Journal of Hazardous Materials 175 (2010) 510–517

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

Reconsideration of anaerobic fermentation from excess sludge at pH 10.0 as an eco-friendly process

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ARTICLE INFO

Article history:
Received 5 September 2009
Received in revised form 9 October 2009
Accepted 9 October 2009
Available online 6 November 2009

Keywords:
Anaerobic fermentation
Extracellular polymeric substances
Sludge dewaterability
Total bromate
Volatile fatty acids

ABSTRACT

Volatile fatty acids' (VFA) production from excess sludges during the initial stage (133 h) in the fermentation processes and the disposal risk of sludge were investigated under the real time controlled condition of pH at 5.5 and 10.0. The results showed that the total VFA production at pH 10.0 was markedly higher than that at pH 5.5. Anaerobic fermentation at pH 10.0 was the first be shown having marked reduction effect on total bromate in excess sludge when compared with pH 5.5. Meanwhile, at pH 5.5, sludge dewaterability deteriorated slightly with the fermentation time, whereas at pH 10.0 it deteriorated greatly with the fermentation time. Moreover, the mechanism of VFA improvement was also explored. It was suggested that anaerobic fermentation process at pH 10.0 should be reconsidered to be applied as an eco-friendly material recycling process, based on its sludge dewaterability and total bromate.

1. Introduction

Anaerobic fermentation could convert complex organic substances in excess sludge into value-added products, such as volatile fatty acids (VFA) and other low molecular weight soluble carbon compounds [1]. The value-added products of fermentation could be used as energy and carbon sources for biological nutrients removal (i.e. tertiary treatment of wastewater) [2]. In previous investigations, some researchers had reported that pH 5.5 could inhibit the methanogens and produce only organic acids [3]. Until recently, Chen and co-workers found that pH 10.0 could also inhibit methanogens and produce more VFA from excess sludges than pH 5.5 [4,5]. Moreover, by applying a well-defined fractioning structure of sludge flocs, Yu et al. [6] further revealed that pH 10.0 could markedly improve the VFA production by increasing the effective contact between extracellular organic matters and enzymes and creating a favorable environment for microbes to accumulate VFA. Therefore, if proven technically robust, anaerobic fermentation at pH 10.0 could be part of an appropriate approach for tertiary treatment in wastewater treatment plant (WWTP).

However, it is necessary to further evaluate the potential disposal risk of produced sludge in this fermentation process, such as total organic halogen and sludge dewaterability. Bromate (Br) compounds are widely applied to flame retardants and can enter the wastewater treatment system. However, Br is difficult to remove from excess sludges using conventional treatment technologies, which has led to investigations into novel removal techniques [7]. For example, Butler et al. [7] had shown that biological Br reduction may occur in anaerobic environment. On the other hand, sludge after anaerobic fermentation usually exhibited resistance to mechanical dewatering. Since sludge dewatering has been pointed out as the most expensive process [8], the factors influencing sludge dewatering after anaerobic fermentation need to be evaluated.

In previous investigations, both the VFA improvement and its mechanism were based on the pH adjustment to 10.0 intermittently [4–6]. Since the microbiology of fermentation was complex and delicate, the pH adjustment every day provided an unsteady pH environment for microbes. To our knowledge, few works have been done on the fermentation under the real time controlled pH condition so far, which could provide a steady pH environment for microbes and thereby better understand the VFA improvement at pH 10.0 as well as its underlying mechanism. On the other hand, all the previous investigations involving the VFA improvement at pH 10.0 were 4 days or 5 days of the sampling interval [4–6]. Since the production of VFA and the release of soluble organic matters and extracellular enzymes were occurred in the initial 4 days or 5 days [4–6], the sampling interval in the above-mentioned investigations was believed unsuitable.

In this study, the fermentation experiments, with the real time control of pH at 5.5 and 10.0 and suitable sampling interval, were designed (1) to evaluate the underlying mechanism of VFA

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doi:10.1016/j.jhazmat.2009.10.035

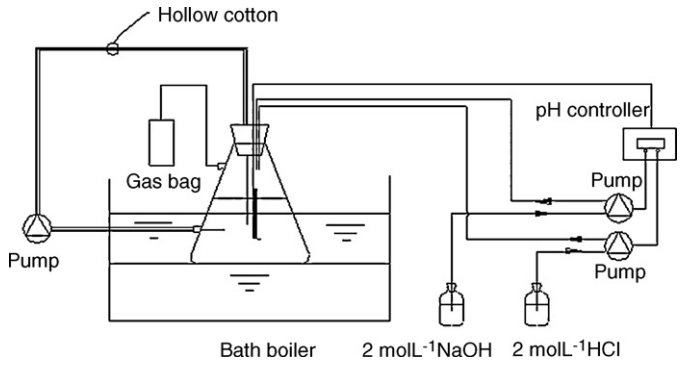

Fig. 1. Schematic of mesophilic digester setup under the real time control of pH.

improvement under the real time control condition of pH at 10.0, (2) to address the fate of total Br under the real time control condition of pH at 5.5 and 10.0, and (3) to compare the sludge dewaterability under the real time control condition of pH at 5.5 and 10.0. The fractioning structure of sludge flocs [9], composed of slime, loosely bound extracellular polymeric substances (LB-EPS), tightly bound-EPS (TB-EPS), and pellet, was applied to address the underlying mechanism. Protease and α-amylase were reported to play essential roles in the hydrolysis of two major fractions of EPS: proteins (PN) and polysaccharides (PS) [10]. Alkaline phosphatase hydrolyzed phosphomonoesters to provide an alternative source of phosphorus for the cells, whereas acid phosphatase was reported to be involved in internal cell metabolism [11]. Therefore, the four extracellular enzymes in conjunction with the particle size distribution (PSD) of excess sludges were selected for this study to evaluate the mechanism of VFA improvement at pH 10.0.

2. Materials and methods

2.1. Sludge samples

Excess sludge samples were collected from the aerated basin of a municipal WWTP in Shanghai, China. The plant treats 75,000 $m^3\,d^{-1}$ of wastewater (93% domestic and 7% industrial sewage) using anaerobic–anoxic–oxic process. The collected excess sludges were transported to laboratory within 2 h after sampling. The sludges were first settled for 1.5 h at 4 °C. The sludge sediments were collected and screened through a 1.2 mm screen. The sludge pH and conductivity were approximately 6.8 and 10.9 $\mu s\,cm^{-1}$, respectively. The chemical oxygen demand (COD) and soluble COD (SCOD) of the sludge were about 17,000 and 140 $mg\,L^{-1}$, respectively, determining by a spectrometer (DR/2000, HACH, USA). Hence, soluble organic matter (i.e. SCOD) in sludge accounted for approximately less than 1%. PN and PS of the sludge were 600 $mg\,g^{-1}$ and 12.7 $mg\,g^{-1}$ volatile suspended solids (VSS), respectively. Therefore, PN and PS were the two major types of organic components in excess sludges, accounting for 60.0% and 1.27% of VSS, respectively. The elemental composition of dried samples was, according to an elemental analyzer (Vario EL III, Germany), as follows: C, 35%; N, 6%; H, 3%; S, 7%.

2.2. Anaerobic fermentation experiments

The two laboratory-scales, batch-continuous stirred tank reactors (CSTR, Fig. 1) had a working volume of 1.0 L and were operated at 37 ± 1 °C through a water bath (HHS-8, Jinkai Science Instrument Ltd. Co., China). Their pH values were controlled at 5.5 ± 0.3 and 10.0 ± 0.3 through two of pH controllers (pH 101, HOTEC, Taiwan), respectively, by adding 2 mol L^{-1} HCl or 2 mol L^{-1} NaOH. A pump was applied to circulate the sludge mixed liquor. Hollow cotton was used to maintain the temperature of the sludge mixed liquor in the circulation tube. Gas bag was used to collect the produced gas. The two reactors were flushed with nitrogen gas (N_2) for 60 s before being sealed. After the start-up, the sludge samples were measured about every 12 h.

2.3. EPS fractionation protocol

Standard procedures for EPS fractionation of sludge samples are detailed elsewhere [9]. In brief, screened sludge sample was allowed to settle for 1.5 h at 4 °C, after which the bulk solution, comprising the supernatant, was collected carefully by a siphon. The sediments were then centrifuged at 2000 × *g* for 15 min. The bulk solution was collected as the slime. The bottom sediments were collected and re-suspended to their original volumes using a pH 7 buffer solution consisting of Na_3PO_4, NaH_2PO_4, NaCl and KCl [12]. The molar ratio of these components was 2:4:9:1. The conductivities of the buffers were adjusted with distilled water to match that of the sludge sediment samples. The suspensions were centrifuged at 5000 × *g* for 15 min, and the bulk solution and solid phase were collected separately. The organic matter in the bulk solution comprised the loosely bound-EPS (LB-EPS). The collected sediments were re-suspended with the aforementioned buffer to the original volumes and then extracted by subjecting the suspension to ultrasound at 20 kHz and 480 W for 10 min. The extracted solutions were centrifuged at 20,000 × *g* for 20 min. Organic matter in the bulk solution comprised the tightly bound-EPS (TB-EPS); the residues (solid phase) were again re-suspended with the aforementioned buffer to their original volumes. This fraction comprised the pellet. Polytetrafluoroethylene (PTFE) membranes (Mosu Scientific Equipment Co., Shanghai, China) with a pore size of 0.45 μm were used to remove the particulates present in the supernatant, slime, LB-EPS, and TB-EPS solutions.

2.4. Molecular weights (MW) distribution

The MW of supernatant after the 0.45 μm-PTFE membranes filtration was determined by a gel permeation chromatography (GPC) (LC-10ADVP, Shimadzu, Japan) equipped with a differential detector (RID-10A) and a TSKgel column (G4000PWXL, TOSOH Co., Japan). The mobile phase was Milli-Q water. Polyethylene glycol/oxides (MW at 1169 kDa; 771 kDa; 128 kDa; 12 kDa; 4 kDa; 620 Da; 194 Da) were used as reference molecules for the calculation.

The weight-averaged molecular weight (M_w) and number-averaged molecular weight (M_n) for the samples were calculated using the following equations [13]:

$$M_n = \frac{\sum_{i=1}^{n} h_i}{\sum_{i=1}^{n} \left(\frac{h_i}{m_i}\right)} \quad (1)$$

$$M_w = \sum_{i=1}^{n} \frac{(h_i \times m_i)}{\sum_{i=1}^{n} h_i} \quad (2)$$

where m_i is the molecular weight at eluted volume *i*, and h_i is the height of the sample GPC curve eluted at volume *i*.

2.5. Particle size distribution

PSD assay of raw and digested sludges was determined using an EyeTech instrument (Ankersmid, USA) with a 300 mm lens which enabled the measurement of particles in the range 0.1–1000 μm. The samples were diluted in filtrated effluent (0.45 μm-PTFE membrane) to avoid multiple scattering. Each sample was gently taken by a wide-mouthed pipet and measured in duplicates. The average

particle size of the sludge flocs was given as the mean based on the volume equivalent diameter (D) [4,3,14]:

$$(\bar{X}_{VM}) = \frac{\sum dM}{\sum dV} = \frac{\sum x^4 \, dN}{\sum x^3 \, dN} \quad (3)$$

Where $\bar{X}_{VM}$ is the average particle size based on the volume equivalent diameter, x is the size of particle, and dN is the number of particle.

2.6. Total Br determination

The sludge samples were dried at 70 °C to constant weight, grinded and passed through the 200 sieve, for the analysis of total Br. Total Br in sludge samples was measured by an analysis equipment that consisted of (1) automatic sample combustion equipment (AQF-100, Mitsubishi) to decompose halogenated compounds into halogen ions, carbon dioxide, and water, and (2) gas adsorption equipment (GA-100) and 3) the IC (ICS-90) to detect Br^- ions.

2.7. Other analytical techniques

Both the chemical analyses and enzymatic assays were carried out in triplicates. Activities of protease and α-amylase were analyzed using the Lowry method [15] with casein as the standard and the method of Bernfeld [16] with glucose as the standard. Alkaline phosphatase and acid phosphatase activities were performed according to the method by Goel et al. [10] with *p*-nitrophenylphosphate di sodium salt (Sigma N 3254) as the standard. PN was determined by the modified Lowry method [12], using casein (Shanghai Sangon Biotechnology Co., Ltd., China) as the standard. PS was measured by the Anthrone method [17], with glucose as the standard. The conductivity was determined by a conductivity meter (DDSJ-308A, Leici Co., Ltd., Shanghai, China). The supernatant of sludge after 0.22 μm polyester filters was measured for VFA (LC-20AD, Shimadzu, Japan). Gas analysis was measured by a gas chromatograph (GC-112A, Shimadzu, Japan) [18]. The carbon or nitrogen percentage in excess sludges was determined by the Elemental Analyzer (Vario EL III, Germany). The sludge dewaterability was evaluated with a capillary suction time (CST) instrument (Model 319, Triton, UK) equipped with an 18-mm diameter funnel and Whatman no. 17 chromatography-grade paper. The CST values were normalized by dividing them by the initial total suspended solids (TSS) concentration and then expressed in units of seconds per liter per gram TSS [10]. Other sludge parameters, including TSS and VSS, were analyzed following the standard methods [19].

3. Results and discussion

3.1. VFA production at the real time pH control of 5.5 and 10.0

Fig. 2 illustrates the production and components of VFA in the mesophilic digesters at the real time pH control of 5.5 and 10.0, respectively. It was noted that the total VFA production was pronouncedly higher at pH 10.0 than that at pH 5.5. At the fermentation time of 1 h, acetic acid was detected only in the digester of pH 10.0. At the fermentation time of 13 h, acetic acid at pH 5.5 was almost equal to that at pH 10.0, with the concentration of about 4 mg L^{-1}. At the fermentation time of 37 h, acetic acids at pH 5.5 and 10.0 mg L^{-1} were 5.9 mg L^{-1} and 16.6 mg L^{-1}, respectively, suggesting that acetic acids at pH 10.0 were about 2.8 times higher than that at pH 5.5. At the fermentation time of 61 h, acetic acids at pH 5.5 and 10.0 were 5.1 mg L^{-1} and 28.7 mg L^{-1}, respectively. Meanwhile, propionic acid with the concentration of about 2.9 mg L^{-1} was also

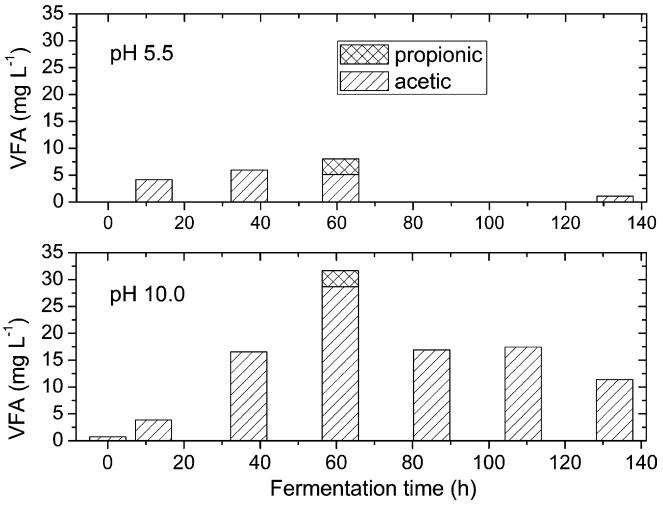

Fig. 2. VFA with fermentation time in mesophilic digesters.

formed at both pHs. At the fermentation time of 85 h and 109 h, acetic acid was found only in the digester of pH 10.0, with the concentration of about 17 mg L^{-1}. At the fermentation time of 133 h, acetic acids at pH 5.5 and 10.0 were 1.1 mg L^{-1} and 11.4 mg L^{-1}, respectively. On the other hand, neither methane nor carbon dioxide was monitored at both pHs during the 133 h of fermentation time.

The VFA improvement was consistent with those of Yu et al. [6] based on the pH adjustment everyday, supporting the idea that the VFA production in hydrolysis and acidification could be markedly improved at pH 10.0 when compared with pH 5.5.

3.2. MW distribution at the real time pH control of 5.5 and 10.0

MW distribution was investigated to further elucidate the organic properties of the supernatant at the real time pH control of 5.5 and 10.0 (Fig. 3). It was noted that two main peaks were detected in the supernatant at pH 5.5 and 10.0 (except for only one peak at 133 h and pH 10.0). Meanwhile, the total area of the two peaks at pH 10.0 was markedly bigger than that at pH 5.5 at the same fermentation time, suggesting that pH 10.0 could improve the concentration of soluble organic matters. More importantly, the MW of soluble organic matters was principally less than 1000 kDa (Fig. 3), which could be directly assimilated by cells [20]. When combined with the VFA production at pH 5.5 and 10.0 (Fig. 2), it could be concluded that pH 10.0 produced more soluble organic matter preferable to cells and thereby improved the VFA production.

The polydispersity (ratio of M_w to M_n) value could be applied to differentiate origin and/or properties of organic matters [13]. Specifically, if it is close to 1 meaning that constituents in organic matters are mostly made up of organic substances with the same/similar origin and/or properties, whereas if it is over 1 indicating that these substances are composite of heterogeneous organic matters with various MWs. Table 1 lists the polydispersity of supernatant with time at the real time pH control of 5.5 and 10.0. It was found that the polydispersities of peak 1 (low MW) in the supernatant at pH 5.5 and 10.0 were in the range from 1.01 to 1.10, whereas as for the peak 2 (high MW) the polydispersities were in the range of 1.95–5.96. The polydispersities results revealed that in the supernatant, the constituents in the low MW peak had the same/similar origin and/or properties, whereas the constituents in the high MW peak were heterogeneous. Therefore, in the supernatant, the low MW peak may come from biotic origin, whereas the high MW peak may come from both biotic and abiotic origins. Moreover, it was also found that as for the high MW peak (peak 2),

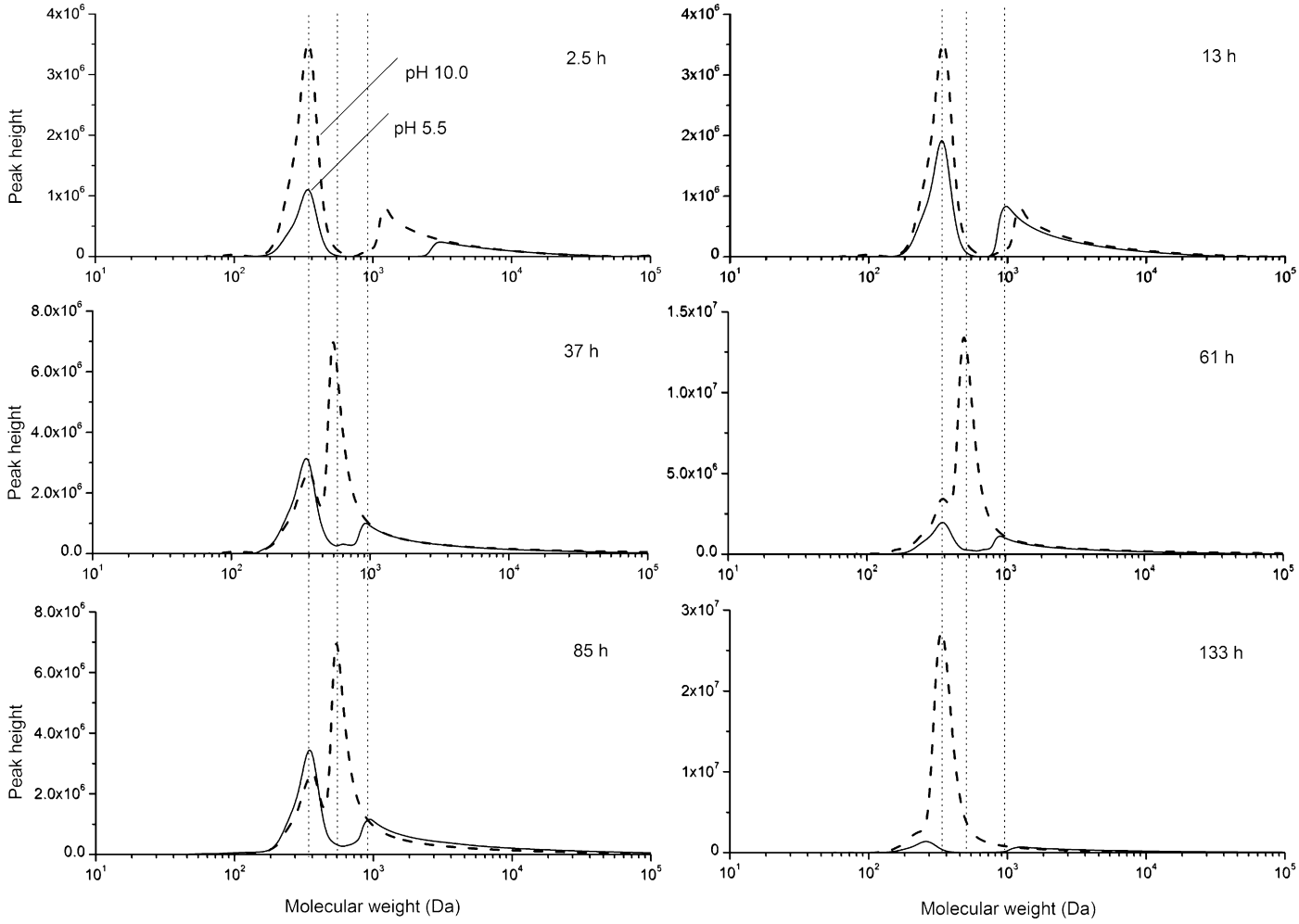

Fig. 3. Molecular weight distribution of supernatant in sludges with fermentation time in mesophilic digesters.

the polydispersities of pH 5.5 were bigger than those of pH 10.0 at the same time. The M_w and M_n of pH 5.5 were bigger than those of pH 10.0 at all the fermentation time, supporting from the micro-view level that the hydrolysis effect at pH 10.0 was better than that at pH 5.5.

Confer and Logan [20] had shown the mechanism for macromolecule degradation in WWTPs incorporated the following features: macromolecules diffuse to the surface of cells where they are hydrolyzed; the hydrolytic fragments are then released and may return to the bulk solution; this process is repeated until the hydrolytic fragments are small enough (<1000 Da) to be directly assimilated into cells. Based on the above macromolecule degradation protocol, the peaks 2 and 1 may be the primary and secondary products of the hydrolytic process, respectively. pH 10.0 had a

Table 1
Polydispersity of supernatant with fermentation time at pH 5.5 and 10.0.

Fermentation time	Peaks	pH 5.5			pH 10.0		
		M_w[a]	M_n[a]	Polydispersities ($\rho = M_w/M_n$)	M_w	M_n	Polydispersities ($\rho = M_w/M_n$)
2.5 h	Peak 1	325	311	1.04	330	316	1.04
	Peak 2	3,279	1,668	1.97	3,093	2,015	1.95
13 h	Peak 1	334	318	1.05	327	314	1.04
	Peak 2	10,290	1,981	5.19	3,341	813	4.11
37 h	Peak 1	335	317	1.06	327	313	1.05
	Peak 2	6,933	1,796	5.53	4,210	761	3.86
61 h	Peak 1	338	309	1.09	308	294	1.05
	Peak 2	10,505	1,764	5.96	1,246	624	2.00
85 h	Peak 1	333	304	1.10	111	109	1.01
	Peak 2	10,436	1,783	5.85	1,260	507	2.48
133 h	Peak 1	253	242	1.04			
	Peak 2	11,240	2,493	4.51	793	361	2.20

[a] Note that M_w and M_n are the weight–averaged molecular weight and number–averaged molecular weight, respectively.

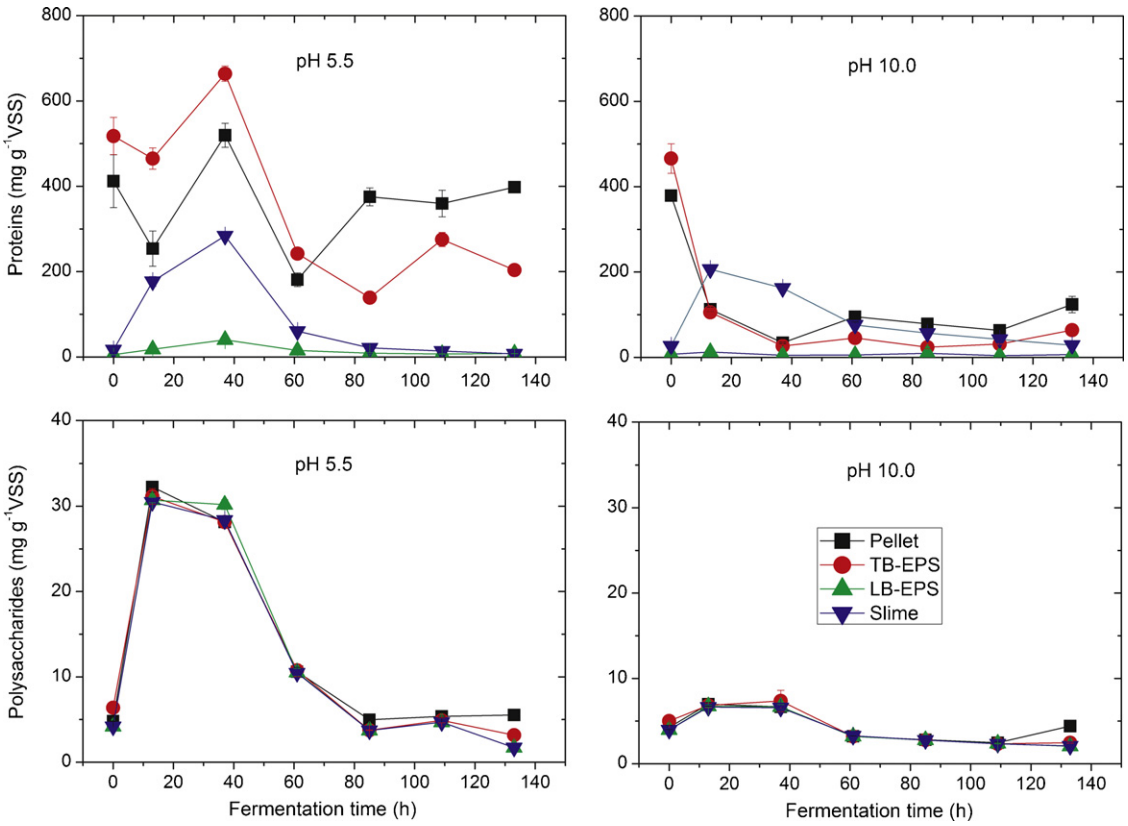

Fig. 4. Proteins and polysaccharides with fermentation time in mesophilic digesters. Error bars represent the standard deviation of triplicate samples.

higher concentration of secondary product than pH 5.5, and thereby had a better hydrolytic performance.

3.3. Degradation of PN and PS at the real time pH control of 5.5 and 10.0

PN and PS were predominant in excess sludges and represented most of organic matters [4,6,9]. More importantly, they were directly related to the production of VFA and other soluble organics [4]. Fig. 4 shows the distribution patterns of PN and PS in the different EPS fractions. PN was mainly distributed in the TB-EPS and pellet fractions, few in the LB-EPS and slime fractions at the initial time, regardless of pHs. In the TB-EPS and pellet fractions, PN changed slightly with time at pH 5.5 but decreased at pH 10.0. In contrast, it increased gradually in the slime fraction at the initial 37 h, regardless of pHs. The PN variations with time in different EPS fractions revealed that during anaerobic fermentation, PN was transferred from the inner fraction (i.e. TB-EPS) to the outer fractions (i.e. slime or LB-EPS) which were available to microbes.

As for PS, it was evenly distributed in every EPS fraction. Meanwhile, the distribution pattern of it had a slight variation with time. Moreover, PS in every EPS fraction increased in the initial 37 h and subsequently decreased until 133 h. The distribution patterns of PN and PS at the real time pH control of 5.5 and 10.0 were similar to those at pH 5.5 and 10.0 of everyday adjustment [6].

Degradation of PS and PN could also be expressed by the variation of carbon and nitrogen percentage in excess sludges (Fig. 5). At the initial time, the carbon and nitrogen percentages in excess sludges were $35.7 \pm 0.2\%$ and $6.2 \pm 0\%$ at pH 5.5, $33.8 \pm 0.4\%$ and $5.2 \pm 0.2\%$ at 10.0, respectively. At the fermentation time of 13 h, they decreased to $23.8 \pm 0.2\%$ and $5.4 \pm 0\%$ at pH 5.5, $11.5 \pm 2.1\%$ and $2.1 \pm 0.4\%$ at pH 10.0. Then, they decreased to $18.8 \pm 1.9\%$ and $3.7 \pm 0.5\%$ at pH 5.5 but ascended to $13.3 \pm 1.5\%$ and $2.5 \pm 0.2\%$ at pH 10.0. In most cases, the carbon and nitrogen percentage in excess sludges at pH 10.0 was significantly lower than that at pH 5.5. Meanwhile, the lowest percentage of carbon and nitrogen at pH 10.0 was 13 h, whereas at pH 5.5 it was 37 h. The results of elemental analysis revealed that the organic matters (i.e. polysaccharides and proteins) were degraded much more and faster at pH 10.0 than at pH 5.5. These trends were supported by the chemical determination results that PN and PS were degraded much more and faster at pH 10.0 than at pH 5.5 (Fig. 4).

3.4. Total Br at the real time pH control of 5.5 and 10.0

Total Br in excess sludges in the fermentation processes at pH 5.5 and 10.0 is investigated and shown in Fig. 6. In the initial excess sludges, the total Br content was about 0.1 mg g^{-1}. After the 13 h of fermentation, it increased to about 0.14 mg g^{-1} at pH 10.0 while decreased to 0.01 mg g^{-1} at pH 5.5. However, it decreased to the level of 0.02–0.08 mg g^{-1} until 133 h at pH 10.0, and increased to the level of 0.11–0.12 mg g^{-1} until 133 h at pH 5.5 (except for 61 h at the level of 0.32 mg g^{-1}). Therefore, pH 10.0 decreased about 20–80% of total Br content in excess sludges after the fermentation time of 133 h, whereas pH 5.5 increased about 10–20% of total Br content in excess sludges after the fermentation time of 133 h. Since the organic matters at pH 10.0 degraded much more than at pH 5.5, it was concluded that the fermentation process at pH 10.0 had marked degradation effects on total Br in excess sludge, whereas the one at pH 5.5 possessed less degradation effects on total Br than on organic matters or had no degradation effect on total Br.

It was noted that sludge samples were centrifuged before the determination of total Br. Therefore, it is reasonable to believe that the reduction of total Br in the sludge samples may be attributable to the transfer of total Br from the solid state to the liquid state owing to the hydrolysis/break of sludge matrix. Thus, this transfer will increase the risk of the produced VFA in the fermentation

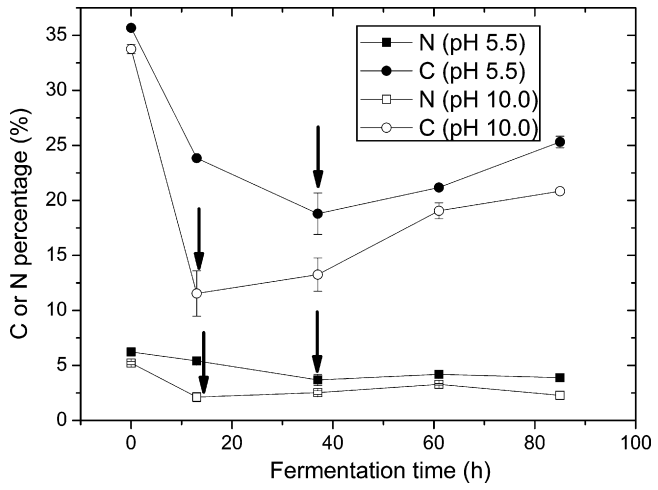

Fig. 5. Carbon and nitrogen percentage in sludges with fermentation time in mesophilic digesters. Error bars represent the standard deviation of triplicate samples.

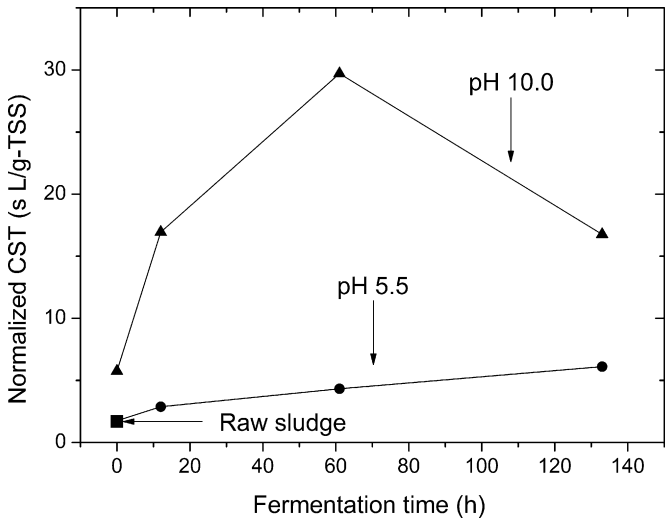

Fig. 7. CST with fermentation time in different digesters.

process at pH 10.0 as the carbon sources of tertiary treatment in WWTP. Our investigations in this study suggested that it is necessary to further explore the risk of the produced VFA as the carbon sources.

A growing awareness of the need to control the nutrient emissions had been reflected in the increasingly stringent regulations [21]. However, if the available carbon source in the raw wastewater is not sufficient to achieve complete nutrient removal, an additional suitable external carbon source must be required. Therefore, the primary driver for a successful nutrient removal is the availability of a suitable carbon source, mainly in the form of VFA. In previous investigations, some investigators had reported that pH 5.5 could produce more VFA than neutral pH [3]. In this study, our results showed that the fermentation process under the real time controlled condition of pH 10.0 is able to greatly improve the VFA production when compared with pH 5.5. However, the produced VFA in the fermentation process at pH 10.0 may increase the risk as the carbon sources of tertiary treatment in WWTP, owing to the total Br transfer from the solid state to the liquid state. Hence, it should be reconsidered whether the process can be applied as an eco-friendly material recycling process and as part of an appropriate solution for tertiary treatment.

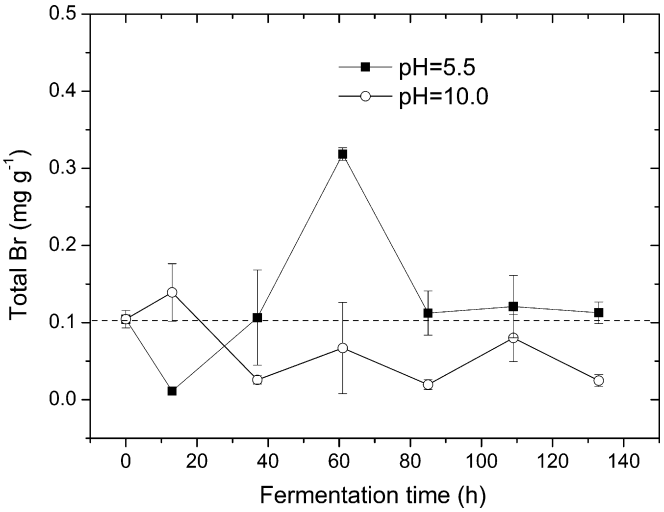

Fig. 6. Total Br with fermentation time in different digesters.

The previous investigations involving the VFA improvement at pH 10.0 did not consider the degradation of total Br. In this study, we suggested the first that pH 10.0 could not only improve VFA but also degrade total Br when compared with pH 5.5. Therefore, the fermentation process at pH 10.0 could reduce the risk of sludge disposal (i.e. land application, incineration) and as a biological Br reduction process.

3.5. Sludge dewaterability at pH 5.5 and 10.0

Sludge dewatering is of major interest in handling properties improvement. Therefore, it is usually an essential unit followed by anaerobic fermentation in most sludge disposal routes [22]. Sludge dewaterability at pH 5.5 and 10.0 was evaluated by the normalized CST (Fig. 7). Both the fermentation processes led to the CST increment to some extents, suggesting that the sludge dewaterability was deteriorated during the fermentation processes. At pH 5.5, the CST value increased slightly with the fermentation time. However, at pH 10.0, the CST climbed rapidly from 5.7 s L g^{-1}TSS for the initial time to about 29.7 s L g^{-1}TSS after 61 h of fermentation. Afterwards, it decreased to 16.8 s L g^{-1}TSS after 133 h of fermentation. The difference between the two pHs could be attributed to the fact that at pH 10.0, the release of organic matters (mainly PN) in excess sludges was higher than at pH 5.5 (Fig. 4). Therefore, the variations of PN might play an important role in sludge dewaterability.

3.6. Mechanism of VFA improvement at pH 10.0

3.6.1. Particle size distribution at pH 5.5 and 10.0

Particle sizes and compositions of excess sludges determine the rate and mechanism of fermentation [23]. Fig. 8 presents the evolution of the PSD with time in both reactors. It was clearly shown that at pH 10.0, particle sizes were smaller than at pH 5.5 throughout the fermentation time. At 13 h, the average particle size at pH 5.5 and 10.0 was about 88 μm. At 37 and 61 h, the average particle size at pH 5.5 was significantly bigger than that at pH 10.0 (84–94 μm vs 43–54 μm). However, at 85 and 133 h, the average particle size at pH 5.5 was similar to that of pH 10.0.

Yu et al. [6] had investigated the PSD after 5 days in mesophilic and thermophilic digesters, in which pH was adjusted everyday by adding 2 mol L^{-1} HCl or 2 mol L^{-1} NaOH. They also found that particle sizes of pH 10.0 were smaller than that of pH 5.5, regardless of temperature.

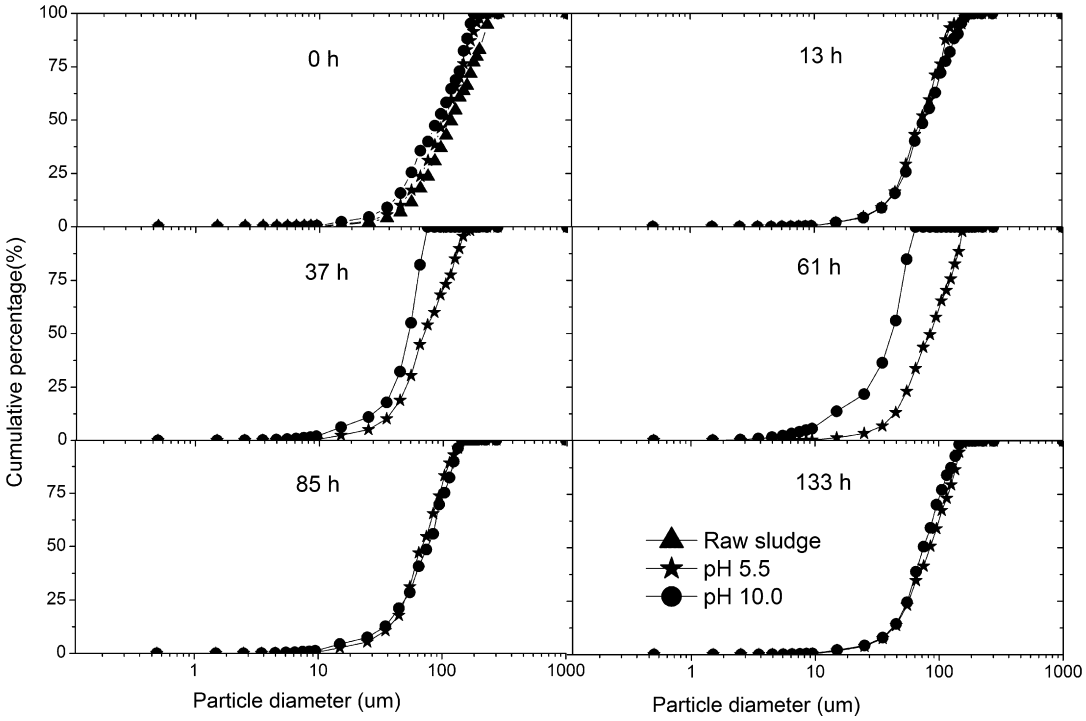

Fig. 8. Particle size distributions with fermentation time based on particle volume in mesophilic digesters.

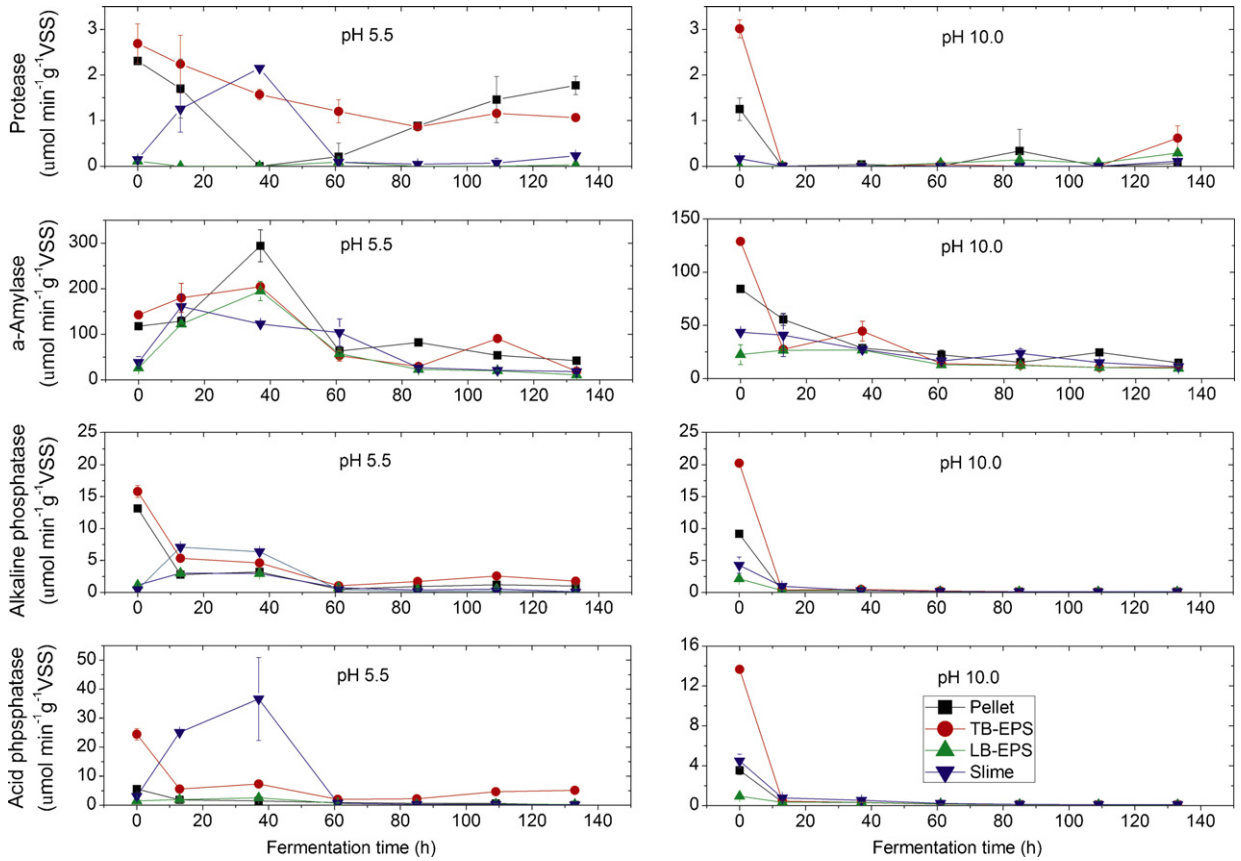

Fig. 9. Enzyme activities with fermentation time in mesophilic digesters. Error bars represent the standard deviation of triplicate samples.

3.6.2. Enzyme activities at pH 5.5 and 10.0

Enzymes play a crucial role in the biological processes [24]. Measurement of enzymes is an alternative method for assessing microbial biomass and activity [6]. Fig. 9 depicts the activity of protease, α-amylase, alkaline phosphatase and acid phosphatase in different fractions of excess sludges. It was found that at the initial day of excess sludges, protease and alkaline phosphatase were mainly distributed in the pellet and TB-EPS fractions, i.e. bound with cells, less distributed in the slime and LB-EPS fractions. Although α-amylase was also principally presented in the TB-EPS and pellet fractions, higher percentage of it was detected in the slime and LB-EPS fractions than other enzymes. As for acid phosphatase, it was mainly distributed in the TB-EPS fraction, few in the pellet, LB-EPS and slime fractions. The results of enzyme distributions in excess sludges were similar to the previous observations [6,25].

As for pH 5.5, the total activities (slime + LB-EPS + TB-EPS + pellet) of enzymes in excess sludges increased with time in the initial 37 h, and subsequently decreased with time. Meanwhile, enzyme activities in the pellet fraction decreased much more than the other fractions (Fig. 9). However, protease activities in the pellet fraction after the fermentation time of 37 h increased with time, probably attributable to the adaptation of microbes secreting protease. The particularly interesting point was that the protease, alkaline phosphatase, and acid phosphatase activities in the slime fraction apparently increased with time and were even higher than those in the pellet fraction at 37 h, indicating that the enzymes originally embedded in the pellet fraction by EPS matrix were released from the inner fraction into the outer one with time. The transfer phenomenon of enzyme activities in excess sludges could be attributed to the degradation of extracellular polymers (mainly PN) and subsequently to the breakage of sludge matrix.

On the other hand, as for pH 10.0, the total activities of enzymes in excess sludges decreased with time. Meanwhile, the total enzyme activities at pH 10.0 were marked lower than those at pH 5.5 during the whole fermentation time of 133 h (Fig. 9). Therefore, the determination of enzyme activities in conjunction with PSD, under the real time controlled condition of pH, clearly demonstrated that the abiotic effect (i.e. alkaline solubility) rather than biotic effect was the leading reason for the VFA improvement at pH 10.0. However, there was also a considerable quantity of enzyme activities in excess sludges at pH 10.0 (Fig. 9). Therefore, the biotic effect also played a role to a certain extent in VFA production. The reasons why enzyme activities at pH 5.5 were higher than those at pH 10.0 may be probably due to the inhibiting role of pH 10.0 on microbes.

4. Conclusions

During the initial stage (133 h) anaerobic fermentation of excess sludge under the real time pH controlled condition, the total VFA production at pH 10.0 was markedly higher than that at pH 5.5. MW distribution further elucidated from a micro-view level that pH 10.0 produced more soluble organic matter (<1000 Da) preferable to cells and thereby improved the VFA production. The fermentation process at pH 10.0 was the first to be shown having marked reduction effects on total Br in excess sludge. Therefore, the fermentation process at pH 10.0 could reduce the risk of sludge disposal (i.e. land application, incineration). However, the produced VFA in the fermentation process at pH 10.0 may increase the risk as the carbon sources of tertiary treatment in WWTP, owing to the total Br transfer from the solid state to the liquid state. The determination of enzyme activities in conjunction with PSD, under the real time controlled condition of pH, clearly demonstrated that the abiotic effect (i.e., alkaline solubility) rather than biotic effect was the leading reason for the VFA improvement at pH 10.0. Sludge dewaterability at pH 10.0 deteriorated greatly during fermentation process, attributable to that pH 10.0 released more organic matters (mainly PN).

Acknowledgements

The authors wish to thank the National Key Project for Water Pollution Control (2008ZX07316-002 and 2008ZX07317-003), the National Hi-Tech Research and Development Program of China (2006AA06Z384) and the Specialized Research Found for Doctoral Program of Higher Education of China (200802470029).

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