Waste Management 48 (2016) 397–403

Contents lists available at ScienceDirect

Waste Management

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

Improved volatile fatty acids anaerobic production from waste activated sludge by pH regulation: Alkaline or neutral pH?

Huijun Ma [a], Xingchun Chen [a], He Liu [a,b,*], Hongbo Liu [a,b], Bo Fu [a,b]

[a] School of Environmental and Civil Engineering, Jiangnan University, 214122 Wuxi, China
[b] Jiangsu Key Laboratory of Anaerobic Biotechnology, 214122 Wuxi, China

ARTICLE INFO

Article history:
Received 30 July 2015
Revised 11 October 2015
Accepted 14 November 2015
Available online 30 November 2015

Keywords:
Waste activated sludge
Anaerobic fermentation
Volatile fatty acids
Pretreatment
Neutral pH
Alkaline pH

ABSTRACT

In this study, the anaerobic fermentation was carried out for volatile fatty acids (VFAs) production at different pH (between 7.0 and 10.0) conditions with untreated sludge and heat-alkaline pretreated waste activated sludge. In the fermentation with untreated sludge, the extent of hydrolysis of organic matters and extent of acidification at alkaline pH are 54.37% and 30.37%, respectively, resulting in the highest VFAs yield at 235.46 mg COD/g VS of three pH conditions. In the fermentation with heat-alkaline pretreated sludge, the acidification rate and VFAs yield at neutral pH are 30.98% and 240.14 mg COD/g VS, respectively, which are higher than that at other pH conditions. With the glucose or bovine serum albumin as substrate for VFAs production, the neutral pH showed a higher VFAs concentration than the alkaline pH condition. The results of terminal restriction fragment length polymorphism (T-RFLP) analysis indicated that the alkaline pH caused low microbial richness. Based on the results in this study, we demonstrated that the alkaline pH is favor of hydrolysis of organic matter in sludge while neutral pH improved the acidogenesis for the VFAs production from sludge. Our finding is obvious different to the previous research and helpful for the understanding of how heat-alkaline pretreatment and alkaline fermentation influence the VFAs production, and beneficial to the development of VFAs production process.

1. Introduction

It has been widely recognized that anaerobic fermentation is an effective method to resolve the large amounts of waste activated sludge (WAS) by reduction of its volume and generation of valuable products (Mahmood and Elliott, 2006; Appels et al., 2008). The volatile fatty acids (VFAs), one kind of main intermediates during anaerobic sludge fermentation, can be used as perfect additional carbon source to enhance biological nitrogen and phosphorous removal in waste water treatment plants (Yang et al., 2014; Longo et al., 2015). Because pH influences many aspects of the anaerobic fermentation, such as the microbial community, hydrolysis rate of sludge and products inhibition it is one the most important factors affecting the VFAs production from WAS by anaerobic fermentation (Chang et al., 2011; Liu et al., 2012; Pratt et al., 2012).

Chen et al. (2007, 2013a,b) investigated the hydrolysis and acidification of WAS at different pH values and reported that alkaline pH could significantly improve the VFAs yield during the anaerobic hydrolysis and acidification. Similarly, Wu et al. (2009) showed that the short-chain fatty acids concentrations at alkaline pH values (pH 8.0, 9.0, 10.0 and 11.0) were significantly higher than that at near neutral pH values (pH 6.0 and 7.0) or acidic pH values (pH 3.0, 4.0 and 5.0). Jie et al. (2014) also demonstrated that pH of 10.0 was the optimum pH condition for VFAs production from WAS. It is well known that the hydrolysis rate is the limiting factor for the anaerobic fermentation from solid wastes (Angelidaki et al., 2009). The reason of enhancement of VFAs production at alkaline pH is alkali improves the disintegration and hydrolysis of organic matters in WAS, resulting in more proteins and polysaccharides release into the liquid and then providing more biodegradable substrates for the acidogenic microorganisms.

Considering the low efficiency of solid waste fermentation, heat-alkaline pretreatment can bring many benefits for the anaerobic fermentation, such as hydrolysis rate and transformation yield increase, so the pretreatment has been accepted as a key method to improve the anaerobic fermentation of WAS (Tan et al., 2012; Cesaro and Belgiorno, 2014). The reason for the enhancement of fermentation by pretreatment is similar to the alkaline fermentation, since it improves the organic matters disintegration and increase of soluble COD due to the release of proteins and polysaccharides from the sludge (Liu et al., 2014; Shahriari et al., 2012).

* Corresponding author at: School of Environmental and Civil Engineering, Jiangnan University, 1800 Lihu Road, Wuxi, China.
E-mail address: liuhe@jiangnan.edu.cn (H. Liu).

http://dx.doi.org/10.1016/j.wasman.2015.11.029
0956-053X/

Generally, the neutral pH is good for the enzyme catalysis and microbial activity for most microorganisms. In the case of heat-alkaline pretreatment and alkaline fermentation, the sludge pH turns into alkalinity, so it may inhibit the activity of acidogenic microorganisms though it improves the hydrolysis reaction of anaerobic fermentation (Lutoslawski et al., 2011; Pandit et al., 2011). Drake et al. (1997), Drake and Steven (2004) reported that neutral pH is favor of the growth and VFAs accumulation pure acetogenesis strain. For example, *Moorella thermoacetica*, the most metabolically diverse acetogen, optimum growth pH value is about 5.7–7.7.

However, there is little literature exploring the interactions among the heat-alkaline pretreatment, suitable pH condition of fermentation and the VFAs production. Based on the above development of VFAs production by anaerobic fermentation from WAS, we propose that the neutral pH, instead of alkaline pH, is in favor of VFAs production from WAS at acidogenesis stage during the anaerobic fermentation. Therefore, the purpose of this study is to (1) explore the suitable pH condition for VFAs production from heat-alkaline pretreated and untreated sludge; (2) explore the mechanism of how neutral (pH 7.0) or alkaline (pH 10.0) condition influences the VFAs production from WAS. The finding of this study will help identify the interaction effects among pH, hydrolysis of sludge and acidogenic microorganisms as well as benefit the process development of VFAs production by anaerobic fermentation from WAS.

2. Materials and methods

2.1. WAS and seeding sludge

Both WAS and seeding sludge were the dewatered sludge obtained from a municipal wastewater treatment plant in Wuxi of China. The collected WAS was stored in 4 °C for subsequent fermentation experiments, and its characteristics were shown in Table 1. The characteristics of diluted WAS by deionized water, which was used for the pretreatment and anaerobic fermentation, were as follows: pH 6.60 ± 0.05, TSS 73.6 ± 0.6 g/L, VSS 45.0 ± 1.2 g/L, SCOD 6193.85 ± 158.62 mg/L, soluble protein 216.67 ± 19.38 mg/L, soluble polysaccharides 85.06 ± 4.91 mg/L. All measurements were conducted in triplicate with average and standard deviation reported.

An upflow anaerobic sludge blanket (UASB) with effective volume about 2.0 L was applied to accumulate and acclimate acidogenic microorganisms. The process of the acclimation and re-reactivation of seeding sludge were followed as the previous literature (Wang et al., 2013). The cultivated seeding sludge was washed twice by deionized water before its application in the fermentation reactors.

2.2. Fermentation experiments

The WAS were pretreated at 90 °C and the pH was adjusted to 11.0 by addition of 4 M NaOH for 2 h to improve the hydrolysis of the organic matters in sludge (Li et al., 2012). Six different experimental groups were set to investigate the impact of pH on the hydrolysis and acidification of WAS. The fermentation substrates were untreated sludge and heat-alkaline pretreated sludge. For each substrate in fermentation, three conditions were tested: pH uncontrolled, pH = 7.0 and pH = 10.0. The pH during fermentation was adjusted every day by adding 2 M HCl or 2 M NaOH. Six 500-ml serum bottles were used and placed in an air-bath shaker at 35 ± 1 °C, 120 rpm for anaerobic fermentation, which numbered R1 (untreated sludge, uncontrolled pH), R2 (untreated sludge, pH = 7.0), R3 (untreated sludge, pH = 10.0), R4 (pretreated sludge, uncontrolled pH), R5 (pretreated sludge, pH = 7.0), R6 (pretreated sludge, pH = 10.0), respectively. All the experiments were operated in batch. Each reactors before the batch fermentation consisted of fermentation substrate and seeding sludge (feed ratio of seeding sludge on VS basis was about 11.5–12.0%) with the effective volume of 400 mL (Zhu et al., 2015). 50 mM 2-Bromoethanesulfonic acid sodium salt (BES) was added in each reactor to inhibit methanogens. Each reactor was closed with a rubber stopper and nitrogen was purged for 15 min to displace the oxygen in the headspace at the beginning and each sample collection. Each time 10.0 mL of the sample was collected and filtered immediately.

2.3. Acidogenic fermentation with glucose and BSA

Bovine serum albumin (BSA) and glucose were used as the models of protein and carbohydrate for the fermentation at pH 10.0 and pH 7.0, respectively. Five experiments were designed as in Table 2. The anaerobic fermentation conditions were as the same in Section 2.2. Samples collection, pH adjustment (except the pH uncontrolled group) and nitrogen sparging were conducted every day. Each time 10.0 mL of the sample was collected and filtered immediately.

2.4. Terminal restriction fragment length polymorphism (T-RFLP) analysis

The total DNA extraction and T-RFLP analysis were the same with the previous study. Further details were according to the literature (Xu et al., 2015).

The PCR mixture (25 μL) consisted of the followings: 2.5 μL of 10 PCR buffer, 2.0 μL of dNTP (10 mM each), 2.0 μL of genomic DNA, 0.25 μL of Bar-PCR primer F (10 μM), 0.25 μL of primer R (10 μM), 0.2 μL of platinum Taq (5 U/μL), and sterile ddH2O to a final volume of 25 μL. For the following T-RFLP, the PCR primers were Bar-PCR primer F(5′-AGAGTTTGATCMTG GCTCAG-3′, and the end of 5′ was labeled with 6-carboxyfluorescein) and primer 1378–1401R (5′-CGGTGTGTACAAGGCCCGGGAA CG-3′). The PCR conditions were as following: 94 °C for 5 min; 30 cycles consisting of 94 °C for 45 s, 54 °C for 1 min, 72 °C for 60 s; a final step of 5 min at 72 °C. The PCR products were evaluated using 1% agarose gels. The PCR products were further purified using a DNA Fragment Purification Kit Ver.2.0 (Sangon Biotech Co. Ltd., Shanghai, China).

Table 1
Characteristics of dewatered sludge.

Parameters	Values
pH	6.60 ± 0.08
Solid content (%)	15.43 ± 0.80
Volatile suspended solids (VSS, g/g TS, %)	60.60 ± 2.62
Total protein (g/g VSS, %)	56.55 ± 2.08
Total carbohydrate (g/g VSS, %)	6.73 ± 0.45
Lipid (g/g VSS, %)	0.25 ± 0.03

Table 2
The experimental design and components of the fermentation substrates.

Group number	Blank	Glu-pH7	Glu-pH10	BAS-pH7	BSA-pH10
Total volume (mL)	300	300	300	300	300
Seeding sludge volume (mL)	30	30	30	30	30
Substrates and concentration (g/L)	Distilled water	Glucose 10	Glucose 10	BSA 10	BSA 10
pH	Uncontrolled	7	10	7	10

2.5. Analytical methods

The concentration of total suspended solid (TSS), volatile solid (VS), volatile suspended solid (VSS), soluble chemical oxygen demand (SCOD) and pH were conducted according to Standard Methods (APHA, 1998). The concentrations of proteins and polysaccharides were measured using the Lowry–Folin (Lowry et al., 1951) and phenol–sulfuric method (Herbert et al., 1971), respectively. The measuring method for the lipid concentration was in accordance with previous studies (Feng et al., 2009a,b). To measure SCOD, soluble protein and soluble polysaccharides, the samples were first centrifuged at 10200*g* for 10 min, and then were filtered with 0.45 μm syringe filters. All the filtered samples were measured immediately after collection.

The calculation of hydrolysis and acidification rates were followed as the formulas:

$$\text{Extent of solubilization } (\%) = (SCOD_{finish} - SCOD_{initial})/\text{total COD} \quad (1)$$

$$\text{Extent of acidification}(\%) = COD_{VFA}/\text{total COD} \quad (2)$$

In formulas, $SCOD_{finish}$ is SCOD concentration at the end of the fermentation, mg/L; $SCOD_{initial}$ is SCOD concentration at 0 d, mg/L; COD_{VFA} is VFAs concentraztion transferred into COD concentration, mg COD/L.

The VFAs concentration in the filtrate samples was detected by a gas chromatograph (GC-2010, Japan), equipped with a flame ionization detector (FID) and a fused-silica capillary column (PEG-20 M, 30 m × 0.32 mm × 0.5 lm, China). The column temperature was maintained at 80 °C initially. While, the highest temperature was 210 °C, and then was held for 2 min. Both the injection port and detector temperatures were 250 °C. Before GC measurement, 4-methyl-valeric acid(acted as internal standard), 3 M phosphoric acid (acidifier) and filtrate sample should be mixed according to the proportion of 1:1:1 (v:v:v). In order to make sure if there was methane during the test, the product spectrum in the headspace, which concluded hydrogen, carbon dioxide and methane, was analyzed by gas chromatography (GC-2010, Japan) outfitted with thermal conductivity detector (TCD) using a stainless steel column.

3. Results and discussion

3.1. VFA production during the fermentation

The VFAs production is the result of a series of complicated physical and biological reactions during the anaerobic digestion. The acidogenesis reaction means the VFAs generating reactions during the anaerobic fermentation, including the primary fermentative acidogenesis, syntrophic acetogenesis and homoacetogenesis (Amani et al., 2010). Fig. 1A showed the VFAs production profiles in all anaerobic fermentation reactors from day 0 to day 10. It can be seen the VFAs concentrations increased rapidly and maintained stably after the 4 d. When the untreated sludge acted as fermentation substrate, VFAs concentration of R3 was the highest and reached 7.78 g/L, indicating alkaline condition was beneficial to producing VFAs from sludge. This consequence was in accordance with previous research (Khiewwijit et al., 2015; Zhao et al., 2015). Some researchers have demonstrated that alkaline pretreatments would disrupt sludge flocs and cells, release inner organic matter, and consequently, improved the substrate concentration for fermentation (Kim et al., 2003; Li et al., 2012). Yuan et al. (2006) reported that under alkaline conditions more soluble substrates was released and supplied as the substrate for acidogenic microorganisms, thus it resulted in high VFAs production.

However, as for the pretreated sludge (R4–R6), VFAs concentration of R5 was the maximal (7.87 g/L), demonstrating that VFAs yield was much higher at neutral pH than the others after heat-alkaline pretreatment. This result was different from previous research which claimed the alkaline pH was suitable for the VFAs production from sludge. Except from the R3 and R5, other reactors showed the similar VFAs concentration during the whole acidogenic fermentation. For the fermentation with heat-alkaline pretreated sludge, the reason of that the neutral pH, instead of the alkaline pH, is the best pH condition for VFAs fermentation is the suitable pH environment and enough supply of substrates for the acidogenic microorganisms (Xiao et al., 2015; Aarle et al. 2015). No matter the alkaline anaerobic fermentation or heat-alkaline pretreatment before anaerobic fermentation, the essence is to enhance the disintegration and solubilization of organic matters from the sludge (Liu et al., 2008).

It can be explained from the SCOD changes of all the fermentations under different pH values (Fig. 1B). From Fig. 1B, after pretreatment, SCOD concentrations of R4, R5, R6 were relatively high and remained at about 30,000 mg/L during the fermentation, which suggesting the heat-alkaline pretreatment was sufficient and almost all soluble organic matters were hydrolyzed and released into the liquid. On the other hand, in the fermentation with untreated sludge, the initial SCOD concentration in R3 was lower than 10,000 mg/L and gradually increased to 33202.08 mg/L, which was similar to the concentrations of the sludge with heat-alkaline pretreatment. In contrast, the SCOD in the R1 and R2 was much lower than that in R3 because of the absence of alkaline pH. The consensus of the SCOD and VFAs concentrations between the R3 and R5 well explained the alkaline pH promoted the disintegration and hydrolysis of organic matters in the sludge and then beneficial for the VFAs production. It should be noted that because of the alkaline fermentation (pH = 10.0) and BES addition to inhibit the methanogens (Chen et al., 2007; Wang et al., 2013), there is almost no methane production during the fermentation process (the data was not shown), so the SCOD values in the reactors with pretreated sludge maintained stable.

According to the VFAs production and SCOD change in Fig. 1, the VFAs yields, the extent of hydrolysis and acidification were calculated and the results were showed in Table 3. The extent of hydrolysis and acidification represent the hydrolysis extent of organic matters in sludge and how much soluble substrate (SCOD) is transformed into VFAs by the acidogenic microorganism, respectively. According to Table 3, in the fermentation with untreated sludge, the R3 fermented at pH 10.0 showed the highest extent of hydrolysis (54.37%) and acidification (30.37%), and finally the VFAs yield (235.46 mg COD/g VS). This is because that the alkaline pH improves the organic matters hydrolysis and provides more substrates for the acidogenic microorganisms for the VFAs production (Park et al., 2014). However, in the fermentation with heat-alkaline pretreated sludge, similar hydrolysis rates were observed in different pH conditions because of the sufficient hydrolysis during the pretreatment. For the acidification rate and VFAs production, the R5 fermented at pH 7.0 showed the highest values (30.98% and 240.14 mg COD/g VS), indicating that neutral pH is favor of the VFAs production though there is similar hydrolysis rates of R4, R and R6. The different trends between the hydrolysis rate and VFAs production of pretreated sludge and untreated sludge fermentation implied there are different impacts of pH on the hydrolysis and acidogenesis for the VFAs production from sludge.

It has been reported that acetate and propionate were the better carbon sources for the enhanced biological nitrogen and phosphorous removal in wastewater treatment plants than other single fatty acid (Chen et al., 2013a,b). The percentages of acetate and propionate in all the six experiments were over 62%, showing high possibility to be carbon source. In Fig. 1 C, the percentages of

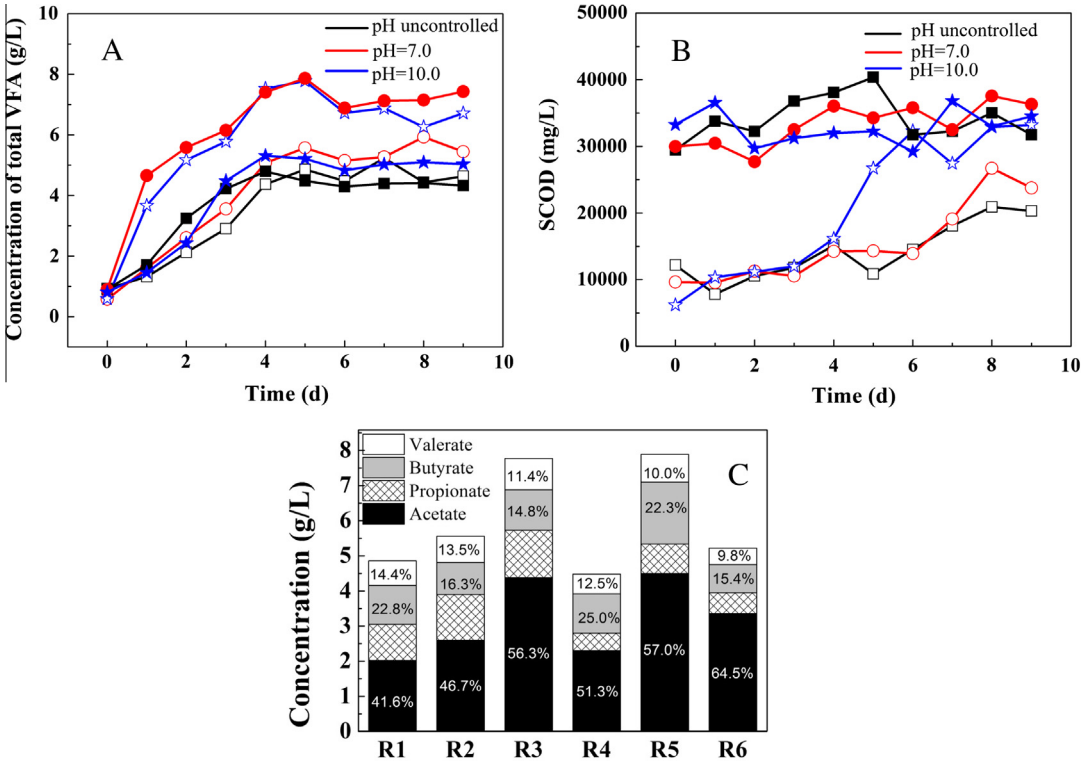

Fig. 1. The production of VFA at different pH values (A), the SCOD profiles during the anaerobic fermentation (B), and the composition of the total VFAs at the end of the fermentation (C). The open symbols in A and B represent un-pretreated sludge and the solid symbols represent the pretreated sludge. The percentages in C represent the single VFAs proportion in the total VFAs.

Table 3
Hydrolysis, acidification rates and VFAs yields during the fermentation.

Parameters	Fermentation with untreated sludge			Fermentation with pretreated sludge		
	R1	R2	R3	R4	R5	R6
Extent of hydrolysis (%)	9.11	18.98	54.37	73.39	75.98	70.18
Extent of acidification (%)	20.45	22.67	30.37	18.26	30.98	19.63
VFAs yield (mg COD/g VS)	158.55	175.77	235.46	141.53	240.14	152.14

acetate in pretreated group (R4, R5 and R6) were higher than untreated group (R1, R2 and R3), demonstrating that the heat-alkaline pretreatment was in favor of acetate production. On the other hand, the alkaline fermentation (73.65% of R3 and 75.81% of R6) was beneficial to acetate and propionate production than neutral pH fermentation (69.98% of R2 and 67.77% of R5) without considering pretreatment. The reason might be that the alkaline pH condition has changed the microbial community structure, and the alkaline-resistant microorganisms survived and produced some certain fatty acids, such as acetate (Liu et al., 2009).

3.2. The change of proteins and polysaccharides during fermentation

The concentration of proteins and polysaccharides were determined to show how the pH influences the substrate utilization by acidogenic bacteria. Fig. 2 showed the changes of soluble polysaccharides and proteins concentrations during fermentation. The change curves of polysaccharides and proteins concentrations in Fig. 2 indicated the substrate release and uptake simultaneously during the acidogenic fermentation.

In the acidogenic fermentation with pretreated sludge, the polysaccharides concentration deceased gradually, which was basically consistent with the increase of VFAs concentration (Fig. 1A). For the neutral fermentation (R5), the polysaccharides concentration decreased dramatically from 2743 mg/L to 1264 mg/L, which is higher than the other two reactors (R4 and R6), suggesting that the acidogenic microbes are suitable at neutral pH condition and more polysaccharides are transformed into VFAs at the pH 7.0. In the acidogenic fermentation with untreated sludge, only the polysaccharides concentration in alkaline pH (R3) increased from 61.16 mg/L to 1302.48 mg/L and its final concentration was similar to R5. The polysaccharides concentrations in R1 and R2 increased slightly and maintained at about 250.00 mg/L during the whole fermentation. The change profiles of polysaccharides concentration in Fig. 2A indicated that the alkaline pH enhanced the release of polysaccharides and the release rate was higher than the uptake rate by the acidogenic microorganisms.

Fig. 2B showed the changes of proteins during fermentation. In the fermentation with pretreated sludge (R4, R5 and R6), the initial soluble proteins concentrations were 9758.33 mg/L, 8538.67 mg/L and 9941.00 mg/L, respectively, which were higher than that of polysaccharides, meaning more proteins were released into the liquid. Many studies demonstrated that the proteins accounted for 50–60% of the total COD and were the major substrates in the sludge (Luo et al., 2013). The soluble proteins in R4 and R6 decreased gradually and R5 showed the largest reduction to final concentration of 3888.68 mg/L. For the proteins in the

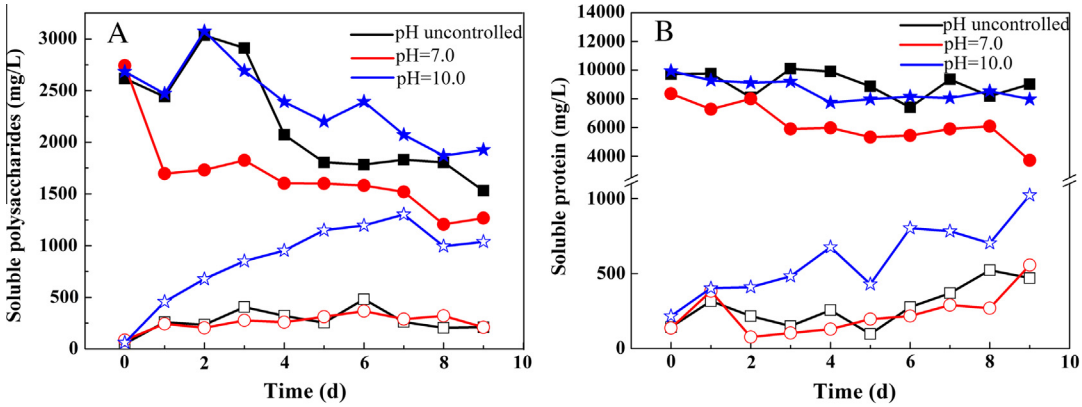

Fig. 2. The daily change of polysaccharides concentration (A) and proteins concentration (B) during the acidogenic fermentation. The open symbols represent un-pretreated sludge and the solid symbols represent the pretreated sludge.

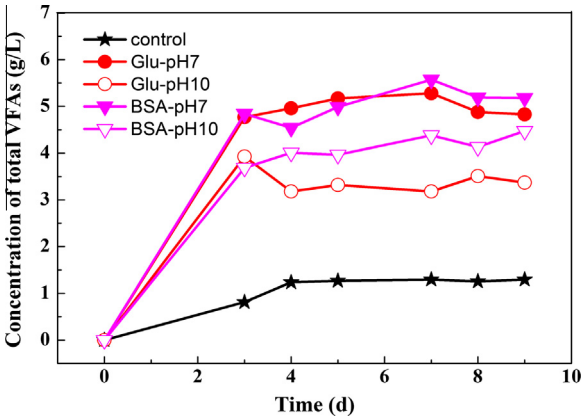

Fig. 3. VFAs concentrations with glucose or BSA as substrate during fermentation.

fermentation with untreated sludge, the change trends are very similar to Fig. 2A and only the sludge fermented at alkaline pH showed the increase trend.

Polysaccharides and proteins were the main substrates for the acidogenic microorganisms to produce VFAs during anaerobic fermentation. Since the VFAs themselves are one part of the SCOD, the profiles of the SCOD change in Fig. 1B cannot show the substrate utilization in response to the pH change. However, the analysis of the change profiles of specific substrates can clearly illustrate the impact of pH on the organic matters hydrolysis and VFAs production. Combining the results of polysaccharides and proteins in Fig. 2, it is clearly indicated that the neutral pH is favor of acidogenesis for the acidogenic microorganisms while the alkaline pH only enhanced the hydrolysis and release of organic matters from the sludge into the liquid, instead of the acidogenesis during the whole VFAs production from sludge.

3.3. Acidogenic fermentation with glucose and BSA as substrates

In order to further explore the pH impact on hydrolysis of organic matters and acidogenesis during VFAs production, glucose or BSA was used as substrate for the acidogenic fermentation.

In Fig. 3, when fermented at pH 7.0, the VFAs concentration maintained at 4.98 g/L with glucose as substrate while at 5.05 g/L with the BSA as substrate. However, when fermented at pH 10.0, the VFAs concentrations with glucose and BSA as substrates were 3.42 g/L and 4.11 g/L, respectively. It can be concluded that the VFAs concentrations of the fermentation at pH 7.0 are higher than that at pH 10.0. Since the glucose and BSA are simple molecules, they will not be hydrolyzed by the extracellular enzymes as the complex organic matters from the sludge. Therefore, it prevents the hydrolysis step and can be used as simple substrates, and helps for the exploration of the pH impact of acidogenesis reaction. According to the results in Fig. 3, it clearly demonstrated that the neutral pH, not the alkaline pH, is favor of VFAs production from the substrate.

By using two pure acid producing bacterial strains of *M. thermoacetica*, JW/B-2 and JW/DB-4, Dorothy et al. (2000) reported that after incubated and cultured for 3 weeks with pH values ranging from 4.0 to 10.0, they grew better at pH between 5.7 and 7.5. And the *Acetobacterium woodii* and *Clostridium aceticum*, which were isolated from Lake Kivu, can oxidize hydrogen and transform carbon dioxide to acetic acid and their optimal growth pH is 6.4 (Leigh et al., 1981). Küsel et al. (2006) also demonstrated *Halodule wrightii* and *Vallisneriaamericana* grew well at neutral pH medium. All these studies verified that neutral pH was better for the growth of most acidogenic bacteria, which were consolidated with our results.

The finding of the neutral pH is the best pH condition for the acidogenic fermentation and the alkaline pH is favor of the hydrolysis of organic matters in sludge provided a deeper understanding of the interaction effects among pH, hydrolysis of sludge and acidogenic microorganisms, and it also will be helpful for the novel process development of VFAs production by anaerobic fermentation from WAS.

3.4. Microbial community structure analysis by T-RFLP

The Terminal Restriction Fragment Length Polymorphism (T-RFLP) was used to explore the microbial community structure of the fermented sludge in different reactors. The fermented sludge in reactors at 5 d was collected for T-RFLP analysis. Fig. 4 depicted the distribution of Terminal Restriction Fragments (TRFs) from different reactors, in which each TRF represents a specific microbial genus (Xu et al., 2010). There were big differences of the microbial community structure among different reactors. In R3 and R6, the TRF of 297 bp was the dominant fragment with the percentage of 64.51% and 63.83%, respectively. Since R3 and R6 are fermented at pH 10.0, the microorganism represented by 297 bp can tolerate the alkaline environment.

For the number of TRFs, in the fermentation with untreated sludge (R1, R2 and R3), it is 9, 10 and 6, respectively, suggesting that with increase of pH the number of microorganisms fall down gradually. This is in accordance with the previous study (Liu et al., 2012). For the evenness of microbial distribution, it can be seen

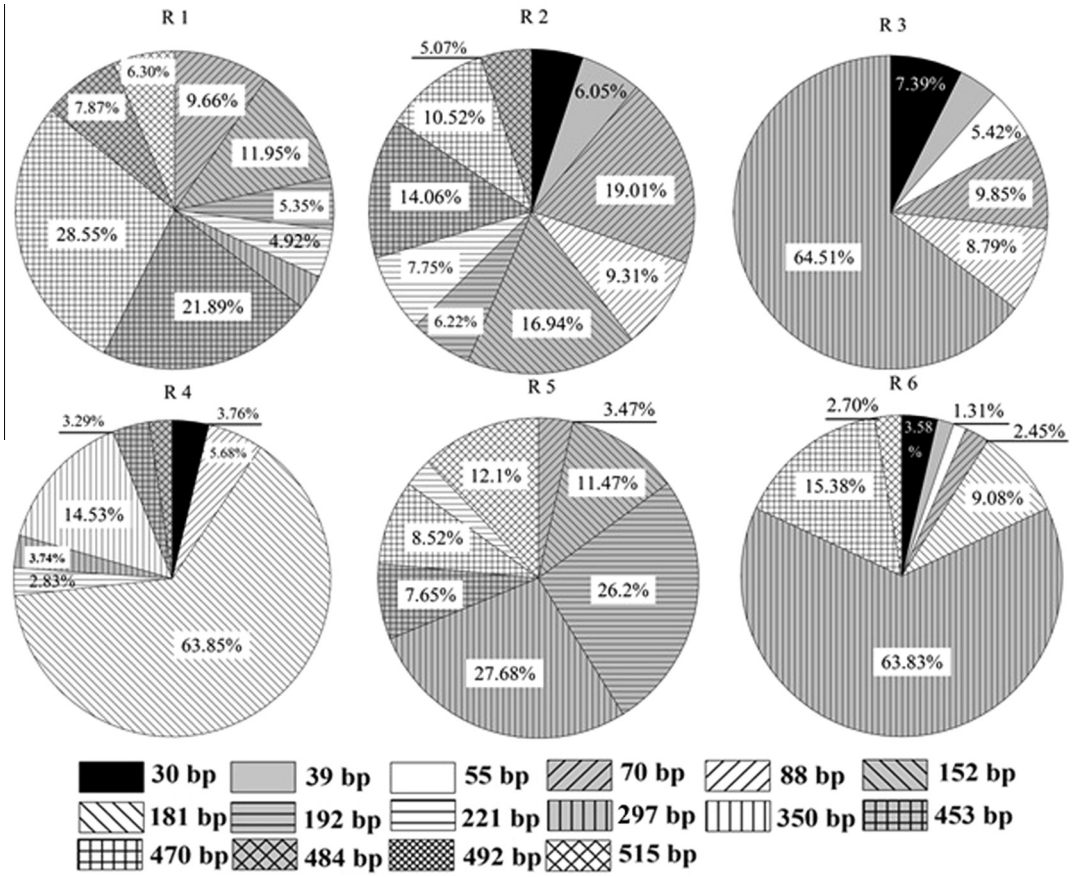

Fig. 4. Distribution of TRFs in the sludge at day 5 by T-RFLP analysis.

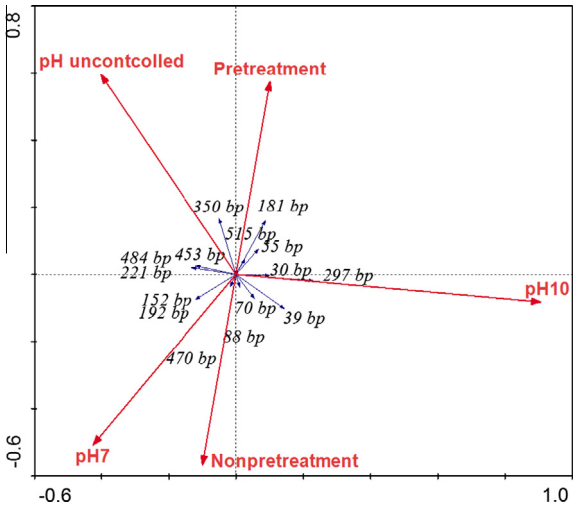

Fig. 5. Redundancy analysis (RDA) of the effect of pH and pretreatment on the presence of different T-RFs. Explanatory variables that contributed significant improvement ($P < 0.05$) to the explanatory power of the RDA models are indicated by red arrows in the ordinations, and the T-RFs are presented by blue arrows. The direction of the arrow indicates the direction of increase of each variable, and the length of the arrow indicates the strength of the correlation with the ordination axis. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

that the fermentation at neutral pH conditions (R2 and R5) showed the highest evenness indices, which is different from the alkaline pH conditions (R3 and R6), indicating that the neutral pH is benefitted to the various microorganism growth.

In order to describe the microbial community structure evolution of fermented sludge in different reactors, the redundancy analysis (RDA) was conducted and the results were showed in Fig. 5. As seen in Fig. 5, the TRF 297 bp showed the maximal positive correlation with pH 10.0, and this was consistent with the results in Fig. 5. It should be noted that the TRF181 bp showed strong positive relationship with pretreatment, which explained why the TRF181 bp accounted for a large percentage in R4 (63.85% in R4) but absence or low percentage in other reactors (Fig. 5). There is a strong positive relationship between TRFs 453 bp, 221 bp, 484 bp, 192 bp and 152 bp with pH 7.0 regardless of pretreatment, thus explaining why these TRFs accounted relative high percentages in R1, R2 and R5.

4. Conclusions

In the anaerobic fermentation with untreated sludge, alkaline pH promotes the disintegration and hydrolysis of organic matters, thus provides high concentration of soluble substrates (SCOD) and this finally increases the VFAs production from sludge.

On the other hand, for the anaerobic fermentation with heat-alkaline pretreated sludge and glucose or BSA, the neutral pH was demonstrated to be favor of the acidogenesis reaction and improves the VFAs production.

Based on the microbial community structure analysis, the number of the microorganisms in alkaline pH is lower than that in the other pH conditions, which indicates the alkaline pH is not suitable for the growth and metabolism of acidogenic microorganisms.

Acknowledgments

This work was financially supported by Joint innovative R&D Program of University and Industry (BY2014023-03), National Key Technology R&D Program of the Ministry of Science and Technology (2014BAD24B03-02) and National Natural Science Foundation of China (51208231).

References

Aarle, I.M., Perimenis, A., Lima-Ramos, J., et al., 2015. Mixed inoculum origin and lignocellulosic substrate type both influence the production of volatile fatty acids during acidogenic fermentation. Biochem. Eng. J. 103, 242–249.
Amani, T., Nosrati, M., Sreekishnan, T.R., 2010. Anaerobic digestion from the viewpoint of microbiological, chemical, and operational aspects — a review. Environ. Rev. 18, 255–278.
Angelidaki, I., Alves, M., Bolzonella, D., et al., 2009. Defining the biomethane potential (bmp) of solid organic wastes and energy crops: a proposed protocol for batch assays. Water Sci. Technol. 59, 927–934.
APHA, 1998. Standard Methods for the Examination of Water and Wastewater. American Public Health Association Inc, Washington, DC.
Appels, L., Baeyens, J., Degrève, J., et al., 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. Prog. Energy Combust. Sci. 34, 755–781.
Cesaro, A., Belgiorno, V., 2014. Pretreatment methods to improve anaerobic biodegradability of organic municipal solid waste fractions. Chem. Eng. J. 240, 24–37.
Chang, C.J., Tyagi, V.K., Lo, S.L., 2011. Effects of microwave and alkali induced pretreatment on sludge solubilization and subsequent aerobic digestion. Bioresour. Technol. 102, 7633–7640.
Chen, Y., Jiang, S., Yuan, H., et al., 2007. Hydrolysis and acidification of waste activated sludge at different pHs. Water Res. 41, 683–689.
Chen, Y., Liu, K., Su, Y., et al., 2013a. Continuous bioproduction of short-chain fatty acids from sludge enhanced by the combined use of surfactant and alkaline pH. Bioresour. Technol. 140, 97–102.
Chen, Y., Li, X., Zheng, X., et al., 2013b. Enhancement of propionic acid fraction in volatile fatty acids produced from sludge fermentation by the use of food waste and *Propionibacterium acidipropionici*. Water Res. 47, 615–622.
Dorothy, E., Byrer, F.A., Rainey, J.W., 2000. Novel strains of *Moorella thermoacetica* form unusually heat-resistant spores. Arch. Microbiol. 174, 334–339.
Drake, H.L., Daniel, S.L., Küsel, K., et al., 1997. Acetogenic bacteria: what are the in situ consequences of their diverse metabolic versatilities? BioFactors 6, 13–24.
Drake, H.L., Steven, L.D., 2004. Physiology of the thermophilic acetogen *Moorella thermoacetica*. Res. Microbiol. 155, 869–883.
Feng, L., Chen, Y., Zheng, X., 2009a. Enhancement of waste activated sludge protein conversion and volatile fatty acids accumulation during waste activated sludge anaerobic fermentation by carbohydrate substrate addition: the effect of pH. Environ. Sci. Technol. 43, 4373–4380.
Feng, L., Wang, H., Chen, Y., et al., 2009b. Effect of solid retention time and temperature on waste activated sludge hydrolysis and short-chain fatty acids accumulation under alkaline condition in continuous-flow reactors. Bioresour. Technol. 100, 44–49.
Herbert, D., Philipps, P.J., Strange, R.E., 1971. Carbohydr. Anal. Methods Enzymol. 5, 265–277.
Jie, W., Peng, Y., Ren, N., et al., 2014. Volatile fatty acids (VFAs) accumulation and microbial community structure of excess sludge (ES) at different pHs. Bioresour. Technol. 152, 124–129.
Khiewwijit, R., Temmink, H., Labanda, A., et al., 2015. Production of volatile fatty acids from sewage organic matter by combined bioflocculation and alkaline fermentation. Bioresour. Technol. 197, 295–301.
Kim, J., Park, C., Kim, T.H., et al., 2003. Effects of various pretreatments for enhanced anaerobic digestion with waste activated sludge. J. Biosci. Bioeng. 95, 271–275.
Küsel, K., Trinkwalter, T., Drake, H.L., et al., 2006. Comparative evaluation of anaerobic bacteria communities associated with roots of submerged macrophytes growing in marine or brackish water sediments. J. Exp. Mar. Biol. Ecol. 337, 49–58.
Leigh, J.A., Mayer, F., Wolfe, R.S., 1981. Acetogenium kivui, a new thermophilic hydrogen-oxidizing, acetogenic bacterium. Arch. Microbiol. 129, 275–280.
Li, H., Li, C., Liu, W., et al., 2012. Optimized alkaline pretreatment of sludge before anaerobic digestion. Bioresour. Technol. 123, 189–194.
Liu, H., Liu, X., Zhang, J., et al., 2009. Acetate accumulation and shift of bacterial community during anaerobic sewage sludge fermentation by pH adjustment. Acta Microbiol. Sin. 49, 1643–1649.
Liu, H., Wang, J., Liu, X., et al., 2012. Acidogenic fermentation of proteinaceous sewage sludge: effect of pH. Water Res. 46, 799–807.
Liu, X., Liu, H., Chen, J., et al., 2008. Enhancement of solubilization and acidification of waste activated sludge by pretreatment. Waste Manage. 28, 2614–2622.
Liu, Y., Li, X., Kang, X., et al., 2014. Short chain fatty acid accumulation and microbial community succession during ultrasonic-pretreated sludge anaerobic fermentation process: effect of alkaline adjustment. Int. Biodeterioration Biodegrad. 94, 128–133.
Longo, S., Katsoua, E., Malamisa, S., et al., 2015. Recovery of volatile fatty acids from fermentation of sewage sludge in municipal wastewater treatment plants. Bioresour. Technol. 175, 436–444.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., et al., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.
Luo, K., Ye, Q., Yi, X., et al., 2013. Hydrolysis and acidification of waste-activated sludge in the presence of biosurfactant rhamnolipid: effect of pH. Appl. Microbiol. Biotechnol. 97, 5597–5604.
Lutoslawski, K., Ryznar, L.A., Cibis, E., et al., 2011. Biodegradation of beet molasses vinasse by a mixed culture of microorganisms: effect of aeration condition and pH control. J. Environ. Sci. 23, 1823–1830.
Mahmood, T., Elliott, A., 2006. A review of secondary sludge reduction technologies for the pulp and paper industry. Water Res. 40, 2093–2112.
Pandit, Santosh, Kim, H.J., Kim, J.E., et al., 2011. Separation of an effective fraction from turmeric against *Streptococcus mutans* biofilms by the comparison of curcuminoid content and anti-acidogenic activity. Food Chem. 126, 1565–1570.
Park, S.K., Jang, H.M., Ha, J.H., et al., 2014. Sequential sludge digestion after diverse pre-treatment conditions: sludge removal, methane production and microbial community changes. Bioresour. Technol. 162, 331–340.
Pratt, S., Liew, D., Batstone, D.J., et al., 2012. Inhibition by fatty acids during fermentation of pre-treated waste activated sludge. J. Biotechnol. 159, 38–43.
Shahriari, H., Warith, M., Hamoda, M., et al., 2012. Anaerobic digestion of organic fraction of municipal solid waste combining two pretreatment modalities, high temperature microwave and hydrogen peroxide. Waste Manage. 32, 41–52.
Tan, R., Miyanaga, K., Uy, D., et al., 2012. Effect of heat-alkaline treatment as a pretreatment method on volatile fatty acid production and protein degradation in excess sludge, pure proteins and pure cultures. Bioresour. Technol. 118, 390–398.
Wang, J., Liu, H., Fu, B., et al., 2013. Trophic link between syntrophic acetogens and homoacetogens during the anaerobic acidogenic fermentation of sewage sludge. Biochem. Eng. J. 70, 1–8.
Wu, H., Yang, D., Zhou, Q., et al., 2009. The effect of pH on anaerobic fermentation of primary sludge at room temperature. J. Hazard. Mater. 172, 196–201.
Xiao, B., Liu, C., Liu, J., et al., 2015. Evaluation of the microbial cell structure damages in alkaline pretreatment of waste activated sludge. Bioresour. Technol. 196, 109–115.
Xu, K., Liu, H., Li, X., et al., 2010. Typical methanogenic inhibitors can considerably alter bacterial populations and affect the interaction between fatty acid degraders and homoacetogens. Appl. Microbiol. Technol. 87, 2267–2279.
Xu, S., Fu, B., Zhang, L., et al., 2015. Bioconversion of H_2/CO_2 by acetogen enriched cultures for acetate and ethanol production: the impact of pH. World J. Microbiol. Biotechnol. 31, 941–950.
Yang, X., Wan, C., Lee, D.J., et al., 2014. Continuous volatile fatty acid production from waste activated sludge hydrolyzed at pH 12. Bioresour. Technol. 168, 173–179.
Yuan, H., Chen, Y., Zhang, H., et al., 2006. Improved bioproduction of short-chain fatty acids (SCFAs) from excess sludge under alkaline conditions. Environ. Sci. Technol. 40, 2025–2029.
Zhao, J., Wang, D., Li, X., et al., 2015. Free nitrous acid serving as a pretreatment method for alkaline fermentation to enhance short-chain fatty acid production from waste activated sludge. Water Res. 78, 111–120.
Zhu, Y.F., Liu, H.B., Liu, H., 2015. Filtration characteristics of anaerobic fermented sewage sludge for fatty acids production. Sep. Purif. Technol. 142, 8–13.