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Methanogenic community dynamics in anaerobic co-digestion of fruit and vegetable waste and food waste

Jia Lin, Jiane Zuo*, Ruofan Ji, Xiaojie Chen, Fenglin Liu, Kaijun Wang, Yunfeng Yang

State Key Joint Laboratory of Environment Simulation and Pollution Control (SKLESPC), School of Environment, Tsinghua University, Beijing 100084, China. E-mail: tsinghuaenv@gmail.com

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Abstract

A lab-scale continuously-stirred tank reactor (CSTR), used for anaerobic co-digestion of fruit and vegetable waste (FVW) and food waste (FW) at different mixture ratios, was operated for 178 days at the organic loading rate of 3 kg VS (volatile solids)/(m^3·day). The dynamics of the Archaeal community and the correlations between environmental variables and methanogenic community structure were analyzed by polymerase chain reactions – denaturing gradient gel electrophoresis (PCR-DGGE) and redundancy analysis (RDA), respectively. PCR-DGGE results demonstrated that the mixture ratio of FVW to FW altered the community composition of Archaea. As the FVW/FW ratio increased, *Methanoculleus*, *Methanosaeta* and *Methanosarcina* became the predominant methanogens in the community. Redundancy analysis results indicated that the shift of the methanogenic community was significantly correlated with the composition of acidogenic products and methane production yield. Different mixture ratios of substrates led to different compositions of intermediate metabolites, which may affect the methanogenic community. These results suggested that the analysis of microbial communities could be used to diagnose anaerobic processes.

Key words: high-solid organic waste; anaerobic co-digestion; methanogenic community structure; denaturing gradient gel electrophoresis (DGGE); redundancy analysis (RDA)

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Introduction

Anaerobic digestion is an efficient method to treat high-solid organic waste to generate renewable energy such as biogas. It is a biological process that can be divided into four main phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis. These reactions are mediated by many microbial species, which could be classified into two categories, acidogenic bacteria and methanogenic Archaea. Characterization of microbial community structure in anaerobic digesters has attracted engineers' interests since understanding the microbial behaviors is essential for improving the performance of digestion processes (Shin et al., 2010). However, the diversity of microbes involved in anaerobic digestion and their responses to changes of management practices and environmental conditions are often overlooked because of the complex microbial ecology involved. As a result, the anaerobic digestion process is often treated as a "black box" (Supaphol et al., 2011). Recently, the development of culture-independent molecular technologies such as 16S rDNA gene analysis has greatly promoted the studies of microbial communities (Demirel and Scherer, 2008). The use of advanced molecular biology techniques is of critical importance for understanding and clarifying the sophisticated reactions which take place in biogas digesters. Particularly, the response of Archaea to the operating conditions of digesters should be clearly understood to achieve stable, efficient reactor management. Therefore, new molecular techniques have been developed in the last decade to support the microbial ecology research. A lot of studies have been carried out on the behavior and activity of methanogens in digesters treating some complex types of industrial wastewaters and simple types of soluble, mostly synthetic substrates (Demirel and Scherer, 2008), but studies of microbial community structure in anaerobic treatment of particulate solid substrates are relatively scarce in literature.

Recently, anaerobic co-digestion, which mixes different wastes (solid or liquid organic wastes) with complementary characteristics in a single digester, has been widely used for production of biogas from various substrates (Bouallagui et al., 2009; Lin et al., 2011). The main advantages of anaerobic co-digestion are as follows: (1) improved methane production yield due to the mixed supply of additional nutrients from co-substrates; (2) more efficient utilization of equipment; and (3) shared costs by processing multiple waste streams in a single facility (Alatriste-Mondragon et al., 2006).

* Corresponding author. E-mail: jiane.zuo@tsinghua.edu.cn

Fruit and vegetable waste (FVW) and food waste (FW) are two major types of municipal organic solid wastes in China. The low cellulose content and C/N ratio of the wastes may accelerate ammonia release, resulting in inhibition of methanogenesis in digesters and low biogas production. To resolve this problem, it has been observed that implementing co-digestion of different waste streams in single-stage anaerobic digestion systems could improve biogas production (Bouallagui et al., 2009; Habiba et al., 2009; Lin et al., 2011). Therefore, it is interesting to investigate the molecular microbial mechanism of co-digestion.

Previous studies have reported that intermediate metabolites produced by acetogens may be important for the growth and community structure of the methanogens in sequential batch systems conducting co-digestion of food waste and biosolids anaerobically (Dearman et al., 2006). Inoculum and loading rates seemed not to affect the diversity of methanogens; instead they affected the concentration of ammonia and volatile fatty acids (VFA). Methanosarcinaceae was dominant at high levels of NH_3 and VFA, while Methanosaetaceae dominated with low levels of NH_3 and VFA (Karakashev et al., 2005). Another study showed that both resource availability and environmental factors are key driving forces in microbial community dynamics of mesophilic anaerobic co-digestion of mixed wastes (Supaphol et al., 2011). Nevertheless, few studies have monitored the community dynamics in the anaerobic co-digestion process at different mixture ratios or analyzed the relationship between microbial diversity and environmental variables.

In this study, the molecular technology PCR-DGGE (denaturing gradient gel electrophoresis) was used to investigate the Archaeal community structural dynamics in the process of anaerobic co-digestion of FVW and FW at different mixture ratios. Redundancy analysis (RDA), a multivariate analysis method based on an iterative process of reciprocal averaging/correspondence analysis ordination and multiple regressions, was used to identify correlations between environmental variables and methanogenic community dynamics in an anaerobic digester. Recently, multivariate statistical techniques have been successfully applied in wastewater treatment systems to study the environmental effects on microbial community structure and the dynamics of microbial populations (Kennedy et al., 2004; Macdonald et al., 2009). For example, the results indicated that Methanobacteriales and Methanosarcinales populations were closely related to chemical properties such as VFAs in anaerobic batch digesters treating swine wastewater, whereas the correlation between Methanomicrobiales and propionate was shown to be different through RDA analysis (Kim et al., 2010).

1 Materials and methods

1.1 Reactor operating conditions

A lab-scale continuously-stirred tank reactor (CSTR) equipped with temperature controller, with a working volume of 4 L, was operated for 178 days at the organic loading rate of 3 kg VS/(m^3·day). The operating temperature was kept at (35 ± 1)°C by the temperature controller. Anaerobic granular sludge with good methanogenesis activity was used as inoculum to the digester, which was taken from a full-scale UASB reactor treating starch-processing wastewater at 35°C in Qinhuangdao City, Hebei, China. Raw FVW were collected from a fruit and vegetable market in Beijing, China in different seasons of 2009; the raw FVW mainly contained residues of vegetables such as Chinese cabbage, carrot, lettuce, and different fruits, such as apple, banana, pear, and watermelon. Raw FW were daily collected for one week from a dining hall at Tsinghua University, Beijing, China. The raw FW mainly contained leftovers of cooked foods, such as meat, fish, rice, bread, noodles and vegetables. The characteristics of FVW and FW are shown in Table 1. The digester was operated with a draw and fill method – the mixed digesting residue was discharged out of the digester and the raw materials were fed into the digester daily with a peristaltic pump. The test mixture ratios of FVW to FW based on volatile solid (VS) contents were 100:0, 67:33, 50:50, 33:67 and 0:100 (m/m), respectively.

1.2 DNA extraction

The broth in the CSTR was homogenized before sampling. Samples with a volume of 50 mL were collected in centrifuge tubes at the end of each mixture ratio–specified stage. The tubes were centrifuged at 15,000 r/min for 20 min at 4°C (CR22G, HITACHI, Japan), with the supernatant removed. Total community DNA was extracted from the solid fraction using the Fast DNA kit for soil (MP, USA). All extraction steps were carried out according to the manufacturer's protocols. The concentration and purity of DNA were measured by a UV spectrophotometer (ND-2000, Nano Drop, USA) at 260 and 280 nm, and checked by 0.8% agarose gel electrophoresis.

1.3 DGGE and phylogenetic analysis

A nested polymerase chain reaction (PCR) approach was used to amplify 16S rRNA genes of Archaea. In the first round, primers ARCH46f and ARCH1017r were applied, and in the second round, primers pARCH344f-GC and UNIV522r were applied (Roling et al., 2006). A touchdown PCR was conducted in a PTC-200 instrument (MJ research, Watertown, MA, USA) as follows: 94°C for 5

Table 1 Characteristics of the fruit and vegetable waste and food waste

	pH	Total solid (%)	Volatile solid (wt.% TS)	Elemental compositions (wt.% TS)				C/N ratio
				C	H	O	N	
FVW	4.24	7.4	88.1	43.3	5.2	38.0	2.8	15.6
FW	3.55	22.2	92.5	51.0	7.3	29.2	3.0	17.2

TS: total solids; FVW: fruit and vegetable waste; FW: food waste.

Table 2 Primers used in PCR amplification of Archaea 16S rRNA genes*

Target	Primer	Sequence (5′–3′)
First round	ARCH46f	YTAAGCCATGCRAGT
	ARCH1017r	GGCCATGCACCWCCTCT
Second round	ARCH344f	HGCAGCAGGCGCGA
	UNIV 522r	GWATTACCGCGGCKGCTG
GC-clamp	On ARCH344f	CGCCCGCCGCGCGCGGCGGG
		CGGGGCGGGGGCACGGGGGG

* Roling et al., 2006.

min, 20 cycles of 94°C for 1 min, annealing at 62 to 52°C (reducing the temperature by 0.5°C per cycle) for 1 min, and extension at 72°C for 1.5 min; additional 5 cycles of 94°C for 1 min, 52°C for 1.5 min, and 72°C for 1.5 min; and final extension at 72°C for 10 min.

Denaturing gradient gel electrophoresis was performed with the Bio-Rad DCode^{TM} system (Bio-Rad, Hercules, CA, USA). The PCR product was loaded on 8% (*W*/*V*) polyacrylamide gels containing a 50% to 70% linear denaturing gradient (100% denaturant contained 7 mol/L urea and 40% (*V*/*V*) deionized formamide). Electrophoresis was performed at 130 V for 6 hr in 1× TAE buffer. The gel was stained with Gel-Red and photographed under UV transillumination (Gel Doc XR, Bio-Rad, Hercules,USA). DGGE images were analyzed via Quantity One (Bio-Rad, Hercules, CA, USA). Dominant DNA bands were excised from the gel, eluted in 30 μL of sterile water, and reamplified with the same primers (without GC-clamp). The resulting fragments were purified and then cloned into the pGEM-T Easy vector (Promega, Madison, WI, USA), followed by sequencing analysis using T7 primer. Subsequently, the sequencing results were compared against the GenBank databases. Sequence alignment and neighbor-joining phylogenetic tree construction were carried out using the MEGA software version 4.0.

1.4 Analytical methods

The following parameters were analyzed: pH, total alkalinity, soluble chemical oxygen demand (sCOD), ammonium concentration, volatile fatty acids (VFAs), total solids (TS), and volatile solids (VS) as described previously (Lin et al., 2011). To quantify the diversity of Archaea, the Shannon index (H') was computed (Nettmann et al., 2008).

1.5 Ordination

The correlation of environmental factors and band occurrence was conducted using Canoco analysis (Canoco 4.5, Biometris, Wageningen, The Netherlands), where the following statistical analysis were undertaken. The band matrices were designated as representing species occurrence and the environmental variables, which were volatile fatty acid (VFA), NH_4^+, methane production yield (MPY) and mixture ratio.

Detrended correlation analysis (DCA) was applied to determine the size of the data gradient and to indicate the best methodology to find the main factors that influence community composition. Once a linear correlation was achieved for the analyzed groups (gradient size lower than 4.0), redundancy analysis (RDA) was used. To verify the significance of environmental variables in the composition of Archaeal communities, the non-parametric Monte Carlo permutation test was applied with 499 random permutations. In addition to P values for significance of each environmental factor, RDA and Monte Carlo permutation tests supplied information about the marginal effects of environmental variables, quantifying the amount of variance explained by each factor.

2 Results and discussion

2.1 Reactor performance

The digester was operated stably and efficiently at five different FVW/FW mixture ratios (100:0, 67:33, 50:50, 33:67 and 0:100, *m*/*m*) and the performances of the reactor at steady state are summarized in Table 3. The NH_4^+-N and total VFA concentrations were estimated to be lower than the inhibitive concentrations reported for methanogens (Anderson et al., 1982, Angelidaki and Ahring, 1993). The maximum methane production yield (MPY) was 0.49 m^3 CH_4/kg VS at the OLR of 3 kg VS/(m^3·day) with the mixture ratio of 50:50 (*m*/*m*). When the ratio of FVW to FW in influent changed to 33:67 (*m*/*m*), the average concentration of NH_4^+-N and total VFA in effluent increased to 1242.1 and 1216.5 mg/L,

Table 3 Summary of performance parameters in different operational phases

	Phase I Day 0–30	Phase II Day 30–60	Phase III Day 60–90	Phase IV Day 90–131	Phase V Day 131–178
Substrates					
FVW (kg VS/(m^3·day))	3	2	1.5	2	0
FW (kg VS/(m^3·day))	0	1	1.5	1	3
TS (%)	7.4	9.4	11.0	13.1	22.2
VS (%)	6.5	8.4	9.7	11.8	20.5
Effluent characteristics					
pH	7.37 ± 0.03	7.40 ± 0.03	7.56 ± 0.03	7.73 ± 0.02	7.07 ± 0.14
NH_4^+-N (mg/L)	585.3 ± 61.7	608.9 ± 27.0	763.9 ± 25.8	1242.1 ± 60.0	2329.7 ± 143.5
VFA (mg/L)	69.7 ± 11.4	181.7 ± 33.6	170.64 ± 44.0	1216.5 ± 77.2	8887.0 ± 754.1
Digester performances					
Biogas production rate (m^3/(m^3·day))	2.17	2.25	2.35	2.45	0.35
Methane production yield (m^3 CH_4/kg VS)	0.42	0.44	0.49	0.49	0.06

respectively. Furthermore, acetate was the major fermentation product, which comprised 92% of total VFA. The VFA production and utilization rates were unbalanced. As a result, the MPY remained unchanged due to the incomplete utilization of acetic acid. Moreover, no anaerobic digestion was observed due to VFAs accumulation when the FW was fed as the only substrate. The NH_4^+-N and TVFAs concentration were 2329.7 and 8887.0 mg/L, respectively. The concentration of each individual acid is as described previously (Lin et al., 2011).

2.2 Methanogenic community analysis

The microbial community involved in the anaerobic digestion of biowaste is affected by changes of environmental conditions. Therefore, it is crucial to relate the microbial community structure to the process of the anaerobic digestion of biowaste, which would be helpful for the operation and optimization of biogas plants (Nettmann et al., 2008). DGGE, followed by phylogenetic analysis, was applied to investigate the dynamic of methanogenic community structures. The total DNAs were extracted from five sludge samples (Sample 1–5) when the reactor was run at a steady state in each mixture ratio and used for PCR targeting Archaeal 16S rDNA genes. After obtaining DNA bands by DGGE (Fig. 1, Table 4), the phylogenetic affiliations of the bands, as shown in Fig. 2, were determined by comparison against the GenBank database.

A total of 22 distinct bands were observed (Fig. 1). The band profiles varied among samples, indicating clear changes of the composition of methanogen communities during the anaerobic digestion at different mixture ratios of FVW to FW. This was confirmed by the community diversity Shannon index, which fluctuated over different mixture ratios and ranged from 0.73 to 0.90. The species composition of the methanogenic community clearly differed between Sample 2 and other samples. Notably, bands 6 and 7 were two dominant bands in Samples 4 and 5.

Band 6 was closely matched to *Methanosaeta* sp., which is widely distributed in nature owing to its specifically high affinity for acetate (Fig. 2). Bands 17–19 were the dominant bands in samples 1 and 3, and two of the dominant bands in Samples 4 and 5. Band 17 showed 99% sequence similarity with *Methanosarcina mazeii*, which was isolated from a broad range of environments, including sediments and digesters (Joulian et al., 1998). *Methanosarcina* was one of the only two genera of methanoarchaea, known to use acetate as substrate for methanogenesis. *Methanosarcina* prefers methylated compounds such as methanol and methylamines as compared to acetate (Shin et al., 2010). Band 19 is related to *Methanoculleus* sp., which has also been previously identified in biogas processes (Feng et al., 2010; Krober et al., 2009).

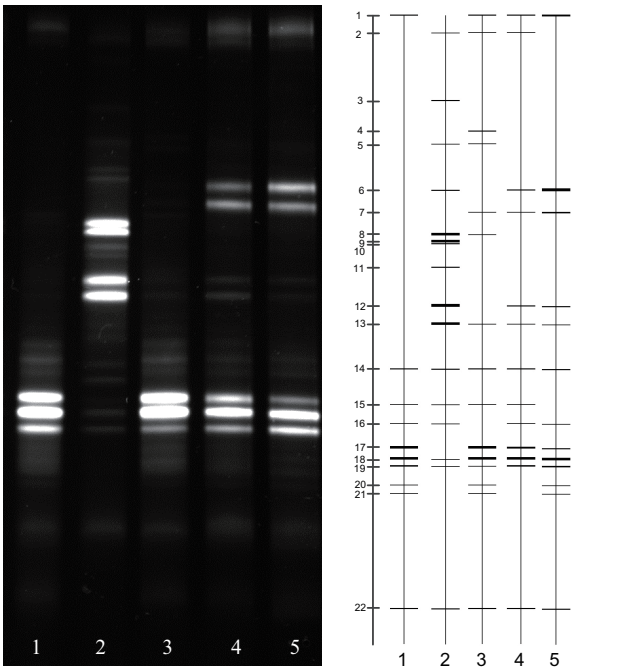

Fig. 1 Archaeal DGGE profiles of the 16S rRNA gene PCR products generated from DNA extracted from bioreactor biomass during the trial. 1–5 mean five sludge samples; 1–22 mean distinct bands.

Bands 8, 9, 12 and 13 were the dominant bands in Sample 2. Band 8 was matched with Methanomicrobiales. The hydrogenotrophic pathway is represented by Methanomicrobiales, which is closely related to extremely halophilic Archaea (Bapteste et al., 2005). Band 12 shares 100% homology with an Archaeal clone isolated from a novel anaerobic digester treating organic waste (Nelson et al., 2010). Band 13 was matched with an uncultured Methanosarcinales achaean in limonene-degrading methanogenic cultures.

Acetate is regarded as the most important intermediate metabolite in anaerobic digestion (Sasaki et al., 2011). Acetate can be directly utilized by aceticlastic methanogens such as *Methanosarcina* spp. *Methanosarcina* are sensitive to turbulence and shearing, and frequently dominate in fixed- and stirred-tank digesters (Liu and Whitman, 2008). The organic fractions of FVW include sugar, cellulose, hemicellulose and lignin, while FW were mainly composed of lipid, protein and starch. When FVW was the only substrate fed into digester, *M. mazeii* and *Methanoculleus* sp. were the dominant methanogens. When FW was added into digester, the lipid and protein were degraded into amino acids and long chain fatty acids (LCFA). More acetate and H_2 were produced in FW due to the high

Table 4 Similarities between the DNA extracted from the bands and known species

Band	Closest match	Match (%)
6	*Methanosaeta* sp. enrichment culture clone A22130 16S ribosomal RNA gene, partial sequence	100
8	Uncultured Methanomicrobiales archaeon gene for 16S rRNA, partial sequence	100
12	Uncultured archaeon clone NBLA23C 16S ribosomal RNA gene, partial sequence	100
13	Uncultured Methanosarcinales archaeon partial 16S rRNA gene, clone LiM 2B-7A	100
17	*Methanosarcina mazeii* strain SarPi 16S ribosomal RNA gene, complete sequence	99
19	Uncultured *Methanoculleus* sp. clone ARK2_4 16S ribosomal RNA gene, partial sequence	100

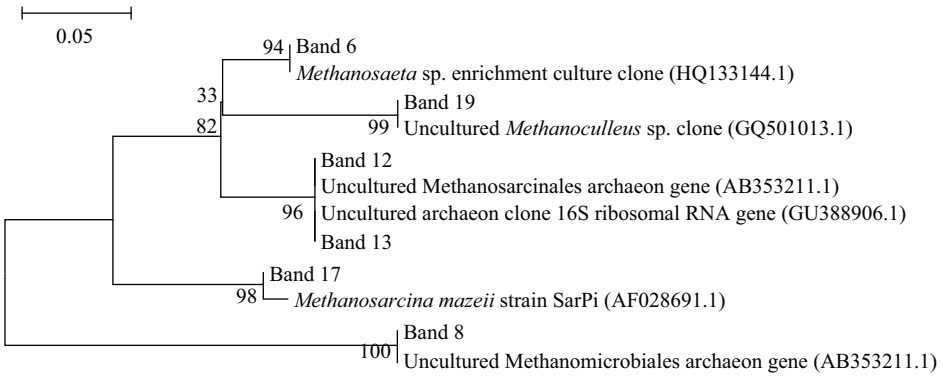

Fig. 2 Neighbor-joining tree showing the phylogenetic affiliation of DGGE band sequences.

contents of lipid and protein. In phase II, with the addition of FW, the TVFA concentration increased to 181.7 mg/L. The dominant species in Sample 2 altered to Methanomicrobiales and Methanosarcinales-like Achaean. This can be explained by the shift in methane production routes. The high H_2 partial pressures in the digester might be one reason for the promotion of the growth of hydrogenotrophic methanogens such as Methanomicrobiales (Liu and Whitman, 2008).

The optimal performance was achieved at the mixture ratio of 50:50 (m/m). The stable performance implies the maintenance of well-established methanogenic consortia throughout the reaction period. The methanogenic community in Sample 3 changed back to nearly the same community structure as in Sample 1. *M. mazeii* has the same metabolic capacity as other *Methanosarcina* spp. (Osumi et al., 2008). The substrates which *M. mazeii* is capable of utilizing are H_2/CO_2, acetate, all methylamines and methanol (Osumi et al., 2008). This differs from *M. acetivorans*, which cannot subsist on H_2/CO_2 utilization for they lack a functional H_2 utiliser (Maeder et al., 2006). In addition, it provides a certain versatility for *M. mazeii*, which allows utilization of a variety of substrates and environments. The high H_2 partial pressure and acetate concentration might be one reason for the variation in culture development. The dominant aceticlastic methanogens made a contribution to the small decrease of VFA concentration despite the fact that the amount of FW increased. It is also likely that the variation was caused by the salinity. Previous studies reported that *M. mazeii* did not aggregate in intermediate salinity, but lower or higher salinity allowed or caused *M. mazeii* to aggregate (Tenchov et al., 2006). This dual behavior is relatively unique even for other *Methanosarcina* spp. because the others do not naturally disaggregate and in fact, have difficulty living singly. The total saline concentration (Na^+, Ca^{2+}, Cl^-) was about 3%. It is noted that a high saline concentration is present in typical Chinese food waste. At the mixture ratio of 67:33 (m/m), the saline concentration increased to an intermediate level due to the addition of FW and caused the disaggregation of *M. mazeii*, resulting in loss of this species in the effluent. When the amount of FW in influent increased to over 50%, the high salinity allowed *M. mazeii* to aggregate. Complex substrates with high acetate concentration and H_2 pressure will support the growth of *M. mazeii*. Acetic acid and propionic acid accumulated when the percentage of FW was over 50%. This was indicative of inhibition of syntrophic propionate-oxidizers as well as inefficient utilization of acetate by methanogens, which was also reflected by a lower methane yield (Ziganshin et al., 2010). Accordingly, hydrogenotrophic methanogens (*Methanoculleus*) were dominant. It is possible that acetate was metabolized by acetotrophic microorganisms via syntrophic acetate oxidation delivering hydrogen and carbon dioxide funneled into hydrogenotrophic methanogenesis (i.e., *Methanoculleus*). In the literature, it was also reported that *Methanoculleus* was dominant in a biogas process containing high levels of ammonium and VFA, where acetate was degraded via syntrophic acetate oxidation (Wagner et al., 2011, Ziganshin et al., 2010). This could explain the dominance of *Methanoculleus* in the last 3 samples.

Usually, only one aceticlastic methanogen group, *Methanosaeta* or *Methanosarcina*, dominates each digester, depending upon the types of waste and digester (Leclerc et al., 2004). *Methanosaeta* have slower growth rates and higher affinity for acetate, while *Methanosarcina* have faster growth rates and lower affinity for acetate

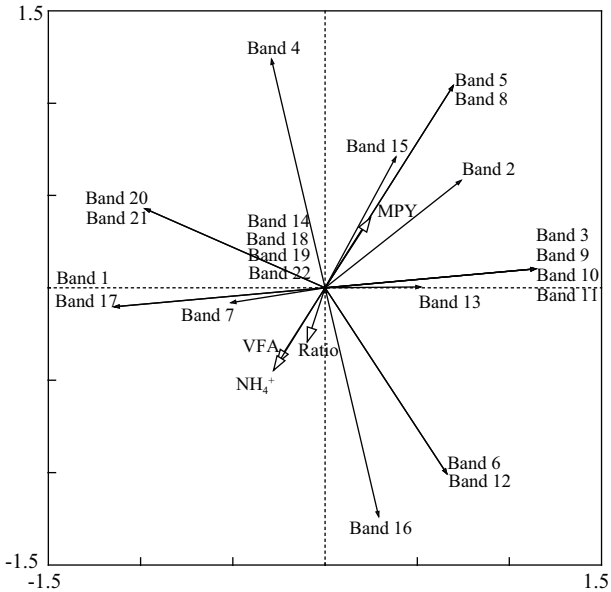

Fig. 3 Redundancy analyses of shifts in the structure of methanogenic community and their correlation between species, VFA, NH_4^+, MPY and mixture ratio.

(Liu and Whitman, 2008). The relative abundance of these two groups is not only regulated by acetate concentrations as seen in other environments, but also by feeding rates (Aiyuk et al., 2006; Conklin et al., 2006). Methanosaeta reside better in digesters with high feeding-rate, such as an upward-flow anaerobic sludge blanket (UASB), presumably due to their efficient adhesion and granulation. In contrast, *Methanosarcina* are more sensitive to turbulence and shear, and frequently dominate in fixed- and stirred-tank digesters (Liu and Whitman, 2008). Band 6 representing *Methanosaeta* of Samples 4 and 5 became brighter than the band 17 representing *Methanosarcina*. These findings contrasted with those of previous studies. One explanation is the adhesion of organism cells. Some researchers reported that the competition between *Methanosaeta* and *Methanosarcina* might be affected by other factors, such as adhesion, and feast and famine conditions (Angenent et al., 2002; Conklin et al., 2006). The individual *Methanosaeta* cells form long filaments in anaerobic biomass, while *Methanosarcina* cells grow as cocci. Lower mixing intensity emerged with higher relative level of *Methanosaeta* and lower relative level of *Methanosarcina* (Hoffmann et al., 2008). In addition, Schmidt's research showed that *Methanosarcina* might serve as inert support material for the growth of *Methanosaeta* (Schmidt and Ahring, 1999). It could form multicellular aggregates to resist the inhibition caused by a high level of VFA, because the slow diffusion rate of the acid limits the concentration of VFA inside the aggregates, which provides another explanation for this phenomenon. It should be mentioned that the cell morphology and acetate accumulation hypotheses are not exclusive and both could play a role in the competition of *Methanosaeta* and *Methanosarcina*. However, further studies are still necessary.

2.3 Correlation between environmental factors and methanogenic community dynamics

To quantify the relationship between environmental factors and changes of the microbial community, RDA was conducted. As shown in Fig. 3, it generated a direct gradient ordination that was related to two sets of variables: the dependent species data and the independent environmental data. For the plot, the eigenvalue was 0.742, meaning that summation of each variable's dispersion could provide an explanatory power of 74.3% for the respective model. The results showed that aceticlastic methanoarchaea (bands 6, 12 and 17) had a positive correlation with VFA, NH_4^+, and mixture ratio, and a negative correlation with MPY. The major factor determining the ability to produce methane is the Methanomicrobiales arrow (band 8) line in RDA. In contrast, *Methanoculleus* had no correlation with those environmental variations. The joint-plot RDA results suggested that the community shifts were more significantly correlated with NH_4^+, VFA and MPY than mixture ratio, implying that the intermediate metabolites generated from hydrolysis and acidification are closely related to the diversity of a microbial community. The results are consistent with the findings of a previous study, which showed that that intermediate metabolites produced by acetogens are important for the community structure of the methanogens (Dearman et al., 2006).

It could be expected that the methanogen community structure would be affected by the composition of acidogenic products, which are further utilized for methanogen growth. This corresponds to the biphasic production of biogas. The results clearly demonstrated that the community structure profiles of methanogens developed in a totally divergent manner during the anaerobic co-digestion trials. This, along with the fact that the functional property of an anaerobic digester is closely related to the relative abundance of microbial populations and the community composition (Lee et al., 2010), suggests that more attention should be paid to bacterial, as well as Archaeal, communities for an in-depth view of the relationship between the microbial communities and environmental factors in methanogenic environments.

3 Conclusions

In this study, the molecular technology PCR-DGGE was used to investigate the Archaea community structural dynamics in anaerobic co-digestion of FVW and FW with different mixture ratios. It demonstrated that the mixture ratio of FVW to FW is a factor affecting changes of the Archaeal community structure. *Methanoculleus*, *Methanosaeta* and *Methanosarcina* became the predominant methanogen with the addition of FW. The methanogenic community shift is significantly correlated with the composition of acidogenic products and the methane production yield. The microbial community analysis could be used to diagnose other anaerobic processes.

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