A new process to improve short-chain fatty acids and bio-methane generation from waste activated sludge

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ABSTRACT

As an important intermediate product, short-chain fatty acids (SCFAs) can be generated after hydrolysis and acidification from waste activated sludge, and then can be transformed to methane during anaerobic digestion process. In order to obtain more SCFA and methane, most studies in literatures were centered on enhancing the hydrolysis of sludge anaerobic digestion which was proved as un-efficient. Though the alkaline pretreatment in our previous study increased both the hydrolysis and acidification processes, it had a vast chemical cost which was considered uneconomical. In this paper, a low energy consumption pretreatment method, *i.e.* enhanced the whole three stages of the anaerobic fermentation processes at the same time, was reported, by which hydrolysis and acidification were both enhanced, and the SCFA and methane generation can be significantly improved with a small quantity of chemical input. Firstly, the effect of different pretreated temperatures and pretreatment time on sludge hydrolyzation was compared. It was found that sludge pretreated at 100°C for 60 min can achieve the maximal hydrolyzation. Further, effects of different initial pHs on acidification of the thermal pretreated sludge were investigated and the highest SCFA was observed at initial pH 9.0 with fermentation time of 6 d, the production of which was 348.63 mg COD/gVSS (6.8 times higher than the blank test) and the acetic acid was dominant acid. Then, the mechanisms for this new pretreatment significantly improving SCFA production were discussed. Finally, the effect of this low energy consumption pretreatment on methane generation was investigated.

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Introduction

Primary and secondary sludges are the two different wastes generated in municipal wastewater treatment plants (WWTP). Primary sludge is produced from a mechanical wastewater treatment process, and, excess secondary sludge is a settling material produced at the secondary sedimentation tank of the wastewater treatment plant after biological treatment. Waste activated sludge (WAS) contains amount of non-hydrolyzable particulate materials and biomass due to the biological metabolism process. The activated sludge technology is used widely as an effective biological method of WWTP, so a large amount of WAS is generated from WWTP annually. The WAS containing high levels of organic matter, it may become a plentiful source of inexpensive organic substrate for fermentative short-chain fatty acid (SCFA) and bio-methane production, by which reduction and stabilization of organic wastes can also be accomplished (Wong et al., 2013). In order to

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prevent secondary environmental pollution caused by WAS and reutilize the sludge as a useful resource, the treatment and disposal methods of WAS have been becoming a more and more interesting topic to many researchers in recent years. Among these different treatments and disposal methods of WAS, the anaerobic fermentation technology is considered as an efficient approach to stabilization and sustainable re-utilization of WAS (Gbosh et al., 1975; Liu et al., 2006).

Considering that SCFA is the key intermediate product that can directly influence the bio-methane generation of WAS during the two-phase anaerobic fermentation process, the strategies to enhance SCFA production have become a very heated research topic to many anaerobic fermentation researchers (Carrere et al., 2010; Tyagi and Lo, 2011; Baier and Schmidheiny, 1997; Cuetos et al., 2010; Eskicioglu et al., 2007; Pilli et al., 2011; Li et al., 2012; Zhang et al., 2009; Frigon et al., 2012; Kampas et al., 2007; Tong and Chen, 2007; Zhang et al., 2008; Zhang and Chen, 2009). It is well acknowledged that the four stages, i.e., hydrolysis, acidogenesis, acetogenesis, and methanogenesis, are contained during the sludge anaerobic fermentation process, so varies of pretreatments to improve the production of SCFA and bio-methane from WAS were conducted by many anaerobic fermentation researchers (Carrere et al., 2010; Tyagi and Lo, 2011), including physical pretreated methods (microwave, ultrasonic, mechanic and thermal pretreatments) (Baier and Schmidheiny, 1997; Cuetos et al., 2010; Eskicioglu et al., 2007; Pilli et al., 2011), chemical pretreated methods (Li et al., 2012; Zhang et al., 2009) and biological pretreatment methods (enzyme pretreatment) (Frigon et al., 2012). However, some studies only focused on the hydrolysis stage, the acidogenesis stage had not been actually taken into consideration during the whole anaerobic fermentation process (Kampas et al., 2007; Tong and Chen, 2007; Zhang et al., 2008; Zhang and Chen, 2009). The final bio-methane generation can be improved by these different sludge pretreatments, but energy consumptions of these pretreatment methods, during which the electric energy, thermal energy and chemical energy were consumed, has been scarcely considered.

It has been studied in our previous publication that the methane generation can be enhanced under alkaline condition (Zhang et al., 2010). With pretreated sludge at pH 10 for 8 days, the methane production was improved more significantly than the traditional thermal pretreatment, initial alkaline pretreatment and thermal-alkaline pretreatment. However, it costs too much energy to apply this method to the practical projects due to the large quantity of alkaline added to sustain the alkaline condition and large quantity of acid for pH neutralization process. In this paper, a low energy consumption method to accelerate both the sludge hydrolysis and acidogenesis for significantly enhancing the SCFA production has been investigated. The mechanisms for improvement in SCFA production of the low energy consumption pretreatment were conducted. In order to investigate the effect of this new sludge pretreated method on methane production, two systems of two-phase anaerobic digested reactors were operated semi-continuously and we come to the conclusion that the energy consumption of this new process was more economical than other pretreatment

methods, which suggested that it was quite meaningful to the engineering projects.

1. Materials and methods

1.1. Sludges

The WAS was withdrawn from the sedimentation tank of a WWTP in Shanghai, China, and then concentrated immediately by settling at 4°C for 24 hr. The main characteristics of raw sludge are as follows: total suspended solids (TSS) 25,162 ± 505 mg/L, volatile suspended solids (VSS) 17,511 ± 96 mg/L, total chemical oxygen demand (TCOD) 24,828 ± 334 mg/L, soluble chemical oxygen demand (SCOD) 99 ± 4.7 mg/L, total carbohydrate (as COD) 2963 ± 132 mg/L, total protein (as COD) 12,634 ± 431 mg/L, soluble carbohydrate (as COD) 5.3 ± 0.2 mg/L and soluble protein (as COD) 43 ± 1.6 mg/L. The anaerobic digestion sludge, which was obtained from the up-flow anaerobic sludge bed (UASB) reactor of a food wastewater treatment plant in Yixing, China, was used as the inoculums. The main characteristics of anaerobic digestion sludge are as follows: TSS 34,471 ± 1034 mg/L, VSS 24,340 ± 430 mg/L, total carbohydrate (as COD) 3318 ± 128 mg/L, and total protein (as COD) 15,287 ± 611 mg/L.

1.2. Comparison of different thermal pre-treatments affecting WAS hydrolyzation

Twenty anaerobic reactors made of Plexiglas, with 2.0 L working volume each, internal diameter of 100 mm and height of 255 mm were applied in the thermal pretreatment trails. A 36 L portions concentrated WAS was divided equally into the 20 anaerobic reactors. The 20 anaerobic reactors were divided equally into four groups (five reactors in each group), and fermentation temperature of group 1 to 4 was autoclaved respectively (Manufacture Belge de Gembloux, Belgium) at 60°C, 80°C, 100°C and 120°C (1 bar), respectively. Further, the raw sludge of reactors 1 to 5 of each group was pretreated respectively for 15, 30, 45, 60 and 75 min. In order to investigate effects of the thermal pretreatment above on sludge hydrolysis, the SCOD of each reactor was investigated. Meanwhile, soluble protein and carbohydrates were analyzed as they were the dominating soluble organic matters of sludge.

1.3. Comparison of different initial pHs affecting WAS acidification

A series of anaerobic reactors made of Plexiglas, with 2.0 L working volume each, internal diameter of 100 mm and height of 255 mm were conducted in the fermentation experiments to compare different initial pHs affecting WAS acidification. All the reactors were controlled at 35 ± 1°C and stirred at 100 r/min to mix the contents. The WAS no less than 16.2 L was pretreated at 100°C for 60 min, and then divided equally into 1–9 reactors so as to make each reactor contain 1.8 L thermal pretreated sludge. The initial pHs of 1–9 reactors were adjusted to 4, 5, 6, 7, 8, 9, 10, 11 and 12 respectively, by adding 4 mol/L HCl and 5 mol/L Ca(OH)$_2$. Reactor 10 which also contained 1.8 L raw sludge, was controlled as blank test without thermal or pH adjustment. Then each of these reactors was filled with 100 mL anaerobic digestion

sludge as inoculums. All experimental reactors were capped with rubber stoppers and sealed after oxygen removal by nitrogen gas flushing for 1 min. Accumulated concentrations of individual SCFA (acetic acid, propionate, iso-butyrate, n-butyrate, iso-valerate and n-valerate) were monitored every two days and the concentrations of organic compounds of sludge were detected before and after the anaerobic fermentation process.

1.4. Semi-continuous-flow experiments for key enzymes and microbial community study

Ten semi-continuous-flow reactors made of Plexiglas, with working volume of 2.0 L each, internal diameter of 100 mm and height of 255 mm were operated to investigate enzyme activity that related to promoting SCFA production and protein and carbohydrate decomposition. Microbial community in the optimal SCFA production reactor would also be tested. 16.2 L thermal pretreated WAS (pretreated at 100°C for 60 min) was divided equally into the 1–9 semi-continuously operated reactors and the initial pHs of 1–9 reactors were adjusted to 4, 5, 6, 7, 8, 9, 10, 11 and 12 respectively, by adding 4 mol/L HCl and 5 mol/L Ca(OH)$_2$. Reactor 10 which also contained 1.8 L raw sludge, was controlled as blank test without thermal or pH adjustment. Then each of these reactors was filled with 100 mL anaerobic digestion sludge as inoculums. The fermentation temperature and sealing operations were the same as above. Every day, 300 mL fermentation mixture was withdrawn under mixing conditions from the reactor manually and the same amount of sludge, which had been pre-treated or un-pretreated the same as described above, was added, which means that the hydrolytic retention time (HRT) of these semi-continuously operated reactors was 6 days. When the SCFA production remained stable after three months running, the analysis of enzyme activity and microbial community was conducted.

1.5. Application of the fermentative SCFA as the carbon source for methane production

During the anaerobic fermentation process, SCFA can be utilized as favorable carbon sources to produce methane. In order to investigate the effect of new sludge pretreated method on methane production, two systems of two-phase anaerobic digested reactors, with 20.0 L working volume each (containing SCFA-production and gas-production reactors with internal diameter of 180 mm and height of 393 mm respectively), were operated semi-continuously (Fig. 1). In system One, before the sludge (8.0 L) was added into SCFA-production reactor, the sludge was pre-treated at 100°C for 60 min and then adjusted the initial pH to 9.0 by adding 5 mol/L Ca(OH)$_2$. 450 mL anaerobic digestion sludge was inoculated. Every day, 1.3 L fermentation sludge was withdrawn from the SCFA-production reactor manually and the same amount of sludge, which had been pre-treated the same as described above, was added, meaning that the HRT was 6 days, while 400 mL fermented sludge from SCFA-production reactor was added to the following gas-production reactor to keep the HRT of gas-production reactor as 20 days. In system Two (controlled as blank test), the raw sludge (8.0 L) was directly added into SCFA-production

Fig. 1 – Semi-continuously operated two-phase anaerobic digested reactor (single SCFA-production or gas-production reactor). SCFA: short-chain fatty acid.

reactor without pretreatment. 450 mL anaerobic digestion sludge was inoculated. Similar as above, 1.3 L fermentation sludge was withdrawn from the SCFA-production reactor manually every day and the same amount of sludge, was added, which means that the HRT was 6 days too, while the 400 mL fermented sludge from SCFA-production reactor was added to the following gas-production reactor to keep the HRT of gas-production reactor as 20 days. The fermentation temperature and sealing operations were controlled the same as above. The SCFA and gas production became stable after 3 months running, then the produced gas was collected by the water displacement method. The gas component was detected by a gastight syringe and a gas chromatograph (Agilent 6890N, USA) which equipped with a thermal conductivity detector (TCD) using nitrogen as the carrier gas. In this study bio-methane yield was reported as the amount (L) of methane generated per m^3 of reactor per day (L-CH$_4$/m^3-reactor/day) unless otherwise stated.

1.6. Analytical methods

The determination of TCOD, SCOD, VSS and TSS were conducted according to the Standard Methods (APHA et al., 1998). The analyses of soluble protein, carbohydrates, SCFA, methane, hydrolytic enzymes (Protease and α-glucosidase) and the enzymes related to acid production including phosphotransbutyrylase (PTB), phosphotransacetylase (PTA), butyrate kinase (BK), acetate kinase (AK), CoA transferase and oxaloacetate transcarboxylase (OAATC) were the same as described in our previous publications (Zhang et al., 2010, 2011).

For purpose of microbial community investigation of the fermentation sludge after the low energy consumption pretreatment, a blended culture sample was centrifuged at 13,000 r/min for 15 min (4°C), and the total genomic DNA was extracted using a Power Soil® DNA Isolation Kit (MOBIO Laboratories, Inc., USA). Quality control of the extracted DNA was carried out by 0.8% agarose electrophoresis using Ethidium Bromide as the dye. The amount and purity of

DNA were checked by ultraviolent spectrophotometer at 260 and 280 nm.

The bacterial 16S rRNA genes were amplified by PCR with primers 7F (5′-CAGAGTTTGATCCTGGCT-3′) and 1540R (5′-AGGAGGTGATCCAGCCGCA-3′). The PCR mixture contained 0.5 μL of each primer (10 μmol/L), 1 μL template DNA, 0.5 μL dNTP mixes (10 mmol/L each), 2.5 μL 10 × Taq reaction buffers, 0.2 μL Taq polymerases (5 U), and sterile deionized water to a final volume of 25 μL. PCR amplification program was performed as follows: initial denaturation step for 5 min at 94°C followed by 35 cycles of 30 s at 94°C, 35 s at 55°C and 1 min at 72°C, combined final extensions for 8 min at 72°C. The PCR products were purified with Gene JET™PCR purification kit (Fermentas), and then ligated into the pMD 18-T Vector (TaKaRa, Dalian, China) and used to transform *Escherichia coli* DH5α competent cells by ampicillin selection and blue/white screening. The white colonies were selected randomly and inserts were amplified using pGEMT-specific primers M13F and M13R. The sequencing was conducted by Sangon Biotech. Co., Ltd. (Shanghai, China) using ABI PRISM 3730 automated DNA sequencer and the sequences with more than 97% similarity were classified into same operational taxonomic units (OTUs). Phylogenetic tree was generated with MEGA 5 (Tamura et al., 2011).

1.7. Statistical analysis

All tests were performed in triplicate and the results were expressed as mean ± standard deviation. An analysis of variance (ANOVA) was used to test the significance of results and $P < 0.05$ was considered to be statistically significant.

2. Results and discussion

2.1. Effect of different pretreatments on WAS hydrolysis, acidification and the composition of SCFA

In this study sludge hydrolysis and acidification were expressed respectively by the changes of observed soluble COD and SCFA (Yuan et al., 2006). The effects of the thermal pretreated temperatures and pretreated time on total WAS hydrolyzation are shown in Fig. 2. With the growth of pretreatment time from 15 to 60 min, the SCOD concentration was enhanced significantly ($F = 17.94$, $F_{crit} = 3.86$, $P_{(0.05)} = 3.88 \times 10^{-4} < 0.05$) except the blank test. No significant increase was observed when pretreatment time reached 75 min ($F = 3.53$, $F_{crit} = 10.13$, $P_{(0.05)} = 0.16 > 0.05$). The concentration of SCOD was also increased significantly as pretreatment temperatures (from 60°C to 120°C) went up ($F = 59.01$, $F_{crit} = 3.86$, $P_{(0.05)} = 3.05 \times 10^{-6} < 0.05$). At lower pretreatment temperatures (60°C and 80°C), the maximal SCOD concentration was only 2540 and 3145 mg/L respectively, but at higher pretreatment temperatures (100°C and 120°C), the maximal SCOD concentration was improved to 4311 and 4197 mg/L respectively, which means that hydrolysis process cannot be sufficiently completed at lower pretreatment temperatures. Obviously, the peak concentration of SCOD was achieved when sludge was pretreated at 100°C for 60 min (4311 mg/L), which was almost 40 times greater than blank test (104 mg/L).
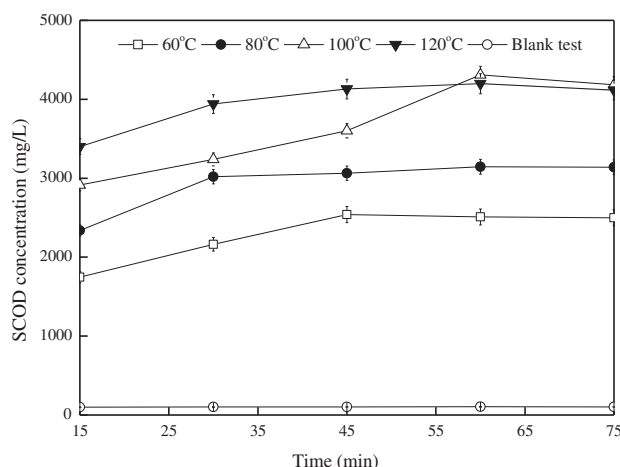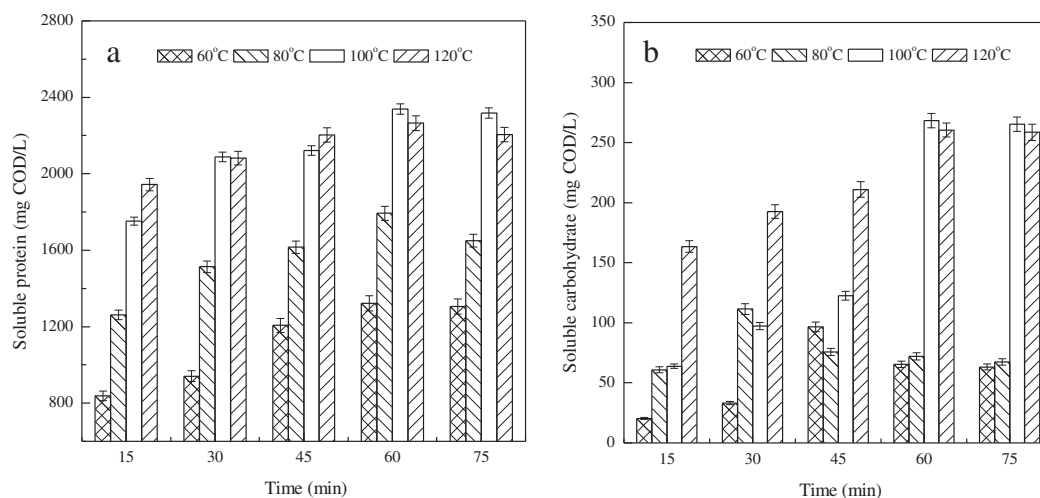

Fig. 2 – Effect of different pretreatment temperatures and pretreated time on soluble chemical oxygen demand concentration. Error bars represent standard deviations of triplicate tests.

Although more SCOD concentration can also be obtained by further increasing the pretreatment time and temperature, such as 75 min and 120°C, it would need more energy consumption to keep the high pretreated temperatures. The effect of different pretreatment temperatures and pretreated time on soluble carbohydrate and protein concentrations were also investigated, which can represent the hydrolyzation of sludge fermentation.

In this study, the raw sludge consisted of 11.9% carbohydrate and 50.8% protein on the basis of TCOD. Thus, the sludge hydrolysis was further investigated by centralizing the soluble carbohydrate and protein. Fig. 3 shows the changes of soluble carbohydrate and protein with pretreatment temperatures and pretreated time. It can be seen in Fig. 3 that both soluble carbohydrate and protein concentrations reached the maximum (268.2 mg COD/L and 2339 mg COD/L respectively) when pretreatment time extended to 60 min at 100°C thermal pretreatment. The maximal soluble carbohydrate and protein concentration of the blank test, by contrast, was only 9.1 and 85 mg COD/L respectively. It is obvious that after sludge thermal pretreatment more soluble carbohydrate and protein can be used to generate SCFA during the acidification process. Taking efficiency and economy into consideration, it seems that the best hydrolysis can be obtained by pretreating the WAS at 100°C for 60 min. Therefore, to investigate the effects of alkaline pretreatment on WAS acidification process, pre-treating WAS at 100°C for 60 min was chosen as the optimal hydrolysis condition in the following experiments. The pre-treatment at 100°C for 60 min has significantly negatively affected the methanogens activity, thus, the methane production volume in this study was actually very low and could be ignored.

The effects of different initial pHs and fermentation time on total SCFA generation from thermal pretreated sludge are illustrated in Fig. 4. The initial total SCFA of the raw sludge was 1.10 mg COD/g VSS (data not shown in Fig. 4). With growth of fermentative time of 6 days, SCFA production was

Fig. 3 – Effect of different pretreatment temperatures and pretreated time on soluble protein (a) and carbohydrate (b) concentrations. Error bars represent standard deviations of triplicate tests.

continuously enhanced except at initial pH 7.0 and 8.0, at which the SCFA had already reached its peak value on the 4th day, and maximal SCFA production was occurred at initial pH 9.0 (348.6 mg COD/g VSS), then no remarkable improvement of SCFA was detected after the 6th day ($F = 2.36$, $F_{crit} = 5.98$, $P_{(0.05)} = 0.18 > 0.05$). Although the production of SCFA at initial pH 11.0 and 10.0 were still increased from fermentative time and reached 271.2 and 301.4 mg COD/g VSS on the 12th day and 14th day respectively, the growth slowed during the following fermentative time and could not reach the maximum value of SCFA concentration (348.6 mg COD/g VSS). It is obvious that the SCFA production was quite low at the extreme initial pH values (such as pH 4.0 or 12.0) during all the fermentative time. The reason may be that these conditions were too toxic for the growth of acidogenic microbes. The active microorganisms were killed or inhibited. Furthermore, this phenomenon might be attributed to the

inhibitory effect of acidogenic bacteria under strong acid or alkali conditions. Therefore, the SCFA generation can be remarkably enhanced and maintained stable by adjusting initial pH to 9.0.

The composition of SCFA was also analyzed. Six SCFA, including acetic, propionic, iso-butyric, n-butyric, iso-valeric and n-valeric acids were detected. It can be seen in Fig. 5 that acetic acid was the dominant SCFA either in pH adjusted experiments or blank test and the acetic acid percentage under alkali conditions was greater than acidic conditions except pH 12.0. When the initial pH was adjusted to 9.0, the acetic acid percentage accounting for total SCFA was 68.2%, which was much greater than other initial pHs with 6-days fermentation time. According to Figs. 4 and 5, the production of individual SCFA of each experiment at fermentation time of
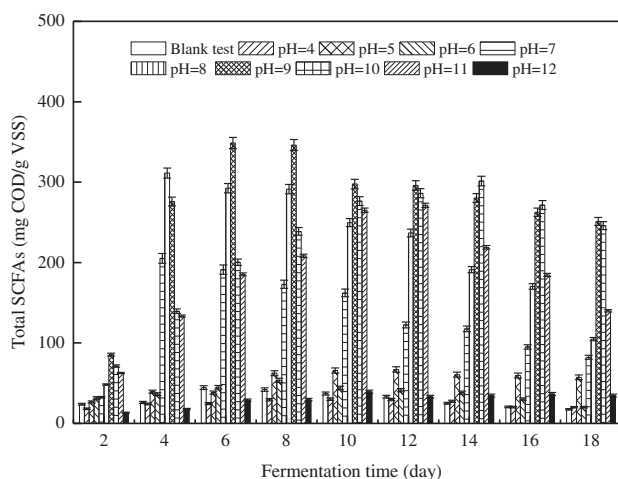
Fig. 4 – Effect of different initial pH and fermentation time on total SCFA production. Error bars represent standard deviations of triplicate tests.
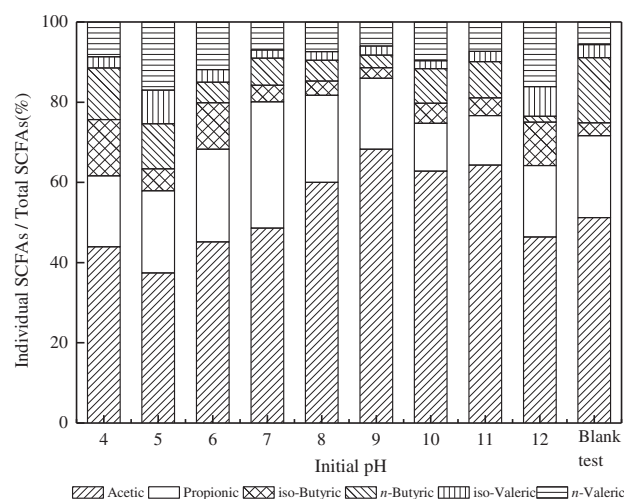

Fig. 5 – Percentage of individual SCFA accounting for total SCFA at different initial pHs with fermentation time of 6 days. The data reported are the averages of triplicate tests.

6 days can be easily calculated. After thermal pretreatment at 100°C for 60 min, by adjusting the initial pH 9.0, the maximum acetic acid production was observed on the 6th fermentation day. Under these suitable conditions, acetic, propionic, iso-butyric, n-butyric, iso-valeric and n-valeric production were 238.1, 61.5, 9.1, 11.1, 7.9 and 20.8 mg COD/g VSS respectively on the 6th fermentation day, while in the blank test the acetic concentration was only 22.8 mg COD/g VSS. The above results showed that the concentration and percentage of acetic acid in SCFA as well as the total SCFA generation can be remarkably enhanced by the low energy consumption pretreatment, i.e. hydrolyzing at 100°C for 60 min and then adjusting initial pH 9.0, meanwhile, it suggested that there was a great potential to generate more methane from low energy consumption pretreated sludge in the next anaerobic fermentation step.

2.2. Effect of low energy consumption pretreatment on the fermentation substrates consumptions

Carbohydrate and protein are two most dominant organic matters of raw sludge, and the consumption of which are related to the formation of total SCFA during anaerobic digestion processes (Feng et al., 2009; Yuan et al., 2006). In this study, the carbohydrate and protein accounted for 11.9% and 50.8% respectively on the basis of sludge TCOD, according to the characteristics of the WAS described above. Therefore, the consumption of organic matters can be represented by the changes of carbohydrate and protein concentrations, and the calculation method was as follows: consumed protein (or carbohydrate) = total protein (or carbohydrate) in-put – total protein (or carbohydrate) out-put. Fig. 6 illustrates the effects of initial pH on the carbohydrate and protein consumptions with fermentation time of 6 days. The results showed the carbohydrate and protein consumptions at initial pH 9.0 were higher than those at other initial pHs, which was consistent with the fact that the total SCFA concentration was the highest at initial pH 9.0. It should be noted that even though the blank test had no thermal pretreatment or pH adjustment,
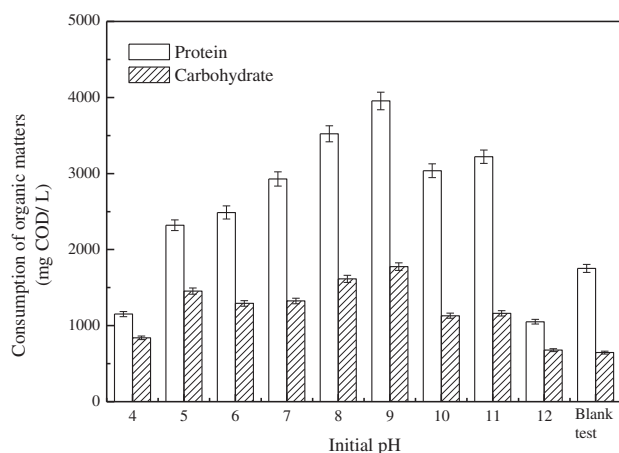
its consumption of organic substrates was still better than the extreme initial pH 4.0 and pH 12.0. It suggests that the inhibiting effect of extreme pH can irreversibly destroy the acidogenic bacteria of sludge to generate SCFA, whereas, the appropriate initial pH can enhance the production of SCFA significantly.

2.3. Effect of low energy consumption pretreatment on the observed activities of key hydrolysis and acidification enzymes

The effect of low energy consumption pretreatment on hydrolysis and acidification can also be expressed from the aspect of enzymes. The proposed metabolic pathways to hydrolysis of protein and carbohydrate and SCFA production from sludge was reported in our previous studies, with some key enzymes labeled (Feng et al., 2009; Zhang et al., 2011). Protease and α-glucosidase are the critical enzymes which are responsible for hydrolyzing polysaccharide and protein to monosaccharide and amino acids respectively, the products then turn into pyruvate and other intermediates. In the metabolic pathway for acidification, acetyl-CoA and butyryl-CoA are first transformed to acetyl and butyryl phosphate by PTA and PTB. These acyl phosphates are then transformed to acetic and butyric acid by AK and BK, respectively. CoA transferase and OAATC are two key enzymes for propionic acid synthesis as OAATC can catalyze the reactions of pyruvate to oxaloacetate and methylmalonyl CoA to propionyl CoA, and CoA transferase can catalyze propionyl CoA to propionic acid and succinic acid to succinyl CoA. The enzymes relevant to hydrolysis and acidification mentioned above were analyzed on the 6th day fermentative time and shown in Table 1. It can be observed that the activity of both the polysaccharide and protein hydrolysis enzymes (α-glucosidase and protease) and key enzymes related to SCFA production (PTA, PTB, AK, BK, CoA transferase and OAATC) were improved after low energy consumption pretreatment except pH 12.0. The highest activity occurred at initial pH 9.0, which were well corresponded with the consumption of organic matters and the production of SCFA mentioned above.

2.4. Microbial community analysis of the anaerobic digestion system after low energy consumption pretreatment

The microbial community in the long-term operated reactor with optimal low energy consumption pretreated sludge (pretreated sludge at 100°C for 60 min and then adjusted initial pH 9.0) was investigated by the PCR-based 16S rRNA gene clone library. A total of 101 clones were obtained and the phylogenetic tree was constructed by using MEGA 5.0 and applying the neighbor-joining method. The simplified neighbor-joining tree of the bacteria with different DNA which were classified according to their genus (Fig. 7), the specific tree of bacteria with whole different DNA can be seen in appendix A Fig. S1. It can be concluded from Fig. 7 that *Porphyromonadaceae*, *Clostridiaceae*, *Ruminococcaceae* and *Clostridiales*, with percentage of 25.74, 21.78, 18.81 and 13.86 respectively, formed a dominant community structure of the reactor under the given condition described above. It was reported that members of the *Porphyromonadaceae* family, which ranked the first place, generate various SCFA from carbohydrates or proteins, *e.g.*, *Petrimonas* produces acetic acid during carbohydrates fermentation and

Fig. 6 – Effect of initial pH on the consumptions of organic matters with fermentation time of 6 days. Error bars represent standard deviations of triplicate tests.
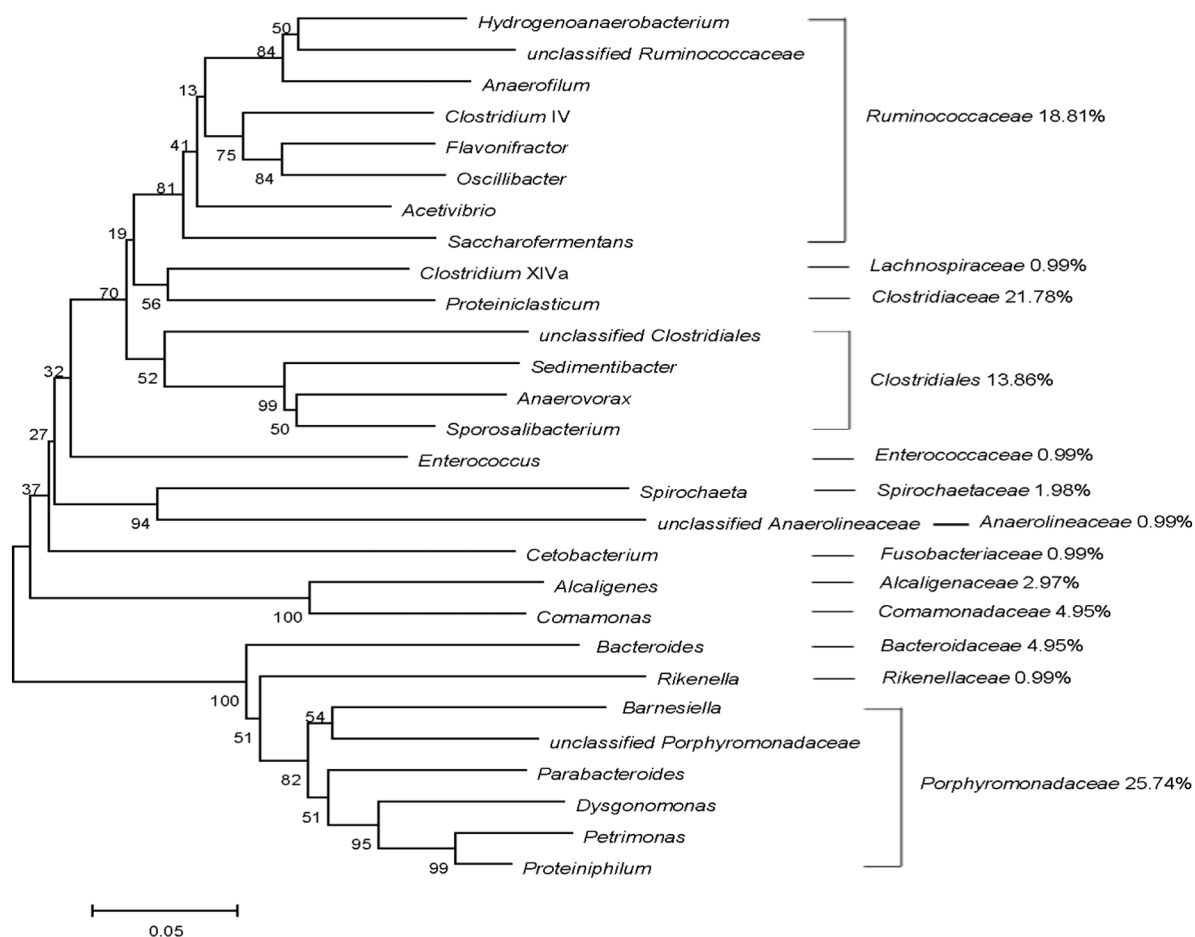
Table 1 – Effect of low energy consumption pretreatment on specific activity of key enzymes involved in hydrolysis and acidification at fermentation time of 6 days [a].

| Initial pH	Protease (U/mg VSS)	α-Glucosidase (U/mg VSS)	PTA (U/mg VSS)	AK (U/mg VSS)	PTB (U/mg VSS)	BK (U/mg VSS)	OAATC (U/mg VSS)	CoA transferase (U/mg VSS)
4.0	0.0017	0.0177	0.1326	1.0467	0.0030	0.0487	0.1653	0.0852
5.0	0.0016	0.0200	0.1435	1.2486	0.0032	0.0536	0.4958	0.1124
6.0	0.0024	0.0212	0.2351	1.3588	0.0033	0.0629	0.7161	0.4068
7.0	0.0025	0.0236	0.3149	2.0382	0.0038	0.0620	0.8447	0.9053
8.0	0.0029	0.0274	0.3788	2.1668	0.0046	0.0793	0.9548	1.0759
9.0	0.0036	0.0305	0.4914	3.4521	0.0052	0.0915	1.4874	1.4222
10.0	0.0033	0.0175	0.3536	1.9464	0.0039	0.0802	0.8814	0.9078
11.0	0.0031	0.0192	0.2914	1.2670	0.0036	0.0786	0.8079	0.8117
12.0	0.0022	0.0127	0.1225	1.0074	0.0023	0.0472	0.1469	0.0788
Blank	0.0028	0.0168	0.1762	1.1936	0.0026	0.0563	0.4407	0.1095

[a] The data are the averages of three different measurements. PTA: phosphotransacetylase, AK: acetate kinase, PTB: phosphotransbutyrylase, BK: butyrate kinase, OAATC: oxaloacetate transcarboxylase.

Proteiniphilum generates acetic and propionic acids from amino acids (Ziganshin et al., 2011). It was observed that 6 genera belong to family *Porphyromonadaceae*. *Clostridiaceae* and *Ruminococcaceae*, which was second and third abundant family and both belong to class *Clostridia*, were also found in anaerobic hydrogen producing systems, some bacteria of which could use different mono-, di-, and oligosaccharides to generate acetic acid (Abreu et al., 2011). The fourth abundant family *Clostridiales* also belongs to class *Clostridia*, which was related to acetic and propionic acids generation (Mitchell, 1997).

Fig. 7 – Simplified neighbor-joining phylogenetic tree of bacteria present in the semi-continuously reactor with low energy consumption pretreated sludge (pretreated at 100°C for 60 min and initial pH 9.0). The scale bar represents 0.05 substitutions per nucleotide position.
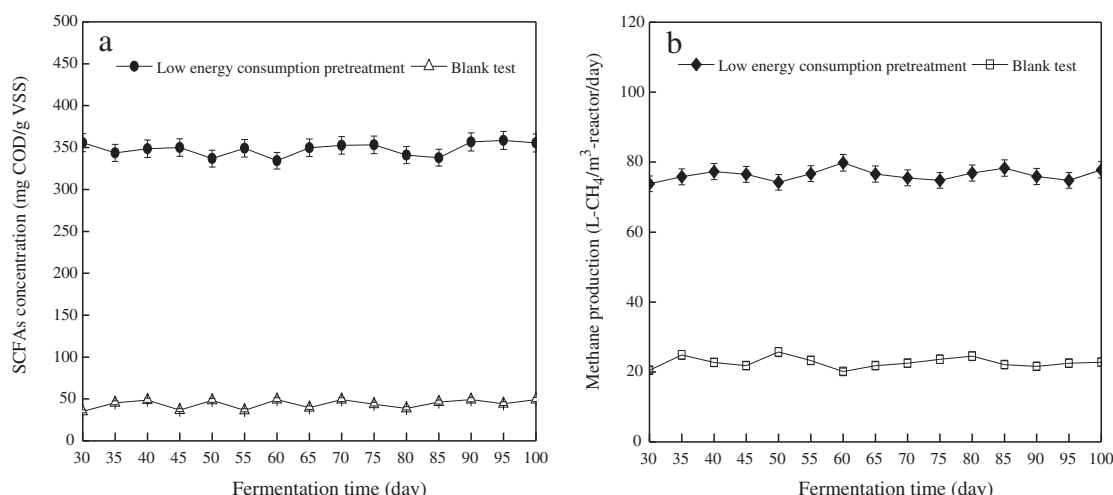
Fig. 8 – SCFA concentration (a) and methane production (b) from low energy consumption pretreated (at 100°C for 60 min and initial pH 9.0) sludge and un-pretreated sludge during the fermentation period of 100 days. Error bars represent standard deviations of triplicate tests.

2.5. Application of the fermentative SCFA by different pretreatment strategies

As mentioned above, the methanogenesis is the last stage of the sludge anaerobic digestion process after the generation of SCFA, which can be used as a favorable carbon source for methane production, therefore, the generation of methane varied from different pretreatment strategies as well as the production of SCFA did. In order to investigate the effect of this low energy consumption pretreated method on sludge methane production, two systems of two-phase anaerobic digested reactors (SCFA-production and gas-production reactors) were operated semi-continuously. Fig. 8 demonstrates the variations of the SCFA concentration and methane production from low energy consumption pretreated sludge (pretreated at 100°C for 60 min

and initial pH of 9.0) and un-pretreated sludge during the fermentation period of 100-d. It can be found that the average SCFA concentration from low energy consumption pretreated sludge was 348.6 ± 13.9 mg COD/g VSS, whereas that of un-pretreated sludge was only 44.5 ± 2.2 mg COD/g VSS (Fig. 8a). On the other hand, the average methane production from low energy consumption pretreated sludge was 76.4 ± 3.1 L-CH$_4$/m^3-reactor/day, whereas that of un-pretreated sludge was only 22.7 ± 0.9 L-CH$_4$/m^3-reactor/day (Fig. 8b). It suggested that the enhanced SCFA from low energy consumption pretreated sludge can be applied to improve methane production. Low energy pretreatment method can significantly enhance the whole three stages (hydrolysis, acidogenesis and methanogenesis) of the anaerobic fermentation process at the same time.

Table 2 – Energy balance of sludge anaerobic digestion by different pretreatment strategies [a].

Pretreatment strategies	Cost (kWh/t-TSS/day)			Income (kWh/t-TSS/day)	Balance (kWh/t-TSS/day)
	Ca(OH)$_2$ [b,c]	HCl [d]	Temperature	Methane [e]	
Low energy consumption pretreatment (at 100°C for 60 min and initial pH 9)	12.3	0	92.8	157.5	52.4
Alkaline pretreatment (pH 10) [f]	245.1	31.8	17.3	169.7	-124.5
Blank test (un-pretreated)	0	0	17.3	19.9	2.6

[a] The unit is (kWh/t-TSS/day). Cost: the cost of different pretreatment strategies, Income: the income of different fermentation strategies, Balance: the balance of cost and income.
[b] The energy consumption of Ca(OH)$_2$ is 729.3 kWh/t. The total added amount of Ca(OH)$_2$ for adjusting the initial pH 9 was 0.101 t/t-TSS, *i.e.* 0.017 t/t-TSS/day (divided by 6 days), and the added amount of Ca(OH)$_2$ in alkaline pretreatment was 0.336 t/t-TSS/day.
[c] The energy consumption of NaOH is 2755.3 kWh/t. When NaOH was used in the reactor of alkaline pretreatment (pH 10 pretreated sludge), the added amount of NaOH was 0.328 t/t-TSS/days, and the balance was −783.1 kWh/t-TSS/day.
[d] The dosage of HCl solution used to adjust pH from 10 to 7 in the reactor of alkaline pretreatment was 0.049 t/t-TSS/day. Its energy consumption is 648.3 kWh/t.
[e] The methane production was 32.4 (low energy consumption pretreated sludge), 34.9 (pH 10 pretreated sludge), and 4.1 m^3/t-TSS/day (un-pretreated sludge), respectively.
[f] See our previous study (Zhang et al., 2010); the alkaline pretreatment method performance better than the ultrasonic, thermal and thermal-alkaline pretreatment strategies.

Although the methane generation can be enhanced significantly from sludge by alkaline pretreatment (pH 10 for 8 days) through improving both of the hydrolysis and acidification processes in our previous study (Zhang et al., 2010), by which the methane production was improved more significantly than the traditional thermal pretreatment, simple alkaline pretreatment and thermal-alkaline pretreatment, the new pretreatment method had a vast of chemical cost which was considered uneconomical. Table 2 illustrates the energy balance of sludge anaerobic digestion by different pretreatment strategies when bio-methane was generated from the low energy consumption pretreated (at 100°C for 60 min and initial pH 9.0) sludges, pH 10.0 pretreated sludge and un-pretreated sludge at the maximal methane production respectively. Although the energy income of methane generation of the low energy consumption pretreated sludge and pH 10.0 pretreated sludge were 157.5 and 169.7 kWh/t-TSS/day (kWh per ton of total suspended sludge solids per day) respectively, the energy cost of chemical and temperature from aforementioned pretreatment strategies was 105.1 and 294.2 kWh/t-TSS/day. Thus, it can be seen clearly that the maximal energy net balance reached 52.4 kWh/t-TSS/day of the low energy consumption pretreated sludge due to the less energy consumption of chemicals ($Ca(OH)_2$ and HCl) and high generation of methane, which was much greater than the alkaline pretreated sludge and the traditional un-pretreated sludge.

3. Conclusions

The present study introduced a new method of efficiently and economically enhanced SCFA and bio-methane generation from WAS by low energy consumption pretreatment. With the optimal conditions of low energy consumption pretreatment, *i.e.*, pretreated sludge at 100°C for 60 min and adjusted initial pH 9.0 then fermented for 6 days, the maximal SCFA generation (348.6 mg COD/g VSS) can be reached, which was higher than that reported previously. Besides, acetic acid was found to be the dominant product of the content of 68.2% during the acidogenesis stage and it can be used as the carbon resource to produce methane in the following stage. Further, mechanisms for the low energy consumption pretreatment significantly improving SCFA production were discussed. The largest carbohydrate and protein consumption and the highest activities of the key relevant enzymes were all observed under the optional conditions of low energy consumption pretreatment. In addition, the 16S rRNA gene clone library demonstrated that *Porphyromonadaceae*, *Clostridiaceae*, *Ruminococcaceae* and *Clostridiales* were the dominant microbial community in this anaerobic digestion system.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jes.2015.10.004.

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