Alkaline fermentation of waste activated sludge stimulated by saponin: volatile fatty acid production, mechanisms and pilot-scale application

Xiangfeng Huang, Tianshuai Mu, Changming Shen, Lijun Lu and Jia Liu

ABSTRACT

Volatile fatty acid (VFA) production stimulated by saponin (SP), an environmentally friendly bio-surfactant, was investigated during sludge alkaline fermentation in laboratory studies and pilot applications. The combined use of SP and pH 9 condition significantly enhanced VFA production to approximately 425 mg COD/g VSS, which was 4.7-fold of raw sludge and 1.5-fold of sole pH 10 adjustment (the optimum pH for alkaline fermentation). Further results indicated that SP & pH 9 condition provided sufficient substrates for acidification and decreased the consumption of VFAs through methanogenesis. Moreover, SP accompanied by moderate alkaline condition (i.e. pH 9) showed weaker inhibitory effects on key enzyme activities and metabolic potential of acidification microorganisms than sole pH 10 adjustment. On this basis, a pilot-scale system involving anaerobic fermentation and anaerobic-anoxic-aerobic step-feed bioreaction tanks was established to study the potential of VFAs as supplementary carbon sources for wastewater treatment. The influent of the pilot system was sanitary wastewater characterized by low C/N ratios from a scenic rural area. After flocculation and nutrient precipitation, the fermentation supernatant was mixed with the influent at a volume ratio of 1:30. With this approach, nitrogen and phosphorus concentrations in effluent fulfilled the first-A wastewater discharge standard in China.

Key words | anaerobic fermentation, metabolic activity, saponin, volatile fatty acids, waste activated sludge

INTRODUCTION

The shortage of influent biodegradable organic carbon sources limits the efficiency of biological nutrient removal in some wastewater treatment plants (WWTPs), particularly in southern China. As external carbon sources, volatile fatty acids (VFAs) generated from anaerobic fermentation of waste activated sludge (WAS) have attracted considerable attention because of their superior capacity toward pure acetic acid (Zhang & Chen 2009).

Physical pretreatments, including ultrasound, microwave or heat, accompanied by process changes, including pH adjustment or the addition of accelerants, such as surfactants, are usually applied to WAS anaerobic fermentation to improve VFA or biogas yield (Lee et al. 2014; Zhang et al. 2015). Many publications proved that pH 10 is a promising fermentation condition to achieve maximum VFA production (Yu et al. 2008; Wu et al. 2010). However, the metabolic activities of acid-producing bacteria were markedly inhibited (Jie et al. 2014), thus restricting VFA enhancement. With regard to surfactants, bio-surfactants have become alternatives for chemical surfactants (Islas et al. 2010) because of their biodegradability, environmental compatibility and high surface/interface activities. Among these bio-surfactants, rhamnolipid (Yi et al. 2015), surfactin and saponin (SP) (Huang et al. 2015) have drawn much attention.

Under this consideration, researchers have combined pH adjustment with bio-surfactants to stimulate VFA yields, among which only rhamnolipid has attracted considerable attention. For instance, Luo et al. (2015) reported that rhamnolipid addition at pH 9 increased VFA concentration to 1.4-fold versus sole treatment of surfactant addition or pH 10 incubation. However, the antimicrobial
effects of rhamnolipid can lead to an evident decrease in bacterial diversity (Zhou et al. 2015). In our previous study, we found that SP, a bio-surfactant extracted from plants, can promote VFA production and lead to less release of nitrogen and phosphorus into the supernatant compared with rhamnolipid (Huang et al. 2015). Therefore, SP could be expected to stimulate VFA production during WAS alkaline fermentation.

To date, few pilot-scale applications of fermented VFAs from WAS have been reported. Li et al. (2011) established a pilot-scale study operation of WAS fermentation at pH 10 and successfully applied the fermentation products to an anaerobic-anoxic-aerobic (A/A/O) municipal wastewater system (10 m³/d). However, low concentrations of VFA production, unwanted release of nitrogen and phosphorus and corrosion effects of alkaline conditions on the equipment restrict the widespread use of sludge-derived VFAs in WWTPs. Moreover, the application of bio-surfactants to pilot-scale works has yet to be reported.

In this study, we investigated an optimized strategy involving the combination of SP and alkaline adjustment for VFA production. Then, a pilot-scale study was conducted based on batch experiments to produce VFA-containing fermentation liquid, which was used as supplementary carbon sources to remove nutrients in an A/A/O step-feed technology.

**MATERIALS AND METHODS**

**Batch experiments**

The WAS used in this study was obtained from a secondary sedimentation tank in a sewage treatment plant (Shanghai, China). The sludge was first screened by an 18-mesh sieve and then concentrated by settling for 24 h at 4 °C. The characteristics of the sludge were as follows: pH 6.73 ± 0.03, total suspended solids (TSS) 15.0 ± 0.2 g/L, volatile suspended solids (VSS) 10.6 ± 0.3 g/L, total chemical oxygen demand (TCOD) 15,380 ± 480 mg/L, soluble COD (SCOD) 129 ± 10 mg/L, soluble NH₄⁺ – N 9.1 ± 0.3 mg/L, soluble phosphorus 19.6 ± 0.5 mg/L, total proteins 8,955 ± 348 mg/L, total saccharides 2,105 ± 136 mg/L and lipid and oil 157 ± 12 mg/L. SP was purchased from Cantin Biotechnology Co., Ltd (Shanghai, China).

Batch laboratory-scale anaerobic fermentation tests were conducted in 500 mL reactors (conical flasks with seals) with 300 mL of WAS. Two groups with pH adjustment were set. One group was combined with SP addition, and the other group was subjected to sole pH adjustment. The pH values of both groups were maintained as 9.0, 10.0 and 11.0, respectively, adjusted with 6 M NaOH every 12 h. Aside from the aforementioned two groups, two other tests were set as follows. One test with sole SP addition was called the SP or the SP test. The SP dosage was 0.10 g/g TSS, which was obtained from dosage gradient tests in our previous study (Huang et al. 2015). The other test without pH adjustment or SP addition (that is, raw sludge) was called the uncontrolled test. The pH values of all reactors were measured daily and shown in the supplementary material (Figure S1, available with the online version of this paper).

Before fermentation, all reactors were purged by nitrogen gas for 5 min and capped with rubber stoppers to eliminate oxygen. Then, the reactors were cultivated under 30 ± 1 °C, 100 rpm for 12 days. Liquid and gas samples were collected at 60 min and at days 1, 2, 3, 4, 5, 6, 8, 10 and 12 during fermentation. Samples at day 6 were selected for Biolog assay. Fermentation liquid samples were centrifuged at 1,000 rpm for 15 min and filtered through a 0.45 μm PTFE membrane. The filtrate was stored at 4 °C and analyzed for the concentrations of proteins, polysaccharides, humic substances, NH₄⁺ – N, total phosphorus (TP), VFAs and its compositions and enzyme activities. All experiments were conducted in triplicate, and the mean values are presented.

**Biolog assay**

Sludge samples were obtained on the 6th day and incubated in Biolog EcoPlates. Pretreatment methods can be found in the supplementary material. The variation of average well color development (AWCD, calculated from the optical density (OD) values) with incubation time was used to evaluate the potential and capability of microbial substrate utilization. Data at 60 h, which was the time point with the largest changes in OD values for all samples, were selected to calculate the Shannon, McIntosh and Simpson diversity indices, which could reflect the microbial diversity and evenness in WAS, as described by Magurran (1988). Normalized data at 60 h were used for principal component analysis (PCA), which was conducted to evaluate the differences in catabolic capabilities of microorganisms among samples (Garland & Mills 1991). The calculations of the AWCD and the three indices and the depiction of the PCA diagram are shown in the supplementary material.

**Outline of the pilot-scale works**

The pilot-scale works served as a modification and upgrade project for a WWTP, which was operated with the oxidation
ditch process in Ji’an City, Jiangxi, China. The configuration was composed of three parts, as shown in Figure 1.

**Anaerobic fermentation system**

The anaerobic fermentation system included one sludge storage tank (1,400 mm × 1,400 mm × 1,600 mm) and two anaerobic fermentation tanks (1,400 mm × 1,400 mm × 1,600 mm each), which were operated at ambient temperature. The operating conditions for sludge fermentation tanks in the fermentation system were determined in advance by conducting a series of conditions (i.e., uncontrolled, pH 9, pH 10, SP, SP & pH 9 and SP & pH 10) according to the batch experiments. The fermentation time was 6 days. The SP dosage was the same as that in the batch experiment (0.10 g/g TSS) and was added in one time into the anaerobic fermentation tanks. After a period of fermentation (i.e., 6 days), another dosage of SP was added. The optimal condition was screened to obtain fermentation liquid. The two fermentation tanks were separately started up in sequence and consequently at an interval of 72 h, which was half of the fermentation time. In this manner, the two tanks could corporately provide continuous supply of fermentation liquid. Fermented WAS consisted of the sludge pumped from the sludge storage tank of the WWTP mentioned previously, as well as the sludge produced from the step-feed process of the pilot-scale project. The main parameters of the sludge in the sludge storage tank of the WWTP during the operating period are as follows: pH 6.74–6.85, TSS 15.7 g/L–17.8 g/L, VSS 5.6 mg/L–6.3 mg/L, TCOD 7,299 mg/L–8,021 mg/L, SCOD 128 mg/L–155 mg/L, soluble TP 9.7 mg/L–14.9 mg/L and soluble NH₄⁺-N 5.8 mg/L–12.1 mg/L.

**Fermentation liquid separation system**

The produced fermentation liquid was pumped into a separation system that employs simultaneous flocculation and nutrient removal to achieve sludge-liquid separation. One clarifier (1,400 mm × 1,400 mm × 1,600 mm), one dewatering equipment and one fermentation liquid storage tank (1,400 mm × 1,400 mm × 1,600 mm) were included in

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Figure 1 | General configuration of the pilot-scale works.
the system. Jar tests using practical fermentation liquid were performed in advance to determine the reagent dosages (polyaluminum chloride (PAC, 120 g/L), cationic polyacrylamide (PAM, 1 g/L) and MgCl₂) in the clarifier. We conducted a comprehensive test in which the mass ratios of PAC/PAM were set at 0:1, 5:1, 10:1, 15:1, 20:1, 30:1, 40:1, 50:1 and 1:0 and the dosages of PAM were 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15% and 16% of the fermentation liquid volume. Then, MgCl₂ dosage was determined under a gradient of 1.6, 2.4, 3.2, 4.8, 8.1, 12.8, 16.0, 19.2, 24.0 and 32.0 mmol/L. The optimal separation conditions were as follows: optimal Mg/P ratio 2.9:1, MgCl₂·6H₂O dosage 0.97 kg/m³, PAC/PAM ratio 20:1 (g/g), PAC dosage 4.7 g/kg TS and PAM dosage 93 g/kg TS. After sludge-liquid separation, the supernatant was pumped into the fermentation liquid storage tank, whereas the residue was first dewatered by the dewatering equipment and then pumped into the fermentation liquid storage tank.

Wastewater treatment system

The influent of the reactor was from the outlet of the rotational flow grit chamber of the WWTP mentioned previously. Basic operational parameters of the A/A/O step-feed reactors in the initial phase are presented in Table S1 (available with the online version of this paper). Three stages were operated successively at an influent flow of 45 m³/d in the A/A/O step-feed system. Stage I was operated with no inflow distribution for 28 days. In stages II and III, the influent was distributed unevenly into the anaerobic zone and the second and third anoxic zones at a volume ratio of 4:2:1. Stage II was operated without supplementation for 24 days. In stage III, VFA-containing fermentation supernatant was pumped from the fermentation liquid storage tank into the regulation pool as the external carbon source at a flow rate of 1.5 m³/d for 62 days, aiming at meeting the first-A wastewater discharge standard in China. The influent and effluent concentrations of COD, NH₄⁺-N, TN and TP in the pilot-scale works were measured three or four times per week.

Analytical methods

For batch experiments and pilot-scale works, measurement methods of proteins, polysaccharides, humic substances, NH₄⁺-N, TP, VFAs and its compositions and enzyme activities were in accordance with those in our previous study (Huang et al. 2015). One-way analysis of variance was used to test the significance of the results, and p < 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Effect of SP on VFA production from WAS alkaline fermentation

Figure 2(a) shows the time course profile of VFA production in the presence of SP during alkaline fermentation. For the group treated by sole pH adjustment, the order of VFA concentrations on the 6th day was pH 10 (approximately 293.0 mg
COD/g VSS) > pH 9 > pH 11 > uncontrolled. VFA yield in the pH 10 test was 3.2-fold of that in the uncontrolled test. This optimal pH value was consistent with the results of most research on alkaline sludge fermentation (Su et al. 2015; Jie et al. 2014). With regard to the group treated by SP combined with different alkaline conditions, VFA concentrations produced were further enhanced. The observed order switched to SP and pH 9 (425.2 mg COD/g VSS) > SP and pH 10 > SP and pH 11 > SP. In the SP and pH 9 test, VFA concentration reached the highest level observed and was approximately 150% of the production in the pH 10 test. After day 6, the VFA concentrations for some of the tests decreased possibly because of the consumption by methanogenesis. VFA production from sludge fermentation in the presence of surfactant and pH adjustment is rarely investigated. Luo et al. (2013) reported that the combined use of rhamnolipid (0.30 g/g TSS) and pH 9 increased VFA production to 313 mg COD/g VSS from 222 mg COD/g VSS at pH 7, which was lower than that in the present study.

According to composition analysis (Figure 2(b)), the top two individual VFAs were acetic and propionic acids after 6 days in all investigated reactors. The concentrations of acetic and propionic acids were the highest in the SP and pH 9 test. Reports have shown that acetic and propionic acids are the most desired substrates for nutrients removal (Elefsiniotis et al. 2004). Thus, VFAs produced at SP and pH 9 were a suitable option as supplementary carbon sources. In addition, the combination of SP and pH 9 represents a decreasing alkaline dosage and, thus, a more moderate condition.

**Mechanisms of VFA production from WAS alkaline fermentation stimulated by SP**

**Solubilization of WAS**

Solubilization of proteins, humic substances and polysaccharides is a prerequisite for acidogenic bacteria to produce VFAs (Riviere et al. 2009). Compared with sole SP addition or sole pH adjustment, the combination significantly increased the concentrations of the aforementioned organic matter (p < 0.05) during the initial 60 min of fermentation (Figure 3(a)). The levels of solubilization were comparable between the SP and pH 9 test and the pH 10 test for proteins (approximately 240 mg/L) and humics (approximately 180 mg/L). SP could effectively detach and disrupt the loosely bound extracellular polymeric substrates and the tightly bound EPS of sludge flocs (Zhou et al. 2013b). Moreover, as a surfactant, SP solutions present a relatively low surface tension and excellent surface activity (Huang et al. 2015), which can cause significant dissolution of organic matter. Under alkaline conditions, the repulsive forces among negatively charged EPS are enhanced and thus the organic matter are more easily solubilized (Wingender et al. 1999).

Nitrogen and phosphorus were also released alongside the solubilization and hydrolysis of organic matter and lysis of microorganisms (Guo et al. 2010), as shown in Figure 3(b). The order of NH$_4^+$-N concentrations was pH 10 > pH 9 > pH 11 > uncontrolled, irrespective of SP addition. In terms of TP concentrations, the SP & pH 9 test and the pH 10 test released both approximately 150 mg/L of TP, which was lower than that in the uncontrolled test (approximately 170 mg/L) and the SP test (approximately 220 mg/L). The ammonium concentrations decreased owing to the release of ammonia gas under strong alkaline conditions, and dissolved phosphorus can also precipitate from liquid (Celen & Turker 2001).

**Relative activities of key enzymes involved in VFA production**

Research has proven that, with the solubilization of organic matter, the immobilized hydrolytic enzymes in EPS are thus released into the fermentation liquid, which will further promote the hydrolysis of organic matter (Chen et al. 2013a). Thus, the activities of enzymes involved in VFA production were measured, and the results are presented in Table 1. In sole pH adjustment tests, the activities of neutral and alkaline protease as well as α-glucosidase were the highest at pH 10. The addition of sole SP resulted in minimal enhancement in hydrolytic enzyme activities. Compared with pH 10 adjustment, SP and pH 9 significantly improved the activities of acidic protease and α-glucosidase (p < 0.05) by 5.8-fold and 1.9-fold, respectively, and simultaneously decreased the activities of alkaline and neutral protease by 20–25%.

Moreover, SP (Huang et al. 2015) and alkaline conditions (Chen et al. 2013b) have been observed to affect the activities of enzymes related to microbial activity (dehydrogenase), acid formation (acetate kinase (AK)) and methanogenesis (coenzyme F$_{420}$). Table 1 shows that alkaline conditions exhibited a strong negative effect on the activities of the three enzymes. The relative activities of dehydrogenase in the SP and pH test were 20% higher than that in the pH 10 test. AK activity was unchanged in the SP and pH 9 test versus the uncontrolled test and was 2.8-fold of that observed in the pH 10 test. Although coenzyme F$_{420}$ activity in the SP and pH test were 60% higher than that of the pH 10 test, CH$_4$ production (Figure S2, available with the online version of this paper) confirmed
the significantly suppressed consumption of VFAs by methanogenesis at SP and pH 9.

**Biolog fingerprints of anaerobic fermentation sludge microbial communities**

The AWCD curves and diversity indices results are illustrated in Figure 4(a) and Table S2 (available with the online version of this paper), respectively. Increased pH resulted in significant inhibition effect on the microbial metabolic potential. Moreover, the decrease in the three diversity indices indicated the decreased capability of microbial species to adapt to and metabolize under strong alkaline conditions (Guo et al. 2010). The addition of sole SP exerted virtually no inhibition effect on metabolic capability, microbial abundance, diversity and evenness. The SP and pH 9 test showed slight effect compared with the uncontrolled test \((p > 0.05)\), but the three indices were one to two times that in the pH 10 test. These results were well-supported by PCA (Figure 4(b)).

Differences in metabolic capability were presented by the distance between the pH 10 test and the SP and pH 9 test.

Based on the results above, the stimulating mechanisms of the SP and pH 9 condition on VFA accumulation can be summarized in three aspects. First, the treatment significantly enhanced the dissolution efficiency of organic matter. Second, SP neutralized the inhibitory effects of pH 9 on certain microbes and enzyme activities. Third, the methanogenesis process was significantly inhibited. In addition, owing to the negligible effects of SP on microbial activity, SP and pH 9 will exert minimal influence on system stability and sludge activity when used in a practical project.

**Performance of the pilot-scale works**

**Influent characteristics of the pilot-scale works**

The influent of the pilot system was sanitary wastewater characterized by low C/N ratios from a scenic rural area.
The characteristics of the influent (without supplementation) during operation were as follows: COD 33 mg/L–95 mg/L (avg. 61 mg/L), TN 11.1 mg/L–29.3 mg/L (avg. 21.2 mg/L), \( \text{NH}_4^+ - \text{N} \) 9.7 mg/L–29.1 mg/L (avg. 20.0 mg/L) and TP 1.18 mg/L–4.61 mg/L (avg. 2.9 mg/L). The low concentrations of COD and TN were due to the mixed combined collection pipelines of rainwater and sewage systems, which consist not only of sanitary wastewater but also of rainwater along the surrounding area and its overflow. Therefore, exceptionally low C/N ratios often occur, particularly during the rainy season in southern China. When the C/N ratio was lower than 3.5, nitrogen and phosphorus concentrations in effluent could not reach the standard by adjusting operating conditions and parameters (Zhu et al. 2008). Thus, the pilot-scale works is aimed to supplement external carbon sources from fermentation liquids to increase the C/N ratio and subsequently enhance the nitrogen removal process.

<table>
<thead>
<tr>
<th>Type of enzymes</th>
<th>uc relative enzymatic activity</th>
<th>uc test ((\times 10^{14}, \text{g VSS}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutral protease</td>
<td>1.00</td>
<td>290.6 (\text{mg min}^{-1})</td>
</tr>
<tr>
<td>Acidic protease</td>
<td>1.00</td>
<td>249.1 (\text{mg min}^{-1})</td>
</tr>
<tr>
<td>Alkaline protease</td>
<td>1.00</td>
<td>124.6 (\text{mg min}^{-1})</td>
</tr>
<tr>
<td>(\alpha)-glucosidase</td>
<td>1.00</td>
<td>73.0 (\mu\text{M min}^{-1})</td>
</tr>
<tr>
<td>Dehydrogenase</td>
<td>1.00</td>
<td>308.6 (\text{mg min}^{-1})</td>
</tr>
<tr>
<td>Acetate kinase</td>
<td>1.00</td>
<td>44.0 (\text{mM min}^{-1})</td>
</tr>
<tr>
<td>Coenzyme F_{420}</td>
<td>1.00</td>
<td>2,404.7 (\mu\text{M})</td>
</tr>
</tbody>
</table>

*The relative enzyme activity of the uncontrolled test is set as 1.00. The values in parentheses are standard deviations.

uc stands for the uncontrolled test.

Figure 4 | AWCD curves (a) and PCA (b) under the effects of SP and alkaline conditions.
Anaerobic fermentation and separation of VFA-containing fermentation liquid

Fermentation conditions were first determined onsite. VFA production at ambient temperature in the pilot-scale study is presented in Figure S3 (available with the online version of this paper). The order of the VFA yields was the same as that in the batch experiments. The SP and pH 9 condition achieved the highest level (approximately 370 mg COD/g VSS, VSS 7.1 g/L) on the 6th day, i.e. 23-fold and 2.3-fold of the yields in the uncontrolled test and the pH 10 test, respectively. Therefore, SP and pH 9 was selected as the optimum anaerobic fermentation condition. The lower operating temperature than that in the batch experiments restricted the VFA yields.

Then, the fermentation liquids were flocculated and the nutrients were removed. The basic characteristics of the fermentation liquid under optimal recovery conditions before and after separation are summarized in **Table 2**. It can be seen that the specific resistance filtration (SRF) was reduced to less than $5 \times 10^{12}$ m/kg, which was beneficial for the dewatering equipment. Approximately 65% and 93% of the NH$_4^+$-N and soluble organic phosphorus (SOP) were recovered from the fermentation liquid, probably in the form of magnesium ammonium phosphate (MgNH$_4$PO$_4$$\cdot$6H$_2$O), as proven by Zhang & Chen (2013). Meanwhile, the separation process reduced only 2% and 12% of SCOD and VFA concentrations, respectively. The COD/N/P ratio reached 660:14:1, indicating that the fermented and separated liquid was of high quality as external carbon sources.

**Operation performance of the wastewater treatment system**

The removal performances of COD, TP, TN and NH$_4^+$-N are shown in **Figure 5**. In stage II (i.e. without supplementary carbon sources), TP concentrations in the effluent were all higher than that permitted by the previously mentioned standard. But this phenomenon occurred for approximately half of the operation time for TN concentrations. In stage III, separated fermentation liquid was used as external carbon sources. When COD in influent was lower than 120 mg/L, fermentation liquid was pumped into the influent to increase the C/N ratio to exceed 3.5. The influent COD concentrations increased owing to fermentation supernatant supplementation. No evident variations in TP concentrations were observed in the influent, whereas TN

**Table 2** | Basic characteristics of liquid before and after fermentation liquid separation

<table>
<thead>
<tr>
<th>Index</th>
<th>Before separation</th>
<th>After separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRF ($\times 10^{12}$ m/kg)</td>
<td>106</td>
<td>4</td>
</tr>
<tr>
<td>VFAs (mg COD/L)</td>
<td>2,631</td>
<td>2,580</td>
</tr>
<tr>
<td>SCOD (mg/L)</td>
<td>3,608</td>
<td>3,153</td>
</tr>
<tr>
<td>NH$_4^+$-N (mg/L)</td>
<td>161</td>
<td>56</td>
</tr>
<tr>
<td>SOP (mg/L)</td>
<td>54.1</td>
<td>3.9</td>
</tr>
</tbody>
</table>

SRF, specific resistance filtration; SOP, soluble organic phosphorus.
and NH$_3$-N concentrations increased by approximately 50%. All effluent concentrations fulfilled the standard during the operating period. The removal efficiencies of TN and TP increased from less than 20% in stages I and II to 34–76% and 80–98% in stage III, respectively. In addition, the sludge volume index was between 90 and 100, indicating favorable sludge settling capability. The activated sludge in bioreactors remained stable without sludge bulking or foaming. The results revealed that the liquids fermented under the SP and pH 9 condition can be efficiently used as the supplementary carbon sources to enhance nitrogen removal efficiency, which may contribute to solving the universal problem of substandard effluent nutrients for WWTPs in southern China.

**CONCLUSIONS**

A combination of SP and alkaline conditions can significantly increase VFA accumulation compared with alkaline treatment or SP addition alone. The highest VFA yield was produced under SP and pH 9, in which the solubilization and hydrolysis of organic matter and the activities of hydrolytic enzymes were increased, metabolic activities of microorganisms were only slightly affected, and methanogenesis was inhibited. In the pilot-scale study, the separated supernatant could be used as high-quality carbon sources to remove nitrogen and phosphorus. The results of this study provide an alternative strategy for generating high-yield VFAs in WAS alkaline fermentation, with negligible negative effects on device operation and the stability of activated sludge. The strategy shows considerable potential for application in WWTPs that lack carbon sources.

**ACKNOWLEDGEMENTS**

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