Chemosphere 144 (2016) 160–167

Contents lists available at ScienceDirect

Chemosphere

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

An efficient and green pretreatment to stimulate short-chain fatty acids production from waste activated sludge anaerobic fermentation using free nitrous acid

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HIGHLIGHTS

- SCFA production was enhanced greatly by FNA pretreatment.
- FNA pretreatment could accelerate EPS and cell envelop disruption.
- FNA pretreatment favored solubilization, hydrolysis and acidification processes.
- FNA pretreatment inhibited methanogenesis process.

ARTICLE INFO

Article history:
Received 10 June 2015
Received in revised form
19 August 2015
Accepted 28 August 2015
Available online xxx

Keywords:
Short-chain fatty acid
Free nitrous acid pretreatment
Waste activated sludge
Anaerobic fermentation

ABSTRACT

Short-chain fatty acid (SCFA) production from waste activated sludge (WAS) anaerobic fermentation is often limited by the slow hydrolysis rate and poor substrate availability, thus a long fermentation time is required. This paper reports a new pretreatment approach, i.e., using free nitrous acid (FNA) to pretreat sludge, for significantly enhanced SCFA production. Experimental results showed the highest SCFA production occurred at 1.8 mg FNA/L with time of day 6, which was 3.7-fold of the blank at fermentation time of day 12. Mechanism studies revealed that FNA pretreatment accelerated disruption of both extracellular polymeric substances and cell envelope. It was also found that FNA pretreatment benefited hydrolysis and acidification processes but inhibited the activities of methanogens, thereby promoting the yield of SCFA. In addition, the FNA pretreatment substantially stimulated the activities of key enzymes responsible for hydrolysis and acidification, which were consistent with the improvement of solubilization, hydrolysis and acidification of WAS anaerobic fermentation.

1. Introduction

Biological nutrient removal (BNR) is an effective technique to mitigate the eutrophication of natural water body (Li et al., 2014; Wang et al., 2012). Nowadays the influent of many municipal wastewater treatment plants (WWTPs) in China, especially in the south of China, is characterized by low organic carbon concentration (Chen et al., 2013a), which limits the performance of BNR. In order to enhance the nutrients removal, methanol and acetic acid are usually used as additional organic carbon source during the wastewater treatment process (Wang et al., 2013a). However, the addition of organic chemicals substantially increases a financial burden for wastewater treatment. Meanwhile, large quantities of waste activated sludge (WAS), as a byproduct of biological wastewater treatment, are inevitably produced (Ni and Yu, 2008a, 2008b). The treatment and disposal of WAS can be up to 60% of the overall costs in WWTPs (Canales et al., 1994). WAS contains high levels of organic substances (such as proteins and polysaccharides), thus it is a plentiful inexpensive source for organic carbon (such as short-chain fatty acid, SCFA) through anaerobic fermentation process (Yuan et al., 2011; Zhang et al., 2012), by

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http://dx.doi.org/10.1016/j.chemosphere.2015.08.076
0045-6535/

which the reduction and recycling of organic wastes are achieved simultaneously (Lee et al., 2014).

It is known that four steps are involved anaerobic sludge fermentation, e.g., solubilization, hydrolysis, acidification, and methanogenesis (Zhao et al., 2010; Chen et al., 2013b). SCFA is generated in acidification step and then consumed in the subsequent methanogenic phase. To make SCFA production maximization, two strategies can be adopted, e.g., accelerating the former three steps and inhibiting the last step (Yuan et al., 2006). Therefore, numerous techniques including chemical, mechanical, thermal and enzymatic, have been applied to improve sludge hydrolysis rate and/or inhibit the activities of methanogens (Lee et al., 2014; Carrère et al., 2010; Zhao et al., 2015a). Although hydrolysis rate and/or the activities of methanogens inhibition can be remarkably improved by those techniques, intensive energy inputs (such as high pressure and/or temperature) or substantial chemicals consumption (such as chlorine, ozone, or alkali) are required (Chu et al., 2008; Cheng et al., 2012; Pijuan et al., 2012), which might have difficulties in application. Also, those approaches are not environmentally friendly. Thus, a cost-effective, efficient and green pretreatment technique for improving SCFA production from WAS is urgently needed.

Recently, free nitrous acid (FNA or HNO_2), protonated form of nitrite, has been demonstrated to be a strong biocidal agent for anaerobic sewer biofilms and microorganisms residing in anaerobic wastewater biofilms and WAS (Pijuan et al., 2012; Jiang et al., 2011; Zhao et al., 2015b). FNA can contribute to the damage of lipids, proteins, carbohydrates and deoxyribonucleic acid in cells/extracellular polymeric substances (EPS) by reacting with them (Yoon et al., 2006; Halliwell et al., 1992). In a recent study, Wang et al. (2014a) demonstrated that both the hydrolysis rate and biochemical methane potential of WAS were greatly enhanced when WAS was pretreated with 2.13 mg/L of FNA for 24 h. Compared with other pretreatments, FNA is easily obtained since FNA can be generated as a by-product of wastewater treatment through nitritation of the anaerobic digestion liquor (Wang et al., 2013c). Thus, FNA seems to be a promising pretreatment technique for WAS anaerobic fermentation. To date, however, the effect of FNA pretreatment on SCFA production from sludge anaerobic fermentation has seldom been reported. Although some scientists found sludge particulate release could be increased with FNA treatment (Ma et al., 2015), the effect of FNA pretreatment on SCFA production from WAS has not been well understood yet.

The aim of this paper is to report a cost-effective, efficient and green WAS pretreatment, e.g., using FNA to treat WAS before anaerobic fermentation. Firstly, the optimization of FNA pretreatment time was conducted. Then the effect of different levels of FNA concentration on SCFA production was investigated. Finally, the mechanisms for the improvement of SCFA production from WAS with FNA pretreatment were studied by analyzing the role of FNA on disintegration of both EPS and cell envelope, the effect of FNA on the processes of solubilization, hydrolysis, acidification, and methanogenesis, and activities of key enzymes.

2. Materials and methods

2.1. Sludge source

Raw WAS was collected from the secondary sedimentation tank of a WWTP with sludge retention time of 20 d in Changsha, China. The sludge was settled at 4 °C in a refrigerator for 24 h with the supernatant discarded. To prevent clogging problems, the sludge was screened with a 1 mm sieve to remove impurities prior to be used. The main characteristics (average value plus standard deviation of three tests) of the concentrated WAS were: pH 6.8 ± 0.1; total suspended solids (TSS), 13.4 ± 0.3 g/L; volatile suspended solids (VSS), 10.2 ± 0.2 g/L; soluble chemical oxygen demand (SCOD), 0.18 ± 0.016 g/L; total chemical oxygen demand (TCOD), 15.2 ± 0.3 g/L; total carbohydrate, 1.5 ± 0.054 g/L; total proteins, 9.4 ± 0.13 g/L; NH_4^+–N, 12.3 ± 0.5 mg/L; soluble PO_4^{3-}–P, 20.1 ± 1.2 mg/L.

2.2. Optimization of FNA pretreatment time experiments

In this investigation, batch laboratory-scale anaerobic fermentation experiments in 5 identical anaerobic fermentation reactors were conducted to optimize FNA pretreatment time. The working volume of anaerobic fermentation reactor was 1.0 L each. The treatment time ranging from 6 to 72 h was chosen and 0, 350, 700, 1050, 1400 mg NO_2^-–N/L was applied to batch reactors, respectively. Nitrogen gas was flushed to remove oxygen, all reactors were capped, sealed, and stirred in an air-bath shaker (120 rpm, revolutions per minute) in a 20 ± 1 °C temperature-controlled room, and the pH was maintained at pH 6.0 ± 0.1 by adding 3.0 M hydrochloric acid (HCl) or 3.0 M sodium hydroxide (NaOH). Then, the temperature, pH and NO_2^-–N concentration applied in this investigation gave rise to FNA concentration of 0, 0.9, 1.8, 2.7 and 3.6 mg/L, which was determined by the formula $FNA = S_{NO_2^- - N}/(K_a \times 10^{pH})$ with K_a value determined by the formular $K_a = e^{(-2300/(T+273))}$ for a given temperature T(°C) (Anthonisen et al., 1976).

2.3. Batch fermentation tests

Batch tests were conducted to assess the effect of FNA pretreatment on SCFA production. For FNA pretreatment, different volumes of 2.0 M $NaNO_2$ stock solution were added to the 5 identical batch reactors (working volume of 1 L) in different volumes to achieve the designated nitrite concentrations of 0, 350, 700, 1050, 1400 mg NO_2^-–N/L, respectively. Each FNA pretreatment test lasted for 48 h, during which pH was controlled at 6.0 ± 0.1 by adding 3.0 M HCl or 3.0 M NaOH solution except for the case of 0 mg NO_2^-–N/L, where pH was not controlled, and this reactor served as blank. All the batch tests were conducted in a 20 °C-controlled room. The NO_2^-–N concentration, pH and temperature applied in this study gave rise to FNA concentration of 0, 0.9, 1.8, 2.7 and 3.6 mg/L (Anthonisen et al., 1976). After FNA pretreatment, the initial fermentation pH was adjusted to around 7.0 ± 0.1, and the fermentation pH was not controlled in all reactors. Each anaerobic reactor was flushed with nitrogen gas for 10 min to provide anaerobic condition, and then capped with rubber stoppers, sealed, and placed in an air-bath shaker (120 rpm) for 15 days.

2.4. Elevation of SCFA consumption for denitrification

Batch tests were conducted to assess the SCFA consumption for denitrification. Synthetic wastewater containing 350, 700, 1050 and 1400 mg NO_2^-–N/L were added to different reactors (working volume: 1 L), respectively. Acetate and propionate (the ratio of acetate to propionate was 2:1, the mass ratio was the same as that in the fermentation liquid) were added into those reactors to serve as carbon source with initial 2000 mg COD/L. 400 mL WAS served as inoculated sludge was evenly distributed in those reactors. The other treatment conditions were the same as those described in Section 2.3. The variations of NO_2^-–N/L and SCFA removal were monitored, then the SCFA consumption for denitrification could be calculated.

2.5. Effect of FNA pretreatment on disruption of EPS and cell envelope

EPS and cell envelop are considered as the main substances to

protect the cell from the detrimental environments, and the disruption of EPS and cell envelope are well related with the sludge sobilization rate, thus, it is necessary to investigate the effect of FNA pretreatment on the on disruption of EPS and cell envelope. Four anaerobic fermentation reactors (working volume: 600 mL) were equally divided into two groups. Group-1 is applied to investigate the effect of FNA pretreatment on the disruption of EPS, whereas, the other Group-2 is used to investigate the influence of FNA pretreatment on the disruption of cell envelop.

To investigate the effect of FNA pretreatment on the disruption of EPS, two reactors fed with 600 mL raw WAS were operated and the operational conditions were the same as the parent reactors described above. The carbohydrate, protein, and COD contents released in liquid phase and remained in EPS were determined periodically during WAS treatment. To assess whether other materials (e.g., intracellular substrates) other than EPS were released, COD mass balance was made according to the soluble substrate content and remnant EPS content.

To compare the effect of FNA on disruption of cell envelope, the sludge used in the Group-2 needed to be without EPS. To obtain no or little EPS sludge, EPS was extracted by a heat extraction method (Li and Yang, 2007), which has been demonstrated to extract both loosely and tightly bound EPS from the raw WAS. In order to assess whether the cell envelope was disrupted or not by EPS extraction method, Adenosine-triphosphate analysis were also conducted. Results showed that insignificant release of adenosine-triphosphate was detected compared with the blank test after this extraction process ($p > 0.05$), suggesting the cell envelope was not significantly destroyed by this EPS extraction method (Takahashi et al., 2009). After extraction, the no or little EPS sludge pellet was resuspended in tap water and then divided equally into two serum bottles. The other operational conditions were the same as parent reactors described above.

2.6. Effect of FNA pretreatment on hydrolysis of solubilized sludge organic matter

The effect of FNA pretreatment on the hydrolysis of solubilized sludge particulate organic carbon was performed in two anaerobic reactors with synthetic wastewater containing bovine serum albumin (BSA molecular weight 67,000, model protein compound) and dextran (molecular weight 23,800, model carbohydrate compound). BSA (6.2 g) and dextran (1.4 g) (the mass ratio of bovine serum albumin and dextran was almost the same as that of protein to carbohydrate in raw WAS) were added into 900 mL tap water and 100 mL raw WAS as inoculum was also added with a final sludge concentration of about 1500 mg/L. The initial FNA concentration in one reactor was controlled at 1.8 mg FNA/L and the FNA pretreatment lasted for 2 days by controlling pH, temperature and initial NO_2^-–N concentration. The other was conducted without FNA pretreatment serving as blank. In addition, 40 mM of 2-bromoethanesulfonate was also added to inhibit the activities of methanogens (Xu et al., 2010).

2.7. Effect of FNA pretreatment on acidification of hydrolyzed products

The investigation of FNA pretreatment influence on the acidification process was similar with that in hydrolysis process except that the substrates in synthetic wastewater were replaced by 3.0 g/L L-alanine (model amino acid compound) and 0.5 g/L glucose (model monosaccharide compound), respectively. Based on the model compounds degradation with time in synthetic wastewater, the effect of FNA pretreatment on acidification process was determined.

2.8. Effect of FNA pretreatment on methanogenesis process

To investigate the effect of FNA pretreatment on the methanogenesis process during WAS anaerobic fermentation, fermentation tests with synthetic wastewater containing sodium acetate (NaAc) were conducted. The chemical composition of synthetic wastewater was the same as Zhou et al. (2013). One reactor was treated with 1.8 mg FNA/L, and the other was set as blank. By measuring the cumulative methane yield, the effect of FNA on methanogenesis process was determined.

2.9. Analytical methods

Sludge samples were centrifuged at a speed of 10,000 rpm/min after fermentation, then filtered through a 0.45 μm cellulose nitrate membrane filter and finally stored at 4 °C prior to analysis. The determinations of TSS, VSS, COD, NO_x^-–N, NH_4^+–N, PO_4^{3-}–P were according to our previous study (Zhao et al., 2015b).

Carbohydrate was measured according to the Anthrone method with glucose as the standard reference, and proteins was determined by the modified Lowry method with BSA as the standard reference (Herbert et al., 1971; Lowry et al., 1951). The COD conversion coefficients are 1.5 g COD/g protein and 1.06 g COD/g carbohydrate (Wang et al., 2015). The measurements of soluble COD (SCOD), SCFA and key enzymes activities were the same as described in the literature (Wang et al., 2013b; Feng et al., 2009). A gas chromatography (GC) was utilized to analyze the composition of SCFA, the total SCFA was calculated as the sum of the determined acetic (HAc), propionic (HPr), n-butyric (n-HBu), iso-butyric (i-HBu), n-valeric (n-HVa) and iso-valeric (i-HVa) acids (Zhou et al., 2014).

2.10. Statistical analysis

All experiments were performed in triplicate. An analysis of variance was used to assess the significance of results, and $p < 0.05$ was considered to be statistically significant.

3. Results and discussion

3.1. Effect of FNA concentration on SCFA production and composition from WAS anaerobic fermentation

Pretreatment time is considered as an important factor for anaerobic fermentation, thus, the optimal FNA pretreatment time for SCFA accumulation was first determined. Figure S1 (Supporting information) shows the variations of SCOD with pretreatment time in the presence of FNA. It was found SCOD substantially increases with time in the range of 0–48 h ($p < 0.05$), however, further increasing pretreatment time gave little benefit to SCOD increase, thus, the optimal FNA pretreatment time was defined to be 48 h.

It should be noted that temperature and pH are the important factors affecting SCFA production from WAS anaerobic fermentation. In this study, temperature was maintained at 20 °C, thus its influence can be eliminated. pH variations were also monitored (Figure S2, Supporting information). It was found that pH change in the FNA pretreated reactors was insignificant ($p > 0.05$) as compared with that in blank. Therefore, the difference of SCFA accumulation in each reactor was mainly caused by FNA pretreatment.

The effect of FNA concentration on the net SCFA generation with fermentation time is illustrated in Fig. 1a. As shown in Fig. 1a, SCFA production increased greatly with time extension to day 6 and then decreased with further increasing fermentation time to day 15 in the presence of FNA. The maximum production of SCFA was

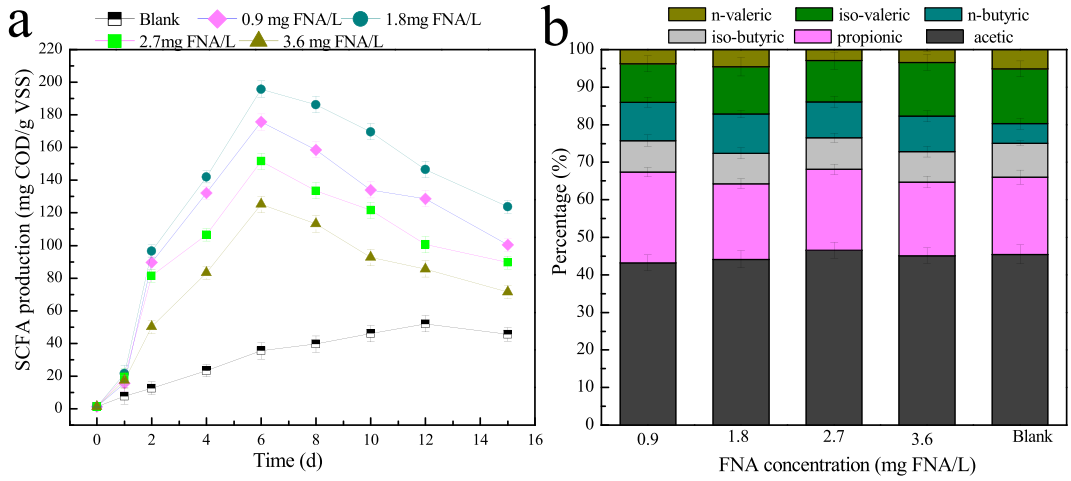

Fig. 1. Effect of different FNA concentration on average SCFA production (a) and the fraction of individual SCFA under their optimal fermentation time during sludge fermentation (b). Error bars represent standard deviations of triplicate tests.

respectively 170.6, 195.7, 151.6 and 125.2 mg COD/g VSS in WAS fermentation reactors with 0.9, 1.8, 2.7 and 3.6 mg FNA/L. However, the maximum SCFA production in blank was 52.0 mg COD/g VSS at the time of day 12. Obviously, the maximum SCFA production in the presence of 1.8 mg FNA/L was 3.7 times higher than that in blank. It should be noted that further increase of FNA concentration over 1.8 mg FNA/L gave a negative effect on SCFA accumulation. For example, when the FNA concentration was 3.6 mg FNA/L, the maximum SCFA production was only 125.2 mg COD/g VSS, which was much lower than that in the reactor with 1.8 mg FNA/L. The negative effect of high FNA concentration on SCFA accumulation might be ascribed to the toxic effect on enzymes responsible for hydrolysis and acidogenesis (Pijuan et al., 2012; Wang et al., 2014a).

The time required for the maximum SCFA production with FNA pretreatment was only 6 days, whereas, the corresponding time was 12 days in blank. It should be emphasized that the SCFA produced in blank at time of day 6 was only 30.5 mg COD/g VSS, which was approximately 1/6 of that produced in the reactor with 1.8 mg FNA/L pretreatment. As seen in Table S1 (Supporting information), NO_2^-–N reduction was observed in all nitrite added reactors. For example, the NO_2^-–N concentration in the 1.8 mg FNA/L pretreated reactor was only 10.3 mg/L at the fermentation time of day 15. It was reported that nitrite can be removed via denitrification during WAS fermentation process (Ma et al., 2015). In order to assess the SCFA consumption for denitrification in the parent reactor, batch tests with synthetic wastewater were conducted and the results were displayed in Table S2 (Supporting information). It was found great SCFAs were used for denitrification, and the SCFA consumptions were 642, 1035, 1186 and 1474 mg COD/L in the 0.9, 1.8, 2.7 and 3.6 mg FNA/L reactors on day 6, respectively. Obviously, even if the SCFA consumption is considered, the highest SCFA generation was also occurred at 1.8 mg FNA/L.

It has been reported that the composition of SCFA in wastewater may have great influence on the activities of microorganisms and thereby affecting the performance of biological nutrient removal (Feng et al., 2009). During WAS anaerobic fermentation, six individual SCFAs (acetic, propionic, n-butyric, iso-butyric, n-valeric and iso-valeric acids) were measured under the optimal conditions and the results were shown in Fig. 1b. It can be seen from Fig. 1b, acetic acid was the most abundant individual SCFA, and propionic acid ranked the second in all FNA pretreated reactors during WAS anaerobic fermentation. Further study showed that there was no significant difference in the percentage of individual SCFA among those reactors ($p > 0.05$). All the above results revealed the FNA pretreatment could not only enhance SCFA production but also greatly accelerate SCFA accumulation rate from WAS anaerobic fermentation. Therefore, it is necessary to investigate the mechanisms for the enhanced SCFA production efficiency and rate with FNA pretreatment from WAS anaerobic fermentation.

3.2. Mechanism investigation for improved SCFA production from WAS with FNA pretreatment

3.2.1. Effect of FNA pretreatment on EPS and cell envelop disruption

It is known that EPS and cell envelop are considered as the key substances protecting cells against hostile environments, thus their disruptions are closely relevant to cell lysis. Fig. 2 illustrates the variation of soluble protein, carbohydrate, and COD during the initial 12 h of treatment. According to the COD mass balance analysis shown in Table S3 (Supporting information), cell envelop was not disrupted during the initial 10 h, because the total amount of soluble COD and remainder COD in EPS showed an insignificant increase as compared with the total COD measured in initial EPS ($p > 0.05$). However, when the sludge was treated with 1.8 mg FNA/L for 12 h, the total amount of soluble COD and remainder COD in EPS showed a significant increase as compared with the total COD measured in initial EPS ($p < 0.05$). The COD mass balance indicated that cell envelop did not disrupt during the initial 10 h of treatment (Supporting information). Obviously, the soluble substances (COD, protein and carbohydrate) in 1.8 mg FNA/L treated reactor were much higher than those in blank, which revealed that FNA was more beneficial to EPS disruption, as compared with the blank.

The effect of FNA on disruption of cell envelop using no/little EPS sludge is shown in Fig. 3. Since the sludge used in this study without EPS, the release of protein and carbohydrate could be applied to indicate the disruption of cell envelope. In addition, VSS reduction was also used to indicate the disruption of cell envelope. From Fig. 3, FNA pretreatment could cause higher cell envelop disruption than uncontrolled condition. Compared with the blank, 1.8 mg FNA/L caused 252% (211%) higher of protein (carbohydrate) release and 123% higher VSS reduction, respectively.

Based on the above experimental results, we can conclude that FNA pretreatment is more effective to the disintegration of both EPS and cell envelope, as compared with the blank. Therefore, more VSS was degraded, and more sludge organic matters such as protein and carbohydrate were released, which thereby provided more soluble substrates for subsequent hydrolysis and acidification.

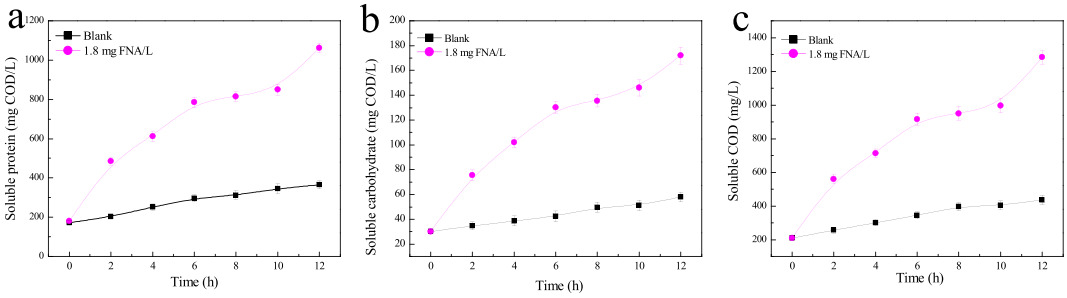

Fig. 2. Variations of soluble protein (a), soluble carbohydrate (b) and soluble COD (c) during the initial 12 h treatment time. Error bars represent standard deviations of triplicate tests.

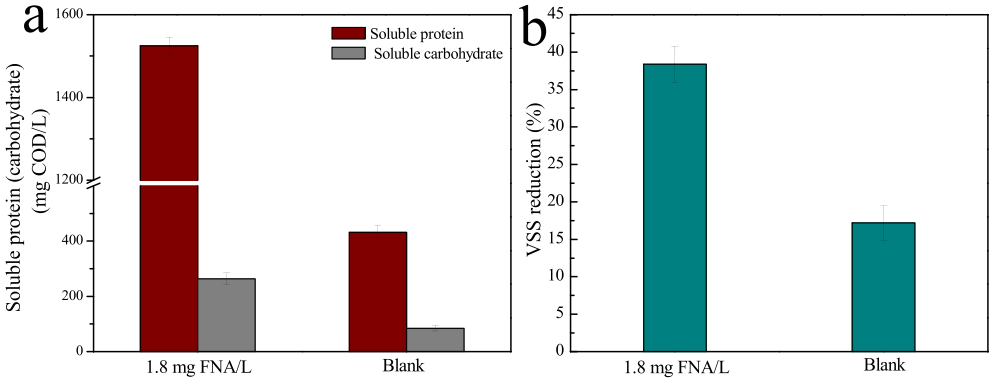

Fig. 3. Comparison of soluble protein and carbohydrate concentrations (a) and VSS reduction (b) between the sole FNA and blank reactors on 2 d. Error bars represent standard deviations of triplicate tests.

3.2.2. *Effect of FNA pretreatment on sludge solubilization*

Four steps-solubilization, hydrolysis, acidification, and methanogenesis – are generally involved in sludge anaerobic fermentation, and solubilization and hydrolysis are considered as rate-limiting steps (Chen et al., 2007; Luo et al., 2015; Zhao et al., 2015c). Generally, protein and carbohydrate, the mainly organic compounds in raw WAS, are needed to be converted to soluble ones for further utilization. Therefore, the sludge organics solubilization could be expressed in terms of soluble protein and carbohydrate concentrations in fermentation liquor. The effect of FNA pretreatment on soluble protein and carbohydrate concentrations is displayed in Fig. 4. The concentrations of soluble protein and carbohydrate in the 1.8 mg FNA/L treated reactor were much higher than those in blank within the initial 6 days. For example, the concentration of soluble protein (carbohydrate) in 1.8 mg FNA/L treated reactor was 2458 (651) mg/L on day 4, whereas, the corresponding data in blank was 552 (124) mg/L. More soluble pretein and carbohydrate achieved in the fermentation liquor of FNA pretreated reactor indicated FNA pretreatment provided more substrates for subsequent SCFA generation.

3.2.3. *Effect of FNA pretreatment on hydrolysis and acidification processes*

The effects of FNA pretreatment on the hydrolysis and acidification processes were studied with synthetic wastewaters containing BSA, dextran, L-alanine and glucose, respectively. Table 1 summarizes the model compounds degradation rate during batch tests. As shown in Table 1, model compounds degradation rates with FNA pretreatment were much higher than those of the blank. For example, the BSA, dextran, L-alanine and glucose degradation rates in 1.8 mg FNA/L pretreatment reactor were respectively 60.5%, 73.5%, 72.6% and 85.6% at the time of day 3 whereas the corresponding date were 38.4%, 53.1%, 59.1% and 63.7% in blank. Those results indicated FNA pretreatment is beneficial for hydrolysis and acidification processes.

3.2.4. *Effect of FNA pretreatment on methanogenesis*

Generally, SCFA, the products of acidification, can be further consumed by methanogen for methane production. If the methanogenesis process could be inhibited, the SCFA production can be further improved. The effect of FNA on methanogenesis step was also investigated with synthetic wastewaters containing acetate. It was found that methane production in 1.8 mg FNA/L reactor was 68.9% of that in the blank at the fermentation time of day 15, which indicated that the activities of methanogens were seriously inhibited by the FNA pretreatment. It should be noted that acid-forming bacteria were not impaired by FNA pretreatment (Wang et al., 2013c). It is well known that the optimal pH for methanogens is around 7.0. As shown in Fig. 5, pH drop was observed in each reactor, for example, the pH was dropped from the initial 7.0 (after FNA pretreatment) to the final 5.2 in the 1.8 mg FNA/L pretreatment reactor at the fermentation time of day 6, whereas pH was dropped from the initial 7.0 to 6.3 in blank. The pH drop posed a negative impact on the activities of methanogens during WAS anaerobic fermentation. In addition, the addition of NO_2^- changed the strict anaerobic condition to anoxic condition during WAS fermentation, which subsequently decreased the activities of methanogens (the obligatory anaerobic microorganisms). It was also reported that nitrite itself could suppress methanogens in the literature (Wang et al., 2014b). In the current system, therefore, it is assumed that the co-function of pH drop and NO_2^- that posed the negative effect on the activities of methanogens. Thus, more SCFA was accumulated by the FNA pretreatment.

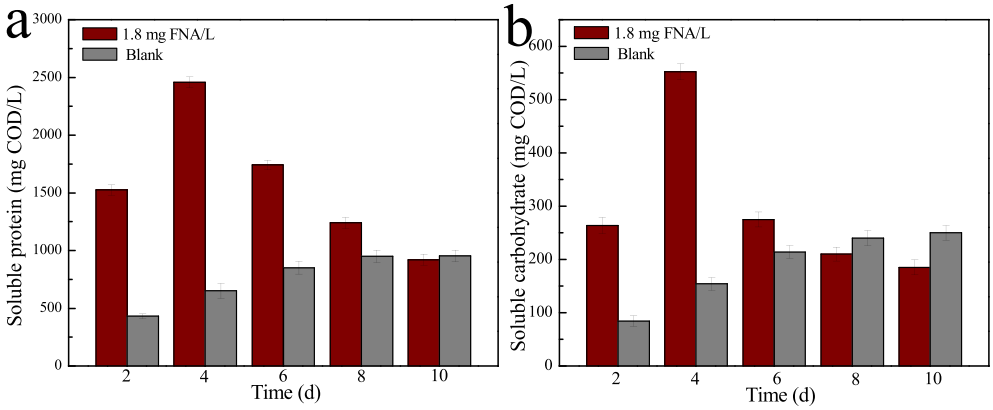

Fig. 4. Effect of FNA pretreatment on sludge solubilization with time. Error bars represent standard deviations of triplicate tests.

Table 1
Effect of FNA pretreatment on degradation rate of model compounds with time[a].

Treatment condition	Time (d)	Degradation rate (%)			
		BSA	Dextran	L-alanine	Glucose
1.8 mg FNA/L	1	28.3 ± 1.1	42.4 ± 1.5	40.3 ± 2.1	32.2 ± 1.5
	2	52.4 ± 2.4	68.9 ± 1.9	63.8 ± 2.3	65.8 ± 2.3
	3	60.5 ± 3.5	73.5 ± 2.3	72.6 ± 2.5	85.6 ± 2.1
Blank	1	16.2 ± 1.1	25.6 ± 1.5	27.9 ± 1.1	27.9 ± 1.5
	2	25.3 ± 1.8	39.4 ± 2.1	48.9 ± 1.5	42.3 ± 2.4
	3	38.4 ± 2.2	53.1 ± 2.6	59.1 ± 1.9	63.7 ± 2.1

[a] Error bars represent standard deviations of triplicate tests.

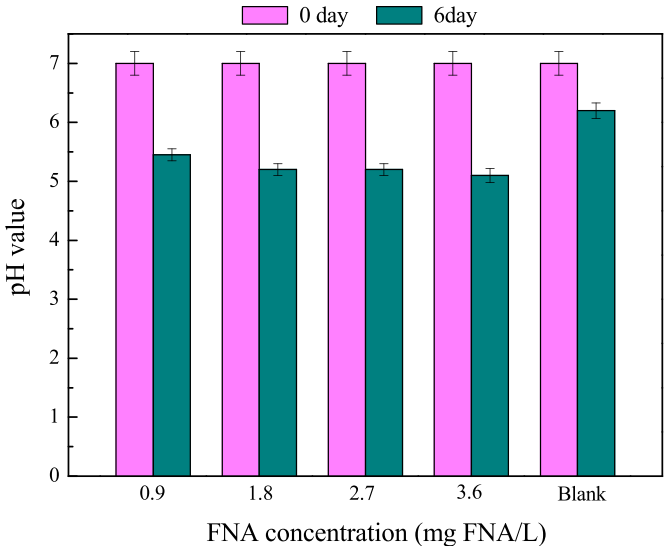

Fig. 5. Changes of pH values during WAS anaerobic fermentation with FNA pretreatment. Error bars represent standard deviations of triplicate tests.

3.2.5. Effect of FNA pretreatment on key enzyme activities related with SCFA

It has been reported that several key enzymes were involved in the metabolic pathway for SCFA formation under anaerobic condition, and their activities were directly related with the production of SCFA (Feng et al., 2009). In this study, the main SCFAs were acetic, propionic (shown in Fig. 1), and the proposed metabolic pathway related with SCFA was exhibited in Figure S3 (Supporting information). Thus, some key SCFA-forming enzymes were selected to investigate the effect of FNA pretreatment on their relative activities (Figure S4, Supporting information). The relative activities of protease, α-glucosidase, phosphotransacetylase (PTA), acetate kinase (AK), oxaloacetate transcarboxylase (OAATC), and CoA transferase in 1.8 mg FNA/L pretreated reactor and blank were shown in Figure S4 (Supporting information). As seen in Figure S4, the activities of protease, α-glucosidase, PTA, AK, OAATC, and CoA transferase in the 1.8 mg FNA/L pretreated reactor were much higher than those in blank, whereas, the activities of F_{420} in the 1.8 mg FNA/L pretreated reactor exhibited the opposite trend. All the observation of enzymes activities were in accordance with the SCFA production shown in Fig. 1. FNA could damage lipids, proteins, carbohydrates in EPS or cells by reacting with them (Yoon et al., 2006). FNA hindered the immobilization of the special enzymes and released them to liquid phase, thereby resulting in solute protein and carbohydrate increase (Zhou et al., 2013).

3.3. Effects of FNA on WAS fermentation by-products: NH_4^+–N and PO_4^{3-}–P

As shown in Fig. 6a, NH_4^+–N concentration substantially increased with fermentation time in the range of day 0-day 6. However, further increase of fermentation gave little benefit to the release of NH_4^+–N. The average NH_4^+–N concentration was 159.3, 184.3, 130.2 and 118.4 mg/L on 6 day when the FNA concentration was 0.9, 1.8, 2.7, and 3.6 mg/L, respectively. However, the NH_4^+–N concentration in blank was only 60.5 mg/L. It has been reported that soluble nitrogen concentration is an indicator for sludge protein hydrolysis (Luo et al., 2011), and the higher protein hydrolysis rate resulted in higher NH_4^+–N concentration. The higher NH_4^+–N concentration observed in FNA pretreatment reactor was consistent with the SCFA production efficiency and rate shown in Fig. 1.

The effect of FNA pretreatment on PO_4^{3-}–P release was shown in Fig. 6b, the average PO_4^{3-}–P concentration was 104.2, 112.1, 96.6 and 81.2 mg/L on day 6 when the FNA concentration was 0.9, 1.8, 2.7

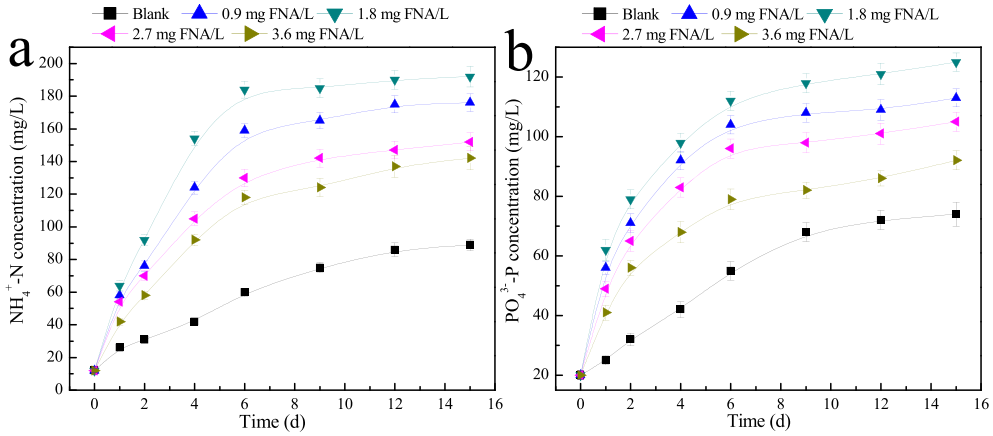

Fig. 6. Effect of FNA concentration on the variation of NH_4^+–N (a) and PO_4^{3-}–P (b) during WAS anaerobic fermentation. Error bars represent standard deviations of triplicate tests.

and 3.6 mg/L, respectively. However, only 55.1 mg PO_4^{3-}–P/L was observed in blank. Those observations were consistent with the high hydrolysis and acidification rate with FNA pretreatment.

It should be noted that NH_4^+–N and PO_4^{3-}–P concentrations decreased when further increased FNA concentration above 1.8 mg FNA/L. The reason for this might be attributed to that the toxicity of higher FNA concentration decreasing the activities of key enzymes involved in hydrolysis and acidification steps. Wang et al. (2013c) also reported high FNA concentration could inhibit sludge hydrolytic enzymes and/or enzymes responsible for acidogenesis.

4. Conclusion

In this study, an efficient and green pretreatment to enhance SCFA production form WAS anaerobic fermentation using FNA was reported. The optimum FNA concentration and fermentation time was 1.8 mg FNA/L and day 6. The highest SCFA production was 195.7 mg COD/g VSS, which was 3.7 times higher than that in blank. Mechanism studies revealed that FNA pretreatment accelerated disruption of both EPS and cell envelope. Moreover, FNA pretreatment could enhance sludge solubilization, hydrolysis and acidification but inhibit the activities of methanogens, thereby maximizing the yield of SCFA.

Acknowledgements

This research was financially supported by the project of National Natural Science Foundation of China (51278175 and 51378188), International Science & Technology Cooperation Program of China (No. 2012DFB30030-03). Dongbo Wang acknowledges the International Postdoctoral Exchange Fellowship Program (China) for partly financial support of this work.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2015.08.076.

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