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Role of surfactants on the hydrolysis and acidogenesis of waste-activated sludge

Lin Xu[a,b], Can Chen[a,c], Na Li[a], Xiuyun Sun[a,*], Jinyou Shen[a], Jiansheng Li[a], Lianjun Wang[a,*]

[a]*Jiangsu Key Laboratory of Chemical Pollution Control and Resources Reuse, School of Environmental and Biological Engineering, Nanjing University of Science and Technology, Nanjing 210094, Jiangsu Province, China, Tel./Fax: +86 25 84315941; emails: sunxyun@mail.njust.edu.cn (X. Sun), wanglj@mail.njust.edu.cn (L. Wang)*
[b]*EIA Section, Nanjing Yuanheng Institute for Environmental Studies Co. Ltd., Nanjing 210049, Jiangsu Province, China*
[c]*Institute of Environmental Engineering Technology, Guangdong Provincial Academy of Enviromental Science, Guangzhou 510045, Guangdong Province, China*

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ABSTRACT

The role of a biosurfactant (i.e. alkyl polyglycosides, APG in short) and a chemical surfactant (i.e. sodium dodecyl sulfate, SDS in short) on the hydrolysis and acidogenesis of waste-activated sludge was investigated in this study. The results showed that both sludge hydrolysis and short-chain fatty acids (SCFAs) accumulation were increased by APG or SDS addition. The maximum SCFAs concentrations were as high as 2,330 mg L^{-1} at SDS dosage of 0.2 g g^{-1} TSS, and 2,222 mg L^{-1} at APG dosage of 0.2 g g^{-1} TSS, while the maximum SCFAs concentration was only 1,212 mg L^{-1} in the blank test. In addition, the distribution of individual SCFA was also influenced significantly at the presence of these two surfactants. With the addition of APG or SDS, α-glucosidase and protease activities were enhanced, while the activity of the hydrolase decreased with the increase in the incubation time. Comparison of sludge surface morphology showed that the sludge matrix could be broken up at the presence of the two surfactants. The enhanced production of SCFAs at the presence of the APG or SDS was mainly caused by biological effect rather than chemical effect. Moreover, the contribution to SCFAs production by the degradation of the two surfactants was negligible.

Keywords: Waste-activated sludge; Hydrolysis; Acidogenesis; Surfactant; Short-chain fatty acid

1. Introduction

Biological treatment of wastewater, such as activated sludge process, has been used widely in the worldwide; however, large amounts of waste-activated sludge (WAS) are produced in this process, causing secondary pollution if improperly treated. The yield of WAS production is estimated to be as high as 0.3–0.5% of sewage treatment capacity (assuming water content 97%). In the WAS, organic matter occupied approximately 50% and biodegradable fraction is often above 40% [1,2]. Therefore, the production of short-chain fatty acids (SCFAs) from WAS has drawn much attention in recent years, since SCFAs are the preferred carbon sources for biological nutrient removal

*Corresponding authors.

process [3,4]. In addition, SCFAs could be alternative raw materials for the synthesis of biodegradable plastics polyhydroxyalkanoates [5].

Hydrolysis of solubilized substrate is the rate-limiting step of sludge anaerobic fermentation [6,7]. SCFAs accumulation can be promoted through increasing the sludge hydrolysis efficiency, which will provide abundant substrate for the production of SCFAs. In order to increase the sludge hydrolysis efficiency, various methods for sludge pretreatment have been developed, such as mechanical treatment [8], chemical treatment [9], thermo-alkaline treatment [10], and ultrasonic-alkaline treatment [11]. However, the application of these methods has been limited by the high energy consumption, complicated operation, or equipment corrosion [12,13].

Surfactant is a kind of material which could be adsorbed on the phase interface at low concentration. Some special properties, such as changing the interfacial properties, significantly reducing the surface tension, and affecting the solubilization at high concentration, have been investigated thoroughly. Due to these special properties, surfactants have been used extensively as detergents, emulsifiers, foaming agents, and dispersants. In recent years, surfactants, especially chemical surfactants, have attract much attention in WAS fermentation since the production of the SCFAs from WAS could be improved at the presence of these surfactants [14]. Compared with other pretreatment methods, improvement of the SCFAs production by surfactants is more economical and feasible, since less facilities are required and less corrosion is applied to the equipment. It has been reported that the addition of surfactant sodium dodecylbenzene sulfonate (SDBS) at a concentration of 0.02 g g^{-1} TSS results in higher SCFAs accumulation during WAS anaerobic fermentation [15,16]. SCFAs production could also be enhanced by sodium dodecyl sulfate (SDS) under room temperature [17]. On the sixth day of fermentation, the concentration of total SCFAs was as high as 2,243 mg COD L^{-1} at SDS dosage 0.1 g g^{-1}, while only 191 mg COD L^{-1} total SCFAs was observed in the control test. However, most surfactants were chemically synthesized using petroleum as the starting material, thus there was unavoidable adverse influence on environment during their manufacture and application. In addition, the biodegradability of the traditional chemical surfactants was usually poor, which would cause a negative environmental impact.

Biosurfactants are generally produced from various carbon sources at the presence of bacteria, yeast, or fungus [18]. Biosurfactants have the same surface activity as chemically synthesized surfactants, but higher biodegradability, lower toxicity, and less hazardous property to the environment [19]. As a result, the biosurfactants are more suitable for the application in environmental engineering. Currently, they are being considered to replace the traditional chemical surfactants because of these favorable aspects. By far, the effect of chemical surfactants on hydrolysis and acidification of WAS has been widely investigated. However, the applications of biosurfactants in terms of the promotion of SCFAs production have been rarely investigated. The key role of biosurfactant rhamnolipid (RL) on hydrolysis and acidification of WAS has been verified [20]. However, the selection of suitable biosurfactants and the promotion mechanisms of SCFAs production from the WAS by the biosurfactants require to be further revealed. Alkyl polyglycosides (APG), as an emerging nonionic biosurfactant with high biodegradability, low toxicity, and good ecological compatibility, is attracting increasing interest due to its application potential. There is hardly any other surfactant comparable with APG in terms of ecological security.

Thus, the present study aimed to investigate the role of the surfactants on the hydrolysis and acidogenesis of WAS. APG and SDS were, respectively, chosen as the model of chemical surfactant and biosurfactant, due to their representativeness and unique merits. The performance of these two surfactants in the hydrolysis and acidogenesis of WAS was compared in this study. At the presence of SDS and APG, not only the production of SCFAs from WAS, but also the main composition of SCFAs produced under different conditions was assayed. Furthermore, the mechanisms of remarkably enhanced SCFAs production with the presence of the two surfactants were studied preliminarily.

2. Materials and methods

2.1. Characteristic of SDS and APG

SDS is an organosulfate consisting of a 12-carbon tail attached to a sulfate group. SDS is a member of alcohol sulfate family. It has been reported that SDS is toxic and the survival of aquatic animals such as fishes, microbes like yeasts, and bacteria could be affected with its presence [21]. It is also toxic to mammals like mice and humans but to a lesser extent [22].

APG is a nonionic surfactant with large sugar-based polar head groups and a hydrophobic hydrocarbon tail. APG can be prepared with raw materials such as sugars and fatty alcohols [23]. They have green chemistry characters such as low toxicity, and low irritation potential in acute toxicity tests [23,24]. APG has

application properties that can make them useful for laundry and dishwashing detergents, cleaning products, cosmetic preparations, and food technology.

2.2. Source and characteristics of WAS

The WAS samples used in this study were taken from the secondary sedimentation tank of a municipal wastewater treatment plant in Nanjing, China. Before use, the sludge was concentrated by settling at 4℃ for 24 h, and the main characteristics of the WAS after settlement were presented in Table 1.

2.3. Batch fermentation experiments

To compare the hydrolysis and acidification of WAS, WAS with the addition of SDS (named after WAS + SDS) and WAS with the addition of APG (named after WAS + APG), WAS without the addition of SDS, or APG were fermented in three identical reactors, with working volume of 250 mL. The reactors was made of plexiglass and maintained at room temperature of 30 ± 2℃. 200 mL WAS described as above was added into each reactor. SDS was added into the reactor 1 with dosage of 0.2 g g^{-1} TSS, while APG was added into the reactor 2 with dosage of 0.2 g g^{-1} TSS as well. According to our previous study, at the surfactant dosage of 0.2 g g^{-1} TSS, high SCFAs production was observed, thus the dosage of 0.2 g g^{-1} TSS was chosen. Three reactors were mixed at a speed of 180 r min^{-1} to sustain homogenous mixing. The hydrolysate and SCFAs concentrations were determined, meanwhile, both the main composition of SCFAs and variation of hydrolase activity under different conditions were analyzed during sludge fermentation.

In order to investigate the mechanism for enhanced SCFAs production in the presence of surfactants, the batch fermentation tests with autoclaved or unautoclaved sludge were conducted in six identical reactors. The reactors 1–3 were sterilized at 121℃ for 20 min, while reactors 4–6 were not. SDS was added into the reactors 1 and 4 at dosage of 0.2 g g^{-1} TSS. APG was added into reactors 2 and 5 at dosage of 0.2 g g^{-1} TSS. Reactors 3 and 6 were operated without the addition of any surfactant.

In order to obviate the possibility that SCFAs was produced from the degradation of surfactant, anaerobic fermentation of the two surfactants, i.e. SDS and APG, was evaluated in two identical reactors. 190 mL SDS and APG solutions at concentration of 10 g L^{-1} were added into the two reactors, respectively. In addition, 10-mL WAS was, respectively, added into the two reactors as inocula. The SCFAs production in these two reactors was evaluated.

Table 1
Characteristics of the WAS used in this study

Parameter	Value
pH	7.6 ± 0.2
TSS (g L^{-1})	28.6 ± 0.3
SCOD (mg L^{-1})	2,748 ± 34
Soluble proteins (mg L^{-1})	680.6 ± 8.2
Soluble carbohydrates (mg L^{-1})	176.3 ± 1.9
SCFAs (mg L^{-1})	801.3 ± 11.7

2.4. Analytical methods

Sludge samples from the reactors were immediately filtered through a 0.45-μm membrane filter. The filtrate was immediately analyzed for soluble carbohydrate, soluble protein, NH_4^+-N, PO_4^{3-}-P, and SCFAs. The determination of soluble carbohydrate was determined using the phenol-sulfuric method with glucose as standard [25]. Soluble protein was quantified using Lowry–Folin method with bovine serum albumin as standard [26]. TSS, NH_4^+-N, and PO_4^{3-}-P were measured according to Standard Methods [27]. The SCFAs were determined through an ion chromatograph (ICS-2100, DIONEX) using Ion Pac® As11-HC (4 × 250 mm) column and a suppressed conductivity detector. The 30-mM NaOH eluent was pumped at a flow rate of 1.5 mL min^{-1}. Enzyme assays for α-glucosidase and protease were carried out using *p*-nitrophenyl-*α*-*D*-glucopyranoside and azocasein as substrates, respectively [28]. The surface tension of sludge liquid was measured using a JYW-200A surface tensiometer [29]. Surface structure of the WAS sample was observed by scanning electron microscope (SEM, JEOLJSM-6380LV, JEOL Ltd, Japan).

3. Results and discussion

3.1. Solubilization of the sludge organic matters

In general, three steps, i.e. hydrolysis, acidification, and methanogenesis, are involved in sludge fermentation process. The initial hydrolysis of particulate organic matter to soluble substance is believed to be the rate-limiting step of anaerobic fermentation. As protein and carbohydrate are the main organic matters of WAS [30], the effect of SDS or APG addition on the hydrolysis process could be expressed by the changes of protein and carbohydrate concentration in the hydrolysate. As was indicated in Fig. 1, at any

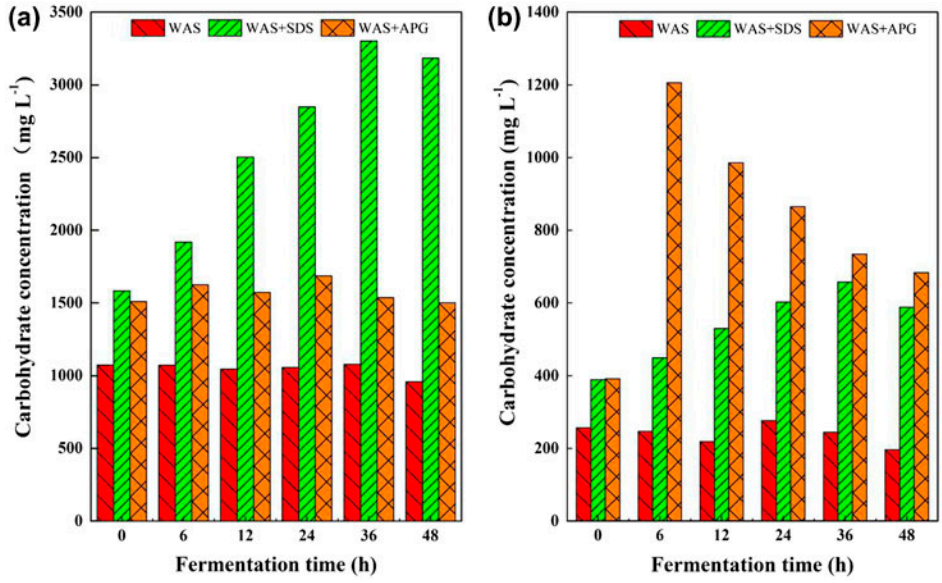

Fig. 1. Changes of protein (a) and carbohydrate (b) concentrations with fermentation time after surfactant addition.

fermentation time, the production of the hydrolysis products, i.e. protein and carbohydrate, was enhanced at the presence of the two surfactants, compared to the sole WAS.

According to Ji et al. [16], in the fermentation system with the addition of SDBS, the maximal soluble protein and carbohydrate at fermentation time of 6 d were as high as 66.7 ± 1.0 and 10.9 ± 0.3 mg COD g^{-1} VSS, which were around 1.7 and 1.9 times of that in the WAS system without the addition of any surfactant. In this study, the protein concentration reached the maximum at fermentation time of 36 h in the WAS + SDS fermentation system, while reached the maximum at fermentation time of 12 h in the WAS + APG fermentation system. In the WAS + SDS fermentation system, the maximal concentration of protein and carbohydrate was as high as 3,302 and 658 mg L^{-1}, and those were as high as 1,687 and 1,206 mg L^{-1} in the WAS + APG fermentation system, which were, respectively, around 3.1 and 2.7, 1.5 and 4.9 times of those in the sole WAS fermentation system. It was interesting to note that protein production could be significantly enhanced at the presence of SDS, while carbohydrate production could be significantly enhanced at the presence of APG. In the WAS + SDS fermentation system, the protein concentrations were relatively higher, but the carbohydrate concentrations were relatively low, indicating the suppression of protein degradation by carbohydrate. This phenomenon could be attributed to the suppression of carbohydrates on the synthesis of the enzymes involved in protein hydrolysis [31].

3.2. SCFAs accumulation and its composition

Usually, more hydrolysis product is in correspondence with higher SCFAs accumulation [32]. As shown in Fig. 1, both protein and carbohydrate increased in the presence of SDS or APG, thus it could be inferred that the SCFAs production could be greatly improved by SDS or APG addition. The total SCFAs concentrations at different fermentation times with or without surfactants were shown in Fig. 2. The maximum SCFAs concentration was as high as 2,313 mg L^{-1} at fermentation time of 48 h in the WAS + SDS fermentation system, and 2,222 mg L^{-1} at

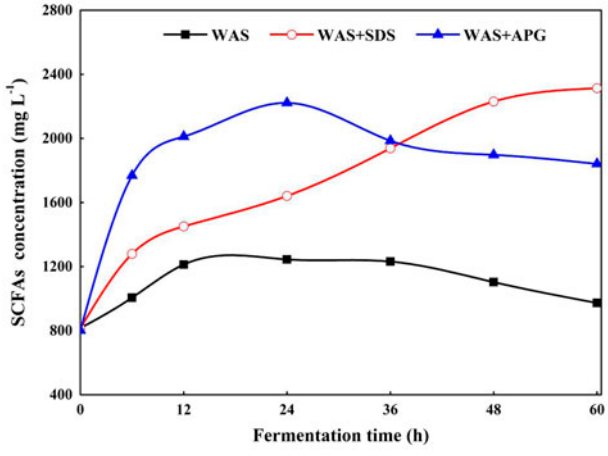

Fig. 2. The effect of SDS and APG addition on SCFAs accumulation.

fermentation time of 24 h in WAS + APG fermentation system; however, it was only 1,212 mg L^{-1} at fermentation time of 12 h in the control WAS fermentation system. What's more, longer fermentation times were required to reach the maximum SCFAs concentration in the WAS + SDS and WAS + APG fermentation systems.

According to the previous study, the maximum total SCFAs production was greatly improved by SDBS and SDS [14]. However, a longer fermentation time was required to reach the maximum SCFAs production in the system with the addition of SDBS and SDS [14]. In addition, as indicated in Fig. 2, obvious SCFAs consumption was observed with the further increase in fermentation time in the WAS fermentation system both with and without surfactants, probably due to the participation of SCFAs consumers, such as methanogens. According to the literature, SDS has been reported to inhibit the activities of methanogens in sludge fermentation [17]. With the dosage of SDS increased from 0.02 to 0.3 g g^{-1}, the inhibition ratio of methane production increased sharply from 3 to 100%. It was also observed that methanogenesis from glucose were reduced to half at SDBS dosage between 20 and 50 ppm during the initial phase of digestion [33]. In addition, it was found that RL possessed antibacterial activity at some extent, which might not only inhibit the methanogenesis, but also slow down the metabolism of other micro-organisms in sludge [34].

Five kinds of SCFAs, i.e. methanoic, cetic, propionic, butyric and valeric acids, were detectable at the presence or absence of the two surfactants. As shown in Fig. 3, the addition of SDS or APG to the WAS fermentation system changed the percentage of the five individual SCFAs significantly. The percentage of acetic acid increased with the fermentation time in the control WAS system; however, it was not so with the addition of the two surfactants. With the further increase in fermentation time, the ratio of propionic acid/total SCFAs decreased in all reactors, regardless of the presence of surfactants. In addition, the addition of APG into the WAS fermentation system resulted in the production of methanoic acid, while SDS did not. It was observed that the percentage of individual SCFA was obviously affected by the carbon/nitrogen ratio (C/N) of fermentation substrate [35]. In addition, it has been reported in the literature that the soluble protein composition was changed with the addition of SDBS at pH 10 [15]. It has been acknowledged that the composition of SCFAs could be mainly affected by the constituent of organic matters and the activities of various micro-organisms in sludge [36]. It can be inferred that bacteria community capable of producing SCFAs can be changed somewhat after the addition of the surfactant, which needs further investigation. Chen et al. [33] indicated that a lower ratio of acetic acid to propionic acid was favorable for the stability of biological phosphorus removal system for the long-term operation. In the WAS treatment system with the addition of APG, the ratio of acetic acid to propionic acid was relatively high, which was advantageous if SCFA produced from WAS was used as an external carbon source for biological phosphorus removal in sewage treatment plants.

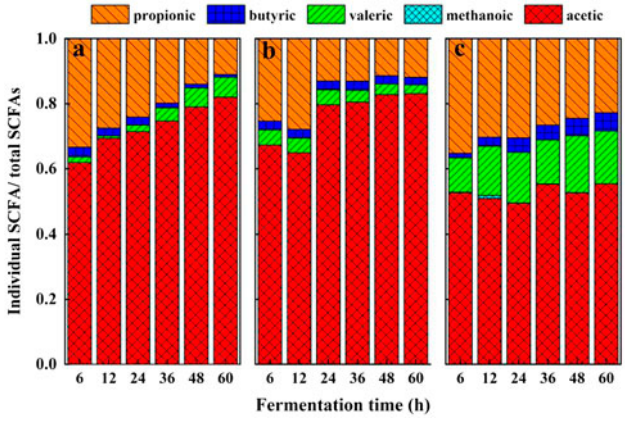

Fig. 3. The percentage of individual SCFA/SCFAs in the system of WAS (a), WAS + SDS (b), and WAS + APG (c) at different fermentation times.

3.3. Release of ammonia and phosphate

For the fermentation of WAS, the release of NH_4^+-N and PO_4^{3-}-P was the key evidence for efficient WAS fermentation. As shown in Figs. 4 and 5, during the process of anaerobic digestion of WAS, more NH_4^+-N and PO_4^{3-}-P were released from WAS with the addition of the two surfactants. However, the release of NH_4^+-N and PO_4^{3-}-P was concentration dependent. When the concentration of SDS and APG was low, the concentration of NH_4^+-N and PO_4^{3-}-P was obviously higher than the blank test. The concentration of NH_4^+-N gradually increased with the extension of the fermentation time. PO_4^{3-}-P concentration showed an rapid increasing trend in the early time and then decreased. In the control WAS system, the NH_4^+-N and PO_4^{3-}-P concentrations at 36 h were 248 and 470 mg L^{-1}, respectively. After the addition of SDS and APG, the NH_4^+-N concentrations were 404 and 473 mg L^{-1} and the PO_4^{3-}-P concentrations were 709 and 649 mg L^{-1}, respectively. The results indicated that NH_4^+-N release at the presence of APG was relatively high compared with SDS.

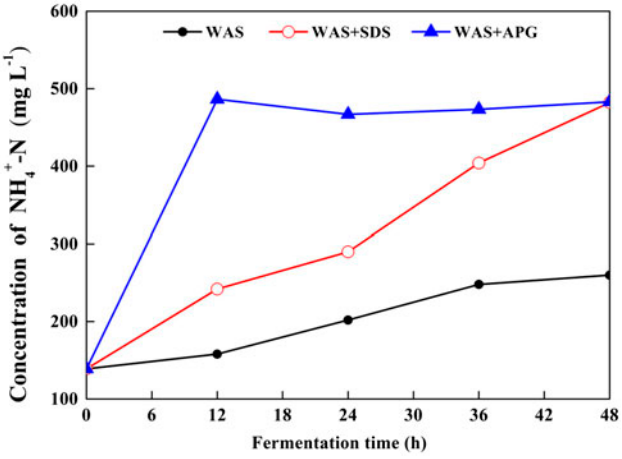

Fig. 4. Release of the NH_4^+-N with the addition of surfactants.

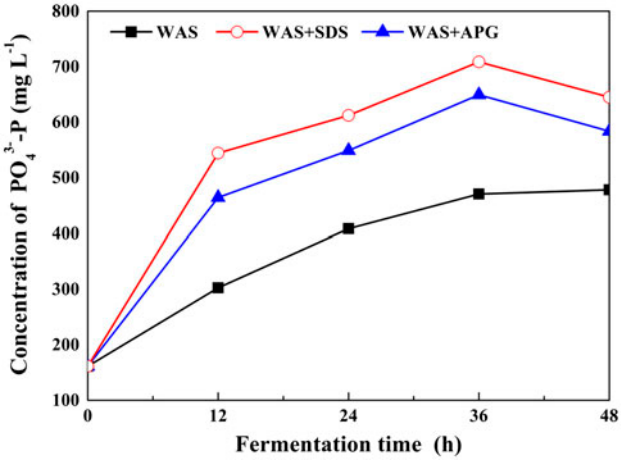

Fig. 5. Release of the PO_4^{3-}-P with the addition of surfactants.

3.4. Mechanism of enhanced production of SCFAs in the presence of surfactants

The effect of SDS and APG on sludge surface tension was demonstrated in Fig. 6. It could be observed that the surface tension of the WAS was reduced with the addition of the both surfactants. The surface tension of the WAS in the initial stage decreased from 60.2 to 20.1 and 24.8, respectively, after the addition of SDS and APG. The obvious decrease in the surface tension could be related to the micelles formed after the addition of the surfactants. The insoluble organic matter entered the micelles firstly, then the insoluble organic matter was released into aqueous phase. During anaerobic fermentation of WAS, hydrolysis was the rate-limiting step, which included solubilization of

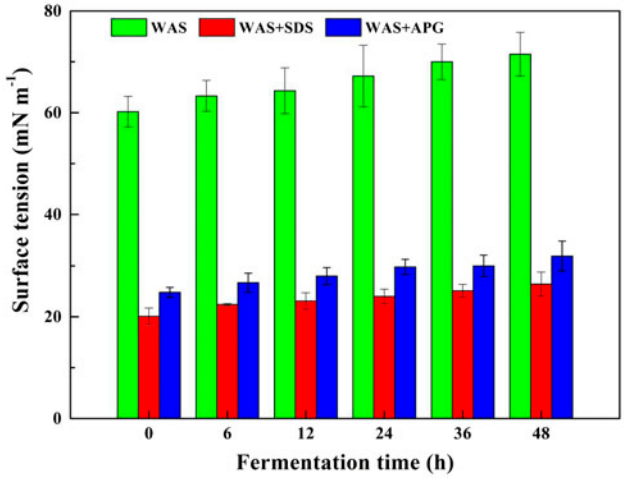

Fig. 6. The effect of SDS and APG on sludge surface tension.

the organic carbon in the sludge particulate and hydrolysis of the solubilized substrate [14]. Solubilization of WAS was the basis of SCAFs production. At the presence of the two surfactants, solubilization of the organic carbon in the sludge particulate could be ameliorated significantly. Thereafter, surface tension increased gradually with time, probably due to the formation of micelle. With the formation of micelle, surfactant monomer, which contributed to the reduced surface tension, decreased gradually [37].

Only when the solubilized substrates were hydrolyzed by extracellular hydrolase into small molecules, such as protein and carbohydrate, SCFAs could be produced from these small molecules in the acidification step [38,39]. Since enzyme activity affected the degradation of the organic matter and the sludge decrement process directly, the investigation of the protease and α-glucosidase activities was highly required. The protein could be digested into peptides by protease, while α-glucosidase could specifically acted on α-1,4-glucoside bond of glucose molecules.

Assuming the relative enzyme activity was 1 in the initial state, we got enzyme activities divided by the initial enzyme activity in different stages of experiments and the detailed information as shown in Fig. 7. The activities of protease and α-glucosidase increased first and then decreased with fermentation time in the three reactors. The addition of the two surfactants into WAS caused a greater increase in both protease and α-glucosidase activities in the WAS fermentation system. For example, the maximum relative activities of protease were 1.55 at the fermentation time of 36 h in the WAS + SDS fermentation system, and 1.48 at the fermentation time of 24 h in the WAS + APG fermentation

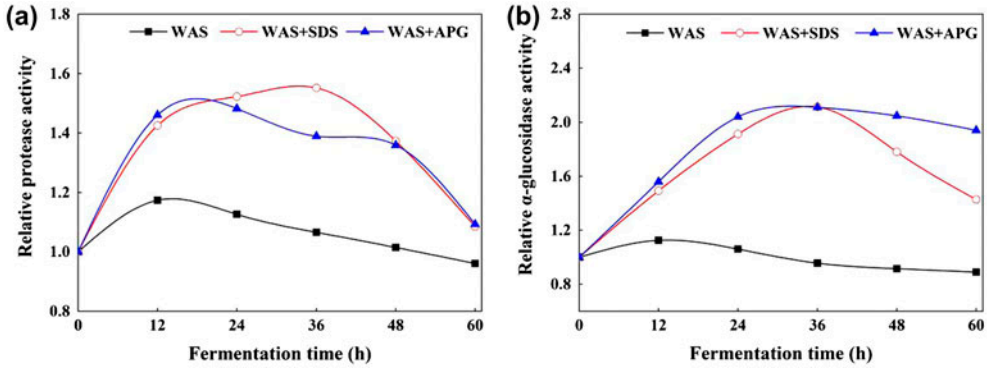

Fig. 7. Variation of (a) protease and (b) α-glucosidase activities with fermentation time.

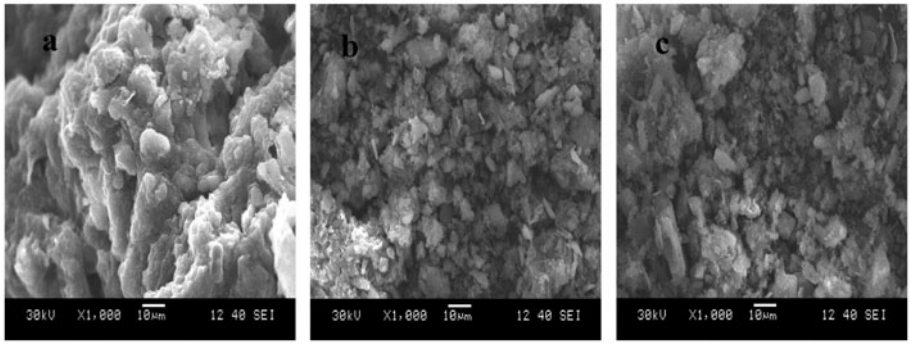

Fig. 8. Comparison of sludge morphology in WAS (a), WAS + SDS (b), and WAS + APG (c) system.

system, while it was only 1.17 at the fermentation time of 12 h in the control WAS fermentation system. The corresponding maximum relative activities of α-glucosidase in the WAS + SDS fermentation system, WAS + APG fermentation system, and the control WAS fermentation system were 2.11, 2.04, and 1.12, respectively. This results indicated that there was no significant difference in the hydrolase activity for both WAS + SDS and WAS + APG fermentation system. The increase in sludge hydrolase activity with surfactants addition was primarily due to two aspects, one was the enlargement of contact surface between microbial cells and solution, and another one was the exudation of hydrolase stimulated by surfactants [40].

During anaerobic hydrolysis process of WAS, broken of the big particles into small particles is crucial but difficult, probably due to the cohesive effect of the extracellular polymeric substances (EPS). In addition, a large number of high polymer compounds are coated by EPS which hinders the diffusion of the substrate and extracellular enzyme. As a result, the hydrolysis and acidification efficiency could be abated afterward [41,42]. As shown in Fig. 8(a), anaerobic sludge was found to possess a multi-layered structure. With the addition of the surfactants, the dimension, shape, and structure of sludge were altered significantly. At the presence of surfactants, the sludge flocs became smaller and looser (Fig. 8(b) and (c)) [43]. Similar results was also observed by Jiang et al. [44], they attributed the enhanced sludge fermentation to the enhanced solubilization of sludge EPS, which was caused by the addition of surfactants.

According to above discussion, the SCFAs production was significantly improved in the presence of SDS or APG. In order to investigate whether the formation of SCFAs was caused by chemical or biological effects, the batch fermentation tests with the addition of autoclaved and unautoclaved sludge were conducted, respectively. As shown in Table 2, at the presence of surfactants, SCFAs concentration in the unautoclaved WAS treatment system was much higher than that in autoclaved one. No obvious increase in the SCFAs concentration could be observed in the autoclaved system, while significant

Table 2
SCFAs production in autoclaved WAS or unautoclaved WAS with and without the addition of surfactants (the unit is mg L^{-1})

Fermentation time		12 h	24 h	36 h	48 h	60 h
Autoclaved	1 (SDS + WAS)	1,118	1,277	1,224	1,286	1,102
	2 (APG + WAS)	1,172	1,283	1,251	1,222	1,167
	3 (WAS)	1,106	1,217	1,146	1,102	904
Unautoclaved	4 (SDS + WAS)	1,474	1,935	2,398	2,514	2,335
	5 (APG + WAS)	2,423	2,057	2,271	2,175	2,064
	6 (WAS)	1,005	1,321	1,237	1,203	1,088

Table 3
SCFAs production from SDS and APG (the unit is mg L^{-1})

Fermentation time	0 h	3 h	6 h	12 h	24 h	36 h	48 h
SDS	37.9	59.2	64.1	64.6	68.4	70.2	65.3
APG	49.2	55.4	67.3	48.7	52.3	39.6	22.5

increase in the SCFAs concentration could be observed in the unautoclaved system. This result indicated that the formation of SCFAs was mainly caused by biological effects rather than chemical effects.

Under anaerobic conditions, SCFAs could also be produced from the microbial degradation of the surfactants. Thus, the contribution of surfactant to the SCFAs production during WAS fermentation has been investigated. It can be seen from Table 3 that in the surfactant fermentation experiment, the SCFAs produced from surfactant fermentation did not exceed 80 mg L^{-1} at any time, indicating that very little SCFAs was generated directly from the two surfactants. Thus, the significant increase in SCFAs production at the presence of the surfactants could be mainly attributed to the enhanced hydrolysis and acidogenesis of the WAS, not the hydrolysis and acidogenesis of the two surfactants added.

4. Conclusions

The key role of biosurfactant APG and chemical surfactant SDS in the hydrolysis and acidogenesis of WAS was investigated in this study. Sludge hydrolysis was significantly enhanced with the addition of SDS or APG. The increase in sludge hydrolysis resulted in the enhanced SCFAs accumulation. The percentages of individual SCFA were also influenced significantly by the two surfactants. The enhanced production of SCFAs at the presence of surfactants was mainly due to biological effects rather than chemical effects. Moreover, the contribution to SCFAs production by the degradation of the two surfactants was negligible.

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