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Enhanced Hydrolysis and Acidification of Waste Activated Sludge by Biosurfactant Rhamnolipid

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Abstract The effect of biosurfactant rhamnolipid (RL) on hydrolysis and acidification of waste activated sludge (WAS) was investigated. The results indicated that RL could greatly reduce the surface tension of sludge, resulting in stimulating the hydrolysis rate of WAS and enhancing the production of short-chain fatty acids (SCFAs). With the increase of RL dosage from 0.2 to 0.5 g/g DS, the maximum soluble chemical oxygen demand (SCOD), protein and carbohydrate concentration increased correspondingly. After 6 h of hydrolysis, SCOD, protein and carbohydrate concentration increased from 371.9, 93.3 and 9.0 mg/l to 3,994.5, 800.0 and 401.4 mg/l at RL 0.3 g/g DS, respectively. Furthermore, the release of NH_4^+-N, PO_4^{3-}-P and the accumulation of SCFAs also improved in the presence of RL. The maximum SCFAs was 1,829.9 mg COD/l at RL 0.3 g/g DS, while it was only 377.7 mg COD/l for the blank test. The propionic acid and acetic acid were the mainly SCFAs produced, accounting for 50–60% of total SCFAs.

Keywords Anaerobic processes · Batch processing · Fermentation · Rhamnolipid · Surface tension · Waste treatment

Notation

RL	Biosurfactant rhamnolipid
SCFAs	Short-chain fatty acids
WAS	Waste activated sludge

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SCOD	Soluble chemical oxygen demand
TCOD	Total chemical oxygen demand
DS	Dry sludge
VSS	Volatile suspended solids
SDBS	Sodium dodecylbenzene sulphonate
SDS	Sodium dodecyl sulphate
EPS	Extracellular polymeric substances
CMC	Critical micelle concentration
ME	Mixed enzyme
TSS	Total suspended solids

Introduction

Although the activated sludge process is an economical and effective wastewater treatment widely used throughout the world, this process generates a large amount of waste activated sludge (WAS), which requires considerable costs to handle and dispose. Considering its pathogen content, unstable and decomposable nature, WAS should undergo some treatment processes prior to final disposal [1].

Anaerobic digestion is a widely applied method for sludge stabilization because of the recovery of usable energy such as biogas and the limited environmental impact. Meanwhile, short-chain fatty acids (SCFAs) and other low weight organic compounds are produced during anaerobic digestion, which could be used as preferred external carbon sources for the biological nutrient removal and the raw materials for the synthesis of biopolymers, like biodegradable plastics polyhydroxyalkanoates [2, 3].

During the anaerobic digestion process, hydrolysis of the particulate compounds is the rate-limiting step [4]. Therefore, various effects have been made to enhance the efficiency of anaerobic digestion, such as chemical (oxidants, odour inhibitors, alkaline, etc.) [5], thermal, ultrasonic, mechanical and enzymatic methods [6, 7]. Recently, the anaerobic digestion process enhanced by surfactant has received more attention. Ewa et al. [8] demonstrated that sludge flocs became smaller and more circular exposed to anionic surfactants (sodium dodecyl sulphate [SDS], sodium dodecylbenzene sulphonate [SDBS] or sodium alkyltrioxyethylene sulphate), and the concentration of volatile suspended solids (VSS) decreased significantly. Jiang et al. [9] also found that SDS could effectively accelerate the solubilization of extracellular polymeric substances (EPS) from WAS and subsequently promote the accumulation of SCFAs.

However, the surfactants used in previous studies, such as Tween 20, Tween 80, Triton X-100, polyethylene glycol and SDBS are chemically synthesized and not biodegradable, therefore making higher toxic to the environment [10]. Biosurfactants, on the contrary, have attracted much attention due to their structural diversity, biodegradability, and effectiveness at extreme temperatures, pH, and salinity [11]. But the available information about biosurfactant in environmental application mainly focused on its effects on oil recovery, hydrocarbon bioremediation and heavy metal removal [12]. And so far, literature about the effect of biosurfactant on WAS hydrolysis and acidification is hardly reported. In this study, rhamnolipid (RL), which was produced from sterilized and centrifuged fermentation broth from the bacterium *Pseudomonas aeruginosa*, was chosen as a model biosurfactant. The mainly purpose of this study was to investigate the influence of RL on sludge protein and carbohydrate solubilization, nitrogen and phosphorus release, and SCFAs production during the fermentation of WAS. In addition, the mechanism of sludge solubilization enhanced by RL was discussed through the changes of sludge surface tension at different RL dosages.

Materials and Methods

WAS and Biosurfactant

The WAS used in this study was obtained from the secondary sedimentation tank of a municipal wastewater treatment plant in Changsha, China. Prior to use, the sludge was concentrated by settling for 4 h, further filtered through a 0.66-mm metal sieve and then stored at 4°C. The characteristics of the sludge were as follows: pH 6.84±0.15, total chemical oxygen demand (TCOD) 9,760±214 mg/l, soluble chemical oxygen demand (SCOD) 371±80 mg/l, total suspended solids (TSS) 9,920±380 mg/l, VSS 6,260±80 mg/l, soluble carbohydrate 9±2 mg/l, soluble protein 93±18 mg/l.

RL was purchased from Yuzhou Biotechnology Ltd. (China), and the raw RL containing 50% of pure RL was brown colored and cream shaped.

Batch Experiments

The batch experiments were performed in five plexiglass reactors with a working volume of 500 ml, each of which contained 300 ml of WAS. To examine the influence of biosurfactant on WAS hydrolysis and acidification, RL was added to the reactors with its dosage to dry sludge ratio being 0, 0.2, 0.3, 0.4 and 0.5 g/g DS, respectively. The reactor with no RL addition was used as the blank test. All reactors were purged by nitrogen gas for 4 min and capped with rubber stoppers so as to maintain strict anaerobic condition. The reactors were then placed in a water-bath shaker (100 rpm) at 30°C.

Analysis Methods

Sludge samples were firstly filtered through Whatmann GF/C glass microfiber filter before analyzing soluble substances. SCOD, NH_4^+-N, PO_4^{3-}-P, TSS and VSS were measured according to standard methods [13]. The carbohydrates were determined using the phenol-sulphuric method with glucose as standard [14] and the proteins were quantified using Lowry–Folin method with bovine serum albumin (BSA) as standard [15]. The SCFAs was analyzed by gas chromatography (Agilent 6890N) using a flame ionization detector (FID) and DB-FFAP column (30 m × 0.25 mm × 0.25 mm) and the total SCFAs was recorded as the sum of measured acetic, propionic, *n*-butyric, *iso*-butyric, *n*-valeric, and *iso*-valeric acids [16]. The surface tension of sludge liquid was measured using a JYW-200A surface tensiometer [11].

Results and Discussion

Effect of RL on Sludge Hydrolysis

Effect of RL on SCOD concentration at different fermentation times was shown in Fig. 1. On the sixth hour of fermentation, the SCOD concentrations at different RL dosages were as follows: 4,591.4 mg/l (0.5 g/g DS) > 4,315.9 mg/l (0.4 g/g DS) > 3,994.5 mg/l (0.3 g/g DS) > 2,938.5 mg/l (0.2 g/g DS) > 734.6 mg/l (blank test). The solubilization of sludge particulate organic carbon was significantly improved by RL in a short time, suggesting that a large amount of particulate organic in sludge flocs was transferred into soluble organic. Different from the initial stage, the SCOD concentrations fluctuated after 6 h, and gradually decreased after about 24 h of hydrolysis.

It is well known that sludge components are cemented together by EPS, which are mainly composed of microbiologically produced biopolymers, such as carbohydrates and proteins [17].

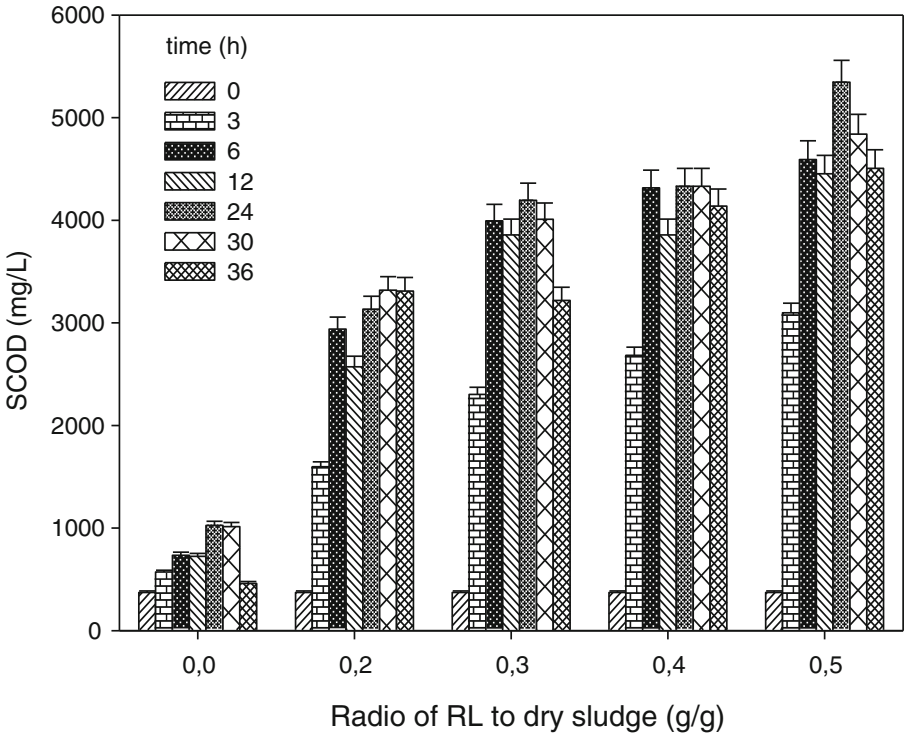

Fig. 1 Effect of RL on SCOD concentration at different fermentation times

The hydrolysis of EPS in the degradation of biodegradable particulate organic matter heavily depends on extracellular enzymes, which are immobilized in sludge flocs by adsorption in the EPS, and they can hydrolyze the molecule polymer loosely bound to sludge surface to monomers or at least to oligomers. However, EPS act as a network confining extracellular enzymes exhibiting its hydrolytic activity [18]. Therefore, more compact structure of the sludge matrix will cause lower hydrolytic rate. A biosurfactant could enhance EPS dissolution and dispersion due to its solubilization, thus causing sludge proteins and polysaccharides to dissolve into aqueous phase. On the other hand, a biosurfactant can change the morphology of sludge flocs, which will become smaller and more circular because of the saponification of the biosurfactant [10]. Disintegration of sludge flocs is beneficial for the release of enzymes embedded in the sludge flocs. Furthermore, biosurfactants weaken the immobilization of the enzymes on the substrate by reducing the binding strength. Thus, the extracellular enzyme might more easily desorb from the binding site after the reaction and move to other binding sites on the substrate [19].

Effect of RL on Protein and Carbohydrate Solubilization

Hydrolysis of WAS particulate organic matter causes sludge protein and carbohydrate release into aqueous phase. Thus, the hydrolysis products can be expressed in terms of soluble proteins and carbohydrates in this study. Figure 2a and b describes the effect of RL on soluble protein concentration at different fermentation times. It can be observed that the concentrations of these two substrates increased greatly with the dosage of RL. As seen in Fig. 2a, the soluble protein

concentration of the raw WAS was only 93.3 mg/l, and increased to 161.6 mg/l for the blank test after 6 h of hydrolysis. Meanwhile, the protein concentration increased greatly to 648.7, 800.0, 818.6 and 855.9 mg/l at RL dosage of 0.2, 0.3, 0.4 and 0.5 g/g DS, which respectively improved by 3.0, 4.0, 4.1 and 4.3 times compared to the blank test. With the hydrolysis time extending to 12 h, no significant increase of protein concentration was observed, and the concentration decreased gradually after 24 h of hydrolysis on the contrary. As shown in Fig. 2b, RL had almost the same effect on soluble carbohydrate concentrations as on soluble protein. For example, the soluble carbohydrate of the raw WAS was only 9.0 mg/l, after 6 h of hydrolysis; it reached the maximum in most cases except for the blank test, in which the maximum amount of carbohydrate appeared after 12 h. The maximum amount of carbohydrate reached 31.4 mg/l in the blank test, 357.1 at RL 0.2 g/g DS, 401.4 at 0.3 g/g, 428.6 at 0.4 g/g and 460.0 mg/l at 0.5 g/g.

As discussed above, during the initial 6 h of hydrolysis the concentration of SCOD (Fig. 1), soluble protein and carbohydrate (Fig. 2a and b) improved significantly when the RL dosage was within 0.30 g/g DS, whereas it did not improve obviously with further increase of RL dosage. Therefore, 0.3 g/g DS appeared to be the optimum dosage in terms of economy and efficiency.

Table 1 summarizes the hydrolysis characteristics in this study and previous literatures. It showed obviously that the RL was more effective in the promotion of sludge hydrolysis. The protein concentration was 800 mg/l at RL 0.3 g/g in this study, which was almost the same as that reported by Jiang et al. [9] (827.7 mg/l) and our previous study [33] (813 mg/l). However, the carbohydrate concentration was 401.4 mg/l, which was much greater than that reported by other studies (Table 1). Moreover, the time to reach the maximum hydrolysates concentration in this study was much shorter than that reported in other studies [9, 27, 28], which might be attributed to the inhibitory effects of the surfactant and strong alkaline conditions on the degradation cascade of the bioploymer [20]. Similarly, in our previous study [33], the ME system could efficiently enhance the hydrolysis of WAS in the short time, but the temperature required should be as high as 50°C, which was much higher than that used in this study.

Therefore, RL appeared to be more effective in the promotion of WAS hydrolysis in terms of economy and efficiency.

Soluble proteins and carbohydrates are two of the most predominant constituents of WAS and compose a large proportion of COD in the sludge. Usually, these sludge proteins and polysaccharides are absorbed onto sludge surface, and they can be solubilized by RL [21]. Meanwhile, the enhanced solubilization of sludge EPS by RL causes the break-up of sludge matrix, resulting in more inner protein and carbohydrate release [16]. Furthermore, the addition of biosurfactant RL could also increase significantly the release of cellular lipopolysaccharides (LPS), which was mainly composed of carbohydrates and lipids [22], thus further increasing the concentration of carbohydrate.

As depicted in Fig. 2a and b, the concentrations of both soluble proteins and carbohydrates increased rapidly in the initial stage, but decreased subsequently. The observed soluble proteins and carbohydrates were the net balance of release and degradation, thus it can be seemed that the initial release rates of the two substrates were higher than their degradation, which made their temporary accumulation [16]. However, in the later stage of hydrolysis, the release rates slowed down and were exceeded by the degradation rates, which resulted in the decrease of observed soluble protein and carbohydrate concentrations, and the increase of NH_4^+-N, PO_4^{3-}-P and SCFAs concentrations (Figs. 3 and 4). In the stage of hydrolysis, proteins convert into polypeptides, two peptides, amino acid, and amino acid further transforms into low molecule organic acid, ammonia and carbon dioxide. Correspondingly, carbohydrates are hydrolyzed into polysaccharides, or even reducing sugar [23].

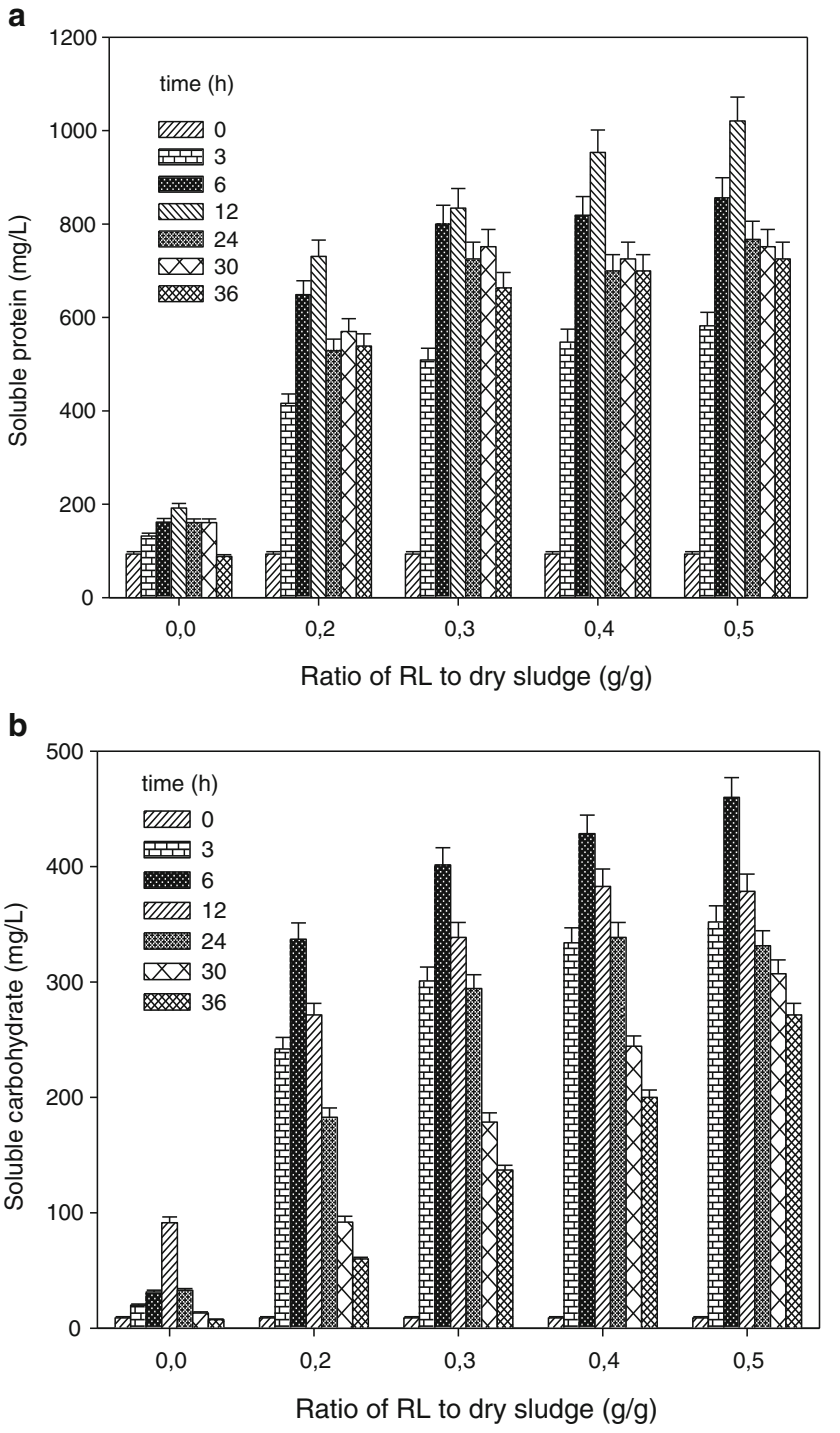

Fig. 2 Effect of RL on soluble protein and carbohydrate concentration at different fermentation times: **a** soluble protein concentration; **b** soluble carbohydrate concentration

Effect of RL on NH_4^+-N and PO_4^{3-}-P Release

During WAS fermentation, the releases of sludge phosphorus and ammonium were also observed (Fig. 3a and b). Instead of increasing rapidly and then decreasing gradually as proteins and carbohydrates during fermentation, the concentrations of NH_4^+-N and PO_4^{3-}-P in the sludge hydrolysis liquid kept increasing during the whole anaerobic digestion period regardless of whether RL was utilized or not. Compared to the blank test, the concentrations of NH_4^+-N and PO_4^{3-}-P were much higher when RL was added. It could be seen from Fig. 3a and b that the released NH_4^+-N concentration in the fermentation liquor increased from 185.7 mg/l in the blank test to 345.2 mg/l at RL 0.5 g/g DS after 36 h of fermentation time. Correspondingly, PO_4^{3-}-P concentration was 51.4 mg/l (blank test) and 91.2 mg/l (0.5 g/g DS). It was interesting to note that the concentration of NH_4^+-N in this study were much higher than that in the literature. For example, at the RL dosage of 0.5 g/g DS, the released NH_4^+-N improved by 3.3 times to the system with SDS 0.10 g/g DS reported by Jiang et al. [9], and 1.9 times to ME system reported in our previous study [7]. It was probably because ammonia accumulation could largely increase under strict closed conditions. Methanogens were likely to cease growth due to ammonia inhibition, and the inhibitor ammonium concentrations that caused 50% reduction in methane production were from 1.7 to 14.0 g/l [24]. The average released ammonia in this study was far below the inhibition concentration; thus, no significant inhibition was observed during the anaerobic digestion.

Phosphorus and nitrogen are important components of cells and EPS of WAS, so lysis of microorganism cells and the degradation of the matters containing phosphorus and nitrogen, such as protein, could cause the release of NH_4^+-N and PO_4^{3-}-P during sludge fermentation [25]. In addition, more EPS could be solubilized by RL and then degraded by hydrolytic enzymes; therefore, much more NH_4^+-N and PO_4^{3-}-P was released.

Effect of RL on SCFAs Accumulation

The generation of SCFAs during sludge acidification stage was associated with the decomposition of sludge protein and carbohydrate [26]; thus, more hydrolysis products were correspondence with higher SCFAs accumulation [27]. Figure 4 shows the variation of SCFAs concentration with fermentation time under different RL dosages, and it was noticeable that SCFAs production was greatly improved by RL. As discussed above, the addition of RL would cause more soluble protein and carbohydrate generation, which could provide more substrates for acidification, and thus more SCFAs were observed. As shown in Fig. 4, the maximum SCFAs concentration was respectively 1,539.1 mg COD/l at RL 0.2 g/g and fermentation time of 2 days, 1,829.9 at 0.3 g/g and 3 days, 1,978.4 at 0.4 g/g and 3 days, 2,159.2 at 0.5 g/g and 4 days, while it was only 377.8 mg COD/l in the blank test after 4 days of fermentation. Apparently, the maximum SCFAs

Table 1 Comparison of hydrolysis characteristics in this study and previous reports

Enhanced conditions	Soluble protein (mg/l)	Soluble carbohydrate (mg/l)	Fermentation time	Reference
SDB (0.1 g/g DS)	827.7	157.6	6 d	Jiang et al. [9]
SDBS (0.02)	484.6	55.7	3 d	Jiang et al. [27]
pH=10	621.3	98.4	2 d	Chen et al. [28]
ME (0.06 g/g DS)+50°C	813	205	1d	Luo et al. [33]
RL (0.3 g/g DS)	800.0	401.4	6 h	This study

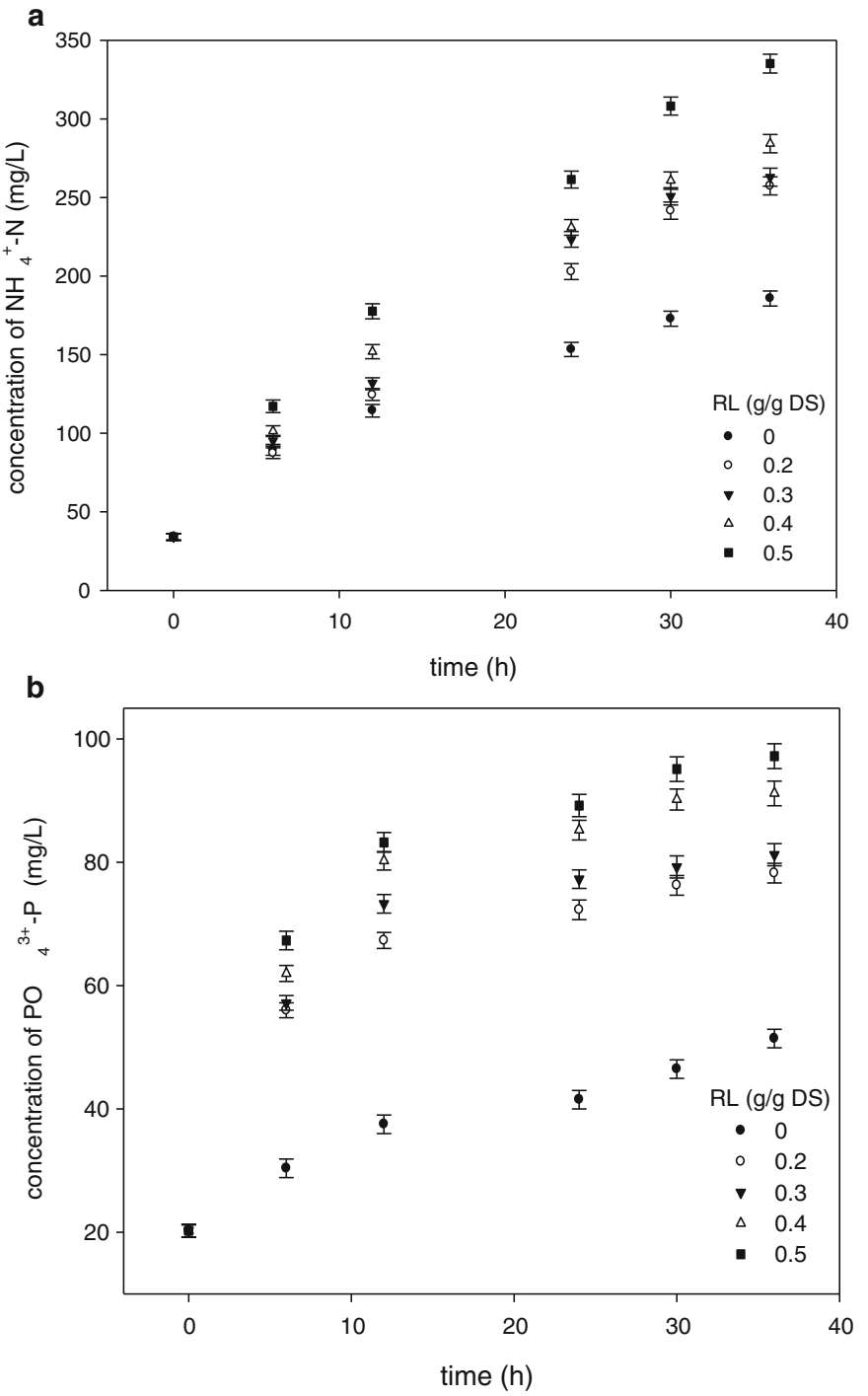

Fig. 3 Variation of NH_4^+-N and PO_4^{3-}-P concentration with hydrolysis time under different rhamnolipid dosages: **a** NH_4^+-N concentration; **b** PO_4^{3-}-P concentration

concentration increased gradually with the dosage of RL increased from 0 to 0.5 g/g. It was also found that the fermentation time to reach the maximum SCFAs concentration in the presence of RL was no more than 4 days, which was shorter than that of chemical surfactant SDBS [28]. It might be attributed to its inhibition to acidogenic bacteria [20]. With the increase of fermentation, the SCFA concentration gradually declined due to methanogenesis. Nevertheless, it gradually approached a stationary value rather than continuously decreasing after about 6 days of fermentation as described in our previous study [16] and in other reports [28]. Methanogen, as one of the four types of anaerobic microorganisms, was known as the least tolerant and the most likely to cease growth due to ammonia inhibition [29]. Consequently, the activity of methanogen was inhibited by the greatly accumulated ammonia, thus reducing the consumption of SCFAs.

Effect of RL on SCFAs Composition

Table 2 shows the composition of maximum SCFAs at different RL dosages during the fermentation process, and it can be found that propionic acid was the dominant SCFA, followed by acetic acid and then by isovaleric acid, and these three SCFAs accounted for 70–90% of total SCFAs for all treatments. Figure 5 illustrates the individual SCFAs profile during the entire fermentation at RL dosage of 0.3 g/g DS for example. After 3 days of fermentation, propionic, acetic and isovaleric acids accounted for 36.58%, 22.97% and 17.31% of total SCFAs, respectively. However, Chen et al. [28] found that acetic acid was the most prevalent product followed by propionic acid during anaerobic digestion at alkaline pH condition.

The reasons for the differences were as follows: acetate production in anaerobic conditions to a large extent depends on the decomposition reaction of alcohols and SCFAs (such as propionic acid, butyric acid) performed by syntrophic acetogenesis, while a large proportion of syntrophic acetogensis are syntrophus, which can only degrade SCFAs when coupled with hydrogenotrophic methanogens [30]. However, a high concentration of ammonia has strong inhibitory effect on the activity of syntrophic acetogensis as well as methanogens. In addition, the propionic

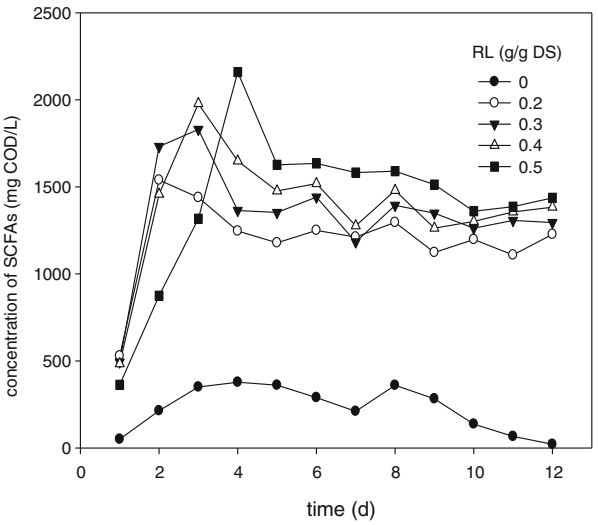

Fig. 4 Variation of SCFAs concentration with hydrolysis time under different rhamnolipid dosages

Table 2 Composition of maximum SCFAs at different RL dosages

RL/dry sludge (g/g)	HAc (%)	HPr (%)	*i*-HBu (%)	HBu (%)	*i*-HVa (%)	HVa (%)
Blank test	30.46	30.70	6.43	0.73	28.32	3.36
0.2	20.01	34.92	10.40	9.36	17.56	7.75
0.3	22.97	36.58	9.25	8.14	17.31	5.75
0.4	19.18	33.66	14.15	10.12	15.96	6.93
0.5	25.05	31.47	10.44	8.88	17.26	6.90

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

acid was found to be the main product during glucose acidification, whereas acetic acid accounted for the highest fraction when protein was fermented [31, 32]. The carbohydrate concentration in this study was larger than that in the literature [9], while the protein was contrary.

Table 3 summarizes the propionic acid/acetic acid ratios in this study and previous reports. In this study, the maximum ratios of HPr to HAc reached 1.59 when the RL dosage was 0.3 g/g DS, which was 7.23 and 1.89 times that in systems with chemical surfactant SDS (0.10 g/g DS) and SDBS (0.02 g/g DS), respectively. Compared to anaerobic fermentation of WAS at pH 10.0, the ratio also improved by 4.42 times. Apparently, the addition of RL was more favorable to form the hydrolysate with a higher ratio of propionic acid to acetic acid. According to Chen et al. [34], the higher ratio of propionic to acetic acid led to superior enhanced biological nutrient and phosphorus removal from real wastewater supplemented with SCFAs.

In addition, it can be easily seen in Fig. 5 that the fraction of branched SCFA (*iso*-butyric and *iso*-valeric) was greater than their corresponding straight SCFA (*n*-butyric and *n*-

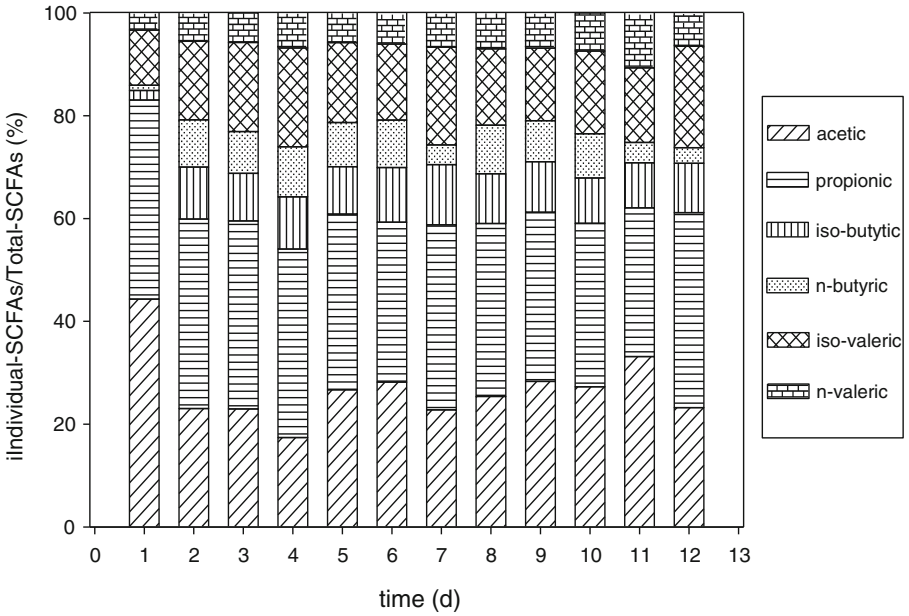

Fig. 5 Profiles of individual SCFAs concentration during the entire fermentation at RL 0.3 g/g

Table 3 Comparison of hydrolysis characteristics in this study and previous reports

Enhanced conditions	HAc (%)	HPr (%)	Ratios of HPr to HAc	Time to reach the maximum SCFA concentration	Reference
SDB (0.1 g/g DS)	53.33	11.54	0.22	6d	Jiang et al. [9]
SDBS (0.02 g/g DS)	27.1	22.8	0.84	6d	Jiang et al. [27]
pH=10	45.84	16.49	0.36	8d	Chen et al. [28]
ME (0.06 g/g DS) + 50°C	44.87	15.13	0.34	6d	Luo et al. [33]
RL (0.3 g/g DS)	22.97	36.58	1.59	3d	This study

valeric), which might be attributed that the decomposition rate of SCFA with a straight-chain (C2–C5) was greater than that of their respective isomer with a branched chain [35].

The Mechanism of RL-Enhanced Solubilization of WAS

The variations of surface tension at different RL dosages are depicted in Fig. 6. As shown in Fig. 6, the surface tension of sludge liquid declined from 57.5 mN/m at RL 0.005 g/g DS to 37.5 mN/m at 0.1g/g DS, and further declined to 34.8 mN/m at 0.5g/g DS, while the surface tension of raw sludge liquid was 79.9 mN/m. Apparently, the RL could greatly reduce the surface tension of sludge liquid at low dosages, whereas it did not improve obviously with RL dosage exceeding 0.1 g/g DS.

Surfactants are amphipathic molecules with hydrophobic and hydrophilic moieties [36]. On the one hand, they can form molecular film at the interface, thus reducing the surface tension of the sludge liquid. On the other hand, they can aggregate into micelle, thus increasing the solubility of insoluble organic matter. It is well known that the surface tension will gradually decrease until the concentration of surfactants achieves the critical micelle concentration (CMC), when the liquid surface is fully surrounded by surfactants molecules with the surface tension reaching the stable value. The RL molecules began to aggregate into micelle when the RL concentration was above CMC; consequently, the hydrophobic moieties could connect with the macromolecules of

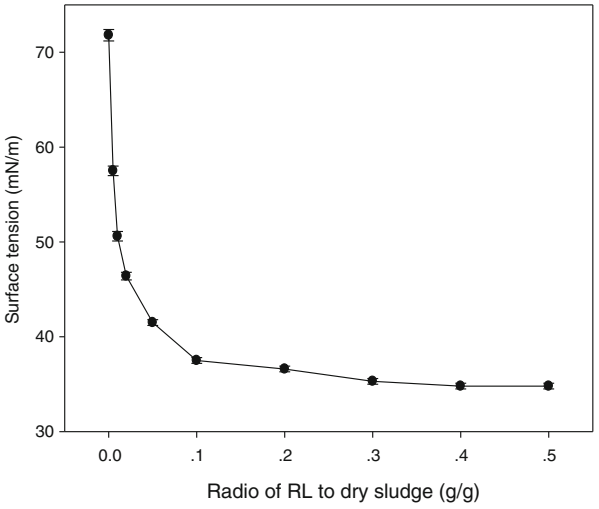

Fig. 6 Effect of RL on the surface tension at different rhamnolipid dosages

sludge surface, while hydrophilic moieties connect with water molecules. Due to the effect of external stirring, the surface macromolecules of sludge, such as proteins and carbohydrates, was desorbed from sludge flocs and dissolved into aqueous phase [21].

It can be easily seen in Fig. 6 that the CMC of this RL biosurfactant in sludge liquid was approximately 0.1 g/g DS, which adequately proved that it was reasonable to set the RL dosages between 0.2 and 0.5 g/g DS.

Conclusion

The biosurfactant RL was effective in the promotion of sludge hydrolysis and acidification. The RL could greatly reduce the surface tension of sludge liquid, thus promoting solubilization of organic solid and release of extracellular enzyme, which further enhanced the SCFA production. After 6 h of fermentation, the concentration of SCOD, proteins and carbohydrates at RL 0.3 g/g DS was respectively 5.4, 4.9 and 12.8 times that in the blank test, which corresponded with higher NH_4^+-N, PO_4^{3-}-P release and SCFAs accumulation. Propionic acid was the prevalent SCFAs, followed by acetic acid at any RL dosages.

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