Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2015.1051127

57 (2016) 12099–12107
June

Taylor & Francis
Taylor & Francis Group

Hydrolysis and acidification of waste activated sludge enhanced by zero valent iron-acid pretreatment: effect of pH

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Received 11 December 2014; Accepted 30 April 2015

ABSTRACT

Zero valent iron (ZVI) is the most commonly applied as a reactive media in remediation of wastewater treatment. In this work, a novel ZVI-acid pretreatment was expected to improve the anaerobic fermentation of waste activated sludge (WAS). The experimental results showed that pH significantly affected the function of ZVI, and the maximum SCOD, soluble protein, and polysaccharides were observed at pH 3.0 in the presence of ZVI. At fermentation time of 4 d, the maximum total volatile fatty acids (VFAs) was 900 mg/L with ZVI dosage of 1 g/g DS, which was 1.53-fold higher than the blank test. The analysis of VFAs composition showed that acetic, propionic, and valeric acids were the three main products at any ZVI dosage, and ZVI dosing contributed to a greater proportion of acetic acid and a lesser proportion of propionic acid. Meanwhile, mechanism investigations showed that the hydrolysis of soluble substrate as well as acidification of hydrolysate was apparently enhanced by ZVI dosing. These results suggested that the ZVI-acid pretreatment was helpful to accelerate and improve anaerobic acidogenesis of WAS.

Keywords: Waste activated sludge; Anaerobic fermentation; Hydrolysis; Acidification; Volatile fatty acids; Zero valent iron

1. Introduction

With the rapid urbanization of many areas of the world, activated sludge process has been used widely for treating municipal and industrial wastewater. However, as the main solid waste in waste water treatment plants (WWTPs), waste activated sludge (WAS) is inevitably produced in large quantities. The conventional treatment and disposal of WAS mainly include landfill and incineration, which is not suitable due to high disposal costs, land scarcity and the increasingly stringent environmental control regulations [1]. Thus, the strategy for sludge disposal is moving toward to more cost-effective and environmentally benign technique.

During the past several years, the anaerobic fermentation of WAS has gained increasingly attention

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due to sludge reduction and biological production of high added-value produced from this process [2]. Recently, the production of volatile fatty acids (VFAs) from WAS has attracted growing concerns since VFAs are preferred additional carbon sources for biological nutrient removal [3–5] or the synthesis of biodegradable plastics polyhydroxyalkanoates (PHAs) [6] during sludge fermentation. It is well known that anaerobic fermentation mainly includes three steps: hydrolysis, acidification, and methane production. Therefore, if the hydrolysis and acidification were enhanced and the methane production of VFAs was inhibited, the accumulation of total VFAs production would increase during anaerobic fermentation. The main strategy for improving the production of VFAs was to enhance the characteristic of sludge, disrupt sludge flocs and bacteria cells, release cellular components, and accelerate subsequent acidification. The influence of temperature, pH, solid retention time, carbon to nitrogen ratio (C/N), and other parameters on sludge fermentation has been intensively studied [7–10], and various methods for sludge pretreatment have been reported, such as mechanical treatment [11], chemical treatment [12], thermal treatment, and biological treatment [13]. In the literature, some researchers also studied the effects of combination of any two of these methods, including ultrasonic-alkaline [14], thermo-alkaline [15], surfactant-enzyme [16], and hybrid microwave (MW)-alkali treatments [17].

As a cheap metallic material, zero valent iron (ZVI) is increasingly used in municipal and industrial wastewater treatment for the removal of less biodegradable pollutants, such as halogenated hydrocarbons (e.g., TCE) [18,19], nitroaromatic compounds [20], synthetic dyes [21], heavy metals (e.g., Ni^{2+}, Hg^{2+}) [22], As(III) [23], and As(V) [24]. Recently, ZVI has attracted much attention due to its reductive property on anaerobic fermentation [25]. ZVI can also serve as an electron donor in the microbial metabolism, which may improve the performance of anaerobic fermentation systems. It has been found that ZVI was readily utilized in anaerobic mixed culture to support methanogenesis and sulfate reduction. Moreover, some publications have reported that ZVI could promote COD removal and accelerate sludge granulation in an anaerobic wastewater process [26].

Despite all these efforts, research on some aspects is still insufficient. Although it has been observed that the addition of ZVI can enhance anaerobic digestion of WAS, the optimal ZVI dosage for maximizing VFAs produced from WAS fermentation remain undetermined. To date, many investigations have indicated that fermentation under alkaline conditions is a preferable technology for VFAs accumulation. However, according to the chemical reaction of Fe^0 with H_2O under anaerobic conditions in Eq. (1):

$$Fe^0 + 2H_2O = Fe^{2+} + H_2 + 2OH^- \quad (1)$$

It is obvious that Fe^{2+} release is strongly affected by the change of pH values, and the reaction is apparently accelerated under acidic condition. Thus, the sole addition of ZVI is not a sustainable pretreatment for the WAS fermentation, and an efficient way to enhance the function of ZVI is needed.

Therefore, in this work, a novel strategy based on ZVI-acid pretreatment to enhance VFAs production was evaluated in laboratory-scale reactors, and the effect of pH on the function of ZVI was also discussed. In addition, the mechanism of VFAs accumulation enhanced by ZVI was investigated.

2. Materials and methods

2.1. Source of WAS

The WAS used in this study was obtained from the secondary sedimentation tank of a municipal WWTP in Nanjing, China. The source WAS was concentrated by settling for 24 h and stored in a fridge at 4℃, and the main characteristics of the concentrated WAS are shown in Table 1.

2.2. ZVI-acid pretreatment with different pHs

The pretreatment of ZVI under acidic conditions was carried out as the following: 750 mL of WAS was evenly distributed into seven batch reactors, ZVI was added into each reactor with a dosage of 1 g/g dry sludge (DS). Meanwhile, the pH value in the reactor was immediately adjusted to 3.0, 4.0, 5.0, 6.0 and uncontrolled, respectively, by adding appropriate dosage of 2 M NaOH or 2 M HCl. All reactors were mixed with an air-bath shaker at 180 rpm, and each pretreatment lasted for 24 h. The SCOD, soluble protein, soluble polysaccharide, and VFAs were measured before and after pretreatment.

2.3. Batch fermentation experiments in the presence of ZVI

Batch laboratory-scale anaerobic fermentation experiments were conducted in 250 mL serum bottles containing 150 mL WAS each. The ZVI was added into the reactors at dosages of 0.5, 1, and 2 g/g DS, respectively. The blank test was conducted in the absence of ZVI simultaneously. According to the discussion below, the pH values of all reactors were

Table 1
Characteristics of WAS

Parameter	Value
Solid content ratio, %	3.0 ± 0.1
pH	6.82 ± 0.05
TSS (total suspended solids), mg/L	27,640 ± 290
VSS (volatile suspended solids), mg/L	11,560 ± 204
STOC, mg/L	1,805.3 ± 10.1
TCOD (total chemical oxygen demand), mg/L	15,200 ± 957.4
SCOD (soluble chemical oxygen demand), mg/L	288.0 ± 4.7
Total VFAs (volatile suspended solids), mg/L	78.3 ± 8.4
Solute carbohydrate, mg/L	82.4 ± 6.2
Solute protein, mg/L	50.3 ± 2.7

adjusted to 3.0 as available pretreatment pH. All reactors were mixed using mechanical stirrers, and each pretreatment lasted for 24 h. The pH values of all reactors were adjusted at 10.0 after ZVI-acid pretreatment according to our previous study [27], and high-pure nitrogen gas (99.999%) was sparged into the reactors for 5 min to maintain strict anaerobic condition, then sealed with rubber plugs and operated at 30℃ on an air-bath shaker at 200 rpm. The samples in all reactors were assayed every certain interval.

2.4. Investigation on the mechanism of Fe^0-enhanced VFAs production

In order to study how the VFAs production from WAS was enhanced by the presence of ZVI, the following experiments were developed.

To understand how the presence of ZVI affects the hydrolysis of solubilized sludge particulate organic-carbon, bovine serum albumin (BSA, M_w 67,000) was used as a model protein compound, dextran (average molecular weight $M_w \sim 20,000$) and soluble starch were used as model polysaccharide compound in this study, respectively. One hundred and fifty milligrams of model compound (BSA, dextran or starch) was dissolved into 130 mL Milli-Q water. After that, 20 mL aliquot of WAS was added to each serum bottle as an inoculums with a final sludge concentration of approximately 4,000 mg/L. ZVI was then added to each reactor at a dosage of 1 g/g DS. The batch reactors were maintained at 30℃, and the other conditions were as the same of the WAS fermentation experiments in the presence of ZVI.

The effect of ZVI on the acidification of hydrolyzed products was also investigated with synthetic wastewater of L-alanine (model amino acid compound used in this study) and glucose (model monosaccharide compound), respectively. One hundred and fifty milligrams of model compound (L-alanine and glucose) was dissolved into 130 mL Milli-Q water, and 20 mL aliquot of WAS was added to each serum bottle as an inoculums with a final sludge concentration of approximately 4,000 mg/L. ZVI was then added to each reactor at a dosage of 1 g/g DS. The batch reactors were maintained at 30℃, and the other conditions were as the same of the WAS fermentation experiments in the presence of ZVI.

2.5. Analysis methods

All sludge samples taken from the reactors were centrifuged at 5,000 rpm for 5 min and then filtered through 0.45 μm PFS membrane prior to analysis. The analyses of pH, VFAs, soluble polysaccharide, soluble protein, SCOD, TSS, and VSS were the same as described in our previous publication [27]. The concentration of soluble iron was determined according to Standard Methods for Examination of Water and Wastewater (APHA, 1998) [28].

Enzyme assays for α-glucosidase, protease, and α-amylase were carried out using p-nitrophenyl-α-D-glucopyranoside, azocasein, and soluble starch as substrates, respectively. The activities of enzymes were determined according to the reported methods [29].

2.6. Statistical analysis

All assays in this study were performed in triplicate, and the results were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was used to detect the significance of results, an LSD test was used to detect any differences between pairs of variables, and $p < 0.05$ was considered to be statistically significant.

3. Results and discussion

3.1. Effect of pH on ZVI-acid pretreatment

The changes in WAS characteristics after 24 h ZVI-acid pretreatment were presented in Fig. 1. After 24 h pretreatment, the total SCOD increased with decreasing of pH values. For the untreated experiment, Fig. 1 showed a slight increase of SCOD (100 mg/L). In contrast, SCOD increased significantly during pretreatment pH decreased from 6.0 to 3.0, and the maximum SCOD was 1,803 mg/L with Fe^0 dosages of 1 g/g DS and pH 3.0, which was approximately six times that initial SCOD. The results implied the degree of cell lysis and/or EPS solubilization may have been improved with ZVI-acid treatment. A similar trend was also observed in the case of soluble proteins and soluble polysaccharides. However, all of experiments are showing that the pretreatment had little or no effect on the accumulation of VFAs, which might be due to the inhibition of strong acidity on the activity of acidogenic bacteria [30].

Moreover, the maximum sludge solubilization efficiency observed in this study was compared with other pretreatment systems reported in literature, and the result was summarized in Table 2 [31–33]. The results showed in this study was higher compared with the reports in other studies. Specially, the improvement of soluble proteins was higher than the single acid pretreatment in the literature (16 times vs. 5.5 times). The reason might be that the addition of ZVI under the acidic condition significantly accelerated the solubilization of WAS, which had more abundant proteins [34].

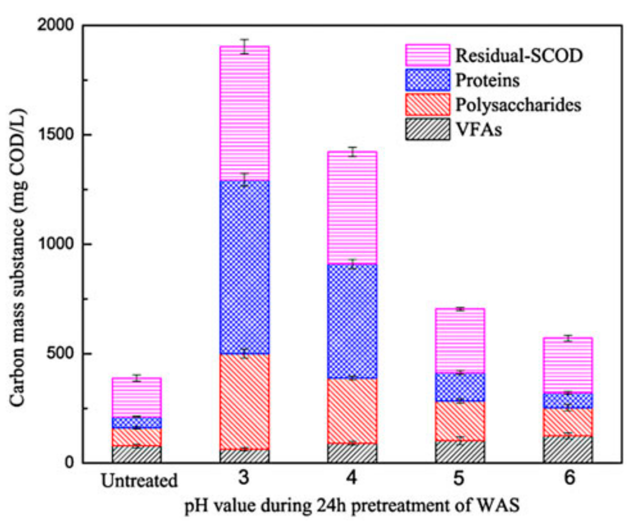

Fig. 1. Carbon mass balance analysis of ZVI-acid pretreatment at different pHs.

The function of Fe^0 could be only made by the reaction: $Fe^0 - 2e^- = Fe^{2+}$, and the Fe^{2+} leaching reflected the intensity of Fe^0 function [35]. Therefore, Fe^{2+} concentration under different pH values was shown in Table 3. The Fe^{2+} concentration at pH 3.0 was significantly higher than other cases, which was around 52 mg/L in aqueous phase. Consequently, the optimum pH for ZVI-acid pretreatment was determined as approximately 3.0 and was selected for the subsequent experiments.

3.2. Effect of ZVI on oxidative–reductive potential (ORP)

It is believed that hydrolysis and acidification of WAS are closely related with ORP. The average ORP value in reactors with different ZVI dosages was shown in Fig. 2. It was obvious that the ORP value decreased greatly to −283.5, −260.5, −291 mV with ZVI dosages of 0.5, 1, 2 g/g DS, respectively, while it was −145 mV in the blank test. The results indicated that ZVI could effectively decrease the ORP and created a favorable anaerobic environment for the growth of anaerobes. Moreover, the decline of ORP could result in the variation of fermentation type [30,36], which subsequently influence VFAs generation. The changes of VFAs composition were discussed in Section 3.4.

3.3. Improvement of total VFAs production on different Fe^0 dosages

The effects of Fe^0 dosages on total VFAs production (including formic, acetic, propionic, valeric, and butyric acids) are shown in Fig. 3. It could be observed that the maximum total VFAs production was improved by the presence of ZVI. As seen in Fig. 3, the total VFAs had a similar trend with fermentation time at all of reactors. The average VFAs concentrations increased from the initial 300 mg/L to their respective maximum on the fourth day. The specific maximum VFAs yields for 4 d fermentation time were 810, 940, and 744 mg/L with ZVI dosages of 0.5, 1, and 2 g/g DS, respectively, while it was only 580 mg/L in the blank test on the 4th day. It indicated that the VFAs production with ZVI dosage of 1 g/g DS was 62% higher than that of no-ZVI dosage. Thus, the results suggested that the optimum conditions for VFAs production were ZVI dosage of 1 g/g DS and fermentation time of 4 d.

Also, it can be observed from Fig. 3 that VFAs were decreased with a further increase in time at all dosages of ZVI due to the activity of some microbes, such as methanogens. Further investigation revealed that the consumption of VFAs with Fe^0 dosages of

Table 2
Effect of different acid pretreatment methods on the solubilization of solid waste

Pretreatment methods	Solid wastes	Pretreatment conditions	Effect of pretreatment			References
			Increase in SP	Increase in SC	Increase in SCOD	
Acid	WAS	HCl, pH 1, 24 h	4.5 times	4 times	4 times	[30]
	Food waste	HCl, pH 3, 4℃, 24 h	25%	20%	28%	[31]
Ultrasound + acid	Food waste	Ultrasonic pretreatment, pH 3, 4℃, 24 h	29%	29%	29%	[31]
Thermo-acid	WAS	H_2SO_4, pH 3, 90℃	430 mg/L	200 mg/L	Not determined	[32]
ZVI-acid	WAS	HCl, pH 3, 1 g/g DS Fe^0 powder	15 times	3 times	5.2 times	This study

Table 3
Fe^{2+} concentration under different pH values pretreatment

pH	Fe^{2+} (mg/L)
3	51.98
4	35.28
5	18.36
6	10.14
Blank	8.86

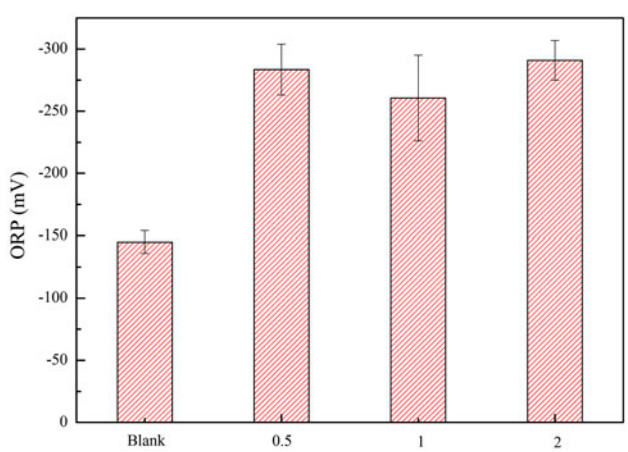

Fig. 2. ORP variation in the presence of ZVI.

2 g/g DS was faster than other three reactors, which might be attributed to the enhancement of higher Fe^0 concentration to methanogenesis [25]. Besides, the VFAs concentration was lower at higher Fe^0 dosage during the initial increase of VFAs. For example, the maximum total VFAs concentration was 900 mg/L at Fe^0 dosage of 1 g/g DS, while it was only 744 mg/L

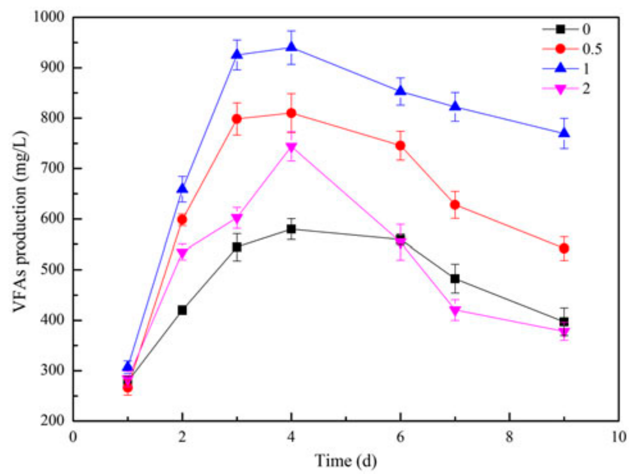

Fig. 3. Effects of Fe^0 dosage and fermentation time on total VFAs production.

at 2 g/g DS, the reason was likely that the toxic effects of higher Fe^0 concentration to microorganism.

3.4. Composition of VFAs on different Fe^0 dosages

The effects of fermentation time and ZVI dosages on the percentage of individual VFA (including formic, acetic, propionic, butyric, and valeric acids) accounting for total VFAs were shown in Fig. 4. As seen in Fig. 4, acetic, propionic, and butyric acids were the three main products at any ZVI dosage investigated in 9-day fermentation time. During the initial 4-day fermentation time, acetic acid was the most prevalent acid, which accounted for approximately 53–64% of the total VFAs in all cases. With further increasing fermentation time, the percentage of acetic acid decreased sharply, and the propionic and valeric

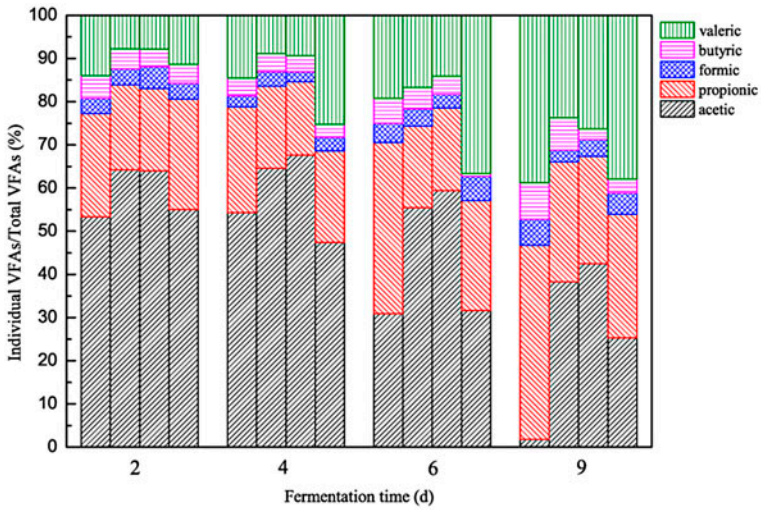

Fig. 4. Percentage of individual VFAs during entire fermentation time in the presence of ZVI (From left to right: Blank, 0.5, 1, and 2 g/g DS).

acids were increased gradually. Compared to the blank text, acetic acid fraction remained relatively stable in the reactors treated with ZVI. For example, the percentages of acetic and propionic were only 1.7 and 45.0%, respectively, in the blank test with fermentation time of 9 d, whereas they were 42.5 and 24.8% at ZVI dosage of 1 g/g DS, indicating that Fe^0 dosing contributed to a greater proportion of acetic acid and a lesser proportion of propionic. The result was in agreement with other literature, in which the conversion of propionate to acetate was enhanced by ZVI addition [30].

3.5. Mechanism of VFAs production enhanced in the presence of ZVI

To further clarify the impacts of Fe^0 on the hydrolysis of protein and polysaccharide, batch tests with synthetic wastewaters of model protein (BSA) and model polysaccharide (dextran and starch) were conducted, respectively, and the hydrolysis of WAS could be expressed as the degradation rate of model protein and polysaccharide in the fermentation liquid. The results were shown in Fig. 5. The degradation rate of BSA was 35.3% at fermentation time of 6 d, but it was only 13.4% in the blank. Similarly, for starch and dextran, the degradation rate was 71.9 and 65.4% with ZVI dosing, respectively. However, it was only 48.9 and 51.8% in the control tests, respectively. Furthermore, the acceleration of hydrolysis could be ascribed to the enhancement of enzyme activity, so the analysis of hydrolytic enzyme activity in the synthetic

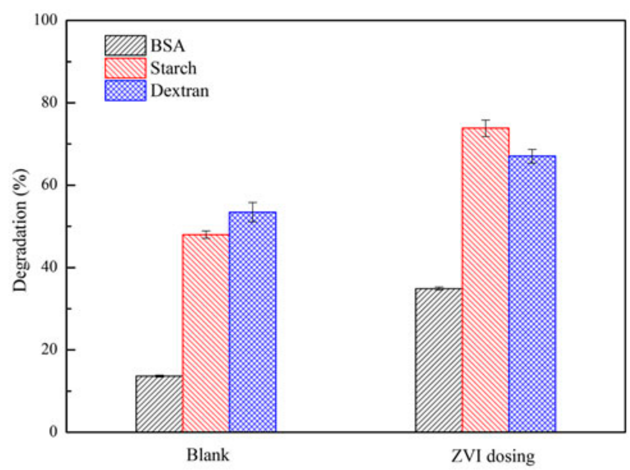

Fig. 5. Effect of ZVI on the degradation of BSA, starch, and dextran.

wastewaters above, including protease, α-glucosidase, and α-amylase, were illustrated in Fig. 6. It was apparent that all of enzymes activities were increased in the presence of ZVI, indicating that the hydrolysis of both protein and polysaccharide was significantly enhanced by ZVI addition, it may attribute to the function of pyruvate–ferredoxin oxidoreductase, which was facilitated by the release of Fe^{2+}.

As is well known, the main hydrolysates, such as monosaccharide and amino acid, are converted to VFAs during WAS acidification stage. The batch experiments with synthetic wastewaters of model monosaccharide (glucose) and model amino acid

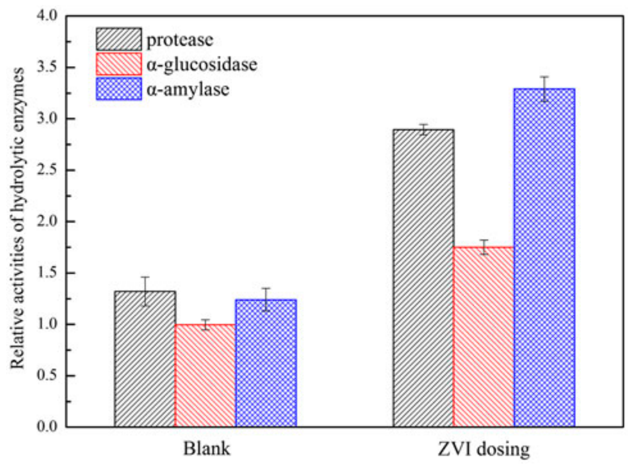

Fig. 6. Effect of ZVI on three hydrolytic enzymes at fermentation time of 3 d.

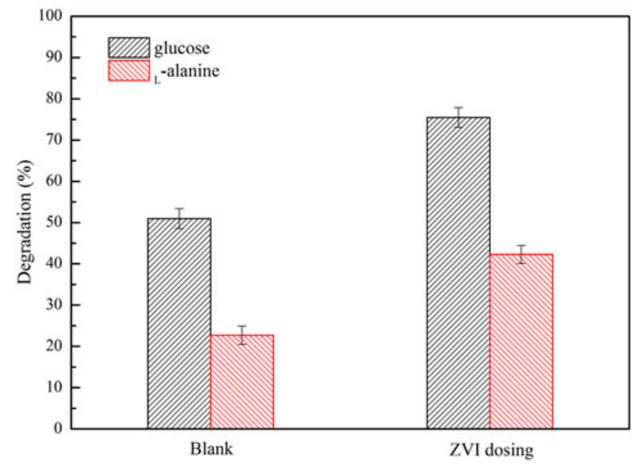

Fig. 7. Effect of ZVI on the degradation of glucose and L-alanine.

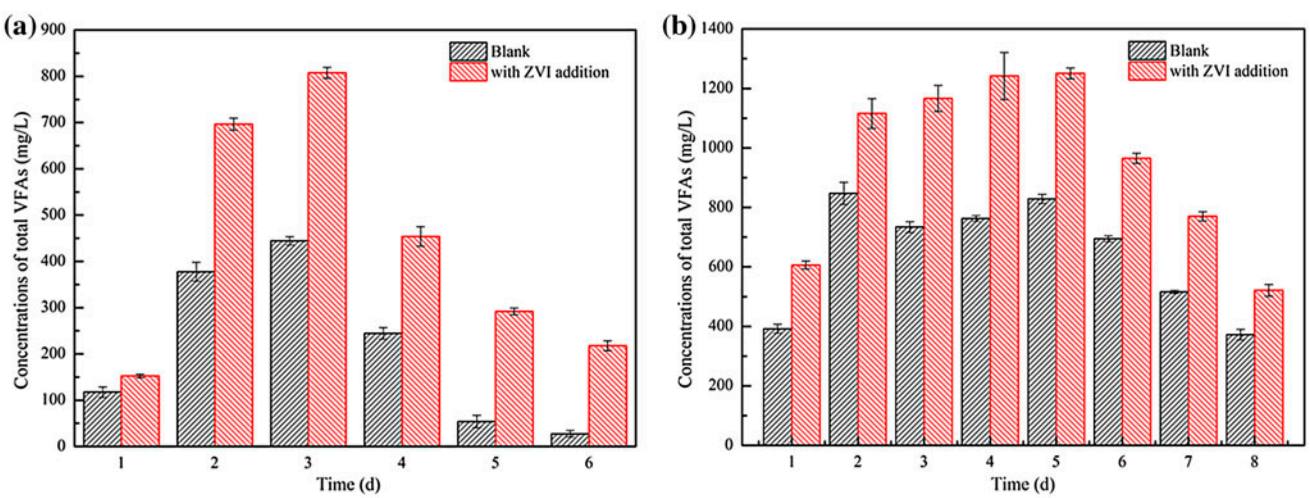

Fig. 8. VFAs production with glucose (a) and L-alanine (b) in the presence of ZVI.

(L-alanine) were performed to estimate the effect of Fe^0 on the acidification process, and the results with acidification time of 4 d are shown in Figs. 7 and 8. The degradation of glucose and L-alanine was, respectively, 79.5 and 48.3% in the presence of ZVI, was higher than that in the blank. From Fig. 7(a) and (b), it was noticed that the VFAs production with glucose and L-alanine in the presence of ZVI was increased compared with that in the absence of ZVI. It may attribute to the growth of hydrogen-consuming microorganisms (e.g., homoacetogens) was enhanced by ZVI [35]. Obviously, both the degradation of glucose and L-alanine and the production of VFAs were improved by ZVI addition.

4. Conclusion

A novel pretreatment of ZVI-acid for enhancing anaerobic fermentation of WAS was successfully investigated in this study. According to the experimental results, using ZVI-acid pretreatment, the accumulation of VFAs production was effectively improved. The pH of 3.0 was the optimal pH for the release of Fe^{2+}, showing a significant degree of cell lysis and/or EPS solubilization. Acetic, propionic, and butyric acids were the prevalent VFAs at any case, and the conversion of propionate to acetate was also accelerated in the presence of ZVI. Additionally, the optimum Fe^0 dosage and fermentation time observed in present work were 1 g/g DS and 4 d, respectively.

The reasons for VFAs being significantly improved in the presence of ZVI were the enhancement of solubilization of WAS particulate organics, hydrolysis of soluble substrate and acidification of hydrolysate. Furthermore, hydrolytic enzymes (protease, α-glucosidase, and α-amylase) were apparently active in the presence of ZVI. These results suggested that ZVI dosing was helpful to the WAS anaerobic acidogenesis and the accumulation of VFAs production.

Acknowledgements

The authors would like to thank the National Natural Science Foundation of China (Contract No. 51278248), and the Jiangsu Key Laboratory of Chemical Pollution Control and Resources Reuse of China for their supports.

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