Toward understanding the mechanism of improving the production of volatile fatty acids from activated sludge at pH 10.0

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ABSTRACT

A well-defined fractionation approach for sludge flocs was applied to a better understanding of the underlying mechanism of improving the production of volatile fatty acids (VFA) in the hydrolysis and acidification processes at pH 10.0. Specifically, sludge flocs were fractionated through centrifugation and ultrasound into four fractions: (1) slime, (2) loosely bound extracellular polymeric substances (LB-EPS), (3) tightly bound EPS (TB-EPS) and (4) pellet. Result showed that during 20 days of fermentation, the total VFA production at pH 10.0 was higher, from 2 to 34 times, than that at pH 5.5. At pH 10.0, however, the enzyme activities (i.e. protease, α-amylase, alkaline phosphatase and acid phosphatase) in all fractions of sludge flocs were all lower than pH 5.5, which strongly suggests that the biotic effect was not the leading cause of the VFA improvement. Further investigation suggests that pH 10.0 could significantly improve the VFA production through the break of sludge matrix which is usually hydrolyzed by the extracellular enzymes embedded in itself, increase the effective contact between extracellular organic matters and enzymes, and create a favorable environment for microbes to accumulate VFA. Hydrolysis and acidification at pH 10.0 can be considered as part of an appropriate solution for tertiary treatment and contribute to the headway toward the goal of sustainable water treatment technologies.

1. Introduction

Hydrolysis and acidification can convert complex organic substances in sludge flocs into volatile fatty acids (VFA) and other low molecule weight soluble carbon compounds (Elefsiniotis and Oldham, 1994). The soluble organic products of hydrolysis and acidification can be used as energy and carbon sources for biological nutrients removal (i.e. tertiary treatment of wastewater) (Barnard, 1983). Some investigators had indicated that pH 5.5 could inhibit the methanogens and produce only organic acids (Elefsiniotis et al., 1996; Fang and Yu, 2002). Until recently, a few investigators found that pH 10.0 could also inhibit methanogens and produce more VFA than pH 5.5 (Chen et al., 2007; Yuan et al., 2006). If proven technically robust, hydrolysis and acidification at pH 10.0 could be part of an appropriate approach for tertiary treatment in wastewater treatment plant (WWTP).

Understanding the underlying mechanism of the VFA improvement occurring at pH 10.0 will help to achieve a better control over the hydrolysis and acidification processes and

promote the processes as part of an appropriate solution to tertiary treatment in WWTP. Yuan et al. (2006) found that the enzyme activities of the un-autoclaved sludge were higher than those of the autoclaved sludge during hydrolysis and acidification at pH 10.0. Based on these results, they believed that the improvement mechanism should be biotic effects rather than abiotic effects (i.e. alkaline hydrolysis). However, since no experiment was conducted to indicate that the enzyme activities at pH 10.0 were higher than those at pH 5.5, the aforementioned results could only illustrate the fact that biotic effects played a role to certain extent rather than predominantly, as regards VFA improvement.

In this study, a well-defined fractioning structure of sludge flocs (Yu et al., 2008), composed of slime, loosely bound extracellular polymeric substances (LB-EPS), tightly bound EPS (TB-EPS), and pellet, was applied to address the variations and distributions of enzyme activities and organic matters among them. The hydrolysis of EPS and/or cells within sludge flocs, conducted by extracellular enzymes, was believed to limit the rate and extent of sludge biodegradation (Higgins and Novak, 1997). Since EPS represent the major organic fraction and determine the structure, integrity and strength of sludge flocs, the disruption of EPS matrix can enhance the rate and extent of the hydrolysis and acidification (Park and Novak, 2007). Sludge pretreatment such as mechanical, alkaline and ultrasonic can disintegrate EPS matrix, release the extracellular proteins (PN), polysaccharides (PS), and enzymes from TB-EPS and pellet fractions to slime or LB-EPS fractions, and subsequently enhance the effective contact among them (Yu et al., 2008). Therefore, it is surmised that as a tactic of sludge pretreatment, the alkaline pretreatment may break EPS matrix and improve the efficacy of hydrolysis and acidification.

It is surmised that at pH 10.0, the distribution patterns of extracellular PN, PS and enzymes in sludge flocs should be different from pH 5.5 during fermentation. This difference would further affect the rate and mechanism of hydrolysis. To our best knowledge, no previous work has been done on the effects of the distribution patterns on hydrolysis and acidification yet. The purposes of this work were to systematically evaluate the fate of extracellular PN, PS and enzymes in the different fractions of sludge flocs, so as to develop the underlying mechanism of VFA improvement at pH 10.0. Variation of the particle size distribution (PSD) of sludge flocs with time was also considered. Protease and α-amylase were reported to play essential roles in the hydrolysis of two major fractions of EPS: PN and PS (Goel et al., 1998). Alkaline phosphatase hydrolyzed phosphomonoesters provide an alternative source of phosphorus for the cells, while acid phosphatase was reported to be involved in internal cell metabolism (Kloeke and Geesey, 1999). Therefore, the four extracellular enzymes were selected for this study.

2. Materials and methods

2.1. Sludge samples

Activated 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 samples were transported to laboratory within 2 h after sampling. The sludge was first settled for 1.5 h at 4 °C. The sludge sediments were collected and screened through a 1.2 mm screen with characteristics listed in Table 1. As shown in Table 1, soluble organic matter in sludge accounted for approximately only 1%. Meanwhile, PN and PS were the two major types of organic components in sludge, accounting for 66.7% and 9.6% of volatile suspended solids (VSS), respectively.

2.2. Hydrolysis and acidification experiments

Four laboratory-scaled, batch–continuous stirred tank reactors (CSTR), having a working volume of 1.0 L for each, were operated at 100 revolutions per minute (rpm) through a shaking cultivating chamber (SPX-2500-Z-S, Shanghai Yuejin Medical Instruments Factory, China); the pH was adjusted to 5.5 or 10.0 every day by adding 2 mol L^{-1} HCl or 2 mol L^{-1} NaOH, respectively. Two of the reactors were maintained at 37 °C, while the other two were maintained at 55 °C by the shaking cultivating chamber. The four reactors were flushed with nitrogen gas (N_2) for 2 min before being sealed. After start-up, sludge was sampled every 5 days.

2.3. Fractioning protocol

Sludge fractioning protocol was modified according to the method previously described by Yu et al. (2007, 2008). In brief, the sludge sediments (Table 1) were centrifuged at 2000 g for 15 min. The bulk solution was collected as the slime, representing the part able to be removed by soft centrifugation. The collected bottom sediments were re-suspended to their original volumes using a pH 7 buffer solution consisting of Na_3PO_4, NaH_2PO_4, NaCl and KCl (Frølund et al., 1995). The molar ratios of these chemical constituents were 2:4:9:1. The conductivities of the buffers were then adjusted with distilled water to those of the sludge sediment samples. The suspensions were centrifuged again at 5000 g for 15 min with the bulk solution and the solid phase collected separately. The organic matter in the bulk solution was the LB-EPS. The collected sediments were re-suspended again with the aforementioned buffer solution to the original volumes and then extracted using ultrasound at 20 kHz and 480 W for 10 min. The extracted solutions were centrifuged at 20,000 g

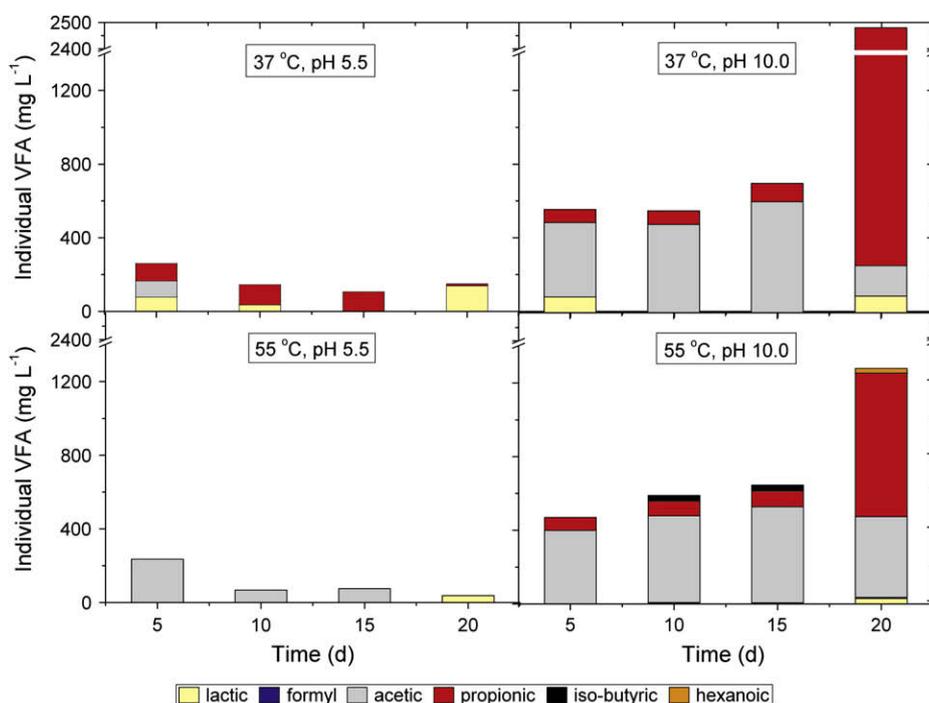| Table 1 – Characteristics of sludge sediment sample			
Parameter	Content	Parameter	Content
pH	6.4 ± 0.1	SCOD (mg L^{-1})	94 ± 5
TSS (mg L^{-1})	7075 ± 375	Proteins (mg eq casein L^{-1})	4460 ± 293
VSS (mg L^{-1})	6683 ± 50	Polysaccharides (mg eq glucose L^{-1})	639 ± 42
COD (mg L^{-1})	9105 ± 378	Conductivity ($\mu S\ cm^{-1}$)	331 ± 14

Fig. 1 – Individual VFA production with time in different reactors.

for 20 min. The organic matter in the bulk solution was the TB-EPS, while the residues (solid phase) re-suspended again with the aforementioned buffer solution to the original volumes were the pellet. 0.45 μm polytetrafluoroethylene (PTFE) membranes (Shanghai Mosu Scientific Equipment Co., China) were used to remove the particulates present in the supernatant, slime, LB-EPS and TB-EPS solutions.

2.4. Analytical techniques

Both the chemical analyses and enzymatic assays were carried out in triplicate. Protease activity was analyzed using Lowry et al.'s (1951) method with casein as the standard. The α-amylase activity was determined using Bernfeld's (1955) method and glucose was the standard. Alkaline phosphatase and acid phosphatase activities were performed according to Goel et al. (1998) with p-nitrophenyl phosphate disodium salt (Sigma N 3254) as the standard.

PSD assay of the sludge flocs 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 pipette and measured in duplicate. The average particle size of the sludge flocs was given as the mean based on the number equivalent diameter (D) [1, 0] (Ankersmid, 2006).

All chemical analyses were carried out using chemicals of analytical grade. PN was determined by the modified Lowry method (Frølund et al., 1995), using casein (Shanghai Sangon Biotechnology Co., Ltd, China) as the standard. PS was measured by the Anthrone method (Gaudy, 1962), with glucose as the standard. The COD of the filtrate was referred to

as SCOD. The SCOD analyses were done using HACH DR/2000 Spectrometer. 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) (Zhang et al., 2008). Other sludge parameters, including total suspended solids (TSS) and VSS, were analyzed following the standard methods (APHA et al., 1998).

3. Results and discussion

3.1. Improvement of VFA production at pH 10.0

Fig. 1 illustrates the production and components of VFA under different temperature and pH conditions. It was noted that the total VFA production was always pronouncedly higher, from 2 to 34 times, at pH 10.0 than that at pH 5.5, regardless of the mesophilic or thermophilic reactors. This result was consistent with that of Yuan et al. (2006), supporting the idea that the VFA production in hydrolysis and acidification could be markedly improved at pH 10.0. Additionally, this result suggested that the pH had a more important influence on VFA production than temperature. Fang and Yu (2001) also indicated that the VFA production was sensitive to pH rather than to temperature.

The total VFA production in the mesophilic reactor was slightly higher than that in the thermophilic reactor at the same pH (Fig. 1). The increases of total VFA production with time were observed at pH 10.0. In contrast, the total VFA production decreased with time at pH 5.5 (Fig. 1).

At pH 5.5, VFA components in the mesophilic reactor were more diverse than those in the thermophilic reactor, whereas at pH 10.0 they were less than in the thermophilic reactor. In addition, lactic acid in the mesophilic reactor was markedly higher than that in the thermophilic reactor. During the first 15 days, acetic acid was the main acidification product, accounting for 72%–100%, except in the reactor at 37 °C and pH 5.5 where propionic acid was the major acidification product. The next important VFA species was propionic acid ranging 7%–100% except in the reactor at 55 °C and pH 5.5, which produced only acetic acid. At 20th day, lactic acid was the predominant acidification product at pH 5.5, while propionic acid was the major acidification product at pH 10.0.

VFA production was significantly enhanced and maintained stable at pH 10.0 (Fig. 1). Also, an obvious VFA consumption was observed at pH 5.5. These results were consistent with those of Yuan et al. (2006).

3.2. Decline of gas production at pH 10.0

Gas production and components were strongly influenced by pH (Fang and Yu, 2001). In this study, neither methane nor carbon dioxide was monitored at pH 10.0. Meanwhile, only after 5 days some gas production was measured at pH 5.5 (data

not shown). These results suggested that pH 10.0 could block the methane generation pathway. Yuan et al. (2006) observed that there was no methane production at pH 10.0. Therefore, pH 10.0 was favorable for acidogenic bacteria and meanwhile repressing for methanogens.

3.3. Biotic effect of VFA improvement

Enzymes play a crucial role in the biological processes (Teuber and Brodisch, 1977). Measurement of enzymes is an alternative method to assess microbial biomass and activity (Nybroe et al., 1992). Fig. 2 depicts the activity of protease, α-amylase, alkaline phosphatase and acid phosphatase in different fractions of sludge flocs. It was found that in the raw sludge flocs, protease and acid phosphatase were mainly distributed in the pellet fraction, i.e. bound with cells, less distributed in the TB-EPS fraction, and almost no in the LB-EPS and slime fractions. However, α-amylase was almost uniformly distributed in the different fractions of sludge flocs. As for alkaline phosphatase, it was mainly distributed in the pellet and TB-EPS fractions, few in the LB-EPS and slime fractions. The results of enzyme distributions in sludge flocs were consistent with the previous observation (Yu et al., 2008).
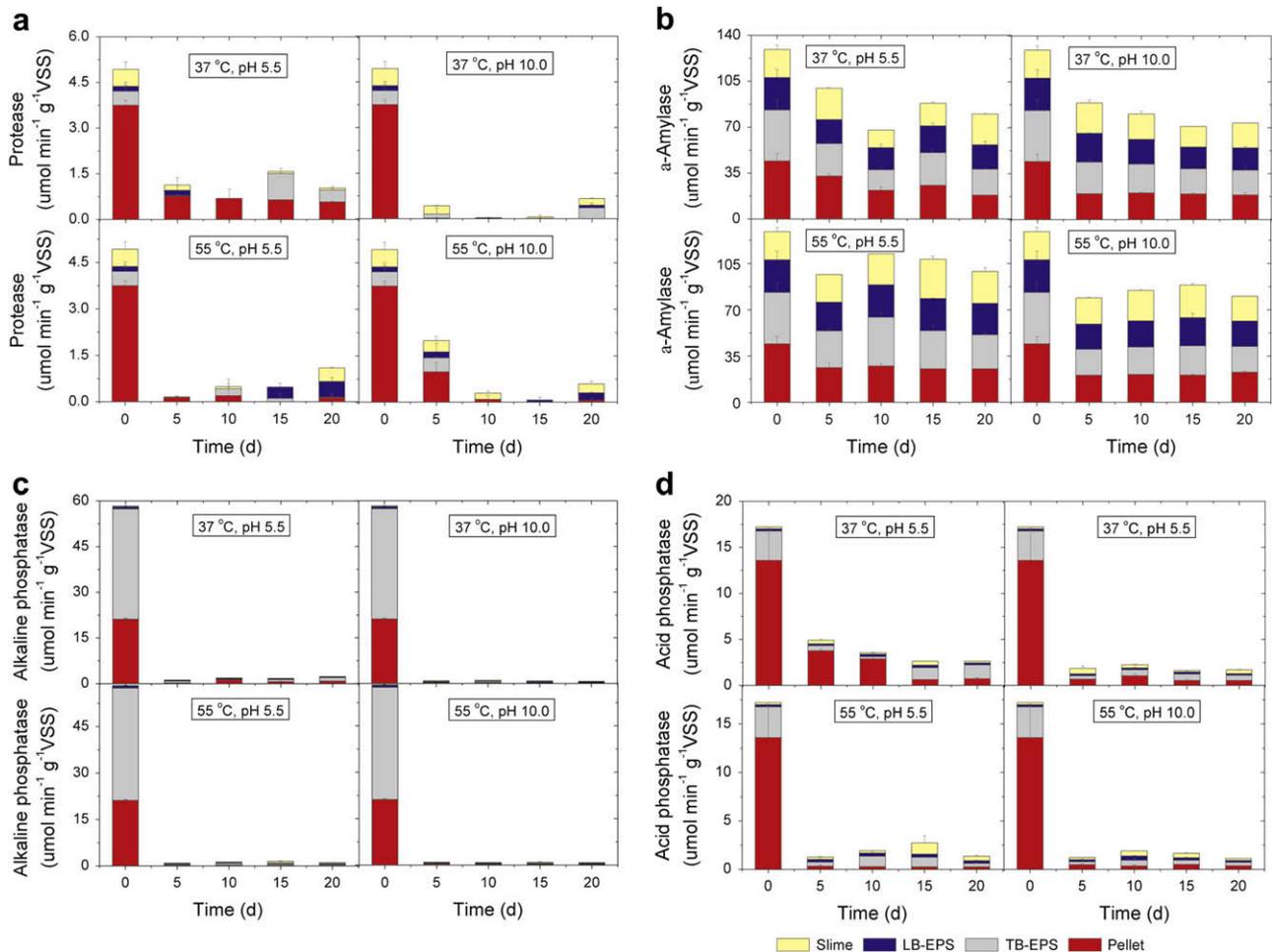

Fig. 2 – Enzyme activities with time in different reactors. (a) protease, (b) α-amylase, (c) alkaline phosphatase, (d) acid phosphatase. Error bars represent the standard deviation of triplicate samples.

The total activities (slime + LB-EPS + TB-EPS + pellet) of enzymes in sludge flocs decreased with time, regardless of pH and temperature. It was noted that the total enzyme activities at pH 5.5 were higher than those at pH 10.0 (Fig. 2). As for the pellet fraction, enzyme activities decreased much more than the other fractions (Fig. 2). The particularly interesting point was the fact that the protease and acid phosphatase activities in the slime fraction apparently increased with time and were even higher than those in the pellet fraction at 20th day, 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 sludge flocs could be attributed to the degradation of extracellular polymers (mainly PN) and subsequently to the breakage of sludge matrix.

The data clearly demonstrated that the enzyme activities at pH 10.0 were lower than those at pH 5.5, suggesting that the biotic effect was not the leading reason of the VFA improvement at pH 10.0. However, there was also a considerable quantity of enzyme activities in sludge flocs at pH 10.0 (Fig. 2). 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.

3.4. Abiotic effect of VFA improvement

Particle sizes and compositions of sludge flocs determine the rate and mechanism of hydrolysis (Morgenroth et al., 2002). Fig. 3 presents the variation of the PSD with time in different reactors. It was clearly shown that at pH 10.0, particle sizes were smaller than at pH 5.5, regardless of the temperature. In the thermophilic reactor, they were smaller than in the mesophilic reactors at the same pH. At 5th, 10th and 15th day,

the order of average particle size in the four reactors followed the same sequence, i.e. reactor at 37 °C and pH 5.5 (13.67 μm) > reactor at 55 °C and pH 5.5 (10.35 μm) > reactor at 37 °C and pH 10.0 (10.22 μm) > reactor at 55 °C and pH 10.0 (6.29 μm). However, at 20th day, the average particle size in the reactor at 55 °C and pH 5.5 was bigger than that in the reactor at 37 °C and pH 5.5.

Decrease of particle size could also be characterized by the variations of soluble organic matters. Fig. 4 shows the release of soluble organic matters and decrease of VSS in sludge flocs. SCOD was low in raw sludge, corresponding to 94 mg L^{-1}, while at 5th day, it climbed rapidly to 1200–2000 mg L^{-1} and 2200–2700 mg L^{-1} at pH 5.5 and 10.0, respectively. Afterwards, at 10th day, it approached the plateau value of 2100–2500 mg L^{-1} and 4400–4700 mg L^{-1} at pH 5.5 and 10.0, respectively. SCOD in the reactors at different temperature but of same pH had no marked differences, indicating that compared with pH, temperature had a slight influence on sludge solubilization. In addition, the decrease of VSS had a similar trend with the release of hydrolysis product (i.e. SCOD). Vlyssides and Karlis (2004) examined the variation of SCOD production and VSS reduction in the anaerobic digestion with alkaline pretreatment and showed that the SCOD production had a good balance with VSS reduction. In their investigations, the SCOD production and VSS reduction had a similar trend with our results reported in this paper. Meanwhile, Cokgor et al. (2009) also showed the similar ratio of SCOD production and VSS reduction with our results.

PN and PS are predominant in sludge flocs and represent most of SCOD (Yu et al., 2007, 2008; Yuan et al., 2006). More important, they are directly related to the production of VFA (Yuan et al., 2006). The PN variations with time in different EPS fractions revealed that during hydrolysis and acidification, PN was transferred from the inner fraction (i.e. TB-EPS) to the
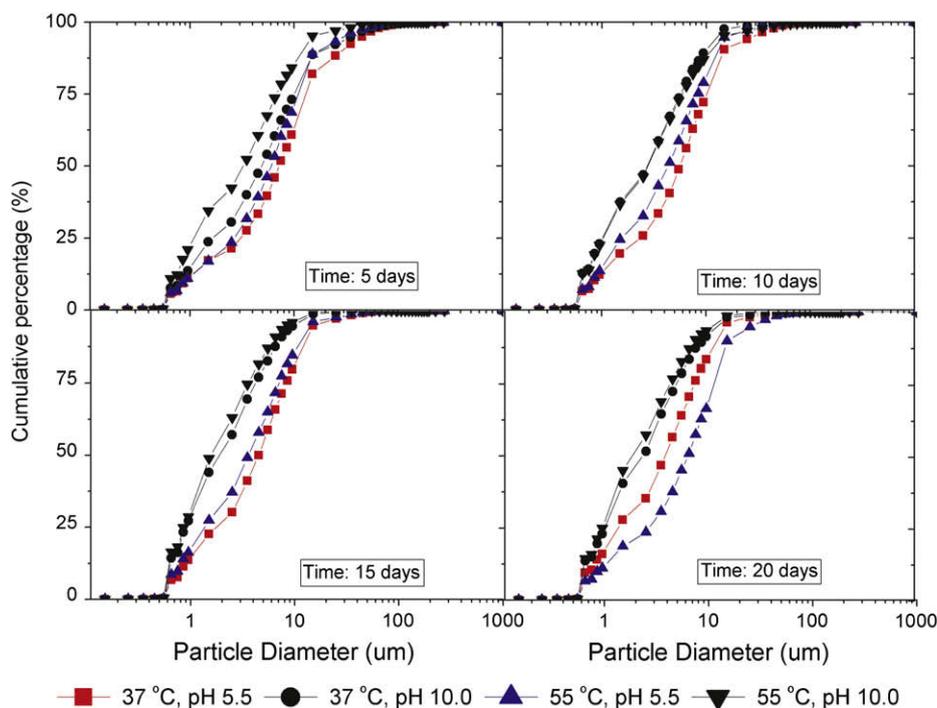

Fig. 3 – Particle size distribution with time based on number in different reactors.
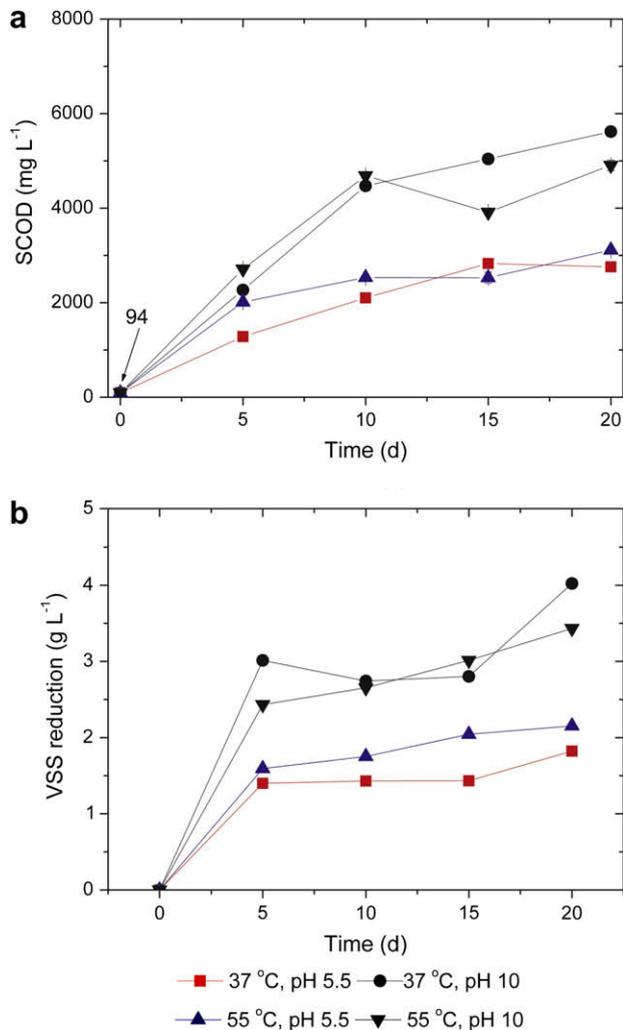
Fig. 4 – SCOD production and VSS reduction with time in different reactors. (a) SCOD production. (b) VSS reduction. Error bars represent the standard deviation of triplicate samples.

outer fraction (i.e. slime or LB-EPS) which was available to microbes. As for PS, it had a slight variation with time in different EPS fractions.

When combining the trends of protease, PN, PSD and SCOD in sludge flocs (Figs. 2–5), it could be concluded that the intensive PN degradation at pH 10.0 should be mainly attributed to the alkaline solubilization rather than to the biotic effect. Meanwhile, it was noted that PN in the slime fraction increased with time, suggesting that the transfer of PN from the TB-EPS fraction to the slime fraction was concomitant with the degradation of PN leading to VFA production. As a result, SCOD increased with time (Fig. 4). However, PS was almost uniformly distributed in sludge flocs and changed little with time.

3.5. Biotic versus abiotic mechanism

Soluble organic matters are easily degradable and available to microbes. However, the hydrolysis rate of particulate organic matter is determined by effective adsorption of hydrolytic

enzymes to biodegradable surface sites (Veeken and Hamelers, 1999; Yu et al., 2003). In raw sludge flocs, the particulate organic matters were predominant, accounting for about 99% (Table 1). When sludge flocs are treated by alkaline, the different EPS fractions are stripping from themselves due to the bond breakage of PN and PS. As a result, an amount of extracellular enzymes and organic matters originally embedded in the pellet fraction is released into the solution (Figs. 2, 3 and 5). Then, the effective contact between soluble organic matters and soluble extracellular enzymes increases, and subsequently improves the VFA production. The experimental results from PSD also support this conclusion, because the decrease of particle size with time followed in a step-by-step way rather than fall apart (Fig. 3). Therefore, it seems that the different fractions of sludge flocs were stripped by alkaline with time. The results also suggest that the different EPS fractions play an essential role in maintaining the integrity and stability of spatial structure. Additionally, a transfer phenomenon of extracellular PN, PS and enzymes in different sludge fractions occurred when sludge flocs were treated by alkaline (Figs. 2 and 5).

Since enzyme activities at pH 10.0 were lower than those at pH 5.5 (Fig. 2), high pH might suppress the microbes to produce VFA. However, pH 10.0 could produce more soluble organic matters than pH 5.5 (Fig. 4). Therefore, the VFA improvement may be mainly driven by the quantity of soluble organic matters which were easily degradable and available to the VFA producer. As a result, the VFA is less inhibitive at high pH.

3.6. Implication for tertiary treatment

A growing awareness of the need to control the nutrient emissions had been reflected in the increasingly stringent regulations (Oehmen et al., 2007). 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. Alkaline treatment could enhance the production of VFA during the hydrolysis of sludge (Chen et al., 2007; Kim et al., 2007; Yuan et al., 2006). In addition, alkaline treatment approach had been used in Belgium (Neyens et al., 2003). In this study, our results indicated that hydrolysis and acidification processes at pH 10.0 are able to greatly improve the VFA production. Additionally, since the technology relies on local materials and renewable resources, it can contribute to the headway toward sustainable water treatment technologies. Therefore, the process can be considered as an eco-friendly material recycling process and as part of an appropriate solution for tertiary treatment. Meanwhile, it may further affect the implementation of sludge management strategy.

Tong and Chen (2007) had shown that no inhibitory effect was observed when the fermentation liquid produced under alkaline conditions was added to the tertiary treatment process. On the other hand, Ang and Elimelech (2008) showed that the effluent including high VFA at high pH could decline the rate of membrane flux. Therefore, further investigation needs to be conducted on the possible risk of VFA applied to the tertiary treatment.
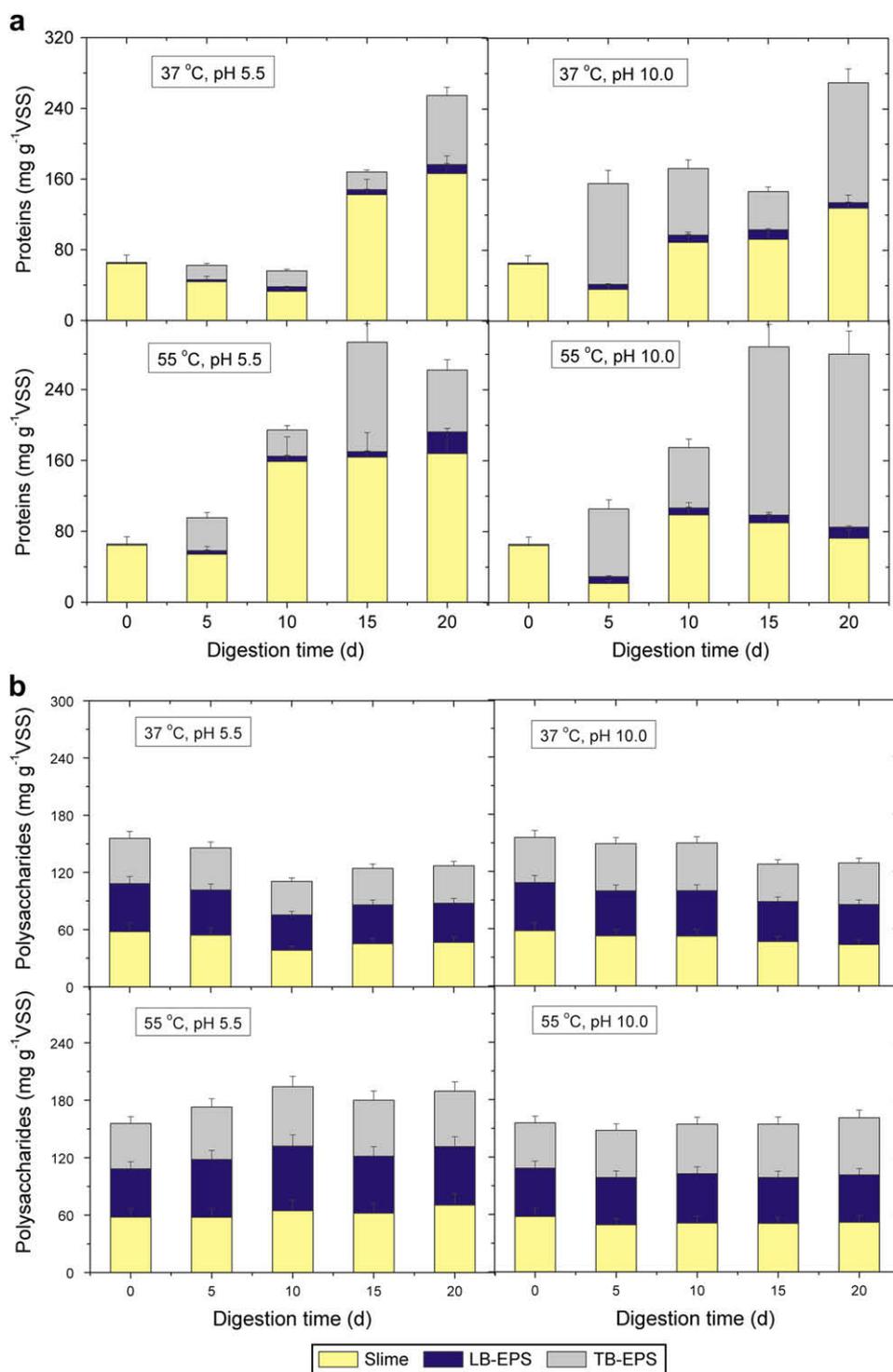
Fig. 5 – Chemical compositions with time in different reactors. (a) proteins. (b) polysaccharides. Error bars represent the standard deviation of triplicate samples.

4. Conclusions

During 20 days of fermentation, the total VFA production at pH 10.0 was markedly higher, from 2 to 34 times, than that at pH 5.5. Meanwhile, gas production was negligible at pH 10.0. The results of enzyme activities (i.e. protease, α-amylase,

alkaline phosphatase and acid phosphatase) in all fractions of sludge flocs strongly suggested that the biotic effect was not the leading cause of the VFA improvement. Further investigation suggested that pH 10.0 could significantly improve the VFA production through the break of sludge matrix which is usually hydrolyzed by the extracellular enzymes embedded in itself, increase the effective contact between extracellular

organic matters and enzymes, and create a favorable environment for microbes to accumulate VFA. Since hydrolysis and acidification processes at pH 10.0 rely on local materials and renewable resources, it can contribute to the headway toward sustainable water treatment technologies. Therefore, the process can be considered as an eco-friendly material recycling process and as part of an appropriate solution for tertiary treatment.

REFERENCES

Ang, W.S., Elimelech, M., 2008. Fatty acid fouling of reverse osmosis membranes: implications for wastewater reclamation. Water Res. 42 (16) 4393–4403.

Ankersmid, 2006. http://ankersmid.instrument.com.cn.

APHA, AWWA, WEF, 1998. Standard Methods for the Examination of Water and Wastewater, 20th ed. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC, USA.

Barnard, J.L., 1983. Background to biological phosphorus removal. Water Sci. Technol. 15 (3–4), 1–13.

Bernfeld, O., 1955. Amylases, alpha and beta. In: Colowick, S.O., Kaplan, N.O. (Eds.), Methods in Enzymology. Academic Press Inc Publishers, New York, pp. 149–158.

Chen, Y.G., Jiang, S., Yuan, H.Y., Zhou, Q., Gu, G.W., 2007. Hydrolysis and acidification of waste activated sludge at different pHs. Water Res. 41 (3), 683–689.

Cokgor, E.U., Oktay, S., Tas, D.O., Zengin, G.E., Orhon, D., 2009. Influence of pH and temperature on soluble substrate generation with primary sludge fermentation. Bioresour. Technol. 100 (1), 380–386.

Elefsiniotis, P., Oldham, W.K., 1994. Anaerobic acidogenesis of primary sludge: the role of solids retention time. Biotechnol. Bioeng. 44 (1), 7–13.

Elefsiniotis, P., Wareham, D.G., Oldham, W.K., 1996. Particulate organic carbon solubilization in an acid-phase upflow anaerobic sludge blanket system. Environ. Sci. Technol. 30 (5), 1508–1514.

Fang, H.H.P., Yu, H.Q., 2001. Acidification of lactose in wastewater. J. Environ. Eng. 127 (9), 825–831.

Fang, H.H.P., Yu, H.Q., 2002. Mesophilic acidification of gelatinaceous wastewater. J. Biotechnol. 93 (2), 99–108.

Frølund, B., Keiding, K., Nielsen, P., 1995. Enzymatic activity in the activated sludge flocs matrix. Appl. Microbiol. Biotechnol. 43 (4), 755–761.

Gaudy, A.F., 1962. Colorimetric determination of protein and carbohydrate. Ind. Water Wastes 7, 17–22.

Goel, R., Mino, T., Satoh, H., Matsup, T., 1998. Enzyme activities under anaerobic and aerobic conditions in activated sludge sequencing batch reactor. Water Res. 32 (7), 2081–2088.

Higgins, M.J., Novak, J.T., 1997. Characterization of exocellular protein and its role in bioflocculation. J. Environ. Eng. 123 (3), 479–485.

Kim, T.H., Kim, T.H., Yu, S., Nam, Y.K., Choi, D.K., Lee, S.R., Lee, M. J., 2007. J. Ind. Eng. Chem. 13 (7), 1149–1153.

Kloeke, F.V., Geesey, G.G., 1999. Localization and identification of populations of phosphatase-active bacterial cells associated with activated sludge flocs. Microb. Ecol. 38 (3), 201–214.

Lowry, O.H., Rosebrough, N.J., Farn, A., Randall, R., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.

Morgenroth, E., Kommedal, R., Harremoes, P., 2002. Processes and modeling of hydrolysis of particulate organic matter in aerobic wastewater treatment – a review. Water Sci. Technol. 45 (6), 25–40.

Neyens, E., Baeyens, J., Creemers, C., 2003. Alkaline thermal sludge hydrolysis. J. Hazard Mater. 97, 295–314.

Nybroe, O., Jorgensen, P.E., Henze, M., 1992. Enzyme activities in waste water and activated sludge. Water Res. 26 (5), 579–584.

Oehmen, A., Lemos, P.C., Carvalho, G., Yuan, Z., Keller, J., Blackall, L.L., Reis, M.A.M., 2007. Advances in enhanced biological phosphorus removal: from micro to macro scale. Water Res. 41 (11), 2271–2300.

Park, C., Novak, J.T., 2007. Characterization of activated sludge exocellular polymers using several cation-associated extraction methods. Water Res. 41 (8), 1679–1688.

Teuber, M., Brodisch, K.E.U., 1977. Enzymatic activities of activated sludge. Eur. J. Appl. Microbiol. 4 (3), 185–194.

Tong, J., Chen, Y.G., 2007. Enhanced biological phosphorus removal driven by short-chain fatty acids produced from waste activated sludge alkaline fermentation. Environ. Sci. Technol. 41 (20), 7126–7130.

Veeken, A., Hamelers, B., 1999. Effect of temperature on hydrolysis rates of selected biowaste components. Bioresour. Technol. 69 (3), 249–254.

Vlyssides, A.G., Karlis, P.K., 2004. Thermal-alkaline solubilization of waste activated sludge as a pre-treatment stage for anaerobic digestion. Bioresour. Technol. 91 (2), 201–206.

Yuan, H.Y., Chen, Y.G., Zhang, H.X., Jiang, S., Zhou, Q., Gu, G.W., 2006. Improved bioproduction of short-chain fatty acids (SCFAs) from excess sludge under alkaline conditions. Environ. Sci. Technol. 40 (6), 2025–2029.

Yu, H.Q., Zheng, X.J., Hu, Z.H., Gu, G.W., 2003. High-rate anaerobic hydrolysis and acidogenesis of sewage sludge in a modified upflow reactor. Water Sci. Technol. 48 (4), 69–75.

Yu, G.H., He, P.J., Shao, L.M., Lee, D.J., 2007. Enzyme activities in activated sludge flocs. Appl. Microbiol. Biotechnol. 77 (3), 605–612.

Yu, G.H., He, P.J., Shao, L.M., Zhu, Y.S., 2008. Extracellular proteins, polysaccharides and enzymes impact on sludge aerobic digestion after ultrasonic pretreatment. Water Res. 42 (8–9), 1925–1934.

Zhang, H.H., He, P.J., Shao, L.M., 2008. Methane emissions from MSW landfill with sandy soil covers under leachate recirculation and subsurface irrigation. Atmos. Environ. 42 (22), 5579–5588.