Bioreactor performance and microbial community dynamics in a production-scale biogas plant in northeastern China

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Abstract: In cold regions, heating is necessary to maintain the continuous and steady year-round operation of biogas fermentation. In this study, changes in the liquid composition, biogas production, and microbial diversity in heated- and unheated-phase samples were evaluated in a production-scale biogas plant that was fed continuously with cattle manure as a mono-substrate in Heilongjiang province in northeastern China. The volatile solid (VS) and volatile fatty acid (VFA) contents both gradually decreased in the heated and unheated fermentation processes. The chemical oxygen demand (COD) removal efficiency in the unheated phase sampled on June 15 (s-6-15) and October 15 (a-10-15) and in the heated phase sampled on January 15 (w-1-15) was 63.35%, 44.2% and 44.0%, respectively. The biogas production yields were in agreement with the results obtained for the VS and VFA contents and COD removal efficiency. The performance of the reactor in the heated phase was less efficient than that in the unheated phase, and the biogas production efficiency in June-August was higher than that in the other months. However, the CH_4 content in the biogas remained similar all year. Moreover, ARDRA (Amplified Ribosomal DNA Restriction Analysis) was used to study the microbial community composition in the fermentation process. The results showed that the methanogenic archaeal consortium consisted mainly of members of the genera *Methanomicrobiales* and *Methanosarcinales*. In the heated phase, hydrogenotrophic methanogens represented the dominant methanogen in w-1-15 feedstock. After fermentation, the strict aceticlastic methanogen *Methanosaeta* became the dominant methanogen. In the unheated phase, the hydrogenotrophic methanogens and aceticlastic methanogens were equivalent in s-6-15 feedstock and effluent, and aceticlastic methanogens were dominant in both a-10-15 feedstock and effluent. Assessments of the bacteria diversity of the microbial communities revealed that the common strains in the feed and effluent of the three samples included the rumen bacteria, *Bacteroides, Clostridium*, *Ruminococcaceae* and *Proteobacteria*.

Keywords: biogas production, production-scale plant, dairy manure, microbial community, northeast of China

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1 Introduction

The search for renewable resources for energy production, including biogas, has been promoted by national programs in many countries to solve the environmental pollution and energy crises, especially in China[1-3]. As a large agricultural country, there are abundant renewable resources and organic wastes in China, such as crop straw, forest residue, livestock and

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poultry manure, etc.[4] In recent years, increasing amounts of livestock and poultry manure are being produced with the fast development of livestock farming, which not only results in the pollution of the surrounding environment but also negatively impacts human and animal health. Anaerobic digestion is one of the most appropriate technologies to solve these problems. The biogas could be considered as a valuable source of energy and electricity[5]. According to statistics in 2009, biogas produced from manure resources was estimated to be approximately 120 billion m^3, and biogas produced from manure resources in large and medium livestock farms was approximately 24 billion m^3, which is equivalent to approximately 13.5 billion m^3 of CH_4[6]. Thus, biogas projects have become an important means of environmental protection and energy structure adjustment in China.

In the biogas fermentation process, various organic matters are decomposed by microorganisms under anaerobic conditions, and part of the material is converted to CH_4 and CO_2 in extremely complex biochemical processes that involve the interaction of numerous microbial species. In the fermentation system, the microbial diversity is related to the stability and efficiency of the fermentation system and plays an important function. Our knowledge of biogas reactors is still limited, and many technical and microbial aspects and the interactions have not yet been investigated. Moreover, the linkage between the digester performance and its microbial content and community changeability is still not fully understood[7,8]. To study the biodiversity and monitor the microbial community shifts in anaerobic digesters, many different molecular techniques have been used, such as 16S rDNA clone library construction, PCR-denatured gradient gel electrophoresis (PCR-DGGE), and high-throughput sequencing, etc.[9-11] Many studies have focused on the microbiological diversity in laboratory scale biogas fermenters supplied with different substrates[12-16], but only a few studies have investigated the microbiological diversity of fermenters in a production-scale biogas plant[17-21]. Biogas projects adopting different biogas fermentation reactors and various materials have resulted in differences in the biogas production efficiency and microbial diversity. Thus, further research examining bacterial and archaeal diversity and changeability in a production-scale biogas plant fermenter during anaerobic digestion is still required and could help improve CH_4 production in the fermentation process.

Large and medium-sized biogas fermentation projects in China have multiple functions, including energy production, elimination of pollution, and biogas residue utilization, to achieve comprehensive energy, environmental and economic benefits. However, there are currently a series of problems, such as a low yield of biogas production and unstable operations, especially in large and medium-sized biogas fermentation projects. Temperature was the main factor affecting the biogas yield and concentration, low temperatures would lead to lower gas production efficiencies, unstable operations, and abnormal processes of fermentation[22]. In cold regions, the average annual temperature is low, even below –20°C in the winter, and there is a large difference in temperature between day and night. Therefore, it is necessary to comprehensively investigate these biogas projects under different temperature conditions. Heating is a necessary means to maintain the continuous and steady operation of year-round biogas fermentation. In the present study, the bioreactor performance and microbial dynamics of a medium-sized biogas fermentation project in a cold region in Northeast China that adopted heating means during December – March and no heating at other times was monitored. The biogas project was a typical project in a cold region, and it can run continuously even below –30°C. The material obtained in the heated phase was sampled on January 15, 2013 (average air temperature of –18.6°C), and the material obtained during the unheated phase was sampled at two time points: June 15, 2012 (average air temperature of 20.6°C) and October 15, 2012 (average air temperature of 5°C). The research results will be expected to provide a basis for further improving the efficiency of biogas plants in cold regions. A further understanding of the microbiology will contribute to our growing knowledge regarding biomass conversion and biofuel production processes in biogas projects.

2 Materials and methods

2.1 Profile of a production-scale biogas plant in Heilongjiang province in northeastern China

The production-scale biogas plant (Figure 1) adopted the full-scale mixed anaerobic fermentation bioreactor located in Fenglin village in Yi'an country in Heilongjiang Province, China. The full-scale mixed anaerobic fermentation bioreactor was constructed with steel, and the effective volume was 1000 m^3. The bioreactor was anaerobically operated with dairy manure as a mono-substrate. Dairy manure was collected from a cooperatives dairy farm in which 700 cows were raised. The cow manure was diluted with water before feeding into the fermentation tank. Biogas project integrated insulation and warming techniques and two-phase anaerobic fermentation, which were suitable for the cold region. Solar and combustion heat from corn stalks were served as a heat source for anaerobic warming. The equipment within the entire station area was run by centralized monitoring and visualization technology. The pretreatment tank and aerobic tanks were heated from December to March each year, and no heating was applied during other times. The biogas was used for cooking by 560 families in Fenglin.

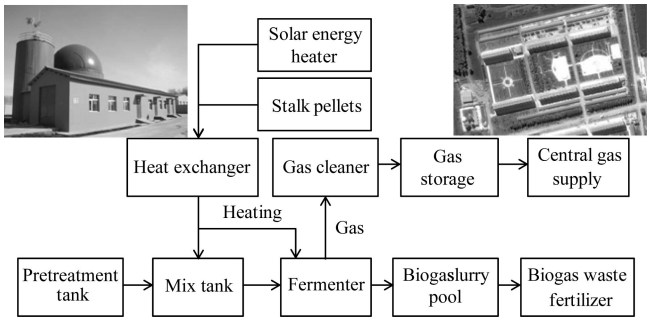


Figure 1 Flow chart of biogas fermentation production process in Yi'an county

The following parameters were used for the reactor in the biogas plant. The daily processing capacity of the cow manure was approximately 10.5 t, with an annual average daily water intake of 21.4 t/d, an average annual total solids (TS) of approximately 5%, a meso-temperature fermentation temperature of 35°C –37°C, a fermenter volume of 1000 m^3, and storage cabinets of 500 m^3. The energy used to fuel the biogas fermentation included energy for stirring and energy for feeding. The motor power used for fermentation liquid stirring was 7.5 kW, and the stirring time was 1 h/d. The motor power for feeding was 7.5 kW, and the duration of the feeding operation was 0.5 h. The motor power for pretreatment stirring was 7.5 kW, and the duration of stirring was 0.5 h/d.

2.2 Sampling

The fermentation samples were collected from the first biogas fermenter in the biogas plant. The full-scale mixed anaerobic fermentation bioreactor was operated at a mesophilic temperature of 35°C. The bioreactor was fed every 14 days, and the biogas volume and composition were measured every day. Approximately 30-50 mL of feedstock, effluent and fermentation liquid materials were sampled from the reactor during the heated phase on January 15, 2013 (average air temperature of –18.6°C), named the January 15 sample (w-1-15), and the unheated phase on June 15, 2012 (average air temperature of 20.6°C) and October 15, 2012 (average air temperature of 5°C), named as June 15 sample (s-6-15) and October 15 sample (a-10-15), respectively. A fraction of each sample was stored in TE solution for DNA extraction, and the remainder was used to analyze the physical and chemical characteristics.

2.3 Physical and chemical analyses

The volatile solids (VS), chemical oxygen demand (COD), and volatile fatty acids (VFA) content were investigated. The VS content was measured using the weight loss method. The COD was determined by potassium dichromate titration[23]. The VFA content was measured by the titration method[23]. The CH_4 content was determined using a biogas analyzer (Model ADG, Landtec, Colton/California, USA). The average daily feed amount, cumulative total biogas production, and CH_4 content were measured and recorded at 12 pm. every day during the heated and unheated phases.

2.4 DNA extraction and conventional PCR

A total of six samples of feedstock, including effluent liquid materials collected at June 15, 2012, October 15, 2012 and January 15, 2013, were stored in TE buffer at –20°C until analysis. The DNA was isolated from the samples using the benzyl chloride method[24]. The DNA was examined by electrophoresis in a 1% agarose gel

before PCR amplification. Archaeal and bacterial 16S rRNA genes were generated by PCR amplification using the archaeal primers 21F (5′-TCCGGTTGATCCYGSCRG-3′) and 915R (5′-GTGCTCCCCCGCCAATTCCT-3′) and the bacterial primers 27F (5′-AGTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). The PCR reaction mixture contained the following components: 5 μL 10× buffer, 1 μL 10 mM dNTP, 0.5 μL primer 27F or primer 21F (50 μM), 0.5 μL primer 1492R or primer 915R (50 μM), 1 μL template DNA, 0.5 μL Taq DNA polymerase (5 units/μL), up to 50 μL with DD water. The PCR conditions were an initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation (95°C, 1 min), annealing (55°C for archaeal; 52°C for bacterial, 1 min) and primer extension (72°C, 1 min for archaeal, 1.5 min for bacterial) with a final extension at 72°C for 10 min. The PCR products were analyzed by electrophoresis before further analysis.

2.5 Amplified ribosomal DNA restriction analysis (ARDRA)

The PCR products of the 16S rRNA genes were excised from the gel and eluted using a QIAquick gel purification Kit (Qiagen, UK). The purified DNA amplicons were ligated into pGEM-T plasmids, and the ligation products were transformed into *Escherichia coli* TOP 10 competent cells. The positive clones were randomly selected using blue-white selection from overnight LB plates containing 20 mg/mL X-gal and 200 mg/mL IPTG. The plasmid inserts were amplified by PCR as described above using the universal vector primers M13-47 (5′-CGC CAG GGT TTT CCC AGT CAC GAC-3′) and RV-M (5′-GAG CGG ATA ACA ATT TCA CAC AGG-3′). The PCR products from the insert-containing clones were digested using *Hinf* Ⅰ and *Msp* Ⅰ and analyzed by electrophoresis in 2.0% agarose gels. Next, they were grouped according to the DNA fingerprinting results based on their restriction profiles, and representatives were chosen for gene sequencing by the Huada Genomics Company (China).

2.6 Phylogenetic classification

The resultant sequence data were compared with the nucleotide databases using the basic local alignment search tool (BLASTn) (http://www.ncbi.nlm.nih.gov/blast/) as described previously[25]. Multiple alignments of sequences were performed using the CLUSTALX program, and trees were constructed with MEGA 4.0 software using the neighbor-joining method[26]. The robustness of the phylogeny was tested by bootstrap analysis with 1000 iterations. The partial 16S rRNA gene sequences determined in this study, were deposited in GenBank under accession numbers AY231301-AY231364.

3 Results and discussion

3.1 Bioreactor performance in the heated and unheated phases

Changes in the content of solid matter can reflect the effect of solid matter removal by anaerobic digestion. The biogas plant produced in an anniversary operation, consisting of feedstock, effluent and fermentation liquid material from a bioreactor system sampled at three different time points in heated and unheated phases, were analyzed (Table 1). During the anaerobic digestion process in s-6-15, a trend toward a decrease was observed for the concentrations of VS in the liquid. The initial VS in the feedstock and effluent were 3.8% and 1.8%, respectively, demonstrating a 2-fold increase in the former compared with the latter. The results showed a trend toward a decrease in the total volatile solid content in liquid during the entire fermentation process in a-10-15, but the extent of the decrease in this sample was not obvious compared with that in s-6-15. These results indicated that the decomposition of VS was deficient, which could be related to the variation in temperature during the fermentation process caused by the large temperature difference in a-10-15. During the anaerobic digestion process in the heated phase, the decreasing trend in the total concentration of VS in liquid was clearly observed. The initial VS in the feedstock and fermentation were 4.5% and 1.64%, respectively, demonstrating a 2.5-fold increase in the former compared with the latter in w-1-15. The total concentrations of VS in the fermentation and effluent were equivalent. The improved solid content removal might have been due to the complete fermentation of the feedstock by the additional thermal preservation in the heated phase.

Table 1 Concentration of VS in liquid in different samples

%

Material	Unheated phase		Heated phase
	s-6-15	a-10-15	w-1-15
Feedstock	3.8	3.45	4.50
Fermentation	3.0	2.89	1.64
Effluent	1.8	2.10	1.57

COD is an important index to evaluate whether the fermentation system has the ability to provide stable degradation of organic waste. The concentrations of the feedstock, effluent, and fermentation COD in the three samples are shown in Figure 2a. The COD for all of the samples revealed a trend toward a decrease in the unheated phase in s-6-15 and a-10-15, and during the heated phase in w-1-15, with feedstock and effluent values from 15183.24 mg/L to 5565.11 mg/L, 9866.06 mg/L to 6454.88 mg/L, and 12323.62 mg/L to 6898.00 mg/L, respectively. The COD removal efficiencies in the unheated phase in s-6-15 and a-10-15 and in the heated phase in w-1-15 were 63.4%, 44.2%, and 44.0%, respectively. The COD removal efficiency in s-6-15 was significantly higher than that in a-10-15 and w-1-15, and thus, more organic wastes were degraded during the unheated phase in s-6-15 compared with a-10-15 and the heated-phase w-1-15 with respect to methane fermentation. The results obtained for COD removal indicated that temperature had a great effect on fermentation.

In anaerobic fermentation, VFA was an important index of the biogas production yield. The concentrations of VFA in the feedstock, effluent and fermentation liquid material from the bioreactor system in the three samples are shown in Figure 2b. In the unheated phase, the concentration of VFA gradually decreased during the fermentation process from 231.66 mmol/L in the feedstock to 111.45 mmol/L in the effluent in s-6-15, and from 115.14 mmol/L in the feedstock to 48.48 mmol/L in the effluent in a-10-15. In the unheated phase, the concentration of VFA also decreased from 131.48 mmol/L in the feedstock to 60.11 mmol/L in the effluent in w-1-15. However, the concentration of VFA in the fermentation and effluent did not show a clear change, which indicated that the microbes could efficiently utilize various types of acid in early phases but fewer types of acid in later phases. Therefore, we deduced that the biogas production yield could be reduced in the later phase, and the whole fermentation process in the heated phase in w-1-15 and in the unheated phase in a-10-15 would demonstrate less instability compared with s-6-15 from the unheated phase.

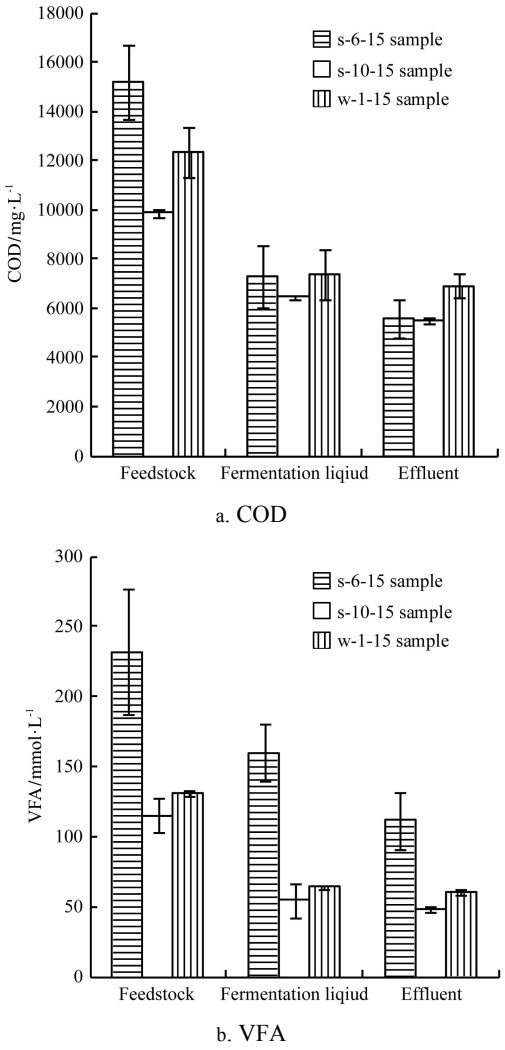


Figure 2 Concentrations of COD and VFA in the feedstock, effluent and fermentation liquid material from the bioreactor system sampled on June 15, October 15, and January 15

The mean biogas production yield per day and CH_4 content in the biogas are shown in Table 2. The mean biogas production yield per day was highest during the unheated phase from June-August, reaching 640 m^3. Values reaching 410 m^3 were documented in other months without heating, as compared with 250 m^3 during the heated phase from December to March. The CH_4 content in the biogas was similar between the heated and unheated phases. The results for the biogas production yield were consistent with the data obtained for the VS, VFA and COD removal efficiencies. The performance

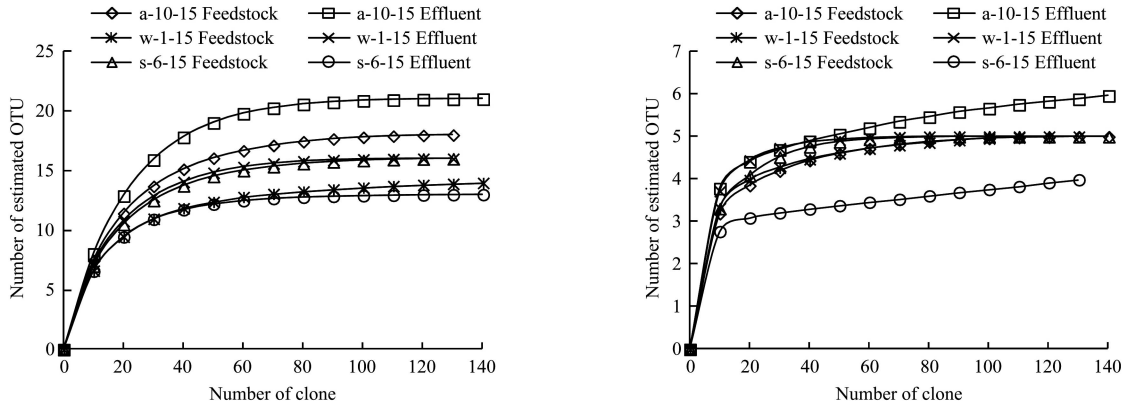
of the reactor (i.e., removal of VFA and biogas production) in the heated and October 15 unheated phases were less efficient than that in the June 15 unheated phase.

Table 2 Total biogas production yield and CH_4 content in the heated and unheated phases

Phase	Month	Total biogas production yield /$\times 10^4 m^3$	CH_4 content /%
Unheated phase	June-August	5.76	54.5
	Other months	6.15	56.9
Heated phase	December - March	3	55.5

3.2 Bacterial community composition and dynamics

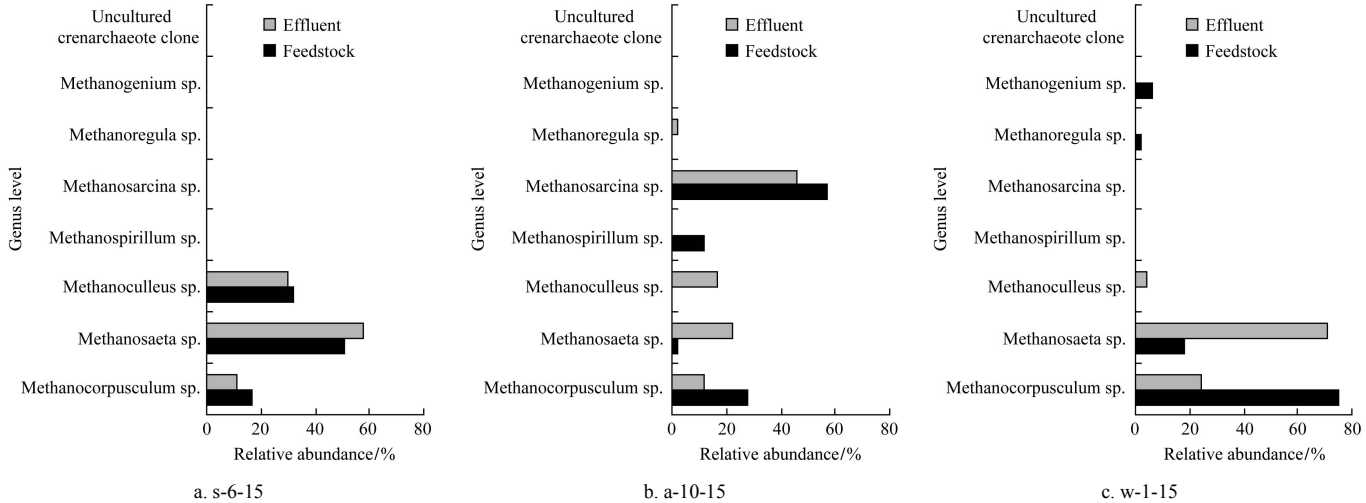
During anaerobic fermentation, microbial species are abundant, including methane archaea, cellulose-degrading bacteria, organic matter-degrading bacteria, etc. Feedstock and effluent samples from a bioreactor system in the unheated and heated phases were investigated by constructing a 16S rRNA gene library. The adequacy of the sample size for the determination of diversity within the 16S rDNA clone library was evaluated by rarefaction analysis[27]. As shown in Figure 3, the calculated rarefaction curve achieved clear saturation for 80 bacterial clones and 60 archaeal clones. More than 100 clones were selected for analysis in of all ARDRA, and thus, the analysis covered diverse microbes capable of anaerobic fermentation.



a. Bacteria b. Archaea

Figure 3 Rarefaction curves for the 16S rDNA sequences from bacteria and archaea

Regarding archaeal diversity, 150, 155 and 126 clones were selected from the 16S rDNA clone libraries for feedstock in s-6-15 and a-10-15 unheated and w-1-15 heated phase, respectively. Five different RFLP patterns were detected in each sample and designated as operational taxonomic units (OTU, 97.0% similarity). For the bacterial diversity, 131, 146, 136 clones were selected from the 16S rDNA clone libraries for feedstock in s-6-15 and a-10-15 unheated phases and w-1-15 heated phase, respectively. In these samples, 4, 6, and 5 different RFLP patterns, respectively, were detected and designated as OTUs. The dynamics of the archaeal communities in the feedstock and effluent of the three samples are shown in Figure 4.



a. s-6-15 b. a-10-15 c. w-1-15

Figure 4 Archaeal community composition in the feedstock and effluent of s-6-15, a-10-15 and w-1-15

The results of the principal component analysis (PCA) showed that the archaeal community structure in s-6-15 feedstock, s-6-15 effluent, and w-1-15 effluent were similar but remarkably different from a-10-15 feedstock, a-10-15 effluent and w-1-15 feedstock (Figure 5).

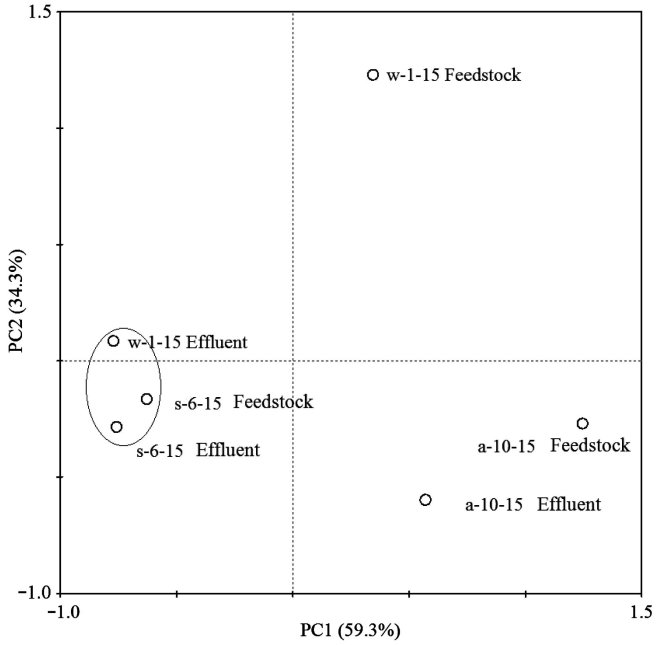


Figure 5 Principal component analysis (PCA) of the 16S rDNA clone library of archaeal communities in the different samples

According to the sequencing results, the methanogenic archaeal community composition was dominated by *Methanomicrobiales* and *Methanosarcinales*. The dominant methanogenic archaeal component was *Methanosarcinales*, which is one of the main members in the anaerobic digestion of cattle manure and maize straw[16]. Furthermore, *Methanomicrobiales* has been reported to be the most abundant archaeal component in an agricultural biogas reactor operating under wet fermentation conditions[21], and *Methanoculleus* have also been shown to play a dominant role in methanogenesis in a production-scale biogas plant fed with renewable primary products[18], a production-scale biogas plant fed with maize silage, green rye and liquid manure[19] and in the anaerobic digestion of cattle manure and maize straw[16].

In s-6-15, there was a small change in the archaeal community composition in the feedstock and effluent, whereas in w-1-15 and a-10-15, there was a large change in the archaeal community composition in the feedstock and effluent. *Methanocorpusculum* and *Methanosaeta* were present in the feedstock and effluent in the unheated and heated phases. In s-6-15 unheated phase sample, hydrogenotrophic methanogens and aceticlastic methanogens in the feedstock accounted for 49% and 51% of the community, respectively. The aceticlastic methanogens in the effluent increased slightly to 58%, which suggested that methanogenesis occurred via the aceticlastic and hydrogenotrophic pathways in s-6-15. In a-10-15 unheated phase sample, aceticlastic methanogens were dominant both in the feedstock and effluent and included *Methanosaeta*, *Methanosarcina*, and *Methanosaeta* in the effluent, which increased from 2% to 22%. In w-1-15 heated phase sample, hydrogenotrophic methanogens were found to be the dominant methanogen in the feedstock at 75%. After fermentation, the strict aceticlastic methanogen *Methanosaeta* became the dominant methanogen, accounting for 71% of the community. These results indicated that aceticlastic methanogenesis was the main methanogenic pathway in w-1-15 heated phase and a-10-15 unheated phase samples. The dynamics of the archaeal community in the different samples affected gas production, with a smaller change in the archaeal community resulting in greater gas production and thus improved reactor function. In addition, the archaeal communities in the feedstock in different samples were quite different. The archaeal communities in the effluent in s-6-15 and w-1-15 were similar, but these samples differed from a-10-15 effluent sample.

In the assessments of the diversity of the bacterial microbial community, the bacterial community in the feedstock and effluent in the three samples showed significant dynamic changes (Figure 6). There were 10 and 14 types of bacterial species in the feedstock in s-6-15 and a-10-15 unheated phase samples, which was greater than that detected in the effluent (8 and 9 types of bacterial species, respectively). In w-1-15 heated phase sample, the diversity of bacterial species in the effluent was greater than that in the feedstock, demonstrating 7 and 11 types, respectively. The PCA results showed that the bacterial community structure was remarkably different among these samples (Figure 7).

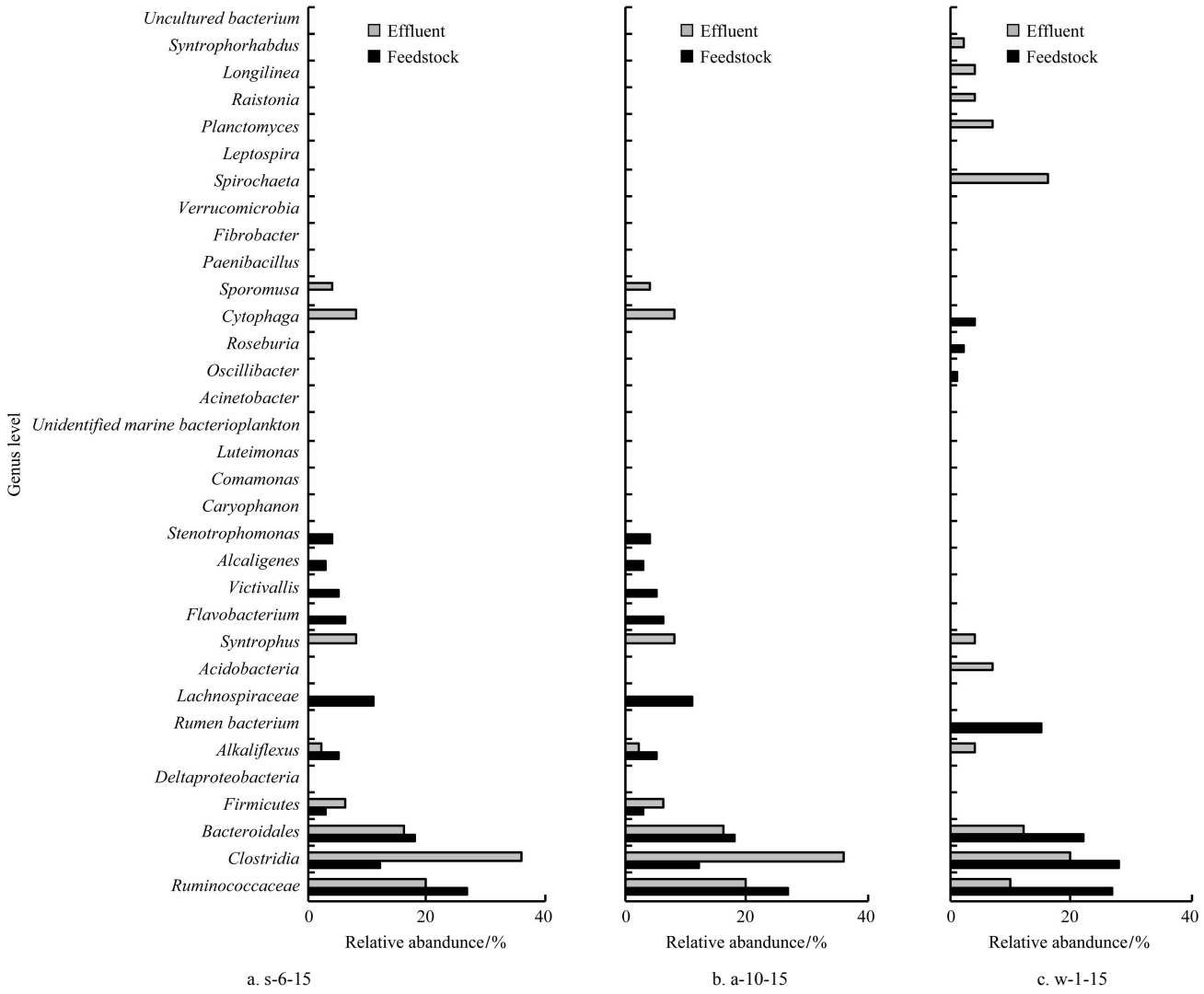


Figure 6 Bacterial community composition in the feedstock and effluent of s-6-15, a-10-15, and w-1-15

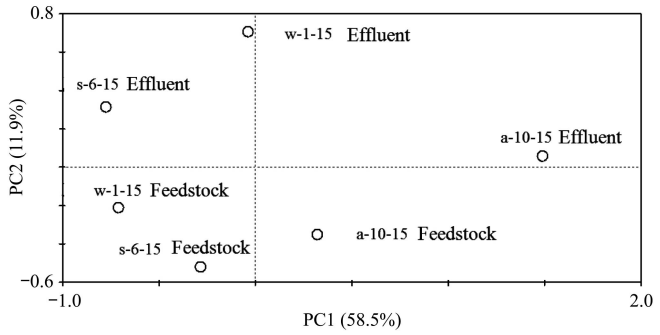


Figure 7 Principal component analysis (PCA) of the 16S rDNA clone library of bacterial communities in the different samples

The common strain in the feed and effluent during the entire fermentation process in the three samples was *Bacteroidales*; other dominant bacteria in the three samples included *Ruminococcaceae*, *Clostridium*, rumen bacteria, and *Deltaproteobacteria*. In mesophilic biogas-producing anaerobic batch fermentation using straw and hay as co-substrates, metagenome and metaproteome analyses revealed that *Clostridiales* and *Bacteroidales* were prevalent in the community[28]. In a production-scale biogas plant fed with maize silage, green rye and liquid manure, microbial community analyses revealed the phylum *Firmicutes* and the most abundant classes *Clostridia* and *Bacteroidetes*[19]. Other bacteria have also been detected in biogas plant fermentation. *Spirochaeta* and *Leptospira* were present in the effluent in a-10-15 and w-1-15. *Spirochetes* are helical cells with axial filaments. Four types of cultured species are heterotrophic, obligate and facultative anaerobes, and they are able to ferment carbohydrates. Spirochetes are widely distributed in nature and in animals[29]. Members of the phyla *Spirochaetes* have also been detected, as represented by two clonal sequences in a biogas-producing, completely stirred tank reactor that was fed continuously with fodder beet silage as a mono-substrate[30].

The related *Bacteroidales* participate in the anaerobic

fermentation of sludge to produce CH_4. *Bacteroidales* are a class of microbes that decompose plant polysaccharides and starches and produce small molecules that can be used by other microorganisms to produce CH_4. The *Bacteroidales* taxon is related to microorganisms that have been previously characterized as biomass degraders[11]. The proportions of bacteria were reduced in the feedstock and effluent in a-10-15 and w-1-15. However, the proportion of bacteria in the effluent increased in the feedstock in a-10-15, which suggests that the material in a-10-15 was fully converted.

Ruminococcaceae, a strict anaerobe, is typically present in the rumen of ruminant animals and is the main bacteria responsible for cellulose degradation via the secretion of cellulase[31]. *Ruminococcaceae* accounted for a certain percentage in the feedstock and decreased in effluent, which were not detected in a-10-15 effluent sample.

Most of the species of *Clostridium* were strict anaerobes, and a few were micro-aerobic bacteria. *Clostridium* plays an important role in fermentation reactions. They can utilize the glucose in the culture medium to produce large amounts of H_2 and CO_2, as well as small amounts of lactic acid, acetic acid, butyric acid, ethanol and butanol, which can greatly promote the production of CH_4. *Clostridium* showed an increasing trend in s-6-15 feedstock and effluent samples, and it was detected in the feedstock but not the effluent of a-10-15. A decreasing trend was identified in the feedstock and effluent of w-1-15.

Deltaproteobacteria are present in sludge, and most are facultative or obligate anaerobic and heterotrophic bacteria that utilize photosynthesis to store energy. Most *Deltaproteobacteria* possess a purple pigment, and they have been reported in biogas fermentation and lignocellulolytic microbial communities[30,32]. This type of bacteria was detected in the feedstock and effluent of a-10-15 and the effluent of w-1-15.

The results obtained for the microbial dynamics in anaerobic fermentation processes in the feedstock and effluent of the three samples indicated the participation of a high diversity of microbial communities with different functions in the biogas fermentation process in the heated and unheated phase. The bacteria mainly decomposed precursor substances in the fermentation process into organic substances, which were utilized by methanogens to produce the biogas. Methanogenic archaea and bacteria synergistically maintain the stability of the entire fermentation process. Moreover, the microorganisms display clear seasonal variations. These phenomena may be largely explained by the changes in temperature. Despite the differences among the three samples, the community profiles were very similar at least in the higher taxonomic ranks, which illustrated that core community taxa play key functions in biomass decomposition and CH_4 synthesis. Additionally, to accurately analyze the bioreactor microbial communities and their functions, gene functions should be taken into account, and the quantitative analytic methods should be adopted, such as real-time PCR.

4 Conclusions

The bioreactor performance and production efficiency of a biogas plant in a cold region in northern China was higher during the unheated phase in comparison with the heated phase. These results indicated that temperature was a main factor for biogas production, and the heating measure and the efficient heat preservation measure play a key role for biogas production in cold region, especially for production in winter. In this year-round operation of biogas plant, heating measures, including solar energy heater and stalk pellets, were used. The microbial community dynamics results showed that the archaeal and bacterial community structure of three samples in heated phase and unheated phase were different, although some common microbial species were identified. Moreover, the change of microbial community dynamics in heated phase samples were more remarkable. In the heated phase, the dominant methanogen hydrogenotrophic methanogens in feedstock converted to the strict aceticlastic methanogen *Methanosaeta* after fermentation. In the unheated phase, the hydrogenotrophic methanogens and aceticlastic methanogens were equivalent in s-6-15 feedstock and effluent, and aceticlastic methanogens were dominant in both a-10-15 feedstock and effluent. The obvious

change of methanogen community dynamics was in accordance with the low production efficiency in winter in cold region. So, additional measures remain to be established in future studies to improve the performance of the bioreactor, including adjustments of the microbial community. The system analysis of bioreactor performance and microbial community dynamics in the production-scale biogas plant fermenter during anaerobic digestion provide the basis for further improving the efficiency of biogas plants in cold regions.

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