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Evaluating the potential impact of hydrochar on the production of short-chain fatty acid from sludge anaerobic digestion

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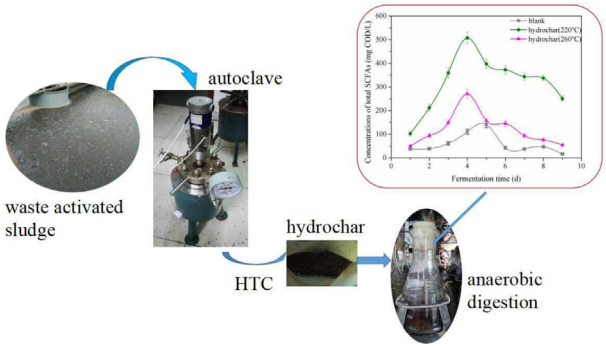
HIGHLIGHTS

- The hydrochar shortened the time to achieve the maximal production of SCFA.
- The production of SCFA and acetic acid kept stable in the presence of hydrochar.
- Hydrochar produced in 220 °C contained more organic matters.
- Humic substances in hydrochar improved the SCFA production.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, waste activated sludge (WAS) was used as feedstock to generate hydrochars at different temperatures (220 °C and 260 °C) and their effect on sludge anaerobic digestion was evaluated. Experimental results showed that the maximum yield of short-chain fatty acid (SCFA) enhanced by hydrochar (220 °C) and hydrochar (260 °C) were 507.33 and 270.80 mg chemical oxygen demand (COD)/L respectively, which were much higher than that in blank (141.49 mg COD/L). Mechanism investigation confirmed that hydrochar remarkably accelerated the solubilization and hydrolysis of organic matters, enhanced the acidification of hydrolyzed products, and inhibited the activity of methanogenic bacteria as well as promoted the activities of key enzymes. Meanwhile, the organic matters especially humic substances existed in the hydrochar played an important role during anaerobic digestion.

1. Introduction

Biological wastewater treatment has been commonly used in the world, but waste activated sludge (WAS), as a byproduct of this process, is produced in huge quantities annually (Zhao et al., 2016a). For example, in China, the production of WAS approximately was 30 million metric tons in 2012 and increased to 34 million metric tons in 2015 (Feng et al., 2015). WAS needs to be stabilized to reduce its potential environmental risks, however, the disposal costs are expensive and the cost of sludge treatment and disposal accounts for about 8% total operation cost of the wastewater treatment plant (WWTP) in China (Feng et al., 2015). Meanwhile, the sludge contains considerable organic matters, such as protein and carbohydrate, which makes it possible to produce useful resources like short-chain fatty acid (SCFA) from WAS (Zhao et al., 2015). It is reported that SCFA is the preferred carbon

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source for biological nutrient removal and the raw material to produce the biodegradable plastics polyhydroxyalkanoates (PHAs) (Lemos et al., 2006). Therefore the utilization of WAS to produce valuable product SCFA has become a promising way for the treatment and disposal of WAS.

Anaerobic digestion of WAS generally includes four continuous steps, namely solubilization, hydrolysis, acidification, and methanogenesis (Yang et al., 2010; Zhao et al., 2016b). When the methanogenesis is inhibited, the accumulation of SCFA will happen. It is well-known that the steps of solubilization and hydrolysis are the rate-limiting steps during anaerobic digestion (Li et al., 2016b; Zhao et al., 2015). In order to promote the production of SCFA, pretreatments such as biological and mechanical (Carrere et al., 2010), chemical (Li et al., 2016a), and enzymatic (Luo et al., 2011) methods, are always employed to break down sludge flocs and tough cell walls/membrane. However, those methods need to consume either intensive energy or large amounts of chemical reagents, which is not sustainable in practical applications (Li et al., 2016b). Therefore, it is essential to develop a new cost-effective and environmental method to improve the solubilization and hydrolysis of sludge.

Preparation of hydrochar through hydro-thermochemical treatment of sludge from WWTP is a good way to realize the reuse of sludge. Compared with the traditional raw materials (wood or plant), hydrochar prepared from sludge has the advantages of low cost, high recovery rate, waste utilization, eliminating pathogens and potentially organic contaminants (Peng et al., 2016). Through hydrothermal carbonization (HTC), it can convert great amount of wet input material into carbonaceous solids and less gases than other carbonization process (Mumme et al., 2011). Recently, researchers began investigating the impact of hydrochar on anaerobic digestion. Mumme et al. explored the effect of hydrochar converted from thermophilic wheat straw digestate on the anaerobic digestion (Mumme et al., 2014). They indicated that the increased production of methane was mainly due to the degradation of readily available carbon provided by the hydrochar. Reza et al. determined the effect of hydrochar on the gas production in anaerobic digestion (Reza et al., 2015). They used hydrochar derived from microcrystalline cellulose as support media to form biofilms. In their study, hydrochar inhibited the production of gas compared with the control. However, these researches emphasized on the characterization of hydrochar, thus the specific mechanism of inhibition to anaerobic digestion were not investigated.

The aim of this work was to assess the impact of hydrochars on anaerobic digestion of WAS. Firstly, the characteristic of hydrochar and its behavior in sludge digestion system were expounded. Secondly, the effect of hydrochars produced at different temperatures (220 °C and 260 °C) on the production of SCFA was compared. Finally, the influence of hydrochars on hydrolysis and acidification of WAS were explored by stages. The findings achieved in this work might provide one promising method for the enhancement of SCFA generation from WAS.

2. Materials and methods

2.1. Waste activated sludge

The WAS used in this work was obtained from a secondary sedimentation tank of a municipal WWTP in Xiangtan, China. The sludge was concentrated by settling at 4 °C for 24 h, and its main characteristics are as follows: pH 6.86 ± 0.15, total suspended solids (TSS) 35,550 ± 156 mg/L, volatile suspended solids (VSS) 12,335 ± 71 mg/L, volatile solids (VS) 12,200 ± 58 mg/L, total chemical oxygen demand (TCOD) 17,875 ± 356 mg/L, soluble chemical oxygen demand (SCOD) 172 ± 8 mg/L, carbohydrate 1430 ± 91 mg COD/L, protein 9831 ± 340 mg COD/L. Clearly, protein and carbohydrate are the two major organic compounds in WAS, which account for approximately 65% of the total volatile suspended solids.

2.2. Preparation of hydrochar

It was reported that when the sludge was treated by HTC below 240 °C, the weight loss rate of organic matter is low, indicating that most of the organic matters are not broken down (Dümpelmann et al., 1991). Therefore, 220 °C and 260 °C were selected to produce the hydrochar, and the products named as hydrochar (220 °C) and hydrochar (260 °C), respectively.

The WAS was kept at 105 °C for 24 h in oven to completely remove water, then grounded and sieved into fine powders (<0.25 mm) for use. The sludge particles and deionized water (at the ratio of 1:9) were mixed evenly and then were placed in a 500 mL 316 stainless steel reactor equipped with an automatic temperature controller and mixing device. The reactor was heated up with a heating rate of approximately 4 °C/min, and then held at specified temperatures (220 °C or 260 °C) for 1 h. After the HTC experiments, the reactor was cooled down to room temperature. Vacuum filtration apparatus with microfiltration filters was used to separate solid and liquid products. The chars were dried at 105 °C overnight, grounded into fine powders (<0.25 mm) and stored in glass bottle with stopper.

2.3. Batch experiments of SCFA production from WAS digestion in the presence of hydrochar

The batch experiments were carried out in five conical flasks (working volume of 500 mL). Firstly, three conical flasks were added 300 mL of sludge, and then two of them were fed with 3.6 g hydrochars produced at different temperatures (220 °C and 260 °C). Another conical flask without hydrochar served as blank. In contrast, the remaining two conical flasks was added 270 ml of tap water, 30 mL of WAS and 3.6 g hydrochar prepared at a temperature of 220 °C or 260 °C respectively to investigate the contribution of hydrochar degradation to the yield of SCFA. The initial pH in all conical flasks was controlled at 7.0 ± 0.1 with 4M sodium hydroxide or 4M hydrochloric acid. Afterwards, those conical flasks were flushed with high purity nitrogen gas for 60 s to remove the air. Finally, all conical flasks were capped with rubber stoppers, sealed, and placed in an air-bath shaker (150 rpm) at 35 ± 1 °C. The above experiments were repeated triple.

2.4. Effects of hydrochar on the process of hydrolysis

The impact of hydrochar on the hydrolysis was investigated using synthetic wastewater containing bovine serum albumin (BSA, average molecular weight 67,000, model protein compound) and dextran (average molecular weight 23,800, model polysaccharide compound). Firstly, 270 mL synthetic wastewater containing 3.2 g BSA and 0.8 g dextran was respectively added in three reactors, and then each reactor was inoculated with 30 mL of sludge. Afterwards, two reactors received 3.6 g hydrochar produced at 220 °C or 260 °C and another reactor without hydrochar served as blank test. All other operation conditions were the same as above batch experiment. The hydrolysis rate was determined by detecting the degradation rate of BSA or dextran.

2.5. Effects of hydrochar on the process of acidification

This batch test was performed with the same method described in the Section 2.4 except that L-alanine (model amino acid compound) and glucose (model monosaccharide compound) replaced

the BSA and dextran, respectively to verify the role of hydrochar in acidification process. The influence was reflected by the variation of SCFA after 2 days.

2.6. Effects of hydrochar on the process of methanogenesis

The effect of hydrochar on the methanogenesis was also studied using synthetic wastewater with 1.0 g/L acetate as the fermentation substrate. All other operation conditions were the same as depicted above. The methane production rate was determined by detecting the degradation rate of acetate.

2.7. Contribution of humic substance to the SCFA production

There are plenty of humic substances, including fulvic acid and humic acid, in hydrochar (Wilén et al., 2003). In order to investigate the contribution of humic substances to the yield of SCFA from sludge acidification, the batch test was performed according to the procedure and conditions described in the Section 2.5 except that the hydrochar was replaced by the humic substances, briefly, 1.08 g of humic acid or 0.12 g of fulvic acid, which were purchased from Shanghai Macklin Biochemical Company and Shanghai Duoyu Biochemical Company, respectively. Another reactor did not add the humus as the blank.

2.8. Analytical methods

TSS, VSS were measured according to the Standard Methods (APHA (American Public Health Association), 2005). Protein and polysaccharide were detected using Lowry's method and phenol-sulfuric acid method with BSA and glucose as standard solution, respectively (Zhao et al., 2016a). SCFA was determined by gas chromatography, and the detailed determination procedures were same as our previous study (Luo et al., 2011). The total content of SCFA was calculated as the sum of acetic, propionic, n-butyric, *iso*-butyric, n-valeric, and *iso*-valeric acid. The ultimate and proximate analysis of hydrochar was performed by 2400 Series II CHNS Analyzer, PerkinElmer, USA. Fourier transform infrared spectrometer (FTIR) spectra were collected on an IR Prestige-21 spectrometer (Shimadzu, Japan) at the room temperature by the standard KBr disk method. The content of trace metals was determined by ICP-OES (Perkin Elmer Optima 5300DV). The extracellular polymeric substance (EPS) was measured by Three-dimensional excitation-emission matrix (3D-EEM) (Yang et al., 2017). The activities of key enzymes that were associated with hydrolysis and acidification of WAS were assayed. Protease and α-glucosidase were measured on the third day of digestion and acetate kinase (AK), phosphotransacetylase (PTA), oxaloacetate transcarboxylase (OAATC), and CoA transferase were measured on their optimal conditions. The method was according to Feng et al. (Feng et al., 2009).

2.9. Statistical analysis

All measurements were conducted in triplicate and results were expressed as mean ± standard deviation. An analysis of variance was used to evaluate the significance of results, and $p < 0.05$ was considered to be statistically significant.

3. Results and discussion

3.1. Characteristics of hydrochars

3.1.1. Proximate and ultimate analysis of the hydrochars

The proximate and ultimate analysis of two hydrochars was listed in Table 1. It was clear that as the hydrothermal temperature raised, the yield of hydrochar decreased and the ash content increased, which should attribute to the complete decomposition of organics existed in the feedstock at higher hydrothermal temperature. The same reason can also explain the variation of volatile matter. The percentage of fixed carbon and H/C of hydrochar (260 °C) is higher than that of hydrochar (220 °C), indicating the better carbonization at higher temperature, which suggested that the former was more stable and thus provided less organic material to the digestion.

3.1.2. FTIR

FTIR analysis is a qualitative approach to detect specific molecular structures and chemical groups of hydrochar (Liu et al., 2015). The FTIR spectra of two hydrochars were similar but the intensity had little difference for different carbonization. The peaks around 3600 cm^{-1} were assigned to the stretching vibration band of hydroxyl (OH) and C—H (Uchimiya et al., 2011). There were two obvious peaks around 2800 and 2900 cm^{-1}, suggesting the existence of aliphatic CH_n groups (Peng et al., 2016). The adsorption peak at 2400 cm^{-1} may be the C—H peak of the weak C≡C stretching vibration peak and peak around 1400 cm^{-1} was belong to the stretching of —C=C. Due to the stretching of C—O—C, there was an adsorption peak at 1080 cm^{-1} (Peng et al., 2016), in which the intensity of two hydrochars existed greater difference. The peak at 1600 $cm^{-1}$was due to the stretching vibration of aromatic C=C bond (Trazzi et al., 2016).

3.1.3. Fluorescence excitation emission matrix spectra of hydrochars

In order to further characterize the hydrochars produced at different temperatures, the three-dimensional fluorescence technique was applied and the results were shown in Fig. 1(I and II). The main peak (Peak A) located at longer wavelengths (excitation wavelengths was longer than 280 nm and emission wavelengths was longer than 380 nm) was related to humic acid-like organics (Chen et al., 2003), which was beneficial to the production of SCFA during sludge anaerobic digestion (Liu et al., 2015). Peak B located at excitation wavelengths shorter than 250 nm and emission wavelengths shorter than 350 nm was related to some aromatic protein-like substances such as tyrosine and tryptophan (Ahmad and Reynolds, 1999), and was only detected in fluorescence spectra of hydrochar (220 °C). These observations suggested that hydrochar produced at 220 °C contains more organic matters than another hydrochar.

3.2. Effects of hydrochars on the production of SCFAs from sludge digestion

Fig. 2A presents the production curve of SCFA from WAS anaerobic digestion in the presence or absence of hydrochars. It can be found that the yield of SCFA firstly rose and then decreased, and the maximal SCFA production of 141.49 mg COD/L was obtained at 5th day in the blank test, which was similar to that documented in the literature (Zhang et al., 2009). After 5 days, the accumulation of SCFA gradually decreased, which was mainly attributed to the consumption of SCFA serving as digestive substrate to generate the methane. However, when hydrochars appeared in the digestion system, as shown in Fig. 2A, the time for the maximal SCFA production was shortened to 4 days. The corresponding SCFA yields were respectively 507.33 mg COD/L and 270.80 mg COD/L in the presence of hydrochars (220 °C) and hydrochars (260 °C), which were significantly greater than that obtained in the blank test (141.49 mg COD/L) ($p < 0.05$). This difference is likely associated with the different characteristics of hydrochars produced at these two temperatures (Dümpelmann et al., 1991). The SCFA production from hydrochar (220 °C) and hydrochar (260 °C) was only 26.70 mg COD/L and 23.44 mg COD/L respectively, which

Table 1
Proximate and ultimate analyses of the hydrochars produced at different temperatures.

	Yield (%)	Ash (%)	VM	FC	Elemental analysis (wt%)					Atomic ratio	
					C	H	O	N	S	H/C	O/C
Hydrochar (220 °C)	62.63	73.05	16.81	9.97	19.68	3.10	2.82	1.32	0.59	1.83	0.11
Hydrochar (260 °C)	58.51	65.17	23.14	11.69	24.49	3.68	4.51	1.59	0.57	1.80	0.14

Abbreviations: VM: volatile matter; FC: fixed carbon.

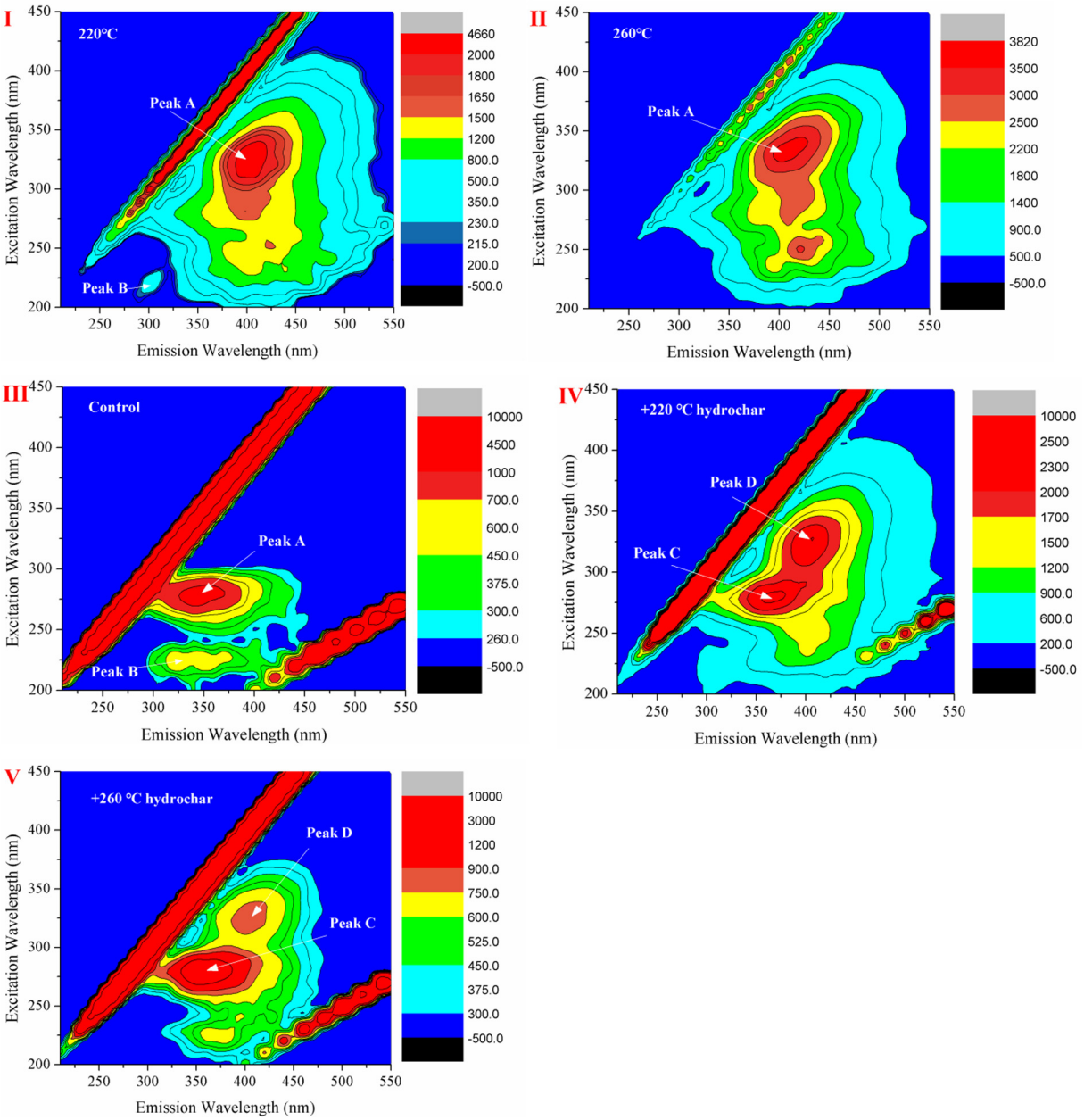

Fig. 1. Fluorescence excitation emission matrix spectra of hydrochar produced at 220 °C (I); hydrochar produced at 260 °C (II); EPS from sludge without hydrochar (III); EPS from sludge with hydrochar produced at 220 °C (IV); EPS from sludge with hydrochar produced at 260 °C (V).

accounted for below 10% of total SCFA production and was negligible. Thus, the SCFA production in this study was mainly derived from the degradation of WAS rather than the degradation of hydrochar. These results clearly showed that hydrochars could not only greatly improve SCFA production but also significantly accelerate SCFA accumulation. The shortening of the time for SCFA accumulation in practical engineering can bring many benefits, such as reducing the volume of the reactor.pH is also an important factor affecting the production of SCFA from sludge anaerobic digestion (Zhang et al., 2009), and the variation of pH was also detected. It was found that the pHs in all reactors were around 7.0 during the entire digestion process, and further investigation showed there was no significant difference of pH variation among those reactors ($p > 0.05$). During anaerobic digestion, pH should decrease

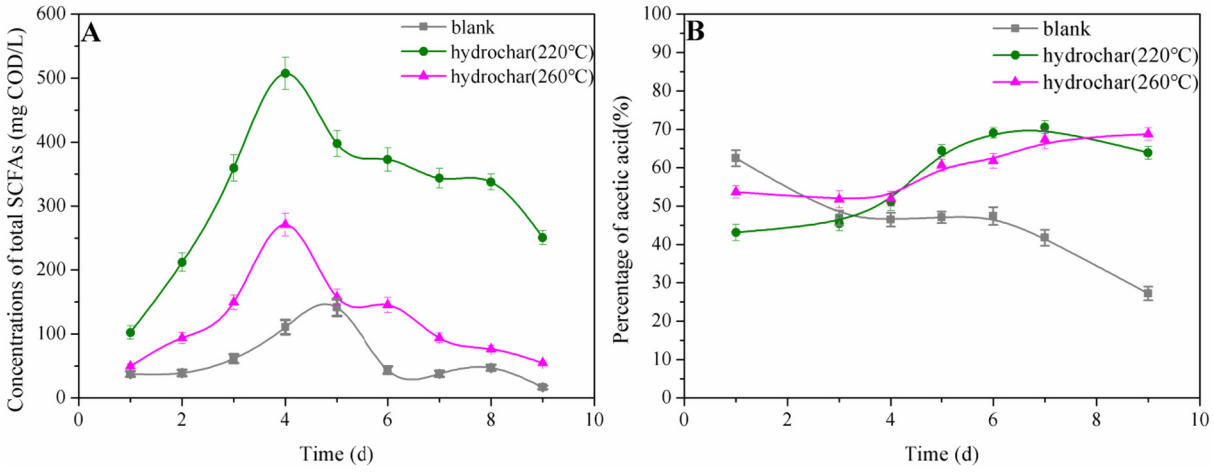

Fig. 2. Effects of hydrochars on the production of total SCFAs (A) and acetic acid (B). Error bars represent standard deviations of triplicate tests.

due to the accumulation of SCFA. In present study, pH kept 7 during the first 9 day, suggesting that the presence of hydrochar provides greater buffering capacity.

The compositions of SCFA in wastewater have a great effect on the activities of microorganisms in WWTPs, thereby affecting the performance of biological nutrient removal (Li et al., 2016b). Thus, it is necessary to clarify the percentage of individual SCFA during these digestion reactors. The mainly individual SCFA was acetic acid for both hydrochars. In blank, the percentage of acetic acid decreased from 62% to 40% when the time extended to 4 d (Fig. 2B). However, the percentage of acetic acid in the presence of hydrochar (220 °C) increased firstly from 43% to 64% within 5 days digestion and kept around 65% after 5 days. The variation trend for hydrochar (260 °C) was similar, but the percentage of acetic acid was slight lower than that of hydrochar (220 °C).

3.3. The details of how hydrochar affects the yield of SCFA from sludge digestion

3.3.1. Effect of hydrochars on sludge solubilization

Sludge anaerobic digestion consists of four consecutive steps, including solubilization, hydrolysis, acidification and methanogenesis. Usually, sludge components are cemented and flocculated together by extracellular polymeric substance (EPS) which are mainly composed of microbiologically produced protein and carbohydrate. Thus, their solubilization and hydrolysis are considered as rate-limiting steps. In this study, the soluble protein and carbohydrate concentrations in digestion represented the solubilization of sludge organic matters. Fig. 3 illustrated the changes of soluble protein and carbohydrate. During the initial three days of digestion, the levels of soluble protein and polysaccharide showed an upward trend whether the hydrochar existed or not, and the concentrations of soluble protein and polysaccharide at any time were in the sequence of hydrochar (220 °C) > hydrochar (260 °C) > blank. For instance, the maximum concentration of protein (carbohydrate) in blank was 82.85 (44.98) mg/L, whereas, the highest concentration of soluble protein (carbohydrate) for hydrochar (220 °C) and hydrochar (260 °C) were 393.62 (76.46) mg/L and 142.85 (52.85) mg/L, respectively. The higher soluble substances provide a more substrate for subsequent utilization of acid-forming bacteria, which is one of the reasons for the enhancement of SCFA production in the presence of hydrochar.

EPS is a complex high molecular-weight mixture of polymers (Mw > 10,000) excreted by microorganisms, produced from cell lysis and hydrolysis, and adsorbed organic matter from wastewater (Zhao et al., 2016a). 3D-EEM is a useful method to characterize the fluorescence compounds in EPS and expound the potential mechanisms of sludge solubilization improvement (Luo et al., 2013). The fluorescence spectra of EPS extracted from raw WAS and sludge treated by hydrochars was displayed in Fig. 1(III and IV). Two peaks were readily identified from fluorescence spectra EEM in the raw sludge (Fig. 1(III)). The first main peak located at excitation/emission wavelengths (Ex/Em) of 275/350 (Peak A) and another main peak located at Ex/Em of 225/(325–370) were related to the protein-like substance (Ahmad and Reynolds, 1999), which were accordance with the previous studies that soluble microbial by-product has strong fluorescence intensity at the excitation/emission wavelengths of 250–280/<380 nm (Chen et al., 2003). Compared with blank, hydrochar (220 °C) had obvious positive effect to sludge solubilization. In the presence of hydrochar (220 °C), two peaks were detected (Fig. 1(IV)): Peak C (Ex = 250–280, Em < 380) was more enhanced than the control, indicating that the EPS dissolution were improved; Peak D (Ex > 280, Em > 380) related to the humic substance (Chen et al., 2003) also had numerous quantity. The similar phenomenon occurred in presence of hydrochar (260 °C), while these two similar peaks were weaker than that of hydrochar (220 °C). The above experimental results revealed that hydrochar was more beneficial to the sludge solubilization.

Usually, the EPS adhered on the surface of sewage sludge can be dissolved into the water phase under the action of certain substances (such as surfactants) (Jiang et al., 2007). It has been reported that some humic acids have the characteristics of surfactant and then promote the dissolution of organic matters (Lovely et al., 1996). The characterization results of two hydrochars confirmed that hydrochar (220 °C) contained more organic matters such as humic acids than hydrochar (260 °C). The experimental results of the enhanced sludge solubilization by hydrochars, especially by hydrochar (220 °C) could attribute to its own humic acid.

3.3.2. Effect of hydrochars on hydrolysis and acidification processes

The effects of hydrochar on the hydrolysis and acidification processes were investigated using synthetic wastewater containing model substrates and the results were summarized in Table 2. It can be seen that the degradation rate of BSA in the presence of hydrochar was higher than that in the blank, which was consistent with the results shown in Fig. 3. Contrarily, the presence of hydrochar has insignificant impact on the degradation of polysaccharides compared with that in blank ($p > 0.05$). For example, the degradation rate of polysaccharides in blank at the 3rd day was

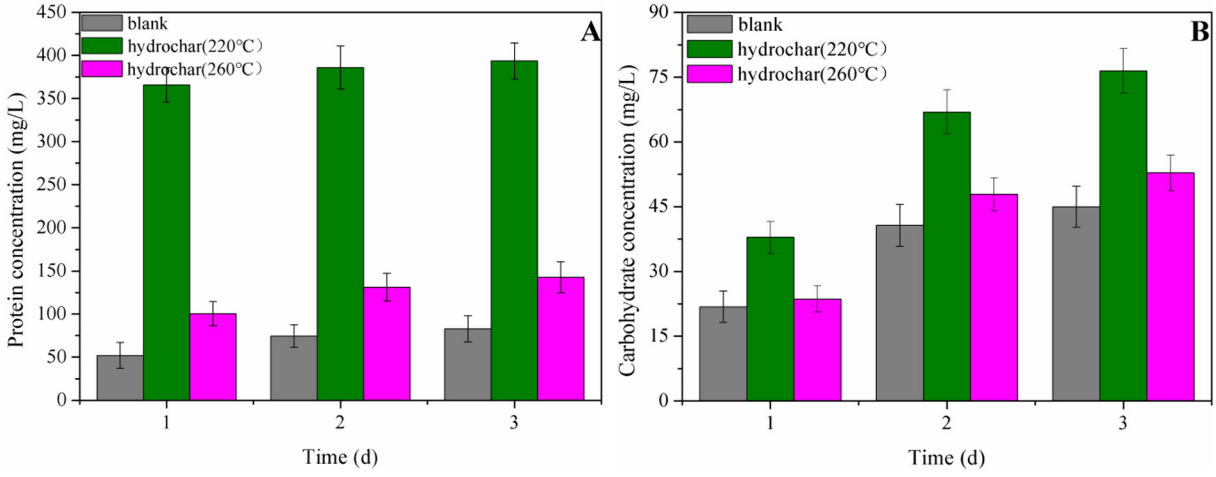

Fig. 3. Effect of hydrochars on the solubilization of protein (A) and carbohydrate (B). Error bars represent standard deviations of triplicate tests.

Table 2
Effect of hydrochars on the degradation of model compounds.

Hydrochar	Degradation rate (%)[a]				
	BSA	Dextran	L-alanine	Glucose	Acetate
Without	14.1 ± 1.1	12.1 ± 1.1	23.7 ± 2.9	24.1 ± 2.2	79.6 ± 3.2
Hydrochar (220 °C)	26.2 ± 1.3	13.9 ± 1.3	82.9 ± 3.7	79.3 ± 2.9	45.2 ± 2.1
Hydrochar (260 °C)	20.3 ± 1.1	12.9 ± 1.1	60.9 ± 3.5	56.9 ± 2.4	56.9 ± 2.8

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

12.1%, and the corresponding data in the presence of hydrochar (220 °C) and hydrochar (260 °C) were 13.9% and 12.9%. Similar results were also observed at first two days.

The impact of hydrochar on the acidification process can be seen from Table 2. The presence of hydrochar also can promote the degradation of L-alanine and glucose. The degradation rate of L-alanine in the presence of hydrochar (220 °C) and hydrochar (260 °C) was 82.9% and 60.9%, which were much higher than that in blank (23.7%). The degradation of glucose was similar. In addition, the degradation rate of model compounds enhanced by hydrochar (220 °C) was higher than those by hydrochar (260 °C). Those observations indicated that hydrochar could accelerate the degradation of glucose and L-alanine, and then improve the production of SCFA. Those results adequately consolidated the positive function of hydrochar on the hydrolysis and acidification processes. It was reported that some humic acids can significantly enhance the production of SCFA from WAS (Liu et al., 2015) because they can act as an electron acceptor and then improved the production of acetic acid (Lovely et al., 1996).

3.3.3. Effect of hydrochars on methanogenesis processes

Methanation is the last step in the anaerobic process, where SCFA was consumed as a digestive substrate by methanogenic bacteria to produce the methane. Methanogens are sensitive to environmental condition, and the environment changes have a great impact on the activities of methanogenic bacteria (Li et al., 2016b).

Table 2 showed the influence of hydrochar on the methanogenesis process. It was clear that the degradation rate of acetate was 79.6% in blank at the 2nd day, whereas the corresponding degradation rate was 45.2% in the presence of hydrochar (220 °C), which was around half of that in blank. Similar experimental results were observed at whole methanogens processes. The experimental results showed that the activities of methanogenic archaea were seriously inhibited in the presence of hydrochar. As shown in Fig. 1, humus was an important part of hydrochar. Humic substances can compete with methanogenic bacteria for the limited electrons in the reaction system, and posed a negative impact on the reaction of acetyl-CoA→5-methyl-THMPT, which caused less SCFA being consumed (Liu et al., 2015).

3.4. Effect of hydrochars on key enzyme activities related with SCFA

Sludge anaerobic digestion is a biological reaction, which was adjusted by a large number of biological enzymes. Therefore, the change of enzyme activities can also reflect the impact of hydrochar on sludge anaerobic digestion process. As for hydrolytic enzymes, proteases break down proteins into amino acids and α-glucosidase is responsible for the decomposition of polysaccharide to monoses. As for acid-forming enzymes, PTA could decompose acetyl-CoA to acetyl and then further converted to acetate with the function of AK (Feng et al., 2009). OAATC could supply the carbon flux from the central carbon metabolism to propionic acid (Feng et al., 2009). CoA transferase could catalyze the reactions of succinic acid to succinyl CoA and propionyl CoA to propionic acid (Feng et al., 2009). The activities of key enzymes were measured at the 3rd day and acid-forming enzymes were measured on their optimal conditions. The blank test was set as 100%. It was clear that the activity of protease and α-glucosidase in the presence of hydrochar was 1.9 and 1.6 times of that in blank, respectively. The increase of hydrolase activities in the presence of hydrochar indicated that the hydrolysis process was improved, which was consistent with the experimental results shown in Fig. 3. As to acid-forming enzymes, hydrochar benefited to the enhancement of their activities, which were almost 1.7 times as much as that in blank. Those observations suggested that hydrochar could remarkably promote the activity of key enzymes responsible for hydrolysis and acidification process. The reason might be as follows: due to the improved solubilization led by

hydrochar, the key enzymes originally existed in the pellet fraction of EPS matrix were released (Yu et al., 2008) and then improved the hydrolysis and acidification process. The reason for the improvement of key enzymes activities with hydrochar addition might be attributed to that hydrochar can decompose sludge floc and release the key enzymes originally existed in the pellet fraction of EPS matrix. Additionally, hydrochar can provide an attachment site for the biological enzyme due to its porous properties, which increases the contact between key enzyme and fermentation substrate.

Although hydrochar is less easily decomposed than uncarbonized materials (Mumme et al., 2014), it can still provide suitable amount of easily bioavailable nutrients such as proteins and diverse volatile organic compounds during anaerobic digestion (Becker et al., 2013; Reza et al., 2014), which can be confirmed by the results of proximate and ultimate analysis in this study. Some scientists pointed out that the hydrochar produced at lower temperature (220 °C) could retain the majority of organic matters such as protein and humic acid (Dümpelmann et al., 1991). However, when the temperature is higher than 240 °C, the loss rate of these organic matters would significantly increase. That is to say, hydrochar produced at 220 °C retains the majority of organic matters, while the other hydrochar loses a lot. Organic matters in hydrochar might play an important role in the SCFA production. According to the fluorescence excitation emission matrix spectra of hydrochars (Fig. 1), lots of humic substance was detected and the stronger fluorescence peaks was obtained in hydrochar produced at 220 °C than the other one, which indicated that more humic substances were detected in hydrochars produced at 220 °C. It has been documented that humic acid could improve the production of SCFAs but inhibit methane production during sludge anaerobic digestion (Cervantes et al., 2000; Liu et al., 2015).

Humus generally contains two categories humic acid and fulvic acid, so it is necessary to distinguish which is the key factor that gives rise to the accumulation of SCFA. Experimental results with synthetic wastewater showed that the presence of fulvic acid can promote the production of SCFA, and the production of SCFA was 31.88 mg COD/L, which was around 1.11-fold of that in blank (28.71 mg COD/L). However, the presence of humic acid can better improve the accumulation of SCFA, and the yield of SCFA reached to 130.95 mg COD/L. Both humic acid and fulvic acid in hydrochar all can accelerate the dissolution of sludge, and the effect of humic acid on the acidification was strong.

4. Conclusion

In sludge anaerobic digestion, the production of SCFA was significantly improved by adding hydrochars. The mechanism of improving SCFA production is that the hydrochars accelerate the solubilization and hydrolysis of particulate organic matters in sludge, enhance the acidification of hydrolyzed products, and inhibit the methanogenic bacteria. Further study showed that the numerous organic matters such as humic acid existed in the hydrochar played more important role, and the effect of inorganic substances such as heavy metals during anaerobic digestion was insignificant. The degradation experiment suggested that the SCFA production derived from the degradation of hydrochar could be ignored. This study provides a promising way to reuse WAS.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2017.07.051.

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