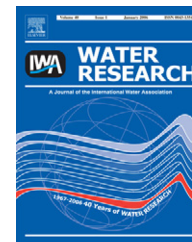
Available at www.sciencedirect.com

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



Biological short-chain fatty acids (SCFAs) production from waste-activated sludge affected by surfactant

Su Jiang, Yinguang Chen, Qi Zhou, Guowei Gu*

State Key Laboratory of Pollution Control and Resources Reuse, School of Environmental Science and Engineering, Tongji University, 1239 Siping Road, Shanghai 200092, China

ARTICLE INFO

Article history:
Received 22 December 2006
Received in revised form
29 March 2007
Accepted 30 March 2007
Available online 17 May 2007

Keywords:
Waste-activated sludge (WAS)
Short-chain fatty acids (SCFAs)
Fermentation
Surfactant
Enhanced biological phosphorus removal (EBPR)

ABSTRACT

Short-chain fatty acids (SCFAs), the preferred carbon sources for biological nutrient removal, are the important intermediate products in sludge anaerobic fermentation. Sodium dodecylbenzene sulfonate (SDBS) is a widespread used surfactant, which can be easily found in waste-activated sludge (WAS). In this investigation, the effect of SDBS on SCFAs production from WAS was investigated, and the potential of using fermentative SCFAs to promote enhanced biological phosphorus removal (EBPR) was tested. Results showed that the total SCFAs production increased significantly in the presence of SDBS at room temperature. At fermentation time of 6 days, the maximum SCFAs was 2599.1 mg chemical oxygen demand (COD)/L in the presence of SDBS 0.02 g/g, whereas it was only 339.1 mg (COD)/L in the absence of SDBS. The SCFAs produced in the case of SDBS 0.02 g/g and fermentation time 6 days consisted of acetic acid (27.1%), propionic acid (22.8%), *iso*-valeric acid (20.1%), *iso*-butyric acid (11.9%), *n*-butyric acid (10.4%) and *n*-valeric acid (7.7%). It was found that during sludge anaerobic fermentation, the solubilization of sludge particulate organic-carbon and hydrolysis of solubilized substrate as well as acidification of hydrolyzed products were all increased in the presence of SDBS, while the methane formation was decreased, the SCFAs production was therefore remarkably improved. Further investigation showed that the production of SCFAs enhanced by SDBS was caused mainly by biological effects, rather than by chemical effects and SDBS decomposition. With the fermentative SCFAs as the main carbon source, the EBPR maintained high phosphorus removal efficiency (~97%).

© 2007 Elsevier Ltd. All rights reserved.

1. Introduction

Biological wastewater treatment has been used widely in the world, but large amounts of sludge (including primary sludge and waste-activated sludge (WAS)) are produced in this process. Rapid urbanization in many areas of the world has resulted in a drastic increase of waste sludge with a typical person generating over 50 g of dry solids every day (Hudson, 1995). Sludge disposal by landfilling may not be appropriate in the near future due to land scarcity and increasingly stringent environmental control regulations. One strategy for sludge management is moving towards reutilization of sludge as useful resources (Mossakowska et al., 1998).

With the development of industry, many scientists are concerned about the depletion of petroleum, the main source of chemicals and fuels. Using waste sludge to biologically produce short-chain fatty acids (SCFAs) has drawn much

*Corresponding author. Tel.: +86 21 65981263; fax: +86 21 65986313.
E-mail address: yg2chen@yahoo.com (Y. Chen).

0043-1354/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.watres.2007.03.039

attention in recent years, since SCFAs are the preferred carbon sources for biological nutrient removal microbes (Aravinthan et al., 2001; Elefsiniotis et al., 2004; Moser-Engeler et al., 1998; Thomas et al., 2003). In addition, it is well known that the SCFAs are the raw materials for the synthesis of biodegradable plastics polyhydroxyalkanoates (Lemos et al., 2006).

SCFAs can be accumulated by the optimization of reactor design, and operational and environmental parameters, such as the hydrolytic retention time, sludge retention time, temperature, pH, etc. (Chanona et al., 2006; Elefsiniotis et al., 1996; Moser-Engeler et al., 1998; Yu et al., 2003; Yuan et al., 2006). During sludge anaerobic digestion, the following four steps: solubilization of sludge particulate organic-carbon, hydrolysis of solubilized substrate, acidification of hydrolyzed products and formation of methane are usually included. If the first three stages could be accelerated, and the last step was prevented or reduced, the accumulation of SCFAs would be increased. Recently, it was observed in our investigation that when a surfactant (linear alkylbenzene sulfonate (LAS))-containing sludge was digested, the production of SCFAs was improved significantly in the case of LAS content exceeding a certain amount.

LAS is a major anionic surfactant accounting for approximately 25–30% of the world total of synthetic surfactants, and used widely in household and industrial detergent formulations due to its excellent dispersive properties and relatively low cost (Larson et al., 1993). After application LAS usually is discharged to the sewer system and appears in municipal wastewater treatment plants. The majority of these plants are of the activated sludge type where some of the LAS which cannot be biodegraded is adsorbed to the sludge and subsequently removed with the wasted sludge. Concentrations of LAS in sludge are rather high ranging from 5 to 15 mg/g dry sludge due to its widespread usage and strong sorption on sludge during treatment (Jensen, 1999), but the LAS levels in sludge depend on the raw sewage characteristics and the treatment method employed. For example, the sodium dodecylbenzene sulfonate (SDBS, one type of LAS) content in WAS can reach up to 31 mg/g dry sludge in a daily-use products wastewater treatment plant in Shanghai, China.

Surfactants have been applied widely to enhance the remediation or removal of environmental contaminants, among which the SDBS has been attracting much attention (Chun et al., 2002; Kim et al., 2004; Pandit and Basu, 2004; Tien et al., 2000; Yang et al., 2006) probably due to its widespread usage and low cost. As to our knowledge, however, enhanced SCFAs bioproduction from WAS by surfactant has not been recognized, although there is a significant amount of surfactant (such as SDBS) in sludge.

In the presence of SDBS, a model surfactant selected in this paper, the bioproduction of SCFAs from WAS at room temperature was firstly reported, and the main composition of SCFAs produced under optimal conditions was assayed. The mechanism of SCFAs production enhanced by SDBS was investigated. Also, the variations of SDBS with time during WAS fermentation were studied. Finally, the fermentative SCFAs were used as the carbon source to drive enhanced biological phosphorus removal (EBPR).

2. Materials and methods

2.1. *Source of WAS*

The WAS used in this study was obtained from the secondary sedimentation tank of a municipal wastewater treatment plant in Shanghai, China. The sludge was concentrated by settling at 4 °C for 24 h, and its main characteristics after settlement are shown in Table 1. Apparently, protein and carbohydrate are the two predominant organic compounds in WAS, which account for about 73% of the total chemical oxygen demand (TCOD).

2.2. *Batch fermentation experiments of SCFAs production from WAS in the presence of SDBS*

Experiments of influence of SDBS on WAS fermentation were carried out in 14 identical reactors, which were made of plexiglass and each had a liquid volume of 2.0 L. All reactors were equipped with stainless-steel stirrers with blades for mixing the contents, and were maintained at 21 ± 1 °C. SDBS was added to the reactors with its dosage to dry sludge ratio being 0, 0.01, 0.02, 0.05, 0.1, 0.15 and 0.2 g/g, respectively. A duplicate reactor was prepared for each SDBS dosage test: one reactor was used for liquid sampling, and another one for methane sampling. By the analysis of SCFAs concentration in fermentation system, the optimum SDBS dosage for efficient SCFAs production could be determined.

2.3. *Investigation on the mechanism of SDBS-enhanced SCFAs production*

The effect of SDBS on sludge particulate organic-carbon solubilization was obtained by assaying the soluble protein and polysaccharide contents in fermentation liquor in above batch fermentation experiments. Also, according to the production of methane at different SDBS dosages, the influence of SDBS on the methanogenesis of sludge could be understood.

To understand how the presence of SDBS affects the hydrolysis of solubilized sludge particulate organic-carbon, the batch fermentation test with synthetic wastewater of bovine serum albumin (BSA, M_w 67,000, model protein

Table 1 – Characteristics of the concentrated WAS

Parameter	Value
pH	6.48 ± 0.13
TSS (total suspended solids) (mg/L)	12421 ± 137
VSS (volatile suspended solids) (mg/L)	10 807 ± 86
SCOD (soluble chemical oxygen demand) (mg/L)	136 ± 9
TCOD (total chemical oxygen demand) (mg/L)	14 878 ± 365
Carbohydrate (mg (COD)/L)	855 ± 73
Protein (mg (COD)/L)	9940 ± 310
Lipid and oil (mg (COD)/L)	169 ± 11
SDBS (mg/g (SS))	3.19 ± 0.12

compound used in this study) and dextran (model polysaccharide compound, average molecular weight M_w~23,800) was conducted, respectively. The model compound (BSA or dextran) of 1000 mg was dissolved into 900 mL of tap water, and 100 mL aliquot of WAS was added to each reactor as an inoculum with a final sludge concentration of 1200 mg/L. SDBS was then added to a final concentration of 50 mg/L in each reactor. The batch reactors were maintained at $21\pm1\,^{\circ}$C.

The effect of SDBS on the acidification of hydrolyzed products was investigated with synthetic wastewater of L-alanine (model amino acid compound used in this study) and glucose (model monosaccharide compound), respectively. The model compound (L-alanine or glucose) of 1000 mg was dissolved into 900 mL of tap water, and a 100 mL aliquot of WAS was added to each reactor as an inoculum with a final sludge concentration of 1200 mg/L. SDBS was then added to a final concentration of 50 mg/L in each reactor. The batch reactors were maintained at $21\pm1\,^{\circ}$C.

To examine whether the formation of SCFAs during sludge fermentation in the presence of surfactant was caused mainly by chemical or biological effects, batch tests using autoclaved sludge were compared with the unautoclaved sludge. Duplicated experiments were conducted. A 4 L portion of WAS was divided equally into eight 1 L Erlenmeyer flasks. Four of them were autoclaved at $121\,^{\circ}$C for 20 min, and another four were not autoclaved. The SDBS dissolved in autoclaved tap water was added to the two autoclaved and two unautoclaved flasks with a dosage of SDBS/dry sludge of 0.02 g/g in each flask. The addition of SDBS to the autoclaved flasks was conducted in a sterilized cabinet. The two flasks that were not autoclaved and no SDBS addition were set as the control. Oxygen in the flasks was removed from the headspace by nitrogen gas sparging for 30 s. The flasks were capped with rubber stoppers. All eight flasks were then placed in an air-bath shaker (120 rpm) at $21\pm1\,^{\circ}$C. The SCFAs concentration and the activities of protease and α-glucosidase in all flasks were assayed.

2.4. SDBS mass balance during SCFAs production

In order to investigate whether SDBS degraded during WAS fermentation, mass balance was made according to the SDBS contents in liquid and solid phases. One liter of WAS was put into the fermentative reactor with a final sludge concentration of approximately 12,000 mg/L, and then SDBS was added with its amount to dry sludge of 0.02 g/g. The reactor was then maintained at $21\pm1\,^{\circ}$C, and SDBS contents in aqueous and sludge phases were determined periodically during WAS fermentation.

2.5. Fermentative SCFAs driving EBPR in sequencing batch reactor (SBR)

Before the SCFAs-containing fermentation liquor was used to drive EBPR, most of the released phosphorus and partial ammonia was recovered in the form of struvite ($MgNH_4PO_4\cdot6H_2O$) (Jaffer et al., 2002). One liter of fermentation liquor was added to a beaker, and its pH was adjusted to 10.0 with 2 M NaOH. $MgCl_2$ was added to the fermentation liquor as Mg^{2+} source at the dosage of Mg/SOP = 1.8/1 (mol/mol). The mixture was stirred for 10 min and then filtered. The filtrate was used as the main carbon source of EBPR microorganisms.

The operation of SBR for EBPR was almost the same as described in our previous publication (Chen et al., 2004) with minor revisions. The SBR, with working volume of 3.5 L, was seeded with biomass from a wastewater treatment plant in Shanghai, China, which is operated with a biological nutrient removal. It was maintained at $21\pm1\,^{\circ}$C and operated on three 8-h cycles per day, with each cycle consisting of 2 h anaerobic and 3 h aerobic periods, followed by 1 h settling, 5 min decanting and remaining idle phase. The reactor was constantly mixed with a magnetic stirrer except during the settling and decanting periods. Aerobic conditions were provided by delivering air to the mixed liquor at a flow rate of 3 L/min. Such an airflow rate provided a dissolved oxygen (DO) concentration about 6.0 mg/L. At the end of the aerobic period, but before mixing was stopped, 115 mL of mixed liquor was wasted from the system to maintain the solids retention time at approximately 10 days. After the setting period, 2.75 L of the supernatant was discharged, resulting in a hydraulic retention time of 10.2 h, and replaced with fresh 2.75 L of wastewater in the initial 10 min of the anaerobic period. The initial pH in the SBR was adjusted to 7.2 by adding either 0.5 M HCl or 0.5 M NaOH. After more than 50 days' acclimatization, the phosphorus anaerobic release and aerobic uptake as well as net removal in the SBR reached steady state.

The feed was prepared daily from stock solutions called "concentrated feed" and "P-water" (constituting 30 mL per 10 L synthetic wastewater, respectively). The concentrated feed was adapted from Smolders et al. (1994), and the fermentation liquor after phosphorus and ammonia recovery was used as the carbon source. The initial COD concentration in SBR was increased progressively over a 20-day period from around 100 mg/L to approximately 425 mg/L. The P-water consisted of (g/L) 23.47 KH_2PO_4 and 17.60 K_2HPO_4, and the pH was adjusted to 10.0 with 2 M NaOH. The beginning COD:P ratio was 20 and it increased to a final value of 25 when the SBR was working at 425 mg (COD)/L.

2.6. Analytical methods

The determination of SCFAs, carbohydrate, protein, lipid, methane, COD, total suspended solids (TSS), volatile suspended solids (VSS), soluble ortho-phosphate (SOP), total phosphate (TP) and ammonia nitrogen, and EBPR sludge volume index (SVI) was the same as described in our previous publications (Chen et al., 2001, 2004; Yuan et al., 2006). The amino acid content was determined with an automatic analyzer (Biochrom 20, Pharmacia Biotech, Piscataway, NJ, USA).

Enzyme assays for α-glucosidase and protease were carried out using *p*-nitrophenyl-α-D-glucopyranoside and azocasein as substrates, respectively. The analytic procedure was according to the method described by Goel et al. (1998).

The measurement of SDBS was based on the method described by Feijtel et al. (1995). Aqueous and solid phases were separated by centrifugation at 4000 rpm for 15 min. Aqueous samples were evaporated to dryness on a stream of

nitrogen. The residue was redissolved in 25 mL of methanol. The extract was passed over a strong anion exchange (SAX) column and the SDBS was then eluted with 2 mL methanol/HCl (80:20, v/v). The eluate was brought to a volume of 50 mL with suprapure water after adjusting the pH to 7 with 1 M NaOH. This solution was then passed over a C18 solid phase extraction (SPE) column, washed with 2 mL methanol/water (30:70, v/v) and subsequently eluted with 5 mL methanol. Wet sludge samples were dried at 80 °C and then were Soxhlet extracted with 150 mL methanol for 12 h. The extracts were partly evaporated on a steambath under a stream of nitrogen and adjusted to 100 mL with methanol. This methanol extract was passed over a SAX column and a C18 column, respectively, as described earlier. The methanol eluate, containing the SDBS, was evaporated to dryness under a stream of nitrogen at 40 °C. The residue was then redissolved in mobile phase and analyzed by high-performance liquid chromatography (HPLC). The HPLC mobile phase consisted of a suprapure water/methanol (16:84, v/v) mixture containing 0.0875 M sodium perchlorate. The HPLC column (Hypersil ODS 250 × 4.6 mm) was operated at a flow rate of 1 mL/min. Detection was by a fluorescence detector operated at an excitation wavelength of 232 nm and an emission wavelength of 290 nm. A five-point calibration curve was made from SDBS solutions prepared in mobile phase at concentration levels between 0 and 20 mg/L. Recoveries ranged from 83% to 98% for the SDBS. The reproducibility, expressed as the standard deviation of the results, was less than 9%.

3. Results and discussion

3.1. *The effects of SDBS dosage on total SCFAs production*

The total SCFAs production at various SDBS dosages and fermentation times are shown in Fig. 1. The individual SCFAs were converted to COD using appropriate conversion factors (Eastman and Ferguson, 1981). As seen in Fig. 1, the maximum total SCFAs production was greatly improved by SDBS. However, a longer fermentation time was required to reach the maximum SCFAs in the case of higher SDBS dosages. For example, the maximum total SCFAs concentration was 2599.1 mg (COD)/L at SDBS dosage of 0.02 g/g and fermentation time of 6 days, 3285.2 at 0.1 g/g and 12 days, and 2674.5 at 0.2 g/g and 15 days, while it was only 339.1 mg (COD)/L in the blank test at fermentation time of 6 days. During the first 6-day time, the total SCFAs production increased significantly with SDBS in the range of SDBS to dry sludge ratio of 0–0.02 g/g, but it kept almost the same at SDBS dosage of 0.02–0.15 g/g and then decreased with further increasing SDBS to 0.2 g/g. Thus, the optimum conditions for SCFAs production were SDBS dosage of 0.02 g/g and fermentation time of 6 days.

Also, as shown in Fig. 1, an obvious SCFAs consumption was observed with the increase of fermentation time at SDBS dosage less than 0.1 g/g due to the participation of SCFAs consumers, such as methanogens, but higher surfactant dosages, such as 0.15 and 0.2 g/g, could reduce or inhibit the methanogens activity, which will be discussed in the following text. It can also be seen from Fig. 1 that the SCFAs concentration was lower at higher surfactant dosages during the initial stage of fermentation, which might be attributed to the toxic effects of higher surfactant concentration to acidogenic bacteria (Feitkenhauer, 2003).

3.2. *Composition of SCFAs at SDBS dosage of 0.02 g/g*

Six kinds of SCFAs, including acetic, propionic, *n*-butyric, *iso*-butyric, *n*-valeric and *iso*-valeric acids, were detectable no matter in the presence or absence of SDBS. Figs. 2 and 3 show the composition of SCFAs at SDBS dosage of 0.02 g/g during the entire fermentation time. As seen in Fig. 2, the maximum concentration of each SCFAs all occurred on the sixth day. During the initial 6-day fermentation time, acetic acid was the most prevalent product and propionic acid was the second major product, and their maximum concentration was 703.2 and 593.2 mg (COD)/L, respectively. With further increasing fermentation time, both acetic and propionic acids concentrations decreased sharply, and their percentages accounting

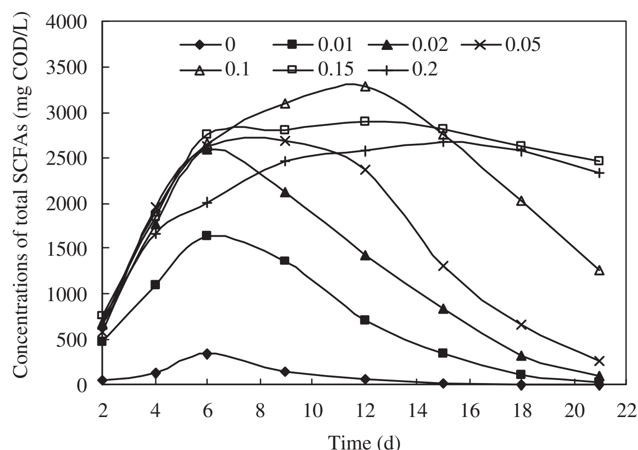


Fig. 1 – Effects of SDBS dosage and fermentation time on total SCFAs production.

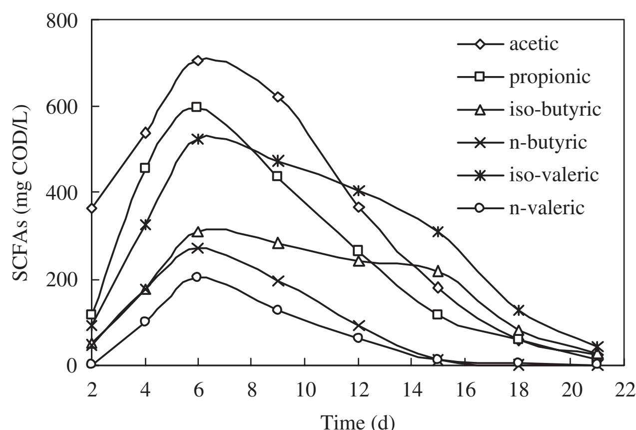


Fig. 2 – Profiles of individual SCFAs concentration during entire fermentation time at SDBS 0.02 g/g.

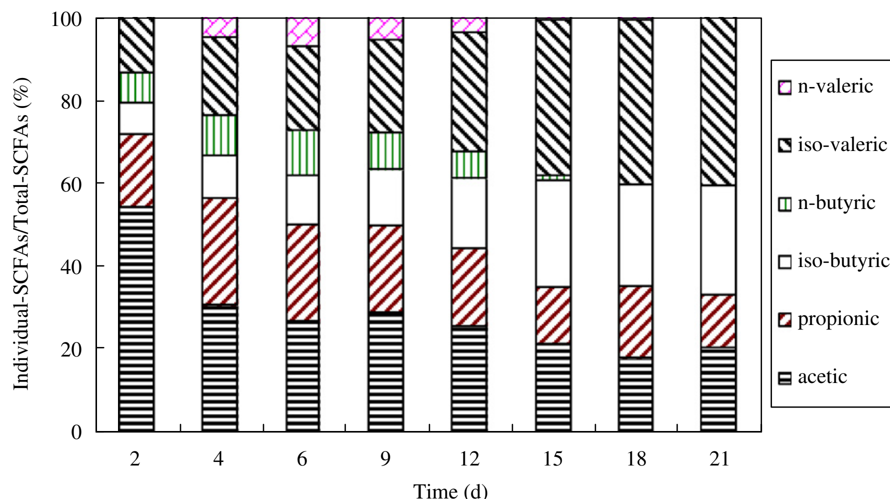


Fig. 3 – Percentage of individual SCFAs during entire fermentation time at SDBS 0.02 g/g.

for total SCFAs also decreased (Fig. 3). Also, concentrations of *iso*-butyric and *iso*-valeric acids decreased after the sixth day of fermentation. However, due to their slower decrease rate (Fig. 2), *iso*-valeric acid became the first one after 12 days and *iso*-butyric acid was the second one after 15 days. The two SCFAs present in the smallest amounts were *n*-butyric and *n*-valeric acids in the entire fermentation process, and their maximum concentration was 271.3 and 200.1 mg (COD)/L, respectively, which was around 10.4% and 7.7% of the total SCFAs.

3.3. *Mechanism of SCFAs production enhanced in the presence of SDBS*

During sludge anaerobic fermentation, hydrolysis (including sludge particulate organic-carbon solubilization and solubilized substrate hydrolysis), acidification and methanogenesis are usually involved. SCFAs are the important intermediate products in the anaerobic digestion. Therefore, in this investigation, the effects of SDBS on the solubilization of sludge particulate organic-carbon, hydrolysis of solubilized substrate, acidification of hydrolyzed products and the formation of methane were examined.

Protein and carbohydrate are the main constituents of WAS, which account for, respectively, 67% and 6% of sludge TCOD (Table 1). Their degradation during sludge anaerobic fermentation has been proved to relate to the formation of SCFAs (Yu et al., 2003). Thus, in this study, the solubilization of sludge particulate organic-carbon was expressed in terms of soluble protein and polysaccharide concentrations in fermentation liquor. Fig. 4 describes the effect of SDBS on soluble protein and polysaccharide concentrations on the third day of WAS fermentation. The concentration of soluble protein and polysaccharide in the blank test was 84.0 and 23.7 mg/L, respectively, which was 484.6 and 55.7 mg/L when the dosage of SDBS was only 0.02 g/g. With the increase of SDBS dosage in the range 0.01–0.2, both the

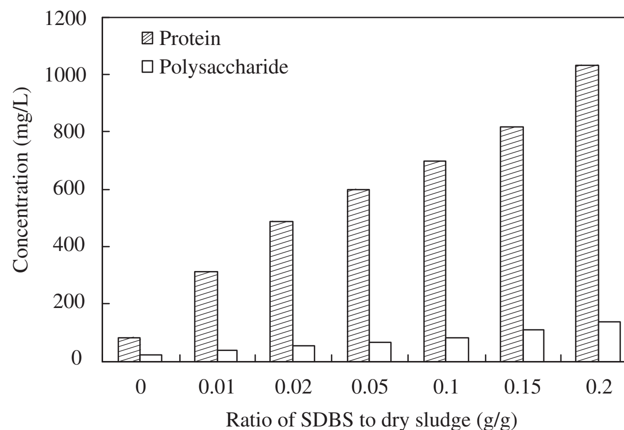


Fig. 4 – Effect of SDBS dosage on soluble protein and polysaccharide concentrations at WAS fermentation time of 3 days.

soluble protein and polysaccharide linearly increased ($y_{protein} = 3662.3$ SDBS+311, $R^2 = 0.92$; $y_{polysaccharide} = 474.3$ SDBS+40, $R^2 = 0.99$). The increase of SDBS dosage resulting in the increase of soluble protein and polysaccharide could be made at any other fermentation time (data not shown). The solubilization of sludge particulate organic-carbon was significantly improved by SDBS.

It is well known that sludge components are cemented together by extracellular polymeric substances (EPS), which are mainly composed of microbiologically produced polymers, such as polysaccharide and protein (Liu and Fang, 2002). Usually, these sludge protein and polysaccharide are absorbed onto sludge surface, but they can be solubilized by SDBS and dissolve into aqueous phase because surfactant has the feature of solubilization. Also, the enhanced solubilization of sludge EPS by SDBS caused the break-up of sludge matrix, which resulted in more sludge inner protein and polysaccharide released. The improved solubilization of sludge protein and polysaccharide by surfactant has also been observed in our previous studies (Chen et al., 2001).

The effect of SDBS on the hydrolysis of solubilized sludge particulate organic-carbon was investigated with synthetic wastewaters of protein (BSA) and polysaccharide (dextran), and the results with hydrolysis time of 3 days are shown in Fig. 5. The degradation rate of BSA was 64.3% in the presence of SDBS, but it was only 15.2% in the control. In dextran, the degradation rate was 82.4% and 60.7% with and without SDBS addition, respectively. The same observations could be made at any other fermentation time. Although partial BSA and dextran might be adsorbed by sludge, from their difference it can be concluded that SDBS significantly enhanced the hydrolysis of both protein and polysaccharide.

The hydrolyzed products, such as amino acid and monosaccharide, are converted to SCFAs in acidification step. The experiments of the effect of SDBS on acidification of synthetic wastewater of L-alanine (model amino compound) and glucose (model monosaccharide compound) were conducted. The results showed that in the presence of SDBS the degradation of glucose and L-alanine was, respectively, 1.22 and 1.44-fold than that in the absence of SDBS. And the SCFAs production with glucose and L-alanine in the presence of SDBS was also increased by 25.7% and 74.3%, respectively. Obviously, both the degradation of glucose and L-alanine, and the production of SCFAs were improved by SDBS.

During sludge fermentation, the effect of SDBS on methanogens activity expressed by methane production is shown in Fig. 6. At lower SDBS dosage, such as 0.01 g/g, there was only slight decrease of methane production with no significant lag-time appeared. However, the lag-phase of methane generation increased from 2 days at SDBS 0.02 g/g to 8 days at SDBS 0.1 g/g. The lag-phase was so long that there was no methane detectable at SDBS 0.15 and 0.2 g/g even on the last day of fermentation. As shown in Fig. 6, the methane production decreased sharply with the increase of SDBS. Apparently, the activity of methanogens was inhibited by surfactant, which has also been observed by Khalil et al. (1988).

It can be seen from Figs. 1 and 6 that the decrease of methane always coincides with the increase of SCFAs production, which indicates that one main reason for improved SCFAs accumulation in WAS fermentation was the decreased SCFAs consumption by methanogens. Due to the measurement of SCFAs and methane carried out in two separated reactors (one for liquid sampling and another for methane sampling, see Section 2), the observed starting time of SCFAs decrease in Fig. 1 did not exactly coincide with that of methane appearing in Fig. 6. For example, the time before methane appeared in the blank test and at SDBS 0.01 and 0.05 g/g was shorter in the gas sampling reactors (Fig. 6) than that before SCFAs began to decrease in the liquid sampling reactors (Fig. 1).

According to above discussion, the SCFAs production was significantly improved in the presence of SDBS. In order to examine whether the formation of SCFAs was caused directly by chemical (SDBS) or biological effects, the batch fermentation test with autoclaved and unautoclaved sludge was conducted, respectively. As shown in Table 2, in the presence of SDBS, the concentration of total SCFAs with the

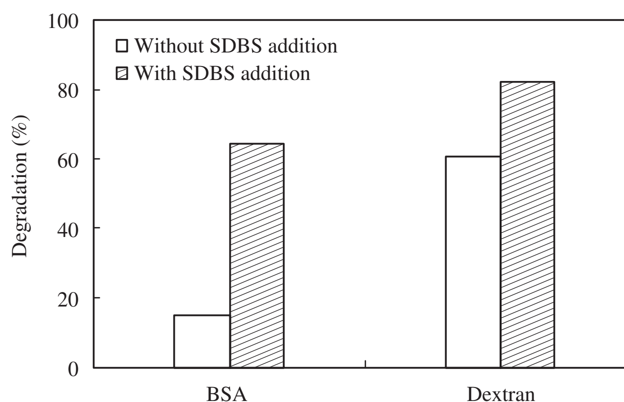


Fig. 5 – Effect of SDBS on BSA and dextran degradation at fermentation time of 3 days.

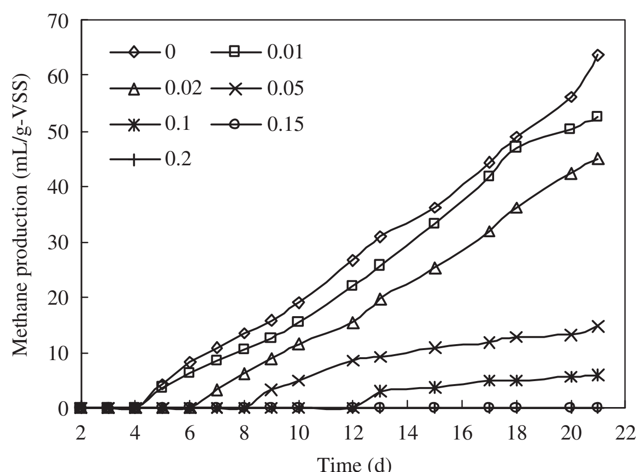


Fig. 6 – Methane production during WAS fermentation at different SDBS dosages.

Table 2 – SCFAs production and enzyme activity in autoclaved and unautoclaved WAS fermentation tests with and without SDBS addition at fermentation time of 6 days

	Total SCFAs (mg (COD)/L)	Enzyme relative activity (%)[a]	
		Protease	α-Glucosidase
Unautoclaved sludge (control)	234.1	100	100
Unautoclaved sludge+SDBS	1759.3	151	106
Autoclaved sludge+SDBS	53.6	0	0
Autoclaved sludge	51.4	0	0

[a] The relative activity of enzyme with the unautoclaved sludge in the absence of SDBS is set to be 100%.

unautoclaved sludge was much higher than that with the autoclaved one (1759.3 versus 53.6 mg (COD)/L), and the enzymes, such as protease and α-glucosidase, lost their activities in the autoclaved sludge test, but these enzymes showed increased activities in the unautoclaved sludge plus SDBS test. Thus, the formation of SCFAs in the presence of SDBS was caused mainly by microbiological activity. Nevertheless, the exact reasons for SDBS increasing the activities of protease and α-glucosidase are unclear right now and need further investigation. It can also be seen from Table 2 that the concentration of SCFAs with the autoclaved sludge was 53.6 mg (COD)/L in the presence of SDBS, while it was 51.4 mg (COD)/L in the absence of SDBS. It seems that there was very little amount of SCFAs generated from the decomposition of SDBS. The above observations could be made at any other fermentation time.

Table 3 – Variations of SDBS content in liquid and sludge phases with fermentation time at SDBS 0.02 g/g ($n = 3$)

	SDBS content at different time (d)[a]		
	1 d	5 d	21 d
Aqueous phase	47.08±3.65	47.86±2.95	49.72±3.48
Sludge phase	195.37±8.74	194.03±9.22	189.68±9.14
Total	242.45±8.63	241.89±7.88	239.40±9.43

[a] The unit of SDBS content is mg.

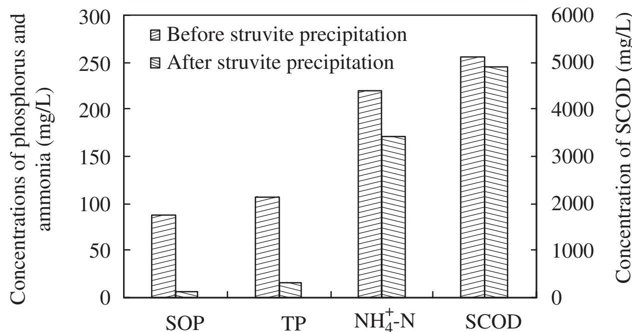


Fig. 7 – Variations of phosphorus, ammonia nitrogen and SCOD before and after struvite precipitation.

3.4. *Variations of SDBS during SCFAs production*

During WAS anaerobic fermentation, the negligible contribution of SDBS decomposition to SCFAs production was further investigated by making the mass balance of SDBS content in fermentation system. As seen in Table 3, there was very little variation of SDBS content in both aqueous and sludge phases during 21-day time. The SDBS increased slightly from 47.08 to 49.72 mg in aqueous phase, and decreased marginally from 195.37 to 189.68 mg in sludge phase. The total SDBS amount in the fermentation system decreased from 242.45 to 239.40 mg, which indicated that there was 1.26% SDBS removed during 21-day time, and the biodegradation of SDBS under anaerobic conditions was negligible in this investigation. The negligible removal of SDBS under anaerobic conditions has also been reported by other researchers, such as Garcia et al. (2005). Therefore, most of SCFAs produced during WAS fermentation was not from the biodegradation of SDBS, although SDBS enhanced SCFAs production significantly.

3.5. *Fermentative SCFAs driving EBPR in SBR*

During sludge fermentation, some sludge phosphorus and ammonia were released. Ideally, before the SCFAs rich effluent from fermented WAS is used to promote biological phosphorus removal, the released phosphorus and ammonia should be removed. Several methods can be used to remove high concentrations of phosphorus from SCFAs-containing fermentation liquor, but simultaneous recovery of phosphorus and ammonia in the form of struvite shows a distinct advantage over other methods because the precipitate can be utilized as fertilizer. The concentrations of SOP, TP, ammonia nitrogen and SCOD before and after struvite precipitation are illustrated in Fig. 7. According to Fig. 7, around 93% SOP and 84% TP with 22% ammonia nitrogen and 4% SCOD could be removed from the fermentation liquor by the struvite precipitation method. After phosphorus recovery, the SCFAs-enriched fermentation liquor was applied to drive EBPR in this investigation.

With fermentative SCFAs as the main carbon sources, Table 4 shows the average variations and removal efficiency of SCOD, SDBS, SOP and TP in one cycle of anaerobic and aerobic SBR. The total SCFAs content in the influent was

Table 4 – The transformation and removal efficiency of SCOD, SOP and TP in the experiment of EBPR driven by fermentative SCFAs[a,b] ($n = 5$)

	SCOD	SDBS	SOP	TP
Influent	425±18	4.57±0.29	15.13±1.26	16.64±1.18
Anaerobic end	107±10	N.D.[c]	127.37±0.41	128.91±9.06
Aerobic end or effluent	71±9	0.04±0.003	0.44±0.03	0.52±0.02
Removal efficiency	83±3	99.12±0.11	97.05±0.40	96.85±0.36

[a] The data reported are the average of five different measurements conducted on day 12, 30, 65, 102 and 155 after the SBR reached steady state.
[b] The unit of removal efficiency is %, and all others are mg/L.
[c] N.D.: not determined.

208 mg (COD)/L, in which the concentrations of acetic, propionic, *n*-butyric, *iso*-butyric, *n*-valeric and *iso*-valeric acids were 56.2, 47.4, 21.7, 24.7, 16.0 and 42.0 mg (COD)/L, respectively. As seen in Table 4, most of the influent COD was consumed in the anaerobic stage and then further decreased in the aerobic phase, which resulted in its removal efficiency of 83%. It has been observed in our experiments that all influent SCFAs were taken up and converted to polyhydroxyalkanoates (PHAs) during the anaerobic time, and the anaerobic synthesized PHAs were then utilized in the aerobic time (data not shown in Table 4). From the influent, anaerobic end and aerobic end data of SOP and TP in Table 4, it is obvious that there were significant phosphorus anaerobic release and aerobic uptake, and the latter was greater than the former. The removal efficiency of SOP and TP, as shown in Table 4, was 97.05% and 96.85%, respectively. It was more than 6 months right now since the SBR reached steady state, and the phosphorus removal efficiency maintained around 97% during this period, and the activated sludge SVI value was between 65 and 78 mL/g.

As seen in Table 4, the influent and effluent SDBS was 4.57 and 0.04 mg/L, respectively, and around 99.12% SDBS was removed in the EBPR process. In addition, it was observed that there was no obvious increase in EBPR sludge SDBS content (data not shown). The easy biodegradation of SDBS under aerobic conditions has also been observed by other researchers (Temmink and Klapwijk, 2004). It seems that the fermentative SCFAs can be supplied as the main carbon source to promote EBPR.

4. Conclusions

The SCFAs production from WAS was increased significantly in the presence of SDBS at room temperature. However, a longer fermentation time was required to reach the maximum SCFAs in the case of higher SDBS dosages. The optimum conditions for SCFAs production were SDBS dosage of 0.02 g/g and fermentation time of 6 days. The three main components in the fermentative SCFAs were acetic, propionic and *iso*-valeric acids with the fraction of 27.1%, 22.8% and 20.1%, respectively.

The reasons for SCFAs production being significantly improved in the presence of SDBS were the increase of solubilization of sludge particulate organic-carbon, hydrolysis of solubilized substrate and acidification of hydrolyzed products, and the decrease of methanogenic bacteria activity. Comparison of SCFAs concentration and enzyme activites between autoclaved and unautoclaved WAS fermentation in the presence of SDBS indicated that the production of SCFAs enhanced by SDBS was caused mainly by biological effects, rather than by chemical effects. And the negligible removal of SDBS over the whole fermentation period suggested that the increase of SCFAs production was not due to the degradation of SDBS. The produced SCFAs showed good applicability to EBPR with a high phosphorus removal efficiency of around 97% and an efficient SDBS removal efficiency of about 99% during 6 months running time.

Acknowledgments

This work was financially supported by the National Science Foundation of China (50678125), the Fok Ying Tung Education Foundation (101080), the Program for NCET (06-0373) in University, and the Doctoral Foundation of Ministry of Education of China (20060247006).

REFERENCES

Aravinthan, V., Mino, T., Takizawa, S., Satoh, H., Matsuo, T., 2001. Sludge hydrolysate as a carbon source for denitrification. Water Sci. Technol. 43 (1), 191–199.

Chanona, J., Ribes, J., Seco, A., Ferrer, J., 2006. Optimum design and operation of primary sludge fermentation schemes for volatile fatty acids production. Water Res. 40 (1), 53–60.

Chen, Y., Yang, H., Gu, G., 2001. Effect of acid and surfactant treatment on activated sludge dewatering and settling. Water Res. 35 (11), 2615–2620.

Chen, Y., Randall, A.A., McCue, T., 2004. The efficiency of enhanced biological phosphorus removal from real wastewater affected by different ratios of acetic to propionic acid. Water Res. 38 (1), 27–36.

Chun, C.L., Lee, J.J., Park, J.W., 2002. Solubilization of PAH mixtures by three different anionic surfactants. Environ. Pollut. 118 (3), 307–313.

Eastman, J.A., Ferguson, J.F., 1981. Solubilization of particulate organic carbon during the acid phase of anaerobic digestion. J. Water Pollut. Control Fed. 53 (3), 352–366.

Elefsiniotis, P., Wareham, D.G., Oldham, W.K., 1996. Particulate organic carbon solubilization in an acid-phase upflow anaerobic sludge blanket system. Environ. Sci. Technol. 30 (5), 1508–1514.

Elefsiniotis, P., Wareham, D.G., Smith, M.O., 2004. Use of volatile fatty acids from an acid-phase digester for denitrification. J. Biotechnol. 114 (3), 289–297.

Feijtel, T.C.J., Matthijs, E., Rottiers, A., Rijs, G.B.J., Kiewiet, A., de Nijs, A., 1995. AIS/CESIO environmental surfactant monitoring programme. Part 1: LAS monitoring study in “de Meern” sewage treatment plant and receiving river “Leidsche Rijn”. Chemosphere 30, 1053–1066.

Feitkenhauer, H., 2003. Anaerobic digestion of desizing wastewater: influence of pretreatment and anionic surfactant on degradation and intermediate accumulation. Enzyme Microb. Technol. 33, 250–258.

Garcia, M.T., Campos, E., Ribosa, I., Latorre, A., Sanchez-Leal, J., 2005. Anaerobic digestion of linear alkyl benzene sulfonates: biodegradation kinetics and metabolite analysis. Chemosphere 60, 1636–1643.

Goel, R., Mino, T., Satoh, H., Matsuo, T., 1998. Enzyme activities under anaerobic and aerobic conditions in activated sludge sequencing batch reactor. Water Res. 32 (7), 2081–2088.

Hudson, J.A., 1995. Treatment and disposal of sewage sludge in mid-1990s. J. Chart. Inst. Water Environ. Manag. 9, 93–100.

Jaffer, Y., Clark, T.A., Pearce, P., Parsons, S.A., 2002. Potential phosphorus recovery by struvite formation. Water Res. 36 (7), 1834–1842.

Jensen, J., 1999. Fate and effects of linear alkylbenzene sulfonates (LAS) in the terrestrial environment. Sci. Total Environ. 226, 93–111.

Khalil, E.F., Whitmore, T.N., Gamal-El-Din, H., El-Bassel, A., Lloyd, D., 1988. The effects of detergents on anaerobic digestion. Appl. Microbiol. Biotechnol. 29, 517–522.

Kim, H., Soh, H.E., Annable, M.D., Kim, D.J., 2004. Surfactant-enhanced air sparging in saturated sand. Environ. Sci. Technol. 38 (4), 1170–1175.

Larson, R.J., Rothgeb, T.M., Shimp, R.J., Ward, T.E., Ventullo, R.M., 1993. Kinetics and practical significance of biodegradation of linear alkylbenzene sulfonate in the environment. J. Am. Oil Chem. 70 (7), 645–657.

Lemos, P.C., Serafim, L.S., Reis, M.A.M., 2006. Synthesis of polyhydroxyalkanoates from different short-chain fatty acids by mixed cultures submitted to aerobic dynamic feeding. J. Biotechnol. 122 (2), 226–238.

Liu, H., Fang, H.H.P., 2002. Extraction of extracellular polymeric substances (EPS) of sludges. J. Biotechnol. 95 (3), 249–256.

Moser-Engeler, R., Udert, K.M., Wild, D., Siegrist, H., 1998. Products from primary sludge fermentation and their suitability for nutrient removal. Water Sci. Technol. 38 (1), 265–273.

Mossakowska, A., Hellström, B.G., Hultman, B., 1998. Strategies for sludge handling in the Stockholm region. Water Sci. Technol. 38 (2), 111–118.

Pandit, P., Basu, S., 2004. Removal of ionic dyes from water by solvent extraction using reverse micelles. Environ. Sci. Technol. 38 (8), 2435–2442.

Smolders, G.J.F., van der Meij, J., van Loosdrecht, M.C.M., Heijnen, J.J., 1994. Model of the anaerobic metabolism of the phosphorus removal process, stoichiometry and pH influence. Biotechnol. Bioeng. 43 (6), 461–470.

Temmink, H., Klapwijk, B., 2004. Fate of linear alkylbenzene sulfonate (LAS) in activated sludge plants. Water Res. 38 (4), 903–912.

Thomas, M., Wright, P., Blackall, L., Urbain, V., Keller, J., 2003. Optimisation of Noosa BNR plant to improve performance and reduce operating costs. Water Sci. Technol. 47 (12), 141–148.

Tien, T.H., Bettahar, M., Kumagai, S., 2000. Optimization of the surfactant/alcohol formulations for the remediation of oily contaminated porous media. Environ. Sci. Technol. 34 (18), 3977–3981.

Yang, K., Zhu, L., Xing, B., 2006. Enhanced soil washing of phenanthrene by mixed solutions of TX100 and SDBS. Environ. Sci. Technol. 40 (13), 4274–4280.

Yu, H.Q., Zheng, X.J., Hu, Z.H., Gu, G.W., 2003. High-rate anaerobic hydrolysis and acidogenesis of sewage sludge in a modified upflow reactor. Water Sci. Technol. 48 (4), 69–75.

Yuan, H., Chen, Y., Zhang, H., Jiang, S., Zhou, Q., Gu, G., 2006. Improved bioproduction of short-chain fatty acids (SCFAs) from excess sludge under alkaline conditions. Environ. Sci. Technol. 40, 2025–2029.