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Alkaline fermentation of primary sludge for short-chain fatty acids accumulation and mechanism

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ABSTRACT

Short-chain fatty acids (SCFAs) are the intermediate products of anaerobic digestion. The alkaline fermentation of primary sludge (PS) for ambient accumulation of SCFAs and mechanism were investigated in the present study. The results showed that the maximum SCFAs yields from PS fermentation was 312.9 mg COD/g VSS at pH 10.0–11.0 after 5 days reaction time, which was 1.8 times of that at neutral and acid pHs. The composition and distribution of SCFAs generated from PS anaerobic fermentation were studied, the maximum ratio of acetic, propionic, iso-butyric, *n*-butytic, iso-valetic and *n*-valetic acids was determined as 49.4%, 34.4%, 14.6%, 12.2%, 17.9% and 6.3%, respectively. In addition, the accumulation of SCFAs related with soluble organic compounds fermentation and methane production during PS fermentation was also investigated. The concentration of SCFAs generated from PS fermentation was found to be positive with the concentration of soluble organic compounds but negative with the production of methane. The accumulation of SCFAs at alkaline pHs can be attributed to the enhanced hydrolysis of PS at alkaline pHs which lead to more soluble protein and carbohydrate generation. At the same time, the activity of methanogens could be inhibited at alkaline pHs which resulting in less SCFAs consumption.

1. Introduction

It has been well acknowledged that biological removal of nutrients (phosphorus and nitrogen) from wastewater is an effective technique to mitigate the eutrophication of water body. Nowadays the influent of many municipal WWTPs in China, particularly in the south of China, is characterized by very low organic carbon concentration, which significantly limits the nutrients removal efficiency. In order to enhance the nutrients removal, methanol, ethanol and acetic acid are normally used as organic carbon source during the wastewater treatment process [1–3]. However, the addition of organic chemicals as carbon source increases the operation cost of WWTP.

During the past several years, waste containing rich of readily biodegradable COD has been proposed as extra available organic carbon source for the enhanced nutrients removal [4]. It has been reported that SCFAs could be produced from the fermentation of primary or waste activated sludge [5–10]. PS generating from primary settling tank in WWTP is quite distinct from the WAS produced at the secondary sedimentation tank. The PS normally involves a high portion of organic matter such as vegetables, fruits, feces, paper, textiles, etc. [11]. Additionally, it owns different biodegradation characteristics from WAS. According to the literature, PS contained more easily biodegradable organic matter and more living biomass was involved in the biodegradable fraction of VSS in PS compared to that of WAS [12]. Therefore, it can be feasible to produce more SCFAs as carbon source using PS as fermentation substrate.

In the sludge anaerobic fermentation process, pH is an important factor which can significantly affect the hydrolysis of sludge as well as the production of SCFAs. Yuan et al. [8] reported that high SCFAs production was obtained under the alkaline condition during WAS fermentation process. It was proposed that the high SCFAs production was due to more SCOD was generated and methanogens was inhibited under alkaline pH. Our previous study also indicated that alkaline condition was beneficial for SCFAs production during PS fermentation process [13]. However, the mechanism of SCFAs accumulation for PS fermentation under alkaline pH is still unclear up to now.

The aim of the present study was to investigate the alkaline fermentation of PS for SCFAs accumulation and to explore the mechanism of SCFAs accumulation under alkaline pH. In this study, the production of SCFAs from PS fermentation at different pHs was systematically investigated. The composition and the distribution of SCFAs generated from the PS fermentation were studied. In addition, the accumulation of SCFAs related with soluble

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Nomenclature

BNR	biological nutrient removal
BSA	bovine serum albumin
$C_{protein}$	total protein (mg COD/L)
COD	chemical oxygen demand (g/L)
EBPR	enhanced biological phosphorus removal
GC	gas chromatography
HCl	hydrochloric acid (M (mol/L))
IN	inorganic nitrogen (mg/L)
NaOH	sodium hydroxide (M (mol/L)
NH_4^+-N	ammonia nitrogen (mg/L)
NO_2^--N	nitrite nitrogen (mg/L)
NO_3^--N	nitrate nitrogen (mg/L)
PS	primary sludge
SCFAs	short-chain fatty acids (mg COD/L)
SCOD	soluble chemical oxygen demand (g/L)
SRT	solid retention time
TCD	thermal conductivity detector
TKN	total Kjeldahl nitrogen (mg/L)
TSS	total suspended solids (g/L)
VSS	volatile suspended solids (g/L)
WAS	waste activated sludge
WWTP	wastewater treatment plant
$Y_{total\ protein}$	total protein concentration (mg COD/L)
$Y_{total\ SCFAs}$	total SCFAs concentration (mg COD/L)

organic compounds fermentation and methane production during PS fermentation was also investigated. Finally, the mechanism of SCFAs accumulation under alkaline pH was discussed. Hopefully, the present study could provide guidance for the preparation of extra available organic carbon source from the PS fermentation for enhanced nutrients removal.

2. Materials and methods

2.1. PS source

The PS used in this study was obtained from the primary settling tank of a municipal WWTP in Shanghai, China. The source PS was concentrated by settling for 24 h and stored in a fridge at 4 °C. Three samples of concentrated PS were analyzed and the main characteristics of the concentrated PS are shown in Table 1. It can be seen from Table 1 protein and carbohydrate were two main organic compounds in the PS with approximately 63.0% of VSS.

2.2. Batch experiments

In order to investigate SCFAs production from PS at different pHs and to dig out the reasons of alkaline pH increasing SCFAs production, the following experiments (each with triplicate) were developed.

Table 1
Characteristics of the concentrated PS.

Parameters	Mean ± SD[a]
pH	5.54 ± 0.03
TSS (g/L)	27.45 ± 0.42
VSS (g/L)	18.98 ± 0.22
SCFAs (mg COD/L)	141.60 ± 9.49
SCOD (g/L)	5.86 ± 0.38
Total COD (g/L)	29.27 ± 1.69
Total carbohydrate (g/L)	3.77 ± 0
Total protein (g/L)	8.09 ± 0.16
Lipid and oil (g/L)	0.67 ± 0.04

[a] SD, standard deviation of triplicate analyses.

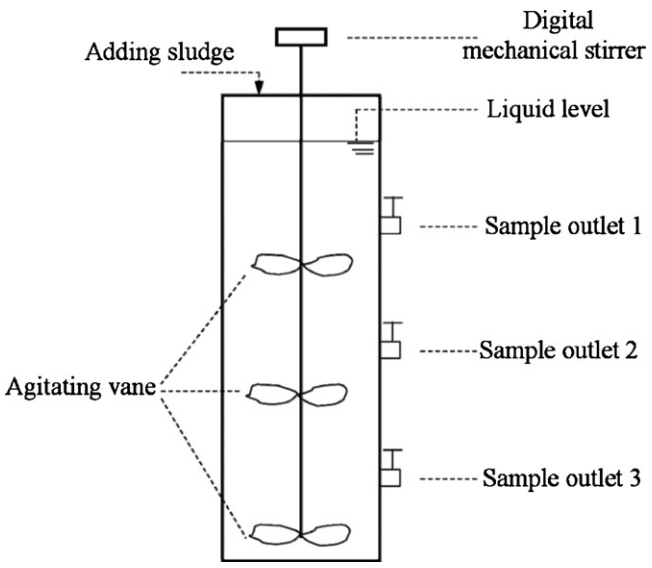

Fig. 1. Schematic diagram of experimental set-up.

2.2.1. PS fermentation at different pHs

To investigate the SCFAs production from PS fermentation at different pHs, 10 identical anaerobic reactors (Fig. 1) were used in this study. The reactors were made of plexiglass and each reactor had a working volume of 3.0 L (120 mm internal diameter, 300 mm height and 3.4 L total volume). All reactors were equipped with stainless-steel stirrers for mixing the sludge. A portion of settled PS was taken and diluted to 30 L with tap water (controlling VSS concentration at 11,000 mg/L), and then 30 L prepared PS solution was equally divided into 10 anaerobic reactors. During the fermentation test, all reactors were continuously stirred at an approximate speed of 70 rpm in order to sustain homogenous mixing. The temperature of the reactors was maintained at room temperature (25 ± 1 °C). We defined the first reactor as blank reactor which was with no pH control, the natural pH was tested at 5.5. The pH in another 9 reactors was controlled at 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0, respectively, by adding appropriate dosage of 2 M NaOH or 2 M HCl. The NaOH and HCl solution was automatically added according to the pH changes of the PS solution.

2.2.2. Comparison between alkaline and acidic pH affecting SCFAs accumulation with synthetic wastewater of protein and carbohydrate

In order to explore whether the production of SCFAs was directly related to the fermentation of protein and carbohydrate under acidic or alkaline conditions, batch fermentation experiment with BSA (model protein compound used in this study) and glucose (model carbohydrate compound) were conducted at two representative pH of 5.0 and 10.0, respectively. Four tapered glass bottles with tight covers and working volume of 1000 mL were used as reactors instead of the anaerobic reactors mentioned above. A certain amount of BSA (456.0 or 1262.7 mg) or glucose (73.8 or 183.2 mg) was dissolved into 900 mL of tap water before 100 mL PS was added to each bottle as inoculums. The initial concentration of BSA or glucose in the four bottles was controlled at 684 mg COD/L BSA (pH 5 reactor), 79 mg COD/L glucose (pH 5 reactor), 1894 mg COD/L BSA (pH 10 reactor) and 196 mg COD/L glucose (pH 10 reactor), respectively, which approximated to the COD concentration of soluble protein or carbohydrate at pH 5.0

or 10.0. Then the pH in the four bottles was adjusted to 5.0 or 10.0 by adding 2 M NaOH and 2 M HCl solution. Finally, the four bottles were put in a thermostatic water bath shaker for mixing and sustaining constant temperature (25 ± 1 °C) in the whole fermentation process. The concentrations of SCFAs, BSA and glucose in the four bottles were detected at different fermentation time.

2.2.3. Methane production during PS fermented at different pHs

Reduced activity of methanogens could lead to an increase of SCFAs as well as a reduction of methane production [14]. Batch experiment on methane production from PS fermentation was conducted in 10 identical glass culture bottles with tight rubber stoppers, each bottle was with a working volume of 250 mL. A 2.5 L PS solution was equally divided into ten 250 mL bottles, the gas in the headspace of the bottle was removed by nitrogen gas sweeping for 30 s. A thermostatic water bath shaker was used to mix sludge (stirring speed of 70 rpm) and the temperature was maintained at room temperature (25 ± 1 °C). One bottle with no pH controlling was defined as the blank reactor, the pH in another bottles was controlled at 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0, respectively.

2.3. Analytical methods

All analyses were conducted in triplicate. Sludge samples taken from the reactors were centrifuged at 3500 rpm for 10 min and then filtered through normal quantitative paper. The filtrate was promptly analyzed for SCOD, soluble carbohydrate and soluble protein. TSS, VSS, total COD were measured according to standard methods [15]. For the quantification of SCFAs, the filtrate was further filtered through a Whatmann GF/C glass microfiber filter (0.45 μm pore size) and acidified with 3% H_3PO_4 to pH 2.0–3.0 before analyzed by GC [16]. An Agilent 6890N GC with flame ionization detector and DB-WAXETR column (30 m × 1.0 μm × 0.53 mm) was used for the analysis of SCFAs. Nitrogen was the carrier gas with a flow rate of 25 mL/min. The temperature of the injection port and the detector were maintained at 220 and 250 °C, respectively. The initial oven temperature was programmed at 110 °C and remained for 2 min, and then the oven temperature was increased to 200 °C at a rate of 10 °C/min and held at 200 °C for 2 min. The injection volume of the sample was 1.0 μL.

Carbohydrate was measured by the anthrone method with glucose as standard [17]. Soluble protein was determined by the Lowry–Folin method with BSA as standard [18]. Sludge lipid was extracted by the Bligh–Dyer method from the acidified sample, and then was measured gravimetrically after the solvent was evaporated at 80 °C [15]. The total protein content of sludge was estimated from the corresponding TKN concentration by subtracting IN (NH_4^+-N, NO_3^--N, NO_2^--N, etc.) concentration and dividing the difference by 0.16 (conversion factor of protein to NH_4^+-N), then multiplying the result by 1.5 (COD conversion factor of protein) [19], Eq. (1). TKN was determined by titrimetric method after distillation from Chinese standard methods [20].

$$C_{\text{protein}} = (\text{TKN} - \text{IN}) \times \frac{1.5}{0.16} \quad (1)$$

The methane production was analyzed by GC (Model 14B, Shimadzu, Japan) equipped with a TCD and a 3 m stainless column packed with Porapak Q (80/10 mesh). The temperature of the injection port, oven column and detector were set at 40, 50 and 90 °C, respectively. Nitrogen was used as the carrier gas at a flow rate of 30 mL/min.

3. Results and discussion

3.1. Effect of pH on total SCFAs production

According to previous reports, pH can significantly affect the digestion efficiency of the digester for treating high concentration of solids and played an important role during the anaerobic solubilization of domestic PS. In this study, the effects of pH and fermentation time on total SCFAs accumulation are shown in Fig. 2. The total SCFAs (expressed by mg COD/g VSS) includes acetic, propionic, iso-butytic, *n*-butyric, iso-valeric and *n*-valeric acids, they are converted to COD by using appropriate conversion factors as 1.07, 1.51, 1.82, 1.82, 2.04 and 2.04, respectively [10]. The initial total SCFAs concentration in the reactor was about 4.5 mg COD/g VSS (not shown in Fig. 2). As seen in Fig. 2, the production of SCFAs had a similar trend with fermentation time at different pHs except at pH 11.0. When pH was between 3.0 and 10.0, the average concentration of SCFAs increased rapidly with fermentation time and reached individual maximum on the 5th day, which followed the order: pH 10.0 (301.6 mg COD/g VSS) > pH 9.0 (291.2 mg COD/g VSS) > pH 8.0 (288.7 mg COD/g VSS) > pH 7.0 (269.3 mg COD/g VSS) > pH 5.0 (217.8 mg COD/g VSS) > pH 6.0 (206.3 mg COD/g VSS) > pH 4.0 (145.4 mg COD/g VSS) > pH 3.0 (83.1 mg COD/g VSS). Further increasing the fermentation time did not result in the increase of total SCFAs. Instead, the total SCFAs decreased with fermentation time after 5 days in most cases except at pH 11.0. Further investigation revealed that the total SCFAs concentration ($Y_{\text{total SCFAs}}$) was linearly increased with pH from 3.0 to 5.0 (Eq. (2)) and from 7.0 to 10.0 (Eq. (3)) on the 5th day of fermentation. After 5 days, however, total SCFAs concentration in pH 7.0–10.0 was linearly decreased with fermentation time (Eq. (4)–(7)).

$$Y_{\text{total SCFAs}} = 67.3\text{pH} + 14.1, \quad R^2 = 0.99 \quad (2)$$

$$Y_{\text{total SCFAs}} = 9.9\text{pH} + 262.8, \quad R^2 = 0.91 \quad (3)$$

$$Y_{\text{total SCFAs (pH 7.0)}} = -45.9t + 312.9, \quad R^2 = 0.99 \quad (4)$$

$$Y_{\text{total SCFAs (pH 8.0)}} = -43.3t + 326.3, \quad R^2 = 0.99 \quad (5)$$

$$Y_{\text{total SCFAs (pH 9.0)}} = -35.1t + 317.4, \quad R^2 = 0.98 \quad (6)$$

$$Y_{\text{total SCFAs (pH 10.0)}} = -35.5t + 344.6, \quad R^2 = 0.98 \quad (7)$$

As seen in Fig. 2, the SCFAs concentration at pH 11.0 was still increased with time from 5 to 13 days. At pH 11.0, the average of SCFAs concentrations was 215.2, 249.7 and 312.9 mg COD/g VSS on the 5th, 9th and 13th days, respectively, and then decreased slightly with the extension of fermentation time. In the blank test, it was found that the SCFAs concentration complied with the tendency as that at pH 3.0–10.0, but on the 9th day it attained the maximum.

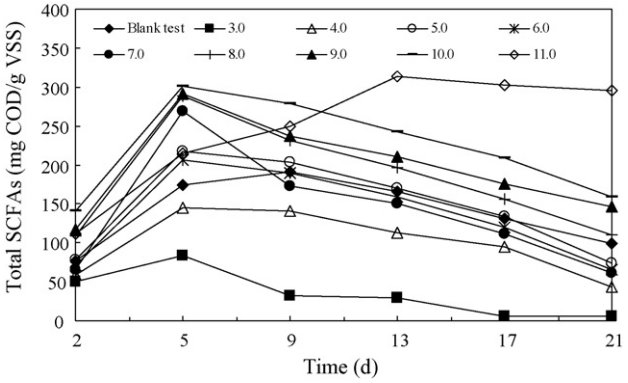

Fig. 2. Effect of pH and fermentation time on total SCFAs accumulation.

Moreover, during the entire fermentation time, the SCFAs concentration in the blank test was greater than that at pH 3.0 and 4.0, and similar with that at pH 7.0 at the end of fermentation. It was also found that the pH in the blank test changed from 5.0 to 6.0 and then near to 7.0 in the late phrase of fermentation. Apparently, more SCFAs produced at pH 5.0–7.0 and in the blank test than that at pH 3.0–4.0. Additionally, the SCFAs produced at pH 3.0 was obviously much less than that at other pHs and even decreased to zero at the end of operation, which indicated that low pH (less than or equal to 3.0) was unsuitable for PS fermentation to produce SCFAs.

The above results revealed that when the fermentation pH was controlled at 10.0, SCFAs yield could be significantly enhanced and maintained stable, the SCFAs yield observed in this study was also higher than that reported in the previous literature. Yuan et al. [8] reported that high SCFAs production of 256.2 mg COD/g VSS was obtained at pH 10.0 on the 8th day of WAS fermentation, which was 3–4 times of SCFAs production at pH 5.0 or uncontrolled pH. Compared to WAS, more SCFAs with 301.6 mg COD/g VSS was produced from PS fermentation at a shorter fermentation time (5 days). In other words, the PS fermentation tank just needs a small SRT to generate the same amount of SCFAs. Thus, smaller volume of fermentation tank was required when designed and less operation cost was consumed. From the above, it seemed that for more SCFAs accumulation the suitable condition could be controlled at pH 10.0–11.0 and with a fermentation time of 5–9 days in the present study, the optimum conditions were still considered as pH 10.0 and fermentation time 5 days. The reason for less SCFAs produced at pH 11.0 or 3.0 during the initial 5 days might be attributed to the toxic inhabitation of extreme alkaline or acidic condition to acidogenic bacteria. As observed in Fig. 2, an obvious SCFAs consumption was observed after 9 days of fermentation at pH between 3.0 and 10.0 as well as in the blank test, which might be due to part of SCFAs consumed by methanogens in the fermentation.

3.2. Compositions of SCFAs produced at the representative pH of 10.0

Increasing evidence suggests that the performance of EBPR relied on not only the total amounts but also the composition of SCFAs. According to the result of Mclnerney [21], SCFAs produced from PS fermentation consisted mainly of straight-chain or branch-chain SCFAs from 2 to 5 carbon atoms, i.e., acetic, propionic, iso-butyric, *n*-butyric, iso-valeric, and *n*-valeric acids. Figs. 3(a) and (b) shows the distribution of individual SCFAs and the percentage of individual SCFAs accounting for the total SCFAs at pH 10.0, respectively. As seen from Figs. 3(a) and (b), acetic acid was the most prevalent species in the initial 17 days with an average of 103.4 mg COD/g VSS or 43.8% of SCFAs. Its average production increased rapidly from 62.6 to 136.0 mg COD/g VSS when the time increased from 5 to 9 days, and then decreased to 67.5 mg COD/g VSS on the 17th day, which was less than that of propionic acid. The average percentage of acetic acid accounting for total SCFAs, during the initial 9 days, increased from 44.4% to 49.4%, which was higher than that of any other SCFA. However, it was found that the proportion of acetic acid began to decline and lower than that of propionic acid after 17 days. Rapid decrease of acetic acid might be the result of its fast utilization by acidogenic bacteria.

Propionic acid was the second major SCFA, its concentration increased from the beginning of fermentation and the average was more than 70.0 mg COD/g VSS in the fermentation of 5–17 days. It can be seen from Fig. 3(a) that the average content of propionic acid was higher than that of acetic acid in the fermentation of 17–21 days. Its maximum production occurred on the 5th day (75.0 mg COD/g VSS or 24.9% of total SCFAs). Instead of occurring at the 5th day, it was on the 17th day of fermentation that the maximum proportion (34.0%) of propionic acid was achieved. Higher

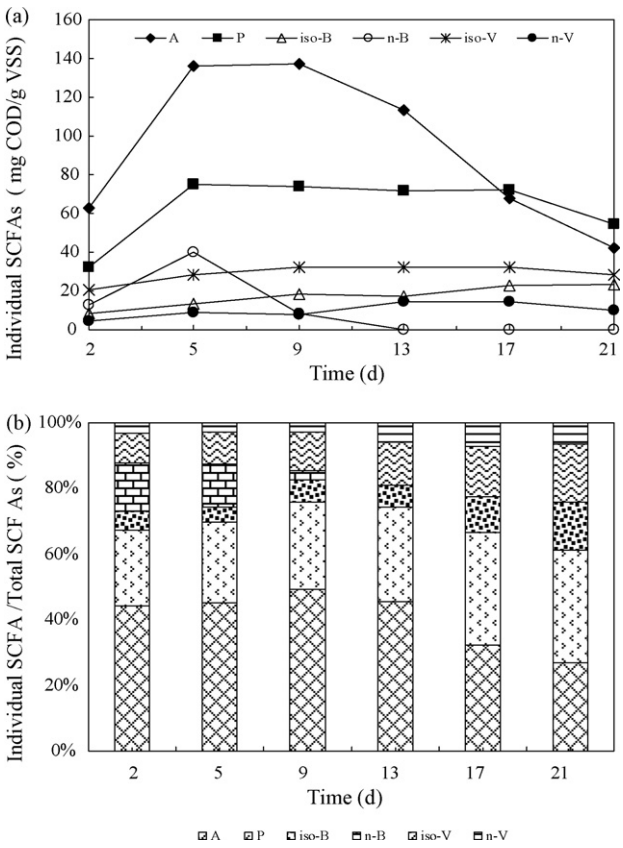

Fig. 3. Composition of SCFAs at pH 10.0: (a) individual SCFAs production and (b) percentage of individual SCFAs accounting for total SCFAs (acetic (A), propionic (P), iso-butyric (iso-B), *n*-butyric (*n*-B), iso-valeric (iso-V), and *n*-valeric (*n*-V)).

ratio of propionic acid (24.9–34.4%) in SCFAs produced from PS fermentation, far different from the result of less than 20.0% from WAS fermentation, could significantly promote EBPR performance if fermentation liquid with more propionic acid was used as carbon source of BNR in the light of previous reports by Chen et al. [16,22].

Iso-valeric acid and iso-butyric acid were relatively stable during the entire fermentation process and their maximum values were 32.5 mg COD/g VSS on the 9th day and 23.2 mg COD/g VSS on the 21st day, respectively, whereas their individual maximum percentage reached 17.9% and 14.6% on the 21st day. The maximum concentration of *n*-butyric and *n*-valeric acids was 39.9 and 14.6 mg COD/g VSS, respectively, or 13.2% and 7.0%. The higher percentages of iso-valeric and iso-butyric acids over *n*-butyric and *n*-valeric acids were observed, which might be due to their lower biodegradation rate compared with the corresponding SCFAs with linear chain.

3.3. Mechanisms of SCFAs accumulation during alkaline fermentation

Theoretically, during anaerobic fermentation SCFAs with 3–6 carbon atoms can be readily decomposed to acetic acid and acetic acid is gradually degraded into CH_4 and CO_2 by methanogens, which will cause less SCFAs accumulation. In this study, however, the reverse results were observed. It was found that higher pH of 8.0–10.0 caused higher SCFAs accumulation as compared to that at pH 3.0–7.0, which was different from the previous studies performed on the study of PS fermentation. Therefore, it was very necessary to investigate the reasons for SCFAs accumulation from PS fermentation under alkaline conditions.

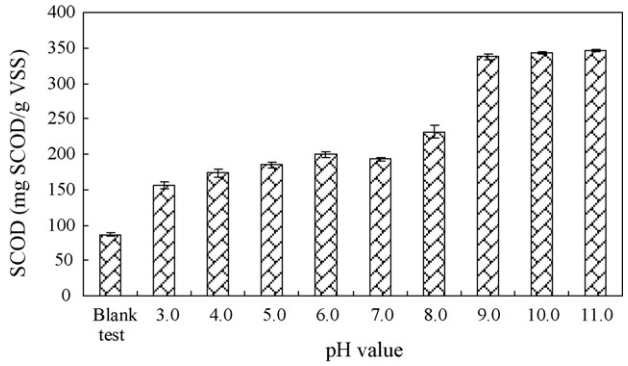

Fig. 4. Changes of SCOD during the sludge hydrolysis process at a 5-day fermentation time.

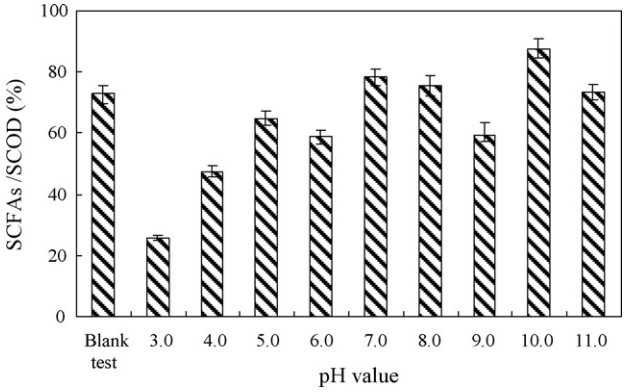

Fig. 6. Percentage of SCFAs in SCOD at different pHs on the 5th day of fermentation.

During anaerobic fermentation of particulate organic matter, the first step (hydrolysis) is usually the rate limiting in the overall degradation sequence [23,24]. Hydrolysis can be expressed by the change of SCOD [25]. Fig. 4 shows the changes of SCOD during the sludge hydrolysis process at a 5-day fermentation time. The average SCOD concentration increased significantly under alkaline conditions: pH 11.0 (346.8 mg SCOD/g VSS) > pH 10.0 (343.3 mg SCOD/g VSS) > pH 9.0 (337.7 mg SCOD/g VSS) > pH 8.0 (231.2 mg SCOD/g VSS) > pH 6.0 (199.6 mg SCOD/g VSS) > pH 7.0 (192.8 mg SCOD/g VSS) > pH 5.0 (185.1 mg SCOD/g VSS) > pH 4.0 (173.2 mg SCOD/g VSS) > pH 3.0 (156.2 mg SCOD/g VSS) > blank test (87.0 mg SCOD/g VSS). Hence, alkaline sludge hydrolysis was of more advantage than acidic one.

Domestic sludge usually contains three main constituents: protein, carbohydrate and lipid [26], and the formation of SCFAs during sludge anaerobic fermentation has been supposed to be related to the fermentation of these organic compounds [27]. In this study protein and carbohydrate were the two major constituents involved in the PS with the average ratio of 42.6% for protein and 19.9% for carbohydrate accounting for VSS. Thus, the compositions of SCOD, mainly on soluble protein and soluble carbohydrate, were further analyzed. Fig. 5 shows the changes of soluble protein and carbohydrate under different pHs at sludge fermentation time of 5 days. As seen in Fig. 5, higher productions of both soluble protein and carbohydrate under alkaline conditions were obtained compared to those under neutral pH or in the blank test, and especially at pH 9.0, 10.0 and 11.0, the values of soluble protein and carbohydrate observed were higher than that at any other condition. At any pH from 3.0 to 11.0, more soluble protein produced as comparison with carbohydrate. The concentration of soluble protein and carbohydrate was 150.0 and 19.5 mg COD/g VSS at pH 10.0, 189.3 and 28.4 mg COD/g VSS at pH 11.0, respectively. At the same pH 10.0 and 11.0, individual hydrolysis rate could be improved to 31.2% and 41.4% for soluble protein, 64.7% and 97.9% for soluble carbohydrate. The higher hydrolysis rate obtained during alkaline fermentation might be attributed to predominant association of protease and phosphatase enzyme activities with the organic particulate matter of the sewage sludge and a broad range of proteolytic activities with prominent enzyme activity at pH 10.0 [28]. At pH 10.0, more hydrolysis products, soluble protein and carbohydrate, would provide inevitably substrates for the subsequent acidification to produce more SCFAs.

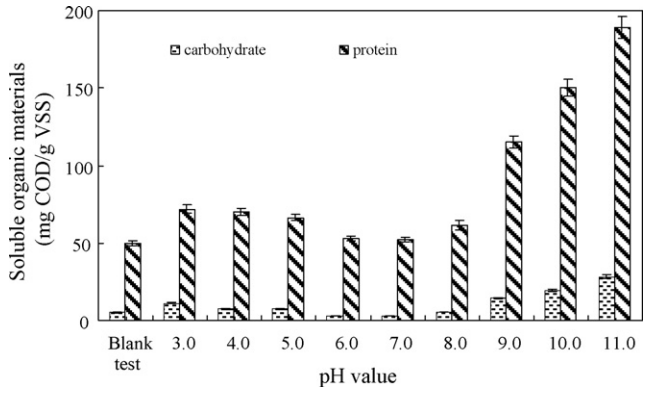

Fig. 5. Changes of soluble protein and carbohydrate under different pHs at sludge fermentation time of 5 days.

It is well known that the percentage of SCFAs in SCOD can be deemed as an indicator of acidogenesis activity [29]. SCFAs/SCOD ratio varied greatly according to previous studies conducted using PS [30,31]. The percentage of SCFAs in SCOD at different pHs on the 5th day of fermentation is shown in Fig. 6. It was observed that in most cases the ratio of SCFAs/SCOD under alkaline conditions was much higher than that under acidic conditions. This proved degree of acidification to be greater under alkaline conditions than others. For fermentation at pH 7.0 and in the blank test, however, the relatively high SCFAs/SCOD ratios did not cause more SCFAs accumulation because of the lower initial SCOD concentration produced in the hydrolysis process. At pH 10.0, it had a maximum SCFAs/SCOD percentage of 87.6% which indicated that a great amount of SCOD generated from PS hydrolysis was converted to more SCFAs via acidification. Therefore, alkaline condition especially at pH 10.0 was similarly helpful to sludge acidogenesis compared to other conditions in this study.

To further investigate whether the produced SCFAs were directly originated from the conversion of sludge soluble protein and carbohydrate, batch tests with BSA (a simulation compound of protein) and glucose (a model carbohydrate) were performed (Fig. 7), respectively. As shown in Fig. 7, SCFAs generation was observed when sole BSA or glucose was fermented at either pH 5.0 or pH 10.0. The SCFAs concentrations increased initially and then decreased with time, which might be caused by the consumption of some sludge microbes after 4 or 6 days of fermentation. Apart from fermentation with BSA as substrate at pH 10.0, the curves of SCFAs variation with time in other three cases had a similar trend. The observed SCFAs concentration at pH 10.0 was significantly higher than that at pH 5.0. Also, the data in Fig. 7 showed that BSA had higher SCFAs accumulation than glucose at either pH 5.0 or 10.0. The above results revealed that even at pH 10.0 the production of SCFAs from PS was associated with its fermentation of protein and carbohydrate, but more protein was contributed to produce more SCFAs due to its higher content in PS.

In theory, if particular strategies could be adopted to reduce or prevent the conversion of SCFAs to methane during sludge fermentation, it would lead to more SCFAs accumulation as well. The

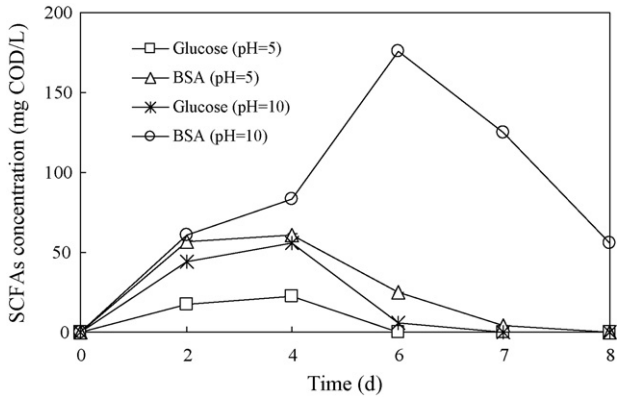

Fig. 7. Comparison between SCFAs production from BSA and glucose.

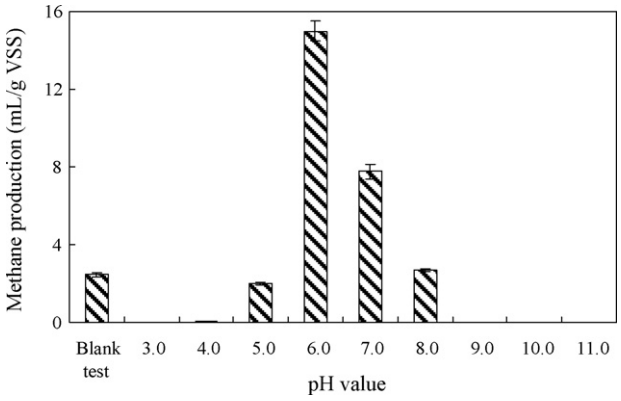

Fig. 8. Methane production at different pHs at the fermentation of 5 days.

methane production at different pHs is shown in Fig. 8. As seen in pH 3.0–6.0, the methane production increased with the increase of pH, but it decreased with further increasing pH from 7.0 to 10.0. Much higher methane yields were observed at pH 6.0 and 7.0 than other conditions. This might be caused by most methanogenic archaea functioning in a neutral or near neutral pH range [32,33]. Also, further investigation revealed that the methane production ($Y_{methane}$) linearly decreased with pH from 6.0 to 9.0 expressed by Eq. (8).

$$Y_{methane} = -5.0\text{pH} + 43.9, \quad R^2 = 0.96 \tag{8}$$

In the blank test, about 2.5 mL/g VSS of methane, similar to that at pH 5.0, was achieved. The reason generating similar amount of methane in these two cases might be attributed to almost the same amount of SCFAs previously produced being consumed in the blank test as well as at pH 5.0. Also, it can be seen in Fig. 8 that there was almost no methane generated at pH 10.0, 11.0 and 3.0. Thus, alkaline pH or acid pH was not beneficial to methane generation. The activity of methanogens decreased drastically or even completely lost at pH 10.0 and 11.0. Therefore, less SCFAs were consumed, which resulted in the significant accumulation of SCFAs.

From the mechanism aforementioned, it was not difficult to understand the reason of significant SCFAs accumulation in this study. Alkaline fermentation significantly promoted the hydrolysis of PS which provided more soluble fermentation substrates to produce more amounts of SCFAs via inhibiting or preventing its conversion to methane.

4. Conclusion

The present study indicated that alkaline pHs could enhance the PS anaerobic fermentation and SCFAs accumulation, the suitable condition for more SCFAs accumulation in PS anaerobic fermentation was determined at pH 10.0–11.0 and with a fermentation time of 5–9 days. The maximum yields of SCFAs at pH 10.0 and 11.0 was achieved 301.6 and 312.9 mg COD/g VSS, respectively. Soluble organic compounds concentration was found to be increased with the increase of pH from 7.0 to 11.0. The average concentration of SCOD at pH 10.0 and 11.0 was 343.3 and 346.8 mg SCOD/g VSS, respectively. The concentration of SCFAs generated from PS fermentation was found to be positive with the concentration of soluble organic compounds. It can be concluded that more SCFAs accumulation at alkaline pHs was because more soluble protein and carbohydrate were available to generate more SCFAs at alkaline pHs than that at neutral or acidic pHs. Additional no methane production was detected for the case at pH 10.0–11.0, which indicated that the activity of methanogens could be inhibited and less SCFAs be consumed at alkaline pHs.

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References

[1] N.R. Louzeiro, D.S. Mavinic, W.K. Oldham, A. Meisen, I.S. Gardner, Methanol-induced biological nutrient removal kinetics in a full-scale sequencing batch reactor, Water Res. 36 (2002) 2721–2732.
[2] S. Hallin, C.F. Lindberg, M. Pell, Microbial adaptation, process performance and a suggested control strategy in a pre-denitrification system with ethanol dosage, Water Sci. Technol. 34 (1996) 91–99.
[3] J.C. Akunna, C. Bizeau, R. Moletta, Nitrate and nitrite reductions with anaerobic sludge using various carbon sources: glucose, glycerol, acetic acid, lactic acid and methanol, Water Res. 27 (1993) 1303–1312.
[4] N. Bernet, F. Habouzit, R. Moletta, Use of an industrial effluent as a carbon source for denitrification of a high strength wastewater, Appl. Microbiol. Biotechnol. 46 (1996) 92–97.
[5] P. Elefsiniotis, W.K. Oldham, Acid-phase anaerobic digestion of primary sludge and its role in the biological phosphorus removal process, Water Pollut. Res. J. Can. 28 (1993) 513–528.
[6] A. Chu, D.S. Mavinic, H.G. Kelly, W.D. Ramey, Volatile fatty acid production in thermophilic aerobic digestion of sludge, Water Res. 28 (1994) 1513–1522.
[7] K.S. Min, A.R. Khan, M.K. Kwom, Y.J. Jung, Z. Yun, Y. Kiso, Acidogenic fermentation of blended food-waste in combination with primary sludge for the production of volatile fatty acids, J. Chem. Technol. Biotechnol. 80 (2005) 909–915.
[8] H.Y. Yuan, Y.G. Chen, H.X. Zhang, S. Jiang, Q. Zhou, G.W. Gu, Improved bioproduction of short-chain fatty acids (SCFAs) from excess sludge under alkaline conditions, Environ. Sci. Technol. 40 (2006) 2025–2029.
[9] Y.G. Chen, S. Jiang, H.Y. Yuan, Q. Zhou, G.W. Gu, Hydrolysis and acidification of waste activated sludge at different pHs, Water Res. 41 (2007) 683–689.
[10] E.U. Cokgor, S. Oktay, D.O. Tas, G.E. Zengin, D. Orhon, Influence of pH and temperature on soluble substrate generation with primary sludge fermentation, Bioresour. Technol. 100 (2009) 380–386.
[11] H.B. Choi, K.Y. Hwang, E.B. Shin, Effects on anaerobic digestion of sewage sludge pretreatment, Water Sci. Technol. 35 (1997) 207–211.
[12] Z.C. Arnai, J.C. Gutierrez, J. Lebrato, Biomass stabilization in the anaerobic digestion of wastewater sludges, Bioresour. Technol. 97 (2006) 1179–1184.
[13] H.Y. Wu, D.H. Yang, Q. Zhou, Z.B. Song, The effect of pH on anaerobic fermentation of primary sludge at room temperature, J. Hazard. Mater. 172 (2009) 196–201.
[14] O. Pahl, A. Firth, I. MacLeod, J. Baird, Anaerobic co-digestion of mechanically biologically treated municipal waste with primary sewage sludge—a feasibility study, Bioresour. Technol. 99 (2008) 3354–3364.

[15] APHA (American Public Health Association), AWWA (American Water Works Association), WEF (Water Environment Federation), Standard Methods for the Examination of Water and Wastewater, 20th ed., 1999, Washington, DC.
[16] Y.G. Chen, Y.S. Chen, Q. Xu, Q. Zhou, G.W. Gu, Comparison between acclimated and unacclimated biomass affecting anaerobic-aerobic transformations in the biological removal of phosphorus, Process Biochem. 40 (2005) 723–732.
[17] D. Jenkins, M.G. Richard, G.T. Daigger, Manual on the Causes and Control of Activated Sludge Bulking and Foaming, 2nd ed., Lewis Publishers, Boca Raton, FL, 1993.
[18] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.
[19] Y. Miron, G. Zeeman, J.B van Lier, G. Lettinga, The role of sludge retention time in the hydrolysis and acidification of lipids, carbohydrates and proteins during digestion of primary sludge in CSTR systems, Water Res. 34 (5) (2000) 1705–1713.
[20] National Environmental Protection Agency of China, Monitoring and Determination Methods for Water and Wastewater (in Chinese), 4th ed., China Environmental Science Press, Beijing, China, 2002.
[21] M.J. Mclnerney, Anaerobic hydrolysis and fermentation of fats and proteins, in: A.J.B. Zehnder (Ed.), Biology of Anaerobic Microorganisms, Wiley, New York, 1988.
[22] Y.G. Chen, Y. Liu, Q. Zhou, G.W. Gu, Enhanced phosphorus biological removal from wastewater-effect of microorganism acclimatization with different ratios of short-chain fatty acids mixture, Biochem. Eng. J. 27 (2005) 24–32.
[23] J.A. Eastman, J.F. Ferguson, Solubilisation of particulate organic carbon during the acid phase anaerobic digestion, J. Water Pollut. Control Fed. 53 (1981) 352–366.
[24] S.G. Pavlostathis, E. Giraldo-Gomez, Kinetic of anaerobic treatment, Water Sci. Technol. 24 (1991) 35–59.
[25] G.J. Hatziconstantinou, P. Yannakopoulos, A. Andreadakis, Primary sludge hydrolysis for biological nutrient removal, Water Sci. Technol. 34 (1996) 417–423.
[26] S. Tanaka, T. Kobayashi, K. Kamiyama, M.N. Bildan, Effects of thermochemical pretreatment on the anaerobic digestion of waste activated sludge, Water Sci. Technol. 35 (1997) 209–215.
[27] H.Q. Yu, X.J. Zheng, Z.H. Hu, G.W. Gu, High-rate anaerobic hydrolysis and acidogenesis of sewage sludge in a modified upflow reactor, Water Sci. Technol. 48 (2003) 69–75.
[28] C.G. Whiteley, P. Heron, B. Pletschke, P.D. Rose, S. Tshivhunge, F.P. Van Jaarsveld, K. Whittington-Jones, The enzymology of sludge solubilisation utilizing sulphate reducing systems-Properties of proteases and phosphatases, Enzyme Microbial Technol. 31 (2002) 419–424.
[29] A. Banerjee, P. Elefsiniotis, D. Tuhtar, The effect of addition of potato-processing wastewater on the acidogenesis of primary sludge under varied hydraulic retention time and temperature, J. Biotechnol. 72 (1999) 203–212.
[30] A.K. Gupta, W.K. Oldham, P.F. Coleman, The effects of temperature, pH and retention time on volatile fatty acid production from primary sludge, in: S. Harper (Ed.), Proceedings, New Directions and Research in Waste Treatment and Residual Management, University of British Columbia, Vancouver, 1985, pp. 376–392.
[31] C. Perot, M. Sergent, P. Richard, R. Phan Tar Luu, N. Millet, The effects of pH, temperature and agitation speed on sludge anaerobic hydrolysis-acidification, Environ. Technol. 9 (1988) 741–752.
[32] M. Cai, J. Liu, Y. Wei, Enhanced biohydrogen production from sewage sludge with alkaline pretreatment, Environ. Sci. Technol. 38 (2004) 3195–3202.
[33] J.J. Lay, Y.Y. Li, T. Noike, Influences of pH and moisture content on the methane production in high-solids sludge digestion, Water Res. 31 (1997) 1518–1524.