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Anaerobic fermentation of excess sludge at different pHs: characterize by acidogenesis capability and microbial communities structure shift

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ABSTRACT

MiSeq high-throughput sequencing and quantitative polymerase chain reaction was applied to characterize the shift of microbial communities in a bench-scale anaerobic reactor for excess sludge fermentation. Without pH adjustment, no significant change occurred in the diversified microbes that originally existed in the excess sludge. When pH was adjusted to 10, the bacterial diversity in the sludge clearly decreased. The disappearance of 99% original bacteria was accompanied by the stimulated growth of acidogenesis bacteria, especially *Bacillus* and *Anaerobacillus* (which collectively constituted 71% of relative dominance) contributing to high short-chain fatty acids (SCFAs) yields. This extinction and regrowth process of bacteria in pH 10 reactor was quickly achieved on the first day of fermentation. Compared with operating without pH adjustment, the optimum fermentation at pH 10 resulted due to: first, the predominance of acidogenesis bacteria for the maximized SCFAs production and second, low consumption of substrates by other bacteria.

Keywords: Excess sludge fermentation; Alkaline environment; Biodiversity variation; Succession of microbial community; Short-chain fatty acids production

1. Introduction

Currently, most urban sewage treatment plants adopt an activated sludge biological treatment process where organic carbon source is required for denitrification and anaerobic phosphorus release. The type and concentration of the carbon source directly influence the effects of the denitrification and phosphorus removal in the biological system [1], especially in sewage treatment plants with low C/N and low C/P. To resolve the problem, several methods were proposed, such as addition of carbon source [2], optimization of the biological treatment process [3], and utilization of internal carbon [4]. Among these methods, pioneers proposed that the short-chain fatty acids (SCFAs), the most important carbon source in the nitrogen and phosphorus removal, could be produced through the anaerobic fermentation of excess sludge [5], and the utilization of internal carbon from the excess sludge is a promising and feasible technology.

To improve the SCFAs yields in the process of the anaerobic fermentation of excess sludge, researchers have explored methods to enhance SCFAs production by controlling the temperature (25°C–55°C), adjusting the pH value, and the sludge retention time [6–8]. The results indicated that the conditions of pH 10 and a temperature of 35°C are most favourable for producing SCFAs during sludge fermentation [9]. In addition, studies also demonstrated that SCFAs production could be remarkably improved when sludge was treated by ultrasonication, high temperature, and high pressure [10].

It is believed that pH adjustment is an effective and general measure to enhance SCFAs accumulation in the sludge fermentation process. The results showed that sludge fermentation to

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produce SCFAs depends on the species and relative abundance of the microbial communities in the fermentation system [11]. In fact, changes of the environmental conditions, especially the pH and salinity, substantially affect microbial community structures [12,13]. Researchers have revealed that the formation of SCFAs production at pH 10.0 was dominated by biological effects rather than by chemical hydrolysis, however, the characteristics of microbial communities distribution and reproductive process have not been explored [5], meanwhile, the mechanisms of the effects of the pH on SCFAs promotion still remain poorly understood at present. According to the principles of microbial growth and propagation, the reasons for the significant distinction at various pH values may be inferred as follows: (1) the alkaline pH condition was beneficial for acidogenesis bacteria propagation and enrichment, which inhibits the growth of other microbes in the system. The growth rate of acidogenesis bacteria increased as a result of reduced competition for nutrients, and the produced SCFAs could not be consumed by other bacteria; (2) A large amount of the original microbial communities might decrease dramatically or disappear during sludge fermentation, and a new microbial community, which was well-suited to SCFAs production, could be cultivated in the alkaline system; (3) A combination of the above two reasons.

By considering these factors, the microbial community structures and acidogenesis bacteria in the excess sludge fermentation system under alkaline and neutral pH conditions were quantitatively investigated in this study using the MiSeq high-throughput sequencing method and quantitative polymerase chain reaction (qPCR). It is worth noting that the MiSeq sequencing method is a high-throughput analytical method that can generate huge amounts of DNA reads through a massively parallel sequencing-by-synthesis approach on an Illumina MiSeq platform [14]. The technology is advantageous in elucidating the characteristics of microbial community via a more accurate approach [15], which have been broadly applied to analyze the microbial community structures in marine water, soil, and wastewater treatment by activated sludge and biofilms [16–21]. By using those methods to unravel the growth processes of bacteria, the structures, and total population of microbial communities in different fermentation systems were compared with the original inhabitants in the initial sludge. Meanwhile, the reasons of the SCFAs accumulation at the alkaline pH value were analyzed. This study is aiming to elucidate the microbial community structure and comparison of microbial shift in acidogenic process of excess sludge under different pH conditions.

2. Experimental setup

2.1. Characteristics of sludge

Excess sludge in this study was taken from Xi'an No. 5 Sewage Treatment Plant, China. The sludge was preserved at a temperature of 4°C for 12 h before being placed in a bench-scale bioreactor in the laboratory, and the primary initial characteristics are shown in Table 1.

Table 1
Parameters for the basic characteristics of the excess sludge in this experiment

Parameter	Excess sludge
pH	6.97
TSS (total suspended solid), mg/L	22,710.6
VSS (volatile suspended solid), mg/L	13,355.7
Total carbohydrate, mgCOD/L	1,783.4
Total protein, mgCOD/L	10,041.2
Lipid and oil, mgCOD/L	136.5
TCOD (total chemical oxygen demand), mgCOD/gVSS	19,495.3
SCOD (soluble chemical oxygen demand), mgCOD/gVSS	33.32

2.2. Sequencing batch reaction of excess sludge fermentation

The suspended solids of the excess sludge were regulated to approximately 10,000.0 mg/L. The SCFAs concentration of the different reactors at the initial time (0 d) was approximately 17.00 mgCOD/gVSS. Ten similar glass bioreactors (all with a volume of 1 L) were filled with excess sludge, and the pH values in the first nine reactors were adjusted to 3, 4, 5, 6, 7, 8, 9, 10, and 11, by adding 1 mol/L of NaOH and 1 mol/L of HCl, and the tenth reactor was a blank with no-pH-adjustment. In order to guarantee the stabilized pH values in the different reactors, pH adjustment was operated every 3 h. To ensure uniform fermentation of the sludge in the reactors, the sludge was subjected to low-speed stirring for 24 h without interruption, and the retention time for the sludge fermentation was 8 d. To ensure sufficient anaerobic fermentation, the reactor openings were sealed with plastic wrap. All the glass bioreactors were put in a water bath kettle to ensure a steady temperature at 35°C ± 1°C for the sludge fermentation.

2.3. TSS, VSS, SCOD, and TCOD

Total suspended solid (TSS), volatile suspended solid (VSS), soluble chemical oxygen demand (SCOD), and total chemical oxygen demand (TCOD) were measured in accordance with standard methods of APHA [22]. All the sludge samples were filtered through a 0.45 μm filter before detecting the SCOD.

2.4. Short-chain fatty acids

Phosphoric acid (3%) was added dropwise to regulate the pH of the samples to approximately 4, then the solution of samples was transferred into a 1.5 mL gas chromatography (GC) vial, then a flame ionization detector of GC (Shimadzu GC-2014) was used to detect the SCFAs of the filtered solution. A DB-FFAP-123-3233 capillary column (30 m × 0.5 μm × 0.32 mm), with a sample size of 1 μL per sampling, was adopted for the detection of the SCFAs. The temperature of the sample injection port was set to 200°C, and the detector temperature was set to 230°C. The procedures to increase the temperature of the instrument (oven of GC) were as follows: the initial column temperature of 100°C maintained for 2 min; the temperature was increased to 120°C at a rate of 10°C/min and later kept at 120°C for 2 min; the temperature was increased to 200°C at a speed of 6°C/min and remained at 200°C for 2 min; the total elapsed time of the

temperature-increasing protocol was 21.33 min. The SCFAs concentration was characterized by the chemical oxygen demand (COD) concentration: 1.07 for acetic acid, 1.51 for propionic acid, 1.82 for butyric and isobutyric acid, and 2.04 for valeric and isovaleric acid [1].

2.5. DNA extraction and PCR amplification

The DNA of bacteria communities in the sludge with pH 10 and no-pH-adjustment on the fourth day of fermentation were sequenced. The results of this study indicate that the excess sludge reached the maximum SCFAs production on the fourth day of fermentation with better acid-producing ability and microbial activity. For comparison, the unfermented initial sludge was sequenced. Amplicon libraries were constructed using the V3 and V4 region primers of bacterial 16S rRNA genes, and high-throughput sequencing was carried out on the MiSeq platform.

Microbial DNA was extracted from three types of sludge samples using the E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's protocols. The V3–V4 region of the bacterial 16S ribosomal RNA gene was amplified by PCR (95°C for 3 min, followed by 27 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s and a final extension at 72°C for 10 min) using primers 338F 5′-ACTCCTACGGGAGGCAGCA-3′ and 806R 5′-GGACTACHVGGGTWTCTAAT-3′, where the barcode is an eight-base sequence unique to each sample. The PCR reactions were performed in triplicate using a 20 μL mixture containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM deoxyribonucleoside triphosphate, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA.

2.6. Illumina MiSeq sequencing

Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions and were quantified using QuantiFluor™-ST (Promega, USA). The purified amplicons were pooled in equimolar and paired-end sequence (2 × 250) on an Illumina MiSeq platform according to the standard protocols.

2.7. Processing of the sequencing data

Raw fastq files were demultiplexed and quality filtered using QIIME software (version 1.17) with the following criteria: (i) The 250 bp reads were truncated at any site receiving an average quality score of <20 over a 10 bp sliding window, discarding the truncated reads that were shorter than 50 bp. (ii) Exact barcode matching, two nucleotides mismatched in the primer matching and reads containing ambiguous characters were removed. (iii) Only sequences that overlapped longer than 10 bp were assembled according to their overlap sequence. Reads which could not be assembled were discarded.

The operational taxonomic units (OTUs) were clustered with a 97% similarity cutoff using UPARSE, and the chimeric sequences were identified and removed using UCHIME. The microbial abundance was mapped using the heatmap modules in R software, vegan statistical package, and using Verdict software to achieve distance calculation [23]. In a heatmap, the relative abundances of the microbes is represented visually by certain colours of different shades; the similarities and the differences of the community composition in the sludge samples at the genus level are represented by colour gradients. A Venn diagram was generated using the Mothur utility [24]. In the Venn diagram, the samples are represented by different colours; the overlapping areas of the circles represent sequences of colour-corresponding samples divided into the same OTU; and the numbers in the overlapping areas represent the OTU numbers of the microbial communities that are shared between the samples.

2.8. Quantitative polymerase chain reaction

qPCR was performed using an Applied Biosystems 7500 qPCR system (Applied Biosystems, Forster City, USA). The universal bacteria primer set and representative strain (*E. coli* K12, DSM 1607) [25] were used for qPCR amplification and fluorescence detection to quantify the total bacteria 16S rRNA gene copy numbers in this study. The analysis of blind samples was repeated three times and the results of 16S rRNA gene copy numbers was produced using the arithmetic mean C_t values, and when the relative standard deviation between the parallel samples was less than 25%, the qPCR data were credible. The 20 μL qPCR reaction mixture was prepared in triplicate using 6.8 μL of PCR-grade water, 10 μL TaKaRa SYBR® Premix Ex Taq™ (TaKaRa Bio Inc., Japan), 0.4 μL of each primer (final concentration 0.1 μM), 0.4 μL of 50 × ROX reference dye, 20 ng of template DNA. The thermal cycler protocol was as follows: initial step for 10 s at 95°C followed by 40 cycles of 95°C for 5 s, then maintained at 56°C for 10 s, finally maintained at 72°C for 27 s.

3. Results and discussion

3.1. Effect of pH on total SCFAs production

The effects of the different pH values on the SCFAs production during the 8 d excess sludge fermentation are shown in Fig. 1. SCFAs concentration in this study was described as mgCOD/gVSS to define the acid-producing ability of per unit organics. It was obvious that the change of the SCFAs

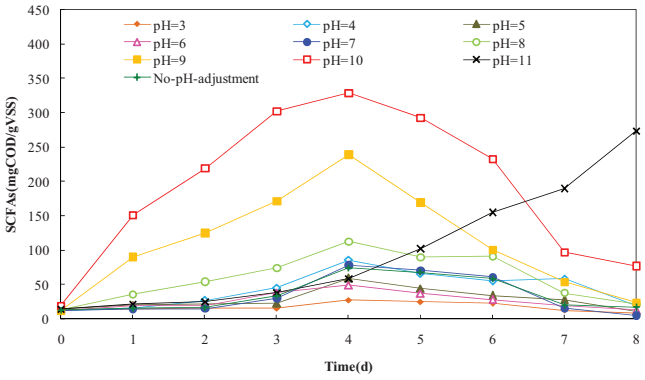

Fig. 1. Effect of pH on total SCFAs production at different fermentation times (0 d indicates the initial time when the excess sludge has not been fermented).

concentration at different pHs showed a similar trend except at pH of 11.0. The concentration of the SCFAs increased from the first to the fourth day, and on the fourth day the SCFAs reached the maximum. After the fourth day, the SCFAs concentration decreased. However, for the excess sludge at pH 11.0, the SCFAs continued to increase with time, but the production speed of SCFAs was much lower than that at neutral and pH 10.0. On the fourth day only 58.36 mgCOD/gVSS was produced, and on the eighth day the SCFAs concentration reached 273.29 mgCOD/gVSS, which was close to the maximum SCFAs production at pH 10.0. Researchers have revealed that the methanogenesis would consume the SCFAs, while the activity of methanogens would be prohibited at the alkaline condition, therefore, the accumulation of SCFAs would be easily achieved at the pH 10.0 condition.

It is obvious that the concentration of the SCFAs in the fermentation process under the alkaline condition was remarkably higher than those under the neutral and acidic conditions. The concentration of the SCFAs was 73.69 mgCOD/gVSS on the fourth day of fermentation in the no-pH-adjustment sludge, however, at pH 10.0, the SCFAs concentration was 328.30 mgCOD/gVSS on the fourth day, which was the maximum amount produced among all the conditions in the process of sludge fermentation. The yield of SCFAs was approximately 300 mgCOD/gVSS at alkaline condition in the previous studies [5,9], and it was in accordance to the results in this study. The concentration of the SCFAs in excess sludge at pH 10 was 4.46 times higher than that under the condition of no-pH-adjustment on the fourth day.

Table 2 depicts the compositions of the TCOD including the SCOD and the suspended COD in the fermentation reactors at pH 10 and no-pH-adjustment, where SCFAs were included in the SCOD. The suspended COD was the major constituent in the initial sludge, constituting more than 95% of the TCOD. Under the condition of no-pH-adjustment, the concentration of the SCOD and SCFAs accounted for 8.7% and 3.3% of the TCOD, respectively, after the 4-d fermentation, indicating that only a small amount of the suspended COD converted to soluble substance. However, the concentration of the SCOD and SCFAs was much higher in the pH 10 sludge accounting for 38.2% and 22.3% of TCOD, respectively. It can be therefore concluded that the condition of pH 10 was beneficial for the conversion of the suspended substance to SCOD and SCFAs during the excess sludge fermentation. Researchers have demonstrated that the activities of the acid-forming enzymes were observed to be much higher at pH 10 than those at other pHs, meanwhile, the microbial activities would be significant [9], therefore, the distribution characteristics would be revealed in the following discussions.

3.2. Compositions of the microbial community

The pyrosequencing yielded a total of 28,365 bacterial 16S rDNA sequences in the initial excess sludge sample; while on the fourth day of fermentation, there were 32,307 and 30,647 bacterial 16S rDNA sequences in the sludge at no-pH-adjustment and pH 10, respectively. Fig. 2 summarizes the microbial compositions at the phylum level for the three samples and the bacteria with a relative abundance higher than 1% were presented. In the initial excess sludge sample, *Bacteroidetes* and *Proteobacteria* were the main microbes with relative abundances of 32.0% and 31.96%, respectively, followed by *Chloroflexi* with a relative abundance of 8.07%. Under the condition of no-pH-adjustment on the fourth day of fermentation, *Bacteroidetes* and *Proteobacteria* were also

Table 2
Variation of compositions in TCOD in two excess sludge fermentation systems at the initial time and the fourth day of fermentation

pH	Time (d)	TCOD (mg/L)	SCOD (mg/L)	VFA (mg/L)	COD loss (mg/L)
No-pH-adjustment	0	9,032.89	205.19	67.21	
	4	8,945.19	626.39	292.19	87.7
pH 10	0	8,783.75	195.03	67.36	
	4	8,598.45	3,287.53	1,917.28	185.3

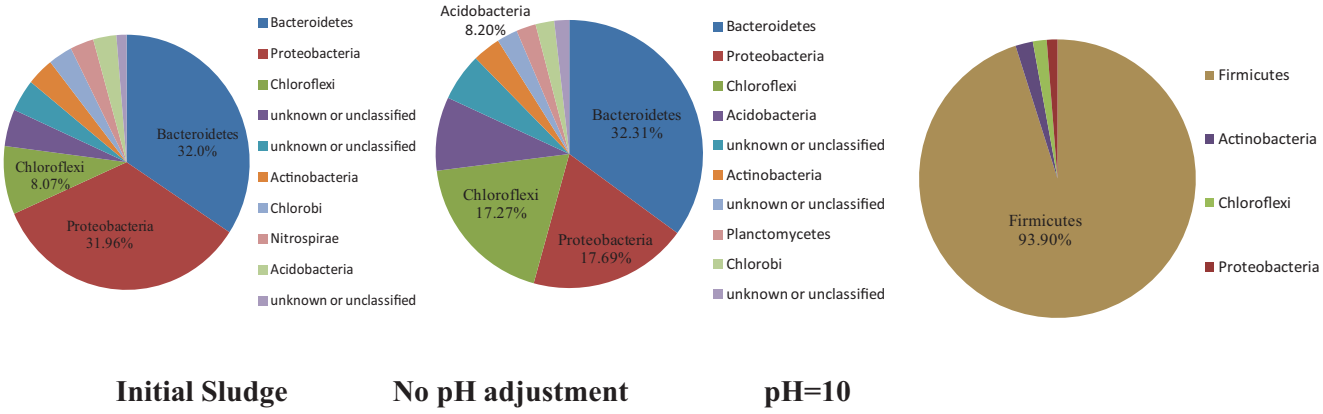

Fig. 2. The relative abundance of microbes at the phylum level in the three samples.

the main microbes, with relative abundances of 32.31% and 17.69%, respectively, followed by *Chloroflexi* (17.27%). Thus, it can be observed that the species and the relative abundance of the bacteria in the excess sludge remained almost the same during the anaerobic fermentation with no-pH-adjustment. The previous study showed that *Bacteroidetes* could convert proteins and carbohydrates to propionate and acetate during the sludge anaerobic fermentation [26], and *Proteobacteria* was the major phylum in the hydrolysis and acidification process of the sludge fermentation [27], indicating that the no-pH-adjustment sludge had the ability to produce SCFAs.

However, the microbial communities in the excess sludge at pH 10 were significantly different from those in the initial excess sludge. *Firmicutes* were the most abundant bacteria, and its relative abundance was up to 93.90%. It is reported that *Firmicutes* could produce ectoenzymes which metabolize protein, fat, and carbohydrates [28,29]. Because the relative abundance of *Firmicutes* was less than 1% in the initial sludge sample and in the no-pH-adjustment excess sludge sample, it could be deduced that *Firmicutes* were enriched in the alkaline bioreactor, and they could adapt to the condition of pH = 10 and become the predominant bacteria in the pH 10 fermentation system. With the exception of *Firmicutes,* the relative abundance of other bacteria was less than 7% under alkaline condition, thus, *Firmicutes* could use more nutrients to grow rapidly, and the propagation of other bacteria was inhibited by the enrichment of *Firmicutes*. Due to the small amount of other non-predominant bacteria competing for nutrients, the *Firmicutes* were stimulated to grow. In general, the anaerobic sludge fermentation process is usually included: hydrolysis, acidification, and methane generation, and for accumulating more SCFAs, the increasing of hydrolysis rate to produce more soluble substrates for acidification should be adopted [9]. Based on the pyrosequencing results, the hydrolysis bacteria (*Firmicutes* and *Bacteroidetes*) were enriched under the alkaline condition which would promote SCFAs producing, meanwhile the hydrolysis effect would be decreased under the acidic condition, therefore, the concentration of SCFAs was accumulated in the pH 10 condition, which was in accordance with the discussion in Fig. 1.

3.3. Bacterial diversity and acidogenesis bacteria

As a measure of bacterial diversity, the numbers of shared OTUs among the initial sludge sample, the no-pH-adjustment and the pH 10 sludge samples were calculated using a Mothur's Venn diagram analysis as shown in Fig. 3(a). The total number of OTUs were 1,145 and 1,043 in the initial sludge and the no-pH-adjustment sludge on the fourth day of fermentation, respectively, and there were 929 OTUs in common in the two samples (81.14% and 89.07% of the total OTUs, respectively), so the majority of OTUs in the initial sludge and the no-pH-adjustment sludge on the fourth day of fermentation were basically consistent, indicating that the species had no significant change with the no-pH-adjustment condition during the 4-d fermentation. There were only 424 OTUs in the pH 10 sludge on the fourth day of fermentation, which was significantly lower than the number of OTUs in the initial sludge sample. In order to state the bacterial diversity in the different sludge, the Shannon index was applied in this study. The Shannon diversity index values of the initial excess sludge and the no-pH-adjustment excess sludge on the fourth day of fermentation were 5.52 and 5.39, respectively; however, the Shannon index of the pH 10 sludge on the fourth day of fermentation was only 2.73, indicating the decrease of bacterial diversity under the alkaline condition. Hence, it could be concluded that a large number of species disappeared during the 4-d fermentation at pH 10. Compared with the initial sludge sample, there were 73 different OTUs found in the pH 10 sludge sample, indicating that some new bacterial species emerged. Based on the above results, it could be concluded that many original bacterial species were found to be disappeared accompanying with a growth of some new species.

To investigate the detailed changes of the bacteria during sludge fermentation under the pH 10 condition, heatmap

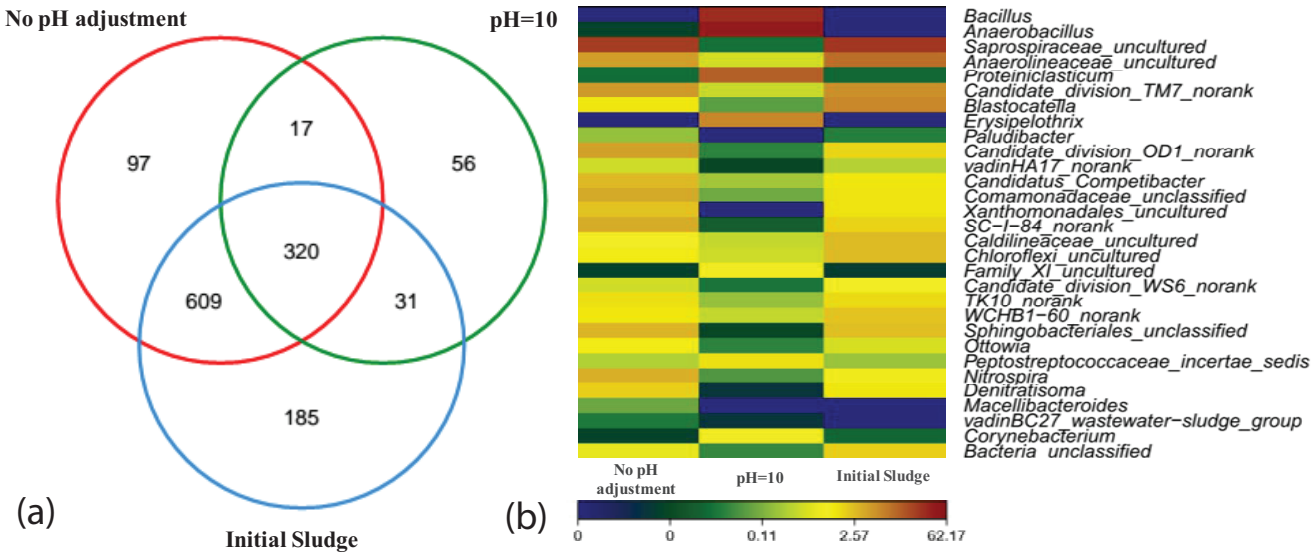

Fig. 3. (a) Mothur's Venn diagram analysis. (b) Heatmap (the above figure shows 30 abundant genes of microbes in the three samples).

software was used to analyze the species and the relative abundance of bacteria at the genus level in the initial sludge and the pH 10 excess sludge. As shown in Fig. 3(b), the relative abundance (0%–62.17%) of microbes are represented by gradient colours from dark blue to dark red, and each stripe represents one type of microbial community. The colours of the stripes are almost the same in the initial excess sludge and the no-pH-adjustment excess sludge on the fourth day of fermentation, and most microbes are represented by blue or yellow. According to the result of pyrosequencing, the bacteria with the top three relative abundances in the initial excess sludge were *Saprospiraceae*, *Candidate_division_TM7*, and *Anaerolineaceae*; and the relative abundance of most microbes in the initial sludge were less than 2%. The relative abundance of microbial communities in the excess sludge at pH 10 are mostly represented in dark red or dark blue, indicating that there were significant change in the relative abundance of the microbial communities during the 4-d sludge fermentation under the pH 10 condition. The relative abundance of the top three genes in the pH 10 excess sludge were *Anaerobacillus, Bacillus*, and *Proteiniclasticum* accounting for about 80%. It can thus be observed that the microbial community structures in the excess sludge at pH 10 varied substantially from the other two samples.

The acidogenesis bacteria played a crucial role in the sludge fermentation and SCFAs-producing process. Table 3 shows the acidogenesis bacteria at the genus level in different excess sludge samples. The total relative abundance of the acidogenesis bacteria at pH 10 was up to 79.78%, which was significantly higher than that in the no-pH-adjustment sludge (1.735%). This result might explain why the alkaline condition enhanced the SCFAs accumulation in the fermentation. *Bacteroidetes* played crucial roles in hydrolysis and acetogenesis steps [32], and they were the dominant functional acidogenesis bacteria in the no-pH-adjustment sludge, but the relative abundance of *Bacteroidetes* was only 1.45%. *Bacillus* and *Anaerobacillus* could convert organic matter to lactic or acetic acid [11,30], and they were the most abundant acidogenesis bacteria in the pH 10 sludge. The relative abundances of these two bacteria were 29.92% and 41.29%, respectively. *Erysipelothrix* and *Peptostreptococcaceae* were also enriched in the pH 10 sludge, and they could also consume the substrate to release the acetate and carbon dioxide [31,33]. However, these bacteria mentioned above could not be found in the initial sludge on the fourth day of fermentation. As seen from the results, the reproduction of the same phylum level of bacteria at a pH value of 10, including *Bacillus, Anaerobacillus, Erysipelothrix*, and *Peptostreptococcaceae*, was attributed to the enrichment of *Firmicutes*. Thus, it could be concluded that many acidogenesis species emerged at pH 10 during the 4-d fermentation, and they could adapt to the alkaline condition.

3.4. The disappearance of original bacteria and propagation of new microflora

In order to show the comparison of microbial communities and understand the mechanism of the SCFAs production under pH 10 and no-pH-adjustment condition during the excess sludge fermentation process, changes in 16S rRNA gene concentration of total bacteria was detected by qPCR. Combining with the diversity analysis of bacteria, the detail information of bacterial reproduction processes measured by qPCR could be obtained.

As shown in Fig. 4, the concentration of 16S rRNA gene copies (copies/mL) had no significant change in the no-pH-adjustment excess sludge during the fermentation process, and the concentration was approximately 2.73×10^{10} which was in consistence with the result reported previously [25]. The concentration of 16S rRNA gene copies under the pH 10 condition decreased to 2.33×10^{8} during the first 5 h, then increased rapidly to an amount which was much larger than 2.73×10^{10}, indicating that the majority of the original bacteria could not adapt to the alkaline condition, resulting in 99% of the bacterial disappearance during sludge fermentation at pH 10. After 24 h, the concentration of 16S rRNA gene copies at pH 10 reached 1.03×10^{11} and remained stable afterwards. Based on the analysis of the microbial communities, it could

Table 3
The relative abundance of the acidogenesis bacteria

Genus level	Initial sludge		No-pH–adjustment		pH 10	
	Reads	%	Reads	%	Reads	%
Bacillus	0	0	0	0	12,754	29.92
Anaerobacillus	0	0	0	0	17,602	41.29
Erysipelothrix	0	0	0	0	2,920	6.85
Bacteroidetes	0	0	469	1.45	1	0.002
Clostridium	0	0	32	0.099	8	0.018
Clostridiaceae	0	0	0	0	55	0.13
Lachnospiraceae	0	0	2	0.0061	5	0.01
Peptostreptococcaceae	0	0	57	0.18	667	1.56
Macellibacteroides	22	0.08	0	0	0	0
Streptococcus	9	0.03	0	0	1	0.002
Veillonellaceae	2	0.0067	0	0	0	0
Zymomonas	50	0.18	0	0	1	0.002
Total relative abundance	83	0.2967	560	1.735	34,012	79.78

be concluded that new bacteria that could adapt to alkaline condition emerged under the pH 10 condition during the sludge fermentation. With the disappearance of most of the original bacteria, there were few bacteria (less than 1%) to compete consuming the nutrients at pH 10, and the new bacteria could grow rapidly to become abundant in the sludge fermentation system. Combining with the information of acidogenesis bacteria in the three samples, it could be concluded that there were more acidogenesis bacteria in the pH 10 sludge. Due to the enrichment of acidogenesis bacteria, the SCOD and SCFAs yield were increased in the fermentation system at pH 10.

Based on the discussion above, a schematic graph describing the growth processes of microbial communities during the excess sludge fermentation at different pH values was constructed as shown in Fig. 5. In summary, there were

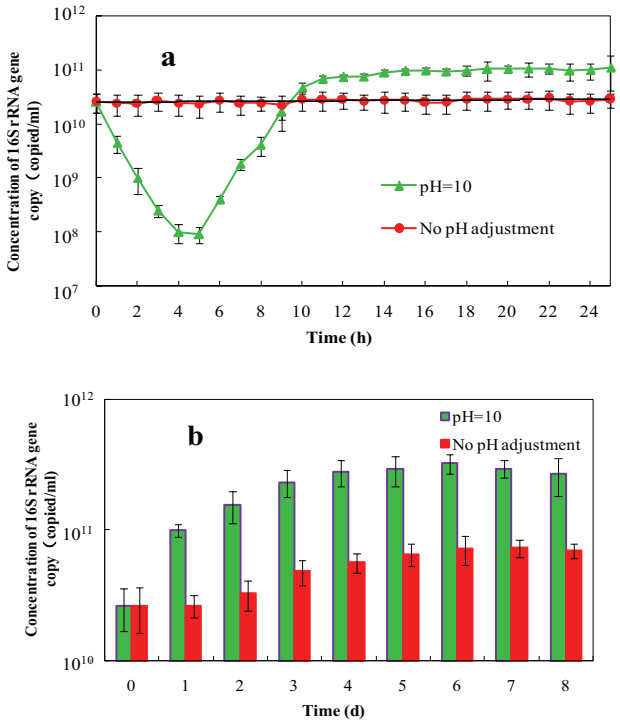

Fig. 4. Variation of 16S rRNA gene concentration of total bacteria at first day (a) and during 8-d fermentation (b).

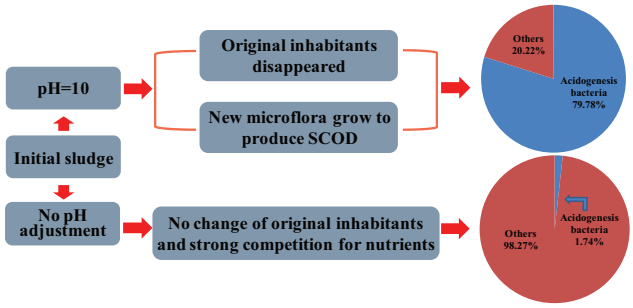

Fig. 5. Growth process of microbial communities in the sludge system.

various species of bacteria under the no-pH-adjustment condition during the sludge fermentation, and due to the competition for nutrients among the bacteria, the acidogenesis bacteria could not propagate rapidly. However, 99% of the original bacteria could not adapt to the alkaline environment and disappeared during sludge fermentation. As a result, acidogenesis bacteria were stimulated to grow under alkaline condition, and SCOD and SCFAs yields were enhanced and SCFAs were accumulated in the system. By combining a variety of advanced technologies, this study unveiled the mechanism of succession of microbial community and stimulated growth of acidogenesis bacteria in alkaline environment during excess sludge fermentation, which therefore could be of great significance for improving the excess sludge fermentation process in future.

4. Conclusions

The shift of bacteria under different pH conditions was studied during an 8-d fermentation experiment. During the early stages of fermentation, 99% of the original bacteria observed in the initial sludge disappeared quickly at pH 10 (in only 5 h). Meanwhile the acidogenesis bacteria such as *Bacillus* and *Anaerobacillus* emerged in the absence of other bacteria competing for nutrients, and the total relative abundance of acidogenesis bacteria reached 79.78%. Because of the enrichment of acidogenesis bacteria, the concentration of the SCOD and SCFAs at pH 10 reached the maximum of all pH values.

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