Bioresource Technology 143 (2013) 512–518

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Molecular characterization of bacterial and archaeal communities in a full-scale anaerobic reactor treating corn straw

Jiang-Tao Qiao [a,b], Yan-Ling Qiu [a,*], Xian-Zheng Yuan [a], Xiao-Shuang Shi [a], Xiao-Hui Xu [a], Rong-Bo Guo [a,*]

[a] Key Laboratory of Biofuels, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao, Shandong Province 266101, PR China
[b] University of Chinese Academy of Sciences, Beijing 100049, PR China

HIGHLIGHTS

- The anaerobic degradation pattern of corn straw for biogas production was investigated.
- Besides volatile acids, aromatic compounds are intermediates in anaerobic degradation.
- Hydrolytic and fermentative microorganisms dominate the bacterial community.
- High proportion of syntrophic propionate and aromatic acids degrading bacteria were detected.
- Hydrogenotrophic methanogens were more dominant than aceticlastic methanogens.

ARTICLE INFO

Article history:
Received 28 April 2013
Received in revised form 3 June 2013
Accepted 5 June 2013
Available online 13 June 2013

Keywords:
Anaerobic degradation
Corn straw
Microbial community
16S rRNA gene clone library

ABSTRACT

A 16S rRNA gene-based method was used to characterize the structure of bacterial and archaeal communities in a full-scale, anaerobic reactor treating corn straw. Degradability experiment indicated biogas slurry had high microbial activity, the TS removal rate was 53% and the specific methanogenic activity was 86 mL CH_4 g VSS^{-1} d^{-1}. During anaerobic degradation of corn straw, volatile acids and aromatic compounds (*p*-cresol, phenylpropionate, phenol and benzoate) were detected as transient intermediates. Phylogenetic analysis revealed bacterial community exhibited high diversity, 69 bacterial phylotypes in 13 phyla were identified. *Firmicutes* (48.3%), *Chloroflexi* (20.1%), *Actinobacteria* (9.1%), *Bacteroidetes* (7.7%), and *Proteobacteria* (7.2%) represented the most abundant bacterial phyla. Hydrolytic and fermentative bacteria were major bacterial populations. Moreover, a relatively high proportion of syntrophic propionate and aromatic compounds degrading bacteria were detected. In the archaeal clone library, 11 archaeal phylotypes affiliated with two phyla of *Crenarchaeota* (10%) and *Euryarchaeota* (90%) were identified.

1. Introduction

Crop residues, such as corn straw, wheat straw, and rice straw are abundant and important renewable biomass resources. Anaerobic digestion is a promising alternative for the treatment of organic wastewater and solid waste, as the process combines waste treatment and energy recovery. Currently, anaerobic digestion has been widely applied for the treatment of most of biodegradable waste, e.g., municipal sewage sludge, kitchen waste and animal manures. Anaerobic digestion of straw has been studied for nearly a century (Acharya, 1935). However, the implementation of full scale biogas plants used straw as the sole feedstock has not yet been demonstrated. The major reason is that straw consists mainly of cellulose, hemicellulose and lignin, which are extremely recalcitrant to degradation. Numerous lab-scale experiments have focused on the pretreatment of lignocellulose to break down the structural integrity of lignocellulosic biomass and to enhance enzymatic action on cellulose, determination of optimal parameters of anaerobic straw digestion, and co-digestion with other wastes (Dinuccio et al., 2010; Zhong et al., 2011, 2012). Whereas, the knowledge of the microbial community involved in anaerobic digestion system for the treatment of crop residues is still limited.

Anaerobic bioconversion of lignocellulosic biomass to methane needs cooperation of complex microbial populations, including hydrolytic (celluloytic), saccharolytic, homoacetogenic, syntrophic hydrogen-producing bacteria and methanogenic archaea. The anaerobic decomposition of crop residues occurs naturally, the process and the functional groups of microorganisms participated in anaerobic degradation of soil organic matter have been revealed, especially on methanogens in anoxic rice fields (Großkopf et al.,

* Corresponding authors. Address: Key Laboratory of Biofuels, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, No. 189 Songling Road, Qingdao, Shandong Province 266101, PR China. Tel./fax: +86 532 80662750.
E-mail addresses: qiuyl@qibebt.ac.cn (Y.-L. Qiu), guorb@qibebt.ac.cn (R.-B. Guo).

0960-8524/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.06.014

1998; Lu et al., 2000) and straw-rotting soils (Conrad et al., 2012). Furthermore, several artificial microbial systems treating lignocellulosic biomass have also been investigated, such as an anaerobic mixed fermentation reactor fed with maize silage, green rye and liquid manure (Kröber et al., 2009), an anaerobic microbial system decomposing poplar wood chips (van der Lelie et al., 2012). However, no studies have characterized the microbial community in a pilot-scale or full-scale biogas reactor with corn straw as the sole feedstock. In this study, the structure of bacterial and archaeal communities in a full-scale biogas reactor treating corn straw was investigated by 16S rRNA sequencing analysis. In addition, the characteristics of anaerobic corn straw degradation and intermediates production were analyzed.

2. Methods

2.1. Source of biogas slurry

Anaerobic biogas slurry was taken from a full-scale mesophilic (35 °C), anaerobic completely stirred tank reactor (CSTR) (volume, 500 L) constructed in china. The reactor was fed with untreated corn straw as the sole feedstock for seven months, and with cow manure as the original inoculum. Corn straw contained 94.4% total solids (TS), of which 36.8%, 27.7% and 8.0% were cellulose, hemicellulose and lignin, respectively. The reactor was operated at an organic loading rate (OLR) of 1.2 kg TS m^{-3} d^{-1} and hydraulic retention time (HRT) of 40 days. The volatile suspended solids (VSS) of the biogas slurry was 6682 mg L^{-1}. The fermentation pH was about 7.0. Volatile fatty acids (VFA) concentration in the biogas slurry was 590 mg L^{-1} acetate, 374 mg L^{-1} propionate, 130 mg L^{-1} *n*-butyrate and 50 mg L^{-1} *i*-butyrate at the time for investigating the microbial communities, respectively. The biogas slurry was washed and suspended with phosphate buffer (10 mM, pH 7.2) for several times. The suspension solution was centrifuged at 5000 rpm for 10 min, and the pellets were collected and used for DNA extraction.

2.2. Degradability experiment

To investigate the degradability of corn straw and methanogenic activity of biogas slurry, batch experiments were carried out in 60 mL (liquid volume, 20 mL) serum vials at 37 °C with anaerobic biogas slurry as the inoculum without shaking. The medium used for cultivation was prepared as described previously (Sekiguchi et al., 2000). A total volume of 100 mL of biogas slurry was washed and suspended with medium for several times. The suspension solution was centrifuged at 5000 rpm for 10 min, and the pellets were collected, and resuspended in aliquots in 20 mL of medium without substrate. The cell suspension was then inoculated into bottles containing medium supplemented with corn straw (TS, 1%) as sole carbon source. Naturally harvested and sun-dried corn stalks were chopped into 1–2 cm pieces using pruning shears and dried at 105 °C for 48 h prior to use. In order to investigate the accumulation of metabolic intermediates, 5 mM 2-bromoethanesulfonate (BES), a specific inhibitor of methanogenesis, was added. The incubation and inhibition experiments were performed in duplicate. Concentrations of fermentation products in batch experiments were represented as the mean of duplicate experiments. The TS of corn straw was measured before and after the degradation assay. Productions of methane, volatile acids and aromatic compounds were measured periodically.

2.3. Analytical methods

The concentrations of the aromatic compounds were analyzed by high pressure liquid chromatography (HPLC) using a zorbax SB-C18 reverse-phase column (5 μm 0.5 × 150 mm, Agilent Technologies Inc, Shanghai, China) held at 25 °C and a UV detector set at 210 and 220 nm. The mobile phase was a 70:30 (v/v) mixture of methanol and 1% aqueous acetic acid at a flow rate of 0.8 mL/min. VFA, methane and hydrogen were measured as described previously (Yuan et al., 2011).

2.4. DNA extraction, PCR amplification and construction of 16S rRNA gene library

Genomic DNA was extracted based on the indirect extraction method (Gabor et al., 2006). PCR amplification, cloning, and sequencing procedures for constructing 16S rRNA gene clone libraries were performed as previously reported (Sekiguchi et al., 1998) with slight modifications. For construction of the 16S rRNA gene clone library, the following primer set for PCR amplification of bacterial and archaeal 16S rRNA genes was used: EUB8F (5′-AGAGTTTGATCMTGGCTCAG-3′; positions 8–27 in the *Escherichia coli* gene), and the reverse primer UNIV1492R (5′-TACGGYTACCTTGTTACGACTT-3′; positions 1492–1513 in *E. coli*) for the domain *Bacteria*, and ARC109F (5′-ACKGCTCAGTAACACGT-3′; positions 109–125 in *E. coli*) and UNIV1492R for the domain *Archaea*, respectively. The amplification conditions were: denaturing step of 95 °C for 9 min and 20 cycles of denaturation at 95 °C for 50 s, annealing at 50 °C for 1 min, and extension at 72 °C for 2 min; the final step was followed by post extension at 72 °C for 10 min. The PCR products were purified with a TIAN quick MiDi purification kit (Tiangen Inc., Beijing, China) and resuspended in nuclease-free water. Purified bacterial and archaeal PCR products were ligated into a pGEM-T easy vector with the TA cloning kit (Promega Inc., Beijing, China) following the manufacturer's instructions and transformed into *E. coli* DH5α competent cells, respectively. White colonies were selected to conduct colony PCR with the vector-specific primers M13F and M13R. Approximately 200 bacterial clones and 100 archaeal clones were randomly picked and screened by comparing restriction fragment length polymorphism (RFLP) patterns with *Hae*III and *Hha*I restriction endonucleases. Clones were grouped according to RFLP banding patterns, and scanning image analyses were performed manually. Then unique phylotypes were identified. Chimeric sequences were identified using the CHIMERA CHECK program of RDP (Cole et al., 2003) and excluded from subsequent analysis. Multiple alignments of the sequences from this study and reference sequences were performed using CLUSTAL X (Thompson et al., 1997). Phylotype was defined as a group of cloned sequences with >97% identity. The phylogenetic tree was constructed by the neighbor-joining method implemented in the MEGA5 computer software program. The confidence values of branches in the phylogenetic tree were determined using bootstrap analysis based on 1000 resamplings. The relative abundance of each phylotype in the library was calculated by dividing the number of cloned belonging to this phylotype by total number of cloned in each library.

2.5. Nucleotide sequence accession numbers

The GenBank/EMBL/DDBJ accession number of the 16S rRNA gene sequence of bacterial and archaeal clones are AB780888 to AB780956 and AB780957 to AB780967, respectively.

3. Results and discussion

3.1. Methanogenic activity of biogas slurry

To assess the methanogenic activity of biogas slurry, batch experiments were performed with corn straw (TS, 1%) as sole

carbon source. Corn straw was converted to methane within 40 days of incubation, and the specific methane yield was 180 mL CH_4 g TS^{-1} (Fig. 1a). The TS removal rate was 53% and the specific methanogenic activity of the anaerobic biogas slurry was 86 mL CH_4 g VSS^{-1} d^{-1}, indicating the biogas slurry had high levels of microbial activity.

During anaerobic degradation of corn straw, acetate, propionate, *n*-butyrate and *i*-butyrate were found to be main metabolic intermediates. Additionally, aromatic compounds *p*-cresol (a maximum of 1.3 mM), phenylpropionate (0.5 mM), phenol (130 μM) and benzoate (63 μM) were also detected as transient intermediates. The formation of aromatic compounds was not affected by BES, the maximum concentrations of these aromatic compounds were about at the same level in both cultures with and without BES (Fig. 1b and d). However, the maximum concentrations of volatile acids in cultures with BES were obviously higher than those in cultures without BES. At the end of degradation, 300 mg L^{-1} acetate, 70 mg L^{-1} propionate, 40 mg L^{-1} *n*-butyrate, and 15 mg L^{-1} *i*-butyrate were detected in cultures without BES (Fig. 1a), however, 1500 mg L^{-1} acetate, 300 mg L^{-1} propionate, 70 mg L^{-1} butyrate, 20 mg L^{-1} *i*-butyrate were observed in BES addition cultures (Fig. 1c). VFA and aromatic compounds were detected as intermediates in the cultures without BES, which were further degraded by the methanogenic consortia. Several reports described the inhibitory effect of aromatic compounds on the anaerobic biogas process. Fedorak and Hrudey (1984) reported methanogenic inhibition of phenol at 1000 mg L^{-1} and of *p*-cresol at 500 mg L^{-1} (Fedorak and Hrudey, 1984). In this study, all aromatic compounds accumulated in distinctly smaller levels than the reported toxic concentrations of aromatic compounds, which further demonstrated that the biogas slurry had relatively high microbial activity.

To monitor anaerobic process, pH, alkalinity, VFA concentration, methane production rate and biogas composition are commonly used as indicators. Recently, several studies indicated that aromatic compounds could be important intermediates during methanogenic degradation of lignocellulose, protein and fat rich waste, which could originate from anaerobic degradation of the lignin–carbohydrate complexes or the aromatic amino acids (Hecht and Griehl, 2009; DeAngelis et al., 2011). A previous study showed that aromatic compounds benzoate, phenylpropionate, and a small amount of phenylacetate were produced as natural by-products in the anaerobic rhizosphere of rice field soil (Glissmann et al., 2005). Hecht and Griehl (2009) found that the anaerobic degradation of kitchen waste also led to the accumulation of aromatic acids, especially with higher substrate loading. Phenylacetic acid, phenylpropionic acid, phenylalanine, indoleacetic acid, hydroxyphenylacetic acid and hydroxyphenylpropionic acid were detected during anaerobic kitchen waste fermentation, and phenylacetate could be a preferred substance indicator for showing imminent process failure (Hecht and Griehl, 2009). In this study, *p*-cresol, phenylpropionate, phenol and benzoate were detected as transient intermediates during anaerobic degradation of corn straw. Combined our results with these previous studies, it could be assumed that aromatic compounds are important intermediates during methanogenic degradation of plant biomass, and different substrate could result in the difference in the type and concentration of aromatic acids.

3.2. Overview of 16S rRNA gene clone libraries

To investigate the microbial community in a full-scale anaerobic reactor treating corn straw, bacterial and archaeal 16S rRNA gene clone libraries were constructed. In total, 209 bacterial clones and 90 archaeal clones were randomly picked and screened by comparing RFLP patterns with *Hae*III and *Hha*I restriction endonucleases, resulting in 69 phylotypes for the domain *Bacteria* and 11

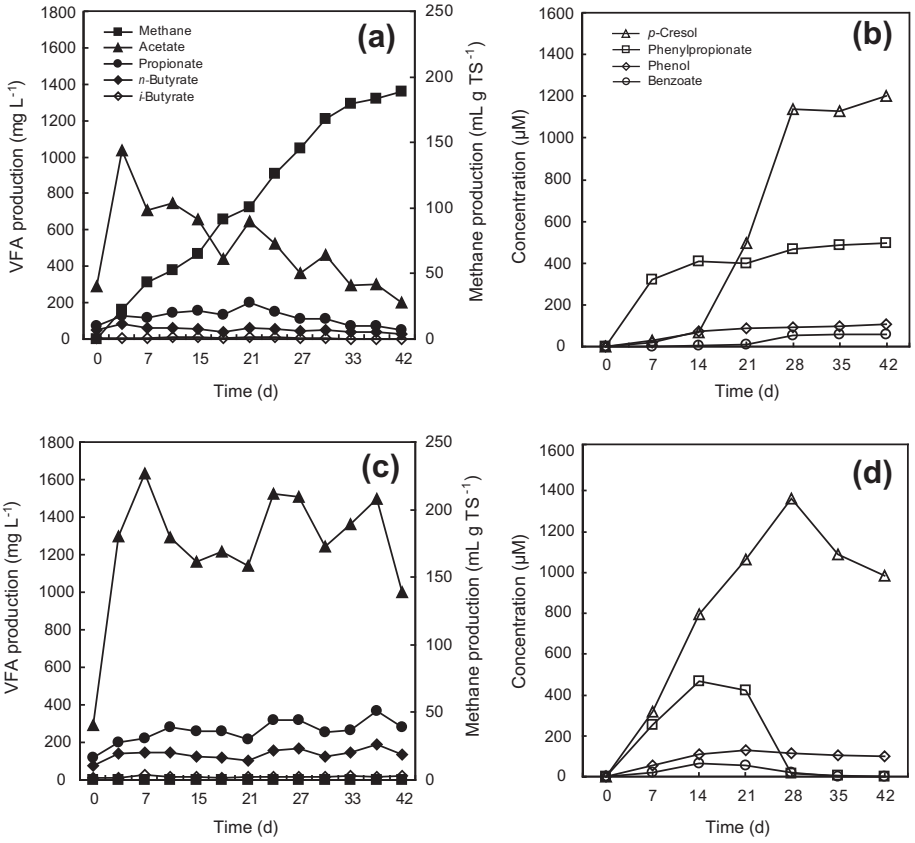

Fig. 1. Cumulative methane yields, VFA and aromatic compounds production during anaerobic corn straw degradation in the absence (a, b) and presence (c, d) of BES.

for the *Archaea*. Almost complete 16S rRNA gene sequences of the phylotypes were sequenced and subjected to detailed phylogenetic analyses. Rarefaction analysis showed the accumulation of phylotypes within the domain *Bacteria* did not reach a clear saturation, suggesting that the analysis of 209 clones had partially covered the diversity (Fig. 2a). In contrast, the number of archaeal clones sequenced had covered most of the diversity in the archaeal library (Fig. 2b). The coverage of the bacterial and archaeal libraries was 85.6% and 96.7%, respectively, indicating the clone libraries were large enough for further analysis.

3.3. Phylogenetic analysis of bacterial clone library

Anaerobic degradation of lignocellulosic biomass to methane is a multi-step process mediated by *Bacteria* and *Archaea*. In this study, the phylogenetic structure of the bacterial and archaeal communities in a full-scale mesophilic corn straw-treating biogas reactor was investigated by 16S rRNA gene-cloning analysis. Within the domain *Bacteria*, 13 distinct phyla and more than 40 genera were detected through analysis of 209 total bacterial clones. *Firmicutes*, *Chloroflexi*, *Actinobacteria* represented the most abundant bacterial phyla, and the other phyla *Planctomycetes*, *Synergistetes*, *Thermodesulfobacteria*, *Verrucomicrobia*, *Acidobacteria* and three uncultured candidate phyla of WS6, OD1, WWE3 were also detected in low frequency in bacterial clone library (Fig. 3a; Table S1). Apparently, the bacterial library exhibited high phylogenetic diversity at phylum level and genus level, suggesting that a variety of microorganisms involved in anaerobic corn straw degradation.

The *Firmicutes* represented the most abundant bacterial phylum (48.3%), which was affiliated with three orders of *Clostridiales* (80.2%), *Lactobacillales* (6.9%), *Bacillales* (4%) and unclassified environmental clones (8.9%). The most dominant phylotype in this phylum (12.9%) was related to *Catabacter hongkongensis* (93% similarity), an anaerobic arabinose, glucose, mannose, and xylose utilizing bacterium (Lau et al., 2007). Cellulolytic and acidogenic bacteria in *Firmicutes*, such as *Acetivibrio multivorans*, *Clostridium thermocellum*, *Clostridium algidixylanolyticum* and *Parasporobacterium paucivorans* were detected in low frequency. The results suggested that hydrolytic and fermentative microorganisms are major players in anaerobic microbial community decomposing corn straw. In addition to hydrolytic and fermentative bacteria, anaerobic aromatic compound-degrading bacteria were also detected. Of this phylum, 3% was related to *Pelotomaculum isophthalicicum* (93% similarity), a phthalate isomers and benzoate degrading bacterium isolated from anaerobic sludge treating phthalates wastewater (Qiu et al., 2006), and 4% was related to *Cryptanaerobacter phenolicus* (96% similarity), a phenol and 4-hydrobenzoate degrading bacterium (Juteau et al., 2005) (Supplementary material, Fig. S1). Under methanogenic conditions, anaerobic aromatic compounds degrading bacteria mostly belong to two phyla of *Firmicutes* and *Preoteobacteria* (Qiu et al., 2008; Peng et al., 2012). In this study, all the clones related to anaerobic aromatic compounds degrading bacteria were affiliated with the *Firmicutes*. In corn straw degradation experiment, aromatic compounds *p*-cresol, phenylpropionate, phenol and benzoate were detected as transient intermediates. Microbial community analysis agreed well with the degradation test, indicating that aromatic compounds were degraded by syntrophic aromatic compound-degrading bacteria coupling with the methanogens. It was assumed that aromatic compounds could be a breakdown product of lignin–carbohydrate complexes (Glissmann et al., 2005). DeAngelis investigated the anaerobic lignin-degrading microbes in tropical forest soil, indicating that phenol oxidase and peroxidase enzyme activities were significantly elevated in lignin-amended beads compared with the unamended beads (DeAngelis et al., 2011).

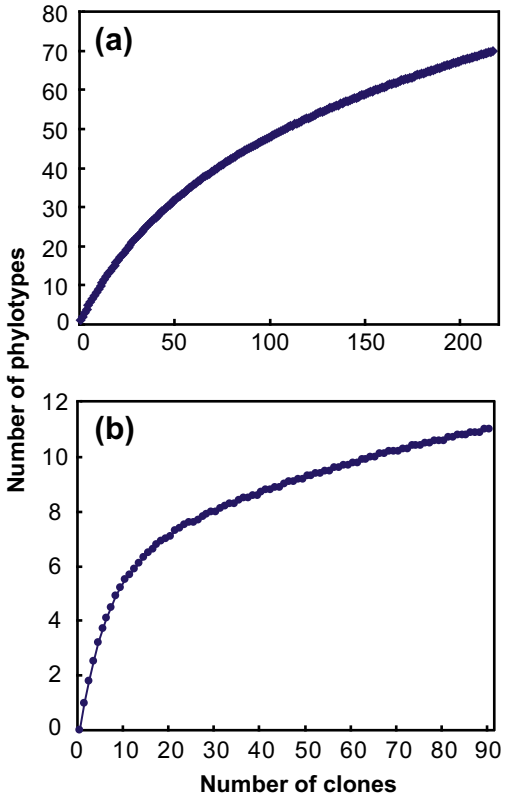

Fig. 2. Rarefaction curves of bacterial (a) and archaeal (b) 16S rRNA gene clones from corn straw treating biogas slurry.

The *Chloroflexi* was the second abundant bacterial community, comprising 20.1% of the total bacterial clones. The most dominant phylotype in *Chloroflexi* (26.2%) showed 89% similarity with *Caldilinea aerophila*, a thermophilic carbohydrates and amino acids utilizing facultative aerobe isolated from a hot spring sulfur-turf (Sekiguchi et al., 2003). Additionally, clones related to *Levilinea saccharolytica*, *Thermanaerothrix daxensis*, *Leptolinea tardivitalis* and *Dehalogenimonas lykanthroporepellens* in *Chloroflexi* were also detected (Table S1).

The *Actinobacteria* was the third dominant bacterial community, accounting for 9.1% of the total bacterial clones. The most predominant phylotype in this phylum (36.8%) was related to *Streptomyces hypolithicus*, which could use glucose and D-galactose anaerobically (Le Roes-Hill et al., 2009), but the similarity was only 84%. Additionally, *Solirubrobacter soli*, *Gaiella occulta*, and *Eggerthella lenta* in *Actinobacteria* were also observed.

The *Bacteroidetes* was the fourth dominant bacterial community, representing 7.7% of the total bacterial clones. Within this phylum, 62.5% belonged to the order *Bacteroidales* of class *Bacteroidia*, and the remaining clones were clustered with unclassified environmental clones. The most numerically dominant phylotype was related to *Acetomicrobium faecale* (90% similarity), a thermophilic anaerobe isolated from sewage sludge, which could ferment a variety of hexoses and pentoses (Winter et al., 1987).

The *Proteobacteria* was the fifth dominant bacterial community, representing 7.2% of total bacterial clones. The proteobacterial clones grouped into three subdivisions of *Alpha-*, *Delta-* and *Gamma-proteobacteria*. The most abundant phylotype in this phylum (60%) was related to *Smithella propionica* (91% similarity), an anaerobic, syntrophic, propionate-oxidizing bacterium isolated from an anaerobic sludge (Liu et al., 1999). In addition, fermentative bacteria such as *Pelagibacterium luteolum* and *Desulfuromonas michiganensis* were detected in *Proteobacteria*.

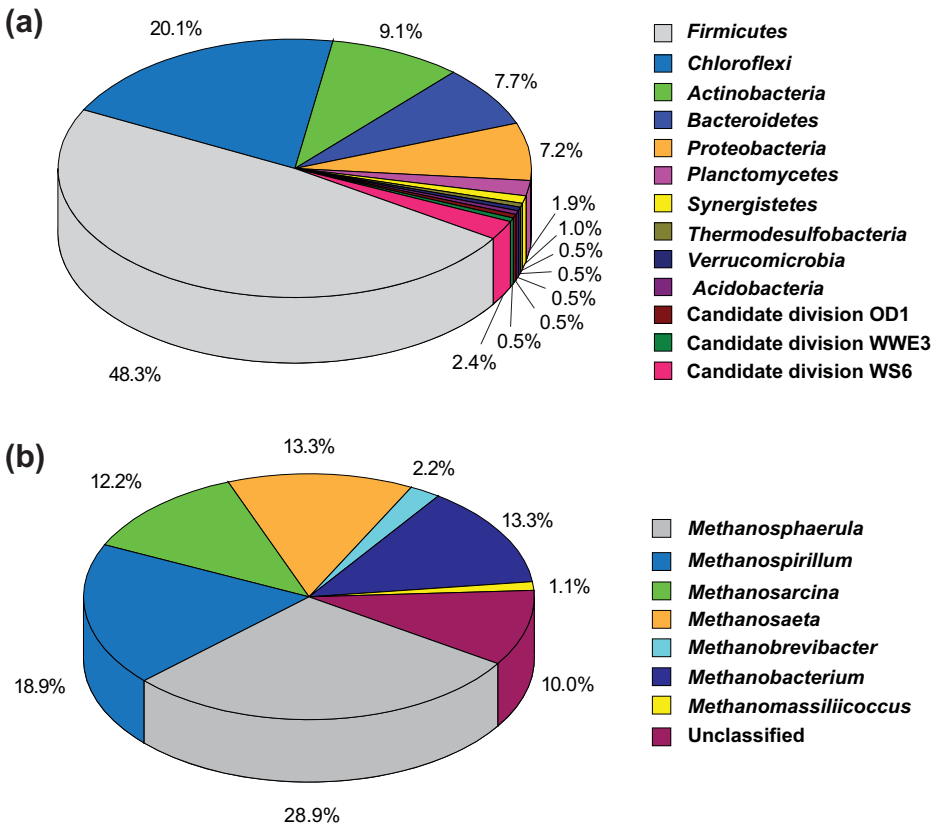

Fig. 3. Bacterial composition at the phylum level (a) and archaeal composition at the genus level (b) retrieved from corn straw treating biogas slurry.

The other phyla of *Planctomycetes* (1.9%), *Synergistetes* (1.0%), *Thermodesulfobacteria* (0.5%), *Verrucomicrobia* (0.5%), *Acidobacteria* (0.5%) and three uncultured candidate phyla WS6 (2.4%), OD1 (0.5%), WWE3 (0.5%) were also detected in low frequency in bacterial clone library. In the bacterial clone library, a considerable proportion of sequences (76%) showed a <97% sequence similarity with known species in GenBank. In addition, uncultured phyla of OD1, WWE3 and WS6 were also detected, which further demonstrated that numerous species residing in the biogas slurry treating corn straw were unknown.

3.4. Phylogenetic analysis of archaeal clone library

In the archaeal clone library, 2 phylotypes in archaeal phyla of *Crenarchaeota* and 9 phylotypes in *Euryarchaeota* were identified through analysis of 90 archaeal clones (Table S2). All the phylotypes in *Crenarchaeota* were related to environmental clones with no cultured representatives. All the 9 phylotypes in *Euryarchaeota* were distributed in the families *Methanobacteriaceae* (3 phylotypes), *Methanospirillaceae* (2), *Methanosaetaceae* (1), *Methanosarcinaceae* (1), *Methanoregulaceae* (1) and unclassified *Methanomicrobia* (1), respectively (Supplementary material, Fig. S2). The families *Methanobacteriaceae*, *Methanospirillaceae* and *Methanoregulaceae* are known to comprise of methanogens utilizing H_2/CO_2 or formate (Garrity and Holt, 2001). The families *Methanosarcinaceae* and *Methanosaetaceae* are known to include aceticlastic *Methanosarcina* and *Methanosaeta* (Garrity and Holt, 2001).

All the archaeal clones were grouped into seven genera of *Methanosphaerula*, *Methanospirillum*, *Methanosarcina*, *Methanosaeta*, *Methanobacterium*, *Methanobrevibacter*, *Methanomassiliicoccus* and one unclassified *Crenarchaeota* at the genus level (Fig. 3b). *Methanosphaerula* was the most dominant genus (28.9%), and all the clones were related to *Methanosphaerula* palustris (96% similarity), a hydrogenotrophic methanogen isolated from a minerotrophic fen peatland (Cadillo-Quiroz et al., 2009). The genus *Methanospirillum* was the second abundant genus (18.9%), and all the clones were related to *Methanospirillum hungatei* (96% similarity), a hydrogenotrophic methanogen isolated from sewage sludge (Ferry et al., 1974). Both acetoclastic *Methanosarcina* (12.2%) and *Methanosaeta* (13.3%) were detected in the archaeal clone library. Syntrophic acetate-degrading bacteria were not observed in the bacterial library, indicating that acetate could mainly be degraded by aceticlastic methanogenic archaea during anaerobic corn straw degradation.

The 16S rRNA gene analysis has been used widely to monitor the diversity, structure, and dynamics of microbial communities in methanogenic systems. Due to the difference in the composition of the feedstock, inocula, and the process conditions, such as OLR, HRT, temperature and pH, the microbial composition varied greatly among different systems. So far, only a few researches reported microbial community involved in lignocellulosic biomass degradation. Shi et al. (2013) compared microbial community dynamics during solid-state anaerobic digestion of corn stover at mesophilic and thermophilic conditions. The DGGE analysis revealed that both bacterial and archaeal communities underwent considerable successions, reflecting the selection for some bacteria and archaea while against other members of the initial inoculum. However, the microbes responsible for anaerobic corn stover degradation were not identified by sequencing analysis. Wang et al. (2009) compared the bacterial communities in three CSTRs co-digesting cow manure with grass silage, oat straw, or sugar beet tops, respectively. Their results indicated that straw treating community exhibited most phylogenetic diversity at class level, and clones affiliated with *Deltaproteobacteria* and *Acidobacteria* were only detected in the oat straw decomposing bacterial community, but not in that of grass silage and sugar beet tops. In the current

study, corn straw treating bacterial community also showed high diversity at phylum level and genus level, which was in good agreement with the results of oat straw. Moreover, the dominant bacterial groups were much similar between corn straw and oat straw treating communities, *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, and *Proteobacteria* were found to be abundant in both corn straw and oat straw treating systems. The major difference was that *Chloroflexi* was detected as dominant bacterial group in our corn straw treating bacterial community, but not in that of oat straw system. Another study also demonstrated that *Firmicutes*, *Bacteroidetes*, *Chlorolfexi* and an unclassfied bacterial group were major groups in a biogas plant fed with maize silage, green rye and liquid manure by clone analysis and metagenomic analysis (Kröber et al., 2009). A recent study showed *Firmicutes*, *Proteobacteria* and *Bacteroidetes* dominated anaerobic poplar wood chips decomposing bacterial community (van der Lelie et al., 2012). Combined previous studies with our results, it could be assumed that bacterial phyla of *Firmicutes*, *Bacteroidetes*, *Proteobacteria* and *Actinobacteria* were dominant in lignocellulose decomposing microbial systems, and *Chlorolfexi* was especially detected in corn straw decomposing microbial community.

4. Conclusions

Principal conclusions drawn from this study are summarized as follows:

- Corn straw biogas slurry showed high specific methane yield and TS removal rate, suggesting crop residues are promising alternative feedstocks for biogas production. Besides VFA, aromatic compounds *p*-cresol, phenylpropionate, phenol and benzoate were detected as intermediates during anaerobic corn straw degradation, which could originate from the decomposition of lignin–carbohydrate complexes.
- Clone analysis indicated bacterial community exhibited high phylogenetic diversity. Fermentative heterotrophs dominated the bacterial library, demonstrating hydrolytic and fermentative microorganisms are major players in anaerobic corn straw decomposition. Furthermore, anaerobic syntrophic propionate and aromatic compounds degrading bacteria were observed in relatively high proportion. Hydrogenotrophic methanogens were more dominant than aceticlastic methanogens in the corn straw decomposing archaeal community.

Acknowledgements

We thank Meng Dai in Qingdao Institute of Bioenergy and Bioprocess Technology for providing the biogas slurry and reactor performance. This work was supported by the National Natural Science Foundation of China (51078344), National Key Technology Research and Development Program of the Ministry of Science and Technology of China (2010BAC67B03), Knowledge Innovation Program of the Chinese Academy of Sciences (KSCX2-EW-J-10), Program Foundation for the Talent Introduction by Qingdao City (11-2-4-15-YX), 863 Program (2011AA060905), and Taishan Scholar Program of Shandong Province.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2013.06.014.

References

Acharya, C.N., 1935. Studies on the anaerobic decomposition of plant materials. Biochem. J. 29, 1459–1467.

Cadillo-Quiroz, H., Yavitt, J.B., Zinder, S.H., 2009. *Methanosphaerula palustris* gen. nov., sp. nov., a hydrogenotrophic methanogen isolated from a minerotrophic fen peatland. Int. J. Syst. Evol. Microbiol. 59, 928–935.

Cole, J.R., Chai, B., Marsh, T.L., Farris, R.J., Wang, Q., Kulam, S., Chandra, S., McGarrell, D., Schmidt, T.M., Garrity, G.M., 2003. The Ribosomal Database Project (RDP-II): previewing a new autoaligner that allows regular updates and the new prokaryotic taxonomy. Nucl. Acids Res. 31, 442–443.

Conrad, R., Klose, M., Lu, Y.L., Chidthaisong, A., 2012. Methanogenic pathway and archaeal communities in three different anoxic soils amended with rice straw and maize straw. Front. Microbiol. 3, 1–12.

DeAngelis, K.M., Allgaier, M., Chavarria, Y., Fortney, J.L., Hugenholtz, P., Simmons, B., HazenT, C., 2011. Characterization of trapped lignin-degrading microbes in tropical forest soil. PLoS One 6, e19306.

Dinuccio, E., Balsari, P., Gioelli, F., Menardo, S., 2010. Evaluation of the biogas productivity potential of some Italian agro-industrial biomasses. Bioresour. Technol. 101, 3780–3783.

Fedorak, P.M., Hrudey, S.E., 1984. The effects of phenol and some alkyl phenolics on batch anaerobic methanogenesis. Water Res. 18, 361–367.

Ferry, J.G., Smith, P.H., Wolfe, R., 1974. *Methanospirillurn*, a new genus of methanogenic bacteria, and characterization of *Methanospirillurn hungatii* sp. nov.. Int. J. Syst. Bacteriol. 24, 465–469.

Gabor, E.M., Vries, E.J., Janssen, D.B., 2006. Efficient recovery of environmental DNA for expression cloning by indirect extraction methods. FEMS Microbiol. Ecol. 44, 153–163.

Garrity, G.M., Holt, J.G., 2001. Phylum All. *Euryarchaeota* phy. nov.. In: Boone, D.R., Castenholz, R.W., Garrity, G.M. (Eds.), Bergey's Manual of Systematic Bacteriology. Springer, Berlin, pp. 211–355.

Glissmann, K., Hammer, E., Conrad, R., 2005. Production of aromatic compounds during methanogenic degradation of straw in rice field soil. FEMS Microbiol. Ecol. 52, 43–48.

Großkopf, R., Janssen, P.H., Liesack, W., 1998. Diversity and structure of the methanogenic community in anoxic rice paddy soil microcosms as examined by cultivation and direct 16S rRNA gene sequence retrieval. Appl. Environ. Microbiol. 64, 960–969.

Hecht, C., Griehl, C., 2009. Investigation of the accumulation of aromatic compounds during biogas production from kitchen waste. Bioresour. Technol. 100, 654–658.

Juteau, P., Côté, V., Duckett, M.F., Beaudet, R., Lépine, F., Villemur, R., Bisaillon, J.G., 2005. *Cryptanaerobacter phenolicus* gen. nov., sp. nov., an anaerobe that transforms phenol into benzoate via 4-hydroxybenzoate. Int. J. Syst. Evol. Microbiol. 55, 245–250.

Kröber, M., Bekel, T., Diaz, N.N., Goesmann, A., Jaenicke, S., Krause, L., Miller, D., Runte, K.J., Viehöver, P., Pühler, A., 2009. Phylogenetic characterization of a biogas plant microbial community integrating clone library 16S-rDNA sequences and metagenome sequence data obtained by 454-pyrosequencing. J. Biotechnol. 142, 38–49.

Lau, S.K.P., McNabb, A., Woo, G.K.S., Hoang, L., Fung, A.M.Y., Chung, L.M.W., Woo, P.C.Y., Yuen, K.Y., 2007. *Catabacter hongkongensis* gen. nov., sp. nov., isolated from blood cultures of patients from Hong Kong and Canada. J. Clin. Microbiol. 45, 395–401.

Le Roes-Hill, M., Rohland, J., Meyers, P.R., Cowan, D.A., Burton, S.G., 2009. *Streptomyces hypolithicus* sp. nov., isolated from an antarctic hypolith community. Int. J. Syst. Evol. Microbiol. 59, 2032–2035.

Liu, Y.T., Balkwill, D.L., Aldrich, H.C., Drake, G.R., Boone, D.R., 1999. Characterization of the anaerobic propionate-degrading syntrophs *Smithella propionica* gen. nov., sp. nov. and *Syntrophobacter wolinii*. Int. J. Syst. Bacteriol. 49, 545–556.

Lu, W., Chen, W., Duan, B., Guo, W., Lu, Y., Lantin, R., Wassmann, R., Neue, H., 2000. Methane emissions and mitigation options in irrigated rice fields in southeast China. Nutr. Cycl. Agroecosyst. 58, 65–73.

Peng, X.X., Zhang, Z.L., Zhao, Z.L., Jia, X.S., 2012. 16S ribosomal DNA clone libraries to reveal bacterial diversity in anaerobic reactor-degraded tetrabromobisphenol A. Bioresour. Technol. 112, 75–82.

Qiu, Y.L., Sekiguchi, Y., Hanada, S., Imachi, H., Tseng, I.C., Cheng, S.S., 2006. *Pelotomaculum terephthalicum* sp. nov. and *Pelotomaculum isophthalicum* sp. nov.: two anaerobic bacteria that degrade phthalate isomers in syntrophic association with hydrogenotrophic methanogens. Arch. Microbiol. 185, 172–182.

Qiu, Y.L., Hanada, S., Ohashi, A., Harada, H., Kamagata, Y., Sekiguchi, Y., 2008. *Syntrophorhabdus aromaticivorans* gen. nov., sp. nov., the first cultured anaerobe capable of degrading phenol to acetate in obligate syntrophic associations with a hydrogenotrophic methanogen. Appl. Environ. Microbiol. 74, 2051–2058.

Sekiguchi, Y., Kamagata, Y., Syutsubo, K., Ohashi, A., Harada, H., Nakamura, K., 1998. Phylogenetic diversity of mesophilic and thermophilic granular sludges determined by 16S rRNA gene analysis. Microbiology 144, 2655–2665.

Sekiguchi, Y., Kamagata, Y., Nakamura, K., Ohashi, A., Harada, H., 2000. *Syntrophothermus lipocalidus* gen. nov., sp. nov., a novel thermophilic, syntrophic, fatty-acid-oxidizing anaerobe which utilizes isobutyrate. Int. J. Syst. Evol. Microbiol. 50, 771–779.

Sekiguchi, Y., Yamada, T., Hanada, S., Ohashi, A., Harada, H., Kamagata, Y., 2003. *Anaerolinea thermophila* gen. nov., sp. nov. and *Caldilinea aerophila* gen. nov., sp. nov., novel filamentous thermophiles that represent a previously uncultured

lineage of the domain bacteria at the subphylum level. Int. J. Syst. Evol. Microbiol. 53, 1843–1851.
Shi, J., Wang, Z.J., Stiverson, J.A., Yu, Z.T., Li, Y.B., 2013. Reactor performance and microbial community dynamics during solid-state anaerobic digestion of corn stover at mesophilic and thermophilic conditions. Bioresour. Technol. 136, 574–581.
Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl. Acids Res. 25, 4876–4882.
van der Lelie, D., Taghavi, S., McCorkle, S.M., Li, L.L., Malfatti, S.A., Monteleone, D., Donohoe, B.S., Ding, S.Y., Adney, W.S., Himmel, M.E., 2012. The metagenome of an anaerobic microbial community decomposing poplar wood chips. PLoS One 7, e36740.
Wang, H., Lehtomäki, A., Tolvanen, K., Puhakka, J., Rintala, J., 2009. Impact of crop species on bacterial community structure during anaerobic co-digestion of crops and cow manure. Bioresour. Technol. 100, 2311–2315.
Winter, J., Braun, E., Zabel, H.P., 1987. *Acetomicrobium faecalis* spec, nov., a strictly anaerobic bacterium from sewage sludge, producing ethanol from pentoses. Syst. Appl. Microbiol. 9, 71–76.
Yuan, X.Z., Shi, X.S., Zhang, P.D., Wei, Y.L., Guo, R.B., Wang, L.S., 2011. Anaerobic biohydrogen production from wheat stalk by mixed microflora: kinetic model and particle size influence. Bioresour. Technol. 102, 9007–9012.
Zhong, W., Zhang, Z., Qiao, W., Fu, P., Liu, M., 2011. Comparison of chemical and biological pretreatment of corn straw for biogas production by anaerobic digestion. Renewable Energy 36, 1875–1879.
Zhong, W., Zhang, Z., Luo, Y., Qiao, W., Xiao, M., Zhang, M., 2012. Biogas productivity by co-digesting Taihu blue algae with corn straw as an external carbon source. Bioresour. Technol. 114, 281–286.