

Reactor performances and microbial communities of biogas reactors: effects of inoculum sources

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Abstract Anaerobic digestion is a very complex process that is mediated by various microorganisms, and the understanding of the microbial community assembly and its corresponding function is critical in order to better control the anaerobic process. The present study investigated the effect of different inocula on the microbial community assembly in biogas reactors treating cellulose with various inocula, and three parallel biogas reactors with the same inoculum were also operated in order to reveal the reproducibility of both microbial communities and functions of the biogas reactors. The results showed that the biogas production, volatile fatty acid (VFA) concentrations, and pH were different for the biogas reactors with different inocula, and different steady-state microbial community patterns were also obtained in different biogas reactors as reflected by Bray-Curtis similarity matrices and taxonomic classification. It indicated that inoculum played an important role in shaping the microbial communities of biogas reactor in the present study, and the microbial community assembly in biogas reactor did not follow the niche-based ecology theory. Furthermore, it was found that the microbial communities and reactor performances of parallel biogas reactors with the same inoculum were different, which could be explained by the neutral-based ecology theory and stochastic factors should played important roles in the microbial community assembly in the biogas reactors. The Bray-Curtis similarity matrices

analysis suggested that inoculum affected more on the microbial community assembly compared to stochastic factors, since the samples with different inocula had lower similarity (10–20 %) compared to the samples from the parallel biogas reactors (30 %).

Keywords Anaerobic digestion · Biogas production · Inoculum · Microbial community

Introduction

Anaerobic digestion for biogas production has been widely used in the treatment of organic wastes, and it is a very complex microbial process involving different microorganisms (Werner et al. 2011). The degradation of organic wastes includes four sequential steps: hydrolysis (conversion of organic wastes into oligomers and monomers), fermentation (conversion of oligomers and monomers into acetate, CO₂, and H₂), acetogenesis (conversion of CO₂ and H₂ to acetate), and methanogenesis (conversion of CO₂, H₂, and acetate into methane) (Schluter et al. 2008). The first three steps are mediated by bacteria, while the last step is mediated by archaea (also known as methanogens). The syntrophic relationship between bacteria and archaea is essential for the efficiency and stability of the biogas process (Luo et al. 2015).

The understanding of microbial community assembly in biogas reactors is crucial for effective biodiversity management and maintenance. Ecologists have studied the microbial community assembly in different ecosystems, and now it is known that deterministic factors (such as environmental selection and interspecies interactions) and stochastic factors (stochastic processes of birth, death, colonization, extinction, and speciation) affect microbial community assembly (Pagaling et al. 2014; Zhou et al. 2013). However, the relative

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importance of these factors is still unclear. There are controversial views on the microbial community assembly. The traditional niche-based theory supports the view that the community is shaped mainly by deterministic factors and thereby asserts that community composition should converge toward a single pattern under similar environmental conditions from diverse starting species sets (Fargione et al. 2003). The view has been demonstrated in several ecosystems including activated sludge bioreactors treating wastewater (Ayarza and Erijman 2011) and sediment slurry microcosms (Kurtz et al. 1998). On the contrary, the neutral-based theory assumes that many natural different community patterns can be generated under similar environmental conditions by stochastic factors, which was demonstrated in several ecosystems including microbiomes from microbial fuel cells, wetlands et al. (Baptista et al. 2008; Chave 2004; Zhou et al. 2013). The stochastic factors are impossible to manipulate or reconstruct in the ecosystems (Chase 2003).

The microbial community in biogas reactors has been studied for several decades, and our understanding of the microbiomes in biogas reactors has been increased greatly with the establishment of culture-independent molecular methods including PCR-DGGE, PCR-cloning, and the recently developed PCR-high-throughput sequencing (Luo and Angelidaki 2014; Luo et al. 2013; Riviere et al. 2009; Sundberg et al. 2013). The roles of predictable factors including temperature, reactor configuration, feedstock et al., on the microbial communities in biogas reactors have been studied previously (Nielsen et al. 2004; Rincón et al. 2008). However, the microbial community assembly in biogas reactors is still not well exploited based on the ecological theories. The importance of inoculum sources on the biogas production has been demonstrated previously in batch experiments treating different organic wastes, and the suitable inoculum sources are essential for efficient biogas production (Dhamodharan et al. 2015; Gu et al. 2014; Lopes et al. 2004). However, inoculum sources should not be important for biogas production based on the niche-based theory, where similar microbial communities should be shaped under similar environmental conditions from diverse inoculum sources. It should be noted that all the abovementioned studies were conducted under batch conditions (lasting for around 1 month), and stable microbial communities were not achieved. Therefore, it is not appropriate to evaluate the importance of inoculum source for biogas production only based on the results from batch experiments, and the role of inoculum in shaping the microbial communities in biogas reactors remains to be elucidated.

Based on the above considerations, the present study aims to reveal the role of inoculum sources in shaping the microbial communities in biogas reactors. Five different inocula were obtained from different sources and inoculated in continuously running biogas reactors with the same operational conditions. In addition, one of the above inocula was inoculated into

three parallel biogas reactors in order to see the reproducibility of both microbial communities and functions of the biogas reactors. The steady-state microbial communities in the biogas reactors were analyzed by 16S rRNA genes high-throughput sequencing.

Material and methods

Inoculum and feed composition

The inocula used in the present study were obtained from five different sources, including the digested stillage from mesophilic continuously stirred tank reactor (CSTR) treating stillage in an ethanol plant (pH 7.1, total solid (TS) 23.2 g/L, volatile solid (VS) 16.5 g/L), the anaerobic granular sludge from a mesophilic up-flow anaerobic sludge blanket (UASB) reactor treating paper mill wastewater (pH 7.2, TS 72.8 g/L, VS 43.5 g/L), the digested manure from mesophilic CSTR reactor treating cattle manure (pH 7.8, TS 35.2 g/L, VS 20.6 g/L), the raw cattle manure (pH 7.5, TS 65.4 g/L, VS 44.3 g/L), and the digested sewage sludge from mesophilic CSTR reactor in a wastewater treatment plant (pH 7.2, TS 24.5 g/L, VS 13.7 g/L). The feed was composed of cellulose and basic anaerobic (BA) medium. The cellulose concentration in the feed was 7.5 g/L. The BA medium was prepared based on a previous literature (Angelidaki and Sanders 2004). The NaHCO₃ concentration in the feed was 5 g/L. The feed was sterilized in an autoclave to reduce the influence of microorganisms that might be grown in the non-sterilized feed.

Experimental setup

Seven identical 2 L CSTR reactors with working volume of 1.8 L were used in the present study, and all the reactors were continuously stirred by magnetic stirrer at a stirring speed of 200 rpm to achieve well mixing of the reactors. All the reactors were equipped with thermal jackets in order to maintain a temperature of 37 °C. The produced biogas was collected by gas bags. The HRT of the reactors was 15 days. Each inoculum was added to one CSTR reactor except for digested manure, which was added to three parallel CSTR reactors. All the reactors were initially filled with the inoculum, BA medium, and feed (200 mL), and the VS concentration of inoculum in all the reactors was 5 g/L. In the beginning, all the reactors were run in batch condition without feeding, and the continuous feeding was started once the biogas production from all the reactors achieved maximum values. The evolved biogas was collected with gas bag, and the amount of biogas was determined periodically using syringe. The parameters such as biogas production, volatile fatty acid (VFA) concentration, and pH were monitored during the whole operational phase. The steady state in this study was defined as a stable biogas

production with daily variation of lower than 10 % for a period at least 10 days. Liquid samples from all the reactors were collected once the steady-state conditions were reached in all the reactors. The reactors were named as 1 (inoculated by digested stillage), 2 (inoculated by anaerobic granular sludge), 3a (inoculated by digested manure), 3b (inoculated by digested manure), 3c (inoculated by digested manure), 4 (inoculated by raw cattle manure), and 5 (inoculated by digested sewage sludge).

DNA extraction, 16S rRNA sequencing, and analysis

Twelve microbial samples were obtained including five inocula and seven liquid samples from all the biogas reactors in steady states. All the samples were centrifuged at 10,000 rpm for 10 min, and the resulting supernatant was discarded. The solid residues were used for DNA extraction. Total genomic DNA of the collected samples was extracted using QIAamp DNA Stool Mini Kit (QIAGEN 51504) according to the manufacturer's instructions. The extracted DNA was PCR amplified with the universal primers 515f and 806r targeting the V4 region of the 16S rRNA gene according to previous studies (Bates et al. 2011; Regueiro et al. 2015), and the PCR products were purified using the QIAquick spin columns (QIAGEN) to remove the excess primer dimers and dNTPs. The concentration of PCR amplicons was measured by NanoDrop spectrophotometer. The PCR products were then sent out for sequencing on an Ion Torrent PGM machine at Shanghai Shenggong. The raw sequences were quality-checked by removing the low-quality sequences without exact matches to the forward and reverse primers, with length shorter than 100 bp, and containing any ambiguous base calls by RDP tools (Cole et al. 2009). Chimeras were removed from the data by using the Find Chimeras web tool. The numbers of high-quality sequences are shown in Table 1 with an average length around 265 bp. The sequences were then normalized to the same sequencing depths (20,000 sequences) by MOTHUR program to facilitate the comparison. The normalized sequences were phylogenetically assigned to taxonomic classifications by RDP Classifier with a confidence threshold of 50 %. The sequences were clustered into operational taxonomic units (OTU) by setting a 0.03 distance limit by MOTHUR program. Rarefaction curve, Shannon diversity index, species richness estimator of Chao1, and dendrogram based on Bray-Curtis similarity matrix were also generated by MOTHUR program. All the sequence data were submitted to NCBI (Bioproject PRJNA293546).

Analytical methods

The concentrations of acetate, propionate, isobutyrate, butyrate, iso-valerate, and valerate were determined by HPLC, and they were separated with a 7.8×300 Aminex HPX-87-H

Table 1 Summary of the obtained sequences

Sample ID	Raw sequences	Average length (bp)
1I	44403	266
1	48003	266
2I	30959	267
2	39789	266
3I	38137	267
3a	43420	261
3b	37248	262
3c	25184	265
4I	41602	261
4	21171	266
5I	45781	268
5	35818	268

column (Bio-Rad) at 55 °C with a refractive index detector at 50 °C. The mobile phase was 5 mmol H₂SO₄, at a flow rate of 0.4 mL/min. The biogas composition was analyzed by GC-TCD equipped with a 2-m stainless column packed with Porapak T (50/80mesh). Helium was used as carrier gas. The operational temperatures of the injection port, the oven, and the detector were 120, 120, and 110 °C, respectively. TS, VS, and COD were analyzed according to APHA (APHA 1995).

Results

Reactor performances

All the biogas reactors were operated for 8 months, and steady-state reactor performances were obtained in all the reactors. The steady-state reactor performances were summarized in Table 2. The biogas production from the seven reactors was different. Reactors 1 and 5 had relatively lower biogas production (below 320 mL/day) compared to the other reactors (all above 450 mL/day). The lower biogas production in reactors 1 and 5 also corresponded to the lower methane content, pH, and COD removal efficiencies. Acetate and propionate were the main constituents in volatile fatty acids (VFA). The higher acetate and propionate concentrations in reactors 1 and 5 showed that the microorganisms in reactors 1 and 5 could not effectively convert the organics into methane. Reactors 3a, 3b, and 3c were all inoculated with the same digested manure; however, the steady-state reactor performances were not the same. The biogas production in reactors 3a and 3c was significantly higher than that in reactor 3b. Lower methane content, pH, and COD removal efficiency were also

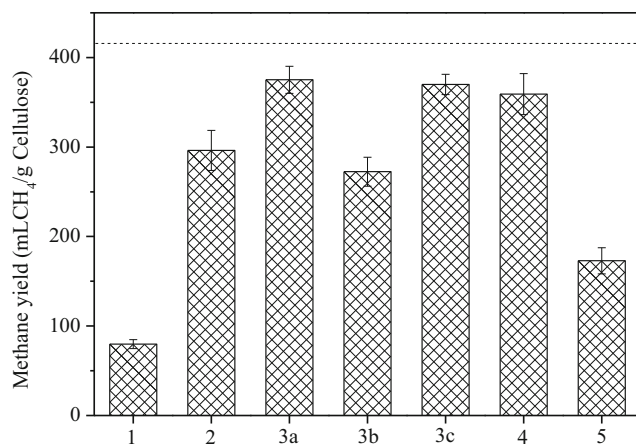
Table 2 Summary of the steady-state reactors performances

	1	2	3a	3b	3c	4	5
Biogas production (mL/d)	223.3 ± 11.5	523.3 ± 15.3	566.7 ± 20.8	483.3 ± 15.3	570 ± 10	573.3 ± 15.3	316.7 ± 20.8
Methane content (%)	32.2 ± 1.5	50.9 ± 2.3	56.6 ± 1.4	50.7 ± 1.8	56.4 ± 0.8	54.5 ± 2.4	49.1 ± 1.4
Methane production (mL/day)	71.8 ± 4.4	266.6 ± 20	320.7 ± 13.8	245.2 ± 14.6	321.7 ± 10.1	312.4 ± 20.7	155.6 ± 13.1
pH	6.68 ± 0.13	7.06 ± 0.06	7.21 ± 0.04	7.13 ± 0.04	7.2 ± 0.05	7.17 ± 0.08	6.97 ± 0.08
Acetate (mM)	44.8 ± 2.4	9 ± 2.1	3 ± 2.4	9.15 ± 1.48	2 ± 0.85	2.9 ± 1.13	19.6 ± 3.39
Propionate (mM)	2.9 ± 0.1	0.55 ± 0.07	0.23 ± 0.2	0.55 ± 0.07	0.15 ± 0.07	0.2 ± 0.11	1.35 ± 0.07
COD removal (%)	24.5 ± 2.4	79.6 ± 3.2	90.3 ± 2.5	73.8 ± 2.5	88.2 ± 3.1	87.7 ± 2.1	51.2 ± 3.3

observed in reactor 3b compared to reactors 3a and 3c. The methane yields from all the reactors were shown in Fig. 1. The theoretical methane yield was calculated to be 413 mL CH₄/g cellulose, and the higher methane yields were obtained from reactors 3a, 3c, and 4, which were around 86 % of the theoretical value.

Diversity of the microbial communities

Both the inocula and the steady-state microbial communities of all the reactors were analyzed by the high-throughput sequencing of the 16S rRNA genes. The obtained sequences were quality checked and normalized to 20,000 sequences for the following analysis. The parameters related to the diversity of the microbial communities are shown in Table 3. All the inocula had higher species richness compared to the samples from steady-state reactors as reflected by the higher numbers of OTUs and CHAO1. The Shannon diversity index, which provides both species richness and evenness of the species among all the species in the community (Lu et al. 2012), also showed that the diversity of microbial communities in the inocula was higher than the samples from the biogas reactors. The rarefaction curves of all the samples at 0.03 distance are shown in Fig. 2. For samples 2I and 5I, the numbers of OTUs tended to increase faster compared to the other

**Fig. 1** Steady-state methane yields of different biogas reactors

samples, which suggested that the sequencing depth of 20,000 was still not enough to cover the whole diversity. However, the higher coverage values (80 %) for all the samples clearly indicated that the dominant OTUs were detected in the present study.

Structure of the microbial communities

The dendrograms from Bray-Curtis similarity matrices that took into account the abundance of sequences in each OTU were generated to assess the differences among all the samples, and the result is shown in Fig. 3. It was obvious that the microbial community of the sample from each biogas reactor and its corresponding inoculum were clustered together (except 5 and 5I), although the similarity (between 10 and 20 %) was not very high. The phylogenetic classification of the sequences was conducted by Ribosomal Database Project classifier, and both bacteria and archaea classifications were obtained since universal primers for PCR were used in the present study. Figure 4 shows the phylogenetic classification of sequences assigned to bacteria. Different distributions of the sequences at phylum level were observed for the five different inocula. For example, *Proteobacteria* were found to be dominant in 2I and 4I, while *Firmicutes* were dominant in 1I, 3I, and 5I. The different phylum level distributions of the inocula resulted in the different similarity as shown in Fig. 3. For the samples obtained in the present study from the biogas reactors, the dominant phyla were *Firmicutes* and *Bacteroidetes*. The higher abundance of *Firmicutes* in 3a compared to 3b and 3c resulted in the different distribution of microorganism in the samples from parallel biogas reactors, which also supported the observation in Fig. 4. Standing in the class level, it was further found that the relative abundance of *Clostridia*, belonging to *Firmicutes*, were usually higher in the samples from biogas reactors compared to the inocula.

The order level identification of the sequences belonging to archaea is shown in Fig. 5. The sequences were mainly assigned to three orders (*Methanobacteriales*, *Methanomicrobiales*, and *Methanosarcinales*) mediating hydrogenotrophic and acetoclastic methanogenesis. Different order level distributions of the sequences obtained from all the

Table 3 Comparison of the richness and diversity of the 16S rRNA gene libraries based on 0.03 distance

Sample ID	Clusters	CHAO1	Shannon diversity	Coverage (%)	Bacteria (%)	Archaea (%)
1I	2906	6641	5.74	88.8	99.57	0.43
1	2025	4632	5.22	92	97.99	2.01
2I	3927	9561	6.49	85.6	92.01	7.99
2	2034	4340	5.19	92.9	95.25	4.75
3I	2686	6450	5.96	88.4	95.81	4.19
3a	2068	3802	5.44	91.3	95.53	4.47
3b	1715	2948	5.14	92.5	96.36	3.64
3c	1581	2920	4.68	92.7	96.17	3.83
4I	2442	5112	5.76	91.6	61.85	38.15
4	2091	4449	5.18	90	92.70	7.30
5I	4215	10347	6.89	80.9	94.00	6.00
5	1659	3648	4.58	92.6	96.95	3.06

inocula were observed. Only *Methanobacteriales* and *Methanomicrobiales* were detected in 1I, while the three orders were all detected in 2I, 3I, 4I, and 5I though their relative abundances were different in each sample. The samples from the biogas reactors also had different order level distributions of the sequences, and *Methanosarcinales* belonging to acetoclastic methanogens were found in all the samples. *Methanomicrobiales* were absent in sample 4 but present in all the other samples from the biogas reactors. Samples 3a, 3b, and 3c had the same dominant orders (*Methanobacteriales*, *Methanomicrobiales*, and *Methanosarcinales*) and genus (*Methanosarcina*, *Methanoculleus*, and *Methanobacterium*) but with different relative abundances, which also might contribute to their difference in Fig. 3.

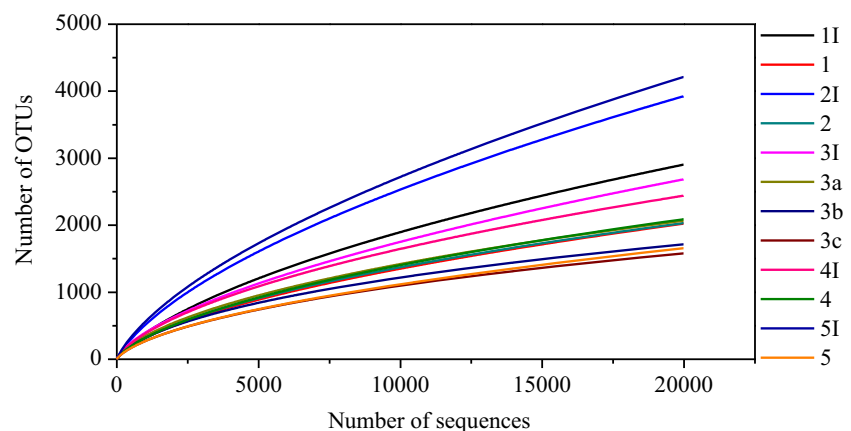
Discussion

The results obtained from the performances of the long-term operated continuous reactors indicated that the inoculum had significant effects on the biogas production from cellulose, and different reactor performances can be obtained with the

same inoculum and operational conditions. The differences between actual and theoretical methane yields (Fig. 1) were due to the organics left in the effluent, which included unconverted VFA, residual cellulose, and also the produced biomass.

The microbial community analysis showed that the inocula generally had higher diversity than the samples from the biogas reactors, and it could be due to that the microbial communities accumulated in the biogas reactors are special for the anaerobic degradation of pure cellulose, while the inocula had more diverse microorganisms to degrade complex substrates including stillage, manure, paper milling wastewater, et al. (Sundberg et al. 2013).

The Bray-Curtis similarity analysis showed that inoculum played an important role in shaping the microbial communities in biogas reactors treating cellulose since the sample from each biogas reactor was clustered together with its corresponding inoculum. Samples 3a, 3b, and 3c were obtained from parallel biogas reactors; however, they only had around 30 % similarity, and it could be related to the different reactor performances as mentioned before. The results also showed that different microbial community patterns could be

Fig. 2 Rarefaction curves of all the samples at 0.03 distance

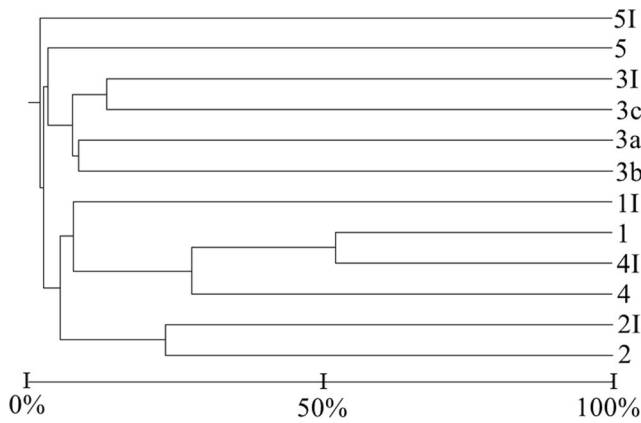


Fig. 3 Dendrograms comparing the level of similarity among all the samples at 0.03 distance

generated under similar environmental conditions. Although reactors 2, 3a, 3b, 3c, and 4 had relatively higher methane yields compared to the other biogas reactors, the microbial communities only had 5 % similarity. It suggested that the efficient biogas production from cellulose could be obtained with different microbial community patterns. Sample 5I (raw

cattle manure) had the lowest similarity to all the other samples, which was expected since all the other samples were obtained from biogas reactors treating different feedstock.

The different phylum level distributions of the inocula showed that the inocula chosen in the present study had different microbial community compositions, which ensured the possibility to elucidate the general relationship of microbial communities between various inocula and their final accumulated microbial communities. *Firmicutes* and *Bacteroidetes* were dominant in all the samples, and they can degrade a wide range of complex organics including proteins and carbohydrates (Sundberg et al. 2013), and their dominance was also reported in several studies focusing on biogas production (Luo et al. 2015; Riviere et al. 2009). The class *Clostridia* was found in all the reactors, and it is a highly versatile class that could degrade both carbohydrates and proteins, and their dominance was found in biogas reactors treating both manure and sewage sludge (Lee et al. 2008; Schluter et al. 2008; Sundberg et al. 2013). The enrichment of *Clostridia* in the present study might be related with their capacity to hydrolyze cellulose substrates and further produce volatile fatty acids

Fig. 4 Taxonomic classification of the bacterial communities at phylum and class level. Phyla making up less than 1 % of total composition in all the samples were classified as “others”

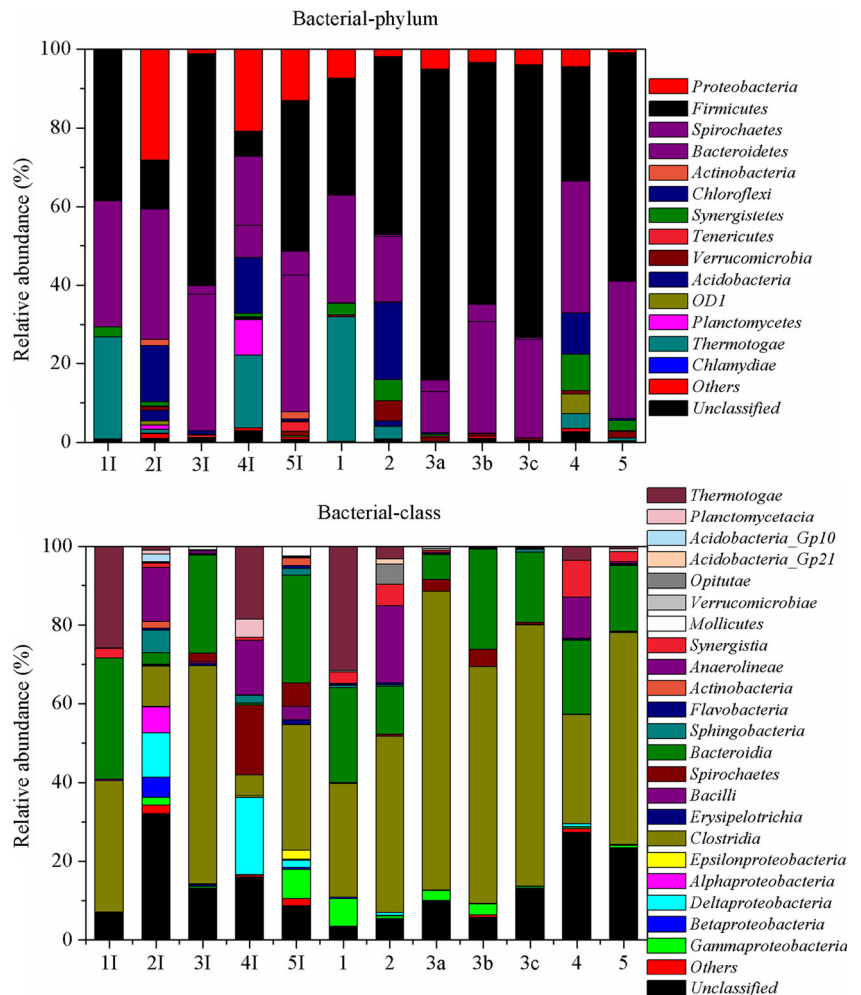
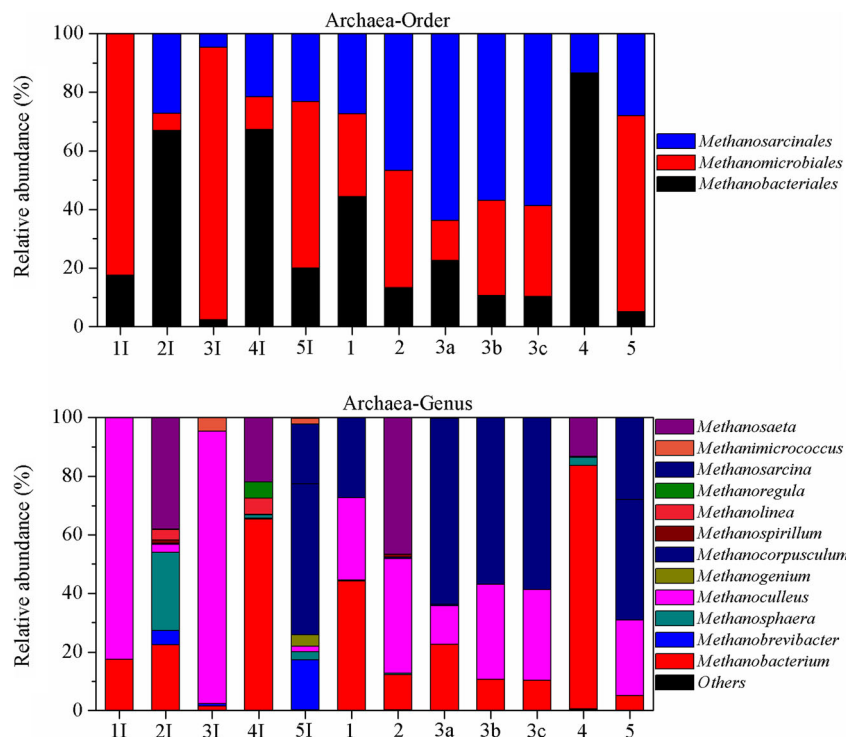


Fig. 5 Taxonomic classification of the archaea communities at order and genus levels. Order and genus making up less than 1 % of total composition in all the samples were classified as “others”



and alcohols (Kratat et al. 2011). *Thermotogae*, which was mainly found in II and 1, can utilize different complex carbohydrates to produce hydrogen and volatile fatty acids (Nguyen et al. 2008). They were thought to be thermophilic microorganisms; however, recently, some *Thermotogae* existing in mesophilic temperatures has been identified (Nesbø et al. 2010). Their dominance in reactor 1 may be related to their existence in the inoculum, which further showed that inoculum had considerable effects in shaping the microbial communities of the biogas reactors.

The results from the analysis of archaea showed that *Methanosarcinales* were found in sample 1 but not in the inoculum II, which indicated different microbial communities could be formed when the cultivation conditions were changed. The lower relative abundances of *Methanosarcinales* (<30 %) in reactors 1 and 5 could be related with the higher concentrations of VFA and lower pH, and it resulted in the dominance of syntrophic acetic acid oxidation and higher relative abundance of hydrogenotrophic methanogens (Fotidis et al. 2013; Fotidis et al. 2014). *Methanobacteriales* had a high relative abundance (85 %) in sample 4 that indicated syntrophic acetic acid oxidation occurred in reactor 4, which was not expected since reactor 4 was not running in an extreme conditions such as high ammonia or VFA concentration (Lü et al. 2013). The result might suggest syntrophic acetic acid oxidation could be present in biogas reactors running in smooth conditions, which deserved further investigation.

The present study clearly showed that inoculum had an important role in shaping the microbial community as well as their functions. Previous studies also showed that inoculum affected the biogas production from organic wastes in batch experiments (Dhamodharan et al. 2015; Gu et al. 2014; Lopes et al. 2004), which was consistent with the results from continuous experiments in the present study. However, our study, for the first time, not only investigated the effects of various inocula on the biogas production from cellulose in long-term operated biogas reactors but also made the microbial community analysis. Karakashev et al. (2005) demonstrated that inoculum population appeared to have no influence on the eventual population of the manure-digesting plants. However, we found that inoculum had considerable influence on the microbial community in the biogas reactor treating cellulose since the inocula and the accumulated samples in the biogas reactors were well clustered together as shown in Fig. 3. The difference might be resulted from the feedstock. In the study of Karakashev et al. (2005), manure was the feedstock for the biogas reactors, which were known to be rich in various microorganisms (also demonstrated in our study as shown in Fig. 4), and therefore the influence of the inoculum could be ignored after long-term operation. However, only sterilized cellulose was used in our study and therefore the initial microorganisms in the inoculum were crucial for the biogas production. Some previous studies showed that common environmental selection recruits the same or similar species from diverse starting species sets to produce similar final community structures, which follows the niche-based theory (Ayarza and

Erijman 2011; Kurtz et al. 1998). However, all the biogas reactors in our study were operated under identical operational conditions (temperature, substrate, reactor configuration), and different final steady-state microbial communities with different functions (i.e., biogas production, VFA concentration et al.) were generated. It indicated that niche-based theory might not be applicable to the microbial community assembly in biogas reactors in the present study, and inoculum played an important role in the microbial community assembly in the biogas reactors. The results also suggested that for the wastes/wastewater (stillage, wastewater from papermaking plant) that lack microorganisms relating with anaerobic digestion, the selection of proper inoculum is important to get efficient biogas production, while it might not be important to get the proper inoculum for biogas production from the wastes including manure and sewage sludge, which already contained various microorganisms.

In addition, the present study also found that different microbial communities and reactor performances were obtained from the parallel biogas reactors 3a, 3b, and 3c, which were all operated under similar conditions (temperature, feedstock, reactor configuration, and even the inoculum). It could be explained by the neutral theory, where stochastic processes of birth, death, colonization, extinction, and speciation can lead to alternative communities with distinct functions under similar environmental conditions (Chave 2004). Zhou et al. (2013) demonstrated that the reactor performances (electricity, H₂, pH, et al.) in parallel microbial electrolysis cells were different under the same operational conditions (temperature, substrate feeding, applied voltage, reactor structure, and materials), which was similar to our results. Another study found that four parallel biogas reactors with glucose as substrate, which were seeded with the inoculum already accumulated to glucose, had replication of function but without replication of community structures (Fernandez et al. 2000), which could be also related to the stochastic assembly of the microbial communities in the four parallel biogas reactors. Furthermore, they also observed a glucose-fed biogas reactor that experienced a shift in community structure with no detectable change in performance (Fernández et al. 1999). The above results showed that microbial community assembly in biogas process is very complex, and not only the inoculum source but also the stochastic factors played important roles in the microbial ecosystem development. The results had important practical implications for biogas research, and parallel biogas reactors should be operated in order to ensure the reliability of the data obtained, which was already applied in batch experiments for biogas production but not common in continuous biogas reactors. In our study, the inoculum seems to affect more on the microbial community assembly, since the samples with different inocula had lower similarity (10–20 %) compared to the samples (3a, 3b, and 3c) with the same inocula (30 %).

Overall, the present study investigated the microbial community assembly in biogas reactors treating cellulose with various inocula. Different reactor performances (biogas production, VFA concentration, and pH) were obtained from the reactors with different inocula, and it was consistent with the different microbial community patterns as reflected by Bray-Curtis similarity matrices and taxonomic classification. The results also showed that the microbial community assembly in biogas reactors did not follow the niche-based ecology theory, and inoculum played an important role in shaping the microbial communities in the present study. By comparison of the results from parallel reactors with the same inoculum, we further found that stochastic assembly contributed to the different microbial community patterns with distinct functions of the biogas reactors with similar operational conditions.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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