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ENVIRONMENTAL BIOTECHNOLOGY

Hydrolysis and acidification of waste-activated sludge in the presence of biosurfactant rhamnolipid: effect of pH

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Abstract In this investigation, the effect of pH (4.0–11.0) on waste-activated sludge (WAS) hydrolysis and acidification in the presence of a biosurfactant rhamnolipid (RL) were studied. The results showed that the hydrolysis and acidification of WAS in the presence of RL at alkaline pH values were more efficient than that at acidic and near-neutral pH values. After 6 h of hydrolysis, the soluble protein and carbohydrate were 1,654.7 and 675.9 mg/L (pH 11.0), and 825.6 and 376.0 mg/L (pH 7.0), whereas the values were only 315.0 and 84.0 mg/L at pH 4.0 and 164.1 and 32.0 mg/L for the blank, respectively. After 2 or 3 days of fermentation, the accumulated short-chain fatty acids (SCFAs) reached the highest and then decreased with a further increase in time at all investigated pH values. The analysis of SCFA compositions showed that acetic, propionic, and iso-valeric acids were the three main products at any pH value. A higher pH contributed to a greater proportion of acetic acid and a lesser proportion of iso-valeric acid; a lower pH resulted in a greater proportion of iso-valeric and lesser proportion of acetic acid in the initial fermentation. The proportions of acetic acid for the system with biosurfactant RL addition were 16.65, 36.33, and 62.94 %, respectively, at pH 4.0, 7.0, and 11.0 after 1 day. Correspondingly, the proportions were 40.34, 12.60, and 11.01 % for iso-valeric acid.

Keywords Acidification · Biosurfactant · Hydrolysis · pH · Rhamnolipid · Waste-activated sludge

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Introduction

Rapid urbanization in many areas of the world has resulted in an increase of waste-activated sludge (WAS) from wastewater treatment plants (WWTPs), which has become a serious environmental issue (Suthar 2009). The costs for traditional treatment and disposal of WAS are quite expensive and would account for up to 60 % of the total operating cost of WWTPs (Low et al. 2000; Wei et al. 2003). Land scarcity and the increasingly stringent environmental control regulations are moving sludge management toward the reutilization of sludge as useful resources (Mossakowska et al. 1998).

Anaerobic digestion is a widely applied method for sludge stabilization and biogas production, in which three steps—hydrolysis, acidification, and methanogenesis—are generally involved. Recently, researchers have paid more attention to the hydrolysis and acidification of WAS, by which sludge reduction and short-chain fatty acid (SCFAs) production are simultaneously accomplished. On the one hand, it is well known that the initial hydrolysis of particulate organic matter to soluble substances is the rate-limiting step of anaerobic digestion (Eastman and Ferguson 1981). On the other hand, the hydrolysis products (mainly SCFAs), as the potentially renewable carbon sources, could be utilized to produce biogas (Wang et al. 2003), generate electricity (Min and Logan 2004), and remove biological nutrient (Li et al. 2011).

In order to accelerate the degradation of organic matters, several efforts have been made to improve the rate of sludge hydrolysis, including thermal (Phothilangka et al. 2008), thermochemical (Tanaka and Kamiyama 2002), mechanical (Nah et al. 2000), ultrasonic (Kang et al. 2011), and enzymatic methods (Yang et al. 2010). It has been reported that surfactants can apparently increase the solubilization and dispersion of the hydrocarbon and change the affinity between the microbial cells and the hydrocarbons by inducing increases in cell surface hydrophobicity, thus accelerating the rate of non-aqueous phase substance dissolution into the aqueous phase (Mayer et al. 1999; Zhang and Miller 1992). However, most of the surfactants, such as Span 20, Span 60, Triton X-100, polyethylene glycol, and sodium dodecyl sulfate (SDS), are chemically synthesized and not biodegradable, thereby posing a significant threat to the environment. Recently, biosurfactants have attracted much attention due to their lower toxicity, better environmental compatibility, biodegradability, and effectiveness in a wide range of temperatures and pH (Mulligan 2005), and they are considered as potential substitutes of synthetic chemicals. Liu et al. (2006) found that rhamnolipid (RL), one of the most important biosurfactants, can noticeably improve the production of cellulase and xylanase in rice straw–bran–sawdust solid substrate fermentation. Zhang et al. (2009a) reported that RL can reduce the requirement of cellulases and promote its recycling process in the rice straw hydrolysis bioprocess.

The available information mainly focused on the effects of biosurfactant RL on the cellulose hydrolysis process. As to our knowledge, however, the effects of biosurfactant RL on WAS hydrolysis and acidification, especially at different pH values, have not been documented in the literature. In a previous literature, pH has been proven to be an important parameter influencing the hydrolysis and acidification of WAS (Chen et al. 2007). In addition, the physical properties of RL solutions including the morphology and surface tension reduction were sensitive to pH (Zhang and Miller 1992). Therefore, the main purpose of this study was to investigate whether the hydrolysis of WAS can be enhanced by biosurfactant RL, the effect of pH on the hydrolysis and acidification of WAS in the presence of biosurfactant RL according to the solubilization of the protein and the carbohydrate, the release of $NH_4^+ - N$ and $PO_4^{3-} - P$, and the accumulation of SCFAs during the anaerobic digestion of WAS.

Material and methods

WAS and biosurfactant

The WAS used in this study was collected from the secondary sedimentation tank of the second municipal WWTPs 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 the sludge in this study are presented in Table 1.

The RL, selected as the model biosurfactant in this study, was purchased from Yuzhou Biotechnology, Ltd. (China) and was produced from sterilized and centrifuged fermentation broth from the bacterium *Pseudomonas aeruginosa* (ATCC 9027). The raw RL containing 50 % of pure RL was brown-colored and cream-shaped.

Batch experiments

Batch fermentation experiments of WAS hydrolysis in the presence of biosurfactant RL were carried out in five identical 250-mL reactors which were made of Plexiglass; each had a sludge volume of 100 mL, and RL was added into the reactors at dosages of 0, 0.20, 0.30, 0.40, and 0.50 g/g dry sludge (DS), respectively. To maintain strict anaerobic condition, nitrogen gas was blown into the reactors for 4 min before batch experiments. All reactors capped with rubber stoppers were agitated in a water bath shaker at 100 rpm and 30 °C for 6 h.

Experiments of WAS hydrolysis and acidification in the presence of biosurfactant RL at different pH values were conducted as the following: RL was added into each flask with a dosage of 0.30 g/g DS; meanwhile, the pH value in the reactor was immediately adjusted to 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, and 11.0, respectively, by adding 2 M sodium hydroxide (NaOH) or 2 M hydrochloric (HCl). The batch experiment without RL addition and pH-controlled was set as the blank. The reactors under strict anaerobic conditions were placed in water bath shakers (100 rpm) for 9 days and the temperature kept constantly at 30 °C. The samples in all reactors were assayed every certain interval.

Table 1 Characteristics of the raw WAS

Parameters	Value
pH	6.93±0.15
TSS (mg/L)	10,900±200
VSS (mg/L)	6,260±80
SCOD (mg/L)	360±10
TCOD (mg/L)	9,760±254
Carbohydrate (mg (COD)/L)	880±65
Protein (mg (COD)/L)	6,560±185

COD mass equivalents of carbohydrate and protein were 1.07 and 1.50 gCOD/g, respectively (Miron et al. 2000)

TSS total suspended solids, *VSS* volatile suspended solids, *SCOD* soluble chemical oxygen demand, *TCOD* total chemical oxygen demand

Analytical methods

Sludge samples from the reactors were firstly filtered through a 0.45-μm membrane filter (Whatmann, USA). The filtrate was immediately analyzed for soluble chemical oxygen demand (SCOD), $NH_4^+ - N$, $PO_4^{3-} - P$, carbohydrate, protein, and SCFAs, and the filter residue was assayed for total suspended solids (TSS) and volatile suspended solids (VSS). The analyses of SCOD, $NH_4^+ - N$, $PO_4^{3-} - P$, TSS, and VSS were conducted in accordance with standard methods (Eaton et al. 2005). The determination of carbohydrate, protein, and SCFAs was the same as described in our previous publications (Luo et al. 2011; Yang et al. 2010).

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

Results

Effect of biosurfactant RL on WAS hydrolysis

In this study, WAS hydrolysis was expressed as the changes of SCOD in fermentation liquid. As seen in Fig. 1, the SCOD value of the raw WAS was only 734.0 mg/L after 6 h of hydrolysis, which increased to 3,994.5 and 4591.4 mg/L at RL dosages of 0.30 and 0.50 g/g DS, respectively. Similarly, it could be observed from Fig. 1 that the concentrations of the soluble protein and soluble carbohydrate linearly increased to respectively 802.5 and 401.4 mg/L in the range of RL dosage from 0.10 to 0.30 g/g DS, whereas they remained almost the same with a further increase of the RL dosage to 0.50 g/g DS.

Effect of pH on WAS hydrolysis in the presence of biosurfactant RL

WAS hydrolysis

As discussed above, the optimal RL dosage could be observed as 0.30 g/g DS; thus, the reactors with RL 0.30 g/g DS were operated at different pH values (in the range of 4.0–11.0) to investigate the effect of pH on WAS hydrolysis in the presence of biosurfactant RL. The effect of pH on SCOD concentration in the presence of RL at different hydrolysis times is shown in Fig. 2. Obviously, the SCOD concentration in each reactor increased gradually with time. However, the pH had a different effect on the hydrolysis of WAS. The SCOD values at alkaline pH values (pH 9.0, 10.0, and 11.0) were significantly higher than those at near-neutral pH values (pH 6.0, 7.0, and 8.0) or acidic pH values (pH 4.0 and 5.0), or in the blank test at different hydrolysis times. After 6 h of hydrolysis, the SCOD was 5,656.6 at pH 11.0, 827.9 mg/L at pH 4.0, 3,872.4 mg/L at pH 7.0, and 729.2 mg/L for the blank test.

Protein and carbohydrate release

As shown in Fig. 3, pH had almost the same effect on the concentrations of soluble protein and carbohydrate as SCOD for WAS hydrolysis in the presence of RL. The concentrations of the two hydrolysis products under alkaline pH values were greater than in the other conditions. After

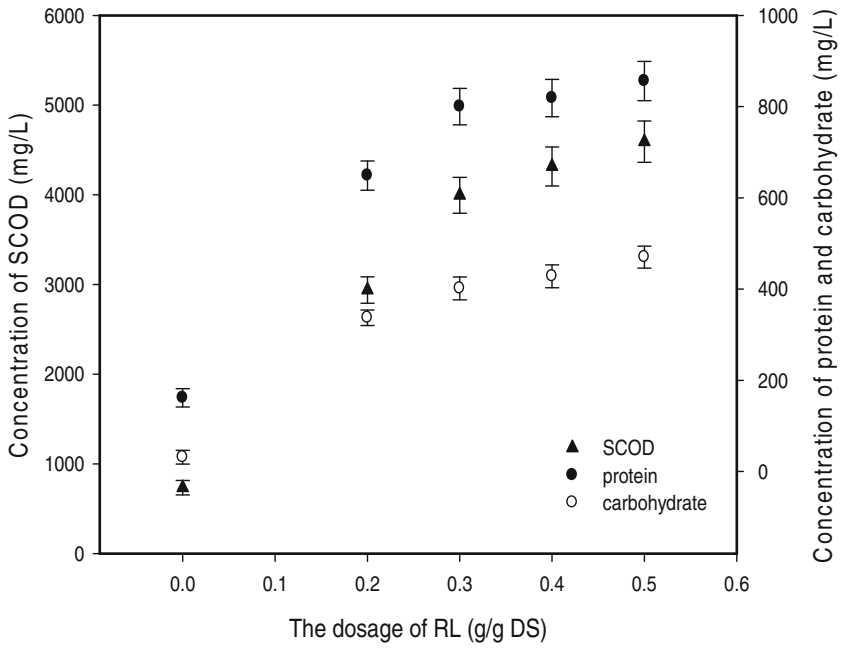

Fig. 1 Variation of SCOD and hydrolysis product concentrations at different RL dosages (T=30 °C, t=6 h)

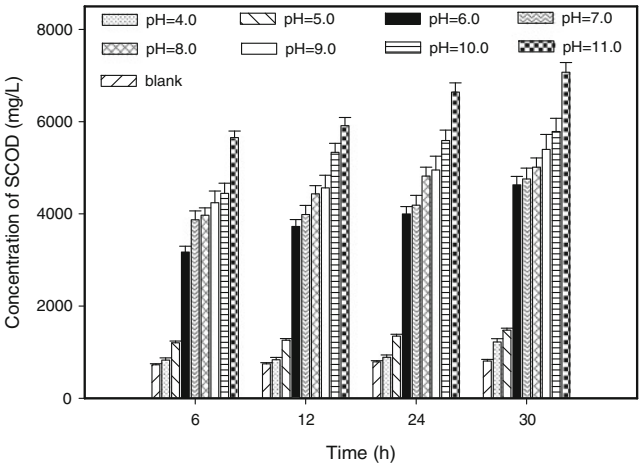

Fig. 2 Effect of pH on SCOD in the presence of biosurfactant RL

6 h of hydrolysis, the soluble protein and carbohydrate were 1,654.7 and 675.9 mg/L (pH 11.0), and 825.6 and 376.0 mg/L (pH 7.0), whereas the values were only 315.0 and 84.0 mg/L at pH 4.0 and 164.1 and 32.0 mg/L for the blank, respectively.

$NH_4^+ - N$ and $PO_4^{3-} - P$ release

The hydrolysis of WAS resulted in significant increases of $NH_4^+ - N$ and soluble phosphorus ($PO_4^{3-} - P$; Fig. 4). As seen in Fig. 4a, the concentration of $NH_4^+ - N$ in the presence of biosurfactant RL was the highest at alkaline pH (pH 9.0 and 10.0) and the lowest at acidic pH (pH 4.0 and 5.0). As shown in Fig. 4b, significant amounts of soluble phosphorus ($PO_4^{3-} - P$) were released in the presence of biosurfactant RL at various pH values. However, the influence of pH on $PO_4^{3-} - P$ concentrations was a little different from that on $NH_4^+ - N$. The concentration of $PO_4^{3-} - P$ was the highest at near-neutral (pH 6.0 and 7.0) and slightly alkaline conditions (pH 8.0 and 9.0) and the lowest at acidic pH (pH 4.0 and 5.0).

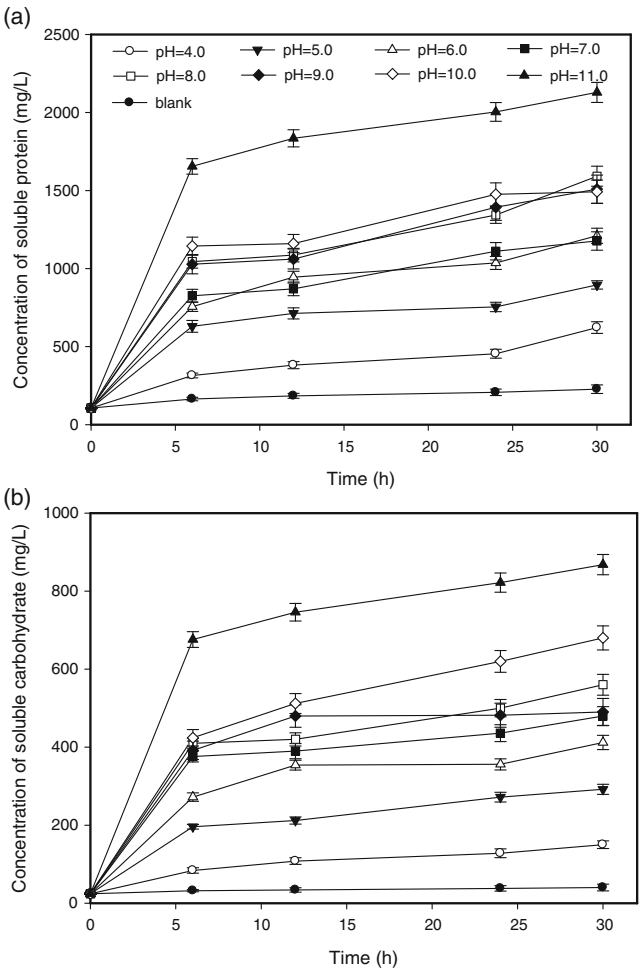

Fig. 3 Effect of pH on soluble protein (**a**) and carbohydrate (**b**) in the presence of biosurfactant RL

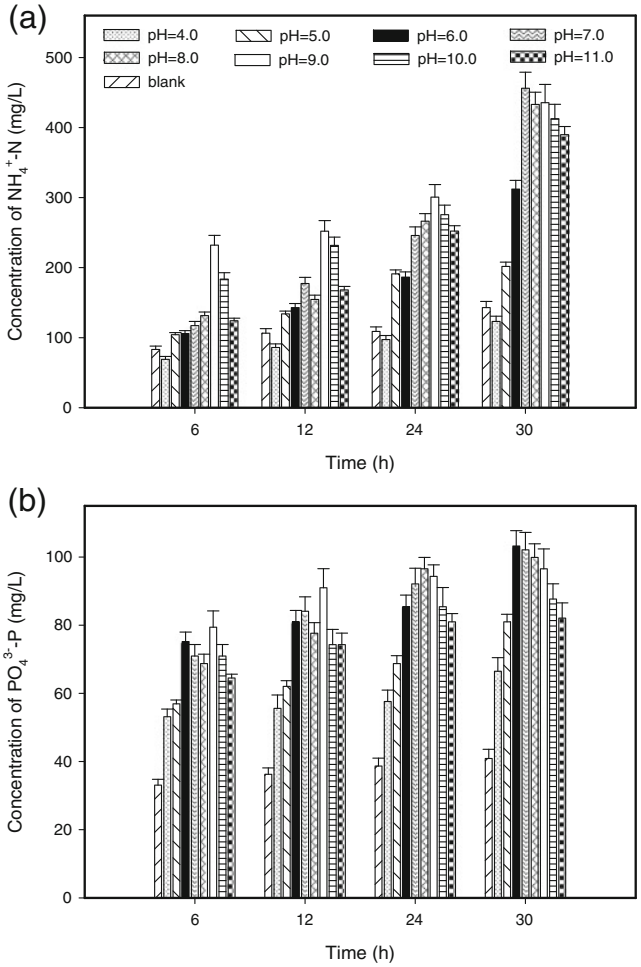

Fig. 4 Releases of $NH_4^+ - N$ (**a**) and $PO_4^{3-} - P$ (**b**) during WAS hydrolysis in the presence of biosurfactant RL at various pH values

Effect of pH on WAS acidification in the presence of biosurfactant RL

SCFA accumulation

The total SCFA production in the presence of biosurfactant RL at different pH values and fermentation times is shown in Fig. 5. SCFA accumulation with biosurfactant RL addition was higher than that without RL addition, and SCFA concentration under alkaline conditions was greater than that under acidic and neutral ones. It was observed that the accumulated SCFAs reached the highest after 2 or 3 days and then decreased with a further increase in time at all pH values, except for the blank. The mean SCFA production was 0.222 g chemical oxygen demand (COD) per gram VSS when the pH was not controlled (pH 7.0), 0.144 gCOD/g VSS at pH 4.0, 0.202 at pH 5.0, 0.313 at pH 9.0, 0.296 at pH 10.0, and 0.270 at pH 11.0.

SCFA composition

Short-chain fatty acids (C_2–C_5) are usually the main acidification products of WAS. Table 2 summarizes the percentage of individual SCFA accounts for total SCFAs at different pH values at the initial 4 days of fermentation. The data indicated that acetic, propionic, and iso-valeric acids were the three main products in the range of pH investigated. At pH 4.0, the individual SCFA concentration was in the following order: iso-valeric>propionic>acetic> iso-butyric>(*n*-valeric, *n*-butyric); it was acetic>propionic> iso-valeric>iso-butyric>(*n*-valeric, *n*-butyric) for the blank. However, acetic acid was the top fraction on the first day, and propionic acid beyond it, and became the first one after 2 days at pH 7.0 in the presence of biosurfactant RL. As for pH 11.0, acetic acid was the most prevalent product at all times, and iso-valeric acid beyond propionic acid, and became the second one after 2 days of fermentation.

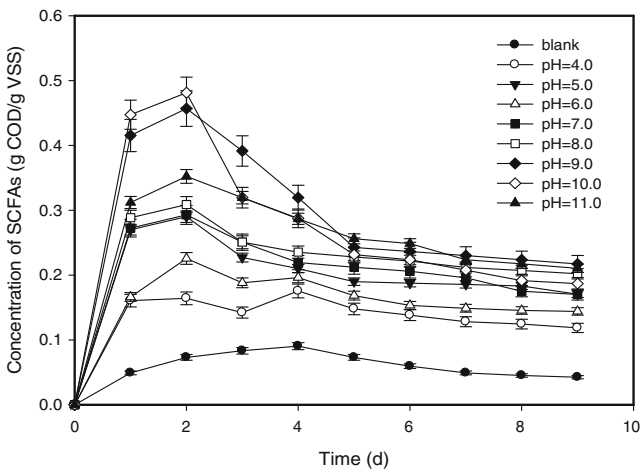

Fig. 5 Effect of pH on total SCFAs at biosurfactant RL 0.30 g/g DS

Discussion

Effect of biosurfactant RL on WAS hydrolysis

WAS hydrolysis could be expressed as the changes of SCOD in the fermentation liquid. It was obvious that the SCOD was greatly increased with the addition of RL (Fig. 1), suggesting that RL caused a large amount of colloidal and insoluble organic in sludge flocs transfer into soluble organic. As reported, protein, carbohydrate, and lipid are the main constituents of domestic sludge (Tanaka and Kamiyama 2002); thus, the observed soluble carbohydrate and protein could be regarded as the main hydrolysis products. The concentrations of SCOD, protein, and carbohydrate were increased to 3,994.5, 802.5, and 401.4 mg/L after 6 h at a RL dosage of 0.30 g/g DS, respectively. Compared with the WAS hydrolysis enhanced by a chemical surfactant, protein (827.73 mg/L) and carbohydrate (157.63 mg/L) at an SDS dosage of 0.10 g/g DS after 6 days reported by Jiang et al. (2007a) and the protein (698.5 mg/L) and carbohydrate (83.6 mg/L) at an SDBS dosage of 0.10 g/g DS at WAS fermentation time of 3 days reported by Jiang et al. (2007b), the carbohydrate in this study was largely improved and the fermentation time was greatly reduced.

The bacteria in WAS are mainly Gram-negative bacteria, of which the outer membrane is composed of lipopolysaccharides (LPS) and phospholipids, covalently linked to the peptidoglycan layer through hydrophobic interactions (Sikkema et al. 1995). Biosurfactants can reduce surface tension and ultimately affect the aqueous dispersion of the solution; therefore, they have the potential to enhance the biodegradation of solid organics (Jain et al. 1992; Vinson et al. 1991). Sotirova et al. (2009) proved that the release of LPS from the cell surface may be due to the solubilization of the outer membrane by the binding of the aggregated biosurfactant RL to the membrane followed by the removal of the LPS component. The disruption of the outer membrane observed in certain zones allows for an increased passage of hydrophobic compounds, thus contributing to the increased cell permeability and increasing significantly the levels of extracellular proteins and carbohydrates.

Effect of pH on WAS hydrolysis in the presence of biosurfactant RL

Previous work has shown that the effect of biosurfactant RL on surface tension and solute dispersion was a function of pH (Zhang and Miller 1992). As seen from Fig. 2, it could be concluded that an acidic pH condition inhibited WAS hydrolysis in the presence of RL, whereas an alkaline pH promoted the process, which had also been observed by other researchers for the system that only controlled pH (Vlyssides and Karlis 2004; Chen et al. 2007). The structure of biosurfactant RL was progressively smaller as the pH increased, and its morphology changed from lamellar, to vesicles, to micelles

Table 2 Percentage of individual SCFA accounted for the total SCFAs at various pH values

	HAc (%)	HPr (%)	i-HBu (%)	HBu (%)	i-HVa (%)	HVa (%)
pH 4.0						
1st day	16.65	25.19	11.74	4.78	40.34	1.30
2nd day	14.05	24.10	13.49	4.90	40.65	2.82
3rd day	15.81	22.98	13.57	5.43	38.53	3.69
4th day	17.32	23.87	10.90	5.41	38.02	4.47
pH 7.0						
1st day	36.33	28.50	9.40	9.39	12.60	3.79
2nd day	25.93	32.26	10.32	11.77	15.15	4.58
3rd day	22.82	29.53	9.51	13.85	16.66	7.62
4th day	22.65	28.99	9.78	13.59	16.77	8.21
pH 11.0						
1st day	62.94	21.11	2.29	0.28	11.01	2.37
2nd day	40.27	15.84	11.83	5.52	25.59	0.95
3rd day	39.76	16.73	11.02	6.95	24.40	1.14
4th day	39.04	15.43	11.29	6.17	25.01	1.67
Blank						
1st day	50.67	25.64	3.88	1.90	15.44	2.47
2nd day	38.45	26.76	4.91	2.14	25.04	2.70
3rd day	24.98	23.41	23.49	2.58	22.51	3.03
4th day	24.46	22.92	23.00	2.52	22.04	5.04

HAc acetic, *HPr* propionic, *i-HBu* iso-butyric, *HBu n*-butyric, *i-HVa* iso-valeric, *HVa n*-valeric

(Champion et al. 1995); thus, it could largely enhance the aqueous dispersion and biodegradation of less soluble organic waste components, which contributed to the high efficiency of WAS hydrolysis at alkaline pH values.

The pH had almost the same effect on the concentrations of soluble protein and carbohydrate as SCOD for WAS hydrolysis in the presence of RL (Fig. 3a, b). It might be expected that the cell surface became more hydrophobic in the presence of biosurfactant RL (Sotirova et al. 2009), and the alkaline conditions could improve the solubilization function of biosurfactant RL, thus increasing cell permeability and accelerating significantly the levels of extracellular proteins and carbohydrates. Furthermore, the main compositions of the extracellular polymeric substances (EPS), protein, and carbohydrate were more easily released at alkaline pH values due to the dissociation of the acidic groups in EPS and the repulsions between the negatively charged EPS (Wingender et al. 1999).

The concentrations of the two hydrolysis products (protein and carbohydrates) presented a quick increase at the beginning of hydrolysis and an obvious platform afterwards. The reason was that soluble protein and carbohydrate were the result of a net balance between competing rates of release and degradation. The initial release rates of soluble protein and carbohydrate were much faster than the degradation rates under the solubilization function of biosurfactant RL at different pH values, whereas the degradation rates with complete solubilization of EPS gradually were equal to the release rates with the increase in time.

As protein was one of the main components of WAS, thus, the release of $NH_4^+ - N$ was mainly from the hydrolysis of sludge protein. Biosurfactant RL can alter microorganism cell structure by making the cell materials leave the attached surface and dissolving them in the aqueous solution (Sotirova et al. 2009). Moreover, they hinder the immobilization of the enzymes on the substrate by reducing the binding strength. Thus, it could be concluded that the interaction between RL and the enzymes improve enzyme stability and activity. Since

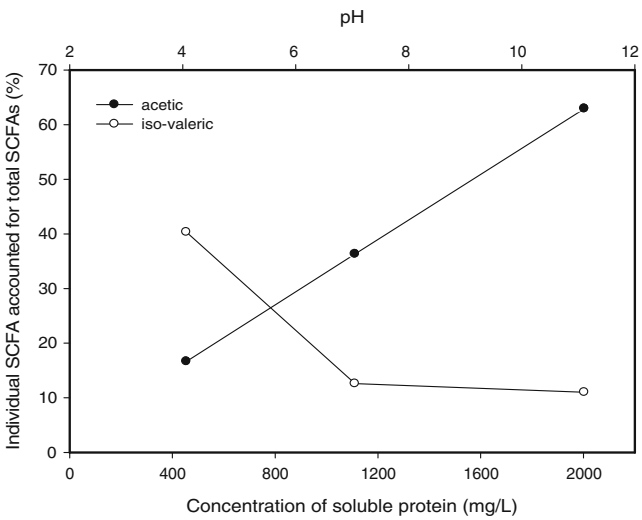

Fig. 6 Relationship between protein concentration and individual SCFA accounted for total SCFAs

stronger acidic and alkaline conditions would decrease the activities of sludge hydrolytic enzymes, such as protease, peptidase, etc.—meanwhile the WAS hydrolysis could be enhanced at alkaline condition—thus, the concentration of $NH_4^+ - N$ in the presence of biosurfactant RL was the highest at alkaline pH values (pH 9.0 and 10.0). The release of $NH_4^+ - N$ was greater than that of $PO_4^{3-} - P$ in most cases (Fig. 4), which might be attributed to the fact that the particulate nitrogenous materials were readily degraded during the acid phase of anaerobic digestion (Eastman 1977) and protein was one of the main components of WAS. Unlike $NH_4^+ - N$, $PO_4^{3-} - P$ was not in agreement with protein and carbohydrate releases. The reasons were the following: firstly, $PO_4^{3-} - P$ release was mainly related to the phospholipids in the outer membrane of the bacteria, which could be released due to the disruption of the outer membrane by biosurfactant RL (Miron et al. 2000). Secondly, according to the distribution formulas of weak acid, $H_2PO_4^-$, HPO_4^{2-}, and PO_4^{3-} accounted for 98.6, 99.4, and 83.1 % of the total phosphorus at pH 4.0, 10.0, and 13.0, respectively; thus, HPO_4^{2-} and PO_4^{3-} existed simultaneously at pH 11.0. PO_4^{3-} could be reacted or co-precipitated with the positive ions in the sludge, such as Fe^{2+}, Zn^{2+}, Cu^{2+}, thus lowering the PO_4^{3-} produced at pH 11.0 than that at pH 9.0 and 10.0.

Effect of pH on WAS acidification in the presence of biosurfactant RL

SCFA accumulation during WAS acidification in the presence of biosurfactant RL was inhibited when the pH was too high or too low. The mean SCFA production was 0.313 gCOD/g VSS at pH 9.0, while it was only 0.144 at pH 4.0, 0.270 at pH 11.0, and 0.222 gCOD/g VSS when the pH was not controlled (pH 7.0). As in the thermo-alkali treatment reported by Zhang et al. (2009b), the mean SCFA production was 0.182 gCOD/g VSS when pH was not controlled and was only 0.042 at pH 4.0 and 0.037 at pH 11.0. The higher SCFA production under alkaline conditions might be attributed to the following three reasons: one is that alkaline conditions could improve the solubilization function of biosurfactant RL, thus producing more soluble substrates for acidification. Secondly, alkaline pH is effective in increasing the hydrolysis rate of WAS. The third is that alkaline conditions could decrease or inhibit the activity of methanogens (Yan et al. 2010). The reason for the greatest SCFAs produced at pH 10.0 instead of pH 11.0 might be attributed to the toxic effects of stronger alkaline conditions to acidogenic bacteria, which further suggested that the overdose of alkaline was harmful for the microbial environment and acidification activity.

Short-chain fatty acids (C_2–C_5) are usually the main acidification products of WAS. Acetic, propionic, butyric, and isobutyric acids can be the result of the digestion of carbohydrates, proteins, and lipids, and the higher-molecular-weight SCFAs such as valeric and iso-valeric acids are mainly associated with the digestion of proteins (McInerney 1988). An interesting phenomenon could be noted from Table 2 in which a higher pH contributed to a greater proportion of acetic acid and lesser proportion of iso-valeric, and a lower pH resulted in a greater proportion of iso-valeric and lesser proportion of acetic acid in the initial fermentation. It might be expected that more soluble proteins were provided for acidification to produce more acetic under alkaline conditions. As shown in Fig. 6, with the increase of pH from 4.0 to 11.0, the soluble protein increased from 454.3 to 2,003.7 mg/L. Correspondingly, the proportions of acetic acid were 16.65, 36.33, and 62.94 %, respectively, for the system at pH 4.0, 7.0, and 11.0 and were 40.34, 12.60, and 11.01 % for iso-valeric after 1 day of fermentation for the system in the presence of biosurfactant RL. The results were consistent with the findings that acetic acid was the top fraction when the protein was fermented (Suwannakham and Yang 2005). In addition, alkaline sludge hydrolysis was more efficient and complete than acidic and neutral conditions (Chen et al. 2007); thus, higher-molecular-weight SCFAs tended to generate lower-molecular-weight SCFAs, leading to the accumulation of acetic acid in a high ratio.

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