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Revealing the anaerobic acclimation of microbial community in a membrane bioreactor for coking wastewater treatment by Illumina Miseq sequencing

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ABSTRACT

The dynamic change of microbial community during sludge acclimation from aerobic to anaerobic in a MBR for coking wastewater treatment was revealed by Illumina Miseq sequencing in this study. The diversity of both Bacteria and Archaea showed an increase–decrease trajectory during acclimation, and exhibited the highest at the domestication interim. Ignavibacteria changed from a tiny minority (less than 1%) to the dominant bacterial group (54.0%) along with acclimation. The relative abundance of Betaproteobacteria kept relatively steady, as in this class some species increased coupled with some other species decreased during acclimation. The dominant Archaea shifted from Halobacteria in initial aerobic sludge to Methanobacteria in the acclimated anaerobic sludge. The dominant bacterial and archaeal groups in different acclimation stages were indigenous microorganisms in the initial sludge, though some of them were very rare. This study supported that the species in "rare biosphere" might eventually become dominant in response to environmental change.

Introduction

Coking wastewater is generated during high-temperature carbonation, coal gas purification, and chemical refining in coking plants. It contains high concentration of ammonia, and recalcitrant organic pollutants like phenol, benzene, polycyclic aromatic hydrocarbons, and nitrogen-, oxygen-, sulfur- heterocyclic compounds (Jiang et al., 2016; Li et al., 2016), resulting in this wastewater as one of the most refractory wastewaters. Since high efficiency and low investment, biological treatment technology is the most acceptable and widely used one for coking wastewater treatment. Thereinto, some anaerobic, anoxic, and oxic combined processes, such as anaerobic/anoxic/oxic (A^2/O), have been applied to deal with the ammonia and recalcitrant organic pollutants in coking wastewater, however, the treatment performance needs to be improved.

Membrane bioreactor (MBR) realizes complete biomass retention, and maintains higher mixed liquid suspended solids (MLSS), thus can resist to influent loading shock as well as improve effluent quality. With the development of membrane technology and the increasing pressure on industrial wastewater treatment, MBR, specifically, oxic MBR (OMBR), has been gradually applied to industrial wastewater biological treatment process (Chen et al., 2008; You et al.,

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2008). A lab scale anaerobic-anoxic-OMBR process has been developed for coking wastewater treatment (Zhu et al., 2013). Though the pollutant removal of the combined process was acceptable, the anaerobic unit, however, could hardly remove any chemical oxygen demand (COD), and the biodegradability of the anaerobic effluent also needs to be improved. Anaerobic membrane bioreactor (AnMBR), which attracted increasing research interest in recent several years (Kanai et al., 2010; Van Zyl et al., 2008), possesses the same advances of OMBR, i.e., complete biomass retention, and higher MLSS, and thus achieves a better hydrolytic acidification performance, improves the biodegradability of wastewater, as well as removes part of the COD. Therefore, an AnMBR was developed in this study to replace the anaerobic unit and enhance the anaerobic treatment performance in the anaerobic-anoxic-OMBR process for coking wastewater treatment.

Microorganism is the key factor affecting the biological treatment process. High throughput sequencing has been widely applied to understand microbial communities in bioreactors (Gentile et al., 2007; Ma et al., 2013). To reach a satisfactory treatment performance, the start-up phase of anaerobic bioreactor is considered to be a critical point (Ike et al., 2010). Several studies have investigated the microbial community succession during the start-up phase of anaerobic bioreactor treating synthetic wastewater (Calli et al., 2005; Leclerc et al., 2001), molasses wastewater (Gong et al., 2005), dairy wastewater (Liu et al., 2002), diluted livestock wastewater (Ren et al., 2010), and aromatic compounds (Stoffels et al., 1998). However, there is no clear study reporting the microbial succession in the start-up phase of an anaerobic bioreactor for coking wastewater treatment.

In this study, the sludge was collected at different stages of the AnMBR during the acclimation period from aerobic to anaerobic. Adopting Illumina Miseq sequencing, this study is aiming to reveal the anaerobic acclimation of microbial community during the start-up phase of the AnMBR, and uncover the relationship between the aerobic and anaerobic microbial communities for coking wastewater treatment.

1. Materials and methods

1.1. Experimental setup

The lab-scale AnMBR was made of plexiglass, with a working volume of 4.9 L. It was composed by an anaerobic reactor and external cross-flow membrane pool, two of which were connected by an external circulating pump (Fig. S1). Water batch insulating layer was set out of the anaerobic reactor to keep inner temperature of the reactor constant at about 37°C. Polyvinylidene fluoride (PVDF) hollow fiber membrane with lining was used in the AnMBR. The pore size was 0.02 μm, and the maximum membrane flux used in this experiment was 3.24 L/(m^2·hr).

1.2. Seed sludge and influent

The AnMBR was seeded with the sludge collected from an aerobic tank of a coking wastewater treatment plant in Hebei, China. The initial MLSS was about 10 g/L. The influent was collected from the regulation pool of the same coking wastewater treatment plant. The influent quality is shown in Table 1. The COD of influent during 21–94 days was lower than that during 95–125 days.

1.3. Operation conditions and performance

The influent was pumped into the AnMBR through a peristaltic pump. The mixed liquor was circulated between membrane pool and anaerobic reactor by circulating pump to make sludge mixed adequately. Meanwhile, the cross-flow generated by mixed liquor circulation could attenuate membrane fouling. The effluent of the AnMBR was also pumped out by a peristaltic pump.

From day 1 to day 20, the AnMBR was run in anaerobic condition with no influent. From day 21 to day 94, the AnMBR was run in sequencing batch mode. Each batch was about 500 mL per week. As the sludge became manure and the performance became steady, the AnMBR was operated in continuous feeding mode from day 95. During continuous feeding period (day 95–125), HRT of AnMBR was kept at 49 hr and influent load was improved. Sludge was not discharged during the whole period, only a small amount of sludge lost during membrane offline cleaning.

The pollutants removal performance is shown in Fig. S2. During the sequencing batch operation period (21–94 days), the influent COD was kept relatively low. The average COD and total organic carbon (TOC) as well as total nitrogen (TN) of effluent were a little higher than the influent since the sludge was under acclimation. The ammonia nitrogen (NH_3-N) was also higher than the influent, because of the acclimation and ammonification. During continuous feeding period, the average COD and TOC of influent were 1482 and 330.2 mg/L, the average COD and TOC of effluent were 1106 and 240.9 mg/L, and the removal rate of COD and TOC were 20.3% and 26.5%, respectively. Thus, the organic pollutants removal rate was improved and the sludge in AnMBR has started to degrade organic pollutant. The acclimation of sludge from aerobic to anaerobic has basically completed.

1.4. Sample collection

A total of 5 sludge samples were collected on days 5, 21, 49, 95, and 125 and named as S1, S2, S3, S4, and S5, respectively. S2 was collected before the sequencing batch mode started, and S4 was collected before the continuous feeding mode started. For each sample, sludge was collected after centrifuging mixed liquor 5 min at 3000 r/min and supernatant discarded. Two aliquots of 3 mL sludge were stored at −80°C until DNA

Table 1 – Influent quality of AnMBR during acclimation period.

Time (day)	COD	TOC	NH_3-N	TN	TP	pH
21–94	993 ± 353	230 ± 77	75 ± 9	191 ± 13	<0.3	7.4 ± 0.1
95–125	1482 ± 227	330 ± 64	74 ± 18	191 ± 6	<0.3	7.7 ± 0.2

AnMBR: anaerobic membrane bioreactor; COD: chemical oxygen demand; TOC: total organic carbon; TN: total nitrogen; TP: total phosphorus.

extraction. The two subsamples of Sx (S1 to S5) were named as Sx-1 and Sx-2.

1.5. DNA extraction and PCR amplification

The total DNA was extracted from about 0.25 g of each sub-sample with TIANamp Soil DNA Kit (TIANGEN, China) according to the manufacturer's protocol. The extracted DNA was checked with 1% agarose gel electrophoresis. The bacterial and archaeal 16S rRNA genes were amplified the DNA samples with barcode linked specific primers.

The primers and thermal programs are listed in Table S1. The PCRs were conducted on a GeneAMP® 9700 (ABI, USA) with a TransStart Fastpfu DNA Polymerse (TransGen, China). Triplicate PCRs were conducted for each sample. The mixture of the triplicate PCR products was tested with 2% agarose gel electrophoresis, extracted by AxyPre DNA Gel Extraction Kit (Axygen, USA), and then quantified by a QuantiFluor™ -ST (Promega, USA).

1.6. Illumina Miseq sequencing and data processing

The purified amplicons were drawn in equimolar and paired-end sequenced on an Illumina MiSeq platform according to the standard protocols. Raw sequencing files were processed with QIIME as follow. Firstly, by checking quality score over a 50 bp sliding window for each read, the reads were truncated at the sites with an average score < 20. All the truncated reads with a length shorter than 10 bp were discarded. Subsequently, by screening and barcode matching, the reads containing any ambiguous characters or 2 mismatched nucleotides were removed from further analysis. The remained sequences were assembled with an overlap of 10 bp and longer, and the assemble-failed reads were discarded.

The assembled reads were clustered into operational taxonomic units (OTUs) with a 97% similarity cutoff adopting UPARSE, after chimeras removed by UCHIME. The singletons were discarded to avoid potential sequencing errors. To avoid biases for alpha diversity comparison, equal sequences were subsampled from each sequencing library, based on the lowest sequencing depth among all samples (30,348 for bacterial 16S rRNA gene libraries; 44,846 for archaeal 16S rRNA gene libraries). Chao 1, Shannon indexes were calculated based on subsampled sequence-sets. All subsampled sequences were compared against to the SILVA database to assign taxonomy, by RDP Classifier with a confidence threshold of 70%. The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: SRP093350).

2. Results and discussion

2.1. Richness and diversity of bacterial/archaeal libraries

Ten bacterial 16S rRNA gene libraries and ten archaeal 16S rRNA gene libraries were constructed by Illumina Miseq sequencing. In total, 303,480 valid reads of bacterial 16S rRNA and 448,460 valid reads of archaeal 16S rRNA were produced from 358,739 and 545,367 trimmed reads after subsampling (Table 2), respectively. All the rarefaction curves show gentle slopes under current sequencing depth (Fig. S3), which indicates that the sequencing libraries could well reflect the microbial communities. The rarefaction curves and Shannon curves (Fig. S3) as well as Chao 1 and Shannon index (Table 2) demonstrated the diversity and richness of Bacteria in samples were significantly higher than that of Archaea. Indicated by the change of Chao 1 and Shannon index from S1 to S5, both richness and diversity of Bacteria and Archaea increased firstly and decreased subsequently during the acclimation. Sample S3, the sludge collected on day 49, possessed the highest bacterial and archaeal diversity among all the samples.

In the first period (1–21 days), the microbial diversity increased with influent loading kept constant. With the loading improved in batch operation period (21–95 days), the microbial diversity increased from day 21 (S2) to day 49 (S3), but decreased from day 49 (S3) to day 95 (S4). In the followed continuous feeding period (95–125 days), the loading was further improved, while the diversity of microbial community decreased. The variation of microbial diversity was not consistent with the change of nutrient concentration. Therefore, the influent loading was not the main reason for microbial diversity variation. During the acclimation, partial microorganisms in the aerobic seed sludge who could not adapt to anaerobic condition would be gradually replaced by the ones who favor anaerobic environment. The microbial diversity is expected to achieve the highest in the intermediate period of acclimation, when the unadapted microbes have not completely disappeared yet, and the new dominant microbial species have appeared, as day 49 in this study. A similar increase–decrease trajectory of bacterial and archaeal diversity was reported by Goux et al. (2016) during the start-up phase of an anaerobic reactor.

2.2. Taxonomic composition of the bacterial community

Bacterial composition of the samples at phylum level is shown in Fig. S4. It shows that the relative abundance of Proteobacteria (26.2%–49.9%) was relatively high in all samples. The relative abundance of Bacteroidetes decreased from average 32.2% in S1 to average 20.6% in S2, then rebounded to 36.2% in S3. In S4 and S5, Bacteroidetes kept in relative low abundance of 10.3% and 6.8%, respectively. The relative abundance of Firmicutes increased from a much lower level in S1 (1.4%) to a relatively higher level in S2 and S3 (16.3% and 15.4%), and then decreased to 5.3% in S4 and 8.8% in S5. Chlorobi was the phylum with the greatest change of relative abundance in bacterial community, which was only 10^{-3}–10^{-5} in S1, S2, and S3, but increased to 23.2% in S4 and 48.4% in S5.

Bacterial composition of the samples at class level is shown in Fig. 1a. Ignavibacteria, belong to the phylum Chorobi (Iino et al., 2010), changed from a tiny minority (less than 1%) in S1, S2, and S3 to a dominant group in S4 (23.2%) and S5 (48.4%), which illustrated that almost all Chorobi in the bioreactor was Ignavibacteria. The change of relative abundance also indicated Ignavibacteria's preference of anaerobic environment. Betaproteobacteria was the main class of Proteobacteria in the samples, with abundance kept relatively

Table 2 – Characteristics of sequencing libraries.

Target gene	Sample	No. of trimmed sequences	No. of subsampled sequences	No. of OTUs	Chao 1 value	Shannon index
Bacterial 16S rRNA	S1–1	39360	30348	255	282	3.20
	S1–2	32718	30348	208	235	2.56
	S2–1	35012	30348	376	421	3.46
	S2–2	30873	30348	365	416	3.50
	S3–1	36925	30348	431	491	3.60
	S3–2	38834	30348	439	493	3.67
	S4–1	38704	30348	427	516	3.42
	S4–2	30348	30348	421	456	3.33
	S5–1	40450	30348	205	284	2.41
	S5–2	35515	30348	216	288	2.20
Archaeal 16S rRNA	S1–1	44846	44846	47	62	0.47
	S1–2	51151	44846	46	64	0.44
	S2–1	51553	44846	125	150	1.42
	S2–2	56434	44846	102	126	1.38
	S3–1	51758	44846	185	196	1.99
	S3–2	60737	44846	175	190	1.78
	S4–1	63974	44846	192	215	1.69
	S4–2	47119	44846	174	194	1.44
	S5–1	55546	44846	123	134	1.08
	S5–2	62249	44846	147	174	1.23

OTUs: operational taxonomic units.

stable (17.4%–31.8%) during the whole domestication period. It could probably be explained by the oxygen adaptability of this class of bacteria was very wide, or another possibility that partial bacteria of this class was suitable for aerobic environment but the other part was suitable for anaerobic environment. Through the comparison of the OTU composition of Betaproteobacteria in each sample (Fig. S5), the second case was proved. The proportions of OTUs in Betaproteobacteria changed dynamically, but the total relative abundance of Betaproteobacteria was relatively stable. Another proteobacterial class, Gamaproteobacteria, only showed a high relative abundance in S4 (21.9%), but kept in low proportion (1.5%–2.8%) in other samples. Sphingobacteriia in phylum Bacteroidetes was one of the main class in S1, with a relative abundance of 14.5%, but almost disappeared in S5. Bacteroidia in phylum Bacteroidetes and Clostidia in phylum Firmicutes were two representative classes showed increase in early domestication, but decrease in late domestication. They were two of the dominant classes in the domestication interim, with relative abundances of 12.3% and 15.9% in S2, 27.4% and 15.2% in S3, respectively.

The bacterial genera in the samples were shown in Fig. 1b. The relative abundance of partial bacterial genera increased, such as *Myroides*, *Desulfitobacterium*, *Alcaligenes*, *Weeksella*, *Arcobacter*, *Sedimentibacter*, *Comamonas*, *Pseudomonas*, an unclassified genus of Comamonadaceae. *Desulfitobacterium* was reported as an anaerobic reductively dechlorinating bacterium (Christiansen and Ahring, 1996), and could for its ability to degrade chlorinated phenols (Field and Sierra-Alvarez, 2007), PCBs (Macherzyński et al., 2014). *Alcaligenes* is a genus commonly present in coking wastewater (Bai et al., 2009; Liu et al., 2012), and previous studies have demonstrated that it can degrade a variety of pollutants, including phenol (Essam et al., 2010), crude oil (Lal and Khanna, 1996), and polychlorinated biphenyls (PCBs) (Furukawa et al., 2014). *Weeksella* is common in the aerobic treatment processes of dyeing wastewater (Shao et al., 2014) and coking wastewater (Zhu et al., 2015). *Sedimentibacter* was known to relate to degradation of a great number of hydrocarbons, such as phenol (Qiu et al., 2008), methylphenol and naphthol (Huang et al., 2016), under anaerobic conditions (Ailijiang et al., 2016). Consistently, *Sedimentibacter* significantly increased along with anaerobic acclimation in this study. *Comamonas* was reported widespread in coking wastewater sludge (Bai et al., 2008; Ma et al., 2015), and was a versatile aromatic degrader for polycyclic aromatic hydrocarbons (PAHs) (Guazzaroni et al., 2013) and heterocyclic aromatics, such as phenol (Felföldi et al., 2010; Yap et al., 1999), naphthalene and phenanthrene (Goyal and Zylstra, 1997), and quinoline (Cui et al., 2004). The denitrifying degradation of aromatic compounds by unclassified genus of Comamonadaceae was also reported (Boon et al., 2001; Ma et al., 2015). The most dominant genus in S5, PHOS-HE36 was uncultured and reported to survive under anaerobic and anoxic condition, which has been found in various wastewater treatment processes (Koenig et al., 2005; Qiao et al., 2009). It can be seen that most of these genera could adapt to anaerobic and organic pollution environment, and good at PAHs and heterocyclic aromatic degradation. The degradation function and environmental adaptability is the possible reason for the increase of these bacterial groups.

On the other hand, a large number of abundant genera in S1 lost dominance after anaerobic domestication. Among them, the dominance of *Nitrosomonas* (Zhu et al., 2013), *Thiobacillus* (Felföldi et al., 2010; Zhang et al., 2015), and uncultured Chitinophagaceae (Joshi et al., 2016) in aerobic activated sludge has been reported. Oxygen preference seems to be the key reason for the decrease of these genera during anaerobic acclimation.

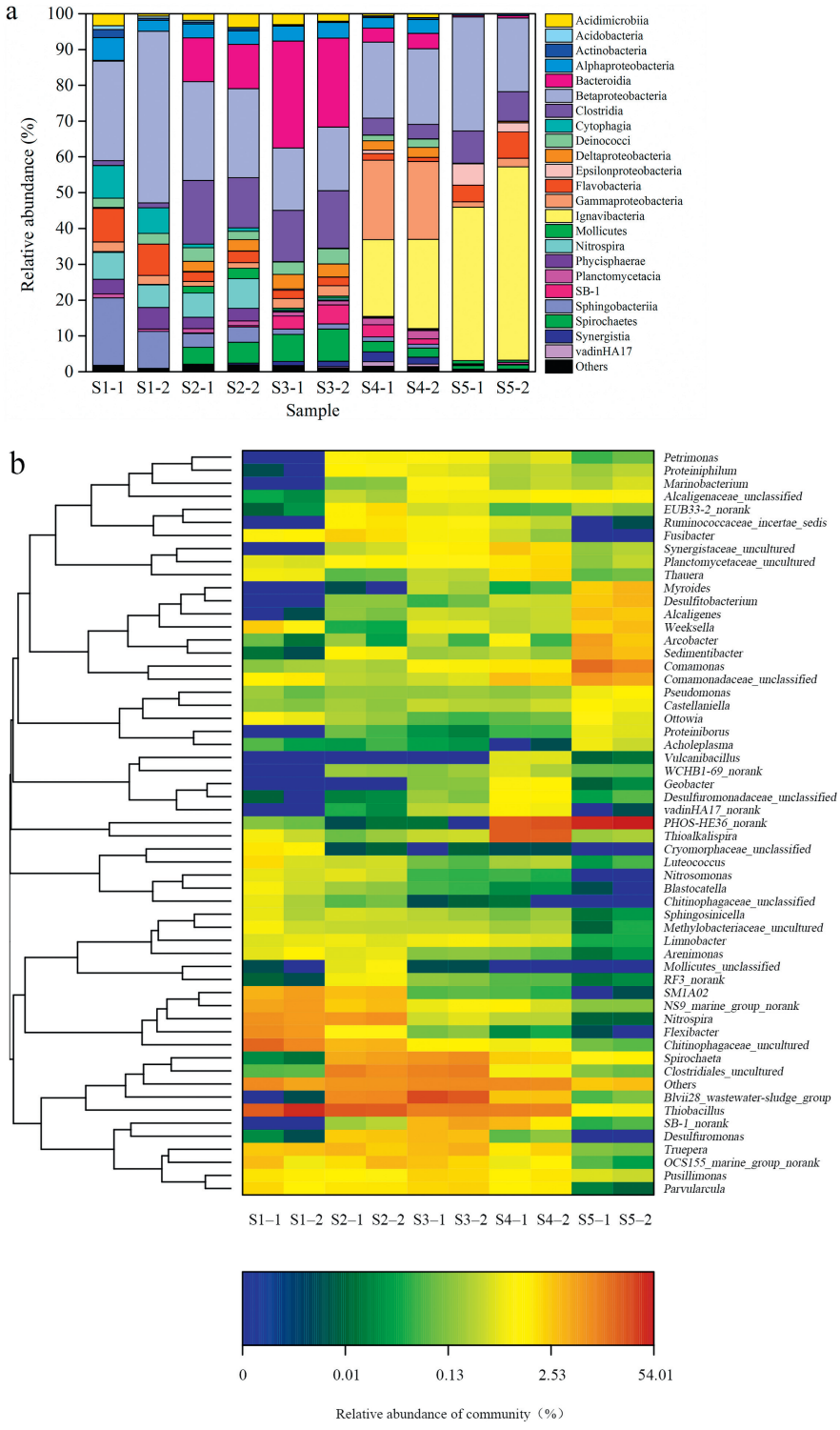

Fig. 1 – Relative abundance of (a) main bacterial classes and (b) main bacterial genera in the samples. Classes and genera had a relative abundance <1% were assigned to 'others'.

2.3. *Taxonomic composition of the archaeal community*

Almost all of Archaea in the samples were Euryarchaeota (relative abundance >99%, shown in Table S2). The relative abundance of Thaumarchaeota, which commonly exists in the marine environment (Swan et al., 2014; Zhang et al., 2014), accounts for less than 0.1% in all samples. The Archaeal classes and genera of the samples were shown in Fig. 2a and b, respectively. At both class and genus level, the components of Archaea were low in each phase, while the dominant population changed significantly. At the class level, Halobacteria was the predominant group in primary stage (account for

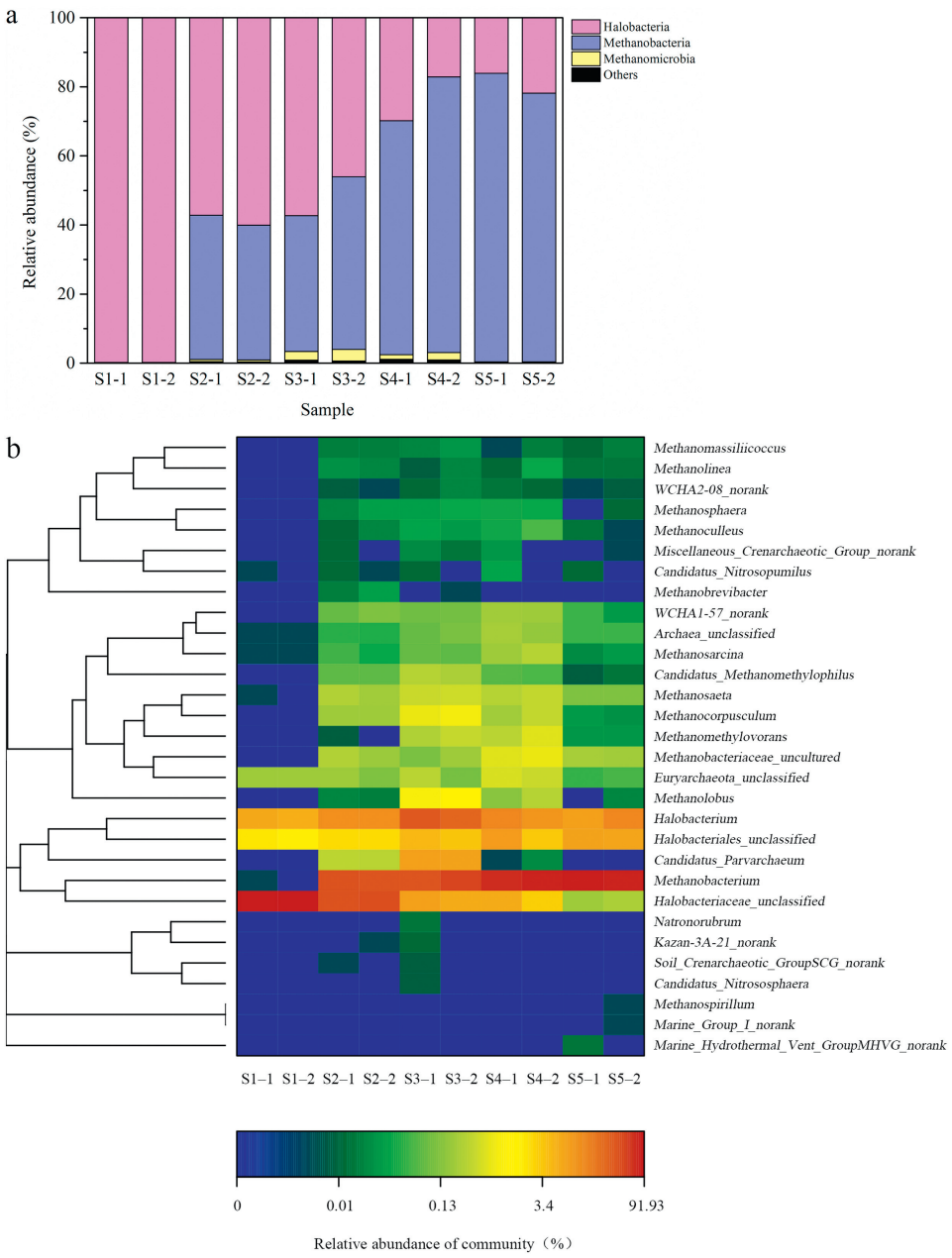

Fig. 2 – Relative abundance of (a) main archaeal classes and (b) main archaeal genera in the samples. Classes and genera had a relative abundance <1% were assigned to 'others'.

99.8% in S1) but decreased gradually along with acclimation, and accounts for 19.0% in acclimated sludge (S5) while Methanobacteria grew from less than 0.1% in S1 to the dominant group (account for 80.6%) in S5. Methanomicrobia was a minority in each phase, with the relative abundance increased from less than 0.1% in S1 to 2.9% in S3, and then decreased to 0.2% in S5.

At the genus level, only 9 genera were detected in the initial sludge (S1), in which an unclassified genus of Halobacteriaceae was the predominant one with relative abundance of 91.4%. *Halobacterium* and an unclassified genus of Halobacteriales were the other main genera in S1, with relative abundance of 6.4% and 2.0%, respectively. The remained genera only accounted for 0.2% in S1. Much more archaeal genera appeared in the sludge (S2) after 21 days of acclimation, and remained in later stage sludge (S3, S4, S5). An unclassified genus of Halobacteriaceae decreased gradually from 91.4% in S1 to less than 0.1% in S5, which could be explained by the fact that the family Halobacteriaceae are generally aerobic chemoheterotrophic (Oren, 2013). *Methanobacterium* was the dominant genus in the anaerobic acclimated sludge, which gradually increased from less than 0.1% in S1 to 80.4% in S5. The increase of *Methanobacterium* was mainly because of the anaerobic as well as mesophilic condition, since studies (Patel et al., 1990; Zeikus and Henning, 1975) have proved that the optimum temperature range for growth of many species of

Methanobacterium is mesophilic. An unclassified genus of Halobacteriales also showed a gradual increase along with acclimation, from 2.0% in S1 to 7.4% in S5. Moreover, the relative abundance of *Halobacterium* and *Candidatus_Parvarchaeum* increased at early domestication stage and then decreased. The highest relative abundance of *Halobacterium* and *Candidatus_Parvarchaeum* was 32.7% and 7.7%, respectively, both observed in S3. *Halobacterium* consists of some species that have adapted to aerobic environment and others favor anaerobic ones (Muller and DasSarma, 2005; Oren and Litchfield, 1999), which might be the reason for the increase–decrease trajectory of its abundance.

2.4. OTU-based comparison of microbial communities

Principal coordinates analysis (PCoA) was conducted based on the Bray–Curtis distance of OTU distribution of each sample (Fig. 3a and b). The duplicated sample located close in the figure indicated the good repeatability and stability of the experiment process. In both bacterial and archaeal community comparison, the samples from different dates separated away from each other, illustrating the striking differences of both bacterial and archaeal community between each two sample. While the sequential distribution of S1–S5, as shown by the arrow lines in Fig. 3a and b, indicated that the communities changed gradually. Consistently, the larger Bray–Curtis distance was observed between two samples with longer acclimation (Fig. 3c and d). It means the microbial community succession persisted during acclimation.

Meanwhile, it can be seen that the change of bacterial community structure kept steady during the acclimation period, as the distribution of the samples was nearly uniform in PCoA figure (Fig. 3a) and the Bray–Curtis distance increased gradually. Of archaeal community, however, the change from S1 to S2 was the biggest (Fig. 3b), and the changing rate slowed down after day 95, as the Bray–Curtis distance of S4 and S5 was the lowest (Fig. 3d).This result indicated that the bacterial community in the bioreactor has not reached a stable composition yet and might show further changes with later operation of the bioreactor, while the archaeal community was becoming a stable structure for coking wastewater treatment under this anaerobic condition.

For shared OTU analysis of the libraries from different samples, the duplicated sequencing libraries were mixed, library Sx-1 and library Sx-2 were mixed to a library Sx for example. The shared OTU number of bacterial and archaeal

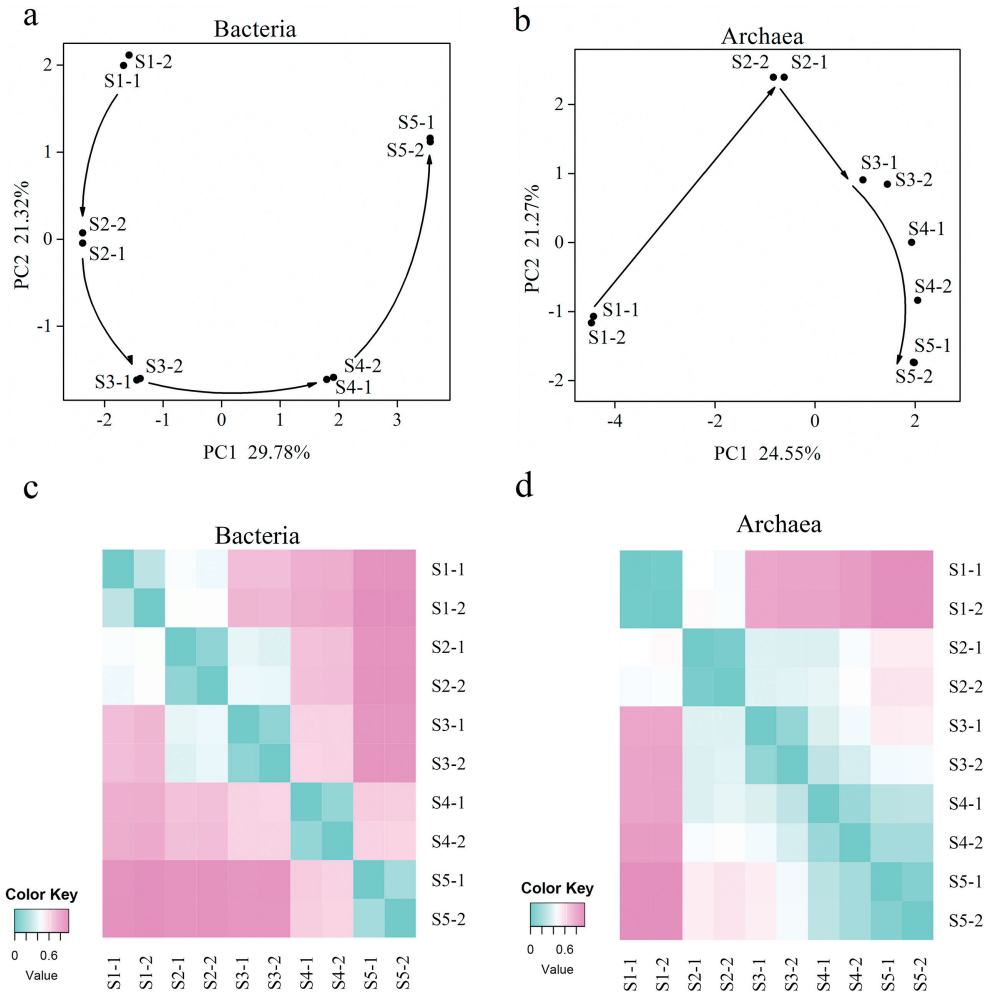

Fig. 3 – PCoA analysis of (a) bacterial and (b) archaeal community based on Bray–Curtis distance. Heatmap of Bray–Curtis distance matrix of (c) bacterial and (d) archaeal communities. PCoA: principal coordinates analysis.

16S rRNA gene libraries was shown in Fig. 4a and b, respectively. The sample-unique OTUs, the OTUs detected in only one sample, accounted for low percentages of the total OTUs in the libraries. Among them, the sample-unique bacterial 16S rRNA OTUs accounted for 2.4%–9.5% of the total OTU amount of the corresponding bacterial libraries. While the sample-unique archaeal OTUs accounted for 3.9%–18.4% of the total OTU amount of the corresponding archaeal libraries. The relative abundances of the sample-unique OTUs (sample-unique reads/total reads in the library × 100%) were much lower, 0.115%–0.623% of bacterial 16S rRNA libraries and 0.011%–0.303% of archaeal 16S rRNA libraries (Tables S3 and S4).

One hundred two OTUs were shared by the five mixed bacterial 16S rRNA libraries, and accounted for 38.6%, 24.8%, 21.0%, 21.6%, and 40.9% of total bacterial 16S rRNA OTU number of S1, S2, S3, S4, and S5, respectively. The taxonomic composition of the five bacterial 16S rRNA libraries shared OTUs is shown in Fig. 4c. The relative abundance of the shared bacterial OTUs decreased from 84.9% in S1 to 57.6% in S4, and then increased to 88.4% in S5. The change trajectory was almost consistent with that of shared OTU percentage. Twenty nine OTUs were shared by the five mixed archaeal 16S rRNA libraries, and accounted for 46.0%, 19.0%, 12.7%, 12.2%, and 17.3% of total archaeal 16S rRNA OTU number of S1, S2, S3, S4, and S5, respectively. The taxonomic composition of the five archaeal 16S rRNA libraries shared OTUs is shown in Fig. 4d. The highest relative abundance of the shared archaeal OTUs was observed in S1 (99.7%). In S2, though the percentage of shared archaeal OTUs was 19.0%, substantially declined from 46.0% in S1, the relative abundance of the shared archaeal OTUs (96.0%) was close to that in S1. In S3, S4, and S5, the relative abundance of the shared archaeal OTUs decreased and kept stable in a range of 81.5%–85.5%.

The same phenomenon was observed in both bacterial and archaeal communities: though, a minority of bacterial and archaeal 16S rRNA OTUs were shared by the five samples, a majority of bacteria and archaea were contained in these OTUs. It indicated that the main bacterial and archaeal species in each sample existed in all the acclimation stages of the sludge. However, the relative abundances of these bacterial and archaeal species changed (Fig. 4c and d). It revealed that these groups of bacteria and archaea were indigenous microorganism in all samples, and the dominant Bacteria changed with variations of environmental conditions in the reactor.

The relative abundance of bacterial and archaeal OTUs are shown in Fig. S6. In bacterial libraries, the relative abundance of OTU407, classified as Ignavibacteria, increased from 0.1% in S1 to 48.3% in S5. The relative abundance of OTU533, classified as Betaproteobacteria, also showed an increased trajectory of 0.2% in S1 to 10.2% in S5. These two OTUs were two dominant

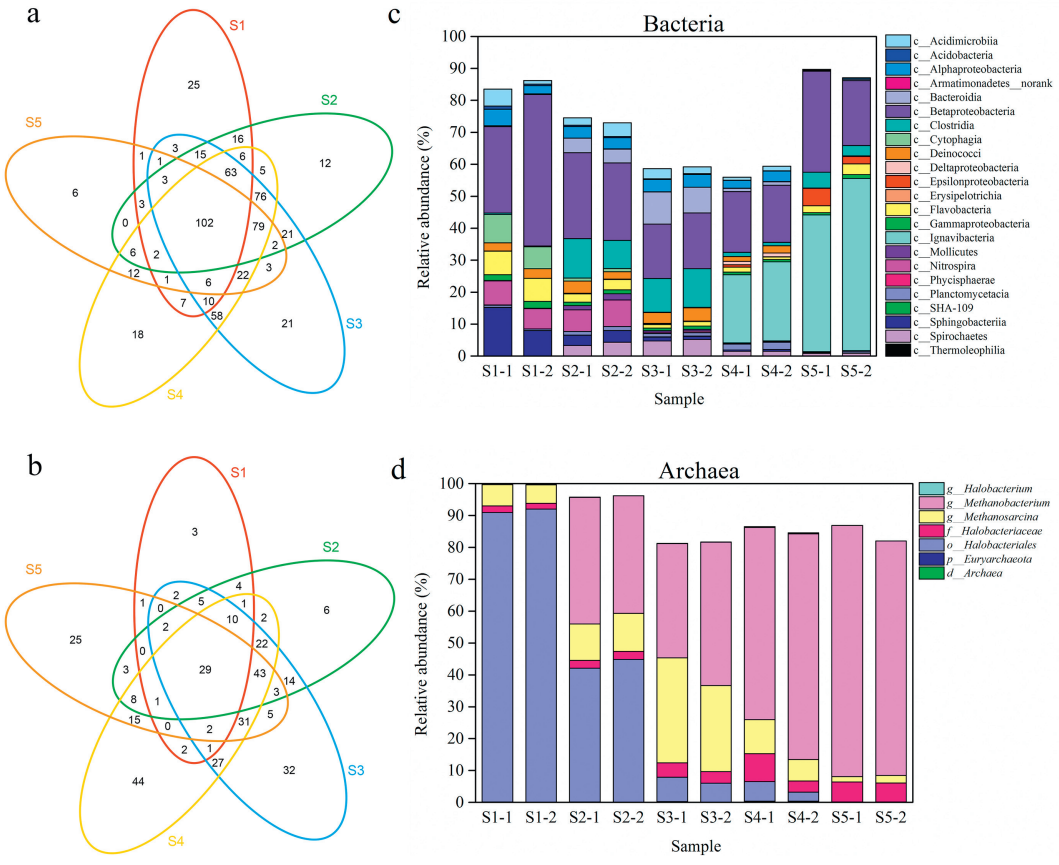

Fig. 4 – (a) Venn of the bacterial communities; (b) Venn of the archaeal communities; (c) Taxonomic composition of the bacterial OTUs shared by all the five samples; (d) Taxonomic composition of the archaeal OTUs shared by all the five samples.

ones in S5. In archaeal libraries, the OTU295, classified as *Halobacteria*, decreased from 90.3% in S1 to 0.1% in S5, while OTU176, classified as Methanobacteria, increased from 0.001% in S1 to 76.2% in S5, along with the acclimation. It reveals that there was one group of bacteria (OTU407) and one group of archaea (OTU176) absolutely predominated in bacterial and archaeal community, respectively, in the acclimated sludge (S5).

In general, through the analysis of shared and sample-unique OTUs, it was found that the majority of bacteria and archaea did always exist in the sludge, though some of them shifted from dominants to rare ones or from rare ones to dominants. This phenomenon was well consistent with the behaviors of "rare biosphere": low-abundance population might eventually become dominant in response to environmental change; dominant populations can correspond to low-abundance populations at a second environment (Sogin et al., 2006). We suspect that the so-called "sample-unique OTUs" were not truly sample-unique. They might be just too rare to be detected in the other samples under this sequencing depth (Shade et al., 2012).

3. Conclusions

This study showed the microbial community succession during the acclimation from aerobic to anaerobic in an AnMBR for coking wastewater treatment. The diversity of both Bacteria and Archaea showed an increase–decrease trajectory during acclimation, and exhibited the highest at the domestication interim. The diversity and richness of Bacteria were significantly higher than that of Archaea in each acclimation stage. Ignavibacteria and Methanobacteria were the dominant bacteria and archaea, respectively, in the acclimated anaerobic sludge. The bacterial community might continue to change rapidly along with further operation, while the archaeal community was becoming stable after 125 days acclimation.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jes.2017.06.003.

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