Feasibility of enhancing short-chain fatty acids production from waste activated sludge after free ammonia pretreatment: Role and significance of rhamnolipid

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ABSTRACT: This study reported a new, renewable and high-efficient strategy for anaerobic fermentation, i.e., using free ammonia (FA) to pretreat waste activated sludge (WAS) for 1 d and then combining with rhamnolipid (RL), by which the short-chain fatty acids (SCFA) production was remarkably improved. Experimental results showed the maximal SCFA production of 324.7 ± 13.9 mg COD/g VSS was achieved at 62.6 mg FA/L pretreatment combined with 0.04g RL/g TSS, which was respectively 5.95-fold, 1.63-fold and 1.41-fold of that from control, FA pretreatment and RL pretreatment. Mechanism investigations revealed that FA + RL enhanced sludge solubilization and hydrolysis, providing more organics for subsequent SCFA production. It was also found that the combined method inhibited acidogenesis and methanogenesis, but the inhibition to methanogenesis was much severer than that to acidogenesis. Finally, the feasibility of NH_4^+ -N and PO_4^{3-} -P, released in fermentation liquor, being recovered as magnesium ammonium phosphate (MAP) was confirmed.

Keywords: Waste activated sludge, Short-chain fatty acids production, Free ammonia Pretreatment, Rhamnolipid, Energy and resource recovery

1. Introduction

Biological nutrient removal (BNR) is an effective and widely used technique for treating municipal wastewater (Zhao et al., 2015a; Wang et al., 2015a; Wang et al., 2017a). However, in real-world operations of wastewater treatment plants (WWTPs), the available carbon sources (e.g., short-chain fatty acids (SCFA)) in the wastewater usually cannot meet the needs of BNR (Wang et al., 2017b; Wang et al., 2017c). The shortage of carbon sources inevitably affects the performance of BNR. To achieve satisfied BNR performance, additional carbon sources (e.g., acetate, propionate, alcohol, and glycerol) are usually added during the wastewater treatment process (Guerrero et al., 2012; Wang et al., 2012; Wang et al., 2013a). The method can indeed effectively alleviate the shortage of carbon sources and then enhance the nutrient removal. However, it inevitably increases the cost of wastewater treatment, which puts great challenges to long-term operation of WWTPs.

As a major byproduct of BNR, huge amount of waste activated sludge (WAS) are daily produced (Li et al., 2016a; Wang et al., 2015b; Raheem et al., 2017; Shi et al., 2018). For instance, about 35 million tons of WAS were produced in China in 2015 (Xu et al., 2017). Continuous high yield and high disposal costs (accounting for up to 60% total operation cost of WWTPs) put also great challenges to long-term operation of WWTPs (Appels et al., 2008). Moreover, large amounts of biodegradable organic substrates (e.g., carbohydrates and proteins) are contained in the WAS, therefore WAS recovery and reuse, considered a more preferable way, have attracted growing attention (Tyagi and Lo, 2013; Wang et al., 2013b; Wang, 2017d; Xu et al., 2018a). Recently, WAS anaerobic fermentation in WWTPs can in-situ produce SCFA, which could be used as supplementary carbon sources to enhance BNR (Zhao et al., 2015b; Zhao et al., 2016; Wang et al., 2017e). Thus, the production of SCFA from WAS anaerobic fermentation has attracted more and more attention, during which not only sludge reduction can be achieved but also the operation cost of WWTPs can

be effectively reduced.

Generally, WAS anaerobic fermentation mainly includes four steps such as solubilization, hydrolysis, acidogenesis and methanogenesis (Wang et al., 2017f; Wang et al., 2018a). Solubilization and hydrolysis, providing small molecular organics for subsequent generation of SCFA, are considered the major rate-limiting steps for SCFA production (Li et al., 2016b). SCFA is produced in acidogenesis step and then rapidly consumed by methanogens (Zhao et al., 2016; Wang et al., 2018b). Therefore, improving SCFA production could be conducted by enhancing the first two steps and suppressing the last step. In fact, for this purpose, many pretreatment methods and/or fermentation control strategies such as thermal, ultrasonic, Fenton, acid and alkaline have been tested and applied to pretreat sludge (Yuan et al., 2006; Yan et al., 2010; Zhao et al., 2015b). Although these methods can effectively enhance the SCFA production, most of them are cost intensive because of high energy inputs (i.e., high pressure and/or temperature) and/or substantial chemical requirements (i.e., acid and alkali), which will inevitably limit its large-scale application. Moreover, those methods may pose negative consequences on environment.

Recently, it was reported that free ammonia (FA), a renewable chemical generated in situ directly from anaerobic fermenter effluent, could effectively improve sludge solubilization and suppress the activities of methanogens (Wang et al., 2018c). FA, at parts per million (ppm) levels, exerts a powerful biocidal impact on broad microbes (Park et al., 2015), resulting in significant improvement of sludge solubilization. Previous study also showed that 40 mg FA/L posed 80% inhibition to the activity of methanogens (McCarty and McKinney, 1961). When 178.4 mg FA/L was applied to pretreat sludge 3 d, the maximum SCFA production (267.2 mg COD/g VSS) was achieved at fermentation time of 8 d, which was 3.2-fold of that from the control (without FA pretreatment) (Zhang et al., 2018). Although FA was an effective and validated pretreatment method with significant contribution, the maximal SCFA production required long fermentation time (8 d) and

more than 60% of VSS could not be degraded during anaerobic fermentation, which inevitably, to some extent, weakens the its values, hindering its further application in real-world situations. Therefore, considering high costs of WAS disposal and supplementary carbon source, any improvement of SCFA production under FA pretreatment would have important economic and ecological benefits.

Rhamnolipid (RL), as an eco-friendly bio-surfactant, has been demonstrated to accelerate the sludge solubilization and hydrolysis, providing small molecular organics and therefore benefit to subsequent SCFA production (Huang et al., 2015). Previous studies also found RL exert a strong inhibition on methane production (Zhou et al., 2015). Moreover, it was reported that RL can be in situ generated by the bacterium *Pseudomonas aeruginosa* during WAS anaerobic fermentation (Zhou et al., 2013). According to the information reported above, it is presumed that the RL-supported method, i.e., combining FA pretreatment with RL might further enhance sludge solubilization/hydrolysis while inhibit methanogenesis simultaneously, thus achieving more SCFA accumulation. Considering that RL and FA are all renewable substances that can be generated in situ during WAS anaerobic fermentation, if above hypothesis is feasible, this combined method will enhance the values of each solo technology and expand its further application, thus achieving significant economic and practical benefits. Until now, however, it is unknown whether FA pretreatment combined with RL causes a synergistic effect on SCFA production from WAS. Furthermore, it is also unknown that the potential effect of the combined method on each process involved in anaerobic fermentation.

The aim of this work was to assess the feasibility of whether FA + RL enhances SCFA production from WAS and to understand the underlying details. Firstly, the effect of different level of FA pretreatment (35.4, 62.6, 116.9 and 171.3 mg/L) and RL (0.04 g/g TSS) on SCFA production from WAS was assessed. Then, the underlying details of how this combined method affect the processes such as solubilization, hydrolysis, acidogenesis, and methanogenesis were investigated via a series of batch test as well as the analysis of key

enzyme activity involved in WAS anaerobic fermentation. The findings reported in this work could promote the application of FA-based strategy in real WWTPs, which might support long-term sustainable operation of WWTPs and even mitigate the “nutrient removal-energy and resource recovery” challenge faced by WWTPs.

2. Materials and methods

2.1 Sources of WAS and bio-surfactant RL

The WAS used in this study was taken from the secondary sedimentation tank of a WWTP, running with anaerobic-anoxic-aerobic (A²/O) system, Changsha, China. The obtained WAS was firstly screened with a 1 mm × 1 mm sieve and then thickened by settling for 24 h at 4 °C prior to use. The relevant characteristics of WAS used in this study are as follows: pH 6.79 ± 0.05 , total suspended solids (TSS) 14750 ± 230 mg/L, volatile suspended solids (VSS) 10320 ± 150 mg/L, total chemical oxygen demand (TCOD) 14150 ± 210 mg/L, soluble chemical oxygen demand (SCOD) 184 ± 18 mg/L, total protein 7825 ± 280 mg COD/L, total carbohydrate 1190 ± 115 mg COD/L, total nitrogen in sludge supernatant 22.8 ± 0.4 , total phosphorus in sludge supernatant 8.2 ± 1.1 . The bio-surfactant RL was purchased from Baisheng Chemical Tech Co., Ltd. (Shenzhen, Guangdong, China). The purity of RL was 99.5%.

2.2 Batch experiments for SCFAs production

Batch experiments were conducted to assess the feasibility of FA + RL enhancing SCFA production from WAS. In this experiment, eight identical anaerobic reactors with working volume of 1L were conducted at 25 ± 1 °C, and each of them was fed with 500ml of the concentrated sludge. Eight reactors were named as reactors 1-8. It should be emphasized that no extra NH_4^+ -N was not added into the reactors 1-3 and 22.8 mg/L NH_4^+ -N was the background value. Different volume of ammonium chloride (NH_4Cl) stock solution (4.0 M) were added into reactors 4-8 to obtain initial 97, 172, 322, 472 and 172 mg NH_4^+ -N/L, respectively. Among these reactors, reactor 1 was set as the control without any pretreatment. Reactor 2, reactor 3 and reactor 8

were respectively used to assess the effect of pH 9, RL and FA pretreatments to the production of SCFA, while reactors 4–7 were conducted to evaluate the effect of different FA +RL on SCFA production from WAS. The detailed operation conditions for these reactors were shown in Table 1.

pH in reactors 2-8 were controlled at 9.0 ± 0.1 for 1 d by adding 4 M hydrochloric acid (HCl) or 4 M sodium hydroxide (NaOH). The concentration of $\text{NH}_4^+\text{-N}$, pH levels and temperature contributed to initial FA concentration of 0.1 (the control), 8.26, 8.26, 35.4, 62.6, 116.9, 171.3 and 62.6 mg /L, which was calculated by the formula $S_{(\text{NH}_3\text{-N}+\text{NH}_4^+\text{-N})} \times 10^{\text{pH}} / (K_b/K_w + 10^{\text{pH}})$, where $S_{(\text{NH}_3\text{-N}+\text{NH}_4^+\text{-N})}$ represents the concentration of $\text{NH}_3\text{-N} + \text{NH}_4^+\text{-N}$, K_b represents the ionization constant of the ammonia equilibrium equation, and K_w represents the ionization constant of water (Kayhanian, 1999). The value of K_b/K_w was calculated via the formula of $K_b/K_w = e^{6,344/(273+T)}$ (Kayhanian, 1999). The pretreatment time was 1 d according to the previous study (Wei et al., 2017). After the 1 d pretreatment, a RL dosage of 0.04 g/g TSS was added into reactors 3-7 (Zhou et al., 2013). Finally, all the reactors were aerated with N_2 (99.99%) for 10 min to remove oxygen, sealed, and placed in a water-bath shaker at stirring speed of 150 rpm.

2.3 Evaluating the impacts of FA + RL on the process of hydrolysis, acidogenesis and methanogenesis

Solubilization process, releasing and providing soluble substrates, is one of the major rate-limiting steps for anaerobic fermentation of WAS. Therefore, the mechanism of how FA+RL improves SCFA production from WAS is firstly investigated in sludge solubilization, which is often evaluated via the variations of soluble proteins in the fermentation liquor and VSS reduction.

Apart from sludge solubilization, hydrolysis, acidogenesis and methanogenesis were also involved in anaerobic fermentation, which affect SCFA production significantly (Xu et al., 2017). To evaluate impact of FA + RL on these processes, the following batch tests were performed with synthetic wastewater containing model compounds as fermentation substrates. In the tests, 12 serum bottles with a working volume of 500mL,

divided into three groups (namely, Hydrolysis Test, Acidogenesis Test and Methanogenesis Test) with four in each, were conducted. The operating steps for these tests were described below.

Hydrolysis Test: Firstly, 0.45 g dextran (average molecular weight 23800, a model carbohydrate compound) were added to 1500 mL Milli-Q water to formulate synthetic wastewater used in this batch test. Then, 360 mL of synthetic wastewater were respectively added into four bottles and finally each reactor was inoculated with 40 mL of same inoculum, which was collected from an anaerobic sludge digester operated in our laboratory. Among there bottles, one reactor was defined as the control without any pretreatment. For comparison, the other three bottles were set as 62.6 mg/L FA, 0.04g RL/g TSS and 62.6 mg/L FA+ 0.04g RL/g TSS, respectively. All other experiment conditions were the same as those described in Section 2.2. The hydrolysis rate was indicated by the degradation rates of dextran.

Acidogenesis Test: The operation of this test was operated with the same method described in Hydrolysis Test except that the organic compounds (i.e., dextran) in the synthetic wastewater were replaced by glucose. The impact of FA +RL on acidogenesis process could be obtained by comparing the degradation efficiency of glucose.

Methanogenesis Test: The operation of this group was performed the same as depicted in Hydrolysis Test except that the organic compounds (i.e., dextran) were replaced with 4 g/L sodium acetate. By analyzing the degradation rate of acetate in these reactors, the effect of FA +RL on the process of methanogenesis could be assessed.

2.4. Phosphorus and ammonium recovery as MAP

Large amounts of NH_4^+ -N and PO_4^{3-} -P were released and observed in this study. To assess the feasibility of the released NH_4^+ -N and PO_4^{3-} -P being recovered as MAP, the supernatant of the anaerobic fermentation sludge was collected. The theoretical molar mass proportions of Mg^{2+} , NH_4^+ and PO_4^{3-} for MAP precipitation was

1:1:1 (Tong and Chen, 2007). Since a certain amount of NH_4^+ -N and PO_4^{3-} -P were released, Mg^{2+} is needed to add additionally to obtain MAP. First of all, 200 mL supernatant of FA+RL reactor was added into 400 mL beaker and pH was maintained at 9.0 ± 0.05 by adding 2.0 M hydrochloric acid or 2.0 M sodium hydroxide during the entire time. Secondly, magnesium sulfate ($\text{MgSO}_4 \cdot 6\text{H}_2\text{O}$) was added and the corresponding molar ratio of Mg/P was 1.5, which was enough for nitrogen and phosphorus crystallization as MAP. Finally, the mixture was filtered using a $0.45\mu\text{m}$ filter membrane. The collected precipitate was washed thoroughly with deionized water for several times and then dried for 48 h. The test was conducted in a temperature-controlled room ($25 \pm 0.5^\circ\text{C}$) with the stirring rate of 150 rpm/min (Xu et al., 2018b).

2.5. Analytical methods

The determinations of COD, NH_4^+ -N, PO_4^{3-} -P, TSS, and VSS were conducted according to standard methods (APHA, 1998). Protein was measured using the Lowry's method with BSA as the standard (Lowry et al., 1951). SCFA was performed by gas chromatography, and the operation procedures were according to our previous study and detailed in Supplementary data (Chen et al., 2018). The activity of key hydrolytic enzymes (protease) and acetate-forming enzymes (acetate kinase (AK)) were measured according to previous study (Goel et al., 1971; Feng et al., 2009). The analytical procedure of coenzyme F420 was according to previous study (Zhao et al., 2017). The morphology and crystal structure of the collected precipitate was respectively analyzed by emission scanning electron microscopy (SEM, Hitachi S-4800) and X-ray diffractometer (XRD, Bruker AXS D8 Advances) (Chen et al., 2017a; Chen et al., 2017b).

2.6. Statistical analysis

All assays in this study were performed in triplicate. An analysis of variance was used to evaluate the significance of results, and $p < 0.05$ ($p > 0.05$) was considered statistically significant (insignificant).

3 Results and discussion

3.1. SCFA productions under different treatment conditions

The SCFA accumulation profile from anaerobic fermentation of WAS with fermentation time under different treatment conditions was shown in Fig. 1a. In this study, it could be found that SCFA production was remarkably enhanced by either FA pretreatment or the addition of RL, as compared with the control (without any pretreatment). However, when FA pretreatment and RL were combined, the SCFA production further increased. For instance, under the addition of RL (0.04 g/g TSS), with the increase of FA level from 35.4 to 62.6 mg/L, the maximal SCFA production increased from 267.4 ± 11.2 to 324.7 ± 13.9 mg COD/g VSS. When the content of FA further increased to 171.3 mg/L, the SCFA production decreased to 279.7 ± 14.9 mg COD/g VSS. Thus, the optimal FA pretreatment concentration for SCFA production under the addition of RL was 62.6 mg/L, and the corresponding maximum SCFA production (324.7 ± 13.9 mg COD/g VSS) was about 1.63, 1.41 and 5.95 times higher than that of 62.6 mg FA/L (198.8 ± 10.5 mg COD/g VSS), 0.04 g RL/g TSS (230.6 ± 12.1 mg COD/g VSS) and control (54.5 ± 2.7 mg COD/g VSS), respectively. Moreover, it should be noted that the fermentation time was 5 d (1 d pretreatment + 4 d fermentation) for maximal SCFA production with FA + RL, which was shorter than that of sole FA (6 d) and control (8 d), respectively. It was obvious that SCFAs production and fermentation time were all promoted by the addition of RL. Previous study reported that SCFA production from WAS could be remarkably enhanced when the sludge was pretreated with FA for 3 d, and the corresponding maximum SCFA production was 267.2 mg COD/g VSS at 8 d (3 d pretreatment + 5 d fermentation). Though only about 19.3% increase of SCFA production was achieved under 62.6 mg FA/L + 0.04 g RL/g TSS compared to previous study, the pretreatment time and fermentation time of maximal SCFA production were shortened by 2 d and 3 d, respectively. It should be emphasized that FA pretreatment requires a certain amount of alkalis to control pH, thus shortening pretreatment time from 3 d to 1 d would save at least 50% of alkalis. Apparently, the combined method (FA+RL) not only accelerated the SCFA production, but

also was more cost-effective and eco-friendly.

The composition of SCFA could impact the efficiency of BNR, thus the percentage of six individual SCFA, i.e., acetic, propionic, n-butyric, iso-butyric, n-valeric and iso-valeric, under different treatment conditions at the optimum fermentation time should be investigated. From Fig.1b, it can be found that the acetic acid and propionic acid, considered as the prior carbon source in WWTPs, were the two highest individual SCFA in these reactors with a total percentage of 70.1-77.7%.

3.2. Effect of FA pretreatment combined with RL on the sludge solubilization

Generally, the WAS solubilization was considered the first and also one of the major rate-limiting process in the anaerobic fermentation. Thus, the effect of different treatment conditions on the solubilization was first evaluated and indicated by soluble protein content in fermentation liquor and VSS reduction. Fig. 2 shows the changes of soluble proteins in fermentation liquor during the initial 56 h. It can be found that the soluble proteins increased with time in all reactors. For example, the concentration of soluble proteins in the control without any pretreatment increased to 506 ± 41.3 mg COD/L. When FA or RL was applied to pretreat sludge, the release of soluble proteins was enhanced, and the corresponding concentrations increased to 1709 ± 91.5 mg COD/L and 1902 ± 119.1 mg COD/L, respectively. However, soluble proteins further increased when the integration method (FA + RL) was applied, resulting in more protein release and reaching to 2725 ± 103.3 mg COD/L, which was 5.38, 1.59 and 1.43 times higher than that of the control, solo FA and solo RL, respectively.

It was known that VSS reduction is also relevant to the solubilization of sludge. It was observed that 4.1%, 17.2% and 18.6% of VSS reduction was respectively obtained in the control, solo FA and solo RL reactor after 56 h fermentation, whereas 24.7% of VSS reduction was achieved in FA + RL reactor (see Supplementary material). It was reported that FA and RL could enhance the disruption of EPS and cell

envelop of sludge cells (Wang et al., 2018d; Wu et al., 2017). In this study, RL was added after 1 d of FA pretreatment. Therefore, the combined method could accelerate the lysis of sludge cells compared with solo treatment condition. It seems that FA pretreatment combined with RL have a positive synergistic effect on sludge solubilization, releasing more soluble substrates, which was beneficial for the subsequent production SCFA.

3.3. How does FA pretreatment combined with RL affect hydrolysis, acidogenesis and methanogenesis?

After sludge solubilization, the solubilized products (i.e., macromolecular organics) will be further hydrolyzed into small molecule organics, which are SCFA-producing substrates. Therefore, the underlying mechanism of how FA pretreatment combined with RL affect hydrolysis process was subsequently investigated, which was conducted with synthetic wastewater containing dextran. As shown in Table 2, compared with the control ($70.1 \pm 3.8\%$), the degradation efficiency of dextran was decreased to $61.4 \pm 3.0\%$ in the presence of FA whereas the dextran degradation efficiency significantly increased to $94.5 \pm 4.2\%$ under the addition of RL. FA suppressed the solubilized substrates hydrolysis mainly due to cell inactivation caused by the diffusion of FA through the cell membrane (Kayhanian 1999), whereas RL could accelerate the disintegration of cell membrane/EPS to release hydrolysis enzymes to enhance hydrolysis (Zhou et al., 2015). Moreover, RL as glycolipid bio-surfactant could increase the C/N ratio of fermentation substrates. And the higher C/N ratio is conducive to the conversion of organic substances (Feng et al., 2009). The cumulative effect was achieved when two different working principles were combined, resulting in a $82.6 \pm 2.9\%$ dextran degradation efficiency in the combined reactor, which was higher than that of the control. Consequently, the results suggested that FA + RL enhanced hydrolysis process.

After hydrolysis, the hydrolyzed products will undergo acidogenesis process and then convert to SCFA. To evaluate the impact of FA + RL on acidogenesis process, batch test was carried out with synthetic

wastewater containing glucose, and the corresponding result was illustrated in Table 2. The degradation efficiency of glucose in the FA+ RL reactor was lower than those of the control, solo FA (62.6 mg/L) and solo RL (0.04g/g TSS). For instance, the degradation efficiency of glucose in the FA + RL reactor was $28.1 \pm 1.8\%$ on 1 d, whereas the corresponding data in the control reactor, sole FA (62.6 mg/L) reactor and solo RL (0.04g/g TSS) reactor were $40.5 \pm 1.4\%$, $35.2 \pm 2.4\%$ and $33.9 \pm 3.2\%$, respectively. The same observation was also made on 3 d. The results above indicated that FA combined with RL suppressed acidogenesis processes.

In general, SCFA produced in acidogenesis process can be further bio-converted to methane in methanogenesis process. Therefore, the impact of the combined method on this process was also evaluated. As shown in Table 2, compared with the control reactor, the degradation efficiency of acetate in the sole FA (62.6 mg/L) reactor and solo RL (0.04g/g TSS) reactor were seriously inhibited. For instance, $26.8 \pm 3.9\%$ and $21.3 \pm 2.3\%$ of acetate was degraded on 3 d fermentation in the sole FA (62.6 mg/L) reactor and solo RL (0.04g/g TSS) reactor, the corresponding degradation efficiency in the control reactor was $45.5 \pm 3.9\%$. However, only $10.3 \pm 2.4\%$ of acetate was degraded on 3 d fermentation when FA and RL were combined, which indicated the methanogenesis was further inhibited. According to previous studies, both FA and RL could suppress the activities of methanogens during the anaerobic fermentation, which made it possible to understand the synergistic inhibitory effect on methanogenesis process when FA and RL are combined. Therefore, the greater the inhibition on methanogenesis process, the higher the SCFA production.

3.4. Effect of FA pretreatment combined with RL on the activities of key enzymes responsible for SCFA production

The production of SCFA from WAS anaerobic fermentation was mainly caused by biological effects, which could be determined by the activities of key enzymes. Therefore, the activities of key enzymes related to hydrolysis, acidogenesis and methanogenesis microbes were also evaluated. In this study, three kinds of

representative key enzymes such as protease, acetate kinase and coenzyme F₄₂₀, which are respectively responsible for hydrolysis (i.e. protein hydrolysis), acidogenesis (i.e. SCFA production) and methanogenesis (i.e. methane production), were selected to be assessed. As shown in Fig.3, compared with the control reactor and solo FA reactor, it can be found that the activity of protease was enhanced by RL reactor whereas acetate kinase and coenzyme F₄₂₀ were inhibited. The same observation was also observed in FA combined RL reactor, however, the activity of acetate kinase was further inhibited and greater inhibition to the coenzyme F₄₂₀. For instance, FA + RL caused 29.5% of inhibition to acetate kinase whereas 76.8% of inhibition to coenzyme F₄₂₀. All these observations were in accord with the results of Hydrolysis Test, Acidogenesis Test and Methanogenesis Test reported above, strengthening the understanding of underlying mechanisms.

3.5. Phosphorus and ammonium recovery as MAP

During WAS fermentation, the released NH₄⁺-N and PO₄³⁻-P in the fermentation liquor were also measured and shown in Fig. 4. The concentrations of NH₄⁺-N and PO₄³⁻-P increased regardless of whether sludge was treated. Compared to the control reactor, FA reactor and RL reactor, the level of released NH₄⁺-N and released PO₄³⁻-P were much higher when FA was combined with RL. For instance, the released NH₄⁺-N concentration was 296 ± 17.8 mg/L in the combined reactor. However, the corresponding data were respectively 81 ± 6.1 mg/L, 235 ± 15.7 mg/L and 164 ± 10.3 mg/L in the control, RL and FA, which were improved by 3.65, 1.26 and 1.81 time to the integration system. Same observation was also made for released PO₄³⁻-P. It was well known that nitrogen and phosphorus are important components of cells and EPS of WAS, lysis of microbial cells and the degradation of the organic matters could release NH₄⁺-N and PO₄³⁻-P during WAS fermentation (Yi et al., 2013). Therefore, releasing more NH₄⁺-N and PO₄³⁻-P in the combined reactor suggested higher sludge solubilization efficiency, which therefore consolidated the results reported above and shown in Fig.2.

To assess the feasibility of released nitrogen and phosphorus from WAS recovery as MAP, the

experiment of MAP preparation using the supernatant of FA + RL reactor and Mg^{2+} was conducted. SEM and XRD were respectively used to describe the morphology and structure characteristics of the prepared precipitate while the corresponding data were shown in Supplementary material. It can be observed that prepared precipitate showed typical orthorhombic shapes, which was in agreement with the morphology of struvite reported previously (Dunn et al., 2004). Furthermore, the diffraction peaks exist on the XRD pattern of the prepared precipitate can be clearly identified, which was similar to position and intensity of the reference struvite (see Supplementary material). The above results confirmed that the prepared precipitate in this study was MAP, suggesting that the combined method (FA + RL) not only improve SCFA production but also realize and enhance resource (i.e., nitrogen and phosphorus) recovery as MAP from WAS.

3.6 Implication for WWTPs

Nowadays, the society is gradually realizing that WWTPs can be a source of valuable resources, not "waste". The function change of WWTPs, from BNR to obtain desirable BNR as well as energy/resource recovery simultaneously, has recently attracted growing attention. However, insufficient carbon sources in wastewater and large amounts of sludge generated as by-product during wastewater treatment inevitably increase the operating costs of wastewater treatment plants, making it difficult to achieve functional changes. Therefore, it is urgent to develop appropriate technology to alleviate and/or even solve the above two problems, and finally promote the functional transformation of WWTPs. Anaerobic fermentation has attracted more and more attention, which not only can in situ obtain the value-added product (i.e., SCFA) to enhance the performance of BNR, but also realize the reuse and reduction of WAS. However, it is often upset by low production of SCFA from WAS, thus limiting its large-scale application. As described in Introduction, although many methods were applied to improve SCFA yield from WAS, most of them are at the expense of high energy and/or substantial chemical input, which is neither environmentally friendly nor not conducive to

its long-term sustainable application. In comparison, the strategy (FA + RL) reported for the first time in this study demonstrated that SCFA production from WAS could also be significantly improved while FA and RL used in this study could be in situ obtained during anaerobic fermentation and recycled in the sludge process without affecting wastewater treatment (see below for details), which not only save energy and/or chemical but also has no negative impact on the environment. Moreover, the underlying mechanisms of how FA combined with RL improves SCFA production are also investigated in this study. It was observed that the combined method accelerated sludge solubilization and hydrolysis and inhibited severely SCFA consumption process (i.e., methanogenesis), which thereby enhanced SCFA production from WAS. Therefore, desirable SCFA production with low cost and eco-friendly as well as scientific understanding of the effect of FA combined with RL on anaerobic fermentation of WAS are beneficial to the application of the strategy (FA + RL), generating great significance for the operation of WWTPs.

According to the findings reported in this paper, we apply FA combined with RL method to propose a concept of “biological nutrient removal-energy and resource recovery” for the operation of a WWTP (Fig.5). In developing countries (e.g., China), when the influent carbon source in the wastewater is insufficient under the traditional operating mode (see Supplementary material), additional carbon sources (i.e., acetate and propionate) are usually added to ensure the desirable BNR. Moreover, WAS rich in useful substances (i.e., organic matter and phosphorus) is usually directly buried after dewatering, which caused serious waste of resources and increased operating costs. In the concept with combined FA and RL presented in this study, the concentrated WAS is first pretreated with FA for 24 h. Then, both pretreated WAS and in situ generated RL are added to the anaerobic fermenter for fermentation, considerable SCFA can be generated with high WAS reduction during anaerobic fermentation. After fermentation and settling, the fermentation liquor (rich in generated SCFA, released organic matters, $\text{NH}_4^+\text{-N}$ and $\text{PO}_4^{3-}\text{-P}$) is introduced to RL production, where the

corresponding fermented sludge is transferred to dewatering and disposal. The production of RL from fermentation liquor mainly depends on the bacterium *Pseudomonas aeruginosa*. The produced RL is centrifuged and added to the anaerobic fermenter and then the fermentation liquor is transferred to phosphorus and nitrogen recovery system, where the magnesium salts are added to recover phosphorus and nitrogen as MAP by coagulation/precipitation process. Phosphorus and nitrogen recovery not only reduces the loading of wastewater treatment, but also obtains valuable resource (i.e., MAP) to reduce operating costs of WWTPs. Finally, the fermentation liquor is returned and applied to pretreat WAS, and the SCFA is used to supplement the influent carbon source of WWTPs. Based on above operational paradigm, desirable BNR, energy and resource recovery as well as considerable WAS reduction could be simultaneously achieved. The combined method proposed in this study could enhance effectively energy and resource recovery, providing supports for sustainable operation of WWTPs.

Given the large quantities of WAS daily generated worldwide, the proposed FA combined RL strategy could have significant benefits as described above. However, the dosage of RL was according to the previous (Zhou et al., 2013). Moreover, the proposed strategy has only been tested on a laboratory scale so far. Since the aim of this study is to identify the feasibility of enhancing SCFA yield from WAS using combined FA with RL, technical optimization was not conducted. Therefore, it is necessary to conduct a comprehensive optimization under real WWTPs when the strategy will be applied in the future.

4. Conclusion

The feasibility of enhancing SCFA production from anaerobic fermentation with a new, renewable and high-efficient method, FA + RL, has been successfully verified, and the underlying mechanisms of the combined method affecting SCFA are also evaluated in this study. Results showed that a synergy effect on WAS anaerobic fermentation occurred when the FA + RL were applied, obtaining a high SCFA yield of 324.7

± 13.9 mg COD/g VSS after 5 d anaerobic fermentation. The combined method significantly accelerated organics release and then facilitated the solubilization and hydrolysis processes while suppressed the acidogenesis and methanogenesis process.

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Supporting Information

This file contains additional analytical methods and 3 figures.

Notes

The authors declare no competing financial interest.

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Figure legends

Fig. 1. The total SCFA production under different operational conditions during sludge anaerobic fermentation (a) and the percentage of individual SCFA under the optimal condition (b). Error bars represent standard deviations of triplicate tests.

Fig. 2. Effect of different treatment conditions on the release of soluble protein during the initial 56 h. Error bars represent standard deviations of triplicate tests.

Fig. 3. Effect of different treatment conditions on the relative activities of key enzymes in the long-term operated reactors. Error bars represent the standard deviations of triplicate measurements.

Fig. 4. The release of $\text{NH}_4^+\text{-N}$ and $\text{PO}_4^{3-}\text{-P}$ under different operational conditions during WAS anaerobic fermentation.

Fig. 5. The proposed FA pretreatment combined RL addition method for enhancing resource and energy recovery in a WWTP.

Table legends

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Table 1

The operation conditions applied in this study.

Reactor	NH ₄ ⁺ -N (mg/L)	FA (mg/L)	pH	Pretreatment time (d)	Temperature (°C)	RL (mg/g TSS)
1	22.8 ^a	0.1	NC	0	25	0
2	22.8 ^a	8.26	9	1	25	0
3	22.8 ^a	8.26	9	1	25	0.04
4	97.8	35.4	9	1	25	0.04
5	172.8	62.6	9	1	25	0.04
6	322.8	116.9	9	1	25	0.04
7	472.8	171.3	9	1	25	0.04
8	172.8	62.6	9	1	25	0

^a The value was the background concentration of ammonium.^b NC = Non-controlled.

Table 2Effect of different conditions on degradation rate of model compounds with time. ^a

Treatment condition	Time(d)	Degradation rate (%)		
		Dextran	Glucose	Acetic
Control	1	37.6 ± 1.6	40.5 ± 1.4	20.3 ± 2.1
	3	70.1 ± 3.8	79.6 ± 2.3	45.5 ± 3.9
62.6 mg FA/L	1	29.8 ± 2.5	35.2 ± 2.4	10.3 ± 1.2
	3	61.4 ± 3.0	69.4 ± 4.0	26.8 ± 3.9
RL	1	51.2 ± 3.6	33.9 ± 3.2	9.5 ± 1.4
	3	94.5 ± 4.2	68.2 ± 2.9	21.3 ± 2.3
62.6 mg FA/L +RL	1	46.3 ± 3.1	28.1 ± 1.8	4.3 ± 1.5
	3	82.6 ± 2.9	58.5 ± 4.0	10.3 ± 2.4

^a Data are shown as arithmetic mean of three replicates ± standard deviation.

Highlights:

- FA pretreatment combined RL enhanced SCFA production and shortened fermentation time.
- FA combined with RL caused a positive synergetic effect on SCFA production and sludge solubilization.
- FA combined with RL severely inhibited methanogenesis process.
- A potential and practical concept of enhancing energy and resource recovery from WWTPs was proposed.

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Feasibility of enhancing short-chain fatty acids production from waste activated sludge after free ammonia pretreatment: Role and significance of rhamnolipid

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