methanogenesis took place mainly *via* an aceticlastic pathway. Hydrogenotrophic methanogens were also supported in high-solid anaerobic digestion of food waste through syntrophism with syntrophic bacterium. Microbial community shifts showed good agreement with the performance parameters in anaerobic digestion, implying the possibility of diagnosing a high-solid anaerobic digestion process by monitoring microbial community shifts. On the other hand, the batch results could be relevant to the start-up period of a continuous system and could also provide useful information to set up a continuous operation.

Keywords: Anaerobic digestion, high-solid, food waste, microbial community

Introduction

The preferred disposal method for municipal solid wastes in most countries is sanitary landfill, but this is not necessarily the most effective option [25]. There is an ever-increasing rate (higher than 10% every year) of food waste with the rapid development of the population and progress of urbanization in China [9]. In China, food waste accounts for 40–50% of the weight of municipal solid waste (MSW) and is an attractive substrate for the production of biogas compared with other organic matters owing to its relatively high methane production potential. Anaerobic digestion is considered to be the most promising alternative and the most cost-effective technology to treat the municipal solid wastes for renewable energy generation, such as biogas [5, 21, 25, 26]. Traditionally, low-solid anaerobic digestion is usually used to recover methane from food waste.

High-solid anaerobic digestion is usually characterized by a high total solid content of the feedstocks, typically more than 15% (w/w) [32]. In recent years, high-solid technology has become attractive compared with traditional low-solid anaerobic digestion for several reasons, such as higher gas production efficiency, higher volumetric organic loading rates, lower energy requirements for heating, and less material handling [15]. Therefore, a few studies on various wastes as feedstocks in high-solid anaerobic digestion were conducted, including agricultural wastes [7], food wastes [9], bio-wastes [14], industrial wastes [31], and the organic fraction of municipal solid wastes (OFMSW) [5].

Anaerobic digestion of organic materials is greatly affected by the biological processes mediated by microorganisms *via* three main steps: hydrolysis, fermentation, and

methanogenesis [37]. The first two reactions and the last reaction are mediated by bacterial groups producing hydrogen and organic acids and archaeal populations producing methane, respectively. Therefore, it is crucial to comprehensively understand the microbial behavior for the fundamental improvement of the anaerobic digestion process [18]. Although the diversity of microbes involved in anaerobic digestion [34] and the relationship of the microbial community structure with metabolic functions have been well documented [10], what is missing in the literature relates to microbial community dynamics in the batch high-solid anaerobic digestion of food waste, or the relationship between microbial diversity and metabolic function.

The aim of this study was to investigate microbial community dynamics during the batch high-solid anaerobic digestion of food waste under mesophilic conditions. Monitoring microbial community shifts in relation to changes in process performance data can provide valuable information that can be used to elucidate the high-solid anaerobic digestion of food waste in a dynamic state. For this purpose, denaturing gradient gel electrophoresis (DGGE) of bacterial and archaeal 16S rRNA genes was conducted to identify major bacterial and archaeal species throughout the batch process. Simultaneously, the quantitative changes in bacterial and methanogenic populations were monitored using real-time PCR technology.

Materials and Methods

Experimental Set-Up

An anaerobic completely stirred tank reactor (CSTR), with a working volume of 6 L, was operated in a batch mode. The rotation speed was set at a rate of 60 rpm (rotations per minute) with 8 min stirring and 2 min break, continuously. Food waste (FW) was collected from a dining room at Tongji University in Shanghai. After removing bones, shells, and other indigestible materials, the food waste was finely smashed using an electrical crusher. The total solid (TS) of the food waste was 20.3% (w/w) and volatile solid (VS) accounted for 92.4% of TS. The mesophilic seed sludge was obtained from a full-scale anaerobic digester at the Bailonggang municipal wastewater treatment plant (WWTP) (Shanghai, China). It had TS of 15% (w/w) and VS was 36% of TS. Characteristics of the substrate and inoculums are listed in Table 1. On the first day of the experiments, 2 L food waste and 4 L seed sludge were added into the anaerobic reactor, which was operated in batch mode at 35 ± 1°C. The experiments continued from March 1 to 27 in 2013 when biogas production stopped. High-solid AD food waste samples retrieved from the reactor were taken with a sterile glass beaker using a food discharging valve for microbial community analysis.

Table 1. Characteristics of the substrates and inoculums.

Parameters	TS[a] (% (w/w))	VS[b]/TS (%)	pH	TAN[c] (mg/l)
Food waste	20.3 ± 0.9	92.4 ± 3.2	5.6 ± 0.3	563 ± 109
Inoculums	15.0 ± 1.8	35.9 ± 1.8	7.8 ± 0.1	291 ± 18

[a]TS, total solid.

[b]VS, volatile solid.

[c]TAN, total ammonia nitrogen.

DNA Extraction

A 5 g fresh sample collected from the reactor was transferred into a 50 ml microcentrifuge tube using wide-mounted pipette tips (allowing transfer of large particles in the samples) and centrifuged at 16.000 ×*g* for 5 min. The supernatant was decanted and the pellet was washed with 4 ml of phosphate buffer (0.12 mol/l, pH = 8) and centrifuged again in the same manner to ensure the maximal removal of potential PCR-interfering material. The supernatants were carefully removed, and the genomic DNA was extracted in the pellet following the recommendations of the MOBIO Ultra Clean Soil DNA isolation kit. Total nucleic acid was quantified and checked for purity with a Nandrop Spectrophotometer and stored at −20°C.

PCR Amplification of Bacterial and Archaeal 16S rRNA Genes

Bacterial and archaeal 16S rRNA gene fragments were amplified by PCR from DNA samples with the oligonucleotide primers specific for bacteria and archaea [1, 16, 29, 33] (Table 2). The 5′-ends of 341F and PARCH344F were added with a 40 bp GC-clamp (5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGG G-3′) to stabilize the melting behavior of the PCR products. In order to increase the sensitivity and to facilitate the DGGE by analyzing fragments of the same length, two nested PCR amplifications were applied. The first round of PCR amplifications with primer pairs 27F-1492R and ARCH201F-ARCH1041R were conducted as has been described previously ([12] and [6, 11], respectively; Table 2). A touchdown PCR in the second amplification reaction was conducted in a thermal cycler (PTC-100; MJ Research Inc., Watertown, MA, USA), according to the same protocol as previously described [37]. The annealing temperature for archaea was 52°C.

Denaturing Gradient Gel Electrophoresis Analysis and Band Identification

DGGE analysis was carried out using a D-Code universal mutation detection system (Bio-Rad Laboratories, Richmond, CA, USA). Both second PCR products of bacteria and archaea were loaded onto an 8% (w/v) acrylamide gel containing a 35−60% denaturant gradient, where 100% was defined as 7 M urea with 40% (v/v) formamide. Electrophoresis was run at 85 V for 16 h in 1× TAE buffer. After electrophoreses, the silver-stained gel was scanned under UV transillumination and the bands of interest

Table 2. Primers used in PCR amplification of 16S rRNA genes with DGGE and real-time PCR methods.

Target group	Primer name[a]	Sequence (5′-3′)	Reference
Bacteria	27F	AGAGTTTGATCCTGGCTCAG	[12]
	1492R	TACCTTGTTACGACT	
	341F	ACTCCTACGGGAGGCAGCAG	[14]
	534R	ATTACCGCGGCTGCTGG	[29]
	BAC338	ACTCCTACGGGAGGCAG	[42]
	BAC805R	GACTACCAGGGTATCTAATCC	
Archaea	ARCH21F	TTCCGGTTGATCCYGCCGGA	[6]
	ARCH1041R	GGCCATGCACCWCCTCTC	[11]
	PARCH344F	HGCAGCAGGCGCGA	[1]
	UNIV522R	GWATTACCGCGGCKGCTG	[33]
	ARC787F	ATTAGATACCCSBGTAGTCC	[42]
	ARC1059R	GCCATGCACCWCCTCT	
Methanobacteriales	MBT857F	CGWAGGGAAGCTGTTAAGT	[42]
	MBT1196R	TACCGTCGTCCACTCCTT	
Methanococcales	MCC495F	TAAGGGCTGGGCAAGT	
	MCC832R	CACCTAGTYCGCARAGTTTA	
Methanomicrobiales	MMB282F	ATCGRTACGGGTTGTGGG	
	MMB832R	CACCTAACGCRCATHGTTTAC	
Methanosarcinales	MSL812F	GTAAACGATRYTCGCTAGGT	
	MSL1159R	GGTCCCCACAGWGTACC	

[a]F and R indicate forward primer and reverse primer, respectively.

were carefully excised and eluted with sterilized distilled water at 4°C. The eluted solution was further amplified with PCR using the corresponding primers without the GC-clamp. The PCR products were purified from a 0.8% agarose gel and cloned into the PMD 18-T Vector (TaKaRa, Dalian, China) according to the manufacturer's instructions. The cloned 16S rRNA gene fragments were sequenced by Beijing AuGCT Biotechnology Co., Ltd. (Beijing, China). After sequencing, reads were first trimmed to remove the vector by scanning of the individual chromatograms using Chromas software ver. 2.23 (Technelysium, Shanghai, China). Only the sequences between forward primers and reverse primers were obtained and checked with the Mothur chimera check program in Mothur ver. 1.31.2. The sequencing results were compared against 16S rRNA gene sequences held in the GenBank database at NCBI (National Centre for Biotechnology Information) and the RDP database to determine their approximate phylogenetic affiliation.

Real-Time Quantitative PCR Analysis

Quantitative real-time PCR was performed using a 7500 Real-Time PCR system (Applied Biosystems, USA) with six primer sets (Table 2). The four order sets should cover most methanogens in anaerobic digesters [42]. SYBR-Green I fluorophore was used to detect the change in target DNA according to manufacturer's instructions. Each 16S rRNA gene amplification was carried out in a 50 µl reaction mixture containing 25 µl of ABI PRISM 7500 Real-time SYBR-Green I Premix Ex Taq (TaKaRa Biotechnology, Dalian, China), 200 nM of Primers, 1 µl of ROX Reference Dye, 18 µl of sterile Milipore water, and 4 µl of diluted template DNA. All amplifications were performed in triplicate and a set of standard curves for the primer sets used were constructed as described previously [17]. Sterile Milipore water (no template) was used as a negative control throughout. Amplification was performed in a two-step thermal cycling procedure as previously described [37] and a melting curve was obtained by heating the products to 95°C. Fluorescence signals were measured once each cycle at the end of the denaturation step. The specificity of the qPCR products was confirmed by melting analysis as has been described previously [17].

Analytical Methods

Volumes of produced biogas were measured by wet gas meters daily. Biogas and digested samples were taken out to determine chemical and physical properties such as total solid (TS), volatile solid (VS), volatile fatty acid (VFA), and total organic carbon (TOC). Total solid, VS, and TOC were determined according to standard methods [2]. The methane content of the biogas was measured by a gas chromatograph (GC) (Agilent Technologies 6890N, CA, USA) with a thermal conductivity detector equipped

with Hayseq Q mesh and Molsieve 5A columns. The VFA was measured according to the procedures as previously described [9]. The degradation or removal level based on VS (*i.e.*, VS reduction) was calculated by the same formula as reported previously [13]. All experimental analyses were performed in triplicate.

Nucleotide Sequence Accession Numbers

Bacterial nucleotide sequences obtained in this study are available in the GenBank database under accession numbers KFO38387–KFO38411. Archaeal nucleotide sequences obtained in this study are available in the GenBank database under accession numbers KFO38412–KFO38424.

Results and Discussion

Anaerobic Digestion Properties

Total biogas yield and daily biogas yield during high-solid anaerobic digestion of food waste are shown in Fig. 1. Biogas production started at day 0.08 with about a 63% CH_4 content, and the anaerobic digestion process lasted for 27 days. The total amount of biogas produced in the high-solid anaerobic digester was 219.28 L and the corresponding average biogas yield per VS removed in this digester was 0.82 L/g $VS_{removal}$, which was within the normal range 0.75–1.12 L/g $VS_{removal}$ [3]. The average methane content ranged from 62.5% to 64.3%, which was higher than that in some research on the anaerobic digestion of food waste [9, 19].

Fig. 2 represents the changes in the physicochemical profile at different times during the high-solid anaerobic digestion. When biogas production ceased, the VS of the initial amounts were removed at 44.5% (Fig. 2A), which was higher than the known data in some references [19, 37], but can be considered low when compared with values found during steady state processes for other organic wastes [22, 30], which was probably due to differences in the substrate composition and digestion approach. The VS reduction efficiency was the highest during the early stage of the anaerobic digestion and about 47% of the total VS reduction (*i.e.*, 21% of the initial VS concentration) was achieved by day 2.21. However, the TOC concentration removal throughout the reaction was notably high (*i.e.*, 83.1% of the total TOC reduction) when compared with the VS removal (Fig. 2A).

As for the total VFA (TVFA), it increased rapidly up to 30 g/l at day 7 and then decreased continuously until day 27 when biogas production ceased (Fig. 2B). In this experiment, acetate, propionate, and *n*-butyric acid were the most abundant acidogenic products. The sum of these three acids was more than 94% of the TVFA throughout the incubation period. Acetate was accumulated up to 22 g/l at day 6.0, which corresponds to 34% equivalence of the

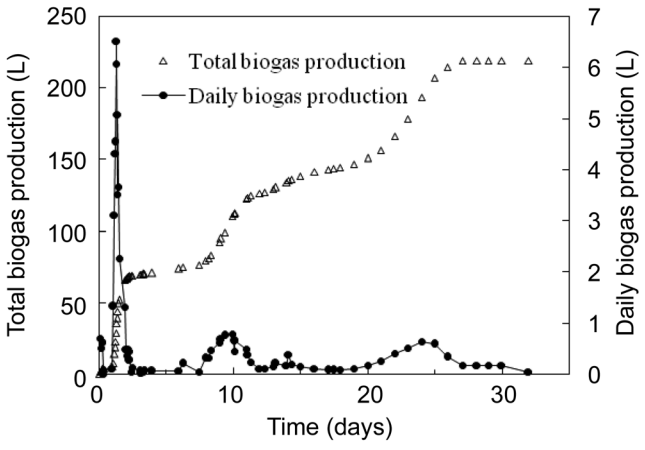

Fig. 1. Total and daily biogas yields of a mixture during anaerobic digestion.

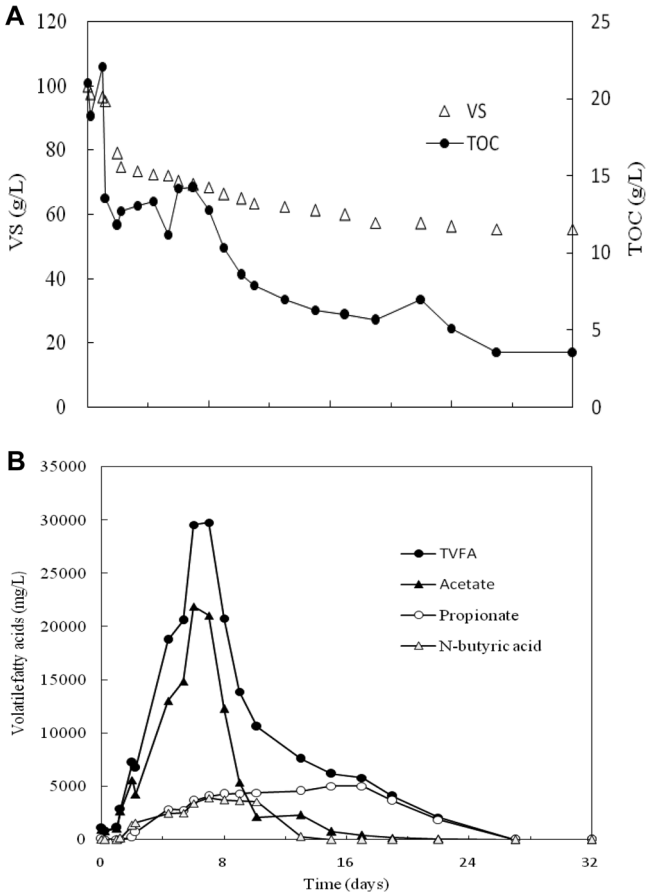

Fig. 2. Physicochemical properties of a mixture at different times of anaerobic digestion.

VS, volatile solid; TOC, total organic carbon; TVFA, total volatile acid.

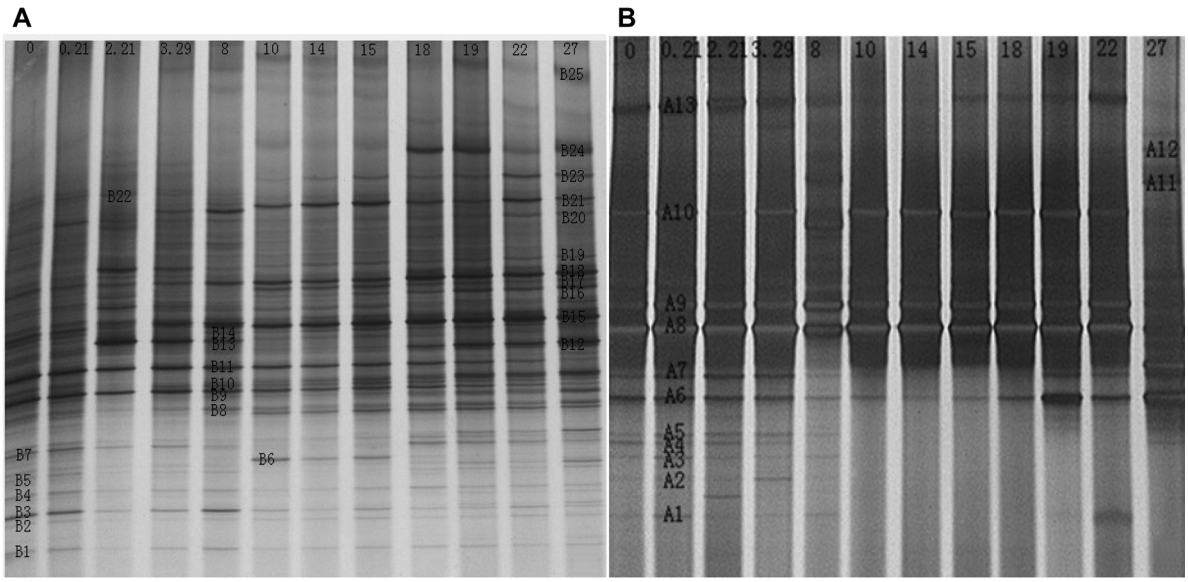

Fig. 3. DGGE profiles of (**A**) bacterial and (**B**) archaeal 16S rRNA gene fragments.
Numbers at the top designate the incubation time in days of each lane.

overall biogas production. Propionate and *n*-butyric acid concentrations peaked at 5.1 g/l at day 17 and 3.9 g/l at day 7, respectively. After 22 days of incubation, acetate and butyrate were completely degraded. Propionate was the last organic acid, which was just in agreement with that in the anaerobic digestion of secondary sludge [37].

Bacterial Community Structure Shifts

PCR-DGGE targeting on bacterial and archaeal 16S rRNA genes was performed in order to investigate changes in microbial community structure. The DGGE band profiles varied among samples, indicating clear changes of the bacterial and archaeal community structures during the batch high-solid anaerobic digestion of food waste. In total, 27 bacterial DGGE bands designated as B1–27 and 15 archaeal bands designated as A1–15 were visually detected (Fig. 3) and excised from the gels for subsequent sequencing analyses. The affiliations of the 16S rRNA sequences were determined by comparison with the GenBank and RDP databases (Table 3).

Firmicutes (*e.g.*, *Clostridium*), *Symbiobacterium*, and *Bacteroidetes* were found to be the dominant organisms during the incubation period. Bands B1, B3, and B4, observed throughout the reaction (Fig. 3A), had high sequence similarities of 98–99% in the full-length 16S rRNA gene with the phylum *Firmicutes*. The *Firmicutes* (*e.g.*, *Clostridium*) are known to produce cellulases, lipases, proteases, and other extracellular enzymes [21].

B8-9 and B19, which were detected during the entire anaerobic digestion process, showed high similarities (98–100%) to the genus *Clostridium*. *Clostridium* is a cellulolytic and proteolytic bacterium, and the fermentation products are acetate and butyrate [38]. B13 and B22, which were only observed during the early period, were closely related to *Clostridium tertium* and *Clostridium tetani*, respectively. The prevalence of organisms belonging to these two species corresponded with the rapid decrease of VS concentration in this period. In fact, *C. tertium* is saccharolytic and produces acetate, butyrate, and lactate from peptone-yeast-glucose (PYG) broth [38]. *C. tetani* is an anaerobic bacterium that can be found in a variety of places, such as the soil and intestinal flora of domestic animals and humans, and is able to metabolize a wide variety of compounds to produces acetate and butyrate [38].

B11, which showed a high similarity (100%) to the thermophilic commensal organism *Symbiobacterium*, was also detected during the entire anaerobic digestion process. A previous study showed that *Symbiobacterium* could utilize glycerol, glucose, and amino acids to form syntrophic relationships with other microorganisms [41]. B7 and B17 were not detected during the middle incubation period and before 10 days of the anaerobic digestion process, respectively. Both of them were assigned to Bacteroidetes with high similarities (99% and 100%). The phylum *Bacteroidetes* contatins proteolytic bacteria that participate in the degradation of proteins and are able to ferment amino

Table 3. Phylogenetic affiliations of the 16S rRNA gene sequences from bacterial and archaeal DGGE bands.

Band (s)	Nearest sequence	Accession No.	% similarity
B1, 3-6[a]	Uncultured *Firmicutes* bacterium QEDT2CD07	GQ995192.1	99-100
B2, 14-15, 18, 20-21, 24	Uncultured bacterium clone LC17	FJ982817.1	95-100
B7, 17	Uncultured *Bacteroidetes* bacterium BC_COM558	HQ727610.1	99-100
B8, 9, 24	Uncultured *Clostridium* sp. clone BRK1_4E	GQ995189.1	98-100
B10	*Peptostreptococcus russellii* strain 37-1	AY167963.1	100
B11	Uncultured *Symbiobacterium* sp. clone SHBZ964	EU639356.1	100
B12, 16	Uncultured prokaryote 07122804-ZSS_Z7_EU_3_3_64	HQ155616.1	99-100
B13	*Clostridium tertium* strain E089	JX267105.1	99
B19	*Clostridium ultunense* strain DSM 10521	GQ461825.1	99
B22	*Clostridium tetani* strain NCTC 279	NR_029260.1	99
B23	Uncultured *Mollicutes* bacterium clone SAO3 B24	JN998160.1	99
B25	Uncultured bacterium clone ncd2310g01c1	JF198254.1	98
A1[b]	Uncultured methanogenic archaeon clone: H08	KC505323.1	100
A2, A3, A5	Uncultured archaeon isolate DGGE gel band 4	JX542582.1	99
A4, A9-12	Uncultured *Methanosarcina* sp. clone KD384	KC582645.1	99-100
A6-A8, A13	Uncultured *Methanosarcina* sp. clone A1_77_12F	GQ995107.1	100

[a]Bands labeled B were detected from the lane in the bacterial DGGE profile.

[b]Bands labeled A were detected from the lane in the archaeal DGGE profile.

acids to acetate and NH_3 [34]. The latter was also detected and shown in other studies to constitute a major microbial component in anaerobic reactors [21, 40]. In addition, B10, which is closely related to *Peptostreptococcus russellii* at a 100% similarity, was detected during the entire anaerobic digestion except on the second day. *Peptostreptococcus russellii* is Gram-reaction-positive, an obligately anaerobic coccus-shaped bacterium, and is weakly saccharolytic. The major end-product from glucose metabolism was acetate, with smaller amounts of lactate, formate, and citrate, and trace amounts of succinate detected. B23, which showed a high similarity (99%) with uncultured *Mollicutes*, was detected from 10 days to the end of anaerobic digestion. However, many aspects of Mollicutes catabolism, including energy conservation in some groups, are poorly understood, and metabolism has only been studied in relatively few species [27].

The dominance of *Clostridium*, *Symbiobacterium*, and *Bacteroidetes* throughout the AD process reflects their ability to metabolize a variety of substrates. Interestingly, their band intensity decreased gradually with the consumption of VS concentration (Fig. 3 and Table 2). These results suggested that they were involved in hydrolysis and acidogenesis reactions and may have been mainly responsible for the organic matter catabolism in this system.

Archaeal Community Structure Shifts

Fig. 3B shows the response of the archaeal community structure during the high-solid anaerobic digestion of food waste (taxa identities are reported in Table 3). Overall, the archaeal band patterns were less complicated than the bacterial results, which was in accordance with the result of the batch anaerobic digestion of secondary sludge [37]. Nine bands showed 99–100% sequence similarities with the genus *Methanosarcina* (Table 3). It is generally known that the concentration of DGGE bands represents the number of microorganisms in DGGE analysis. Among these bands, bands A8 and A9 were the dominant methanogens throughout the entire incubation process, and were particularly abundant before 22 days of the anaerobic digestion (Fig. 3B). The dominance of genus *Methanosarcina*, presented in Fig. 3B, shows that aceticlastic methanogens utilizing acetate mainly contributes to biogas production, and aceticlastic methanogenesis is the major methanogenic pathway in the high-solid anaerobic digestion of food waste.

Interestingly, archaeal DGGE bands related to the hydrogenotrophic methanogens Methanomicrobiales and Methanobacteriales were not identified by DGGE throughout the entire anaerobic digestion process, although these two groups were successfully quantified with real-time PCR (Fig. 4). The relatively low abundance of these groups (<13

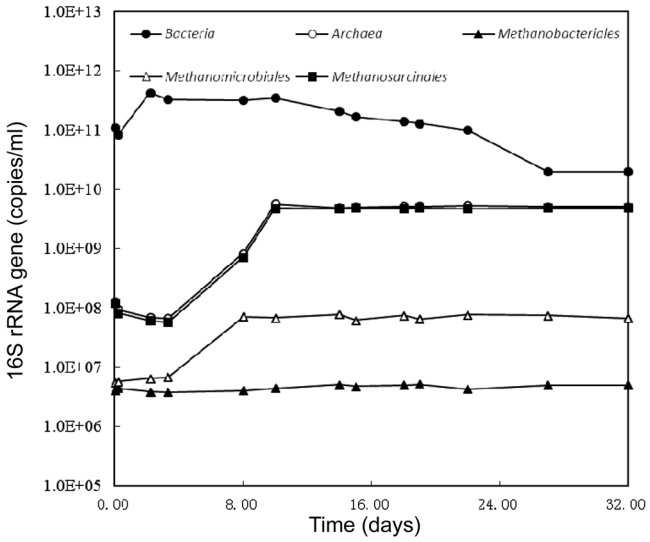

Fig. 4. Quantitative changes in bacterial, archaeal, and methanogenic 16S rRNA gene concentrations determined by real-time PCR.

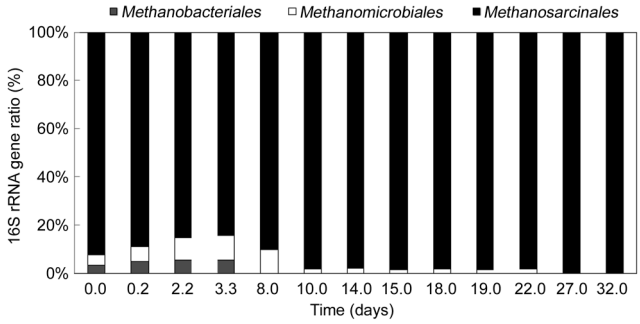

Fig. 5. Relative abundance of the three methanogenic orders based on 16S rRNA gene concentrations.

of the sum of methanogenic orders) within the domain Archaea could be the reason for the lack of visible bands in the domain-level analysis [37]. In fact, many other reports have demonstrated the prevalence of the hydrogenotrophic pathway in anaerobic digestion processes with different substrates [18]. The key factor determining the major pathway could be the existence of high levels of inhibitory ions, which in general have more severe effects on aceticlastic methanogens [37]. Na^+ was considered a potential factor influencing FW digestion systems. It was reported that high concentrations of Na^+ could affect microbial activity by interfering with their metabolism processes [27]. It could be deduced that the high-solid anaerobic digestion of food waste may reduce inhibition levels of Na^+ and help maintain satisfactory stability during the conversion of substrates into biogas. Aceticlastic methanogens are more significant during high-solid anaerobic digestion processes, perhaps because aceticlastic methanogens are not inhibited by the Na^+ level and the accumulation of volatile fatty acids.

Changes in the Abundance of Bacterial and Archaeal Communities

Fig. 4 shows the significant changes in the amounts of bacterial, archaeal, and order-level methanogenic populations during the whole incubation period as estimated using real-time PCR of the 16S rRNA genes, respectively. The bacterial 16S rRNA gene concentration initially reached its peak (*i.e.*, 4.3×10^{11} copies/ml) at 2.21 days, and then decreased continuously down by 95% to 2×10^{10} copies/ml at the end of the reaction. This rapid change corresponded well to the substrate consumption profiles shown in Fig. 2A. However, the concentration of bacterial 16S rRNA genes did not change significantly, remaining at a relatively high level of 10^{10} copies/ml. This persistence was assumed to be because bacterial populations were active and playing various roles (*e.g.*, syntrophic propionate oxidation and saturated fatty acid oxidation) even during the active methanogenic period [26]. The concentration of archaeal 16S rRNA genes increased sharply from 1.3×10^8 to 5.7×10^9 copies/ml (a 43-fold increase) on day 10. After day 10, the archaeal 16S rRNA gene level also did not change significantly, remaining above 5.0×10^9 copies/ml. This corresponded to the 16S rRNA gene levels of methanogens or archaea quantified in another report [42].

The aceticlastic methanogens, including the *Methanosarcinales* order and hydrogenotrophic methanogens, *Methanomicrobiales*, *Methanobacteriales*, and *Methanococcales*, behaved differently in terms of their abundance (Fig. 4). The 16S rRNA gene level of *Methanosarcinales* increased from 3.2×10^8 to 4.9×10^9 copies/ml (a 15.3-fold increase) between days 6 and 27. During this period, the acetate concentration gradually decreased to zero (Fig. 2B). The *Methanosarcinales* level increased steeply between days 8 and 10, corresponding to the period when the biogas production rate increased sharply (Fig. 1). The concentration of *Methanomicrobiales* 16S rRNA genes increased sharply during the early incubation period. This trend coincided with the elevation of VFA concentrations. Similarly with the archaeal result, the *Methanomicrobiales* gene concentration did not change significantly after day 8, remaining above 7.0×10^7 copies/ml. No distinct growth of *Methanobacteriales* was observed, and Methanococcales were not detected throughout the incubation period, which was in accordance with the result

for the batch anaerobic digestion of secondary sludge [37]. The relative abundance of methanogenic populations are visualized in Fig. 5. The seed inoculums contained 5.4×10^6 and 1.1×10^8 copies/ml of methanogenic 16S rRNA genes with *Methanomicrobiales* and *Methanosarcinales* as the major populations, respectively. The aceticlastic *Methanosarcinales* was the most abundant population when the substrate and seed inoculums were mixed, and dominated throughout the incubation process, though the *Methanomicrobiales* gene concentration increased sharply during the initial incubation period (Fig. 4). The 16S rRNA gene ratio of *Methanosarcinales* revealed its maximum of 98.6% at day 15 and remained greater than 84% thereafter (Fig. 5).

Acetate is regarded as the most important intermediate metabolite in anaerobic digestion [35] and can be directly utilized by aceticlastic methanogens. *Methanosarcina* and *Methanosaeta* are the only genera known to utilize acetate as a substrate for methanogenesis [20]. *Methanosarcinales* was the most abundant methanogenic group (Fig. 5), and the genus *Methanosarcina* (Band A4, A6–13) was the only aceticlastic methanogen visualized in the archaeal DGGE profile (Table 2), which suggests that this acetate-utilizing (acetoclastic) methanogen was mainly driving methanogenesis in this high-solid anaerobic digestion of food waste. Some previous studies have also reported the dominance of aceticlastic methanogens in mesophilic anaerobic digestion [21, 40]. However, it is noteworthy that *Methanosaeta* was dominant in mesophilic anaerobic digestion in other studies [39]. Generally, the relative abundance of these two groups is mainly regulated by acetate concentrations as seen in environmental samples [9]. *Methanosarcina* strains have faster growth rates and a lower affinity for acetate, whereas *Methanosaeta* have slower growth rates and a higher affinity for acetate [21, 23, 26]. The predominance of *Methanosarcina* over *Methanosaeta* is a consequence of the high acetate concentration. The acetate concentration increased rapidly and was obviously higher than other volatile fatty acids during most of the incubation period in this system (Fig. 2B). *Methanosarcina*, with its faster growth rates in high acetate concentrations, managed to out-compete *Methanosaeta*. The relative abundance of these two groups is also regulated by feeding rates to the bioreactor [9]. *Methanosaeta* strains reside better in digesters with a high feeding rate, such as an upward-flow anaerobic sludge blanket (UASB), presumably due to their efficient adhesion and granulation [23]. In contrast, *Methanosarcina* strains are more sensitive to turbulence and shear, and frequently dominate in fixed- and stirred-tank digesters [24].

Hydrogenotrophic methanogenesis is also one of the major methanogenic pathways in anaerobic digesters [37]. Low hydrogen partial pressure must be maintained for syntrophic consortia to utilize various intermediates because of the thermodynamic limitations of hydrogen-mediated metabolism [4]. The organic fractions of the FW are mainly composed of lipids, proteins, and starch, and the lipids and proteins are degraded into amino acids and long-chain fatty acids (LCFA). More acetate and H_2 are produced in high-solid FW owing to the high contents of lipids and proteins. Therefore, the efficient removal of hydrogen by hydrogen-utilizing microorganisms is necessary for acidogenesis and/or acetogenesis to occur. In this study, hydrogenotrophic methanogens *Methanomicrobiales* and *Methanobacteriales* increased steeply during the initial incubation period and were detected by real-time PCR throughout the entire incubation process, although these two groups were not detected in the archaeal DGGE profile (Fig. 3B). Note that this bias could have been generated by the method of DNA extraction, the choice of the primer set for PCR, and the drawbacks of the DGGE method itself, to name a few reasons. It is speculated that at least one hydrogen methanogen existed in this system and was in part responsible for the methanogenesis through syntrophism with hydrogen-producing bacteria (*e.g.*, the syntrophic bacterium corresponding to B11).

This study has helped to illustrate the changes in microbial community structure and dynamics that occur during the batch high-solid anaerobic digestion of food waste. The microbial community shifts correlated well with the performance data during the whole incubation period, suggesting the possibility of diagnosing an anaerobic digestion process by monitoring microbial community shifts. Because the end-of-batch quantitative structure of the methanogenic community was stable, the operation of this batch reaction could provide an efficient means for the enrichment of methanogens during the start-up period of a continuous system. However, because this study was carried out on a single substrate and with no changes in operating conditions, it was not possible to evaluate which factors were more decisive for shaping the microbial community. This suggests that it is necessary to conduct quantitative comparisons and correlations of the effects of plural factors on reactor microbial communities in further studies. In isolation, the batch results are relevant to the start-up period of a continuous system and can also provide useful information for the establishment of a continuous operation.

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