Bioresource Technology 102 (2011) 7103–7110

Contents lists available at ScienceDirect

Bioresource Technology

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

Combined effect of sodium dodecyl sulfate and enzyme on waste activated sludge hydrolysis and acidification

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ARTICLE INFO

Article history:
Received 18 January 2011
Received in revised form 7 April 2011
Accepted 8 April 2011
Available online 15 April 2011

Keywords:
Acidification
Enzyme
Hydrolysis
Sodium dodecyl sulfate (SDS)
Waste activated slude (WAS)

ABSTRACT

The combined effect of sodium dodecyl sulfate (SDS) and enzyme system on hydrolysis and acidification of waste activated sludge (WAS) was investigated. The results showed that the combined system was more effective in the promotion of sludge hydrolysis than sole SDS and sole enzyme, and the SDS + mixed-enzymes (ME) system had better hydrolysis performance than SDS + single enzyme system. Compared with SDS + protease and SDS + amylase systems, the soluble protein concentration in SDS + ME system increased respectively by 20.0% and 44.4%, and the soluble carbohydrate concentration increased by 78.3% and 37.0%, respectively. During the WAS acidification stage, the SDS, ME and SDS + ME system could make the maximum short-chain fatty acids (SCFAs) concentration increased by 1.82 (6th day), 2.04 (5th day), 2.32 (7th day) times, respectively. The composition analysis of SCFAs produced in SDS + ME system indicated that acetic acid was the most prevalent product and propionic acid was the second one.

1. Introduction

The increasing amounts of WAS derived from wastewater treatment process had become a serious environmental issue concerned by modern society due to fast urbanization (Suthar, 2009). The annual production of WAS reached 25 million tons (calculated by 80% of water content) in China in recent years, which might pose a significant threat to ecology system if it was not properly disposed (Yang et al., 2010). In most developed countries, like European countries, WAS were conventionally treated by concentration and anaerobic digestion, which would account for 60% of the total operating cost of WWTPs (Campos et al., 2009), and that could not be accepted by many developing countries. Therefore, cost effective or towards reuse technologies for WAS disposal need to be developed.

Anaerobic digestion, in which three steps (hydrolysis, acidification and methanogenesis) are generally involved, is widely applied to stabilize organic matters of the sludge and thereby prevent or slow the release of harmful chemicals into environment (Bouskova et al., 2005). And in these steps, the hydrolysis and acidification of WAS have received more attention, since the products are rich in readily biodegradable substances, which are potentially renewable carbon sources, and had been utilized to produce biogas (Guo et al., 2008), generate electricity (Jiang et al., 2009), and been used as preferred external carbon source for nitrogen and phosphorus removal (Tong and Chen, 2009).

The hydrolysis of complex organic molecules in the degradation of biodegradable particulate organic matter depends heavily on hydrolytic enzymes, e.g., glucosidases, lipases and proteases (Ayol, 2005). The additional commercially available enzymes could promote matric hydrolysis, leading to increased rate of WAS disintegration. The enhancement of anaerobic digestion by either endogenous enzymes or industrial enzymes had been demonstrated. Roman et al. (2006) investigated the application of hydrolytic enzymes (cellulase and pronase E) in decreasing solids and subsequently improving sludge digestibility. Our previous studies also showed that a combination of protease and α-amylase could accelerate the solubilization rate of WAS (Yang et al., 2010). However, enzymatic treatment of WAS contributes significantly to the overall process cost due to the high enzyme cost and difficult enzyme recycling (Eriksson et al., 2002). The enzyme cost for enzymatic hydrolysis of WAS (TSS 8270 mg/L) would be approximately $3/m^3 according to our previous investigation (Yang et al., 2010). Therefore, it is proposed that those enzymes already present within the floc matrix, and those which are cell-membrane surface bound, be exploited.

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doi:10.1016/j.biortech.2011.04.023

Surfactant can cause an apparent increase in the aqueous solubility (Myers, 2006), thus accelerate the rate of nonaqueous phase substance dissolution into the aqueous phase, which may be able to compromise the floc integrity, liberate the trapped enzymes (within the floc matrix and on the cell-surface) and also expose more substrate. Jiang et al. (2007) found that surfactant could enhance the hydrolysis and acidification of WAS, and the hydrolysate (such as protein and carbohydrate) in aqueous phase were significantly improved, which contributed to higher SCFAs accumulation. Fu et al. (2009) investigated the effect of surfactants on WAS dewaterability and settleability, and also elucidated the action mechanism of cationic surfactant.

In consideration of the special characteristics of both enzyme and surfactant, the combination of surfactant and enzyme might exhibit a positive synergetic effect on waste solids hydrolysis. And it had been used for promoting the hydrolysis of newspaper and corn stover (Eriksson et al., 2002). However, there are very limited studies about the combined effect of surfactant and enzyme on WAS hydrolysis. Furthermore, the initial hydrolysis of particulate organic matter to soluble substance is believed to be the rate-limiting step of anaerobic digestion process (Eastman and Ferguson, 1981). Thus, acceleration of the sludge hydrolysis rate by applying surfactant together with enzyme might improve the production rate of SCFAs, and leading to an enhancement of WAS hydrolysis and acidification. Therefore, the main purpose of this study is to investigate the effect of a combination of surfactant and enzyme on protein and carbohydrate solubilization, NH_4^+–N release and SCFAs accumulation during the anaerobic digestion of WAS. Sodium dodecyl sulfate (SDS), protease and α-amylase are selected as the model surfactant and enzymes, respectively.

2. Methods

2.1. WAS and enzymes

WAS were obtained from the secondary sedimentation tank of the second municipal wastewater treatment plant in Changsha, China. Fresh sludge was concentrated by settling for 4 h, further filtered through a 0.71 mm metal sieve and then stored at 4 °C for later use. The characteristics of sludge were as followings: pH 6.74 ± 0.15, TCOD 8426 ± 254 mg/L, SCOD 120 ± 10 mg/L, TSS 8824 ± 200 mg/L, VSS 6050 ± 80 mg/L, carbohydrate 590 ± 65 mg/L, protein 5561 ± 185 mg/L. Apparently, protein and carbohydrate were the two predominant organic compounds in the WAS, which accounted for about 75% of the total chemical oxygen demand (TCOD).

The enzymes were preparations of commercial neuter protease and α-amylase (both purchased from solarbio biotechnology Ltd.). The activities of neuter protease and α-amylase were 6000 and 3700 U g^{-1}, respectively. Correspondingly, the optimal temperature of neuter protease was 40–50 °C, and the optimal temperature of α-amylase was 50–70 °C. According to our previous study (Yang et al., 2010), 50 °C was observed to be the optimum temperature for enhancing sludge hydrolysis by mixed-enzymes system.

2.2. Batch experiments

Experiments of the combined effect of SDS and single enzyme (protease or α-amylase) on WAS hydrolysis were carried out in 18 identical 250 mL reactors, which were made of plexiglass and each had a sludge volume of 100 mL. These reactors were averagely divided into three groups, and SDS was added into the six reactors of identical group at the dosages of 0, 0.02, 0.05, 0.10, 0.15 and 0.20 g/g dry sludge (DS), respectively. After that, the three groups were respectively added protease 0.06 g/g DS, α-amylase 0.06 g/g DS and without enzyme addition, in which the last group was set as the reference group.

To optimize the mixture ratios of protease and α-amylase in the presence of SDS, WAS were firstly pre-treated with SDS 0.10 g/g DS and then with enzymes 0.06 g/g DS, according to above tests and our previous study (Yang et al., 2010). The enzymes were the preparations of protease and α-amylase with the mixture ratios of 1:1, 1:2, 2:1, 1:3 and 3:1 (w/w), respectively.

Three batch experiments were undertaken to investigate the combined effect of SDS and mixed-enzymes (mixture of protease and α-amylase) on WAS hydrolysis and acidification. (I) SDS (SDS 0.10 g/g DS). (II) ME (mixed-enzymes 0.06 g/g DS, protease: α-amylase = 3:1). (III) SDS + ME (SDS 0.10 g/g DS, mixed-enzymes 0.06 g/g DS, protease: α-amylase = 3:1). The batch experiment without SDS and ME addition was set as the blank.

After adding various amounts of SDS and enzymes, all reactors in each experiment were capped with rubber stoppers, and oxygen in the flasks was removed from the headspace by nitrogen gas sparging for 4 min to maintain strict anaerobic condition. The flasks were then placed in a water-bath shaker (100 rpm) at 50 °C. All tests were conducted in triplicate, and the data shown in this paper were the average based on three independent experiments.

2.3. Analytical methods

Sludge samples from the reactors were firstly filtered through Whatmann GF/C glass microfiber filter. The filtrate was analyzed for SCFAs, carbohydrate and protein, and the filter residue was assayed for TSS and VSS. The analyses of TSS and VSS were conducted in accordance with Standard Methods (APHA, AWWA, WEF, 2005). Hydrolysis of WAS particulate organic matter causes sludge protein and carbohydrate release into aqueous phase. Therefore, the hydrolysis products can be expressed in terms of soluble protein and carbohydrate in this study. Soluble carbohydrate was measured by the phenol–sulfuric method with glucose as standard (Herbert et al., 1971). Soluble protein was determined by the Lowry–Folin method with bovine serum albumin (BSA) as standard (Lowry et al., 1951).

For the quantification of SCFAs, the filtrate was acidified with 3% H_3PO_4 to pH 4.0, and collected in a 1.5 mL gas chromatography (GC) vial before assaying on an Agilent 6890N GC with flame ionization detector (FID) and the automatic liquid sampler. The GC was equipped with a capillary free fatty acid phase (polarity) column (DB-FFAP, 30 m × 0.25 mm × 0.25 mm). The temperatures of the injector and detector maintained at 250 and 300 °C, respectively. The initial temperature of the oven was 70 °C for 3 min, followed with a ramp of 20 °C/min for 5.5 min and with a final temperature of 180 °C for 3 min. N_2 was used as carrier gas with a flow rate of 2.6 mL/min. The total SCFAs were recorded as the sum of measured acetic, pro-pionic, *n*-butyric, iso-butyric, *n*-valeric and iso-valeric acids.

Each sample was analyzed in triplicate and the standard deviations of all analyses were always less than 5%, unless noted in the text.

2.4. Data analysis

Descriptive statistics and correlation analysis were applied to treat the analytical data using SPSS 13.0 for Windows® (SPSS Inc., 2004). One-way ANOVA analysis was performed to assess and analyze the correlations among the three methods (SDS, SDS + protease and SDS + amylase) on hydrolysis at various dosages of SDS.

3. Results and discussion

3.1. Combined effect of SDS and single enzyme on WAS hydrolysis

Since foam would be generated due to the decrease of surface tension in the presence of surfactants, which might deteriorate the WAS treatment processe to a certain extent. According to the preliminary experiments, there was no obviously negative impact caused by foam on the WAS hydrolysis process at SDS dosage less than 0.20 g/g DS. As a result, a limitation of 0.20 g/g DS for SDS dosage was set for further research.

As seen in Fig. 1a and b, the concentration of soluble protein linearly improved with SDS dosage increasing from 0.02 to 0.20 g/g DS, and the carbohydrate concentration improved when the SDS dosage was within 0.10 g/g DS, whereas it did not improve obviously with further increase of SDS dosage. Surfactants can alter microorganism cell structure by making cell materials leave the attached surface and dissolve them in aqueous solution (Jiang et al., 2007). Furthermore, they hinder the immobilization of the enzymes on the substrate by reducing the binding strength. Thus, when surfactant work together with enzyme, the enzyme might more easily desorb from the binding site after reaction and move to other binding sites on the substrate (Helle et al., 1993). However, higher concentration of surfactant might contribute to negative effect on enzyme activity. The relative activities of the two enzymes depicted in Fig. 2 showed that protease activity increased 2.30-time at SDS dosage of 0.10 g/g DS, while further increase of SDS dosage resulted in a little decrease. As for α-amylase, lower dosage of SDS was beneficial for the enhancement of its activity. The activity increased 1.13-time at SDS amount of 0.02 g/g DS, whereas it was inhibited when SDS dosage was higher than 0.05 g/g DS.

As depicted in Fig. 1a and b, the SDS + single enzyme system had better hydrolysis performance on WAS than sole SDS and sole

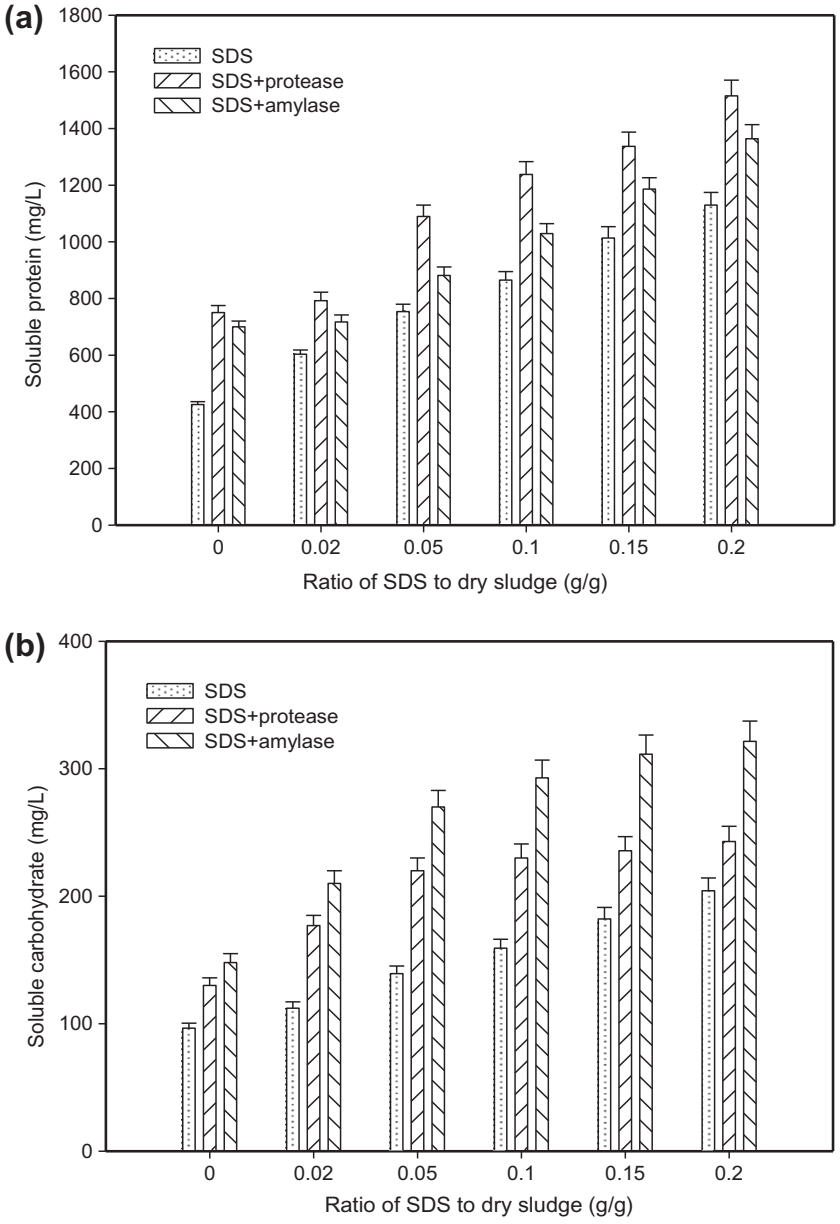

Fig. 1. Combined effect of SDS and single enzyme system on soluble protein and carbohydrate concentration (T = 50 °C, t = 4 h): (a) soluble protein concentration; (b) soluble carbohydrate concentration.

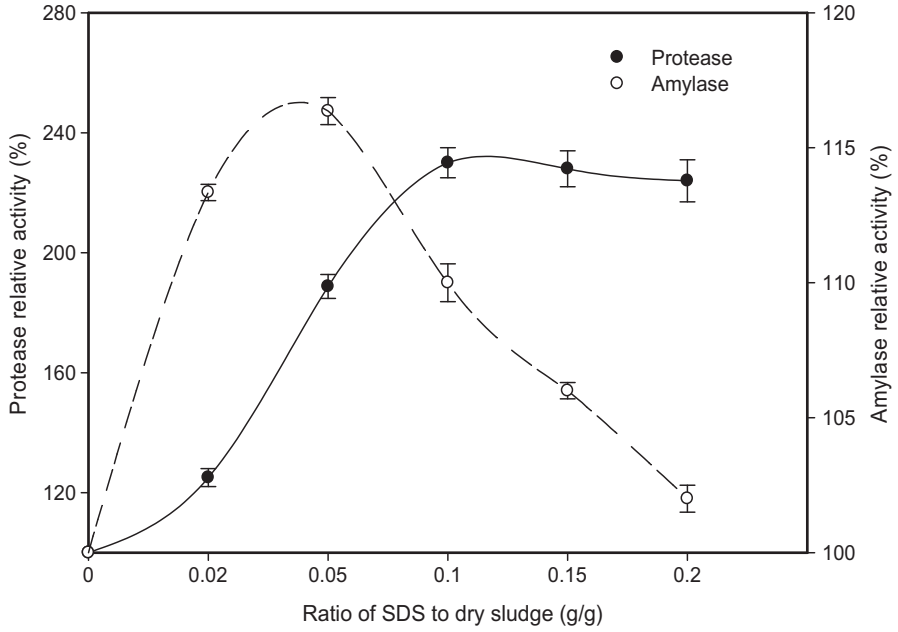

Fig. 2. Effect of SDS dosage on enzyme activities (T = 50 °C, t = 4 h).

enzyme. The ANOVA results (Table 1) further indicated there were significant different effects among the three hydrolysis methods (SDS, SDS + protease and SDS + amylase) for protein and carbohydrate releasing at various dosages of SDS ($F > F_{0.05}(2, 3)$, $P < 0.05$). Compared with the system at SDS dosage of 0.10 g/g DS, the soluble protein concentration in the SDS + protease and SDS + amylase systems increased by 43.2% and 19.0%, respectively, and the carbohydrate increased by 44.4% and 83.9%, respectively. Furthermore, in comparison with the system at protease dosage of 0.06 g/g DS (SDS + protease, SDS 0 g/g DS), SDS + protease (SDS 0.10 g/g DS, protease 0.06 g/g DS) system improved by 65.1% and 47.0% for soluble protein and carbohydrate concentration, respectively. Correspondingly, SDS + amylase (SDS 0.10 g/g DS, amylase 0.06 g/g DS) system improved by 77.0% and 97.9%, respectively, compared with the system at amylase dosage of 0.06 g/g DS (SDS + amylase, SDS 0 g/g DS).

3.2. Combined effect of SDS and ME on WAS hydrolysis

Single enzyme had limited hydrolytic activity while mixed-enzymes consisted of a variety of enzymes be capable of numerous catalytic functions, which could exhibit a synergistic effect on WAS hydrolysis. Considering the special characteristics of both mixed-enzymes and surfactant, the combined effect of SDS and ME on the WAS hydrolysis was studied. Based on the variation of soluble carbohydrate, protein and NH_4^+–N concentrations under different enzyme mixture ratio systems in the presence of SDS (Fig. 3), the mixture ratio of 3:1 for protease and α-amylase was found to have better hydrolysis performance on WAS. Therefore, the mixture ratio of 3:1 for protease and α-amylase was selected.

As seen in Fig. 4a and b, the concentration of soluble protein and carbohydrate increased gradually in the initial stage, but decreased later. The maximum soluble protein concentration reached 868.7, 1120.0 and 1486.0 mg/L, respectively for ME, SDS and SDS + ME system. Correspondingly, the maximum soluble carbohydrate concentration reached 187.5, 305.0 and 455.8 mg/L, respectively. Compared to the test without pretreatment (blank), soluble protein and carbohydrate improved by 2.42 and 3.05 times respectively to the system with SDS 0.10 g/g DS reported by Jiang et al. (2007), 1.78 and 2.54 times to ME system reported in our previous study (Yang et al., 2010), whereas improved by respectively, 4.10 and 5.06 times to SDS + ME system (Fig. 4a and b). Moreover, the SDS + ME system was observed to have better effect than that of SDS + single enzyme system in the promotion of WAS hydrolysis. The soluble protein concentration increased by 20.0% and 44.4%, respectively, and the soluble carbohydrate concentration increased by 78.3% and 37.0%, respectively, in comparison with the SDS + protease and SDS + amylase systems.

Soluble protein and carbohydrate are the result of a net balance between competing rates of release and degradation. Apparently, soluble protein and carbohydrate increased significantly under the collective function of SDS and ME (Fig. 4a and b). Thus the initial release rate of this substrate was higher than the degradation rate, which made the accumulation temporarily increase. However, in the later stage of digestion, the release rate slowed down and was exceeded by the degradation rate, which resulted in the decrease of soluble protein and carbohydrate concentrations, and the increase of NH_4^+–N and SCFAs concentrations (Figs. 4c and 5).

Table 1
Correlations among the three methods (SDS, SDS + protease and SDS + amylase) on hydrolysis at various dosages of SDS by ANOVA analysis.

Parameters	ANOVA		
	SDS(g/g DS)	F	P
Soluble protein	0	81.419	0.002
	0.02	15.573	0.026
	0.05	27.122	0.012
	0.10	25.273	0.013
	0.15	13.808	0.031
	0.20	15.015	0.027
Soluble carbohydrate	0	20.35	0.018
	0.02	39.342	0.007
	0.05	42.789	0.006
	0.10	36.617	0.008
	0.15	42.566	0.006
	0.20	21.384	0.017

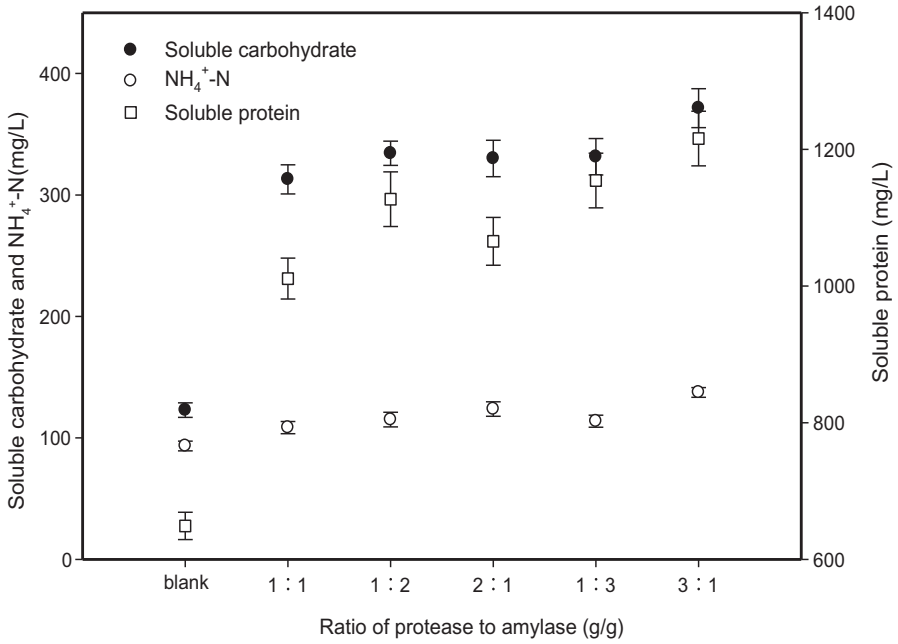

Fig. 3. Variation of soluble carbohydrate, protein and NH_4^+–N concentrations under different enzyme mixture ratio systems in the presence of SDS (T = 50 °C, t = 4 h).

3.3. NH_4^+–N release during WAS acidification

During the acidification stage of anerobic digestion, ammonia is produced by biological degradation of nitrogenous substrates, mostly in the form of sludge protein in this study, which is hydrolyzed to amino acids and further degraded to ammonia. The NH_4^+–N production in the digestion liquor kept increasing during the whole anerobic digestion period regardless of the treatment (Fig. 4c). Compared to the sole SDS and sole ME systems, the concentration of NH_4^+–N for SDS + ME increased by 30.0% and 44.9%, respectively at the digestion time of 72 h. Meanwhile, compared with the test without pretreatment (blank), NH_4^+–N concentration improved by 2.2 times to the system with SDS 0.10 g/g DS reported by Jiang et al. (2007), and 1.78 times to ME system reported in our previous study (Yang et al., 2010), whereas improved by 2.50 times to SDS + ME system (Fig. 4c). It could be seen from Fig. 4a and c that the great decrease of protein coincided with the increase of NH_4^+–N production, which also indicated the improved NH_4^+–N accumulation was mostly from the decreased protein.

Ammonia was one of the inhibitory substances to anaerobic digestion when it was present in substantial concentration in anaerobic reactors (Chen et al., 2008). Generally, the ammonia concentrations below 200 mg/L were beneficial to anaerobic process since nitrogen was an essential nutrient for anerobic microorganisms (Liu and Sung, 2002). Methanogens were likely to cease growth due to ammonia inhibition, the inhibitor ammonia concentrations that caused 50% reduction in methane production were from 1.7 to 14.0 g/L under different substrates, inocula and environmental conditions (temperature, pH) (Chen et al., 2008). In this study, the average released ammonia was no more than 224.94 mg/L for SDS + ME treatment, thus there was no significant inhibition observed.

3.4. SCFAs accumulation during WAS acidification

The formation of SCFAs during sludge acidification stage was associated with the digestion of sludge protein and carbohydrate (Yu et al., 2003), thus more hydrolysis products were correspondence with higher SCFAs accumulation. As Fig. 5 illustrated, SDS + ME system contributed more SCFAs accumulation than both sole SDS and sole ME systems. As discussed above, the addition of SDS and ME would cause more soluble protein and carbohydrate generation, which could provide more substrates for acidification and thus more SCFAs were observed. The maximum SCFAs concentration in the blank was 628.07 mg (COD)/L on the 6th day. In contrast, the SDS, ME and SDS + ME system could make the maximum SCFAs concentration increased by 1.82 (6th day), 2.04 (5th day), 2.32 (7th day) times, respectively. With the increase of digestion time, the SCFAs concentration gradually declined with methanogens fermentation.

Fig. 6 illustrated the individual SCFAs profile during the whole anerobic digestion process for SDS + ME system, and it could be found that acetic acid was the most prevalent product and propionic acid was the second one, which was consistent with the report of Zhang et al. (2009). However, Ahn and Speece (2006) found that propionic acid was the largest SCFA, and the lowest SCFA was isovaleric during primary sludge mesophilic or thermophilic digestion. It was mainly due to that the digestion substrates and treatment methods used in this study were different from that in the literature. Moreover, the propionic acid was found to be the mainly product during glucose acidification, whereas acetic acid was the top fraction when protein was fermented (Feng et al., 2009; Suwannakham and Yang, 2005).

The SCFAs can be utilized by methanogens, among which acetic and propionic acids are more easily biodegraded than slightly long-chain acid (such as iso-valeric acid) in the anaerobic digestion system. Therefore, the concentrations of both acetic acid and propionic acid were observed to decrease sharply with the increase of digestion time. However, due to its slow biodegradation rate, iso-valeric acid beyond propionic acid and became the second one after 10 days. Furthermore, the fraction of branched SCFA (iso-butyric and iso-valeric) obtained was much greater than their corresponding straight SCFA (*n*-butyric and *n*-valeric). The main reason might be that the decomposition rate of SCFA with a straight-chain (C2–C5) was greater than that of their respective isomer with a branched chain (Wang et al., 1999).

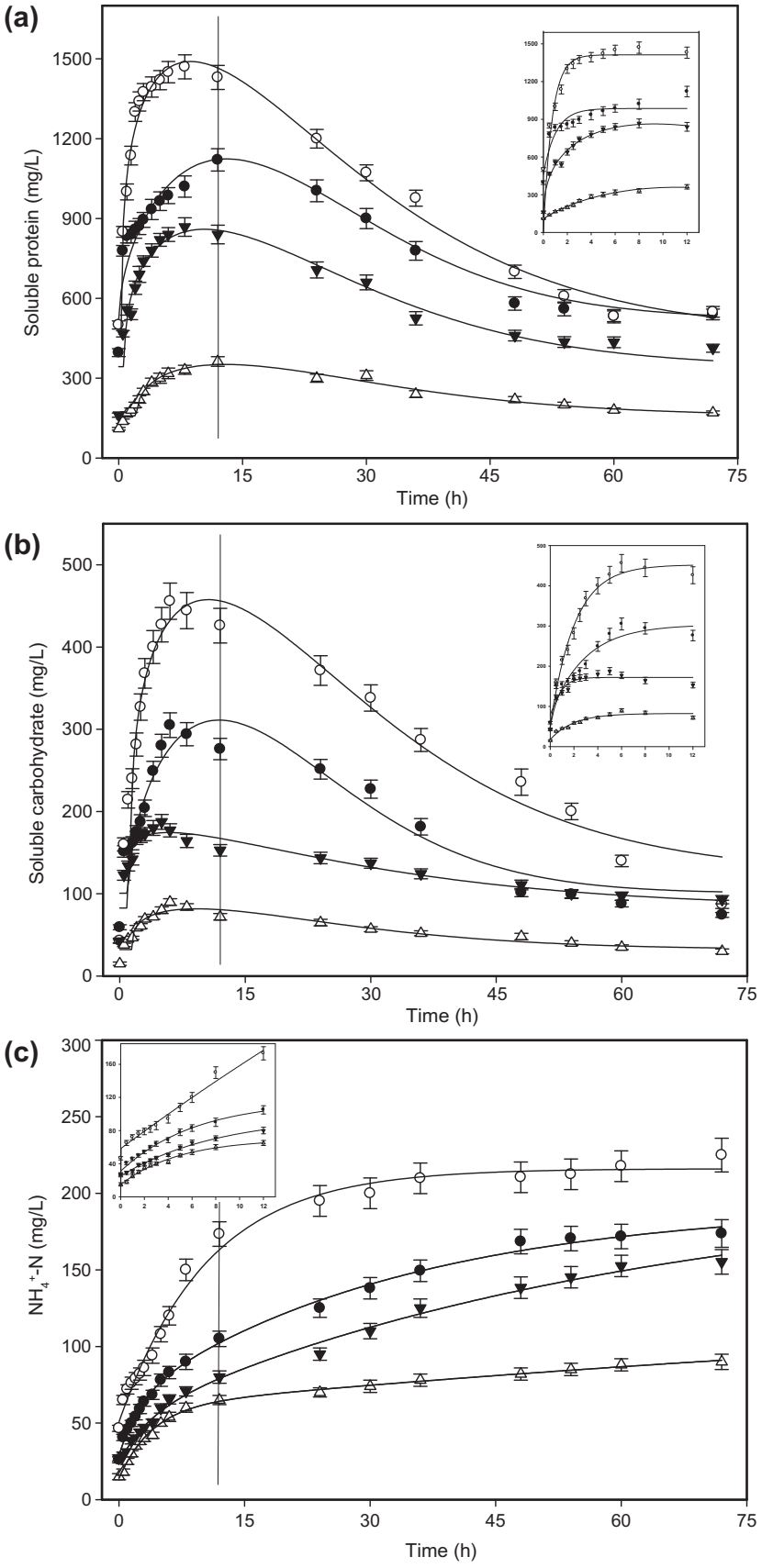

Fig. 4. Combined effect of SDS and ME on soluble protein, carbohydrate and NH_4^+–N concentrations (T = 50 °C, t = 72 h): (a) soluble protein concentration; (b) soluble carbohydrate concentration; (c) the NH_4^+–N release. SDS + ME (○), SDS (●), ME (▼), blank (△).

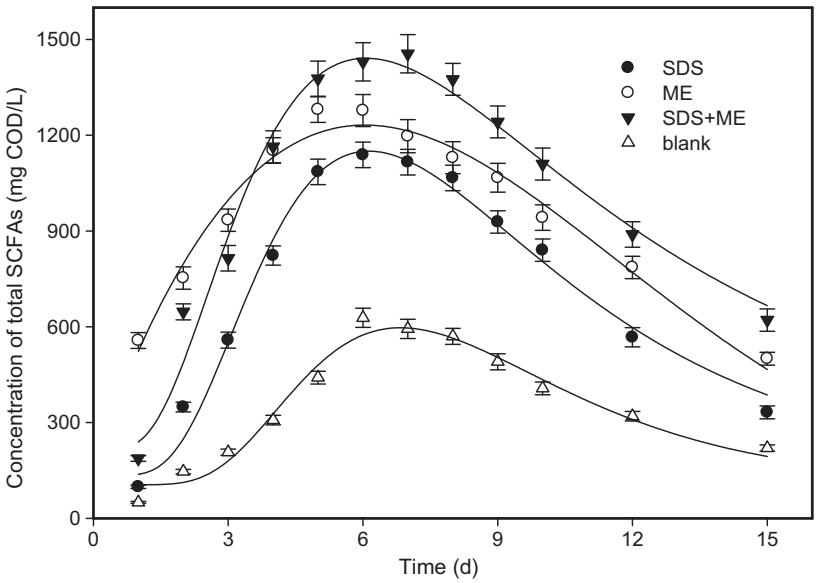

Fig. 5. Total SCFAs accumulation during acidification stage (T = 50 °C, t = 15 d).

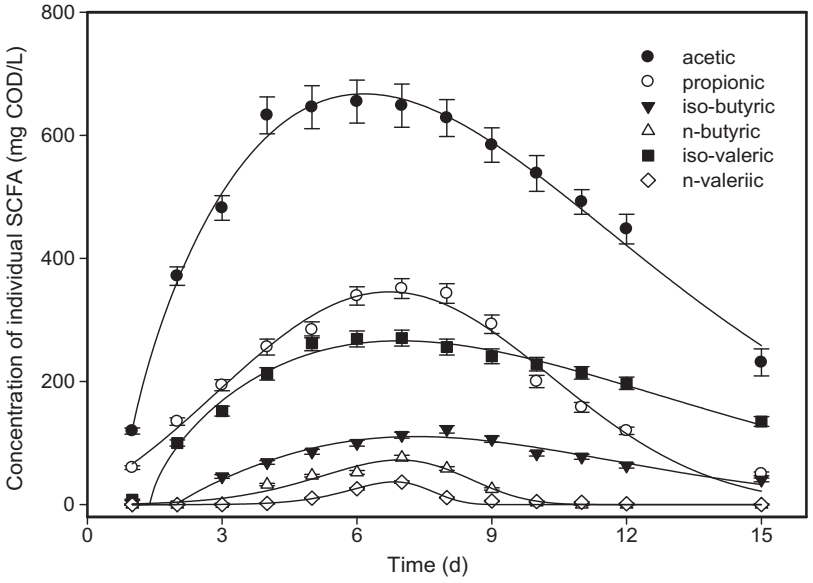

Fig. 6. Profiles of individual SCFAs concentration during the whole digestion process for SDS + ME system (T = 50 °C, t = 15 d).

4. Conclusions

The combination of SDS and enzyme was proved to be very effective in the promotion of sludge hydrolysis and acidification. The SDS + ME system had better hydrolysis performance than SDS, ME and SDS + single enzyme system at the temperature of 50 °C. The soluble protein and carbohydrate concentrations respectively increased from 362.5 and 90.0 mg/L (blank) to 1486.0 and 455.8 mg/L for SDS + ME system (SDS 0.10 g/g DS, mixed-enzymes 0.06 g/g DS, protease: α-amylase = 3:1). More hydrolysate was correspondence with higher NH_4^+–N release and SCFAs accumulation during WAS acidification stage. Acetic, propionic and iso-valeric acids were the main SCFAs in WAS hydrolysate.

Acknowledgements

This research was financially supported by the project of National Natural Science Foundation of China (Grant No. 51078128), Hunan Key Scientific Research Project (2009FJ1010), Hunan Provincial Natural Science Foundation of China (10JJ7005), and Planned Science and Technology Project of Hunan Province, China (Grant No. 2009WK3048).

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